Better ‘Tools’ for Investigative Monitoring under the Water Framework Directive

by

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Abstract

Current approaches to sampling and analysis are thought to be unsuitable for investigative monitoring under the auspices of the European Union's Water Framework Directive. During this study, new sampling and analytical techniques were developed and tested that provide a 'toolkit' that can be utilised by most laboratories engaged in regulatory analysis of water samples. The techniques developed include;

i) Targeted screening methodology based on passive sampling in-conjunction with comprehensive two-dimensional chromatography mass spectrometry was developed and successfully applied to the broad-scope detection and determination of non-polar emerging pollutants and related contaminants in environmental waters. This method was found to be superior to existing approaches based on spot sampling and one dimensional gas chromatography.

ii) Modifications to the Chemcatcher® passive sampler to allow for the sampling of polar pollutants present in the water environment. Statistical analysis of the data obtained demonstrated that sampler performance was equivalent to that of the established POCIS passive sampler but with greater physical robustness together with simplified preparation and extraction procedures.

iii) Targeted screening methodology based on modifications to the existing Chemcatcher® passive sampler in-conjunction with liquid chromatography-high resolution mass spectrometry techniques for the identification of polar pharmaceutical residues present in the effluents of waste water treatment

plants located in south west Wales. Identification of analytes was strengthened through the development, validation and application of a novel accurate mass data-independent acquisition method and the high degree of analyte identification assurance obtained confirmed it to be analogous to traditional collision-induced dissociation transitions.

iv) The targeted application was supplemented through the development and application of a novel in silico methodology for retention time prediction which was successfully used to identify additional compounds in an extract obtained from a Chemcatcher passive sampler, and, thus, the preliminary identification of potential emerging contaminants.

Overall, this work has furthered the knowledge and capability of the sampling and analysis of existing and newly emerging contaminants in environmental waters.

Contents

List of Tables xiii

List of Figures xvii

List of Abbreviations xxviii

Acknowledgements xxxiii

Dissemination xxxiv

Chapter 1 1

Literature review

1.1 The European Union's Water Framework Directive (WFD) 2

1.2 Monitoring under the WFD 4

1.3 Existing approaches to WFD investigative monitoring of organic chemicals 5

1.4 Sampling techniques for the determination of organic contaminants in the aquatic environment 6

1.5 Extraction techniques for organic contaminants in in the aquatic environment 8

1.5.1 Liquid/Liquid extraction 8

1.5.2 Solid phase extraction 8

1.5.3 Other extraction techniques 11

1.6 Passive Sampling 12

1.6.1 Passive sampling devices for organic compounds 16

1.6.1.1 The semi-permeable membrane device (SPMD) 16

1.6.1.2 The silicon rubber or polydimethylsiloxane sampler 18

1.6.1.3 The polar organic chemical integrative sampler (POCIS) 19

1.6.1.3.1 Types of sorbent used with POCIS 21

1.6.1.4 The Chemcatcher® 23

1.6.1.4.1 Non-polar Chemcatcher® 24

1.6.1.4.2 Polar Chemcatcher® 26

1.6.1.5 Other types of passive sampler for organic compounds 35

1.6.2 Uptake of analytes by passive samplers and determination of aqueous TWA concentrations compounds 36

1.7 Analytical separations and detection techniques 38

1.7.1 One-dimensional gas chromatography 38

1.7.2 Multi-dimensional gas chromatography 44

1.7.3 Comprehensive two-dimensional gas chromatography (GCxGC) 44

1.7.3.1 Enabling GCxGC capability with existing GC chromatographs 46

1.7.3.2 Coupling of GCxGC to time-of-flight mass spectrometry (GCxGC TOF-MS) 47

1.7.3.3 Comprehensive GCxGC and low energy or soft electron ionisation 55

1.7.3.4 Soft ionisation techniques 56

1.7.3.5 Chemical ionisation 57

1.7.3.6 Supersonic molecular beams (SMB) and soft cold EI 58

1.7.3.7 Variable electron ionisation source 63

1.7.4 High Performance Liquid Chromatography (HPLC) 65

1.7.4.1 HPLC with high resolution mass spectrometry (HPLC HRMS) 67

1.7.4.2 Matrix effects 69

1.7.4.3 TOF and Orbitrap mass spectrometric detectors 70

1.7.4.4 LC HRMS libraries and databases for organic pollutants in water 72

1.7.4.5 Data independent MS/MS acquisition 75

1.7.5 Retention time prediction 76

1.8 Conclusions from the literature review 81

1.9 Aims and objectives 84

Chapter 2 86

Identification of pollutants in non-polar passive sampling devices using comprehensive two-dimensional gas chromatography and variable energy electron ionisation time-of-flight mass spectrometry

2.1 Introduction 87

2.2 Aims and objectives 89

2.3 Experimental 90

2.3.1 Reagents and standards 90

2.3.2 Deployment of SPMDs 93

2.3.3 Extraction of SPMDs 95

2.3.4 Conventional one-dimension GC analysis (experimental conditions) 97

2.3.5 GCxGC TOF-MS analysis (experimental conditions) 98

2.3.6 GCxGC TOF-MS analysis with Select eV (experimental conditions) 99

2.4 Results and discussion 101

2.4.1 Compounds identified from conventional one dimensional GC analysis 101

2.4.2 Deconvolution 112

2.4.3 Comprehensive GCxGC analysis 112

2.4.4 Pesticides identified with GCxGC analysis 112

2.4.5 PAHs identified with GCxGC analysis 122

2.4.6 PCB analysis 132

2.4.7 Emerging contaminants 140

2.4.7.1 Polycyclic musks, UV sunscreen filters and phosphate fire retardants 140

2.4.8 Variable energy electron ionisation 145

2.4.8.1 Molecular ion intensity and signal-to-noise ratio improvements for the molecular ions of pesticides 145

2.4.8.2 Signal-to-noise ratio improvements for the molecular ions of emerging contaminants 151

2.4.8.3 Reduced spectral 'noise' 157

2.5 Conclusions 157

Chapter 3 160

Development of a novel 'all ions' MS/MS screening method for the reliable identification of pharmaceuticals in polar passive sampler extracts

3.1 Introduction 161

3.2 Aims and objectives 163

3.3 'All ions' MS/MS 163

3.3.1 Find by formula algorithm 168

3.3.2 Formation of the database 168

3.4 Experimental 177

3.4.1 Reagents and reference materials 177

3.4.2 Intermediate and working standard solution mixes 179

3.4.3 Preparation of the mobile phase for LC Q-TOF-MS analysis 179

3.4.4 Preparation of POCIS samplers 179

3.4.4.1 Preparation and cleaning of membranes for POCIS 179
3.4.4.2 Cleaning of the OASIS HLB phase sorbent 180
3.4.4.3 Assembly of POCIS samplers 181
3.4.5 Cleaning of the Chemcatcher® bodies, rings and transport caps 183
3.4.6 Conditioning of the HLB-L disks for the polar Chemcatcher® 183
3.4.7 Preparation of membranes for of the Chemcatcher® 184
3.4.8 Chemcatcher® assembly 184
3.4.9 Deployment of the samplers at the waste water treatment plant 185
3.4.10 Extraction of POCIS samplers 185
3.4.11 Extraction of HLB-L disks from the Chemcatcher® samplers 190
3.4.12 Creation of the searchable database using the Agilent personal compound database and library (PCDL) software 193
3.4.13 LC Q-TOF-MS analysis 201
3.4.14 Data analysis 204
3.4.15 Waste water treatment plants chosen for the deployments 205
3.5 Results and discussion 206
3.5.1 Identification of pharmaceuticals in MS scan mode 206
3.5.2 Peak area responses for the cardiovascular drugs at the Carmarthen and Llanelli waste water treatment plants 207
3.5.3 Identification of pharmaceuticals using the ‘all ions’ MS/MS technique 208
3.5.4 Mass accuracy 211
3.6 Conclusions 230

Chapter 4 232

Comparison of the sampling efficiency of two passive samplers for organic compounds in effluents: Polar Chemcatcher® versus the polar organic chemical integrative sampler (POCIS)

4.1 Introduction 233

4.2 Aims and objectives 234

4.3 Experimental 235

4.3.1 Data acquisition and analysis 235

4.3.2 LC Q-TOF-MS analysis 235

4.3.3 Peak extraction of targeted pharmaceuticals 236

4.3.4 Feature finding and peak extraction of untargeted features 239

4.3.5 Correction of peak areas obtained from POCIS devices 243

4.3.6 Mass profiling of 'unknowns' present in Chemcatcher® and POCIS samplers 247

4.4 Results and discussion 248

4.4.1 Comparison of uptakes of 68 pharmaceuticals by Chemcatcher® and POCIS 254

4.4.2 Analysis of variance of pharmaceuticals identified in co-deployments undertaken at the Gowerton site 255

4.4.3 Least squares and orthogonal regression analysis of the averaged pharmaceutical Chemcatcher® and POCIS data 258

4.4.4 Statistical analysis of 'unknown' features or peaks 264

4.4.5 Comparison of uptakes of 'unknown' compounds by Chemcatcher® and POCIS 265

4.4.6 Analysis of variance of 'unknowns' identified in co-deployments undertaken at the Gowerton WWTP 267

4.4.7 Least squares and orthogonal regression analysis of the 'unknowns' data obtained from all replicates of Chemcatcher® and POCIS 269

4.4.8 Least squares and orthogonal regression analysis of the averaged 'unknowns' data obtained from Chemcatcher® and POCIS 274

4.4.9 Mass and retention time profiling of the polar passive sampler extracts 281

4.5 Conclusions 294

Chapter 5 296

In-silico prediction of liquid chromatographic retention times to aid in the identification of unknown or suspect compounds

5.1 Introduction 297

5.2 Aims and objectives 298

5.3 Experimental 298

5.3.1 Reagents and standards 298

5.3.2 LC Q-TOF-MS analysis 299

5.3.3 Formation of database 300

5.4 Results and discussion 302

5.4.1 The correlation plot obtained from the 'training set' of compounds 302

5.4.2 Retention time prediction from the testing set of compounds 303

5.4.3 Comparison of chromatographic similarity between regression plots 313

5.4.4 The effects of matrix on measured retention times in passive sampling extracts 316

5.4.5 Tentative identification of three metabolites of the pharmaceutical irbesartan without reference standards 317

5.5 Conclusions 322

Chapter 6 324

Conclusions and recommendations for further work

6.1 Overall conclusions 325

6.2 Recommendations for future work 328

Chapter 7 329

References

Appendices 394

Appendix A 395

Appendix B 401

Appendix C 405

Appendix D 415

UPR 16 Form (Research Ethics Review Checklist) 480

Declaration

Whilst registered as a candidate for the above degree, I have not been registered for any other research award. The results and conclusions embodied in this thesis are the work of the named candidate and have not been submitted for any other academic award.

Word count: Approximately 51,085 not including ancilliary data

List of Tables

Table 1.1 A comparison of R_s values obtained from Petrie et al. (64) for the polar Chemcatcher against published sampling rates for the POCIS sampler.

Table 1.2 Overview of the technical specifications, characteristics and performance of hybrid TOF and Orbitrap HRAM systems.

Table 2.1 List of 117 compounds investigated in SPMD samplers using one dimensional and GCxGC TOF-MS techniques.

Table 2.2 Comparison of compounds identified using GC TOF-MS and GCxGC TOF-MS from a deployed SPMD sampler, extracted and spiked with 55 pesticides.

Table 2.3 NIST library match factors obtain from different data analysis techniques for pesticides, PAHs and PCBs.

Table 2.4 PAH assignment based on the spiked SPMD extract analysis.

Table 2.5 GCxGC PCB assignment based on the spiked SPMD sample analysis.

Table 2.6 NIST library match factors obtain from different data analysis techniques for emerging contaminants.

Table 2.7 Signal-to-noise ratios of the molecular ions of pesticides spiked into the SPMD extract at different electron ionisation energies.

Table 2.8 Signal-to-noise ratios for the molecular ions of the emerging contaminants identified in the SPMD extract at different electron ionisation energies.

Table 3.1 Co-elution scores for the individual trimethoprim fragment ions, together with scores for the precursor mass accuracy, mass and retention time differences.

Table 3.2 Full list of pharmaceuticals for which reference materials were purchased for Q-TOF-MS analysis.

Table 3.3 Ratios of POCIS sorbent recovered against the original added.

Table 3.4 MS adducts and MS/MS fragment ion species for the pharmaceuticals in the personal compound database and library.

Table 3.5 Chromatographic conditions and mass spectrometer settings.

Table 3.6 Pharmaceuticals identified in extracts at the three sites using the MS scan method (positive and negative ion modes).

Table 3.7 Pharmaceuticals identified in extracts at the three sites using the 'all ions' MS/MS technique.

Table 3.8 Co-elution scores for the individual irbesartan fragment ions, together with scores for the precursor mass accuracy, mass and retention time differences.

Table 3.9 Co-elution scores for the individual fexofenadine fragment ions, together with scores for the precursor mass accuracy, mass and retention time differences.

Table 3.10 Co-elution scores for the individual clarithromycin fragment ions, together with scores for the precursor mass accuracy, mass and retention time differences.

Table 3.11 Mass accuracy for compounds identified using the 'all ions' MS/MS method.

Table 3.12 Summary of the mass accuracy statistics obtained for each site.

Table 4.1 Batch Targeted Feature Extraction parameters.

Table 4.2 Profinder Batch Recursive Feature Extraction parameters.

Table 4.3 Mass of Oasis® HLB sorbent recovered from POCIS devices from all replicates at all sites.

Table 4.4 RSD's obtained for 68 pharmaceutical compounds identified in Chemcatcher® and POCIS samplers.

Table 4.5 Principal component analysis for pharmaceuticals identified in the co-deployments undertaken at the Gowerton site.

Table 4.6 Two-way ANOVA with sample repeat and passive sampler type for pharmaceuticals identified in the co-deployments undertaken at the Gowerton site.

Table 4.7 Output from least squares regression analysis of the averaged pharmaceutical Chemcatcher® and POCIS data.

Table 4.8 Output from the orthogonal regression analysis of the averaged pharmaceutical Chemcatcher® and POCIS data.

Table 4.9 Principal component analysis of 'unknowns' identified in co-deployments undertaken at the Gowerton WWTP.

Table 4.10 Two-way ANOVA with sample repeat and passive sampler type for co-deployments undertaken at the Gowerton WWTP.

Table 4.11 Output from least squares regression analysis of 'unknowns' in all replicates of POCIS and Chemcatcher®.

Table 4.12 Output from orthogonal regression analysis of 'unknowns' in all replicates of POCIS and Chemcatcher®.

Table 4.13 Output from least squares regression analysis of the averaged Chemcatcher® and POCIS 'unknowns' data.

Table 4.14 Output from orthogonal regression analysis of the averaged Chemcatcher® and POCIS ‘unknowns’ data.

Table 5.1 Distribution of retention time errors from both prediction models.

Table A1 Output from least squares regression analysis of the averaged Log_{10} transformed Chemcatcher® and POCIS pharmaceutical data.

Table A2 Output from the orthogonal regression analysis of the averaged Log_{10} transformed Chemcatcher® and POCIS pharmaceutical data.

Table B1 Output from least squares regression analysis of the averaged Chemcatcher® and POCIS ‘unknowns’ data after application of DFITS.

Table B2 Output from orthogonal regression analysis of the averaged Chemcatcher® and POCIS ‘unknowns’ data after standardised residuals filtering.

Table C1 Output from least squares regression analysis of Log_{10} transformed data for ‘unknowns’ in all POCIS and Chemcatcher® replicates.

Table C2 Output from orthogonal regression analysis of Log_{10} transformed data for ‘unknowns’ in all POCIS and Chemcatcher® replicates.

Table C3 Output from least squares regression analysis of the averaged Log_{10} transformed Chemcatcher® and POCIS ‘unknowns’ data.

Table C4 Output from orthogonal regression analysis of the averaged Log_{10} transformed Chemcatcher® and POCIS ‘unknowns’ data.

Table D1 List of prescribed items by British National Formulary Chemical (BNF) name exceeding 25,000 items dispensed by GPs in Wales during 2014.

Table D2 Compounds for the entire ‘training set’ database

List of Figures

Figure 1.1 Structure of the patented divinylbenzene-N-vinylpyrrolidone copolymer used in Waters Oasis® HLB SPE products. Reproduced with permission from (57) copyright 2008, John Wiley and Sons.

Figure 1.2 Number of publications on passive sampling of the aquatic environment between 2000 and 2017 (from an advanced search using SCOPUS using search terms “passive-sampling, water, river, groundwater and effluent” in title, abstract or key words of article).

Figure 1.3 Plot of the three phases of passive sampler uptake, i) linear phase, ii) curvilinear phase and iii) equilibrium phase. Time is given in half lives. Adapted with permission from (44), copyright 2006, Springer.

Figure 1.4 The polar organic chemical integrated sampler (POCIS) for sampling water soluble organic micropollutants from aquatic environments. Photo taken in author’s laboratory in 2014.

Figure 1.5 Process of compound diffusion and barriers to chemical uptake into the POCIS and polar Chemcatcher® samplers. The line shows the chemical concentration gradient in the water through each barrier. Reproduced with permission from (98), copyright 2007, Elsevier.

Figure 1.6 A plot of Log10 RSi - Pi (as a function of the sampling rate) versus Log10 Kow for the non-polar Chemcatcher from a series of nine calibration experiments using a third order polynomial function. Reproduced with permission from (110), copyright 2007, Elsevier.

Figure 1.7 A bio-fouled SPMD (on holder) retrieved from a watercourse after a deployment period of one month (left) and an unused SPMD (right). Photo taken by the author in 2014.

Figure 1.8 Simulation of a typical GCxGC chromatogram with typical peak widths of 0.05–0.10 s, highlighting the requirement for high speed detection systems. Adapted with permission from Leco UK.

Figure 1.9 Simple modifications to an existing 1-D gas chromatographs to enable its use for GCxGC. Adapted with permission from Leco UK.

Figure 1.10 Number of publications between 2000 and 2016 for GCxGC obtained from advanced SCOPUS search.

Figure 1.11 A schematic diagram of the supersonic molecular beam interface coupled to a Varian 1200 GCMS (top) and a detailed zoomed in section of the interface (bottom). Reproduced with permission from (241), copyright 2008, Elsevier.

Figure 1.12 A comparison of cold EI mass spectrum of a linear chain hexadecane (n-C16H34) (upper trace) with its NIST library mass spectrum (bottom trace). Reproduced with permission from (262) copyright 2008, John Wiley and Sons.

Figure 1.13 Comparison of 'Soft Cold EI' mass spectrum (upper trace), 'Cold EI' mass spectrum (middle trace) and NIST library mass spectrum (bottom trace) of squalene. The 'Cold EI' mass spectrum shown in the middle trace was obtained at 70 eV electron energy while the 'Soft Cold EI' mass spectrum shown in the upper trace was obtained at 18 eV electron energy. Reproduced with permission from (223) copyright 2008, John Wiley and Sons.

Figure 1.14 Schematic diagram showing the effects of different electron ionisation energies of ion flow (a) operation of a conventional ion source at 70 eV, (b) operation of a conventional ion source at lower ionisation energies' (c) operation of the variable-energy source showing how it overcomes the space-charge effect at low ionisation energy [reproduced with permission from Select-eV® technical note (244)].

Figure 1.15 Process of ‘data independent MS/MS acquisition’ for a Q-TOF-MS where the collision cell rapidly alternates between low and high collision energies. Diagram used with permission from Bruker UK Ltd.

Figure 2.1. Satellite image showing the locations of the waste water treatment plant, final effluent discharge point into the river and the downstream SPMD deployment site (scale not given). Taken from Imagery© 2016, Bluesky, DigitalGlobe, Getmapping plc, Infoterra Ltd & Bluesky, Landsat, The GeoInformation Group Map data© Google.

Figure 2.2 Total ion chromatogram of 1-D GC MS-TOF analysis of spiked SPMD extract.

Figure 2.3 Total ion chromatogram of spiked SPMD extract analysed by one dimensional GC TOF-MS showing the highly complex nature of the sample.

Figure 2.4 Extracted ion profiles for the chlorinated biphenyl compounds obtained from the analysis of the spiked SPMD extract. Highlighted peaks at 16.90 min in the trichlorinated and tetrachlorinated biphenyl traces exhibit perfect co-elution of analytes.

Figure 2.5 GC Image rendered contour plot obtained from GCxGC analysis of the extract from the SPMD field blank for the deployment at Garnswllt WWTP.

Figure 2.6 GC Image rendered contour plot obtained from GCxGC analysis of the extract from the un-spiked SPMD (deployed at Garnswllt WWTP).

Figure 2.7 GC Image rendered contour plot obtained from GCxGC analysis of the extract from the spiked SPMD (deployed at Garnswllt WWTP).

Figure 2.8 3D view of the boxed area in Figure 3.7 illustrating sample complexity and significant number of co-eluting peaks that occur in the first dimension.

Figure 2.9 GC Image rendered contour plot obtained from GCxGC analysis of the extract from the spiked SPMD (deployed at Garnswllt WWTP) with pesticides circled.

Figure 2.10 GC Image rendered contour plot and 3D surface plot for extracted ion 219 (±0.5 amu) showing separation of the four stereoisomers of hexachlorocyclohexane for the spiked SPMD extract analysis.

Figure 2.11 NIST 2011 library match and 3-D rendered peak for Parathion taken from the spiked SPMD extract analysis.

Figure 2.12 NIST 2011 library match and 3-D rendered peak for Chloropyriphos-methyl taken from the spiked SPMD extract analysis.

Figure 2.13 NIST 2011 library match and 3-D rendered peak for Irgarol 1051 taken from the spiked extract sample analysis.

Figure 2.14 GC Image rendered contour plots of the spiked SPMD extract with PAHs circled. Wrapped compounds are highlighted in red.

Figure 2.15 Spectral comparison of NIST 2011 library spectra of four 4-ring PAHs to illustrate the spectral similarities.

Figure 2.16 GC Image-rendered surface for the extracted ion 228 (±0.5 amu) of the 4-ring PAHs present in the spiked SPMD extract analysis.

Figure 2.17 NIST 2011 library match and 3-D rendered peak for acenaphthylene taken from the spiked extract sample analysis.

Figure 2.18 NIST 2011 library match and 3-D rendered peak for 3,6-dimethylphenanthrene taken from the spiked extract sample analysis.

Figure 2.19 NIST 2011 library match and 3-D rendered peak for 6-ethylchrysene (reported as 5-ethyl because the structure is not in the NIST library) taken from the spiked SPMD extract analysis.

Figure 2.20 NIST 2011 library match and 3-D rendered peak (extracted ion 252) for benzo[k]fluoranthene taken from the spiked extract sample analysis.

Figure 2.21 GC Image-rendered contour plots of the spiked SPMD extract samples with chlorinated biphenyls coloured by chlorination level. The red oval highlights what would be a co-elution in 1-D GC (1-D chromatogram in Figure 3.4).

Figure 2.22 GC Image 3-D surface plot for PCB extracted ions 222, 256, 292, 326, 360 and 394 (±1 amu) for the spiked extract. Inset highlight possible presence of two more PCB congeners.

Figure 2.23 NIST 2011 library match and 3-D rendered peak for a tetrachlorinated biphenyl taken from the spiked extract sample analysis.

Figure 2.24 NIST 2011 library match and 3-D rendered peak for a pentachlorinated biphenyl taken from the spiked extract sample analysis.

Figure 2.25 NIST 2011 library match and 3-D rendered peak for a hexachlorinated biphenyl taken from the spiked extract sample analysis.

Figure 2.26 NIST 2011 library match and 3D-rendered peak for a heptachlorinated biphenyl taken from the spiked extract sample analysis.

Figure 2.27 Diastereoisomers of HHCB and AHTN separated using GCxGC.

Figure 2.28 Combined two-dimensional extracted ion chromatogram for m/z 213 and 159, showing the identification of the polycyclic musks AHTN and HHCB, and related structural analogues of HHCB.

Figure 2.29 Comparison of signal-to-noise ratios (S/N) for the molecular ions of the organo-phosphate pesticides dichlorvos and mevinphos present in the SPMD extract at 70 eV and 12 eV ionisation energies.

Figure 2.30 Comparison of signal-to-noise ratios (S/N) for the molecular ions of the organo-chlorine pesticides 2,4'-TDE and 4,4'-DDE present in the SPMD extract at 70 eV and 12 eV ionisation energies.

Figure 2.31 Comparison of signal-to-noise ratios (S/N) for the molecular ion of the organo-phosphate chlorpyrifos methyl present in the SPMD extract at 70 eV and 12 eV ionisation energies.

Figure 2.32 Comparisons of the mass spectra obtained for Homosalate and Octinoxate, present in the SPMD extract, measured at three different ionisation energies (12, 14 and 70 eV).

Figure 2.33 Comparisons of the mass spectra obtained for HHCB and Octocrylene, present in the SPMD extract, measured at three different ionisation energies (12, 14 and 70 eV).

Figure 2.34 Mass spectrum of Amgard TMCP, present in the SPMD extract, at electron ionisation energies of 70, 14 and 12eV.

Figure 3.1 Mass spectra for trimethoprim at 0 eV (a), averaged mass spectrum from collision energies of 0, 20 and 40e V (b) and summed 'cleaned' MS/MS spectrum of the higher collision energies (c) for the Chemcatcher® sampler at the Carmarthen WWTP.

Figure 3.2 Overlaid un-normalised extracted ion chromatograms for the fragment ions of trimethoprim (a) and normalized ratios of the fragment ions to the precursor ion intensity (m/z 291.1452) plotted versus retention time and displayed in a co-elution plot (b).

Figure 3.3 Preparation of in-house POCIS samplers.

Figure 3.4 Fully assembled POCIS sampler.

Figure 3.5 Prototype version of the polar Chemcatcher® placed in the protective housing.

Figure 3.6 Location of the Gowerton deployments in the final effluent stream.

Figure 3.7 Sorbent within the POCIS sampler after disassembly of one of the Gowerton ‘1’ (a) and Gowerton ‘2’ (b) samplers showing uneven distribution of material.

Figure 3.8 HLB-L disks from retrieved Chemcatcher® samplers at the Gowerton WWTP.

Figure 3.9 Apparatus used for the elution of adsorbed compounds from the Chemcatcher® HLB-L disk.

Figure 3.10 PCDL software showing the accurate mass MS/MS spectrum of meloxicam acquired in positive ionisation mode with a collision energy of 20 eV.

Figure 3.11 Overlay of precursor and fragment ion extracted ion chromatograms for irbesartan in a Chemcatcher® from Llanelli WWTW (a), high collision energy scan displaying all fragment ions (b) and cleaned high collision energy scan displaying only irbesartan fragment ions (c).

Figure 3.12 Overlay of precursor and fragment ion extracted ion chromatograms for fexofenadine in a Chemcatcher® from Llanelli WWTP (a), high collision energy scan displaying all fragment ions (b) and cleaned high collision energy scan displaying only fexofenadine fragment ions (c).

Figure 3.13 Overlay of precursor and fragment ion extracted ion chromatograms for clarithromycin in a Chemcatcher® from Llanelli WWTP (a), high collision energy scan displaying all fragment ions (b) and cleaned high collision energy scan displaying only clarithromycin fragment ions (c).

Figure 4.1 Results obtained from batch recursive feature extraction. [a] compound group table, [b] compound details table, [c(i) and c(ii)] overlaid extracted ion chromatograms for Chemcatcher® and POCIS respectively, (d) Mass spectra for POCIS.

Figure 4.2 Overlaid scatter plots with regression lines of the averaged peak areas for pharmaceuticals identified in POCIS and Chemcatcher® at all sites.

Figure 4.3 Overlaid scatter plots with regression lines of the averaged Log_{10} peak areas for pharmaceuticals identified in POCIS and Chemcatcher® at all sites.

Figure 4.4 Fitted line regression and plots of standardised residuals obtained from least squares regression analysis of the averaged pharmaceutical Chemcatcher® and POCIS data.

Figure 4.5 Fitted line regression and plots of standardised residuals obtained from the orthogonal regression analysis of the averaged pharmaceutical Chemcatcher® and POCIS data.

Figure 4.6 Overlaid scatter plots with regression lines of the averaged peak areas for ‘unknowns’ obtained from POCIS and Chemcatcher® for all WWTPs.

Figure 4.7 Overlaid scatter plots with regression lines of the averaged Log_{10} peak areas for ‘unknowns’ obtained from POCIS and Chemcatcher® for all WWTPs.

Figure 4.8 Fitted line and plots of standardised residuals obtained from least squares regression analysis of ‘unknowns’ in all replicates of POCIS and Chemcatcher®.

Figure 4.9 Fitted line and plots of standardised residuals obtained from orthogonal regression analysis of ‘unknowns’ in all replicates of POCIS and Chemcatcher®.

Figure 4.10 Fitted line and plots of standardised residuals obtained from least squares regression analysis of the averaged Chemcatcher® and POCIS 'unknowns' data.

Figure 4.11 Fitted line and plots of standardised residuals obtained from orthogonal regression analysis of the averaged Chemcatcher® and POCIS 'unknowns' data.

Figure 4.12 Plot of mass versus retention time for features identified in the Chemcatcher® (a) and POCIS (b) samplers deployed at the Carmarthen WWTP.

Figure 4.13 Plot of mass versus retention time for features identified in the Chemcatcher® (a) and POCIS (b) samplers deployed at the Gowerton '1' WWTP.

Figure 4.14 Plot of mass versus retention time for features detected in the Chemcatcher® (a) and POCIS (b) samplers deployed at the Gowerton '2' WWTP.

Figure 4.15 Plot of mass versus retention time for features detected in the Chemcatcher® (a) and POCIS (b) samplers deployed at the Llanelli WWTP.

Figure 4.16 'Zoomed-in' section of the mass versus retention time plot (14.5–20 min, 420–900 Da) for the Chemcatcher® (a) and POCIS (b) samplers deployed at the Llanelli WWTP.

Figure 4.17 Plot of mass versus retention time for features detected in the Chemcatcher® (a) and POCIS (b) field blanks used at the Carmarthen WWTP.

Figure 4.18 Retention time/feature (a) and mass/feature (b) histograms for the Chemcatcher® and POCIS samplers deployed at the Carmarthen WWTP.

Figure 4.19 Retention time/feature (a) and mass/feature (b) histograms for the Chemcatcher® and POCIS samplers deployed at the Gowerton '1' WWTP.

Figure 4.20 Retention time/feature (a) and mass/feature (b) histograms for the Chemcatcher® and POCIS samplers deployed at the Gowerton '2' WWTP.

Figure 4.21 Retention time/feature (a) and mass/feature (b) histograms for the Chemcatcher® and POCIS samplers deployed at the Llanelli WWTP.

Figure 5.1 Correlation between experimental and calculated retention times of 1453 compounds in the 'training set' database.

Figure 5.2 Distribution of retention time errors from both prediction models.

Figure 5.3 Regression plots of calculated versus measured retention times using the best 50 compounds fit. a) = forced through the origin, b) = origin not included.

Figure 5.4 Regression plots of calculated versus measured retention times using the all compounds fit. a) = forced through the origin, b) = origin not included.

Figure 5.5 Structures and chemical formula for Irbesartan and three of its metabolites (M4, M5 and M6) tentatively identified in a Chemcatcher® passive sampler extract.

Figure 5.6 Accurate mass extracted ion chromatograms for Irbesartan and three of its common metabolites (Irbesartan M4, M5 and M6).

Figure 5.7 Structure and chemical formula for the common fragment ion of Irbesartan and three of its metabolites.

Figure A1 Fitted line regression and plots of standardised residuals obtained from least squares regression analysis of the averaged Log_{10} transformed Chemcatcher® and POCIS pharmaceutical data.

Figure A2 Fitted line regression and plots of standardised residuals obtained from the orthogonal regression analysis of the averaged Log_{10} transformed Chemcatcher® and POCIS pharmaceutical data.

Figure B1 Fitted line and plots of standardised residuals obtained from least squares regression analysis of the averaged Chemcatcher® and POCIS 'unknowns' data after application of DFITS.

Figure B2 Fitted line and plots of standardised residuals obtained from orthogonal regression analysis of the averaged Chemcatcher® and POCIS 'unknowns' data after standardised residuals filtering.

Figure C1 Fitted line and plots of standardised residuals obtained from least squares regression analysis of Log_{10} transformed data for 'unknowns' in all POCIS and Chemcatcher® replicates.

Figure C2 Fitted line and plots of standardised residuals obtained from orthogonal regression analysis of Log_{10} transformed data for 'unknowns' in all POCIS and Chemcatcher® replicates.

Figure C3 Fitted line and plots of standardised residuals obtained from least squares regression analysis of the averaged Log_{10} transformed Chemcatcher® and POCIS 'unknowns' data.

Figure C4 Fitted line and plots of standardised residuals obtained from orthogonal regression analysis of the averaged Log_{10} transformed Chemcatcher® and POCIS 'unknowns' data.

List of abbreviations

WFD	Water Framework Directive
EU	European Union
GC	Gas chromatography
GC-MS	Gas chromatography mass spectrometry
LLE	Liquid-liquid extraction
SPE	Solid phase extraction
TWA	Time-weighted average
USEPA	United States Environmental Protection Agency
PSDVB	Polystyrene divinylbenzene
HLB	Hydrophilic-lipophilic balanced
MIP	Moleculary imprinted polymers
SPME	Solid phase micro-extraction
MS	Mass spectrometry
ECD	Electron capture detector
NPD	Nitrogen phosphorous detector
SPMD	Semi-permeable membrane device
LDPE	Low density polyethylene
K_{ow}	Octanol water partition coefficient
SR	Silicone rubber
PDMS	Polydimethylsiloxane
ASE	Accelerated solvent extraction
PAHs	Polynuclear aromatic hydrocarbons
PCBs	Polychlorinated biphenyls

POCIS	Polar organic chemical integrative sampler
PES	Polyethersulphone
PPCPs	Pharmaceuticals and personal care products
PTFE	Polytetrafluoroethylene
CA	Cellulose acetate
C_8	Octyl (alkyl) carbon chain
C_{18}	Octadecyl (alkyl) carbon chain
OC	Organochlorine
PRCs	Performance reference compound
R_s	Rate of sampling (uptake rate)
DGT®	Diffusive gradients in thin-films
o-DGT	Organic DGT
HETP	Height equivalent to a theoretical plate
GCxGC	Two-dimensional comprehensive gas chromatography
1-D	One-dimensional
2-D	Two-dimensional
SCCPs	Short chained chlorinated paraffins
PBDEs	Polybrominated diphenyl ethers
PCTs	Polychlorinated terphenyls
TOF-MS	Time-of-flight mass spectrometry
EI	Electron Ionisation
eV	Electron Volts
PCDDs	Polychlorinated dibenzo-p-dioxins
PCDFs	Polychlorinated dibenzofurans
NIST	National Institute of Standards and Technology

CI	Chemical ionisation
PI	Photo-ionisation
FI	Field ionisation
SMB	Supersonic molecular beams
PFM	Pulsed flow modulation
LC	Liquid chromatography
HPLC	High Performance Liquid Chromatography
HRMS	High resolution mass spectrometry
MS/MS	Tandem mass spectrometry
ESI	Electrospray ionisation
APCI	Atmospheric pressure chemical ionisation
APPI	Atmospheric pressure photo-ionisation
QQQ	Triple-quadrupole
HRAM	High resolution accurate mass
CID	Collision induced dissociation
Q-TOF-MS	Quadrupole based collision cell for TOF-MS
HMDB	Human Metabolome Database
QSRR	Quantitative structure–retention relationship
ppm	parts per million
RP	Resolution power
WWTP	Waste water treatment plant
UV	Ultra-violet
DEHP	Diethyl hexyl phthalate
PTV	Programmable Temperature Vapouriser
CAS	Chemical Abstract Service

UHPLC	Ultra-high performance liquid chromatography
Auto-MS/MS	Automated tandem mass spectrometry
CAS RN	Chemical Abstract Service Registry Number
GPs	General Medical Practitioners
IUPAC	International Union of Pure and Applied Chemistry
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
UHP	Ultra-high purity
MTBE	Methyl tertiary butyl ether
PCDL	Personal compound database and library
CE	Collision energy
RT	Retention time
m/z	mass to charge ratio
Iso. abund.	Isotope abundance
Iso. spacing	Isotope spacing
BRFE	Batch Recursive Feature Extraction
MFE	Molecular Feature Extractor
ANOVA	Analysis of variance
PC	Principal component
R-sq	R-squared
R-sq(adj)	R-squared adjusted
R-sq(pred)	R-squared predicted
PRESS	Prediction sum of squares
DF	Degrees of freedom
Seq SS	Sequential sum of squares

SE Coef	Standard error of the coefficient
Adj SS	Adjusted sums of squares
Adj MS	Adjusted mean of squares
VIF	Variance inflation factor
NMR	Nuclear magnetic resonance
bbCID	Broadband collision-induced dissociation
SMILES	Simplified molecular line entry system strings
LogD,	Log of dissociation constant
MW	Molecular weight
MV	Molecular volume
MR	Molar refractivity
PSA	Polar surface area
NDon	Number of proton donors
NAcc	Number of proton acceptors
WADA	World Anti-doping Agency

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Dissemination

List of publications:

1. Petrie, B., Gravell, A., Mills, G. A., Youdan, J., Barden, R. & Kasprzyk-Hordern, B. 2016. In Situ Calibration of a New Chemcatcher Configuration for the Determination of Polar Organic Micropollutants in Wastewater Effluent. Environ Sci Technol, 50, 9469-78.

2. Cole, R. F., Mills, G. A., Bakir, A., Townsend, I., Gravell, A. & Fones, G. R. 2016. A simple, low cost GC/MS method for the sub-nanogram per litre measurement of organotins in coastal water. MethodsX, 3, 490-6.

3. Schumacher, M., Castle, G., Gravell, A., Mills, G. A. & Fones, G. R. 2016. An improved method for measuring metaldehyde in surface water using liquid chromatography tandem mass spectrometry. MethodsX, 3, 188-94.

4. Vrana, B., Smedes, F., Prokeš, R., Loos, R., Mazzella, N., Miege, C., Budzinski, H., Vermeirssen, E., Ocelka, T., Gravell, A. & Kaserzon, S. 2016. An interlaboratory study on passive sampling of emerging water pollutants. TrAC Trends in Analytical Chemistry, 76, 153-165.

5. Mills, G. A., Gravell, A., Vrana, B., Harman, C., Budzinski, H., Mazzella, N. & Ocelka, T. 2014. Measurement of environmental pollutants using passive sampling devices - an updated commentary on the current state of the art. Environ Sci Process Impacts.

List of platform presentations:

1. 12th International Symposium on Hyphenated Techniques in Chromatography (HTC-12), Bruges, Belgium, 1st – 3rd February 2012.

Screening of complex passive sampling extracts using hyphenated gas chromatographic and mass spectrometric techniques.

2. RSC Separation Science Group and RSC Environmental Chemistry Group Meeting, 28th February 2013, London, UK. Recent Advances in the Analysis of Complex Environmental Matrices.

Screening for pharmaceuticals and personal care products in effluents and surface water using LC-Q-TOF.

3. 13th International Symposium on Hyphenated Techniques in Chromatography (HTC-13), Bruges, Belgium, 28th – 31st January 2014.

High performance screening of environmental pollutants in water.

4. RSC Separation Science Group and RSC Environmental Chemistry Group Meeting, London, UK. New Developments in the Analysis of Complex Environmental Matrices, 6th February 2015.

Monitoring of polar pollutants in surface waters using Chemcatcher-based passive sampling methods.

5. Royal Society of Chemistry, Water Science Forum, Sheffield, UK. Emerging contaminants in waters and soils, practical considerations: Sampling, analysis and consequences, 4th March 2015.

Active and Passive Sampling for Pollutants of Emerging Concern.

(Joint Oral Presentation) Graham Mills[1] and Anthony Gravell[2]

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List of posters:

1. 4th International Passive Sampling Workshop and Symposium (IPSW 2011) - 11th to 14th May 2011.

Screening of Complex Passive Sampling Extracts using Comprehensive Two-Dimensional Gas Chromatography Time of Flight Mass Spectrometry

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2. 6th International Passive Sampling Workshop (IPSW 2013), Bordeaux, France, 26th -29th June 2013.

GC×GC–TOF MS analysis for the screening of emerging contaminants in SPMD passive sampling extracts

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Screening of Pharmaceuticals in Effluents using POCIS Samplers with LCMS Q-TOF analysis

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"There are *known knowns*. These are things we know that we know. There are *known unknowns*. That is to say, there are things that we know we don't know. But there are also *unknown unknowns*. There are things we don't know we don't know."

Donald Rumsfeld
United States Secretary of Defense (2001-2006)

Chapter 1

Literature review

1.1 The European Union's Water Framework Directive

In December 2000, the European Union's Water Framework Directive (WFD) came into force across the community and formally became part of law in the United Kingdom in December 2003 (1). The WFD has the key mandated objective of providing for the planning and delivery of a better aquatic environment across the European Member States through the application of river basin management plans (2, 3). The directive sets out three cycles of 'River Basin Planning and Management' leading towards the eventual achievement of 'good ecological status'. The WFD aims to help protect and further enhance the quality of surface freshwater (including lakes, rivers and streams), groundwater, estuaries and coastal waters up to one nautical mile off shore. Various types of monitoring activities are described including: investigative, operational and surveillance (1, 4-6). In addition to improving the ecological health of water bodies, the WFD sets out to achieve chemical quality standards that will help to deal with diffuse and point source pollution that remain a serious environmental concern (7, 8). Furthermore, the Marine Strategy Framework Directive came into force in June 2008, and its key aims are similar; to protect and enhance the environmental status of the marine waters bounding the Member States by 2020 (9-12).

Most of the strategies currently used for the identification of pollutants in water bodies, within the context of the WFD, focus on the measurement of the concentrations of substances specified in a priority pollutant list. In 2008, an initial list of 33 priority chemicals and eight other pollutants were specified in "Directive 2008/105/EC on environmental quality standards in the field of water policy" (13).

The same directive introduced eleven additional compounds and two compound groups (polychlorinated biphenyls and dioxins) for possible identification as priority substances or priority hazardous substances in any future directive.

A subsequent EU directive 2013/39, introduced in 2013, incorporated many of these compounds into mandatory operational or surveillance monitoring programmes, and introduced the concept of a 'Watch list' of substances selected from amongst those for which the information available indicates that they may pose a significant risk to the aquatic environment and for which current monitoring data are insufficient (14). 17-alpha-ethinylestradiol, 17-beta-estradiol and diclofenac were included in the first WFD watch list, which was extended by a further 13 compounds in 2015 through EU Decision 2015/495 (15).

Substance or compound lists in the various EU directives are mainly organic compounds together with the following metals: cadmium, lead, mercury and nickel. The measured concentrations in water samples are compared with the prescribed environmental quality standards set by the European Commission for each of these pollutants, however, each subset (e.g. polyaromatic hydrocarbons, specific classes of pesticides) of pollutants may require a separate water sample to be taken in the field and the combined cost of these analyses can prove labour intensive and hence costly. In such surveillance monitoring campaigns, many compounds will, however, remain unidentified, but which could have a significant toxicological impact on the fauna and flora within the given aquatic environment resulting in poor ecological status. This could mean that the environmental objectives for a water body are unlikely to be met and further investigative monitoring may have to be undertaken to gather additional

information on the likely reason for the failure (13). Further, within the WFD, the practice of taking low volume (1 – 5 L) bottle, grab or spot samples of water followed by their laboratory analysis may not always provide a useful indication of the environmental status of a water course and alternative approaches (e.g. bio-monitoring, sensors, passive sampling) and strategies may be warranted (16-19).

1.2 Monitoring under the WFD

Monitoring programmes under the auspices of the WFD can be of three types, as described below.

(i) Surveillance monitoring: This is undertaken to provide information for the assessment of long-term changes resulting from widespread anthropogenic activity. In addition, information gained from surveillance monitoring is used to supplement and validate the impact assessment procedure detailed in the WFD and allow for the efficient and effective design of future monitoring programmes.

(ii) Operational monitoring: This is to determine the status of water bodies identified as being at risk of failing to meet their environmental objectives and assess any changes in the status of such bodies resulting from the programmes of measures.

(ii) Investigative monitoring: This is carried out when reasons of any exceedance for ecological and chemical status is unknown and where surveillance

monitoring indicates that the objectives for a water body are not likely to be achieved. Investigative monitoring therefore tries to determine the causes and ascertain the magnitude and impacts of unknown pollution.

1.3 Existing approaches to WFD investigative monitoring of organic chemicals

No clear guidance exists for this type of monitoring as it must be tackled on a 'case-by-case' basis and for several years has involved the analysis of spot samples of water for unknown (non-target) organic chemical pollutants. This is usually accomplished by low resolution gas chromatographic-mass spectrometric (GC-MS) analytical methods using simple mass spectral library searching routines (20-22). GC-MS is a powerful technique for the separation and determination of volatile and semi-volatile compounds, yet even with the use of high resolution capillary columns, it is unable to resolve the multitude of compounds that can be present in complex environmental samples (23-25). Screening of samples via low unit mass resolution GC-MS is also susceptible to interference from other compounds of a similar molecular mass (21, 26-29). This implies that when using conventional single quadrupole GC-MS techniques many compounds will remain unidentified, but which could have a significant aquatic toxicity (30-33). It is, therefore, desirable to introduce other instrumental techniques to improve the quality of environmental assessments and to benefit from resource saving as further analytical developments become available (17, 34, 35).

1.4 Sampling techniques for the determination of organic contaminants in the aquatic environment

Regulatory water monitoring programmes for directives such as the WFD, typically entail active sampling at specific locations and points of time as defined in the monitoring programme. Active sampling refers to techniques or methods that require physical intervention or external energy input for sample collection, extraction or trapping of compounds in the exposure medium. e.g. the taking of samples in specific bottles or containers more commonly known as spot sampling (36-38). Spot samples are nearly always extracted with organic solvents i.e., liquid-liquid extraction (LLE) or solid-phase extraction (SPE) and where necessary clean-up is employed to remove potential interferences. Spot sampling techniques are well developed and are known to provide very good accuracy with well documented quality control procedures (39). Spot sampling methods do, however, suffer from potential problems with sample preservation such as losses due to volatilisation, sorption to container walls, chemical degradation and loss of analyte during filtration (40).

Spot sampling is arguably the most widely used and validated sampling technique, but it has many shortcomings. For example, spot water sampling provides only a 'snapshot' of the situation at the set time of sampling and episodic events such as storm water runoff, pesticide runoff or accidental spills are often missed as contaminants can dissipate between sampling intervals (41, 42). Spot sampling therefore fails to detect and account for temporal variation in pollutant concentrations and may not provide a truly representative picture of the extent of contamination (43).

Despite allowing for the determination of total contaminant spot sampling fails to consider the bioavailability of pollutants in water and especially so for compounds with high Log_{10} K_{ow}'s which readily partition onto suspended particles present in the watercourse. Bioavailability, and the accumulation of sediment contaminants in aquatic organisms, depends upon desorption and diffusion processes into and from the sediment particles, partitioning into water and into biological membranes. Therefore, the fraction of the total waterborne residues characterised by the dissolved phase or bioavailable phase is not distinguished from other waterborne phases by spot sampling (44).

Sample sizes, typically ≤ 2 L, may also be inadequate for the analysis of ultra-trace levels of contaminants as specified in EU Decision 2015/495 (15), e.g. Cypermethrin and Hepatchlor (plus its epoxide) which have EQS values of 8.5×10^{-5} and 2.0×10^{-7} respectively. As spot sampling in a watercourse is generally relevant only to a few points in time (i.e. monthly sampling), it does not provide time-weighted average (TWA) concentrations which are useful indicators of organism exposure to pollutants (45). However, measurement of TWA concentrations over specified time periods requires continuous, additive extraction (i.e., integrative sampling). Notwithstanding the significant financial costs in purchasing automatic sampling systems that can take sufficient repetitive samples to formulate estimates of TWA concentrations, their installation and operation in remote areas can present significant logistical and site security difficulties (43).

1.5 Extraction techniques for organic contaminants in water

1.5.1 Liquid/Liquid Extraction

Liquid–liquid extraction is an important extraction technique for environmental, clinical, and industrial laboratories where the target or compounds of interest partition between two immiscible phases. One phase is usually aqueous and the other phase is an organic solvent (e.g. dichloromethane or hexane) and because the phases are immiscible they form two layers, with the denser phase on the bottom. After extraction, the target compound is present in the organic phase with the percentage extracted determined by the equilibrium constant between the two phases. The extraction efficiency for acidic and basic compounds can be influenced by the pH of the aqueous phase and acidification of the aqueous phase is generally undertaken prior to extraction to prevent degradation of compounds during storage. Despite the introduction of alternative extraction techniques for aqueous samples, such as SPE, liquid-liquid extraction methods, including regulatory methods such as those used by the United States Environmental protection Agency (USEPA) remain very popular for the extraction of non-polar compounds (46-49).

1.5.2 Solid-Phase Extraction

SPE uses a solid sorbent to remove analytes of interest from various liquid samples which can include surface waters, effluents and biological samples and is based on specific interactions between the sorbent particles, which contains the stationary phase, and the analyte present in the matrix of interest (50). There is a vast number of different types of sorbents available allowing the processing of many different sample

types and the nature of the sorbent stationary phase dictates the mode of SPE whether it be normal-phase, reverse-phase, ion-exchange (51-53).

Extraction of aqueous samples is typically performed in reversed-phase mode where a non-polar sorbent is used to retain mid to non-polar compounds from a polar matrix such as water. Compound retention is achieved through non-polar interactions such as van der Waal forces. Ion-exchange SPE is also used for the extraction of compounds from water and involves electrostatic interactions between charged functional groups on the compound to be retained and oppositely charged groups on the sorbent surface. Ion-exchange is therefore used for the extraction of acids and bases with a wide range of pK_a values. Sorbents can either contain cation exchange groups such as sulphonic acid, which are used to extract bases from water, or anion exchange groups such as amines to extract acids from water.

The past decade has seen an emergence of polymeric sorbents that allow retention of a wider range of compounds through a combination of different interactions (54). A common polymeric sorbent is polystyrene divinylbenzene (PSDVB) whose surface can be modified to provide varying functionality to allow mixed mode retention of compounds (55, 56). These polymeric sorbents allow for the retention of neutral and some charged species through a variety of interactions including π-π, dipole-dipole and van der Waals forces. The most frequently used polymer sorbent for the extraction of compounds from aqueous environmental samples is the patented divinylbenzene-N-vinylpyrrolidone copolymer which allows the retention of compounds across a wide polarity range. The commercial product employing this polymer is the Waters Oasis® hydrophilic-lipophilic balanced (HLB) sorbent which

is made from a balanced ratio of two monomers, the hydrophilic N-vinylpyrrolidone and the lipophilic divinylbenzene; its structure is shown in Figure 1.1 (57).

A non-targeted analytical method such as qualitative screening should extract as many compounds within a single sample as possible, allowing for a maximum representation of the sample components to be obtained (58). The Waters OASIS® HLB sorbent, and other similar sorbents are ideal for qualitative non-target screening in addition to its application to quantitative analysis of target compounds.

Of increasing use in environmental based SPE are the molecularly imprinted polymers (MIP's) which have robust molecular recognition elements which can mimic natural recognition entities such as biological receptors, and are therefore useful to trap and separate target compounds from highly complex matrices such as biological fluids and environmental samples (59). These would cover a limited range of compounds with similar structures and have limited application to non-target screening.

The SPE approach also includes the 3M range of Empore™ disks and Horizon Technology's range of Atlantic® SPE disks (60-62). These disks or membranes consist of a Teflon fibril (Empore™ disks) or glass fibre matrix (Atlantic® SPE disks) loaded with the SPE sorbents but the particle size of these sorbents is typically smaller than those used with standard SPE cartridges. In cases where water samples contain significant particulates, they are generally filtered through glass fibre filters first to avoid clogging of SPE cartridges during extraction. The presence of particulates has much less of an impact when using SPE disks as the surface area in contact with the

water is considerably higher. The 3M range of Empore™ disks have been used extensively with the Chemcatcher passive sampler since its development in 2000, and more recently Horizon Technology's Atlantic® SPE disk (containing Waters Oasis® HLB Sorbent) has seen use with the Chemcatcher for the determination of polar organic micro-pollutants in wastewater effluents (42, 63, 64).

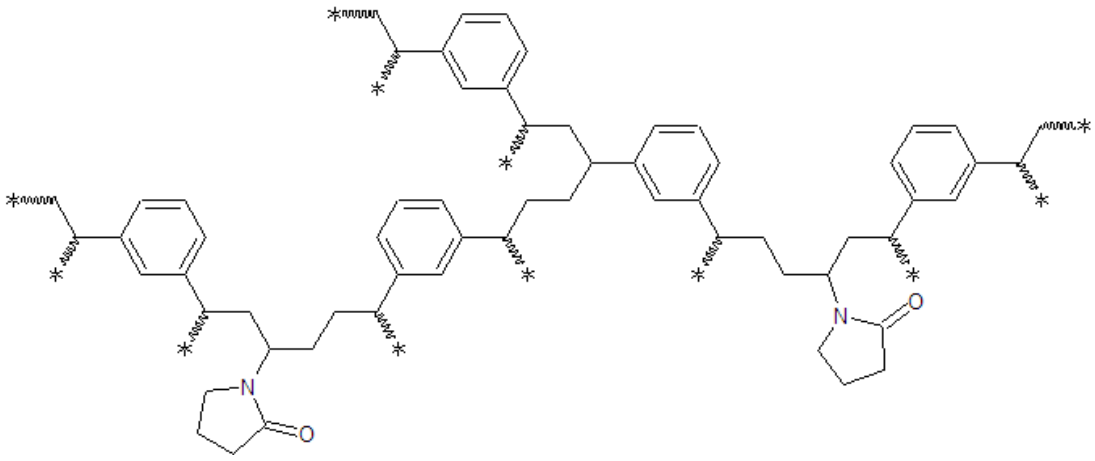


Figure 1.1 Structure of the patented divinylbenzene-N-vinylpyrrolidone copolymer used in Waters OASIS® HLB SPE products. Reproduced with permission from (57) copyright 2008, John Wiley and Sons.

1.5.3 Other extraction techniques

Solid phase micro-extraction (SPME) methods have been used extensively in environmental analysis since its introduction in the early 1990's (65-68). The SPME approach has several advantages over active sampling methods, which includes the elimination of the SPE filtration step or LLE typically required for sample preparation, and allows for the direct introduction and desorption of the adsorbed compounds into a GC for analysis. However, the use of SPME methods for

monitoring contaminants at very low ng/L concentrations in water is very challenging (69).

Aliquots of the prepared extracts obtained from LLE and SPE are typically introduced into a gas or liquid chromatograph for analysis whilst SPME allows for the direct injection of the total sample extracted into the chromatographic system. Various detection systems are used including mass spectrometry (MS) and elemental selective detectors such as the electron capture detector (ECD) for halogen containing compounds, or the nitrogen phosphorous detector (NPD) for phosphorous and nitrogen containing compounds (70).

1.6 Passive sampling

According to Górecki and Namieśnik, passive sampling can be defined as "any sampling techniques based on free flow of analyte molecules from the sampled medium to a collecting medium, as a result of a difference in chemical potential of the analytes between the two media" (71).

Prior to the mid-1980's, passive sampling was mainly used for gas and air sampling and it was not until 2002 that the number of publications for passive sampling in water exceeded ten. Figure 1.2 shows the number of publications on passive sampling of the aquatic environment between 2000 and 2017. The increase in publications was mainly driven by the capability of passive samplers to achieve pg/L time weighted average concentrations in the aqueous environment and to identify new and emerging contaminants which had previously remained unidentified (72).

Passive sampling devices can be used for the determination of both organic and inorganic compounds in various matrices, including water. Passive methods may generally be classified as either based on adsorption or absorption with adsorptive methods involving the transfer of analytes usually through a membrane and the physical or chemical retention of contaminants by the surface of the adsorbent (71, 73). The chemical retention may be based on Van der Waal, π-π, ion-exchange or hydrogen bonding interactions. Absorptive methods involve not only involve surface phenomena, but also analyte permeation in the interceding material, hence providing the possibility of compound discrimination due to the membrane's physicochemical characteristics.

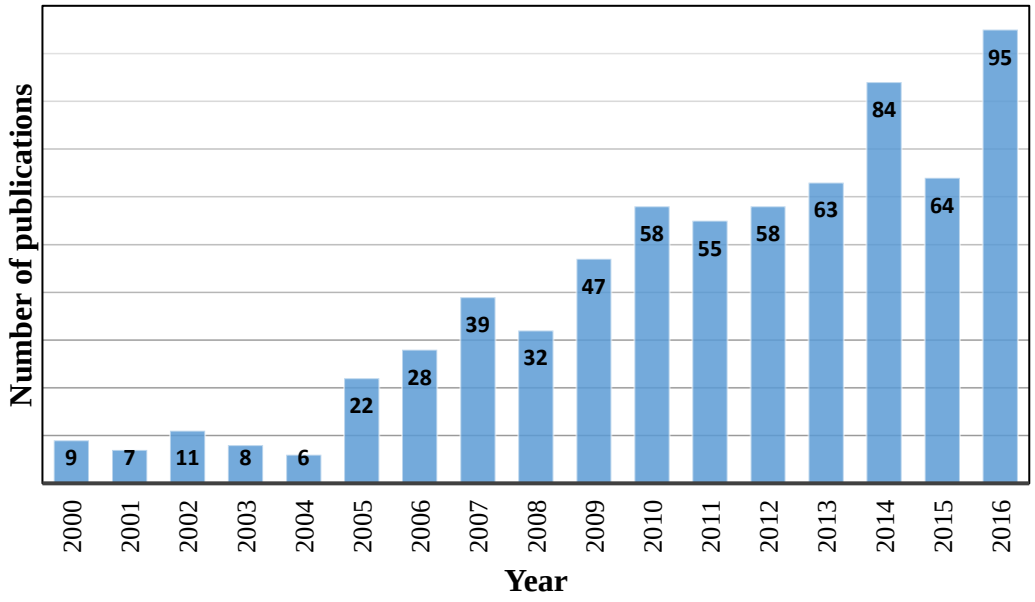


Figure 1.2 Number of publications on passive sampling of the aquatic environment between 2000 and 2017 (from an advanced search using SCOPUS using search terms "passive-sampling, water, river, groundwater and effluent" in title, abstract or key words of article).

Membranes used in absorptive passive sampling are the most critical component of the sampler and mass transfer of compounds through the membrane is greatly

influenced by the nature of the membrane material (74, 75). Compound molecules flow from one medium to the other until equilibrium is reached in the sampler or until the sampling deployment is terminated, thus the quantity of the compound collected by the sampler is dependent on both its concentration in the sampled medium and the exposure time (71). The ratio of analyte distribution between the two media involved, or the experimental calibration of the device, can then be used to determine the analyte's concentration.

The use of passive samplers can provide an estimate of TWA concentrations of pollutants of interest and permits sequestration of residues from episodic events commonly not detected with grab sampling. In addition, the technique can allow the concentration of ultra-trace, yet, toxicologically relevant contaminant mixtures to be determined over extended periods of time (43).

The uptake of compounds from water into a passive sampler can be described by a first-order, one compartment mathematical model as shown in Equation 1.1 (76).

$$C_s(t) = C_w \frac{k_1}{k_2}(1 - e^{-k_2 t}) \quad - \quad 1.1$$

Where $C_s(t)$ is the concentration of the analyte in the sampler at time t, Cw is the concentration of the analyte in the aqueous environment and k_1 and k_2 are the upload and offload rate constants respectively. This equation can be further simplified depending on whether the sampler used is in the linear, curvilinear or equilibrium phase as shown in Figure 1.3 (44).

For equilibrium passive samplers, where a rapid achievement of equilibrium between contaminants in the water to be sampled and contaminants inside the passive sampler is established, Equation 1.1 reduces to Equation 1.2 (77).

$$C_s = C_w \frac{k_1}{k_2} = C_w K_{sw} \quad - \quad 1.2$$

Where K_{sw} is the sampler-water partition coefficient. When K_{sw} is known it is possible to estimate the concentration of the dissolved analytes and as such determine the TWA for the water body in question (78).

For integrative passive samplers (also termed kinetic passive samplers), it is assumed that the rate of mass transfer between both mediums is linearly proportion to the difference in chemical activity of the analyte for both media. Equation 1.1 can then be reduced to,

$$C_s(t) = C_w k_1 t \quad - \quad 1.3$$

This can in turn be rearranged to an equivalent relationship,

$$M_s(t) = C_w R_s t \quad - \quad 1.4$$

Where $M_s(t)$ is the mass of analyte accumulated in the receiving phase as exposure for length of time (t), R_s is the sampling rate which is the product of the first order rate constant for uptake of pollutant (k_1) and the volume of water that gives the same chemical activity as the volume of the receiving phase. When R_s is known, C_w (the

TWA concentration of a compound in the water phase) may be calculated from the sampling rate R_s, the amount $M_s(t)$ of the analyte trapped by the receiving phase and the exposure time (t). For most devices operating in the linear mode, R_s does not vary with C_w but is affected by water flow, turbulence, temperature and biofouling (43, 76, 79). A passive sampling device is left in the water for a few days to several weeks, during which it sequesters hydrophobic or hydrophilic water-borne contaminants depending on the sampler design and, at the end of the period, the sampler is removed and then analysed for the contaminants. Passive samplers combine sampling, selective analyte isolation, pre-concentration and in some cases, speciation preservation in one step; reducing sample preparation costs and the potential for contamination with spot samples which must typically undergo several extraction and concentration steps in the laboratory (80). Moreover, decomposition of the sample during transport and storage and/or changes during sample enrichment are also minimised (74).

1.6.1 Passive sampling devices for organic compounds

Four types of commonly used passive samplers for organic compounds are discussed in the following section.

1.6.1.1 The semipermeable membrane device

The semi-permeable membrane device (SPMD) was developed by Huckins et al. as technique for the monitoring of lipophilic compounds and is the most widely used passive sampler used for investigative monitoring in the aqueous environment (81). The SPMD is a bi-phasic sampler for water comprising of a lay-flat low density

polyethene (LDPE) which is a non-porous material with no fixed pores having only transient cavities with typical size of 1 nm tubing enclosing a thin film of high purity synthetic triolein (82).

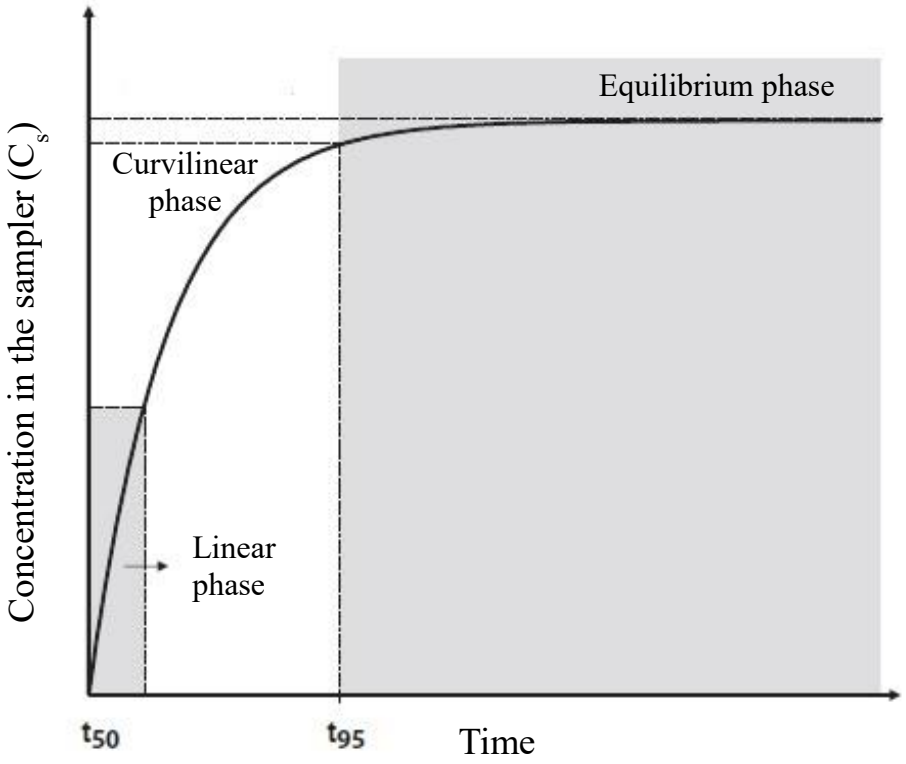


Figure 1.3 Plot of the three phases of passive sampler uptake, i) linear phase, ii) curvilinear phase and iii) equilibrium phase. Time is given in half lives. Adapted with permission from (44), copyright 2006, Springer.

In an aquatic environment, SPMDs passively accumulates hydrophobic organic compounds with partition coefficients (Log_{10} K_{ow}) in the range of 3.0 to 6.0, such as polynuclear aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organotin compounds plus various pesticides (including organochlorines, organophosphates and synthetic pyrethroid pesticides) and many other non-polar chemicals (83-86). In general, any hydrophobic compound with Log_{10} K_{ow} > 3 and a cross-sectional diameter less than 10 Å (1 nm) can be sampled by SMPDs in water

(87). Uptake of contaminants by SPMDs is strongly dependent on the compound's physical and chemical properties and can be greatly influenced by factors such as temperature, flow rate and biofouling (86).

SPMDs have several advantages over spot sampling, these include long sampling times without approaching equilibrium and accurate representation of the freely dissolved fraction of the analyte in water (74). However, its main shortcoming is the lengthy and complex sample preparation procedure required to recover the accumulated compounds from the receiving phase including dialysis of the SPMD followed by size exclusion chromatography; this latter step is required to remove the triolein which would otherwise interfere with the GC analysis (44, 88). The entire procedure also consumes large amounts of hazardous high-purity solvents and is seen by many as environmentally unfriendly.

1.6.1.2 The silicon rubber or polydimethylsiloxane sampler

The silicon rubber (SR) sampler consists of a single phase based on polydimethylsiloxane (PDMS) and, like other hydrophobic samplers such as the SPMD, are suitable for compounds with Log_{10} Kows greater than 3 (89). The surface area and thickness of the sampler can be varied to adjust the sampling rate and, for compounds with Log_{10} Kows up to 4 or 5, equilibrium can be reached with long deployment times (90). Nine cm x 5cm is the most commonly used size for deployments, but Allan et al. produced a SR sampler with the same dimensions and surface area as that of an SPMD, allowing the use of the same holders and protective cages (91). The SR samplers are cheap, robust, and have a huge advantage over the

SPMD in that it can be used several times, making it a very cost effective sampler. The samplers must, however, be thoroughly pre-extracted with an organic solvent such as ethyl acetate to remove oligomers before they can be used otherwise contamination or damage to gas chromatography column can occur and even low concentration of oligomers can cause interference with gas chromatographic analysis (92). Extraction of the absorbed substances from the silicone rubber sampler after exposure is straightforward, usually involving Soxhlet extraction with methanol, acetonitrile or mixtures of polar and non-polar solvents (93). Brockmeyer et al. successfully used accelerated solvent extraction (ASE) to facilitate the pre-cleaning and extraction process of silicone sheets and significantly decreased time and solvent usage compared to the commonly used Soxhlet extraction methods (92).

Silicone rubber samplers have seen a sharp rise in their use over the past decade for the monitoring of hydrophobic pollutants in rivers and in marine waters and have been successfully applied for the monitoring of PAHs, PCBs, hexachlorobenzene as well as organophosphate pesticides (86, 94, 95). Allan et al. tested silicone rubber and LDPE strips for the screening of a wide range of chemicals and showed that the former is less discriminating than LDPE for polar substances such as organophosphate compounds (89).

1.6.1.3 The polar organic chemical integrative sampler

The polar organic chemical integrative sampler (POCIS), shown in Figure 1.4, was developed by Alvarez in 1999 for sampling water soluble (polar or hydrophilic) organic micropollutants from aquatic environments (96). The POCIS is an adsorption type sampler and typically consists of 200 mg of sorbent enclosed between two

microporous polyethersulphone (PES) diffusion-limiting membranes. To prevent sorbent loss, two compression rings are used to firmly sandwich the PES membranes. The rings are made of either aluminium, as in the original design, but stainless-steel is now the preferred material as, unlike aluminium, it resists corrosion. The rings are firmly secured in place using a combination of thumb screws, bolts, nuts or clips. PES is a porous membrane characterised by fixed pores of about 0.1 µm diameter which allow molecules to diffuse through it (based on size exclusion) to the sorbent where they are adsorbed; larger materials, with cross-sectional diameters of less than 0.1 µm, such as particulate material, macromolecules and bacteria are therefore excluded (96, 97). Figure 1.5 shows most of the barriers to chemical uptake by the POCIS sorbent. Mass transfer of the solutes from the water include the following steps: movement of solutes from the water and diffusion through the water boundary layer, diffusion through the water filled membrane pores and through the membrane matrix, and finally, diffusion through any water boundary layers associated with the inside of the membrane and sorbent particles (98).

POCIS has an effective surface area of 45.8 cm^2 and contains a sorbent mass of 200 mg and typically adsorbs organic contaminants with Log_{10} K_{ow} values between approximately 0.1 and 3, although compounds with higher Log_{10} Kows, such as hormones and steroids, are also known to accumulate within POCIS devices (99, 100).

1.6.1.3.1 Types of sorbents used with POCIS

Two configurations of POCIS are commonly used, each containing a different sorbent(s). A configuration for pesticides, hormones and wastewater related chemicals consists of a triphasic admixture of Isolute ENV^{+} polystyrene divinylbenzene resin (80% by weight) and Ambersorb 572 carbon, lightly dispersed on S-X3 Biobeads (20% by weight) (41, 101).

Figure 1.4 The polar organic chemical integrated sampler (POCIS) for sampling water soluble organic micropollutants from aquatic environments. Photo taken in authors laboratory in 2014.

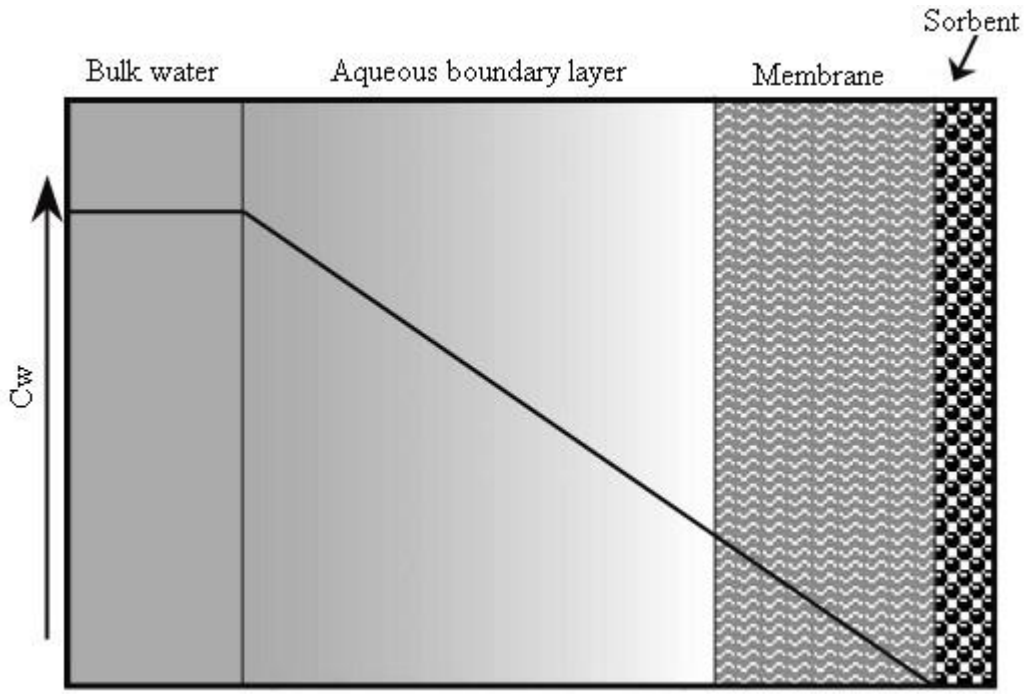


Figure 1.5 Process of compound diffusion and barriers to chemical uptake into the POCIS and polar Chemcatcher® samplers. The line shows the chemical concentration gradient in the water through each barrier. Reproduced with permission from (98), copyright 2007, Elsevier.

The alternative configuration termed 'Pharmaceutical POCIS' contains 200 mg of a single hydrophilic-lipophilic balanced sorbent (Waters Oasis® HLB) which is a water-wettable reversed phase sorbent comprising of a specific ratio of two linked monomers namely N-vinylpyrrolidone (hydrophilic) and divinylbenzene (lipophilic) (102). This mixture forms a macroporous poly(dinylbenzene-co-N-vinylpyrolidone) polymer that exhibits both hydrophilic and lipophilic retention characteristics capable of adsorbing both polar and non-polar compounds. Oasis® HLB sorbent is characterised by enhanced retention of polar analytes and a relative hydrophobic retention capacity that is about three times higher than that of traditional silica based SPE sorbents (98). Oasis HLB is by far the most commonly used sorbent incorporated into POCIS and has found wide application for sampling pesticides, pharmaceuticals and personal care products (PPCP's) plus many industrial chemicals in water (103-

106). POCIS has also been used as a targeted screening tool for determining the possible sources and relative amounts of organic contaminants in surface water and wastewater and drinking water (102). POCIS samplers remain in the integrative phase of sampling for much shorter exposure periods than SPMD or SR samplers with typical deployment periods of around 14 days but longer periods of at least 30 days have been reported (98, 102).

1.6.1.4 The Chemcatcher®

The Chemcatcher® passive sampler was developed in 2000 (42) and has been adapted for various types of water contaminants (e.g., trace metals, organometallics, pesticides, pharmaceutical residues and various emerging contaminants) making it the most flexible of all passive samplers currently used (63, 107, 108).

As the Chemcatcher® body is manufactured from PTFE it has a low sorption capacity for most environmental contaminants allowing it to be used to sample a wide variety of contaminants (42). PTFE is also a robust material allowing it to be used in any environment and they have been reused over a hundred times in the author's laboratory keeping operational monitoring and replacement costs low.

The Chemcatcher® retains a membrane layer covering a solid receiving phase in the form of a 47 mm SPE disk and the receiving phase or solid sorbent within the disk is immobilised in a PTFE fibril matrix (as is the case with the 3M Empore™ disk) (63, 109) but can also be incorporated into a glass fibre matrix as with the Horizon Technology, HLB disk (64). A comprehensive range of commercially available

sorbents (within disks) can be chosen for increasing the range of compounds sampled or for making highly selective phases for use with the Chemcatcher® (63).

There are two configurations of Chemcatcher® on the market; organic and inorganic both having an active sampling area of 17.5 cm^2 (110). For the organic Chemcatcher®, both polar and non-polar organic compounds can be sampled and this method has found many applications with deployment periods generally ranging from 7 to 30 days, with 14 days being common enabling the measurement of TWA concentrations (111). The accumulation of compounds from the surrounding environments into the Chemcatcher® is influenced by the choice of a favourable adsorbent receiving phase. Selected analytes permeate through the diffusion membrane phase before being adsorbed or absorbed onto the receiving phase (107). The diffusion membrane used for the Chemcatcher® depends on the nature of the analyte to be sampled with LDPE being used in many cases to sample for hydrophobic contaminants (112) while a cellulose acetate (CA) membrane can be used for organo-metallic species such as the organo-tins (113), and a polyethersulphone (PES) membrane for polar compounds (114).

1.6.1.4.1 Non-polar Chemcatcher®

The non-polar Chemcatcher® is an integrative passive sampler, consisting of a C_8 or C_{18} Empore® disk receiving phase saturated with *n*-octanol and fitted with a low-density polyethylene diffusion membrane, which can be calibrated for the measurement of time-weighted average concentrations of hydrophobic micropollutants, including PAHs, PCBs and OCs, in the aqueous environment (112).

Following retrieval of the sampler, the disassembly of the sampler and extraction of the disks is very straightforward using relatively low volumes (≤ 20 mL) of organic solvent (115).

In calibration experiments for non-polar compounds the effect of physicochemical properties e.g. compound hydrophobicity, water turbulence and temperature etc, have been investigated and it has been found that the rate of uptake of compounds from water to the receiving phase is related to the rate at which they offload to the water (42, 116). This enables the use of performance reference compounds (PRCs), pre-loaded on to the receiving phase disk, to be used and to adjust uptake rates for the effects of temperature and hydrodynamic conditions in the field (107). Calibration data is available for many non-polar chemicals and it is also possible to predict uptake rates based on a model produced by Vrana et al. (110). A non-linear regression was performed for the Log_{10} of the sampling rate from a series of nine calibration experiments using a third order polynomial function of Log_{10} K_{ow}. The plot obtained (Figure 1.6) showed good correlation for sampling rates of compounds with Log_{10} K_{ow} in the range from 3.7 to 6.8. Further details on calibration are mentioned in section 1.6.2.

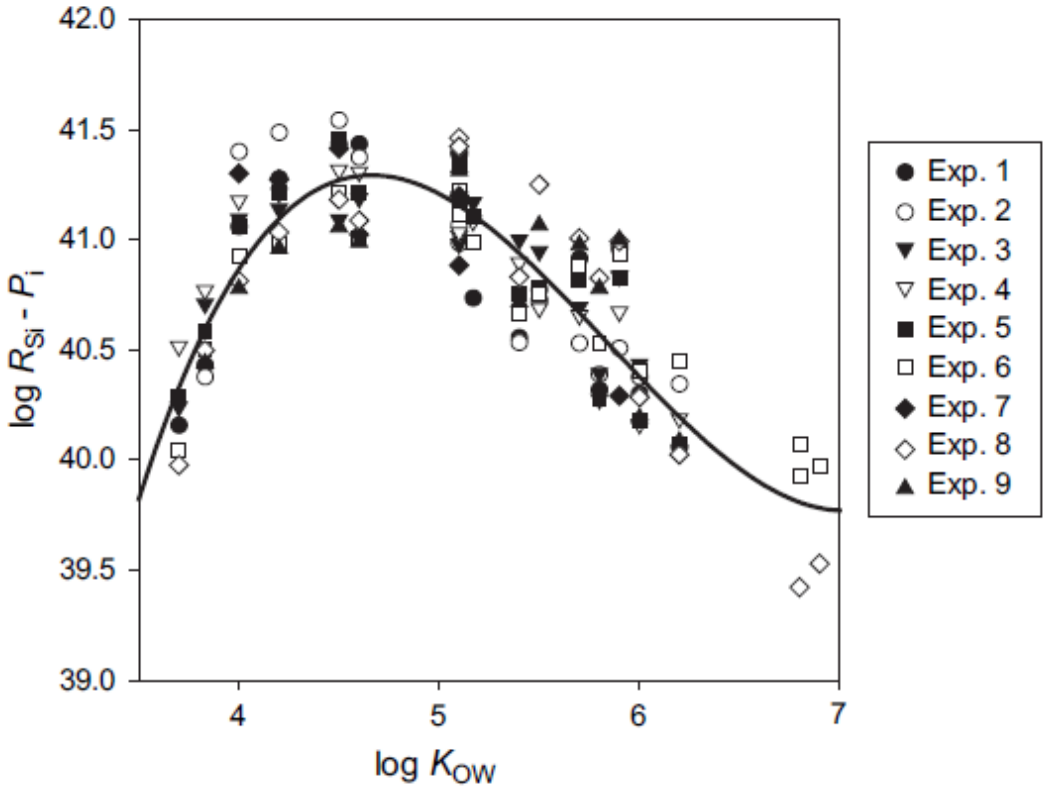


Figure 1.6 A plot of Log$_{10}$ R_{Si} - Pi (as a function of the sampling rate) versus Log$_{10}$ K_{ow} for the non-polar Chemcatcher from a series of nine calibration experiments using a third order polynomial function. Reproduced with permission from (110), copyright 2007, Elsevier.

1.6.1.4.2 Polar Chemcatcher®

The Chemcatcher® is typically used with a modified styrene-divinylbenzene (SDVB) adsorbent for polar to medium polarity compounds such as pesticides, pharmaceuticals and industrial chemicals with most researchers using the SDVB-RPS or SDVB-XC adsorbent bound in a PTFE matrix disk with the exposed surface normally covered with a thin diffusion-limiting polyethersulphone (PES) membrane (86, 109, 111, 117, 118).

Recently Petrie et al. (2016), investigated the suitability of the Chemcatcher® sampler for monitoring organic compounds, including pharmaceuticals, in wastewater effluent containing a Horizon Technology, Atlantic HLB disk for the first time (64). The existing PTFE body was modified to accommodate the thicker HLB disk, which is approximately 3–4 mm thick, without altering the existing sampling area or hydrodynamics of the sampler; the modified sampler was named the polar Chemcatcher®.

The Atlantic disk is estimated to contain a very similar amount of Waters Oasis® HLB sorbent to that used with the POCIS sampler. Using the HLB sorbent is an obvious choice for the receiving material because it is the preferred sorbent for analytical methods involving extracting a broad range of polar organic pollutants in spot or composite water samples (119-121). This has been demonstrated in numerous previous studies using POCIS (≥ 21), with > 90 individual pharmaceuticals detected (122); moreover, the Chemcatcher® with a HLB receiving phase is highly desirable because it combines the proven ability of HLB as a sorbent for a broad range of polar organic pollutants with the handling benefits of the Chemcatcher®.

The Atlantic HLB disk configuration was preferred over POCIS for this work (which contains an approximate equivalent mass of sorbent as a powder) because loose sorbent within POCIS can sag toward the base of the device during deployment in the vertical plane, potentially reducing the active sampling surface area and increasing variability in uptake rate (123). As the sorbent is immobilized in the HLB disk, variability of field data should be minimized while improving ease of use; this configuration will also overcome potential issues with the loss of sorbent during

assembly, deployment or subsequent extraction. The polar Chemcatcher® devices were deployed in the final effluent stream of a waste water treatment plant (WWTP) in the south west of England.

In-situ calibration was undertaken and is recommended for determining representative uptake rates because it can be conducted in the exact location where future measurements are to be taken. This approach can be considered as essential for quantitative purposes because it accounts for site-specific factors (e.g., matrix composition) that cannot be adequately replicated under laboratory conditions (122). Once calibrated in-situ, the determined uptake rates can be applied to estimate pollutant concentrations in future studies at the same site. *In-situ* calibration offers other advantages as an extensive experimental laboratory setup need not be required and maintained. Moreover, it avoids the need to purchase relatively large quantities of target micropollutants for laboratory experiments, which may be cost-prohibitive (123). From the 66 compounds detected in composite samples during the 8-day calibration study, 65 were found in every composite sample. Furthermore, the majority exhibited inter-day concentration variations of <20% (n = 8). Linear uptake was observed for most of the detected compounds over the 9 days of deployment and sampling rates (R_s), determined for 59 compounds, were generally in the range of 0.01-0.10 L/day.

The concentration of compounds measured using passive samplers was generally within ±20% of that determined from composite samples taken during the 9-day deployment. The concentrations for many of the micropollutants determined by the passive samplers were underestimated (52 of 62), albeit not greatly (maximum =

−25%). This may have been due to a lag between deployment and the start of micropollutant uptake.

A comparison of R_s values obtained from Petrie and co-workers work was made against sampling rates for the POCIS gathered from published values; the data are presented in Table 1.1. As the Chemcatcher® has a sampling area of 3.01 less than the POCIS (sampling areas of 15.2 cm^2 and 45.8 cm^2 respectively) the R_s values for the Chemcatcher® were adjusted accordingly to also allow a comparison based on sampler surface area. R_s values obtained from Petrie et al. were in very good agreement with the literature values for 5 of the 16 pharmaceuticals, these were Atenolol, Caffeine, Diclofenac, Ketoprofen and Naproxen. Seven others fell between the lowest and highest literature values and 5 others fell outside literature values.

The comparison between samplers is complicated by the fact that the POCIS typically uses a 0.1 µm PES membrane whilst the Chemcatcher employs a 0.2 µm PES membrane. This is not expected to make a significant difference in uptake rates as most molecules under 1000 Da are expected to pass through the smaller pores unhindered. The use of a protective cage for deployment may also affect the uptake rate as well as the orientation of the sampler.

Table 1.1 A comparison of *Rs* values obtained from Petrie et al (64) for the polar Chemcatcher against published sampling rates for the POCIS sampler

Compound	Compound class	R_s ± SD L/d	Corrected R_s ± SD L/d	Type of calibration	Ref
Estrone	Hormone	0.071[c] (Chemcatcher)	0.214	*In-situ* (WWTP effluent)	(64)
		0.120 (±0.018)		Laboratory	(124)
		0.699 (±0.087)		Laboratory	(125)
		0.636 (±0.068)		Laboratory	(125)
		0.601 (±0.022)[b]		Laboratory	(125)
		0.363 (±0.065)[a]		Laboratory	(125)
		0.28		*In-situ* (WWTP effluent)	(126)
Ketoprofen	NSAID	0.037[c] (Chemcatcher)	0.111	*In-situ* (WWTP effluent)	(64)
		0.135 (±0.035)		Laboratory	(127)
		0.083 (±0.078)[a]		Laboratory	(127)
Ibuprofen		0.048[c] (Chemcatcher)	0.144	*In-situ* (WWTP effluent)	(64)
		0.348 (±0.052)		Laboratory	(125)
		0.254 (±0.019)		Laboratory	(125)
		0.204 (±0.004)[b]		Laboratory	(125)
		0.197 (±0.013)[a]		Laboratory	(125)
Naproxen		0.048[c] (Chemcatcher)	0.144	*In-situ* (WWTP effluent)	(64)
		0.392 (±0.024)		Laboratory	(125)
		0.298 (0.016)		Laboratory	(125)
		0.239 (±0.009)[b]		Laboratory	(125)
		0.200 (±±0.037)[a]		Laboratory	(125)
		0.116 (±0.053)		Laboratory	(127)

continued

Compound	Compound class	R_s ± SD L/d	Corrected R_s ± SD L/d	Type of calibration	Ref
Diclofenac		0.044[c] (Chemcatcher)	0.132	*In-situ* (WWTP effluent)	(64)
		0.166 (±0.052)		Laboratory	(127)
		0.092 (±0.055)[a]		Laboratory	(127)
		0.12		*In-situ* (river downstream)	(126)
		0.16		*In-situ* (WWTP effluent)	(126)
Atenolol	Beta-blocker	0.034[c] (Chemcatcher)	0.102	*In-situ* (WWTP effluent)	(64)
		0.094 (±0.015)		Laboratory	(125)
		0.087 (±0.003)[b]		Laboratory	(125)
		0.073 (±0.013)[a]		Laboratory	(125)
		0.040 (±0.070)		Laboratory	(127)
		0.037 (±0.064)[a]		Laboratory	(127)
		0.090 (±0.064)		*In-situ*	(128)
Metoprolol		0.050[c] (Chemcatcher)	0.151	*In-situ* (WWTP effluent)	(64)
		0.465 (±0.039)		Laboratory	(125)
		0.309 (±0.106)		Laboratory	(125)
		0.156 (±0.034)[a]		Laboratory	(125)
		0.599 (±0.270)		Laboratory	(127)
		0.097 (±0.066)[a]		Laboratory	(127)
		0.270 (±0.140)		*In-situ*	(128)
Propranolol		0.114[c] (Chemcatcher)	0.343	*In-situ* (WWTP effluent)	(64)
		0.917 (±0.084)		Laboratory	(125)
		0.646 (±0.029)		Laboratory	(125)
		0.484 (±0.063)[b]		Laboratory	(125)

continued

Compound	Compound class	R_s ± SD L/d	Corrected R_s ± SD L/d	Type of calibration	Ref
		0.271 (±0.066)[a]		Laboratory	(125)
		0.980 (±0.345)		Laboratory	(127)
		0.147 (±0.129)[a]		Laboratory	(127)
		0.06		*In-situ* (river downstream)	(126)
		0.12		*In-situ* (WWTP effluent)	(126)
		0.250 (±0.133)		*In-situ*	(128)
Venlafaxine	Antidepressants	0.065[c] (Chemcatcher)	0.196	*In-situ* (WWTP effluent)	(64)
		0.521 (±0.033)		Laboratory	(125)
		0.388 (±0.038)		Laboratory	(125)
		0.167 (±0.065)[b]		Laboratory	(125)
		0.104 (±0.039)[a]		Laboratory	(125)
Fluoxetine		0.032[c] (Chemcatcher)	0.096	*In-situ* (WWTP effluent)	(64)
		0.974 (±0.045)		Laboratory	(125)
		0.694 (±0.009)		Laboratory	(125)
		0.484 (±0.012)[b]		Laboratory	(125)
		0.433 (±0.058)[a]		Laboratory	(125)
		1.37 (±0.35)		Laboratory	(127)
		0.223 (±0.130)[a]		Laboratory	(127)
Sertraline		0.116[c] (Chemcatcher)	0.349	*In-situ* (WWTP effluent)	(64)
		0.868 ±0.054)		Laboratory	(125)
		0.622 (±0.026)		Laboratory	(125)
		0.602 (±0.036)[b]		Laboratory	(125)
		0.471 (±0.044)[a]		Laboratory	(125)

continued

Compound	Compound class	R_s ± SD L/d	Corrected R_s ± SD L/d	Type of calibration	Ref
Citalopram		0.069[c] (Chemcatcher)	0.208	*In-situ* (WWTP effluent)	(64)
		0.758 (±0.033)		Laboratory	(125)
		0.735 (±0.015)		Laboratory	(125)
		0.354 (±0.020)[b]		Laboratory	(125)
		0.314 (±0.086)[a]		Laboratory	(125)
Desmethyl-citalopram		0.149[c] (Chemcatcher)	0.448	*In-situ* (WWTP effluent)	(64)
		0.707 (±0.024)		Laboratory	(125)
		0.598 (±0.044)		Laboratory	(125)
		0.401 (±0.082)[b]		Laboratory	(125)
		0.355 (±0.035)[a]		Laboratory	(125)
Carbamazepine	Anti-convulsant	0.045[c] (Chemcatcher)	0.135	*In-situ* (WWTP effluent)	(64)
		0.561 (±0.024)		Laboratory	(125)
		0.397 (±0.018)		Laboratory	(125)
		0.230 (±0.016)[b]		Laboratory	(125)
		0.235 (±0.046)[a]		Laboratory	(125)
		0.348 (±0.116)		Laboratory	(127)
		0.112 (±0.023)[a]		Laboratory	(127)
		0.1		*In-situ* (river downstream)	(126)
		0.21		*In-situ* (WWTP effluent)	(126)
Temazepam	Benzodiazepine	0.326[c] (Chemcatcher)	0.981	*In-situ* (WWTP effluent)	(64)
		0.421 (±0.101)		Laboratory	(127)
		0.128 (±0.062)[a]		Laboratory	(127)

continued

Compound	Compound class	R_s ± SD L/d	Corrected R_s ± SD L/d	Type of calibration	Ref
Caffeine	Stimulant	0.037[c] (Chemcatcher)	0.111	*In-situ* (WWTP effluent)	(64)
		0.127 (±0.021)		Laboratory	(125)
		0.151 (±0.018)		Laboratory	(125)
		0.096 (±0.008)[b]		Laboratory	(125)

Key

[a] Unstirred condition

[b] Temperature ≤10 °C

[c] Temperature of effluent 8.4 ± 0.5 °C

Sampling rate (*Rs*) for POCIS with 45.8 cm^2 exposure and HLB sorbent. Standard conditions: unsalted water, stirred, between 15 °C and 25 °C, except when specified.

1.6.1.5 Other types of passive sampler for organic compounds

The DGT is constructed from two layers and compound uptakes and kinetics are different. During the deployment of DGT's, the analyte in solution diffuses through the diffusive boundary layer and a material diffusion layer comprising of a filter membrane and hydrogel; finally, the analyte is taken up by a binding layer. As the diffusion layer is thicker than the typical thickness of the diffusive boundary layer, this makes the DGT measurement insensitive to the hydrodynamic conditions of the water.

In 2012 Chen et al. developed the first configuration of DGT (diffusive gradients in thin-films) for measuring organic chemicals (termed the o-DGT) (129) and, in subsequent research, investigated the fate and behaviour of 40 antibiotics in the environment by deploying o-DGT's in a UK WWTP (130). Of the 14 antibiotics detected with spot samples, 10 were detected in the o-DGT devices deployed for more than 7 days. o-DGT has also been use to investigate various other organic compounds including pesticides, phenols, bisphenols using various binding gels such as activated charcoal, Oasis® HLB, Oasis® HLB-MAX, and a molecularly imprinted polymer (131-135). Despite having normalized surface area sampling rates of 0.54−5.74 mL/d/cm^2, which are comparable with other polar passive samplers, o-DGT is less sensitive than active sampling methods due to its low sampling rate; however, sensitivity could be increased by pooling binding layers from parallel samplers. However, a significant advantage of the DGT over other passive samplers is that calibration is not required, rather, the diffusional characteristics are obtained in the laboratory as fundamental physical properties.

1.6.2 Uptake of analytes by passive samplers and determination of aqueous TWA concentrations

To obtain TWA concentrations of target compounds extensive calibrations are required to characterise the uptake of various compounds into passive samplers (113, 136, 137). Compound uptake rates depend on their physico-chemical properties and on the sampler design and can also be influenced by environmental variables such as flow rate, (a measure of turbulence) that affects the thickness of the water boundary layer, temperature and bio-fouling (123, 138). These variables pose a challenge when comparing estimated water concentrations of pollutants sequestered by non-polar and polar passive samplers deployed at different sites experiencing different sets of environmental conditions (88). Figure 1.7 compares an SPMD that has been bio-fouled, after deployment in a river for a period of a month, and an unused SPMD.

Booij et al. described a procedure for estimating the uptake kinetics in non-polar samplers by spiking them prior to deployment with several (PRCs) that did not occur in nature but had very similar properties to the target compounds, (e.g. mass labelled analogues). These PRCs can compensate for all of the environmental variables described above and by doing so, the *in-situ* sampling rates of target compounds can be determined more accurately than with laboratory calibration (139). Ideally, the incorporation of PRCs into POCIS and Chemcatcher samplers could yield accurate *in-situ* uptake rates and therefore correct estimates of compound TWAs. However, estimation of TWAs using the PRC approach is complicated by strong compound sorption to the sampler sorbent and desorption or offload kinetics are thought to be non iso-kinetic with the uptake.

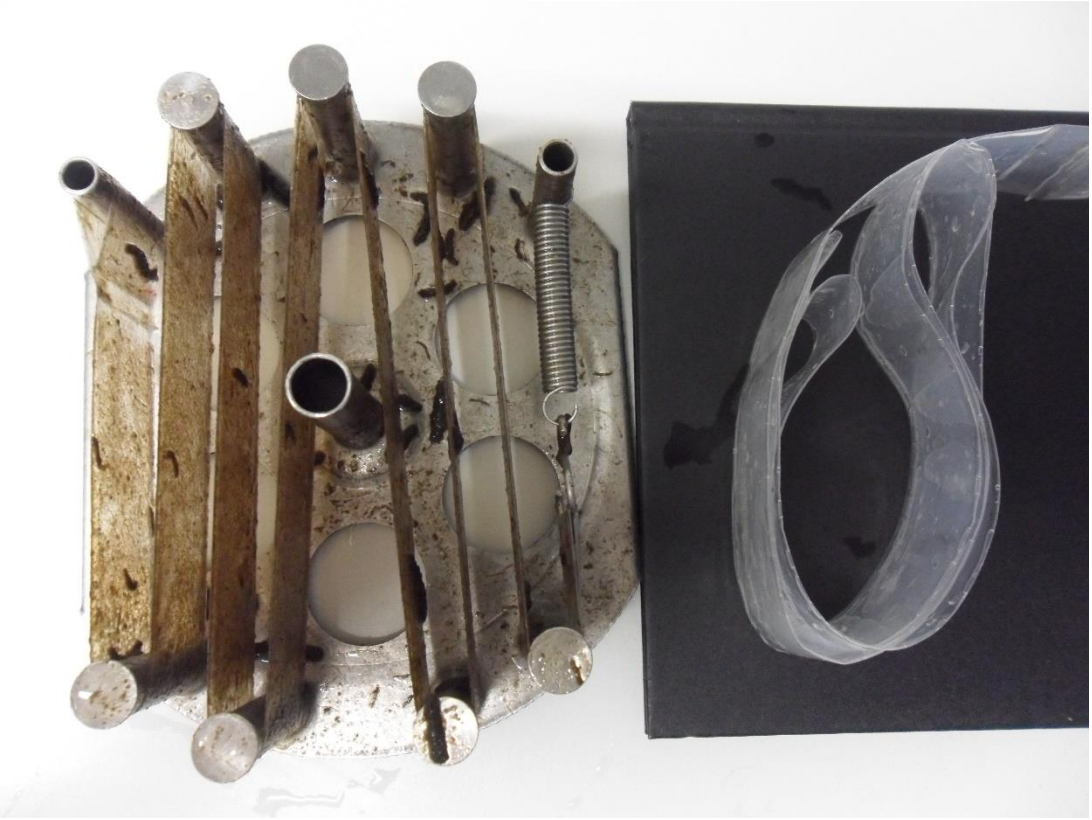


Figure 1.7 A bio-fouled SPMD (on holder) retrieved from a watercourse after a deployment period of one month (left) and an unused SPMD (right). Photo taken by the author in 2014.

Attempts have been made to use PRCs with POCIS and polar Chemcatcher samplers and work undertaken by Shaw et al. with the Chemcatcher concluded that while compound uptake was linear and reproducible, PRC loss was not linear, indicating that the dissipation rates of the PRCs could not be exploited to estimate field exposure conditions on uptake rates (118). An alternative *in-situ* calibration technique tested using PRC loaded polydimethylsiloxane (PDMS) disks deployed alongside the Chemcatcher samplers showed some promise (118). Vermeirssen et al. tested the uptake and release kinetics of 22 polar organic chemicals in the Chemcatcher and concluded that as sorption and desorption did not show isotropic kinetics, it was not

possible to develop robust PRCs for adsorbent material such as SDB disks (109). Kaserson et al. unsuccessfully used PRC's with their work on per-fluorinated chemicals (PFCs) confirming widespread concern as to their applicability with polar samplers such as POCIS (140). Only Fauvelle et al. were able to successfully employ a PRC (deuterated deisopropylatrazine) for 10 herbicides in river waters using POCIS containing HLB sorbent (141); therefore reliable calculation of *in-situ* compound uptake rates using the PRC approach for adsorption based passive samplers may only be possible for a very limited number of compounds (e.g. compounds within a specific group or those with very similar structures). Although the parameters in a laboratory calibration may not be completely identical to field conditions, uptake rates for most compounds will continue to be determined in this manner for adsorption based passive samplers (123). A further drawback of the polar passive samplers is that, for all compounds, the sampling rate has to be determined separately with all sorbents for every application (142).

1.7 Analytical separations and detection techniques

1.7.1 One-dimensional gas chromatography

Gas chromatography is a technique that has continually evolved since its inception in 1951 (144) and the introduction of the first commercial systems by Griffin and George (London, UK) in 1954. It is used for the separation of volatile and semi-volatile compounds with minimal degradation and this includes compounds which can be volatilised within the temperature range of the gas chromatograph, typically ambient to 400 °C but can go as low as -25 °C with cryogenic cooling. GC can also be used to analyse semi-volatile and non-volatile compounds which are initially derivatised to

make them volatile. e.g. compounds that contain active and/or labile hydrogens such as carboxylic acids, amides, amines and thiols (145-149).

A sample or aliquot of an extract is introduced into a heated injector (typically 200–350 °C) using a syringe, where the sample is vaporised (if a liquid) and the gas transferred onto the chromatographic column via a carrier gas such as helium, nitrogen and more recently hydrogen. The carrier gas transports the sample through the column which is housed within a heated compartment or oven that is either maintained at a constant temperature (isocratic) or temperature programmed. The sample is separated inside the column - usually a long silica based column with small internal diameter and the internal wall coated with a thin polymeric layer or liquid.

The column dimensions vary significantly in length and diameter, typically 10–30 m in length, 0.1–0.53 mm internal diameter and a stationary phase thickness of 0.1–5.0 µm. The stationary phase can vary greatly from non-polar columns such as polydimethylsiloxane to highly polar columns which employ ionic liquids and the choice of phase depends on the chemistry and properties of the compound(s) being analysed. The individual components of the sample are separated by differential partition between the mobile and stationary phases as they travel down the column, based on their relative vapour pressure and solubility in the immobilised liquid stationary phase.

The ‘partition or distribution coefficient’ (K) measures the tendency of an analyte to be attracted to the stationary phase as expressed in Equation 1.5.

$$k = \frac{Cs}{Cm} = k\beta \quad - \quad (1.5)$$

where *Cs* and *Cm* are the equilibrium concentration of analyte in the stationary phase and in the mobile phase (carrier gas) respectively. The partition coefficient can be calculated using the retention factor (k) and the phase ratio (β). Analytes which have a higher affinity for the stationary phase have a larger distribution coefficient and will therefore take longer to elute from the column (i.e. they will have a longer retention time). As analytes partition coefficients are temperature dependent, using oven temperature programmes is highly effective in obtaining effective and efficient separations as coefficients can vary enormously from highly volatile to semi-volatile compounds such as those commonly found in GC-MS screening methods.

The partition coefficient is determined from the phase ratio of the column and from a compounds retention factor with the latter describing the elution rate of an analyte through a column and can be determined from the analytes retention time and the retention time of an un-retained species such as air (Equation 1.6).

$$k = \frac{t_r - t_m}{t_m} \quad - \quad (1.6)$$

The phase ratio, β, is the ratio of the volume of the carrier gas to the volume of the stationary phase as shown in (Equation 1.7) where the phase ratio can be calculated using the column internal radius (r_c) and the film thickness (d_f).

$$\beta = \frac{v_m}{v_s} = \frac{r_c}{2d_f} \quad - \quad (1.7)$$

On elution from the column, the analytes pass into a detector, which responds to some physicochemical property of the analyte and generates an electronic signal measuring the amount of analyte present. A data system then produces an integrated chromatogram of response against retention time.

There are many types of detector available for gas chromatography which can be described as universal or selective which only responds to certain elements (such as halogens) or compound types (aromatics). The most common types of universal detector used in environmental analysis are the mass spectrometer, the thermal conductivity detector and the flame ionisation detector which responds to compounds containing carbon. Examples of selective detectors used in environmental analysis include the electron capture detector which is selective to compounds such as pesticides that contain halogens as well as some organo-phosphate pesticides; the nitrogen phosphorous detector which is specific for nitrogen and phosphorous; the flame photometric detector which is selective for sulphur, phosphorous and tin.

The most important factor in GC, as well as other chromatographic techniques, is to obtain resolution in the minimum amount of time. Resolution (RS) is calculated using the separation of two adjacent analyte peaks relative to their peak widths as described in Equation 1.8.

$$R_S = \frac{(t_{R2}-t_{R1})}{(w_{b1}+w_{b2})/2} = 2\frac{(t_{R2}-t_{R1})}{(w_{b1}+w_{b2})} \quad - \quad (1.8)$$

where t_{R1} and t_{R2} are the retention times of the earlier and later eluting analytes respectively; w_{b1} and w_{b2} are the peak widths of the earlier and later eluting analytes

respectively at the base. The width at the base of each peak is the segment of the peak base intercepted by the tangents drawn to the inflexion points on either side of the peaks and, in the case of two adjacent peaks, it may be assumed that the peak widths at the base are equal and thus the width of the second peak may be substituted for the average value. A resolution of 1.5 or greater between two peaks will ensure good separation, but can be improved by maximising the three terms in Equation 1.9: where *N* is the degree of column efficiency also known as the theoretical number of plates, α is the selectivity factor and *k* is the retention factor.

$$R_S = \frac{1}{4}\sqrt{N}\, x\, \frac{\propto -1}{\propto}\, x\, \frac{k}{1+k} \qquad - \qquad (1.9)$$

The plate number (*N*) is primarily a measure of the peak dispersion in the GC column, reflecting the columns performance. *N* is derived from fractional distillation, where the column or 'fractioning tower' is divided into 'theoretical plates', and can be calculated from the analytes retention time (t_R) and its peak width (w_b) at the baseline as shown in Equation 1.10.

$$N = 16\left(\frac{t_r}{w_b}\right)^2 \qquad - \qquad (1.10)$$

Similarly, for a fractioning tower of a given length (L) the higher the number of plates, the lower will be the distance between each plate. Therefore, for high efficiency separations the plate number (*N*) will be high and the plate height (H) low. H is also described in the literature as height equivalent to a theoretical plate or HETP. The two terms are related through the expression, H=L/N, therefore column efficiency, and

thus resolution, can be improved by increasing the column length, L. Very often this will increase the analysis time required especially when dealing with a complex matrix as efficiency has a square root dependence (Equation 1.9). Therefore, a four-fold increase in efficiency (i.e. quadrupling the length of the column) will result only in a two-fold improvement in resolution. Changing the column's stationary phase, thus altering the interactions between the phase and the analytes, is another means to improve the resolution.

A final measure of column efficiency is peak capacity and is simply the theoretical maximum number of separated peaks that can be fitted into a chromatogram of defined retention time between the column void volume and the last eluting peak of interest at a specified resolution. The column capacity is calculated using equation 1.11.

$$n_c = \frac{t_r,\max - t_m}{w_b\, R_s} \qquad - \qquad (1.11)$$

Where $t_{r,}\,max$ is the last eluting peak, t_m is the column void volume or dead time, w_b is the width at the base and R_s is the resolution. Due to the random distribution of peaks, which can lead to many overlapping peaks occupying the same space, the maximum peak capacity cannot be realised and a complex environmental sample would quickly exceed the available peak capacity of a one dimensional single column technique (150). Even for a moderately complex analysis such as the separation of all 209 PCB congeners, no single GC column has yet been developed to separate them all. Therefore, a multidimensional approach involving the use of more than one column is required (151-155).

1.7.2 Multidimensional gas chromatography

The capability of multidimensional separation in terms of peak capacities and the analysis of complex samples has been recognised for decades and has come to be unsurpassed by any other analytical approach (156). The theory behind multidimensional separations was mainly developed by Giddings and co-workers in the 1980's – 1990's and continues to be discussed over 20 years later (153, 157).

Multidimensional separations based on gas chromatography are categorised as either comprehensive or heart-cutting with the latter only sampling a fraction of the analytes present in the sample passed on for further separation (158-165). Comprehensive multidimensional separations, on the other hand, subjects the whole injected sample to each separation dimension so that the resulting chromatogram can be considered as being representative of the entire sample being analysed (166-168). Heart-cutting can therefore be considered as being suitable only for target based analysis, whereas comprehensive multidimensional separations can be considered as suitable for sample profiling, target based studies plus general screening (169-171).

1.7.3 Comprehensive two-dimensional gas chromatography

Two-dimensional comprehensive gas chromatography (GCxGC) was first introduced by Liu and Phillips in 1991 (172) and has been used extensively since then for the analysis of various complex mixtures where conventional 1-D separations are typically used, for example, crude oil and petroleum products (173-177), forensics (170, 178, 179), clinical (180-182), metabolomics (183, 184) and environmental

analysis (185-190). A comprehensive 2-D separation can separate many more components of a complex mixture in less time than a 1-D separation and GCxGC allows for improved separation and resolution due to its inherent superior peak capacity. Peak capacity for 1-D separations is equal to the capacity of a single column (n_c), whereas in GCxGC theoretical peak capacity is equal to the product of the individual peak capacities for the primary and secondary columns ($n_{c1} \times n_{c2}$).

To put the potential peak capacity gain of GC × GC into some perspective relative to 1-D GC; if one was to try and obtain a 10-fold peak capacity gain from a 40 m column its length would have to be 100 times longer at 4 km, the inlet pressure at around 1400 psi (with helium carrier gas) and the analysis time 1.5 months instead of approximately 1 hour. A modest 2-fold peak capacity increase would require a 160 m long column, 280 psi inlet pressure, and a minimum 8 h analysis (191). The separation power gap, that exists between GCxGC and 1-D GC, is probably even bigger than between the capillary and packed column (153).

In addition to increased peak capacity, GCxGC offers improvements in sensitivity through increased signal-to-noise ratios for analytes due to interfering compounds being separated from the analytes of interest. Improvements in analyte detection are also seen due to the high peak compression occurring at the modulator (see section 1.7.3.1) resulting in very sharp peaks, as shown in Figure 1.8, especially with thermal/cryogenic modulators but less so with pneumatic and valve modulators (192). Moreover, structurally related compounds such as PAHs, PCBs, short chained chlorinated paraffins (SCCPs), polychlorinated terphenyls (PCT's), polybrominated diphenyl ethers (PBDE's) elute with highly distinctive patterns resulting in ordered

chromatograms which can greatly assist in sample characterisation and compound identification (34, 80, 152, 187, 193-198).

1.7.3.1 Enabling GCxGC capability with existing GC chromatographs

As shown in Figure 1.9, enabling existing 1-D gas chromatographs for GCxGC use involves some relatively straightforward modifications to the oven compartment. These include a modulator, a secondary column and a small secondary oven but the latter is not always considered a prerequisite for successful GCxGC chromatography (157). Typically, the two columns used in GCxGC are of different selectivity, where the column in the first dimension is non-polar (e.g. dimethyl polysiloxane or 5 % phenyl methyl polysiloxane) whilst the second column is generally polar (e.g. 50 % diphenyl dimethyl polysiloxane). As long as the columns provide orthogonal selectivity, analysts are not confined to this configuration, and polar/non-polar, chiral/polar and other specialised combinations have been used (199-202). Columns used in the first dimension are of similar dimensions to those used in conventional 1-D GC while columns used in the second dimension are much shorter (0.5–2 m) with narrower internal diameters, typically 0.1–0.25 mm when used with the common thermal or cryogenic modulator. The modulator is positioned at the junction of the dual column set and depending on the type of modulator, cryogenic cooling and / or heating is used to trap (and compress) the eluting bands of analytes from the first-dimension column and transfer them as a narrow ‘packets’ to the second-dimension column. When a ‘packet’ is analysed in the second dimension column, the modulator prevents further elution from the first column and therefore segregates analytes into fractions according to the frequency of modulation which is typically 1–5 s. Ideally,

the modulator will collect a sufficient number of fractions over the duration of the eluting peak to preserve the separation achieved in the first dimension and, as for most chromatographic techniques, achieve ten data points which is considered a minimum for quantitative work (203). Separation of analytes in the second dimension must be undertaken quickly so that each transferred 'packet' has completely eluted from the secondary column before the following 'packet' is introduced to prevent a phenomenon called wrap-around from occurring. This happens when the analytes retention time in the second dimension exceeds the modulation period (153, 191).

1.7.3.2 Coupling of GCxGC to time-of-flight mass spectrometry (GCxGC TOF-MS)

Narrow second dimension peaks obtained from cryogenic modulation require a mass spectrometer capable of very fast scanning rates (> 50 Hz) for acceptable compound identification (204). Therefore, the coupling of fast-scanning TOF-MS with comprehensive 2-D GC provides the capabilities necessary for identification of compounds within a complex matrix and with the addition of automatic spectral deconvolution, co-eluting compounds can be distinguished based on mass spectral differences (153, 205). The ability of TOF-MS instruments to collect data over a wide mass range (> 1000 Da) gives them the potential to undertake non-targeted screening in addition to the simultaneous determination of targeted compounds (194, 206). Obtaining useful information from the analysis of these mixtures, which may contain literally thousands of different compounds, requires a reliable and relatively rapid technique to identify compounds of concern.

The suitability of TOF-MS for the detection of extremely narrow peaks was demonstrated in the later part of the 1990's by Van Ysacker et al., in which 14 volatile compounds were separated in about 40 s with a spectral acquisition rate of 20 Hz (207). In 2000, van Deursen et al. used a reflectron-type TOF-MS (Leco Pegasus II) in an ultra-fast GC experiment where 10 volatile solvents were separated in 500 ms on a microbore column using a spectral acquisition rate of 500 Hz over a 40–200 Da mass range. Even though peak widths were extremely narrow at around 12 ms, software peak re-construction was excellent. The system deconvolution algorithm could separate two partially co-eluting compounds, (octane and cis-1,4-dimethylcyclohexane) proving its ability to successfully deconvolute unresolved peaks based on mass spectral differences. Excellent library similarity factors (798-957) were obtained for most of the compounds when compared against the NIST library clearly demonstrating the effectiveness of the system under challenging conditions (208). Unlike quadrupole based MS systems that scan from high to low mass, TOF-MS achieves high-speed full-spectrum acquisition, without mass spectral skewing (i.e. spectral patterns do not change across the chromatographic peak) allowing for accurate mass library search results (209, 210).

In contrast, quadrupole based MS systems operated under relatively fast-scanning conditions in a series of GC×GC experiments by Shellie and Marriott in 2003 could acquire spectra only at a rate of 20 scans/s in a reduced range of 41–228.5 Da for the detection of oxygenated sesquiterpenes in ginseng. The authors reported that reliable quantitative data could not be derived with only three to four data points per peak but did allow for identification. However, higher molecular weight compounds could not be identified due to the limited mass range used (211).

In 2004, Debonneville and Chaintreau reported the first example of quantitation in a GCxGC application using a quadrupole based MS systems for fragrance allergens, by monitoring selected ions in pre-defined retention-time windows. A detection frequency of 30.7 Hz was reported and affirmed to be sufficient for peak quantitation as GCxGC data were in good agreement with those data derived from a 1-D GC-MS system (212). Despite being successfully applied to known target analytes, the sampling rate is insufficient for the proper construction of the narrower GCxGC analyte bands (50–100 ms).

In 2005, Adahchour et al., described the principles, practicability, and potential of rapid-scanning quadrupole instrumentation in GCxGC by employing a Shimadzu QP2010, whose detector was characterized by a scan speed of up to 10,000 amu/s and could even reach 50 spectra/s, but this work was again over a restricted mass range of only 95 Da (213). Even in 2016, the maximum scan speeds attainable from a quadrupole mass spectrometer was only 20,000 amu/s (214).

As well as being applied for targeted analysis GCxGC has been used for the screening of non-target compounds (215-220). The highly structured chromatograms obtained from GCxGC can be used very effectively as fingerprints to characterize sources of pollution with detailed congener specific data provided for pollutant classes such as polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and non-ortho substituted polychlorinated biphenyls (PCBs) (217). However, the use of GCxGC MS in the forensic domain has remained relatively scarce since its introduction by Liu and Phillips in 1991 (172). A review of GCxGC MS applications in forensics investigations undertaken by Sampat et al. in 2016

recorded only 36 peer reviewed articles by 12 research groups between 2001 and 2015 (170). Figure 1.10 shows a plot obtained from an advanced SCOPUS search on the number of publications employing GCxGC MS analysis between 2000 and 2016 using the following search criteria.

i) GCxGC MS (all matrices)

ii) GCxGC MS (environmental matrices, targeted compound analysis only)

iii) GCxGC MS Screen (all matrices including environmental, forensic, petrochemical, toxicology and biology)

iv) GCxGC MS Screen (environmental matrices only)

The output from the search (Figure 1.10, identified a total of 1315 publications using GCxGC MS (numbers for TOF-MS detection in parentheses) for the analysis of all matrix types with 957 (601) for GCxGC MS target compound analysis in all matrices, 172 (115) for targeted environmental analysis, 148 (103) for GCxGC MS screening in all matrices but only 38 (29) publications for the screening of environmental samples. The number of publications peaked in 2011 - 2012 with only 6 and 5 publications respectively and fewer than this have appeared in subsequent years. Moreover, only11 publications were found for the target and screening analysis of passive sampler extracts using GCxGC MS between 2000 – 2016.

Despite the unparalleled peak capacity that GCxGC MS offers over 1-D GCMS and the highly successful determination of complex environmental, petrochemical and biological matrices for targeted compounds, there has been a tremendous underutilisation of the technique for screening of environmental matrices. This may

be due to the higher costs associated with TOF-MS systems compared with quadrupole MS systems; this however is likely to change rapidly with the recent introduction of bench-top GC-TOF-MS systems by Markes International (Llantrisant, UK) and LECO (Saint Joseph, MI, USA) which are in the same price range (£120,000 – 150,000) as triple quadrupole mass spectrometers.

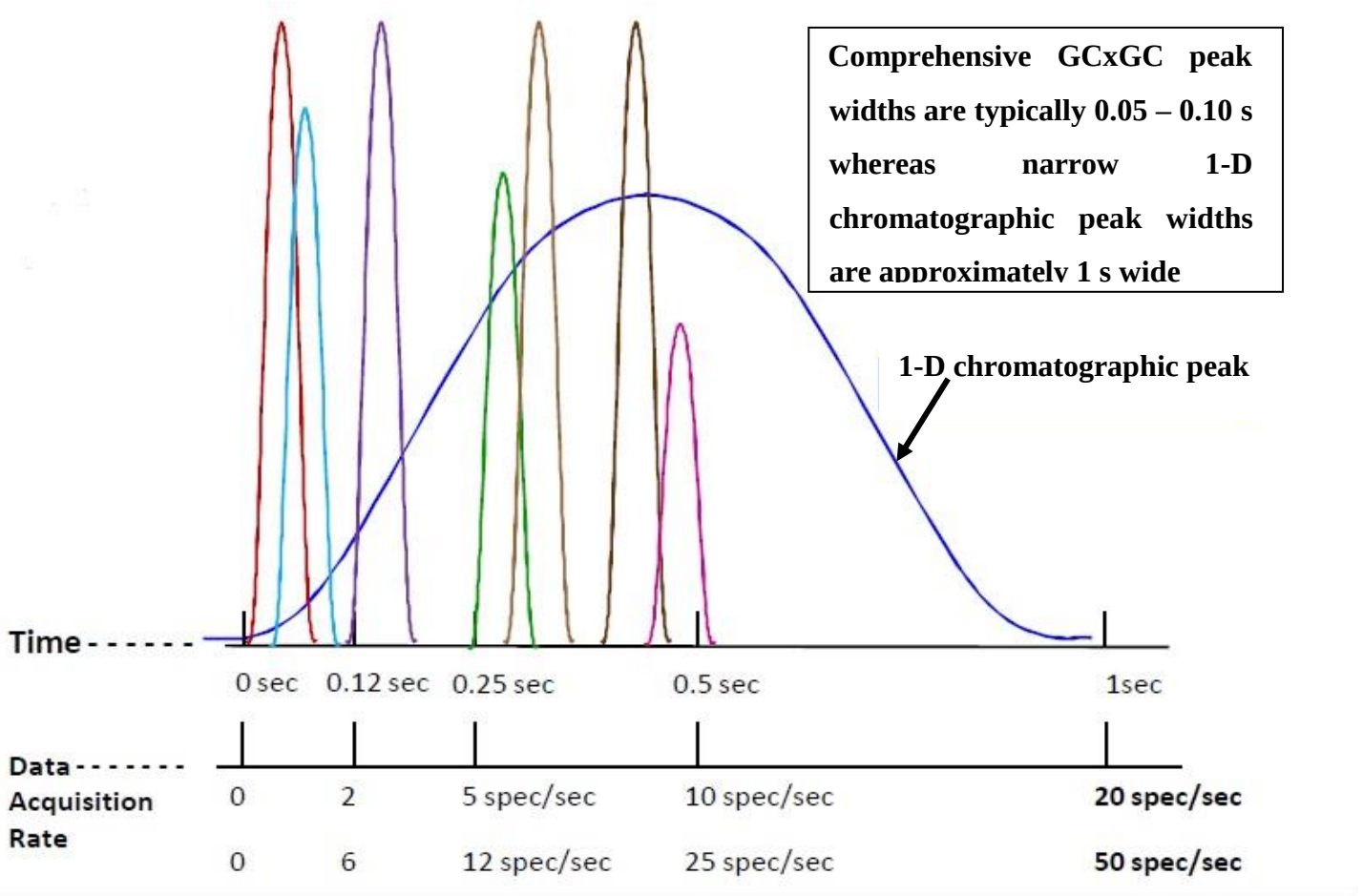


Figure 1.8 **Simulation of a typical GCxGC chromatogram with typical peak widths of 0.05–0.10 sec, highlighting the requirement for high speed detection systems. Adapted with permission from Leco UK.**

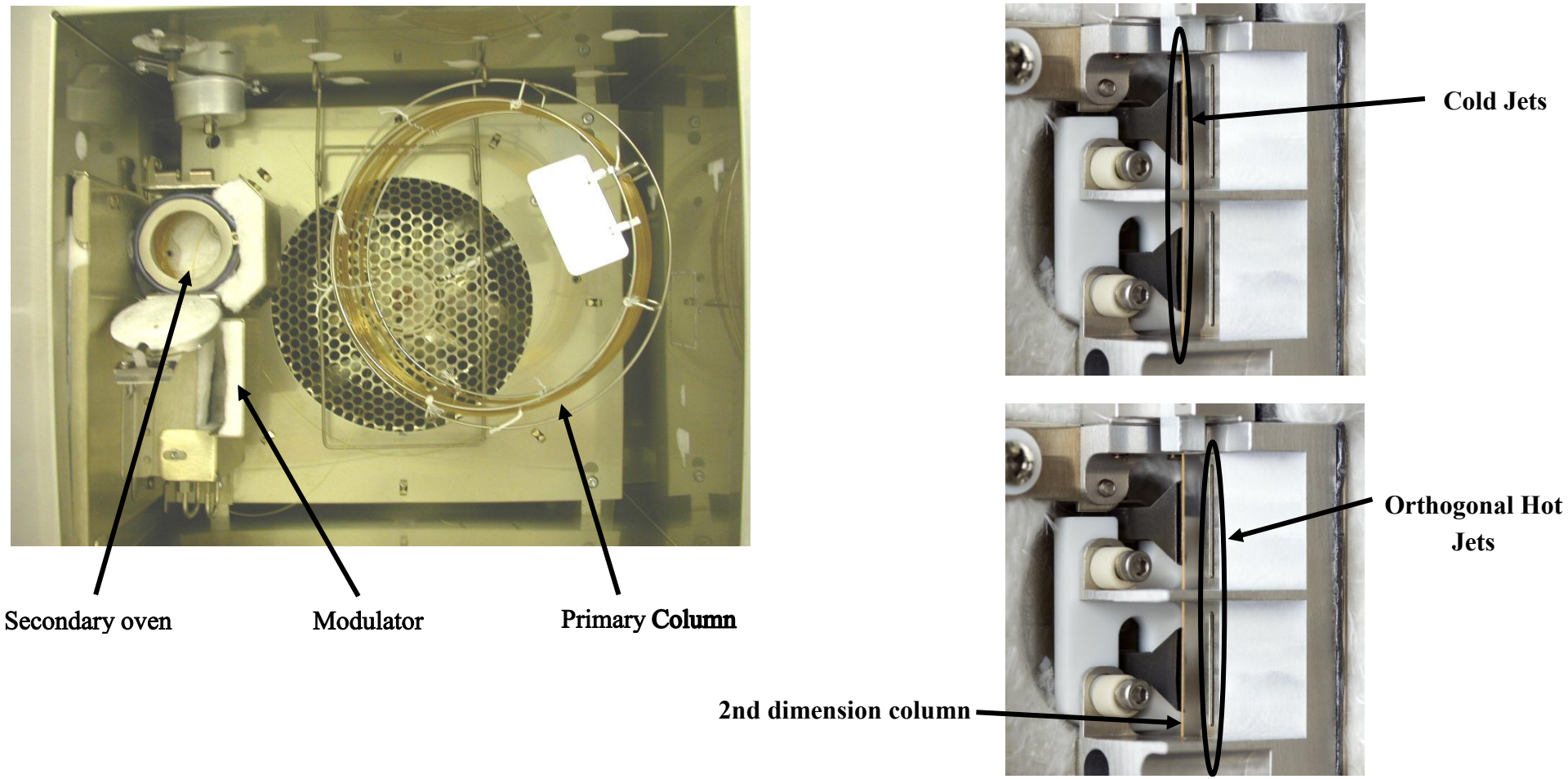


Figure 1.9 **Simple modifications to an existing 1-D gas chromatographs to enable its use for GCxGC. Adapted with permission from Leco UK.**

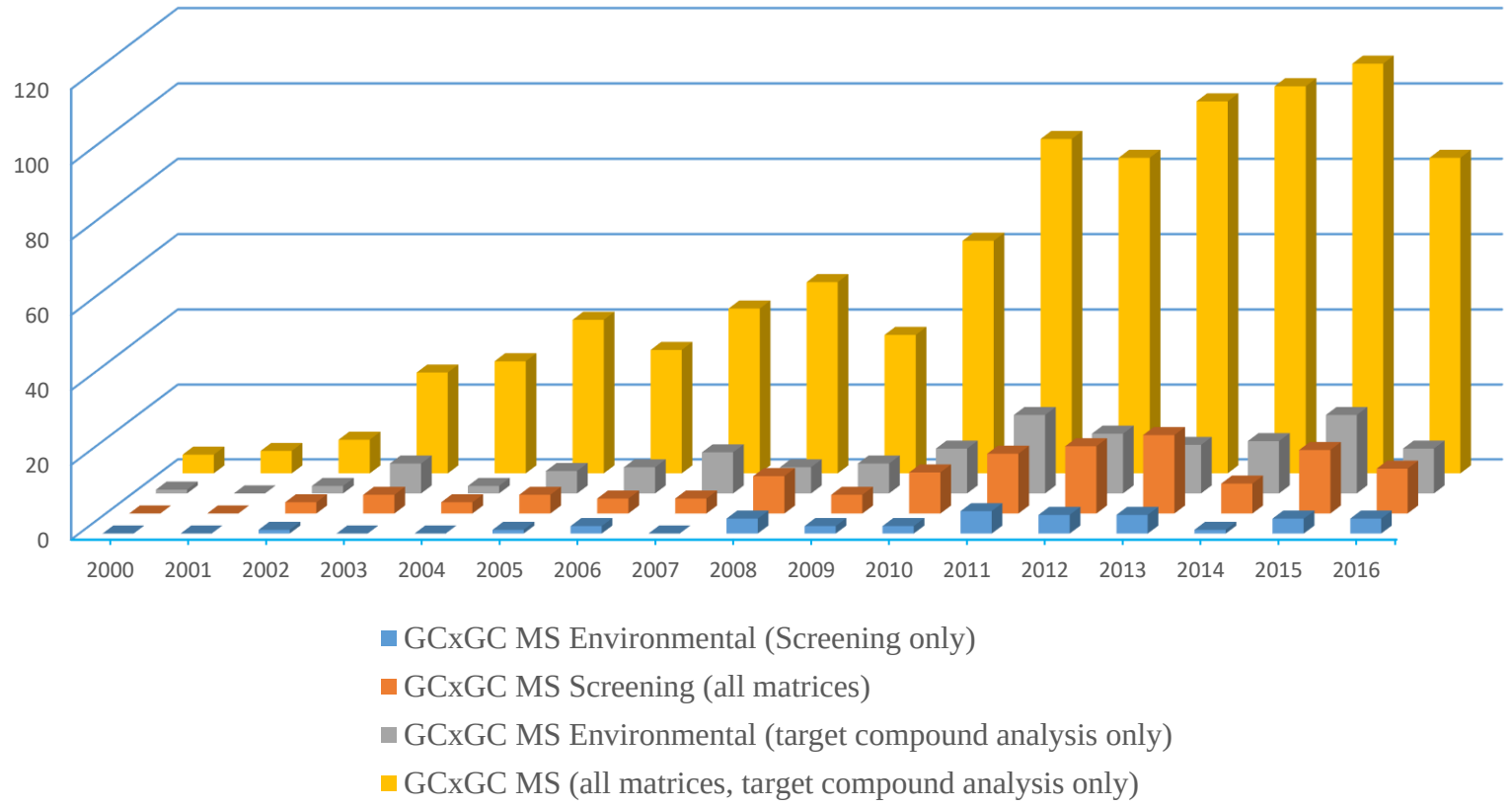


Figure 1.10 **Number of publications between 2000 and 2016 for GCxGC obtained from advanced SCOPUS search using criteria listed**

1.7.3.3 Comprehensive GCxGC and low energy or soft electron ionisation

Mass spectrometers when used in conjunction with gas chromatography typically employ electron impact (EI) ionisation at 70 eV which imparts a large amount of energy onto the molecule when ionised, usually resulting in extensive but spectral rich fragmentation patterns (21, 22). The benefit of such fragmentation is that the spectra are easily compared with mass spectral libraries such as NIST and/or Wiley for compound identification (22). However, some compounds remain difficult to identify at 70 eV due to absent molecular ions or their similar spectra. With electron ionisation, the molecular ion is weak or absent in about 30% of the compounds in the NIST library and this substantially decreases the confidence in assigning the molecular mass of a compound (221). The lack of the molecular ion signal and non-specific fragmentation patterns of many organic species, especially aliphatic hydrocarbons and their many isomers, make them spectrally indistinguishable (222).

Electron ionisation is considered as a hard ionisation, however, by suitable control of the main causes leading to dissociation of the molecular ion, EI can be controlled to deliver a highly informative soft ionisation technique (223). According to Amirav et al., "The electron ionization process imparts up to several electron volts of intra-molecular-ion internal energy that leads to molecular ion dissociation. This added vibrational energy can originate via direct excitation of high lying vibrational states of the lowest electronic state of the molecular ion or via the excitation of additional high lying electronic states of the molecular ion which undergo internal conversion into highly excited vibrational states of the ground electronic state of the molecular ion thus leading to dissociation" (223). The higher lying electronic states, though, can become inaccessible at lower electron energies therefore reducing the amount of

dissociation of the molecular ion. Nevertheless, lowering the electron energy for certain compounds (e.g. herbicides which contain many heteroatoms) does not affect the compounds mass spectrum as the higher lying electronic states are inaccessible but the ionisation yield is significantly reduced. Thus, there is a continual demand for robust soft (low energy) ionisation techniques for MS, in order to retain the molecular ion signal and aid identification of spectra.

1.7.3.4 Soft ionisation techniques

Common soft ionisation methods are listed below and all have been subject to recent review (224-226). Chemical ionisation (CI) is the most common of all soft ionisation techniques employed for GC-MS and GCxGC MS amenable compounds and takes place when the analyte molecules are ionised by chemical ion-molecule reactions initiated by ionised reagent gases such as methane, isobutane and ammonia (227-233). Photo-ionisation (PI) and field ionisation (FI) have also been coupled with GCxGC MS, however, both ionisation techniques are rarely used in environmental analysis and are mainly limited to specialised applications within petroleum and particulate composition analysis (222, 226, 234-237). Until recently few of these soft ionisation MS techniques have been coupled to GC×GC (235, 238-241) but with the arrival of powerful computing systems and the introduction of relatively inexpensive, reliable, easy to use modulators and soft ionisation sources, researchers are finding it easier to undertake routine complex multi-dimensional analysis (222, 242-244).

1.7.3.5 Chemical ionisation

Chemical ionisation (CI) has been a widely used analytical technique since its discovery by Munson and Field over 50 years ago (245-247) and has developed into a powerful and flexible technique for the identification of organic molecules. Positive and negative CI are soft ionisation techniques which produce significantly less fragmentation than EI, and in the vast majority of cases retains the *pseudo*-molecular ion and structural information typically absent with EI (248). Fragmentation can be controlled in CI mode by using reagent gases with different proton affinities such as ammonia, iso-butane and methane, with the former having the highest proton affinity leading to little or no fragmentation and methane having the lowest proton affinity and consequently the greatest amount of fragmentation. Iso-butane offers a good compromise between the two but readily fouls the ion source and shortens the lifetime of the electron filaments to hours or days of use. Methane on the other hand can be used for weeks or months before a filament requires replacement (232). Other parameters such as ion source temperature and pressure can be adjusted to control fragmentation where high ion source temperature favours fragmentation diminishing the relative intensity of the quasi-molecular ion. High reagent gas pressures reduces the relative intensities of fragment ions enhancing the relative abundancies of the quasi-molecular adduct ions (249).

When using ammonia CI, it is imperative to use the highest purity available so that amount of water present can be kept to an absolute minimum (≤ 5 ppm) as the presence of water in ammonia will result in very short filament lifetimes. Ammonia gas also reacts with water in the oil to raise the pH and attack the pump seals so it is necessary to ballast the rough pump daily to remove any residual ammonia gas and frequent

vacuum system maintenance is necessary. Little et al. found a CI reagent gas mixture of 3 % methylamine in methane to be very useful in obtaining molecular weight information with positive ion GCMS as some labile compounds, even when using ammonia CI, fragmented extensively by elimination and substitution reactions yielding no molecular weight information (250).

CI has, in-conjunction with gas chromatography, found extensive applications in fields of chemistry and biochemistry including the determination of pesticides, endocrine disruptors, illicit drugs, anabolic steroids in food, body fluids and various environmental matrices (228, 251-258).

1.7.3.6 Supersonic molecular beams (SMB) and soft cold EI

Since the late 1990's, Amirav and co-workers have developed and explored the capabilities of a supersonic molecular beam (SMB), which interfaces the outlet of a gas chromatograph to a mass spectrometer, is a process that produces vibrationally cold sample molecules (i.e. molecules cooled by supersonic expansion through a shaped nozzle) which then enter the mass spectrometers for ionisation and detection. With ionisation taking place at 70 eV, SMB is not classed as a soft ionisation technique but compound MS spectra typically have strong molecular ions together with library searchable EI fragment ions and is best described as 'Cold EI'. The SMB has become a commercial product, successfully interfaced with various mass spectrometer systems and typically used for petroleum, food and environmental analysis (221, 223, 240, 259). Figure 1.11 shows a schematic diagram of such a SMB interfaced with a pulsed flow comprehensive 2-D GC system (241) whilst Figure 1.12

shows a ‘Cold EI’ mass spectrum (obtained from a SMB) of a linear chain hexadecane (n-$C_{16}H_{34}$) along with its NIST library mass spectrum obtained at 70 eV (240). Despite a reduction in the match factor, the probability match is slightly higher for the cold EI spectrum than for the NIST spectrum due to a much larger response for the molecular ion and reduced fragmentation between 150–200 m/z (240, 260). Kochmann et al. observed a similar reduction in the match factors obtained for a range of pesticides with standard 70 eV libraries (such as NIST), unless cold EI MS library spectra were included within the library itself. However, superior identification probabilities were obtained as the likelihood of the same or a similar spectrum within the NIST library for the pesticides was reduced (261). Cold ionisation is often seen as the only soft ionisation method that is compatible with library based identification as it improves library identification probabilities.

Amirav also explored SMB ‘Cold EI’ at low electron energies (18 eV), a process he described as ‘Soft Cold EI’ where the spectra for squalene ($C_{30}H_{62}$) and tetracosane (n-$C_{24}H_{50}$) showed little to no fragmentation (223). Figure 1.13 compares the ‘Cold EI’ plus ‘Soft Cold EI’ mass spectra obtained for squalene together with its NIST library mass spectrum obtained at 70 eV.

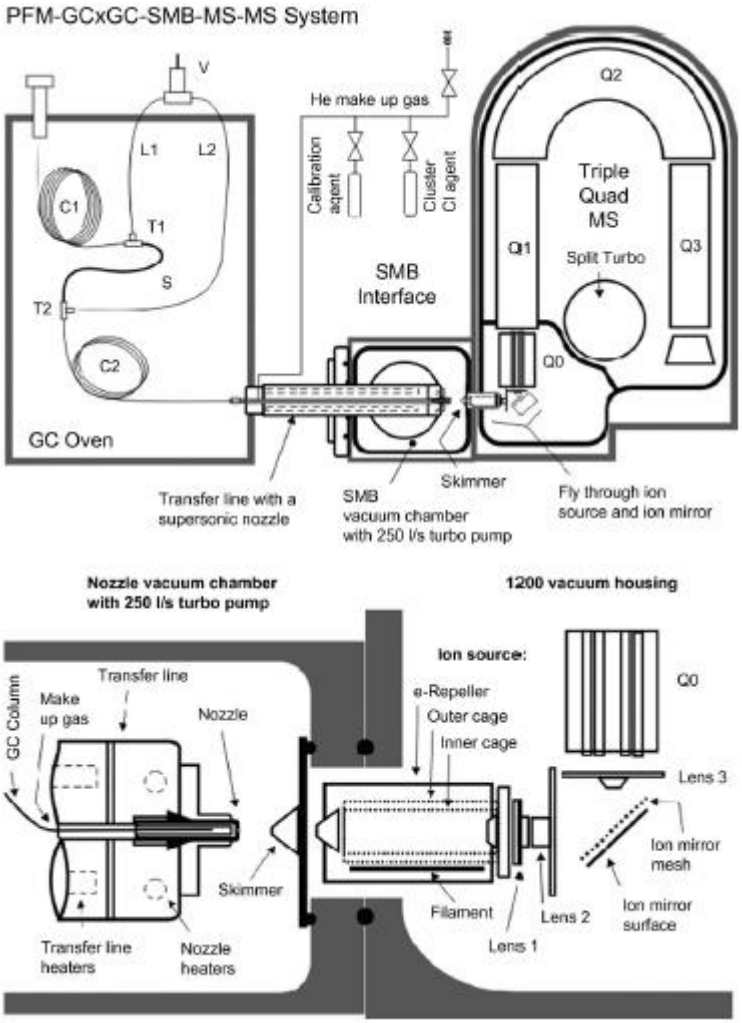


Figure 1.11 A schematic diagram of the supersonic molecular beam interface coupled to a Varian 1200 GCMS (top) and a detailed zoomed in section of the interface (bottom). Reproduced with permission from (241), copyright 2008, Elsevier.

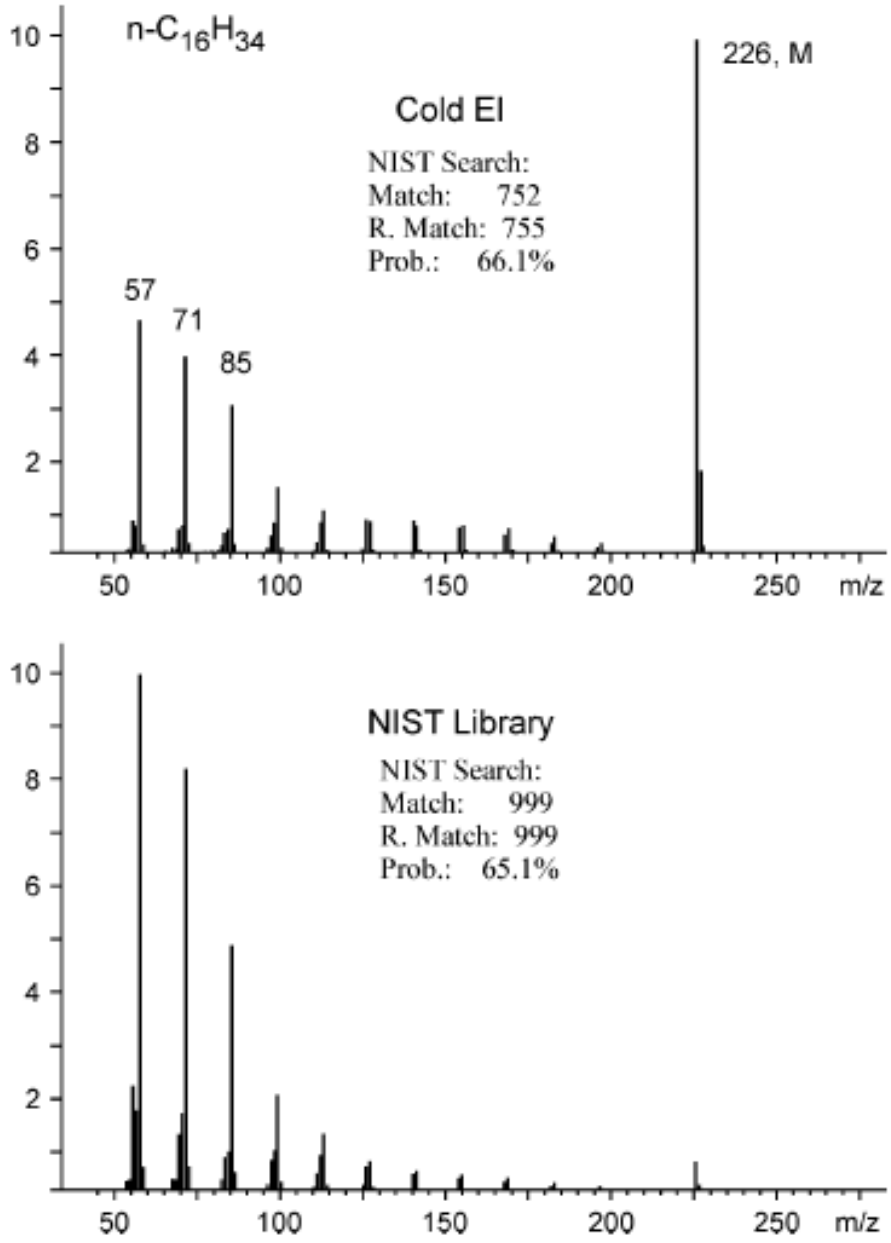


Figure 1.12 A comparison of cold EI mass spectrum of a linear chain hexadecane (n-$C_{16}H_{34}$) (upper trace) with its NIST library mass spectrum (bottom trace). Reproduced with permission from (262) copyright 2008, John Wiley and Sons.

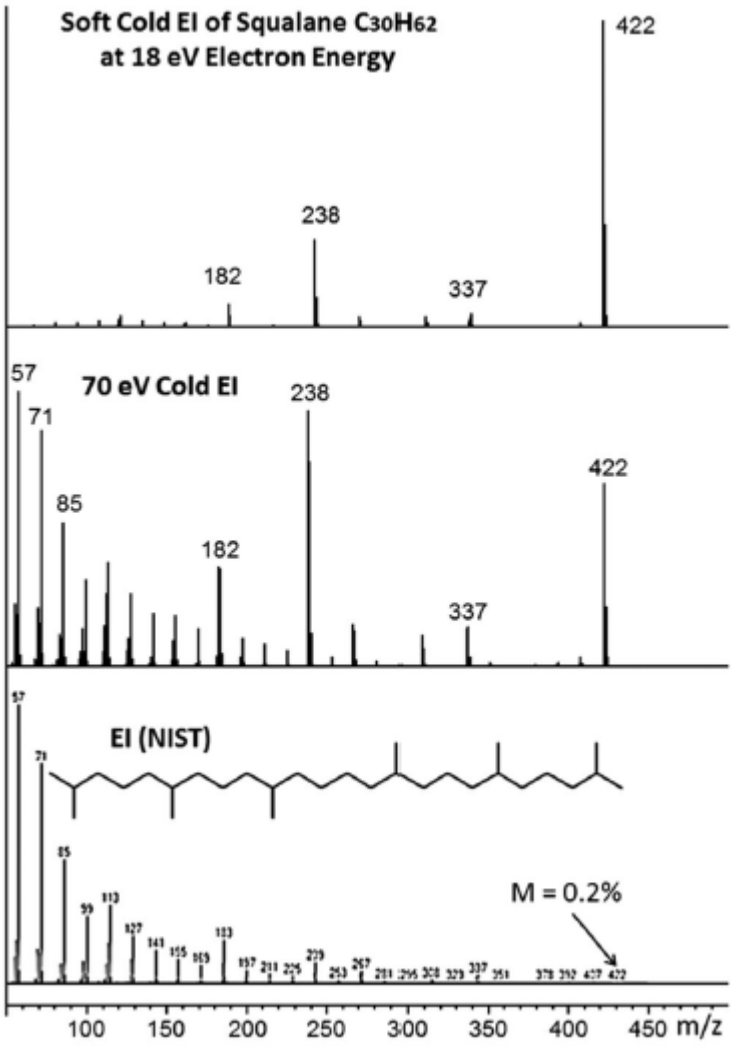


Figure 1.13 Comparison of 'Soft Cold EI' mass spectrum (upper trace), 'Cold EI' mass spectrum (middle trace) and NIST library mass spectrum (bottom trace) of squalene. The 'Cold EI' mass spectrum shown in the middle trace was obtained at 70 eV electron energy while the 'Soft Cold EI' mass spectrum shown in the upper trace was obtained at 18 eV electron energy. Reproduced with permission from (223) copyright 2008, John Wiley and Sons.

1.7.3.7 Variable electron ionisation source

As stated earlier in this chapter, in a conventional electron source there is a potential difference of 70 V between the positively charged ion chamber and the negatively charged filament ('electron gun') (21). This potential difference accelerates thermionic electrons away from the surface of the filament and through an aperture in the ion chamber wall (Figure 1.14a) for efficient ionisation of analytes eluting from the GC. Achieving low electron energies with a conventional EI ion source requires a lower accelerating potential, which is insufficient to draw electrons into the ion chamber and results in the clustering of electrons around the filament known as the 'space-charge limitation' (Figure 1.14b). This inefficient channelling of electrons into the ion chamber results in a small number of ions being generated leading to an unacceptable loss of sensitivity. To overcome this, a new ion source technology called Select-eV® has been developed which uses a high potential difference to accelerate the electrons away from the filament, but then reduces their energy before they arrive in the ion chamber (Figure 1.14c). By introducing an additional electrostatic element between the filament and the ion chamber, the electron ionisation energy can be varied from the conventional value of 70 eV to lower energies without loss of sensitivity (244). This 'lower energy' EI provides repeatable, simplified spectra, containing enhanced diagnostic and molecular ions, which retain enough fragmentation to allow robust library searching and isomer speciation.

The new ion source also allows the ionisation energy to be tuned between 10 and 70 eV and unlike CI and FI, no reagent gases, adjustments in pressure, or switching of ionisation sources is required (244).

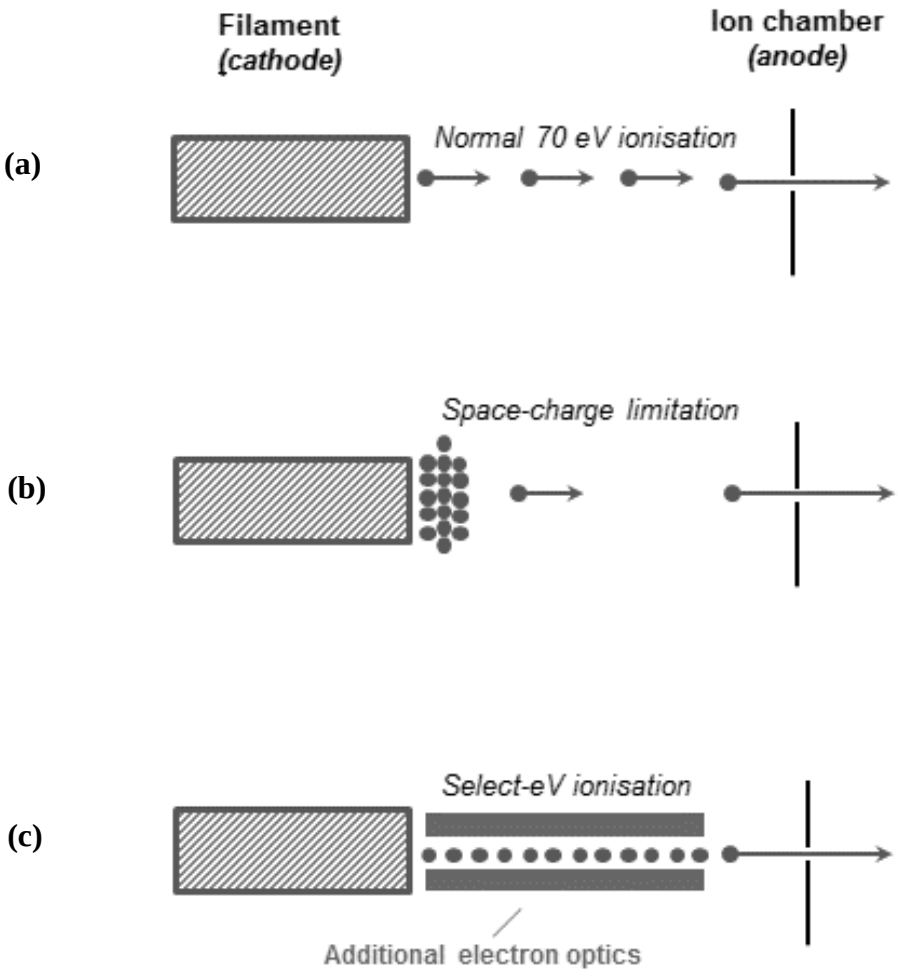


Figure 1.14 Schematic diagram showing the effects of different electron ionisation energies of ion flow (a) operation of a conventional ion source at 70 eV, (b) operation of a conventional ion source at lower ionisation energies' (c) operation of the variable-energy source showing how it overcomes the space-charge effect at low ionisation energy [reproduced with permission from Select-eV® technical note (244)].

1.7.4 High Performance Liquid Chromatography

High Performance Liquid Chromatography (HPLC) is a separation technique which has gained immense popularity in applications covering pharmaceuticals, foods, life sciences and environmental analysis. As the name suggests HPLC uses a liquid mobile phase and are generally mixtures of solvents of compatible polarities whereas in gas chromatography the mobile phase is a single high purity gas.

The fundamental resolution equation for gas chromatography described in section 1.5.1, and shown below, equally applies to liquid chromatography and is affected by the same three important parameters; selectivity, efficiency and retention.

$$R_S = \frac{1}{4}\sqrt{N}\ x\ \frac{\propto -1}{\propto}\ x\ \frac{k}{1+k}$$

The most effective and convenient way to alter the retention factor of a compound peak is to adjust the 'solvent strength' of the chromatographic mobile phase. This is typically achieved in reverse phase chromatography (the most commonly used technique in analytical HPLC) by altering the amount of organic solvent in the mobile phase. Reversed phase HPLC columns have a non-polar stationary phase, where increasing the polarity of the mobile phase (low organic but high aqueous content) will increasingly repel the hydrophobic (non-polar) sections of the analyte molecules into the stationary phase and the analyte will be retained for longer on the column. The converse is also true where decreasing the polarity of the mobile phase (low aqueous but high organic content) will reduce analyte retention on the column. When retention factors are very high or very low (e.g. < 1), the quality of the separation is

reduced and the separation will be poor. As with GC, the largest gain in resolution is achieved when the k value is between 1 and 5.

The selectivity of a separation in HPLC is highly dependent upon the chemistry of the analyte, mobile phase and column stationary phase and all of these factors may be altered in order to change or optimise the selectivity of a HPLC separation. The mobile phase pH can have a drastic effect on the selectivity of a separation especially when acidic or basic compounds are analysed as the degree of ionisation affects their hydrophobicity and therefore their retention on the column stationary phase. In addition, parameters such as solvent strength, column stationary phase and temperature will also impact on selectivity.

Gas chromatography separations are mainly carried out on compounds ranging in molecular weights up to a few hundred Daltons and compounds separate on differences in their volatilities. On the other hand, compounds separated using HPLC have higher molecular weights ranging from a few hundred daltons to several million for large polymers and biomolecules.

HPLC separations are typically carried out at ambient or close to ambient temperatures on columns which have much shorter and wider dimensions in comparison to GC columns. Typical HPLC columns used in environmental analysis are between 50-250 mm in length and 2-4 mm in diameter. HPLC column packings are typically 1.7-5 µm in diameter and give significantly greater resistance to flow of liquids in comparison to gases. The current trend is towards faster analysis so columns

used for HPLC can be as short as 1 cm in length and contain particles as small as 1.5 µm in diameter.

Detectors used with HPLC include ultra-violet, refractive index, photodiode array detectors, conductivity and mass spectrometry; the latter detector is the most commonly used for routine targeted and non-targeted environmental analysis.

1.7.4.1 HPLC with high resolution mass spectrometry (HPLC HRMS)

Several approaches have been adopted for routine targeted and non-targeted environmental analysis using liquid chromatography in combination with mass spectrometry (LC/MS or LC/MS/MS) employing various atmospheric pressure ionisation sources including electrospray ionisation (ESI), atmospheric pressure chemical ionisation (APCI) and atmospheric pressure photo-ionisation (APPI) (103, 263-267). All the above mentioned ionisation source can be used for general screening but ESI is by far the most commonly used for the identification of small molecules (268).

The application of single-stage quadrupole or ion-trap mass spectrometers for the purposes of screening is limited and not recommended because of disturbances by high matrix burden and co-eluting peaks which have similar or identical masses to those of targeted compounds. By using triple-quadrupole (QQQ) mass spectrometers many of these problems have been resolved but can only be performed as multi-targeted screening which means that compounds can be detected only if they are included in the methods (269). The number of substances in such a procedure is limited by the minimum dwell time for each compound's MS/MS (or fragmentation)

transition included in one measurement cycle. This process is commonly termed a multiple reaction monitoring (MRM) experiment and is accomplished by specifying the parent mass of the compound for MS/MS fragmentation and then specifically monitoring for a single fragment ion.

Nevertheless, powerful screening procedure with hundreds of compounds in a single chromatographic run have been developed by Dresen et al. who used a hybrid QQQ linear ion-trap mass spectrometer together with information dependent acquisition and enhanced product ion scan (at three different three collision energies) to obtain a library searchable mixed mass spectrum (270). This procedure, however, requires the approximate retention time of the compound to be known and placed within discrete two-minute time windows so that sufficient time is allocated to each compound for fragmentation.

Generally, LC QQQ screening with MS/MS identification consists of a survey scan to detect the analytes and a dependent scan for measurement of the corresponding MS/MS spectra which are library searched for identification. Survey scan and dependent scan can be accomplished within the same analytical run by automatic selection of precursor ions and measurement of the MS/MS spectra immediately after their detection in the survey scan. In ‘data-dependent acquisition’ this is determined only by the actual MS data whereas ‘information-dependent acquisition’ is restricted to a list of preselected precursors included in the method (271-275).

1.7.4.2 Matrix effects

LC-MS/MS analysis has been shown to be influenced by matrix effects which alters ionisation efficiency due to the presence of co-eluting substances in the sample extract and is considered to be a major drawback to qualitative and quantitative analyses using ESI (276). Matrix effects can lead to signal suppression or enhancement of the analyte signal, and ultimately to relatively high detection limits and reduced method repeatability (277). Ion suppression refers to the phenomenon of the analyte signal being suppressed by apparent competition in the ionisation process and subsequent reduction in the analyte MS signal. The phenomenon of signal enhancement can also occur, where the analyte signal is increased due to the presence of the interfering species (277). Natural organic matter, salts and non-target contaminants have all been shown to cause ion suppression effects and the complexity of the matrix influences the degree of matrix effect observed and can differ from analyte to analyte within the same sample due to differences in the ability of the compound to ionise.

There are several approaches that can be applied in order to compensate for any apparent matrix effect, these include improving the sample extraction and chromatographic separation, limiting the concentration factor in SPE, reducing the injection volume, diluting the sample and applying a variety of calibration techniques (278, 279). Suitable calibration techniques can help to minimise the matrix effects and can be achieved by means of ‘matrix-matched’ external calibration, standard addition or internal standard calibration using either structurally similar or mass labelled analogues (280, 281).

1.7.4.3 TOF and Orbitrap mass spectrometric detectors for HPLC

The availability of accurate mass TOF and Orbitrap mass spectrometers in the past decade or so has been a real game changer, with much improved mass resolution and mass accuracy and have provided new possibilities in the use of LC–MS for toxicological, forensic and environmental screening (282-284). TOF, TOF with a quadrupole-based collision cell (Q-TOF) and Orbitrap instruments enable comprehensive recording of all data within a specified mass range, and there is no limitation of the compounds included in the search procedure as long as they elute within the chromatogram and have a measurable response. Table 1.2 provides an overview of the technical specifications, characteristics and performance of hybrid TOF and Orbitrap HRAM systems currently used for accurate mass analysis. The increased mass resolution of recently introduced systems such as Bruker's Impact QTOF and the Sciex Triple TOF 6600, also provide high selectivity for overlapping peaks in complex matrices. The biggest advantage is that molecular formulae of compounds are directly available from the accurate molecular mass and the isotope peak pattern, this therefore allows for the development of very large theoretical databases which can be used for substance identification using the compound's molecular formula (282, 285-287). Databases of toxicologically relevant compounds with up to 50,500 substances including metabolites (288) or 'in-house' databases containing many hundreds of substances have been created (289-294).

However, the molecular formula of an eluted unknown substance in a chromatogram is only a first step of the identification, because of the huge number of possible isomers, as can easily be shown by use of chemical software. For instance, according to 'Molgen' (on-line molecular structure generation software) for the nominal

molecular mass of 149 Da, 27 different molecular formulas are theoretically possible if only the elements C, H, N, and O are included (295). HRAM with mass accuracy < 3 ppm (i.e. absolute mass error < 0.45 mDa) can clearly distinguish between these 27 possibilities one of which is $C_9H_{11}NO$ with a monoisotopic mass of 149.084060. Based on the rules of chemical bonds, 25,895,621 structural isomers (stereoisomers not included) can be theoretically calculated, and 'Chemspider' (an on-line searchable database) shows the structural formulae of 1270 compounds with the molecular formula $C_9H_{11}NO$. Among them are 4-(dimethylamino)benzaldehyde, 1-methyl-1,5,6,7-tetrahydro-4H-indol-4-one and norephedrone an amphetamine type stimulant found in the shrub catha edulis (296).

It is very important to be aware of this almost unlimited structural diversity of organic chemistry when commencing systematic screening analysis, therefore, much more evidence is required in order to distinguish between isomers. It is for this reason that retention times under defined chromatographic conditions have been measured for hundreds of substances in some 'in-house' libraries (292, 294). Structure-specific information should also be obtained from collision-induced dissociation (CID) fragment spectra (in-source or from collision cells) and even special software can also been used for fragmentation prediction in order to differentiate between structural isomers (297).

If no previous information is available, identification of compounds based only on accurate mass and mass fragmentation data will be very challenging and the amount of accurate mass spectral information for environmental contaminants in currently available accurate mass libraries and databases is far from comprehensive but is

steadily improving. The situation is quite different for EI mass spectra; the current NIST 14 reference library contains approximately 276,259 EI mass spectra of 242,477 compounds (298), whereas the Wiley Registry (eleventh edition) contains 775,500 spectra from 599,700compounds (299). Therefore, the approach to suspect or non-target screening will have to rely on general chemical databases and deal with the lack of MS library information.

1.7.4.4 LC HRMS libraries and databases for organic water pollutants

As already mentioned, a simple but reliable approach for the successful identification and confirmation of unknowns is the comparison of measured accurate product/fragment ion mass spectra with accurate mass spectra of authentic reference compounds, as it provides additional selectivity instead of using only the exact mass and the corresponding isotopic pattern.

A variety of commercially available and user-defined mass spectral libraries have been developed for certain MS instrument types and settings but, as various collision energies are applied in tandem mass spectra, the relative intensities of ions can differ considerably. Consequently, mass spectral matching fails if the signal intensity is a criterion, but is successful if only the fragment ion pattern is considered. Several studies have shown the potential for transferability of tandem mass spectra for use with multiple instrument types and that instrument-independent tandem mass spectra can be obtained by application of multiple collision energies for fragmentation (300, 301).

A library of CID spectra of 319 substances measured with an LC Q-TOF-MS instrument at ten collision energies was described by Pavlic et al. (302) and Broecker et al. developed a library of CID accurate mass spectra of more than 2,500 compounds for systematic toxicological analysis by LC Q-TOF-MS (269). In 80 % of all cases, the mass spectrum of an unknown compound could be assigned to a structure if it was compared with two or more reference mass spectra recorded with different instruments or with different collision energies (303-305). Hence, a considerable collection of tandem mass spectra obtained with different collision energies and with different instruments can improve the overall performance of a successful library search (275).

Several commercially and publicly available spectral libraries aim to identify compounds independently of the instrument type and settings. Most of the LC-MS libraries have been developed and published by researchers in life sciences (e.g. proteomics and metabolomics). A recent review on computational MS for metabolomics summarized existing compound libraries containing ESI mass spectra, which include the commercially available NIST reference library, the freely accessible metabolite libraries METLIN, Human Metabolome Database (HMDB) and MassBank (268). Only the NIST, METLIN, and 'MassBank' reference libraries contain unit-resolution mass spectra and accurate mass spectral data. The 'NIST' reference library, released in 2014, contains 183,068 high and low resolution tandem mass spectra of 14,835 different ions from 7,692 compounds and also includes environmentally relevant compounds.

METLIN is a metabolite database currently containing accurate tandem mass spectra from over 14,000 compounds and metabolites; the tandem mass spectra are recorded with an Agilent Technologies Q-TOF-MS instrument, in both positive and negative ESI modes, using four different collision energies (0, 10, 20, and 40 eV) (268). As of August 2015, 'MassBank' contained nearly 28,000 high resolution MS/MS spectra in negative mode obtained with different types of instruments, settings, and ionisation modes (306). It has many options for searching for a mass spectrum, such as by peak, compound name, exact mass, molecular formula, substructure, instrument type, single or multiple fragmentation, and type and mode of ionisation. One of the major advantages of 'MassBank' is its free accessibility and the possibility to upload both nominal and accurate mass spectra in common and different data formats. This allows the collection of a considerable amount of useful mass spectral information from a broad research community, which might help to improve the overall performance of successful suspect or non-target analysis (306).

Although some data entries on metabolites are useful for research on emerging contaminants, the mass spectral information associated with environmental pollutants is rather scarce. Nevertheless, computational techniques and tools for a reliable library search are well developed, and the spectrum-matching tools and search functions are already optimized for ESI MS/MS library search. It is therefore possible to extend databases with information on environmental pollutants and 'MassBank' has been significantly expanded in recent years with data for environmental pollutants collected by the NORMAN network of reference laboratories (307).

A database for water pollutants with emphasis on non-target screening is the DAIOS database, which contains numeric information on the nominal and accurate masses of precursor and product ions and, as with 'MassBank' mentioned above, the mass spectral data can be searched for precursor and product ions (308).

1.7.4.5 Data independent MS/MS acquisition

Data independent MS/MS acquisition, such as all-fragment-ion techniques have recently gained attention in the analysis of small molecules (309-311). Novel mass spectrometers with fast duty cycles and acquisition times up to 100 MS/MS scans per second over a wide mass range and at high resolution has allowed for the development of these techniques (312). Figure 1.15 illustrates the process of data independent MS/MS acquisition for a Q-TOF-MS where the collision cell rapidly alternates between low and high collision energies. One advantage is that very low intensity precursor ions are fragmented, even if they would not trigger intensity thresholds (as in data-dependent MS/MS). Even if there are co-eluting molecules with higher intensities (that are usually triggered first in data-dependent MS/MS), low abundant ions are still fragmented. Hence, in principle, all molecules in data-independent MS/MS will undergo fragmentation. The obvious disadvantage for all-fragment-ion techniques analyses is that the direct link between a specific precursor ion and its corresponding product ions is broken, therefore, mixed product ion spectra are generated, that originate from multiple precursor ions. Precursor ion determination when using the all-fragment-ion technique requires mass spectral deconvolution on the MS2 level and retention time information. However, when working with suspect lists, where the compound's monoisotopic mass and potential adducts (arising from

API techniques) and fragment ions are known, then the task of identification is made considerably easier. When used in-conjunction with retention time prediction software the technique becomes a very powerful tool for the identification of compounds suspected to be present in samples.

1.7.5 Retention time prediction

As stated earlier, large libraries of high resolution, accurate mass spectra (including MS/MS fragment ion data) have been developed are now becoming commercially available for use with automated library searching and comparison tools for suspect pollutant screening (313). Differentiation of structural isomers, however, is not possible by accurate mass measurement alone. Fragment ions obtained from collision induced dissociation can provide useful verification that a suspect compound is present but one major weakness in 'suspect' or 'known unknown' screening approaches is the lack of chromatographic retention times associated with these libraries. These are essential for tentative compound identification but unequivocal identification, can only be provided by analysing a reference standard under the same chromatographic conditions (284, 314).

The lack of reference standards for new pharmaceuticals, metabolites, pesticides and the plethora of industrial chemicals, however, forces the analyst to find alternative tools for tentative compound identification (314). A review of the literature indicated that *in-silico* tools can help in predicting the suspect compounds retention time in the chromatographic system employed.

Extensive information about retention phenomena can be obtained from quantitative structure–retention relationship (QSRR) models (315, 316). The aim of these models is to discover the relation between the molecular descriptors, calculated from the chemical structure, and retention. QSRR models describe chromatographic retention in single chromatographic systems. QSRR analyses can identify the most useful structural descriptors in a molecule, detect the molecular mechanism of retention of a given compound, compare the separation mechanisms of various chromatography columns, calculate the physicochemical properties of the analytes, and estimate biological activity of xenobiotics. QSRR models have been used for predicting the retention times of drug compounds in order to determine their retention behaviours (317). Despite the advantages of QSRR models, they have not yet become part of routine LC method development or of compound identification. A crucial challenge is to select the most informative molecular descriptors from a large number of possibilities; Dragon software (Talete, Milano, Italy) for example, calculates almost 5000 molecular descriptors (318). The evaluation of prediction performance is an important and critical phase in model validation and the understanding and use of QSRR requires personnel fully conversant with computational modeling. Several researchers have produced ‘in-house’ software based on QSRR models but none have so far been employed in routine analysis. There is therefore a need for easy to use retention time prediction software for routine use with liquid chromatography mass spectrometry for the identification of suspect compounds where a reference material is unavailable or prohibitively expensive to purchase or synthesise.

Table 1.2 Overview of the technical specifications, characteristics and performance of hybrid TOF and Orbitrap HRAM systems.

Analyser Type	Manufacturer	Instrumental name	Resolving power (FWHM) at defined m/z value	Approximate resolution (Δm/z)	Mass Accuracy (ppm)		m/z range	Acquisition speed (Hz)
					Internal calibration	External calibration		
Q-TOF	Bruker Daltonics	MicroOTOF-Q II	20,000 (m/z 922)	0.05	<2	<5	50-20,000	20
		Maxis Impact	50,000 (m/z 922),	0.01	<1	<3	50-20,000	50
		Maxis 4G	60,000 (m/z 1222)	0.02	<0.6	<2	50-20,000	30 (MS), 10 (MS/MS)
	Waters	XEVO G2 Q-TOF	22,500 (m/z 956)	0.04	<1	-	20-16,000	30
		Synapt G2-S HDMS	50,000 (m/z 956)	0.02	<1	-	20-100,000	30
	Agilent	6500 Q-TOF series	42,000 (m/z 922)	0.02	<1	-	50-10,000	50
	Sciex	Triple TOF 4600	30,000 (full m/z range)	-	<0.5	<1	5-40,000	100
		Triple TOF 5600	35,000 (full m/z range)	-	<0.5	<2	5-40,000	100
		Triple TOF 6600	40,000 (full m/z range)	-	<0.5	<2	5-40,000	100
IT-TOF	Shimadzu	LC-MS-IT-TOF	10,000 (m/z 1000)	0.1	3	5	50-5,000	10

continued

Q-Orbitrap	Thermo Fisher Scientific	Q-Exactive	140,000 (m/z 200)	0.001	<1	<5	50-4,000	12 (at RP of 17,500)
LTQ-Orbitrap		Orbitrap Elite	240,000 (m/z 400)	0.002	<1	<3	50-4,000	8 (at RP of 17,500)
Tribrid-Orbitrap		Orbitrap Fusion Lumos Tribrid	500,000 (m/z 200)	0.0004	<1	<3	50-4,000	18 (at RP of 17,500)

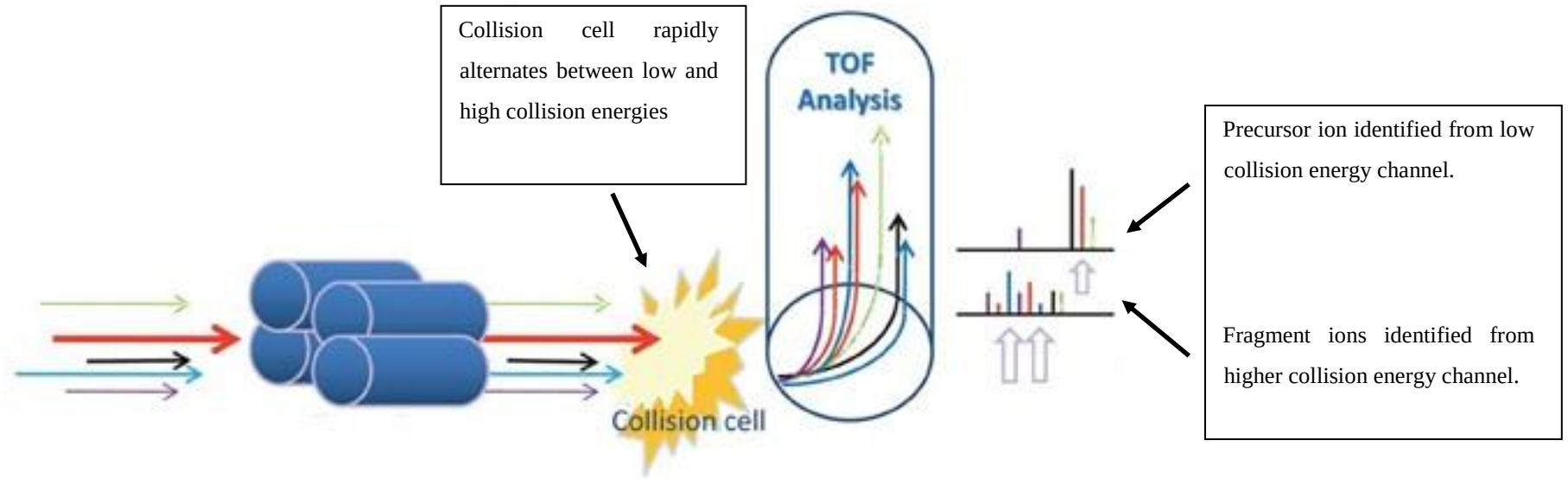


Figure 1.15 Process of 'data independent MS/MS acquisition' for a Q-TOF-MS where the collision cell rapidly alternates between low and high collision energies. Diagram used with permission from Bruker UK Ltd.

1.8 Conclusions from the literature review

The occurrence of contamination of the environment by pollutants continues to be actively researched with greater knowledge required on their presence especially within environmental waters where very low concentrations of pollutants such as pesticides, pharmaceuticals and industrial chemicals are known to be present but remain undetected with traditional sampling and analytical techniques. Despite their low concentration many have a deleterious effect on various flora and fauna. Therefore, the targeted approach to the analysis of aqueous environmental samples probably leads to an underestimation of the true breadth of contamination.

It can be concluded from the preceding sections that spot sampling has many shortcomings and that alternatives such as passive sampling may offer a better alternative. Even though passive sampling is not specifically mentioned as a monitoring method in the WFD, the guidance document on surface water chemical monitoring does refer to passive sampling as a complementary method that can be used for both surveillance and investigative monitoring. The WFD states that when no analysis methods are available that fulfil the minimum 'performance criteria', the best available techniques not entailing excessive costs must be used. Passive sampling may be the best available technique for very low concentrations of pollutants that are not detectable in spot samples. In addition, passive sampling can also be used in conjunction with spot sampling to confirm or refute the results obtained from the latter technique and particularly in situations where pollutant concentrations fluctuate considerably over time; passive sampling can also play this role in investigative monitoring. An ongoing issue is that the compliance checking of water quality under the WFD with respect to organic compounds is based on total water concentrations

while passive sampling measures the freely dissolved (bio-available) concentration that is needed for risk assessments. Many emerging contaminants such as pharmaceuticals and pesticides are polar in nature and passive samplers have been developed to sample them from the aquatic environment. A popular polar passive sampler is the POCIS, but one of its shortcomings is the lack of robustness for deployment in rivers without considerable protection to avoid puncturing the membrane, which leads to a loss of sorbent. The sorbent is sandwiched between two membranes but will sag to the bottom of the sampler presenting an inconsistent surface area for pollutants to be adsorbed. The sampler can also lose sorbent during deployment or when disassembling in the laboratory. An alternative passive sampler is the polar Chemcatcher® which uses a disk containing an identical sorbent to that used with the POCIS (Oasis® HLB). The sorbent within the disk is immobilised within a glass fibre matrix presenting a consistent surface area during the entire deployment period. As the disk is supported on a PTFE base, the possibility of puncturing the membrane is reduced and there is very little chance of damaging the disk and losing the sorbent.

Despite the unparalleled peak capacity that GCxGC MS offers over 1-D GCMS and the highly successful determination of complex environmental, petrochemical and biological matrices for targeted compounds, there has been a tremendous underutilisation of the technique for screening of environmental matrices. This may be due to the higher costs associated with TOF-MS systems compared to quadrupole MS systems; this, however, is likely to change rapidly with the recent introduction of bench-top GC-TOF-MS systems by Markes International (Llantrisant, UK) and

LECO (Saint Joseph, MI, USA) which are in the same price range as triple quadrupole mass spectrometers.

The emerging use of HRMS and combined use of targeted and suspect screening/non-targeted approaches have been shown to be essential complementary approaches to allow capture and identification of high concentration and potentially environmentally relevant compounds. The acquisition of high-resolution accurate-mass full-scan data permits the ability to perform retrospective analysis, in which the data can be reprocessed at a later date without the need to reanalyse samples. The use of HRMS will also allow a historical picture of pollutant release and environmental exposure of a potentially unlimited number of contaminants to be gained over an unlimited number of years. Retrospective analysis can also be performed after the completion of the proposed solution, allowing a before and after overview of the system to be gained; this is of huge benefit to investigative monitoring.

QSRR models have been used for predicting the retention times of compounds in order to determine their retention behaviours with both liquid and gas chromatography. Despite the advantages that retention time prediction brings, they have not yet become part of routine LC method development or of compound identification. There is a need for easy to use retention time prediction software for routine use with liquid chromatography mass spectrometry to aid in the identification of suspect compounds and reduce the enormous burden of determining which compounds are likely to be present in sample chromatograms.

1.9 Aims and objectives

The aim of this work was to investigate alternative sampling and analysis techniques for investigative monitoring. The main objectives are discussed below:

i) Investigate the use of comprehensive GCxGC-MS analysis for the analysis of complex SPMD passive sampling extracts and establish if the technique would identify more targeted pollutants, and emerging contaminants thought to be present in the SPMD, with greater confidence than one dimensional GC-MS. In addition, explore the use of standard and soft electron ionisation in-conjunction with comprehensive GCxGC-MS analysis as a complimentary pollutant identification and confirmation tool, by analysing targeted pollutants, and emerging contaminants thought to be present in the SPMD extracts.

ii) Develop an accurate mass database and fragment ion library for the identification of pharmaceutical residues and personal care products in passive sampling extracts obtained from POCIS and a prototype 'polar' Chemcatcher sampler using a new and novel LC Q-TOF-MS technique. In addition, evaluate the ease of use of both samplers for deployment and subsequent extraction and analysis.

iii) Determine, via a combination of LC Q-TOF-MS analysis and statistical tools, the reproducibility of chromatographic peak areas of targeted pharmaceuticals identified in extracts obtained from two polar passive samplers (POCIS and polar Chemcatcher) deployed in a waste water effluent stream. In addition,

statistically determine whether the relative uptake rates, and range of molecular masses for ‘unknown’ compounds were similar for both samplers.

iv) Investigate retention time prediction software, in conjunction with LC Q-TOF-MS analysis, and evaluate its applicability for the accurate prediction of retention times for pharmaceutical metabolites.

Chapter 2

Identification of pollutants in non-polar passive sampling devices using comprehensive two-dimensional gas chromatography and variable energy electron ionisation time-of-flight mass spectrometry

2.1 Introduction

The purpose of this chapter was to address the analytical challenges associated with the tentative identification of 'suspect' or 'known unknowns' pollutants, present in non-polar passive samplers deployed in the aquatic environment, using gas chromatography mass spectrometric techniques.

Analysis of water sample extracts for unknown (non-target) organic chemical pollutants is usually undertaken by full scan one-dimensional gas chromatography-mass spectrometry (GC-MS) using simple mass spectral library searching routines (20, 319). However, GC-MS is unable to resolve the many thousands and large variety of compounds that can be present in water samples and a more sophisticated approach is therefore required to identify any potential substances that may be responsible for ecological failures. Recently, several advanced instruments have been developed to identify pollutants in water samples, for example, comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GCxGC TOF-MS) which offers enhanced separation capacity with highly sensitive full scan detection.

Electron ionisation at 70 eV has been adopted as the standard for GC-MS analysis, due to the production of reproducible, fragment-rich mass spectra that are searchable against 'in-house' or large commercially available libraries. However, some compounds remain difficult to identify at 70 eV due to their similar spectra, complex fragmentation or weak/absent molecular ions. With electron ionisation, the molecular ion is weak or absent in about 30 % of the compounds in the NIST library and this substantially decreases the confidence in assigning the molecular mass of a

compound. Chemical ionisation may overcome this but spectra typically contain only the molecular ion, making speciation of isomers and library searching impossible.

Due to a potential difference of 70 V between the positively charged ion chamber and the negatively charged filament ('electron gun') in a conventional EI source, electrons are accelerated from the surface of a negatively charged filament toward a positively charged ion chamber thereby efficiently ionizing compounds eluting from GC column. Reducing this potential difference (for soft ionisation) typically results in a dramatic loss of sensitivity due to the inefficiency of drawing electrons into the ion chamber and the clustering of electrons around the filament. In a recently developed variable ionisation source, named 'Select eV®', ion optics are used to retain the high potential difference, thus accelerating the electrons away from the filament but reducing their energy prior to their arrival at the ion chamber. This allows the ionisation energy to be tuned between 10 and 70 eV.

This chapter explored the use of GCxGC-TOF MS with variable-energy EI, to assess its use in detecting priority and emerging contaminants in complex extracts obtained from the deployment of passive samplers in river water. To the author's knowledge, this is the first use of this novel analytical technology for the analysis of complex extracts from passive samplers as part of a water quality investigation. The future potential of this approach for investigative monitoring within the context of the WFD was explored.

2.2 Aims and objectives

i) Using automated deconvolution and library search algorithms, establish if comprehensive GCxGC-MS analysis of a complex passive sampling sample extract would identify a known list of target compounds with greater confidence than the traditional one dimensional GC-MS screening approach. This would be undertaken by analysing extracts (spiked with solutions of various pollutants at known concentrations) obtained from semi-permeable membrane devices deployed in a water course and compare the data obtained from both techniques. In addition, establish if comprehensive GCxGC-MS analysis would identify several emerging contaminants thought to be present in the SPMD with greater confidence than one dimensional GC-MS.

ii) Explore the use of soft electron ionisation as a complimentary pollutant identification and confirmation tool to standard 70 eV electron ionisation by analysing extracts (spiked with solutions of various pollutants at known concentrations and known to contain emerging contaminants) from semi-permeable membrane devices deployed in a water course using comprehensive two-dimensional gas chromatography time of flight mass spectrometer under standard electron ionisation and soft ionisation conditions.

2.3 Experimental

2.3.1 Reagents and standards

All reagents and solvents were of analytical reagent grade and pesticide residue grade respectively. Acetone, dichloromethane, *n*-hexane, propan-2-ol, hydrochloric acid (36 % w/v), anhydrous sodium sulphate, were obtained from Fisher Scientific UK Ltd. (Loughborough, Leicestershire, UK). Ultrapure water was obtained from an in-house source (ELGA Purelab Ultra) and was used in all laboratory procedures (Elga Process Water, Marlow, Buckinghamshire, UK). The ultrapure water system was equipped with a UV lamp, carbon and membrane filter to remove trace organic compounds, ionic species and particulates. Custom mixes of polychlorinated biphenyls (Restek Corp., Bellefonte, PA, USA), polyaromatic hydrocarbons (Spex Certiprep Group LLC, NJ, USA), organochlorine, organophosphate and triazine pesticides (Restek Corp.) were used to prepare a standard solution (10 ng/µL) for which the full list of compounds appears in Table 2.1. The solution facilitated in the optimisation of the 2-D GC acquisition method and ensuring that compounds of interest would be seen in the resulting 1-D and 2-D GCxGC TOF-MS chromatograms obtained from the work detailed in section 2.3.4 and 2.3.5. Also, many compounds present in the standard solution, including organophosphate pesticides, have weak molecular ions and were chosen as representative compounds from which improvements in the signal-to-noise ratio of the molecular ion could be assessed when using low electron ionisation energies as detailed in section 2.3.6. SPMDs (91.4 cm × 2.5 cm) containing 1 mL of ultra-high purity triolein, spiked with performance reference compounds (10 µg fluorene-d_{10}, phenanthrene-d_{10} and pyrene-d_{10}) were obtained from Environmental Sampling Technologies (St. Joseph, MO, USA). SPMDs were shipped in gas-tight metal containers flushed with argon and kept at < –18 °C until deployment.

Table 2.1 List of 117 compounds investigated in SPMD samplers using one dimensional and GCxGC TOF-MS techniques.

Compound	CAS No.	Compound	CAS No.	Compound	CAS No.
Pentachlorobenzene	608-93-5	2,3,5-trimethylnaphthalene	2245-38-7	2,2',3,4,4',5,5'-Heptachlorobiphenyl	35065-29-3
Alpha-HCH	319-84-6	1,4,6,7-tetramethylnaphthalene	13764-18-6	3,3',4,4',5,5'-Hexachlorobiphenyl	32774-16-6
Hexachlorobenzene	118-74-1	1-methylfluorene	1730-37-6	2,2',3,3',4,4',5-Heptachlorobiphenyl	35065-30-6
Beta- HCH	319-85-7	9-n-propylfluorene	4037-45-0	Chlorpyrifos methyl	5598-13-0
Gamma- HCH	58-89-9	Dibenzothiophene	132-65-0	Parathion methyl	298-00-0
Delta- HCH	319-86-8	1,7-dimethylfuorene	442-66-0	Fenitrothion	122-14-5
Triallate	2303-17-5	2-methyldibenzothiophene	20928-02-3	Pirimiphos ethyl	23505-41-1
Aldrin	309-00-2	1,2-dimethyldibenzothiophene	31317-14-3	Malathion	121-75-5
Isodrin	465-73-6	1-methylphenanthrene	832-69-9	Fenthion	55-38-9
Endosulfan I	959-98-8	3-methylphenanthrene	832-71-3	Chlorpyrifos	2921-88-2
Dieldrin	60-57-1	3,6-dimethylphenanthrene	1576-67-6	Parathion	56-38-2
Endrin	72-20-8	2-methylfluoranthene	33543-31-6	Chlorfenvinphos	470-90-6
Endosulfan II	33212-65-9	1,2,6-trimethylphenanthrene	30436-55-6	Iodofenphos	18181-70-9
2,4’-DDT	789-02-6	1,2,6,9-tetramethylphenanthrene	204256-39-3	Triazophos	24017-47-8
4,4’-DDT	50-29-3	Triphenylene	217-59-4	Carbophenothion	786-19-6
2,4’-DDD	53-19-0	Benz(b)anthrance	92-24-0	Azinphos ethyl	2642-71-9
4,4’-DDD	72-54-8	2,4,7-trimethyldibenzothiophene	216983-03-8	Azinphos methyl	86-50-0
2,4’-DDE	3424-82-6	Perylene	198-55-0	Pirimiphos methyl	29232-93-7

continued

4,4’-DDE	72-55-9	1-methylchrysene	3351-28-8	Ethion	563-12-2
Hexachlorobutadiene	87-68-3	6-ethylchrysene	2732-58-3	Buprimate	41483-43-6
Acenaphthene	83-32-9	1,3,5-trimethylchrysene	1586755-28-3	Coumaphos	56-72-4
Acenaphthylene	208-96-8	Benzo(e)pyrene	192-97-2	Fenchlorphos	299-84-3
Anthracene	120-12-7	6-n-butylchrysene	6901-71-9	Desisopropylatrazine	1007-28-9
Benz(a)anthrance	56-55-3	2,4'-Dichlorobiphenyl	34883-43-7	Desethylatrazine	6190-65-4
Benzo(a)pyrene	50-32-8	2,4',5-Trichlorobiphenyl	16606-02-3	Simazine	122-34-9
Benzo(b)fluoranthene	205-99-2	2,4,4'-Trichlorobiphenyl	7012-37-5	Atrazine	1912-24-9
Benzo(g,h,i)perylene	191-24-2	2,3,3'-Trichlorobiphenyl	38444-84-7	Propazine	139-40-2
Benzo(j)fluoranthene	205-82-3	2,2',5,5'-Tetrachlorobiphenyl	35693-99-3	Trietazine	1912-26-1
Benzo(k)fluoranthene	207-08-9	3,3',4-Trichlorobiphenyl	37680-69-6	Desmetryn	1014-69-3
Chrysene	218-01-9	2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2	Prometryn	7287-19-6
Dibenz(ah)anthracene	53-70-3	3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	Terbutryn	886-50-0
Fluoranthene	206-44-0	2,2',3,4',5',6-Hexachlorobiphenyl	38380-04-0	Cyanazine	21725-46-2
Fluorene	86-73-7	2,3',4,4',5-Pentachlorobiphenyl	31508-00-6	Irgarol 1501	28159-98-0
Indeno(1,2,3-c,d)pyrene	193-39-5	2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	Dichlorvos	62-73-7
Naphthalene	91-20-3	2,3,3',4,4'-Pentachlorobiphenyl	32598-14-4	Mevinphos	7786-34-7
Phenanthrene	85-01-8	2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2	Dimethoate	60-51-5
Pyrene	129-00-0	3,3',4,4',5-Pentachlorobiphenyl	57465-28-8	Fonofos	944-22-9
1-methylnaphthalene	90-12-0	2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3	Propemtamphs	31218-83-4
1,3-dimethylnaphthalene	575-41-7	2,3,3',4,4',5-Hexachlorobiphenyl	38380-08-4	Diazinon	333-41-5

2.3.2 Deployment of SPMDs

Duplicate SPMD passive samplers were deployed for 28 days between the 8th August and 6th September 2012 at a site 1.3 km downstream (51.7606, -4.0080) of a waste water treatment plant (51.7706, -3.9998). An SPMD field blank was also used at this location. The waste water treatment plant was located near to the village of Garnswllt in South West Wales with a population equivalent of approximately 35,000. The works is a conventional activated sludge plant with the effluent discharging via an outfall to the River Loughor (Figure 2.1).

SPMDs, held in argon flushed 300 mL solvent rinsed steel tins, were transported to the deployment sites in cool boxes containing frozen ice packs. The SPMDs were attached to stainless-steel holders and then placed inside a protective stainless-steel cage which was rapidly placed beneath the surface of the river so as to minimize exposure to atmospheric contaminants. The protective stainless-steel cage was secured to a nearby tree trunk using chains and padlocks.

After the defined exposure, the SPMDs were retrieved, sealed in the shipping containers and transported to the laboratory in cool boxes and stored frozen at < –18 °C until extracted. Field blank SPMDs were used at both sites to account for contamination during transport, both to and from study sites, and exposure to airborne contaminants during deployment and retrieval of exposed SPMDs. The field blank was treated in exactly the same way as the deployed SPMD with the exception that they were not exposed to the matrix of interest at the study sites and were stored frozen during the exposure period.

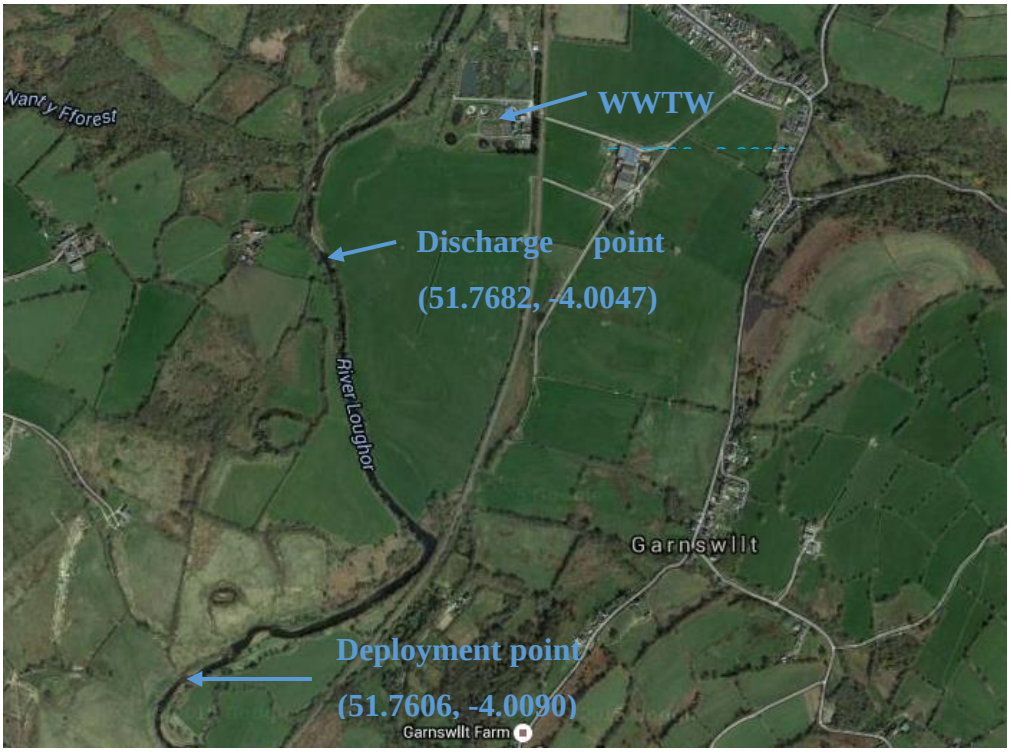


Figure 2.1. Satellite image showing the locations of the waste water treatment plant, final effluent discharge point into the river and the downstream SPMD deployment site (scale not given). Taken from Imagery© 2016, Bluesky, DigitalGlobe, Getmapping plc, Infoterra Ltd & Bluesky, Landsat, The GeoInformation Group Map data© Google.

2.3.3 Extraction of SPMDs

The extraction procedure is based on the procedure developed by Huckins et al. (44). The 250 mL steel tins containing the SPMDs were removed from the freezer and allowed to thaw. Surface bio-fouling on the exposed SPMDs was removed by adding 5 mL of hexane to each tin and shaking for five seconds before discarding the hexane. The SPMDs were removed from the tins and placed in a sequence of 500 mL wide necked glass jars for 20-30 s containing 150 mL of the following solvents:

i) Water

ii) 1 molar hydrochloric acid

iii) Water

iv) Acetone

v) Propan-2-ol

Following the final propan-2-ol rinse, the SPMDs were removed from the jar, allowed to air dry in a laminar fume cupboard for 10 min and transferred to individual glass 500 mL beakers containing 250 mL of hexane. The beakers were covered with aluminium foil, sealed using rubber bands, and placed in an incubator for 18 h at 20 °C. The hexane was poured into a pre-rinsed large (250 mL) glass tubes and the hexane evaporated with the aid of a Turbo-Vap nitrogen blow down evaporator (set at 40 °C) to approximately 3-5 ml (Turbovap, Biotage AB, Uppsala, Sweden). A further 250 mL of hexane was dispensed into the 500 mL glass beaker containing the SPMDs and sealed in the same manner as previously outlined and returned to the incubator for a further six hours. Following the second six-hour dialysis the second 250 mL extracts were poured into the same turbo-vap tubes, containing the initial 3-

5 mL dialysate, evaporated to a final volume of 5 mL and dried over anhydrous sodium sulphate. The extracts were then transferred to glass vials.

To remove triolein, which is incorporated into the SPMD, dialysates were cleaned-up using a size-exclusion chromatography system comprised of a quaternary pump, auto-sampler, variable wavelength detector and a fraction collector (Agilent 1200 series HPLC, Agilent Technologies, Wilmington, DE, USA). The preparative clean-up column was a 300 mm × 21.2 mm i.d. PL Gel column (10 µm particle size, 100 Å pore size), equipped with a 50 mm × 21.2 mm i.d. PL Gel guard column (Agilent Technologies). The mobile phase was 1:1 (*v/v*) *n*-hexane–dichloromethane (5 mL/min). The clean-up column was calibrated using a mixture of corn oil, diethyl hexyl phthalate (DEHP) and perylene. The collection time for the eluate was set just prior to the elution of DEHP and one minute after the elution of perylene which resulted in a total eluate volume of 60 mL which was evaporated to a final volume of 5 mL as described above.

Aliquots of the extracts (3.6 mL) were loaded onto the clean-up system and the fraction containing compounds of low molecular mass (typically <1000 Da) collected and evaporated to approximately 500 µL, as described previously, and then made up to 1 mL using *n*-hexane. The field blank samplers were processed identically. The extracts from the duplicate SPMDs were split into two equal aliquots; one aliquot was transferred to an auto-sampler vial and the second aliquot spiked (5 µL) with the standard solution prepared in section 2.3.1. One duplicate would be used for the analysis outlined in section 3.6 and 3.7 (one dimensional GC and GCxGC) and the second duplicate used for the analysis outlined in section 3.8 (GCxGC TOF-MS analysis with Select eV).

2.3.4 Conventional one dimension GC analysis (experimental conditions)

SPMD extracts, including the un-spiked, spiked and field blanks together with a mixed compound standard solution, were analysed using a BenchTOF-dx® TOF MS (ALMSCO International, Llantrisant, U.K.) coupled to a 7890A GC (Agilent Technologies, Santa Clara, CA, USA).

Injector: PTV; Liner: 1.8 mm (i.d.) baffled; Carrier gas: He, constant flow at 1.5 mL/min; Mode: Splitless for 2 min (then 100 mL/min purge); Temperature: 60 °C for 0.05 min, 300 °C/min to 320 °C (1 min), 720 °C/min to 360 °C (cleaning phase) held to end of run; Septum purge: On, 3 mL/min; Injection volume: 1µL; Column: SGE BPX5 (30 m × 0.25 mm × 0.25 µm film thickness); Oven temperature programme: 50 °C (2 min), 12 °C/min to 320 °C (8 min), total run time: 32.5 min.

TOF MS: Filament voltage: 1.6 V; Ion source: 300 °C; Transfer line: 300 °C; Mass range: 45–400 amu; Data rate: 4 Hz.

Data files were processed using Target View which utilises dynamic baseline compensation followed by peak deconvolution. The dynamic baseline correction algorithm selectively eliminated ions due to chromatographic background noise (e.g. column bleed). This minimised interference in the total ion chromatogram and improved compound spectra related data. After eliminating the background, a deconvolution algorithm was applied to distinguish between co-eluting compounds and assigned each respective mass ion to the appropriate individual compound. Principal component analysis was then applied to the de-convoluted spectra to highlight characteristic ion fragmentation patterns, which were then compared with

compound spectra within the NIST target library to determine matches. A minimum library match factor of 650 (out of a 1000) was used for compound identification. In addition, data files were processed without peak deconvolution to determine its effectiveness on the quality of the library match factors.

2.3.5 GCxGC TOF-MS analysis (experimental conditions)

SPMD extracts, including un-spiked, spiked and field blanks together with a mixed compound standard solution, were analysed using a BenchTOF-dx® TOF MS (ALMSCO International, Llantrisant, U.K.) coupled to a 7890A GC (Agilent Technologies, Santa Clara, CA, USA) fitted with a ZX1 cryogenic modulator (Zoex Corp., Houston, TX, USA). Oven programming and modulation parameters were adjusted to yield wide analyte volatility range and high peak capacity.

Injector: PTV; Liner: 1.8 mm (i.d.) baffled; Carrier gas: He, constant flow at 1.5 mL/min; Mode: Splitless for 2 min (then 100 mL/min purge); Temperature: 60 °C for 0.05 min, 300 °C/min to 320 °C (1 min), 720 °C/min to 360 °C (cleaning phase) held to end of run; Septum purge: On, 3 mL/min; Injection volume: 1µL.

Column set: 1st dimension: SGE BPX5, (27 m × 0.25 mm × 0.25 µm film thickness), 2nd dimension: SGE BPX50, (1.95 m × 0.1 mm × 0.1 µm film thickness) (SGE Analytical Science Pty. Ltd.)

Modulation loop: Column set: As for 2nd dimension; equivalent pneumatic impedance to 60 m × 0.2 mm (calculated from impedance factor look-up charts for 1st- and 2nd-dimension columns used).

GC×GC temperature programme and modulation: Main oven: 50 °C (2.0 min), 5 °C/min to 320 °C (8 min); Secondary oven: Not applicable; Hot jet: 150 °C (2.0 min), 5 °C/min to 400 °C (hold time matched to total run time); Cold jet: Dewar fill high 60%, Dewar fill low 50%; Cold jet flow: 15 L/min, Modulation period: 5 s; Hot-jet pulse 350 ms; Total run time: 64 min.

TOF MS: Filament voltage: 1.6 V; Ion source: 300 °C; Transfer line: 300 °C; Mass range: 40–500 amu; Data rate: 50 Hz.

Software: The platform-neutral software package GC Image (GC Image LLC, Lincoln, NE, USA) was used to visualize and process the GCxGC-TOF-MS data. Default method parameters were used and the peak mass spectra were searched against the NIST 11 mass spectral library with a library match factor of 650 used for compound identification.

2.3.6 GCxGC-TOF MS analysis with Select eV (experimental conditions)

SPMD extracts, including un-spiked, spiked and field blanks together with a mixed compound standard solution, were analysed using a Bench TOF-Select-eV® TOF MS, (Markes International, Llantrisant, UK) coupled to a 7890A GC (Agilent Technologies) fitted with a ZX1 thermal loop modulator (Zoex Corp.). Oven programming and

modulation conditions were adjusted to permit analysis of a wide volatility range of analytes and give a high peak capacity as detailed below.

Injector: PTV; Liner: 1.8 mm (i.d.) baffled; Carrier gas: He, constant flow at 1.5 mL/min; Mode: Pulsed splitless for 1.5 min (then 100 mL/min purge); Temperature: 60 °C min, 720 °C/min to 320 °C (1 min), 720 °C/min to 360 °C (cleaning phase) held to end of run; Septum purge: On, 3 mL/min. Injection volume: 1 µL.

Column set: 1st dimension: SGE BPX-5, (27 m × 0.25 mm × 0.25 µm film thickness), 2nd dimension: SGE BPX-50, (1.95 m × 0.1 mm × 0.1 µm film thickness) (SGE Analytical Science Pty Ltd., Victoria, Australia) Modulation loop: As for 2nd dimension (0.95 m).

GC×GC temperature programme and modulation: Main oven: 60 °C (2.0 min), 2.5 °C/min to 320 °C (10 min); Secondary oven: Not applicable; Hot jet: 150 °C (2.0 min), 2.5 °C/min to 410 °C (hold time matched to total run time); Cold jet: Dewar fill high 60%, Dewar fill low 50%; Cold jet flow: 15 L/min, Modulation period: 8 s; Hot-jet pulse 350 ms; Total run time: 110 min.

TOF MS: Filament voltage: 1.8 V; Ion source: 280 °C; Transfer line: 300 °C; Mass range: 40–500 amu; Data rate: 50 Hz; Electron ionisation energy: variable (10–70 eV).

Software: The platform-neutral software package GC Image (GC Image LLC, Lincoln, NE, USA) was used to visualize and process the GCxGC-TOF-MS Select-eV® data.

Default method parameters were used and peak mass spectra were searched against the NIST 14 mass spectral library.

2.4 Results and discussion

2.4.1 Compounds identified from conventional one dimensional GC analysis

Spiking values equivalent to an aqueous sample concentration of 50 ng/L (assuming an uptake rate of 1–2 L per day) were chosen to reflect the concentrations of many pollutants that could be present in a passive sampler deployed for a period of approximately one month. For compounds, such as the PCBs and PAHs, which typically have strong responses under 70eV conditions, aqueous concentrations as low as 0.01–0.03 ng/L can therefore be detected using the applied SPMD full scan approach for the screening of pollutants.

Of the 56 pesticides added to the SPMD extract, 23 were identified (using the criteria set in section 3.3.4) by one dimensional chromatography respectively with eight pesticides remaining unidentified. The list of identified compounds from each technique are shown in Table 2.2 with the library match factors obtained shown in Table 2.3.

Seventeen of the 33 pesticides not identified by one dimensional GC have molecular ions which are ≥ 30 % of the base peak whilst the remaining 15 have molecular ions which are ≤ 8 % of the base peak in the 70 eV mass spectrum. This result would suggest that the presence of an abundant molecular ion in a compounds EI spectrum is not a prerequisite for accurate identification in a complex extract, such as that

obtained from the SPMD sampler, when using the traditional one dimensional GC approach. This observation is indicative of the enhanced matrix interferences of the SPMD extract having an impact on the deconvolution process and subsequent library match as the extract may contain several thousand compounds many of which could co-elute and share identical masses with the analytes of interest resulting in poor library matches. In addition, the SPMD extract also contains residues of triolein, methyl oleate and its degradation compound, oleic acid plus numerous polyethylene oligomers which can suppress target compound ionisation if present at significant concentrations (high µg/SPMD). Figure 2.2 shows the total ion chromatogram obtained from the one-dimensional GC analysis of the spiked SPMD extract with several large peaks clearly visible. Figure 2.3 shows the view of the boxed area in Figure 2.2 illustrating the highly complex one-dimensional GC MS-TOF chromatogram containing a profusion of co-eluting peaks many of which have very large peak responses. Four-hundred and eighty peaks were identified using the standard peak extraction software, however, when using the deconvolution option, a further 298 compounds were identified proving the usefulness of the advanced peak picking algorithm.

The molecular ions for pentachlorobenzene and cyanazine, which were not identified using either automated technique, were observed by manually extraction their respective ions from the chromatograms tentatively confirming their presence in the SPMD extract. Of the 33 compounds not identified, 14, 9 and 10 are classed as organo-phosphate, organo-nitrogen and organo-chlorine pesticides respectively and no single class of compound appears to be more readily identifiable than another.

Despite several pairs of co-eluting compounds, 24 of the 42 PAHs present in the SPMD extract returned library search values of greater than 650 and these are highlighted in Table 3.4. Only 18 PAHs were fully resolved in the 1st dimension with benzo(k)fluoranthene and benzo(j)fluoranthene co-eluting and only partially resolved from benzo(b)fluoranthene, however all returned library match scores greater than 650. Chrysene and triphenylene also co-eluted with both returning high match scores greater than 800 and dibenz(a,h)anthracene, which co-eluted with indeno(1,2,3-cd)pyrene, was misidentified for dibenz(a,j)anthracene as their MS spectra are identical. Despite PAHs having strong molecular ions, most of the alkyl substituted 5–6 ring PAHs were not identified and this may be in part due to the complex mass spectrum obtained from alkylated PAHs making individual ions difficult to de-convolute from the matrix background.

All 19 PCBs added to the SPMD extract were correctly identified by one dimensional chromatography with 2,4',5-Trichlorobiphenyl and 2,4,4'-Trichlorobiphenyl co-eluting but both congeners returned library match scores above 650. The presence of strong molecular ions with intense chlorine isotope patterns makes the deconvolution and subsequent identification of PCBs far easier than for compounds which do not contain chlorine or other halogens. However, with 209 possible congeners many PCBs will have very similar properties making conventional one dimensional GC analysis highly challenging. Despite the introduction of speciality GC columns (e.g. SGE HT-8) many congeners remain difficult to resolve from each other and will often co-elute leading many regulatory laboratories to undertake multiple one dimensional GC analyses on different columns in order to separate and identify all 209 congeners. Figure 2.4 gives a graphic illustration, from the one-dimensional GC analysis

undertaken, of the potential for chlorinated biphenyls to co-elute. Even with deconvolution the highlighted peak at 16.90 min from the spiked blank analysis would report as either a trichlorinated biphenyl, with a match penalty due to the presence of an ion cluster at m/z 292, or a tetrachlorinated biphenyl with a match penalty due to an over-abundance of ions clustered around m/z 256.

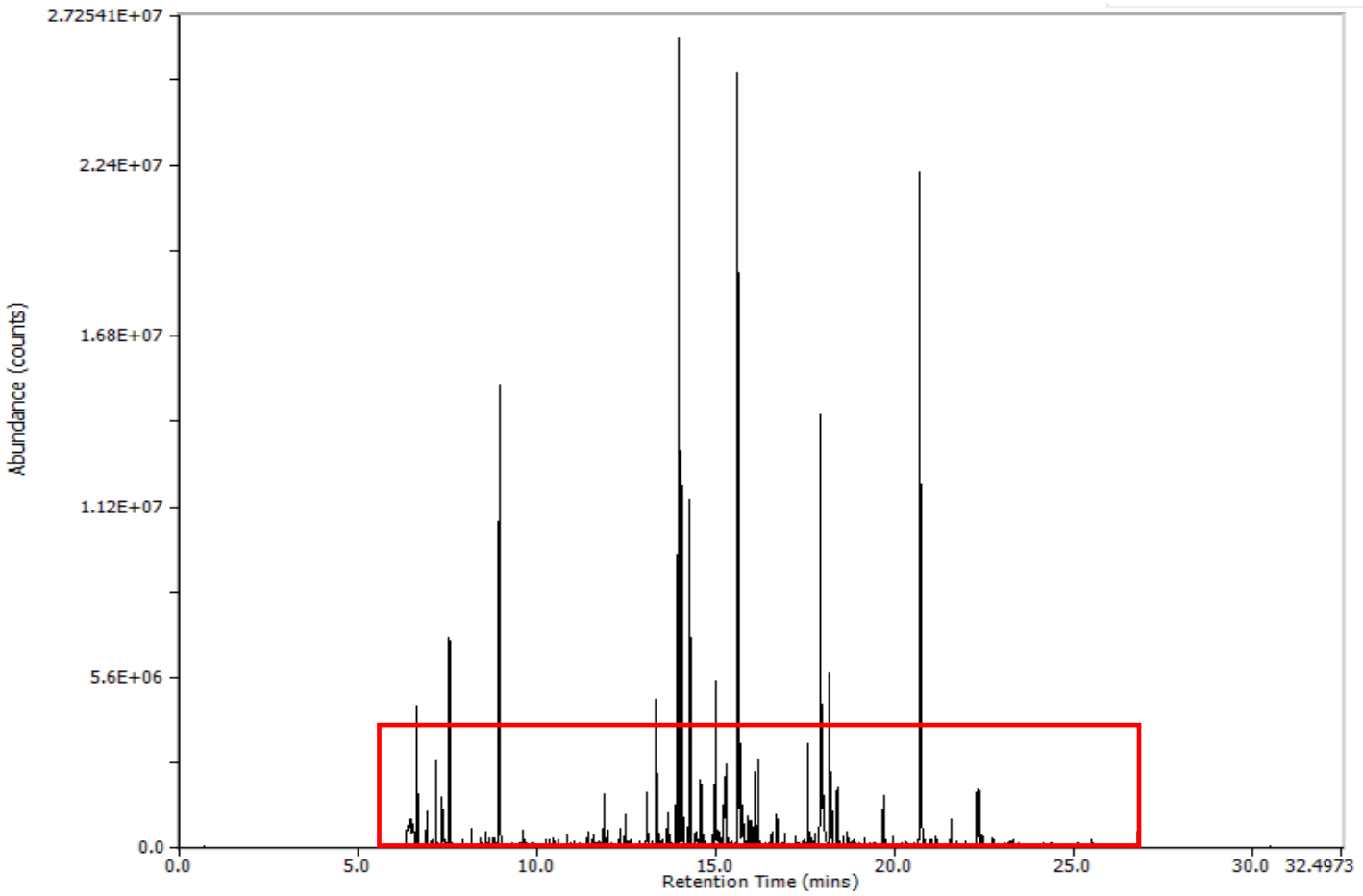


Figure 2.2 Total ion chromatogram of 1-D GC MS-TOF analysis of spiked SPMD extract.

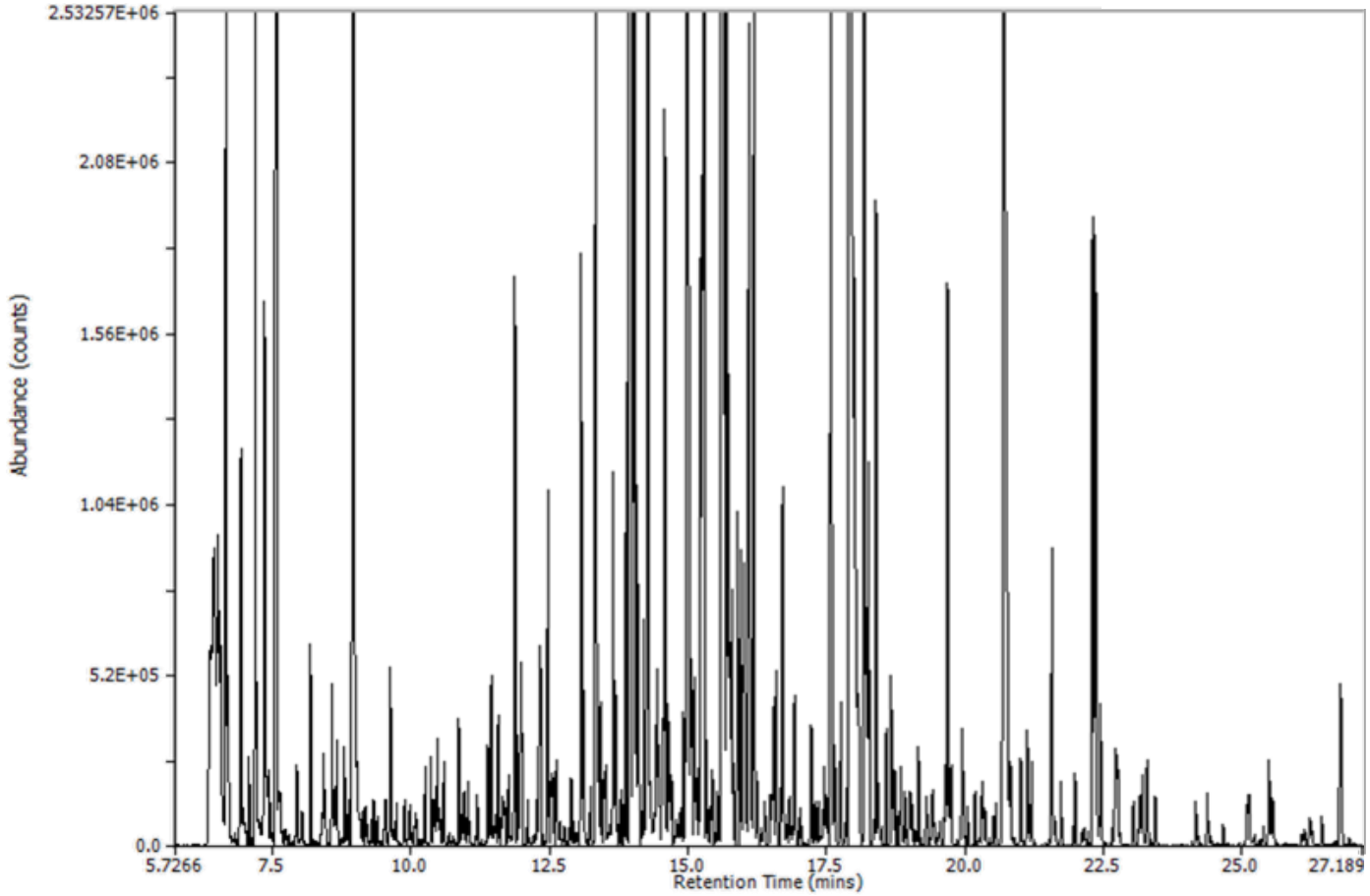


Figure 2.3 Total ion chromatogram of spiked SPMD extract analysed by one dimensional GC TOF-MS showing the highly complex nature of the sample.

Table 2.2 Comparison of compounds identified using GC TOF-MS and GCxGC TOF-MS from a deployed SPMD sampler, extracted and spiked with 55 pesticides.

Elution order	Pesticide	CAS No.	GC TOF MS Retention time (min)	GC×GC–TOF MS First-dimension (min)	GC×GC–TOF MS Second-dimension (s)	Intensity of molecular ion relative to base peak in mass spectrum at 70 eV (%)	Intensity of molecular ion relative to base peak in mass spectrum at 12 eV (%) 'Select eV'
1	Hexachlorobutadiene	87-68-3	10.45	23.76	1.16	34	85
2	Dichlorvos	62-73-7	10.97	24.51	2	5	15
3	Mevinphos	7786-34-7	13.58	29.43	2.14	3	9
4	Desethylatrazine	6190-65-4	ni	35.35	2.62	32	57
5	alpha-BHC	319-84-6	16.99	36.51	2.08	<1	<1
6	Hexachlorobenzene	118-74-1	17.15	36.68	1.82	bp	bp
7	Simazine	122-34-9	17.41	37.1	2.44	bp	bp
8	Atrazine	1912-24-9	17.54	37.18	2.18	55	bp
9	Propazine	139-40-2	—	37.26	1.94	58	bp
10	Propemtamphos	31218-83-4	—	37.43	1.78	<1	1
11	Diazinon	333-41-5	18.16	37.6	1.68	61	67
12	Trietazine	1912-26-1	—	37.6	2.02	53	bp
13	gamma-BHC	319-85-7	17.6	37.93	2.3	1	1
14	beta-BHC	58-89-9	17.6	38.01	2.22	1	1
15	Fonofos	944-22-9	—	38.01	2.18	33	73

continued

16	Desisopropylatrazine	1007-28-9	—	38.6	2.55	bp	bp
17	delta-BHC	319-86-8	16.99	39.35	2.48	<1	<1
18	Desmetryn	1014-69-3	—	39.76	2.58	bp	bp
19	Chlorpyrifos methyl	5598-13-0	—	39.93	2.3	2	9
20	Parathion methyl	298-00-0	—	40.35	2.44	90	bp
21	Prometryn	7287-19-6	—	40.52	2.1	bp	bp
22	Fenchlorphos	299-84-3	—	40.6	2.06	<1	1
23	Pirimiphos methyl	29232-93-7	—	40.76	2.04	76	bp
24	Terbutryn	886-50-0	19.67	41.01	2.24	53	73
25	Fenitrothion	122-14-5	19.73	41.18	2.44	70	bp
26	Malathion	121-75-5	19.96	41.18	2.2	<1	<1
27	Chlorpyrifos	2921-88-2	20.18	41.6	2.02	10	nd
28	Fenthion	55-38-9	—	41.93	2.58	bp	bp
29	Parathion	56-38-2	—	42.02	2.1	bp	bp
30	Pirimiphos ethyl[a]	23505-41-1	20.63	42.1	1.9	bp	bp
31	Aldrin	309-00-2	20.02	42.1	1.88	1	2
32	Chlorfenvinphos	470-90-6	—	43.18	2.26	<1	1
33	Isodrin	465-73-6	—	43.35	2.1	7	11
34	Irgarol 1051	28159-98-0	—	43.52	2.46	89	bp
35	2,4'-DDE	3424-82-6	—	44.43	2.28	43	bp
36	Iodofenphos	18181-70-9	—	45.27	2.7	<1	<1
37	Buprimate	41483-43-6	—	45.52	2.32	30	83
38	4,4'-DDE	72-55-9	21.45	45.6	2.16	93	bp
39	2,4'-DDD (TDE)	53-19-0	—	45.93	2.46	2	4
40	Dieldrin	60-57-1	—	46.1	2.34	4	10
41	Ethion	563-12-2	23	46.85	2.5	14	14

continued

42	Endrin	72-20-8	22.56	46.93	2.6	4	10
43	4,4'-DDT	50-29-3	22.25	47.27	2.52	<1	2
44	Triazophos	24017-47-8	23.24	47.6	3.53	15	38
45	Carbophenothion	786-19-6	—	48.02	2.68	32	46
46	Azinphos methyl	86-50-0	—	51.85	4.61	<1	<1
47	Azinphos ethyl	2642-71-9	—	52.77	4.29	<1	<1
48	Coumaphos	56-72-4	26.68	53.69	3.81	bp	bp
49	2,4'-DDT	789-02-6	ni	ni	ni	<1	2
50	4,4'-DDD	75-54-8	ni	ni	ni	<1	2
51	Cyanazine[b]	21725-46-2	ni	ni	ni	34	32
52	Dimethoate	60-51-5	ni	ni	ni	8	8
53	Endosulfan I	959-98-8	ni	ni	ni	<1	<1
54	Endosulfan II	33212-65-9	ni	ni	ni	<1	4
55	Pentachlorobenzene[b]	608-93-5	ni	ni	ni	bp	bp
56	Triallate	2303-17-5	ni	ni	ni	<1	<1

ni = identified

nd = not determined

bp = base peak

[a] = equal in response to fragment ion in compound mass spectrum

[b] = library match factor < 650 but seen in the manually extracted ion chromatogram for the molecular ion

Table 2.3 NIST library match factors obtain from different data analysis techniques for pesticides, PAHs and PCBs.

Compound	CAS No.	Class	GCxGC	1D GC (no deconvolution)	1D GC (with deconvolution)
Acenaphthylene	208-96-8	PAH	932	722	Not found
Fluoranthene	206-44-0	PAH	944	936	927
3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	PCB	870	819	800
2,2',3,4',5',6-Hexachlorobiphenyl	38380-04-0	PCB	844	795	776
4,4'-DDT	50-29-3	Organo-chlorine pesticide	835	801	790
Dieldrin	60-57-1	Organo-chlorine pesticide	842	Not found	Not found
Atrazine	1912-24-9	Organo-nitrogen pesticide	842	680	Not found
Desmetryn	1014-69-3	Organo-nitrogen pesticide	817	Not found	Not found
Chlorpyrifos	2921-88-2	Organo-phosphorous pesticide	824	701	684
Parathion methyl	298-00-0	Organo-phosphorous pesticide	803	693	Not found

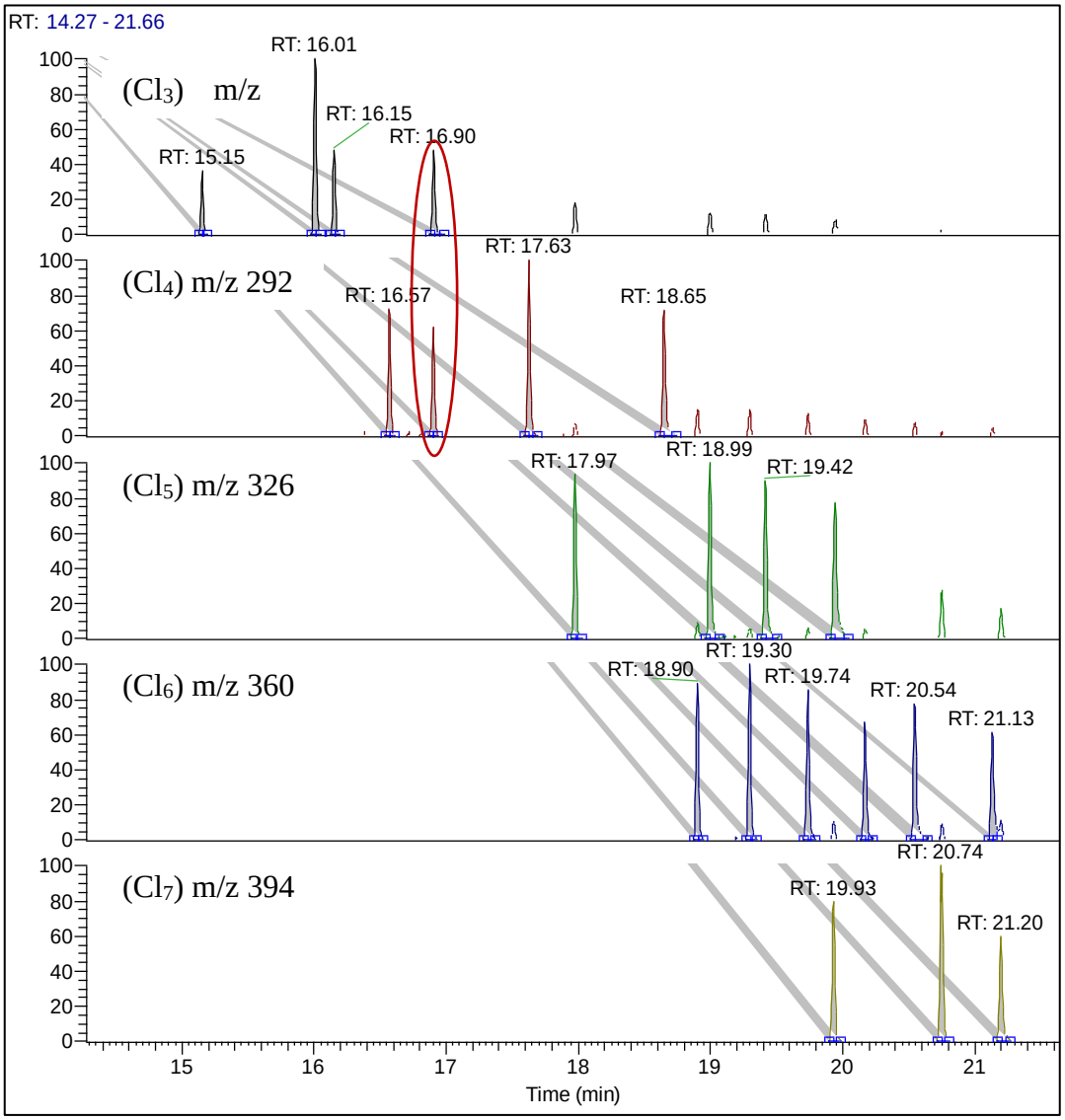


Figure 2.4 Extracted ion profiles for the chlorinated biphenyl compounds obtained from the analysis of the spiked SPMD extract. Highlighted peaks at 16.90 min in the trichlorinated and tetrachlorinated biphenyl traces exhibit perfect co-elution of analytes.

2.4.2 Deconvolution

Table 2.2 shows the library search results for selected compounds from various pollutants classes with and without deconvolution. For the majority of compounds only small improvements are observed when using deconvolution but for two compounds, acenaphthylene and atrazine, they were only identified above the set threshold only when using the deconvolution algorithm. Deconvolution removes chemical noise, and significantly improves the detectability of the two compounds in the SPMD extract; this can also be viewed as increasing signal-to-noise ratio through improved selectivity versus the background.

2.4.3 Comprehensive GC×GC analysis

Following the conventional one dimensional GC analysis, the column set was changed, as detailed in the experimental section, and the analyses repeated. Figures 2.5 to 2.7 show the GC Image-rendered GCxGC contour plots of the field blank, un-spiked and spiked SPMD extracts. Figure 2.8 shows a three dimensional view of the boxed area in Figure 2.7 illustrating extreme sample complexity with a concentrated number of co-eluting peaks occurring in the first dimension.

2.4.4 Pesticides identified with GCxGC analysis

Of the 56 pesticides spiked to the SPMD extract, 48 were correctly identified by comprehensive GCxGC with the same 8 pesticides, listed in Table 2.1, remaining unidentified from both techniques. GCxGC identified over twice as many compounds as one dimensional GC and this was a result of its orthogonal separation capability

and superior resolving power which resulted in the improved separation of the interfering matrix from the pesticides of interest resulting in significantly improved library match factors.

Figure 2.9 shows an expanded view of a GC Image-rendered contour plot for the spiked SPMD extract, with the pesticides circled using the 'blob' tool in GC Image. The compounds are well separated in both dimensions with the more polar pesticides exhibiting longer retention times due to the use of a polar column in the second dimension. For example, the polar atrazine metabolite, desethylatrazine, has a short first dimension retention time of 35.35 min but a relatively long second dimension retention time of 2.62 s when compared to diazinon which has a slightly longer first dimension retention time but a much shorter second dimension retention time of 1.68 s.

There are several pesticides that have multiple stereoisomers and hexachlorocyclohexane (HCH) is one such pesticide, having alpha-, beta-, gamma- and delta- isomers, which are relatively indistinguishable spectrally. Figure 2.10 shows the GC Image-rendered contour plot and 3D surface plot for extracted ion 219 (±0.5 amu) showing clear separation of the four stereoisomers of HCH in the spiked SPMD extract analysis. The separation in the 2nd dimension is again based on isomer polarity.

Figures 2.11 to 2.13 give examples of NIST library spectral matches for a range of pesticides along with the peak shape recorded in GC Image. Excellent peak shapes and spectral quality together with high scores for the library matches were obtained

for 3 pesticides that were not identified using one-dimensional GC. This is reflected in the reverse fit library match scores of 810, 811 and 867 and probability scores of 88.4 %, 91.5 % and 94.8 obtained for parathion, irgarol 1051 and chlorpyrifos-methyl respectively.

Eight pesticides out of 56 remained unidentified although the molecular ions for pentachlorobenzene and cyanazine could be seen by manual extraction of their respective ions, tentatively confirming their presence in the SPMD extract. Five of the eight pesticides have molecular ions which are < 1 % of the base peak and of these endosulfan I, endosulfan II and cyanazine have mass spectra which are highly fragmented presenting considerable difficulties in matching compound spectra to those contained in the reference NIST library.

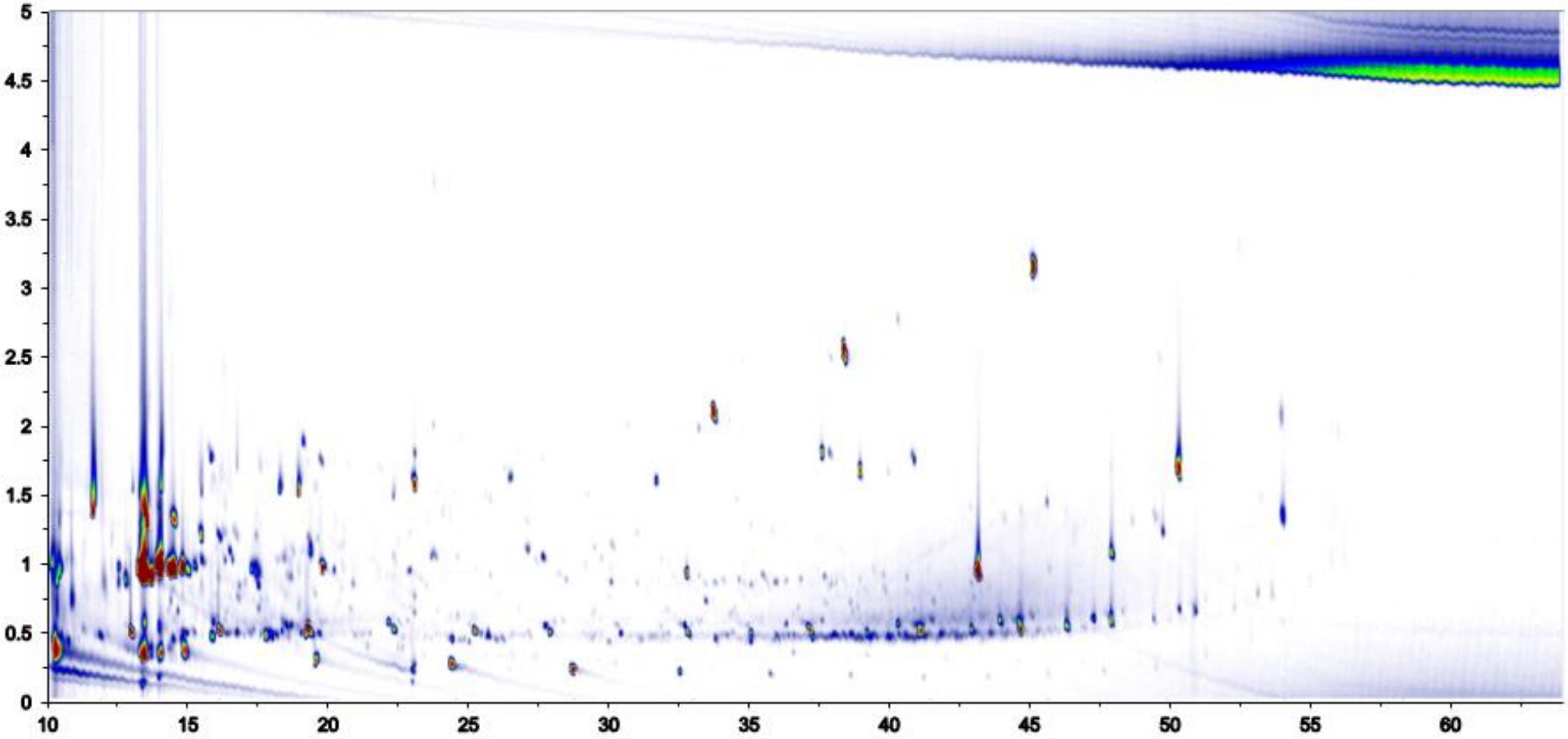


Figure 2.5 GC Image rendered contour plot obtained from GCxGC analysis of the extract from the SPMD field blank for the deployment at Garnswllt WWTP.

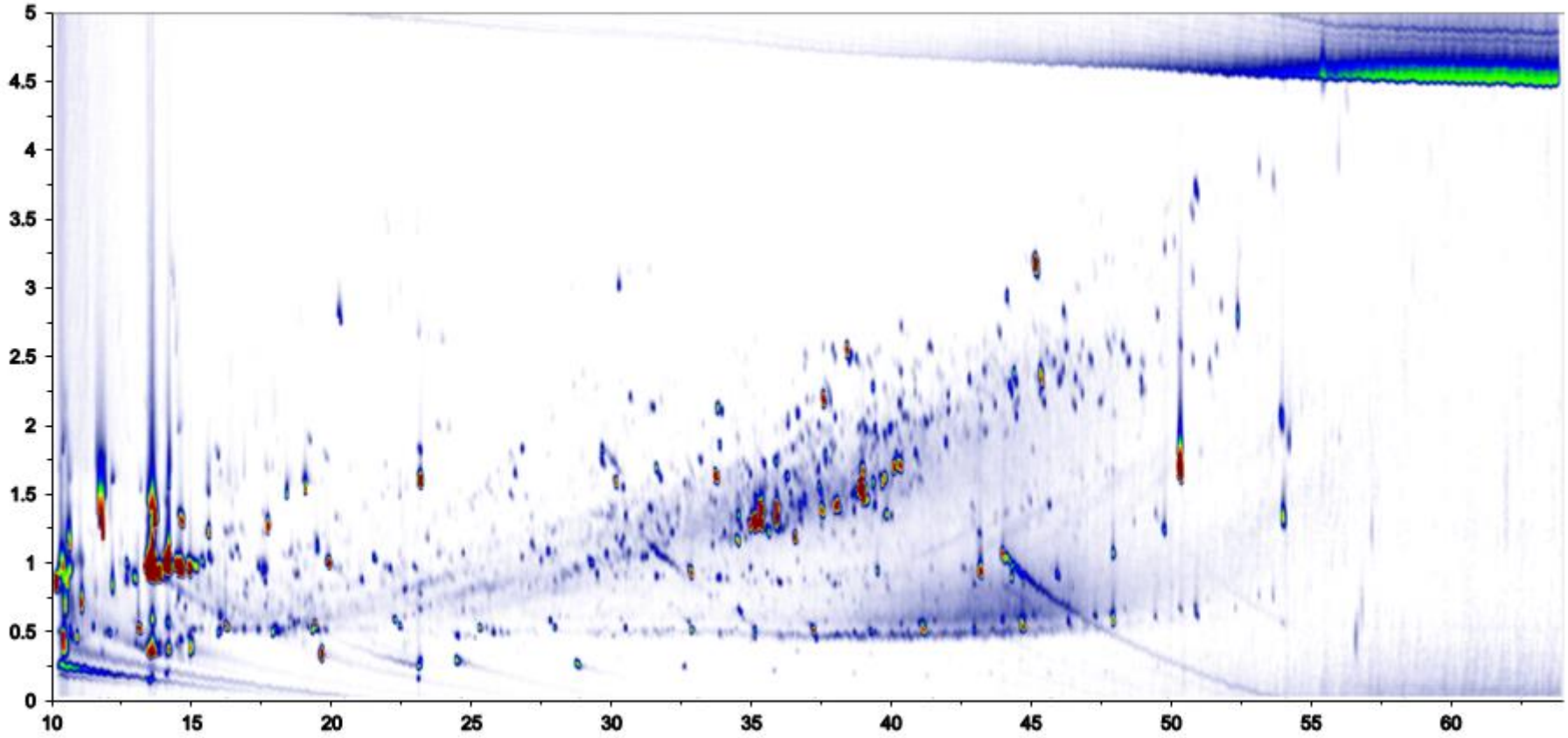


Figure 2.6 GC Image rendered contour plot obtained from GCxGC analysis of the extract from the un-spiked SPMD (deployed at Garnswllt WWTP).

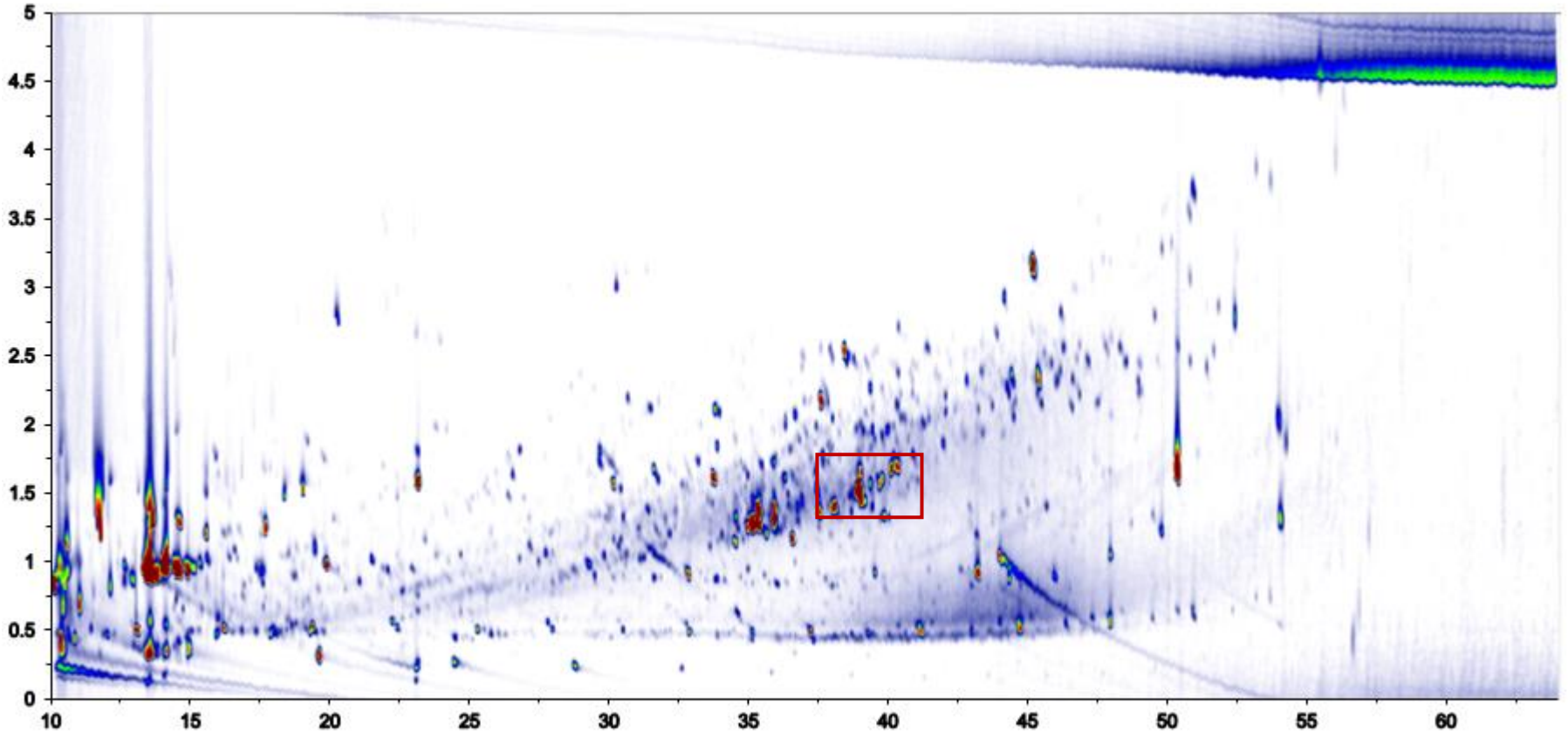


Figure 2.7 GC Image rendered contour plot obtained from GCxGC analysis of the extract from the spiked SPMD (deployed at Garnswllt WWTP).

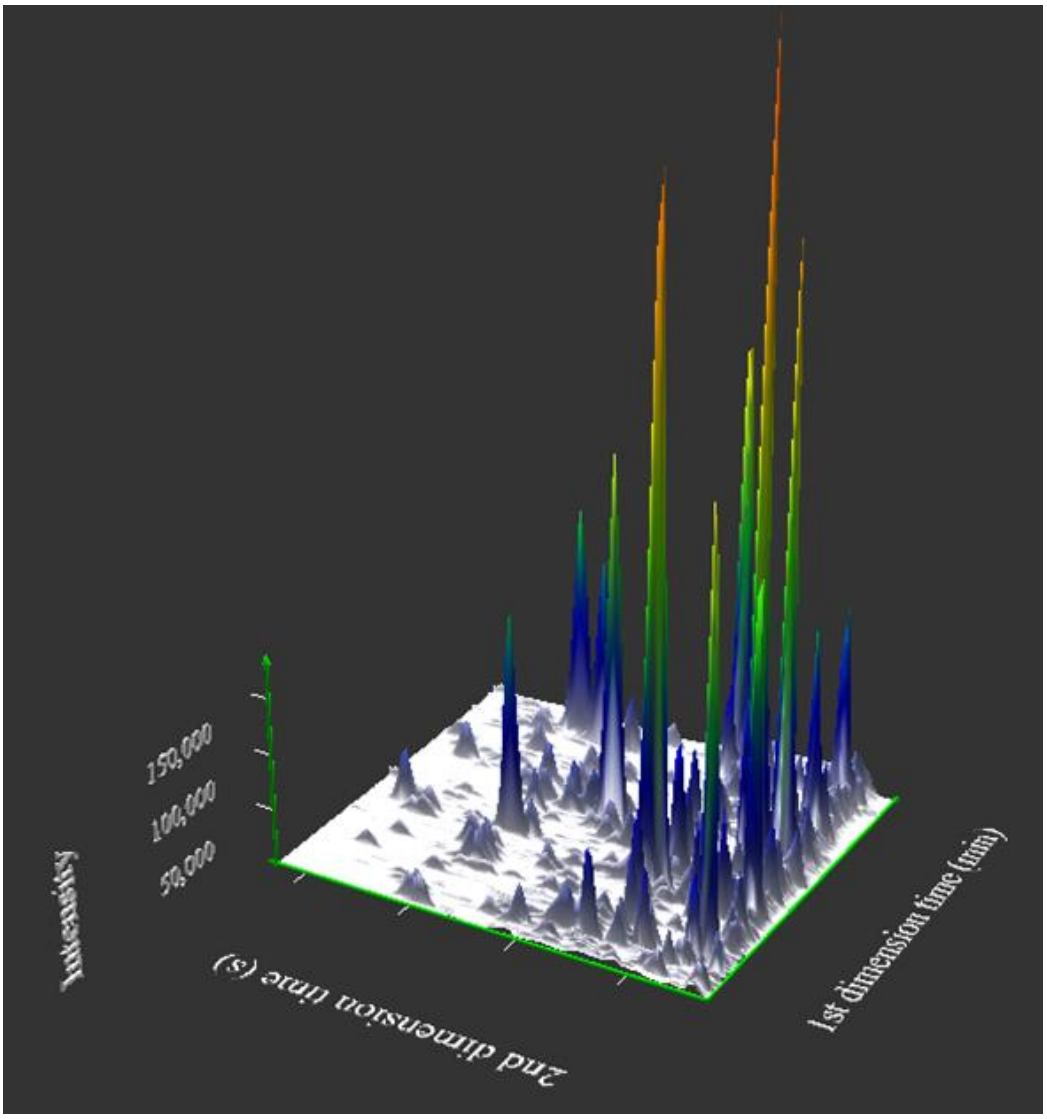


Figure 2.8 3D view of the boxed area in Figure 3.7 illustrating sample complexity and significant number of co-eluting peaks that occur in the first dimension.

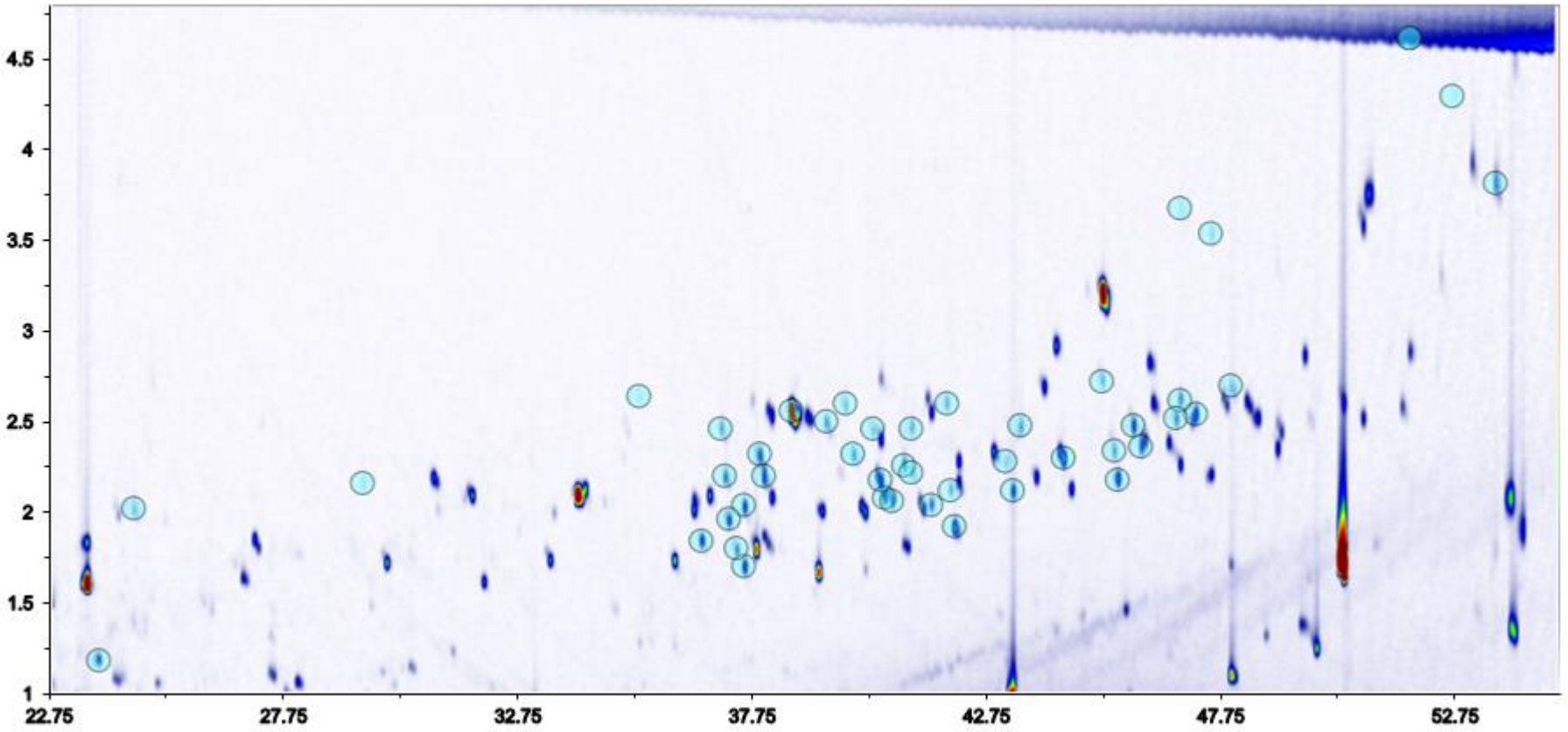


Figure 2.9 GC Image rendered contour plot obtained from GCxGC analysis of the extract from the spiked SPMD (deployed at Garnswllt WWTP) with pesticides circled.

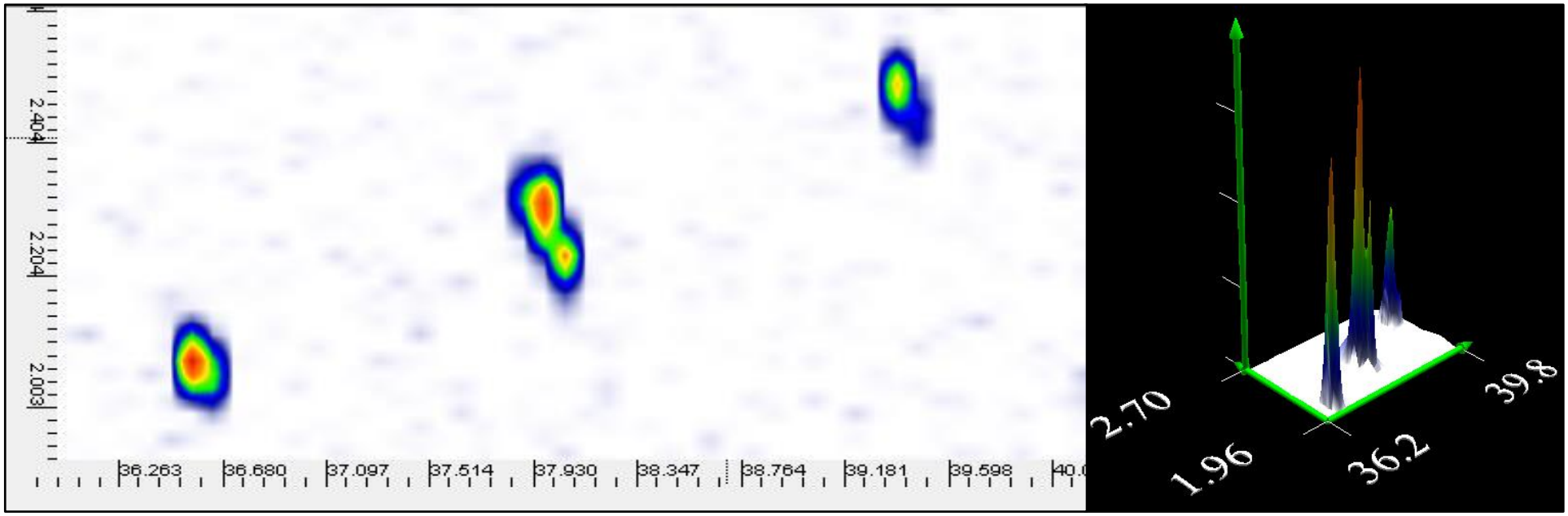


Figure 2.10 GC Image rendered contour plot and 3-D surface plot for extracted ion 219 (±0.5 amu) showing separation of the four stereoisomers of hexachlorocyclohexane for the spiked SPMD extract analysis.

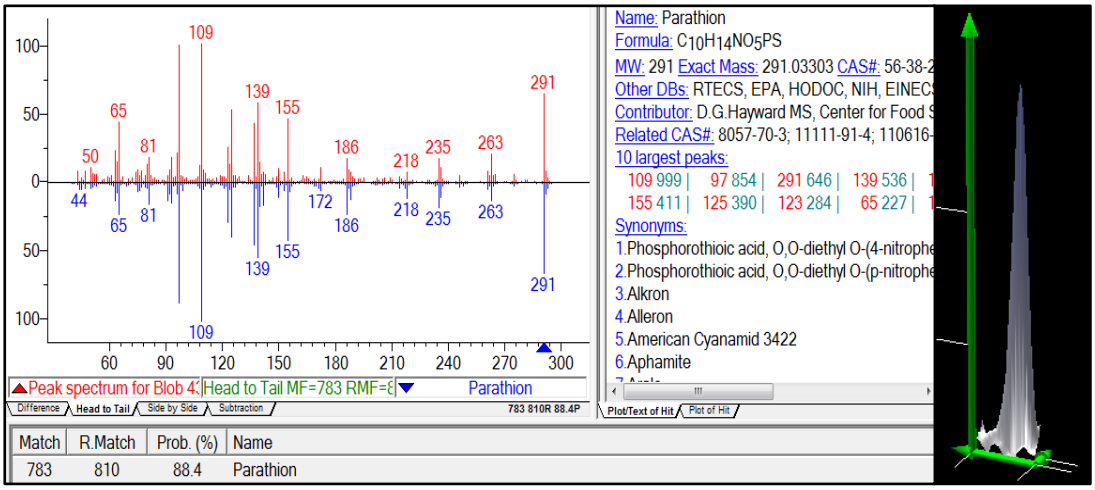


Figure 2.11 NIST 2011 library match and 3-D rendered peak for parathion taken from the spiked SPMD extract analysis.

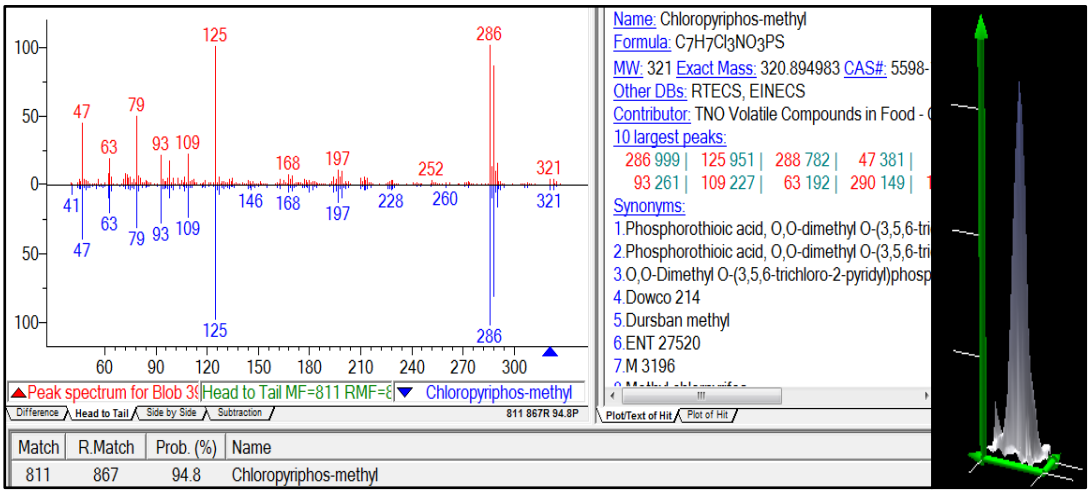


Figure 2.12 NIST 2011 library match and 3-D rendered peak for chloropyriphos-methyl taken from the spiked SPMD extract analysis.

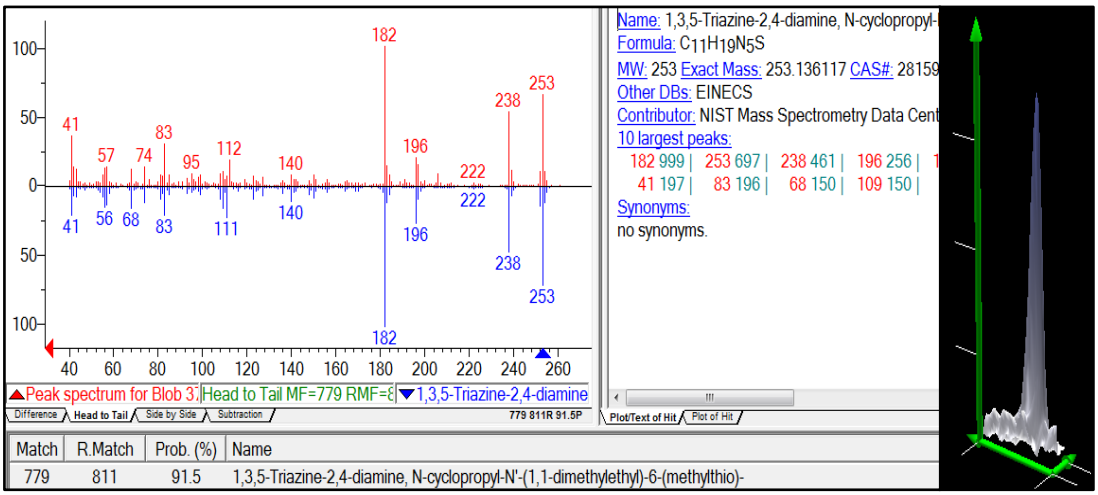


Figure 2.13 NIST 2011 library match and 3-D rendered peak for irgarol 1051 taken from the spiked extract sample analysis.

2.4.5 PAHs identified with GCxGC analysis

Forty out of 42 PAHs were correctly identified using GCxGC with 37 being fully resolved in the 2nd dimension. Table 2.4 gives a detailed description of the assignments made, including retention times in both dimensions and the general elution order. Five PAHs that co-eluted in the first and second dimension, but achieved a library match score of 650, are included in Table 2.4 as being tentatively identified. Two PAHs, spiked into the SPMD extract, were not identified and these were 2,4,7-trimethyldibenzothiophene, which is not in the NIST 11 database, and perylene, which has a molecular ion of m/z 252, common to numerous PAHs. In all probability, perylene could not be distinguished from benzo(a)pyrene and the benzo(b)/(k)/(j)fluoranthenes isomers due to co-elution. It was not, however, reported as a potential candidate compound in the library search.

Figure 2.14 shows an expanded view of a GC Image-rendered contour plot for the spiked SPMD extract, with the PAHs circled using the 'blob' tool in GC Image. Three compounds; indeno(1,2,3-cd)pyrene, benzo(ghi)perylene and dibenz(a,h)anthracene exhibited a phenomenon called 'wrap-around' and these are highlighted in red. Wrap-around occurs when compounds have not eluted from the secondary column before the end of the modulation period and appear in the following modulation. Compounds that have 'wrapped' in the second dimension typically show peak broadening. 'Wrap-around' can be avoided by increasing the oven temperature gradient but this will lead to higher first-dimension elution temperatures, thus decreasing secondary retention times if the primary and secondary columns are in the same oven or subject to the same temperature. To maintain resolution in the first dimension, a dual oven system can be employed to house the secondary column and this is used with a positive temperature offset in the second dimension reducing the possibility of 'wrap around'.

Examples of 4-ring PAHs are given in Figure 2.15 and because of their similar structure almost identical retention times are obtained in the first dimension. Fragmentation patterns, obtained under electron ionisation conditions, are very similar and accurate assignment without running individual standards to determine retention times is very difficult. It is for this reason that benz[a]anthracene, benz[b]anthracene, chrysene and triphenylene have been labelled as 'tentative assignment' in Table 2.4. Figure 2.16 shows a surface plot for the entire separation space of extracted ion 228 (the molecular ion for the above PAHs'). As can be seen, there are only two significant peaks in the separation space area, with one being approximately three times larger in volume than the other, suggesting that, with the spiking levels being the same, two or more of these compounds co-elute. Figures 2.17

to 2.20 show the inherent spectral quality obtained from GCxGC analysis with examples of the NIST library matches obtained for the 3-, 4-, and 5-ring PAHs.

Table 2.4 PAH assignment based on the spiked SPMD extract analysis.

Elution order	PAH	CAS No.	Col. I RT (min)	Col. II RT (s)
1[a]	Naphthalene	91-20-3	23.5086	1.8229
2[a]	1-Methylnaphthalene	90-12-0	27.0932	1.843
3[a]	1,3-Dimethylnaphthalene	575-41-7	29.9276	1.7027
4[a]	Acenaphthalene	208-96-8	30.9279	2.1835
5[a]	Acenaphthene	83-32-9	31.7616	2.0833
6[a]	2,3,5-Trimethylnaphthalene	2245-38-7	33.4288	1.7228
7[a]	Fluorene	86-73-7	34.179	2.1031
8	1,4,6,7-Tetramethylnaphthalene	13764-18-6	36.0965	1.7728
9	1-Methylfluorene	1730-37-6	36.8647	2.0833
10	9-n-Propylfluorene	4037-45-0	38.0138	1.843
11[a]	Dibenzothiophene	132-65-0	38.1806	2.524
12	Phenanthrene	85-01-8	38.7641	2.524
13[a]	Anthracene	120-12-7	38.9308	2.524
14	1,7-Dimethylfluorene	442-66-0	39.2643	1.9832
15	2-Methyldibenzothiophene	20928-02-3	40.5147	2.3838
16	3-Methylphenanthrene	832-71-3	41.5151	2.6242
17[a]	1-Methylphenanthrene	832-69-9	41.5985	2.5441
18[a]	3,6-Dimethylphenanthrene	1576-67-6	42.9323	2.3237

continued

19[a]	1,2-Dimethyldibenzothiophene	31317-14-3	44.016	2.6843
20[a]	Fluoranthene	206-44-0	44.2661	2.9047
21[a]	Pyrene	129-00-0	45.35	3.185
22	2-Methylfluoranthene	33543-31-6	46.2668	2.8245
23[a]	1,2,6-Trimethylphenanthrene	30436-55-6	46.3502	2.5841
24[a]	1,2,6,9-Tetramethylphenanthrene	204256-39-3	48.3509	2.6042
25[b]	Benz[a]anthracene	56-55-3	50.8518	3.5657
26[a,b]	Benz[b]anthracene	56-55-3	50.8518	3.5657
27[a,b]	Chrysene	218-01-9	50.8518	3.5657
28[a,b]	Triphenylene	217-59-4	50.9351	3.746
29[a]	1-Methylchrysene	3351-28-8	53.186	3.9063
30	6-Ethylchrysene	2732-58-3	53.6861	3.8061
31[c]	Benzo[a]pyrene	50-32-8	55.5201	4.5673
32[c]	Benzo[e]pyrene	192-97-2	55.5201	4.5673
33	6-n-Butylchrysene	6901-71-9	56.0203	3.9463
34	1,3,5-Trimethylchrysene	na	56.2704	4.4471
35[a,d]	Benzo[b]fluoranthene	205-99-2	56.6039	0.4607
36[a,d]	Benzo[k]fluoranthene	207-08-9	56.8539	0.6611
37[a,d]	Benzo[j]fluoranthene	205-82-3	57.1874	1.1218
38[d]	Indeno[1,2,3-cd]pyrene	193-39-5	61.1391	1.1819
39[a,d,e]	Dibenz[a,h]anthracene	53-70-3	61.7583	1.211
40[d]	Benzo[ghi]perylene	191-24-2	63.4396	3.7059

continued

na	2,4,7-trimethyldibenzothiophene	na	ni	ni
na	Perylene	198-55-0	ni	ni

[a]Identified with 1-D GC chromatography [b]Tentative assignment, potential co-elution; [c]Tentative assignment, potential co-elution; [d]Wrap-around observed, [e]Mis-identified as dibenz[a,j]anthracene, [na]not applicable – not in NIST library, [ni]not identified

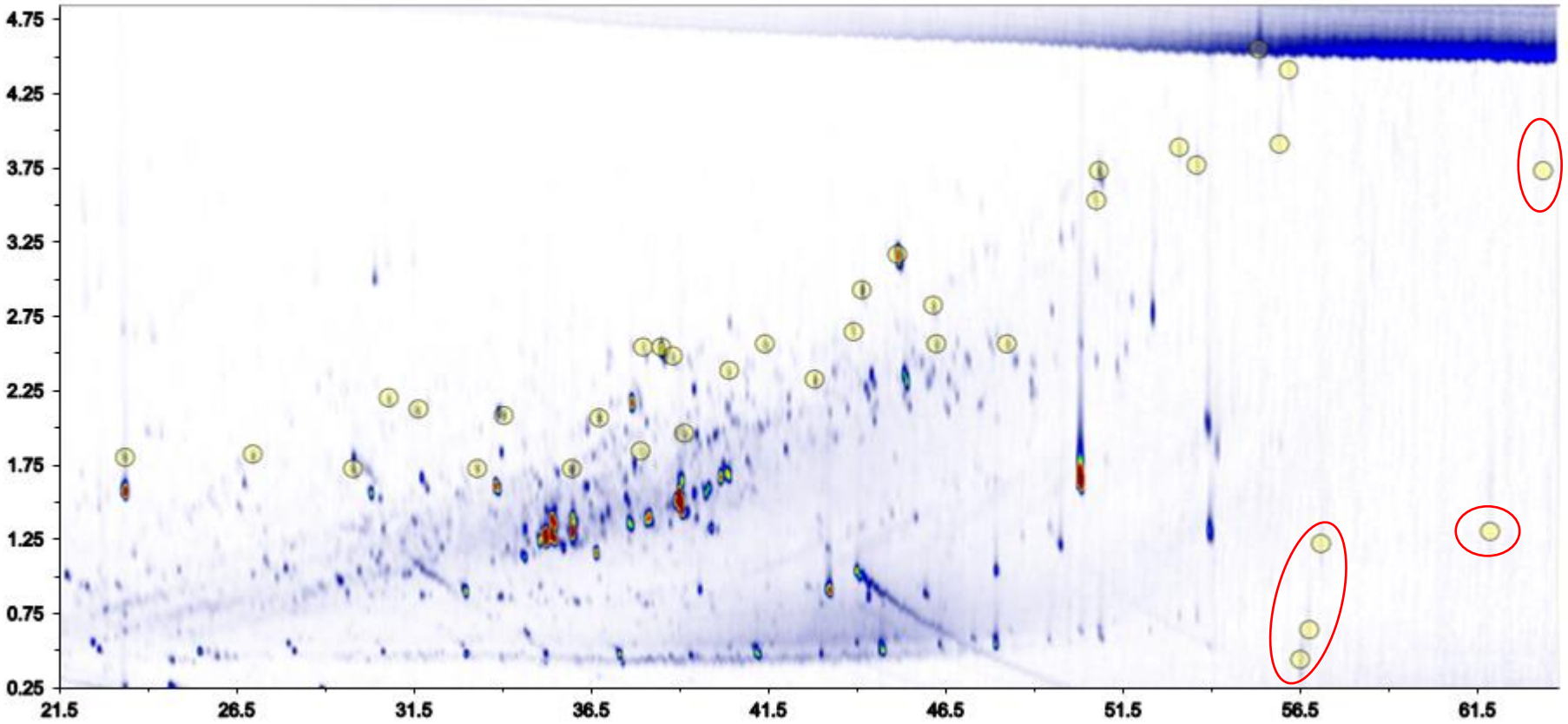
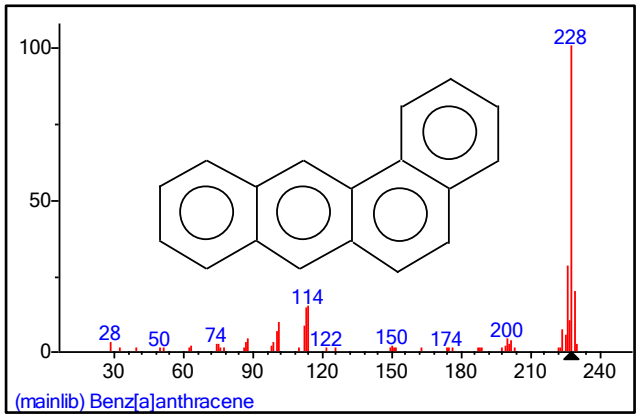
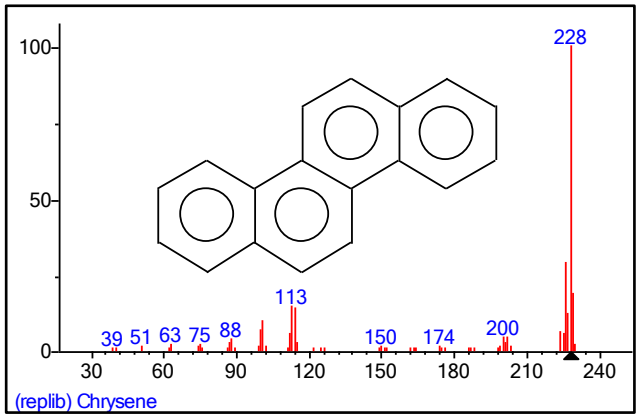


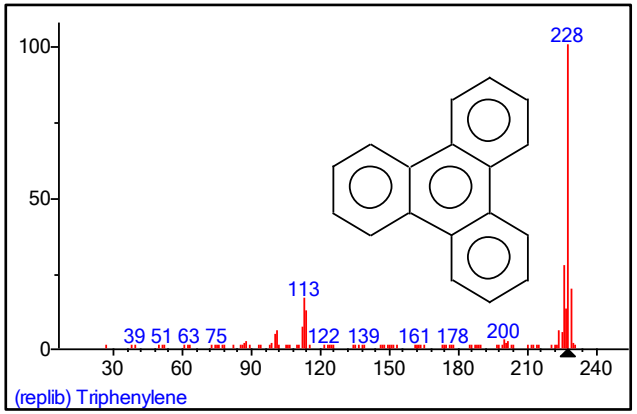
Figure 2.14 GC Image rendered contour plots of the spiked SPMD extract with PAHs circled. Wrapped compounds are highlighted in red.



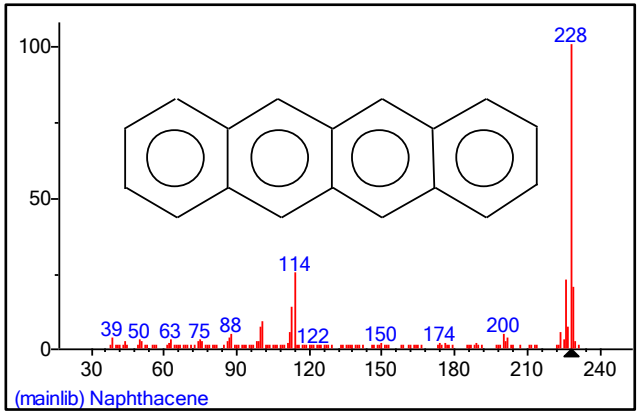
Benz[a]anthracene



Chrysene



Triphenylene



Benz[b]anthracene

Figure 2.15 Spectral comparison of NIST 2011 library spectra of four 4-ring PAHs to show the spectral similarities.

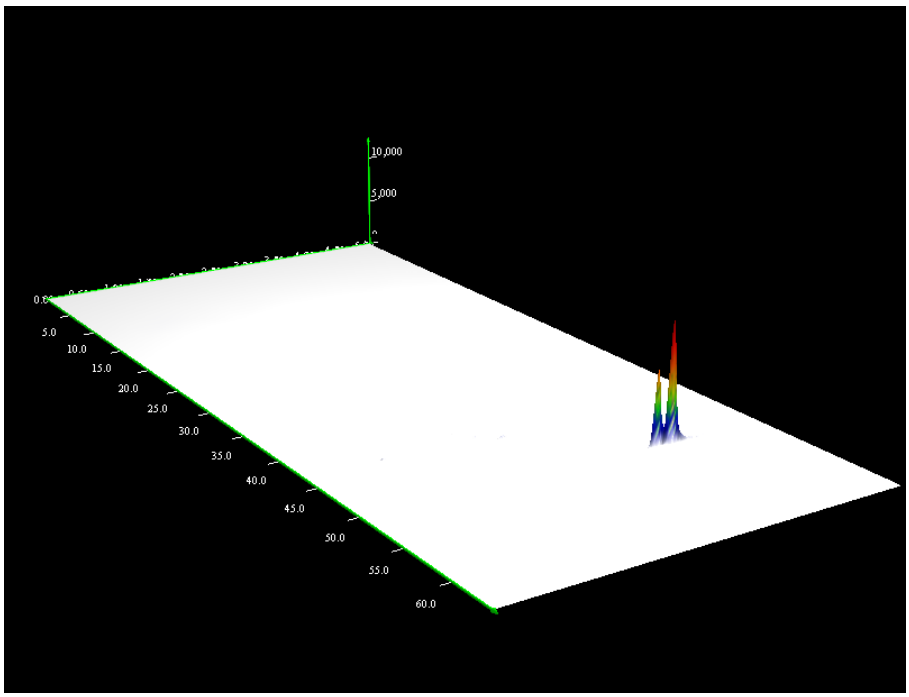


Figure 2.16 GC Image-rendered surface for the extracted ion 228 (±0.5 amu) of the 4-ring PAHs present in the spiked SPMD extract analysis.

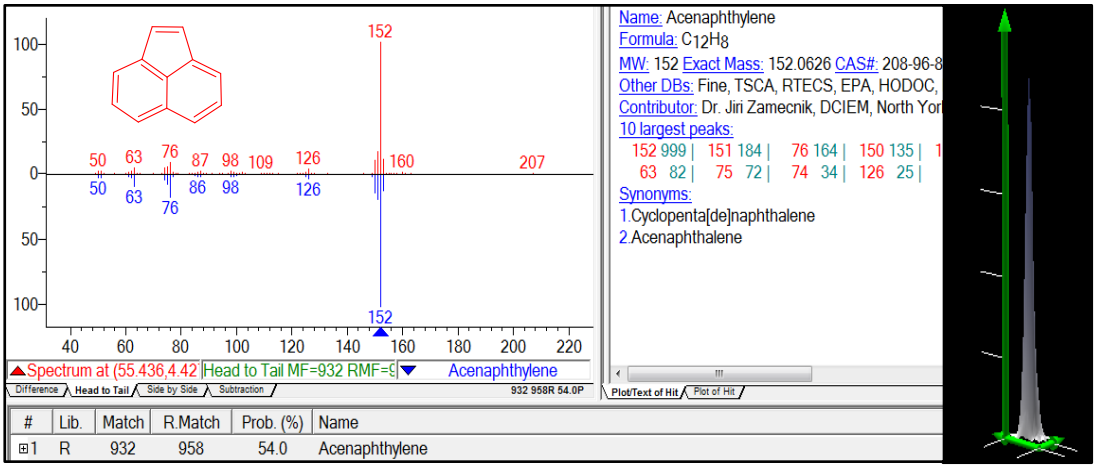


Figure 2.17 NIST 2011 library match and 3-D rendered peak for acenaphthylene taken from the spiked extract sample analysis.

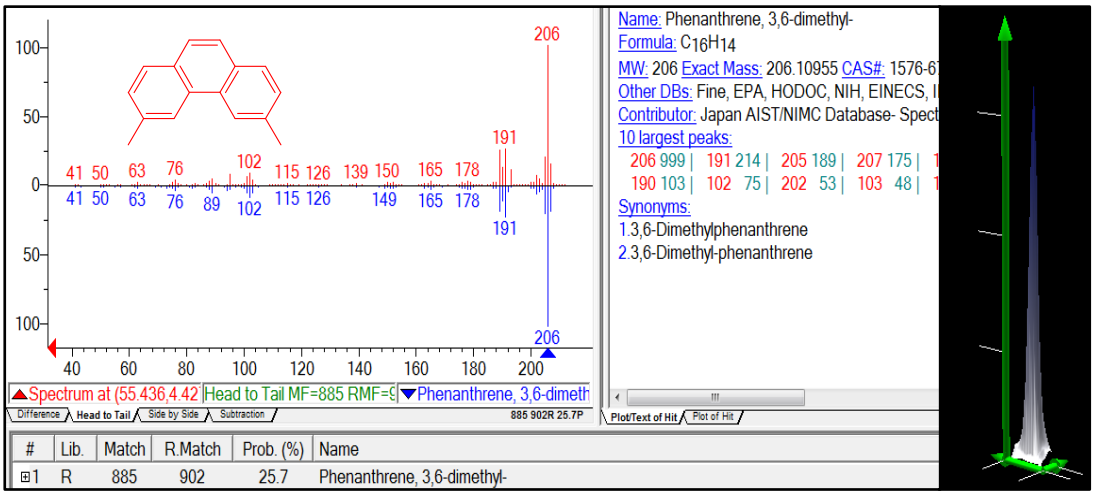


Figure 2.18 NIST 2011 library match and 3-D rendered peak for 3,6-dimethylphenanthrene taken from the spiked extract sample analysis.

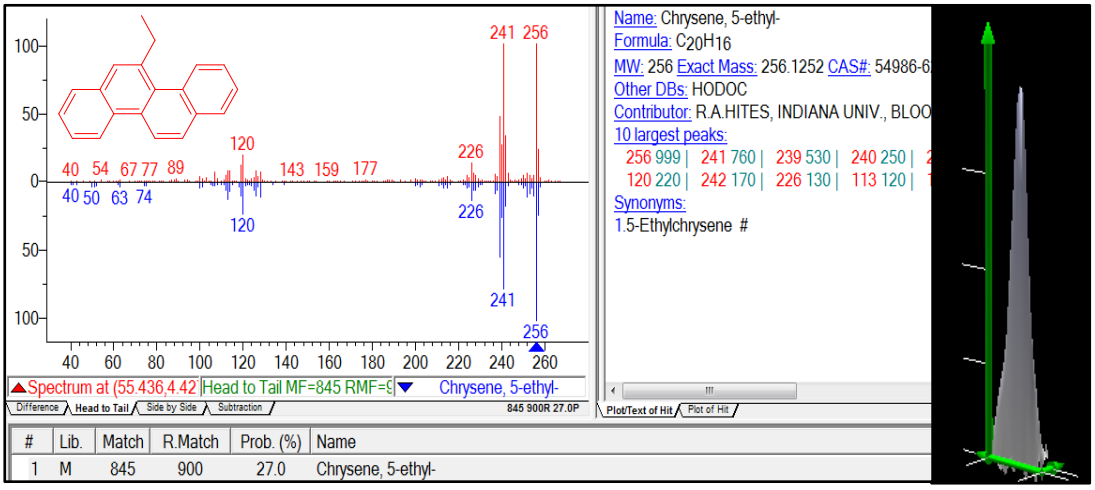


Figure 2.19 NIST 2011 library match and 3D-rendered peak for 6-ethylchrysene (reported as 5-ethyl because the structure is not in the NIST library) taken from the spiked SPMD extract analysis.

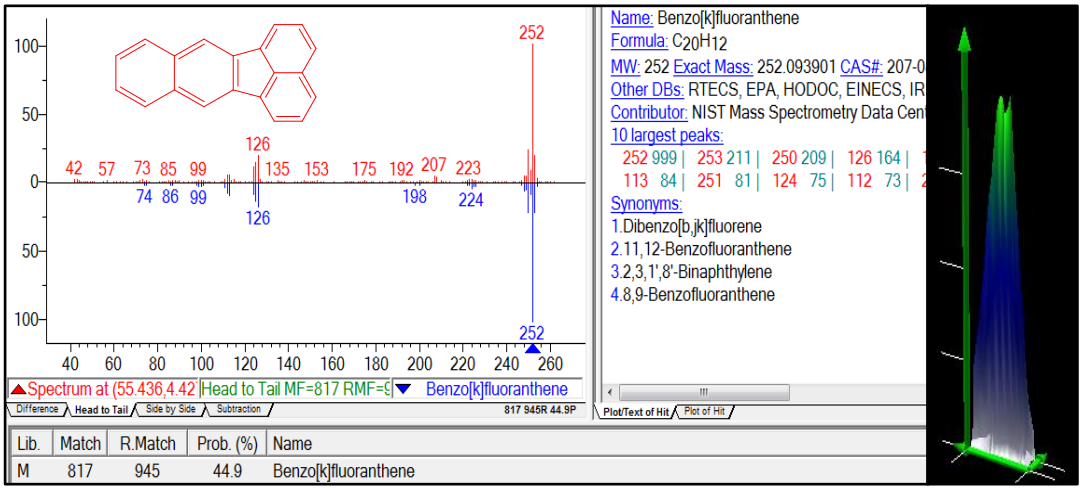


Figure 2.20 NIST 2011 library match and 3D-rendered peak (extracted ion 252) for benzo[k]fluoranthene taken from the spiked extract sample analysis.

2.4.6 PCB analysis

Twenty-two PCBs were identified in the analysis with only19 PCBs present in the standard solution used to spike the SPMD extract. Of the three extra non-targeted PCBs identified, two were tetrachlorinated and one was heptachlorinated. Table 2.5 gives a detailed description of the assignments made, including retention times in both dimensions and the general elution order. The spectra for congeners from the same level of chlorination are very similar; however, it has been shown that by using a combination of molecular ion and ion ratio information it is possible to confidently identify individual congeners. However, specifying individual congeners within a chlorination level is more difficult without prior knowledge of elution orders. For the three non-targeted PCBs the chlorine substitution pattern assignment was based on a linear retention index (320).

Figure 2.21 shows an expanded view of GC Image-rendered contour plots for the spiked extract samples, with the PCBs labelled by level of chlorination. Figure 2.22 shows a 3D-rendered image based on the extracted molecular ions of the PCBs detected with potentially two additional PCBs present highlighted in the inset included in Figure 2.22, however, the separation is not sufficiently optimised to confirm this with certainty. Enhanced chemometric peak deconvolution may be successful in separating co-eluting congeners but use of specialised GC columns, in both the first and second dimensions may be the better option to separate closely co-eluting PCB pairs (200).

Comprehensive GC×GC has provided the resolution necessary to separate the two PCBs that previously co-eluted during the 1D analysis. This co-elution may have been alleviated, in the 1-D analysis, using different oven temperature programming during the separation, but this may in turn have then led to other compounds co-eluting.

Figures 2.23 to 2.26 again show the inherent spectral quality obtained from GCxGC analysis for the PCBs with examples of spectral library matches obtained from NIST, together with an inset of the excellent 3-D peak shape from GC Image.

Table 2.5 GCxGC PCB assignment based on the spiked SPMD sample analysis.

Elution order	PCB	CAS No.	Col. I RT (min)	Col. II RT (s)
1	2,4'-Dichlorobiphenyl	34883-43-7	36.5133	2.0032
2	2,4',5-Trichlorobiphenyl	16606-02-3	38.1806	2.0633
3	2,4,4'-Trichlorobiphenyl	7012-37-5	40.1813	1.9832
4	2,3,3'-Trichlorobiphenyl	38444-84-7	40.5147	2.1635
5	2,2',5,5'-Tetrachlorobiphenyl	35693-99-3	41.4317	2.0032
6	3,3',4-Trichlorobiphenyl	37680-69-6	42.1820	2.1434
7	2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2	44.5995	2.1034
8	3,3',4,4'-Tetrachlorobiphenyl	32598-13-3	46.1834	2.3838
9	2,2',3,4',5',6-Hexachlorobiphenyl	38380-04-0	46.6836	2.3638
10	2,3',4,4',5-Pentachlorobiphenyl	31508-00-6	46.9337	2.2436
11	2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1	47.6006	2.1835
12	2,3,3',4,4'-Pentachlorobiphenyl	32598-14-4	47.9341	2.5841
13	2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2	48.6010	2.5040
14	3,3',4,4',5-Pentachlorobiphenyl	57465-28-8	49.1012	2.4239
15	2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3	49.6013	2.8446
16	2,3,3',4,4',5-Hexachlorobiphenyl	38380-08-4	50.4350	2.5841
17	2,2',3,4,4',5,5'-Heptachlorobiphenyl	35065-29-3	50.8518	2.5040

continued

18	3,3',4,4',5,5'-Hexachlorobiphenyl	32774-16-6	51.6854	2.5641
19	2,2',3,3',4,4',5-Heptachlorobiphenyl	35065-30-6	51.8521	2.8646

Untargeted analytes – Chlorination level assignment based on molecular ion data; substitution pattern assignment based on linear retention index.

i.	2,2',4,6'-Tetrachlorobiphenyl	68194-04-7	42.1820	2.2636
ii.	3,3',5,5'-Tetrachlorobiphenyl	33284-52-5	43.8493	2.1835
iii.	2,2',3,3',5,6,6'-Heptachlorobiphenyl	52663-64-6	49.0178	2.3438

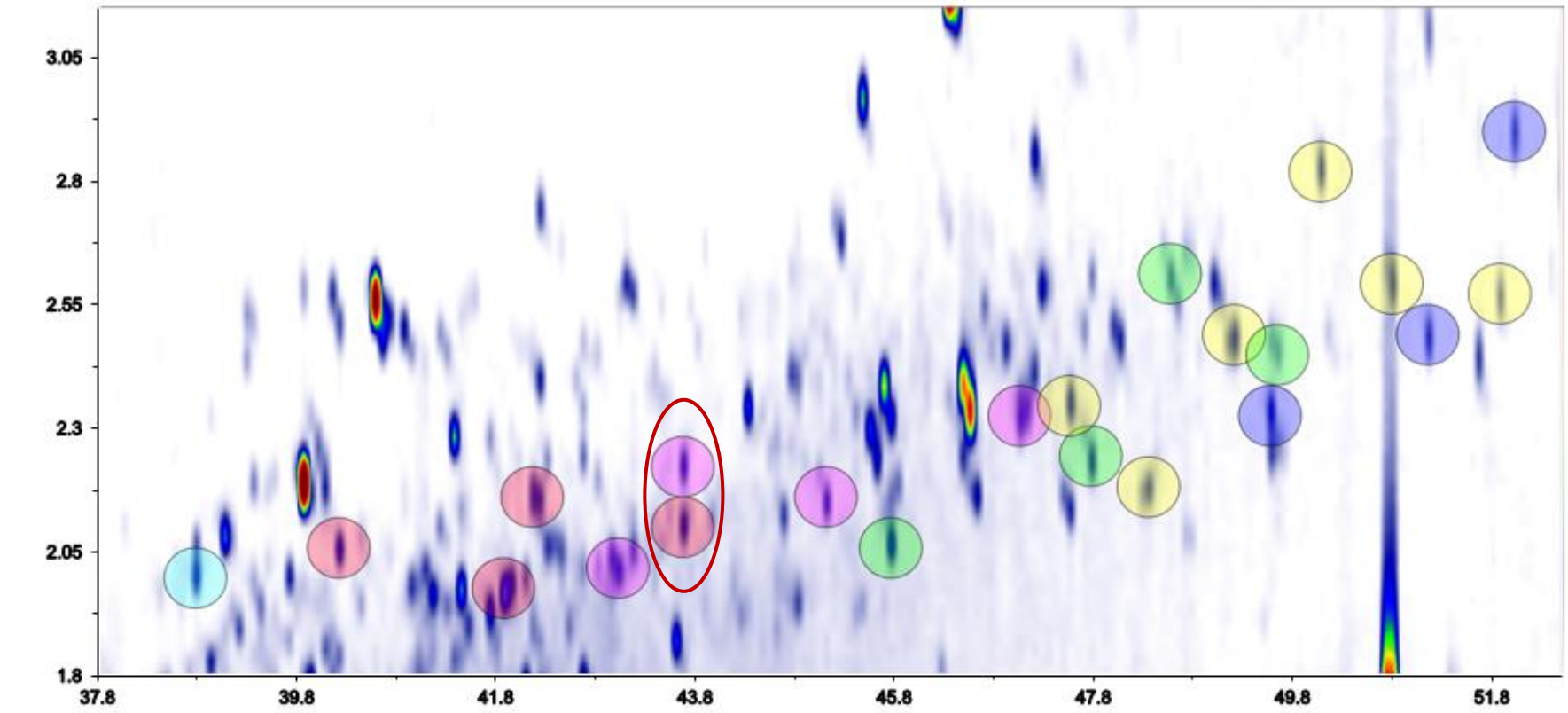


Figure 2.21 GC Image rendered contour plots of the spiked SPMD extract samples with chlorinated biphenyls coloured by chlorination level. The red oval highlights what would be a co-elution in 1-D GC (see Figure 3.4 for 1-D chromatogram).

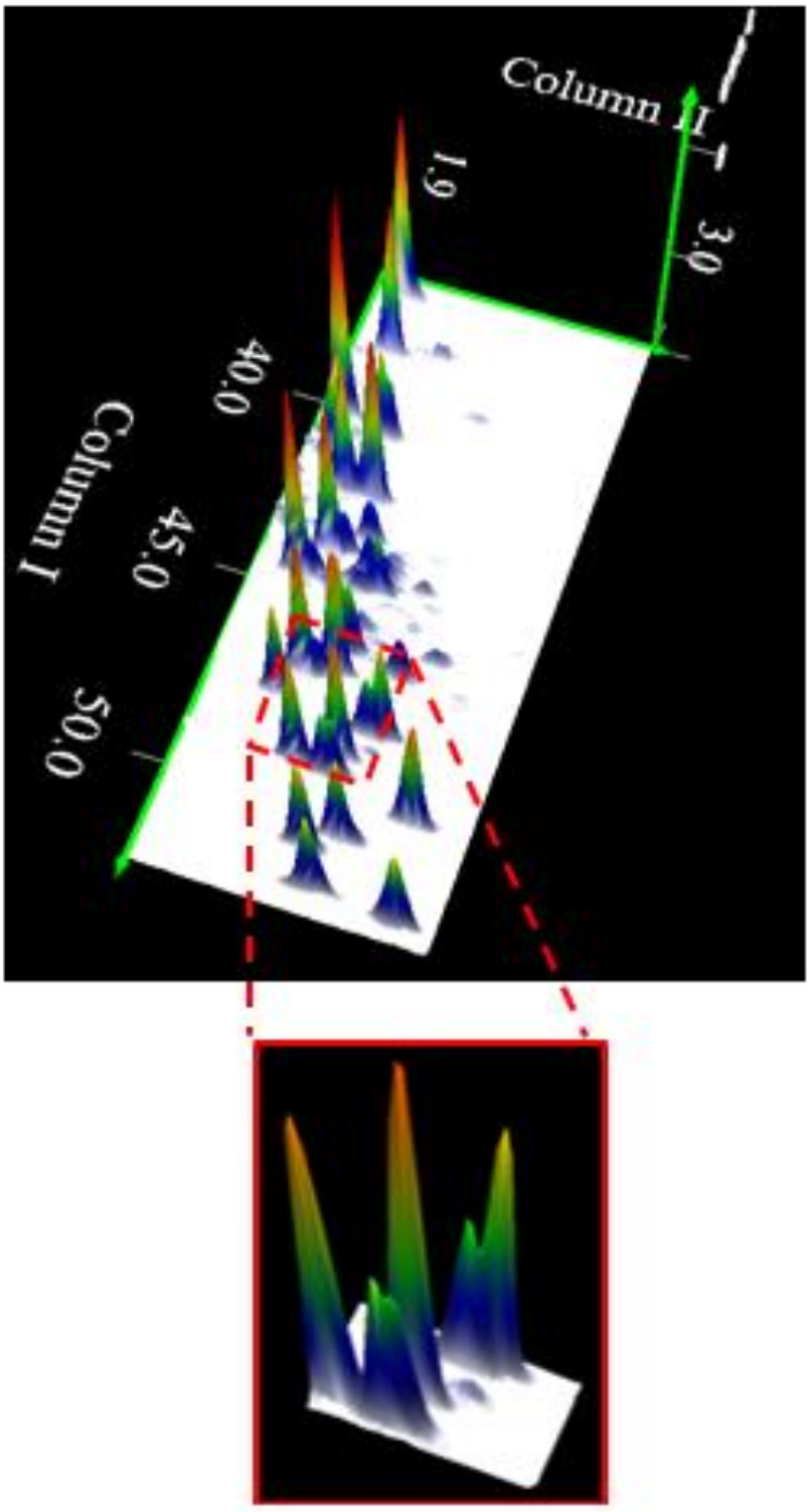


Figure 2.22 GC Image 3-D surface plot for PCB extracted ions 222, 256, 292, 326, 360 and 394 (±1 amu) for the spiked extract. Inset highlight possible presence of two more PCB congeners.

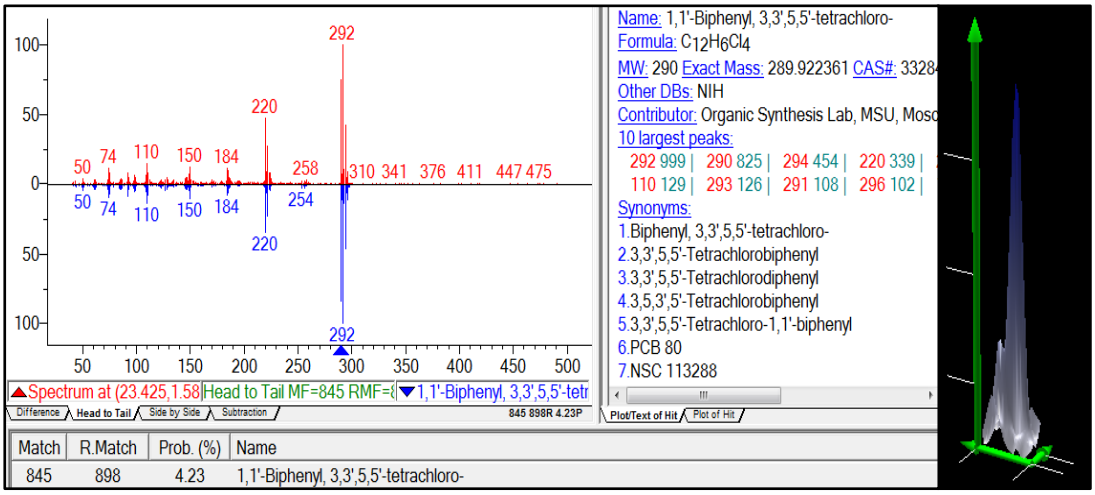


Figure 2.23 NIST 2011 library match and 3-D rendered peak for a tetrachlorinated biphenyl taken from the spiked extract sample analysis.

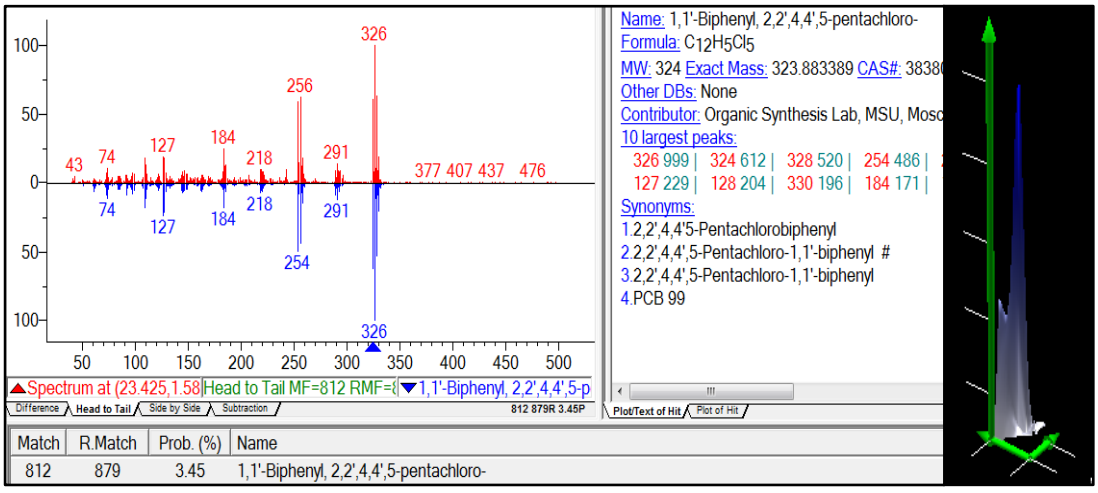


Figure 2.24 NIST 2011 library match and 3-D rendered peak for a pentachlorinated biphenyl taken from the spiked extract sample analysis.

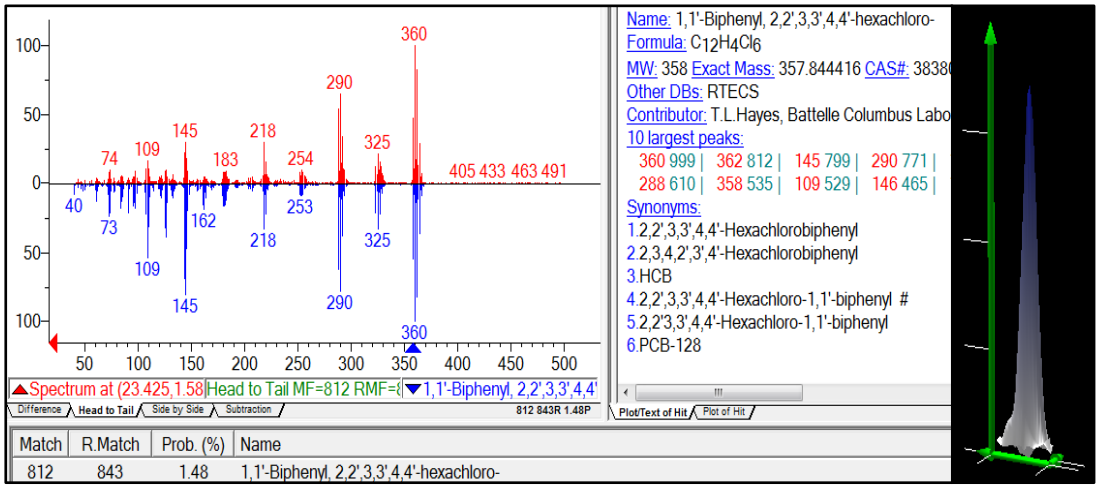


Figure 2.25 NIST 2011 library match and 3-D rendered peak for a hexachlorinated biphenyl taken from the spiked extract sample analysis.

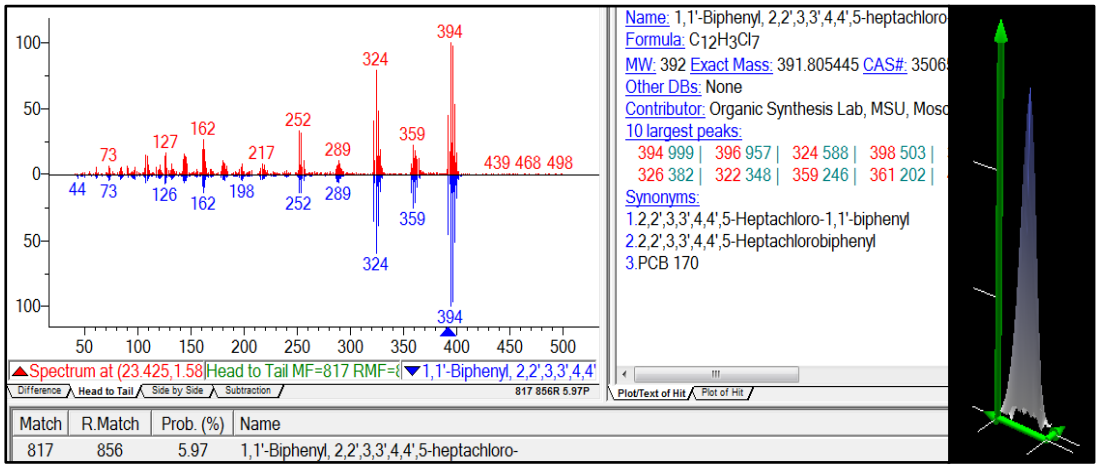


Figure 2.26 NIST 2011 library match and 3-D rendered peak for a heptachlorinated biphenyl taken from the spiked extract sample analysis.

2.4.7 Emerging contaminants

The focus of this work was also to evaluate GCxGC for the identification of compounds of emerging concern, such as those from personal care products and industrial chemicals. Many of the emerging contaminants (not spiked into the extract) were present at a low concentration in the SPMD extract, making identification more complicated. The emerging contaminants referred to below were not spiked into the SPMD extracts.

2.4.7.1 Polycyclic musks, UV sunscreen filters and phosphate fire retardants identified in the SPMD extract

Polycyclic musks are widely used in detergents and personal hygiene products and are considered as emerging aquatic pollutants which are very toxic to aquatic life and are known to induce oxidative and genetic damage in the zebra mussel (321). HHCB (4,6,6,7,8,8-Hexamethyl-1,3,4,6,7,8-hexahydrocyclopenta[g]isochromene) and AHTN (1-(3,5,5,6,8,8-hexamethyl-5,6,7,8-tetrahydro-2-naphthalenyl)ethanone) are polycyclic musks that are commonly found in waste water treatment effluents and the former has several diastereoisomers (see Figure 2.27) and structural analogues at much lower concentration than the intended product, and all have similar mass spectra (322).

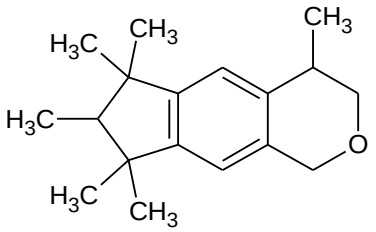
As AHTN has the same molecular formula, a similar mass spectrum, and an almost identical first dimension retention time, separation from HHCB is very difficult using traditional 1-D GC. Even sophisticated mass spectrometry (high resolution or tandem mass spectrometry) would not provide an adequate separation with 1-D GC as all the

compounds concerned are isomers, thus isobaric signals (peaks from compounds with identical mass) would be obtained. However, GCxGC in this work, was used to successfully separate the five diastereoisomers of HHCB plus AHTN as shown in Figure 2.28. Excellent library match scores of 879 and 809 were obtained for HHCB and AHTN respectively as shown in Table 2.6. With one dimensional GC and deconvolution a library match score just above the set threshold of 650 was obtained. Without deconvolution, however, AHTN was not identified and this may have been due to its almost complete co-elution with HHCB in the first dimension. As HHCB produced a larger response than AHTN, and shares the same molecular ion, the mass spectrum without peak deconvolution would predominantly be that of HHCB and would probably explain why only HHCB was identified in the library search.

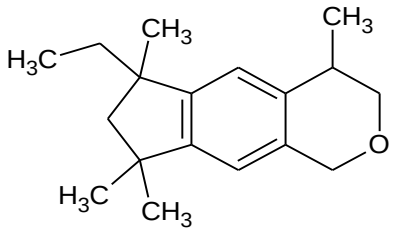
UV sunscreen filters, Octinoxate (2-ethylhexyl (2E)-3-(4-methoxyphenyl) acrylate), which was not identified using one dimensional GC, Homosalate (3,3,5-trimethylcyclohexyl salicylate) and Octocrylene (2-ethylhexyl 2-cyano-3,3-diphenylacrylate) were confidently identified with high (> 870) library match factors when using GCxGC as shown in Table 2.6. However, small differences were observed in the library match factors for both Octocrylene and Homosalate using either GC technique in this work. The organo-phosphate fire retardants, amgard TMCP **[**tris(2-chloro-1-methylethyl) phosphate] and octicizer **[**2-ethylhexyl diphenyl phosphate, both considered to be toxic to aquatic organisms and/or potential carcinogens (323), were also identified in the SPMD extracts as shown in Table 2.6. Despite extensive fragmentation, and the lack of a molecular ion, very little difference was observed in the library match factors for amgard TMCP and octicizer using either GC technique.

Table 2.6 NIST library match factors obtain from different data analysis techniques for emerging contaminants identified in the SPMD extract.

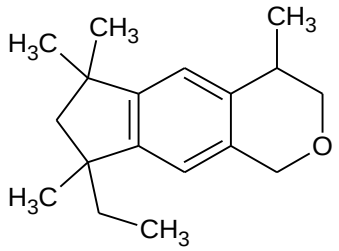
Compound	CAS No.	Class	GCxGC	1-D GC (no deconvolution)	1-D GC (with deconvolution)
HHCB	1222-05-5	Polycyclic musk	879	835	835
AHTN	21145-77-7	Polycyclic musk	809	ni	651
Octinoxate	5466-77-3	UV sunscreen filter	875	ni	ni
Octocrylene	6197-30-4	UV sunscreen filter	880	857	868
Homosalate	118-56-9	UV sunscreen filter	899	893	903
Amgard TMCP	13674-84-5	Organo-phosphate fire retardant	869	817	844
Octicizer	1241-94-7	Organo-phosphate fire retardant	858	857	865



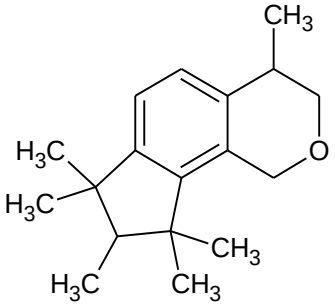
4,6,6,7,8,8-Hexamethyl-1,3,4,6,7,8-hexahydrocyclopenta[g]isochromene; [HHCB]; CAS No. 1222-05-5



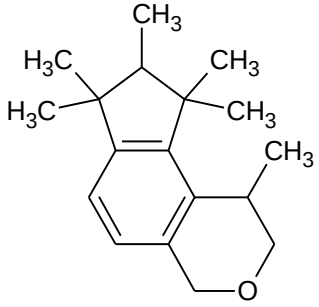
6-Ethyl-4,6,8,8-tetramethyl-1,3,4,6,7,8-hexahydrocyclopenta[g]isochromene; CAS No. 78448-48-3



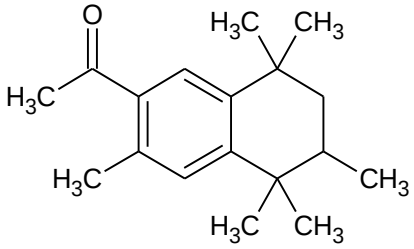
8-Ethyl-4,6,6,8-tetramethyl-1,3,4,6,7,8-hexahydrocyclopenta[g]isochromene; CAS No. 78448-49-4



4,7,7,8,9,9-Hexamethyl-1,3,4,7,8,9-hexahydrocyclopenta[h]isochromene; CAS No. 114109-63-6



1,7,7,8,9,9-Hexamethyl-1,2,4,7,8,9-hexahydrocyclopenta[f]isochromene; CAS No. 114109-62-5



1-(3,5,5,6,8,8-hexamethyl-5,6,7,8-tetrahydro-2-naphthalenyl)ethanone [AHTN] CAS No. 21145-77-7

Figure 2.27 Diastereoisomers of HHCB and AHTN separated using GCxGC.

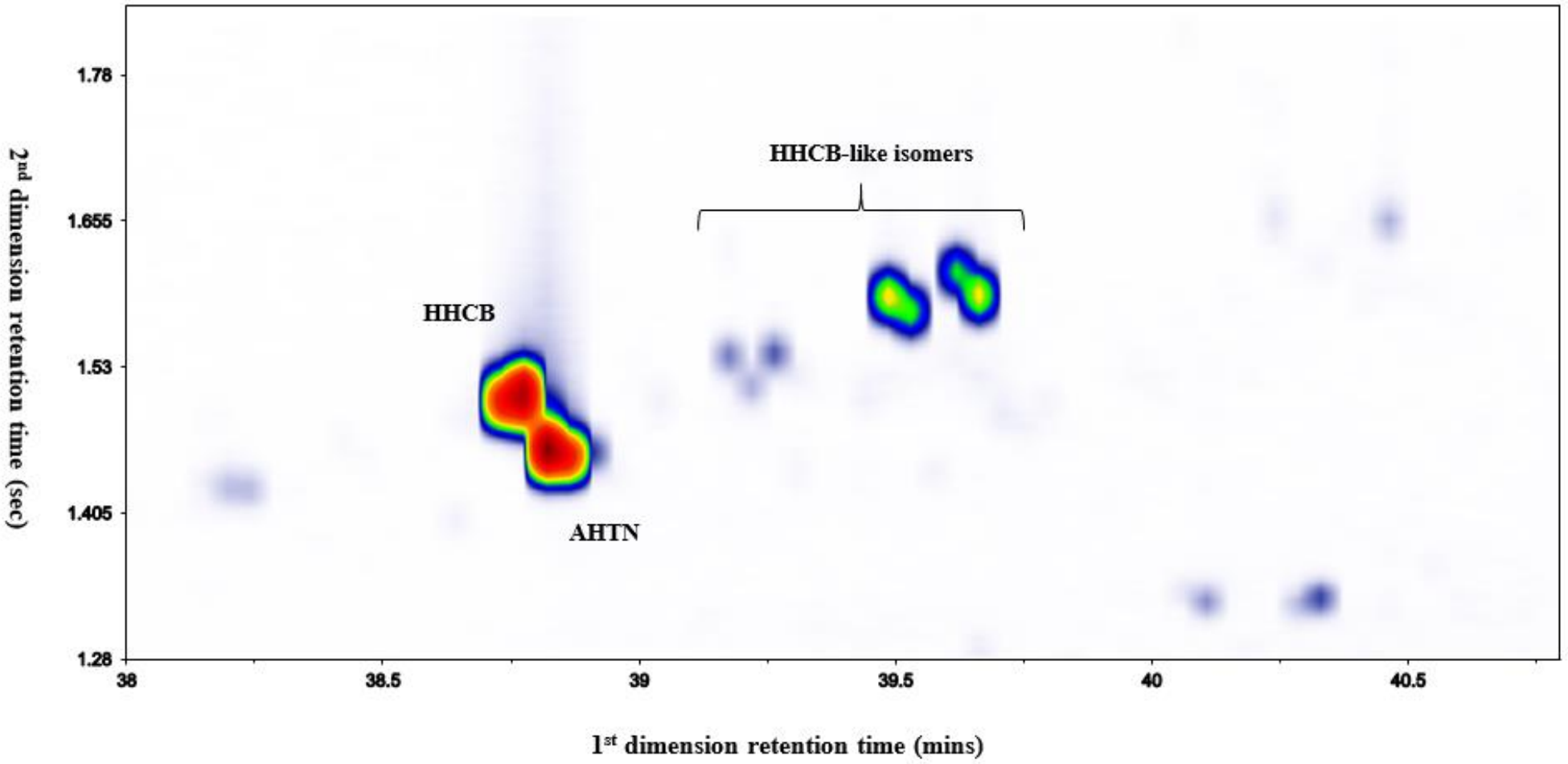


Figure 2.28 Combined two-dimensional extracted ion chromatogram for m/z 213 and 159, showing the identification of the polycyclic musks AHTN and HHCB, and related structural analogues of HHCB in the SPMD extract.

2.4.8 Variable-energy electron ionisation

The use of GCxGC in-conjunction with soft electron ionisation as a complimentary identification and confirmation tool was investigated for the emerging contaminants and the pesticides identified in the SPMD extract. A few changes were made to the GCxGC method outlined in section 2.3.6 in order to prevent 'wrap around' occurring in the second dimension and these included a longer modulation period of 8 s and the following oven temperature ramp: 60 °C (2.0 min), 2.5 °C/min to 320 °C (10 min). The changes resulted in a significantly longer chromatographic run time of 110 min over the original 64 min. Automated deconvolution in-conjunction with library searching was not possible as, to the best of the author's knowledge, no commercial library yet exists for spectra obtain at lower electron ionisation energies. If attempted, low energy spectra searched against the NIST library would return very low match scores as many fragment ions would be missing or their ratios significantly changed under low ionisation energies. Improvements in using lower ionisation energies were therefore demonstrated by calculating the signal-to-noise ratios for the molecular ions and comparing to those obtained using 70eV in the same analytical run. This was possible due to the fast ionisation energy switching capability of the Select-eV ionisation source.

2.4.8.1 Molecular ion intensity and signal-to-noise ratio improvements for the molecular ions of pesticides

Table 2.2 (in section 2.4.1) shows the percentage intensity of the molecular ions for the pesticides relative to the base peak for the mass spectra acquired at 70 and 12 eV. Large increases of around 200 – 400 % in the intensities of the molecular ions were observed

at 12 eV for many pesticides especially where the intensity of the molecular ion was between 2–5 % in the 70 eV spectrum. These compounds included dichlorvos and mevinphos (Figure 2.29), 2,4-TDE and 4,4-DDE (Figure 2.30), with chlorpyrifos-methyl having the largest increase from around 2 to 9 % for the molecular ion in the 12 eV spectrum as can be clearly seen in Figure 2.31. Simplified fragment ions spectra were obtained for all compounds and especially so for aromatic compounds such as hexachlorobenzene and hexachlorobutadiene. More modest improvements were obtained for desethylatrazine, carbophenothion, diazinon, terbutryn and isodrin. Generally, little to no improvements were seen for compounds where the molecular ion was $\leq$ 1 % of the base peak, examples include azinphos ethyl, azinphos methyl, dimethoate, iodofenphos and triallate. Cyanazine even showed a slight drop of 2 % in the molecular ion response compared to the base peak at 12 eV. There was no clear relationship, in terms of class of compound, where the largest increases in molecular ion intensity were observed.

Pesticides which showed the largest increases in molecular ion intensity also showed the largest improvements in the signal-to-noise ratios at an ionisation energy of 12 eV. Mevinphos, an organophosphate pesticide, showed the largest improvement (70-fold), whilst 2,4-TDE and dichlorvos showed improvements of >20 fold. Even 4,4-DDE with a relative abundance of >50% for the molecular ion exhibited a >10-fold improvement in its signal-to-noise ratio as shown in Table 2.8. The dramatic increase in the response of the molecular ion at 12 eV and the noticeable decrease in low mass fragment ions for all the aforementioned compounds, and especially for 4,4'-DDE, are clearly observed in Figure 3.30.

Table 2.7 Signal-to-noise ratios of the molecular ions of pesticides spiked into the SPMD extract at different electron ionisation energies.

Compound (m/z)	Electron ionisation energy	
	70 eV	12 eV
Mevinphos (224)	8	563
2,4 TDE (318)	59	1,392
Methyl chlorpyrifos (321)	62	671
Dichlorvos (220)	157	3,425
4,4 DDE (316)	2,551	26,270

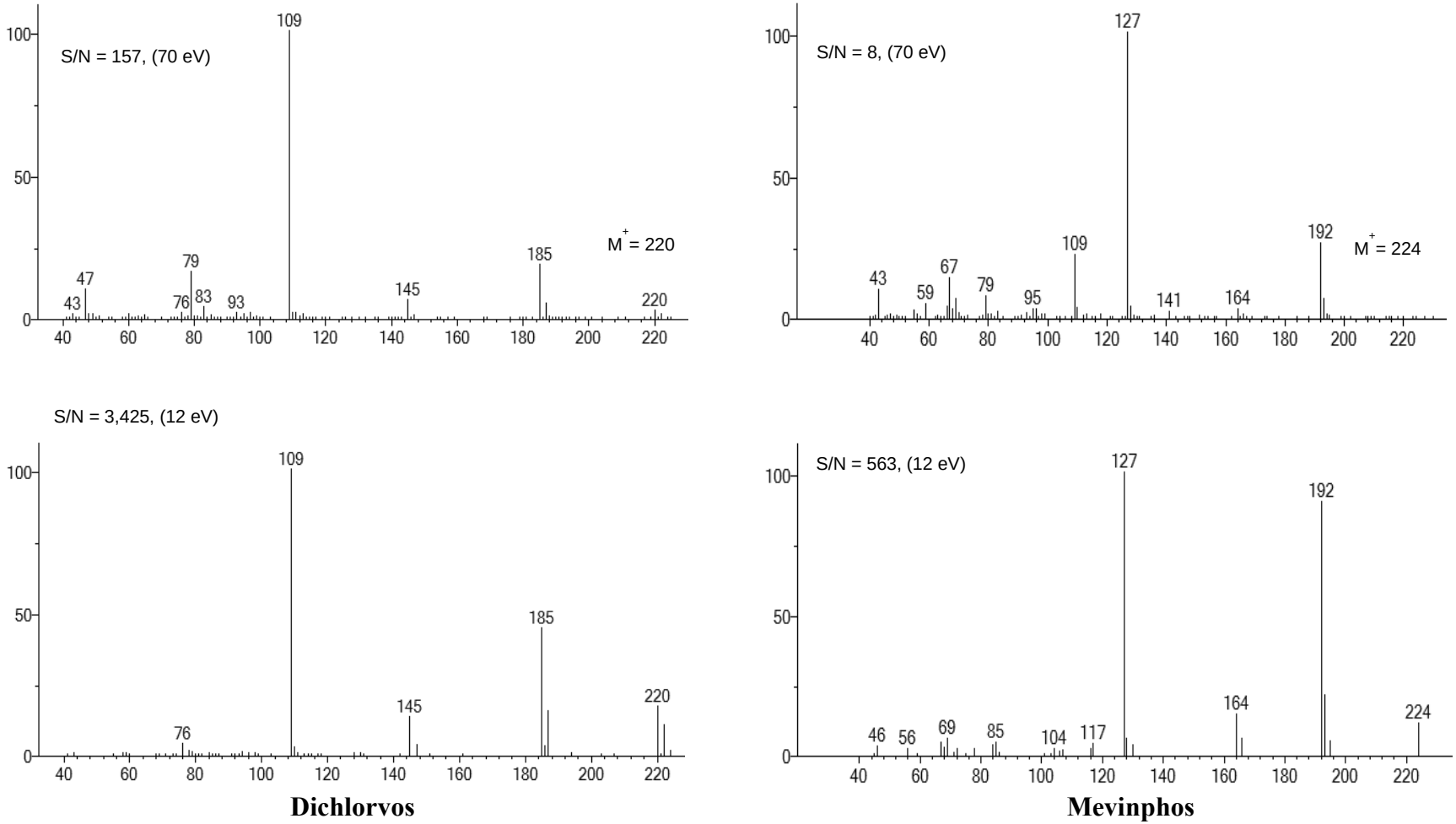


Figure 2.29 Comparison of signal-to-noise ratios (S/N) for the molecular ions of the organo-phosphate pesticides dichlorvos and mevinphos present in the SPMD extract at 70 eV and 12 eV ionisation energies.

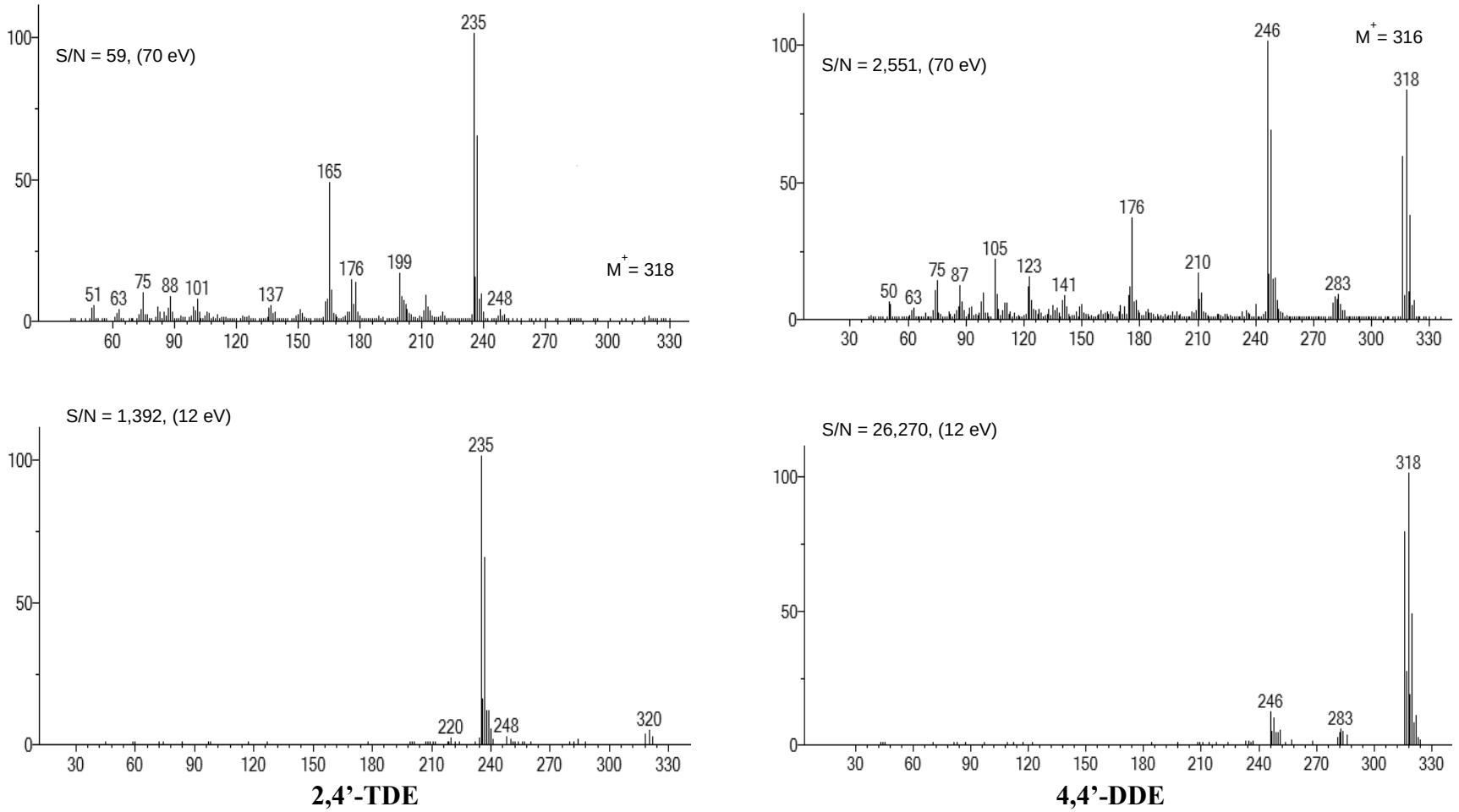


Figure 2.30 Comparison of signal-to-noise ratios (S/N) for the molecular ions of the organo-chlorine pesticides 2,4'-TDE and 4,4'-DDE present in the SPMD extract at 70 eV and 12 eV ionisation energies.

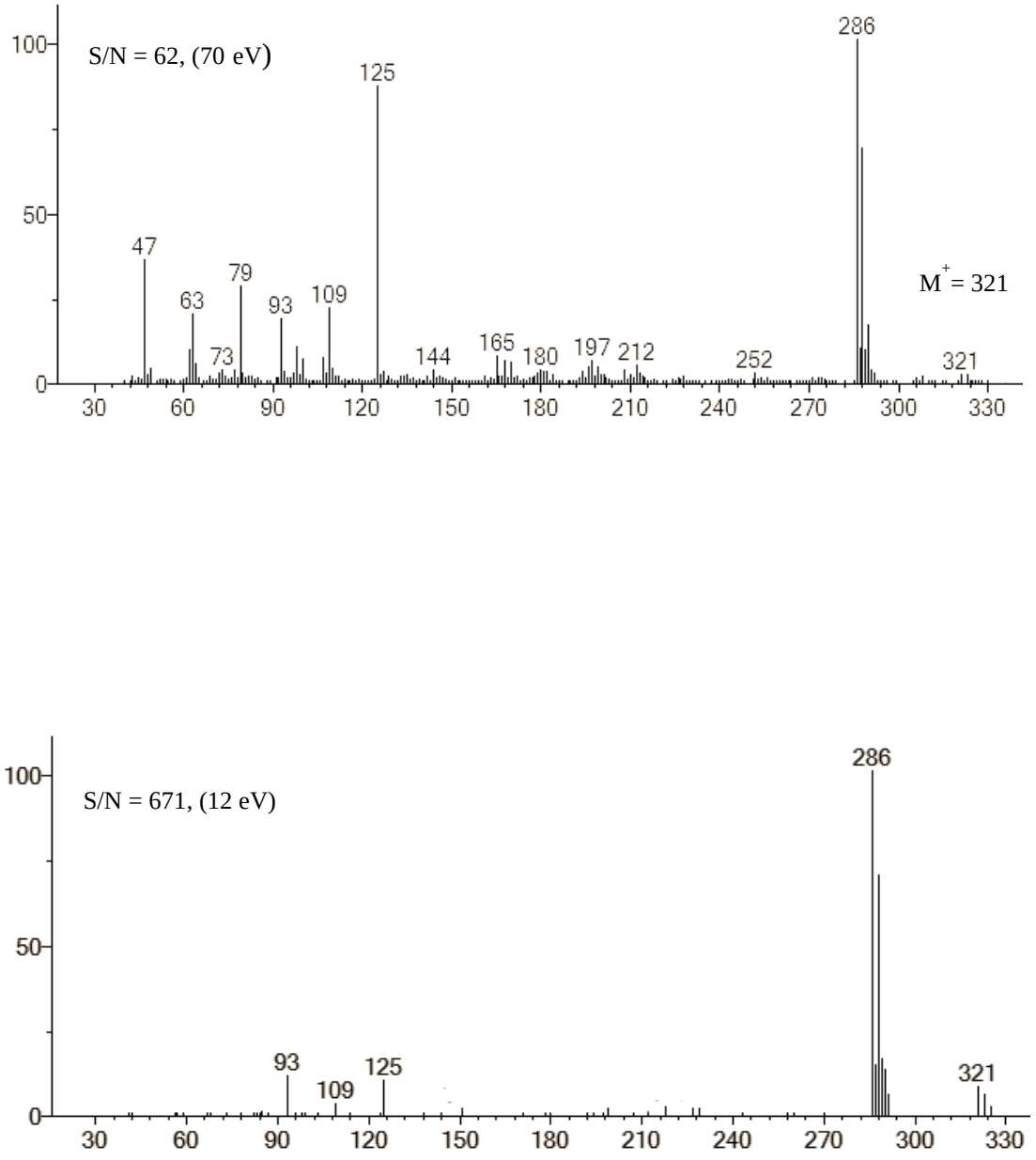


Figure 2.31 Comparison of signal-to-noise ratios (S/N) for the molecular ion of the organo-phosphate chlorpyrifos methyl present in the SPMD extract at 70 eV and 12 eV ionisation energies.

2.4.8.2 Signal-to-noise ratio improvements for the molecular ions of emerging contaminants

The molecular ions for the UV filters Homosalate, Octinoxate and Octocrylene (m/z 262, 290 and 360 respectively) are relatively weak when compared to the base peak in the 70 eV spectrum. By lowering the ionisation energy stepwise from 70 eV to 12 eV the response of the molecular ion for all three compounds (mass spectra shown in Figures 2.32 and 2.33) increased relative to the base peak, but especially so for Octinoxate where a ten-fold improvement in the signal-to-noise ratio was observed. Similar significant increases in the signal-to-noise ratios for Homosalate and Octocrylene were also observed at lower ionisation energies (> 7 and > 4 respectively) as shown in Table 2.7. The molecular ion for HHCB was approximately 20 % of the base peak in the 70 eV spectrum but rose to > 80 % at 12 eV as shown in Figure 2.33 and at 10 eV the molecular ion becomes the base peak.

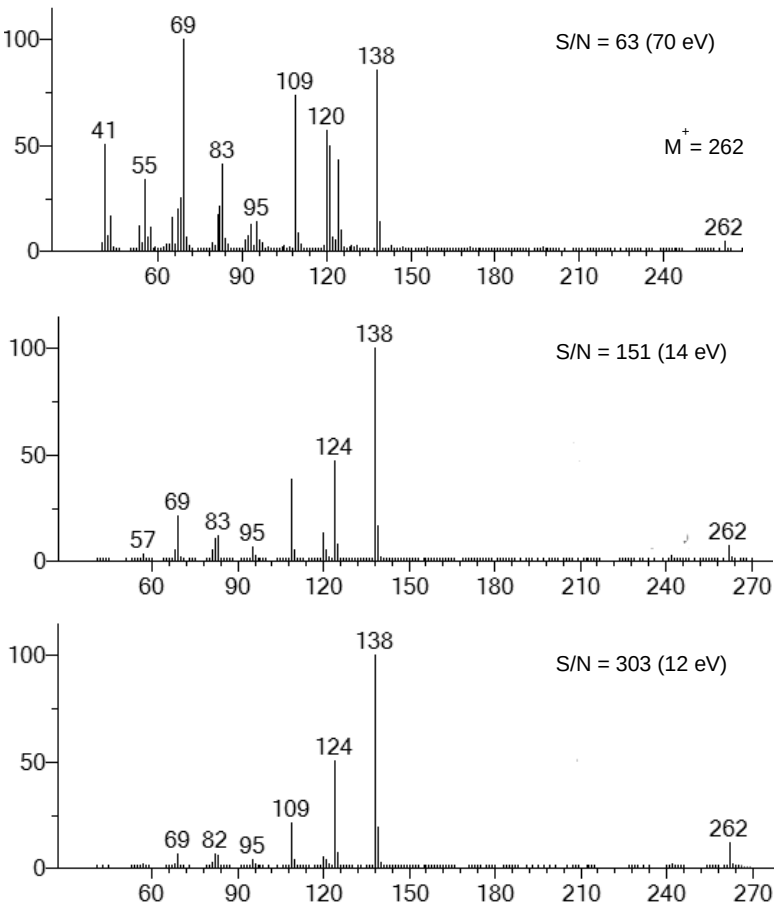
Extensive fragmentation of alkylated phosphates occurs under 70 eV ionisation potential which can compromise their accurate identification. Positive ion chemical ionisation has been used in conjunction with GC-MS but the technique requires a high degree of operator experience and precludes the use of direct mass spectral searching of commercial libraries such as NIST within the same chromatographic run. Amgard TMCP, identified in the SPMD extract, does not produce a molecular ion even at 12 eV but the fragment ion at m/z 291, due to the loss of a single chlorine atom, shows an increase of approximately 90-fold in its signal-to-noise ratio when compared with 70eV. As the mass spectrum at 12 eV was extensively fragmented it was searched against the NIST library and returned a search score of 910. The ion at m/z 291 when

used in-conjunction with the other fragment ions (as shown in Figure 2.34), was therefore highly influential in confirming the compounds identity.

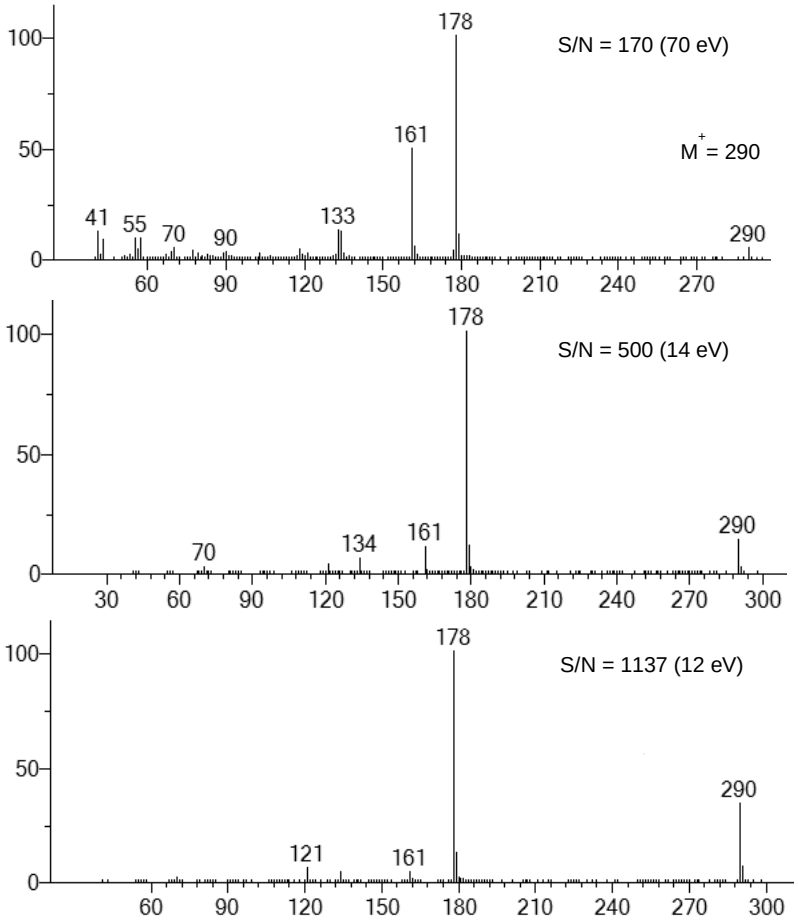
Table 2.8 Signal-to-noise ratios for the molecular ions of the emerging contaminants identified in the SPMD extract at different electron ionisation energies.

Compound (m/z)	Electron ionisation energy		
	70 eV	14 eV	12 eV
Octocrylene (361)	61	252	270
Homosalate (262)	63	151	303
Octinoxate (290)	170	500	1137
Amgard TMCP (291)[a]	46	381	4154
HHCB (258)	1308	5855	6960

[a] = fragment ion

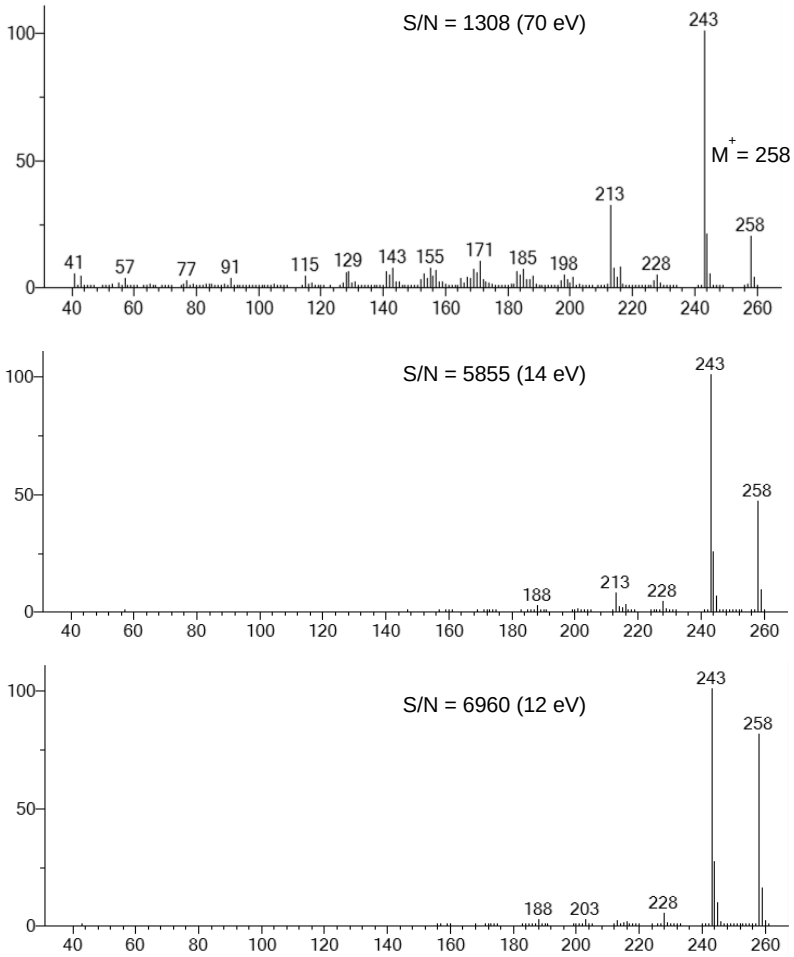


Homosalate (3,3,5-Trimethylcyclohexyl salicylate)

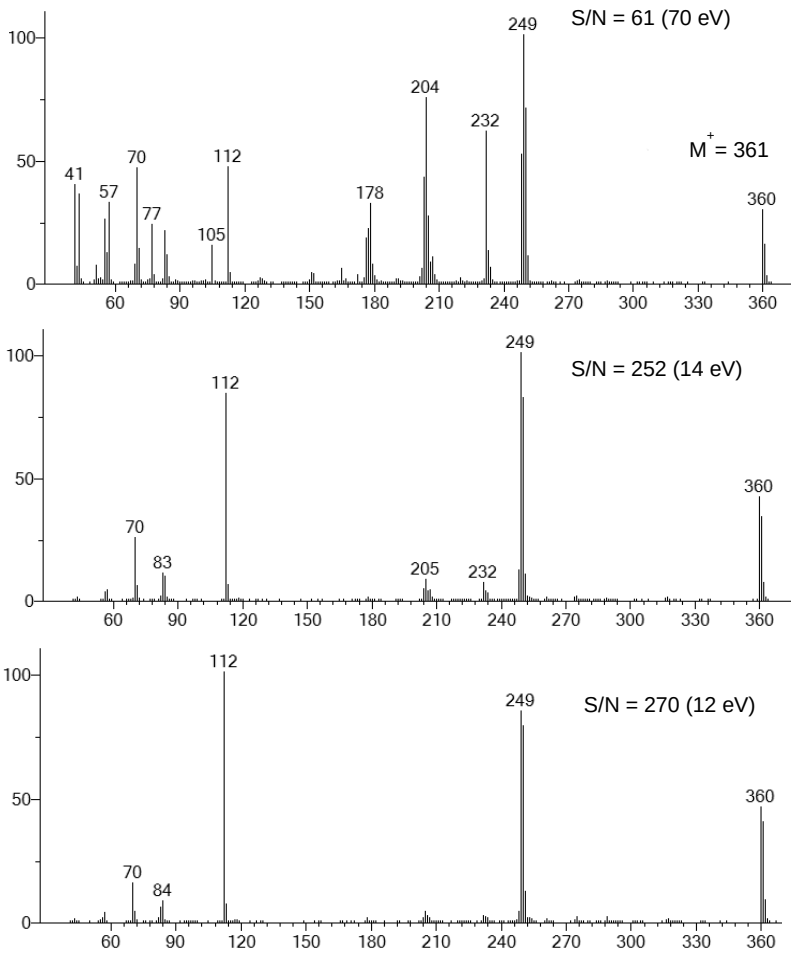


Octinoxate (2-Ethylhexyl (2E)-3-(4-methoxyphenyl)acrylate)

Figure 2.32 Comparisons of the mass spectra obtained for Homosalate and Octinoxate, present in the SPMD extract, measured at three different ionisation energies (12, 14 and 70 eV).



HHCB (4,6,6,7,8,8-Hexamethyl-1,3,4,6,7,8-hexahydrocyclopenta[g]isochromene)



Octocrylene (2-Ethylhexyl 2-cyano-3,3-diphenylacrylate)

Figure 2.33 Comparisons of the mass spectra obtained for HHCB and Octocrylene, present in the SPMD extract, measured at three different ionisation energies (12, 14 and 70 eV).

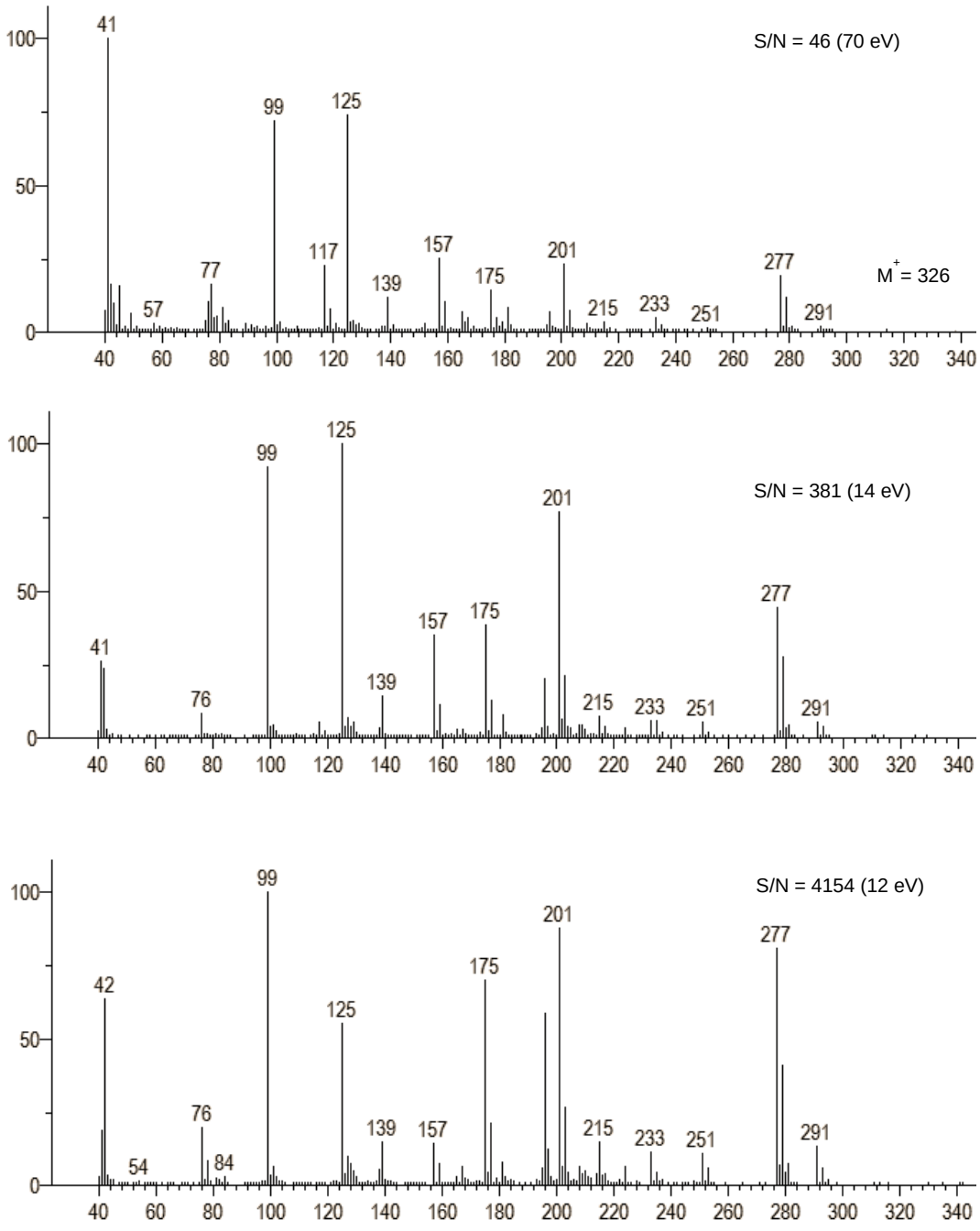


Figure 2.34 Mass spectrum of Amgard TMCP, present in the SPMD extract, at electron ionisation energies of 70, 14 and 12eV.

2.4.8.3 Reduced spectral 'noise'

At lower energies, ionisation of carrier and background gases were eliminated, resulting in much lower spectral 'noise'. In the 12 eV spectrum for the pesticide chlorpyrifos methyl, shown in Figure 2.31, carbon dioxide $CO_2^{+\bullet}$ (m/z 44) and oxygen $O_2^{+\bullet}$ (m/z 32) are entirely absent. The greatly reduced interference from ionised carrier and background gases, along with the reduction in fragmentation of analytes, results in much cleaner spectra at lower energies. The lower incidence of commonly encountered smaller fragment ions, means that closely eluting target compounds can be distinguished far more readily than at 70 eV.

2.5 Conclusions

The work undertaken in this chapter has demonstrated that, when chosen carefully, the GCxGC analytical technique can enhance the inherent advantages of passive sampling of watercourses over more traditional 1-D GCMS. In particular, the higher compound loadings resulting from the use of SPMDs were satisfactorily dealt with by GC×GC, allowing separation of compounds that would otherwise co-elute. The high data rate and sensitivity of TOF MS, in-conjunction with GC×GC, produced high-quality mass spectra enhancing confidence in compound identification.

Of the 56 pesticides added to a SPMD extract, 23 and 48 were identified by 1-D and GCxGC respectively and results suggested that the presence of significant matrix interference could only be dealt with efficiently by GCxGC's higher peak capacity and increased resolution capability. Dieldrin, which was not identified using 1D GC (with or without deconvolution) was readily identified using GCxGC with a high match factor of 842. Other pesticide compound classes including organo-chlorine and

organo-nitrogen pesticides, were confidently detected with atrazine and chlorpyrifos seeing significant improvements in library match factors.

Of 42 PAHs added to the SPMD extract only 24 were identified by 1-D GCMS and of these only 18 were fully resolved. GCxGC, on the other hand, identified 40 PAHs with 37 fully resolved. 22 PCBs were identified using GCxGC, three of which were not identified with 1-D GCMS. Improved resolution and higher library match scores were obtained with GCxGC for the polycyclic musks which have been the focus of recent studies into emerging contaminants. The above results clearly demonstrate the superior separation of the technique by separating target and non-target analytes from interfering compounds with similar mass spectra.

The variable-energy ionisation technology described in this work has been shown to be a reliable technique that provided significant increases in the intensity of the molecular ion and also produced considerably higher signal-to-noise ratios for all classes of pollutants including the emerging contaminants in the SPMD extract at lower ionisation energies. The ability to easily switch between hard and soft electron ionisation within the same chromatographic run allows regular 70 eV spectra to be generated for matching against existing libraries alongside lower-energy spectra, generating complementary information on the molecular ion and structurally significant fragment ions.

The use of GCxGC together with the variable-energy ionisation source can be seen as increasing the 'dimensionality' of the analysis thereby increasing confidence in the identification of pollutants when undertaking investigations into emerging contaminants or target lists monitoring programmes under the auspices of the water framework directive. Furthermore, the reduced fragmentation of analytes, matrix

interferences and carrier gases significantly improves signal-to-noise ratios for target substances, and should allow lower limits of detection to be achieved.

Chapter 3

Development of a novel ‘all ions’ MS/MS screening method for the reliable identification of pharmaceuticals in polar passive sampler extracts

3.1 Introduction

Pharmaceuticals are ubiquitous in surface waters because of continuous discharges from municipal wastewater treatment plants and we still do not know which pharmaceuticals (including those not currently monitored) are reaching the environment, the size of the problem for exposed fauna, nor what the effects, if any, of that exposure may be. Of the 200 different pharmaceuticals that have been reported in river waters globally, the >5000 pharmaceuticals approved for use in Europe represents just a snapshot of the total, i.e. < 4 % of pharmaceuticals have been analysed for or detected in surface waters (324). It demonstrates a clear need for future research to be expanded across the less well studied compounds, particularly those which may pose environmental risks (56, 325, 326).

The purpose of this chapter was to develop and test a new approach to suspect target screening where full scan MS and MS/MS spectra, acquired within the same analytical run, are used in conjunction with an 'in-house' database to identify pharmaceutical residues present in passive samplers deployed in the final effluent of a sewage treatment works.

Targeted LC-MS analytical methods employing triple quadrupole mass spectrometers are being increasingly complemented by high resolution accurate mass (HRAM) systems including time-of-flight (TOF), quadrupole time-of-flight (Q-TOF) and Orbitrap systems. This is mainly due to an increasing interest in the occurrence of contaminants of emerging concern and the need for high confidence in reporting results for regulatory compliance. For most of the LC-MS amenable compounds specified in the European Union's Water Framework and Drinking Water Directives, method detection limits in the low ng/L range can now be achieved with HRAM systems (327). The cost of purchasing such systems has also reduced significantly in recent years making the purchase of such high-performance systems more feasible.

TOF-MS, Q-TOF-MS and Orbitrap instruments satisfy the need for full spectrum acquisition with high sensitivity but only TOF and Q-TOF systems can acquire data at high sampling rates above 3 Hz without sacrificing resolution. High resolution is essential to obtain information on molecular ions, isotope patterns, and fragments with narrow peaks (≤ 1 s) obtained from modern ultra-high performance liquid chromatography (UHPLC) systems.

Two different approaches are currently used for compound identification in environmental screening analysis in combination with high resolution accurate mass LC-MS, these are targeted MS/MS and auto-MS/MS (328). These two approaches have significant disadvantages, namely: targeted MS/MS operation performs MS/MS only on what is included in the target list and never on unknown or unexpected targets and with auto MS/MS desired precursors can be missed where multiple adducts are formed by adduction of an ionic species to a molecule (typically the protonated molecule but they can also originate from alkali metals) are formed in highly complex sample extracts. This chapter looks at alternative methodology based on 'all ions' MS/MS which overcomes the disadvantages of the other two approaches.

3.2 Aims and objectives

i) Identify and obtain the most commonly prescribed pharmaceuticals in Wales for 2014 based on prescription cost analysis data produced by Welsh Government.

ii) Develop an accurate mass and fragment ion database for the pharmaceuticals identified in 3.2 (i) for use with a novel LC Q-TOF-MS method for the identification of pharmaceuticals and personal care product ingredients in the aquatic environment.

iii) Evaluate a prototype 'polar' Chemcatcher® alongside POCIS samplers in the effluent streams of three local waste water treatment plants and identify pharmaceutical residues present in the extracts.

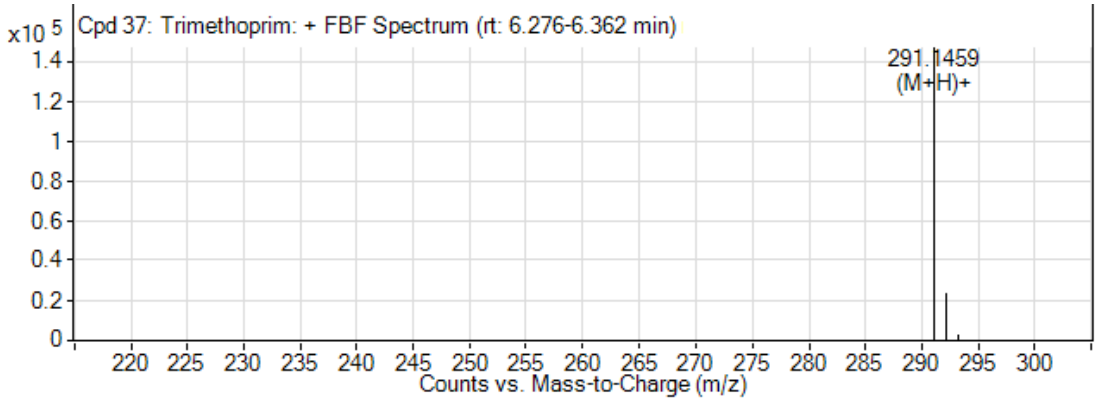
3.3 'All ions' MS/MS

With the 'all ions' MS/MS technique, high resolution accurate mass data is acquired using a minimum of two different collision energies, formed of a low value and one or more higher energy values. Target compounds are fragmented without precursor selection in very fast sequential steps and accurate mass precursor and fragment data are recorded for all collision energies. The result of alternating the collision energy is a data file with a low energy channel that contains predominantly precursor ions and one or several high-energy channels that contain precursor and fragment ions. Only the most stable compounds will have a precursor ion present at the highest collision energy setting. Figure 3.1a shows a spectrum of trimethoprim from a passive sampler extract with a collision energy of 0 eV where the precursor ion at 291.1459 is

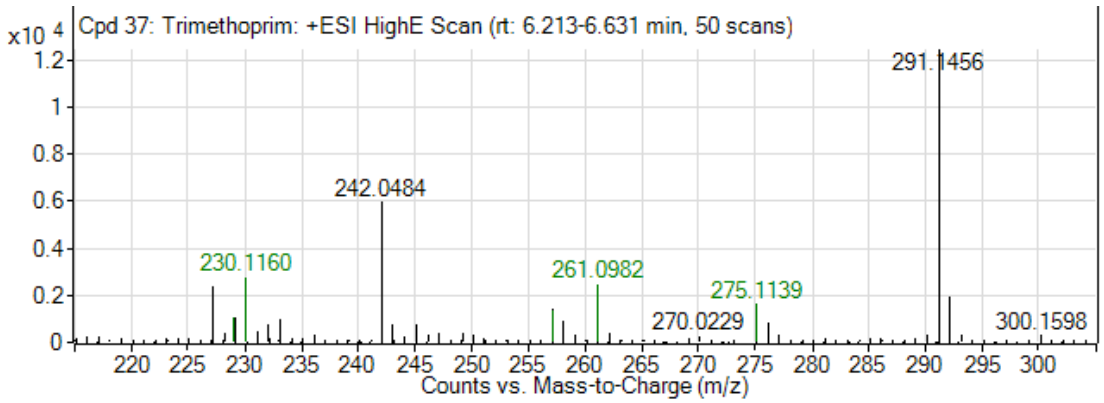
prominently featured. Figure 3.1b shows an averaged spectrum of collision energies at 0, 20 and 40 eV for trimethoprim in the same extract which contains the precursor as well as several fragment ions (displayed in green). Figure 3.1c shows a summed 'cleaned' MS/MS spectrum of the higher collision energies which shows only the known fragment ions obtained for trimethoprim. The known MS/MS fragment ions for trimethoprim at the various collision energies were obtained experimentally from analysing pure standards that were then entered into a searchable pharmaceutical compound database or library.

Analysis of data files acquired using the 'all ions' MS/MS technique involves correlating the elution profile of the precursor ion in the low energy channel (0 eV) with those of the fragments generated under higher energy conditions (20 eV and 40 eV). The fragments from MS/MS spectra in the searchable pharmaceutical compound database were used to form individual extracted ion chromatograms that were overlaid with the precursor extracted ion chromatogram. A co-elution score was derived for each fragment ion from an algorithm that calculates a number based on abundance, peak shape (symmetry), peak width and retention time as shown in Table 3.1. The normalized ratio of the fragment ions to the precursor ion intensity are plotted over the retention time and displayed in a co-elution plot as seen in Figure 3.2 for trimethoprim that is from the same passive sampling extract as that shown in Figure 3.1.

a



b



c

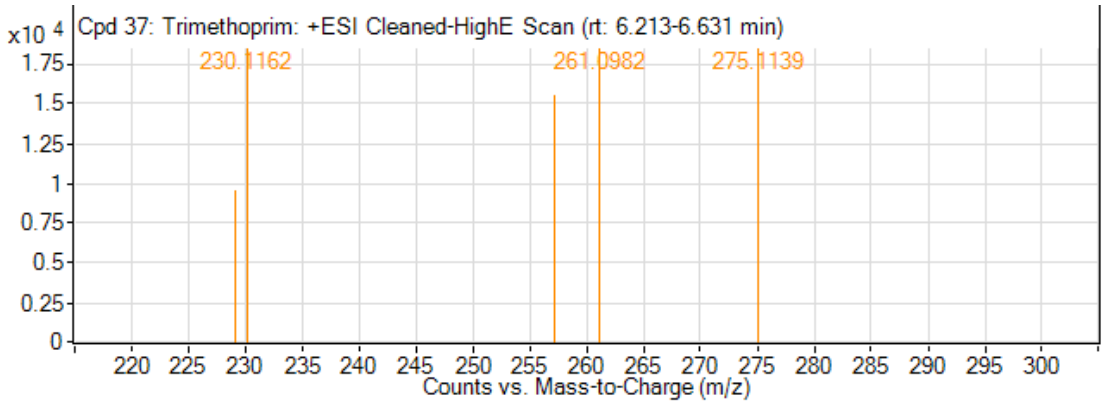


Figure 3.1 Mass spectra for trimethoprim at 0 eV (a), averaged mass spectrum from collision energies of 0, 20 and 40e V (b) and summed 'cleaned' MS/MS spectrum of the higher collision energies (c) for the Chemcatcher® sampler at the Carmarthen site.

Table 3.1 Co-elution scores for the individual trimethoprim fragment ions, together with scores for the precursor mass accuracy, mass and retention time differences.

Name	Formula	m/z	Mass	Mass (Tgt)	Diff (ppm)	Score (Tgt)	RT	RT (Tgt)	RT Diff	Score (RT)	Species
Trimethoprim	$C_{14}H_{18}N_4O_3$	291.1459	290.1385	290.1379	-2.27	98.68	6.31	6.306	0.004	99.87	$(M+H)^+$

Coelution Score	CE	Flags(FIs)	Height	m/z	Name	RT	RT Diff	SNR
99.4	20	Qualified	29948.4	230.1162	Trimethoprim	6.319	0.009	48.9
99.8	40	Qualified	27805.2	261.0982	Trimethoprim	6.302	0.009	86.3
98.1	40	Qualified	9595.2	229.1084	Trimethoprim	6.302	0.009	17.4
99.1	40	Qualified	15527.5	257.1033	Trimethoprim	6.302	0.009	37.3
99.4	20	Qualified	18458	275.1139	Trimethoprim	6.319	0.009	27.2

Score (iso. abund)	Score (mass)	Score (MS)	Score (iso. spacing)	Height	Species	m/z
98.97	96.76	98.11	99.78	147135.8	$(M+H)^+$	291.1459

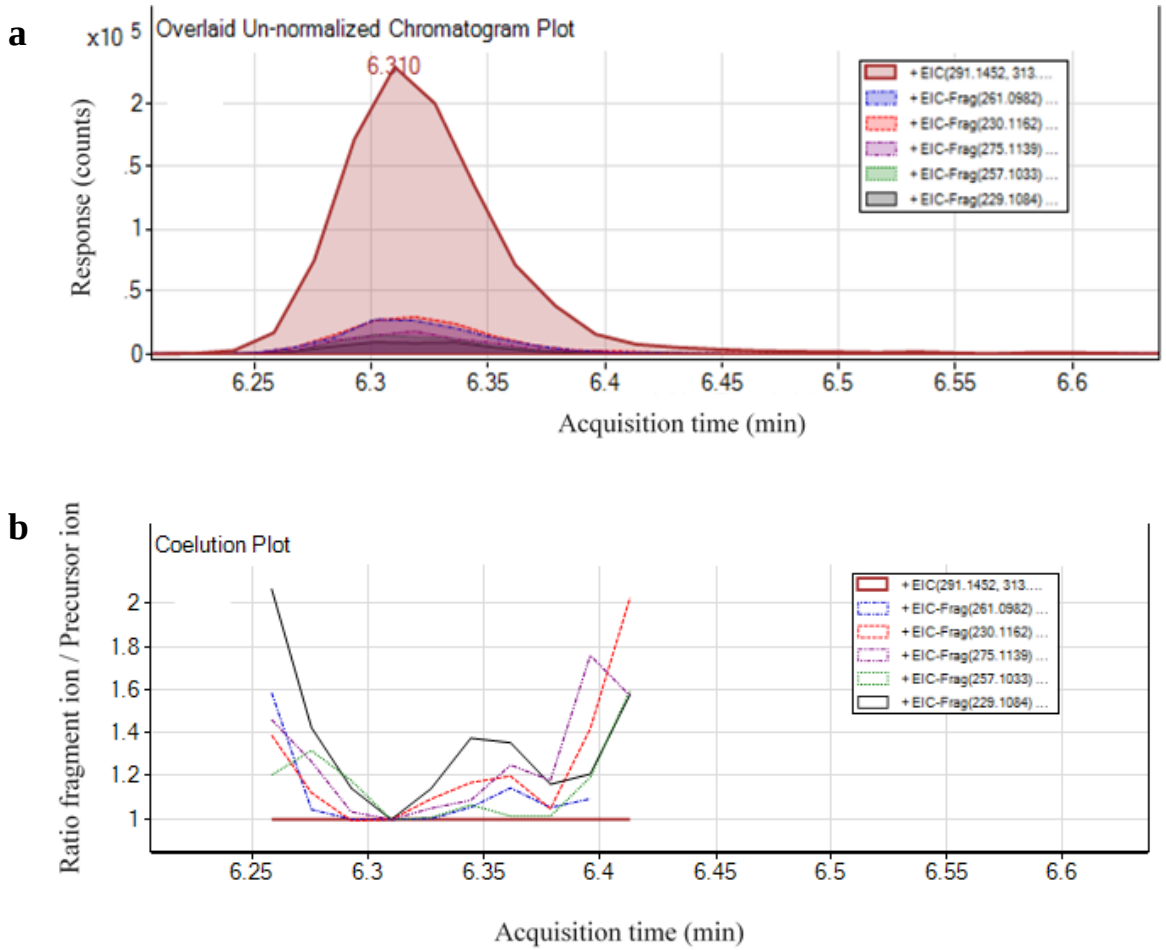


Figure 3.2 Overlaid un-normalised extracted ion chromatograms for the fragment ions of trimethoprim (a) and normalized ratios of the fragment ions to the precursor ion intensity (m/z 291.1452) plotted versus retention time and displayed in a co-elution plot (b).

3.3.1 Find by formula algorithm

The find by formula algorithm in Mass Hunter qualitative analysis software is used in conjunction with the 'all ions' MS/MS technique. The algorithm starts with the formula of a compound and calculates the monoisotopic mass and isotope pattern. If the compound is suspected to be present in the data file, the find by formula algorithm verifies that the mass accuracy, isotope patterns and isotope spacing are consistent with the database formula. It then constructs extracted ion chromatograms based on the most abundant isotopes for each selected charge carrier (or adduct) including those for multimers, integrates and groups them. Scoring criteria for mass accuracy, isotope patterns and isotope spacing can be manually set providing a cut-off point where the analyst can be satisfied that the compound is indeed present.

3.3.2 Formation of the database

To curate the database that would be used with the LC Q-TOF-MS system, a search was undertaken to identify the most prescribed pharmaceuticals in Wales in 2014 that may be present in final effluents and watercourses receiving discharges from sewage works. Information on prescription items dispensed in 2014, which were prescribed by general medical practitioners (GPs) in Wales, was obtained from the Welsh government website http://gov.wales/statistics-and-research/prescriptions-dispensed-community/. This information is produced by Welsh government to provide statistical information for current prescribing policies in Wales.

For reasons of practicality it was decided to include only individual pharmaceuticals (by chemical name) in the database where the total number of prescriptions for each exceeded 25,000 that year; these appear in Table D1 (Appendix D). Prescribed items

such as prosthetic medical devices, dressings (including those that contained antimicrobials or other active drugs) were excluded. The list was further refined by excluding compounds that were not amenable to reversed phase liquid chromatography conditions typically used in LC-MS methods and those exhibiting very poor solubility in water or organic solvents. These compounds included sugars, metal-based compounds, vitamins and other dietary supplements, oils, oxidisers, solvents, insulin and common inorganic salts. Pharmaceuticals previously analysed at the authors laboratory (for the UKWIR Chemical Investigation Programme) were also included giving a total of 164 compounds; the full list of pharmaceuticals for which reference materials were purchased appear in Table 3.2.

Table 3.2 Full list of pharmaceuticals for which reference materials were purchased for Q-TOF-MS analysis.

Pharmaceutical class	Compound determined	CAS RN	Chemspider ID	Systematic (IUPAC) name for compound determined - obtained from PubChem
Analgesic	Sumatriptan	103628-46-2	5165	1-[3-[2-(dimethylamino)ethyl]-1H-indol-5-yl]-N-methylmethanesulfonamide
	Tramadol	27203-92-5	31105	(1R,2R)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexan-1-ol
Antacid	Ranitidine	66357-35-5	571454	(E)-1-N'-[2-[[5-[(dimethylamino)methyl]furan-2-yl]methylsulfanyl]ethyl]-1-N-methyl-2-nitroethene-1,1-diamine
Anti-arrhythmic	Amiodarone	1951-25-3	2072	(2-butyl-1-benzofuran-3-yl)-[4-[2-(diethylamino)ethoxy]-3,5-diiodophenyl]methanone
	Flecainide	54143-55-4	3239	N-(piperidin-2-ylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)benzamide
Anti-asthmatic	Cromolyn	16110-51-3	2779	5-[3-(2-carboxy-4-oxochromen-5-yl)oxy-2-hydroxypropoxy]-4-oxochromene-2-carboxylic acid
	Ipratropium (as the cation)	60205-81-4	3615	[(1S,5R)-8-methyl-8-propan-2-yl-8-azoniabicyclo[3.2.1]octan-3-yl] 3-hydroxy-2-phenylpropanoate
	Salbutamol	18559-94-9	1999	4-[2-(tert-butylamino)-1-hydroxyethyl]-2-(hydroxymethyl)phenol
	Salmeterol	89365-50-4	4968	2-(hydroxymethyl)-4-[1-hydroxy-2-[6-(4-phenylbutoxy)hexylamino]ethyl]phenol
Anti-biotic	Amoxicillin	26787-78-0	31006	(2S,5R,6R)-6-[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
	Cefalexin	15686-71-2	25541	(6R,7R)-7-[[(2R)-2-amino-2-phenylacetyl]amino]-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid
	Chloramphenicol	56-75-7	5744	2,2-dichloro-N-[(1R,2R)-1,3-dihydroxy-1-(4-nitrophenyl)propan-2-yl]acetamide
	Chlortetracycline	57-62-5	10469370	(4S,4aS,5aS,6S,12aR)-7-chloro-4-(dimethylamino)-1,6,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4,4a,5,5a-tetrahydrotetracene-2-carboxamide
	Clarithromycin	81103-11-9	4447591	(3R,4S,5S,6R,7R,9R,11R,12R,13S,14R)-6-[(2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-14-ethyl-12,13-dihydroxy-4-[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-7-methoxy-3,5,7,9,11,13-hexamethyl-oxacyclotetradecane-2,10-dione
	Doxycycline	564-25-0	10469369	(4S,4aR,5S,5aR,6R,12aR)-4-(dimethylamino)-1,5,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4a,5,5a,6-tetrahydro-4H-tetracene-2-carboxamide
	Erythromycin	114-07-8	12041	(3R,4S,5S,6R,7R,9R,11R,12R,13S,14R)-6-[(2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-14-ethyl-7,12,13-trihydroxy-4-[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-3,5,7,9,11,13-hexamethyl-oxacyclotetradecane-2,10-dione
	Nitrofurantoin	67-20-9	5036498	1-[(E)-(5-nitrofuran-2-yl)methylideneamino]imidazolidine-2,4-dione
	Nystatin	34786-70-4	10468627	(1S,3R,4R,7R,9R,11R,15S,16R,17R,18S,19E,21E,25E,27E,29E,31E,33R,35S,36R,37S)-33-[(3-Amino-3,6-dideoxy-beta-D-mannopyranosyl)oxy]-1,3,4,7,9,11,17,37-octahydroxy-15,16,18-trimethyl-13-oxo-14,39-dioxabic yclo[33.3.1]nonatriaconta-19,21,25,27,29,31-hexaene-36-carboxylic acid
	Ofloxacin	82419-36-1	4422	9-Fluoro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid
	Oxytetracycline	79-57-2	10482174	(4S,4aR,5S,5aR,6S,12aR)-4-(dimethylamino)-1,5,6,10,11,12a-hexahydroxy-6-methyl-3,12-dioxo-4,4a,5,5a-tetrahydrotetracene-2-carboxamide
	Penicillin V	87-08-1	6607	(2S,5R,6R)-3,3-dimethyl-7-oxo-6-[(2-phenoxyacetyl)amino]-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid
	Trimethoprim	738-70-5	5376	5-[(3,4,5-trimethoxyphenyl)methyl]pyrimidine-2,4-diamine

continued

Anti-coagulant	Dipyridamole	58-32-2	2997	2-[[2-[bis(2-hydroxyethyl)amino]-4,8-di(piperidin-1-yl)pyrimido[5,4-d]pyrimidin-6-yl]-(2-hydroxyethyl)amino]ethanol
	Warfarin	81-81-2	10442445	4-hydroxy-3-(3-oxo-1-phenylbutyl)chromen-2-one
Anti-convulsant	Carbamazepine	298-46-4	2457	Benzo[b][1]benzazepine-11-carboxamide
	Lamotrigine	84057-84-1	3741	6-(2,3-dichlorophenyl)-1,2,4-triazine-3,5-diamine
	Phenytoin	57-41-0	1710	5,5-diphenylimidazolidine-2,4-dione
Anti-depressant	Amitriptyline	50-48-6	2075	3-(5,6-dihydrodibenzo[2,1-b:2',1'-f][7]annulen-11-ylidene)-N,N-dimethylpropan-1-amine
	Citalopram	59729-33-8	2669	1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-3H-2-benzofuran-5-carbonitrile
	Clomipramine	303-49-1	2699	3-(2-chloro-5,6-dihydrobenzo[b][1]benzazepin-11-yl)-N,N-dimethylpropan-1-amine
	Dosulepin	113-53-1	4445580	(3Z)-3-(6H-benzo[c][1]benzothiepin-11-ylidene)-N,N-dimethylpropan-1-amine
	Fluoxetine	54910-89-3	3269	N-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]propan-1-amine
	Lofepramine	23047-25-8	3810	1-(4-chlorophenyl)-2-[3-(5,6-dihydrobenzo[b][1]benzazepin-11-yl)propyl-methylamino]ethanone
	Mirtazapine	61337-67-5	4060	2-Methyl-1,2,3,4,10,14b-hexahydropyrazino[2,1-a]pyrido[2,3-c][2]benzazepine
	Paroxetine	61869-08-7	39888	(3S,4R)-3-(1,3-benzodioxol-5-yloxymethyl)-4-(4-fluorophenyl)piperidine
	Sertraline	79617-96-2	61881	(1S,4S)-4-(3,4-dichlorophenyl)-N-methyl-1,2,3,4-tetrahydronaphthalen-1-amine
	Trazodone	19794-93-5	5332	2-[3-[4-(3-chlorophenyl)piperazin-1-yl]propyl]-[1,2,4]triazolo[4,3-a]pyridin-3-one
	Venlafaxine (Venlaxafine)	93413-69-5	5454	1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexan-1-ol
Anti-diabetic	Gliclazide	21187-98-4	3356	1-(3,3a,4,5,6,6a-hexahydro-1H-cyclopenta[c]pyrrol-2-yl)-3-(4-methylphenyl)sulfonylurea
Anti-diarrhoeal	Loperamide	53179-11-6	3818	4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-N,N-dimethyl-2,2-diphenylbutanamide
Anti-emetic	Cyclizine / Marzine	82-92-8	6470	1-benzhydryl-4-methylpiperazine
	Metoclopramide	364-62-5	4024	4-amino-5-chloro-N-[2-(diethylamino)ethyl]-2-methoxybenzamide
	Scopolamine	51-34-3	10194106	(1R,2R,4S,5S,7s)-9-Methyl-3-oxa-9-azatricyclo[3.3.1.0~2,4~]non-7-yl (2S)-3-hydroxy-2-phenylpropanoate
Anti-estrogen	Tamoxifen	10540-29-1	2015313	2-[4-[(Z)-1,2-diphenylbut-1-enyl]phenoxy]-N,N-dimethylethanamine
Anti-fungal	Clotrimazole	23593-75-1	2710	1-[(2-chlorophenyl)-diphenylmethyl]imidazole
	Fluconazole	86386-73-4	3248	2-(2,4-difluorophenyl)-1,3-bis(1,2,4-triazol-1-yl)propan-2-ol
	Ketoconazole	65277-42-1	401695	1-[4-[4-[[(2S,4R)-2-(2,4-dichlorophenyl)-2-(imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazin-1-yl]ethanone
	Miconazole	22916-47-8	4044	1-[2-(2,4-dichlorophenyl)-2-[(2,4-dichlorophenyl)methoxy]ethyl]imidazole
	Terbinafine	91161-71-6	1266005	(E)-N,6,6-trimethyl-N-(naphthalen-1-ylmethyl)hept-2-en-4-yn-1-amine
Anti-glaucoma	Latanoprost	130209-82-4	4470740	Propan-2-yl (Z)-7-[(1R,2R,3R,5S)-3,5-dihydroxy-2-[(3R)-3-hydroxy-5-phenylpentyl]cyclopentyl]hept-5-enoate

continued

Anti-histamine	Cetirizine	83881-51-0	2577	2-[2-[4-[(4-chlorophenyl)-phenylmethyl]piperazin-1-yl]ethoxy]acetic acid
	Chlorpheniramine	132-22-9	2624	3-(4-chlorophenyl)-N,N-dimethyl-3-pyridin-2-ylpropan-1-amine
	Fexofenadine	83799-24-0	3231	2-[4-[1-hydroxy-4-[4-[hydroxy(diphenyl)methyl]piperidin-1-yl]butyl]phenyl]-2-methylpropanoic acid
	Hydroxyzine	68-88-2	3531	2-[2-[4-[(4-chlorophenyl)-phenylmethyl]piperazin-1-yl]ethoxy]ethanol
	Loratadine	79794-75-5	3820	Ethyl 4-(8-chloro-5,6-dihydrobenzo[1,2]cyclohepta[2,4-b]pyridin-11-ylidene)piperidine-1-carboxylate
	Promethazine	60-87-7	4758	N,N-dimethyl-1-phenothiazin-10-ylpropan-2-amine
	Cinnarizine	298-57-7	1264793	1-benzhydryl-4-[(E)-3-phenylprop-2-enyl]piperazine
Anti-hypertensive	Alfuzosin	81403-80-7	2008	N-[3-[(4-amino-6,7-dimethoxyquinazolin-2-yl)-methylamino]propyl]oxolane-2-carboxamide
	Amlodipine	88150-42-9	2077	3-O-ethyl 5-O-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate
	Atenolol	50-78-2	2162	2-[4-[2-hydroxy-3-(propan-2-ylamino)propoxy]phenyl]acetamide
	Bisoprolol	66722-44-9	2312	1-(propan-2-ylamino)-3-[4-(2-propan-2-yloxyethoxymethyl)phenoxy]propan-2-ol
	Candesartan	139481-59-7	2445	2-ethoxy-3-[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]benzimidazole-4-carboxylic acid
	Carvedilol	72956-09-3	2487	1-(9H-carbazol-4-yloxy)-3-[2-(2-methoxyphenoxy)ethylamino]propan-2-ol
	Celiprolol	56980-93-9	2563	3-[3-acetyl-4-[3-(tert-butylamino)-2-hydroxypropoxy]phenyl]-1,1-diethylurea
	Clonidine	4205-90-7	2701	N-(2,6-dichlorophenyl)-4,5-dihydro-1H-imidazol-2-amine
	Diltiazem-Cis	42399-41-7	35850	[(2S,3S)-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3-dihydro-1,5-benzothiazepin-3-yl] acetate
	Doxazosin	74191-85-8	3045	[4-(4-amino-6,7-dimethoxyquinazolin-2-yl)piperazin-1-yl]-(2,3-dihydro-1,4-benzodioxin-3-yl)methanone
	Enalapril	75847-73-3	4534998	(2S)-1-[(2S)-2-[[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]pyrrolidine-2-carboxylic acid
	Felodipine	72509-76-3	3216	5-O-ethyl 3-O-methyl 4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate
	Irbesartan	138402-11-6	3618	2-butyl-3-[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]-1,3-diazaspiro[4.4]non-1-en-4-one
	Labetalol	36894-69-6	3734	2-hydroxy-5-[1-hydroxy-2-(4-phenylbutan-2-ylamino)ethyl]benzamide
	Lisinopril	76547-98-3	4514933	(2S)-1-[(2S)-6-amino-2-[[(1S)-1-carboxy-3-phenylpropyl]amino]hexanoyl]pyrrolidine-2-carboxylic acid
	Losartan	114798-26-4	3824	[2-butyl-5-chloro-3-[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]imidazol-4-yl]methanol
	Metoprolol	37350-58-6	4027	1-[4-(2-methoxyethyl)phenoxy]-3-(propan-2-ylamino)propan-2-ol
	Moxonidine	75438-57-2	4645	4-chloro-N-(4,5-dihydro-1H-imidazol-2-yl)-6-methoxy-2-methylpyrimidin-5-amine
	Nebivolol	99200-09-6	64421	1-(6-fluoro-3,4-dihydro-2H-chromen-2-yl)-2-[[2-(6-fluoro-3,4-dihydro-2H-chromen-2-yl)-2-hydroxyethyl]amino]ethanol
	Nifedipine	21829-25-4	4330	Dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate
	Oxprenolol	6452-71-7	4470	1-(propan-2-ylamino)-3-(2-prop-2-enoxyphenoxy)propan-2-ol

continued

	Perindopril	82834-16-0	96956	(2S,3aS,7aS)-1-[(2S)-2-[[(2S)-1-ethoxy-1-oxopentan-2-yl]amino]propanoyl]-2,3,3a,4,5,6,7,7a-octahydroindole-2-carboxylic acid
	Propranolol	525-66-6	4777	1-naphthalen-1-yloxy-3-(propan-2-ylamino)propan-2-ol
	Ramipril	87333-19-5	4514937	(2S,3aS,6aS)-1-[(2S)-2-[[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]-3,3a,4,5,6,6a-hexahydro-2H-cyclopenta[b]pyrrole-2-carboxylic acid
	Sotalol	3930-20-9	5063	N-[4-[1-hydroxy-2-(propan-2-ylamino)ethyl]phenyl]methanesulfonamide
	Telmisartan	144701-48-4	59391	2-[4-[[4-methyl-6-(1-methylbenzimidazol-2-yl)-2-propylbenzimidazol-1-yl]methyl]phenyl]benzoic acid
	Timolol	26839-75-8	31013	(2S)-1-(tert-butylamino)-3-[(4-morpholin-4-yl-1,2,5-thiadiazol-3-yl)oxy]propan-2-ol
	Valsartan	137862-53-4	54833	(2S)-3-methyl-2-[pentanoyl-[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]amino]butanoic acid
	Verapamil	52-53-9	2425	2-(3,4-dimethoxyphenyl)-5-[2-(3,4-dimethoxyphenyl)ethyl-methylamino]-2-propan-2-ylpentanenitrile
Anti-infective	Chlorhexidine	55-56-1	2612	(1E)-2-[6-[[amino-[(E)-[amino-(4-chloroanilino)methylidene]amino]methylidene]amino]hexyl]-1-[amino-(4-chloroanilino)methylidene]guanidine
Anti-malarial	Quinine	130-95-0	84989	(R)-[(2S,4S,5R)-5-ethenyl-1-azabicyclo[2.2.2]octan-2-yl]-(6-methoxyquinolin-4-yl)methanol
Anti-neoplastic	Anastrozole	120511-73-1	2102	2-[3-(2-cyanopropan-2-yl)-5-(1,2,4-triazol-1-ylmethyl)phenyl]-2-methylpropanenitrile
Anti-obesity	Orlistat	96829-58-2	2298564	[(2S)-1-[(2S,3S)-3-hexyl-4-oxooxetan-2-yl]tridecan-2-yl] (2S)-2-formamido-4-methylpentanoate
Anti-platelet	Clopidogrel	113665-84-2	54632	Methyl (2S)-2-(2-chlorophenyl)-2-(6,7-dihydro-4H-thieno[3,2-c]pyridin-5-yl)acetate
Anti-psychotic	Amisulpride	71675-85-9	2074	4-amino-N-[(1-ethylpyrrolidin-2-yl)methyl]-5-ethylsulfonyl-2-methoxybenzamide
	Chlorpromazine	50-53-3	2625	3-(2-chlorophenothiazin-10-yl)-N,N-dimethylpropan-1-amine
	Haloperidol	52-86-8	3438	4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-1-(4-fluorophenyl)butan-1-one
	Risperidone	106266-06-2	4895	3-[2-[4-(6-fluoro-1,2-benzoxazol-3-yl)piperidin-1-yl]ethyl]-2-methyl-6,7,8,9-tetrahydropyrido[1,2-a]pyrimidin-4-one
Anti-rheumatic / Anti-malarial	Hydroxychloroquine	118-42-3	3526	2-[4-[(7-chloroquinolin-4-yl)amino]pentyl-ethylamino]ethanol
Anti-malarial	Sulfasalazine	599-79-1	10481900	(3Z)-6-oxo-3-[[4-(pyridin-2-ylsulfamoyl)phenyl]hydrazinylidene]cyclohexa-1,4-diene-1-carboxylic acid
Anti-spasmodic	Mebeverine	630-20-3	3891	4-[ethyl-[1-(4-methoxyphenyl)propan-2-yl]amino]butyl 3,4-dimethoxybenzoate
	Oxybutynin	5633-20-5	4473	4-(diethylamino)but-2-ynyl 2-cyclohexyl-2-hydroxy-2-phenylacetate
	Procyclidine	77-37-2	4750	1-cyclohexyl-1-phenyl-3-pyrrolidin-1-ylpropan-1-ol
Bronchodilator	Terbutaline	23031-25-6	5210	5-[2-(tert-butylamino)-1-hydroxyethyl]benzene-1,3-diol
	Theophylline	58-55-9	2068	1,3-dimethyl-7H-purine-2,6-dione
Cardiotonic drug	Digoxin	20830-75-5	2006532	3-[(3S,5R,8R,9S,10S,12R,13S,14S,17R)-3-[(2R,4S,5S,6R)-5-[(2S,4S,5S,6R)-5-[(2S,4S,5S,6R)-4,5-dihydroxy-6-methyloxan-2-yl]oxy-4-hydroxy-6-methyloxan-2-yl]oxy-4-hydroxy-6-methyloxan-2-yl]oxy-12,14-dihydroxy-10,13-dimethyl-1,2,3,4,5,6,7,8,9,11,12,15,16,17-tetradecahydrocyclopenta[a]phenanthren-17-yl]-2H-furan-5-one
Cholinergic antagonist	Alverine	150-59-4	3550	N-ethyl-3-phenyl-N-(3-phenylpropyl)propan-1-amine

continued

Corticosteroid	Betamethasone	378-44-9	9399	(4aR,4bS,5S,6aS,6bS,9aR,10aS,10bS)-6b-Glycoloyl-5-hydroxy-4a,6a-dimethyl-8-propyl-4a,4b,5,6,6a,6b,9a,10,10a,10b,11,12-dodecahydro-2H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-2-one
	Betamethasone-17-valerate	2152-44-5	15673	[(8S,9R,10S,11S,13S,14S,16S,17R)-9-fluoro-11-hydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-17-yl] pentanoate
Diuretic	Amiloride	2609-46-3	15403	3,5-diamino-6-chloro-N-(diaminomethylidene)pyrazine-2-carboxamide
	Bendroflumethiazide	73-48-3	2225	3-benzyl-1,1-dioxo-6-(trifluoromethyl)-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide
	Bumetanide	28395-03-1	2377	3-(butylamino)-4-phenoxy-5-sulfamoylbenzoic acid
	Furosemide	54-31-9	3322	4-chloro-2-(furan-2-ylmethylamino)-5-sulfamoylbenzoic acid
	Hydrochlorothiazide	58-93-5	3513	6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide
	Indapamide	26807-65-8	3574	4-chloro-N-(2-methyl-2,3-dihydroindol-1-yl)-3-sulfamoylbenzamide
	Spironolactone	52-01-7	5628	S-[(7R,8R,9S,10R,13S,14S,17R)-10,13-dimethyl-3,5'-dioxospiro[2,6,7,8,9,11,12,14,15,16-decahydro-1H-cyclopenta[a]phenanthrene-17,2'-oxolane]-7-yl] ethanethioate
Dopamine agonist	Ropinirole	91374-21-9	4916	4-[2-(dipropylamino)ethyl]-1,3-dihydroindol-2-one
Laxative	Bisacodyl	603-50-9	2299	[4-[(4-acetyloxyphenyl)-pyridin-2-ylmethyl]phenyl] acetate
	Dioctyl sulfosuccinate	10041-19-7	10862	1,4-bis(2-ethylhexoxy)-1,4-dioxobutane-2-sulfonate
Lipid regulator	Atorvastatin	134523-00-5	54810	(3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid
	Bezafibrate	41859-67-0	35728	2-[4-[2-[(4-chlorobenzoyl)amino]ethyl]phenoxy]-2-methylpropanoic acid
	Fenofibrate	49562-28-9	3222	Propan-2-yl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoate
	Pravastatin	81093-37-0	49398	(3R,5R)-7-[(1S,2S,6S,8S,8aR)-6-hydroxy-2-methyl-8-[(2S)-2-methylbutanoyl]oxy-1,2,6,7,8,8a-hexahydronaphthalen-1-yl]-3,5-dihydroxyheptanoic acid
	Simvastatin	79902-63-9	49179	[(1S,3R,7S,8S,8aR)-8-[2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl]-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl] 2,2-dimethylbutanoate
Local anasthetic	Lidocaine	137-58-6	3548	2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide
Nicotinic antagonist	Varenicline	249296-44-4	4470510	(1R,12S)-5,8,14-Triazatetracyclo[10.3.1.0~2,11~.0~4,9~]hexadeca-2,4,6,8,10-pentaene
Nootropic agent	Donepezil	120014-06-4	3040	2-[(1-benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one
NSAID	Acetaminophen	103-90-2	1906	N-(4-Hydroxyphenyl)acetamide
	Acetylsalicylic acid	50-78-2	2157	2-Acetoxybenzoic acid
	Benzindamine	642-72-8	12036	3-(1-benzylindazol-3-yl)oxy-N,N-dimethylpropan-1-amine
	Celecoxib	169590-42-5	2562	4-[5-(4-methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl]benzenesulfonamide
	Diclofenac	15307-86-5	2925	2-[2-(2,6-dichloroanilino)phenyl]acetic acid
	Ibuprofen	15687-27-1	3544	2-[4-(2-methylpropyl)phenyl]propanoic acid

continued

	Ketoprofen	22071-15-4	3693	2-(3-benzoylphenyl)propanoic acid
	Mefenamic acid	61-68-7	3904	2-(2,3-dimethylanilino)benzoic acid
	Meloxicam	71125-38-7	10442740	4-hydroxy-2-methyl-N-(5-methyl-1,3-thiazol-2-yl)-1,1-dioxo-1,2-benzothiazine-3-carboxamide
	Naproxen	22204-53-1	137720	(2S)-2-(6-methoxynaphthalen-2-yl)propanoic acid
	Piroxicam	36322-90-4	10442653	4-Hydroxy-2-methyl-N-(2-pyridinyl)-2H-1,2-benzothiazine-3-carboximidic acid 1,1-dioxide
	Salicylic acid	69-72-7	331	2-hydroxybenzoic acid
PDE5 inhibitor	Sildenafil	139755-83-2	5023	5-[2-ethoxy-5-(4-methylpiperazin-1-yl)sulfonylphenyl]-1-methyl-3-propyl-4H-pyrazolo[4,3-d]pyrimidin-7-one
Proton pump inhibitor	Lansoprazole	103577-45-3	3746	2-[[3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methylsulfinyl]-1H-benzimidazole
	Omeprazole	73590-58-6	4433	6-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methylsulfinyl]-1H-benzimidazole
	Pantoprazole	102625-70-7	4517	6-(difluoromethoxy)-2-[(3,4-dimethoxypyridin-2-yl)methylsulfinyl]-1H-benzimidazole
Steroidal anti-androgen	Cyproterone acetate	427-51-0	9496	(1R,3aS,3bR,7aR,8aS,8bS,8cS,10aS)-1-Acetyl-5-chloro-8b,10a-dimethyl-7-oxo-1,2,3,3a,3b,7,7a,8,8a,8b,8c,9,10,10a-tetradecahydrocyclopenta[a]cyclopropa[g]phenanthren-1-yl acetate
Steroidal anti-asthmatic	Beclomethasone	4419-39-0	19276	(8S,9R,10S,11S,13S,14S,16S,17R)-9-chloro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-3-one
	Beclomethasone dipropionate	08-09-34	20396	[2-[(8S,9R,10S,11S,13S,14S,16S,17R)-9-chloro-11-hydroxy-10,13,16-trimethyl-3-oxo-17-propanoyloxy-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-17-yl]-2-oxoethyl] propanoate
Steroidal anti-inflammatory	Budesonide	51333-22-3	36566	(4aR,4bS,5S,6aS,6bS,9aR,10aS,10bS)-6b-Glycoloyl-5-hydroxy-4a,6a-dimethyl-8-propyl-4a,4b,5,6,6a,6b,9a,10,10a,10b,11,12-dodecahydro-2H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-2-one
	Clobetasol propionate	25122-46-7	30399	[(8S,9R,10S,11S,13S,14S,16S,17R)-17-(2-chloroacetyl)-9-fluoro-11-hydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-17-yl] propanoate
	Clobetasone-17-butyrate	25122-57-0	64481	[(8S,9R,10S,13S,14S,16S,17R)-17-(2-chloroacetyl)-9-fluoro-10,13,16-trimethyl-3,11-dioxo-7,8,12,14,15,16-hexahydro-6H-cyclopenta[a]phenanthren-17-yl] butanoate
	Dexamethasone	50-02-2	5541	(8S,9R,10S,11S,13S,14S,16R,17R)-9-fluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-3-one
	Dexamethasone-21-acetate	1177-87-3	206624	[2-[(8S,9R,10S,11S,13S,14S,16R,17R)-9-fluoro-11,17-dihydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-17-yl]-2-oxoethyl] acetate
	Flumethasone	2135-17-3	15632	(6S,8S,9R,10S,11S,13S,14S,16R,17R)-6,9-difluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16-trimethyl-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-3-one
	Fluticasone-17-Propionate	80474-14-2	49297	[(6S,8S,9R,10S,11S,13S,14S,16R,17R)-6,9-difluoro-17-(fluoromethylsulfanylcarbonyl)-11-hydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-17-yl] propanoate
	Fusidic acid	1859-24-0	2271900	(2Z)-2-[(3R,4S,5S,8S,9S,10S,11R,13R,14S,16S)-16-acetyloxy-3,11-dihydroxy-4,8,10,14-tetramethyl-2,3,4,5,6,7,9,11,12,13,15,16-dodecahydro-1H-cyclopenta[a]phenanthren-17-ylidene]-6-methylhept-5-enoic acid

continued

	Hydrocortisone	50-23-7	5551	(8S,9S,10R,11S,13S,14S,17R)-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-2,6,7,8,9,11,12,14,15,16-decahydro-1H-cyclopenta[a]phenanthren-3-one
	Hydrocortisone-21-acetate	50-03-3	5542	[2-[(8S,9S,10R,11S,13S,14S,17R)-11,17-dihydroxy-10,13-dimethyl-3-oxo-2,6,7,8,9,11,12,14,15,16-decahydro-1H-cyclopenta[a]phenanthren-17-yl]-2-oxoethyl] acetate
	Mometasone furoate	83919-23-7	390091	[(8S,9R,10S,11S,13S,14S,16R,17R)-9-chloro-17-(2-chloroacetyl)-11-hydroxy-10,13,16-trimethyl-3-oxo-6,7,8,11,12,14,15,16-octahydrocyclopenta[a]phenanthren-17-yl] furan-2-carboxylate
	Prednisolone	50-24-8	5552	(8S,9S,10R,11S,13S,14S,17R)-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13-dimethyl-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a]phenanthren-3-one
Steroidal contraceptive	Desogestrel	54024-22-5	37400	(8S,9S,10R,13S,14S,17R)-13-ethyl-17-ethynyl-11-methylidene-1,2,3,6,7,8,9,10,12,14,15,16-dodecahydrocyclopenta[a]phenanthren-17-ol
Steroidal estrogen	17-alpha-estradiol	57-91-0	61840	(8R,9S,13S,14S,17R)-13-methyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[a]phenanthrene-3,17-diol
	17-beta-estradiol	50-28-2	5554	(8R,9S,13S,14S,17S)-13-methyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[a]phenanthrene-3,17-diol
Steroidal hormone	Progesterone	57-83-0	5773	(8S,9S,10R,13S,14S,17S)-17-acetyl-10,13-dimethyl-1,2,6,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-3-one
Steroidal progestin	Medroxyprogesterone-17-acetate	71-58-9	6043	[(6S,8R,9S,10R,13S,14S,17R)-17-acetyl-6,10,13-trimethyl-3-oxo-2,6,7,8,9,11,12,14,15,16-decahydro-1H-cyclopenta[a]phenanthren-17-yl] acetate
	Nor-ethisterone (19-Norethindrone)	68-22-4	5994	(8R,9S,10R,13S,14S,17R)-17-ethynyl-17-hydroxy-13-methyl-1,2,6,7,8,9,10,11,12,14,15,16-dodecahydrocyclopenta[a]phenanthren-3-one
Steroidal reductase inhibitor	Finasteride	98319-26-7	51714	(1S,3aS,3bS,5aR,9aR,9bS,11aS)-N-tert-butyl-9a,11a-dimethyl-7-oxo-1,2,3,3a,3b,4,5,5a,6,9b,10,11-dodecahydroindeno[5,4-f]quinoline-1-carboxamide
Vasodilator	Betahistine	5638-76-6	2276	N-methyl-2-pyridin-2-ylethanamine
α1a adrenergic receptor antagonist	Tamsulosin	106133-20-4	114457	5-[(2R)-2-[2-(2-ethoxyphenoxy)ethylamino]propyl]-2-methoxybenzenesulfonamide

Key

CASRN - Chemical Abstracts Service Registry Number (https://www.cas.org/content/chemical-substances)
Chemspider - Royal Society of Chemistry (http://www.chemspider.com/)
IUPAC - International Union of Pure and Applied Chemistry (http://iupac.org/)
PubChem - National Center for Biotechnology Information (https://pubchem.ncbi.nlm.nih.gov/)

3.4 Experimental

3.4.1 Reagents and reference materials

All reagents of analytical reagent grade otherwise specified. All solvents were of LC-MS or HPLC grade. Acetone, ammonium acetate, ammonium formate, dichloromethane, formic acid, methanol and methyl-tert-butyl ether (MTBE) were purchased from Fisher Scientific UK (Loughborough, Leicestershire, UK) or Sigma-Aldrich (Gillingham, Dorset, UK). Ultrapure water was obtained from an 'in-house' source (ELGA Purelab Ultra) and was used in all laboratory procedures (Elga Process Water, Marlow, Buckinghamshire, UK). The ultrapure water system was equipped with a UV lamp, carbon and membrane filter to remove trace organic compounds, ionic species and particulates. Supor® 200 polyethersulphone membrane was obtained as a 30 cm by 15 m roll from Pall Corporation (Portsmouth, Hampshire, UK). Oasis HLB sorbent was obtained from Waters Ireland as a lose powder (Santry, Dublin, Ireland) and '316' grade stainless-steel rings, for the preparation of POCIS devices, were manufactured by A.T. Engineering (Tadley, Hampshire, UK). All glassware was silanised to reduce surface activity and potential loss of analytes through adsorption onto glass surfaces. The glassware was rinsed initially with methanol followed by toluene before being soaked in Sylon CT™ solution (5% dimethylchlorosilane in toluene) according to the instructions provided. After soaking the glassware was rinsed twice with toluene, methanol and then allowed to air dry in a fume hood.

For acquisition of high quality accurate mass spectra, all pharmaceutical reference compounds were purchased as secondary reference materials from Sigma-Aldrich in 5 mg quantities with a purity of at least 95%. Stock standard solutions were prepared

by dissolving the reference compounds in either acetone, acetonitrile, DMF, DMSO, ethanol, methanol, sodium hydroxide solution or water depending on the physicochemical properties of the substance (329).

17-alpha-estradiol, 17-beta-estradiol, Beclomethasone di-propionate, Betamethsone-17-valerate, Bisacodyl, Cinnarizine, Clobetasone-17-butyrate, Cyproterone acetate, Dexamethasone, Felodipine, Fenofibrate, Flumethasone, Hydrocortisone-21-acetate, Hydroxyzine, Ketoprofen, Medroxyprogesterone -17-acetate, Mometasone furoate, Naproxen, Nifedipine, Nitrofurantoin, Norethisterone and Warfarin were dissolved in 5 mL of acetone. Carvedilol, Diltiazem-cis, Dipyridamole, Dosulepin, Finasteride, Hydrocortisone, Losartan, Progesterone, Promethazine, Salbutamol, Scopolamine, Simvastatin, Spironolactone, Terbutaline and Verapamil were dissolved in 5 mL of ethanol. Cetirizine, Dioctyl sulfosuccinate, Flecainide, Hydroxychloroquine, Penicillin V and Trazadone were dissolved in 5 mL of water. Chlorhexidine and Donepezil were dissolved in 5 mL of acetonitrile. Nystatin, Doxazosin and Mefenamic acid were dissolved in 5 mL of dimethylformamide, dimethylsulfoxide and 1 M sodium hydroxide respectively. All remaining pharmaceutical compounds were dissolved in 5mL of methanol.

Many of the reference compounds were available only as salts e.g. chloride or acetate. All subsequent dilutions of the individual stock solutions were corrected taking into consideration the percentage of salt and water of crystallization present in each solution thus ensuring accurate preparation of standard solutions ahead of LC-MS analysis. The individual stock standard solutions were stored until use at –18 °C.

3.4.2 Intermediate and working standard solution mixes

An intermediate stock standard solution mix was prepared in methanol at a concentration of 1 ng/uL and was found to be stable for at least one month at 3-5 °C. The solution was diluted 100-fold in mobile phase and used as a working standard solution mix. The working solution was prepared as and when required.

3.4.3 Preparation of the mobile phase for LC Q-TOF-MS analysis

Ten M ammonium acetate was prepared by dissolving 77.083 g in 90 mL of water and making up to 100 mL with water in a volumetric flask. Mobile phase components A and B were prepared by adding 200 µL of the 10 M ammonium acetate solution and 100 µL of formic acid. to individual HPLC mobile phase reservoir bottles (1.2 L) containing exactly 1 L of water and methanol respectively.

3.4.4 Preparation of POCIS samplers

3.4.4.1 Preparation and cleaning of membranes for POCIS

Supor® 200 polyethersulphone membrane was cut into 9 cm by 18 cm rectangular pieces and folded in two. Twenty-five cut membranes were placed into a cleaned, solvent rinsed 2 L beaker containing 1.5 L of 20% methanol in water. The membranes were dispersed in the solvent and prevented from sticking together in a large mass with the use of a large glass rod. The beaker was covered with aluminium foil and allowed to soak for 24 h in an incubator at 40 ºC to aid in the cleaning. The solvent was discarded and the above step repeated with a second aliquot of 1.5 L of 20%

methanol in UHP water. The solvent was discarded the entire procedure repeated twice using 1.5 L of methanol. The methanol was discarded and the membranes placed on a piece of solvent rinsed aluminium foil to air dry (~ 8 h). Once dry they were placed in a solvent rinsed aluminium foil envelope and stored in a clean metal can flushed with argon gas and kept in a freezer at -18ºC until POCIS devices were ready to assemble.

3.4.4.2 Cleaning of the Oasis® HLB phase sorbent

Fifty mL of methanol was placed into a solvent rinsed, 19 mm × 61 cm, glass chromatography column with a fritted disk and stopcock. Six g of Oasis® HLB sorbent (enough for 30 POCIS devices) was slowly added to the column and allowed to settle on the fritted disk. The stopcock was opened and the methanol allowed to percolate through the sorbent until the meniscus was approximately 10 mm from the surface of the sorbent and the stopcock closed. The sorbent was allowed to soak for 30 min and a further 200 mL of methanol was slowly added to the column before the stopcock was opened again and the methanol allowed to percolate through the sorbent. The stopcock was closed just as the methanol entered the sorbent. The procedure was repeated using 250 mL aliquots of methyl-tertiary-butyl ether (MTBE) and dichloromethane followed by a final 250 mL aliquot of methanol and allowed to drain completely out of the column after the final 30 min soak. The sorbent was forced out of the column and into a glass flask by applying positive pressure with nitrogen gas at the outlet of the column. The flask was placed into a Genevac Rocket™ centrifugal rotary vacuum evaporator set at medium boiling point and the clean sorbent dried for a period of 1 h. The cleaned and dried sorbent was placed into a

clean solvent rinsed glass jar and covered with aluminium foil and sealed with a PTFE lined screw cap lid.

3.4.4.3 Assembly of POCIS samplers

Two-hundred ± 2 mg, of the purified Oasis® HLB was weighed out for each POCIS device required and transferred into a clean glass weighing boat. The folded membrane was put over one clean solvent rinsed stainless-steel POCIS ring and a sharp steel awl used to make holes in the membrane over the three bolt holes as shown in the left-hand image in Figure 3.3. The folded membrane was opened and sorbent placed on one half of the membrane as shown in the right-hand image in Figure 3.3. The membrane was folded back over and the second solvent rinsed POCIS ring placed on top ensuring the holes for the two POCIS rings were aligned and bolts pushed through each hole in the rings and folded membrane. A stainless-steel nut was placed on the end of each bolt and tightened as much as possible to obtain the assembled POCIS device as shown in Figure 3.4. Excess membrane was removed with a scalpel blade and the POCIS device completed. The prepared devices were wrapped in aluminium foil and stored frozen in clean 5 L metal cans until they were ready to be deployed.

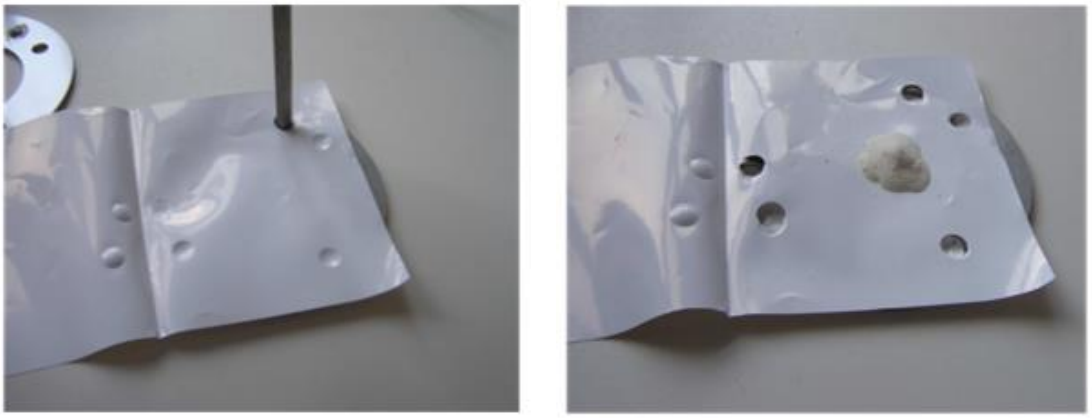


Figure 3.3 Preparation of in-house POCIS samplers.

Figure 3.4 Fully assembled POCIS sampler.

3.4.5 Cleaning of the Chemcatcher® bodies, rings and transport caps

Chemcatcher® bodies were fully disassembled and all components soaked overnight in 10% Decon® detergent in tap water overnight in a 10 L plastic bucket. The Chemcatcher® components were removed from the detergent solution and rinsed thoroughly with copious quantities of tap water ensuring that all traces of the detergent were removed. All components were then rinsed with de-ionised water and dried with clean paper towels before being placed in a clean solvent rinsed glass container containing methanol for 5 min. All Chemcatcher® bodies and components were removed from the beaker and allowed to dry on a large piece of solvent rinsed aluminium foil.

3.4.6 Conditioning of the HLB-L disks for the polar Chemcatcher®

Twenty Horizon Technology, Atlantic HLB-L disks were soaked in 1 L of methanol overnight in a clean solvent rinsed 2 L glass beaker after which the methanol was discarded and the disks allowed to air dry on aluminium foil.

Acetone rinsed PTFE disk supports and glass support bases were mounted onto a stainless-steel vacuum manifold. Pre-soaked and dried Atlantic HLB-L disks were centred onto the disk supports and the glass reservoir secured onto the glass support base with the aid of a metal sprung clamp. Fifty mL of methanol was added to each reservoir and allowed to soak into the disk under gravity until a small volume of methanol remained on the disk. Fifty mL of ultra-pure water was then added to each disk and a slight vacuum was applied to draw the first 5 - 10 mL of water into the disk with the remaining water allowed to flow completely though the disk under

gravity. The clamp was removed and, with the aid of tweezers, the conditioned Atlantic HLB disks were removed from the manifold and placed in ultra-pure water until assembled into the Chemcatcher® body.

3.4.7 Preparation of membranes for the Chemcatcher®

Supor® 200 polyethersulphone membrane was cut into 9 cm x 9 cm square pieces and a piece of clean aluminium foil was placed in between each layer of PES membrane to prevent them sticking to each other. A 51 mm diameter wad-punch, together with a hammer, was then used to 'punch-out' a sufficient number of PES membranes for the deployments. PES membranes were cleaned and stored using the same procedure as outlined in section 3.4.4.1 (Preparation and cleaning of membranes for POCIS).

3.4.8 Chemcatcher® assembly

Conditioned HLB-L disks were placed on a Chemcatcher® body and a clean PES membrane centred on the top of the disk. A PTFE retaining ring, which holds the disk and membrane in place, was screwed onto the Chemcatcher® body and firmly hand tightened. The fully assembled Chemcatcher® samplers were placed in a large Tupperware box containing deionised water and stored refrigerated at 3–5ºC until they were ready to be deployed.

3.4.9 Deployment of samplers at the waste water treatment plants

Triplicate Chemcatcher® and POCIS samplers were attached to stainless-steel holders prior to being placed in protective steel housings approximately 30 cm tall. Figure 3.5 shows the prototype version of the polar Chemcatcher® used in the deployments. Permission to deploy the samplers was obtained from the site operators Dwr Cymru Welsh Water and all samplers were deployed at all three sites on the 4th of August 2014 and were retrieved on the 25th of August 2014, a deployment period of 21 days. Five mm thick chain was used to securely fasten the deployment cages to the stainless-steel platform covering the final effluent channels at the three sites. Figure 3.6 shows the location of the Gowerton co-deployments where the sampler housing was placed into the final effluent stream via an access hatch.

3.4.10 Extraction of POCIS samplers

On return to the laboratory the housings containing the POCIS samplers were removed from the holder and cleaned under a gentle flow of running tap water to remove any sediment. Excess water was removed with laboratory blue roll and the sampler was allowed to dry overnight at room temperature before extraction. The two membrane layers were separated and the exposed sorbent was allowed to air dry before being removed from the membrane by carefully brushing into a tared 15 mL glass vial with the aid of a glass filter funnel. Figure 3.7a and Figure 3.7b clearly shows that the sorbent within the two sets of POCIS samplers (deployed at Gowerton WWTP) is not evenly distributed between the two membranes and tends to ‘flow’ when the membrane layers are separated leading to potential losses. As the POCIS

sorbent is electrostatic when dried, precautions were taken to minimise these losses by discharging any static build up by ‘earthing’ a large sheet of aluminium foil that was used for holding any glass vials, containing the dried sorbent, and other equipment prior to weighing. Once all the sorbent had been removed from both membranes the glass vial was weighed and the mass of sorbent recovered from each POCIS sampler is shown below in Table 3.3. Significantly lower amounts of sorbent were recovered from the Carmarthen A and Llanelli B POCIS devices, 103.8 mg and 97.0 mg, respectively. An overall average of 69.6% of sorbent was recovered out of the original 200 mg added with an RSD of 15.4%.

The sorbent from each sampler was then transferred into a labelled methanol rinsed 15 mL polypropylene SPE cartridge fitted with a PTFE frit at the bottom. Once the sorbent was transferred a second PTFE frit was gently pushed on top of the sorbent. A stainless-steel solvent guide was placed at the bottom of a flow control valve located on a 24 position SPE vacuum box. The male Luer fitting of the SPE tube was inserted firmly into the female hub of the flow control valve which was set to the fully ‘open’ position. A labelled 50 mL screw cap glass vial was placed inside the vacuum box with the stainless-steel solvent guide approximately 1 cm into the vial. This was done in order to catch the eluate from the SPE cartridge. Five mL of methanol was added to the glass vial to transfer any remaining sorbent onto the SPE cartridge. A disposable glass Pasteur pipette was used to transfer the methanol to the SPE cartridge. Once the methanol had percolated, under gravity, into the sorbent a further 5 mL of methanol was added to the SPE cartridge and this was again allowed to percolate into the sorbent. The flow control valve then turned to the fully ‘closed’ position.

A 75 mL methanol rinsed polypropylene reservoir was then attached to the top of the SPE cartridge using an adaptor. Thirty mL of methanol was poured into the reservoir. The flow control valve was opened slowly and the flow of eluate, dripping into the glass vial, adjusted to approximately 1 mL/min. The entire elution solvent was allowed to pass through the cartridge under gravity flow. Once all the eluent has been passed through the cartridge, a syringe with an airtight seal was attached to the SPE cartridge in order to push any remaining solvent out of the sorbent and into the 50 mL glass vial.

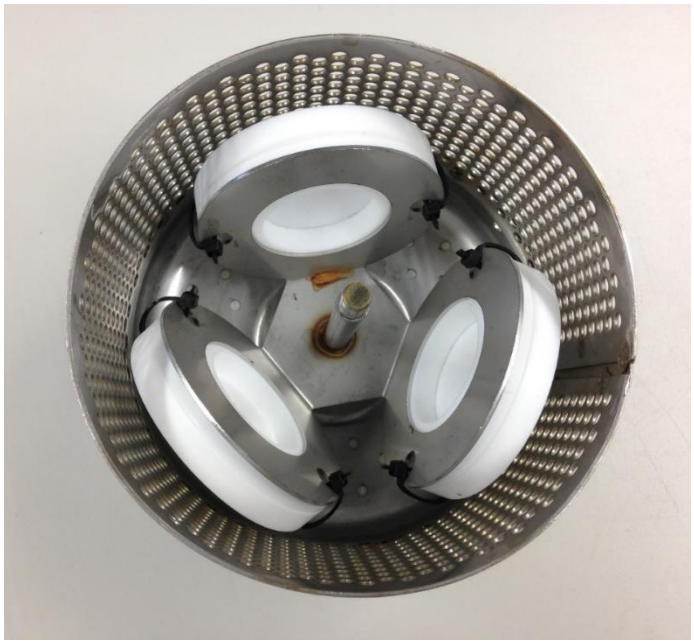


Figure 3.5 Prototype version of the polar Chemcatcher® placed in the protective housing.

Figure 3.6 Location of the Gowerton deployments in the final effluent stream.

a

b

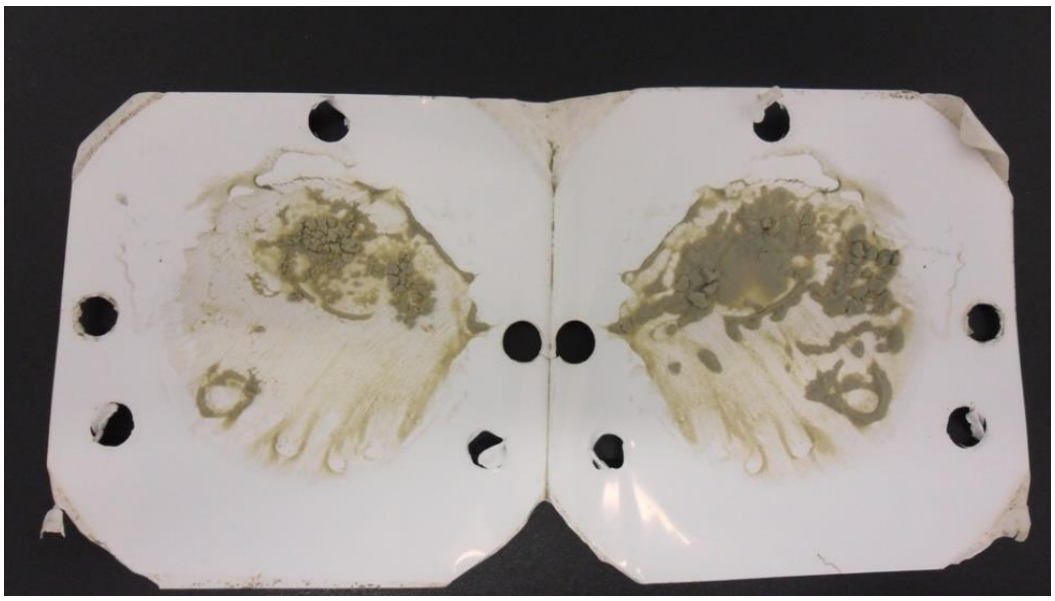


Figure 3.7 Sorbent within the POCIS sampler after disassembly of one of the Gowerton '1' (a) and Gowerton '2' (b) samplers showing uneven distribution of material.

Table 3.3 Ratios of POCIS sorbent recovered against the original added.

Site	Mass of sorbent added (mg) A	Mass of sorbent recovered (mg) B	% of sorbent recovered
Carmarthen A	200 ± 2	103.8	52.0
Carmarthen B	200 ± 2	140.9	70.5
Carmarthen C	200 ± 2	146.0	73.0
Gowerton 1A	200 ± 2	144.0	72.0
Gowerton 1B	200 ± 2	145.5	72.8
Gowerton 1C	200 ± 2	126.2	63.1
Gowerton 2A	200 ± 2	147.0	73.5
Gowerton 2B	200 ± 2	151.4	75.7
Gowerton 2C	200 ± 2	142.7	71.4
Llanelli A	200 ± 2	177.5	88.8
Llanelli B	200 ± 2	97.0	48.5
Llanelli C	200 ± 2	146.7	73.4

3.4.11 Extraction of HLB-L Disks from Chemcatcher® samplers

On return to the laboratory the Chemcatcher® samplers were cleaned under a gentle flow of running tap water to remove any sediment. The Chemcatcher® samplers were disassembled using the reverse process as outlined in section 3.7 and the HLB-L disk (Figure 3.8) placed into the polypropylene disk elution apparatus. The elution apparatus used, which is positioned on top of a vacuum box, prevented the loss of eluate that can typically occur with vacuum manifold where the SPE disk is secured to a glass reservoir with a sprung clamp. A stainless-steel solvent guide was placed at the bottom of a flow control valve located on a 24 position SPE vacuum box. The

male Luer fitting of the disk elution apparatus was inserted firmly into the female hub of the flow control valve as shown in Figure 3.9. The flow control valve was set to the fully ‘open’ position. Full vacuum was applied to the vacuum box for 1 h to dry the HLB-L disk.

The HLB-L disk was eluted in a similar manner to that of the SPE cartridge in section 4.3–4.4. Five mL of methanol was added to the disk and once it had percolated, under gravity, into the disk a further 5 mL of methanol was added and this was again allowed to percolate into the disk. The flow control valve then turned to the fully ‘closed’ position. Thirty mL of methanol was poured into the reservoir and the flow control valve opened fully. The entire elution solvent was allowed to pass through the disk under gravity flow. Once the entire eluent has been passed through the disk, slight vacuum was applied to the vacuum box in order to pull any remaining solvent out of the disk and into the labelled 50 mL glass vial.

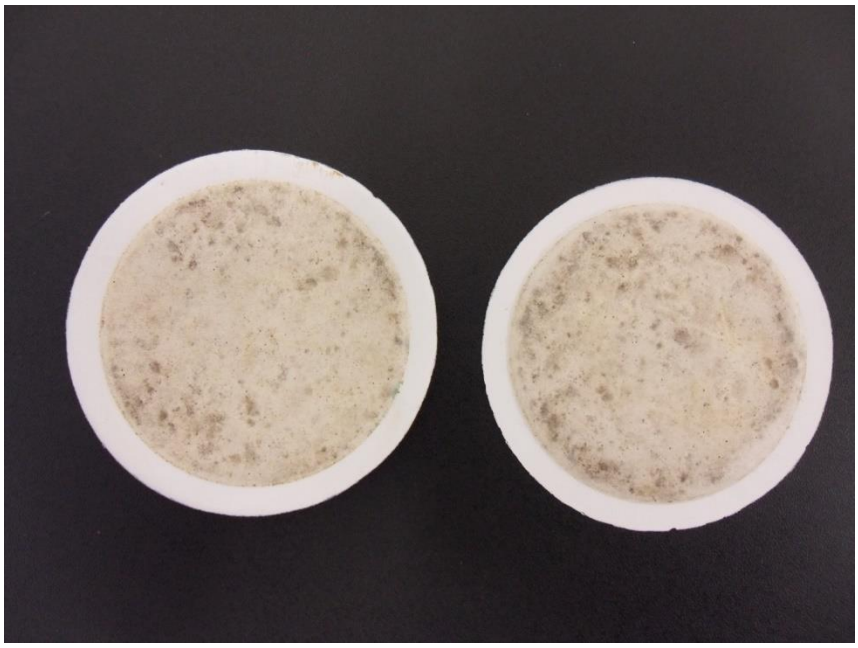


Figure 3.8 HLB-L disks from retrieved Chemcatcher® samplers at the Gowerton WWTP.

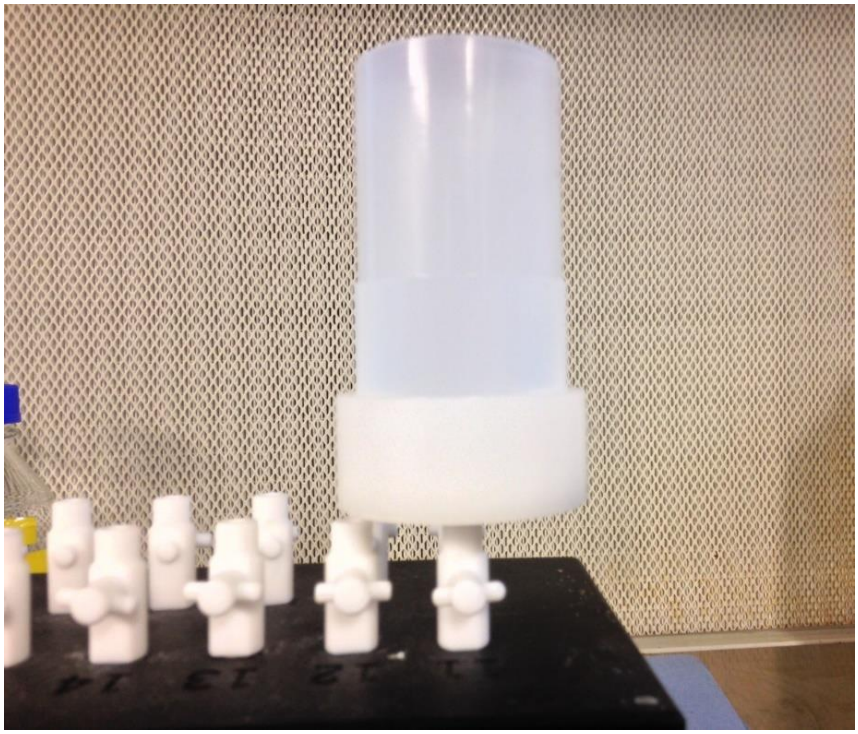


Figure 3.9 Apparatus used for the elution of adsorbed compounds from the Chemcatcher® HLB-L disk.

3.4.12 Creation of the searchable database using Agilent personal compound database and library (PCDL) software

Accurate mass MS spectra for the protonated adducts $[M+H]^+$ in positive ion mode and de-protonated adducts $[M-H]^-$ for each compound were acquired using flow injection in MS mode with a collision energy of 0eV. Accurate mass spectra were also acquired for the $[M+Na]^+$ and $[M+K]^+$ adducts in positive ion mode, or $[M-HCOO]^-$ and $(M+CH_3COO]^-$ adducts in negative ion mode, when they were observed in significant abundance. Accurate mass fragment ion spectra for the protonated and de-protonated adducts were again acquired using flow injection in MS/MS mode with a collision energy of 20 eV and 40 eV. Fragment ion spectra for other adducts, especially those for sodium and potassium were not always obtained as they tend to be unstable (330, 331). To eliminate mass assignment errors, fragment masses in the acquired spectra were compared with the theoretical fragment formulas and where necessary corrected to their theoretical masses and possible structures. Theoretical masses for fragment ions and potential structures were generated using ACD Labs Mass Fragmenter software that calculates theoretical fragmentation pathways for compounds under specific ionisation conditions (332). MS/MS spectra below 1% of the base peak for each collision energy were removed from the database. Full details of all the MS adducts and MS/MS fragment ion species are shown in Table 3.4.

A searchable database (PCDL) was created by populating it with the accurate mass MS and MS/MS data, acquired above, for the 164 target compounds. In addition, the compound database library was populated with compound information including the name, formula, accurate mass, structure, database identifiers such as the CAS number and Chemspider ID number and retention times which were obtained from

chromatographic analysis of the working standard solution mix of pharmaceuticals. Figure 3.10 shows a screen capture of the PCDL Manager software along with the accurate mass MS/MS spectrum of Meloxicam acquired in positive ionisation mode with a collision energy of 20 eV.

The spectra of the following pharmaceuticals, obtained from the analysis of pure standards, showed significant abundances of adducts of potassium and ammonium that were at least 50% of the base peak (typically the sodiated adduct): Bendroflumethiazide, calcipotriol, cefalexin, chloramphenicol, digoxin, docusate, hydrochlorthiazide, hydrocortisone-21-acetate, ketoprofen, nitrofurantoin, orlistat, pravastatin and spironolactone.

However, with the exception of one compound, docusate, the use of adducts of potassium and ammonium when screening for pharmaceuticals in POCIS or Chemcatcher® extracts against an accurate mass database did not result in an increase in the number of compounds identified or in the score obtained over the use of only protonated and sodiated adducts. Use of only adducts of potassium and ammonium in the positive ion accurate mass database search produced low scores, typically well below the set acceptable threshold. Also, the spectra for the above compounds in POCIS and Chemcatcher® extracts did not show the same ratio of sodium to potassium and ammonium as observed in standards. It was decided, therefore not to include potassium and ammonium adducts in any further investigations.

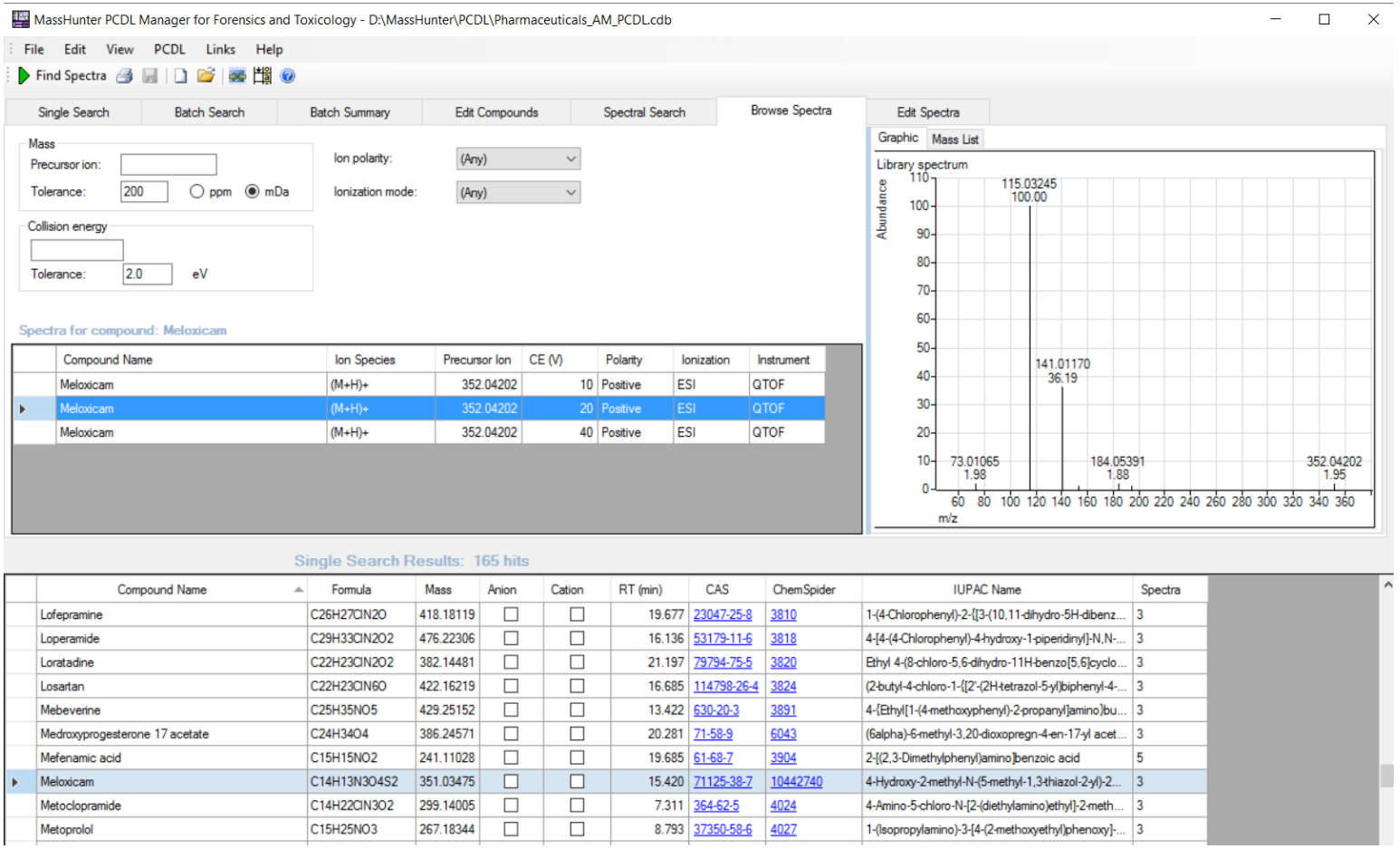


Figure 3.10 PCDL software showing the accurate mass MS/MS spectrum of meloxicam acquired in positive ionisation mode with a collision energy of 20 eV.

Table 3.4 MS adducts and MS/MS fragment ion species for the pharmaceuticals in the personal compound database and library.

Compound determined	Molecular formula	Monoisotopic Mass	Retention time (min)	$(M-H)^-$	$(M+HCOO)^-$	$(M+CH_3COO)^-$	Most abundant fragment ions (CE 20eV)	Most abundant fragment ions (CE 40eV)
			negative ionisation mode					
Chloramphenicol	C11H12Cl2N2O5	322.0123	10.41	321.0051			152.0353, 151.0269, 194.0459, 321.0051, 148.0404	151.0269, 152.0353, 148.0404, 122.0248, 135.0326
Fusidic acid	C31H48O6	516.3451	23.21	515.3378			515.3378, 455.3167, 473.3272, 425.3061, 321.1707	455.3167, 473.3272, 193.1598, 139.1128, 205.1598
Dioctyl sulfosuccinate	C20H38O7S	422.2338	22.66	421.2265			Unstable fragment ions observed	Unstable fragment ions observed
Hydrochlorothiazide	C7H8ClN3O4S2	296.9645	4.34	295.9572			295.9572, 268.9463, 204.9844, 231.9953, 216.9844	204.9844, 202.9687, 203.9766, 123.9960, 189.9735
Ibuprofen	C13H18O2	206.1307	17.39	205.1234	**251.1289**	265.1445	159.1179, 161.1336, 205.1234	Fragmentation too extensive
Mefenamic acid	C15H15NO2	241.1103	19.69	240.1030			196.1132, 240.1030, 194.0975	196.1132, 194.0975, 120.0819, 144.0819, 118.0662
Pravastatin	C23H36O7	424.2461	16.38	423.2388			321.1707, 217.1234	161.0972, 217.1234, 227.1289
Salicylic acid	C7H6O3	138.0317	5.50	137.0244			No fragment ions observed	No fragment ions observed
Compound determined		**Monoisotopic Mass**	**Retention time (min)**	$(M+H)^+$	$(M+Na)^+$	$(M+K)^+$	**Most abundant fragment ions (CE 20eV)**	**Most abundant fragment ions (CE 40eV)**
			positive ionisation mode					
17-alpha-estradiol	C18H24O2	272.1776	18.07	273.1849	295.1669		No fragmentation observed	145.0659, 239.1441, 253.1598, 269.1547, 183.0815
17-beta-estradiol	C18H24O2	272.1776	17.79	273.1849	295.1669		No fragmentation observed	145.0659, 239.1441, 253.1598, 269.1547, 183.0815
Acetaminophen	C8H9NO2	151.0633	4.44	152.0706			110.0600, 93.0335, 65.0386, 92.0495, 152.0706	65.0386, 109.0522, 80.0495, 92.0495, 67.0417
Acetylsalicylic acid	C9H8O4	180.0423	8.29	181.0495	203.0315		93.0346, 59.0139, 137.0244	93.0346, 59.0139, 65.0397, 75.0240
Alfuzosin	C19H27N5O4	389.2063	10.84	390.2136			235.1190, 156.1019, 71.0491, 275.1503	71.0491, 235.1190, 156.1019, 219.0877, 220.0955
Alverine	C20H27N	281.2144	14.47	282.2216			91.0542, 164.1434, 119.0855	91.0542, 65.0386, 58.0651
Amiloride	C6H8ClN7O	229.0479	5.24	230.0552			171.0068, 60.0570, 143.0119, 116.0010, 230.05516	116.0010, 60.0570, 63.9949, 108.0431, 88.9901
Amiodarone	C25H29I2NO3	645.0237	21.13	646.0310			100.1121, 58.0651, 86.0964, 73.0886, 72.0808	58.0651, 100.1121, 86.0964, 72.0808, 73.0886
Amisulpride	C17H27N3O4S	369.1722	6.63	370.1795			242.0482, 112.1121, 196.0063, 129.1386, 155.1179	242.0482, 196.0063, 112.1121, 214.0169, 149.0471
Amitriptyline	C20H23N	277.1831	15.34	278.1903			233.1325, 91.0542, 105.0699, 117.0699, 191.0855	91.0542, 105.0699, 117.0699, 191.0855, 203.0855
Amlodipine	C20H25ClN2O5	408.1452	15.50	409.1525	431.1344		238.0629, 206.0367, 294.0892, 220.0524, 377.1263	170.0600, 165.0102, 208.0609, 149.0153, 220.0524
Amoxicillin	C16H19N3O5S	365.1045	7.22	366.1118	388.0938		114.0008, 134.0600, 160.0427, 70.0651, 208.0400	114.0008, 134.0600, 86.0059, 70.0651, 107.0491
Anastrozole	C17H19N5	293.1641	12.97	230.0246			225.1386, 210.1152	157.0886, 168.0808, 129.0699, 115.0542, 131.0855
Atenolol	C14H22N2O3	266.1630	4.15	267.1703			190.0863, 145.0648, 74.0600, 116.1070, 72.0808	145.0648, 56.0495, 74.0600, 133.0648, 91.0542
Atorvastatin	C33H35FN2O5	558.2530	19.11	559.2603			440.2220, 466.2013, 422.2115, 292.1496, 250.1027	250.1027, 276.1156, 292.1496, 380.1657, 362.1551
Beclomethasone	C22H29ClO5	408.1704	16.77	Absent	431.1596		395.1829, 431.1596, 377.1723	Unstable fragment ions observed
Beclomethasone-dipropionate	C28H37ClO7	520.2228	21.18	521.2301	**543.2120**		503.2195, 393.2060, 429.1827, 373.1565, 411.2166	147.0804, 135.0804, 185.0961, 121.0648, 159.0804
Bendroflumethiazide	C15H14F3N3O4S2	421.0378	13.38	422.0451	444.0270		No fragmentation observed	341.0566, 405.0185
Benzindamine	C19H23N3O	309.1841	14.11	310.1914			86.0964, 58.0651, 265.1335, 174.0788	86.0964, 58.0651, 91.0542, 146.0475, 174.0788

continued

Betahistine	C8H12N2	136.1001	2.35	137.1073			94.0651, 106.0651, 78.0338, 53.0386	Fragmentation too extensive
Betamethasone	C22H29FO5	392.1999	16.49	393.2072	**415.1891**		319.1704, 337.1798, 279.1755, 301.1598, 147.0804	147.0804, 185.0961, 159.0804, 135.0804, 153.0910
Betamethasone-17-valerate	C27H37FO6	476.2574	20.77	477.2647	**499.2466**		279.1743, 337.1798, 319.1693, 147.0804, 291.1743	147.0804, 171.0804, 263.1430, 159.0804, 279.1743
Bezafibrate	C19H20ClNO4	361.1081	17.03	362.1154	**384.0973**		138.9945, 121.0648, 316.1099, 276.0781, 161.0961	138.9945, 121.0648, 110.9996, 93.0699, 77.0386
Bisacodyl	C22H19NO4	361.1314	17.16	**362.1387**	384.1206	400.0946	184.0757, 226.0863, 320.1281, 167.0730	184.0757, 167.0730, 183.0679, 156.0808, 166.0651
Bisoprolol	C18H31NO4	325.2253	11.63	326.2326			116.1070, 74.0600, 72.0808, 98.0964, 56.0495	74.0600, 56.0495, 107.0491, 72.0808, 116.1070
Budesonide	C25H34O6	430.2355	19.16	431.2428			147.0804, 413.2323, 173.0961, 323.1642, 225.1274	147.0804, 173.0961, 121.0648, 171.0804, 225.1274
Bumetanide	C17H20N2O5S	364.1093	16.05	365.1166	**387.0985**		240.1383, 184.0757, 284.12812, 196.0730, 212.1434	Fragmentation too extensive
Candesartan	C24H20N6O3	440.1597	22.14	441.1670			263.1305, 207.0917, 235.1190, 192.0808, 352.1094	192.0808, 207.0917, 190.0651, 180.0808, 165.0699
Carbamazepine	C15H12N2O	236.0950	14.60	237.1022			194.0964, 192.0808, 193.0886, 179.0730	193.0886, 194.0964, 179.0730, 192.0808, 165.0699
Carvedilol	C24H26N2O4	406.1893	13.69	407.1965			224.1281, 100.0757, 222.0913, 283.1441, 180.1019	100.0757, 56.04948, 194.0964, 222.0913, 184.0757
Cefalexin	C16H17N3O4S	347.0940	16.09	348.1013			Unstable fragment ions observed	Unstable fragment ions observed
Celecoxib	C17H14F3N3O2S	381.0759	19.01	382.0832			362.0769, 303.1065, 300.0831, 302.0987, 301.0909	362.0769, 282.0925, 281.0847, 301.0909, 300.0831
Celiprolol	C20H33N3O4	388.1554	10.63	389.1626			251.1179, 307.1652, 74.0600, 306.1812, 324.1918	74.0600, 56.0495, 74.0964, 100.0757, 72.0444
Cetirizine	C21H25ClN2O3	388.1554	16.72	**389.1627**	411.1446		201.0466, 166.0777, 165.0699, 187.1077	166.0777, 201.0466, 165.0699, 199.0309
Chlorhexidine	C22H30Cl2N10	504.2032	14.20	505.2105			336.1698, 184.1557, 201.1822, 353.1964, 170.0480	170.0480, 125.1073, 184.1557, 167.1291, 159.1604
Chlorpheniramine	C16H19ClN2	274.1237	11.88	275.1310			230.0731, 167.0730, 202.0418	167.0730, 230.0731, 201.0340, 118.0651, 125.0153
Chlorpromazine	C17H19ClN2S	318.0958	16.32	319.1030			86.0964, 58.0651, 246.0139, 239.0763, 274.0452	58.0651, 86.0964, 214.0418, 246.0139, 211.0450
Chlortetracycline	C22H23ClN2O8	478.1143	11.03	479.1216	**501.1035**		444.0845, 462.0950, 154.0504, 479.1216, 371.0317	98.0605, 154.0504, 303.0419, 275.0470, 301.0262
Cinnarizine	C26H28N2	368.2253	17.90	369.2325			167.08553, 165.0699, 152.0621, 117.0699	167.0855, 165.0699, 152.0621, 166.0777, 151.0542
Citalopram	C20H21FN2O	324.1638	12.52	325.1711			109.0448, 262.1027, 234.0714, 116.0495, 58.0663	109.0448, 234.0714, 116.0495, 247.0792, 246.0714
Clarithromycin	C38H69NO13	747.4769	17.23	748.4842			158.1176, 590.3899, 558.3637, 116.1070, 83.0491	158.1176, 83.0491, 116.1070, 116.0706, 98.0964
Clobetasol propionate	C25H32ClFO5	466.1922	19.70	467.1995			278.1677, 355.1459, 263.1442, 279.1755, 147.0816	263.1442, 147.0816, 171.0804, 121.0659, 159.0816
Clobetasone-17-butyrate	C26H32ClFO5	478.1922	20.52	479.1995			71.0491, 343.1459, 279.1380, 371.1409, 329.1303	266.1665, 251.1430, 261.1274, 159.0804, 71.0491
Clomipramine	C19H23ClN2	314.1550	16.64	315.1623			86.0964, 58.0651, 242.0731, 270.1044, 235.1356	58.0651, 86.0964, 227.0496, 242.0731, 220.1121
Clonidine	C9H9Cl2N3	229.0174	5.22	230.0246			No fragmentation observed	159.9715, 144.9606, 123.9949, 132.9606, 135.9949
Clopidogrel	C16H16ClNO2S	321.0590	20.65	322.0663			184.0519, 212.0473, 152.0257, 183.0207, 155.0258	155.0258, 125.0153, 152.0257, 91.0542, 183.0207
Clotrimazole	C22H17ClN2	344.1080	20.22	345.1153	367.0972		277.0779, 165.0699, 242.1090, 241.1012, 199.0309	165.0699, 241.1012, 199.0309, 242.1090, 239.0855
Cromoglicic acid	C23H16O11	468.0693	8.54	**469.0765**	491.0585		245.0445, 207.0288, 263.0550, 451.0660, 57.0335	207.0288, 245.0445, 57.0335, 163.0390, 217.0495
Cyclizine / Marzine	C18H22N2	266.1783	13.23	267.1856			167.0855, 165.0699, 152.0621, 166.0777	152.0621, 165.0699, 167.0855, 166.0777, 151.0542
Cyproterone acetate	C24H29ClO4	416.1755	19.68	417.1827			417.1827, 357.1616, 321.1849, 313.1354, 315.1510	277.1587, 313.1354, 237.1274, 321.1849, 159.0804
Desogestrel	C22H30O	310.2297	24.48	311.2369			311.2369, 293.2264, 159.1168, 177.1274, 185.1325	119.0855, 159.1168, 185.1325, 199.1479, 177.1274
Dexamethasone	C22H29FO5	392.1999	16.48	393.2072	**415.1891**		237.1274, 147.0804, 355.1904, 279.1743, 337.1798	147.0804, 121.0659, 171.0804, 119.0855, 222.1039
Dexamethasone-21-acetate	C24H31FO6	434.2105	17.99	435.2177			237.1274, 147.0804, 291.1743, 319.1693, 227.1430	147.0804, 121.0659, 171.0804, 237.1274, 159.0804
Diclofenac	C14H11Cl2NO2	295.0167	18.99	296.0240			215.0496, 214.0418, 250.0185	214.0418, 215.0496, 180.0808, 179.0730, 178.0651
Digoxin	C41H64O14	780.4296	17.31	781.4369	803.4188	**819.3928**	97.0648, 113.0597, 131.0703, 391.2479, 373.2373	97.0648, 113.0597, 131.07023, 95.0491, 69.0335
Diltiazem-Cis	C22H26N2O4S	414.1613	13.96	415.1686			178.0321, 370.1108, 310.0896, 150.0372, 312.1053	178.0321, 150.0372, 109.0107, 72.0808, 137.0597

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Dipyridamole *	C24H40N8O4	504.3173	17.48	505.3245		No fragmentation observed	429.2721, 385.2459, 487.3140, 460.2905, 399.2589
Donepezil	C24H29NO3	379.2147	12.13	380.2220		91.0542, 362.2115, 288.1509, 243.1380, 151.0992	91.0542, 65.0386, 243.1380, 151.0754, 105.0699
Dosulepin	C19H21NS	295.1395	14.13	296.1467		223.0576, 218.1090, 225.0733, 251.0889, 58.0651	203.0855, 221.0420, 217.1012, 178.0777, 202.0777
Doxazosin	C23H25N5O5	451.1856	14.25	452.1928		344.1717, 247.1190, 290.1612	344.1717, 247.1190, 290.1612, 221.1033, 98.0600
Doxycycline	C22H24N2O8	444.1533	11.59	445.1605	**467.1425**	428.1340, 429.1418, 430.1371, 410.1234, 154.0499	98.0600, 267.0652, 154.0499, 201.0546, 321.0758
Enalapril	C20H28N2O5	376.1998	13.86	377.2071		234.1489, 303.1703, 160.1121, 130.0863, 117.0699	117.0699, 91.0542, 56.0495, 160.1121, 130.0863
Erythromycin	C37H67NO13	733.4612	15.45	734.4685		158.1176, 576.3742, 558.3637, 83.0491, 522.3425	158.1176, 83.0491, 116.1070, 116.0706, 98.0964
Felodipine	C18H19Cl2NO4	383.0691	20.06	384.0764	**406.0583**	338.0345, 352.0502, 324.0189, 340.0371, 384.0764	278.0134, 243.0445, 324.0189, 288.0422, 242.0367
Fenofibrate	C20H21ClO4	360.1128	22.07	361.1201		233.0364, 138.9945, 121.0284, 235.0309, 234.0442	138.9945, 121.0284, 110.9996, 140.9949, 93.0335
Fexofenadine	C32H39NO4	501.2879	15.08	502.2952		484.2846, 466.2741, 171.1168, 262.1590, 131.0855	171.1168, 466.2741, 131.0855, 262.1590, 484.2846
Finasteride	C23H36N2O2	372.2777	18.87	373.2850		317.2224, 318.2216, 305.2587, 72.0444, 57.0699	72.0444, 317.2224, 57.0699, 305.2587, 69.0335
Flecainide	C17H20F6N2O3	414.1378	12.76	415.1451		398.1185, 98.0964, 301.0294, 81.0710, 232.0968	301.0294, 98.0964, 81.0699, 398.1185, 232.0968
Fluconazole	C13H12F2N6O	306.1041	9.77	307.1113		238.0787, 220.0681, 169.0460, 239.0802, 289.1008	70.0400, 139.0354, 127.0354, 121.0448, 151.0354
Flumethasone	C22H28F2O5	410.1905	16.04	411.1978	**433.1797**	253.1223, 121.0648, 277.1587, 235.1117, 237.1274	121.0648, 235.1117, 135.0804, 253.1223, 91.0542
Fluoxetine	C17H18F3NO	309.1341	15.61	310.1413		148.11208	Fragmentation too extensive
Fluticasone-17-Propionate	C25H31F3O5S	500.1844	19.74	501.1917	**523.1737**	293.1536, 275.1430, 313.1598, 205.0659, 265.1587	121.0648, 275.1430, 107.0855, 179.0855, 171.0804
Furosemide	C12H11ClN2O5S	330.0077	11.98	Absent	352.9969	310.9888, 328.9993, 233.9622, 251.0344, 313.9884	249.0187, 214.0499, 231.0082, 168.0444, 170.0003
Gliclazide	C15H21N3O3S	323.1304	16.62	324.1376	**346.1196**	110.0964, 127.1230, 91.0542, 153.1022, 155.0161	91.0542, 110.0964, 127.1230, 111.1043, 153.1022
Haloperidol	C21H23ClFNO2	375.1401	13.45	376.1474		165.0710, 358.1369, 123.0241, 194.0868, 95.0292	123.0241, 165.0710, 95.0292
Hydrocortisone	C21H30O5	362.2093	15.24	363.2166		121.0648, 327.1955, 309.1849, 267.1743, 97.0648	121.0648, 97.0648, 105.0699, 145.1012, 91.0542
Hydrocortisone-21-acetate	C23H32O6	404.2199	16.89	405.2272	**427.2091**	327.1955, 309.1849, 111.0441, 241.1587, 129.0546	111.0441, 55.0542, 83.0491, 121.0648, 101.0597
Hydroxychloroquine	C18H26ClN3O	335.1764	5.55	336.1837		247.0997, 158.1539, 102.0913, 179.0366, 191.0366	179.0366, 247.0997, 102.0913, 191.0366, 69.0699
Hydroxyzine	C21H27ClN2O2	374.1761	15.33	375.1834		201.0466, 166.0777, 165.0699, 173.1285	166.0777, 201.0466, 165.0699, 199.0309
Indapamide	C16H16ClN3O3S	365.0601	13.23	366.0674		132.0808, 91.0542, 117.0573, 133.0886	91.0542, 132.0808, 117.0573, 118.0651, 65.0386
Ipratropium (cation)	C20H30NO3	332.2226	7.04	332.2226		166.1590, 124.1121, 290.1751, 93.0699, 96.0808	124.1121, 93.0699, 166.1590, 96.0808, 91.05423
Irbesartan	C25H28N6O	428.2325	17.94	429.2397		207.0917, 195.1492, 386.2214, 401.2336, 180.0808	207.0917, 180.0808, 206.0839, 192.0754, 84.0808
Ketoconazole	C26H28Cl2N4O4	530.1488	18.15	531.1560		489.1455, 82.0526	82.0526, 489.1455, 255.0082, 112.0757, 177.1022
Ketoprofen	C16H14O3	254.0943	16.38	255.1016		105.0335, 209.0961, 77.0386, 177.0546, 194.0726	77.0386, 105.0335, 103.0542, 51.0229, 165.0699
Labetalol	C19H24N2O3	328.1787	11.22	329.1860		162.0550, 294.1489, 91.0542, 311.1754, 207.1128	91.0542, 162.0550, 134.0600, 106.0651, 147.0441
Lamotrigine	C9H7Cl2N5	255.0079	9.25	256.0151		210.9824, 166.0292, 171.9715, 186.9824, 158.9763	144.9606, 58.0400, 156.9606, 158.9763, 210.9824
Lansoprazole	C16H14F3N3O2S	369.0759	16.09	370.0832	**392.0651**	252.0330, 119.0615, 205.0731, 234.0195, 136.0768	136.0768, 234.0195, 119.0615, 107.0741, 204.0653
Latanoprost	C26H40O5	432.2876	20.74	433.2949	455.2768	337.2162, 207.1380, 379.2632, 131.0855, 171.1168	131.0855, 133.1012, 171.1168, 147.1168, 145.1012
Lidocaine	C14H22N2O	234.1732	7.27	235.1805		86.0964, 58.0651	86.0964, 58.0651
Lisinopril	C21H31N3O5	405.2264	5.89	406.2336		114.0561, 386.2085, 91.0553, 289.1558	114.0561, 91.0553, 68.0506
Lofepramine	C26H27ClN2O	418.1812	20.38	419.1885		224.0837, 196.0524, 208.1121, 168.0575, 236.1434	125.0153, 208.1121, 196.0524, 168.0575, 193.0886
Loperamide	C29H33ClN2O2	476.2231	16.36	477.2303		266.1539, 210.1277, 267.1510	266.1539, 210.1277, 72.0449, 238.1226, 115.0542
Loratadine	C22H23ClN2O2	382.1448	20.96	383.1521		337.1102, 267.0809, 259.1356, 294.1044, 281.0966	267.0809, 259.1356, 266.0731, 280.0888, 281.0966

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Losartan	C22H23ClN6O	422.1622	16.50	423.1695		179.0866, 127.0064, 157.0533, 187.0630, 156.0455	127.0064, 157.0533, 179.0866, 187.0630, 113.9985
Mebeverine	C25H35NO5	429.2515	13.68	430.2588		149.0961, 121.0648, 100.1121, 165.0546, 430.2588	121.0648, 149.0961, 91.0542, 93.0699, 165.0546
Medroxyprogesterone-17-acetate	C24H34O4	386.2457	20.13	387.2530	**409.2349**	123.0804, 327.2319, 285.2213, 97.0648, 267.2107	123.0804, 97.0648, 214.1352, 145.1012, 95.0855
Meloxicam	C14H13N3O4S2	351.0348	15.21	352.0420		115.0325, 141.0117, 73.0107, 184.0539, 194.0270	115.0325, 141.0117, 73.0107, 88.0216, 153.0005
Metoclopramide	C14H22ClN3O2	299.1401	7.59	300.1473		227.0582, 184.0160, 183.0315, 212.0347	184.0160, 212.0347, 183.0315, 227.0582, 140.9976
Metoprolol	C15H25NO3	267.1834	9.05	268.1907		74.0600, 116.1070, 72.0808, 98.0964, 56.0495	56.0495, 103.0542, 74.0600, 91.0542, 105.0699
Miconazole	C18H14Cl4N2O	413.9860	22.03	414.9933		158.9745, 160.9668, 69.0447, 227.0132, 159.9823	158.9745, 160.9668, 159.9823, 69.0447, 99.0445
Mirtazapine	C17H19N3	265.1579	10.15	266.1652		195.0917, 72.0808, 209.1073, 235.1230, 223.1230	195.0917, 194.0839, 72.0808, 92.0495, 209.1073
Mometasone furoate	C27H30Cl2O6	521.1492	19.78	521.1492		355.1459, 263.1430, 279.1743, 278.1665, 503.1387	95.0128, 263.1430, 147.0804, 135.0804, 121.0648
Moxonidine	C9H12ClN5O	241.0730	4.09	242.0803		206.1036, 199.0381, 56.0495, 137.0822, 149.0822	56.0495, 136.0505, 199.0381, 150.0536, 69.0447
Naproxen	C14H14O3	230.0943	16.87	231.1016		185.0961, 170.0726, 153.0699, 154.0777, 155.0855	141.0699, 153.0699, 170.0726, 115.0542, 152.0621
Nebivolol	C22H25F2NO4	405.1752	15.50	406.1824		406.1824, 151.0554, 388.1719, 224.1081, 238.1238	151.0554, 123.0605, 406.1824, 125.0397, 177.0710
Nifedipine	C17H18N2O6	346.1165	16.10	347.1238		254.1050, 239.0815, 253.0972, 195.0917, 211.0866	195.0917, 194.0839, 223.0866, 193.0760, 253.0972
Nitrofurantoin	C8H6N4O5	238.0338	6.75	239.0411	261.0230	77.0033, 152.0102, 124.0040, 64.0193, 99.0200	77.0033, 64.0193, 65.0033, 50.0162, 119.0224
Nor-ethisterone	C20H26O2	298.1933	17.72	299.2006		109.0648, 231.1743, 83.0491, 119.0855, 171.1168	109.0648, 91.0542, 79.0542, 83.0491, 105.0699
Nystatin	C47H75NO17	925.5035	19.82	926.5108	**948.4927**	908.5002, 691.3715, 727.3985, 371.0941, 673.3641	355.0660, 357.0632, 341.0139, 297.1274, 356.0679
Ofloxacin	C18H20FN3O4	361.1438	7.51	362.1511		318.1612, 261.1034, 344.1405, 316.1456, 58.0651	261.1034, 221.0709, 219.0564, 58.0651, 205.0408
Omeprazole	C17H19N3O3S	345.1147	14.97	346.1220		198.0583, 151.0992, 136.0757, 180.0478, 168.1019	136.0757, 180.0478, 150.0913, 121.0886, 108.0808
Orlistat	C29H53NO5	495.3924	25.20	496.3997	**518.3816**	319.2995, 160.0968, 337.3101, 193.1951, 142.0863	114.0913, 123.1168, 319.2995, 193.1951, 142.0863
Oxprenolol	C15H23NO3	265.1678	5.14	266.1751		72.0808, 116.1070, 56.0495, 74.0600, 98.0964	72.0808, 56.0495, 74.0600, 58.0651, 105.0699
Oxybutynin	C22H31NO3	357.2304	16.32	358.2377		124.1121, 142.1226, 72.0808, 97.0284, 54.0338	72.0808, 58.0651, 142.1226, 105.0335, 54.0338
Oxytetracycline	C22H24N2O9	460.1482	8.59	461.1555	**483.1374**	426.1183, 381.0605, 444.1289, 443.1449, 337.0666	201.0506, 283.0561, 98.0574, 350.0381, 337.0666
Pantoprazole	C16H15F2N3O4S	383.0751	14.66	384.0824		138.0550, 200.0376, 153.0784, 107.0730, 152.0706	138.0550, 136.0393, 107.0730, 200.0369, 152.0706
Paroxetine	C19H20FNO3	329.1427	14.41	330.1500		192.1183, 70.0651, 151.0390, 123.0605, 163.0918	70.0651, 135.0605, 109.0448, 123.0605, 56.0495
Penicillin V	C16H18N2O5S	350.0936	13.90	351.1009	**373.0829**	160.0427, 114.0372, 192.0655, 229.0641, 142.0321	114.0372, 160.0427, 142.0321, 116.0342, 107.0491
Perindopril	C19H32N2O5	368.2311	15.23	369.2384		172.1332, 98.0964, 170.1176, 295.2016, 72.0808	98.0964, 72.0808, 56.0495, 172.1332, 124.1121
Phenytoin	C15H12N2O2	252.0899	13.83	253.0972	**275.0791**	182.0964, 104.0495, 225.1022, 105.0335, 132.0444	104.0495, 77.0386, 182.0964, 132.0444, 79.0542
Piroxicam	C15H13N3O4S	331.0627	14.15	332.0700		95.0604, 121.0396, 164.0818, 78.0338, 210.0213	95.0604, 121.0396, 78.0338, 153.0005, 105.0421
Prednisolone	C21H28O5	360.1937	15.21	361.2010	**383.1829**	147.0804, 343.1904, 173.0961, 307.1693, 325.1798	147.0804, 121.0648, 173.0961, 159.0804, 135.0804
Procyclidine	C19H29NO	287.2249	14.48	288.2322		84.0808, 270.2216	84.0808, 55.0542, 56.0495, 91.0542
Progesterone	C21H30O2	314.2246	20.30	315.2319		97.0648, 109.0648, 297.2213, 123.0804, 279.2107	109.0648, 97.0648, 81.0699, 79.0542, 123.0804
Promethazine	C17H20N2S	284.1347	14.03	285.1420		86.0964, 198.0372, 240.0842, 71.0730, 56.0495	86.0964, 198.0372, 71.0730, 56.0495, 154.0651
Propranolol	C16H21NO2	259.1572	12.01	260.1645		116.1070, 183.0804, 74.0600, 72.0808, 56.0495	56.0495, 58.0651, 127.0542, 129.0699, 74.0600
Quinine	C20H24N2O2	324.1838	10.87	325.1911		307.1805, 160.0757, 184.0757, 253.1335, 81.0699	81.0699, 79.0542, 172.0757, 160.0757, 184.0757
Ramipril	C23H32N2O5	416.2311	16.87	417.2384		234.1489, 343.2016, 130.0863, 160.1121, 156.1019	117.0699, 130.0863, 160.1121, 91.0542, 56.0495
Ranitidine	C13H22N4O3S	314.1413	4.25	315.1485		176.0488, 130.0559, 125.0056, 98.0839, 102.0372	102.0372, 125.0056, 81.0335, 97.0760, 130.0559
Risperidone	C23H27FN4O2	410.2118	11.45	411.2191		191.11789	191.1179, 110.0598, 69.0335, 82.0651
Ropinirole	C16H24N2O	260.1889	6.90	261.1961		114.1277, 160.0757, 86.0964, 132.0808, 72.0808	132.0808, 86.0964, 114.1277, 72.0808, 117.0573

continued

Salbutamol	C13H21NO3	239.1521	4.06	240.1594			148.0757, 57.0699, 121.0648, 166.0863, 130.0651	91.0542, 121.0648, 77.0386, 133.0522, 130.0651
Salmeterol	C25H37NO4	415.2723	16.24	416.2795			380.2584, 398.2690, 232.1696, 91.0542, 248.1645	91.0542, 55.0542, 133.0648, 135.0804, 230.1539
Scopolamine	C17H21NO4	303.1471	5.42	**304.1543**	326.1363		138.0913, 156.1019, 121.0648, 110.0964, 304.1543	103.0542, 138.0913, 79.0542, 110.0964, 77.0386
Sertraline	C17H17Cl2N	305.0738	16.29	306.0811			158.9763, 275.0389, 129.0699, 91.0542, 196.9919	158.9763, 129.0699, 122.9996, 128.0621, 91.0542
Sildenafil	C22H30N6O4S	474.2049	15.04	475.2122			58.0651, 100.0995, 99.0917, 311.1496, 283.1190	58.0651, 100.0995, 99.0917, 283.1183, 70.0651
Simvastatin	C25H38O5	418.2719	22.42	**419.2792**	441.2611		199.1481, 173.1325, 225.1638, 201.1121, 243.1743	Fragmentation too extensive
Sotalol	C12H20N2O3S	272.1195	3.61	273.1267			213.0692, 133.0760, 255.1162, 134.0839, 176.1308	133.0760, 106.0651, 107.0730, 116.0495, 134.0839
Spironolactone	C24H32O4S	416.2021	17.68	Absent	439.1914	**455.1653**	341.2111, 187.1117	341.2111, 187.1117, 205.1223, 107.0855, 323.2006
Sulfasalazine	C18H14N4O5S	398.0685	13.59	399.0758			381.0652, 317.1033, 223.0496, 213.0652, 241.0635	119.0128, 94.0526, 223.0496, 147.0189, 91.0178
Sumatriptan	C14H21N3O2S	295.1355	5.03	296.1427			58.0651, 251.0849, 157.0886, 158.0964, 201.1386	58.0651, 157.0886, 156.0808, 130.0685, 158.0964
Tamoxifen	C26H29NO	371.2249	19.56	372.2322			72.0808, 129.0699, 58.0651, 91.0542, 70.0651	72.0808, 70.0651, 129.0699, 91.0542, 57.0573
Tamsulosin	C20H28N2O5S	408.1719	10.25	409.1792			228.0689, 271.1111, 200.0376, 148.0883, 147.0804	200.0376, 148.0883, 147.0804, 228.0682, 120.0444
Telmisartan	C33H30N4O2	514.2369	19.79	515.2442			497.2336, 305.1761, 276.1370	497.2336, 276.1370, 289.1448, 211.0754, 305.1761
Terbinafine	C21H25N	291.1987	18.54	292.2060			141.0699, 93.0699, 91.0542, 105.0699, 79.0542	141.0699, 115.0542, 77.0386, 91.0542, 79.0542
Terbutaline	C12H19NO3	225.1365	3.87	226.1438			152.0706, 125.0597, 107.0491, 57.0699, 135.0441	107.0491, 77.0386, 79.0542, 57.0699, 125.0597
Theophylline	C7H8N4O2	180.0647	5.96	181.0720			124.0505, 96.0556, 69.0447, 137.0822, 108.0556	Fragmentation too extensive
Timolol	C13H24N4O3S	316.1569	8.89	317.1642			74.0600, 261.1016, 244.0750, 188.0488, 57.0699	74.0600, 56.0495, 57.0699, 144.0226, 188.0488
Tramadol	C16H25NO2	263.1885	8.79	264.1958			58.06513	58.06513
Trazodone	C19H22ClN5O	371.1513	11.84	372.1586			176.0818, 148.0524, 78.0338, 237.1135	148.0524, 176.0818, 78.0338, 133.0760, 93.0447
Trimethoprim	C14H18N4O3	290.1379	6.60	291.1452			230.1162, 261.0982, 123.0665, 275.1139, 258.1111	123.0665, 110.0587, 81.0447, 229.1084, 257.1033
Valsartan	C24H29N5O3	435.2270	17.79	436.2343	**458.2163**		207.0917, 235.0951, 291.1479, 306.1686, 180.0781	207.0917, 180.0808, 190.0651, 206.0839, 192.0768
Varenicline	C13H13N3	211.1109	5.59	212.1182			212.1182, 169.0760, 183.0917, 195.0917, 168.0682	168.0682, 169.0760, 183.0917, 180.0682, 195.0917
Venlafaxine	C17H27NO2	277.2042	11.55	278.2115			58.0651, 121.0648, 215.1430, 147.0804, 260.2009	58.0651, 121.0648, 91.0542, 147.0804, 79.0542
Verapamil	C27H38N2O4	454.2832	14.06	455.2904			165.0910, 303.2067, 150.0675, 260.1645, 166.0948	165.0910, 150.0675, 105.0699, 133.0648, 303.2067
Warfarin	C19H16O4	308.1049	17.57	309.1121			251.0703, 163.0390, 147.0804, 173.0233, 183.0804	121.0284, 163.0390, 173.0233, 155.0855, 77.0386

Key

Masses in bold and underlined are strong and ≥ 10-30 % of the base ion(s)

* Strong precursor ion in 40eV CE spectrum

3.4.13 LC Q-TOF-MS analysis

Chromatographic separation of compounds was carried out using an Agilent 1290 Infinity ultra-high performance liquid chromatography (UHPLC) system consisting of a vacuum degasser, binary pump, high performance auto-sampler and thermostated column compartment. The chromatographic column used was an Atlantis T3 Column 2.1 mm (i.d.) × 150 mm, 3.5 μm particle size (Waters, Elstree, UK).

The UHPLC system was coupled to an Agilent G6540A quadrupole time-of-flight mass spectrometer (Q-TOF-MS) equipped with a dual sprayer Agilent Jet Stream electrospray source. A reference mass solution was delivered to the reference sprayer via a pressurised glass bottle (nitrogen gas at 5 psi) containing the reference mass solution. The flow rate was approximately 10 μL/min. The second sprayer was used to deliver the column eluent to the ESI source.

The Q-TOF was operated in 2 GHz extended dynamic range mode and data was acquired in two different modes. Firstly, sample extracts were analysed using positive and negative electrospray ionisation in MS only mode and secondly in 'all ions' MS/MS mode using positive electrospray ionisation only. The use of the 'all ions' acquisition resulted in alternating spectra comprised of a low energy channel (0eV) containing the precursor ion, and two high energy channels (20eV and 40eV) which contained the product ions. The entire chromatographic system was controlled using Agilent Mass Hunter acquisition software (rev. B.06.01). Table 3.5 shows the chromatographic conditions and major MS settings.

Table 3.5 Chromatographic conditions and mass spectrometer settings.

Analytical column	Waters, Atlantis T3 Column 2.1 × 150 mm, 3.5 μm	
Column temperature	40 °C	
Mobile phase	A)	2 mM ammonium acetate + 0.01% formic acid in water
	B)	2 mM ammonium acetate + 0.01% formic acid in methanol

Gradient programme	Time (min)	% B
	00.0	5
	25.0	100
	30.0	100
	30.1	5

Stop time	30.0 min
Post time	10.0 min
Injection volume	20 μL

Mass spectrometer settings

Nebulizer (psi)	50	45
Sheath gas temperature (°C)	350	300
Sheath gas flow (L/min)	12	11
Capillary voltage (V)	3,000	2,000

continued

Table 3.5 continued

Nozzle voltage (V)		
	1,000	750
Fragmentor	135	110
Skimmer 1	65	55
Octopole RF Peak	750	750
<u>MS only mode</u>		
Data acquisition scan rate (Hz)	1	1
Mass range (Da)	50-1,100	50-1,100
<u>All Ions MS/MS mode</u>		
Data acquisition scan rate (Hz)	3	3
Mass range (Da)	50- 1,100	50-1,100

3.4.14 Data Analysis

The sample data was evaluated using Mass Hunter Qualitative Analysis software (B.07.00). A retention time window of ±1 min was specified for each target compound against the experimentally derived retention times. This was required to compensate for potential retention time shifts due to matrix variability and any target compound that fell outside this window was deemed to be absent. Positive identification of pharmaceutical compounds was established by using the find-by-formula data mining algorithm with a mass error setting of no greater than 10 ppm for the molecular ion adduct with additional confirmation obtained from comparing the isotope abundance pattern and isotope spacing mass accuracy. A minimum peak area threshold of 1,000 was set for the detection of the molecular ion (adduct) in MS scan only mode. Weightings were set for the individual criteria used to generate an overall score for identified compounds, these were 100, 60, 50 and 100 for mass accuracy, isotope abundance score, isotope spacing and retention time respectively. An overall score of 60 was set as a threshold for the the positive identification of a compounds molecular adduct ion in the extract, this threshold together with fragment ion confirmation ensured that the potential for false positive reporting was remote.

Fragment ion confirmation utilised a maximum of five of the most abundant ions (when available) from the personal compound database library with a minimum of one required for compound identification. A minimum threshold of five was set for the signal-to-noise ratio for each of the confirmatory ions and all had to be within 6 s of the precursor's retention time to achieve the minimum co-elution score of 80 required for peak compound confirmation. A mass error setting of no greater than 10 ppm was also set for the fragment ions obtained from the precursor ion.

3.4.15 Waste water treatment plants chosen for the deployments

Three WWTPs were chosen for the deployments with all three sites situated in South West Wales serving the areas of Carmarthen, Gowerton (west Swansea) and Llanelli. These were:

i) Carmarthen sewage works, Parc-y-Splott, Llansteffan Road, Carmarthen, latitude 51.835780 longitude -4.3267572. The population equivalent of the works as of 2013 was approximately 22,000 but receives waste water from Glangwili General Hospital, Carmarthen located approximately 2.8 miles north of the works.

ii) Gowerton sewage works, Victoria Road, Gowerton, Swansea, latitude 51.6545 longitude -4.0323. The population equivalent of the works as of 2013 was approximately 50,000.

iii) Llanelli sewage works, Bynea, Llanelli, latitude 51.6636 longitude -4.1098. The population equivalent of the works as of 2013 was approximately 55,000.

All three WWTPs employ identical treatment processes including bar screens to filter solids, fats, greases and large objects, before primary treatment which is a based on conventional activated sludge. Population equivalent data for the three waste water treatment plants was obtained from the operators, Dwr Cymru Welsh Water (DCWW).

3.5 Results and discussion

3.5.1 Identification of pharmaceuticals in MS scan mode

Of the 164 pharmaceuticals targeted using MS scan mode a total of 72 were identified with a mass accuracy of better than 10 ppm with additional confirmation coming from isotope pattern and spacing matching. The retention times of all compounds were within ± 0.2 min of the library retention time. Of the 47 pharmaceutical compound classes listed in Table 3.1, 30 were identified in the extracts of both samplers and, of the classes not found, the majority only contained one compound thereby limiting the possibility of detecting compounds within that class. The number of detections at each site were 71, 68 and 69 for Carmarthen, Gowerton and Llanelli respectively with an identical number identified for both samplers but not all were found at all three sites. Atorvastatin, terbinafine and naproxen were found only at the Carmarthen site whilst Piroxicam was found only at the Llanelli site.

Of the 72 pharmaceuticals identified, 71 were found using positive ionisation and 19 using negative ionisation with 57 and 5 detected in positive ion and negative ion modes only respectively. Of the five pharmaceuticals (ibuprofen, indapamide, naproxen mefenamic acid and salicylic acid) identified in negative ion mode only, all are acidic and, with the exception of indapamide, do not ionise efficiently in positive ion mode due to their very low proton affinities. Ibuprofen, naproxen, mefenamic acid and salicylic acid form strong anions due to the dissociation of a hydrogen atom resulting in a strong response with negative ion electrospray. Indapamide contains two ionisation sites, the sulfamoyl group and the amide functionality, and hydrogen abstraction at both groups is possible with the former process more likely to form a strong anion at a mobile phase pH of 4 under negative ion electrospray conditions.

Indapamide, however, was not observed in positive ion mode despite it containing a primary amine group that, for most compounds, would result in a strong response using positive ion electrospray ionisation. Two possibilities for its non-detection in positive ionisation mode are that indapamide was subject to interference from another compound of similar mass in positive ion mode or its concentration was too low in the passive sample extract to be detected. According to Salgado et al., indapamide has a removal rate of almost 100% during waste water treatment over 6 days so this is the most likely reason for its absence from the positive ion chromatogram (333).

An unexpected observation was that a larger peak area response was obtained for salicylic acid with the Chemcatcher® sampler. A probable explanation for this observation is that salicylic acid, being an anion, adsorbs onto the positively charged groups on the surface of the glass fibre matrix (of the HLB-L disk) through an ion exchange mechanism which is absent from the POCIS sampler. A summary of the results obtained from MS scan mode are shown in Table 3.6.

3.5.2 Peak area responses for cardiovascular drugs at the Carmarthen and Llanelli waste water treatment plants

The peak area response for Valsartan at the Carmarthen site was approximately 20 times higher than for the Gowerton site and twice the response observed at the Llanelli site. Also, the peak area response for dipyridamole, furosemide, verapamil and losartan at Carmarthen were approximately 5–6 times higher than observed at Gowerton and twice that of Llanelli. Both the Carmarthen and Llanelli treatment works receive effluents from hospitals with Carmarthen being the largest and having a specialist cardiac unit serving west Wales; Gowerton, however, does not receive

any hospital effluent. As all of the above drugs are used in cardiovascular treatment the most likely reason for their higher peak area response, especially at Carmarthen, is that the treatment works receive significant inputs of these pharmaceuticals from nearby hospitals.

3.5.3 Identification of pharmaceuticals using the 'all ions' MS/MS technique

Analysis of data files acquired using the 'all ions' MS/MS technique involved correlating the elution profile of the targets precursor ion in the low energy channel (0 eV) with those of the fragments generated under higher energy conditions (20 eV and 40 eV). The fragments within the searchable pharmaceutical compound database were used to form individual extracted ion chromatograms that were then overlaid with those of the precursors. A co-elution score was calculated for each fragment ion based on abundance, peak shape (symmetry), peak width and retention time. In addition, signal-to-noise ratios and mass accuracy calculations for the peak spectra for both molecular ions and fragment ions were performed and assessed against the criteria set in the data analysis software.

Fifty of the 72 pharmaceuticals identified using the MS scan only were unequivocally identified (qualified) using the 'all ions' MS/MS technique, but differences in the number of compounds qualified were observed between the samplers and between sites. The individual number of compounds identified by each sampler at each site are shown in Table 3.7 with the highest number of qualified pharmaceuticals present at the Llanelli site (45) and the lowest at Gowerton (33). A lower number of pharmaceuticals were qualified with the Chemcatcher® sampler and this observation

was strongly correlated with lower signal-to-noise ratios for those compounds that returned scores of less than the set threshold of 60 in the data analysis workflow. The difference in the number of pharmaceuticals identified between sampler types at each site varied between 6 and 8 but only one more compound was identified in the POCIS sampler over the Chemcatcher® for the Gowerton 2 co-deployment.

Unlike selective reaction monitoring on triple quadrupole mass spectrometers, no mass filtering (i.e. selection of precursor ion) takes place ahead of the collision cell with the 'all ions' MS/MS acquisition method. Therefore, other non-target precursor ions in the collision cell will also form fragment ions which may have very similar masses to those of the target compounds leading to a reduction in signal-to-noise ratio as well as a reduction in mass accuracy. The consequence of this is that there is a net loss in the signal-to-noise ratio when fragment ions are formed from the precursor ion potentially decreasing the rate of detection for compounds present in sample extracts at low concentrations. As the Chemcatcher® has a lower uptake rate, a lower mass of compound would be adsorbed onto the HLB-L disk and hence a lower signal-to-noise ratio was obtained using the 'all ions' MS/MS method. Concentrating the Chemcatcher® extract to a lower volume, or making a smaller dilution of the extract, before analysis would improve peak height (or area) and therefore signal-to-noise ratio and detectability.

Figure 3.11(a) shows the overlaid extracted ion chromatograms for the precursor and fragment ions obtained for irbesartan present in the Chemcatcher® deployed at the Llanelli site. Figure 3.11(b) shows the fragment ion mass spectra obtained from the high-energy channels for irbesrtan (shown in green) as well as for other co-eluting

compounds, whilst in Figure 3.11(c) the background fragment ions have been removed thus allowing for the mass accuracy and signal-to-noise ratio calculations to be performed for the irbesrtan fragment ions which were searched for within the compound database. The co-elution score corresponding to each of the fragment ions of irbesartan, under collision energies of 20 eV and 40 eV, are shown in Table 3.8 and all are above the minimum threshold value of 80. In addition, the signal-to-noise ratios for the qualified fragment ions are above the minimum threshold value of 10 and the mass accuracy for the peak spectra for both molecular ions and fragment ions was better than 10 ppm as set in the data analysis software. The extracted ion chromatograms obtained for the compounds fexofenadine and clarithromycin, also present in the Chemcatcher® deployed at the Llanelli site, are shown in Figures 3.12 and 3.13 respectively. The co-elution scores, signal-to-noise ratio values and mass accuracy information for the two compounds are shown in Tables 3.9 and 3.10 respectively. For most of the qualified targeted compounds, at least one and up to five specific fragment ions within the compound database library were observed as qualifier ions.

A further seven pharmaceuticals were identified using the 'all ions' MS/MS method over the MS scan only method. Four of the seven compounds, amiloride, felodipine, mebeverine and scopolamine were identified in the 20 eV collision energy channel together with qualifying fragment ions in at least one of the three sites. The most likely reason for their identification in the 20 eV channel is that the higher collision energy resulted in the fragmentation or reduction in the intensity of compounds of similar mass which prevented their identification when using the MS scan only method. The remaining three compounds, dosulepin, meloxicam and sildenafil were

identified only in the low energy channel but as a higher accelerating voltage is applied to the ions leaving the ion source, when using the 'all ions' MS/MS method, it is thought this was sufficient to increase the number of ionised molecules reaching the detector. Of the 72 compounds identified in MS scan mode, only betamethasone and trazadone, were not identified. Ibuprofen, indapamide, mefenamic acid, naproxen and saliyclic acid were not qualified using the negative ion 'all ions' MS/MS method due to an instrument fault which was traced to one of the amplifier boards.

The results obtained from the screening of pharmaceuticals using the 'all ions' MS/MS technique are summarised in Table 3.7.

3.5.4 Mass accuracy

Mass accuracy calculations were undertaken on the molecular adduct ions in the low energy channel of the 'all ions' MS/MS method for each compound identified, and at each site including the co-deployment at Gowerton. The results including the average for each site are presented in Table 3.11. The mass accuracy of the molecular adducts ions for all identified compounds was generally better than 5 ppm (85 %), with 39 % and 75 % better than 1 ppm and 3 ppm respectively. The average across the three sites varied from 1.85 ppm for Carmarthen (POCIS) to 2.53 ppm at Gowerton (Chemcatcher®). A summary of the mass accuracy statistics obtained for each site is shown in Table 3.12 and a Welch's t-test established that the means (of the mass accuracy) for the Chemcatcher® was not significantly different from the mean of the POCIS ($t = 1.47$, $pr = 0.141$) at the 0.05 level of significance.

Table 3.6 Pharmaceuticals identified in extracts at the three sites using the MS scan method (positive and negative ion modes).

Compound	Carmarthen Chemcatcher®	Carmarthen POCIS	Gowerton 1 Chemcatcher®	Gowerton 1 POCIS	Gowerton 2 Chemcatcher®	Gowerton 2 POCIS	Llanelli Chemcatcher®	Llanelli POCIS
Alfuzosin								
Alverine								
Amisulpride								
Amitriptyline								
Atenolol								
Atorvastatin								
Betamethasone 17 valerate								
Bezafibrate								
Bisoprolol								
Carbamazepine								
Cefalexin								
Celiprolol								
Cetirizine								
Chlorpheniramine								
Citalopram								
Clarithromycin								
Clopidogrel								
Cyclizine								
Diclofenac								
Diltiazem (Cis)								

Continued

Key

Compound present, Compound absent, [a] Analysed in negative ion MS scan

Dipyridamole								
Doxazosin								
Erythromycin								
Fexofenadine								
Flecainide								
Fluconazole								
Furosemide								
Gliclazide								
Irbesartan								
Ketoconazole								
Ketoprofen								
Labetalol								
Lamotrigine								
Lansoprazole								
Lidocaine								
Loperamide								
Loratadine								
Losartan								
Metoclopramide								
Metoprolol								
Mirtazapine								
Nifedipine								
Omeprazole								
Oxprenolol								

Continued

Key

Compound present Compound absent, [a] Analysed in negative ion MS scan

Pantoprazole								
Phenytoin								
Piroxicam								
Procyclidine								
Propranolol								
Quinine								
Ranitidine								
Sulfasalazine								
Salbutamol								
Sertraline								
Sotalol								
Sumatriptan								
Tamsulosin								
Telmisartan								
Terbinafine								
Timolol								
Tramadol								
Trazodone								
Trimethoprim								
Valsartan								
Venlafaxine								
Verapamil								
Warfarin								
Ibuprofen[a]								

Continued

Key

Compound present, Compound absent, [a] Analysed in negative ion MS scan

Indapamide[a]								
Naproxen[a]								
Mefenamic acid[a]								
Salicylic acid[a]								

Key

Compound present, Compound absent, [a] Analysed in negative ion MS scan

Table 3.7 Pharmaceuticals identified in extracts at the three sites using the 'all ions' MS/MS technique.

Compound	Carmarthen Chemcatcher®	Carmarthen POCIS	Gowerton 1 Chemcatcher®	Gowerton 1 POCIS	Gowerton 2 Chemcatcher®	Gowerton 2 POCIS	Llanelli Chemcatcher®	Llanelli POCIS
Alfuzosin								
Alverine								
Amiloride								
Amisulpride								
Amitriptyline								
Atenolol								
Atorvastatin								
Bezafibrate								
Bisoprolol								
Carbamazepine								
Cefalexin								
Celiprolol								
Cetirizine								
Chlorpheniramine								
Citalopram								
Clarithromycin								
Clopidogrel								
Cyclizine								

continued

Key

Compound present in low energy MS channel of 'all ions MS/MS' method,

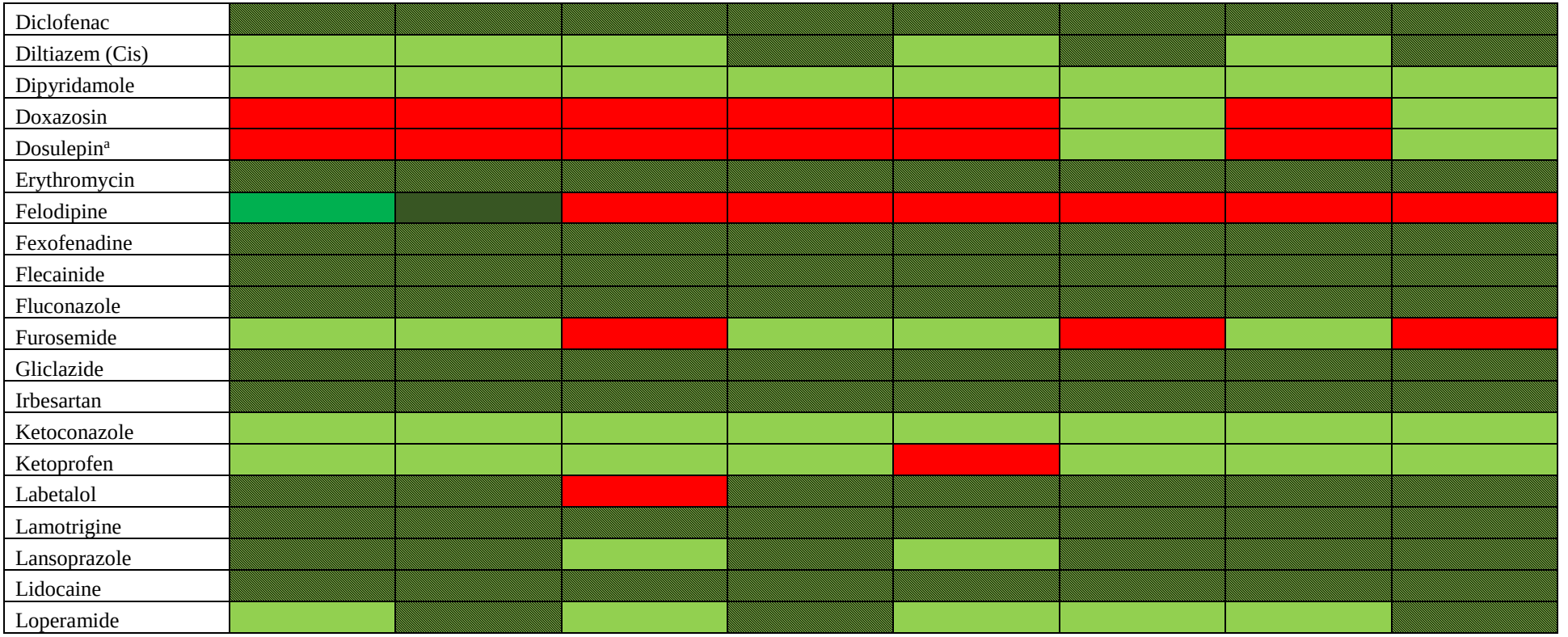
Compound identified in the 20eV CE channel of 'all ions MS/MS' method

Qualifying fragment ions present from 'all ions MS/MS' method,

Molecular adduct ion identified in the 20eV CE channel with qualifying fragment ions

Adduct ion of compound absent

[a] Not identified in MS Scan only analysis



continued

Key

Compound present in low energy MS channel of 'all ions MS/MS' method,

Compound identified in the 20eV CE channel of 'all ions MS/MS' method

Qualifying fragment ions present from 'all ions MS/MS' method,

Molecular adduct ion identified in the 20eV CE channel with qualifying fragment ions

Adduct ion of compound absent

[a] Not identified in MS Scan only analysis

Loratadine								
Losartan								
Mebeverine								
Meloxicam[a]								
Metoclopramide								
Metoprolol								
Mirtazapine								
Nifedipine								
Omeprazole								
Oxprenolol								
Pantoprazole								
Phenytoin								
Piroxicam								
Procyclidine								
Propranolol								
Quinine								
Ranitidine								
Sulfasalazine								
Salbutamol								
Scopolamine								

continued

Key

Compound present in low energy MS channel of 'all ions MS/MS' method,

Qualifying fragment ions present from 'all ions MS/MS' method,

Adduct ion of compound absent

Compound identified in the 20eV CE channel of 'all ions MS/MS' method

Molecular adduct ion identified in the 20eV CE channel with qualifying fragment ions

[a] Not identified in MS Scan only analysis

Sertraline								
Sildenafil[a]								
Sotalol								
Sumatriptan								
Tamsulosin								
Telmisartan								
Terbinafine								
Timolol								
Tramadol								
Trimethoprim								
Valsartan								
Venlafaxine								
Verapamil								
Warfarin								
No. of compounds identified using 'all ions' MS/MS method	33	39	30	38	33	34	39	45

Key

Compound present in low energy MS channel of 'all ions MS/MS' method,

Qualifying fragment ions present from 'all ions MS/MS' method,

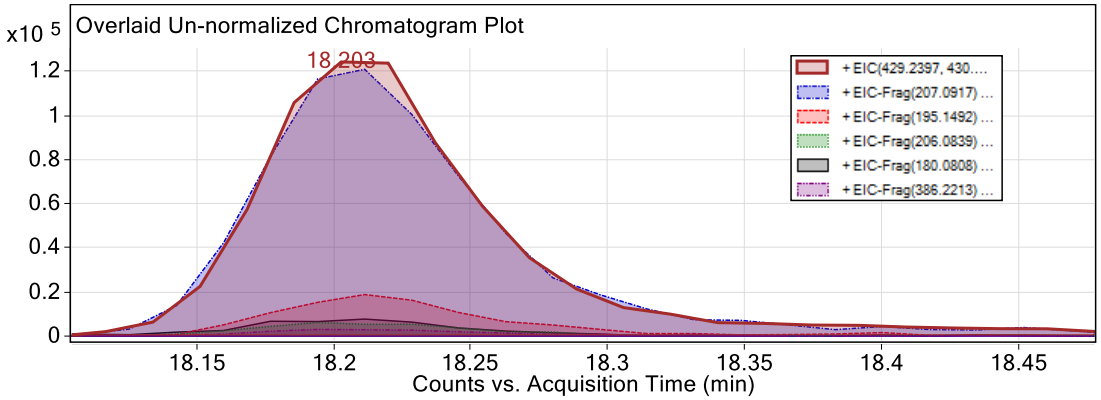
Adduct ion of compound absent

Compound identified in the 20eV CE channel of 'all ions MS/MS' method

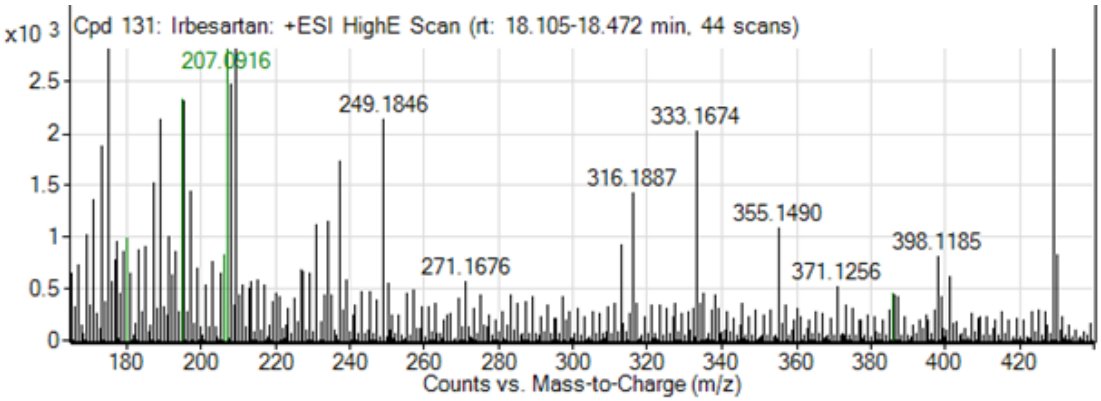
Molecular adduct ion identified in the 20eV CE channel with qualifying fragment ions

[a] Not identified in MS Scan only analysis

a



b



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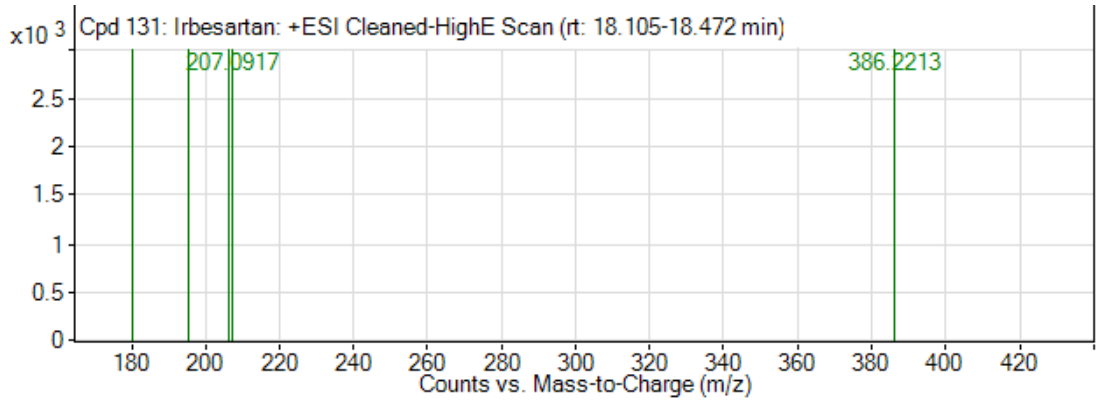


Figure 3.12 Overlay of precursor and fragment ion extracted ion chromatograms for irbesartan in a Chemcatcher® from Llanelli WWTW (a), high collision energy scan displaying all fragment ions (b) and cleaned high collision energy scan displaying only irbesartan fragment ions (c).

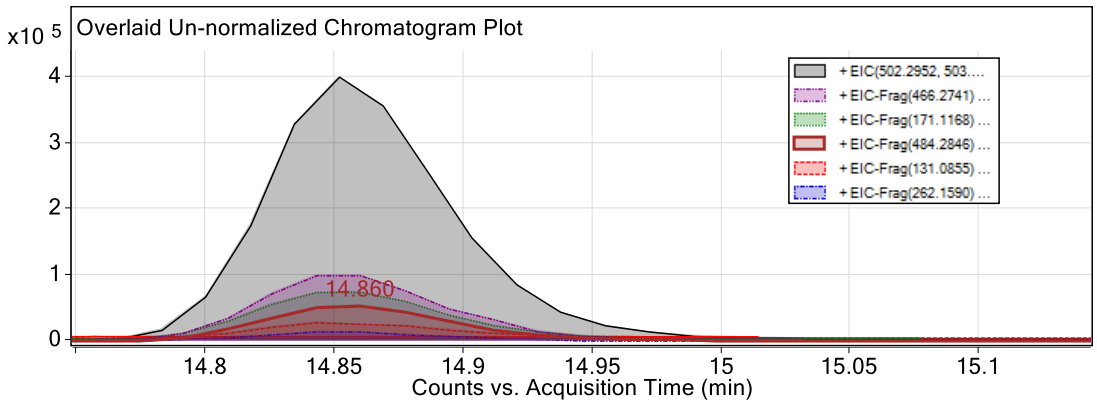
Table 3.8 Co-elution scores for the individual irbesartan fragment ions, together with scores for the precursor mass accuracy, mass and retention time differences.

Name	Formula	m/z	Mass	Mass (Tgt)	Diff (ppm)	Score (Tgt)	RT	RT (Tgt)	RT Diff	Score (RT)	Species
Irbesartan	C_{25} H_{28} N_6 O	429.2399 451.2216	428.2326	428.2325	-0.29	99.61	18.203	18.2	0.003	99.94	$(M+H)^+$ $(M+Na)^+$

Coelution Score	CE	Flags(FIs)	Height	m/z	Name	RT	RT Diff	SNR
98.9	20	Qualified	18802.7	195.1492	Irbesartan	18.211	0.009	44.4
99.5	40	Qualified	120821	207.0917	Irbesartan	18.211	0.009	37.3
97	20	Qualified	3011	386.2213	Irbesartan	18.194	0.009	12.9
96.7	40	Qualified	7705.2	180.0808	Irbesartan	18.211	0.009	11.7
98.1	40	Qualified	6189.7	206.0838	Irbesartan	18.194	0.009	11.5

Score (iso. abund)	Score (mass)	Score (MS)	Score (iso. spacing)	Height	Species	m/z
98.33	99.87	99.46	99.98	69055.4	$(M+H)^+$	429.2399

a



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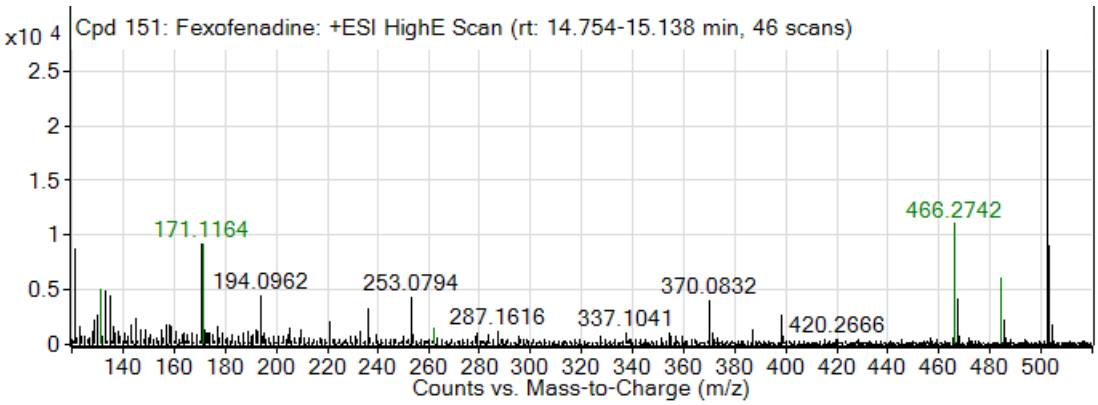


c

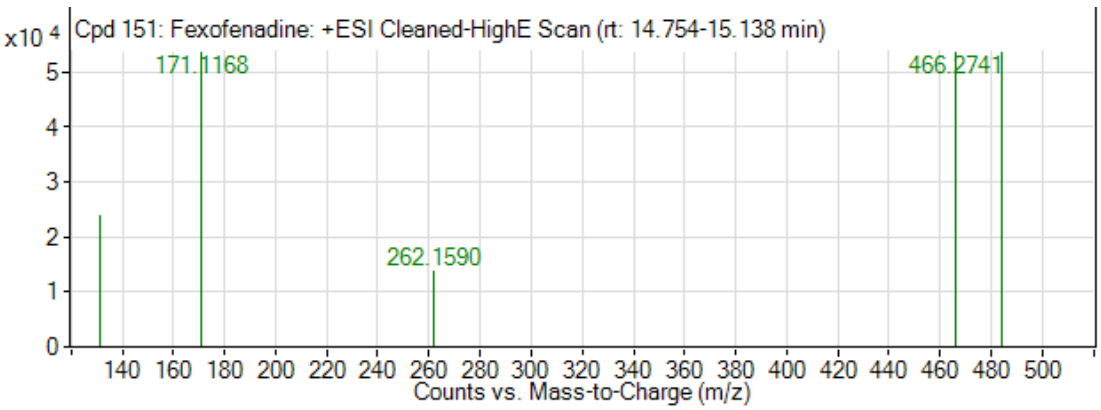


Figure 3.13 Overlay of precursor and fragment ion extracted ion chromatograms for fexofenadine in a Chemcatcher® from Llanelli WWTW (a), high collision energy scan displaying all fragment ions (b) and cleaned high collision energy scan displaying only fexofenadine fragment ions (c).

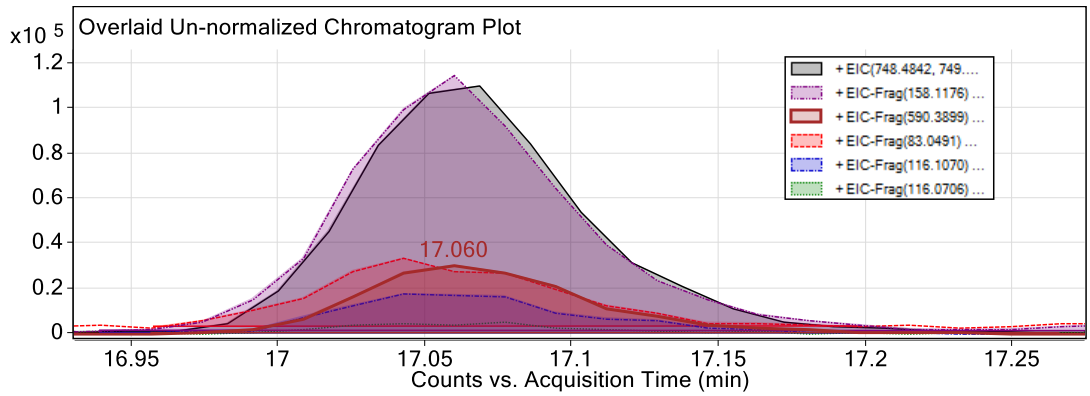
Table 3.9 Co-elution scores for the individual fexofenadine fragment ions, together with scores for the precursor mass accuracy, mass and retention time differences.

Name	Formula	m/z	Mass	Mass (Tgt)	Diff (ppm)	Score (Tgt)	RT	RT (Tgt)	Score (RT)	Species
Fexofenadine	C_{32} H_{39} N O_4	502.2959 524.2784	501.2885	501.2879	-1.11	97.5	14.851	14.87	97.44	$(M+H)^+$ $(M+Na)^+$

Coelution Score	CE	Flags(FIs)	Height	m/z	Name	RT	RT Diff	SNR
99.6	20	Qualified	53886.5	484.2846	Fexofenadine	14.86	0.009	139.6
99.5	40	Qualified	99160.2	466.2741	Fexofenadine	14.843	0.009	156.2
99.1	40	Qualified	72359.9	171.1168	Fexofenadine	14.86	0.009	34.1
98.9	40	Qualified	13738.1	262.159	Fexofenadine	14.843	0.009	30.5
98.9	40	Qualified	23843.8	131.0855	Fexofenadine	14.843	0.009	7.3

Score (iso. abund)	Score (mass)	Score (MS)	Score (iso. spacing)	Height	Species	m/z
94.67	98.67	97.53	98.68	209463.1	$(M+H)^+$	502.2959

a



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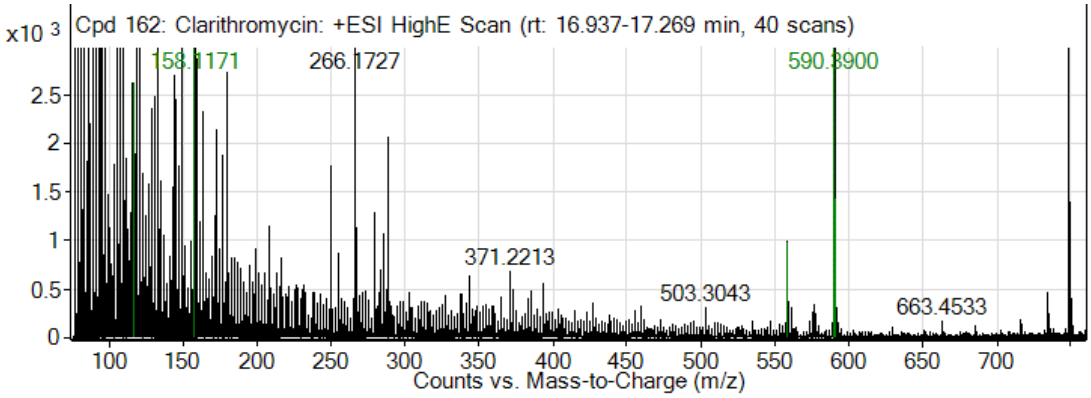


c

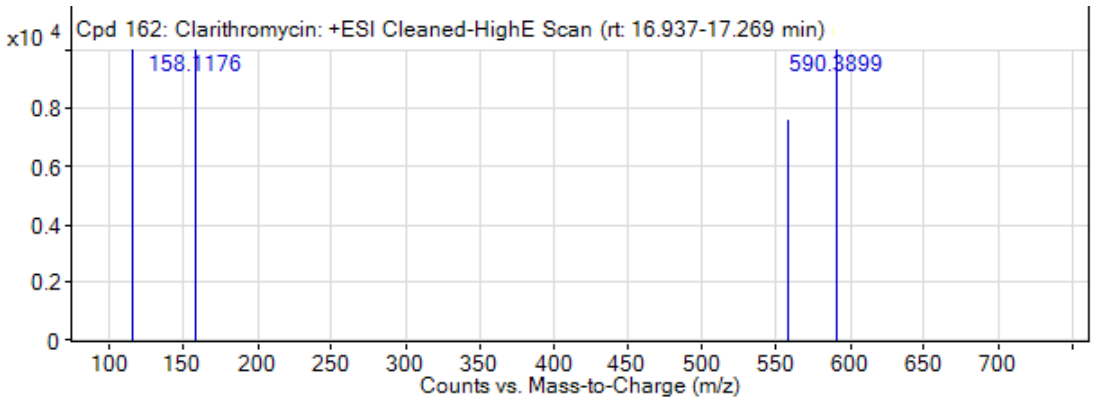


Figure 3.14 Overlay of precursor and fragment ion extracted ion chromatograms for clarithromycin in a Chemcatcher® from Llanelli WWTW (a), high collision energy scan displaying all fragment ions (b) and cleaned high collision energy scan displaying only clarithromycin fragment ions (c).

Table 3.10 Co-elution scores for the individual clarithromycin fragment ions, together with scores for the precursor mass accuracy, mass and retention time differences.

Name	Formula	m/z	Mass	Mass (Tgt)	Diff (ppm)	Score (Tgt)	RT	RT (Tgt)	Score (RT)	Species
Clarithromycin	C_{38} H_{69} N O_{13}	748.4846	747.4771	747.4769	-0.34	99.72	17.068	17.068	100	$(M+H)^+$

Coelution Score	CE	Flags(FIs)	Height	m/z	Name	RT	RT Diff	SNR
99.5	20	Qualified	30344	590.3899	Clarithromycin	17.06	0.009	131.9
99.3	40	Qualified	113402	158.1176	Clarithromycin	17.06	0.009	44
98.2	40	Qualified	17603	116.107	Clarithromycin	17.043	0.026	21.5
92.5	40	Qualified	30561	83.0491	Clarithromycin	17.043	0.026	10.7
		Low S/N ratio	5060	116.0706	Clarithromycin	17.077	0.009	4

Score (iso. abund)	Score (mass)	Score (MS)	Score (iso. spacing)	Height	Species	m/z
99.45	99.84	99.58	99.23	56577	$(M+H)^+$	748.4846

Table 3.11 Mass accuracy (in ppm) for compounds identified using the 'all ions' MS/MS method.

Compound	Carmarthen Chemcatcher®	Carmarthen POCIS	Gowerton 1 Chemcatcher®	Gowerton 1 POCIS	Gowerton 2 Chemcatcher®	Gowerton 2 POCIS	Llanelli Chemcatcher®	Llanelli POCIS
Alfuzosin	-2.90	-3.00	-3.10	-2.93	-6.96	-4.53	-7.95	-2.89
Alverine	-2.71	-1.63	-9.65	-2.77	-8.42	-6.65	-2.57	-2.73
Amiloride	3.74	-0.08	-1.55	-5.95	nd	-5.80	1.81	-1.63
Amisulpride	1.97	1.68	1.09	1.12	0.78	1.34	0.55	1.07
Amitriptyline	0.42	-0.32	-0.41	-0.75	-0.74	-0.28	-0.45	-0.91
Atenolol	1.23	1.23	0.71	0.32	0.83	0.55	0.35	1.11
Atorvastatin	1.46	1.76	nd	nd	nd	nd	nd	nd
Bezafibrate	3.47	1.25	5.08	1.27	2.22	1.18	0.49	-2.08
Bisoprolol	-2.05	-3.37	-1.09	-1.51	-1.24	-1.00	-0.83	-2.21
Carbamazepine	1.41	-1.27	1.02	0.63	1.00	0.41	1.68	0.02
Cefalexin	6.79	5.90	nd	6.20	6.49	4.70	4.95	3.08
Celiprolol	-0.26	-1.03	-0.21	-0.60	-0.14	-0.24	0.32	0.11
Cetirizine	0.94	0.18	1.01	0.78	0.95	0.25	1.00	0.60
Chlorpheniramine	8.00	5.66	3.97	9.12	2.33	5.54	nd	4.64
Citalopram	0.86	0.25	1.15	1.47	0.81	1.35	0.97	1.10
Clarithromycin	1.00	1.41	0.19	0.38	0.50	0.35	0.34	1.07
Clopidogrel	0.51	0.21	1.08	-0.25	0.48	0.03	0.49	0.26
Cyclizine	-2.29	-1.85	-2.75	-1.29	-2.96	-1.99	-1.55	-1.50
Diclofenac	-1.44	-2.35	-0.05	-0.42	-0.60	-0.95	-0.85	-2.25
Diltiazem (Cis)	5.57	4.29	6.30	0.96	8.09	3.50	8.81	0.75
Dipyridamole	-1.15	-1.99	-1.54	-2.31	-5.59	-3.03	-1.80	-2.49
Dosulepin	nd	nd	nd	nd	nd	2.62	nd	5.34

continued

Doxazosin	nd	nd	nd	nd	nd	-0.22	nd	-3.96
Erythromycin	0.29	0.03	-0.09	0.38	0.52	0.26	0.23	0.23
Felodipine	-3.37	nd	nd	nd	nd	nd	nd	nd
Fexofenadine	1.07	0.98	1.42	1.17	1.18	1.11	1.11	0.77
Flecainide	-0.01	-0.03	-0.20	0.18	-0.30	-0.09	1.26	-0.33
Fluconazole	0.62	1.59	0.61	1.09	3.35	1.74	1.30	-0.21
Furosemide	7.28	-0.49	nd	2.19	1.07	nd	1.61	nd
Gliclazide	2.01	1.64	1.57	1.93	1.98	2.30	2.14	0.99
Irbesartan	0.17	-0.10	0.56	0.64	0.41	0.66	0.29	0.45
Ketoconazole	0.19	1.54	1.79	-0.16	1.46	1.35	0.21	1.02
Ketoprofen	2.26	4.34	6.11	7.31	nd	2.66	1.97	5.53
Labetalol	-0.08	0.41	nd	0.08	1.82	0.98	0.75	0.89
Lamotrigine	1.24	1.47	1.49	1.86	1.02	2.21	1.67	1.56
Lansoprazole	5.38	5.77	6.53	5.55	4.16	4.90	4.13	2.74
Lidocaine	-0.01	0.30	-0.72	-1.47	-1.63	-1.27	-0.47	-0.28
Loperamide	3.05	2.20	0.08	0.03	3.28	2.33	5.90	0.84
Loratadine	2.61	3.31	3.95	1.01	1.59	1.54	7.12	4.24
Losartan	1.87	0.26	3.95	2.86	7.47	7.48	5.64	5.55
Mebeverine	nd	nd	-7.03	nd	-0.38	nd	-3.45	-3.88
Meloxicam	0.74	0.62	0.30	8.13	-0.42	-2.55	3.11	7.23
Metoclopramide	1.24	0.86	2.34	1.81	2.01	2.25	1.78	1.22
Metoprolol	0.46	-0.76	-0.81	-0.40	-0.34	-0.18	-0.27	0.59
Mirtazapine	-0.50	-1.02	-0.51	-0.48	-0.56	-0.56	-0.99	-0.01
Nifedipine	0.25	nd	nd	nd	nd	nd	0.50	2.29
Omeprazole	-4.37	-1.22	-0.18	1.33	5.09	2.41	9.75	6.29
Oxprenolol	9.98	0.90	1.65	0.37	0.75	0.71	0.76	0.90
Pantoprazole	nd	2.06	6.04	nd	nd	2.30	9.12	1.61

continued

Phenytoin	0.67	-1.36	-0.51	-1.42	-0.48	-0.47	-0.55	-1.00
Piroxicam	nd	nd	nd	nd	nd	nd	1.51	-0.04
Procyclidine	-0.29	6.15	7.60	7.86	7.42	7.79	7.09	6.06
Propranolol	9.75	0.23	0.65	1.43	0.82	1.47	0.09	0.85
Quinine	0.16	-1.36	-8.30	-4.38	-9.63	nd	-1.77	-1.59
Ranitidine	-1.05	3.62	2.09	0.84	4.61	3.71	2.32	4.79
Salbutamol	4.74	-0.84	-1.15	-0.04	0.18	-2.64	0.54	0.99
Scopolamine	nd	0.49	nd	0.38	-2.07	-0.63	nd	nd
Sertraline	-0.71	-0.75	0.67	0.49	0.51	0.04	0.75	-0.97
Sildenafil	9.81	7.89	5.35	2.97	5.16	nd	6.14	5.85
Sotalol	1.39	1.52	1.87	1.35	1.39	1.78	1.46	0.96
Sulfasalazine	1.73	-1.35	-0.07	0.70	-0.36	0.43	1.18	-0.69
Sumatriptan	-0.59	0.21	1.29	0.22	0.79	1.07	1.05	0.81
Tamsulosin	nd	nd	1.03	4.85	1.83	1.02	nd	9.97
Telmisartan	-0.30	0.01	0.50	-0.15	0.37	-0.55	0.69	-0.11
Terbinafine	nd	-5.65	nd	nd	nd	nd	nd	nd
Timolol	nd	1.09	nd	nd	4.56	-9.83	nd	nd
Tramadol	0.34	-5.27	-3.43	-3.18	-4.10	0.17	-3.53	-5.94
Trimethoprim	-7.67	1.64	2.42	2.08	2.32	2.56	2.32	2.08
Valsartan	2.34	0.09	9.17	6.66	nd	nd	3.72	2.00
Venlafaxine	1.09	-4.10	-9.46	-9.97	-9.89	-9.99	-9.11	-9.01
Verapamil	9.87	-2.45	-1.50	nd	-4.94	-2.44	-0.71	-1.16
Warfarin	-0.18	-1.18	3.28	1.07	1.16	-2.94	0.38	-4.69

Key
nd not detected

Table 3.12 Summary of the mass accuracy statistics (in ppm) obtained for each site.

Compound	Carmarthen Chemcatcher®	Carmarthen POCIS	Gowerton 1 Chemcatcher®	Gowerton 1 POCIS	Gowerton 2 Chemcatcher®	Gowerton 2 POCIS	Llanelli Chemcatcher®	Llanelli POCIS
Mean	2.41	1.85	2.50	2.16	2.53	2.22	2.35	2.29
Standard Error	0.35	0.22	0.34	0.32	0.34	0.29	0.33	0.28
Standard Deviation	2.76	1.81	2.66	2.48	2.62	2.31	2.60	2.26
Sample Variance	7.63	3.28	7.09	6.17	6.86	5.32	6.75	5.13
Number of compounds detected	63	66	60	61	61	63	63	67

3.6 Conclusions

A searchable accurate mass compound database containing all relevant ion species and selective qualifier ions for 164 pharmaceuticals was developed for the screening of pharmaceuticals in extracts of polar passive samplers using an UHPLC coupled to a Q-TOF-MS system. Chemcatcher® and POCIS passive samplers were deployed for a period of almost four weeks at three sewage treatment works in west Wales in 2014. Following retrieval and subsequent processing of the passive samplers the extracts obtained were screened using the newly developed 'all ions' MS/MS technique and a total of 79 pharmaceuticals were identified with high confidence. Fifty were unequivocally confirmed through the use of selective qualifier ions using the accurate mass compound database as the fragment ion source, and evaluating the five most specific ions from the MS/MS spectral library.

Differences in the number of pharmaceuticals identified were observed between the MS scan only method and the 'all ions' MS/MS technique with seven additional pharmaceuticals identified using the new method but two compounds missed which were present in the MS scan data. Excellent mass accuracy was obtained from the analysis and was strongly correlated with chromatographic peak area or height. 75 and 85% of compounds were identified with errors of less than 3 and 5 ppm respectively. Clean-up of the extracts using selective sorbents to remove potential interfering compounds, which lead to large mass measurement errors, would be highly beneficial. Further optimisation of the Q-TOF-MS methods may also result in a higher number of compounds being detected.

The method takes full advantage of UHPLC separations to increase the chromatographic resolution and benefits from the sensitivity and selectivity of a modern Q-TOF-MS instrument. The developed technique is an ideal complement for existing target and suspect screening methods. Applying both MS scan and the 'all ions' MS/MS methods resulted in a highly efficient workflow for the analysis of the passive sampling extracts. The 'all ions' MS/MS acquisition method is very fast and the acquired data can be re-interrogated at a later time for compounds that were outside the scope of the analysis during measurement allowing for retrospective data analysis for new emerging contaminants such as pharmaceutical metabolites, their transformation products plus personal care products without the need to reacquire data from old samples. It may also be possible to transfer the method to other instrument vendor systems which employ high resolution accurate mass detection. The results obtained clearly show the value of the approach developed and when combined with an efficient data analysis workflow should prove invaluable for investigative monitoring purposes within the remit of the WFD.

Chapter 4

Comparison of the sampling efficiency of two passive samplers for organic compounds in effluents: Polar Chemcatcher® versus the polar organic chemical integrative sampler (POCIS)

4.1 Introduction

The purpose of this chapter was to compare the sampling efficiency of two passive samplers, namely the polar Chemcatcher® and the polar organic chemical integrative sampler (POCIS), for organic compounds in effluents using an identical solid phase extraction sorbent commonly used in sample preparation.

The use of quality-controlled, commercially available receiving phases within the Chemcatcher® device allows for high reproducibility and ease of use when compared with some other passive sampling devices. Both Chemcatcher® and POCIS samplers employ a solid sorbent sandwiched between polyethersulphone membranes which expose a sampling area of 45.8 cm^2 for the POCIS sampler and 15.2 cm^2 for the Chemcatcher, a factor of 3.01. With regards to the Chemcatcher® the immobilised receiving phase, within a disk of PTFE fibrils or glass fibre, ensures that the active sampling area of the device remains constant during field deployments and laboratory calibration uptake experiments. The sorbent in the POCIS sampler is in the form of a free-flowing powder and may not therefore have the same effective sampling area throughout the deployment period. In addition, unless care is taken during assembly of POCIS devices, losses of sorbent from POCIS devices can occur which, if significant, could lead to irreproducible results (334-336).

Until recently the sorbent used by the POCIS sampler (Waters Oasis® HLB) was unavailable in the 47mm disk format used in the Chemcatcher®. However, the recent introduction of a commercially available SPE disk (Horizon Technology Atlantic range), which incorporates the same phase in the 47mm format, has allowed the Chemcatcher® to be used with one of the most commonly used and versatile solid

phase extraction sorbent phases used in environmental analysis. It was hypothesised that both polar passive samplers would behave in a similar manner.

4.2 Aims and objectives

i) Determine, via a combination of LC Q-TOF-MS analysis and statistical tools, the reproducibility of chromatographic peak areas of targeted pharmaceuticals identified in extracts obtained from two passive samplers deployed in a waste water effluent stream.

ii) Determine statistically, using compound peak areas obtained from LC Q-TOF-MS analysis, whether the relative uptake rates of the targeted pharmaceuticals and 'unknown' peaks (features) differed between samplers at different sites and between co-deployed samplers at one site.

iii) Determine through the use of mass profiling and statistical software, whether the range of molecular masses for 'unknown' compounds adsorbed by both samplers was similar.

4.3 Experimental

4.3.1 Data acquisition and analysis

The following workflow, typically used in LC-MS based metabolomics, was used to acquire and process the known and 'unknowns' data and involved the following steps (337).

i. Data acquisition using high resolution accurate mass LC-MS in MS only mode.
ii. Compound and feature extraction from targeted and untargeted analysis.
iii. Retention time alignment.
iv. Integration of peak area.
v. Export of data for statistical evaluation using Agilent Mass Profinder and Mass Profiler software in conjunction with Microsoft Excel and Minitab statistical software.

4.3.2 LC Q-TOF-MS analysis

Chromatographic separation of compounds present in the passive sampler extracts was carried out using an identical set-up as described in Chapter 3. In summary, an Agilent 1290 Infinity UHPLC system was coupled to an Agilent G6540A Quadrupole Time-of-Flight LC/MS System equipped with a dual sprayer Agilent Jet Stream electrospray source and operated using Agilent Mass Hunter acquisition software (rev. B.06.01). The Q-TOF MS instrument acquired data in 2 GHz extended dynamic range mode using positive and negative ion electrospray for the targeted pharmaceuticals and also for the 'unknowns' analysis. Sample data was acquired

using MS (scan mode) only at an acquisition rate of 1 Hz and corrected using reference lock mass correction with all data stored in profile mode. A mass axis calibration was undertaken at the beginning of every chromatogram using a dedicated tuning solution of known masses spanning the mass range of 118–922 Da. The 'all ions' feature used in Chapter 3 was not used in this chapter as only the molecular adduct ions and their isotopes were required with a metabolomics based workflow. All 24 sample extracts from the deployments (12 Chemcatcher® and 12 POCIS as described in Chapter 3) were analysed along with a standard mix of pharmaceuticals as used in Chapter 3.

4.3.3 Peak extraction of targeted pharmaceuticals

The personal compound database library developed of 164 pharmaceuticals in Chapter 3 was used in conjunction with Agilent Mass Hunter Profinder software to identify pharmaceuticals in the Chemcatcher® and POCIS extracts. A process within the software called 'Batch Targeted Feature Extraction' was used to extract compounds from the within the PCDL based on their chemical formulae. The approach is similar to the find by formula process described in Chapter 3 but as no fragment ion confirmation was employed a narrower retention time window of 0.2 min was used for accurate retention alignment of peaks across all data files. A higher score threshold of 90 was also used to ensure that only high quality data would be obtained. Isotope ions were also used for confirming the presence of the targeted pharmaceuticals in the extracts. The parameters used are listed in Table 4.1.

Table 4.1 Batch Targeted Feature Extraction parameters.

Source of formulas to confirm	
Database:	Pharmaceuticals
Values to match:	Mass and retention time
Charge carriers (adducts)	
Positive Ions	Negative Ions
H^+ (protonated)	H^- (deprotonated)
Na^+ (sodiated)	$HCOO^-$ (formate)
	CH_3COO^- (acetate)
Isotope grouping	
Peak spacing tolerance:	0.0025 m/z, plus 7 ppm
Isotope model:	Common organic molecules
Charge state maximum:	1
Matching tolerances and scoring	
Mass match tolerance:	+/- 10 ppm
Retention time tolerance:	+/- 0.2 min
Contribution to overall score	
Mass score:	100
Isotope abundance score:	60
Isotope spacing score:	50
Retention time score:	100
Matching criteria	

Do not match if score is less than:	90

Spectra to include

Average scans:	At 25% of peak height
Peak spectrum background:	Average of spectra at peak start and end

Post processing filters

Minimum height:	1000 counts
Minimum filter matches:	Compound must be present across all data files

4.3.4 Feature finding and peak extraction of untargeted features

Untargeted data acquisition on the LC Q-TOF MS was performed using the set up as described in section 3.4.13 in Chapter 3 (LC Q-TOF-MS analysis). Feature extraction, combined with chromatographic alignment across multiple data files, is a critical step in the peak finding and data reduction workflow that minimizes the possibility of both false positive and false negative features by 'binning' the features in the chromatographic time domain (337). In this chapter the term feature is used to describe chemical entities or peaks that are found as a result of using an untargeted (unbiased) peak finding algorithm. Features only become compounds when a molecular formula is assigned to the feature either using a molecular formula generator or via a database search.

A process within the Agilent MassHunter Profinder software called 'Batch Recursive Feature Extraction' (BRFE) was used to de-convolute (or extract) the data. This utilises an in-built molecular feature extractor which is an untargeted data mining algorithm which removes constant background ions and locates the co-variant ions in a chromatogram. It then tests for chemically logical relationships and looks for user specified adducts, specifically the protonated and sodiated adduct ions in this work, and groups together isotopes to form a single feature or peak with unique neutral mass and retention time values. Batch recursive feature extraction provides for missing feature recovery and is achieved by 'binning' and aligning compound features in the first-pass molecular feature extractor, after which a composite spectrum for all found ions is created for each consensus feature. The composite compound feature list is then used as a target list for the second-pass 'find by ion' feature extraction. By implementing two-pass batch feature extraction the number of false negatives is

reduced significantly resulting in decreased variability within sample group replicates. The find by ion algorithm therefore has a significant positive impact on statistics derived from the mass spectrometric data. Figure 4.1 shows the results of Profinder BRFE for data acquired from Chemcatcher® and POCIS extracts. The compound group table (Figure 4.1a) displays the compound information grouped and summarised across multiple data files. The individual file details of a selected compound group are shown in the compound details table (Figure 4.1b), integrated extracted ion chromatogram (Figure 4.1c), and MS spectrum (Figure 4.1d) windows. The parameters used by the find by ion algorithm for this work are listed in Table 4.2.

The data outputs from both targeted batch feature extraction and batch recursive feature extraction contained features that were only found in all 24 sample extracts and were in the form of comma separated variable files which were further processed using Microsoft Excel and Minitab® statistical analysis software.

Table 4.2 Profinder Batch Recursive Feature Extraction parameters.

Charge carriers (adducts)

Positive Ions	Negative Ions
H^+ (protonated)	H^- (deprotonated)
Na^+ (sodiated)	$HCOO^-$ (formate)
	CH_3COO^- (acetate)

Isotope grouping

Peak spacing tolerance:	0.0025 m/z, plus 7 ppm
Isotope model:	Common organic molecules
Charge state maximum:	1

Compound ion count threshold

Two or more ions

'Binning' and alignment tolerances

Retention time window:	+/- 0.2 min
Mass window:	10ppm

Contribution to overall score

Mass score:	100
Isotope abundance score:	60
Isotope spacing score:	50
Retention time score:	100

Matching criteria

Do not match if score is less than:	90

Spectra to include

Average scans:	At 50% of peak height
Peak spectrum background:	Average of spectra at peak start and end

MFE post processing filters

Minimum height:	1000 counts
Score MFE:	90
Minimum filter matches:	Compound must be present across all data files

Find by Ion -Matching tolerances and scoring

Expansion of values for chromatographic extraction:	Symmetric +/- 20 ppm
Limit extracted ion chromatogram extraction range:	+/- 5 min

4.3.5 Correction of peak areas obtained from POCIS devices

Losses of sorbent occurred during deployment and/or when disassembling the POCIS devices at the laboratory and varied considerably between devices across all sites. The literature review which accompanies this thesis did not identify any published papers which mention the loss of sorbent when disassembling POCIS samplers or weighing the sorbent following retrieval to check for sorbent loss. A few authors mention the use of stainless-steel rings to sandwich the two membranes together to prevent the loss of sorbent prior to deployment (334, 336) and others have mentioned placing the samplers in the horizontal plane to prevent potential loss of sorbent (338). From the author's own significant experience with POCIS, loss of sorbent does occur during deployment as the steel rings seldom form a perfect seal when fully tightened. The POCIS sorbent is also electrostatic when dry and precautions are required to prevent static build up which, as far as the author of this thesis is aware, are not mentioned in any published papers.

It is generally recommended that the PES membrane from POCIS deployments be placed in glass funnels and methanol used to wash the sorbent off into a suitable glass receptacle (96). This may, however, result in the desorption of compounds which have been adsorbed onto the PES membrane during deployment (117). As POCIS is considered to be a monophasic sampler, equations for the calculation of uptake rates consider only what is adsorbed onto the sorbent. Inaccurate results could therefore be obtained if compounds adsorbed onto the membrane are also measured and included in any calculations. In contrast, the Chemcatcher® considers only compounds that have passed through the membrane and subsequently adsorbed onto the sorbent and glass fibre matrix of the disk.

The mass of sorbent recovered, after overnight drying to remove moisture, from each device was recorded using an analytical balance and appear in Table 4.3. An overall average of 69.6% of sorbent was recovered out of the original 200 mg added to each device with an RSD of 15.4%. Significantly lower amounts of sorbent, 103.8 mg and 97.0 mg, were recovered from the Carmarthen (i) and Llanelli (ii) POCIS devices respectively. As POCIS sorbent is electrostatic when dried, precautions were taken to minimise these losses by discharging any static build up by 'earthing' a large sheet of aluminium foil that was used for holding any glass vials, containing the dried sorbent, and other equipment prior to weighing. As losses occurred from POCIS devices, peak areas obtained from peak (feature) integration in sections 4.3.4 were corrected for by applying the factors shown in Table 4.3. The corrected peak areas obtained would allow for the comparison of the compound uptake in both samplers. It was assumed that losses would not occur with the disks during deployments as the sorbent is immobilised within the glass fibre matrix.

Table 4.3 Mass of Oasis HLB sorbent recovered from POCIS devices from all replicates at all sites.

Site	Mass of sorbent added (mg) A	Mass of sorbent recovered (mg) B	% of sorbent recovered	Recovery factor A/B
Carmarthen A	200 ± 2	103.8	52.0	1.927
Carmarthen B	200 ± 2	140.9	70.5	1.419
Carmarthen C	200 ± 2	146.0	73.0	1.370
Gowerton 1A	200 ± 2	144.0	72.0	1.390
Gowerton 1B	200 ± 2	145.5	72.8	1.375
Gowerton 1C	200 ± 2	126.2	63.1	1.585
Gowerton 2A	200 ± 2	147.0	73.5	1.361
Gowerton 2B	200 ± 2	151.4	75.7	1.320
Gowerton 2C	200 ± 2	142.7	71.4	1.127
Llanelli A	200 ± 2	177.5	88.8	1.127
Llanelli B	200 ± 2	97.0	48.5	2.062
Llanelli C	200 ± 2	146.7	73.4	1.363

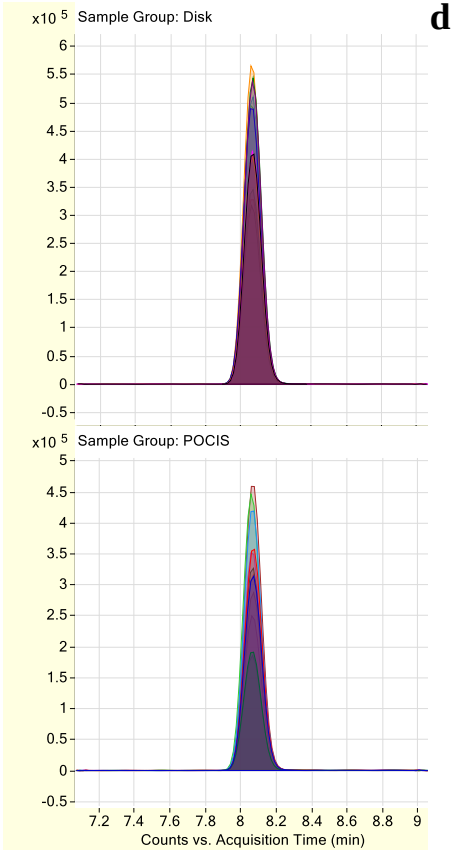
a

Group	RT (Tgt)	RT (med)	Found	Misse
2	25.148	25.155	24	
3	18.021	18.024	24	
1	24.91	24.909	24	
4	25.753	25.757	24	
7	8.065	8.07	24	
8	21.497	21.505	24	
9	8.065	8.068	24	
5	24.256	24.258	24	
6	22.263	22.264	24	
13	23.818	23.823	24	
14	19.963	19.967	24	
15	10.751	10.749	24	
10	18.019	18.023	24	
11	6.514	6.512	24	

b

Min Z	Mass	m/z	Score (MFE)
1	396.0143	457.0255	100
1	396.0144	395.0074	100
1	396.0143	443.0096	100
1	396.0143	395.0073	100
1	396.0144	455.0283	100
1	396.0144	457.0257	100
1	396.0144	457.0257	100
1	396.0144	443.0098	100
1	396.0145	793.0188	100
1	396.0142	395.0073	100
1	396.0142	457.0255	100

c(i) **c(ii)**



d

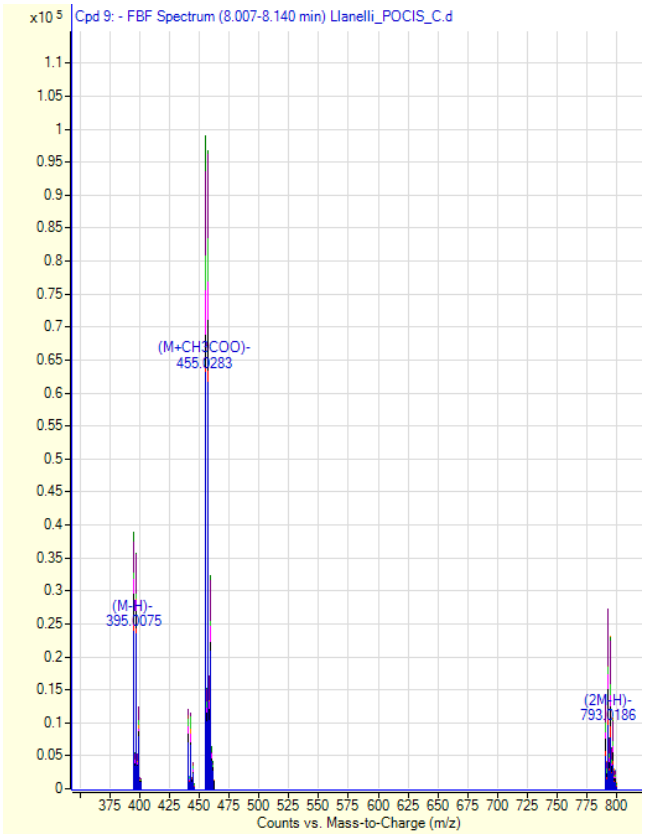


Figure 4.1 Results obtained from batch recursive feature extraction. [a] compound group table, [b] compound details table, [c(i) and c(ii)] overlaid extracted ion chromatograms for Chemcatcher® and POCIS respectively, (d) Mass spectra for POCIS.

4.3.6 Mass profiling of 'unknowns' present in Chemcatcher® and POCIS samplers

Mass profiling of 'unknown' features present in both samplers was undertaken to determine whether the range of compounds sequestered by both samplers was similar. Feature plots of 'unknown' compounds detected in Chemcatcher® and POCIS samplers were produced from the UHPLC Q-TOF-MS data files using Agilent Mass Profiler software. The settings used were identical to those used with Mass Profinder (Table 4.2) with the exception that for a feature to be confirmed it had to be found in at least two of the three replicates in any one sampler type but independent of any deployment site. This allowed for an unbiased approach to establishing the molecular mass range of the features detected in each sampler type and determination of any differences between sampler types and between sites.

Histograms of mass against frequency of detection were created, using the data analysis function incorporated within Microsoft Excel, for the Carmarthen, Llanelli and Gowerton sites; the latter site used to determine any differences obtained between the co-deployments at this site. The form of the histograms was obtained by splitting the range of the data obtained into 47 equal-sized, non-overlapping mass intervals (20 Da wide) along the horizontal axis and the number of points from the data set that fell into each interval represented on the vertical axis. In addition, histograms of feature detection against retention time were made using 40 distinct non-overlapping time intervals of 30 s. The form of the histograms initially used were based on Scott's normal reference rule (339) (the automatic option with Excel) which resulted in mass bin widths of approximately 55 Da and retention time bin widths of approximately

1.6 min. These were considered as too wide as it was felt that the underlying trend in the data would be missed.

4.4 Results and discussion

A total of 72 pharmaceuticals were identified in the extracts and 68 pharmaceuticals were identified at all three sites and in both types of sampler. Of these 72 pharmaceuticals atorvastatin, naproxen and terbinafine were found only at the Carmarthen site whilst piroxicam was found only at the Llanelli site. Sixty-seven and 19 pharmaceuticals were identified in positive ion and negative ion modes with 53 and 5 pharmaceuticals identified only in positive and negative ion modes respectively. Of the five pharmaceuticals identified in negative ion mode only, four are acidic and do not ionise efficiently in positive ion mode due to their very low proton affinities. Peak areas obtained from positive ion mode were generally higher than obtained from negative ion mode for both samplers and were therefore chosen for statistical analysis.

The peak areas obtained for each pharmaceutical that were common to all replicate samplers (both Chemcatcher® and POCIS) and at all sites were averaged, percentage relative standard deviations (% RSD) were calculated and are presented in Table 4.4. Percentage RSD's varied significantly between compounds and between samplers with less variation observed between sites for the individual compounds. Percentage RSD's obtained from positive ion analysis were generally higher for the Chemcatcher® sampler at two sites (Carmarthen and Gowerton) but considerably lower than POCIS for the Llanelli site. Lower recoveries of the sorbent obtained from the POCIS samplers for the Llanelli site may have contributed to the higher RSD's

for POCIS but this would seem an unlikely reason as larger peak area responses were nevertheless obtained from the POCIS sampler because of its larger sampling area.

Fixty-six and 32 compounds exceeded 15 % RSD in the Chemcatcher® and POCIS extracts respectively, the higher RSD's in the Chemcatcher® strongly correlated with peak area response which were approximately 3 times lower in the Chemcatcher. Only 10 compounds exceeded 30% RSD in both samplers with the largest error observed for the POCIS sampler at 165% RSD for the compound loratadine. The highest RSD observed for loratadine in the Chemcatcher® was only 23%.

RSD's obtained from negative ion analysis were generally lower for the Chemcatcher® sampler across all sites with five and three compounds exceeding 15 % RSD in the Chemcatcher® and POCIS extracts respectively. Only one compound, naproxen in the POCIS sampler, exceeded 30% RSD in negative ion mode.

Table 4.4 % RSD's obtained for 68 pharmaceutical compounds identified in Chemcatcher® and POCIS samplers.

Compound	Carmarthen Chemcatcher®	Carmarthen POCIS	Gowerton 1 Chemcatcher®	Gowerton 1 POCIS	Gowerton 2 Chemcatcher®	Gowerton 2 POCIS	Llanelli Chemcatcher®	Llanelli POCIS
Salicylic acid[a]	8.7	15.1	13.3	2.9	12.6	11.4	3.9	14.1
Ibuprofen[a]	6.2	10.4	4.8	7.5	5.0	7.8	6.2	11.7
Naproxen[a]	10.7	34.6	nd	nd	nd	nd	nd	nd
Mefenamic acid[a]	11.0	2.9	8.6	5.7	7.3	3.2	2.4	5.1
Indapamidea	4.9	9.0	3.2	7.1	3.1	4.5	2.7	1.3
Alfuzosin	27.9	12.5	1.8	6.4	16.2	5.7	17.4	1.5
Alverine	12.9	7.4	11.6	5.1	5.6	3.8	27.2	8.1
Amisulpride[b]	12.8	5.7	9.1	5.8	6.5	2.6	3.9	3.4
Amitriptyline	15.8	18.7	9.0	3.5	6.2	4.9	4.8	14.2
Atenolol	7.5	7.2	8.4	3.4	4.6	1.9	3.5	3.1
Atorvastatin	21.8	32.4	nd	nd	nd	nd	nd	nd
Betamethasone 17 valerate	23.1	32.7	14.6	19.2	15.2	31.5	12.4	8.1
Bezafibrate	18.3	6.9	8.0	6.6	6.9	3.1	3.2	10.1
Bisoprolol	13.9	4.2	9.3	3.7	10.2	3.3	3.6	4.0
Carbamazepine	13.3	6.9	8.9	4.1	7.3	1.8	3.0	3.0
Cefalexin	14.2	4.9	9.9	4.8	11.0	6.1	3.5	9.4
Celiprolol[b]	16.9	6.8	7.6	4.6	7.9	1.5	1.3	2.1
Cetirizine	13.0	4.0	6.2	3.1	7.8	2.5	3.0	4.5
Chlorpheniramine	19.2	8.6	25.3	15.1	5.9	33.2	32.1	20.9
Citalopram	14.1	8.2	7.8	3.8	6.8	4.2	3.8	11.8

continued

Clarithromycin[b]	37.5	5.8	5.2	2.7	4.9	4.7	4.9	15.7
Clopidogrel	12.3	19.2	6.3	3.3	7.2	1.5	9.5	9.5
Cyclizine	13.2	4.5	6.8	1.7	9.1	2.3	3.6	5.8
Diclofenac	11.0	5.9	7.9	4.6	3.8	3.3	2.9	2.0
Diltiazem (Cis)	24.6	7.4	7.1	3.0	8.8	4.2	4.0	12.4
Dipyridamole	24.0	5.0	9.1	6.5	6.2	5.4	5.0	3.7
Doxazosin	49.5	30.2	28.3	14.3	24.0	20.6	2.7	8.3
Erythromycin[b]	27.1	5.4	6.9	3.3	8.3	4.1	0.8	13.5
Fexofenadine[b]	15.1	5.0	8.2	3.8	8.1	3.4	3.4	7.0
Flecainide[b]	25.7	3.9	6.8	4.9	22.5	3.2	5.4	8.2
Fluconazole[b]	8.5	10.7	5.0	7.4	7.4	3.1	9.0	3.5
Furosemide	3.8	15.6	0.6	6.6	7.9	9.4	6.2	12.5
Gliclazide	12.9	9.9	24.5	3.4	6.1	4.5	2.5	2.1
Irbesartan[b]	14.1	3.2	7.0	3.5	7.7	2.7	4.1	4.9
Ketoconazole	26.3	5.8	11.4	3.3	8.3	2.7	3.5	14.2
Ketoprofen	12.4	17.6	6.7	2.4	24.2	14.9	24.6	7.3
Labetalol	12.0	1.1	10.1	3.7	7.1	1.2	0.5	11.0
Lamotrigine	9.5	9.6	10.5	3.6	5.5	1.1	2.0	2.0
Lansoprazole	10.1	8.2	10.4	3.9	8.8	2.2	3.1	9.8
Lidocaine	12.3	7.0	6.8	1.8	8.6	1.8	3.8	3.2
Loperamide	18.3	6.7	14.2	2.5	3.2	5.7	11.7	13.7
Loratadine	23.0	97.7	22.0	164.7	18.2	5.1	15.0	22.1
Losartan	14.4	4.0	10.8	5.4	1.0	6.3	7.0	3.8
Metoclopramide	12.8	8.4	10.3	2.1	4.6	1.8	2.7	3.7
Metoprolol	13.4	10.6	7.5	5.5	9.5	1.2	3.5	3.7
Mirtazapine	11.7	3.6	10.0	4.4	8.5	3.0	3.1	3.6

continued

Nifedipine	32.0	45.7	47.8	18.2	24.0	13.4	9.3	28.5
Omeprazole	16.4	1.8	6.9	3.9	7.4	6.6	6.0	10.3
Oxprenolol[b]	2.5	7.9	6.7	7.1	4.8	4.0	3.1	4.4
Pantoprazole	11.7	12.8	26.6	6.3	20.3	5.1	7.1	8.8
Phenytoin	13.1	13.9	6.5	6.6	8.6	1.2	4.6	4.4
Piroxicam	nd	nd	nd	nd	nd	nd	3.7	3.2
Procyclidine	20.2	8.1	6.0	3.9	7.8	6.5	6.5	7.9
Propranolol	13.6	3.3	7.7	3.8	6.9	2.4	4.4	6.5
Quinine	15.6	4.7	11.3	3.4	14.6	3.7	3.3	5.5
Ranitidine	20.7	32.2	31.2	19.2	16.5	15.8	1.9	21.8
Sulfasalazine	10.9	4.7	6.7	5.7	5.3	0.9	3.8	5.6
Salbutamol	4.2	10.7	9.2	1.4	43.1	4.6	4.7	5.8
Sertraline	20.6	22.1	11.8	3.4	1.7	4.1	6.2	11.2
Sotalol[b]	9.2	8.5	7.9	4.0	5.7	2.5	3.1	2.5
Sumatriptan[b]	11.6	9.0	14.4	5.3	5.2	3.9	2.5	5.9
Tamsulosin	28.4	5.0	29.1	5.7	11.1	15.9	23.5	27.6
Telmisartan	14.9	11.1	7.6	5.2	4.9	1.2	6.0	8.9
Terbinafine	35.8	17.0	nd	nd	nd	nd	nd	nd
Timolol	32.2	19.5	18.5	12.3	22.2	15.8	8.3	10.2
Tramadol[b]	12.8	5.9	8.2	4.4	8.0	1.5	3.5	0.2
Trazodone	27.5	25.0	6.6	3.8	10.3	0.6	6.3	7.0
Trimethoprim	13.4	8.4	7.5	3.6	8.1	0.9	3.8	0.2
Valsartan[b]	23.6	4.5	18.1	17.2	6.2	8.4	5.0	13.1
Venlafaxine	13.5	6.8	7.8	4.3	7.4	2.5	3.4	0.3
Verapamil	31.0	12.2	2.2	2.7	7.4	5.1	3.0	12.7
Warfarin	16.8	9.0	10.1	5.6	6.6	2.5	14.8	8.7

continued

Table 4.4 (continued)

Key
RSDs expressed as a percentage
[a] obtained from negative ion data
[b]identified in both positive and negative ionisation
nd - not identified in extract

4.4.1 Comparison of uptakes of 68 pharmaceuticals by Chemcatcher® and POCIS

The uptake of the range of pharmaceuticals identified at all sites was similar in both samplers and a simple scatter plot of the averaged data, as shown in Figure 4.2, shows that the variance is not constant but increases with the amount of compound accumulated. Individual regression fits for the 3 sites, including the co-deployment at the Gowerton site, are shown below and there appears to be little variation between the sites.

Carmarthen; POCIS (average) = - 115,259 + 2.843 Chemcatcher® (average),r^2 = 97.2 %

Gowerton 1; POCIS (average) = 20,715 + 2.457 Chemcatcher® (average), r^2 = 95.8%

Gowerton 2; POCIS (average) = - 57,104 + 2.288 Chemcatcher® (average), r^2= 98.4%

Llanelli; POCIS (average) = 217,632 + 2.462 Chemcatcher® (average), r^2 = 90.6%

Log transforming the data to Log $base_{10}$ indicated a much smaller deviation from normality as seen in Figure 4.3, with the exception of one unusual value for the compound Loratadine in the POCIS sampler deployed at the Carmarthen site.

A principal component analysis, using standardised variables, (340) for all replicates at all sites indicated that one major component accounted for 98.3% of the total variation. This is what the two samplers have in common whilst the second component shows the contrast between the two samplers and this accounted for only 1.7% of the total variation and was probably based on random variation (see Table 4.5).

4.4.2 Analysis of variance of pharmaceuticals identified in co-deployments undertaken at the Gowerton site

A two-way ANOVA, with sample repeat and passive sampler type was undertaken on the areas obtained for the 68 pharmaceuticals identified in the two sets of passive samplers co-deployed in the final effluent stream at Gowerton. An interaction between sample repeat and sampler type was also calculated. A significant difference was observed between sampler types ($f = 29.71$, $p = <0.001$) but not between the locations ($f = 0.33$, $p = 0.565$) and there was no significant interaction ($f = 0.29$, $p = 0.591$) as shown in Table 4.6.

The ratio of the summed peak areas for the 68 pharmaceuticals obtained from both sets of samplers was approximately 2.53. The ratio of the mean peak areas for the 68 pharmaceuticals from both sets of samplers at the two deployment locations was 2.48 and 2.23 for Gowerton 1 and 2 respectively with an average value of 2.35. Therefore, the sampling rate of POCIS was approximately 2.4 times that of the Chemcatcher® as shown in Table 4.6.

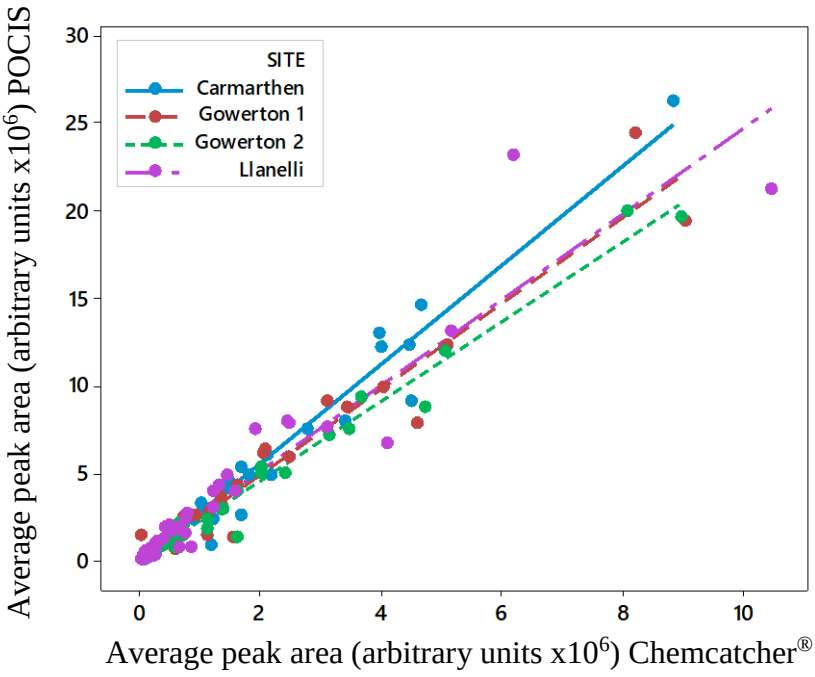


Figure 4.2 Overlaid scatter plots with regression lines of the averaged peak areas for pharmaceuticals identified in POCIS and Chemcatcher® at all sites

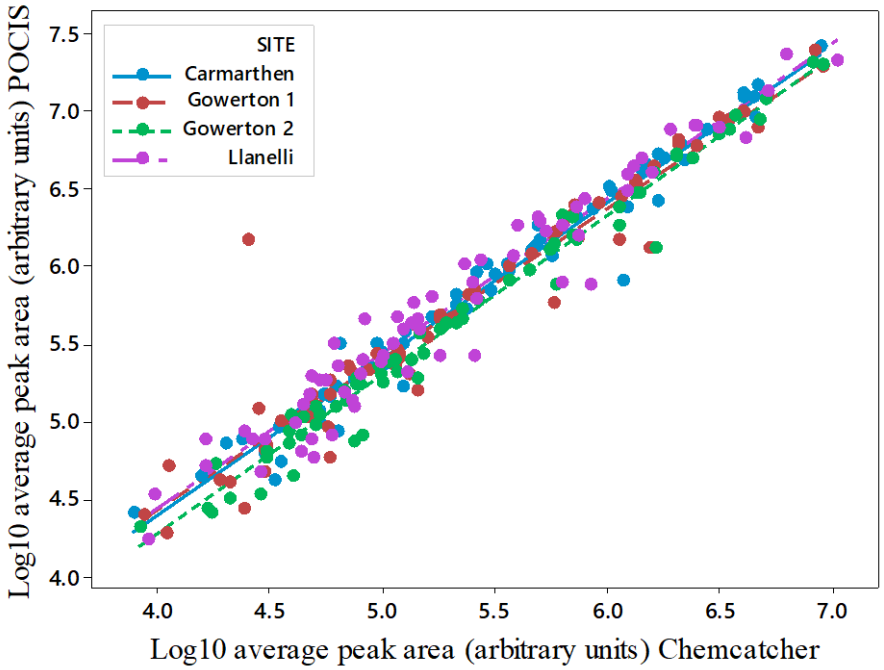


Figure 4.3 Overlaid scatter plots with regression lines of the averaged Log_{10} peak areas for pharmaceuticals identified in POCIS and Chemcatcher® at all sites.

Table 4.5 Principal component analysis for pharmaceuticals identified in the co-deployments undertaken at the Gowerton site.

Eigenanalysis of the correlation matrix

Eigenvalue	1.9166	0.0834
Proportion	0.958	0.042
Cumulative	0.958	1

Variable	PC1	PC2
POCIS	0.707	0.707
Chemcatcher®	0.707	-0.707

Table 4.6 Two-way ANOVA with sample repeat and passive sampler type for pharmaceuticals identified in the co-deployments undertaken at the Gowerton site.

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sampler[a]	1	3.05E+14	3.05E+14	29.71	< 0.001
Location[b]	1	3.40E+12	3.40E+12	0.33	0.565
Sampler * Site	1	2.97E+12	2.97E+12	0.29	0.591
Error	812	8.35E+15	1.03E+13		
Total	815	8.66E+15			

[a]Sampler = Chemcatcher® or POCIS
[b]Location '1' or '2' at Gowerton

Total summed peak areas

POCIS	1816277943
Chemcatcher®	716559868
Ratio	2.53

Tables of means

Location	Chemcatcher®	POCIS	Ratio
Gowerton 1	908781	2253177	2.48
Gowerton 2	900407	2003324	2.23
		Mean	2.35

4.4.3 Least squares and orthogonal regression analysis of the averaged pharmaceutical Chemcatcher® and POCIS data

Orthogonal regression or Deming regression (341) is used where two variables each have an independent error distribution. This is in contrast with ordinary least squares regression where the predictor (x variable) is a fixed variable with no error and all of the error is associated with the dependent variable (y). In least squares regression, the fitted line gives the minimum deviation of the y-values from the fitted line. In orthogonal regression, the fitted line corresponds to the minimum deviations at right angles to the fitted line and is equivalent to the first principal component (341). The interpretation of the regression parameters of an orthogonal regression is similar to that for least squares.

The output from the least squares regression, with the Chemcatcher® data as the predictor, is shown in Table 4.7 with the plot of residuals shown in Figure 4.4. The variation accounted for by the regression (r^2) is 94.7% with a slope of 2.50 (t = 69.33, p = <0.001) and an intercept of 35,028 (t = 0.52, p = 0.61) which was not significantly different from zero since it falls within the confidence range (-98,625, 168,680) that contains zero. This indicates that the sampling rate of POCIS is approximately 2.5 times higher than for the Chemcatcher®. The output from orthogonal regression is shown in Table 4.8, with the plot of residuals shown in Figure 4.5. The slope obtained from orthogonal regression was 2.62 which is significantly different from zero since the approximate 95 % confidence range is 2.7 to 3. The value of 2.62 does, however, fall just outside this range which reflects the lack of normality and homogeneity in the variance. However, since it is close, and the intercept -71,700 is not significantly different from zero it can be taken that the two samplers are behaving in a similar

manner, but with the sampling rate of POCIS being 2.6 times that of the Chemcatcher®. The plots of standardised residuals from the least squares and orthogonal regression plots show 9 large values greater than 3, which are for the compounds fexofenadine, lamotrigine and tramadol at all sites. These large residuals had little influence on the slope as removing them from the data set and performing a least squares regression on the remaining data produced a change in slope of only 0.03 and the intercept was almost unchanged at 34,273.

Least squares and orthogonal regression regression analysis was performed on the averaged Log_{10} transformed Chemcatcher® and POCIS pharmaceutical data. The outputs are discussed in A1.1 and shown in Tables A1 & A2 plus Figures A1 & A2 in Appendix A.

Table 4.7 Output from least squares regression analysis of the averaged pharmaceutical Chemcatcher® and POCIS data.

Regression Equation

POCIS (average) = 35028 + 2.4948 Chemcatcher® (average)

Model Summary

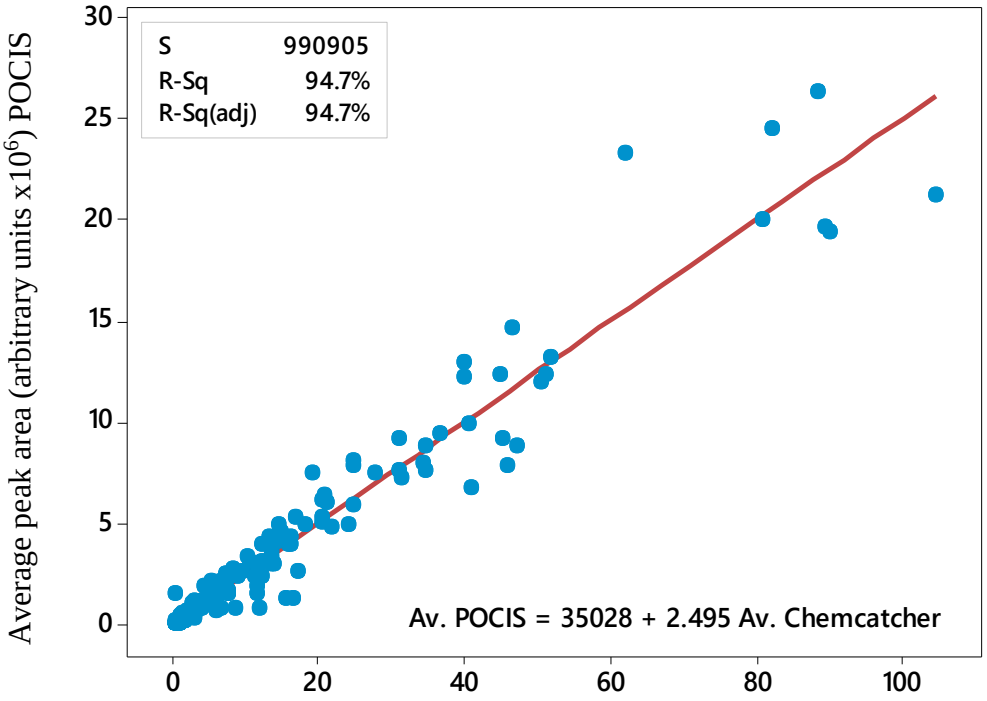
S	R-sq	R-sq(adj)	PRESS	R-sq(pred)
990905	94.68%	94.66%	2.90E+14	94.17%

Analysis of Variance

Source	DF	Seq SS	Contrib	Adj SS	Adj MS	F-Value	P-Value
Regression	1	4.72E+15	94.68%	4.72E+15	4.72E+15	4806.6	< 0.001
Chemcatcher® (average)	1	4.72E+15	94.68%	4.72E+15	4.72E+15	4806.6	< 0.001
Error	270	2.65E+14	5.32%	2.65E+14	9.82E+11		
Total	271	4.98E+15	100.00%				

Coefficients

Term	Coef	SE Coef	95% CI	T-Value	P-Value	VIF
Constant	35028	67886	(-98625, 168680)	0.52	0.606	
Chemcatcher® (average)	2.4948	0.036	(2.4240, 2.5657)	69.33	< 0.001	1.00



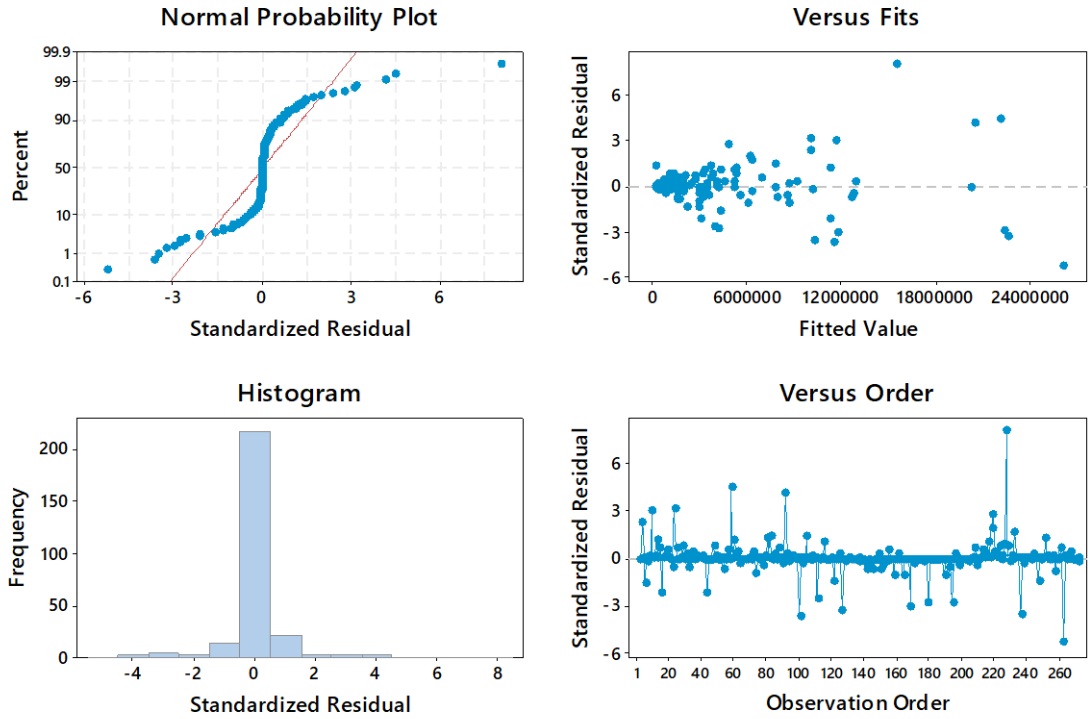


Figure 4.4 Fitted line regression and plots of standardised residuals obtained from least squares regression analysis of the averaged pharmaceutical Chemcatcher® and POCIS data.

Table 4.8 Output from the orthogonal regression analysis of the averaged pharmaceutical Chemcatcher® and POCIS data.

Error Variance Ratio: POCIS (average)/Chemcatcher® (average): 1

Regression Equation

POCIS (average) = -71688 + 2.616 Chemcatcher® (average)

Coefficients

Predictor	Coef	SE Coef	Z	P	Approx 95% CI
Constant	-7.17E+04	69719.2	-1.0282	0.304	(-208335, 64959.1)
Chemcatcher® (average)	2.61636	0.0	69.3239	< 0.001	(3, 2.7)

Error Variances

Variable	Variance
POCIS (average)	1.29962E+11
Chemcatcher® (average)	1.29962E+11

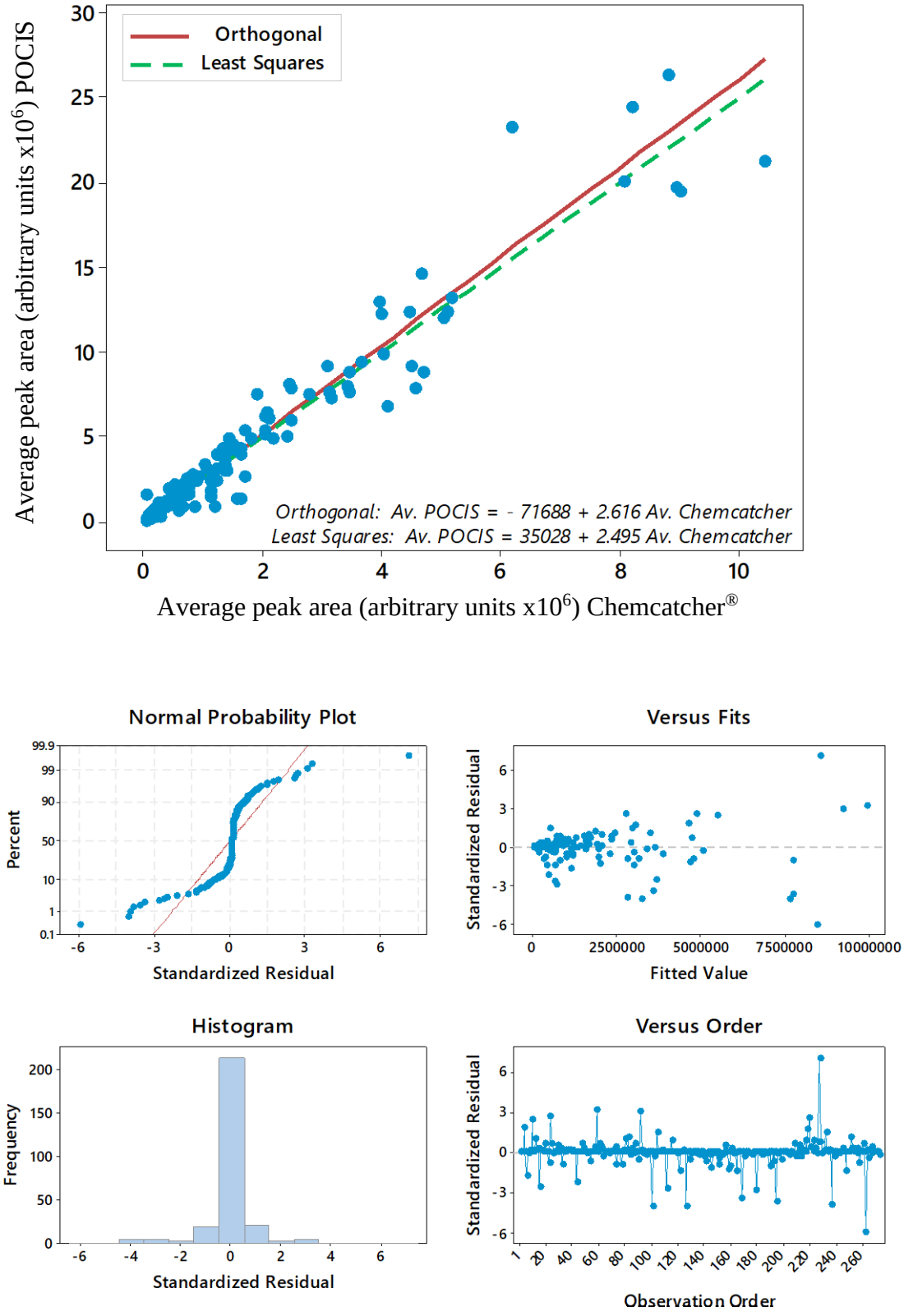


Figure 4.5 Fitted line regression and plots of standardised residuals obtained from the orthogonal regression analysis of the averaged pharmaceutical Chemcatcher® and POCIS data.

4.4.4 Statistical analysis of 'unknown' features or peaks

As stated in 4.1, the POCIS sampler uses the Waters OASIS HLB sorbent which has been used extensively for the determination of pollutants in the aqueous environment with typical Log_{10} K_{ow} values up to 4. The choice of compounds to represent a Log_{10} K_{ow} cut-off of 4 for the data obtained was accomplished by choosing six pH neutral compounds, at the mobile phase pH value of 4.05, contained within the personal compound database library in Chapter 3. Six hormones (five synthetic and one natural); betamethasone-17-valerate, clobetasol propionate, fluticasone propionate, medroxyprogesterone-17-acetate, mometasone furoate and progesterone have calculated Log_{10} K_{ow} values of 3.78, 3.98, 3.73, 4.11, 4.27 and 4.04 as determined using the ACD Labs Percepta Platform PhysChem module available on the Royal Society of Chemistry's Chemspider website (http://www.chemspider.com/) and experimentally determined retention times of 20.77, 19.70, 19.74, 20.13, 19.78 and 20.30 min respectively. Average values of 3.99 and 20.07 min for Log_{10} K_{ow} and retention time respectively represent an appropriate retention time cut-off of 20 min for the evaluation of 'unknowns' data.

Non-targeted metabolomics type studies, such as this work, seek to analyse as wide a range of compounds as possible. The use of LC-MS for this purpose has found a wide range of applications, including drug discovery, disease biomarker discovery, pesticide and wastewater analysis (342). LC-MS, however, suffers from lower reproducibility in comparison to other analytical techniques (e.g. nuclear magnetic resonance (NMR) spectroscopy) for the analysis of 'unknowns', therefore peak areas obtained from features identified in replicate sampler types at each site were averaged and, together with replicate data, subjected to statistical evaluation.

Negative ion data was not used for statistical evaluation as the instrument's high-gain amplifier developed a fault leading to saturation of peaks at low peak area counts with significant mass errors rendering the data unusable.

4.4.5 Comparison of uptakes of 'unknown' compounds by Chemcatcher® and POCIS

The uptake of the range of 2,204 'unknowns' identified at all sites, in positive ion mode, is similar in both samplers and a simple scatter plot with fitted lines was made from averaged data from all sites. The variance is not constant but increases with the amount of compound accumulated as shown in Figure 4.6. Individual least squares regression fits for the three sites, including the co-deployment at the Gowerton site, are shown below and there appears to be little variation between two sites (Gowerton and Llanelli) but a larger variation is observed for the Carmarthen site. Log transforming the data to Log $base_{10}$ indicated a much smaller deviation from normality as seen in Figure 4.7.

Carmarthen, POCIS (average) = 86,481 + 2.116 Chemcatcher® (average), r^2 = 92.2%

Gowerton 1, POCIS (average) = 144,291 + 1.626 Chemcatcher® (average), r^2 = 85.3%

Gowerton 2, POCIS (average) = 89,766 + 1.763 Chemcatcher® (average), r^2 = 93.7%

Llanelli, POCIS (average) = 107,084 + 1.824 Chemcatcher® (average),r^2 = 80.7%

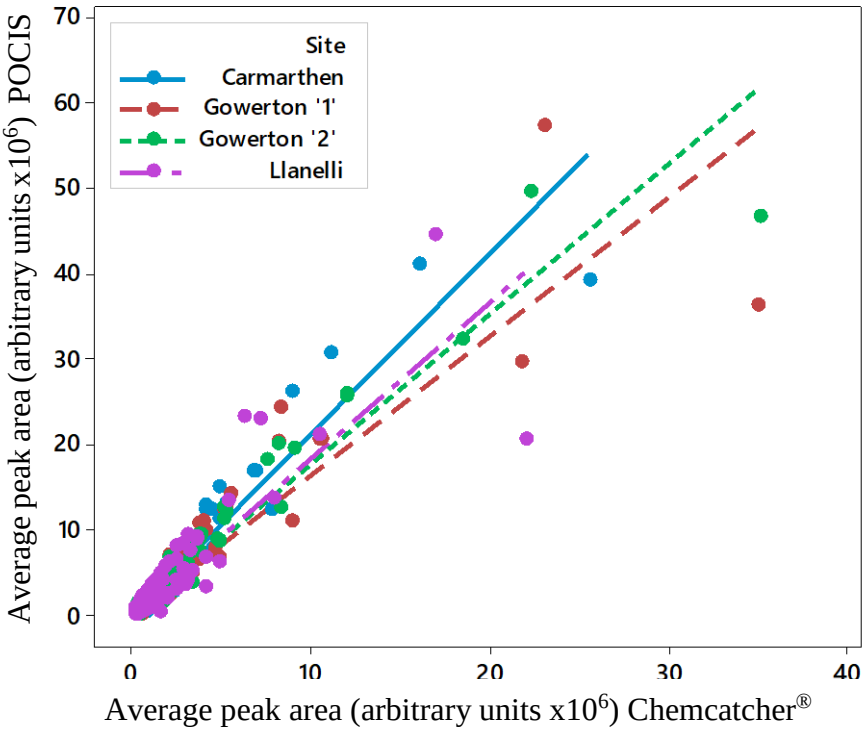


Figure 4.6 Overlaid scatter plots with regression lines of the averaged peak areas for 'unknowns' obtained from POCIS and Chemcatcher® for all WWTPs.

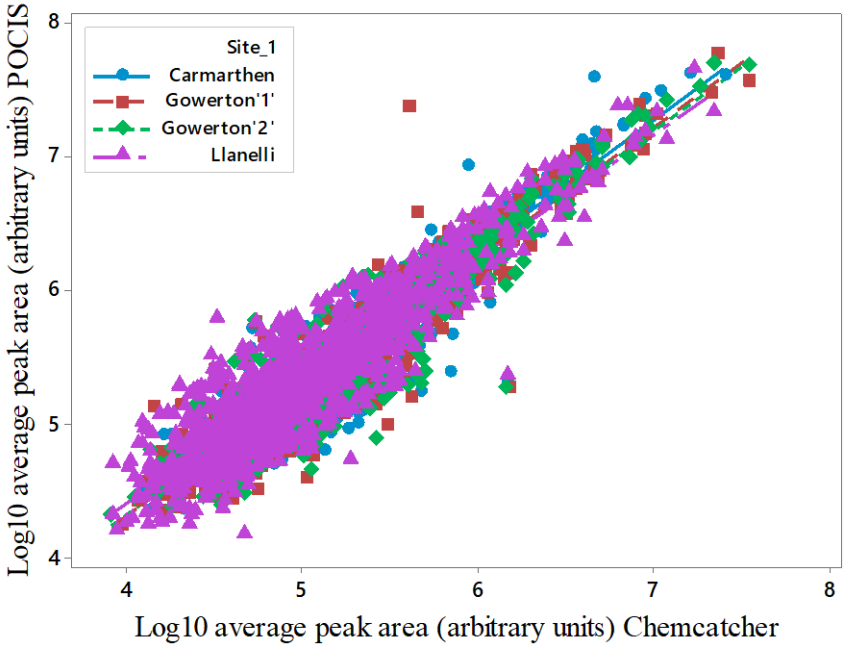


Figure 4.7 Overlaid scatter plots with regression lines of the averaged Log_{10} peak areas for 'unknowns' obtained from POCIS and Chemcatcher® for all WWTPs.

4.4.6 Analysis of variance of 'unknowns' identified in co-deployments undertaken at the Gowerton WWTP

A principal component analysis (using standardised variables) for all replicates at all sites, indicated that one major component accounted for 95.8% of the total variation (Table 4.9), this is what the two samplers have in common whilst the second component shows the contrast between the two samplers and this accounted for only 4.2% of the total variation and was probably based on random variation.

A two-way ANOVA (Table 4.10), with sample repeat and passive sampler type was undertaken on the areas obtained for the 2204 features identified in the two sets of passive samplers co-deployed in the final effluent stream at Gowerton. An interaction between sample repeat and sampler type was also calculated. A significant difference was observed between sampler types but not between samples and there was no significant interaction. The ratio of the summed peak areas obtained from both sets of samplers was approximately 2.05. The ratio of the mean peak areas from both sets of samplers at the two deployment locations was 1.96 and 1.91 for Gowerton 1 and 2 respectively with an average value of 1.93. Therefore, the sampling rate of POCIS is approximately 1.93 times that of the Chemcatcher®.

Table 4.9 Principal component analysis of 'unknowns' identified in co-deployments undertaken at the Gowerton WWTP.

Eigenanalysis of the correlation matrix

Eigenvalue	1.9166	0.0834
Proportion	0.958	0.042
Cumulative	0.958	1

Variable	PC1	PC2
POCIS	0.707	0.707
Chemcatcher®	0.707	-0.707

Table 4.10 Two-way ANOVA with sample repeat and passive sampler type for co-deployments undertaken at the Gowerton WWTP.

Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sampler[a]	1	5.20E+14	5.20E+14	85.88	< 0.001
Site[b]	1	5.87E+10	5.87E+10	0.01	0.922
Sampler*Site	1	2.97E+11	2.97E+11	0.05	0.825
Error	13220	8.01E+16	6.06E+12		
Total	13223	8.06E+16			

[a]Sampler = Chemcatcher® or POCIS
[b]Site = Location '1' or '2' at Gowerton

Total summed peak areas

POCIS	10891662241
Chemcatcher®	5317346067
Ratio	2.05

Tables of means

Location	Chemcatcher®	POCIS	Ratio
Gowerton 1	422152	828402	1.96
Gowerton 2	427417	814710	1.91
		Mean	1.93

4.4.7 Least squares and orthogonal regression analysis of the 'unknowns' data obtained from all replicates of Chemcatcher® and POCIS

The output from the least squares regression is shown in Table 4.11. The variation accounted for by the regression (r^2) is 84.0%. The slope of the regression is 1.76 (t = 263.54, pr = < 0.001) which is significantly different from zero since the approximate 95% confidence interval is 1.75 to 1.78. The intercept of 114,473 (t = 11.1, pr = < 0.001) is not significantly different from zero as it falls within the 95% confidence range of 94,257 to 134,688, it can be taken that the two samplers are behaving in a similar manner, but with the sampling rate of POCIS being 1.76 times that of the Chemcatcher®. The plots of residuals in Figure 4.8 displays significant heteroscedasticity (non-homogeneity) with a few extreme standardised residual values in the range 5–25. The variance is not constant but increases with the amount of compound accumulated.

The output from the orthogonal regression is shown in Table 4.12. The slope of the regression is 2.03 which is significantly different from zero since the approximate 95% confidence interval is 2.0. The intercept of 8,986 is not significantly different from zero since it falls within the 95% confidence range that contains zero, therefore it can be taken that the two samplers are behaving in a similar manner, but with the sampling rate of POCIS being 2.03 times that of the Chemcatcher®. The plots of residuals in Figure 4.9 also displays significant heteroscedasticity with a few extreme standardised residual values in the range 5–35 and the variance is not constant but increases with the amount of compound accumulated.

Table 4.11 Output from least squares regression analysis of 'unknowns' in all replicates of POCIS and Chemcatcher®.

Analysis of Variance

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
Regression	1	9.10E+16	84.01%	9.10E+16	9.10E+16	69451.16	< 0.001
Chemcatcher®	1	9.10E+16	84.01%	9.10E+16	9.10E+16	69451.16	< 0.001
Error	13222	1.73E+16	15.99%	1.73E+16	1.31E+12		
Lack-of-Fit	12898	1.70E+16	15.70%	1.70E+16	1.32E+12	1.35	< 0.001
Pure Error	324	3.18E+14	0.29%	3.18E+14	9.80E+11		
Total	13223	1.08E+17	100.00%				

Model Summary

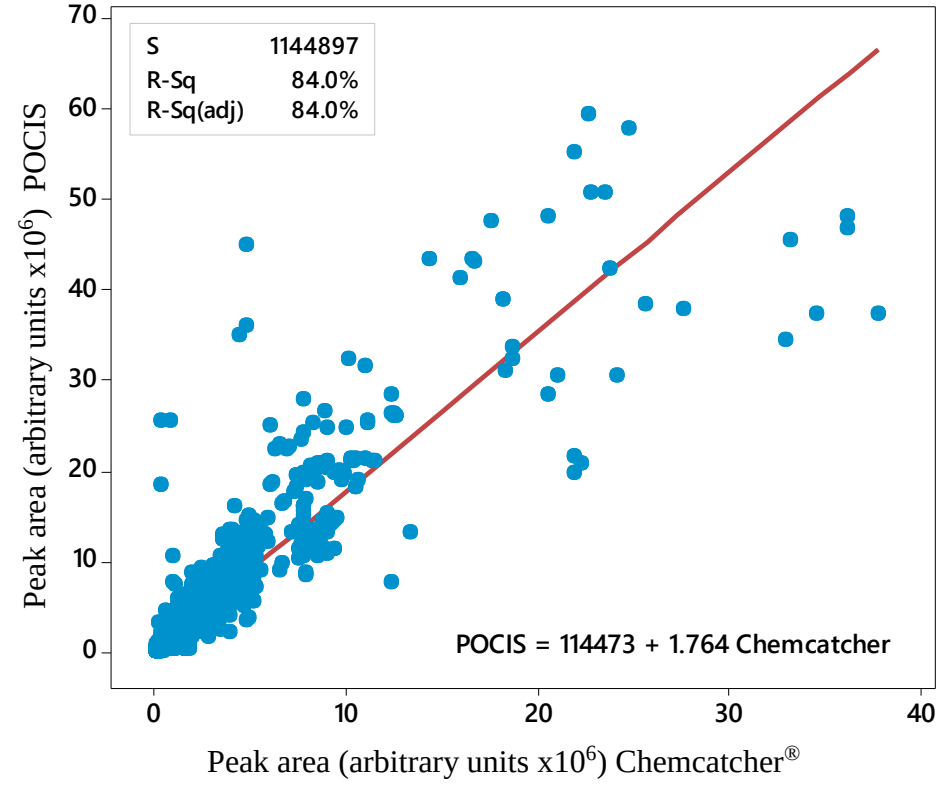
S	R-sq	R-sq(adj)	PRESS	R-sq(pred)
1144897	84.01%	84.01%	1.77E+16	83.66%

Coefficients

Term	Coef	SE Coef	95% CI	T-Value	P-Value	VIF
Constant	114473	10313	(94257, 134688)	11.1	< 0.001	
Chemcatcher®	1.76364	0.00669	(1.75052, 1.77676)	263.54	< 0.001	1.00

Regression Equation

POCIS = 114473 + 1.76364 Chemcatcher®



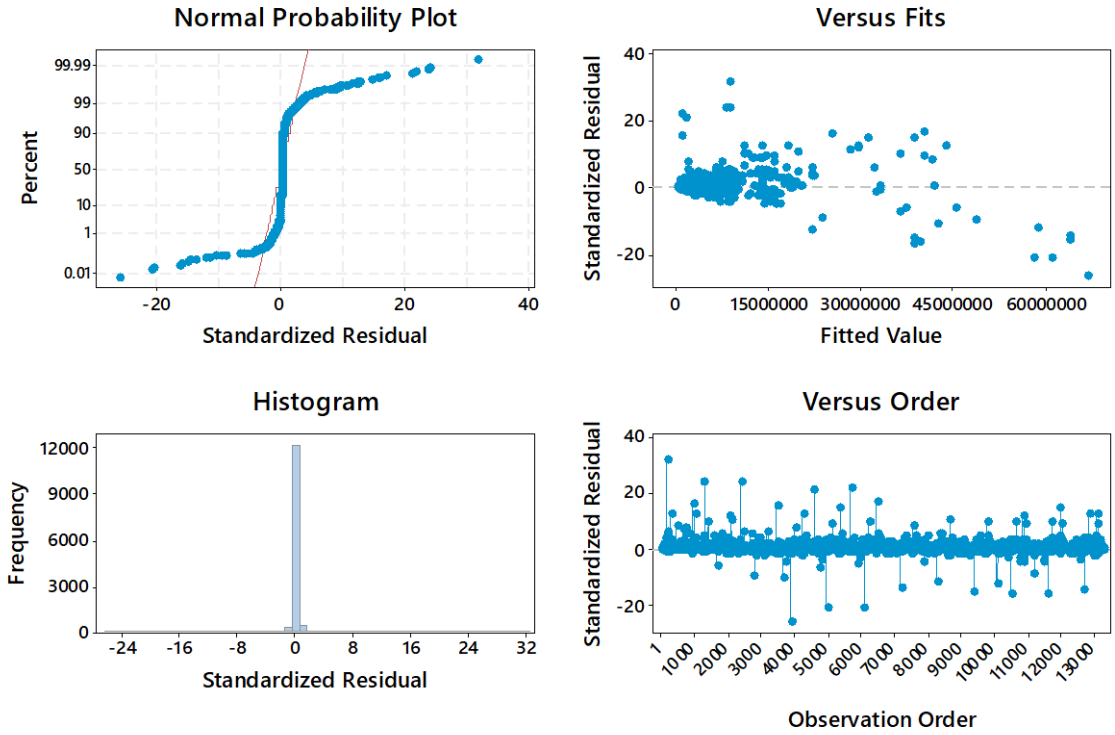


Figure 4.8 Fitted line and plots of standardised residuals obtained from least squares regression analysis of 'unknowns' in all replicates of POCIS and Chemcatcher®.

Table 4.12 Output from orthogonal regression analysis of 'unknowns' in all replicates of POCIS and Chemcatcher®.

Error Variance Ratio (POCIS/Chemcatcher®): 1

Regression Equation

POCIS = 8987 + 2.026 Chemcatcher®

Coefficients

Predictor	Coef	SE Coef	Z	P	Approx 95% CI
Constant	8986.923	10963.5	0.8197	0.412	(-12501.1, 30474.9)
Chemcatcher®	2.02598	0.0	263.5347	< 0.001	(2.0, 2.0)

Error Variances

Variable	Variance
POCIS	2.87E+11
Chemcatcher®	2.87E+11

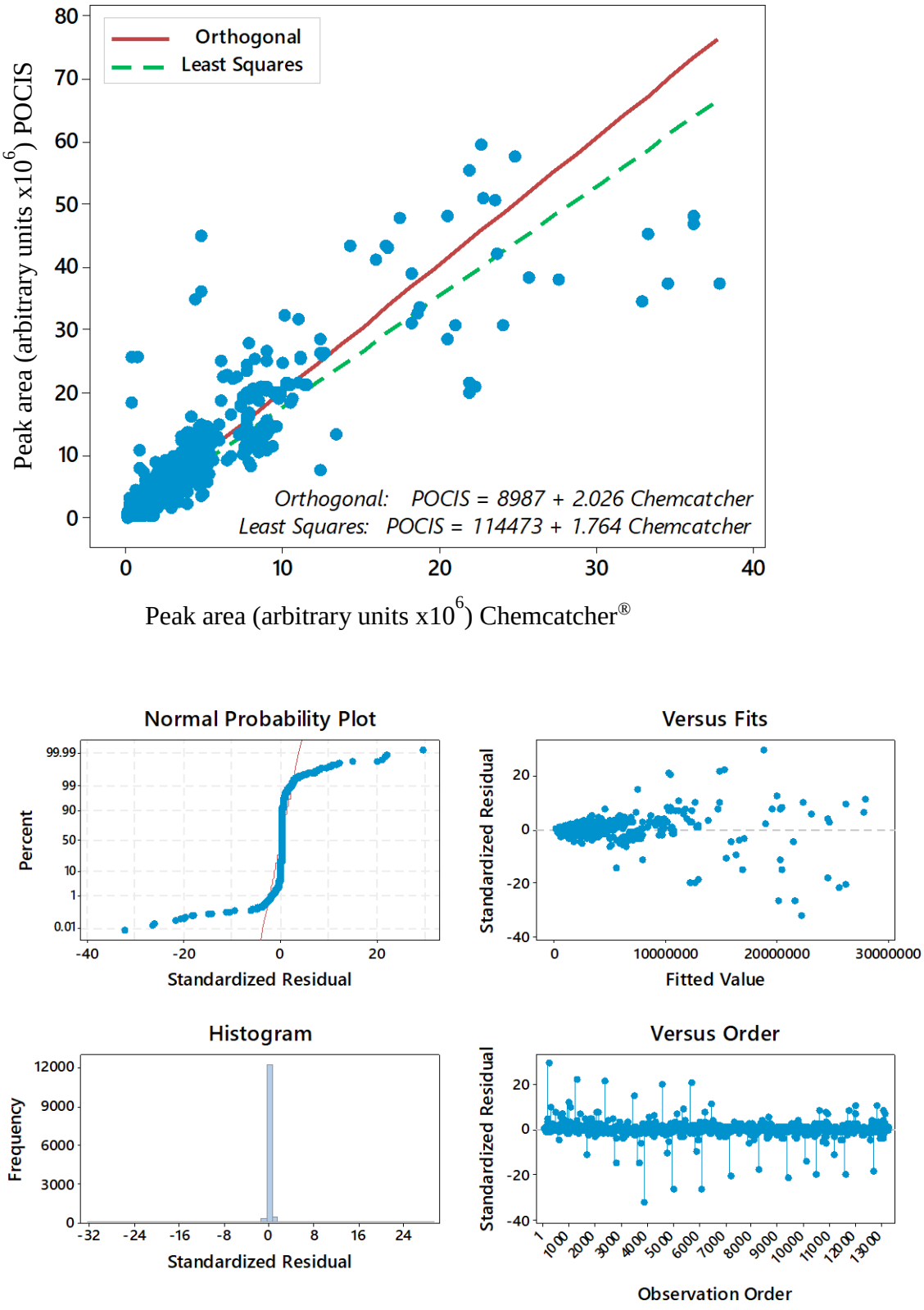


Figure 4.9 Fitted line and plots of standardised residuals obtained from orthogonal regression analysis of 'unknowns' in all replicates of POCIS and Chemcatcher®.

4.4.8 Least squares and orthogonal regression analysis of the averaged 'unknowns' data obtained from Chemcatcher® and POCIS

The output from the least squares regression is shown in Table 4.13. The variation accounted for by the regression (r^2) is 84.8% and the slope of the regression is 1.77 (t = 157.01, pr = < 0.001) which is significantly different from zero since the approximate 95% confidence interval is 1.75-1.79. The intercept of 111,421 (t = 6.42, pr = < 0.001) falls within the 95% confidence range of 77,403 to 145,438 which does not include zero. As it is close to zero it can be taken that the two samplers are behaving in a similar manner, but with the sampling rate of POCIS being 1.77 times that of the Chemcatcher®. The values for the slope and intercept based on the averaged data are very similar to those obtained from the least squares regression for all replicates which indicates that the replicate data does not show significant variability. The plots of residuals in Figure 4.10 displays heteroscedasticity but as expected with fewer extreme standardised residual values. The variance is not constant but increases with the amount of compound accumulated.

The output from the orthogonal regression is shown in Table 4.14. The slope of the regression is 2.02 which is significantly different from zero since the approximate 95% confidence interval is 2.0. The intercept of 11,951 is not significantly different from zero since it falls within the 95% confidence range that contains zero (-24,073.9, 47,975.4), therefore it can be taken that the two samplers are behaving in a similar manner, but with the sampling rate of POCIS being 2.02 times that of the Chemcatcher®. The plots of residuals in Figure 4.11 also displays significant heteroscedasticity but with few extreme standardised residual values in the range 5–30.

To assess the overall impact of the extreme residual values on the least squares regression, a statistical measure called DFITS was used to determine whether an observation was unusual. DFITS uses the leverage and deleted (studentised) residual to calculate the difference between the fitted value calculated with and without the *i*th observation. DFITS represents roughly the number of estimated standard deviations that the fitted value changes when the *i*th observation is removed from the data. According to Belsley, Kuh and Welsch (343), observations with DFITS value greater than $2\sqrt{\frac{k}{n}}$ deserve attention where k is the number of estimated coefficients and n is the sample size. Based on the number of data points (4,408) obtained after averaging, a DFITS value of between -0.031 and 0.031 was calculated and any data points outside this range were removed (a total of 140 or 3.8% of the data set) as they were considered to exhibit significant leverage. The remaining data, within the range -0.031 and 0.031, was re-plotted using least squares regression and the slope obtained compared to the original.

The output from the least squares regression is shown in Table B1 (Appendix B). The variation accounted for by the regression (r^2) is 89.4% and the slope of the regression is 1.9 (t = 7.46, pr = <0.001) which is significantly different from zero since the approximate 95% confidence interval is 1.87-1.91. The intercept of 33154 (t = 6.42, pr = <0.001) falls within the 95% confidence range of 24,440 to 41,868 which does not include zero. As it is close to zero it can be taken that the two samplers are behaving in a similar manner, but with the sampling rate of POCIS being 1.9 times that of the Chemcatcher®. The value for the slope is very similar to that obtained (1.77) from the least squares regression for the averaged data prior to application of DFITS, indicating that the extreme residuals had very little influence on the slope.

The plots of residuals in Figure B1 (Appendix B) displays lower extreme residual values (all less than 8) and almost equally distributed above and below zero. To assess the overall impact of the extreme residual values on the orthogonal regression, standardised residuals were used to assess the regression model. If the normal linear model holds, the standardised residuals have approximately standard normal distributions. Therefore, approximately 95% of them will be between -2 and +2. Standardised residuals greater than 2 were removed (a total of 72 or 1.6% of the data set) and the remaining data re-plotted using orthogonal regression.

The output from the orthogonal regression is shown in Table B2 (Appendix B). The slope of the regression is 2.11 which is significantly different from zero since the approximate 95% confidence interval is between 2.10 and 2.13. The intercept of -16,572 falls within the 95% confidence range of (-27,759 and -5,386) and almost includes zero. It can therefore be taken that the two samplers are behaving in a similar manner, but with the sampling rate of POCIS being 2.1 times that of the Chemcatcher®. The value for the slope is very similar to that obtained (2.02) from the orthogonal regression for the averaged data prior to application of standardised residuals filter, indicating that the extreme residuals had very little influence on the slope. The plots of residuals in Figure B2 (Appendix B) displays lower extreme residual values (all less than 8.5) and almost equally distributed above and below zero.

Least squares and orthogonal regression regression analysis was performed on the averaged Log_{10} transformed Chemcatcher® and POCIS 'unknowns' data. The outputs are discussed in C1.1 and shown in Tables C1 & C2 plus Figures C1 & C2 in Appendix C.

Table 4.13 Output from least squares regression analysis of the averaged Chemcatcher® and POCIS 'unknowns' data.

Analysis of Variance

Source	DF	Seq SS	Contrib.	Adj SS	Adj MS	F-Value	P-Value
Regression	1	3.05E+16	84.84%	3.05E+16	3.05E+16	24652.21	< 0.001
Chemcatcher® (average)	1	3.05E+16	84.84%	3.05E+16	3.05E+16	24652.21	< 0.001
Error	4406	5.45E+15	15.16%	5.45E+15	1.24E+12		
Total	4407	3.59E+16	100.00%				

Model Summary

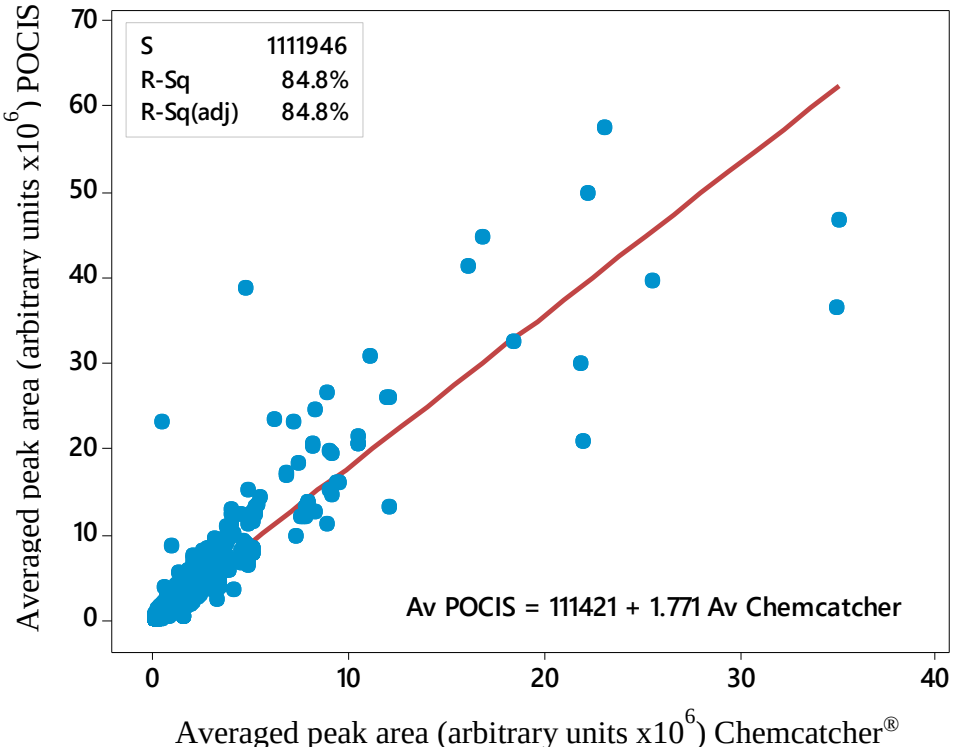
S	R-sq	R-sq(adj)	PRESS	R-sq(pred)
1111946	84.84%	84.83%	5.85E+15	83.71%

Coefficients

Term	Coef	SE Coef	95% CI	T-Value	P-Value	VIF
Constant	111421	17351	(77403, 145438)	6.42	< 0.001	
Chemcatcher® (average)	1.7712	0.0113	(1.7491, 1.7933)	157.01	< 0.001	1.00

Regression Equation

POCIS (average) = 111421 + 1.7712 Chemcatcher® (average)



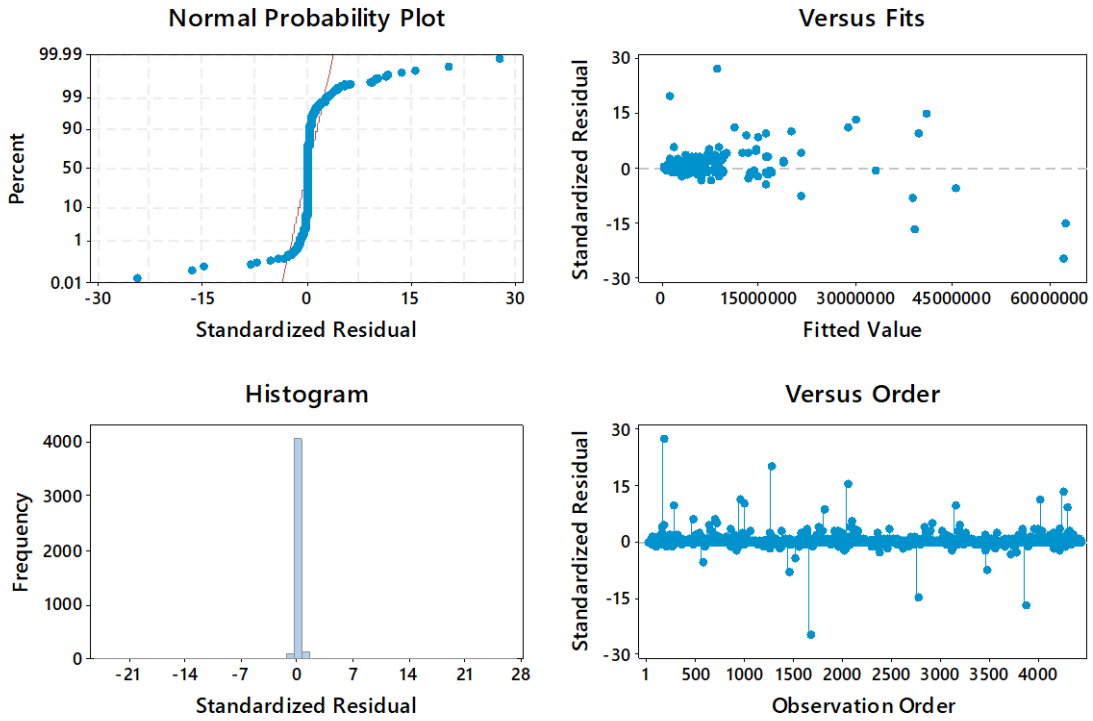


Figure 4.10 Fitted line and plots of standardised residuals obtained from least squares regression analysis of the averaged Chemcatcher® and POCIS 'unknowns' data.

Table 4.14 Output from orthogonal regression analysis of the averaged Chemcatcher® and POCIS 'unknowns' data.

Error Variance Ratio POCIS (average) / Chemcatcher® (average): 1

Regression Equation

POCIS (average) = 11951 + 2.019 Chemcatcher® (average)

Coefficients

Predictor	Coef	SE Coef	Z	P	Approx 95% CI
Constant	11950.75	18380.3	0.6502	0.516	(-24073.9, 47975.4)
Chemcatcher® (average)	2.01861	0.0	157.0083	< 0.001	(2.0, 2.0)

Error Variances

Variable	Variance
POCIS (average)	2.70E+11
Chemcatcher® (average)	2.70E+11

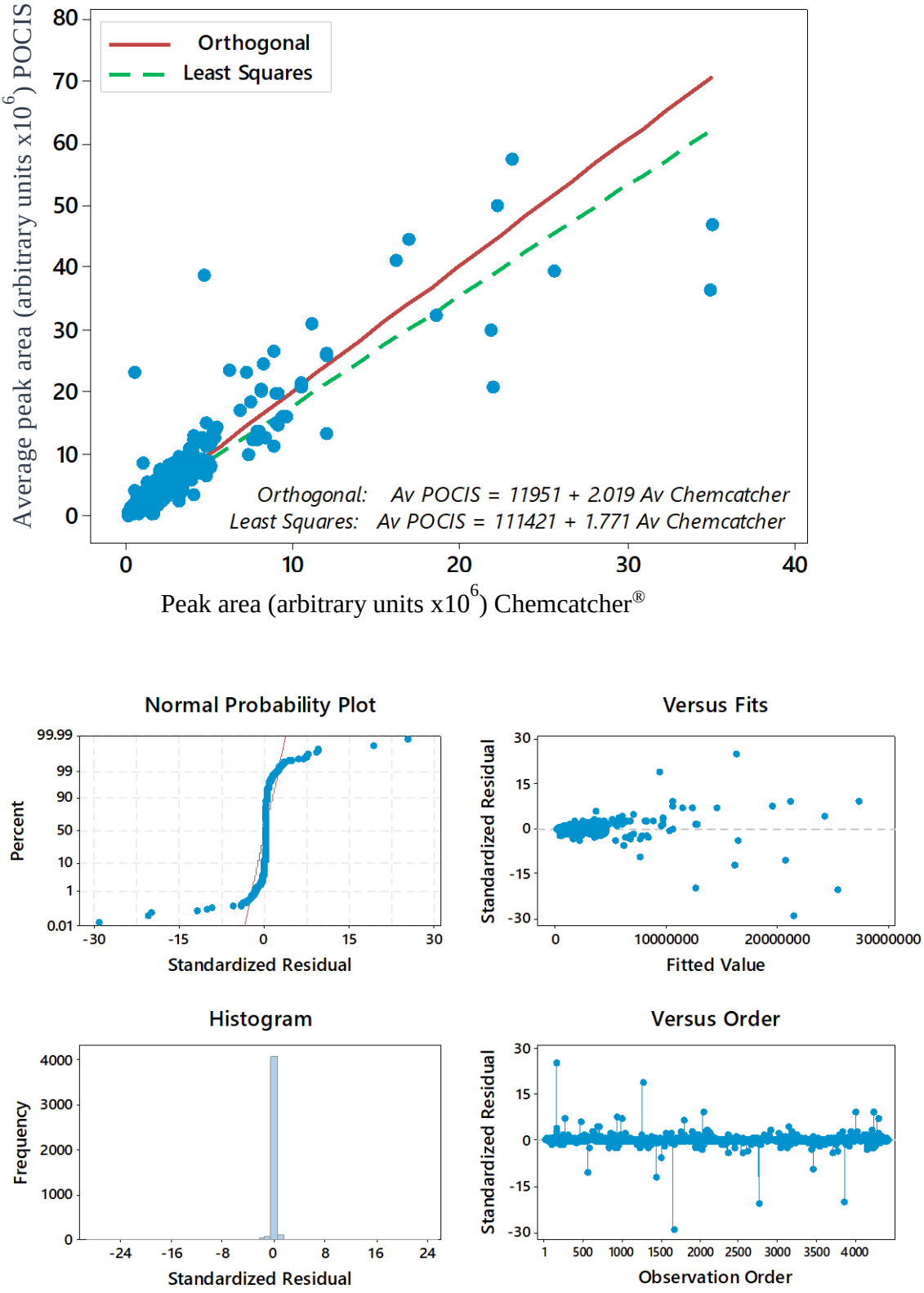


Figure 4.11 Fitted line and plots of standardised residuals obtained from orthogonal regression analysis of the averaged Chemcatcher® and POCIS 'unknowns' data.

4.4.9 Mass and retention time profiling of passive sampling extracts

The mass/retention time profiling plots obtained from the Chemcatcher® and POCIS extracts for the Carmarthen site were almost identical (Figure 4.12) with 4,695 and 4,967 features detected for the Chemcatcher® and POCIS samplers respectively, amounting to 6% more features detected in the POCIS. The mass/feature and retention time/feature histograms in Figure 4.18 clearly shows the similarity obtained from both samplers at the Carmarthen site. This observation was unexpected as the larger surface sampling area of the POCIS was anticipated to sequester more compounds present at lower concentrations in the effluent stream which may have gone undetected in the Chemcatcher® extract. Both samplers can therefore be shown to be behaving in an identical manner at the Carmarthen site.

The overall mass/retention time profiles for the Gowerton site was again almost identical for both samplers (Figures 4.13 and 4.14) with a negligible difference in the number of features found in the co-deployed samplers. The number of features detected were 4,570/4,952 in the Gowerton '1' deployment with 4572/4944 features detected in the Gowerton '2' deployment for the Chemcatcher® and POCIS samplers respectively. This amounted to an average of 8% more features detected in the POCIS samplers. The mass/feature and retention time/feature histograms in Figure 4.19 and 4.20 show the similarity obtained from both samplers site and they can therefore be shown to be behaving in a very similar manner at the Gowerton site.

The mass/retention time profile obtained for the Llanelli site were significantly different between samplers (Figure 4.15). The number of features detected were 3,690 and 4,872 for the Chemcatcher® and POCIS samplers respectively amounting to 32%

more features detected in the POCIS sampler. A clear distinction is seen in the mass histogram/feature histogram in Figure 4.21 and specifically so for features with molecular masses above 500 Da where the number of features detected in the Chemcatcher® was 35% of the number found in the POCIS. This is in stark contrast for features with molecular masses below 500 Da where the number of features found in the Chemcatcher® was 85% of the number found in the POCIS.

The histograms in Figure 4.21 indicate that most of the features with molecular masses above 500 Da elute between 16 and 20 min. A zoomed in section of the mass/retention time profile plot (Figure 4.16) for both samplers in this time range show a very distinct pattern of features which exhibited mass differences of 14 and 44 Da. These are, in all probability, homologous series of polyethoxylated compounds with differing alkyl chain lengths such as those found in domestic and industrial surfactants (344). These compounds readily degrade in waste water treatment plants to lower molecular mass compounds although sufficient concentrations remain intact and are readily detected in spot samples and passive sampling extracts (73, 345). Upon examining the summed peak areas, for features above 500 Da in all the Chemcatcher® extracts, the Llanelli site had the lowest value and was approximately 48% less than the Carmarthen site which had the largest summed peak area. Upon examining the individual peak areas, for features above 500 Da in all the Llanelli Chemcatcher® extracts, many were close to the threshold value set for peak detection and integration. Therefore, the most likely reason for the difference in the molecular mass profiles of the Chemcatcher® and POCIS extracts for the Llanelli site, is that many features in the Chemcatcher® extract, which eluted between 16 and 20 min were below the detection limit of the Q-TOF-MS instrument.

The mass/retention time profiling plots obtained from the Chemcatcher® and POCIS field blank extracts for the Carmarthen site are shown in Figure 4.17 which show the presence of a low number of polyethoxylated compounds but which elute much earlier in the chromatogram than those observed in the actual deployments. In all likelihood, these originated from the polyethersulphone membrane and were not completely removed during the organic solvent cleaning step for the membranes as described in Chapter 3.

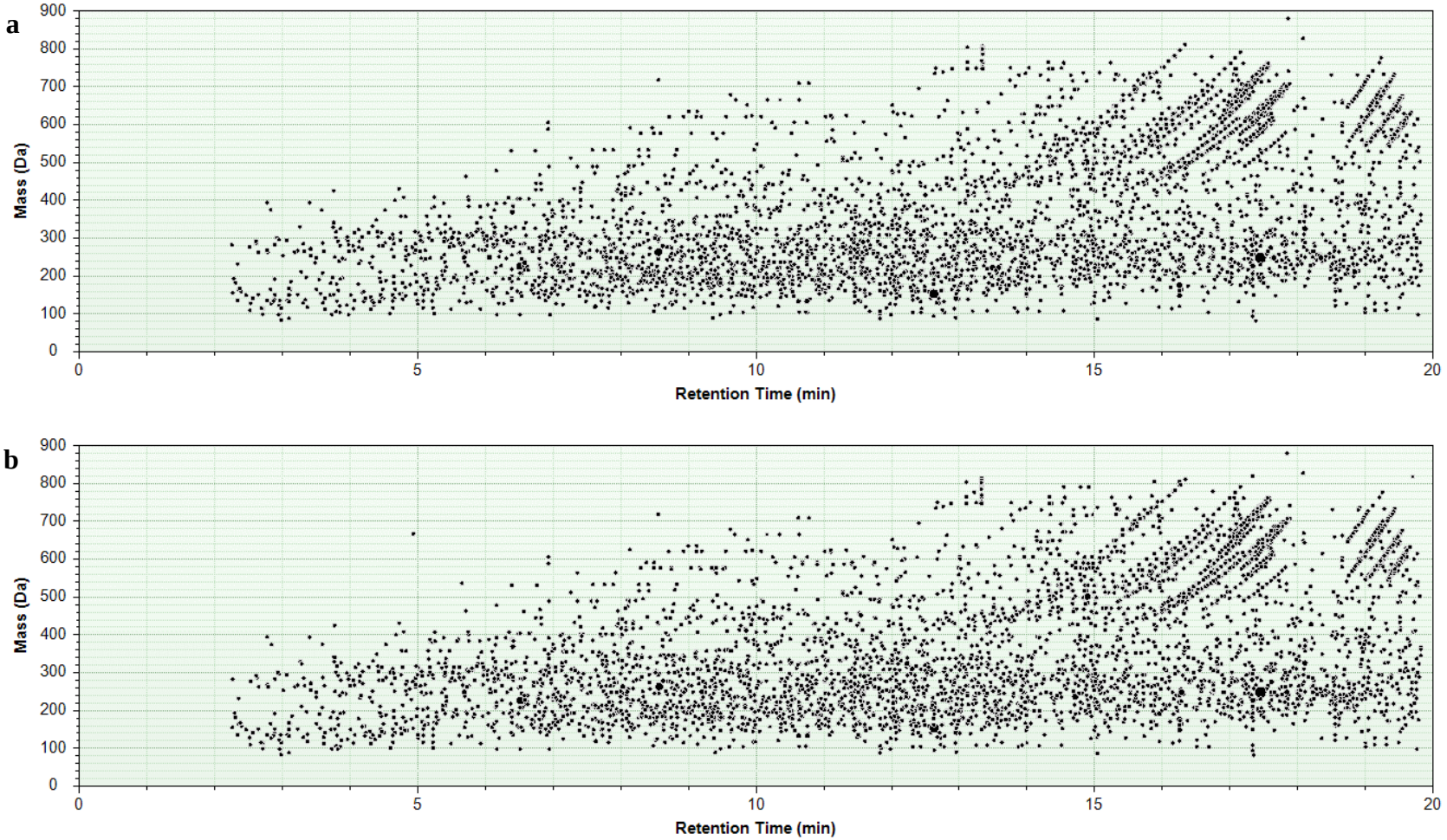


Figure 4.12 Plot of mass versus retention time for features identified in the Chemcatcher® (a) and POCIS (b) samplers deployed at the Carmarthen WWTP.

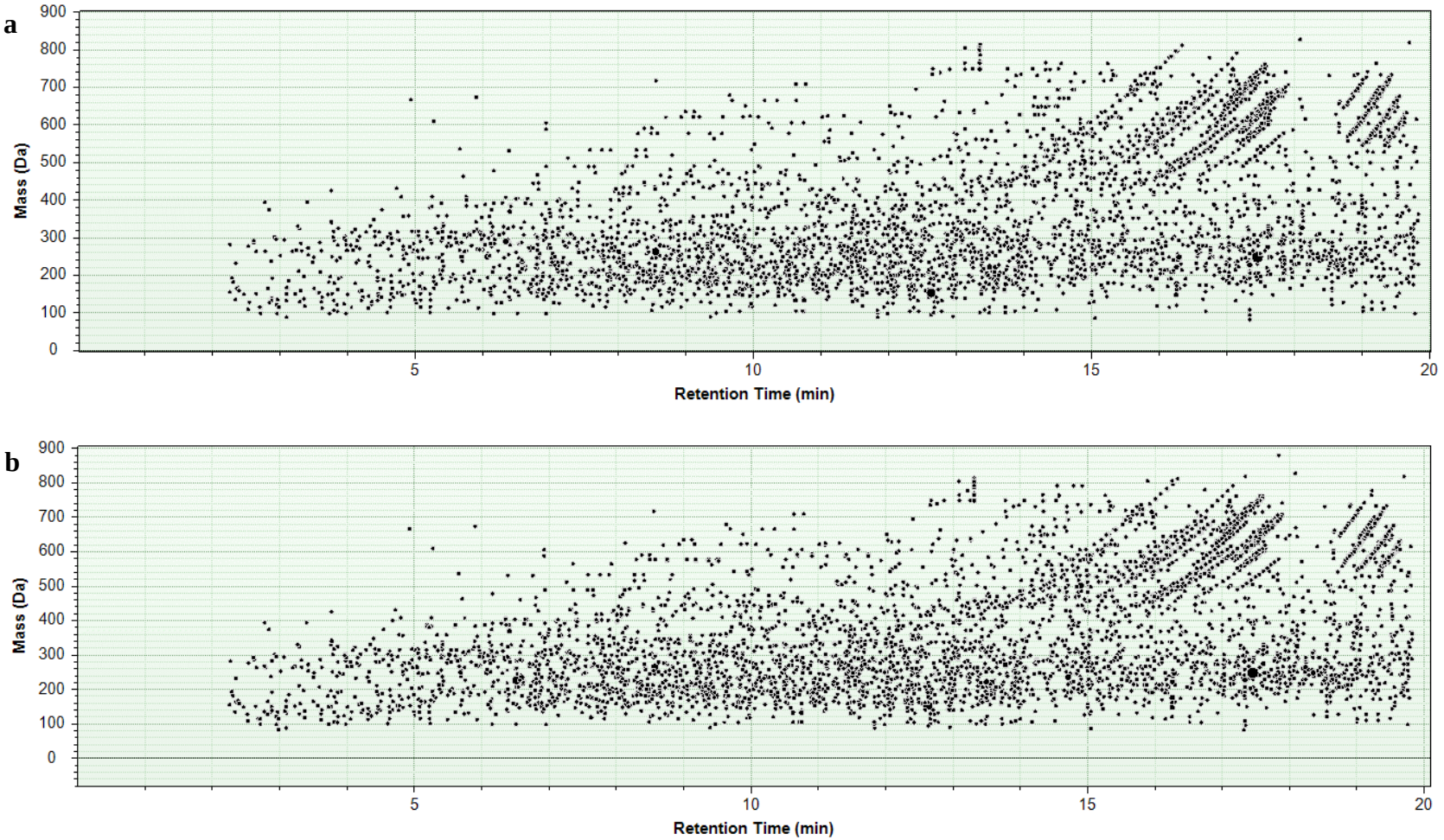


Figure 4.13 Plot of mass versus retention time for features identified in the Chemcatcher® (a) and POCIS (b) samplers deployed at the Gowerton '1' WWTP.

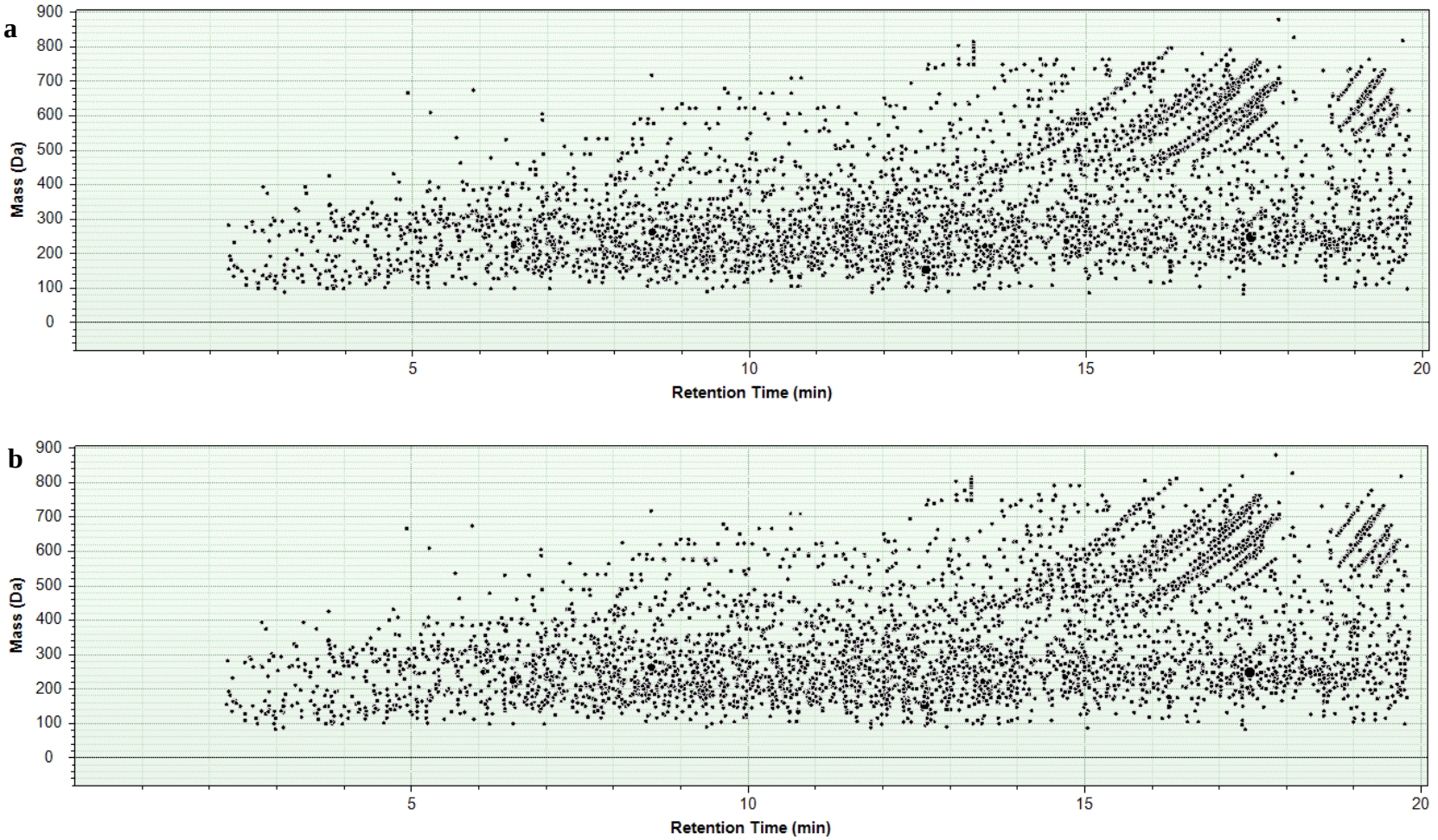


Figure 4.14 Plot of mass versus retention time for features detected in the Chemcatcher® (a) and POCIS (b) samplers deployed at the Gowerton '2' WWTP.

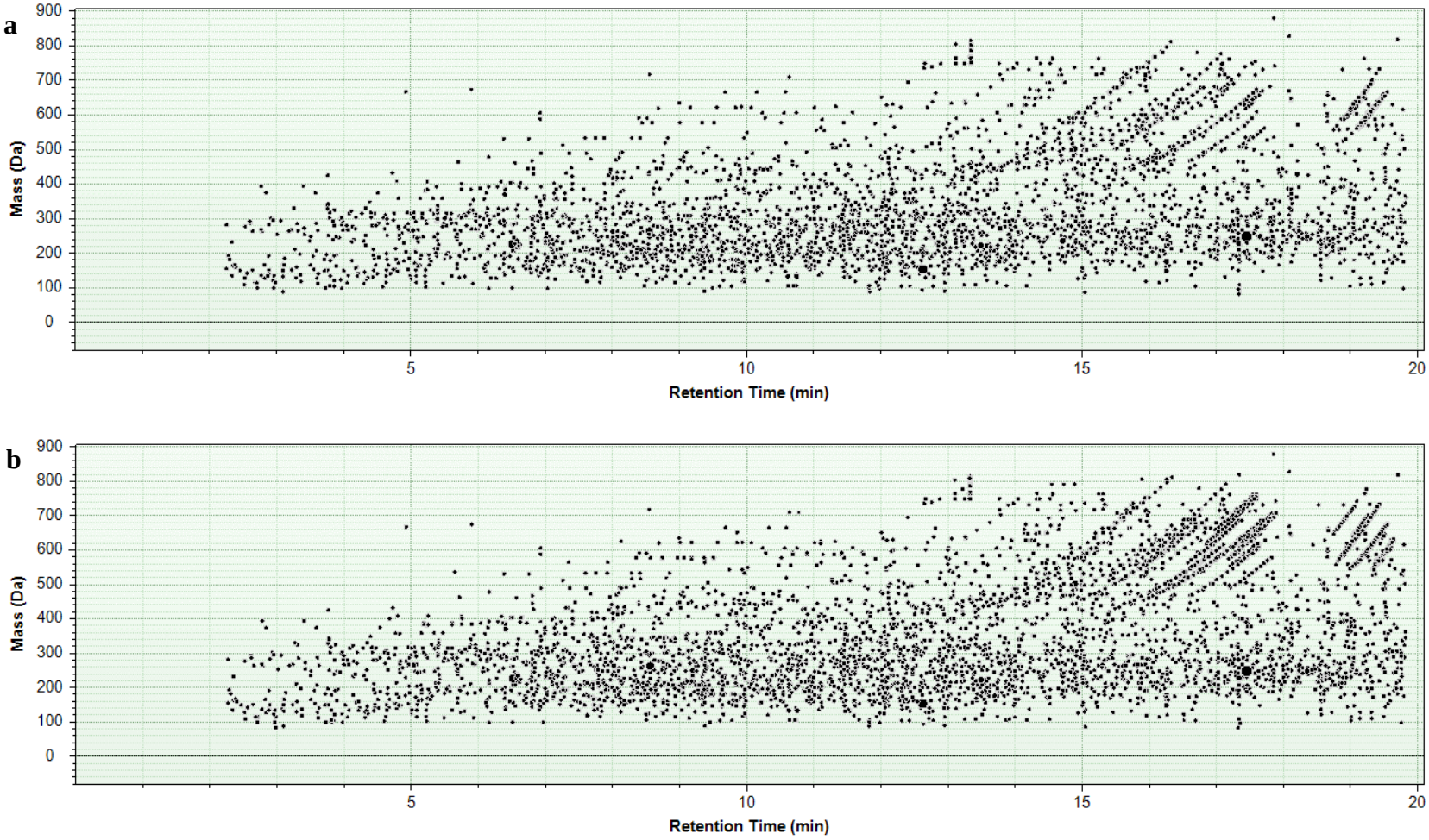


Figure 4.15 Plot of mass versus retention time for features detected in the Chemcatcher® (a) and POCIS (b) samplers deployed at the Llanelli WWTP.

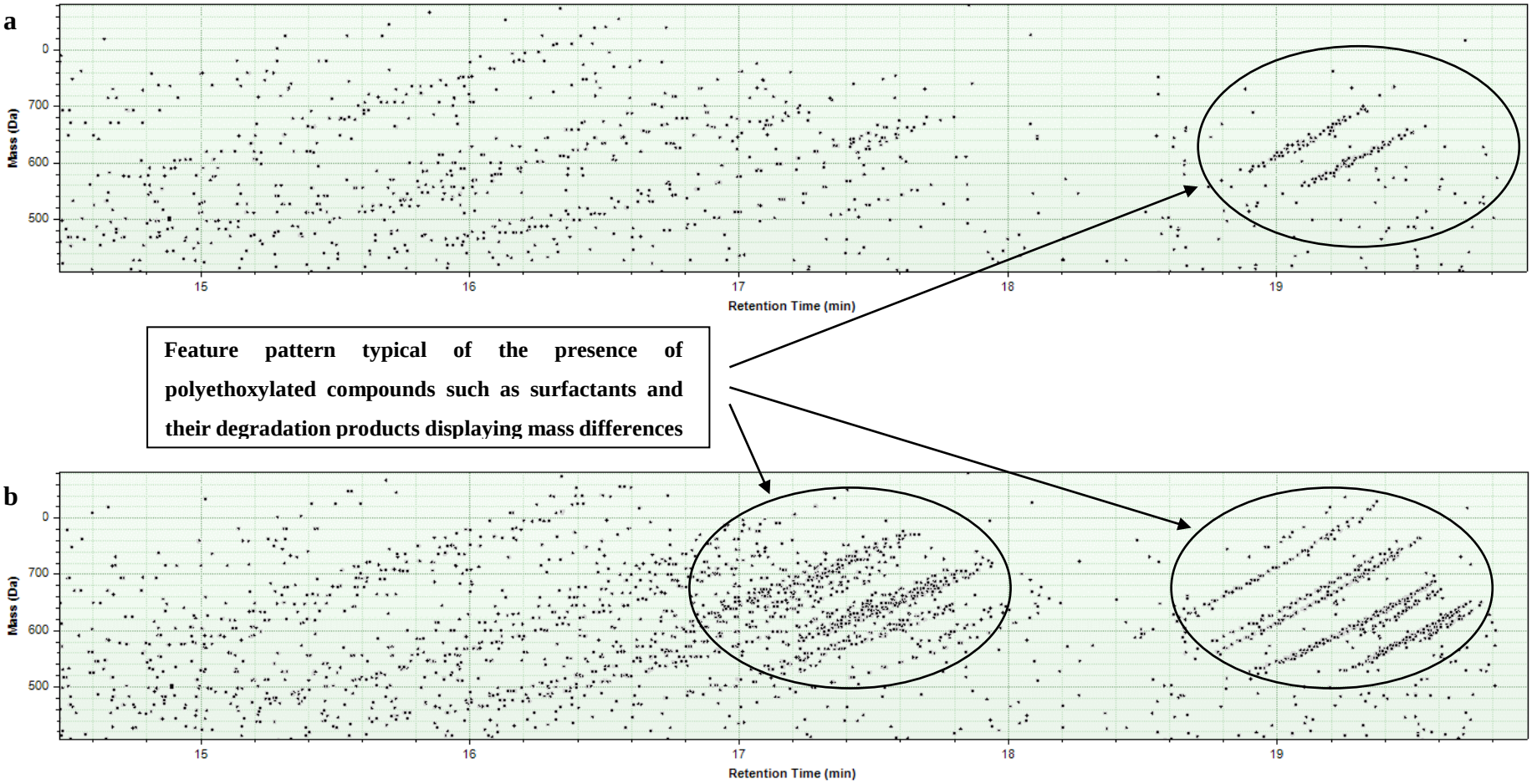


Figure 4.16 'Zoomed-in' section of the mass versus retention time plot (14.5–20 min, 420–900 Da) for the Chemcatcher® (a) and POCIS (b) samplers deployed at the Llanelli WWTP.

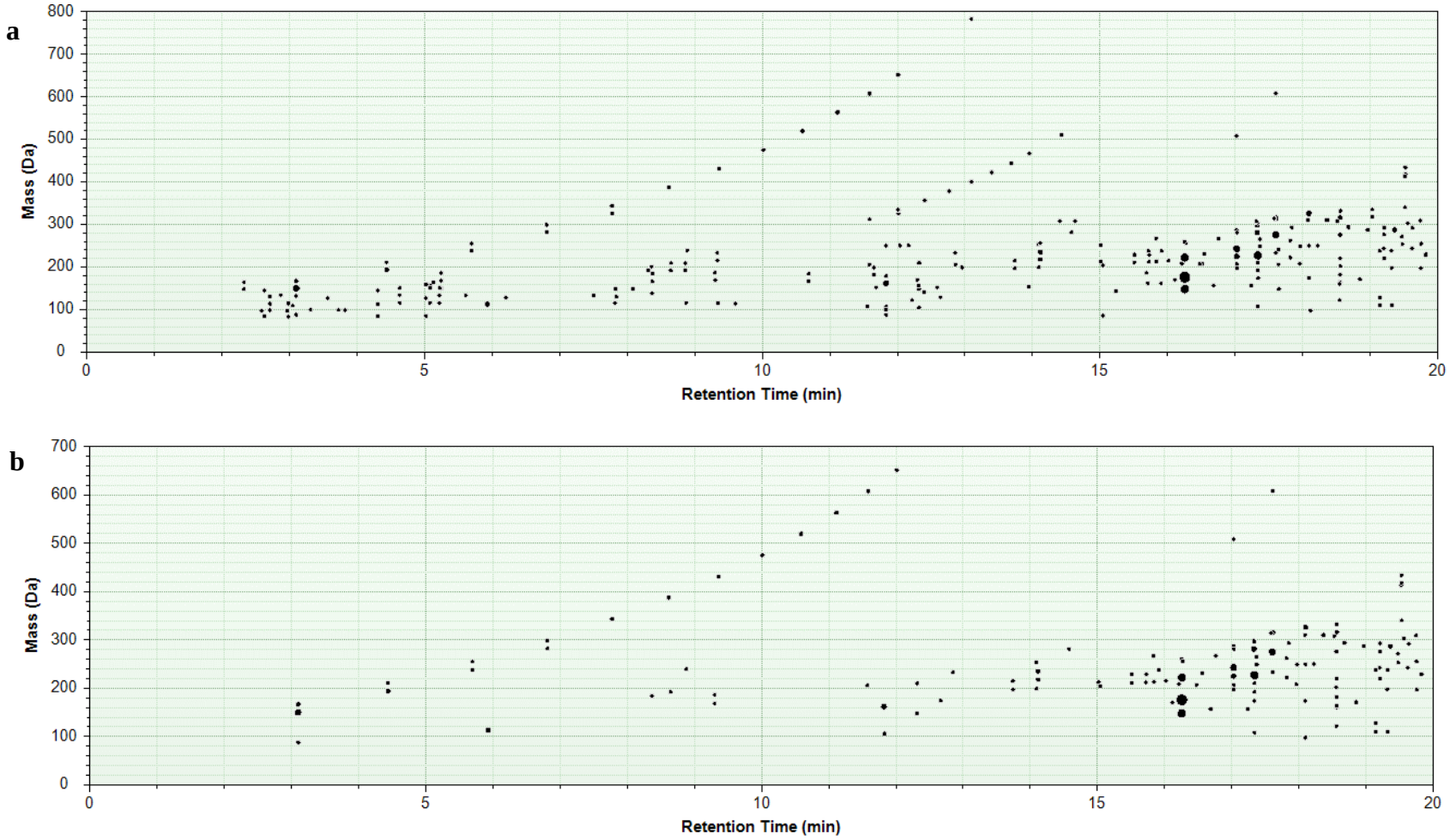


Figure 4.17 Plot of mass versus retention time for features detected in the Chemcatcher® (a) and POCIS (b) field blanks used at the Carmarthen WWTP.

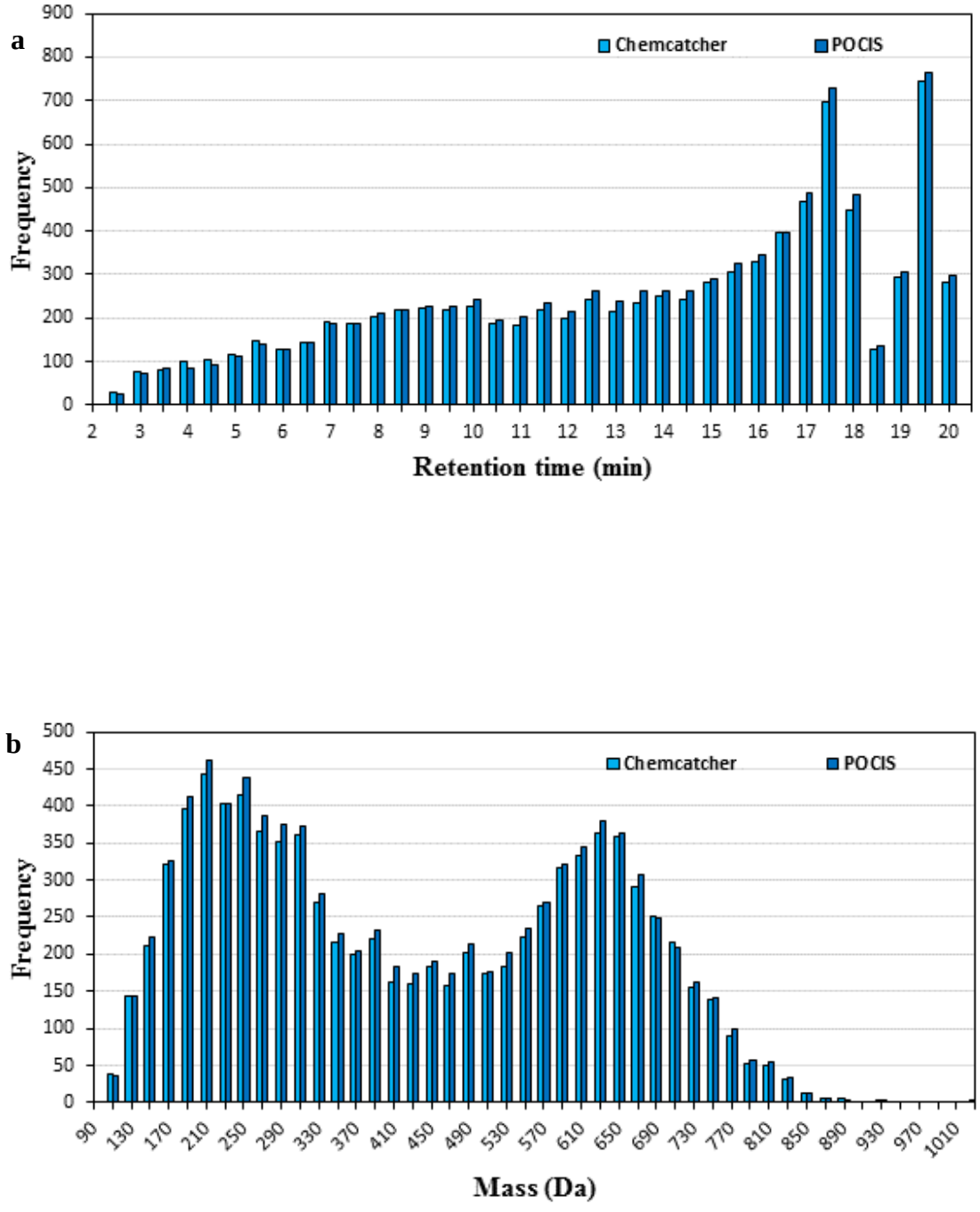


Figure 4.18 Retention time/feature (a) and mass/feature (b) histograms for the Chemcatcher® and POCIS samplers deployed at the Carmarthen WWTP.

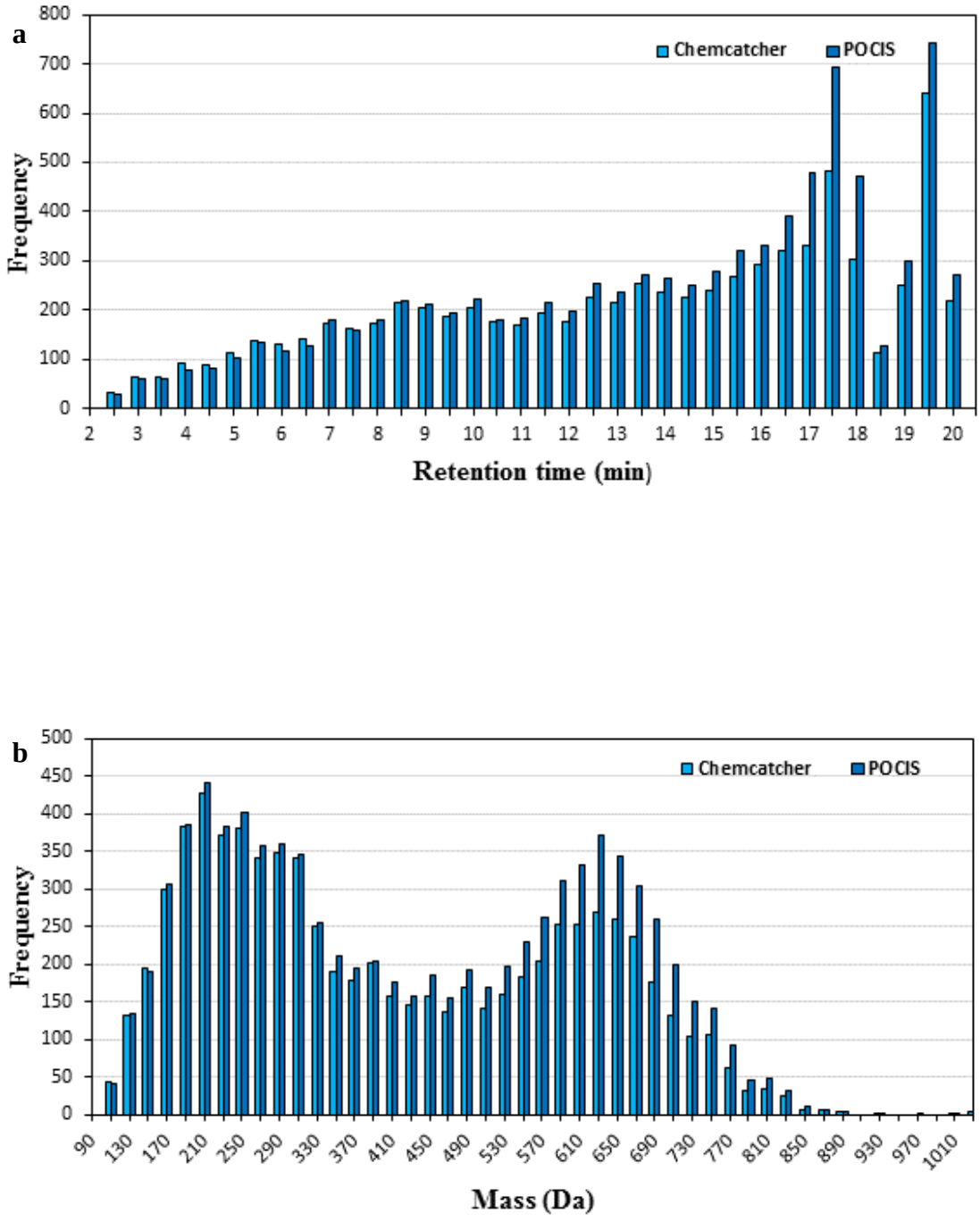


Figure 4.19 Retention time/feature (a) and mass/feature (b) histograms for the Chemcatcher® and POCIS samplers deployed at the Gowerton '1' WWTP.

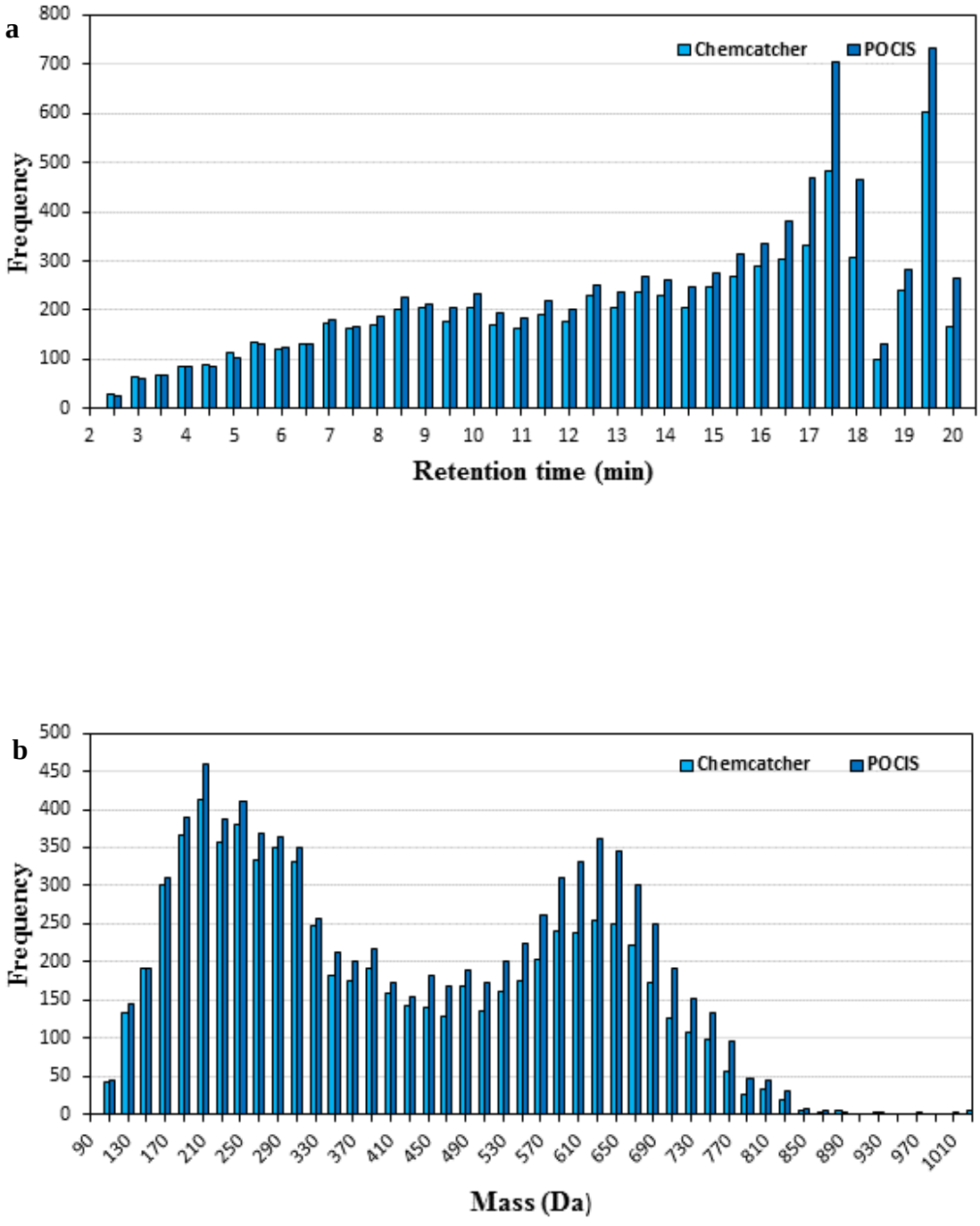
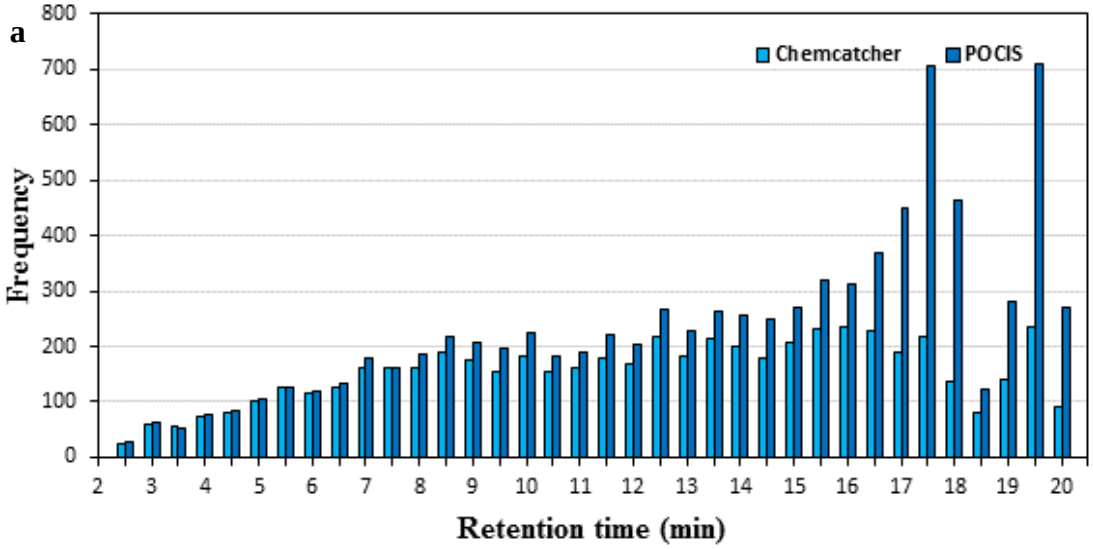


Figure 4.20 Retention time/feature (a) and mass/feature (b) histograms for the Chemcatcher® and POCIS samplers deployed at the Gowerton '2' WWTP.



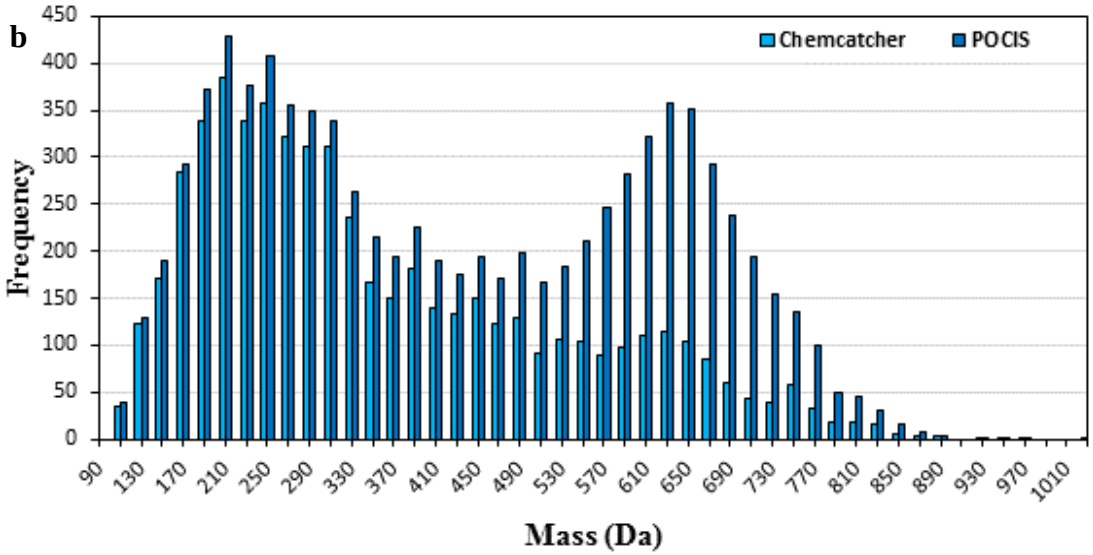


Figure 4.21 Retention time/feature (a) and mass/feature (b) histograms for the Chemcatcher® and POCIS samplers deployed at the Llanelli WWTP.

4.5 Conclusions

Fixty-six and 32 compounds exceeded 15 % RSD in the Chemcatcher® and POCIS extracts respectively, the higher RSD's in the Chemcatcher® strongly correlated with peak area response which were approximately 3 times lower in the Chemcatcher. Only 10 compounds exceeded 30% RSD in both samplers with the largest error observed for the POCIS sampler at 165% RSD for the compound loratadine. The highest RSD observed for loratadine in the Chemcatcher® was only 23%. RSD's obtained from negative ion analysis were generally lower for the Chemcatcher® sampler across all sites with five and three compounds exceeding 15 % RSD in the Chemcatcher® and POCIS extracts respectively. Only one compound, naproxen in the POCIS sampler, exceeded 30% RSD in negative ion mode.

Two-way ANOVA analysis of the pharmaceutical data indicated a significant difference between sampler types for the Gowerton co-deployments but not between location and there was no significant interaction between location and sampler. Principal component analysis for the Gowerton co-deployments indicated that one major component (i.e. the uptake rate) accounted for 98.3% of the total variation. Least squares regression of the data from each site produced slopes between 2.3 (Gowerton '2') and 2.8 (Carmarthen), the differences possibly due to the varying flow rates at each site which may have influenced one sampler more than the other. The slopes obtained from least squares and orthogonal regression of the entire averaged pharmaceutical data set suggests that the Chemcatcher® uptake rate is approximately 2.4 to 2.6 times lower than that for the POCIS.

Two-way ANOVA analysis of the features detected in the 'unknowns' data also indicated a significant difference between sampler types for the Gowerton co-deployments but not between location and there was no significant interaction between location and sampler. Principal component analysis for the Gowerton co-deployments indicated that one major component (i.e. the uptake rate) accounted for 95.8% of the total variation. Individual least squares regression of the averaged data from each site show little variation between the slopes for two sites (1.6, 1.8 and 1.8 for Gowerton '1', Gowerton '2' and Llanelli respectively) but a larger variation was observed for the Carmarthen site with a slope of 2.1. The slopes obtained from least squares and orthogonal regression of the entire averaged 'unknowns' data set suggests that the Chemcatcher® uptake rate is approximately 1.9 to 2.1 times lower than that for the POCIS. The differences in the uptake rates for the pharmaceuticals and the 'unknowns' data is, in all probability, due to the larger sampling area of 45.8 cm^2 for the POCIS sampler compared to 15.2 cm^2 for the Chemcatcher®, a factor of 3.01.

The mass profiling plots obtained for both samplers were almost identical for the Carmarthen site and very similar for the co-deployments at the Gowerton site. However, significant differences were observed between the samplers at the Llanelli site for features with masses above 500 Da. This was attributed to many features, eluting between 16–20 min, in the Llanelli Chemcatcher® sampler extract being just below the limit of detection of the Q-TOF-MS instrument.

It can be concluded from the LC Q-TOF-MS analysis of the extracts from the Chemcatcher® and POCIS extracts and the extensive statistical analysis that the performance of the polar Chemcatcher® is very similar to that of the POCIS.

Chapter 5

In-silico prediction of liquid chromatographic retention times to aid in the identification of unknown or suspect compounds

5.1 Introduction

The purpose of this chapter was to address the analytical challenges associated with the tentative identification of ‘suspect’ or ‘known unknowns’ pollutants present in polar passive samplers deployed in the aquatic environment.

Hyphenated LC/MS techniques allow for the fast and highly sensitive generation of detailed and information rich analytical data on the compounds studied in various environmental matrices. Despite the introduction of large MS/MS libraries and on-line searchable MS/MS databases, a major shortcoming in suspect or screening approaches is the lack of chromatographic retention times which is essential for tentative compound identification. The lack of reference standards for new pharmaceuticals, metabolites, pesticides and the plethora of industrial chemicals, however, forces the analyst to find alternative tools for tentative compound identification.

This chapter therefore focuses on the use of a software package to predict the retention times of compounds based on their structure and physicochemical properties and is evaluated with respect to accuracy and practical application.

5.2 Aims and objectives

i) To develop a complementary approach, based on retention time prediction, for the analysis of suspect pollutants in polar passive sampling extracts.

ii) To build a large comprehensive database of over 1550 compounds for use with liquid chromatographic retention time prediction software. The database would include known environmental pollutants including pharmaceuticals, pesticides, drugs of abuse and their metabolites.

iii) To assess the ability of the liquid chromatographic retention time prediction software to accurately calculate retention times for pharmaceutical residues

5.3 Experimental

5.3.1 Reagents and standards

All reagents and solvents were HPLC, LC/MS or analytical reagent grade. Ammonium formate, formic acid and methanol were purchased from Fisher Scientific UK (Loughborough, Leicestershire, UK) or Sigma-Aldrich (Gillingham, Dorset, UK). Ultrapure water was obtained from an in-house source (ELGA Purelab Ultra) and was used in all laboratory procedures (Elga Process Water, Marlow, Buckinghamshire, UK). The ultrapure water system was equipped with a UV lamp, carbon and membrane filter to remove trace organic compounds, ionic species and particulates. Stock standard solutions, intermediate and working solutions used were prepared as in Chapter 3.

5.3.2 LC Q-TOF-MS analysis

Chromatographic separation of compounds was carried out using a Dionex Ultimate 3000 UHPLC system consisting of a vacuum degasser, binary Pump, high performance auto-sampler and thermostated column compartment. (Thermo Fisher Scientific, Bremen, Germany).

The UHPLC system was interfaced to a Bruker Maxis Impact II Q-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). Chromatographic separation was performed on a Dionex Acclaim RSLC 120 C_{18} analytical column (2.1 i.d. × 100 mm length, 2.2 μm particle size, Thermo Fisher Scientific, Dreieich, Germany). A guard column, Waters VanGuard, Acquity UPLC BEH C_{18} 1.7 μm, (Dublin, Ireland), was placed ahead of the analytical column and both columns thermostated at 30˚C. The entire system was operated with Bruker HyStar acquisition software (rev. 3.2).

The Q-TOF-MS was equipped with an electrospray ionisation source, operating in positive ionisation mode only. The mobile phase was (A) methanol with 5 mM ammonium formate and 0.01 % v/v formic acid and (B) an aqueous solution comprised of 10 % of methanol, 5 mM ammonium formate and 0.01 % formic acid. The gradient and flow elution programme was: 0 min,1 % B, 0.2 mL/min; 3 min, 39 % B, 0.2 mL/min; 14 min, 99.9 % B, 0.4 mL/min; 16 min, 99.9 % B, 0.480 mL/min; 16.1 min, 1% B, 0.480 mL/min; 19 min, 1 % B, 0.480 mL/min; 19.1, 1 % B, 0.2 mL/min; 20 min, 1 % B, 0.2 mL/min.

The Q-TOF-MS operation parameters were: capillary voltage, 2500 V; end plate offset, 500 V; nebulizer pressure, 2 bar (N2); drying gas, 8 L/min (N_2); and drying

temperature, 200 °C. The injection volume was 20 µL. The Q-TOF-MS system was used in broadband collision-induced dissociation (bbCID) acquisition mode and recorded spectra over the range 30−1000 Da at a scan rate of 2 Hz. The Bruker 'bbCID' mode provides MS and MS/MS spectra at the same time, while it works at two different collision energies. A low collision energy of 6 eV was used to acquire MS spectra, and a higher energy setting of 30 eV was used to obtain MS/MS spectra. The higher energy setting was ramped from 80–120 % of its value (i.e from 24-36 eV). Data was collected by the mass spectrometer between 0.1 and 15.0 min.

A mass axis calibration was undertaken at the beginning of every chromatogram by infusing a mixture of 1 mM sodium formate in water/isopropanol/formic acid (1:1:0.01 v/v/v) with a syringe pump into the mass spectrometer ahead of the elution of the first target compound from the analytical column.

5.3.3 Formation of database

An in-house customised database for use with the ACD Labs 'Chrom Genius'LC method development software was compiled from a commercial database 'ToxScreener' (Bruker Daltonics, Bremen, Germany) which contained accurate mass, retention time and fragment ion information for 1556 compounds including pharmaceuticals, pesticides, drugs of abuse and their metabolites. The 'ToxScreener' database was curated by toxicologists at the forensic institutes at the Universities of Freiburg, Germany and Helsinki, Finland. The fragment ions for each compound were obtained from two different collision energies as stated in 7.4. The full list of compounds appears in the Table D2 (Appendix D).

Of the 165 pharmaceuticals that were screened for in Chapter 3, 103 of these appeared in the 'ToxScreener' database. The 103 compounds were removed from the database to avoid any potential bias, and would be used as the 'testing set' of compounds for the determination of the accuracy of the retention time prediction model. Canonical simplified molecular line entry system strings (SMILES) were obtained from the ChemSpider website (Royal Society of Chemistry, UK) or created using ACD Labs ChemSketch (Advanced Chemistry Development, Inc., Toronto, Canada) for the remaining 1453 compounds in the database. These compounds were representative of the 'training set' that would be used to build the retention time prediction model.

The SMILES notations generated for the 'training set' were then imported into ACD Labs ChromGenius software. ChromGenius software bases retention time prediction on physicochemical parameters that are calculated for the 'training set' compounds whose retention times had been measured previously. The parameters used to create a chromatographic model for a reverse phase liquid chromatographic method were: LogP, LogD, molecular weight (MW), molecular volume (MV), molar refractivity (MR), polar surface area (PSA), number of proton donors (NDon) and number of proton acceptors (NAcc). LogD values calculated from the chemical structure using pK_a and LogK_{ow} values, were used to estimate the pH-dependent chromatographic behaviour of each compound under the chromatographic conditions used (mobile phase is approximately pH 4.1). The calculated physicochemical parameters were then used to model the chromatographic method (346).

Multiple linear regression is then used to build a predictive quantitative structure-retention relationship (QSRR) model using a 'training set' database that is most similar to the suspect compound. To find the most similar compounds in the 'training

set' database ChromGenius generates 2D 'fingerprints' and from these 'fingerprints' the software calculates a structural similarity value, with '1' being identical, using the Tanimoto similarity index (347). The accuracy of the resulting QSRR model is dependent on the size of the database and the compounds in the 'training set', with similar structure, are used to build the QSRR model (346). A prediction equation was created and calculated retention times were stored in a method file.

5.4 Results and discussion

5.4.1 The correlation plot obtained from the 'training set' of compounds

The correlation plot between the predicted and measured retention times for the 1453 compounds obtained from the 'training set' is shown in Figure 5.1. A linear fit was used and the equation of the line with the intercept set at zero was y = 0.9781x. A mean absolute error of 0.95 min with a standard deviation of 1.25 min and a coefficient of determination, R^2, of 0.8052 obtained.

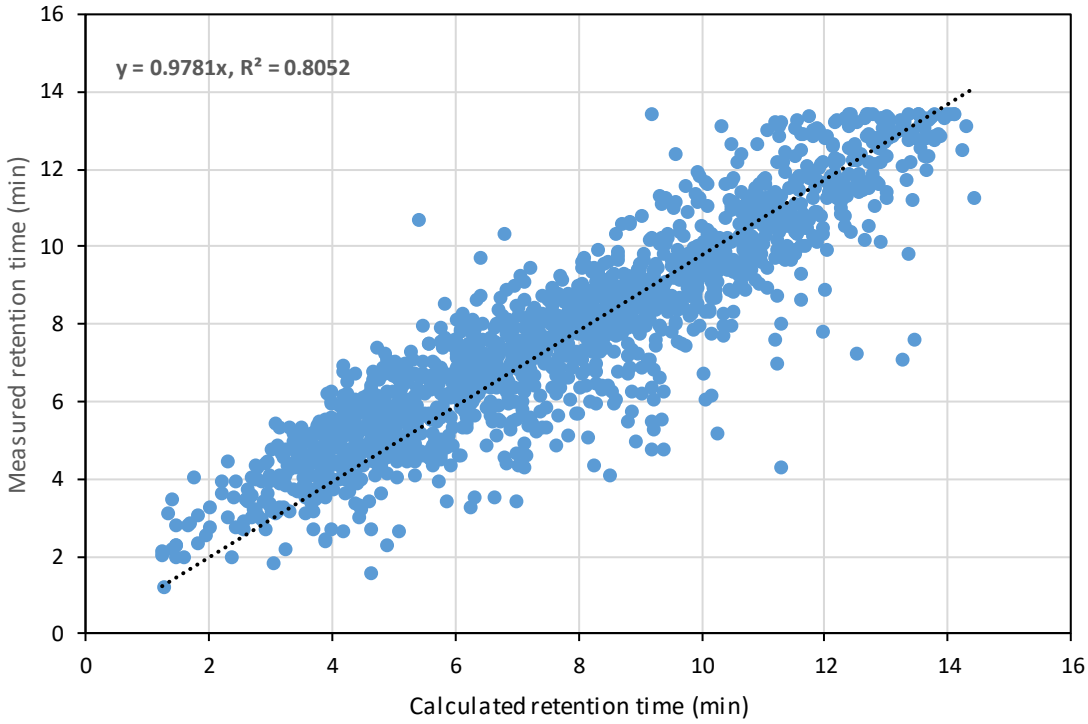


Figure 5.1 Correlation between experimental and calculated retention times of 1453 compounds in the 'training set' database.

5.4.2 Retention time prediction from testing set of compounds

The retention time predictions for each of the 103 compounds in the 'testing set' were obtained using two approaches. The first used the model based on the entire 'training set' (all compounds fit) and the second was performed using 50 of the most similar compounds (best 50 compounds fit) obtained by the Tanimoto coefficient similarity search which is based on chemical structure. As can be observed from the distribution of retention time errors both prediction fits produced similar error values for retention time prediction (Figure 5.2). A larger maximum absolute error value and a higher mean absolute error was obtained using the all compounds fit but this was only due to 4 compounds out of a total of 103 or < 4 % of the entire 'testing set' of compounds.

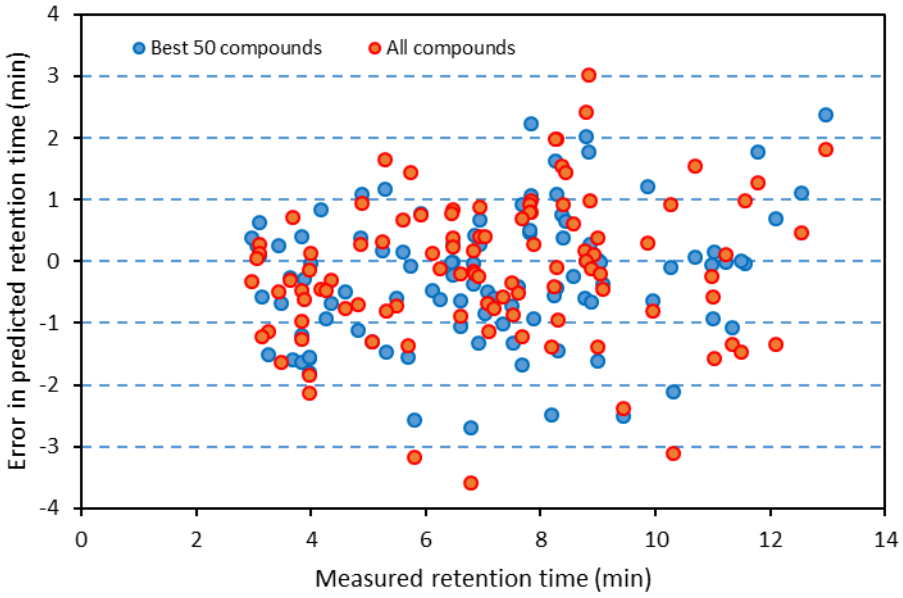


Figure 5.2 Distribution of retention time errors from both prediction models.

The maximum measured retention time for the liquid chromatographic system was 14.5 min (fenbutatin oxide) and the minimum 1.2 min (histidine). For all compounds within the retention time window of 13.3 min, the percenatge mean error in retention time prediction was 6.6 % and 6.2 % for the all compounds fit and best 50 compounds fit respectively. The mean absolute errors and standard deviations obtained were 0.88 ± 0.73 min and 0.82 ± 0.68 min for the all compounds fit and best 50 compounds fit respectively. When looking specifically at the all compounds fit 70 % of the compounds were within one minute and 93 % were within two minutes of their measured retention times with a maximum absolute error of 3.58 min for mebeverine. A very similar set of error values was obtained for the best 50 compounds fit option with 65 % of the compounds within one minute and 92 % within two minutes of their measured retention times with a maximum absolute error of 2.69 min again for mebeverine. The maximum error recorded for the training set was 6.95 min for the

Benzoxonium cation with a calculated retention time of 4.32 min and a measured retention time of 11.27 min. This large error is probably due to the predicted LogK_{ow} value of 1.39 not accurately reflecting the compound's hydrophobicity under the acididc experimental conditions.

Prediction errors were investigated for any apparent trends and predicted retention times for polar compounds eluting in the first 5 min were generally overestimated but the converse was not observed for less polar compounds eluting after 5 min. The all compounds fit and best 50 compounds fit options overestimated the retention time for 21 and 24 compounds respectively within the -1 to -4 min window with only 11 and 12 compounds respectively being underestimated within the same retention time window. With the exception of mebeverine, percentage retention time prediction errors improved after 5 min.

Only eight compounds accounted for retention time prediction errors in excess of 2.0 min for both fits. In retention time order these were; ropinirole, donepezil, mebeverine, erythromycin, cetirizine, salmeterol, irbesartan and telmisartan. A minimum match factor of 0.670 was set within the software which typically allowed for the calculation of similarity coefficient fits for over 30 compounds. The structure for each of the returned compounds was also displayed which allowed for a visual comparison with the compound's structure whose prediction time was being calculated. Similarity coefficient fit values obtained for the best 10 matching structures were on average lower than 0.8 for the eight compounds mentioned above. The lower match values were reflected in the considerable differences between the chemical structures. A fundamental premise of using QSRR models on those

compounds in the ‘training set’ database, whose prediction time were being calculated, is that they will yield more accurate predictions. This was indeed observed for compounds where retention time differences of considerably less than 1 minute were observed between the calculated and measured retention times. Ibuprofen, labetalol, diclofenac, propranolol, flecainide, naproxen, enalapril, atenolol, trimethoprim, progesterone, oxybutynin, hydrocortisone-21-acetate, clomipramine, terbinafine and loratadine all had retention time errors less than 0.10 minute with a minimum of five similar structures within the ‘training set’ database and high similarity coefficient fit values of between 0.95 and 0.85.

Guidelines for compound identification criteria intended for environmental screening analysis by liquid chromatographic retention time are not available. However, a technical document for identification criteria for qualitative assays in doping analysis by the World Anti-doping Agency (WADA) states that the liquid chromatographic retention time of the compound and reference standard should not differ by more than 2 % or ± 0.1 min in the same analysis (348). The Society of Toxicological and Forensic Chemistry guidelines specify an acceptable tolerance of 5 % for relative retention time repeatability as one criterion for positive compound identification (349). Considering that the retention time criteria are for methods where reference materials must be used, a retention time repeatability limit of 10 % should be considered as acceptable for liquid chromatographic analysis where reference materials are unavailable or too expensive to source. In this work the retention times calculated by ChromGenius, using the best 50 compounds fit, were within ± 2 % for 16 % (16), by ± 5 % for 28 % (29) and by ± 10 % for 54 % (56) of the compounds in the ‘testing set’ database. When using the all compounds fit, calculated retention

times were within ± 2 % for 13 % (13), by ± 5 % for 29 % (30) and by ± 10 % for 44 % (45) of the compounds in the ‘testing set’ database. (Number of compounds appear in brackets).

Table 5.1 Distribution of retention time errors from both prediction models.

No.	Compound	Experimental RT[a]	Calculated RT from all compounds fit[a]	Calculated RT from best 50 compounds fit[a]	RT error from all compounds fit[a]	RT error from best 50 compounds fit[a]	% error from all compounds fit	% error from best 50 compounds fit
1	Sotalol	2.96	3.29	2.58	-0.33	0.38	11	-13
2	Terbutaline	3.06	3.01	2.81	0.05	0.25	-2	-8
3	Atenolol	3.09	2.95	3.01	0.14	0.08	-5	-3
4	Salbutamol	3.11	2.84	2.48	0.27	0.63	-9	-20
5	Ranitidine	3.14	4.37	3.72	-1.23	-0.58	39	18
6	Moxonidine	3.26	4.39	4.78	-1.13	-1.52	35	47
7	Sumatriptan	3.43	3.93	3.17	-0.5	0.26	15	-8
8	Acetaminophen	3.48	5.11	4.16	-1.63	-0.68	47	20
9	Varenicline	3.63	3.94	3.9	-0.31	-0.27	9	7
10	Amiloride	3.68	2.97	5.27	0.71	-1.59	-19	43
11	Scopolamine	3.83	4.3	3.44	-0.47	0.39	12	-10
12	Clonidine	3.83	4.81	5.03	-0.98	-1.2	26	31
13	Thephylline	3.84	5.1	5.48	-1.26	-1.64	33	43
14	Amisulpride	3.88	4.5	4.17	-0.62	-0.29	16	7
15	Lisinopril	3.98	4.13	5.55	-0.15	-1.57	4	39
16	Hydroxychloroquine	3.98	5.83	5.78	-1.85	-1.8	46	45
17	Ropinirole	3.98	6.11	5.53	-2.13	-1.55	54	39
18	Trimethoprim	3.99	3.87	4.02	0.12	-0.03	-3	1
19	Ipratropium	4.17	4.63	3.34	-0.46	0.83	11	-20
20	Ofloxacin	4.27	4.75	5.21	-0.48	-0.94	11	22
21	Metoclopramide	4.36	4.66	5.05	-0.3	-0.69	7	16

continued

22	Lidocaine	4.59	5.35	5.09	-0.76	-0.5	17	11
23	Tramadol	4.81	5.52	5.93	-0.71	-1.12	15	23
24	Metoprolol	4.86	4.58	4.48	0.28	0.38	-6	-8
25	Timolol	4.88	3.93	3.79	0.95	1.09	-19	-22
26	Fluconazole	5.06	6.36	6.37	-1.3	-1.31	26	26
27	Celiprolol	5.24	4.93	5.06	0.31	0.18	-6	-3
28	Lamotrigine	5.28	3.63	4.12	1.65	1.16	-31	-22
29	Mirtazapine	5.31	6.12	6.77	-0.81	-1.46	15	27
30	Quinine	5.49	6.21	6.09	-0.72	-0.6	13	11
31	Oxprenolol	5.61	4.94	5.45	0.67	0.16	-12	-3
32	Risperidone	5.69	7.05	7.25	-1.36	-1.56	24	27
33	Labetalol	5.74	4.3	5.82	1.44	-0.08	-25	1
34	Donepezil	5.81	8.98	8.39	-3.17	-2.58	55	44
35	Bisoprolol	5.91	5.15	5.14	0.76	0.77	-13	-13
36	Venlafaxine	6.11	5.97	6.58	0.14	-0.47	-2	8
37	Chlorpheniramine	6.25	6.36	6.87	-0.11	-0.62	2	10
38	Flecainide	6.44	5.67	6.46	0.77	-0.02	-12	0
39	Propranolol	6.48	5.64	6.5	0.84	-0.02	-13	0
40	Trazodone	6.48	6.1	6.21	0.38	0.27	-6	-4
41	Citalopram	6.48	6.24	6.71	0.24	-0.23	-4	4
42	Sulfasalazine	6.61	6.81	7.24	-0.2	-0.63	3	10
43	Indapamide	6.61	7.49	7.67	-0.88	-1.06	13	16
44	Mebeverine	6.79	10.37	9.48	-3.58	-2.69	53	40
45	Bendroflumethiazide	6.82	6.99	7.18	-0.17	-0.36	2	5
46	Enalapril	6.83	6.66	6.87	0.17	-0.04	-2	1
47	Piroxicam	6.84	7.05	6.41	-0.21	0.43	3	-6
48	Carvedilol	6.91	7.16	8.23	-0.25	-1.32	4	19

continued

49	Cyclizine	6.94	6.07	6.28	0.87	0.66	-13	-10
50	Haloperidol	6.94	6.53	6.66	0.41	0.28	-6	-4
51	Verapamil	7.03	6.62	7.88	0.41	-0.85	-6	12
52	Phenytoin	7.08	7.77	7.57	-0.69	-0.49	10	7
53	Meloxicam	7.09	8.23	7.78	-1.14	-0.69	16	10
54	Diltiazem-Cis	7.18	7.95	7.77	-0.77	-0.59	11	8
55	Carbamazepine	7.35	7.93	8.37	-0.58	-1.02	8	14
56	Omeprazole	7.49	7.84	8.21	-0.35	-0.72	5	10
57	Promethazine	7.53	8.4	8.85	-0.87	-1.32	12	18
58	Dosulepin	7.61	8.12	8.02	-0.51	-0.41	7	5
59	Paroxetine	7.68	6.99	6.77	0.69	0.91	-9	-12
60	Fexofenadine	7.68	8.91	9.36	-1.23	-1.68	16	22
61	Procyclidine	7.8	7.01	7.3	0.79	0.5	-10	-6
62	Hydrocortisone	7.82	6.9	7.35	0.92	0.47	-12	-6
63	Erythromycin	7.83	6.84	5.61	0.99	2.22	-13	-28
64	Bumetanide	7.83	7.04	6.77	0.79	1.06	-10	-14
65	Sildenafil	7.88	7.6	8.82	0.28	-0.94	-4	12
66	Losartan	8.19	9.57	10.67	-1.38	-2.48	17	30
67	Amitriptyline	8.23	8.65	8.78	-0.42	-0.55	5	7
68	Hydroxyzine	8.25	6.28	6.63	1.97	1.62	-24	-20
69	Nebivolol	8.28	6.3	7.19	1.98	1.09	-24	-13
70	Ketoprofen	8.28	8.38	8.72	-0.1	-0.44	1	5
71	Nifedipine	8.31	9.27	9.75	-0.96	-1.44	12	17
72	Amlodipine	8.36	6.81	7.6	1.55	0.76	-19	-9
73	Dexamethasone	8.38	7.47	8	0.91	0.38	-11	-5
74	Fluoxetine	8.43	7	7.78	1.43	0.65	-17	-8

continued

75	Ramipril	8.56	7.96	8.81	0.6	-0.25	-7	3
76	Bezafibrate	8.77	8.59	9.37	0.18	-0.6	-2	7
77	Cetirizine	8.79	6.38	6.78	2.41	2.01	-27	-23
78	Hydrocortisone-21-acetate	8.8	8.79	8.8	0.01	0	0	0
79	Salmeterol	8.83	5.81	7.05	3.02	1.78	-34	-20
80	Sertraline	8.85	7.87	8.58	0.98	0.27	-11	-3
81	Chlorpromazine	8.89	9	9.54	-0.11	-0.65	1	7
82	Oxybutynin	8.92	8.81	8.9	0.11	0.02	-1	0
83	Naproxen	8.99	8.61	8.97	0.38	0.02	-4	0
84	Valsartan	9	10.38	10.62	-1.38	-1.62	15	18
85	Clomipramine	9.03	9.24	9.04	-0.21	-0.01	2	0
86	Warfarin	9.08	9.53	9.44	-0.45	-0.36	5	4
87	Irbesartan	9.44	11.82	11.95	-2.38	-2.51	25	27
88	Cinnarizine	9.86	9.56	8.65	0.3	1.21	-3	-12
89	Atorvastatin	9.96	10.77	10.6	-0.81	-0.64	8	6
90	Diclofenac	10.28	9.36	10.37	0.91	-0.09	-9	1
91	Telmisartan	10.31	13.42	12.43	-3.11	-2.12	30	21
92	Ibuprofen	10.68	9.13	10.61	1.55	0.07	-15	-1
93	Terbinafine	10.97	11.22	11.03	-0.25	-0.06	2	1
94	Felodipine	11.01	11.59	11.94	-0.58	-0.93	5	8
95	Tamoxifen	11.02	12.6	10.86	-1.58	0.16	14	-1
96	Progesterone	11.22	11.11	11.23	0.11	-0.01	-1	0
97	Clobetasone-17-butyrate	11.33	12.68	12.4	-1.35	-1.07	12	9
98	Loratadine	11.49	12.95	11.49	-1.46	0	13	0
99	Mefenamic acid	11.55	10.56	11.59	0.99	-0.04	-9	0
100	Clopidogrel	11.78	10.51	10.01	1.27	1.77	-11	-15

continued

101	Amiodarone	12.1	13.44	11.4	-1.34	0.7	11	-6
102	Fenofibrate	12.55	12.09	11.44	0.46	1.11	-4	-9
103	Miconazole	12.97	11.15	10.59	1.82	2.38	-14	-18

[a] values are listed in min

5.4.3 Comparison of chromatographic similarity between regression plots

According to Tropsha et al., (350) for a QSRR model to be considered predictive the slope of the regression through the origin should be within the range 0.85–1.15 and the normalised difference between the coefficients of determination, R^2, (equation 5.1) should be less than 0.1:

$$\Delta R = (R^2 - R_0^2)/R_0^2 \quad - \quad (5.1)$$

where R_0^2 is the coefficient of determination between the observed and predicted retention times where the regression is forced through the origin. An average slope of 0.9428 and a normalised difference between the coefficients of determination of 0.0386 for the best 50 compounds fit was well within the proposed values of 0.85–1.15 and < 0.1; see Figure 5.3. Values of 0.9243 and 0.0999 were obtained for average slope and difference between the coefficients of determinations, respectively using the all compounds fit. These fall just within the proposed values as shown in Figure 5.4. The above data demonstrates that the use of a QSRR model derived from the best 50 compound fit is superior in its ability to accurately predict a suspect's retention time. When ACD Labs ChromGenius is used with the all compounds fit, it can incorporate compound structures with lower similarity coefficient values in the calculation of retention time thereby reducing the accuracy of prediction.

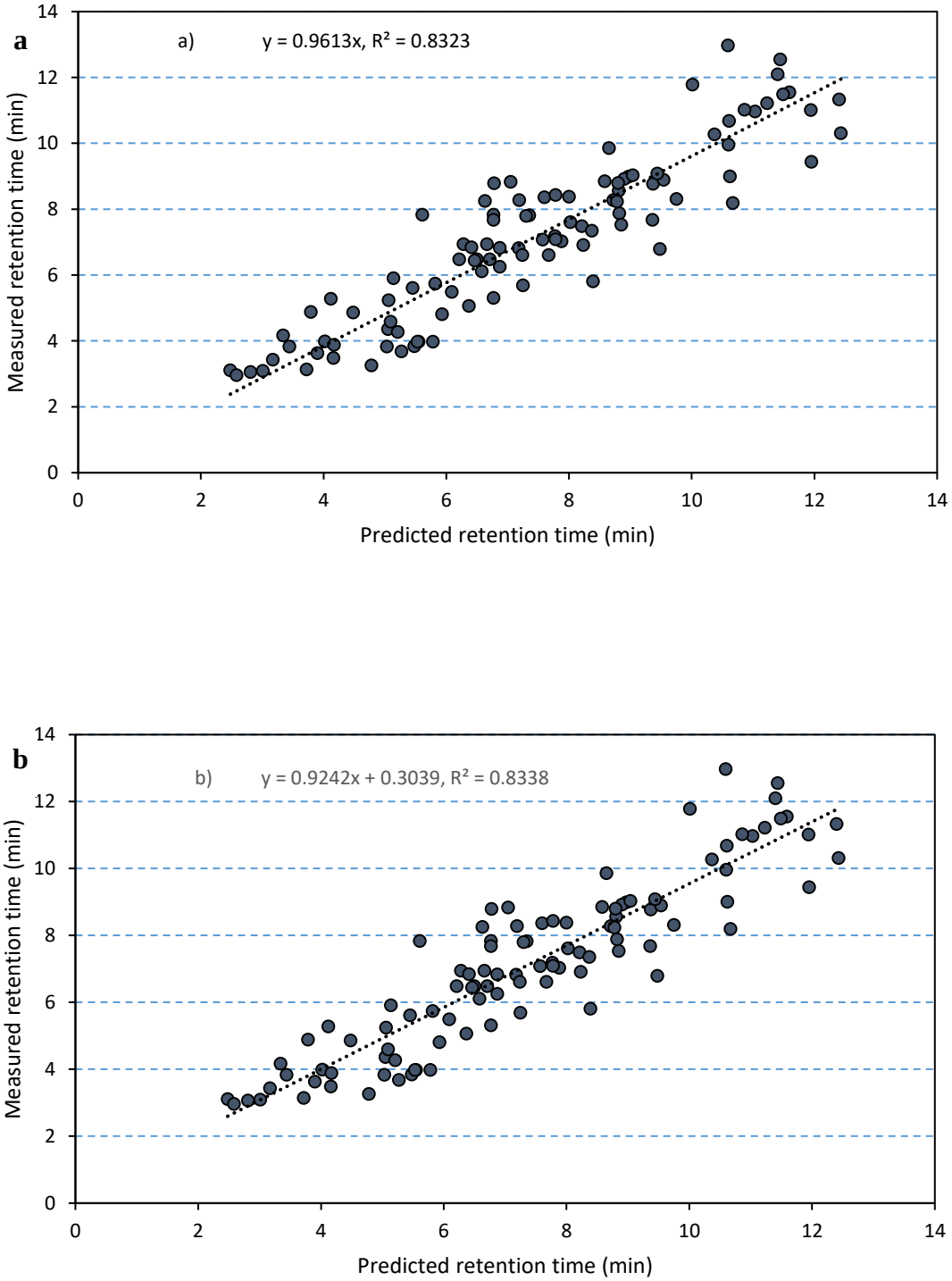


Figure 5.3 Regression plots of calculated versus measured retention times using the best 50 compounds fit. a) = forced through the origin, b) = origin not included.

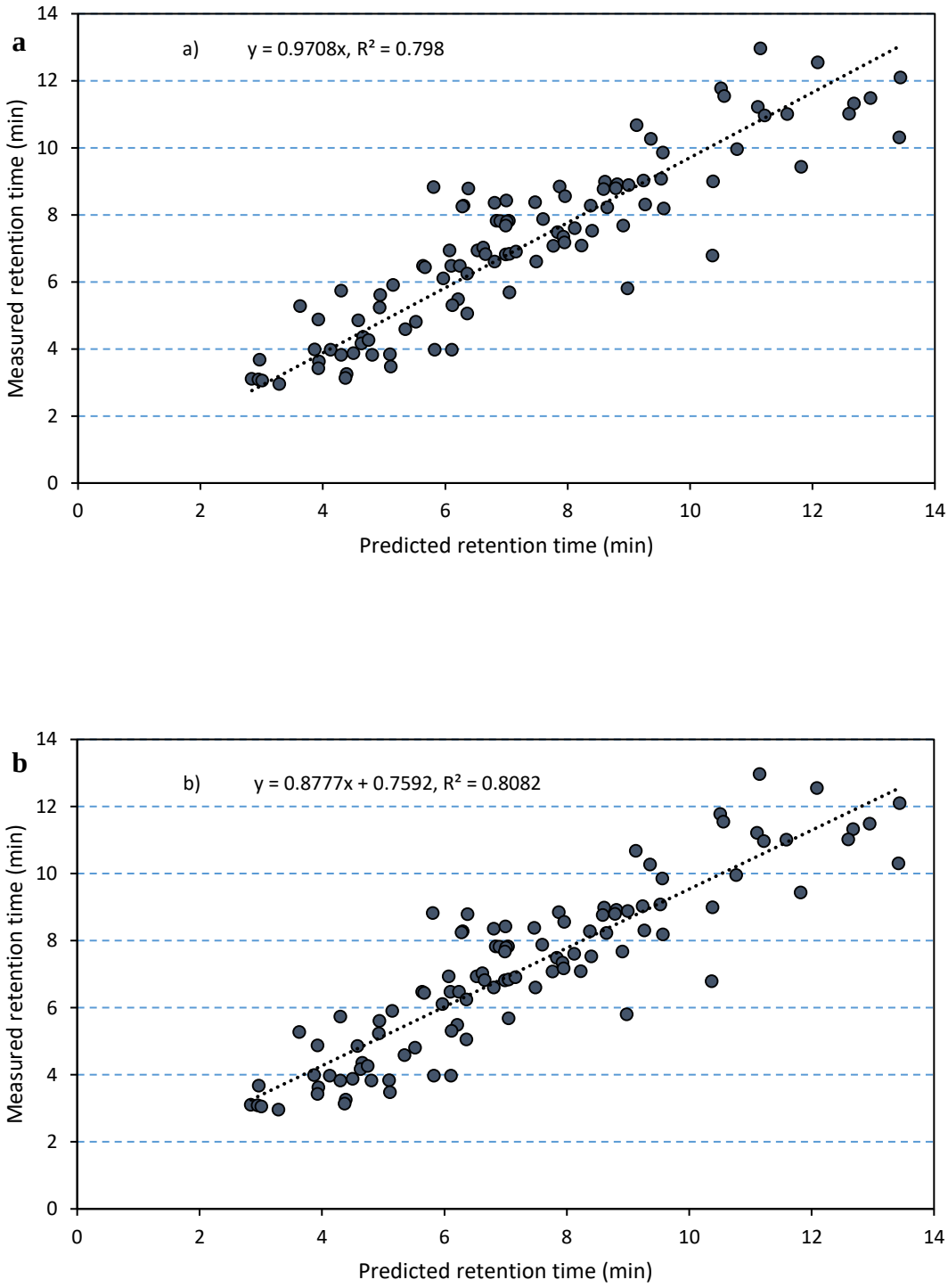


Figure 5.4 Regression plots of calculated versus measured retention times using the all compounds fit. a) = forced through the origin, b) = origin not included.

5.4.4 The effects of matrix on measured retention times in passive sampling extracts

The retention time prediction model developed in this chapter was derived from reference materials prepared in organic solvents and then diluted to appropriate concentrations in mobile phase. In the context of a quantitative targeted method this can be considered as suitable as many method employ clean-up techniques to remove a large proportion of matrix components thereby reducing matrix interferences such as retention time shifting, ion suppression and ion enhancement. Cleaning the sample would also be expected to improve accurate mass measurements and isotope pattern matching.

The use of a passive sampler with a polar polyethersulphone membrane would effectively eliminate any interfering effects from high molecular mass compounds such as lipids as these molecules are too large to pass through the pores of the membrane which is typically 0.1 µm for POCIS and 0.2 µm for the polar Chemcatcher®. Compounds such as the antibiotics, erythromycin, clarithromycin and azithromycin have molecular masses greater than 700 Da but these pass through the membrane unhindered. Bacteria would be prevented from passing through the polar membrane due to their large size. In effect, the polar polyether sulphone membrane acts as a clean-up step, allowing the passage of most known and emerging contaminants onto the sorbent beyond.

Compounds in the testing set, i.e. those listed in Table 5.1, did not appear to be influenced by the presence of matrix from the polar passive sampling extract. Differences between the retention times obtained in mobile phase and the extracts

did not differ by more than 0.05–0.10 min and were therefore not corrected. Retention time differences observed between successive injections on the LC Q-TOF-MS system were on average typically in the range 0.025–0.050 min so any differences obtained from the passive sampling extracts was therefore considered as insignificant.

5.4.5 Tentative identification of three metabolites of the pharmaceutical irbesartan without reference standards

The identification of suspect compounds is very challenging especially in environmental matrices such as waste water effluents where many thousands of compounds are present and reference materials are not available. While ultra-high resolution and accurate mass have gone some way to reduce false positive and false negatives, matrix interferences can have a significant effect on compound identification. The research so far in this thesis has concentrated on parent pharmaceutical compounds, however, many pharmaceuticals are metabolised in humans or degraded within waste water treatment plants. In this respect, the tentative identification of three metabolites of the pharmaceutical irbesartan (M4, M5 and M6) were explored using predictive retention time prediction together with accurate mass and a fragment ion common to irbesartan and its metabolites. As a result of human metabolism, irbesartan has at least nine metabolites and several metabolites have been reported recently in waste waters (264, 351). The structure and chemical formulae for irbesartan and three of its most common metabolites, that arise from human metabolism, are shown in Figure 5.5. The chemical formula for two the metabolites, M4 and M5, are identical and the

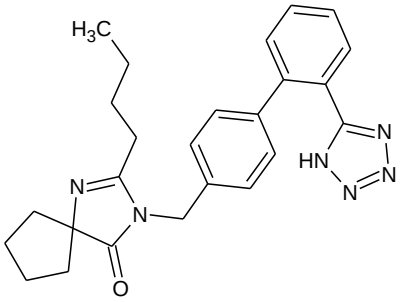
structures for the metabolites M4 and M5, differ only in the position of the hydroxyl group.

A polar Chemcatcher® sample extract from the Carmarthen WWTP, previously screened and known to contain irbesartan (Chapter 3), was re-analysed using the UHPLC Q-TOF-MS instrument set up as described in section 7.4. As with the Q-TOF-MS instrument in Chapter 3, the Bruker Maxis Impact instrument employs two collision energies, one at low (6 eV) and the second at a higher ramped energy of 25 eV. The presence of additional chromatographic peaks in the extracted ion chromatograms from the ramped higher energy setting of 25 eV, for a parent compound) may be indicative of the presence of potential metabolites. This strategy assumes that many metabolites share the same fragmentation pathway with the parent compound (352, 353).

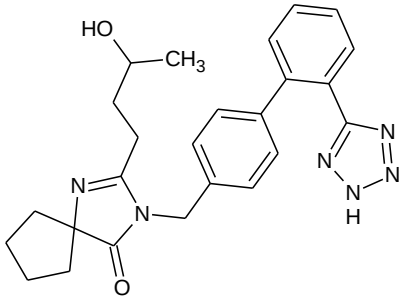
Two chromatographic peaks, for each of the metabolite chemical formulae, were observed at the exact mass of each protonated molecule, m/z 445.2347 for M4 and M5 and m/z 443.2190 for M6. A single but strong irbesartan fragment ion (m/z 207.1907) shown in Figure 5.7 was also observed for the four other peaks which suggested a common skeletal structure. Irbesartan was therefore included in the all compound database as it was expected to improve the accuracy of prediction. Only one precursor and one fragment ion was used but this approach satisfies the requirement for a minimum of three identification points required for authorised and four identification points for banned substances in products of animal origin according to EU Council Directive 96/23/EC (354). For high resolution mass spectrometry two identification points are earned for the precursor ion and 2.5 identification points for each fragment ion. Assuming that

this approach can be adopted for environmental samples, a total of 4.5 identification points was achieved fully meeting the requirement.

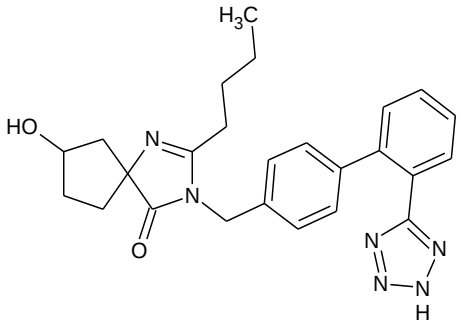
ACD Labs ChromGenius was then used to try and predict the retention times and order of the four suspected metabolite peaks of irbesartan. The predicted retention time for the metabolites M4, M5 and M6 were 9.77, 9.88 and 9.90 min respectively using the best 50 compounds fit. For the peaks corresponding to metabolites M4 and M5 (retention times of 7.92 and 8.08 min respectively) both had prediction errors of less than 2.0 min. For the two peaks corresponding to the metabolite M6 (retention times of 7.84 and 8.16 min respectively) a prediction error of 2.06 min was obtained for the first peak and the second peak was within 2.0 min of the predicted value. It was observed that a very low number of structures (maximum of four) had similarity coefficients above 0.70 which may have significantly influenced the accuracy of prediction. The retention time prediction settings were therefore adjusted and a best 10 compounds fit evaluated. Predicted retention times of 8.28, 8.60 and 8.27 min were obtained which corresponded to maximum prediction errors of 0.36, 0.68 and 0.43 min for the M4, M5 and M6 metabolites. The results clearly demonstrate that for accurate retention time prediction, using a QSRR model, a sufficient number of structures, with high similarity to the suspect compound under investigation, must be present within the retention prediction database. This may not always be possible especially when dealing with unknowns. Greater importance must therefore be placed on the use of very large databases of compounds where the probability of a compound with a similar structure is that much higher.



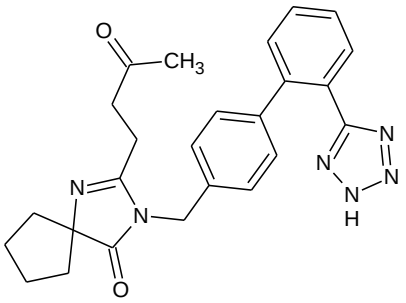
Irbesartan ($C_{25}H_{28}ON_6$)



Irbesartan M4 ($C_{25}H_{28}O_2N_6$)



Irbesartan M5 ($C_{25}H_{28}O_2N_6$)



Irbesartan M6 ($C_{25}H_{26}O_2N_6$)

Figure 5.5 Structures and chemical formula for Irbesartan and three of its metabolites (M4, M5 and M6) tentatively identified in a Chemcatcher® passive sampler extract.

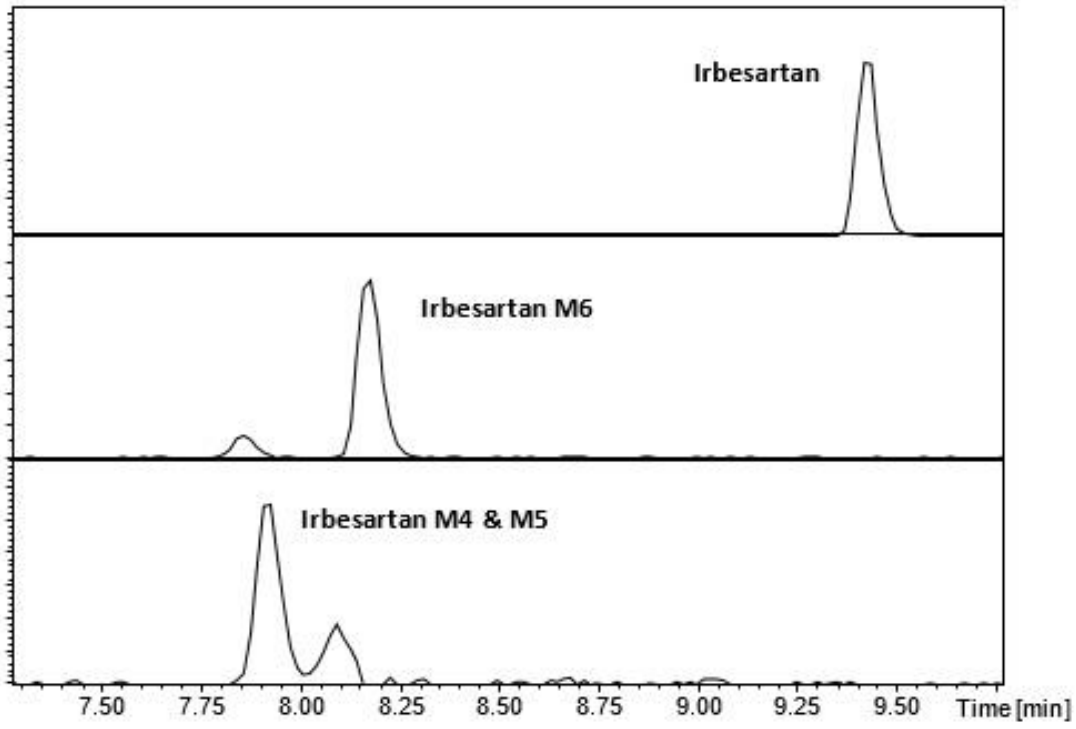
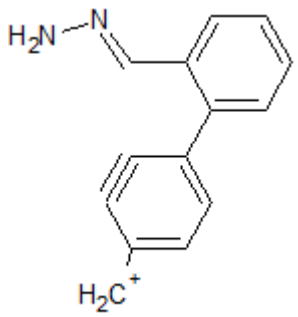


Figure 5.6 Accurate mass extracted ion chromatograms for Irbesartan and three of its common metabolites (Irbesartan M4, M5 and M6).



m/z 207.0917 ($C_{14}H_{11}N_2$)

Figure 5.7 Structure and chemical formula for the common fragment ion of Irbesartan and three of its metabolites.

5.5 Conclusions

This work demonstrates the development and use of a retention time prediction tool based on commercially available software employing QSRR principles. Overall, retention times for 93% of compounds were within 2.0 min of the predicted values using the all compounds fit. Slightly lower accuracy of 92% was obtained using the best 50 compounds fit. Only eight compounds accounted for retention time prediction errors in excess of 2 min for both fits. Fifteen compounds had retention time errors equal to or less than 0.1 min when using the best 50 compounds fit. This fit chooses the best 50 structurally similar compounds, compared to the suspect and forms the basis for the calculation of the predicted retention time.

The database model was successfully applied to suspect compound identification in a Chemcatcher® passive sampler extract, previously deployed in a final effluent stream of a waste water treatment plant. While the examples explained in this chapter show the successful assignment of retention times, 100% confidence with retention time prediction is not possible. Notwithstanding this limitation, significant time can be saved by cutting down the number of chromatographic peaks requiring investigation when prior knowledge of molecular formulae, compound structures and fragment ions are available.

It is recommended that when undertaking large scale screening of samples for unknowns or known unknowns (e.g. with a database of over 1500 compounds as used in this work) it would be sensible to include accurate retention time prediction in the strategy used for identification. This approach could also offer

significant cost advantages when reference standards are either unavailable or may have to be custom synthesised for unequivocal identification.

Chapter 6

Conclusions and recommendations for further work

6.1 Overall conclusions

The work undertaken in this study has demonstrated that the various analytical techniques developed over the course of this study can enhance the inherent advantages of passive sampling of watercourses over more traditional techniques currently employed. The key areas of novelty and its main contributions to knowledge are:

i) The development and successful application of a GCxGC technique in-conjunction with a new and highly innovative variable-energy ionisation source for the screening of target and non-target compounds in an SPMD passive sampler deployed in a river downstream of a WWTP discharge point.

ii) The development and successful application of a LC Q-TOF-MS data-independent acquisition technique for the identification of pharmaceuticals with high confidence and unequivocal compound confirmation using selective qualifier ions.

iii) The introduction of a new SPE disk for use with the Chemcatcher® passive sampler enhancing its ability to sample highly polar compounds and deliver performance very similar to that of the established POCIS sampler.

iv) The development and successful application of a new *in-silico* retention time prediction model based on a commercial LC method development software package.

The use of GCxGC together with the variable-energy ionisation source can be seen as increasing the 'dimensionality' of the analysis thereby increasing confidence in the identification of pollutants when undertaking investigations into emerging contaminants or target lists monitoring programmes under the auspices of the WFD. Furthermore, the reduced fragmentation of analytes, matrix interferences and carrier gases significantly improves signal-to-noise ratios for target substances, and should allow lower limits of detection to be achieved. The variable-energy ionisation technology described in this work has been shown to be a rugged technique that provided significant increases in the intensity of the molecular ion and produced considerably higher signal-to-noise ratios for all classes of pollutants including the emerging contaminants in the SPMD extract at lower ionisation energies. The ability to collect both regular 70 eV spectra for library matching alongside lower-energy spectra provides complementary information on the molecular ion and structurally significant fragment ions.

The development of a suitably broad and reliable 'all ions' LC Q-TOF-MS technique for the analysis of extracts obtained from two polar passive samplers proved successful for the identification of 79 pharmaceuticals with 50 being unequivocally confirmed using selective qualifier ions. The developed technique is an ideal complement for existing target and suspect screening methods and the acquired data can be re-interrogated at a later time allowing for retrospective data analysis for new emerging contaminants such as pharmaceutical metabolites, their transformation products plus personal care products. The results obtained show the value of the approach taken and when combined with an efficient data analysis workflow should prove invaluable for investigative monitoring purposes. It may also be possible to

transfer the method to other instrument vendor systems which employ high resolution accurate mass detection.

It can be concluded from the LC Q-TOF-MS analysis of the extracts from the Chemcatcher® and POCIS extracts and the extensive statistical analysis that the performance of the polar Chemcatcher® is very similar to that of the POCIS sampler. The polar Chemcatcher® samplers themselves have proven to be robust, easy to prepare and the subsequent extraction has proven equally simple to undertake avoiding the difficulties associated with the POCIS sampler. The slopes obtained from least squares and orthogonal regression of the entire averaged pharmaceutical data set suggests that the Chemcatcher® uptake rate is approximately 2.4 to 2.6 times lower than that for the POCIS whereas the slopes obtained from least squares and orthogonal regression of the entire averaged 'unknowns' data set suggests that the Chemcatcher® uptake rate is approximately 1.9 to 2.1 times lower than that for the POCIS. The differences in the uptake rates for the pharmaceuticals and the 'unknowns' data is, in all probability, due to the larger sampling area of 45.8 cm^2 for the POCIS sampler compared to 15.2 cm^2 for the Chemcatcher®, a factor of 3.01. The statistical evidence therefore suggests that the Chemcatcher is more efficient at sampling polar compounds than the POCIS sampler when the surface sampling areas of both samplers are normalised.

The development of a predictive chromatographic retention time model, employing QSRR principles, was shown to be a suitable complimentary tool to facilitate the identification of pharmaceutical residues in polar passive sampling Overall, retention times for 93% of the 164 test compounds were within 2.0 min of the predicted values using the all compounds fit. Slightly lower accuracy of 92% was obtained using the

best 50 compounds fit. Fifteen compounds had retention time errors equal to or less than 0.1 min when using the best 50 compounds fit. The database model was successfully applied to the identification of three metabolites of the pharmaceutical irbesartan in a Chemcatcher® passive sampler extract. It is expected that significant time can be saved by cutting down the number of chromatographic peaks requiring investigation when prior knowledge of molecular formulae, compound structures and fragment ions are available. This approach could also offer significant cost advantages when reference standards are either unavailable or when tentatively identified compounds must be custom synthesised for unequivocal identification.

Overall, this study has furthered the knowledge of the analysis of known and unknown and especially regarding the use of multi-dimensional chromatography, high resolution mass spectrometric techniques and passive sampling for investigative monitoring.

6.2 Recommendations for further work

Ongoing research and development of passive sampling (and particularly for polar devices) will greatly support and further validate the technique for the purposes of future environmental monitoring. Further work to fully evaluate the performance of the polar Chemcatcher® against the POCIS by verifying uptake rates through quantitative analysis is considered as highly beneficial to the passive sampling community. Furthermore, the relevance of passive sampling as a monitoring tool in regulatory applications will be enhanced by future development of a proficiency testing schemes whereby laboratories can further develop their techniques and then use the derived analytical information for wider quality purposes.

Chapter 7

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Appendices

Appendix A

A 1.1 Least squares and orthogonal regression analysis of the averaged Log_{10} transformed Chemcatcher® and POCIS pharmaceutical data

The least squares and orthogonal regression plots obtained from the averaged Log_{10} transformed data indicate a much smaller deviation from normality, and homogeneity of the variances. The variation accounted for by the least squares regression (r^2), as shown in Table A1, is 95.1% and the slope of 1.00 (t = 71.98, pr = <0.001) is significantly different from zero since it falls within the approximate 95% confidence intervals of 0.98 to 1.03. The intercept of 0.37 falls within the approximate 95% confidence intervals of 0.22 to 0.52 and is significantly different from zero (t=4.90, pr = < 0.001). The antilog of 0.37 (2.33) is the value of POCIS when Chemcatcher® is 1. The slope of 1 shows an approximate linear relationship in the unlogged data, and so this indicates that the sampling rate of POCIS is approximately 2.3 times that of the Chemcatcher®. The least squares regression plot is shown in Figure A1.

The slope of 1.03 obtained from the orthogonal regression plot (Table A2) is significantly different from zero and falls within the approximate 95% confidence intervals of 1.00 to 1.06. The intercept of 0.23 falls within the approximate 95% confidence intervals (0.08, 0.38) but zero does not and is therefore significantly different from zero. Since the data are on a Log scale, the intercept corresponds to the Log_{10} POCIS value when the Chemcatcher® value is one, and is equivalent to a POCIS value of 1.68 (the anti-log of 0.23). The plots of standardised residuals from the least squares and orthogonal regression plots show only two large values (greater than three) which are the compounds loratidine and oxprenolol at the Carmarthen and

Gowerton sites and appear to have little influence on the slope. The orthogonal regression plot is shown in Figure A2.

Table A1 Output from least squares regression analysis of the averaged Log_{10} transformed Chemcatcher® and POCIS pharmaceutical data.

Regression Equation

Log_{10} POCIS (average) = 0.3678 + 1.0027 Log_{10} Chemcatcher® (average)

Analysis of Variance

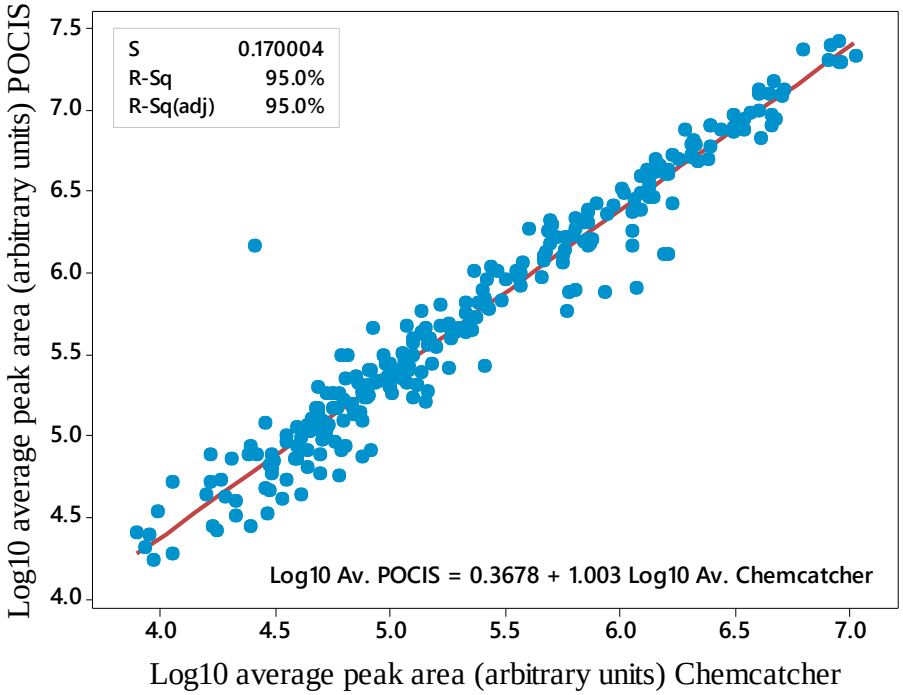
Source	DF	Seq SS	Contrib.	Adj SS	Adj MS	F-Value	P-Value
Regression	1	1.50E+02	95.05%	1.50E+02	1.50E+02	5181.05	< 0.001
Log10 Chemcatcher® (average)	1	1.50E+02	95.05%	1.50E+02	1.50E+02	5181.05	< 0.001
Error	270	7.803	4.95%	7.803	0.029		
Total	271	1.58E+02	100.00%				

Model Summary

S	R-sq	R-sq(adj)	PRESS	R-sq(pred)
0.170004	95.05%	95.03%	7.92E+00	94.97%

Coefficients

Term	Coef	SE Coef	95% CI	T-Value	P-Value	VIF
Constant	0.3678	0.0751	(0.2199, 0.5157)	4.90	< 0.001	
Log10 Chemcatcher® (average)	1.0027	0.0139	(0.9752, 1.0301)	71.98	< 0.001	1.00



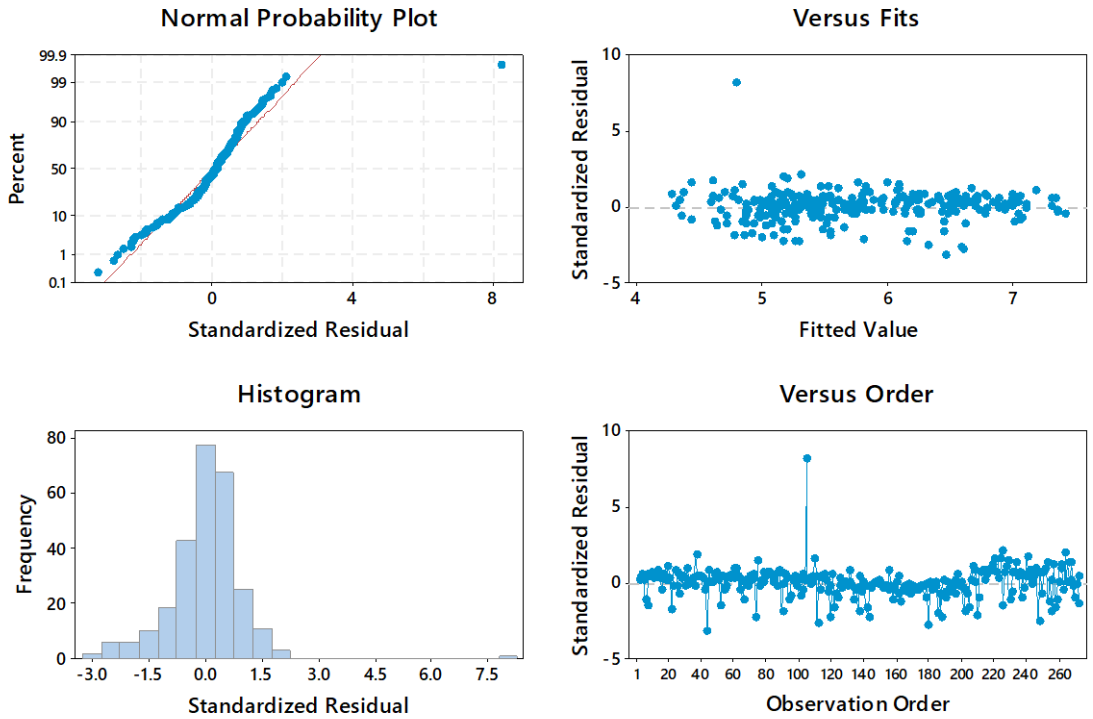


Figure A1 Fitted line regression and plots of standardised residuals obtained from least squares regression analysis of the averaged Log_{10} transformed Chemcatcher® and POCIS pharmaceutical data.

Table A2 Output from the orthogonal regression analysis of the averaged Log_{10} transformed Chemcatcher® and POCIS pharmaceutical data.

Error Variance Ratio: Log_{10} POCIS (average) / Log_{10} Chemcatcher® (average): 1

Regression Equation

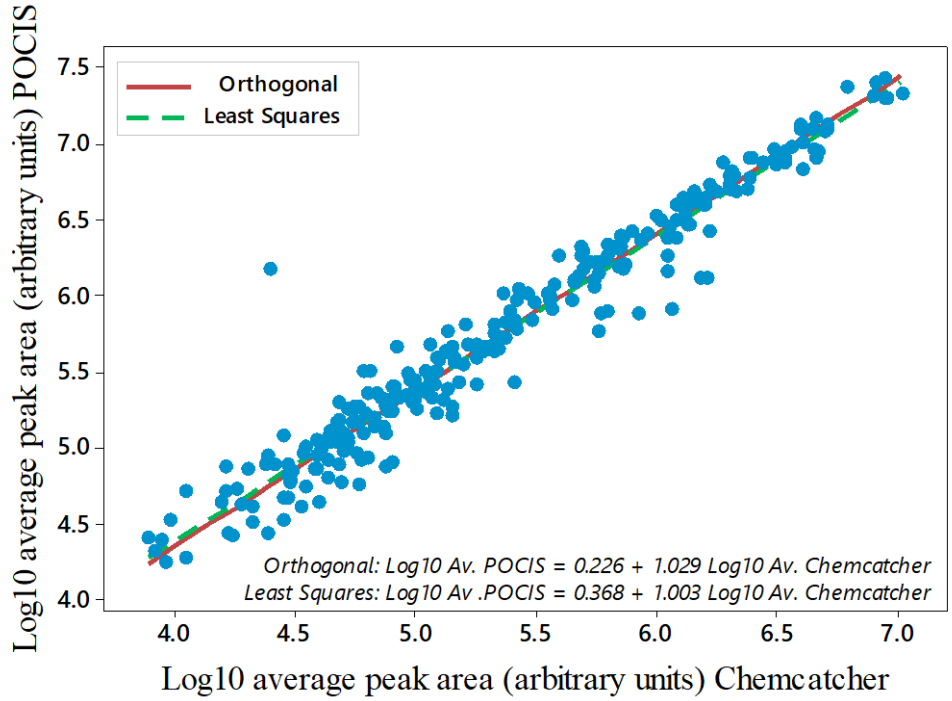
Log_{10} POCIS (average) = 0.226 + 1.029 Log_{10} Chemcatcher® (average)

Coefficients

Predictor	Coef	SE Coef	Z	P	Approx 95% CI
Constant	0.22608	0.077079	2.9331	0.003	(0.07501, 0.37716)
Log10 Chemcatcher® (average)	1.02920	0.014299	71.9777	< 0.001	(1.00117, 1.05723)

Error Variances

Variable	Variance
Log10 POCIS (average)	0.0141710
Log10 Chemcatcher® (average)	0.0141710



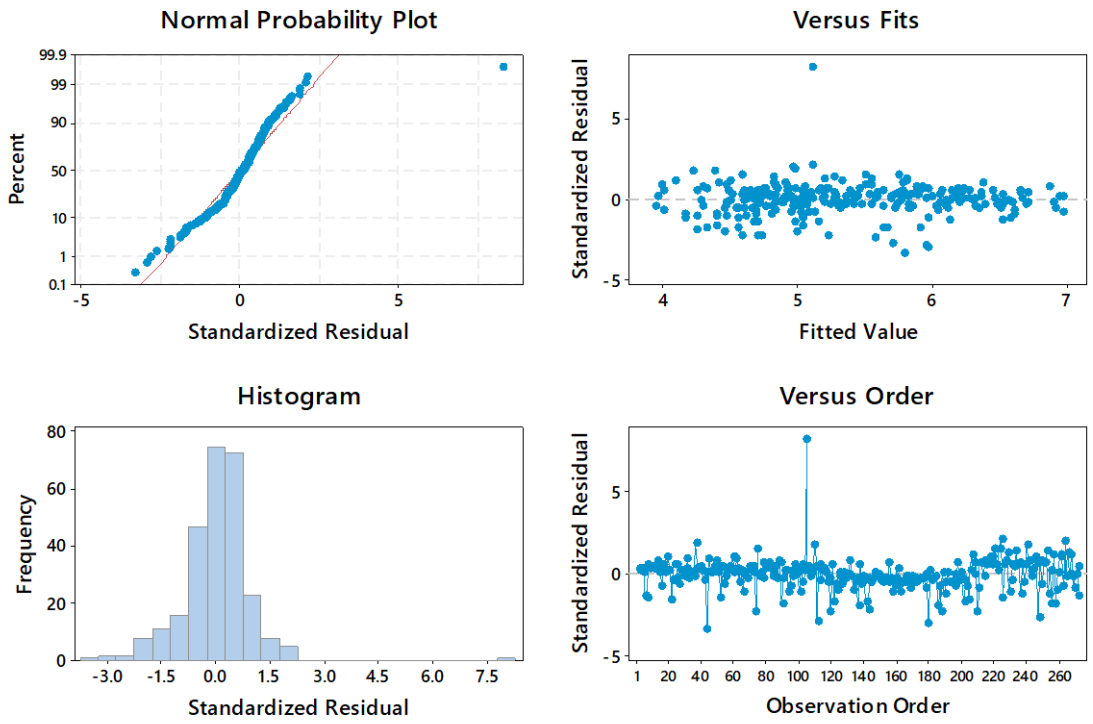


Figure A2 Fitted line regression and plots of standardised residuals obtained from the orthogonal regression analysis of the averaged Log_{10} transformed Chemcatcher® and POCIS pharmaceutical data.

Appendix B

Table B1 Output from least squares regression analysis of the averaged Chemcatcher® and POCIS 'unknowns' data after application of DFITS.

Analysis of Variance

Source	DF	Seq SS	Contrib.	Adj SS	Adj MS	F-Value	P-Value
Regression	1	2.28E+15	89.38%	2.28E+15	2.28E+15	35903.66	< 0.001
Chemcatcher® (average)	1	2.28E+15	89.38%	2.28E+15	2.28E+15	35903.66	< 0.001
Error	4266	2.71E+14	10.62%	2.71E+14	6.35E+10		
Total	4267	2.55E+15	100.00%				

Model Summary

S	R-sq	R-sq(adj)	PRESS	R-sq(pred)
251956	89.38%	89.38%	2.72E+14	89.33%

Coefficients

Term	Coef	SE Coef	95% CI	T-Value	P-Value	VIF
Constant	33154	4445	(24440, 41868)	7.46	< 0.001	
Chemcatcher® (average)	1.886	0.00995	(1.86649, 1.90552)	189.48	< 0.001	1

Regression Equation

POCIS (average) = 33154 + 1.88600 Chemcatcher® (average)

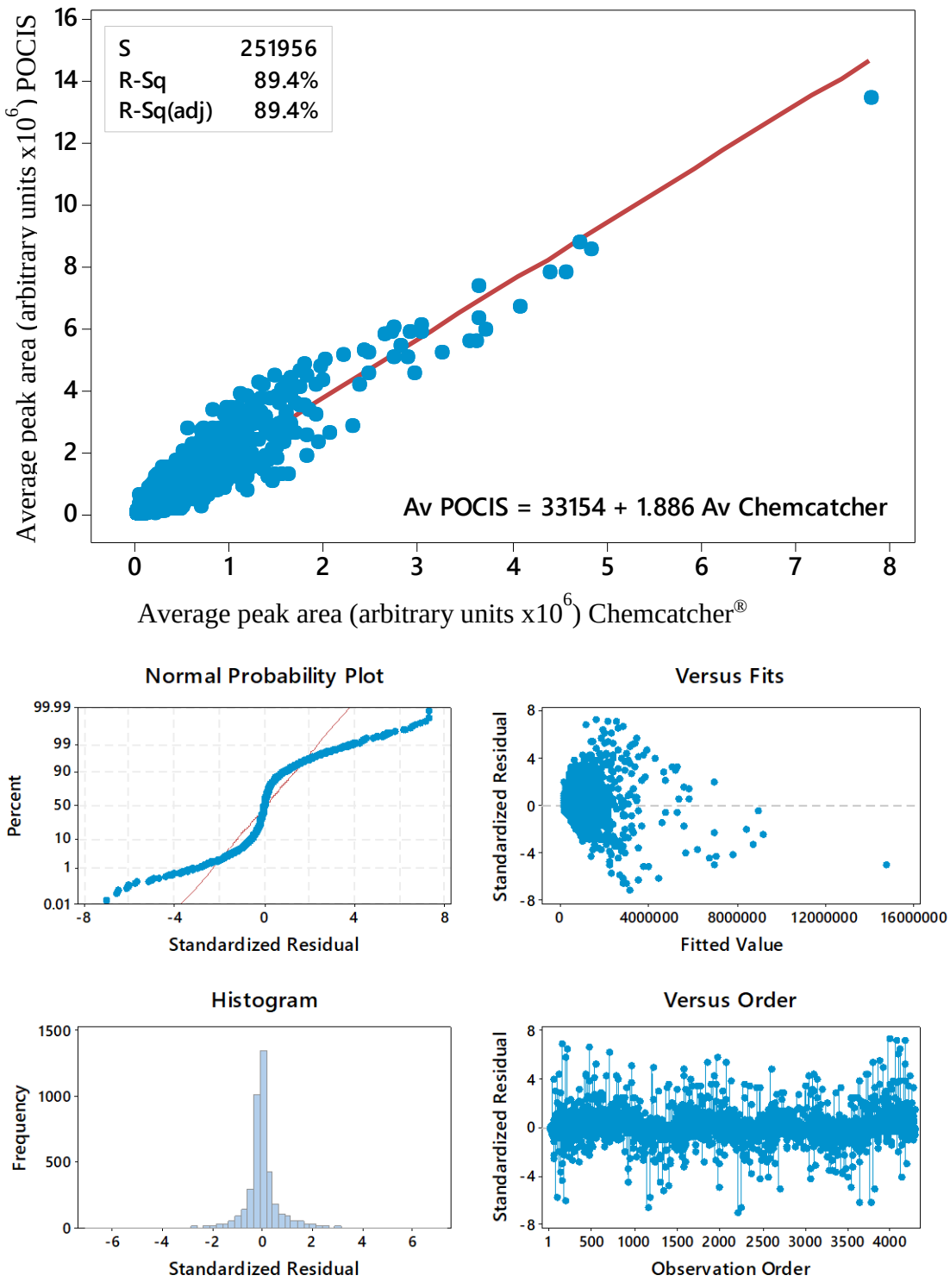


Figure B1 Fitted line and plots of standardised residuals obtained from least squares regression analysis of the averaged Chemcatcher® and POCIS 'unknowns' data after application of DFITS.

Table B2 Output from orthogonal regression analysis of the averaged Chemcatcher® and POCIS 'unknowns' data after standardised residuals filtering.

Error Variance Ratio POCIS (average)/Chemcatcher® (average): 1

Coefficients

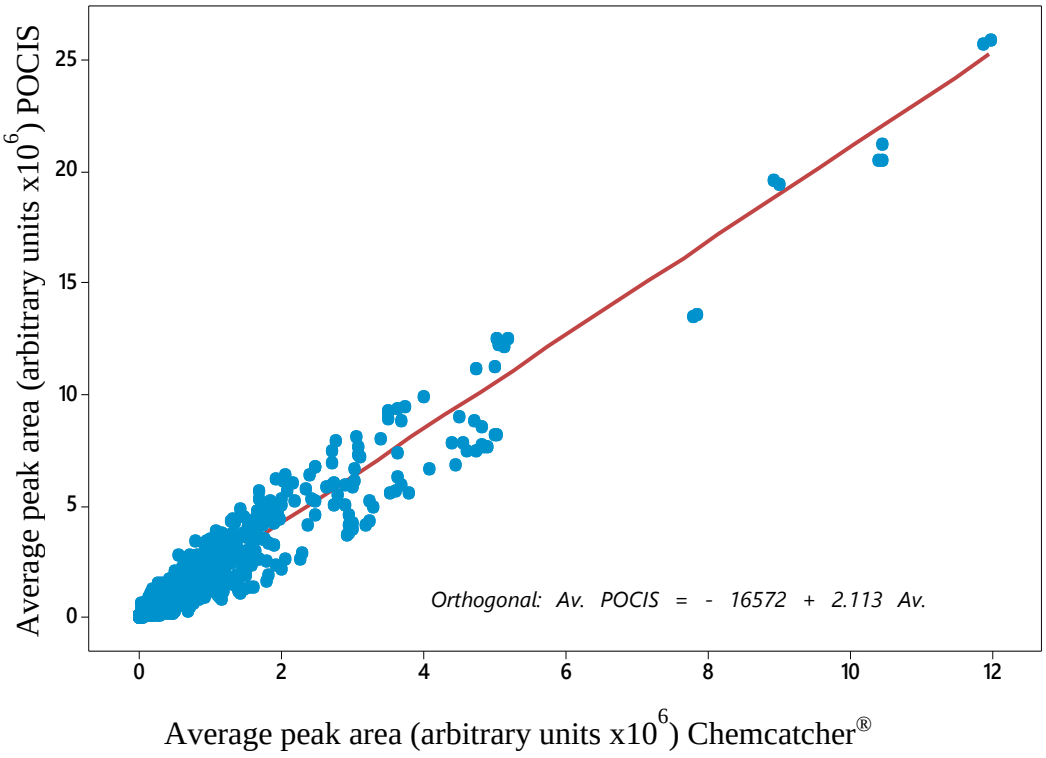
Predictor	Coef	SE Coef	Z	P	Approx 95% CI
Constant	-1.66E+04	5707.39	-2.9037	0.004	(-27758.6, -5386.06)
Chemcatcher® (average)	2.11306	0.01	268.0219	< 0.001	(2.1, 2.13)

Regression Equation

POCIS (average) = - 16572 + 2.113 Chemcatcher® (average)

Error Variances

Variable	Variance
POCIS (average)	2.19E+10
Chemcatcher® (average)	2.19E+10



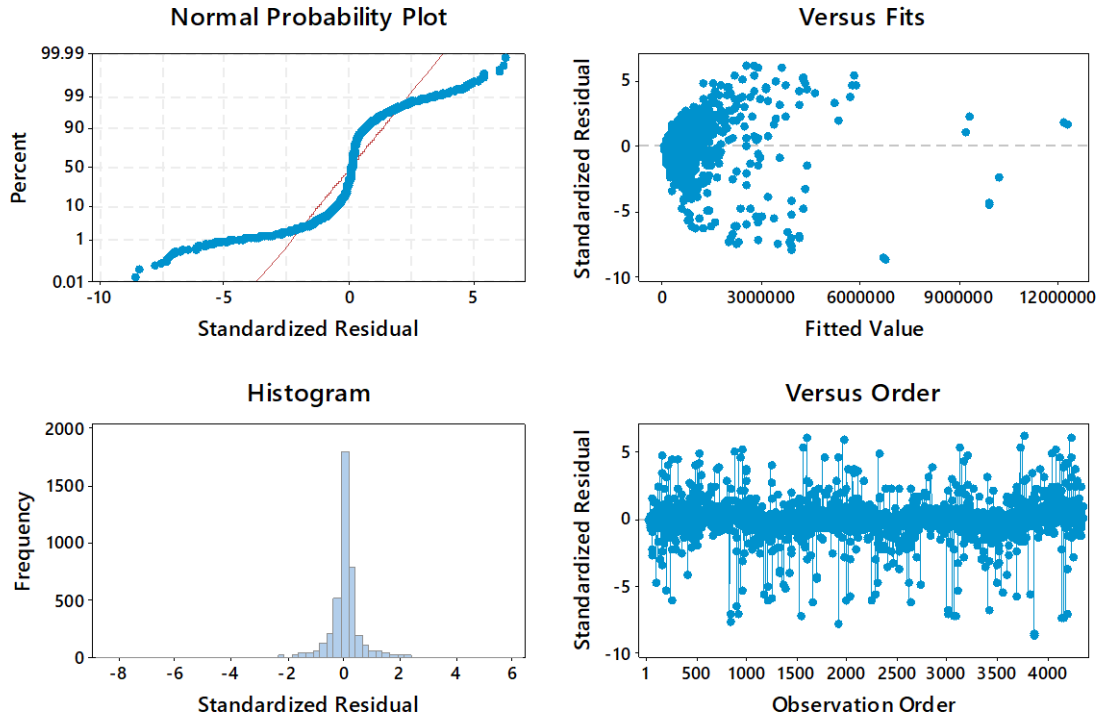


Figure B2 Fitted line and plots of standardised residuals obtained from orthogonal regression analysis of the averaged Chemcatcher® and POCIS 'unknowns' data after standardised residuals filtering.

Appendix C

C1.1 Least squares and orthogonal regression analysis of the Log_{10} transformed data for 'unknowns' in all POCIS and Chemcatcher® replicates

The variation accounted for by the least squares regression (r^2), as shown in Table C1, is 85.8% and the slope of 0.94 (t = 282.33, pr = <0.001) is significantly different from zero and falls within the approximate 95% confidence intervals of 0.93 to 0.95. The intercept of 0.60 (t = 35.03, pr = <0.001) falls within the approximate 95% confidence intervals of 0.57 to 0.63 but zero does not and is therefore significantly different from zero. The least squares regression plot is shown in Figure C1. The antilog of 0.60 (3.98) is the value of POCIS when Chemcatcher® is 1. The slope of approximately one shows an approximate linear relationship in the unlogged data. The plots of residuals in Figure C1 shows some heteroscedasticity with a few standardised residual values in the range 5–8. The variance is not constant but decreases with the amount of compound accumulated, the histogram of residuals, however, closely matches that of a normal distribution.

The output from the orthogonal regression is shown in Table C2. The slope of 1.02 is significantly different from zero and falls within the approximate 95% confidence intervals of 1.01 to 1.02. The intercept of 0.21 falls within the approximate 95% confidence intervals (0.17, 0.25) but zero does not and is thus significantly different from zero. Since the data are on a Log scale, the intercept corresponds to the Log_{10} POCIS value when the Chemcatcher® value is one, and is equivalent to a POCIS value of 1.62 (the anti-log of 0.21). The variance is not constant but decreases with the amount of compound accumulated, the histogram of residuals (Figure C2), however, closely matches that of a normal distribution.

Table C1 Output from least squares regression analysis of Log_{10} transformed data for 'unknowns' in all POCIS and Chemcatcher® replicates.

Analysis of Variance

Source	DF	Seq SS	Contrib.	Adj SS	Adj MS	F-Value	P-Value
Regression	1	3533.62	85.77%	3533.62	3533.62	79708.24	< 0.001
Log10 Chemcatcher®	1	3533.62	85.77%	3533.62	3533.62	79708.24	< 0.001
Error	13222	586.16	14.23%	586.16	0.04		
Lack-of-Fit	12898	572.06	13.89%	572.06	0.04	1.02	0.413
Pure Error	324	14.09	0.34%	14.09	0.04		
Total	13223	4119.78	100.00%				

Model Summary

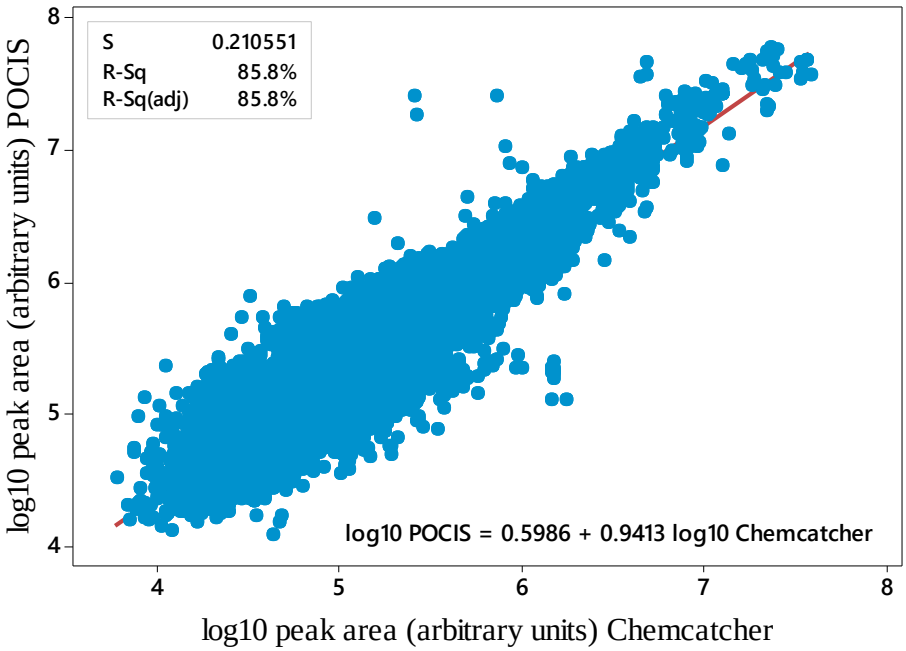
S	R-sq	R-sq(adj)	PRESS	R-sq(pred)
0.210551	85.77%	85.77%	586.334	85.77%

Coefficients

Term	Coef	SE Coef	95% CI	T-Value	P-Value	VIF
Constant	0.5986	0.0171	(0.5651, 0.6321)	35.03	< 0.001	
Log10 Chemcatcher®	0.94128	0.00333	(0.93475, 0.94782)	282.33	< 0.001	1.00

Regression Equation

Log_{10} POCIS = 0.5986 + 0.94128 Log_{10} Chemcatcher®



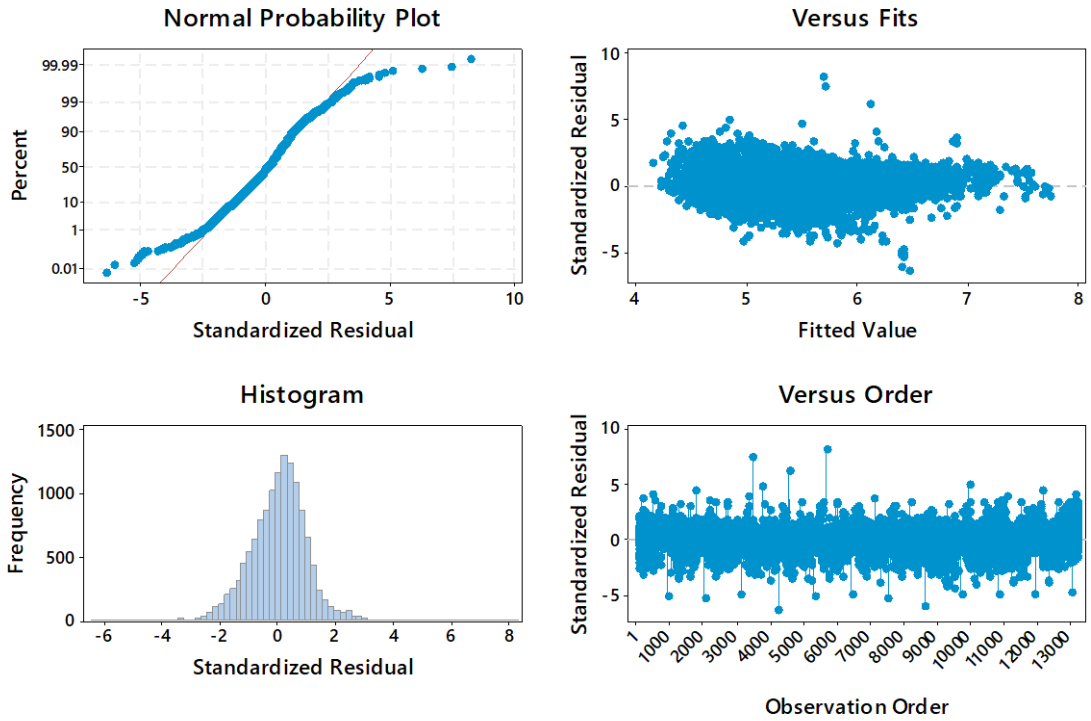


Figure C1 Fitted line and plots of standardised residuals obtained from least squares regression analysis of Log_{10} transformed data for 'unknowns' in all POCIS and Chemcatcher® replicates.

Table C2 Output from orthogonal regression analysis of Log_{10} transformed data for 'unknowns' in all POCIS and Chemcatcher® replicates.

Error Variance Ratio Log_{10} POCIS/Log_{10} Chemcatcher®: 1

Regression Equation

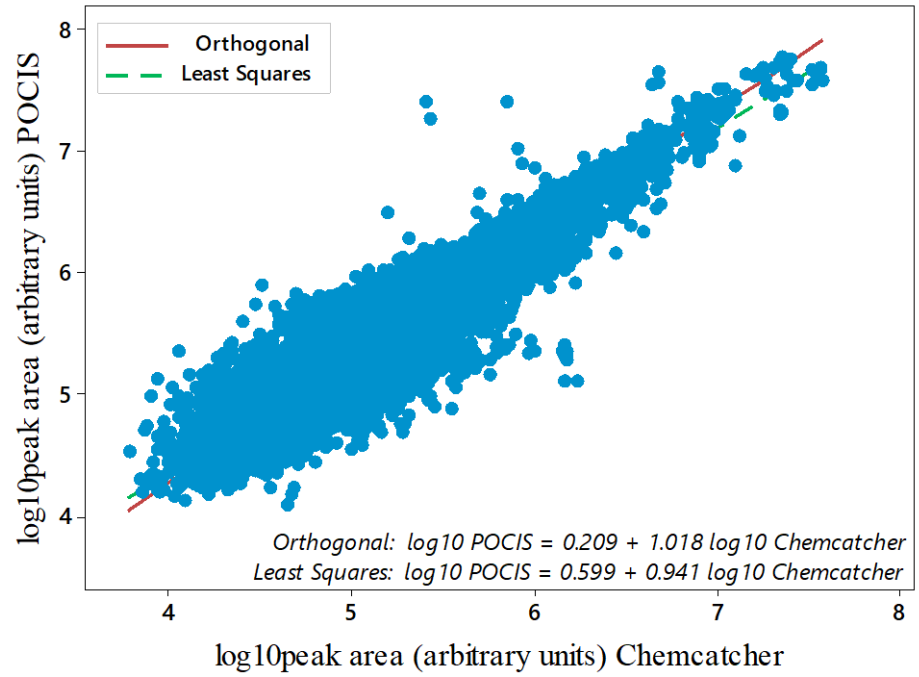
Log_{10} POCIS = 0.209 + 1.018 Log_{10} Chemcatcher®

Coefficients

Predictor	Coef	SE Coef	Z	P	Approx 95% CI
Constant	0.20929	0.018466	11.334	< 0.001	(0.17310, 0.24549)
Log10 Chemcatcher®	1.01768	0.003605	282.3261	< 0.001	(1.01061, 1.02474)

Error Variances

Variable	Variance
Log10 POCIS	0.022641
Log10 Chemcatcher®	0.022641



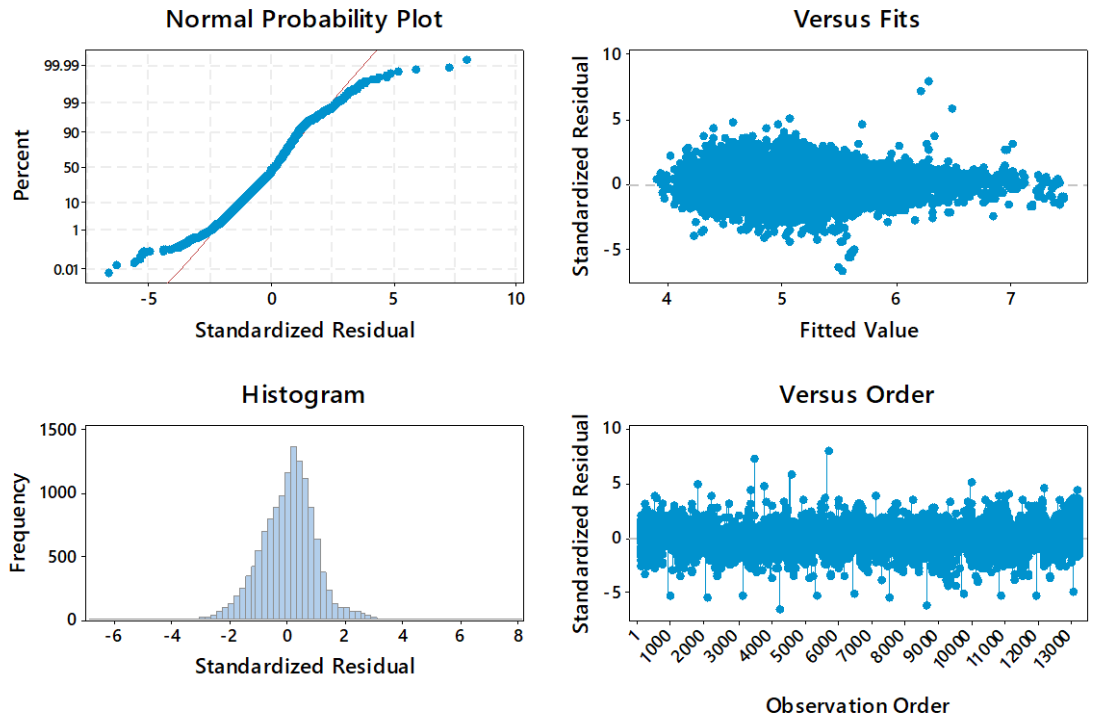


Figure C2 Fitted line and plots of standardised residuals obtained from orthogonal regression analysis of Log_{10} transformed data for 'unknowns' in all POCIS and Chemcatcher® replicates.

C1.2 Least squares and orthogonal regression analysis of the averaged Log10 transformed Chemcatcher® and POCIS 'unknowns' data

The variation accounted for by the least squares regression (r^2), as shown in Table C3, is 87.2% and the slope of 0.95 (t = 173.36, pr = < 0.001) is significantly different from zero and falls within the approximate 95% confidence intervals of 0.94 to 0.96. The intercept of 0.55 (t = 19.53, pr = < 0.001) falls within the approximate 95% confidence intervals of 0.49-0.60 but is significantly different from zero. The antilog of 0.55 (3.55) is the value of POCIS when Chemcatcher® is 1. The slope of approximately one shows an approximate linear relationship in the unlogged data, and so this indicates that the sampling rate of POCIS is approximately 2.3 times that of the Chemcatcher®. The variance is not constant but decreases with the amount of compound accumulated, the histogram of residuals, however, is close to that of a normal distribution (Figure C3).

The slope of 1.02 obtained from orthogonal regression is significantly different from zero and falls within the approximate 95% confidence intervals of 1.01 to 1.03. The intercept of 0.20 falls within the approximate 95% confidence intervals of 0.14 to 0.26 but zero does not and is thus significantly different from zero. Since the data are on a Log scale, the intercept corresponds to the Log_{10} POCIS value when the Chemcatcher® value is one, and is equivalent to a POCIS value of 1.58 (the anti-log of 0.20). See Table C4. The variance is not constant but decreases with the amount of compound accumulated, the histogram of residuals (Figure C4), however, is close to that of a normal distribution.

Table C3 Output from least squares regression analysis of the averaged Log_{10} transformed Chemcatcher® and POCIS 'unknowns' data.

Analysis of Variance

Source	DF	Seq SS	Contrib.	Adj SS	Adj MS	F-Value	P-Value
Regression	1	1188.98	87.21%	1188.98	1188.98	30052.45	< 0.001
Log10 Chemcatcher® (average)	1	1188.98	87.21%	1188.98	1188.98	30052.45	< 0.001
Error	4406	174.32	12.79%	174.32	0.04		
Total	4407	1363.3	100.00%				

Model Summary

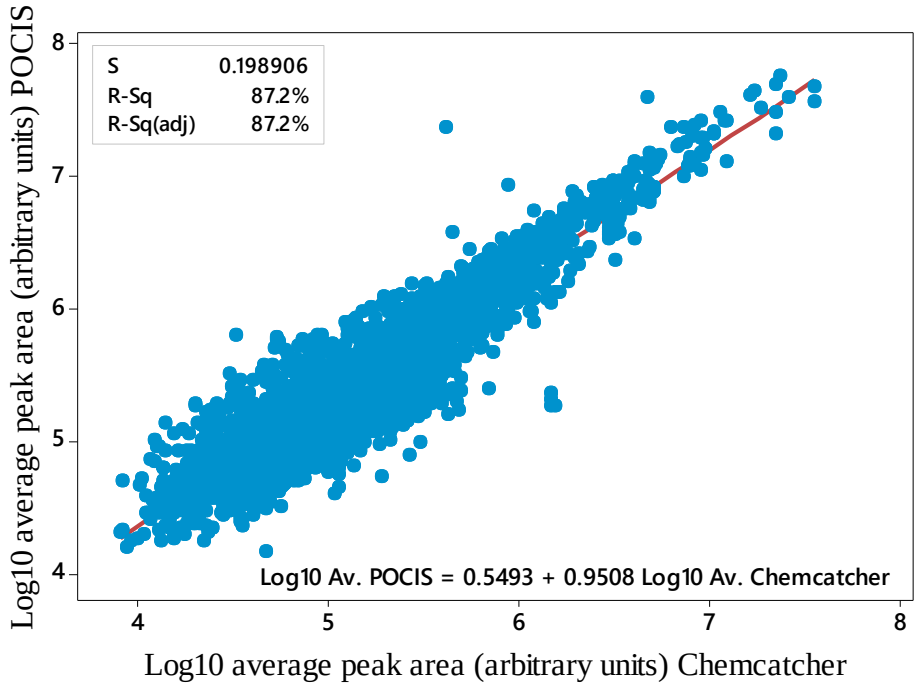
S	R-sq	R-sq(adj)	PRESS	R-sq(pred)
0.198906	87.21%	87.21%	174.472	87.20%

Coefficients

Term	Coef	SE Coef	95%CI	T-Value	P-Value	VIF
Constant	0.5493	0.0281	(0.4942, 0.6045)	19.53	< 0.001	
Log10 Chemcatcher® (average)	0.95082	0.00548	(0.94006, 0.96157)	173.36	< 0.001	1.00

Regression Equation

Log_{10} POCIS (average) = 0.5493 + 0.95082 Log_{10} Chemcatcher® (average)



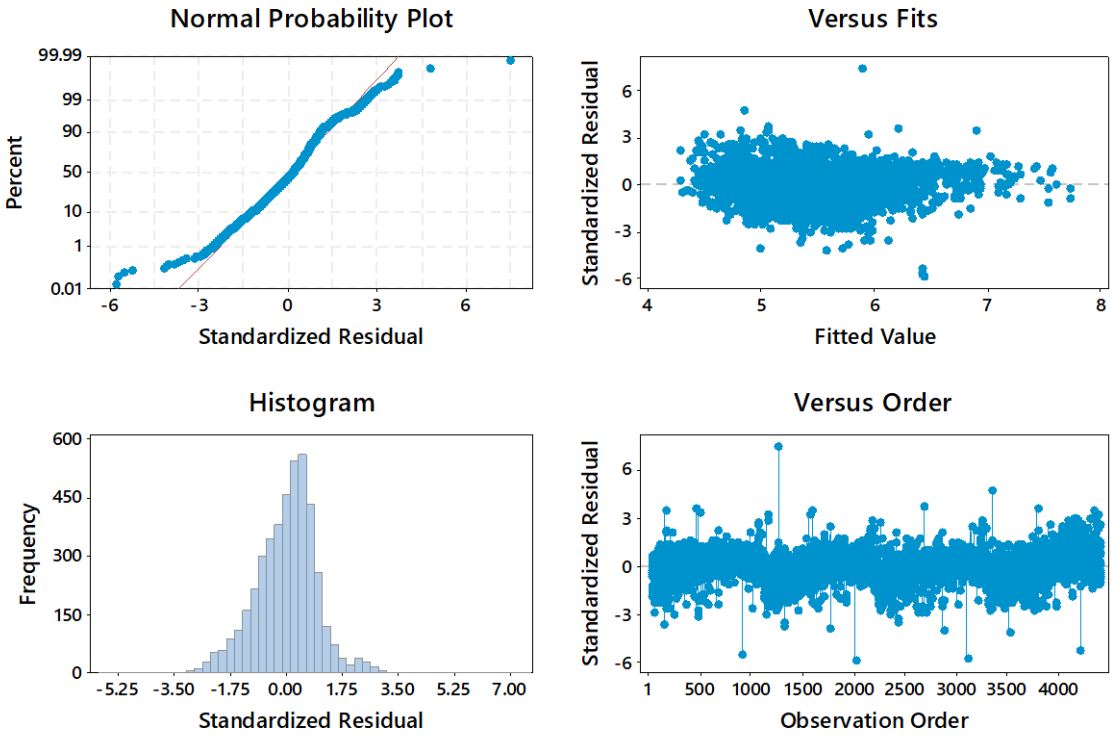


Figure C3 Fitted line and plots of standardised residuals obtained from least squares regression analysis of the averaged Log_{10} transformed Chemcatcher® and POCIS 'unknowns' data.

Table C4 Output from orthogonal regression analysis of the averaged Log_{10} transformed Chemcatcher® and POCIS 'unknowns' data.

Error Variance Ratio Log_{10} POCIS (average) / Log_{10} Chemcatcher® (average): 1

Regression Equation

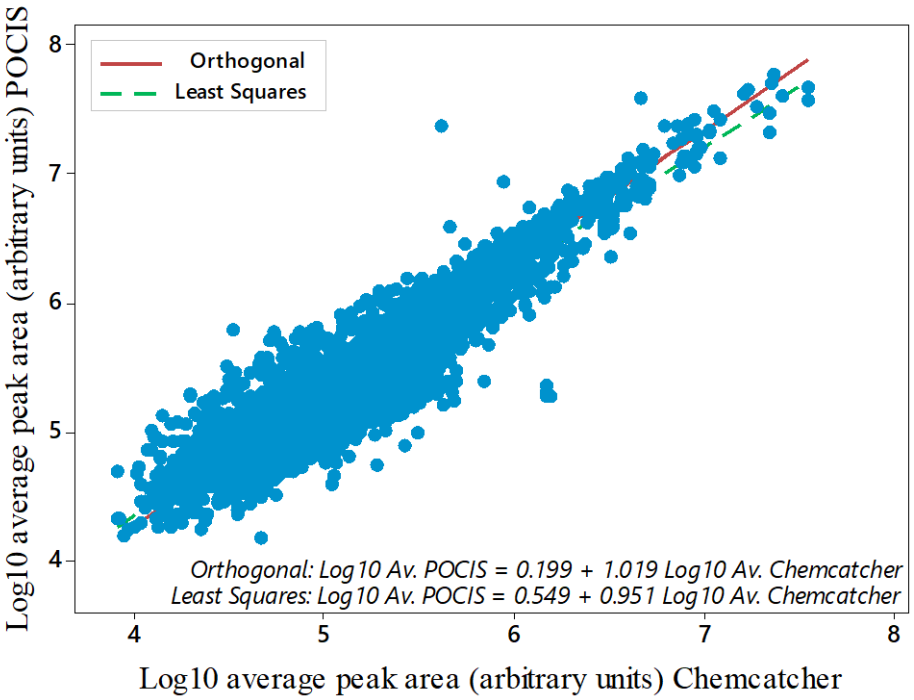
Log_{10} POCIS (average) = 0.199 + 1.019 Log_{10} Chemcatcher® (average)

Coefficients

Predictor	Coef	SECoef	Z	P	Approx95%CI
Constant	0.19941	0.030144	6.6153	< 0.001	(0.14033, 0.25850)
Log10 Chemcatcher® (average)	1.01943	0.005881	173.3557	< 0.001	(1.00790, 1.03095)

Error variances

Variable	Variance
Log10 POCIS (average)	0.020086
Log10 Chemcatcher® (average)	0.020086



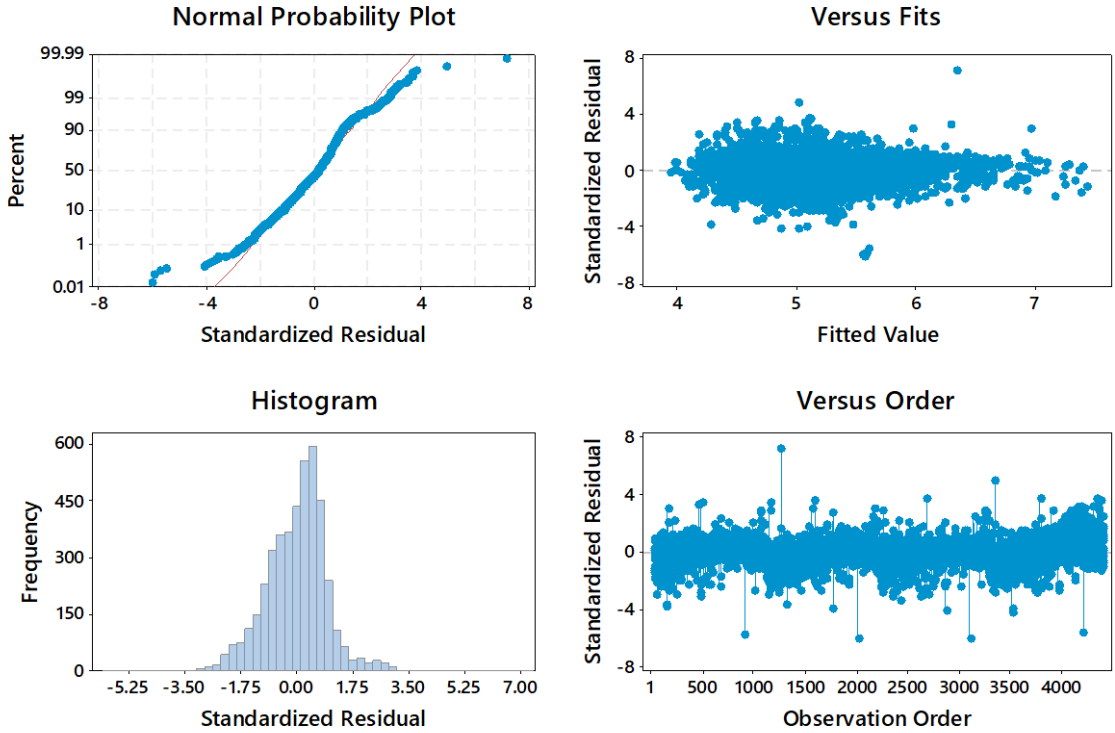


Figure C4 Fitted line and plots of standardised residuals obtained from orthogonal regression analysis of the averaged Log_{10} transformed Chemcatcher® and POCIS 'unknowns' data.

Appendix D

Table D1 List of prescribed items by British National Formulary Chemical (BNF) name exceeding 25,000 items dispensed by GPs in Wales during 2014.

No.	British National Formulary Chemical Name	Number of prescribed items
1	Simvastatin	2875131
2	Omeprazole	2502345
3	Aspirin/Salicylic acid	2283925
4	Levothyroxine Sodium	2281606
5	Ramipril	2079882
6	Co-Codamol (Codeine Phosphate/Paracetamol)	1798399
7	Amlodipine	1792202
8	Salbutamol	1739342
9	Atorvastatin	1619116
10	Lansoprazole	1566649
11	Paracetamol	1506464
12	Bisoprolol Fumarate	1439376
13	Citalopram Hydrobromide	1324650
14	Metformin Hydrochloride	1313226
15	Bendroflumethiazide	1216015
16	Furosemide	1038516
17	Warfarin Sodium	965874
18	Fluticasone Furoate/Propionate	934356
19	Amitriptyline Hydrochloride	866025
20	Amoxicillin	831646
21	Lisinopril	819281
22	Ibuprofen	676924
23	Atenolol	674909
24	Sertraline Hydrochloride	597904
25	Tramadol Hydrochloride	592678
26	Losartan Potassium	588148
27	Diazepam	587903
28	Prednisolone Acetate/Sodium Metasulphobenzoate	557255
29	Naproxen	552789
30	Gliclazide	533018
		continued

31	Mirtazapine	531878
32	Alendronic Acid	521663
33	Fluoxetine Hydrochloride	516680
34	Hydrocortisone Sodium Succinate/Phosphate/Acetate/Butyrate	512454
35	Perindopril Erbumine/Arginine (Diuretic)	507244
36	Clopidogrel	488036
37	Zopiclone	454336
38	Tamsulosin Hydrochloride	437346
39	Tiotropium	433337
40	Cetirizine Hydrochloride	421527
41	Doxazosin Mesilate	421144
42	Budesonide	418084
43	Allopurinol	411959
44	Alginic Acid	401114
45	Gabapentin	395109
46	Morphine Sulphate	345808
47	Ranitidine Hydrochloride	329153
48	Loratadine	305813
49	Candesartan Cilexetil	290698
50	Flucloxacillin Sodium	289770
51	Pregabalin	289725
52	Trimethoprim	286945
53	Diltiazem Hydrochloride	284136
54	Propranolol Hydrochloride	278877
55	Felodipine	278116
56	Betamethasone Dipropionate/Phosphate/Valerate	270426
57	Co-Dydramol (Dihydrocodeine/Paracetamol)	253839
58	Venlafaxine	250625
59	Digoxin	249280
60	Quinine Sulphate	239683
61	Pravastatin Sodium	228578
62	Combined Ethinylestradiol	227480
63	Indapamide	221788
64	Quetiapine	219003
65	Finasteride	203334
66	Temazepam	201342
		continued

67	Latanoprost	200396
68	Thiamine Hydrochloride	200347
69	Duloxetine Hydrochloride	197748
70	Nifedipine	197485
71	Mometasone Furoate	193727
72	Doxycycline Hyclate	190600
73	Sodium Valproate	189815
74	Hypromellose	188870
75	Solifenacin Succinate	188592
76	Spironolactone	187662
77	Colecalciferol	187340
78	Betahistine Hydrochloride	178983
79	Diclofenac Sodium/Potassium	173686
80	Phenoxymethylpenicillin (Penicillin V)	171520
81	Montelukast	171190
82	Mebeverine Hydrochloride	170087
83	Hydroxocobalamin	168837
84	Sildenafil	168799
85	Erythromycin/Ethylsuccinate/Stearate	166866
86	Clobetasone Butyrate/Propionate	165089
87	Buprenorphine	163490
88	Carbamazepine	162220
89	Fexofenadine Hydrochloride	160418
90	Clotrimazole	158855
91	Olanzapine	158534
92	Sitagliptin	158298
93	Rosuvastatin Calcium	154124
94	Clarithromycin	153445
95	Lamotrigine	152070
96	Glyceryl Trinitrate	151246
97	Irbesartan	146398
98	Methotrexate	144034
99	Metronidazole	141415
100	Paroxetine Hydrochloride	141335
101	Codeine Phosphate	140314
102	Dihydrocodeine Tartrate	140277
		continued

103	Nicorandil	137978
104	Loperamide Hydrochloride	137875
105	Dexamethasone Phosphate/Sodium Metasulphobenzoate	134246
106	Chloramphenicol	133536
107	Nitrofurantoin	132764
108	Enalapril Maleate	126081
109	Carbocisteine	125672
110	Co-Amoxiclav (Amoxicillin/ clavulanic acid)	122675
111	Prochlorperazine Maleate	122600
112	Estradiol/Valerate with Progestogen	118878
113	Desogestrel	116469
114	Methadone Hydrochloride	114742
115	Ezetimibe	112920
116	Hyoscine Hydrobromide/Butylbromide	112485
117	Valsartan	111786
118	Oxybutynin Hydrochloride	108441
119	Lercanidipine Hydrochloride	106936
120	Sumatriptan Succinate	106745
121	Levetiracetam	106420
122	Cefalexin	104033
123	Chlorpheniramine Maleate	101372
124	Risperidone	100482
125	Timolol Maleate	95370
126	Domperidone	95335
127	Sodium Cromoglicate	95012
128	Oxycodone Hydrochloride	93901
129	Bumetanide	91105
130	Piroxicam	90659
131	Trazodone Hydrochloride	89401
132	Fusidic Acid	88682
133	Mesalazine	88146
134	Medroxyprogesterone Acetate	86892
135	Lorazepam	86507
136	Brinzolamide	84035
137	Chlorhexidine Gluconate	83894
138	Tadalafil	83242
		continued

139	Lymecycline	80530
140	Promethazine Hydrochloride	79920
141	Beclometasone Dipropionate	79100
142	Aciclovir	78402
143	Bimatoprost	78324
144	Dipyridamole	76807
145	Donepezil Hydrochloride	76522
146	Metoclopramide Hydrochloride	76343
147	Terbutaline Sulphate	76247
148	Fentanyl	75391
149	Tolterodine Tartrate	74497
150	Pioglitazone Hydrochloride	74065
151	Esomeprazole	73307
152	Nitrazepam	72840
153	Letrozole	71250
154	Nicotine/Nicotinates	70224
155	Calcipotriol	69585
156	Benzydamine Hydrochloride	68827
157	Dorzolamide	67170
158	Salmeterol	66670
159	Terbinafine Hydrochloride	63996
160	Methylphenidate Hydrochloride	63867
161	Sulfasalazine	63710
162	Pantoprazole	63157
163	Verapamil Hydrochloride	63150
164	Ipratropium Bromide	62781
165	Procyclidine Hydrochloride	62307
166	Lidocaine/Lidocaine hydrochloride	61941
167	Dosulepin Hydrochloride	61773
168	Cyclizine Hydrochloride	61636
169	Baclofen	60175
170	Fenofibrate	59595
171	Clindamycin Phosphate/Hydrochloride	59578
172	Norethisterone	58961
173	Ciprofloxacin	58339
174	Ketoconazole	58280
		continued

175	Co-Careldopa (Carbidopa/Levodopa)	58094
176	Fluconazole	57124
177	Azathioprine	56886
178	Aripiprazole	56813
179	Phenytoin Sodium	55561
180	Co-Amilofruse (Amiloride Hydrochloride/Frusemide)	54505
181	Saxagliptin	54329
182	Hydroxychloroquine Sulphate	54129
183	Heparinoid	52325
184	Nystatin	51750
185	Clonazepam	51351
186	Meloxicam	50960
187	Liraglutide	50790
188	Alfacalcidol	49430
189	Zolpidem Tartrate	48805
190	Bisacodyl	47784
191	Co-Beneldopa (Benserazide/Levodopa)	47370
192	Carmellose Sodium	46744
193	Risedronate Sodium	45796
194	Theophylline	45448
195	Moxonidine	45335
196	Pizotifen Malate	44827
197	Oxytetracycline	44697
198	Azithromycin	44154
199	Methylprednisolone Acetate	43247
200	Valproic Acid	42755
201	Carvedilol	42032
202	Dutasteride & Tamsulosin Hydrochloride	41970
203	Miconazole Nitrate /Miconazole	41781
204	Alfuzosin Hydrochloride	41446
205	Nebivolol	40742
206	Alverine Citrate	40332
207	Bezafibrate	39761
208	Linagliptin	39741
209	Colchicine	38993
210	Sotalol Hydrochloride	38762
		continued

211	Topiramate	38317
212	Amisulpride	38067
213	Telmisartan	37645
214	Ropinirole	37252
215	Cyanocobalamin	36345
216	Olmesartan Medoxomil/Hydrochlorothiazide/Amlodipine	36252
217	Lacidipine	35811
218	Metoprolol Tartrate	35387
219	Orlistat	34809
220	Escitalopram	34403
221	Cinnarizine	33955
222	Carbimazole	33907
223	Trospium Chloride	33759
224	Tranexamic Acid	33378
225	Glimepiride	33099
226	Tamoxifen Citrate	33001
227	Capsaicin	32154
228	Dimethicone	30862
229	Hydroxyzine Hydrochloride	30813
230	Urea	30649
231	Amiodarone Hydrochloride	30276
232	Estriol	29466
233	Etoricoxib	29288
234	Ivabradine	29217
235	Pneumococcal	29141
236	Travoprost	28603
237	Pancreatin	28225
238	Clonidine Hydrochloride	27400
239	Nortriptyline	27153
240	Anastrozole	26590
241	Mefenamic Acid	26404
242	Co-Amilozide (Amiloride Hydrochloride & Hydrochlorothiazide)	26384
243	Haloperidol Decanoate	26200
244	Flecainide Acetate	25857
245	Neomycin Sulphate	25512
246	Lofepramine Hydrochloride	25266
		continued

247	Eplerenone	25011
248	Clomipramine Hydrochloride	25000

Table D2 Compounds for the entire ‘training set’ database

No.	RT (min)	Name	CAS	Chemspider ID	SMILES Notation
1	1.23	Histidine	(4998-57-6)	752	c1c(nc[nH]1)CC(C(=O)O)N
2	1.23	Diquat (Ion 2+)	(2764-72-9)	6537	c1cc[n+]2c(c1)-c3cccc[n+]3CC2
3	1.24	Carbachol	(51-83-2)	2454	C[N+](C)(C)CCOC(=N)O
4	1.31	Calteridol	(132722-73-7)	54720	CC(CN1CCN(CCN(CCN(CC1)CC(=O)O)CC(=O)O)CC(=O)O)O
5	1.38	Ecgonine methyl ester	(7143-09-1)	220696	CN1C2CCC1C(C(C2)O)C(=O)OC
6	1.39	Metformin	(657-24-9)	3949	CN(C)C(=N)NC(=N)N
7	1.44	Glufosinate	(51276-47-2)	4630	CP(=O)(CCC(C(=O)O)N)O
8	1.44	Vigabatrin	(60643-86-9)	5463	C=CC(CCC(=O)O)N
9	1.44	Amitrole	(61-82-5)	1577	c1[nH][nH]c(=N)n1
10	1.45	Melamine	(108-78-1)	7667	c1(nc(nc(n1)N)N)N
11	1.58	Histamine	(51-45-6)	753	c1c([nH]cn1)CCN
12	1.63	Morphine-3-beta-D-glucuronide	(20290-09-9)	3523765	CN1CCC23c4c5ccc(c4OC2C(C=CC3C1C5)O)OC6C(C(C(C(O6)C(=O)O)O)O)O
13	1.67	Chlormequat	(7003-89-6)	13237	C[N+](C)(C)CCCl
14	1.74	Arecoline	(63-75-2)	13872064	CN1CCC=C(C1)C(=O)OC
15	1.79	Tranexamic acid	(1197-18-8)	5325	C1CC(CCC1CN)C(=O)O
16	1.81	Norfenefrine	(536-21-0)	4379	c1cc(cc(c1)O)C(CN)O
17	1.93	Ethambutol	(74-55-5)	3164	CCC(CO)NCCNC(CC)CO
18	1.99	Dioxethedrin	(497-75-6)	64697	CCNC(C)C(c1ccc(c(c1)O)O)O
19	2.01	Sulfaguanidine	(57-67-0)	5133	c1cc(ccc1N)S(=O)(=O)NC(=N)N
20	2.18	Isoniazide	(54-85-3)	3635	c1cnccc1C(=O)NN
21	2.18	Pyridoxine	(65-23-6)	1025	Cc1c(c(c(cn1)CO)CO)O
22	2.28	Tyramine	(51-67-2)	5408	c1cc(ccc1CCN)O
23	2.28	Hordenine	(539-15-1)	61609	CN(C)CCc1ccc(cc1)O
24	2.34	Aciclovir	(59277-89-3)	1945	c1nc2c(n1COCCO)[nH]c(=N)nc2O

continued

25	2.37	4-Methoxy-1,3-phenylenediamine	(615-05-4)	11481	COc1ccc(cc1N)N
26	2.41	Nicotine	(13890-81-8)	917	CN1CCCC1c2cccnc2
27	2.43	Adenine	(73-24-5)	185	c1[nH]c-2ncnc2c(n1)N
28	2.48	Morphine-6-beta-D-glucuronide	(50444-03-6)	2757229	CN1CCC23c4c5ccc(c4OC2C(C=CC3C1C5)OC6C(C(C(C(O6)C(=O)O)O)O)O)O
29	2.53	Thioguanine	(154-42-7)	2005804	c1[nH]c2c(n1)c(nc(=N)[nH]2)S
30	2.54	Amidephrine	(3354-67-4)	14288	CNCC(c1cccc(c1)NS(=O)(=O)C)O
31	2.59	HHMA (3,4-Dihydroxymethamphetamine)	(15398-87-5)	141547	CC(Cc1ccc(c(c1)O)O)NC
32	2.61	Morphine	(57-27-2)	4103	CN1CCC23c4c5ccc(c4OC2C(C=CC3C1C5)O)O
33	2.61	6-Mercaptopurine	(50-44-2)	580869	c1[nH]c2c(n1)c(ncn2)S
34	2.64	Pramipexole	(104632-26-0)	4716	CCCNC1CCc2c(sc(n2)N)C1
35	2.66	Etilefrine (Etiladrianol)	(709-55-7)	3190	CCNCC(c1cccc(c1)O)O
36	2.66	Dihydromorphine	(509-60-4)	240226	CN1CCC23c4c5ccc(c4OC2C(CCC3C1C5)O)O
37	2.68	Anatabine	(581-49-7)	229558	c1cc(cnc1)C2CC=CCN2
38	2.71	Normorphine	(466-97-7)	380506	c1cc(c2c3c1CC4C5C3(CCN4)C(O2)C(C=C5)O)O
39	2.73	Pholcodine	(509-67-1)	454729	CN1CCC23c4c5ccc(c4OC2C(C=CC3C1C5)O)OCCN6CCOCC6
40	2.73	Nicotinamide	(98-92-0)	911	c1cc(cnc1)C(=N)O
41	2.74	Pilocarpine	(92-13-7)	4653	CCC1C(COC1=O)Cc2cncn2C
42	2.75	Oxymorphone	(76-41-5)	4478	CN1CCC23c4c5ccc(c4OC2C(=O)CCC3(C1C5)O)O
43	2.76	Methamidophos	(10265-92-6)	3954	COP(=O)(N)SC
44	2.78	Cyromazine	(66215-27-8)	43550	C1CC1Nc2nc(nc(n2)N)N
45	2.83	Procainamide	(51-06-9)	4744	CCN(CC)CCNC(=O)c1ccc(cc1)N
46	2.84	Apophedrin (Phenylethanolamine)	(7568-93-6)	975	c1ccc(cc1)C(CN)O
47	2.88	Amrinone	(60719-84-8)	3570	c1cnccc1c2cc(c(=O)[nH]c2)N
48	2.89	Carbuterol	(34866-47-2)	33928	CC(C)(C)NCC(c1ccc(c(c1)NC(=O)N)O)O
49	2.91	Hydromorphone	(466-99-9)	3522	CN1CCC23c4c5ccc(c4OC2C(=O)CCC3C1C5)O
50	2.91	Pholedrine	(370-14-9)	4494	CC(Cc1ccc(cc1)O)NC
51	2.92	Monocrotaline	(315-22-0)	4097	CC1C(=O)OC2CCN3C2C(=CC3)COC(=O)C(C1(C)O)(C)O
52	2.94	Anabasine	(13078-04-1)	21106257	c1cc(cnc1)C2CCCCN2

continued

53	2.96	Sotalol	(3930-20-9)	5063	CC(C)NCC(c1ccc(cc1)NS(=O)(=O)C)O
54	2.96	Nizatidine	(76963-41-2)	2298266	CNC(=C[N+](=O)[O-])NCCSCc1csc(n1)CN(C)C
55	2.98	Sulpiride	(15676-16-1)	5162	CCN1CCCC1CNC(=O)c2cc(ccc2OC)S(=O)(=O)N
56	2.98	P-hydroxyamphetamine [(+)-Paredrine]	(1693-66-9)	3525	CC(Cc1ccc(cc1)O)N
57	2.98	P-Hydroxymethamphetamine (Pholedrine)	(370-14-9)	4494	CC(Cc1ccc(cc1)O)NC
58	2.99	Galanthamine	(357-70-0)	3331	CN1CCC23C=CC(CC2Oc4c3c(ccc4OC)C1)O
59	2.99	Xanthinol	(2530-97-4)	9526	Cn1c2c(c(=O)n(c1=O)C)n(cn2)CC(CN(C)CCO)O
60	3.03	Obidoxime	(7683-36-5)	4588647	c1c(cc[n+](c1)COC[n+]2ccc(cc2)/C=N\O)/C=N\O
61	3.06	Terbutaline	(23031-25-6)	5210	CC(C)(C)NCC(c1cc(cc(c1)O)O)O
62	3.06	Clopyralid	(1702-17-6)	14797	c1cc(nc(c1Cl)C(=O)O)Cl
63	3.09	Atenolol	(29122-68-7)	2162	CC(C)NCC(COc1ccc(cc1)CC(=O)N)O
64	3.09	Apraclonidine	(66711-21-5)	2130	c1c(cc(c(c1Cl)NC2=NCCN2)Cl)N
65	3.11	Salbutamol	(18559-94-9)	1999	CC(C)(C)NCC(c1ccc(c(c1)CO)O)O
66	3.11	Acephate	(30560-19-1)	1905	CC(=O)NP(=O)(OC)SC
67	3.13	Hydroxycotinine	(34834-67-8)	401	CN1C(CC(C1=O)O)c2cccnc2
68	3.14	Ranitidine	(66357-35-5)	4863	CNC(=C[N+](=O)[O-])NCCSCc1ccc(o1)CN(C)C
69	3.14	Formetanate	(22259-30-9)	15150933	CNC(=O)Oc1cccc(c1)/N=C/N(C)C
70	3.14	Adenosine	(58-61-7)	21241906	C1=NC3=C([N]1C2OC(CO)C(C2O)O)N=CN=C3N
71	3.16	Diethylcarbamazine	(90-89-1)	2944	CCN(CC)C(=O)N1CCN(CC1)C
72	3.16	Thiocyclam (Evisekt)	(31895-21-3)	33084	CN(C1CSSSC1)C
73	3.18	O-Desmethylsulpiride	(67381-52-6)	27523917	CCN1CCCC1CNC(=O)c2cc(ccc2O)S(=O)(=O)N
74	3.19	Dorzolamide	(120279-96-1)	3042	CCNC1CC(S(=O)(=O)c2c1cc(s2)S(=O)(=O)N)C
75	3.19	HMMA (4-Hydroxy-3-methoxymethamphetamine)	(117652-28-5)	2338803	CC(Cc1ccc(c(c1)OC)O)NC
76	3.19	Monocrotaline-N-oxide	(35337-98-5)	170755	CC1C(=O)OC2CC[N+]3(C2C(=CC3)COC(=O)C(C1(C)O)(C)O)[O-]
77	3.2	Endothal Peak 1&2	(28874-46-6)	3112	C1CC2C(C(C1O2)C(=O)O)C(=O)O
78	3.21	Procaine	(59-46-1)	4745	CCN(CC)CCOC(=O)c1ccc(cc1)N
79	3.23	HMA (HMA-5)	1428-95-1	1728	C1CCCN(CC1)c2c(nc(c(n2)N)C(=O)NC(=N)N)Cl
80	3.24	Famotidine	(76824-35-6)	3208	c1c(nc(s1)N=C(N)N)CSCC/C(=N/S(=O)(=O)N)/N

continued

81	3.24	Cimetidine	(51481-61-9)	2654	Cc1c([nH]cn1)CSCCN/C(=N/C)/NC#N
82	3.26	Moxonidine	(75438-57-2)	4645	Cc1nc(c(c(n1)Cl)NC2=NCCN2)OC
83	3.28	Tolazoline	(59-98-3)	5303	c1ccc(cc1)CC2=NCCN2
84	3.28	Theobromine	(83-67-0)	5236	Cn1cnc2c1c(nc(=O)n2C)O
85	3.29	Psilocin	(520-53-6)	4807	CN(C)CCc1c[nH]c2c1c(ccc2)O
86	3.34	Practolol	(6673-35-4)	4715	CC(C)NCC(COc1ccc(cc1)NC(=O)C)O
87	3.35	Omethoate	(1113-02-6)	13574	CNC(=O)CSP(=O)(OC)OC
88	3.35	Butocarboxim-sulfoxide	(34681-24-8)	7851180	O=S(C(\C(=N\OC(=O)NC)C)C)C
89	3.36	Dihydrocodeine	(125-28-0)	(N/A)	CN1CCC23C4C1CC5=C2C(=C(C=C5)OC)OC3C(CC4)O
90	3.37	Sulfadiazine	(68-35-9)	5026	c1cnc(nc1)NS(=O)(=O)c2ccc(cc2)N
91	3.39	Nalorphine	(62-67-9)	4270	C=CCN1CCC23c4c5ccc(c4OC2C(C=CC3C1C5)O)O
92	3.4	Codeine	(76-57-3)	2726	CN1CCC23c4c5ccc(c4OC2C(C=CC3C1C5)O)OC
93	3.41	Asulam	(3337-71-1)	17707	CO/C(=N/S(=O)(=O)c1ccc(cc1)N)/O
94	3.41	Tiapride	(51012-32-9)	5268	CCN(CC)CC/N=C(/c1cc(ccc1OC)S(=O)(=O)C)\O
95	3.43	Sumatriptan	(103628-46-2)	5165	CNS(=O)(=O)Cc1ccc2c(c1)c(c[nH]2)CCN(C)C
96	3.43	Acetazolamide	(59-66-5)	1909	CC(=O)Nc1nnc(s1)S(=O)(=O)N
97	3.44	Naloxone	(465-65-6)	(N/A)	C=CCN1CCC23C4C(=O)CCC2(C1CC5=C3C(=C(C=C5)O)O4)O
98	3.48	Paracetamol (Acetaminophen)	(103-90-2)	1906	C/C(=N\c1ccc(cc1)O)/O
99	3.49	Heliotrine-N-oxide	(6209-65-0)	2985574	CC(C)C(C(C)OC)(C(=O)OCC1=CC[N+]2(C1C(CC2)O)[O-])O
100	3.49	Rizatriptan	(144034-80-0)	4900	CN(C)CCc1c[nH]c2c1cc(cc2)Cn3cncn3
101	3.5	Norcodeine	(467-15-2)	(N/A)	COC1=C2C3=C(CC4C5C3(CCN4)C(O2)C(C=C5)O)C=C1
102	3.5	Propamocarb	(24579-73-5)	30114	CCCO/C(=N/CCCN(C)C)/O
103	3.5	Aldicarb-sulfoxide	(1646-87-3)	7843407	CC(C)(/C=N/OC(=O)NC)[S+](C)[O-]
104	3.53	DMPEA (3,4-Dimethoxy phenethylamine)	(120-20-7)	8114	COc1ccc(cc1OC)CCN
105	3.53	Sulfathiazole	(72-14-0)	5148	c1cc(ccc1N)S(=O)(=O)Nc2nccs2
106	3.54	Norephedrine	(14838-15-4)	4622	CC(C(c1ccccc1)O)N
107	3.56	Oxycodone	(76-42-6)	4474	CN1CCC23c4c5ccc(c4OC2C(=O)CCC3(C1C5)O)OC
108	3.56	Reproterol	(54063-54-6)	23898	Cn1c2c(c(=O)n(c1=O)C)n(cn2)CCCNCC(c3cc(cc(c3)O)O)O

continued

109	3.56	Actinoquinol	(15301-40-3)	22136	CCOc1ccc(c2c1nccc2)S(=O)(=O)O
110	3.57	2-Phenethylamine	(64-04-0)	13856352	c1ccc(cc1)CCN
111	3.58	Naltrexone	(16590-41-3)	4275	c1cc(c2c3c1CC4C5(C3(CCN4CC6CC6)C(O2)C(=O)CC5)O)O
112	3.58	Metronidazole	(443-48-1)	4029	Cc1ncc(n1CCO)[N+](=O)[O-]
113	3.59	Cathinone	(71031-15-7)	96940	CC(C(=O)c1ccccc1)N
114	3.59	Dinotefuran	(165252-70-0)	171124	CN/C(=N/[N+](=O)[O-])/NCC1CCOC1
115	3.61	Zolmitriptan	(139264-17-8)	5529	CN(C)CCc1c[nH]c2c1cc(cc2)CC3COC(=O)N3
116	3.62	Alizapride	(59338-93-1)	39202	COc1cc2c(cc1C(=O)NCC3CCCN3CC=C)nn[nH]2
117	3.63	Varenicline	(249296-44-4)	148958	c1cnc2cc3c(cc2n1)C4CC3CNC4
118	3.64	Tizanidine	(51322-75-9)	5287	c1cc(c(c2c1nsn2)NC3=NCCN3)Cl
119	3.64	Dimethylcathinone (Metamfepramone)	(15351-09-4)	64889	CC(C(=O)c1ccccc1)N(C)C
120	3.65	Lycopsamine	(10285-07-1)	21009	CC(C)C(C(C)O)(C(=O)OCC1=CCN2C1C(CC2)O)O
121	3.66	Levetiracetam	(102767-28-2)	53863	CCC(C(=O)N)N1CCCC1=O
122	3.66	Butoxycarboxim	(34681-23-7)	55798	CC(/C(=N/OC(=O)NC)/C)S(=O)(=O)C
123	3.68	Cathine	(492-39-7)	4622	CC(C(c1ccccc1)O)N
124	3.68	Amiloride	(2016-88-8)	15403	c1(c(nc(c(n1)Cl)N)N)/C(=N/C(=N)N)/O
125	3.68	Methcathinone (Ephedrone)	(5650-44-2)	1519	CC(C(=O)c1ccccc1)NC
126	3.69	Hydrocodone	(125-29-1)	364475	CN1CCC23c4c5ccc(c4OC2C(=O)CCC3C1C5)OC
127	3.69	Esculin (Aesculin)	(531-75-9)	4508522	c1cc(=O)oc2c1cc(c(c2)O)OC3C(C(C(C(O3)CO)O)O)O
128	3.69	Methiopropamine	(801156-47-8)	385727	CC(Cc1cccs1)NC
129	3.71	Noroxycodone	(57664-96-7)	544866	COc1ccc2c3c1OC4C35CCNC(C2)C5(CCC4=O)O
130	3.71	Gabapentin	(60142-96-3)	3328	C1CCC(CC1)(CC(=O)O)CN
131	3.71	Phenelzine	(51-71-8)	3547	c1ccc(cc1)CCNN
132	3.71	Sulfapyridine	(144-83-2)	5145	c1ccnc(c1)NS(=O)(=O)c2ccc(cc2)N
133	3.73	Methylephedrine	(552-79-4)	4221	CC(C(c1ccccc1)O)N(C)C
134	3.73	Homatropine	(87-00-3)	3497	CN1C2CCC1CC(C2)OC(=O)C(c3ccccc3)O
135	3.73	Dimethipin (Na adduct in MS spectrum)	(55290-64-7)	37765	CC1=C([S](=O)(=O)CC[S]1(=O)=O)C
136	3.74	Pseudoephedrine	(90-82-4)	4856	CC(C(c1ccccc1)O)NC

continued

137	3.75	6-O-Monoacetylmorphine (MAM)	(2784-73-8)	21537497	CC(=O)OC1C=CC2C3Cc4ccc(c5c4C2(C1O5)CCN3C)O
138	3.75	Cotinine	(75202-09-4)	395	CN1C(CCC1=O)c2cccnc2
139	3.75	Aldicarb-sulfone (Aldoxycarb)	(1646-88-4)	7844561	CC(C)(/C=N/OC(=O)NC)S(=O)(=O)C
140	3.76	Ephedrine	(299-42-3)	4856	CC(C(c1ccccc1)O)NC
141	3.76	Diprophylline	(479-18-5)	3070	Cn1c2c(c(=O)n(c1=O)C)n(cn2)CC(CO)O
142	3.77	Oxamyl	(23135-22-0)	7869433	CNC(=O)O/N=C(/C(=O)N(C)C)\SC
143	3.78	bk-MDDMA (Dimethylone)	No CAS available	7970239	O=C(c1ccc2OCOc2c1)C(N(C)C)C
144	3.79	Dropropizine (Dopropizin)	(17692-31-8)	3057	c1ccc(cc1)N2CCN(CC2)CC(CO)O
145	3.81	MDAI	(132741-81-2)	111694	c1c2c(cc3c1OCO3)CC(C2)N
146	3.81	Methylone (MDMC)	(186028-79-5)	21106350	CC(C(=O)c1ccc2c(c1)OCO2)NC
147	3.83	Scopolamine	(51-34-3)	4997	CN1C2CC(CC1C3C2O3)OC(=O)C(CO)c4ccccc4
148	3.83	Clonidine	(4205-90-7)	2701	c1cc(c(c(c1)Cl)NC2=NCCN2)Cl
149	3.84	Theophylline	(58-55-9)	2068	Cn1c2c(c(=O)n(c1=O)C)nc[nH]2
150	3.86	Tinidazole	(19387-91-8)	5279	CCS(=O)(=O)CCn1c(ncc1[N+](=O)[O-])C
151	3.86	Sulfamerazine	(127-79-7)	5134	Cc1ccnc(n1)NS(=O)(=O)c2ccc(cc2)N
152	3.87	Oxitropium	No CAS available	4466	CC[N+]1(C2CC(CC1C3C2O3)OC(=O)C(CO)c4ccccc4)C
153	3.88	Pregabalin	(148553-50-8)	3902475	CC(C)CC(CC(=O)O)CN
154	3.88	Methylscopolamine	(13265-10-6)	3977	C[N+]1(C2CC(CC1C3C2O3)OC(=O)C(CO)c4ccccc4)C
155	3.88	Amisulpiride	(71675-85-9)	2074	CCN1CCCC1CNC(=O)c2cc(c(cc2OC)N)S(=O)(=O)CC
156	3.88	Mescaline	(54-04-6)	3934	COc1cc(cc(c1OC)OC)CCN
157	3.88	Desethylhydroxychloroquine (Cletoquine)	(4298-15-1)	64850	CC(CCCNCCO)Nc1ccnc2c1ccc(c2)Cl
158	3.89	Physostigmine	(57-47-6)	4646	CC12CCN(C1N(c3c2cc(cc3)OC(=O)NC)C)C
159	3.89	Pindolol	(13523-86-9)	4662	CC(C)NCC(COc1cccc2c1cc[nH]2)O
160	3.9	Nitenpyram	(150824-47-8)	2298774	CCN(Cc1ccc(nc1)Cl)/C(=C/[N+](=O)[O-])/NC
161	3.91	Oxydemeton-methyl	(301-12-2)	4457	CCS(=O)CCSP(=O)(OC)OC
162	3.91	Carteolol	(51781-06-7)	2485	CC(C)(C)NCC(COc1cccc2c1CCC(=O)N2)O
163	3.91	Flephedrone	(447-40-5)	21477355	CC(C(=O)c1ccc(cc1)F)NC
164	3.92	Diaveridine	(5355-16-8)	20162	COc1ccc(cc1OC)Cc2cnc(nc2N)N

continued

165	3.93	Ethylcathinone	(18259-37-5)	403504	CCNC(C)C(=O)c1ccccc1
166	3.94	3-4-DMA (3,4-Dimethoxyamphetamine)	(120-26-3)	8116	CC(Cc1ccc(c(c1)OC)OC)N
167	3.94	DMT (Dimethyltryptamine)	(61-50-7)	5864	CN(C)CCc1c[nH]c2c1cccc2
168	3.94	3-F-Methcathinone	(1049677-77-1)	24958236	CC(C(=O)c1cccc(c1)F)NC
169	3.94	Lycopsamine-N-oxide	(41708-76-3)	35633	CC(C)C(C(C)O)(C(=O)OCC1=CC[N+]2(C1C(CC2)O)[O-])O
170	3.96	Methotrexate	(59-05-2)	3969	CN(Cc1cnc2c(n1)c(nc(n2)N)N)c3ccc(cc3)C(=O)NC(CCC(=O)O)C(=O)O
171	3.96	Sulfaclomide	(4015-18-3)	64308	Cc1c(c(nc(n1)C)NS(=O)(=O)c2ccc(cc2)N)Cl
172	3.97	Bethanidine	(55-73-2)	2278	CNC(=NCc1ccccc1)NC
173	3.97	Dazomet	(533-74-4)	10332	CN1CN(C(=S)SC1)C
174	3.98	Lisinopril	(76547-98-3)	3800	c1ccc(cc1)CCC(C(=O)O)NC(CCCCN)C(=O)N2CCCC2C(=O)O
175	3.98	Ropinirole	(91374-21-9)	4916	CCCN(CCC)CCc1cccc2c1CC(=O)N2
176	3.98	Demeton-S-methylsulfoxide (Oxydemeton-methyl)	(301-12-2)	4457	CCS(=O)CCSP(=O)(OC)OC
177	3.98	5-MeO-DMT (5-Methoxydimethyltryptamine)	(1019-45-0)	1766	CN(C)CCc1c[nH]c2c1cc(cc2)OC
178	3.98	Hydroxychloroquine	(118-42-3)	3526	CCN(CCCC(C)Nc1ccnc2c1ccc(c2)Cl)CCO
179	3.99	Trimethoprim	(738-70-5)	5376	COc1cc(cc(c1OC)OC)Cc2c[nH]c(=N)[nH]c2=N
180	4.01	Retrorsine	(480-54-6)	4509313	C/C=C/1\CC(C(C(=O)OCC2=CCN3C2C(CC3)OC1=O)(CO)O)C
181	4.01	alpha-PPP (alpha-Pyrrolidinopropiophenone)	(19134-50-0)	181124	CC(C(=O)c1ccccc1)N2CCCC2
182	4.01	Pymetrozine	(123312-89-0)	7850487	CC1=NN=C(N(C1)/N=C/c2cccnc2)O
183	4.01	5-MeOT (5-Methoxytryptamine)	(608-07-1)	1767	COc1ccc2c(c1)c(c[nH]2)CCN
184	4.04	Ethylone	(1112937-64-0)	21106271	CCNC(C)C(=O)c1ccc2c(c1)OCO2
185	4.06	O-Desmethyltramadol	(73986-53-5)	115703	CN(C)CC1CCCCC1(c2cccc(c2)O)O
186	4.08	Ethylmorphine	(76-58-4)	3187	CCOc1ccc2c3c1OC4C35CCN(C(C2)C5C=CC4O)C
187	4.08	MDDMA (N,N Dimethyl-3,4-methylenedioxyamphetamine)	(74698-50-3)	479880	CC(Cc1ccc2c(c1)OCO2)N(C)C
188	4.09	Nimorazole	(6506-37-2)	21533	c1c(n(cn1)CCN2CCOCC2)[N+](=O)[O-]
189	4.09	Etofylline	(519-37-9)	1820	Cn1c2c(c(=O)n(c1=O)C)n(cn2)CCO
190	4.1	Imazapyr	(81334-34-1)	49445	CC(C)C1(C(=O)N=C(N1)c2c(cccn2)C(=O)O)C
191	4.11	Amfepramone	(134-80-5)	6762	CCN(CC)C(C)C(=O)c1ccccc1
192	4.11	Sulfamethizole	(144-82-1)	5137	Cc1nnc(s1)NS(=O)(=O)c2ccc(cc2)N

continued

193	4.11	Sulthiame	(61-56-3)	5163	c1cc(ccc1N2CCCCS2(=O)=O)S(=O)(=O)N
194	4.11	Pirenzepine	(28797-61-7)	4682	CN1CCN(CC1)CC(=O)N2c3ccccc3C(=O)Nc4c2nccc4
195	4.13	MDPPP (3',4'-Methylenedioxy-alpha-pyrrolidinopropiophenone)	(783241-66-7)	4936183	CC(C(=O)c1ccc2c(c1)OCO2)N3CCCC3
196	4.14	N-MBZP (1-Benzyl-4-methylpiperazine)	(62226-74-8)	667589	CN1CCN(CC1)Cc2ccccc2
197	4.14	Fenproporex (NARL)	(15686-61-0)	55690	CC(Cc1ccccc1)NCCC#N
198	4.14	Phenmetrazine	(134-49-6)	4598	CC1C(OCCN1)c2ccccc2
199	4.15	Demeton-S-methylsulfone	(17040-19-6)	26248	CCS(=O)(=O)CCSP(=O)(OC)OC
200	4.16	Strychnine	(57-24-9)	21428631	c1ccc2c(c1)C34CCN5C3CC6C7C4N2C(=O)CC7OCC=C6C5
201	4.16	Methomyl	(16752-77-5)	3966	C/C(=N\OC(=O)NC)/SC
202	4.16	TMA (trimethoxyamphetamine)	(1082-23-1)	28771	CC(Cc1ccc(c(c1OC)OC)OC)N
203	4.16	Amphetamine	(300-62-9)	13852819	CC(Cc1ccccc1)N
204	4.16	Chloroquine	(54-05-7)	2618	CCN(CC)CCCC(C)Nc1ccnc2c1ccc(c2)Cl
205	4.16	Methedrone	(530-54-1)	187475	CC(C(=O)c1ccc(cc1)OC)NC
206	4.17	Nalbuphine	(20594-83-6)	4266	c1cc(c2c3c1CC4C5(C3(CCN4CC6CCC6)C(O2)C(CC5)O)O)O
207	4.17	Ipratropium	(22254-24-6)	3615	CC(C)[N+]1(C2CCC1CC(C2)OC(=O)C(CO)c3ccccc3)C
208	4.17	Seneciphylline	(480-81-9)	16093177	C/C=C\1/CC(=C)C(C(=O)OCC2=CCN3C2C(CC3)OC1=O)(C)O
209	4.18	MDMA (Ecstacy)	(42542-10-9)	1556	CC(Cc1ccc2c(c1)OCO2)NC
210	4.19	Methamphetamine	(537-46-2)	1169	CC(Cc1ccccc1)NC
211	4.19	MDA (3,4-Methylenedioxyamphetamine)	(4764-17-4)	1555	CC(Cc1ccc2c(c1)OCO2)N
212	4.19	Furazolidone	(67-45-8)	3317	c1cc(oc1C=NN2CCOC2=O)[N+](=O)[O-]
213	4.19	5-MeO-TMT	(67292-68-6)	45143	Cc1c(c2cc(ccc2[nH]1)OC)CCN(C)C
214	4.21	Brucine	(357-57-3)	191250	COc1cc2c(cc1OC)N3C4C25CCN6C5CC7C4C(CC3=O)OCC=C7C6
215	4.21	Dobutamine	(34368-04-2)	33786	CC(CCc1ccc(cc1)O)NCCc2ccc(c(c2)O)O
216	4.21	Simazine 2-Hydroxy	(2599-11-3)	16505	CC/N=c\1/[nH]/c(=N\CC)/nc([nH]1)O
217	4.22	1-Piperonylpiperazine	(32231-06-4)	85214	c1cc2c(cc1CN3CCNCC3)OCO2
218	4.22	Flonicamid	(158062-67-0)	8010234	c1cncc(c1C(F)(F)F)/C(=N/CC#N)/O
219	4.23	Caffeine	(58-08-2)	2424	Cn1cnc2c1c(=O)n(c(=O)n2C)C
220	4.23	Benzylpiperazine	(2759-28-6)	68493	c1ccc(cc1)CN2CCNCC2

continued

221	4.24	Dimethocaine	(94-15-5)	6909	CCN(CC)CC(C)(C)COC(=O)c1ccc(cc1)N
222	4.24	Tetroxoprim	(53808-87-0)	58910	COCCOc1c(cc(cc1OC)Cc2cnc(nc2N)N)OC
223	4.24	2-Fluoramphetamine	(1716-60-5)	108441	CC(Cc1ccccc1F)N
224	4.25	Thiamethoxam	(153719-23-4)	96828	CN1COCN(C1=N[N+](=O)[O-])Cc2cnc(s2)Cl
225	4.26	Ketobemidone (Ketogan)	(469-79-4)	9697	CCC(=O)C1(CCN(CC1)C)c2cccc(c2)O
226	4.26	Buphedrone	(408332-79-6)	26286946	CCC(C(=O)c1ccccc1)NC
227	4.27	Heliotrine	(303-33-3)	8977	CC(C)C(C(C)OC)(C(=O)OCC1=CCN2C1C(CC2)O)O
228	4.27	Tranylcypromine	(155-09-9)	5329	c1ccc(cc1)C2CC2N
229	4.27	Dimetridazole	(551-92-8)	2980	Cc1ncc(n1C)[N+](=O)[O-]
230	4.27	Ofloxacin	(82419-36-1)	4422	CC1COc2c3n1cc(c(=O)c3cc(c2N4CCN(CC4)C)F)C(=O)O
231	4.27	Baclofen	(1134-47-0)	2197	c1cc(ccc1C(CC(=O)O)CN)Cl
232	4.28	O-Desmethylnortramadol	(N/A)	(N/A)	OC1(CCCCC1CNC)c2ccc(O)cc2
233	4.28	Methazolamide	(554-57-4)	3958	CC(=O)/N=c\1/n(nc(s1)S(=O)(=O)N)C
234	4.28	Apomorphine	(58-00-4)	2129	CN1CCc2cccc-3c2C1Cc4c3c(c(cc4)O)O
235	4.29	Nadolol	(42200-33-9)	4258	CC(C)(C)NCC(COc1cccc2c1CC(C(C2)O)O)O
236	4.3	PMMA (p-Methoxymethamphetamine)	(22331-70-0)	81951	CC(Cc1ccc(cc1)OC)NC
237	4.31	Mefexamide	(1227-61-8)	3905	CCN(CC)CC/N=C(/COc1ccc(cc1)OC)\O
238	4.32	MDAT (6,7-(Methylenedioxy)-2-aminotetralin)	(101625-35-8)	33531	c1c2c(cc3c1OCO3)CC(CC2)N
239	4.33	Retrorsine-N-oxide (Isatidine))	(15503-86-3)	3317818	CC=C1CC(C(C(=O)OCC2=CC[N+]3(C2C(CC3)OC1=O)[O-])(CO)O)C
240	4.33	2-Fluoromethamphetamine	(1017176-48-5)	23900072	CC(Cc1ccccc1F)NC
241	4.34	PMA (4-methoxyamphetamine)	(64-13-1)	29417	CC(Cc1ccc(cc1)OC)N
242	4.34	Pemoline	(2152-34-3)	4561	c1ccc(cc1)C2C(=O)N=C(O2)N
243	4.34	Proxyphylline	(603-00-9)	4806	CC(Cn1cnc2c1c(=O)n(c(=O)n2C)C)O
244	4.34	4-OH-DIPT (4-Hydroxy-N,N-diisopropyltryptamine)	(63065-90-7)	10579819	CC(C)N(CCc2cnc1cccc(O)c12)C(C)C
245	4.34	Butylone (bk-MBDB)	(802575-11-7)	21106270	CCC(C(=O)c1ccc2c(c1)OCO2)NC
246	4.34	DET (Diethyltryptamine)	(61-51-8)	5865	CCN(CC)CCc1c[nH]c2c1cccc2
247	4.36	Bulbocapnine	(298-45-3)	8919	CN1CCc2cc3c(c-4c2C1Cc5c4c(c(cc5)OC)O)OCO3
248	4.36	Metoclopramide	(364-62-5)	4024	CCN(CC)CC/N=C(/c1cc(c(cc1OC)N)Cl)\O

continued

249	4.36	5-MeO-AMT (5-Methoxy-alpha-methyltryptamine)	(1137-04-8)	33864	CC(Cc1c[nH]c2c1cc(cc2)OC)N
250	4.36	O-Desmethyldinortramadol	(N/A)	(N/A)	OC1(CCCCC1CN)c2ccc(O)cc2
251	4.36	Monocrotophos	(6923-22-4)	4522053	CC(=CC(=O)NC)OP(=O)(OC)OC
252	4.36	Atropine	(51-55-8)	3534	CN1C2CCC1CC(C2)OC(=O)C(CO)c3ccccc3
253	4.37	cis-1,2,3,6-Tetrahydrophthalimide	(1469-48-3)	6549	C1C=CCC2C1C(=O)NC2=O
254	4.38	Dibutylone	(802286-83-5)	(N/A)	CN(C)C(CC)C(=O)c1ccc2OCOc2c1
255	4.39	4-Fluoroamphetamine	(459-02-9)	9592	CC(Cc1ccc(cc1)F)N
256	4.39	MDEA (N-Ethyl-3,4-methylenedioxyamphetamine)	(82801-81-8)	10723892	CC(NCC)Cc1ccc2COOc2c1
257	4.39	3-Fluoroamphetamine	(1626-71-7)	108417	CC(Cc1cccc(c1)F)N
258	4.39	Debrisoquine	(1131-64-2)	2860	c1ccc2c(c1)CCN(C2)C(=N)N
259	4.39	3-Fluoromethamphetamine	(1182818-14-9)	27050449	CC(Cc1cccc(c1)F)NC
260	4.4	N-Ethylamphetamine	(457-87-4)	9588	CCNC(C)Cc1ccccc1
261	4.42	Ciprofloxacin	(85721-33-1)	2662	c1c2c(cc(c1F)N3CCNCC3)n(cc(c2=O)C(=O)O)C4CC4
262	4.43	2C-H (2,5-Dimethoxyphenethylamine)	(3600-86-0)	28541669	COc1ccc(cc1CCN)CO
263	4.43	4-Fluoromethamphetamine	(351-03-1)	9919721	Fc1ccc(cc1)CC(NC)C
264	4.44	Biotin	(58-85-5)	248	C1C2C(C(S1)CCCCC(=O)O)NC(=O)N2
265	4.44	Sulfalene	(152-47-6)	8695	COc1c(nccn1)NS(=O)(=O)c2ccc(cc2)N
266	4.44	Alpha-Methyltryptamine (AMT)	(299-26-3)	8930	CC(Cc1c[nH]c2c1cccc2)N
267	4.44	Sulfamethoxypyridazine	(80-35-3)	5139	COc1ccc(nn1)NS(=O)(=O)c2ccc(cc2)N
268	4.44	Mepindolol	(23694-81-7)	64750	Cc1cc2c([nH]1)cccc2OCC(CNC(C)C)O
269	4.46	Acetiamine (trans)	(299-89-8)	4677905	Cc1ncc(c(n1)N)CN(C=O)/C(=C(\CCOC(=O)C)/SC(=O)C)/C
270	4.46	Norfloxacin	(70458-96-7)	4380	CCn1cc(c(=O)c2c1cc(c(c2)F)N3CCNCC3)C(=O)O
271	4.47	Thebacon	(466-90-0)	(N/A)	CC(=O)OC1=CCC2C3CC4=C5C2(C1OC5=C(C=C4)OC)CCN3C
272	4.47	Tetryzoline (Tetrahydrozoline)	(84-22-0)	5226	c1ccc2c(c1)CCCC2C3=NCCN3
273	4.48	2,6-Dichlorobenzamide	(2008-58-4)	15359	c1cc(c(c(c1)Cl)C(=N)O)Cl
274	4.49	MDPBP (3',4'-Methylenedioxy-alpha-pyrrolidinobutyrophenone)	(24622-60-4)	(N/A)	CCC(C(=O)C1=CC2=C(C=C1)OCO2)N3CCCC3
275	4.5	Seneciphylline-N-oxide	(38710-26-8)	(N/A)	CC=C1CC(=C)C(C(=O)OCC2=CC[N+]3(C2C(CC3)OC1=O)[O-])(C)O
276	4.5	Dicrotophos	(141-66-2)	4522051	C/C(=C\C(=O)N(C)C)/OP(=O)(OC)OC

continued

277	4.53	Zonisamide	(68291-97-4)	5532	c1ccc2c(c1)c(no2)CS(=O)(=O)N
278	4.53	Mephedrone	(1189805-46-6)	21485694	Cc1ccc(cc1)C(=O)C(C)NC
279	4.54	Sulfamethoxazole	(723-46-6)	5138	Cc1cc(no1)NS(=O)(=O)c2ccc(cc2)N
280	4.56	Nifenazone	(2139-47-1)	4332	Cc1c(c(=O)n(n1C)c2ccccc2)NC(=O)c3cccnc3
281	4.57	Guanoxan	(2165-19-7)	15704	c1ccc2c(c1)OCC(O2)CNC(=N)N
282	4.59	Captopril	(62571-86-2)	2453	CC(CS)C(=O)N1CCCC1C(=O)O
283	4.59	Lidocaine	(137-58-6)	3548	CCN(CC)C/C(=N\c1c(cccc1C)C)/O
284	4.59	Tolycaine	(3686-58-6)	65111	CCN(CC)CC(=O)Nc1c(cccc1C(=O)OC)C
285	4.61	Ritalinic acid	(19395-41-6)	78360	c1ccc(cc1)C(C2CCCCN2)C(=O)O
286	4.61	Triamterene	(396-01-0)	5345	c1ccc(cc1)c2c(=N)nc-3[nH]c(=N)[nH]c(c3n2)N
287	4.62	Senecionine	(130-01-8)	223408	CC=C1CC(C(C(=O)OCC2=CCN3C2C(CC3)OC1=O)(C)O)C
288	4.62	7-Aminodesmethylflunitrazepam	(894-76-8)	141082	c1ccc(c(c1)C2=NCC(=O)Nc3c2cc(cc3)N)F
289	4.62	Detajmium (1+)	(47719-70-0)	(N/A)	CCC1C2CC3C4C5(CC(C2C5O)[N+]3(C1O)CC(CN(CC)CC)O)C6=CC=CC=C6N4C
290	4.63	Imazamox	(114311-32-9)	77711	CC(C)C1(C(=O)NC(=N1)c2c(cc(cn2)COC)C(=O)O)C
291	4.63	Ethiofencarb-sulfone	(53380-23-7)	106713	CCS(=O)(=O)Cc1ccccc1OC(=O)NC
292	4.63	Phenazone (antipyrine)	(60-80-0)	2121	Cc1cc(=O)n(n1C)c2ccccc2
293	4.64	Chlordimeform	(6164-98-3)	10468746	CC1=C(C=CC(=C1)Cl)N=CN(C)C
294	4.64	Phentermine	(122-09-8)	4607	CC(C)(Cc1ccccc1)N
295	4.64	Nikethamide	(59-26-7)	5296	CCN(CC)C(=O)c1cccnc1
296	4.66	Heroin	(561-27-3)	(N/A)	CC(=O)OC1C=CC2C3CC4=C5C2(C1OC5=C(C=C4)OC(=O)C)CCN3C
297	4.66	Bunitrolol	(34915-68-9)	2379	CC(C)(C)NCC(COC1=CC=CC=C1C#N)O
298	4.66	MBDB	(103818-46-8)	111153	CCC(Cc1ccc2c(c1)OCO2)NC
299	4.66	Mepivacaine	(22801-44-1)	3922	Cc1cccc(c1NC(=O)C2CCCCN2C)C
300	4.66	Phthalylsulfathiazole	(85-73-4)	4641	c1ccc(c(c1)C(=O)Nc2ccc(cc2)S(=O)(=O)Nc3nccs3)C(=O)O
301	4.67	Clenbuterol	(50306-03-1)	2681	CC(C)(C)NCC(c1cc(c(c(c1)Cl)N)Cl)O
302	4.68	Benzoylecgonine	(519-09-5)	2250	CN1C2CCC1C(C(C2)OC(=O)c3ccccc3)C(=O)O
303	4.68	Methylhexanamine (1,3-DMAA)	(105-41-9)	7467	CCC(C)CC(C)N
304	4.68	Ketamine	(100477-72-3)	3689	CNC1(CCCCC1=O)c2ccccc2Cl

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305	4.68	Norfentanyl	(1609-66-1)	227671	CCC(=O)N(c1ccccc1)C2CCNCC2
306	4.69	Methoxyphenamine	(93-30-1)	3974	CC(Cc1ccccc1OC)NC
307	4.69	Carticaine (Articaine)	(23964-58-1)	29837	CCCNC(C)C(=O)Nc1c(csc1C(=O)OC)C
308	4.69	Prilocaine	(721-50-6)	4737	CCCNC(C)C(=O)Nc1ccccc1C
309	4.69	Sulfadoxine	(2447-57-6)	16218	COc1c(ncnc1OC)NS(=O)(=O)c2ccc(cc2)N
310	4.7	Ethiofencarb Sulfoxide	(53380-22-6)	2299504	CCS(=O)Cc1ccccc1OC(=O)NC
311	4.71	Laudanosine	(20412-65-1)	14792	CN1CCc2cc(c(cc2C1Cc3ccc(c(c3)OC)OC)OC)OC
312	4.72	Sulfabenzamide	(127-71-9)	5128	c1ccc(cc1)C(=O)NS(=O)(=O)c2ccc(cc2)N
313	4.73	BDB (1,3-Benzodioxolylbutanamine)	(107447-03-0)	114963	CCC(Cc1ccc2c(c1)OCO2)N
314	4.74	Rivastigmine	(123441-03-2)	4899	CCN(C)C(=O)Oc1cccc(c1)C(C)N(C)C
315	4.74	Meptazinol	(54340-58-8)	37469	CCC1(CCCCN(C1)C)c2cccc(c2)O
316	4.74	4-Methylaminophenazone	(519-98-2)	10173	Cc1c(c(=O)n(n1C)c2ccccc2)NC
317	4.74	4-MEC (4-Methylethcathinone)	(1225617-18-4)	25630091	CCNC(C)C(=O)c1ccc(cc1)C
318	4.74	Disopyramide	(3737-09-5)	3002	CC(C)N(CCC(c1ccccc1)(c2ccccn2)C(=O)N)C(C)C
319	4.75	Atrazine-desisopropyl (Deisopropylatrazine)	(1007-28-9)	13278	CC/N=c\1/[nH]c(=N)nc([nH]1)Cl
320	4.75	Quinmerac	(90717-03-6)	82847	Cc1cc2ccc(c(c2nc1)C(=O)O)Cl
321	4.76	Selegiline	(14611-51-9)	5007	CC(Cc1ccccc1)N(C)CC#C
322	4.76	Indanazoline	(40507-78-6)	59376	c1cc2c(c(c1)NC3=NCCN3)CCC2
323	4.76	Desvenlafaxine (O-Desmethylvenlafaxine)	(93413-62-8)	111300	CN(C)CC(c1ccc(cc1)O)C2(CCCCC2)O
324	4.76	Imidacloprid	(138261-41-3)	77934	c1cc(ncc1CN2CCN=C2N[N+](=O)[O-])Cl
325	4.76	Salicylamide	(65-45-2)	4963	c1ccc(c(c1)C(=N)O)O
326	4.76	Dikegulac	(18467-77-1)	65070	CC1(OCC2C(O1)C3C(O2)(OC(O3)(C)C)C(=O)O)C
327	4.76	4-MePPP	(28117-80-8)	4936084	Cc1ccc(cc1)C(=O)C(C)N2CCCC2
328	4.77	3,5-Diiodotyrosine	(66-02-4)	5946	c1c(cc(c(c1I)O)I)CC(C(=O)O)N
329	4.77	Tacrine	(321-64-2)	1859	c1ccc2c(c1)c(=N)c3c([nH]2)CCCC3
330	4.78	2-5-DMA (2,5-Dimethoxyamphetamine)	(2801-68-5)	56526	CC(Cc1cc(ccc1OC)OC)N
331	4.78	Acebutolol	(37517-30-9)	1901	CCCC(=O)Nc1ccc(c(c1)C(=O)C)OCC(CNC(C)C)O
332	4.78	7-Aminonitrazepam	(4928-02-3)	70996	c1ccc(cc1)C2=NCC(=Nc3c2cc(cc3)N)O

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333	4.79	5-APB (5-(2-aminopropyl)benzofuran)	(286834-80-8) as HCl	8012953	o2c1ccc(cc1cc2)CC(N)C
334	4.81	Tramadol	(27203-92-5)	5322	CN(C)CC1CCCCC1(c2cccc(c2)OC)O
335	4.81	Sulfaethidole	(94-19-9)	6913	CCc1nnc(s1)NS(=O)(=O)c2ccc(cc2)N
336	4.81	5-MeO-DIPT (5-Methoxy-N,N-diisopropyltryptamine)	(4021-34-5)	133247	CC(C)N(CCc1c[nH]c2c1cc(cc2)OC)C(C)C
337	4.81	6-APB [6-(2-Aminopropyl)benzofuran]	(286834-85-3)	7970110	o2c1cc(ccc1cc2)CC(N)C
338	4.83	N-Propylamphetamine	(51799-32-7)	93484	CCCNC(C)Cc1ccccc1
339	4.83	Flumetsulam	(98967-40-9)	82857	Cc1ccn2c(n1)nc(n2)S(=O)(=O)Nc3c(cccc3F)F
340	4.84	Levobunolol	(47141-42-4)	3777	CC(C)(C)NCC(COc1cccc2c1CCCC2=O)O
341	4.84	Naphazoline	(835-31-4)	4283	c1ccc2c(c1)cccc2CC3=NCCN3
342	4.84	Ornidazole	(16773-42-5)	26102	Cc1ncc(n1CC(CCl)O)[N+](=O)[O-]
343	4.86	Metoprolol	(37350-58-6)	4027	CC(C)NCC(COc1ccc(cc1)CCOC)O
344	4.86	Trimethobenzamide	(138-56-7)	5375	CN(C)CCOc1ccc(cc1)CNC(=O)c2cc(c(c(c2)OC)OC)OC
345	4.86	7-Aminoclonazepam	(4959-17-5)	163665	c1ccc(c(c1)C2=NCC(=Nc3c2cc(cc3)N)O)Cl
346	4.86	Amantadine	(768-94-5)	2045	C1C2CC3CC1CC(C2)(C3)N
347	4.86	Cocaine	(50-36-2)	2724	CN1C2CCC1C(C(C2)OC(=O)c3ccccc3)C(=O)OC
348	4.86	Methiocarb-sulfoxide	(2635-10-1)	16568	Cc1cc(cc(c1S(=O)C)C)OC(=O)NC
349	4.87	DiPT (N,N-Diisopropyltryptamine)	(14780-24-6)	25060	CC(C)N(CCc1c[nH]c2c1cccc2)C(C)C
350	4.87	Aceclidine	(827-61-2)	1902	CC(=O)OC1CN2CCC1CC2
351	4.88	Timolol	(26839-75-8)	5278	CC(C)(C)NCC(COc1c(nsn1)N2CCOCC2)O
352	4.88	4-AcO-DIPT (4-Acetoxy-diisopropyltryptamine)	(na)	29760176	CC(C)N(CCc1c[nH]c2c1ccc(c2)OC(=O)C)C(C)C
353	4.88	Minoxidil	(38304-91-5)	4056	[H]/N=c/1\nc(cc(n1O)N)N2CCCCC2
354	4.89	Molindone	(7416-34-4)	22342	CCc1c([nH]c2c1C(=O)C(CC2)CN3CCOCC3)C
355	4.89	5-MeO-DALT (N,N-Diallyl-5-methoxytryptamine)	(928822-98-4)	21106245	COc1ccc2c(c1)c(c[nH]2)CCN(CC=C)CC=C
356	4.89	Pentedrone	(879722-57-3)	26286729	CCCC(C(=O)c1ccccc1)NC
357	4.9	Clothiandin	(210880-92-5)	184723	C/N=C(/NCc1cnc(s1)Cl)\N[N+](=O)[O-]
358	4.9	Quinclorac	(84087-01-4)	82837	c1cc(c(c2c1cc(cn2)Cl)C(=O)O)Cl
359	4.91	Gibberellic acid	(77-06-5)	(N/A)	CC12C(C=CC3(C1C(C45C3CCC(C4)(C(=C)C5)O)C(=O)O)OC2=O)O
360	4.91	Harmane (Harman)	(486-84-0)	4444755	Cc1c2c(ccn1)c3ccccc3[nH]2

continued

361	4.92	Terazosin	(63590-64-7)	5208	COc1cc2c(cc1OC)nc(nc2N)N3CCN(CC3)C(=O)C4CCCO4
362	4.93	Befunolol	(39552-01-7)	2219	CC(C)NCC(COc1cccc2c1oc(c2)C(=O)C)O
363	4.93	Zopiclone	(43200-80-2)	5533	CN1CCN(CC1)C(=O)OC2c3c(nccn3)C(=O)N2c4ccc(cn4)Cl
364	4.93	Methoxetamine	(1239943-76-0)	24721792	CCNC1(CCCCC1=O)c2cccc(c2)OC
365	4.94	Cafaminol	(30924-31-3)	32824	Cn1c2c(nc1N(C)CCO)n(c(=O)n(c2=O)C)C
366	4.96	Methylphenidate	(113-45-1)	4015	COC(=O)C(c1ccccc1)C2CCCCN2
367	4.97	Dicamba	(1918-00-9)	2922	COc1c(ccc(c1C(=O)O)Cl)Cl
368	4.98	Ondansetron	(99614-02-5)	4434	Cc1nccn1CC2CCc3c(c4ccccc4n3C)C2=O
369	4.98	Pheniramine	(86-21-5)	4597	CN(C)CCC(c1ccccc1)c2ccccn2
370	4.98	Moclobemide	(71320-77-9)	4087	c1cc(ccc1C(=O)NCCN2CCOCC2)Cl
371	4.99	Aminoglutethimide	(125-84-8)	2060	CCC1(CCC(=O)NC1=O)c2ccc(cc2)N
372	4.99	Hydroxyquetiapine	(139079-39-3)	116771	c1ccc2c(c1)C(=Nc3ccc(cc3S2)O)N4CCN(CC4)CCOCCO
373	4.99	Pentylone (bk-MBDP)	(698963-77-8)	29786041	CCCC(C(=O)c1ccc2c(c1)OCO2)NC
374	5	Sulcotrione	(99105-77-8)	82858	CS(=O)(=O)c1ccc(c(c1)Cl)C(=O)C2C(=O)CCCC2=O
375	5.01	3-Methylnorfentanyl (N-Methylfentanyl)	(na)	459397	CCC(=O)N(c1ccccc1)C2CCN(CC2)C
376	5.01	5-Carboxybupranolol	(42242-69-3)	2284302	CC(C)(C)NCC(COc1cc(ccc1Cl)C(=O)O)O
377	5.02	Vamidothion	(2275-23-2)	486975	CC(C(=O)NC)SCCSP(=O)(OC)OC
378	5.03	Atrazine 2-Hydroxy (Hydroxyatrazine)	(2163-68-0)	15693	CC/N=c/1\[nH]/c(=N/C(C)C)/[nH]c(n1)O
379	5.04	Vincamine	(1617-90-9)	5466	CCC12CCCN3C1c4c(c5ccccc5n4C(C2)(C(=O)OC)O)CC3
380	5.04	2,4-Dimethylaniline (Metabolite Amitraz)	(95-68-1)	13869462	Cc1ccc(c(c1)C)N
381	5.04	alpha-ET (Alpha-Ethyltryptamine)	(2235-90-7)	8064	CCC(Cc1c[nH]c2c1cccc2)N
382	5.04	4-Methylbuphedrone	(1336911-98-8) as the HCl salt	(N/A)	CCC(C(=O)C1=CC=C(C=C1)C)NC
383	5.05	Brefedrone	(486459-03-4)	8597261	O=C(c1ccc(Br)cc1)C(NC)C
384	5.06	Remoxipride	(117591-79-4)	49611	CCN1CCCC1CNC(=O)c2c(ccc(c2OC)Br)OC
385	5.06	Fenethylline	(3736-08-1)	18398	CC(Cc1ccccc1)NCCn2cnc3c2c(=O)n(c(=O)n3C)C
386	5.06	Tiemonium (1+)	(6252-92-2)	5274	C[N+]1(CCOCC1)CCC(c2ccccc2)(c3cccs3)O
387	5.06	Chlortalidone	(77-36-1)	2631	c1ccc2c(c1)C(=O)NC2(c3ccc(c(c3)S(=O)(=O)N)Cl)O
388	5.06	2-Benzyltetronic acid	(3734-22-3)	21249083	c1ccc(cc1)CC2=C(COC2=O)O

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389	5.06	Fluconazole	(86386-73-4)	3248	c1cc(c(cc1F)F)C(Cn2cncn2)(Cn3cncn3)O
390	5.06	Harmaline	(304-21-2)	4444444	CC1=C2C(=c3ccc(cc3=N2)OC)CCN1
391	5.07	Senecionine-N-oxide	(13268-67-2)	3617285	CC=C1CC(C(C(=O)OCC2=CC[N+]3(C2C(CC3)OC1=O)[O-])(C)O)C
392	5.08	Yohimbine	(146-48-5)	2763	COC(=O)C1C(CCC2C1CC3c4c(c5ccccc5[nH]4)CCN3C2)O
393	5.08	Olanzapine	(132539-06-1)	10442212	Cc1cc2c(s1)Nc3ccccc3N=C2N4CCN(CC4)C
394	5.08	Ethylphenidate	(57413-43-1)	2338571	CCOC(=O)C(c1ccccc1)C2CCCCN2
395	5.08	Alpha-PVP (α-Pyrrolidinopentiophenone)	(14530-33-7)	9324063	CCCC(C(=O)C1=CC=CC=C1)N2CCCC2
396	5.08	Lacosamide	(175481-36-4)	10266281	CC(=O)NC(COC)C(=O)NCc1ccccc1
397	5.08	Sitagliptin	(486460-32-6)	9481667	Fc1cc(c(F)cc1F)CC(N)CC(=O)N3Cc2nnc(n2CC3)C(F)(F)F
398	5.09	Carbofuran-3-hydroxy	(16655-82-6)	26024	CC1(C(c2cccc(c2O1)OC(=O)NC)O)C
399	5.09	Tropisetron	(89565-68-4)	5393	CN1C2CCC1CC(C2)OC(=O)c3c[nH]c4c3cccc4
400	5.1	Tilidine	(51931-66-9)	29969	CCOC(=O)C1(CCC=CC1N(C)C)c2ccccc2
401	5.1	Mevinphos Peak 1 (Z or Trans isomer)	(298-01-1)	4938358	C/C(=C/C(=O)OC)/OP(=O)(OC)OC
402	5.11	Guaifenesin	(93-14-1)	3396	COc1ccccc1OCC(CO)O
403	5.12	Fenuron	(101-42-8)	7279	CN(C)/C(=N\c1ccccc1)/O
404	5.13	3-MBZP (1-benzyl-4-methylpiperazine)	(62226-74-8)	667589	CN1CCN(CC1)Cc2ccccc2
405	5.15	Aminocarb (Metacil)	(2032-59-9)	15416	Cc1cc(ccc1N(C)C)OC(=O)NC
406	5.16	Senkirkine	(2318-18-5)	4516153	C/C=C\1/CC(C(C(=O)OC/C/2=C/CN(CCC(C2=O)OC1=O)C)(C)O)C
407	5.16	Bambuterol	(81732-65-2)	49466	CC(C)(C)NCC(c1cc(cc(c1)OC(=O)N(C)C)OC(=O)N(C)C)O
408	5.16	Dioxacarb	(6988-21-2)	21901	CNC(=O)Oc1ccccc1C2OCCO2
409	5.16	m-CPP (meta-Chlorophenylpiperazine)	(6640-24-0)	1314	c1cc(cc(c1)Cl)N2CCNCC2
410	5.17	Tulobuterol	(41570-61-0)	5404	CC(C)(C)NCC(c1ccccc1Cl)O
411	5.17	Acetamiprid	(135410-20-7)	184719	C/C(=N\C#N)/N(C)Cc1ccc(nc1)Cl
412	5.18	Esmolol	(103598-03-4)	53916	CC(C)NCC(COc1ccc(cc1)CCC(=O)OC)O
413	5.18	Doxylamine	(469-21-6)	3050	CC(c1ccccc1)(c2ccccn2)OCCN(C)C
414	5.18	MDPV (Methylenedioxypyrovalerone)	(687603-66-3)	16788110	CCCC(C(=O)c1ccc2c(c1)OCO2)N3CCCC3
415	5.19	Fenpipramide	(77-01-0)	59016	c1ccc(cc1)C(CCN2CCCCC2)(c3ccccc3)C(=O)N
416	5.19	Ajmaline	(4360-12-7)	1989	CCC1C2CC3C4C5(CC(C2C5O)N3C1O)c6ccccc6N4C

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417	5.21	Remifentanyl	(132875-61-7)	54803	CCC(=O)N(c1ccccc1)C2(CCN(CC2)CCC(=O)OC)C(=O)OC
418	5.21	Difenzoquat (Ion 1+)	(49866-87-7)	36047	Cn1c(cc([n+]1C)c2ccccc2)c3ccccc3
419	5.21	Methocarbamol	(532-03-6)	3964	COc1ccccc1OCC(COC(=O)N)O
420	5.21	Melperone	(3575-80-2)	14646	CC1CCN(CC1)CCCC(=O)c2ccc(cc2)F
421	5.21	Primidone	(125-33-7)	4740	CCC1(C(=NCN=C1O)O)c2ccccc2
422	5.21	Aminophenazone (Amidopyrine)	(58-15-1)	5787	Cc1c(c(=O)n(n1C)c2ccccc2)N(C)C
423	5.22	Bentazone	(25057-89-0)	2238	CC(C)N1C(=O)c2ccccc2NS1(=O)=O
424	5.22	Pethidine	(57-42-1)	3918	CCOC(=O)C1(CCN(CC1)C)c2ccccc2
425	5.23	Dimethoate	(60-51-5)	2973	C/N=C(\CSP(=S)(OC)OC)/O
426	5.23	Trichlorfon (Dylox)	(52-68-6)	5644	COP(=O)(C(C(Cl)(Cl)Cl)O)OC
427	5.23	3-4-DMMC (3,4-Dimethylmethcathinone)	(1081772-06-6)	25630192	Cc1ccc(cc1C)C(=O)C(C)NC
428	5.23	Pentoxifylline	(6493-05-6)	4578	CC(=O)CCCCn1c(=O)c2c(ncn2C)n(c1=O)C
429	5.24	Bamifylline	(2016-63-9)	15401	CCN(CCn1c(nc2c1c(=O)n(c(=O)n2C)C)Cc3ccccc3)CCO
430	5.24	4-MTA (4-Methylthioamphetamine)	(14116-06-4)	133883	CC(Cc1ccc(cc1)SC)N
431	5.24	Celiprolol	(56980-93-9)	2563	CCN(CC)C(=O)Nc1ccc(c(c1)C(=O)C)OCC(CNC(C)(C)C)O
432	5.24	Metamitron	(41394-05-2)	35563	Cc1nnc(c(=O)n1N)c2ccccc2
433	5.26	DPT (N,N-Dipropyltryptamine)	(61-52-9)	5866	CCCN(CCC)CCc1c[nH]c2c1cccc2
434	5.28	Nortilidine	(38677-94-0)	459101	CCOC(=O)C1(CCC=CC1NC)c2ccccc2
435	5.28	Methiocarb-sulfone	(2179-25-1)	15729	Cc1cc(cc(c1S(=O)(=O)C)C)OC(=O)NC
436	5.28	Isoxsuprine	(395-28-8)	3651	CC(COc1ccccc1)NC(C)C(c2ccc(cc2)O)O
437	5.28	Lamotrigine	(84057-84-1)	3741	c1cc(c(c(c1)Cl)Cl)c2c([nH]c(=N)nn2)N
438	5.28	2C-C (4-chloro-2,5-dimethoxyphenethylamine)	(88441-14-9)	21106221	COc1cc(c(cc1Cl)OC)CCN
439	5.29	Quinidine	(56-54-2)	1036	COc1ccc2c(c1)c(ccn2)C(C3CC4CCN3CC4C=C)O
440	5.29	Toliprolol	(2933-94-0)	17050	Cc1cccc(c1)OCC(CNC(C)C)O
441	5.31	Mefenorex	(17243-57-1)	20467	CC(Cc1ccccc1)NCCCCl
442	5.31	Mirtazapine	(61337-67-5)	4060	CN1CCN2c3c(cccn3)Cc4ccccc4C2C1
443	5.31	Desmethyl-Mirtazapine	(61337-68-6)	8642761	n1cccc3c1N4C(c2c(cccc2)C3)CNCC4
444	5.32	Cinoxacin	(28657-80-9)	2660	CCn1c2cc3c(cc2c(=O)c(n1)C(=O)O)OCO3

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445	5.33	Paliperidone (9-OH-Risperidone)	(144598-75-4)	103109	Cc1c(c(=O)n2c(n1)C(CCC2)O)CCN3CCC(CC3)c4c5ccc(cc5on4)F
446	5.34	2 C-D (2-(2,5-Dimethoxy-4-methylphenyl)ethanamine)	(24333-19-5)	119559	Cc1cc(c(cc1OC)CCN)OC
447	5.34	Prazosin	(19216-56-9)	4724	COc1cc2c(cc1OC)nc([nH]c2=N)N3CCN(CC3)C(=O)c4ccco4
448	5.34	Oxomemazine	(3689-50-7)	18281	CC(CN1c2ccccc2S(=O)(=O)c3c1cccc3)CN(C)C
449	5.34	Chloridazon	(1698-60-8)	14790	c1ccc(cc1)n2c(=O)c(c(cn2)N)Cl
450	5.34	Harmine	(442-51-3)	4444445	Cc1c2c(ccn1)c3ccc(cc3[nH]2)OC
451	5.36	Melatonin	(73-31-4)	872	C/C(=N\CCc1c[nH]c2c1cc(cc2)OC)/O
452	5.36	Carbutamide	(339-43-5)	9189	CCCC/N=C(/NS(=O)(=O)c1ccc(cc1)N)\O
453	5.36	Schradan	(152-16-9)	8685	CN(C)P(=O)(N(C)C)OP(=O)(N(C)C)N(C)C
454	5.37	Indoramin	(26844-12-2)	31014	c1ccc(cc1)C(=O)NC2CCN(CC2)CCc3c[nH]c4c3cccc4
455	5.39	Ropivacaine	(84057-95-4)	21513357	CCCC1CCCCN1C(=O)Nc2c(cccc2C)C
456	5.41	7-Aminoflunitrazepam	(34084-50-9)	83325	CN1c2ccc(cc2C(=NCC1=O)c3ccccc3F)N
457	5.42	Nomifensine	(24526-64-5)	4371	CN1Cc2c(cccc2N)C(C1)c3ccccc3
458	5.43	LSD (Lysergic acid diethylamide)	(50-37-3)	3843	CCN(CC)C(=O)C1CN(C2Cc3c[nH]c4c3c(ccc4)C2=C1)C
459	5.43	Fluroxypyr	(69377-81-7)	45757	C(C(=O)O)Oc1c(c(c(c(n1)F)Cl)N)Cl
460	5.44	Pitofenone	(54063-52-4)	108096	COC(=O)c1ccccc1C(=O)c2ccc(cc2)OCCN3CCCCC3
461	5.46	Pentazocine	(359-83-1)	4574	CC1C2Cc3ccc(cc3C1(CCN2CC=C(C)C)C)O
462	5.46	Cocaethylene	(529-38-4)	2723	CCOC(=O)C1C2CCC(N2C)CC1OC(=O)c3ccccc3
463	5.46	Carbendazim	(10605-21-7)	23741	CO/C(=N/c1[nH]c2ccccc2n1)/O
464	5.47	4-Benzamidosalicylic acid	(13898-58-3)	10267	c1ccc(cc1)C(=O)Nc2ccc(c(c2)O)C(=O)O
465	5.49	Carazolol	(57775-29-8)	64783	CC(C)NCC(COc1cccc2c1c3ccccc3[nH]2)O
466	5.49	Dibenzepin	(4498-32-2)	9048	CN1c2ccccc2C(=O)N(c3c1cccc3)CCN(C)C
467	5.49	Quinine	(130-95-0)	1036	COc1ccc2c(c1)c(ccn2)C(C3CC4CCN3CC4C=C)O
468	5.54	Pyritinol	(1098-97-1)	13561	Cc1c(c(c(cn1)CSSCc2cnc(c(c2CO)O)C)CO)O
469	5.54	Ketotifen	(34580-13-7)	3695	CN1CCC(=C2c3ccccc3CC(=O)c4c2ccs4)CC1
470	5.56	Prolintane	(493-92-5)	13930	CCCC(Cc1ccccc1)N2CCCC2
471	5.56	2C-B (2-(4-Bromo-2,5-dimethoxyphenyl)ethanamine)	(66142-81-2)	88978	COc1cc(c(cc1Br)OC)CCN
472	5.57	5-IAI (2-Amino-5-iodoindane)	(132367-76-1)	116224	c1cc2c(cc1I)CC(C2)N

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473	5.58	Benazolin	(3813-05-6)	18521	c1cc2c(c(c1)Cl)n(c(=O)s2)CC(=O)O
474	5.59	Iso-LSD	(2126-78-5)	3843	CCN(CC)C(=O)C1CN(C2Cc3c[nH]c4c3c(ccc4)C2=C1)C
475	5.59	Mexiletine	(31828-71-4)	4034	Cc1cccc(c1OCC(C)N)C
476	5.59	Oxycarboxin	(5259-88-1)	20048	CC1=C(S(=O)(=O)CCO1)/C(=N\c2ccccc2)/O
477	5.6	Cymoxanil	(57966-95-7)	4514714	CCNC(=O)NC(=O)/C(=N\OC)/C#N
478	5.6	Imazethapyr	(81335-77-5)	49447	CCc1cc(c(nc1)C2=NC(C(=O)N2)(C)C(C)C)C(=O)O
479	5.61	Oxprenolol	(6452-71-7)	4470	CC(C)NCC(COc1ccccc1OCC=C)O
480	5.61	Guanabenz	(5051-62-7)	3397	c1cc(c(c(c1)Cl)C=NN=C(N)N)Cl
481	5.62	Thiacloprid	(111988-49-9)	103099	c1cc(ncc1CN\2CCS/C2=N\C#N)Cl
482	5.63	Prohexadione	(88805-35-0)	160745	CCC(=O)C1C(=O)CC(CC1=O)C(=O)O
483	5.66	Dapiprazole	(72822-12-9)	2298190	Cc1ccccc1N2CCN(CC2)CCc3nnc4n3CCCC4
484	5.66	Olsalazine	(15772-48-2)	10642377	C1=CC(=C(C=C1/N=N/C2=CC(=C(C=C2)O)C(=O)O)C(=O)O)O
485	5.67	Acetaminodantrolene (Acetamide, N-[4-[5-[(E)-[(2,4-dioxo-1-imidazolidinyl)imino]methyl]-2-furanyl]phenyl]-) F490	(41515-09-7)	7845047	O=C(Nc3ccc(c2oc(\C=N\N1C(=O)NC(=O)C1)cc2)cc3)C
486	5.67	Nefopam	(13669-70-0)	4295	CN1CCOC(c2ccccc2C1)c3ccccc3
487	5.67	DOC (4-chloro-2,5-dimethoxyamphetamine)	(123431-31-2)	472008	CC(Cc1cc(c(cc1OC)Cl)OC)N
488	5.68	Ethenzamide	(938-73-8)	3167	CCOc1ccccc1C(=O)N
489	5.69	Risperidone	(106266-06-2)	4895	Cc1c(c(=O)n2c(n1)CCCC2)CCN3CCC(CC3)c4c5ccc(cc5on4)F
490	5.69	Chlorothiamid	(1918-13-4)	2016563	c1cc(c(c(c1)Cl)C(=N)S)Cl
491	5.71	Sulfaquinoxaline	(59-40-5)	5147	c1ccc2c(c1)[nH]/c(=N/S(=O)(=O)c3ccc(cc3)N)/cn2
492	5.72	Ramifenazone	(3615-24-5)	4861	Cc1c(c(=O)n(n1C)c2ccccc2)NC(C)C
493	5.72	Relaspium (1+) [Trospium]	(47608-32-2)	5394	c1ccc(cc1)C(c2ccccc2)(C(=O)OC3CC4CCC(C3)[N+]45CCCC5)O
494	5.73	Milnacipran	(92623-85-3)	9797657	CCN(CC)C(=O)C1(CC1CN)C2=CC=CC=C2
495	5.73	Atrazine-desethyl (Deethylatrazine)	(6190-65-4)	21157	CC(C)/N=c\1/[nH]c(=N)nc([nH]1)Cl
496	5.74	Levopropylhexedrine	(6192-97-8)	7277	CC(CC1CCCCC1)NC
497	5.74	Isocarbamide (Azolamide)	(30979-48-7)	32837	CC(C)CNC(=O)N1CCNC1=O
498	5.74	Labetalol	(36894-69-6)	3734	CC(CCc1ccccc1)NCC(c2ccc(c(c2)C(=O)N)O)O
499	5.74	Phencyclidine	(77-10-1)	6224	c1ccc(cc1)C2(CCCCC2)N3CCCCC3

continued

500	5.75	Mevinphos Peak 2 (E-or Cis isomer)	(7786-34-7)	4511751	O=P(OC)(OC)O\C(=C/C(=O)OC)C
501	5.75	Florasulam	(145701-23-1)	9875220	COc1ncc(c2n1nc(n2)S(=O)(=O)Nc3c(cccc3F)F)F
502	5.76	Bupropion	(34911-55-2)	431	CC(C(=O)c1cccc(c1)Cl)NC(C)(C)C
503	5.76	Oxypertine	(153-87-7)	4479	Cc1c(c2cc(c(cc2[nH]1)OC)OC)CCN3CCN(CC3)c4ccccc4
504	5.76	TFMPP	(15532-75-9)	4145	c1cc(cc(c1)N2CCNCC2)C(F)(F)F
505	5.77	Azithromycin	(83905-01-5)	2182	CCC1C(C(C(N(CC(CC(C(C(C(C(C(=O)O1)C)OC2CC(C(C(O2)C)O)(C)OC)C)OC3C(C(CC(O3)C)N(C)C)O)(C)O)C)C)O)(C)O
506	5.78	Pipradrol	(467-60-7)	9681	c1ccc(cc1)C(c2ccccc2)(C3CCCCN3)O
507	5.78	Buspirone	(36505-84-7)	2383	c1cnc(nc1)N2CCN(CC2)CCCCN3C(=O)CC4(CCCC4)CC3=O
508	5.8	Zolpidem	(82626-48-0)	5530	Cc1ccc(cc1)c2c(n3cc(ccc3n2)C)CC(=O)N(C)C
509	5.81	Donepezil	(120014-06-4)	3040	COc1cc2c(cc1OC)C(=O)C(C2)CC3CCN(CC3)Cc4ccccc4
510	5.83	DOM (dl-2,5-Dimethoxy-4-methylamphetamine)	(15588-95-1)	77462	Cc1cc(c(cc1OC)CC(C)N)OC
511	5.84	Clidinium (1+)	(7020-55-5)	2682	C[N+]12CCC(CC1)C(C2)OC(=O)C(c3ccccc3)(c4ccccc4)O
512	5.86	Pyribenzamine (Tripelennamine / Azaron)	(91-81-6)	5385	CN(C)CCN(Cc1ccccc1)c2ccccn2
513	5.86	Phentolamine	(50-60-2)	5571	Cc1ccc(cc1)N(CC2=NCCN2)c3cccc(c3)O
514	5.88	2CT-2 (2,5-Dimethoxy-4-Ethylthiophenethylamine)	(207740-24-7)	16787961	CCSc1cc(c(cc1OC)CCN)OC
515	5.89	Mepyramine (Pyrilamine)	(91-84-9)	4818	CN(C)CCN(Cc1ccc(cc1)OC)c2ccccn2
516	5.89	Pirimicarb-desmethyl	(30614-22-3)	84084	Cc1c(nc(nc1OC(=O)N(C)C)NC)C
517	5.91	Bisoprolol	(66722-44-9)	2312	CC(C)NCC(COc1ccc(cc1)COCCOC(C)C)O
518	5.92	Lisuride	(18016-80-3)	3801	CCN(CC)C(=O)NC1CN(C2Cc3c[nH]c4c3c(ccc4)C2=C1)C
519	5.92	Pyrimethamine	(58-14-0)	4819	CCc1c(c([nH]c(=N)n1)N)c2ccc(cc2)Cl
520	5.93	DOB (2,5-Dimethoxy-4-bromoamphetamine)	(64638-07-9)	55902	CC(Cc1cc(c(cc1OC)Br)OC)N
521	5.93	Phenacetin	(62-44-2)	4590	CCOc1ccc(cc1)/N=C(\C)/O
522	5.93	Tricyclazole	(41814-78-2)	35726	Cc1cccc2c1n3cnnc3s2
523	5.94	Meprobamate	(57-53-4)	3924	CCCC(C)(COC(=O)N)COC(=O)N
524	5.94	Oxybuprocaine	(99-43-4)	4472	CCCCOc1cc(ccc1N)C(=O)OCCN(CC)CC
525	5.94	Chlormezanone	(80-77-3)	2616	CN1C(S(=O)(=O)CCC1=O)c2ccc(cc2)Cl
526	5.94	10-Hydroxycarbamazepine	(29331-92-8)	102704	c1ccc2c(c1)CC(c3ccccc3N2C(=O)N)O
527	5.96	Norbuprenorphine	(78715-23-8)	(N/A)	CC(C)(C)C(C)(C1CC23CCC1(C4C25CCNC3CC6=C5C(=C(C=C6)O)O4)OC)O

continued

528	5.96	Benperidol	(2062-84-2)	15521	c1ccc2c(c1)[nH]c(=O)n2C3CCN(CC3)CCCC(=O)c4ccc(cc4)F
529	5.98	Levomepromazinesulfoxide	(7052-08-6)	145102	CC(CN1c2ccccc2S(=O)c3c1cc(cc3)OC)CN(C)C
530	5.99	Fenfluramine	(458-24-2)	3220	CCNC(C)Cc1cccc(c1)C(F)(F)F
531	5.99	Azatadine	(3964-81-6)	18709	CN1CCC(=C2c3ccccc3CCc4c2ncccc4)CC1
532	6	Isoaminile Peak 1 & 2	(77-51-0)	6236	CC(C)C(CC(C)N(C)C)(C#N)c1ccccc1
533	6.01	Clopamide	(636-54-4)	2702	CC1CCCC(N1NC(=O)c2ccc(c(c2)S(=O)(=O)N)Cl)C
534	6.01	Pirmenol	(68252-19-7)	4687	CC1CCCC(N1CCCC(c2ccccc2)(c3ccccn3)O)C
535	6.01	Fentanyl	(437-38-7)	3228	CCC(=O)N(c1ccccc1)C2CCN(CC2)CCc3ccccc3
536	6.03	Pyrovalerone	(3563-49-3)	13733	CCCC(C(=O)c1ccc(cc1)C)N2CCCC2
537	6.03	Tropicamide	(1508-75-4)	5391	CCN(Cc1ccncc1)C(=O)C(CO)c2ccccc2
538	6.04	Ambroxol	(18683-91-5)	2047	c1c(cc(c(c1CNC2CCC(CC2)O)N)Br)Br
539	6.04	Dexfenfluramine	(3239-44-9)	3220	CCNC(C)Cc1cccc(c1)C(F)(F)F
540	6.04	Carbamazepine-10,11-epoxide (10,11-Dihydro-10,11-epoxycarbamazepine)	(36507-30-9)	2458	c1ccc2c(c1)C3C(O3)c4ccccc4N2C(=O)N
541	6.04	Flumazenil	(78755-81-4)	3256	CCOC(=O)c1c2n(cn1)-c3ccc(cc3C(=O)N(C2)C)F
542	6.06	2C-I (2-(4-Iodo-2,5-dimethoxyphenyl)ethanamine)	(69587-11-7)	8442670	COC1=CC(=C(C=C1CCN)OC)I
543	6.07	Butocarboxim	(34681-10-2)	33840	CC(C(=NOC(=O)NC)C)SC
544	6.07	Alypin (Amydricaine)	(963-07-5)	11921	CCC(CN(C)C)(CN(C)C)OC(=O)c1ccccc1
545	6.08	Phenazocine	(127-35-5)	14031	CC1C2Cc3ccc(cc3C1(CCN2CCc4ccccc4)C)O
546	6.08	Metolazone	(17560-51-9)	4026	Cc1ccccc1N2C(Nc3cc(c(cc3C2=O)S(=O)(=O)N)Cl)C
547	6.08	Benactyzine	(302-40-9)	8966	CCN(CC)CCOC(=O)C(c1ccccc1)(c2ccccc2)O
548	6.08	Carbinoxamine	(486-16-8)	2466	CN(C)CCOC(c1ccc(cc1)Cl)c2ccccn2
549	6.08	Fencamfamine (norcamphane)	(2240-14-4)	13922	CCNC1C2CCC(C2)C1c3ccccc3
550	6.09	Alpha-methylfentanyl	(79704-88-4)	56081	CCC(=O)N(c1ccccc1)C2CCN(CC2)C(C)Cc3ccccc3
551	6.1	Imazaquin	(81335-37-7)	49446	CC(C)C1(C(=O)NC(=N1)c2c(cc3ccccc3n2)C(=O)O)C
552	6.11	Venlafaxine	(93413-69-5)	5454	CN(C)CC(c1ccc(cc1)OC)C2(CCCCC2)O
553	6.11	Pipamperone	(1893-33-0)	4664	c1cc(ccc1C(=O)CCCN2CCC(CC2)(C(=O)N)N3CCCCC3)F
554	6.12	Fedrilate	(23271-74-1)	29485	CC(CCN1CCOCC1)OC(=O)C2(CCOCC2)c3ccccc3

continued

555	6.13	Para-fluorofentanyl	(90736-23-5)	56096	CCC(=O)N(c1ccc(cc1)F)C2CCN(CC2)CCc3ccccc3
556	6.14	Oxyfedrine	(15687-41-9)	25548	CC(C(c1ccccc1)O)NCCC(=O)c2cccc(c2)OC
557	6.14	Butizide	(2043-38-1)	15442	CC(C)CC1Nc2cc(c(cc2S(=O)(=O)N1)S(=O)(=O)N)Cl
558	6.16	Cyclopentolate	(512-15-2)	2802	CN(C)CCOC(=O)C(c1ccccc1)C2(CCCC2)O
559	6.17	Metoxuron	(19937-59-8)	27749	CN(C)C(=O)Nc1ccc(c(c1)Cl)OC
560	6.17	Bromisoval (Bromural)	(496-67-3)	2353	CC(C)C(C(=O)NC(=O)N)Br
561	6.17	Aldicarb	(116-06-3)	7844539	CC(C)(/C=N/OC(=O)NC)SC
562	6.18	Bupivacaine	(38396-39-3)	2380	CCCCN1CCCCC1C(=O)Nc2c(cccc2C)C
563	6.18	Droperidol	(548-73-2)	3056	c1ccc2c(c1)nc(n2C3=CCN(CC3)CCCC(=O)c4ccc(cc4)F)O
564	6.18	Norvenlafaxine	(149289-30-5)	2741972	CNCC(c1ccc(cc1)OC)C2(CCCCC2)O
565	6.19	Pergolide	(66104-22-1)	4583	CCCN1CC(CC2C1Cc3c[nH]c4c3c2ccc4)CSC
566	6.19	Azacyclonol	(115-46-8)	14952	c1ccc(cc1)C(c2ccccc2)(C3CCNCC3)O
567	6.19	Cythioate	(115-93-5)	7992	COP(=S)(OC)Oc1ccc(cc1)S(=O)(=O)N
568	6.2	Crimidine	(535-89-7)	10356	Cc1cc(nc(n1)Cl)N(C)C
569	6.2	Thiabendazole	(148-79-8)	5237	c1ccc2c(c1)[nH]c(n2)c3cscn3
570	6.21	Moxisylyte	(54-32-0)	4110	Cc1cc(c(cc1OC(=O)C)C(C)C)OCCN(C)C
571	6.21	Metyrapone	(54-36-4)	4030	CC(C)(c1cccnc1)C(=O)c2cccnc2
572	6.22	Diflufenzopyr	(109293-97-2)	4816775	C/C(=N\NC(=O)NC1=CC(=CC(=C1)F)F)/C2=C(C=CC=N2)C(=O)O
573	6.23	Doxapram	(309-29-5)	3044	CCN1CC(C(C1=O)(c2ccccc2)c3ccccc3)CCN4CCOCC4
574	6.24	Propipocaine	(3670-68-6)	64027	CCCOc1ccc(cc1)C(=O)CCN2CCCCC2
575	6.24	Thifensulfuron-methyl	(79277-27-3)	66325	Cc1n/c(=N/C(=N/S(=O)(=O)c2ccsc2C(=O)OC)/O)/[nH]c(n1)OC
576	6.25	Chlorpheniramine	(132-22-9)	2624	CN(C)CCC(c1ccc(cc1)Cl)c2ccccn2
577	6.26	Dimethylanilin (N,N-)	(121-69-7)	924	CN(C)c1ccccc1
578	6.33	Phosphamidon (Dimecron)	(13171-21-6)	2297538	CCN(CC)C(=O)/C(=C(\C)/OP(=O)(OC)OC)/Cl
579	6.27	Benzocaine	(94-09-7)	13854242	CCOC(=O)c1ccc(cc1)N
580	6.27	Papaverine	(58-74-2)	4518	COc1ccc(cc1OC)Cc2c3cc(c(cc3ccn2)OC)OC
581	6.27	Clibucaine	(15302-10-0)	59054	CC(CC(=O)Nc1ccc(cc1Cl)Cl)N2CCCCC2
582	6.27	Bucetin (Bucetalon)	(1083-57-4)	13507	CCOc1ccc(cc1)NC(=O)CC(C)O

continued

583	6.27	Beclamide	(501-68-8)	9962	c1ccc(cc1)C/N=C(/CCCl)\O
584	6.28	Colchicine	(64-86-8)	2731	CC(=O)NC1CCc2cc(c(c(c2-c3c1cc(=O)c(cc3)OC)OC)OC)OC)OC
585	6.28	Metipranolol	(22664-55-7)	29193	Cc1cc(c(c(c1OC(=O)C)C)C)OCC(CNC(C)C)O
586	6.28	Triprolidine	(486-12-4)	4445597	Cc1ccc(cc1)/C(=C\CN2CCCC2)/c3ccccn3
587	6.29	Oxadixyl	(77732-09-3)	48518	Cc1cccc(c1N(C(=O)COC)N2CCOC2=O)C
588	6.3	Desoxypipradol	(519-74-4)	141045	c1ccc(cc1)C(c2ccccc2)C3CCCCN3
589	6.31	Metsulfuron-methyl	(74223-64-6)	47883	Cc1n/c(=N\C(=N/S(=O)(=O)c2ccccc2C(=O)OC)\O)/[nH]c(n1)OC
590	6.31	Clobutinol (Pertoxil)	(14860-49-2)	25085	CC(CN(C)C)C(C)(Cc1ccc(cc1)Cl)O
591	6.32	Clobenzepam	(1159-93-9)	13752	CN(C)CCN1c2ccc(cc2Nc3ccccc3C1=O)Cl
592	6.32	Methaphenilene	(493-78-7)	9884	CN(C)CCN(Cc1cccs1)c2ccccc2
593	6.33	Letrozole	(112809-51-5)	3765	c1cc(ccc1C#N)C(c2ccc(cc2)C#N)n3cncn3
594	6.36	Cinosulfuron	(94593-91-6)	83438	COCCOc1ccccc1S(=O)(=O)NC(=O)Nc2nc(nc(n2)OC)OC
595	6.36	Protionamide	(14222-60-7)	579891	CCCc1cc(ccn1)C(=S)N
596	6.36	Paraoxon-methyl	(950-35-6)	13114	COP(=O)(OC)Oc1ccc(cc1)[N+](=O)[O-]
597	6.37	Allidochlor	(93-71-0)	6890	C=CCN(CC=C)C(=O)CCl
598	6.38	Triazamate	(112143-82-5)	77849	CCOC(=O)CSc1nc(nn1C(=O)N(C)C)C(C)(C)C
599	6.38	EDDP (2E)-2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine)	(30223-73-5)	4509491	C/C=C/1\C(CC(N1C)C)(c2ccccc2)c3ccccc3
600	6.41	Deacetyldiltiazem	(42399-40-6)	9243880	CN(C)CCN1C2=CC=CC=C2SC(C(C1=O)O)C3=CC=C(C=C3)OC
601	6.41	Antazoline	(91-75-8)	2115	c1ccc(cc1)CN(CC2=NCCN2)c3ccccc3
602	6.41	Fluanisone	(1480-19-9)	14410	COc1ccccc1N2CCN(CC2)CCCC(=O)c3ccc(cc3)F
603	6.43	Tetracaine	(94-24-6)	5218	CCCCNc1ccc(cc1)C(=O)OCCN(C)C
604	6.44	Flecainide	(54143-55-4)	3239	c1cc(c(cc1OCC(F)(F)F)C(=O)NCC2CCCCN2)OCC(F)(F)F
605	6.46	Oxcarbazepine	(28721-07-5)	31608	c1ccc2c(c1)CC(=O)c3ccccc3N2C(=O)N
606	6.47	Isothipendyl	(482-15-5)	3649	CC(CN1c2ccccc2Sc3c1nccc3)N(C)C
607	6.48	Trazodone	(19794-93-5)	5332	c1ccn2c(c1)nn(c2=O)CCCN3CCN(CC3)c4cccc(c4)Cl
608	6.48	Citalopram	(59729-33-8)	2669	CN(C)CCCC1(c2ccc(cc2CO1)C#N)c3ccc(cc3)F
609	6.48	Propranolol	(525-66-6)	4777	CC(C)NCC(COc1cccc2c1cccc2)O

continued

610	6.48	Fuberidazole	(3878-19-1)	18609	c1ccc2c(c1)[nH]c(n2)c3ccco3
611	6.48	2C-E (2,5-Dimethoxy-4-ethylphenethylamine)	(71539-34-9)	21106222	CCc1cc(c(cc1OC)CCN)OC
612	6.49	TEPP (Tetraethyl pyrophosphate)	(107-49-3)	7585	CCOP(=O)(OCC)OP(=O)(OCC)OCC
613	6.51	Dextromethorphan	(125-71-3)	2901	CN1CCC23CCCCC2C1Cc4c3cc(cc4)OC
614	6.51	Norcitalopram	(144025-14-9)	142424	CNCCCC1(c2ccc(cc2CO1)C#N)c3ccc(cc3)F
615	6.52	Benzoctamine	(17243-39-9)	26444	CNCC12CCC(c3c1cccc3)c4c2cccc4
616	6.52	Oxasulfuron	(144651-06-9)	77958	Cc1cc(nc(n1)NC(=O)NS(=O)(=O)c2ccccc2C(=O)OC3COC3)C
617	6.54	Flurazepam	(17617-23-1)	3276	CCN(CC)CCN1c2ccc(cc2C(=NCC1=O)c3ccccc3F)Cl
618	6.54	Azapropazone	(13539-59-8)	24310	CCCC1C(=O)N2c3cc(ccc3N=C(N2C1=O)N(C)C)C
619	6.54	Chlorsulfuron	(64902-72-3)	43209	Cc1nc(nc(n1)OC)NC(=O)NS(=O)(=O)c2ccccc2Cl
620	6.55	Cyanazine	(21725-46-2)	28552	CC/N=c/1\[nH]c(nc(n1)Cl)NC(C)(C)C#N
621	6.56	Brompheniramine	(86-22-6)	6573	CN(C)CCC(c1ccc(cc1)Br)c2ccccn2
622	6.56	Mefruside	(7195-27-9)	3907	CC1(CCCO1)CN(C)S(=O)(=O)c2ccc(c(c2)S(=O)(=O)N)Cl
623	6.58	Carbetamide	(16118-49-3)	25761	CCNC(=O)C(C)OC(=O)Nc1ccccc1
624	6.58	Talinolol	(57460-41-0)	62014	CC(C)(C)NCC(COc1ccc(cc1)NC(=O)NC2CCCCC2)O
625	6.59	Metolcarb	(1129-41-5)	13684	Cc1cccc(c1)OC(=O)NC
626	6.59	Moperone	(1050-79-9)	4100	Cc1ccc(cc1)C2(CCN(CC2)CCCC(=O)c3ccc(cc3)F)O
627	6.6	Triasulfuron	(82097-50-5)	66025	Cc1nc(nc(n1)OC)NC(=O)NS(=O)(=O)c2ccccc2OCCCl
628	6.61	Indapamide	(26807-65-8)	3574	CC1Cc2ccccc2N1NC(=O)c3ccc(c(c3)S(=O)(=O)N)Cl
629	6.61	Sulfasalazine	(599-79-1)	10481900	C1=CC=NC(=C1)NS(=O)(=O)C2=CC=C(C=C2)N=NC3=CC(=C(C=C3)O)C(=O)O
630	6.61	Zaleplon	(151319-34-5)	5517	CCN(c1cccc(c1)c2ccnc3n2ncc3C#N)C(=O)C
631	6.62	Nicosulfuron	(111991-09-4)	66024	CN(C)C(=O)c1cccnc1S(=O)(=O)N/C(=N/c2nc(cc(n2)OC)OC)/O
632	6.63	Diphenhydramine	(58-73-1)	2989	CN(C)CCOC(c1ccccc1)c2ccccc2
633	6.63	Dimethylphthalate	(131-11-3)	13837329	COC(=O)c1ccccc1C(=O)OC
634	6.64	Tromantadine	(53783-83-8)	57947	CN(C)CCOCC(=O)NC12CC3CC(C1)CC(C3)C2
635	6.64	Trapidil	(15421-84-8)	5330	CCN(CC)c1cc(nc2n1ncn2)C
636	6.64	Thioridazine-5-sulfoxide	(7776-05-8)	22902	CN1CCCCC1CCN2c3ccccc3S(=O)c4c2cc(cc4)SC
637	6.66	Raubasine (Ajmalicine)	(483-04-5)	220386	CC1C2CN3CCc4c5ccccc5[nH]c4C3CC2C(=CO1)C(=O)OC

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638	6.66	Acrivastine	(87848-99-5)	4447574	Cc1ccc(cc1)/C(=C\CN2CCCC2)/c3cccc(n3)/C=C/C(=O)O
639	6.67	N-(2,4-Dimethylphenyl)formamide (Amitraz Metabolite)	(60397-77-5)	83385	Cc1ccc(c(c1)C)NC=O
640	6.67	Enoximon	(77671-31-9)	48492	Cc1c([nH]c(=O)[nH]1)C(=O)c2ccc(cc2)SC
641	6.67	Torasemide	(56211-40-6)	38123	Cc1cccc(c1)Nc2ccncc2S(=O)(=O)NC(=O)NC(C)C
642	6.68	Betaxolol	(63659-18-7)	2279	CC(C)NCC(COc1ccc(cc1)CCOCC2CC2)O
643	6.69	Clomethiazole	(533-45-9)	10327	Cc1c(scn1)CCCl
644	6.69	2C-T-4 (2,5-dimethoxy-4-isopropylthiophenethylamine)	(207740-25-8)	21106232	CC(C)Sc1cc(c(cc1OC)CCN)OC
645	6.71	Alprenolol	(13655-52-2)	2035	CC(C)NCC(COc1ccccc1CC=C)O
646	6.72	Budipine	(57982-78-2)	62021	CC(C)(C)N1CCC(CC1)(c2ccccc2)c3ccccc3
647	6.73	Cisapride	(81098-60-4)	2667	COc1cc(c(cc1C(=O)NC2CCN(CC2OC)CCCOc3ccc(cc3)F)Cl)N
648	6.74	Mesoridazine	(5588-33-0)	3936	CN1CCCCC1CCN2c3ccccc3Sc4c2cc(cc4)S(=O)C
649	6.75	Naptalam (N-1-Naphthylphthalamicacid)	(132-66-1)	8275	c1ccc2c(c1)cccc2NC(=O)c3ccccc3C(=O)O
650	6.76	Tertatolol	(34784-64-0)	33875	CC(C)(C)NCC(COc1cccc2c1SCCC2)O
651	6.76	Modafinil	(68693-11-8)	4088	c1ccc(cc1)C(c2ccccc2)S(=O)CC(=O)N
652	6.77	Amidosulfuron	(120923-37-7)	82874	CN(S(=O)(=O)C)S(=O)(=O)NC(=O)Nc1nc(cc(n1)OC)OC
653	6.79	Mebeverine	(3625-06-7)	3891	CCN(CCCCOC(=O)c1ccc(c(c1)OC)OC)C(C)Cc2ccc(cc2)OC
654	6.8	Oxfendazole	(53716-50-0)	37316	COC(=O)Nc1[nH]c2ccc(cc2n1)S(=O)c3ccccc3
655	6.8	Thiram [Tetramethylthiuramdisulfide]	(137-26-8)	5256	CN(C)C(=S)SSC(=S)N(C)C
656	6.81	Benzthiazide	(91-33-8)	2253	c1ccc(cc1)CSCC2=Nc3cc(c(cc3S(=O)(=O)N2)S(=O)(=O)N)Cl
657	6.82	Fenpiprane	(3540-95-2)	171191	c1ccc(cc1)C(CCN2CCCCC2)c3ccccc3
658	6.82	Bendroflumethiazide	(73-48-3)	2225	c1ccc(cc1)CC2Nc3cc(c(cc3S(=O)(=O)N2)S(=O)(=O)N)C(F)(F)F
659	6.82	Primaquine	(90-34-6)	4739	CC(CCCN)Nc1cc(cc2c1nccc2)OC
660	6.83	Enalapril	(75847-73-3)	3109	CCOC(=O)C(CCc1ccccc1)NC(C)C(=O)N2CCCC2C(=O)O
661	6.84	Piroxicam	(36322-90-4)	10442653	CN1C(=C(c2ccccc2S1(=O)=O)O)/C(=N/c3ccccn3)/O
662	6.85	Mefluidide	(53780-34-0)	37346	Cc1cc(c(cc1NC(=O)C)NS(=O)(=O)C(F)(F)F)C
663	6.86	Dimetindene	(5636-83-9)	20541	CC(c1ccccn1)C2=C(Cc3c2cccc3)CCN(C)C
664	6.86	Normianserine	(71936-92-0)	103070	c1ccc2c(c1)Cc3ccccc3N4C2CNCC4
665	6.88	DOET	(22004-32-6)	25499	CCc1cc(c(cc1OC)CC(C)N)OC

continued

666	6.88	Doxepin	(1668-19-5)	3046	CN(C)CC/C=C\1/c2ccccc2COc3c1cccc3
667	6.89	Entacapon	(130929-57-6)	4444537	CCN(CC)C(=O)/C(=C/c1cc(c(c(c1)O)O)[N+](=O)[O-])/C#N
668	6.89	2-Mercaptobenzothiazole	(149-30-4)	608157	c1ccc2c(c1)[nH]c(=S)s2
669	6.9	Monuron	(150-68-5)	8470	CN(C)C(=O)Nc1ccc(cc1)Cl
670	6.91	Carvedilol	(72956-09-3)	2487	COc1ccccc1OCCNCC(COc2cccc3c2c4ccccc4[nH]3)O
671	6.91	Memantine	(19982-08-2)	3914	CC12CC3CC(C1)(CC(C3)(C2)N)C
672	6.92	Sulfometuron-methyl	(74222-97-2)	47881	Cc1cc(nc(n1)NC(=O)NS(=O)(=O)c2ccccc2C(=O)OC)C
673	6.93	Thiophanate-methyl	(23564-05-8)	2297683	COC(=O)NC(=S)Nc1ccccc1NC(=S)NC(=O)OC
674	6.94	Mianserine	(24219-97-4)	4040	CN1CCN2c3ccccc3Cc4ccccc4C2C1
675	6.94	Apronalide	(528-92-7)	10264	CC(C)C(CC=C)C(=O)NC(=O)N
676	6.94	Cyclizine	(82-92-8)	6470	CN1CCN(CC1)C(c2ccccc2)c3ccccc3
677	6.94	Ketorolac	(74103-06-3)	3694	c1ccc(cc1)C(=O)c2ccc3n2CCC3C(=O)O
678	6.94	Propoxur	(114-26-1)	4775	CC(C)Oc1ccccc1O/C(=N/C)/O
679	6.94	Haloperidol	(52-86-8)	3438	c1cc(ccc1C(=O)CCCN2CCC(CC2)(c3ccc(cc3)Cl)O)F
680	6.95	Levocabastine	(79516-68-0)	3778	CC1CN(CCC1(c2ccccc2)C(=O)O)C3CCC(CC3)(C#N)c4ccc(cc4)F
681	6.96	Noscapine	(128-62-1)	4385	CN1CCc2cc3c(c(c2C1C4c5ccc(c(c5C(=O)O4)OC)OC)OC)OCO3
682	6.96	Norverapamil	(67018-85-3)	94724	CC(C)C(CCCNCCc1ccc(c(c1)OC)OC)(C#N)c2ccc(c(c2)OC)OC
683	6.98	DNOC (4,6-dinitro-o-cresol)	(534-52-1)	10343	Cc1cc(cc(c1O)[N+](=O)[O-])[N+](=O)[O-]
684	6.98	Proguanil	(500-92-5)	4754	CC(C)NC(=N)NC(=N)Nc1ccc(cc1)Cl
685	6.98	Rosiglitazone	(122320-73-4)	70383	CN(CCOc1ccc(cc1)CC2C(=O)NC(=O)S2)c3ccccn3
686	6.98	2C-T-7 (2,5-dimethoxy-4-n-propylthiophenethylamine)	(207740-26-9)	21106233	CCCSc1cc(c(cc1OC)CCN)OC
687	6.99	Oxymetazoline	(1491-59-4)	4475	Cc1cc(c(c(c1CC2=NCCN2)C)O)C(C)(C)C
688	6.99	Cyclamic acid	(100-88-9)	7252	C1CCC(CC1)NS(=O)(=O)O
689	6.99	Carboxyibuprofen	(15935-54-3)	8619532	O=C(O)C(c1ccc(cc1)CC(C(=O)O)C)C
690	7	Ofurace	(58810-48-3)	39084	Cc1cccc(c1N(C2CCOC2=O)C(=O)CCl)C
691	7	Dichlorvos	(62-73-7)	2931	COP(=O)(OC)OC=C(Cl)Cl
692	7.01	Malaoxon	(1634-78-2)	14674	CCOC(=O)CC(C(=O)OCC)SP(=O)(OC)OC
693	7.01	Melphalan	(148-82-3)	3913	c1cc(ccc1CC(C(=O)O)N)N(CCCl)CCCl

continued

694	7.01	Carbofuran	(1563-66-2)	2468	CC1(Cc2cccc(c2O1)O/C(=N/C)/O)C
695	7.01	Dimetotiazine	(7456-24-8)	2979	CC(CN1c2ccccc2Sc3c1cc(cc3)S(=O)(=O)N(C)C)N(C)C
696	7.02	Bendiocarb	(22781-23-3)	2224	CC1(Oc2cccc(c2O1)OC(=O)NC)C
697	7.03	Nordoxepin	(1225-56-5)	4510230	CNCC/C=C\1/c2ccccc2COc3c1cccc3
698	7.03	Verapamil	(52-53-9)	2425	CC(C)C(CCCN(C)CCc1ccc(c(c1)OC)OC)(C#N)c2ccc(c(c2)OC)OC
699	7.06	Nalidixic acid	(389-08-2)	4268	CCn1cc(c(=O)c2c1nc(cc2)C)C(=O)O
700	7.07	Bromacil	(314-40-9)	9040	CCC(C)n1c(=O)c(c([nH]c1=O)C)Br
701	7.07	Simazine	(122-34-9)	5027	CC/N=c\1/[nH]/c(=N\CC)/nc([nH]1)Cl
702	7.07	Bupranolol	(14556-46-8)	2381	Cc1ccc(c(c1)OCC(CNC(C)(C)C)O)Cl
703	7.07	Metribuzin	(21087-64-9)	28287	CC(C)(C)c1c(=O)n(c(nn1)SC)N
704	7.08	Phenytoin	(57-41-0)	1710	c1ccc(cc1)C2(C(=NC(=N2)O)O)c3ccccc3
705	7.08	2-Hydroxyibuprofen	(51146-55-5)	8618954	O=C(O)C(c1ccc(cc1)CC(O)(C)C)C
706	7.09	Aconitine	(302-27-2)	1935	CCN1CC2(C(CC(C34C2C(C(C31)C5(C6C4CC(C6OC(=O)c7ccccc7)(C(C5O)OC)O)OC(=O)C)OC)OC)O)COC
707	7.09	N-Desmethylpropafenone	(86383-21-3) as the maleate salt	114154	c1ccc(cc1)CCC(=O)c2ccccc2OCC(CN)O
708	7.09	Flupirtine	(56995-20-1)	48119	CCOC(=O)Nc1ccc(nc1N)NCc2ccc(cc2)F
709	7.09	Chloropyramine	(59-32-5)	23628	CN(C)CCN(Cc1ccc(cc1)Cl)c2ccccn2
710	7.09	Rimsulfuron	(122931-48-0)	82876	CCS(=O)(=O)c1cccnc1S(=O)(=O)N/C(=N/c2nc(cc(n2)OC)OC)/O
711	7.09	Meloxicam	(71125-38-7)	10442740	Cc1cnc(s1)/N=C(/C2=C(c3ccccc3S(=O)(=O)N2C)O)\O
712	7.09	Hexazinone	(51235-04-2)	36542	Cn1c(nc(=O)n(c1=O)C2CCCCC2)N(C)C
713	7.1	Metosulam	(139528-85-1)	77938	Cc1ccc(c(c1Cl)NS(=O)(=O)c2nc3nc(cc(n3n2)OC)OC)Cl
714	7.11	AM-2233	(444912-75-8)	8401830	CN1CCCCC1CN2C=C(C3=CC=CC=C32)C(=O)C4=CC=CC=C4I
715	7.12	Bromperidol	(10457-90-6)	2354	c1cc(ccc1C(=O)CCCN2CCC(CC2)(c3ccc(cc3)Br)O)F
716	7.13	Norclozapine	(6104-71-8)	14126465	c1ccc2c(c1)C(=Nc3cc(ccc3N2)Cl)N4CCNCC4
717	7.13	Benzthiazuron	(1929-88-0)	15208	CNC(=O)Nc1nc2ccccc2s1
718	7.14	N-Isopropylsalicylamide	(551-35-9)	61658	CC(C)NC(=O)c1ccccc1O
719	7.14	Demoxepam	(963-39-3)	10441314	C1C(=O)NC2=C(C=C(C=C2)Cl)C(=[N+]1[O-])C3=CC=CC=C3
720	7.15	Ancymidol	(12771-68-5)	23841	COc1ccc(cc1)C(c2cncnc2)(C3CC3)O

continued

721	7.16	Methyl 2-dimethoxyphosphinothioylsulfanylacetate (formothion methanolyse)	(1741-11-3)	62992	COC(=O)CSP(=S)(OC)OC
722	7.16	Imazamethabenz-methyl (Imazamethabenz)	(81405-85-8)	49450	Cc1ccc(c(c1)C2=NC(C(=O)N2)(C)C(C)C)C(=O)OC
723	7.17	Piretanide	(55837-27-9)	4683	c1ccc(cc1)Oc2c(cc(cc2S(=O)(=O)N)C(=O)O)N3CCCC3
724	7.17	Thidiazuron	(51707-55-2)	36635	c1ccc(cc1)/N=C(\Nc2cnns2)/O
725	7.18	Diltiazem	(42399-41-7)	2967	CC(=O)OC1C(Sc2ccccc2N(C1=O)CCN(C)C)c3ccc(cc3)OC
726	7.18	Dichlormid	(37764-25-3)	34686	C=CCN(CC=C)C(=O)C(Cl)Cl
727	7.19	Prednisone	(53-03-2)	4731	CC12CC(=O)C3C(C1CCC2(C(=O)CO)O)CCC4=CC(=O)C=CC34C
728	7.19	Histapyrrodine	(493-80-1)	61430	c1ccc(cc1)CN(CCN2CCCC2)c3ccccc3
729	7.19	Chlorpropamide	(94-20-2)	2626	CCC/N=C(/NS(=O)(=O)c1ccc(cc1)Cl)\O
730	7.19	Clozapine	(5786-21-0)	10442628	CN1CCN(CC1)C2=Nc3cc(ccc3Nc4c2cccc4)Cl
731	7.19	Gallopamil	(16662-47-8)	1197	CC(C)C(CCCN(C)CCc1ccc(c(c1)OC)OC)(C#N)c2cc(c(c(c2)OC)OC)OC
732	7.21	Prothipendyl	(303-69-5)	14002	CN(C)CCCN1c2ccccc2Sc3c1nccc3
733	7.21	Nordiltiazem (N-Desmethyldiltiazem)	(85100-17-0)	14600890	CC(=O)OC1C(Sc2ccccc2N(C1=O)CCNC)c3ccc(cc3)OC
734	7.21	Fenamiphos - sulfone	(31972-44-8)	33142	CCOP(=O)(NC(C)C)Oc1ccc(c(c1)C)S(=O)(=O)C
735	7.23	Propyphenazone	(479-92-5)	3646	Cc1c(c(=O)n(n1C)c2ccccc2)C(C)C
736	7.23	Diphenylpyraline	(147-20-6)	2992	CN1CCC(CC1)OC(c2ccccc2)c3ccccc3
737	7.24	P-Hydroxymesocarb	(72460-70-9)	(N/A)	Oc1ccc(cc1)NC(=O)[N-]c2c[n+](no2)C(C)Cc3ccccc3
738	7.25	N.N-Dimethyl-N'-p-tolylsulphamide (DMST)	(66840-71-9)	645289	Cc1ccc(cc1)NS(=O)(=O)N(C)C
739	7.25	Tebuthiuron	(34014-18-1)	5190	CC(C)(C)c1nnc(s1)N(C)C(=O)NC
740	7.26	Aceprometazine	(13461-01-3)	24249	CC(CN1c2ccccc2Sc3c1cc(cc3)C(=O)C)N(C)C
741	7.28	Reboxetine	(98769-81-4)	2289101	CCOc1ccccc1OC(c2ccccc2)C3CNCCO3
742	7.28	Bromazepam	(1812-30-2)	2347	c1ccnc(c1)C2=NCC(=Nc3c2cc(cc3)Br)O
743	7.28	Fenthion-sulfoxide	(3761-41-9)	18444	Cc1cc(ccc1S(=O)C)OP(=S)(OC)OC
744	7.29	Norsildenafil (N-Desmethyl Sildenafil)	(139755-82-1)	4932278	CCCc1c2c(c(=O)nc([nH]2)c3cc(ccc3OCC)S(=O)(=O)N4CCNCC4)n(n1)C
745	7.29	Phenyltoloxamine	(92-12-6)	6810	CN(C)CCOc1ccccc1Cc2ccccc2
746	7.31	Sufentanil	(56030-54-7)	38043	CCC(=O)N(c1ccccc1)C2(CCN(CC2)CCc3cccs3)COC
747	7.33	Terbacil	(5902-51-2)	20830	Cc1c(c(=O)n(c(=O)[nH]1)C(C)(C)C)Cl

continued

748	7.33	Naphyrone	(850352-53-3)	9418039	CCCC(C(=O)C1=CC2=CC=CC=C2C=C1)N3CCCC3
749	7.34	2-C-C-NBoMe (2-(4-Chloro-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine)	(1227608-02-7)	24583389	COc1ccccc1CNCCc2cc(c(cc2OC)Cl)OC
750	7.35	N-Desmethylflunitrazepam (Norflunitrazepam)	(2558-30-7)	453770	c1ccc(c(c1)C2=NCC(=O)Nc3c2cc(cc3)[N+](=O)[O-])F
751	7.35	Propantheline (+)	(298-50-0)	4765	CC(C)[N+](C)(CCOC(=O)C1c2ccccc2Oc3c1cccc3)C(C)C
752	7.36	Quetiapine	(111974-69-7)	4827	c1ccc2c(c1)C(=Nc3ccccc3S2)N4CCN(CC4)CCOCCO
753	7.35	Carbamazepine	(298-46-4)	2457	c1ccc2c(c1)C=Cc3ccccc3N2C(=O)N
754	7.36	Xipamide	(14293-44-8)	24795	Cc1cccc(c1NC(=O)c2cc(c(cc2O)Cl)S(=O)(=O)N)C
755	7.37	Cortisone	(53-06-5)	454849	CC12CCC(=O)C=C1CCC3C2C(=O)CC4(C3CCC4(C(=O)CO)O)C
756	7.37	Valdecoxib	(181695-72-7)	106796	Cc1c(c(no1)c2ccccc2)c3ccc(cc3)S(=O)(=O)N
757	7.39	Asenapine	(65576-45-6)	8079624	CN1CC2C(C1)C3=C(C=CC(=C3)Cl)OC4=CC=CC=C24
758	7.39	Pipotiazine	(39860-99-6)	56598	CN(C)S(=O)(=O)c1ccc2c(c1)N(c3ccccc3S2)CCCN4CCC(CC4)CCO
759	7.41	Benorilate	(5003-48-5)	19846	C/C(=N\c1ccc(cc1)OC(=O)c2ccccc2OC(=O)C)/O
760	7.41	Bromodragonfly	(502759-67-3)	8014776	CC(CC1=C2C=COC2=C(C3=C1OC=C3)Br)N
761	7.42	Trifluperidol	(749-13-3)	5366	c1cc(cc(c1)C(F)(F)F)C2(CCN(CC2)CCCC(=O)c3ccc(cc3)F)O
762	7.44	Flumequine	(42835-25-6)	3257	CC1CCc2cc(cc3c2n1cc(c3=O)C(=O)O)F
763	7.44	Orphenadrine	(83-98-7)	4440	Cc1ccccc1C(c2ccccc2)OCCN(C)C
764	7.45	Periciazine	(2622-26-6)	4585	c1ccc2c(c1)N(c3cc(ccc3S2)C#N)CCCN4CCC(CC4)O
765	7.46	Carbaryl	(63-25-2)	5899	C/N=C(\O)/Oc1cccc2c1cccc2
766	7.46	Chlorazanil	(500-42-5)	9946	c1cc(ccc1Nc2ncnc(n2)N)Cl
767	7.46	Carboxin	(5234-68-4)	20027	CC1=C(SCCO1)C(=O)Nc2ccccc2
768	7.46	2C-P (2-(2,5-Dimethoxy-4-propylphenyl)ethanamine)	(207740-22-5)	21106226	CCCc1cc(c(cc1OC)CCN)OC
769	7.47	Tolpropamine	(5632-44-0)	65115	Cc1ccc(cc1)C(CCN(C)C)c2ccccc2
770	7.47	Acepromazine	(61-00-7)	5852	CC(=O)c1ccc2c(c1)N(c3ccccc3S2)CCCN(C)C
771	7.48	Desloratadine	(100643-71-8)	110575	c1cc2c(nc1)C(=C3CCNCC3)c4ccc(cc4CC2)Cl
772	7.49	Eprosartan	(133040-01-4)	4444504	CCCCc1ncc(n1Cc2ccc(cc2)C(=O)O)C=C(Cc3cccs3)C(=O)O
773	7.49	Omeprazole	(73590-58-6)	4433	Cc1cnc(c(c1OC)C)CS(=O)c2[nH]c3ccc(cc3n2)OC
774	7.52	Cyamemazine	(3546-03-0)	56597	CC(CN1c2ccccc2Sc3c1cc(cc3)C#N)CN(C)C

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775	7.53	Promethazine	(60-87-7)	4758	CC(CN1c2ccccc2Sc3c1cccc3)N(C)C
776	7.54	Nororphenadrine (Tofenacin or Elamol)	(15301-93-6)	23647	Cc1ccccc1C(c2ccccc2)OCCNC
777	7.54	Hexobendine	(54-03-5)	5573	CN(CCCOC(=O)c1cc(c(c(c1)OC)OC)OC)CCN(C)CCCOC(=O)c2cc(c(c(c2)OC)OC)OC
778	7.56	Azadirachtin (Na adduct in MS spectrum)	(11141-17-6)	10619174	C/C=C(\C)/C(=O)OC1CC(C2(COC3C2C14COC(C4C(C3O)(C)C56C7CC(C5(O6)C)C8(C=COC8O7)O)(C(=O)OC)O)C(=O)OC)OC(=O)C
779	7.56	Clonazepam	(1622-61-3)	2700	c1ccc(c(c1)C2=NCC(=O)Nc3c2cc(cc3)[N+](=O)[O-])Cl
780	7.57	Oxypendyl	(5585-93-3)	147734	c1ccc2c(c1)N(c3c(cccn3)S2)CCCN4CCN(CC4)CCO
781	7.57	Fenthion-sulfone	(3761-42-0)	18445	Cc1cc(ccc1S(=O)(=O)C)OP(=S)(OC)OC
782	7.59	Azelastine	(58581-89-8)	2180	CN1CCCC(CC1)n2c(=O)c3ccccc3c(n2)Cc4ccc(cc4)Cl
783	7.59	Fenfuram	(24691-80-3)	81792	Cc1c(cco1)C(=O)Nc2ccccc2
784	7.59	Nitrazepam	(146-22-5)	4350	c1ccc(cc1)C2=NCC(=Nc3c2cc(cc3)[N+](=O)[O-])O
785	7.61	Dosulepin	(113-53-1)	4447605	CN(C)CC/C=C/1\c2ccccc2CSc3c1cccc3
786	7.62	Azimsulfuron	(120162-55-2)	77873	Cn1c(c(cn1)c2nnn(n2)C)S(=O)(=O)NC(=O)Nc3nc(cc(n3)OC)OC
787	7.63	Atomoxetine	(83015-26-3)	49516	Cc1ccccc1O[C@H](CCNC)c2ccccc2
788	7.63	Mianserine-N-oxide	(62510-46-7)	2342189	C[N+]1(CCN2c3ccccc3Cc4ccccc4C2C1)[O-]
789	7.63	Biperidene	(514-65-8)	2289	c1ccc(cc1)C(CCN2CCCCC2)(C3CC4CC3C=C4)O
790	7.64	Amoxapine	(14028-44-5)	2085	c1ccc2c(c1)N=C(c3cc(ccc3O2)Cl)N4CCNCC4
791	7.64	Barverin	(1639-79-8)	175137	c1ccc(cc1)C2(C(=O)N(C(=O)N(C2=O)CCN3CCCCC3)CCN4CCCCC4)N5CCCCC5
792	7.64	Thiodicarb	(59669-26-0)	7875353	C/C(=N\OC(=O)N(SN(C(=O)O/N=C(/SC)\C)C)C)/SC
793	7.65	1-Hydroxymethyltriazolam (alpha-Hydroxy-Triazolam)	(37115-45-0)	1887	c1ccc(c(c1)C2=NCc3nnc(n3-c4c2cc(cc4)Cl)CO)Cl
794	7.66	Fludrocortisone	(127-31-1)	471225	CC12CCC(=O)C=C1CCC3C2(C(CC4(C3CCC4(C(=O)CO)O)C)O)F
795	7.66	Cambendazole	(26097-80-3)	30767	CC(C)OC(=O)Nc1ccc2c(c1)[nH]c(n2)c3cscn3
796	7.68	Paroxetine	(61869-08-7)	4529	c1cc(ccc1C2CCNCC2COc3ccc4c(c3)OCO4)F
797	7.68	Tolbutamide	(64-77-7)	5304	CCCC/N=C(/NS(=O)(=O)c1ccc(cc1)C)\O
798	7.68	Fexofenadine	(83799-24-0)	3231	CC(C)(c1ccc(cc1)C(CCCN2CCC(CC2)C(c3ccccc3)(c4ccccc4)O)O)C(=O)O
799	7.68	Normethadone	(467-85-6)	9687	CCC(=O)C(CCN(C)C)(c1ccccc1)c2ccccc2
800	7.69	Alfentanyl	(71195-58-9)	46451	CCC(=O)N(c1ccccc1)C2(CCN(CC2)CCn3c(=O)n(nn3)CC)COC
801	7.69	Promazine	(58-40-2)	4757	CN(C)CCCN1c2ccccc2Sc3c1cccc3
802	7.7	Dimethirimol	(5221-53-4)	20014	CCCCc1c(nc(nc1O)N(C)C)C

continued

803	7.7	Ethiofencarb	(29973-13-5)	31991	CCSCc1ccccc1OC(=O)NC
804	7.73	Dextropropoxyphene	(469-62-5)	14592	CCC(=O)OC(Cc1ccccc1)(c2ccccc2)C(C)CN(C)C
805	7.73	Fosthiazate	(98886-44-3)	82856	CCC(C)SP(=O)(N1CCSC1=O)OCC
806	7.75	Xylometazoline	(526-36-3)	5507	Cc1cc(cc(c1CC2=NCCN2)C)C(C)(C)C
807	7.76	Diethazine	(60-91-3)	58979	CCN(CC)CCN1c2ccccc2Sc3c1cccc3
808	7.76	Monolinuron	(1746-81-2)	14868	CN(C(=O)Nc1ccc(cc1)Cl)OC
809	7.76	1-Naphthaleneacetic acid (NH4 adduct in MS spectrum)	(86-87-3)	6601	c1ccc2c(c1)cccc2CC(=O)O
810	7.77	Bamipine	(4945-47-5)	65061	CN1CCC(CC1)N(Cc2ccccc2)c3ccccc3
811	7.77	Pirimicarb	(23103-98-2)	29348	Cc1c(nc(nc1OC(=O)N(C)C)N(C)C)C
812	7.78	XMC (Group of peaks!)	(2655-14-3)	16606	Cc1cc(cc(c1)OC(=O)NC)C
813	7.8	Thiofanox	(39196-18-4)	4517040	CC(C)(C)/C(=N\OC(=O)NC)/CSC
814	7.8	Procyclidine	(77-37-2)	4750	c1ccc(cc1)C(CCN2CCCC2)(C3CCCCC3)O
815	7.81	Propafenone	(54063-53-5)	4763	CCCNCC(COc1ccccc1C(=O)CCc2ccccc2)O
816	7.81	Esomeprazole	(119141-88-7)	4433	Cc1cnc(c(c1OC)C)CS(=O)c2[nH]c3ccc(cc3n2)OC
817	7.81	Atraton	(1610-17-9)	14620	CC/N=c/1\[nH]/c(=N/C(C)C)/[nH]c(n1)OC
818	7.81	Hydroxyalprazolam	(30896-57-2)	158315	Cc1nnc2n1-c3ccc(cc3C(=NC2O)c4ccccc4)Cl
819	7.82	Hydrocortisone	(50-23-7)	21240790	CC12CCC(=O)C=C1CCC3C2C(CC4(C3CCC4(C(=O)CO)O)C)O
820	7.83	Opipramol	(315-72-0)	9046	c1ccc2c(c1)C=Cc3ccccc3N2CCCN4CCN(CC4)CCO
821	7.83	Flunitrazepam	(1622-62-4)	3263	CN1c2ccc(cc2C(=NCC1=O)c3ccccc3F)[N+](=O)[O-]
822	7.83	Bumetanide	(28395-03-1)	2377	CCCCNc1cc(cc(c1Oc2ccccc2)S(=O)(=O)N)C(=O)O
823	7.83	Methaqualone	(72-44-6)	6055	Cc1ccccc1n2c(nc3ccccc3c2=O)C
824	7.84	Nordextropropoxyphene (Norpropoxyphene)	(3376-94-1)	17756	CCC(=O)OC(Cc1ccccc1)(c2ccccc2)C(C)CNC
825	7.83	Erythromycin	(114-07-8)	3140	CCC1C(C(C(C(=O)C(CC(C(C(C(C(C(=O)O1)C)OC2CC(C(C(O2)C)O)(C)OC)C)OC3C(C(CC(O3)C)N(C)C)O)(C)O)C)C)O)(C)O
826	7.85	Triclopyr	(55335-06-3)	37801	c1c(c(nc(c1Cl)Cl)OCC(=O)O)Cl
827	7.85	Fluometuron	(2164-17-2)	15702	CN(C)C(=O)Nc1cccc(c1)C(F)(F)F
828	7.86	Trihexyphenidyl (Benzhexol)	(144-11-6)	5371	c1ccc(cc1)C(CCN2CCCCC2)(C3CCCCC3)O
829	7.86	Pizotifen	(15574-96-6)	25497	CN1CCC(=C2c3ccccc3CCc4c2ccs4)CC1
830	7.86	Disulfoton-sulfoxid (oxydisulfoton)	(2497-07-6)	16321	CCOP(=S)(OCC)SCCS(=O)CC

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831	7.87	Pentoxyverine	(77-23-6)	2464	CCN(CC)CCOCCOC(=O)C1(CCCC1)c2ccccc2
832	7.88	Sildenafil	(139755-83-2)	5023	CCCc1c2c(c(=O)[nH]c(n2)c3cc(ccc3OCC)S(=O)(=O)N4CCN(CC4)C)n(n1)C
833	7.89	Tolmetin	(26171-23-3)	5308	Cc1ccc(cc1)C(=O)c2ccc(n2C)CC(=O)O
834	7.89	Clobazam	(22316-47-8)	2687	CN1c2ccc(cc2N(C(=O)CC1=O)c3ccccc3)Cl
835	7.89	Cyproheptadine	(129-03-3)	2810	CN1CCC(=C2c3ccccc3C=Cc4c2cccc4)CC1
836	7.91	Terodiline	(15793-40-5)	21952	CC(CC(c1ccccc1)c2ccccc2)NC(C)(C)C
837	7.92	Furilazole	(121776-33-8)	77743	CC1(N(CC(O1)c2ccco2)C(=O)C(Cl)Cl)C
838	7.93	Imipramine	(50-49-7)	3568	CN(C)CCCN1c2ccccc2CCc3c1cccc3
839	7.93	Trimeprazine	(84-96-8)	5373	CC(CN1c2ccccc2Sc3c1cccc3)CN(C)C
840	7.94	Cilazapril	(88768-40-5)	2649	CCOC(=O)C(CCc1ccccc1)NC2CCCN3CCCC(N3C2=O)C(=O)O
841	7.94	Sulfinpyrazone	(57-96-5)	5149	c1ccc(cc1)N2C(=O)C(C(=O)N2c3ccccc3)CCS(=O)c4ccccc4
842	7.94	Tribenuron-methyl	(101200-48-0)	135649	Cc1nc(nc(n1)OC)N(C)C(=O)NS(=O)(=O)c2ccccc2C(=O)OC
843	7.95	Cyclobenzaprine	(303-53-7)	2792	CN(C)CCC=C1c2ccccc2C=Cc3c1cccc3
844	7.96	Buprenorphine	(52485-79-7)	2382	CC(C)(C)C(C)(C1CC23CCC1(C4C25CCN(C3Cc6c5c(c(cc6)O)O4)CC7CC7)OC)O
845	7.96	Methadone	(76-99-3)	3953	CCC(=O)C(CC(C)N(C)C)(c1ccccc1)c2ccccc2
846	7.97	Benzatropine	(86-13-5)	2254	CN1C2CCC1CC(C2)OC(c3ccccc3)c4ccccc4
847	7.98	Chlorotoluron	(15545-48-9)	25472	Cc1ccc(cc1Cl)/N=C(\N(C)C)/O
848	7.98	Oxyphencyclimine	(125-53-1)	4481	CN1CCCN=C1COC(=O)C(c2ccccc2)(C3CCCCC3)O
849	7.99	Carisoprodol	(78-44-4)	2478	CCCC(C)(COC(=O)N)COC(=O)NC(C)C
850	7.99	Famphur	(52-85-7)	5650	CN(C)S(=O)(=O)c1ccc(cc1)OP(=S)(OC)OC
851	7.99	Protriptyline	(438-60-8)	4805	CNCCCC1c2ccccc2C=Cc3c1cccc3
852	8	Ethirimol	(23947-60-6)	29820	CCCCc1c(nc(nc1O)NCC)C
853	8.01	Paraoxon	(311-45-5)	9026	CCOP(=O)(OCC)Oc1ccc(cc1)[N+](=O)[O-]
854	8.02	Benodanil	(15310-01-7)	25310	c1ccc(cc1)NC(=O)c2ccccc2I
855	8.02	Disulfoton-sulfone	(2497-06-5)	16320	CCOP(=S)(OCC)SCCS(=O)(=O)CC
856	8.03	Ziprasidone	(146939-27-7)	54841	c1ccc2c(c1)c(ns2)N3CCN(CC3)CCc4cc5c(cc4Cl)NC(=O)C5
857	8.03	Astemizole	(68844-77-9)	2160	COc1ccc(cc1)CCN2CCC(CC2)Nc3nc4ccccc4n3Cc5ccc(cc5)F

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858	8.03	25I-NBoMe [2-(4-Iodo-2,5-dimethoxyphenyl)-N-(2-methoxybenzyl)ethanamine]	(919797-19-6)	8427392	COC1=CC=CC=C1CNCCC2=CC(=C(C=C2OC)I)OC
859	8.04	Duloxetine	(116539-59-4)	109024	CNCCC(c1cccs1)Oc2cccc3c2cccc3
860	8.04	Oxeladin	(468-61-1)	4458	CCC(CC)(c1ccccc1)C(=O)OCCOCCN(CC)CC
861	8.05	Griseofulvin	(126-07-8)	3392	CC1CC(=O)C=C(C12C(=O)c3c(cc(c(c3O2)Cl)OC)OC)OC
862	8.05	Triazolam	(28911-01-5)	5355	Cc1nnc2n1-c3ccc(cc3C(=NC2)c4ccccc4Cl)Cl
863	8.06	Rosuvastatin	(287714-41-4)	4859072	CC(C)c1c(c(nc(n1)N(C)S(=O)(=O)C)c2ccc(cc2)F)/C=C/C(CC(CC(=O)O)O)O
864	8.08	Desipramine	(50-47-5)	2888	CNCCCN1c2ccccc2CCc3c1cccc3
865	8.08	Maprotiline	(10262-69-8)	3871	CNCCCC12CCC(c3c1cccc3)c4c2cccc4
866	8.08	Metominostrobin 1 (Z-Isomer)	(133408-51-2)	7850488	O=C(NC)C(=N\OC)/c2ccccc2Oc1ccccc1
867	8.09	Dienogest	(65928-58-7)	10761296	O=C\2CC\C\3=C4/CCC1(C)C(CCC1(O)CC#N)C4CC\C/3=C/2
868	8.09	Tolazamide	(1156-19-0)	5302	Cc1ccc(cc1)S(=O)(=O)NC(=O)NN2CCCCCC2
869	8.09	AM-1220 [(1-{[(2R)-1-Methyl-2-piperidinyl]methyl}-1H-indol-3-yl)(1-naphthyl)methanone]	(134959-64-1)	8105520	CN1CCCCC1CN2C=C(C3=CC=CC=C32)C(=O)C4=CC=CC5=CC=CC=C54
870	8.1	Trimethacarb (3,4,5-) [Shell SD 8530]	(2686-99-9)	16632	Cc1cc(cc(c1C)C)OC(=O)NC
871	8.1	Alimemazine	(84-96-8)	5373	CC(CN1c2ccccc2Sc3c1cccc3)CN(C)C
872	8.11	Glipizide	(29094-61-9)	3359	Cc1cnc(cn1)/C(=N\CCc2ccc(cc2)S(=O)(=O)N/C(=N\C3CCCCC3)/O)/O
873	8.11	alpha-Hydroxy-Alprazolam	(37115-43-8)	142474	c1ccc(cc1)C2=NCc3nnc(n3-c4c2cc(cc4)Cl)CO
874	8.11	Metazachlor	(67129-08-2)	44885	Cc1cccc(c1N(Cn2cccn2)C(=O)CCl)C
875	8.11	Propham (IPC)	(122-42-9)	23083	CC(C)OC(=O)Nc1ccccc1
876	8.11	Vardenafil	(224785-90-4)	99300	CCCc1nc(c2n1nc([nH]c2=O)c3cc(ccc3OCC)S(=O)(=O)N4CCN(CC4)CC)C
877	8.11	Trifloxysulfuron	(145099-21-4)	8131496	COC1=CC(=NC(=N1)NC(=O)NS(=O)(=O)C2=C(C=CC=N2)OCC(F)(F)F)OC
878	8.12	Lethane 384	(112-56-1)	7904	CCCCOCCOCCSC#N
879	8.12	Isoprocarb	(2631-40-5)	16564	CC(C)c1ccccc1OC(=O)NC
880	8.12	Flutriafol	(76674-21-0)	82827	c1ccc(c(c1)C(Cn2cncn2)(c3ccc(cc3)F)O)F
881	8.12	5-(p-Methylphenyl)-5-phenylhydantoin (MPPH)	(51169-17-6)	83358	Cc1ccc(cc1)C2(C(=O)NC(=O)N2)c3ccccc3
882	8.14	Levomepromazine	(60-99-1)	3779	CC(CN1c2ccccc2Sc3c1cc(cc3)OC)CN(C)C
883	8.14	Simetryn	(1014-70-6)	13303	CC/N=c\1/[nH]/c(=N\CC)/nc([nH]1)SC
884	8.14	Estazolam	(29975-16-4)	3146	c1ccc(cc1)C2=NCc3nncn3-c4c2cc(cc4)Cl

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885	8.15	Metobromuron	(3060-89-7)	17276	CN(C(=O)Nc1ccc(cc1)Br)OC
886	8.15	Thioproperazine	(316-81-4)	9058	CN1CCN(CC1)CCCN2c3ccccc3Sc4c2cc(cc4)S(=O)(=O)N(C)C
887	8.16	Atrazine	(1912-24-9)	2169	CC/N=c/1\[nH]/c(=N/C(C)C)/[nH]c(n1)Cl
888	8.17	Dihydroergocristine	(17479-19-5)	2957	CC(C)C1(C(=O)N2C(C(=O)N3CCCC3C2(O1)O)Cc4ccccc4)NC(=O)C5CC6c7cccc8c7c(c[nH]8)CC6N(C5)C
889	8.17	Desmetryn	(1014-69-3)	13302	CC(C)Nc1nc(nc(n1)SC)NC
890	8.19	Lorazepam	(846-49-1)	3821	c1ccc(c(c1)C2=NC(C(=O)Nc3c2cc(cc3)Cl)O)Cl
891	8.19	Losartan	(114798-26-4)	3824	CCCCc1nc(c(n1Cc2ccc(cc2)c3ccccc3c4n[nH]nn4)CO)Cl
892	8.19	Piritramide	(302-41-0)	8967	c1ccc(cc1)C(CCN2CCC(CC2)(C(=O)N)N3CCCCC3)(C#N)c4ccccc4
893	8.2	Terconazole	(67915-31-5)	5211	CC(C)N1CCN(CC1)c2ccc(cc2)OCC3COC(O3)(Cn4cncn4)c5ccc(cc5Cl)Cl
894	8.2	N,N-Diethyl-m-toluamide (DEET)	(134-62-3)	4133	CCN(CC)C(=O)c1cccc(c1)C
895	8.22	Metalaxyl	(57837-19-1)	38839	Cc1cccc(c1N(C(C)C(=O)OC)C(=O)COC)C
896	8.22	Cyprazine	(22936-86-3)	23128	CC(C)Nc1nc(nc(n1)Cl)NC2CC2
897	8.23	Spirapril	(83647-97-6)	(N/A)	CCOC(=O)C(CCC1=CC=CC=C1)NC(C)C(=O)N2CC3(CC2C(=O)O)SCCS3
898	8.23	Amitriptyline	(50-48-6)	2075	CN(C)CCC=C1c2ccccc2CCc3c1cccc3
899	8.24	Sulindac	(38194-50-2)	1265915	CC\1=C(c2cc(ccc2/C1=C\c3ccc(cc3)S(=O)C)F)CC(=O)O
900	8.25	Harpagoside	(19210-12-9)	3430705	CC1(CC(C2(C1C(OC=C2)OC3C(C(C(C(O3)CO)O)O)O)O)O)OC(=O)C=Cc4ccccc4
901	8.25	Propachlor	(1918-16-7)	4762	CC(C)N(c1ccccc1)C(=O)CCl
902	8.26	Tadalafil	(171596-29-5)	3875386	CN1CC(=O)N2C(C1=O)Cc3c4ccccc4[nH]c3C2c5ccc6c(c5)OCO6
903	8.26	Oxazepam	(604-75-1)	4455	c1ccc(cc1)C2=NC(C(=O)Nc3c2cc(cc3)Cl)O
904	8.26	Methabenzthiazuron	(18691-97-9)	27173	CNC(=O)N(C)c1nc2ccccc2s1
905	8.27	N-Desmethyl-Chlordiazepoxide	(7722-15-8)	10441296	Clc1ccc2\N=C(\N)C[N+](/[O-])=C(\c2c1)c3ccccc3
906	8.27	Fensulfothion	(115-90-2)	7991	CCOP(=S)(OCC)Oc1ccc(cc1)S(=O)C
907	8.27	Cyanophos	(2636-26-2)	16569	COP(=S)(OC)Oc1ccc(cc1)C#N
908	8.27	Rabenzazole	(40341-04-6)	35229	Cc1cc(n(n1)c2[nH]c3ccccc3n2)C
909	8.27	Lenacil	(2164-08-1)	15699	C1CCC(CC1)n2c(=O)c3c(nc2O)CCC3
910	8.28	Brotizolam	(57801-81-7)	2357	Cc1nnc2n1-c3c(cc(s3)Br)C(=NC2)c4ccccc4Cl
911	8.28	Fluvoxamine	(54739-18-3)	4481878	COCCCC/C(=N\OCCN)/c1ccc(cc1)C(F)(F)F
912	8.28	Nebivolol	(99200-09-6)	64421	c1cc2c(cc1F)CCC(O2)C(CNCC(C3CCc4cc(ccc4O3)F)O)O

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913	8.28	Ketoprofen	(22071-15-4)	3693	CC(c1cccc(c1)C(=O)c2ccccc2)C(=O)O
914	8.28	Chlorcyclizine	(82-93-9)	2609	CN1CCN(CC1)C(c2ccccc2)c3ccc(cc3)Cl
915	8.25	Hydroxyzine	(68-88-2)	3531	c1ccc(cc1)C(c2ccc(cc2)Cl)N3CCN(CC3)CCOCCO
916	8.29	Moxaverine	(10539-19-2)	64045	CCc1cc2cc(c(cc2c(n1)Cc3ccccc3)OC)OC
917	8.29	Mequitazine	(29216-28-2)	3926	c1ccc2c(c1)N(c3ccccc3S2)CC4CN5CCC4CC5
918	8.31	Etizolam	(40054-69-1)	3191	CCc1cc2c(s1)-n3c(nnc3CN=C2c4ccccc4Cl)C
919	8.31	Nifedipine	(21829-25-4)	4330	CC1=C(C(C(=C(N1)C)C(=O)OC)c2ccccc2[N+](=O)[O-])C(=O)OC
920	8.31	Trimipramine	(739-71-9)	5382	CC(CN1c2ccccc2CCc3c1cccc3)CN(C)C
921	8.32	Benproperine	(2156-27-6)	2236	CC(COc1ccccc1Cc2ccccc2)N3CCCCC3
922	8.32	Isoproturon	(34123-59-6)	33695	CC(C)c1ccc(cc1)NC(=O)N(C)C
923	8.32	Isocarbophos	(24353-61-5)	81690	CC(C)OC(=O)c1ccccc1OP(=S)(N)OC
924	8.32	Kavain	(500-64-1)	4520267	COC1=CC(=O)OC(C1)/C=C/c2ccccc2
925	8.33	Etodroxizine	(17692-34-1)	57011	c1ccc(cc1)C(c2ccc(cc2)Cl)N3CCN(CC3)CCOCCOCCO
926	8.33	Norlevomepromazine	(37819-98-0)	8511272	O(c2cc1N(c3c(Sc1cc2)cccc3)CC(C)CNC)C
927	8.34	3-Hydroxyphenazepam	(70030-11-4)	111897	c1ccc(c(c1)C2=NC(C(=O)Nc3c2cc(cc3)Br)O)Cl
928	8.35	Buturon	(3766-60-7)	18451	CC(C#C)N(C)C(=O)Nc1ccc(cc1)Cl
929	8.35	Thionazin (Zinophos)	(297-97-2)	8915	CCOP(=S)(OCC)Oc1cnccn1
930	8.36	Alprazolam	(28981-97-7)	2034	Cc1nnc2n1-c3ccc(cc3C(=NC2)c4ccccc4)Cl
931	8.36	Amlodipine	(88150-42-9)	2077	CCOC(=O)C1=C(NC(=C(C1c2ccccc2Cl)C(=O)OC)C)COCCN
932	8.37	Triazoxide	(72459-58-6)	84327	c1cc2c(cc1Cl)[n+](nc(n2)n3ccnc3)[O-]
933	8.38	Dexamethasone	(50-02-2)	(N/A)	CC1CC2C3CCC4=CC(=O)C=CC4(C3(C(CC2(C1(C(=O)CO)O)C)O)F)C
934	8.39	Tiagabine	(115103-54-3)	5267	Cc1ccsc1C(=CCCN2CCCC(C2)C(=O)O)c3c(ccs3)C
935	8.39	Nicardipine	(55985-32-5)	4319	CC1=C(C(C(=C(N1)C)C(=O)OCCN(C)Cc2ccccc2)c3cccc(c3)[N+](=O)[O-])C(=O)OC
936	8.4	Trimethacarb (2.3.5-)	(2655-15-4)	23827	Cc1cc(c(c(c1)OC(=O)NC)C)C
937	8.4	Nortriptyline	(72-69-5)	4384	CNCCC=C1c2ccccc2CCc3c1cccc3
938	8.41	Cinchocaine	(85-79-0)	2917	CCCCOc1cc(c2ccccc2n1)/C(=N/CCN(CC)CC)/O
939	8.42	Oxatomide	(60607-34-3)	4454	c1ccc(cc1)C(c2ccccc2)N3CCN(CC3)CCCn4c5ccccc5[nH]c4=O
940	8.43	Difenoxuron	(14214-32-5)	24757	CN(C)C(=O)Nc1ccc(cc1)Oc2ccc(cc2)OC

continued

941	8.43	Fluoxetine	(54910-89-3)	3269	CNCCC(c1ccccc1)Oc2ccc(cc2)C(F)(F)F
942	8.44	1-Hydroxymidazolam	(59468-90-5)	97043	c1ccc(c(c1)C2=NCc3cnc(n3-c4c2cc(cc4)Cl)CO)F
943	8.45	Trinexapac-ethyl	(95266-40-3)	83439	CCOC(=O)C1CC(=O)C(=C(C2CC2)O)C(=O)C1
944	8.47	Reserpine	(50-55-5)	21428561	COc1ccc2c(c1)[nH]c3c2CCN4C3CC5C(C4)CC(C(C5C(=O)OC)OC)OC(=O)c6cc(c(c(c6)OC)OC)OC
945	8.47	Cycluron	(2163-69-1)	15694	CN(C)C(=O)NC1CCCCCCC1
946	8.48	Fensulfothion-sulfone	(14255-72-2)	24774	CCOP(=S)(OCC)Oc1ccc(cc1)S(=O)(=O)C
947	8.49	Propionylpromazine	(3568-24-9)	22768	CCC(=O)c1ccc2c(c1)N(c3ccccc3S2)CCCN(C)C
948	8.49	Chlorphenethazine	(2095-24-1)	56593	CN(C)CCN1c2ccccc2Sc3c1cc(cc3)Cl
949	8.49	Phenazopyridine	(94-78-0)	4592	c1ccc(cc1)/N=N/c2ccc(=N)[nH]c2N
950	8.49	Temazepam	(846-50-4)	5198	CN1c2ccc(cc2C(=NC(C1=O)O)c3ccccc3)Cl
951	8.49	Nortrimipramine	(2293-21-2)	141149	CC(CNC)CN1c2ccccc2CCc3c1cccc3
952	8.49	Heptenophos	(23560-59-0)	56515	COP(=O)(OC)OC1=C(C2C1CC=C2)Cl
953	8.51	Metixene	(4969-02-2)	4023	CN1CCCC(C1)CC2c3ccccc3Sc4c2cccc4
954	8.51	Norflurazon	(27314-13-2)	31131	CNc1cnn(c(=O)c1Cl)c2cccc(c2)C(F)(F)F
955	8.52	Naftifine	(65472-88-0)	43344	CN(C/C=C/c1ccccc1)Cc2cccc3c2cccc3
956	8.53	Metominostrobin 2 (E-Isomer)	(133408-50-1)	4588319	CNC(=O)/C(=N/OC)/c1ccccc1Oc2ccccc2
957	8.53	Forchlorfenuron	(68157-60-8)	84301	c1ccc(cc1)/N=C(\Nc2ccnc(c2)Cl)/O
958	8.54	Bornaprine	(20448-86-6)	28011	CCN(CC)CCCOC(=O)C1(CC2CCC1C2)c3ccccc3
959	8.54	Fendiline	(13042-18-7)	3219	CC(c1ccccc1)NCCC(c2ccccc2)c3ccccc3
960	8.54	Aminopromazine	(58-37-7)	18278	CN(C)CC(CN1c2ccccc2Sc3c1cccc3)N(C)C
961	8.55	Diuron	(330-54-1)	3008	CN(C)/C(=N\c1ccc(c(c1)Cl)Cl)/O
962	8.55	Naled	(300-76-5)	4267	COP(=O)(OC)OC(C(Cl)(Cl)Br)Br
963	8.56	Ramipril	(87333-19-5)	4862	CCOC(=O)C(CCc1ccccc1)NC(C)C(=O)N2C3CCCC3CC2C(=O)O
964	8.56	Flazasulfuron	(104040-78-0)	84440	COc1cc(nc(n1)NC(=O)NS(=O)(=O)c2c(cccn2)C(F)(F)F)OC
965	8.57	Prosulfuron	(94125-34-5)	82849	Cc1nc(nc(n1)OC)NC(=O)NS(=O)(=O)c2ccccc2CCC(F)(F)F
966	8.57	Diphenamid	(957-51-7)	13133	CN(C)C(=O)C(c1ccccc1)c2ccccc2
967	8.57	Desmedipham	(13684-56-5)	23133	CCO/C(=N/c1cccc(c1)O/C(=N/c2ccccc2)/O)/O
968	8.58	Dimethachlor	(50563-36-5)	36319	Cc1cccc(c1N(CCOC)C(=O)CCl)C

continued

969	8.59	Mefloquine	(53230-10-7)	3906	c1cc2c(cc(nc2c(c1)C(F)(F)F)C(F)(F)F)C(C3CCCCN3)O
970	8.6	Phorate-oxon	(2600-69-3)	68291	CCOP(=O)(OCC)SCSCC
971	8.6	Flamprop	(58667-63-3)	39044	CC(C(=O)O)N(c1ccc(c(c1)Cl)F)C(=O)c2ccccc2
972	8.6	Diphacinone	(82-66-6)	6463	c1ccc(cc1)C(c2ccccc2)C(=O)C3C(=O)c4ccccc4C3=O
973	8.61	Lormetazepam	(848-75-9)	12750	CN1c2ccc(cc2C(=NC(C1=O)O)c3ccccc3Cl)Cl
974	8.61	Thiophanat-ethyl	(23564-06-9)	2297684	CCOC(=O)NC(=S)Nc1ccccc1NC(=S)NC(=O)OCC
975	8.61	AM-1220-Azepane	(1348081-04-8)	29341387	CN1CCCCC(C1)n2cc(c3c2cccc3)C(=O)c4cccc5c4cccc5
976	8.62	Fenpropidin	(67306-00-7)	82797	CC(Cc1ccc(cc1)C(C)(C)C)CN2CCCCC2
977	8.63	Aripiprazole	(129722-12-9)	54790	c1cc(c(c(c1)Cl)Cl)N2CCN(CC2)CCCCOc3ccc4c(c3)NC(=O)CC4
978	8.65	Diazinon-O-analog (Diazoxon)	(962-58-3)	13157	CCOP(=O)(OCC)Oc1cc(nc(n1)C(C)C)C
979	8.66	Sibutramine	(106650-56-0)	5021	CC(C)CC(C1(CCC1)c2ccc(cc2)Cl)N(C)C
980	8.68	Norsibutramine (Desmethylsibutramine)	(na)	8374698	Clc1ccc(cc1)C2(C(NC)CC(C)C)CCC2
981	8.68	Agomelatine	(138112-76-2)	74141	CC(=O)NCCc1cccc2c1cc(cc2)OC
982	8.7	Methidathion	(950-37-8)	13115	COc1nn(c(=O)s1)CSP(=S)(OC)OC
983	8.7	Chlorantraniliprole	(500008-45-7)	9446648	CC1=CC(=CC(=C1NC(=O)C2=CC(=NN2C3=C(C=CC=N3)Cl)Br)C(=O)NC)Cl
984	8.73	Flumioxazin	(103361-09-7)	83443	C#CCN1c2cc(c(cc2OCC1=O)F)N3C(=O)C4=C(C3=O)CCCC4
985	8.73	Pimozide	(2062-78-4)	15520	c1ccc2c(c1)nc(n2C3CCN(CC3)CCCC(c4ccc(cc4)F)c5ccc(cc5)F)O
986	8.74	Corticosterone	(50-22-6)	388799	CC12CCC(=O)C=C1CCC3C2C(CC4(C3CCC4C(=O)CO)C)O
987	8.74	Desalkylflurazepam (Norfludiazepam)	(2886-65-9)	4381	c1ccc(c(c1)C2=NCC(=Nc3c2cc(cc3)Cl)O)F
988	8.75	Phenmedipham	(13684-63-4)	23134	Cc1cccc(c1)/N=C(\O)/Oc2cccc(c2)/N=C(\O)/OC
989	8.76	Dimefuron	(34205-21-5)	82721	CC(C)(C)c1nn(c(=O)o1)c2ccc(cc2Cl)NC(=O)N(C)C
990	8.77	Bensulfuron-methyl	(83055-99-6)	49630	COc1cc(nc(n1)NC(=O)NS(=O)(=O)Cc2ccccc2C(=O)OC)OC
991	8.77	Bicalutamide	(90357-06-5)	2284	CC(CS(=O)(=O)c1ccc(cc1)F)(C(=O)Nc2ccc(c(c2)C(F)(F)F)C#N)O
992	8.77	Bezafibrate	(41859-67-0)	35728	CC(C)(C(=O)O)Oc1ccc(cc1)CCNC(=O)c2ccc(cc2)Cl
993	8.77	Captan	(133-06-2)	8287	C1C=CCC2C1C(=O)N(C2=O)SC(Cl)(Cl)Cl
994	8.78	Imazosulfuron	(122548-33-8)	83451	COc1cc(nc(n1)NC(=O)NS(=O)(=O)c2c(nc3n2cccc3)Cl)OC
995	8.79	Melitracen	(5118-29-6)	23697	CC1(c2ccccc2C(=CCCN(C)C)c3c1cccc3)C
996	8.79	Secbumeton	(26259-45-0)	30881	CCC(C)Nc1nc(nc(n1)OC)NCC

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997	8.79	Cetirizine	(83881-51-0)	2577	c1ccc(cc1)C(c2ccc(cc2)Cl)N3CCN(CC3)CCOCC(=O)O
998	8.79	Methfuroxam	(28730-17-8)	31609	Cc1c(oc(c1C(=O)Nc2ccccc2)C)C
999	8.8	Hydrocortisone 21-acetate	(50-03-3)	3515	CC(=O)OCC(=O)C1(CCC2C1(CC(C3C2CCC4=CC(=O)CCC34C)O)C)O
1000	8.8	Perazine	(84-97-9)	4582	CN1CCN(CC1)CCCN2c3ccccc3Sc4c2cccc4
1001	8.81	5-[3-(4-Methoxybenzoyl)-1H-indol-1-yl]pentanoic acid (RCS-4-M-5-COOH-Pentyl)	(1427521-39-8)	29341868	COc1ccc(cc1)C(=O)c2cn(c3c2cccc3)CCCCC(=O)O
1002	8.82	Fenthion-oxon	(6552-12-1)	21567	Cc1cc(ccc1SC)OP(=O)(OC)OC
1003	8.82	Fluridone	(59756-60-4)	39255	Cn1cc(c(=O)c(c1)c2cccc(c2)C(F)(F)F)c3ccccc3
1004	8.83	Salmeterol	(89365-50-4)	4968	c1ccc(cc1)CCCCOCCCCCCNCC(c2ccc(c(c2)CO)O)O
1005	8.83	Midazolam	(59467-70-8)	4047	Cc1ncc2n1-c3ccc(cc3C(=NC2)c4ccccc4F)Cl
1006	8.84	Dicloran	(99-30-9)	7152	c1c(cc(c(c1Cl)N)Cl)[N+](=O)[O-]
1007	8.85	Aprindine	(37640-71-4)	2132	CCN(CC)CCCN(c1ccccc1)C2Cc3ccccc3C2
1008	8.86	Prometon	(1610-18-0)	4759	CC(C)/N=c\1/[nH]/c(=N\C(C)C)/nc([nH]1)OC
1009	8.86	Bispyribac	(125401-75-4)	391332	COc1cc(nc(n1)Oc2cccc(c2C(=O)O)Oc3nc(cc(n3)OC)OC)OC
1010	8.86	Cetirizine (Methyl ester)	(83881-46-3)	528920	COC(=O)COCCN1CCN(CC1)C(c2ccccc2)c3ccc(cc3)Cl
1011	8.87	Benoxacor	(98730-04-2)	56101	CC1COc2ccccc2N1C(=O)C(Cl)Cl
1012	8.85	Sertraline	(79617-96-2)	5014	CNC1CCC(c2c1cccc2)c3ccc(c(c3)Cl)Cl
1013	8.89	Chlorpromazine	(50-53-3)	2625	CN(C)CCCN1c2ccccc2Sc3c1cc(cc3)Cl
1014	8.89	Clomazone (Command)	(81777-89-1)	49469	CC1(CON(C1=O)Cc2ccccc2Cl)C
1015	8.89	Azinphos-methyl (Guthion)	(86-50-0)	2181	COP(=S)(OC)SCn1c(=O)c2ccccc2nn1
1016	8.89	Prenylamine	(390-64-7)	9418	CC(Cc1ccccc1)NCCC(c2ccccc2)c3ccccc3
1017	8.91	Demeton-S	(126-75-0)	23115	CCOP(=O)(OCC)SCCSCC
1018	8.91	Fenpiclonil	(74738-17-3)	82824	c1cc(c(c(c1)Cl)Cl)c2c[nH]cc2C#N
1019	8.91	Nortramadol (N-Desmethyltramadol)	(75377-45-6)	171856	CNCC1CCCCC1(c2cccc(c2)OC)O
1020	8.92	Oxybutynin	(5633-20-5)	4473	CCN(CC)CC#CCOC(=O)C(c1ccccc1)(C2CCCCC2)O
1021	8.93	Phenazepam	(51753-57-2)	36657	c1ccc(c(c1)C2=NCC(=O)Nc3c2cc(cc3)Br)Cl
1022	8.94	Phosmet	(732-11-6)	12367	COP(=S)(OC)SCN1C(=O)c2ccccc2C1=O
1023	8.95	Acifluorfen (as the NH4 adduct)	(50594-66-6)	40113	c1cc(c(cc1C(F)(F)F)Cl)Oc2ccc(c(c2)C(=O)O)[N+](=O)[O-]

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1024	8.96	Clothiapine	(2058-52-8)	15510	CN1CCN(CC1)C2=Nc3ccccc3Sc4c2cc(cc4)Cl
1025	8.98	Embutramide	(15687-14-6)	25547	CCC(CC)(CNC(=O)CCCO)c1cccc(c1)OC
1026	8.98	Phenylbutazone	(50-33-9)	4617	CCCCC1C(=O)N(N(C1=O)c2ccccc2)c3ccccc3
1027	8.99	Furalaxyl	(57646-30-7)	38763	Cc1cccc(c1N(C(C)C(=O)OC)C(=O)c2ccco2)C
1028	8.99	Chlorprotixene	(113-59-7)	580849	CN(C)CC/C=C\1/c2ccccc2Sc3c1cc(cc3)Cl
1029	8.99	Naproxen	(22204-53-1)	1262	CC(c1ccc2cc(ccc2c1)OC)C(=O)O
1030	8.99	2-Amino-5-nitrobenzophenone	(1775-95-7)	14916	c1ccc(cc1)C(=O)c2cc(ccc2N)[N+](=O)[O-]
1031	9	Valsartan	(137862-53-4)	5448	CCCCC(=O)N(Cc1ccc(cc1)c2ccccc2c3n[nH]nn3)C(C(C)C)C(=O)O
1032	9	Terbufos-sulfoxid	(10548-10-4)	23684	CCOP(=S)(OCC)SCS(=O)C(C)(C)C
1033	9.01	Penbutolol	(36507-48-9)	4562	CC(C)(C)NCC(COc1ccccc1C2CCCC2)O
1034	9.01	Terbufossulfone	(56070-16-7)	38067	CCOP(=S)(OCC)SCS(=O)(=O)C(C)(C)C
1035	9.01	Fomesafen	(72178-02-0)	46694	CS(=O)(=O)/N=C(/c1cc(ccc1[N+](=O)[O-])Oc2ccc(cc2Cl)C(F)(F)F)\O
1036	9.02	Azoxystrobin	(131860-33-8)	2298772	CO/C=C(\c1ccccc1Oc2cc(ncn2)Oc3ccccc3C#N)/C(=O)OC
1037	9.03	Quinapril	(85441-61-8)	4830	CCOC(=O)C(CCc1ccccc1)NC(C)C(=O)N2Cc3ccccc3CC2C(=O)O
1038	9.03	Exemestane	(107868-30-4)	3659700	CC12CCC3C(C1CCC2=O)CC(=C)C4=CC(=O)C=CC34C
1039	9.03	Clomipramine	(303-49-1)	2699	CN(C)CCCN1c2ccccc2CCc3c1cc(cc3)Cl
1040	9.04	O.O.O-Triethylphosphorothioate	(126-68-1)	29087	CCOP(=S)(OCC)OCC
1041	9.04	Diethofencarb	(87130-20-9)	82840	CCOc1ccc(cc1OCC)NC(=O)OC(C)C
1042	9.04	Zotepine	(26615-21-4)	5534	CN(C)CCOC1=Cc2ccccc2Sc3c1cc(cc3)Cl
1043	9.05	Fenobucarb	(3766-81-2)	18452	CCC(C)c1ccccc1OC(=O)NC
1044	9.05	Phenprocoumon	(435-97-2)	10441592	CCC(C1=CC=CC=C1)C2=C(C3=CC=CC=C3OC2=O)O
1045	9.08	Dixyrazine (Esocalm)	(2470-73-7)	16265	CC(CN1CCN(CC1)CCOCCO)CN2c3ccccc3Sc4c2cccc4
1046	9.08	Warfarin	(81-81-2)	10442445	CC(=O)CC(C1=CC=CC=C1)C2=C(C3=CC=CC=C3OC2=O)O
1047	9.09	Methohexital	(151-83-7)	8683	CCC#CC(C)C1(C(=O)NC(=O)N(C1=O)C)CC=C
1048	9.09	Sebuthylazine	(7286-69-3)	22172	CCC(C)Nc1nc(nc(n1)Cl)NCC
1049	9.09	Flunixin	(38677-85-9)	34911	Cc1c(cccc1Nc2c(cccn2)C(=O)O)C(F)(F)F
1050	9.09	Imazalil (Enilconazole)	(35554-44-0)	34116	C=CCOC(Cn1ccnc1)c2ccc(cc2Cl)Cl
1051	9.12	Riluzole	(1744-22-5)	4892	c1cc2c(cc1OC(F)(F)F)sc(n2)N

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1052	9.14	Methoprotryne	(841-06-5)	12730	CC(C)Nc1nc(nc(n1)SC)NCCCOC
1053	9.14	Ethofumesate	(26225-79-6)	30816	CCOC1C(c2cc(ccc2O1)OS(=O)(=O)C)(C)C
1054	9.14	Norclomipramine (Desmethylclomipramine)	(303-48-0) as the HCl salt	540947	CNCCCN1c2ccccc2CCc3c1cc(cc3)Cl
1055	9.14	Mizolastine	(108612-45-9)	59315	CN(c1[nH]c(=O)ccn1)C2CCN(CC2)c3nc4ccccc4n3Cc5ccc(cc5)F
1056	9.14	Flurtamone	(96525-23-4)	82853	CNC1=C(C(=O)C(O1)c2ccccc2)c3cccc(c3)C(F)(F)F
1057	9.16	Terbumeton	(33693-04-8)	33617	CC/N=c/1\nc([nH]c(n1)OC)NC(C)(C)C
1058	9.16	Ametryn	(834-12-8)	12705	CC/N=c/1\[nH]/c(=N/C(C)C)/[nH]c(n1)SC
1059	9.16	Propazine	(139-40-2)	4768	CC(C)/N=c\1/[nH]/c(=N\C(C)C)/nc([nH]1)Cl
1060	9.17	Acrinathrin	(101007-06-1)	4859914	CC1(C(C1C(=O)OC(C#N)c2cccc(c2)Oc3ccccc3)/C=C/C(=O)OC(C(F)(F)F)C(F)(F)F)C
1061	9.17	Nuarimol	(63284-71-9)	82786	c1ccc(c(c1)C(c2ccc(cc2)F)(c3cncnc3)O)Cl
1062	9.18	RCS-4-M-5-OH-Pentyl	(1379604-66-6)	29341441	COc1ccc(cc1)C(=O)c2cn(c3c2cccc3)CCCCCO
1063	9.19	Drofenine	(1679-76-1)	3054	CCN(CC)CCOC(=O)C(c1ccccc1)C2CCCCC2
1064	9.19	Albendazole	(54965-21-8)	1998	CCCSc1ccc2c(c1)nc([nH]2)NC(=O)OC
1065	9.19	Fluspirilene	(1841-19-6)	3279	c1ccc(cc1)N2CNC(=O)C23CCN(CC3)CCCC(c4ccc(cc4)F)c5ccc(cc5)F
1066	9.2	Diponium	(58875-33-5)	64834	CC[N+](CC)(CC)CCOC(=O)C(C1CCCC1)C2CCCC2
1067	9.22	Fenamidone	(161326-34-7)	10701914	CS/C2=N/C(C)(C(=O)N2Nc1ccccc1)c3ccccc3
1068	9.22	Triflusulfuron-methyl	(126535-15-7)	83452	Cc1cccc(c1S(=O)(=O)N/C(=N/c2nc(nc(n2)OCC(F)(F)F)N(C)C)/O)C(=O)OC
1069	9.23	Nordiazepam (Desmethyldiazepam)	(1088-11-5)	2890	c1ccc(cc1)C2=NCC(=Nc3c2cc(cc3)Cl)O
1070	9.23	Pioglitazone	(111025-46-8)	4663	CCc1ccc(nc1)CCOc2ccc(cc2)CC3C(=O)NC(=O)S3
1071	9.24	Parathion-methyl	(298-00-0)	3987	COP(=S)(OC)Oc1ccc(cc1)[N+](=O)[O-]
1072	9.25	TCMTB [(1,3-Benzothiazol-2-ylsulfanyl)methyl thiocyanate]	(21564-17-0)	28480	c1ccc2c(c1)nc(s2)SCSC#N
1073	9.25	Ticlopidine	(55142-85-3)	5273	c1ccc(c(c1)CN2CCc3c(ccs3)C2)Cl
1074	9.25	Halofenozide	(112226-61-6)	102925	CC(C)(C)N(C(=O)c1ccccc1)NC(=O)c2ccc(cc2)Cl
1075	9.26	Dimethenamid	(87674-68-8)	82842	Cc1csc(c1N(C(C)COC)C(=O)CCl)C
1076	9.26	Iprindol	(5560-72-5)	20417	CN(C)CCCn1c2ccccc2c3c1CCCCCC3
1077	9.26	Sertindole	(106516-24-9)	54229	c1cc(ccc1n2cc(c3c2ccc(c3)Cl)C4CCN(CC4)CCN5CCNC5=O)F
1078	9.27	Inabenfide	(82211-24-3)	83423	c1ccc(cc1)C(c2cc(ccc2NC(=O)c3ccncc3)Cl)O

continued

1079	9.27	Triflupromazine	(146-54-3)	5367	CN(C)CCCN1c2ccccc2Sc3c1cc(cc3)C(F)(F)F
1080	9.27	Chlorbufam	(1967-16-4)	15261	CC(C#C)OC(=O)Nc1cccc(c1)Cl
1081	9.29	Dabigatran etexilate	(211915-06-9)	4948999	CCCCCCOC(=O)/N=C(\c1ccc(cc1)NCc2nc3cc(ccc3n2C)C(=O)N(CCC(=O)OCC)c4ccccn4)/N
1082	9.3	Chlorimuronethyl	(90982-32-4)	50690	CCOC(=O)c1ccccc1S(=O)(=O)NC(=O)Nc2nc(cc(n2)Cl)OC
1083	9.3	Linuron	(330-55-2)	9130	CN(/C(=N\c1ccc(c(c1)Cl)Cl)/O)OC
1084	9.3	Mexacarbate	(315-18-4)	9043	Cc1cc(cc(c1N(C)C)C)OC(=O)NC
1085	9.31	Clemastine	(15686-51-8)	2679	CC(c1ccccc1)(c2ccc(cc2)Cl)OCCC3CCCN3C
1086	9.32	Ethiprole	(181587-01-9)	8106298	CCS(=O)C1=C(N(N=C1C#N)C2=C(C=C(C=C2Cl)C(F)(F)F)Cl)N
1087	9.32	Ethoxysulfuron	(126801-58-9)	2858750	CCOc1ccccc1OS(=O)(=O)NC(=O)Nc2nc(cc(n2)OC)OC
1088	9.33	JWH-073-M-N-Butanoic acid	(1307803-52-6)	26458429	c1ccc2c(c1)cccc2C(=O)c3cn(c4c3cccc4)CCCC(=O)O
1089	9.34	Methiocarb (Mercaptodimethur)	(2032-65-7)	15417	Cc1cc(cc(c1SC)C)O/C(=N/C)/O
1090	9.34	Thioridazine	(50-52-2)	5253	CN1CCCCC1CCN2c3ccccc3Sc4c2cc(cc4)SC
1091	9.35	Deflazacort	(14484-47-0)	24883	CC1=NC2(C(O1)CC3C2(CC(C4C3CCC5=CC(=O)C=CC45C)O)C)C(=O)COC(=O)C
1092	9.36	Terbuthylazine	(5915-41-3)	20848	CC/N=c/1\[nH]c(nc(n1)Cl)NC(C)(C)C
1093	9.36	Propiverine	(60569-19-9)	4773	CCCOC(c1ccccc1)(c2ccccc2)C(=O)OC3CCN(CC3)C
1094	9.37	Nandrolone	(434-22-0)	191235	CC12CCC3C(C1CCC2O)CCC4=CC(=O)CCC34
1095	9.38	Propanil	(709-98-8)	4764	CCC(=O)Nc1ccc(c(c1)Cl)Cl
1096	9.39	Crotoxyphos	(7700-17-6)	4522061	CC(c1ccccc1)OC(=O)/C=C(\C)/OP(=O)(OC)OC
1097	9.4	Boscalid	(188425-85-6)	184713	c1ccc(c(c1)c2ccc(cc2)Cl)/N=C(\c3cccnc3Cl)/O
1098	9.41	Proadifen	(302-33-0)	4741	CCCC(c1ccccc1)(c2ccccc2)C(=O)OCCN(CC)CC
1099	9.42	Isoxaben	(82558-50-7)	66323	CCC(C)(CC)c1cc(on1)NC(=O)c2c(cccc2OC)OC
1100	9.44	Pyrimethanil	(53112-28-0)	82753	Cc1cc(nc(n1)Nc2ccccc2)C
1101	9.44	Pentifylline	(1028-33-7)	63738	CCCCCCn1c(=O)c2c(ncn2C)n(c1=O)C
1102	9.44	Irbesartan	(138402-11-6)	3618	CCCCC1=NC2(CCCC2)C(=O)N1Cc3ccc(cc3)c4ccccc4c5n[nH]nn5
1103	9.48	Fludioxonil	(131341-86-1)	77916	c1cc(c2c(c1)OC(O2)(F)F)c3c[nH]cc3C#N
1104	9.48	Benthiavalicarb-isopropyl	(177406-68-7)	(N/A)	CC(C)C(C(=O)NC(C)C1=NC2=C(S1)C=C(C=C2)F)NC(=O)OC(C)C
1105	9.49	Paclobutrazole	(76738-62-0)	536024	CC(C)(C)C(C(Cc1ccc(cc1)Cl)n2cncn2)O
1106	9.51	Promecarb	(2631-37-0)	16563	Cc1cc(cc(c1)OC(=O)NC)C(C)C

continued

1107	9.52	Flutolanil	(66332-96-5)	43579	CC(C)Oc1cccc(c1)/N=C(/c2ccccc2C(F)(F)F)\O
1108	9.53	Diazepam	(439-14-5)	2908	CN1c2ccc(cc2C(=NCC1=O)c3ccccc3)Cl
1109	9.55	Perphenazine	(58-39-9)	4586	c1ccc2c(c1)N(c3cc(ccc3S2)Cl)CCCN4CCN(CC4)CCO
1110	9.55	Chlorbromuron	(13360-45-7)	24141	CN(C(=O)Nc1ccc(c(c1)Cl)Br)OC
1111	9.55	WIN-48-098 (Pravadoline)	(92623-83-1)	50942	Cc1c(c2ccccc2n1CCN3CCOCC3)C(=O)c4ccc(cc4)OC
1112	9.56	Methoxyfenozide	(161050-58-4)	94755	Cc1cc(cc(c1)C(=O)N(C(C)(C)C)/N=C(/c2cccc(c2C)OC)\O)C
1113	9.56	JWH-073-M-4-OH-Butyl [1-(4-Hydroxybutyl)-1H-indol-3-yl)(naphthalen-1-yl)methanone]	(335161-14-3)	26458428	c1ccc2c(c1)cccc2C(=O)c3cn(c4c3cccc4)CCCCO
1114	9.59	Malathion	(121-75-5)	3864	CCOC(=O)CC(C(=O)OCC)SP(=S)(OC)OC
1115	9.59	Niflumic acid	(4394-00-7)	4333	c1cc(cc(c1)Nc2c(cccn2)C(=O)O)C(F)(F)F
1116	9.59	Cyclovalone	(579-23-7)	1266902	COc1c(ccc(c1)/C=C\2/C(=O)/C(=C/c3cc(c(cc3)O)OC)/CCC2)O
1117	9.6	Bensultap	(17606-31-4)	78640	CN(C)C(CSS(=O)(=O)c1ccccc1)CSS(=O)(=O)c2ccccc2
1118	9.61	Proquazone	(22760-18-5)	29222	Cc1ccc2c(c1)n(c(=O)nc2c3ccccc3)C(C)C
1119	9.61	Isradipine	(75695-93-1)	3652	CC1=C(C(C(=C(N1)C)C(=O)OC(C)C)c2cccc3c2non3)C(=O)OC
1120	9.61	Suxibuzone	(27470-51-5)	5169	CCCCC1(C(=O)N(N(C1=O)c2ccccc2)c3ccccc3)COC(=O)CCC(=O)O
1121	9.62	Mepronil	(55814-41-0)	37994	Cc1ccccc1/C(=N/c2cccc(c2)OC(C)C)/O
1122	9.62	Flurprimidol	(56425-91-3)	66319	CC(C)C(c1ccc(cc1)OC(F)(F)F)(c2cncnc2)O
1123	9.64	Dicamba-methyl	(6597-78-0)	73143	COc1c(ccc(c1C(=O)OC)Cl)Cl
1124	9.65	Acibenzolar-S-Methyl	(135158-54-2)	77928	CSC(=O)c1cccc2c1snn2
1125	9.68	SWEP /MCC [Methyl (3,4-dichlorophenyl)carbamate]	(1918-18-9)	15173	COC(=O)Nc1ccc(c(c1)Cl)Cl
1126	9.68	Triadimefon	(43121-43-3)	36029	CC(C)(C)C(=O)C(n1cncn1)Oc2ccc(cc2)Cl
1127	9.68	Daimuron (Dymron)	(42609-52-9)	35899	Cc1ccc(cc1)NC(=O)NC(C)(C)c2ccccc2
1128	9.69	Propyzamide (Pronamide)	(23950-58-5)	29822	CC(C)(C#C)/N=C(/c1cc(cc(c1)Cl)Cl)\O
1129	9.69	Terfenadine	(50679-08-8)	5212	CC(C)(C)c1ccc(cc1)C(CCCN2CCC(CC2)C(c3ccccc3)(c4ccccc4)O)O
1130	9.71	Aziprotryne	(4658-28-0)	2297441	CC(C)Nc1nc(nc(n1)SC)N=[N+]=[N-]
1131	9.71	JWH-018-N-Pentanoic acid	(1254475-87-0)	26458423	c1ccc2c(c1)cccc2C(=O)c3cn(c4c3cccc4)CCCCC(=O)O
1132	9.74	Barban (endo) (isomerA)	(101-27-9)	7270	c1cc(cc(c1)Cl)NC(=O)OCC#CCCl
1133	9.74	Anilazine (Dyrene)	(101-05-3)	7260	c1ccc(c(c1)Nc2nc(nc(n2)Cl)Cl)Cl

continued

1134	9.74	Myclobutanil	(88671-89-0)	6096	CCCCC(Cn1cncn1)(C#N)c2ccc(cc2)Cl
1135	9.74	Chlorophacinone	(3691-35-8)	18286	c1ccc(cc1)C(c2ccc(cc2)Cl)C(=O)C3C(=O)c4ccccc4C3=O
1136	9.77	Canrenone	(976-71-6)	282625	CC12CCC(=O)C=C1C=CC3C2CCC4(C3CCC45CCC(=O)O5)C
1137	9.78	Chlorpropham	(101-21-3)	2627	CC(C)OC(=O)Nc1cccc(c1)Cl
1138	9.79	Indinavir	(150378-17-9)	3577	CC(C)(C)NC(=O)C1CN(CCN1CC(CC(Cc2ccccc2)C(=O)NC3c4ccccc4CC3O)O)Cc5cccnc5
1139	9.79	Propetamphos	(31218-83-4)	4939080	CCNP(=S)(OC)O/C(=C\C(=O)OC(C)C)/C
1140	9.79	Ketazolam	(27223-35-4)	31110	CC1=CC(=O)N2CC(=O)N(c3ccc(cc3C2(O1)c4ccccc4)Cl)C
1141	9.79	Zuclopenthixol	(53772-83-1)	4470984	c1ccc2c(c1)/C(=C/CCN3CCN(CC3)CCO)/c4cc(ccc4S2)Cl
1142	9.79	Glibornuride	(26944-48-9)	31034	Cc1ccc(cc1)S(=O)(=O)NC(=O)NC2C3CCC(C2O)(C3(C)C)C
1143	9.79	Pyridaphenthion	(119-12-0)	8078	CCOP(=S)(OCC)Oc1ccc(=O)n(n1)c2ccccc2
1144	9.82	Nabumetone	(42924-53-8)	4256	CC(=O)CCc1ccc2cc(ccc2c1)OC
1145	9.82	Iprovalicarb Peak 1 & 2	(140923-17-7)	5290696	CC1=CC=C(C=C1)C(C)NC(=O)C(C(C)C)NC(=O)OC(C)C
1146	9.83	Capsaicin	(404-86-4)	1265957	CC(C)/C=C/CCCC/C(=N/Cc1ccc(c(c1)OC)O)/O
1147	9.83	Mefenacet	(73250-68-7)	82816	CN(c1ccccc1)C(=O)COc2nc3ccccc3s2
1148	9.84	Flurochloridone	(61213-25-0)	82780	c1cc(cc(c1)N2CC(C(C2=O)Cl)CCl)C(F)(F)F
1149	9.85	Piprozolin	(17243-64-0)	4744588	CCN1C(=CC(=O)OCC)SC(C1=O)N2CCCCC2
1150	9.86	Dimethylvinphos	(2274-67-1)	4938499	COP(=O)(OC)O/C(=C\Cl)/c1ccc(cc1Cl)Cl
1151	9.86	Cinnarizine	(298-57-7)	1299022	c1ccc(cc1)/C=C\CN2CCN(CC2)C(c3ccccc3)c4ccccc4
1152	9.87	Isazophos	(42509-80-8)	35885	CCOP(=S)(OCC)Oc1nc(n(n1)C(C)C)Cl
1153	9.87	Bifenazate	(149877-41-8)	154052	CC(C)OC(=O)NNc1cc(ccc1OC)c2ccccc2
1154	9.88	JWH-200 [{1-[2-(4-Morpholinyl)ethyl]-1H-indol-3-yl}(1-naphthyl)methanone]	(103610-04-4)	8221134	C1COCCN1CCN2C=C(C3=CC=CC=C32)C(=O)C4=CC=CC5=CC=CC=C54
1155	9.89	Molinate	(2212-67-1)	15790	CCSC(=O)N1CCCCCC1
1156	9.89	Butafenacil	(134605-64-4)	10001509	CC(C)(C(=O)OCC=C)OC(=O)c1cc(ccc1Cl)n2c(=O)cc(n(c2=O)C)C(F)(F)F
1157	9.89	Prochlorperazine	(58-38-8)	4748	CN1CCN(CC1)CCCN2c3ccccc3Sc4c2cc(cc4)Cl
1158	9.9	Acemetacin	(53164-05-9)	1904	Cc1c(c2cc(ccc2n1C(=O)c3ccc(cc3)Cl)OC)CC(=O)OCC(=O)O
1159	9.9	Triazophos	(24017-47-8)	29847	CCOP(=S)(OCC)Oc1ncn(n1)c2ccccc2
1160	9.91	JWH-073-M-3-OH-Butyl [1-(3-Hydroxybutyl)-1H-indol-3-yl](1-naphthyl)methanone	1320363-48-1	29341402	CC(CCn1cc(c2c1cccc2)C(=O)c3cccc4c3cccc4)O

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1161	9.92	Fenitrothion	(122-14-5)	28941	Cc1cc(ccc1[N+](=O)[O-])OP(=S)(OC)OC
1162	9.93	Fluphenazine	(69-23-8)	3255	c1ccc2c(c1)N(c3cc(ccc3S2)C(F)(F)F)CCCN4CCN(CC4)CCO
1163	9.93	Chromafenozide	(143807-66-3)	8332992	Cc1cc(cc(c1)C(=O)N(C(C)(C)C)NC(=O)c2ccc3c(c2C)CCCO3)C
1164	9.93	Triadimenol I &2	(55219-65-3)	37749	CC(C)(C)C(C(n1cncn1)Oc2ccc(cc2)Cl)O
1165	9.95	Nefazodone	(83366-66-9)	4294	CCc1nn(c(=O)n1CCOc2ccccc2)CCCN3CCN(CC3)c4cccc(c4)Cl
1166	9.95	Fluoxastrobin	(361377-29-9)	9223963	CO/N=C(\C1=CC=CC=C1OC2=C(C(=NC=N2)OC3=CC=CC=C3Cl)F)/C4=NOCCO4
1167	9.96	Atorvastatin	(134523-00-5)	2163	CC(C)c1c(c(c(n1CCC(CC(CC(=O)O)O)O)c2ccc(cc2)F)c3ccccc3)C(=O)Nc4ccccc4
1168	9.97	Clopenthixol Peaks 1 & 2	(982-24-1)	11945	c1ccc2c(c1)/C(=C\CCN3CCN(CC3)CCO)/c4cc(ccc4S2)Cl
1169	9.98	Glibenclamide	(10238-21-8)	3368	COc1ccc(cc1/C(=N\CCc2ccc(cc2)S(=O)(=O)N/C(=N\C3CCCCC3)/O)/O)Cl
1170	9.98	Captafol	(2425-06-1)	16139	C1C=CCC2C1C(=O)N(C2=O)SC(C(Cl)Cl)(Cl)Cl
1171	9.99	Dicycloverine	(77-19-0)	2934	CCN(CC)CCOC(=O)C1(CCCCC1)C2CCCCC2
1172	9.99	Chloroxuron	(1982-47-4)	15299	CN(C)C(=O)Nc1ccc(cc1)Oc2ccc(cc2)Cl
1173	10.01	Bromhexine	(3572-43-8)	2348	CN(Cc1cc(cc(c1N)Br)Br)C2CCCCC2
1174	10.02	Thiopropazate	(84-06-0)	6504	CC(=O)OCCN1CCN(CC1)CCCN2c3ccccc3Sc4c2cc(cc4)Cl
1175	10.02	Camazepam	(36104-80-0)	34285	CN1c2ccc(cc2C(=NC(C1=O)OC(=O)N(C)C)c3ccccc3)Cl
1176	10.02	Dichlofluanid	(1085-98-9)	13520	CN(C)S(=O)(=O)N(c1ccccc1)SC(F)(Cl)Cl
1177	10.04	JWH-018-M-N-5-OH-Pentyl	(335161-21-2)	26458422	c1ccc2c(c1)cccc2C(=O)c3cn(c4c3cccc4)CCCCCO
1178	10.05	Fenhexamid	(126833-17-8)	184726	CC1(CCCCC1)C(=O)Nc2ccc(c(c2Cl)Cl)O
1179	10.05	Fluquinconazole	(136426-54-5)	77933	c1cc2c(cc1F)c(=O)n(c(n2)n3cncn3)c4ccc(cc4Cl)Cl
1180	10.06	Diphenylamine	(122-39-4)	11003	c1ccc(cc1)Nc2ccccc2
1181	10.06	Flufenacet	(142459-58-3)	77944	CC(C)N(c1ccc(cc1)F)C(=O)COc2nnc(s2)C(F)(F)F
1182	10.06	Prometryn	(7287-19-6)	4760	CC(C)/N=c\1/[nH]/c(=N\C(C)C)/nc([nH]1)SC
1183	10.07	Thenylchlor	(96491-05-3)	391337	Cc1cccc(c1N(Cc2c(ccs2)OC)C(=O)CCl)C
1184	10.07	Penfluridol	(26864-56-2)	31017	c1cc(ccc1C(CCCN2CCC(CC2)(c3ccc(c(c3)C(F)(F)F)Cl)O)c4ccc(cc4)F)F
1185	10.08	JWH-018-M-N-4-OH-Pentyl	(1320363-47-0)	29341417	CC(CCCn1cc(c2c1cccc2)C(=O)c3cccc4c3cccc4)O
1186	10.09	Pethoxamid	(106700-29-2)	4953376	CCOCCN(C(=O)CCl)C(=C(C)C)c1ccccc1
1187	10.11	Ethoxyquin	(91-53-2)	3177	CCOc1ccc2c(c1)C(=CC(N2)(C)C)C
1188	10.12	Climbazole	(38083-17-9)	34752	CC(C)(C)C(=O)C(n1ccnc1)Oc2ccc(cc2)Cl

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1189	10.12	Fenson (as sodium adduct)	(80-38-6)	6384	c1ccc(cc1)S(=O)(=O)Oc2ccc(cc2)Cl
1190	10.13	Triticonazole	(131983-72-7)	4941086	CC1(CC/C(=C/c2ccc(cc2)Cl)/C1(Cn3cncn3)O)C
1191	10.13	Nortetrazepam	(10379-11-0)	145774	c1cc2c(cc1Cl)C(=NCC(=O)N2)C3=CCCCC3
1192	10.13	Fenarimol	(60168-88-9)	39394	c1ccc(c(c1)C(c2ccc(cc2)Cl)(c3cncnc3)O)Cl
1193	10.13	CGA 321113 (Trifloxystrobin Metabolite)	(252913-85-2)	(N/A)	FC(F)(F)c1cccc(c1)C(\C)=N\OCc2ccccc2C(=N\OC)/C(=O)O
1194	10.13	JWH-200-M-4-OH-Ind ({4-Hydroxy-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl}(1-naphthyl)methanone)	(1427325-73-2)	29341839	c1ccc2c(c1)cccc2C(=O)c3cn(c4c3c(ccc4)O)CCN5CCOCC5
1195	10.14	Tetraconazole	(112281-77-3)	72518	c1cc(c(cc1Cl)Cl)C(Cn2cncn2)COC(C(F)F)(F)F
1196	10.14	Altretamine	(645-05-6)	2038	CN(C)c1nc(nc(n1)N(C)C)N(C)C
1197	10.15	Trietazine	(1912-26-1)	15157	CCNc1nc(nc(n1)Cl)N(CC)CC
1198	10.16	Mecarbam	(2595-54-2)	16491	CCOC(=O)N(C)C(=O)CSP(=S)(OCC)OCC
1199	10.16	Ethoprophos	(13194-48-4)	3173	CCCSP(=O)(OCC)SCCC
1200	10.17	Clotiazepam	(33671-46-4)	2709	CCc1cc2c(s1)N(C(=O)CN=C2c3ccccc3Cl)C
1201	10.17	Azinphos-ethyl	(2642-71-9)	16576	CCOP(=S)(OCC)SCn1c(=O)c2ccccc2nn1
1202	10.17	Etaconazole Peak 1 & 2	(60207-93-4)	82776	CCC1COC(O1)(Cn2cncn2)c3ccc(cc3Cl)Cl
1203	10.18	Chlorthion	(500-28-7)	9944	COP(=S)(OC)Oc1ccc(c(c1)Cl)[N+](=O)[O-]
1204	10.18	Napropamide	(15299-99-7)	25304	CCN(CC)C(=O)C(C)Oc1cccc2c1cccc2
1205	10.18	Ditalimfos	(5131-24-8)	19939	CCOP(=S)(N1C(=O)c2ccccc2C1=O)OCC
1206	10.19	Flupentixol	(2709-56-0)	4445173	c1ccc2c(c1)/C(=C/CCN3CCN(CC3)CCO)/c4cc(ccc4S2)C(F)(F)F
1207	10.19	Mepanipyrim	(110235-47-7)	77839	CC#Cc1cc(nc(n1)Nc2ccccc2)C
1208	10.2	Iopodic acid	(5587-89-3)	5051	CN(C)/C=N/c1c(cc(c(c1I)CCC(=O)O)I)I
1209	10.2	Coumachlor	(81-82-3)	10443016	CC(=O)CC(C1=CC=C(C=C1)Cl)C2=C(C3=CC=CC=C3OC2=O)O
1210	10.22	Chlorfenprop-methyl	(14437-17-3)	24868	COC(=O)C(Cc1ccc(cc1)Cl)Cl
1211	10.22	Procymidone	(32809-16-8)	33326	CC12CC1(C(=O)N(C2=O)c3cc(cc(c3)Cl)Cl)C
1212	10.22	Medazepam	(2898-12-6)	3901	CN1CCN=C(c2c1ccc(c2)Cl)c3ccccc3
1213	10.23	Terbutryn	(886-50-0)	12874	CC/N=c/1\nc([nH]c(n1)SC)NC(C)(C)C
1214	10.23	Thiometon	(640-15-3)	12024	CCSCCSP(=S)(OC)OC
1215	10.23	Acetochlor	(34256-82-1)	1911	CCc1cccc(c1N(COCC)C(=O)CCl)C

continued

1216	10.24	Trifluoperazine	(117-89-5)	5365	CN1CCN(CC1)CCCN2c3ccccc3Sc4c2cc(cc4)C(F)(F)F
1217	10.25	Tebutame	(35256-85-0)	83330	CC(C)N(Cc1ccccc1)C(=O)C(C)(C)C
1218	10.26	Epoxiconazole	(133855-98-8)	2564795	c1ccc(c(c1)C2C(O2)(Cn3cncn3)c4ccc(cc4)F)Cl
1219	10.28	Diclofenac	(15307-86-5)	2925	c1ccc(c(c1)CC(=O)O)Nc2c(cccc2Cl)Cl
1220	10.29	Nilvadipine	(75530-68-6)	4338	CC1=C(C(C(=C(N1)C#N)C(=O)OC)c2cccc(c2)[N+](=O)[O-])C(=O)OC(C)C
1221	10.29	Indomethacin	(53-86-1)	3584	Cc1c(c2cc(ccc2n1C(=O)c3ccc(cc3)Cl)OC)CC(=O)O
1222	10.29	Metolachlor	(51218-45-2)	4025	CCc1cccc(c1N(C(C)COC)C(=O)CCl)C
1223	10.31	Telmisartan	(144701-48-4)	59391	CCCc1nc2c(cc(cc2n1Cc3ccc(cc3)c4ccccc4C(=O)O)c5nc6ccccc6n5C)C
1224	10.32	Cyazofamid	(120116-88-3)	8037772	Cc1ccc(cc1)c2c(nc(n2S(=O)(=O)N(C)C)C#N)Cl
1225	10.33	Alachlor	(15972-60-8)	1994	CCc1cccc(c1N(COC)C(=O)CCl)CC
1226	10.34	Drazoxolon	(5707-69-7)	7844674	CC\1=NOC(=O)/C1=N\NC2=CC=CC=C2Cl
1227	10.35	Oryzalin	(19044-88-3)	27326	CCCN(CCC)c1c(cc(cc1[N+](=O)[O-])S(=O)(=O)N)[N+](=O)[O-]
1228	10.36	Nateglinide	(105816-04-4)	4290	CC(C)C1CCC(CC1)C(=O)NC(Cc2ccccc2)C(=O)O
1229	10.37	17-alpha-Methyltestosterone	(58-18-4)	(N/A)	CC12CCC(=O)C=C1CCC3C2CCC4(C3CCC4(C)O)C
1230	10.37	Lonazolac	(53808-88-1)	61957	c1ccc(cc1)n2cc(c(n2)c3ccc(cc3)Cl)CC(=O)O
1231	10.38	Fentin (as the cation triphenylstannylium) (1+)	(668-34-8)	82606	c1ccc(cc1)[Sn+](c2ccccc2)c3ccccc3
1232	10.38	Fenbuconazole	(114369-43-6)	77712	c1ccc(cc1)C(CCc2ccc(cc2)Cl)(Cn3cncn3)C#N
1233	10.39	Fipronil-desulfinyl	(205650-65-3)	11542895	c1c(cc(c(c1Cl)n2c(c(c(n2)C#N)C(F)(F)F)N)Cl)C(F)(F)F
1234	10.41	Thiethylperazine	(1420-55-9)	5245	CCSc1ccc2c(c1)N(c3ccccc3S2)CCCN4CCN(CC4)C
1235	10.43	Diphenoxylate	(915-30-0)	12919	CCOC(=O)C1(CCN(CC1)CCC(C#N)(c2ccccc2)c3ccccc3)c4ccccc4
1236	10.44	Triclopyr-methylester	(60825-26-5)	85360	COC(=O)COc1c(cc(c(n1)Cl)Cl)Cl
1237	10.45	Fenamiphos	(22224-92-6)	28827	CCOP(=O)(NC(C)C)Oc1ccc(c(c1)C)SC
1238	10.45	Tebufenozide	(112410-23-8)	82870	CCc1ccc(cc1)C(=O)NN(C(=O)c2cc(cc(c2)C)C)C(C)(C)C
1239	10.46	Tetrazepam	(10379-14-3)	23551	CN1c2ccc(cc2C(=NCC1=O)C3=CCCCC3)Cl
1240	10.46	Uniconazole	(83657-22-1)	4941231	CC(C)(C)C(/C(=C\c1ccc(cc1)Cl)/n2cncn2)O
1241	10.46	Iprodione	(36734-19-7)	34418	CC(C)/N=C(/N1CC(=O)N(C1=O)c2cc(cc(c2)Cl)Cl)\O
1242	10.46	AM-694 (1-(5-fluoropentyl)-3-(2-iodobenzoyl)indole)	(335161-03-0)	8064843	Ic1ccccc1C(=O)c3c2ccccc2n(c3)CCCCCF
1243	10.47	Rotenone	(83-79-4)	6500	CC(=C)C1Cc2c(ccc3c2OC4COc5cc(c(cc5C4C3=O)OC)OC)O1

continued

1244	10.48	Prazepam	(2955-38-6)	4721	c1ccc(cc1)C2=NCC(=O)N(c3c2cc(cc3)Cl)CC4CC4
1245	10.48	Flusilazole	(85509-19-9)	66326	C[Si](Cn1cncn1)(c2ccc(cc2)F)c3ccc(cc3)F
1246	10.49	Toremifene	(89778-26-7)	2275722	CN(C)CCOc1ccc(cc1)/C(=C(/CCCl)\c2ccccc2)/c3ccccc3
1247	10.5	Fipronil	(120068-37-3)	3235	c1c(cc(c(c1Cl)n2c(c(c(n2)C#N)S(=O)C(F)(F)F)N)Cl)C(F)(F)F
1248	10.5	2-Amino-5-chlorobenzophenone	(719-59-5)	12339	c1ccc(cc1)C(=O)c2cc(ccc2N)Cl
1249	10.5	Dicapthon (American Cyanamid 4124)	(2463-84-5)	16252	COP(=S)(OC)Oc1ccc(cc1Cl)[N+](=O)[O-]
1250	10.51	Bupirimate	(41483-43-6)	35588	CCCCc1c(nc(nc1OS(=O)(=O)N(C)C)NCC)C
1251	10.51	Bensulide	(741-58-2)	12397	CC(C)OP(=S)(OC(C)C)SCCNS(=O)(=O)c1ccccc1
1252	10.51	Crufomate	(299-86-5)	8941	CC(C)(C)c1ccc(c(c1)Cl)OP(=O)(NC)OC
1253	10.56	JWH-073-M-6-OH-Ind [(1-butyl-6-hydroxy-1H-indol-3-yl)(naphthalen-1-yl)-methanone)]	(1307803-48-0)	26458426	CCCCn1cc(c2c1cc(cc2)O)C(=O)c3cccc4c3cccc4
1254	10.58	Diflubenzuron	(35367-38-5)	34065	c1cc(c(c(c1)F)C(=O)NC(=O)Nc2ccc(cc2)Cl)F
1255	10.59	Tetrachlorvinphos (Stirofos)	(22248-79-9)	4447527	COP(=O)(OC)O/C(=C\Cl)/c1cc(c(cc1Cl)Cl)Cl
1256	10.59	Iprobenfos	(26087-47-8)	30753	CC(C)OP(=O)(OC(C)C)SCc1ccccc1
1257	10.59	Fenoxycarb	(79127-80-3)	46739	CCO/C(=N\CCOc1ccc(cc1)Oc2ccccc2)/O
1258	10.62	Nisoldipine	(63675-72-9)	4343	CC1=C(C(C(=C(N1)C)C(=O)OCC(C)C)c2ccccc2[N+](=O)[O-])C(=O)OC
1259	10.63	Pentanochlor	(2307-68-8)	15945	CCCC(C)C(=O)Nc1ccc(c(c1)Cl)C
1260	10.64	Vinclozolin (as Na adduct)	(50471-44-8)	36278	CC1(C(=O)N(C(=O)O1)c2cc(cc(c2)Cl)Cl)C=C
1261	10.64	Beflubutamid	(113614-08-7)	4953638	CCC(C(=O)NCc1ccccc1)Oc2ccc(c(c2)C(F)(F)F)F
1262	10.64	JWH-122-M-N-5-OH-Pentyl	(1379604-68-8)	29341442	Cc1ccc(c2c1cccc2)C(=O)c3cn(c4c3cccc4)CCCCCO
1263	10.66	Dimoxystrobin	(149961-52-4)	9111528	CC1=CC(=C(C=C1)C)OCC2=CC=CC=C2/C(=N\OC)/C(=O)NC
1264	10.67	Clodinafop-propargyl	(105512-06-9)	5291880	CC(C(=O)OCC#C)OC1=CC=C(C=C1)OC2=C(C=C(C=N2)Cl)F
1265	10.67	EPTC (S-Ethyl dipropylthiocarbamate)	(759-94-4)	12428	CCCN(CCC)C(=O)SCC
1266	10.67	Neburon	(555-37-3)	10672	CCCCN(C)C(=O)Nc1ccc(c(c1)Cl)Cl
1267	10.68	Ibuprofen	(15687-27-1)	3544	CC(C)Cc1ccc(cc1)C(C)C(=O)O
1268	10.68	Isoxadifen-ethyl	(163520-33-0)	4953634	CCOC(=O)C1=NOC(C1)(c2ccccc2)c3ccccc3
1269	10.68	Phenthoate	(2597-03-7)	16492	CCOC(=O)C(c1ccccc1)SP(=S)(OC)OC
1270	10.69	Glimepiride	(93479-97-1)	3357	CCC1=C(CN(C1=O)C(=O)NCCc2ccc(cc2)S(=O)(=O)NC(=O)NC3CCC(CC3)C)C

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1271	10.69	Carfentrazone-ethyl	(128639-02-1)	77773	CCOC(=O)C(Cc1cc(c(cc1Cl)F)n2c(=O)n(c(n2)C)C(F)F)Cl
1272	10.7	Fenpropimorph	(67564-91-4)	82798	CC1CN(CC(O1)C)CC(C)Cc2ccc(cc2)C(C)(C)C
1273	10.71	Parathion	(56-38-2)	13844817	CCOP(=S)(OCC)Oc1ccc(cc1)[N+](=O)[O-]
1274	10.71	Diclobutrazol (main peak)	(75736-33-3)	48148	CC(C)(C)C(C(Cc1ccc(cc1Cl)Cl)n2cncn2)O
1275	10.72	Thiazopyr	(117718-60-2)	82873	CC(C)Cc1c(c(nc(c1C(=O)OC)C(F)F)C(F)(F)F)C2=NCCS2
1276	10.73	Fenothiocarb	(62850-32-2)	40198	CN(C)C(=O)SCCCCOc1ccccc1
1277	10.74	Croconazole	(77175-51-0)	2777	C=C(c1ccccc1OCc2cccc(c2)Cl)n3ccnc3
1278	10.75	Fipronil-sulfide	(120067-83-6)	8129550	c1c(cc(c(c1Cl)n2c(c(c(n2)C#N)SC(F)(F)F)N)Cl)C(F)(F)F
1279	10.76	Kresoxim-methyl	(143390-89-0)	4813314	Cc1ccccc1OCc2ccccc2/C(=N\OC)/C(=O)OC
1280	10.76	Alanycarb	(83130-01-2)	7850539	CCOC(=O)CCN(CC1=CC=CC=C1)SN(C)C(=O)O/N=C(/C)\SC
1281	10.77	Sulfotepp	(3689-24-5)	18280	CCOP(=S)(OCC)OP(=S)(OCC)OCC
1282	10.78	Tolyfluanid	(731-27-1)	12364	Cc1ccc(cc1)N(SC(F)(Cl)Cl)S(=O)(=O)N(C)C
1283	10.78	Flufenzine (Diflovidazin)	(162320-67-4)	135715	c1ccc(c(c1)c2nnc(nn2)c3c(cccc3F)F)Cl
1284	10.79	Propaphos	(7292-16-2)	22177	CCCOP(=O)(OCCC)Oc1ccc(cc1)SC
1285	10.8	Edifenphos	(17109-49-8)	26320	CCOP(=O)(Sc1ccccc1)Sc2ccccc2
1286	10.8	Quinalphos	(13593-03-8)	24335	CCOP(=S)(OCC)Oc1cnc2ccccc2n1
1287	10.83	Penconazole	(66246-88-6)	82796	CCCC(Cn1cncn1)c2ccc(cc2Cl)Cl
1288	10.83	Chlorbenzoxamine	(522-18-9)	64700	Cc1ccccc1CN2CCN(CC2)CCOC(c3ccccc3)c4ccccc4Cl
1289	10.84	Flamprop-isopropyl	(52756-22-6)	37022	CC(C)OC(=O)C(C)N(c1ccc(c(c1)Cl)F)C(=O)c2ccccc2
1290	10.84	Anilofos	(64249-01-0)	82790	CC(C)N(c1ccc(cc1)Cl)C(=O)CSP(=S)(OC)OC
1291	10.85	Halcinonide	(3093-35-4)	3432	CC1(OC2CC3C4CCC5=CC(=O)CCC5(C4(C(CC3(C2(O1)C(=O)CCl)C)O)F)C)C
1292	10.85	Norethisterone acetate	(51-98-9)	471291	CC(=O)OC1(CCC2C1(CCC3C2CCC4=CC(=O)CCC34)C)C#C
1293	10.86	Aclonifen	(74070-46-5)	83411	c1ccc(cc1)Oc2ccc(c(c2Cl)N)[N+](=O)[O-]
1294	10.87	Tebuconazole	(107534-96-3)	77680	CC(C)(C)C(CCc1ccc(cc1)Cl)(Cn2cncn2)O
1295	10.87	Penfluron	(35367-31-8)	96832	c1cc(c(c(c1)F)C(=O)NC(=O)Nc2ccc(cc2)C(F)(F)F)F
1296	10.88	Cyanofenphos	(13067-93-1)	23911	CCOP(=S)(c1ccccc1)Oc2ccc(cc2)C#N
1297	10.88	Ebastine	(90729-43-4)	3079	CC(C)(C)c1ccc(cc1)C(=O)CCCN2CCC(CC2)OC(c3ccccc3)c4ccccc4
1298	10.89	Benalaxyl	(71626-11-4)	46525	Cc1cccc(c1N(C(C)C(=O)OC)C(=O)Cc2ccccc2)C

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1299	10.89	Oxetacaine	(126-27-2)	4460	CC(C)(Cc1ccccc1)N(C)C(=O)CN(CCO)CC(=O)N(C)C(C)(C)Cc2ccccc2
1300	10.9	Pyraflufen-ethyl	(129630-19-9)	159109	CCOC(=O)COc1cc(c(cc1Cl)F)c2c(c(n(n2)C)OC(F)F)Cl
1301	10.91	Pyrazoxyfen	(71561-11-0)	83405	Cc1c(c(n(n1)C)OCC(=O)c2ccccc2)C(=O)c3ccc(cc3Cl)Cl
1302	10.93	WIN-55-212-2	(131543-22-1)	5487	Cc1c(c2cccc3c2n1C(CO3)CN4CCOCC4)C(=O)c5cccc6c5cccc6
1303	10.94	Fluvastatin	(93957-54-1)	4510159	CC(C)n1c2ccccc2c(c1/C=C/C(CC(CC(=O)O)O)O)c3ccc(cc3)F
1304	10.96	Etrimfos	(38260-54-7)	34830	CCc1nc(cc(n1)OP(=S)(OC)OC)OCC
1305	10.97	Terbinafine	(91161-71-6)	1266005	CC(C)(C)C#C/C=C/CN(C)Cc1cccc2c1cccc2
1306	10.98	Propiconazole Peak 1 & 2	(60207-90-1)	39402	CCCC1COC(O1)(Cn2cncn2)c3ccc(cc3Cl)Cl
1307	10.99	Benzoylprop-ethyl	(22212-55-1)	28825	CCOC(=O)C(C)N(c1ccc(c(c1)Cl)Cl)C(=O)c2ccccc2
1308	11.01	Fipronilsulfone	(120068-36-2)	2336427	c1c(cc(c(c1Cl)n2c(c(c(n2)C#N)S(=O)(=O)C(F)(F)F)N)Cl)C(F)(F)F
1309	11.01	Felodipine	(72509-76-3)	3216	CCOC(=O)C1=C(NC(=C(C1c2cccc(c2Cl)Cl)C(=O)OC)C)C
1310	11.01	Triphenylphosphate	(115-86-6)	7988	c1ccc(cc1)OP(=O)(Oc2ccccc2)Oc3ccccc3
1311	11.02	Fenthion	(55-38-9)	3229	Cc1cc(ccc1SC)OP(=S)(OC)OC
1312	11.02	Coumaphos	(56-72-4)	2768	CCOP(=S)(OCC)Oc1ccc2c(c(c(=O)oc2c1)Cl)C
1313	11.02	Tamoxifen	(10540-29-1)	2015313	CC/C(=C(\c1ccccc1)/c2ccc(cc2)OCCN(C)C)/c3ccccc3
1314	11.03	Famoxadone	(131807-57-3)	184727	CC1(C(=O)N(C(=O)O1)Nc2ccccc2)c3ccc(cc3)Oc4ccccc4
1315	11.04	Diazinon	(333-41-5)	2909	CCOP(=S)(OCC)Oc1cc(nc(n1)C(C)C)C
1316	11.04	Cyprodinil	(121552-61-2)	77885	Cc1cc(nc(n1)Nc2ccccc2)C3CC3
1317	11.05	Lercanidipine	(100427-26-7)	59276	CC1=C(C(C(=C(N1)C)C(=O)OC(C)(C)CN(C)CCC(c2ccccc2)c3ccccc3)c4cccc(c4)[N+](=O)[O-])C(=O)OC
1318	11.05	Vegadex (Sulfallate)	(95-06-7)	6946	CCN(CC)C(=S)SCC(=C)Cl
1319	11.07	Pyranocoumarin	(518-20-7)	10161	CC1(CC(c2c(c3ccccc3oc2=O)O1)c4ccccc4)OC
1320	11.08	Zoxamide	(156052-68-5)	108892	CCC(C)(C(=O)CCl)NC(=O)c1cc(c(c(c1)Cl)C)Cl
1321	11.1	Chlorfenson	(80-33-1)	6383	c1cc(ccc1OS(=O)(=O)c2ccc(cc2)Cl)Cl
1322	11.1	Mefenpyr-diethyl	(135590-91-9)	9112846	CCOC(=O)C1=NN(C(C1)(C)C(=O)OCC)C2=C(C=C(C=C2)Cl)Cl
1323	11.11	Endosulfan-sulfate (Na)	(1031-07-8)	13338	C1C2C(COS(=O)(=O)O1)C3(C(=C(C2(C3(Cl)Cl)Cl)Cl)Cl)Cl)Cl
1324	11.15	Oxadiargyl	(39807-15-3)	85276	CC(C)(C)c1nn(c(=O)o1)c2cc(c(cc2Cl)Cl)OCC#C
1325	11.16	Pyraclostrobin	(175013-18-0)	4928348	COC(=O)N(c1ccccc1COc2ccn(n2)c3ccc(cc3)Cl)OC
1326	11.18	JWH-018-M-6-OH-Ind	(1307803-44-6)	26458420	CCCCCn1cc(c2c1cc(cc2)O)C(=O)c3cccc4c3cccc4

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1327	11.19	Hexaconazole	(79983-71-4)	59833	CCCCC(Cn1cncn1)(c2ccc(cc2Cl)Cl)O
1328	11.19	Prothioconazole	(178928-70-6)	4953623	c1ccc(c(c1)CC(Cn2c(=S)nc[nH]2)(C3(CC3)Cl)O)Cl
1329	11.21	Phoxim	(14816-18-3)	25076	CCOP(=S)(OCC)ON=C(C#N)c1ccccc1
1330	11.21	Metconazole	(125116-23-6)	77764	CC1(CCC(C1(Cn2cncn2)O)Cc3ccc(cc3)Cl)C
1331	11.21	Endosulfan I / II	(115-29-7)	3111	C1C2C(COS(=O)O1)C3(C(=C(C2(C3(Cl)Cl)Cl)Cl)Cl)Cl
1332	11.21	JWH-073-M-7-OH-Ind [(1-Butyl-7-hydroxy-1H-indol-3-yl)(1-naphthyl)methanone]	(1307803-49-1)	26458427	CCCCn1cc(c2c1c(ccc2)O)C(=O)c3cccc4c3cccc4
1333	11.22	Progesterone	(57-83-0)	4751	CC(=O)C1CCC2C1(CCC3C2CCC4=CC(=O)CCC34C)C
1334	11.22	Butamifos	(36335-67-8)	34329	CCC(C)NP(=S)(OCC)Oc1cc(ccc1[N+](=O)[O-])C
1335	11.22	Benzododecinium (Ion1+)	10328-35-5	8424	CCCCCCCCCCCC[N+](C)(C)Cc1ccccc1
1336	11.22	Dodine [Dodecylguanidine] (Ion1+)	112-65-2	7912	CCCCCCCCCCCCNC(=N)N
1337	11.23	Isofenphos	(25311-71-1)	30459	CCOP(=S)(NC(C)C)Oc1ccccc1C(=O)OC(C)C
1338	11.23	Triflumuron	(64628-44-0)	43172	c1ccc(c(c1)C(=O)NC(=O)Nc2ccc(cc2)OC(F)(F)F)Cl
1339	11.23	AM-2201	(335161-24-5)	24751884	c1ccc2c(c1)cccc2C(=O)c3cn(c4c3cccc4)CCCCCF
1340	11.23	RCS-4-ortho [(2-Methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone]	(1345966-76-8)	29341490	CCCCCn1cc(c2c1cccc2)C(=O)c3ccccc3OC
1341	11.25	Prochloraz	(67747-09-5)	159925	CC(C)N(CCOc1c(cc(cc1Cl)Cl)Cl)C(=O)n2ccnc2
1342	11.26	Isoxathion	(18854-01-8)	27255	CCOP(=S)(OCC)Oc1cc(on1)c2ccccc2
1343	11.26	JWH-018-M-5-OH-Ind [(5-Hydroxy-1-pentyl-1H-indol-3-yl)(1-naphthyl)methanone]	(1307803-43-5)	26458419	CCCCCn1cc(c2c1ccc(c2)O)C(=O)c3cccc4c3cccc4
1344	11.27	Meclofenamic acid	(644-62-2)	3897	Cc1ccc(c(c1Cl)Nc2ccccc2C(=O)O)Cl
1345	11.27	Phosalone	(2310-17-0)	4629	CCOP(=S)(OCC)SCn1c2ccc(cc2oc1=O)Cl
1346	11.27	Benzoxonium (Ion1+)	(23884-64-2)	27484	CCCCCCCCCCCC[N+](CCO)(CCO)Cc1ccccc1
1347	11.27	Bitertanol	(55179-31-2)	82759	CC(C)(C)C(C(n1cncn1)Oc2ccc(cc2)c3ccccc3)O
1348	11.27	Butoxycaine	(3772-43-8)	15997	CCCCOc1ccc(cc1)C(=O)OCCN(CC)CC
1349	11.29	Benzoximate	(29104-30-1)	496542	CCO/N=C(\c1c(ccc(c1OC)Cl)OC)/OC(=O)c2ccccc2
1350	11.29	JWH-073-M-5-OH-Ind	(1307803-47-9)	26458425	CCCCn1cc(c2c1ccc(c2)O)C(=O)c3cccc4c3cccc4
1351	11.31	Meclozine	(569-65-3)	3894	Cc1cccc(c1)CN2CCN(CC2)C(c3ccccc3)c4ccc(cc4)Cl
1352	11.33	Clobetasone butyrate	(25122-57-0)	2690	CCCC(=O)OC1(C(CC2C1(CC(=O)C3(C2CCC4=CC(=O)C=CC43C)F)C)C)C(=O)CCl

continued

1353	11.34	Spinosad A (Spinosyn A)	(168316-95-8)	3685331	CCC1CCCC(C(C(=O)C2=CC3C4CC(CC4C=CC3C2CC(=O)O1)OC5C(C(C(C(O5)C)OC)OC)OC)C)OC6CCC(C(O6)C)N(C)C
1354	11.34	Orbencarb	(34622-58-7)	33829	CCN(CC)C(=O)SCc1ccccc1Cl
1355	11.35	Pirimiphos-methyl	(29232-93-7)	31773	CCN(CC)c1nc(cc(n1)OP(=S)(OC)OC)C
1356	11.37	Phorate	(298-02-2)	4626	CCOP(=S)(OCC)SCSCC
1357	11.38	Pencycuron	(66063-05-6)	82795	c1ccc(cc1)NC(=O)N(Cc2ccc(cc2)Cl)C3CCCC3
1358	11.4	Pyrazophos	(13457-18-6)	24247	CCOC(=O)c1cn2c(cc(n2)OP(=S)(OCC)OCC)nc1C
1359	11.41	Metrafenone	(220899-03-6)	4953549	Cc1cc(c(c(c1C(=O)c2c(c(ccc2OC)Br)C)OC)OC)OC
1360	11.42	Tolclofos-methyl	(57018-04-9)	82767	Cc1cc(c(c(c1)Cl)OP(=S)(OC)OC)Cl
1361	11.44	Thiobencarb	(28249-77-6)	31512	CCN(CC)C(=O)SCc1ccc(cc1)Cl
1362	11.48	Vernolate	(1929-77-7)	15204	CCCN(CCC)C(=O)SCCC
1363	11.49	Cadusafos	(95465-99-9)	82850	CCC(C)SP(=O)(OCC)SC(C)CC
1364	11.49	Fluacrypyrim	(229977-93-9)	8129795	CC(C)OC1=NC(=CC(=N1)OCC2=CC=CC=C2/C(=C\OC)/C(=O)OC)C(F)(F)F
1365	11.49	Loratadine	(79794-75-5)	3820	CCOC(=O)N1CCC(=C2c3ccc(cc3CCc4c2nccc4)Cl)CC1
1366	11.49	Bifenox	(42576-02-3)	35891	COC(=O)c1cc(ccc1[N+](=O)[O-])Oc2ccc(cc2Cl)Cl
1367	11.51	Pebulate	(1114-71-2)	13579	CCCCN(CC)C(=O)SCCC
1368	11.51	Diniconazole	(83657-24-3)	4941232	CC(C)(C)C(/C(=C\c1ccc(cc1Cl)Cl)/n2cncn2)O
1369	11.51	Clofentezine	(74115-24-5)	66321	c1ccc(c(c1)c2nnc(nn2)c3ccccc3Cl)Cl
1370	11.51	JWH-015	(155471-08-2)	3480676	CCCn1c(c(c2c1cccc2)C(=O)c3cccc4c3cccc4)C
1371	11.52	Disulfoton	(298-04-4)	3006	CCOP(=S)(OCC)SCCSCC
1372	11.52	Bromadiolone	(28772-56-7)	10606098	C1=CC=C(C=C1)C(CC(C2=CC=C(C=C2)C3=CC=C(C=C3)Br)O)C4=C(C5=CC=CC=C5OC4=O)O
1373	11.53	Dialifos	(10311-84-9)	23490	CCOP(=S)(OCC)SC(CCl)N1C(=O)c2ccccc2C1=O
1374	11.55	Pyrethrins: Cinerin II	(121-20-0)	4520910	C/C=C/CC1=C(C(CC1=O)OC(=O)C2C(C2(C)C)/C=C(\C)/C(=O)OC)C
1375	11.55	Indoxacarb	(173584-44-6)	8112367	COC(=O)C12CC3=C(C1=NN(CO2)C(=O)N(C4=CC=C(C=C4)OC(F)(F)F)C(=O)OC)C=CC(=C3)Cl
1376	11.55	Chlorthal-dimethyl (as the NH4 adduct)	(1861-32-1)	2839	COC(=O)c1c(c(c(c(c1Cl)Cl)C(=O)OC)Cl)Cl
1377	11.58	JWH-201 [2-(4-Methoxyphenyl)-1-(1-pentyl-1H-indol-3-yl)ethanone]	(864445-47-6)	23256220	CCCCCn1cc(c2c1cccc2)C(=O)Cc3ccc(cc3)OC
1378	11.6	Cerivastatin	(145599-86-6)	2575	CC(C)c1c(c(c(c(n1)C(C)C)C=CC(CC(CC(=O)O)O)O)c2ccc(cc2)F)COC
1379	11.6	Metaclazepam	(65517-27-3)	64398	CN1c2ccc(cc2C(=NCC1COC)c3ccccc3Cl)Br

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1380	11.55	Mefenamic acid	(61-68-7)	3904	Cc1cccc(c1C)Nc2ccccc2C(=O)O
1381	11.6	Benzethonium (Ion1+)	(10172-60-8)	2245	CC(C)(C)CC(C)(C)c1ccc(cc1)OCCOCC[N+](C)(C)Cc2ccccc2
1382	11.6	Trifloxystrobin	(141517-21-7)	9839700	C/C(=N\OCc1ccccc1/C(=N\OC)/C(=O)OC)/c2cccc(c2)C(F)(F)F
1383	11.64	Denaverine	(3579-62-2)	64278	CCC(CC)COC(c1ccccc1)(c2ccccc2)C(=O)OCCN(C)C
1384	11.64	JWH-302 [2-(3-Methoxyphenyl)-1-(1-pentyl-1H-indol-3-yl)ethanone]	(864445-45-4)	9668546	CCCCCN1C=C(C2=CC=CC=C21)C(=O)CC3=CC(=CC=C3)OC
1385	11.65	Cycloate	(1134-23-2)	13698	CCN(C1CCCCC1)C(=O)SCC
1386	11.65	Isofenphos-methyl	(99675-03-3)	113043	CC(C)NP(=S)(OC)Oc1ccccc1C(=O)OC(C)C
1387	11.68	Pyrethrin II	(121-29-9)	4522309	CC1=C(C(=O)CC1OC(=O)C2C(C2(C)C)/C=C(\C)/C(=O)OC)C/C=C/C=C
1388	11.7	Chlorpyriphos-methyl	(5598-13-0)	20493	COP(=S)(OC)Oc1c(cc(c(n1)Cl)Cl)Cl
1389	11.7	EPN [O-Ethyl O-(4-nitrophenyl) phenylphosphonothioate]	(2104-64-5)	15571	CCOP(=S)(c1ccccc1)Oc2ccc(cc2)[N+](=O)[O-]
1390	11.71	Fluoroglycofen-ethyl	(77501-90-7)	48457	CCOC(=O)COC(=O)c1cc(ccc1[N+](=O)[O-])Oc2ccc(cc2Cl)C(F)(F)F
1391	11.72	Diflufenican	(83164-33-4)	82834	c1cc(cc(c1)Oc2c(cccn2)C(=O)Nc3ccc(cc3F)F)C(F)(F)F
1392	11.74	Dithiopyr	(97886-45-8)	82855	CC(C)Cc1c(c(nc(c1C(=O)SC)C(F)(F)F)C(F)F)C(=O)SC
1393	11.74	JWH-122-F-Pentyl [[1-(5-Fluoropentyl)-1H-indol-3-yl](4-methyl-1-naphthyl)methanone]	(1354631-24-5)	28289977	Cc1ccc(c2c1cccc2)C(=O)c3cn(c4c3cccc4)CCCCCF
1394	11.76	Hexaflumuron	(86479-06-3)	82839	c1cc(c(c(c1)F)C(=O)NC(=O)Nc2cc(c(c(c2)Cl)OC(C(F)F)(F)F)Cl)F
1395	11.78	Clopidogrel	(113665-84-2)	2704	COC(=O)C(c1ccccc1Cl)N2CCc3c(ccs3)C2
1396	11.78	Pretilachlor	(51218-49-6)	82747	CCCOCCN(c1c(cccc1CC)CC)C(=O)CCl
1397	11.78	JWH-018-M-7-OH-Ind	(1307803-45-7)	26458421	CCCCCn1cc(c2c1c(ccc2)O)C(=O)c3cccc4c3cccc4
1398	11.8	RCS-4 [(4-Methoxyphenyl)(1-pentyl-1H-indol-3-yl)methanone]	(1345966-78-0)	24769418	CCCCCn1cc(c2c1cccc2)C(=O)c3ccc(cc3)OC
1399	11.81	Diallate	(2303-16-4)	4447452	CC(C)N(C(C)C)C(=O)SC/C(=C/Cl)/Cl
1400	11.82	Chlorfenapyr (as the Na adduct)	(122453-73-0)	82875	CCOCn1c(c(c(c1C(F)(F)F)Br)C#N)c2ccc(cc2)Cl
1401	11.83	Triflumizole	(68694-11-1)	82801	CCCOC/C(=N\c1ccc(cc1C(F)(F)F)Cl)/n2ccnc2
1402	11.83	JWH-250	(864445-43-2)	23256117	CCCCCn1cc(c2c1cccc2)C(=O)Cc3ccccc3OC
1403	11.84	Spinosad D	(131929-63-0)	3685333	CCC1CCCC(C(C(=O)C2=CC3C4CC(CC4C(=CC3C2CC(=O)O1)C)OC5C(C(C(C(O5)C)OC)OC)OC)C)OC6CCC(C(O6)C)N(C)C
1404	11.84	Dinobuton (as the Na adduct)	(973-21-7)	13186	CCC(C)c1cc(cc(c1OC(=O)OC(C)C)[N+](=O)[O-])[N+](=O)[O-]
1405	11.85	Dicofol Fragm 251	(115-32-2)	7970	c1cc(ccc1C(c2ccc(cc2)Cl)(C(Cl)(Cl)Cl)O)Cl

continued

1406	11.87	p,p-Dichlorobenzophenone	(90-98-2)	6767	c1cc(ccc1C(=O)c2ccc(cc2)Cl)Cl
1407	11.88	JWH-073 [(1-Butyl-1H-indol-3-yl)(1-naphthyl)methanone]	(208987-48-8)	8647081	CCCCN1C=C(C2=CC=CC=C21)C(=O)C3=CC=CC4=CC=CC=C43
1408	11.9	Novaluron	(116714-46-6)	84442	c1cc(c(c(c1)F)C(=O)NC(=O)Nc2ccc(c(c2)Cl)OC(C(OC(F)(F)F)F)(F)F)F
1409	11.92	Prosulfocarb	(52888-80-9)	55867	CCCN(CCC)C(=O)SCc1ccccc1
1410	11.92	Benfuracarb	(82560-54-1)	49560	CCOC(=O)CCN(C(C)C)SN(C)C(=O)Oc1cccc2c1OC(C2)(C)C
1411	11.95	Repaglinide	(135062-02-1)	4388	CCOc1cc(ccc1C(=O)O)CC(=O)NC(CC(C)C)c2ccccc2N3CCCCC3
1412	11.96	Butylate	(2008-41-5)	15357	CCSC(=O)N(CC(C)C)CC(C)C
1413	11.96	Triclocarban	(101-20-2)	7266	c1cc(ccc1N/C(=N/c2ccc(c(c2)Cl)Cl)/O)Cl
1414	11.96	JWH-073-M-4-OH-Ind	(1307803-46-8)	26458424	CCCCn1cc(c2c1cccc2O)C(=O)c3cccc4c3cccc4
1415	11.98	CP-55940	(83002-04-4)	3612960	CCCCCCC(C)(C)c1ccc(c(c1)O)C2CC(CCC2CCCO)O
1416	11.98	Tritoqualine	(14504-73-5)	65119	CCOc1c2c(c(c(c1OCC)OCC)N)C(=O)OC2C3c4c(cc5c(c4OC)OCO5)CCN3C
1417	12.01	Emamectin B1b	(121424-52-0)	8299197	O=C4OC7CC/1(OC(C(C)C)C(\C=C\1)C)OC(C/C=C(/C(OC3OC(C(OC2OC(C)C(NC)C(OC)C2)C(OC)C3)C)C(\C=C\C=C5\C6(O)C4/C=C(/C)C(O)C6OC5)C)C)C7
1418	12.02	Nitrothal-isopropyl	(10552-74-6)	39827	CC(C)OC(=O)c1cc(cc(c1)[N+](=O)[O-])C(=O)OC(C)C
1419	12.03	Lovastatin	(74133-25-8)	3825	CCC(C)C(=O)OC1CC(C=C2C1C(C(C=C2)C)CCC3CC(CC(=O)O3)O)C
1420	12.03	Profenophos	(41198-08-7)	35529	CCCSP(=O)(OCC)Oc1ccc(cc1Cl)Br
1421	12.06	Nitrofen (as NH4 adduct)	(1836-75-5)	15010	c1cc(ccc1[N+](=O)[O-])Oc2ccc(cc2Cl)Cl
1422	12.09	Furathiocarb	(65907-30-4)	43456	CCCCOC(=O)N(C)SN(C)C(=O)Oc1cccc2c1OC(C2)(C)C
1423	12.1	Amiodarone	(1951-25-3)	2072	CCCCc1c(c2ccccc2o1)C(=O)c3cc(c(c(c3)I)OCCN(CC)CC)I
1424	12.11	Dioxathion	(78-34-2)	6283	CCOP(=S)(OCC)SC1C(OCCO1)SP(=S)(OCC)OCC
1425	12.11	Oxyfluorfen	(42874-03-3)	35974	CCOc1cc(ccc1[N+](=O)[O-])Oc2ccc(cc2Cl)C(F)(F)F
1426	12.15	Haloxyfop ethoxyethyl ester	(87237-48-7)	82841	CCOCCOC(=O)C(C)Oc1ccc(cc1)Oc2c(cc(cn2)C(F)(F)F)Cl
1427	12.15	Lactofen	(77501-63-4)	56077	CCOC(=O)C(C)OC(=O)c1cc(ccc1[N+](=O)[O-])Oc2ccc(cc2Cl)C(F)(F)F
1428	12.15	JWH-251 [2-(2-Methylphenyl)-1-(1-pentyl-1H-indol-3-yl)ethanone]	(864445-39-6)	9791472	CCCCCN1C=C(C2=CC=CC=C21)C(=O)CC3=CC=CC=C3C
1429	12.16	Pyrethrins: Jasmolin II	(1172-63-0)	4520896	CC/C=C/CC1=C(C(CC1=O)OC(=O)C2C(C2(C)C)/C=C(\C)/C(=O)OC)C
1430	12.18	JWH-203 (2-(2-Chlorophenyl)-1-(1-pentyl-1H-indol-3-yl)ethanone)	(864445-54-5)	23256082	CCCCCn1cc(c2c1cccc2)C(=O)Cc3ccccc3Cl
1431	12.19	Tebufenpyrad	(119168-77-3)	77872	CCc1c(c(n(n1)C)C(=O)NCc2ccc(cc2)C(C)(C)C)Cl
1432	12.2	Tetradifon (as Na adduct)	(116-29-0)	8004	c1cc(ccc1S(=O)(=O)c2cc(c(cc2Cl)Cl)Cl)Cl

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1433	12.21	STS-135	(1354631-26-7)	28189067	c1ccc2c(c1)c(cn2CCCCCF)C(=O)NC34CC5CC(C3)CC(C5)C4
1434	12.23	Esprocarb	(85785-20-2)	82838	CCN(C(C)C(C)C)C(=O)SCc1ccccc1
1435	12.25	Terbufos	(13071-79-9)	23912	CCOP(=S)(OCC)SCSC(C)(C)C
1436	12.25	Tolnaftate	(2398-96-1)	5309	Cc1cccc(c1)N(C)C(=S)Oc2ccc3ccccc3c2
1437	12.25	Buprofezin (Z-isomer)	(953030-84-7)	45678	CC(C)N1/C(=N/C(C)(C)C)/SCN(C1=O)c2ccccc2
1438	12.28	Fluazinam	(79622-59-6)	82831	c1c(cnc(c1Cl)Nc2c(cc(c(c2[N+](=O)[O-])Cl)C(F)(F)F)[N+](=O)[O-])C(F)(F)F
1439	12.28	Isoconazole	(27523-40-6)	3629	c1cc(c(c(c1)Cl)COC(Cn2ccnc2)c3ccc(cc3Cl)Cl)Cl
1440	12.31	JWH-073-2-Methyl [(1-Butyl-1H-indol-3-yl)(2-methyl-1-naphthyl)methanone]	(1427325-61-8)	29341456	CCCCn1cc(c2c1cccc2)C(=O)c3c(ccc4c3cccc4)C
1441	12.31	Picolinafen	(137641-05-5)	2542991	c1cc(cc(c1)Oc2cccc(n2)/C(=N/c3ccc(cc3)F)/O)C(F)(F)F
1442	12.32	Pirimiphos-ethyl	(23505-41-1)	29635	CCN(CC)c1nc(cc(n1)OP(=S)(OCC)OCC)C
1443	12.32	Tebupirimphos	(96182-53-5)	84419	CCOP(=S)(Oc1cnc(nc1)C(C)(C)C)OC(C)C
1444	12.35	Butachlor	(23184-66-9)	29376	CCCCOCN(c1c(cccc1CC)CC)C(=O)CCl
1445	12.35	Oxadiazon	(19666-30-9)	27628	CC(C)Oc1cc(c(cc1Cl)Cl)n2c(=O)oc(n2)C(C)(C)C
1446	12.36	Temephos	(3383-96-8)	5199	COP(=S)(OC)Oc1ccc(cc1)Sc2ccc(cc2)OP(=S)(OC)OC
1447	12.36	Tolfenpyrad	(129558-76-5)	8286062	CCC1=NN(C(=C1Cl)C(=O)NCC2=CC=C(C=C2)OC3=CC=C(C=C3)C)C
1448	12.36	Imibenconazole	(86598-92-7)	84387	c1cc(ccc1CS/C(=N\c2ccc(cc2Cl)Cl)/Cn3cncn3)Cl
1449	12.36	JWH-018-6-Methoxy-Ind [(6-Methoxy-1-pentyl-1H-indol-3-yl)(1-naphthyl)methanone]	(1427325-49-2)	26458653	CCCCCn1cc(c2c1cc(cc2)OC)C(=O)c3cccc4c3cccc4
1450	12.37	Piperonylbutoxide	(51-03-6)	5590	CCCCOCCOCCOCc1cc2c(cc1CCC)OCO2
1451	12.39	JWH-073-3-Methyl [1-(3-Methylbutyl)-1H-indol-3-yl](1-naphthyl)methanone	(1346604-93-0)	26458652	CC(C)CCn1cc(c2c1cccc2)C(=O)c3cccc4c3cccc4
1452	12.4	Emamectin B1a	(119791-41-2)	8410190	CCC(C)C1C(C=CC2(O1)CC3CC(O2)C/C=C(/C(C(/C=C/C=C/C=C/4\COC5C4(C(C=C(C5O)C)C(=O)O3)O)C)OC6CC(C(C(O6)C)OC7CC(C(C(O7)C)NC)OC)OC)\C)C
1453	12.41	JWH-018	(209414-07-3)	8558143	CCCCCN1C=C(C2=CC=CC=C21)C(=O)C3=CC=CC4=CC=CC=C43
1454	12.42	Cinidon-ethyl	(142891-20-1)	4722745	CCOC(=O)/C(=C/c1cc(ccc1Cl)N2C(=O)C3=C(C2=O)CCCC3)/Cl
1455	12.43	Flocoumafen Peak 1 & 2	(90035-08-8)	10469214	C1C(CC2=CC=CC=C2C1C3=C(C4=CC=CC=C4OC3=O)O)C5=CC=C(C=C5)OCC6=CC=C(C=C6)C(F)(F)F
1456	12.45	Ethion	(563-12-2)	3171	CCOP(=S)(OCC)SCSP(=S)(OCC)OCC
1457	12.47	THC-OH (11-OH-THC)	(36557-05-8)	34385	CCCCCc1cc(c2c(c1)OC(C3C2C=C(CC3)CO)(C)C)O
1458	12.48	Lufenuron	(103055-07-8)	64813	c1cc(c(c(c1)F)C(=O)NC(=O)Nc2cc(c(cc2Cl)OC(C(C(F)(F)F)F)(F)F)Cl)F

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1459	12.49	Metenolone acetate	(434-05-9)	3946	CC1=CC(=O)CC2C1(C3CCC4(C(C3CC2)CCC4OC(=O)C)C)C
1460	12.5	THC-COOH (11-COOH-THC or 11-nor-9-Carboxy-THC)	(56354-06-4)	97019	CCCCCc1cc(c2c(c1)OC(C3C2C=C(CC3)C(=O)O)(C)C)O
1461	12.5	Pyrimidifen	(105779-78-0)	4953620	CCc1c(c(ncn1)NCCOc2ccc(c(c2C)C)CCOCC)Cl
1462	12.51	Teflubenzuron	(83121-18-0)	82833	c1cc(c(c(c1)F)/C(=N/C(=N/c2cc(c(c(c2F)Cl)F)Cl)/O)/O)F
1463	12.51	JWH-018-M-4-OH-Ind	(1307803-42-4)	26458418	CCCCCn1cc(c2c1cccc2O)C(=O)c3cccc4c3cccc4
1464	12.52	Fenclofos (Ronnel)	(299-84-3)	8939	COP(=S)(OC)Oc1cc(c(cc1Cl)Cl)Cl
1465	12.52	Pyributicarb	(88678-67-5)	84390	CC(C)(C)c1cccc(c1)OC(=S)N(C)c2cccc(n2)OC
1466	12.55	Dichlofenthion	(97-17-6)	7051	CCOP(=S)(OCC)Oc1ccc(cc1Cl)Cl
1467	12.55	Fenofibrate	(49562-28-9)	3222	CC(C)OC(=O)C(C)(C)Oc1ccc(cc1)C(=O)c2ccc(cc2)Cl
1468	12.61	Pyriproxyfen	(95737-68-1)	82851	CC(COc1ccc(cc1)Oc2ccccc2)Oc3ccccn3
1469	12.62	Hexythiazox	(78587-05-0)	3753586	CC1C(SC(=O)N1C(=O)NC2CCCCC2)c3ccc(cc3)Cl
1470	12.64	JWH-007	(155471-10-6)	8536309	CCCCCN1C(=C(C2=CC=CC=C21)C(=O)C3=CC=CC4=CC=CC=C43)C
1471	12.65	Iodofenphos (Jodfenphos)	(18181-70-9)	26915	COP(=S)(OC)Oc1cc(c(cc1Cl)I)Cl
1472	12.66	Bromophos (Bromophos-methyl)	(2104-96-3)	15572	COP(=S)(OC)Oc1cc(c(cc1Cl)Br)Cl
1473	12.66	Flucythrinate	(70124-77-5)	46213	CC(C)C(c1ccc(cc1)OC(F)F)C(=O)OC(C#N)c2cccc(c2)Oc3ccccc3
1474	12.67	Noviflumuron	(121451-02-3)	8004099	C1=CC(=C(C(=C1)F)C(=O)NC(=O)NC2=CC(=C(C(=C2F)Cl)OC(C(C(F)(F)F)F)(F)F)Cl)F
1475	12.68	Fluazuron	(86811-58-7)	59088	c1cc(c(c(c1)F)C(=O)NC(=O)Nc2ccc(c(c2)Oc3c(cc(cn3)C(F)(F)F)Cl)Cl)F
1476	12.68	JWH-081	(210179-46-7)	8722599	CCCCCN1C=C(C2=CC=CC=C21)C(=O)C3=CC=C(C4=CC=CC=C43)OC
1477	12.68	ORG 27569	(868273-06-7)	22369629	CCc1c2cc(ccc2[nH]c1C(=O)NCCc3ccc(cc3)N4CCCCC4)Cl
1478	12.7	Cannabidiol	(13956-29-1)	454786	CCCCCc1cc(c(c(c1)O)C2C=C(CCC2C(=C)C)C)O
1479	12.71	Chlorpyriphos	(2921-88-2)	2629	CCOP(=S)(OCC)Oc1c(cc(c(n1)Cl)Cl)Cl
1480	12.73	Triallate	(2303-17-5)	5342	CC(C)N(C(C)C)C(=O)SCC(=C(Cl)Cl)Cl
1481	12.77	Sulprofos (Bolstar)	(35400-43-2)	34067	CCCSP(=S)(OCC)Oc1ccc(cc1)SC
1482	12.77	Flucycloxuron	(113036-88-7)	5020876	c1cc(c(c(c1)F)C(=O)NC(=O)Nc2ccc(cc2)CO/N=C(/c3ccc(cc3)Cl)\C4CC4)F
1483	12.77	JWH-412	(1364933-59-4)	28647803	CCCCCn1cc(c2c1cccc2)C(=O)c3ccc(c4c3cccc4)F
1484	12.78	Quinoxyfen	(124495-18-7)	2635909	c1cc(ccc1Oc2ccnc3c2c(cc(c3)Cl)Cl)F
1485	12.8	Pendimethalin	(40487-42-1)	35265	CCC(CC)Nc1c(cc(c(c1[N+](=O)[O-])C)C)[N+](=O)[O-]
1486	12.8	Spiromesifen	(283594-90-1)	8083064	CC1=CC(=C(C(=C1)C)C2=C(C3(CCCC3)OC2=O)OC(=O)CC(C)(C)C)C

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1487	12.83	Propargite	(2312-35-8)	4767	CC(C)(C)c1ccc(cc1)OC2CCCCC2OS(=O)OCC#C
1488	12.83	Etoxazole	(153233-91-1)	135707	CCOc1cc(ccc1C2COC(=N2)c3c(cccc3F)F)C(C)(C)C
1489	12.85	Tiocarbazil	(36756-79-3)	34422	CCC(C)N(C(C)CC)C(=O)SCc1ccccc1
1490	12.86	Flufenoxuron	(101463-69-8)	82863	c1cc(c(c(c1)F)C(=O)NC(=O)Nc2ccc(cc2F)Oc3ccc(cc3Cl)C(F)(F)F)F
1491	12.88	Amorolfine	(78613-35-1)	2083	CCC(C)(C)c1ccc(cc1)CC(C)CN2CC(OC(C2)C)C
1492	12.89	JWH-122	(619294-47-2)	24623066	CCCCCn1cc(c2c1cccc2)C(=O)c3ccc(c4c3cccc4)C
1493	12.9	Pyrethrins: Cinerin I	(25402-06-6)	4722677	C/C=C/CC1=C(C(CC1=O)OC(=O)C2C(C2(C)C)C=C(C)C)C
1494	12.9	Carbophenothion	(786-19-6)	12536	CCOP(=S)(OCC)SCSc1ccc(cc1)Cl
1495	12.91	Aspon	(3244-90-4)	17576	CCCOP(=S)(OCCC)OP(=S)(OCCC)OCCC
1496	12.93	Fenpropathrin	(39515-41-8)	43074	CC1(C(C1(C)C)C(=O)OC(C#N)c2cccc(c2)Oc3ccccc3)C
1497	12.93	JWH-019	(209414-08-4)	24598813	CCCCCCn1cc(c2c1cccc2)C(=O)c3cccc4c3cccc4
1498	12.93	UR-144	(1199943-44-6)	24634882	CCCCCn1cc(c2c1cccc2)C(=O)C3C(C3(C)C)(C)C
1499	12.94	Diafenthiuron	(80060-09-9)	2298854	CC(C)c1cc(cc(c1NC(=S)NC(C)(C)C)C(C)C)Oc2ccccc2
1500	12.95	Pyrethrin I	(121-21-1)	4521834	CC1=C(C(=O)CC1OC(=O)C2C(C2(C)C)C=C(C)C)C/C=C/C=C
1501	12.97	Miconazole	(22916-47-8)	4044	c1cc(c(cc1Cl)Cl)COC(Cn2ccnc2)c3ccc(cc3Cl)Cl
1502	12.99	Cyhalothrin (lambda-)	(91465-08-6)	8519181	Cl\C(=C\C3C(C(=O)OC(C#N)c2cccc(Oc1ccccc1)c2)C3(C)C)C(F)(F)F
1503	12.99	Butralin	(33629-47-9)	33600	CCC(C)Nc1c(cc(cc1[N+](=O)[O-])C(C)(C)C)[N+](=O)[O-]
1504	13	Desoxycortone 21-(3-phenylpropionate)	(14007-50-2)	77265	CC12CCC3C(C1CCC2C(=O)COC(=O)CCc4ccccc4)CCC5=CC(=O)CCC35C
1505	13	Tiocarlide	(910-86-1)	2272774	CC(C)CCOc1ccc(cc1)NC(=S)Nc2ccc(cc2)OCCC(C)C
1506	13.01	Beta-Cyfluthrin (Baythroid)	(68359-37-5)	94690	CC1(C(C1C(=O)OC(C#N)c2ccc(c(c2)Oc3ccccc3)F)C=C(Cl)Cl)C
1507	13.02	Brodifacoum Peak 1 & 2	(56073-10-0)	10444663	C1C(CC2=CC=CC=C2C1C3=C(C4=CC=CC=C4OC3=O)O)C5=CC=C(C=C5)C6=CC=C(C=C6)Br
1508	13.03	Spirodiclofen	(148477-71-8)	17215909	CCC(C)(C)C(=O)OC1=C(C(=O)OC12CCCCC2)c3cc(cc(c3)Cl)Cl
1509	13.03	RCS-8	(1345970-42-4)	24751863	COc1ccccc1CC(=O)c2cn(c3c2cccc3)CCC4CCCCC4
1510	13.08	CP-47947 (2-(3-Hydroxycyclohexyl)-5-(2-methyl-2-octanyl)phenol)	(70434-82-1)	8171613	OC2CC(c1c(O)cc(cc1)C(C)(C)CCCCCC)CCC2
1511	13.15	Fenpyroximate	(134098-61-6)	7850857	CC1=NN(C(=C1/C=N/OCC2=CC=C(C=C2)C(=O)OC(C)(C)C)OC3=CC=CC=C3)C
1512	13.21	Deltamethrin (NH4)	(120710-23-8)	2876	CC1(C(C1C(=O)OC(C#N)c2cccc(c2)Oc3ccccc3)C=C(Br)Br)C
1513	13.22	Chlorfluazuron	(71422-67-8)	82810	c1cc(c(c(c1)F)C(=O)NC(=O)Nc2cc(c(c(c2)Cl)Oc3c(cc(cn3)C(F)(F)F)Cl)Cl)F

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1514	13.24	JWH-210	(824959-81-1)	24617616	CCCCCn1cc(c2c1cccc2)C(=O)c3ccc(c4c3cccc4)CC
1515	13.25	Isopropalin	(33820-53-0)	33636	CCCN(CCC)c1c(cc(cc1[N+](=O)[O-])C(C)C)[N+](=O)[O-]
1516	13.28	JWH-398	(1292765-18-4)	28647395	CCCCCn1cc(c2c1cccc2)C(=O)c3ccc(c4c3cccc4)Cl
1517	13.3	Amitraz	(33089-61-1)	33405	Cc1cc(c(cc1)/N=C/N(/C=N/c2c(cc(cc2)C)C)C)C
1518	13.34	Pyrethrins: Jasmolin I	(4466-14-2)	4521837	CC/C=C/CC1=C(C(CC1=O)OC(=O)C2C(C2(C)C)C=C(C)C)C
1519	13.34	Fenvalerate	(51630-58-1)	3230	CC(C)C(c1ccc(cc1)Cl)C(=O)OC(C#N)c2cccc(c2)Oc3ccccc3
1520	13.34	JWH-147	(914458-20-1)	23277882	CCCCCCn1cc(cc1c2ccccc2)C(=O)c3cccc4c3cccc4
1521	13.35	Proquinazid	(189278-12-4)	9232930	Ic2ccc1\N=C(\OCCC)N(C(=O)c1c2)CCC
1522	13.36	AvermectinB1b (Abamectin)	(65195-56-4)	26456620	CC1/C=C/C=C/2\COC3C2(C(C=C(C3O)C)C(=O)OC4CC(C/C=C(/C1OC5CC(C(C(O5)C)OC6C C(C(C(O6)C)O)OC)OC)\C)OC7(C4)C=CC(C(O7)C(C)C)C)O
1523	13.38	Fenticonazole	(72479-26-6)	46840	c1ccc(cc1)Sc2ccc(cc2)COC(Cn3ccnc3)c4ccc(cc4Cl)Cl
1524	13.39	Tribufos (Merphos oxide or DEF)	(78-48-8)	4944	CCCCSP(=O)(SCCCC)SCCCC
1525	13.4	Pyridaben	(96489-71-3)	82852	CC(C)(C)c1ccc(cc1)CSc2cnn(c(=O)c2Cl)C(C)(C)C
1526	13.41	Tefluthrin (as the Na adduct)	(79538-32-2)	8020661	Cl\C(=C\C2C(C(=O)OCc1c(F)c(F)c(c(F)c1F)C)C2(C)C)C(F)(F)F
1527	13.43	JWH-370	(914458-22-3)	23277889	CCCCCn1cc(cc1c2ccccc2C)C(=O)c3cccc4c3cccc4
1528	13.45	Almitrine	(27469-53-0)	31235	C=CCNc1nc(nc(n1)N2CCN(CC2)C(c3ccc(cc3)F)c4ccc(cc4)F)NCC=C
1529	13.49	Fluvalinate	(79472-91-6)	45805	CC(C)C(C(=O)OC(C#N)c1cccc(c1)Oc2ccccc2)Nc3ccc(cc3Cl)C(F)(F)F
1530	13.51	(C8)-CP 47,497	(70434-92-3)	10442937	OC1CC(CCC1)c2ccc(cc2O)C(C)(C)CCCCCCC
1531	13.53	Bromophos-ethyl	(4824-78-6)	19722	CCOP(=S)(OCC)Oc1cc(c(cc1Cl)Br)Cl
1532	13.53	HU-210	(112830-95-2)	3404012	CCCCCCC(C)(C)c1cc(c2c(c1)OC(C3C2CC(=CC3)CO)(C)C)O
1533	13.53	JWH-073-M-2-OH-Ind	(1427325-54-9)	26458112	CCCCn1c2ccccc2c(c1O)C(=O)c3cccc4c3cccc4
1534	13.59	Bioresmethrin (or Resmethrin)	(28434-01-7)	4877	CC(=CC1C(C1(C)C)C(=O)OCc2cc(oc2)Cc3ccccc3)C
1535	13.6	Nandrolone phenylpropionate	(62-90-8)	4282	CC12CCC3C(C1CCC2OC(=O)CCc4ccccc4)CCC5=CC(=O)CCC35
1536	13.61	Prothiophos (Tokuthion)	(34643-46-4)	33832	CCCSP(=S)(OCC)Oc1ccc(cc1Cl)Cl
1537	13.63	Tralomethrin	(66841-25-6)	7985670	CC1(C(C1C(=O)OC(C#N)C2=CC(=CC=C2)OC3=CC=CC=C3)C(C(Br)(Br)Br)Br)C
1538	13.64	AvermectinB1a (Abamectin)	(65195-55-3)	2175	CCC(C)C1C(C=CC2(O1)CC3CC(O2)CC=C(C(C(C=CC=C4COC5C4(C(C=C(C5O)C)C(=O)O3) O)C)OC6CC(C(C(O6)C)OC7CC(C(C(O7)C)O)OC)OC)C)C
1539	13.65	Cannabinol	(521-35-7)	2447	CCCCCc1cc(c-2c(c1)OC(c3c2cc(cc3)C)(C)C)O
1540	13.68	Fenazaquin	(120928-09-8)	77874	CC(C)(C)c1ccc(cc1)CCOc2c3ccccc3ncn2

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1541	13.69	JWH-018-Adamantoyl (or AB-001)	(1345973-49-0)	26286891	CCCCCn1cc(c2c1cccc2)C(=O)C34CC5CC(C3)CC(C5)C4
1542	13.73	Desoxycortone enanthate	(1420-68-4)	28590147	CCCCCCC(=O)OCC(=O)C1CCC2C1(CCC3C2CCC4=CC(=O)CCC34C)C
1543	13.75	Carbosulfan	(55285-14-8)	37764	CCCCN(CCCC)SN(C)C(=O)Oc1cccc2c1OC(C2)(C)C
1544	13.76	Pyridate	(55512-33-9)	37831	CCCCCCCCSC(=O)Oc1cc(nnc1c2ccccc2)Cl
1545	13.77	Leptophos	(21609-90-5)	28496	COP(=S)(c1ccccc1)Oc2cc(c(cc2Cl)Br)Cl
1546	13.82	Testosterone benzoate	(2088-71-3)	312539	CC12CCC3C(C1CCC2OC(=O)c4ccccc4)CCC5=CC(=O)CCC35C
1547	13.86	Flumethrin	(69770-45-2)	82804	CC1(C(C1C(=O)OC(C#N)c2ccc(c(c2)Oc3ccccc3)F)/C=C(/c4ccc(cc4)Cl)\Cl)C
1548	13.93	Permethrin (cis-)	(61949-76-6)	36845	CC1(C(C1C(=O)OCc2cccc(c2)Oc3ccccc3)C=C(Cl)Cl)C
1549	13.97	THC (Delta9-Tetrahydrocannabinol)	(1972-08-3)	2872	CCCCCc1cc(c2c(c1)OC(C3C2C=C(CC3)C)(C)C)O
1550	14.02	Dibutylchlorendate	(1770-80-5)	191879	CCCCOC(=O)C1C(C2(C(=C(C1(C2(Cl)Cl)Cl)Cl)Cl)Cl)Cl)C(=O)OCCCC
1551	14.08	Etofenprox (as the NH4 adduct)	(80844-07-1)	64377	CCOc1ccc(cc1)C(C)(C)COCc2cccc(c2)Oc3ccccc3
1552	14.09	Bifenthrin (as the NH4 adduct)	(82657-04-3)	4445165	Cc1c(cccc1c2ccccc2)COC(=O)C3C(C3(C)C)/C=C(/C(F)(F)F)\Cl
1553	14.2	Ivermectin B1b (as the NH4 adduct)	(70209-81-3)	23259889	CC1CCC2(CC3CC(O2)C/C=C(/C(C(/C=C/C=C/4\COC5C4(C(C=C(C5O)C)C(=O)O3)O)C)OC6CC(C(C(O6)C)OC7CC(C(C(O7)C)O)OC)OC)\C)OC1C(C)C
1554	14.27	Halfenprox	(111872-58-3)	8315975	CC(C)(COCC1=CC(=CC=C1)OC2=CC=CC=C2)C3=CC=C(C=C3)OC(F)(F)Br
1555	14.4	Ivermectin B1a (as the Na adduct)	(70161-11-4)	3535064	CCC(C)C1C(CCC2(O1)CC3CC(O2)CC=C(C(C(C=CC=C4COC5C4(C(C=C(C5O)C)C(=O)O3)O)C)OC6CC(C(C(O6)C)OC7CC(C(C(O7)C)O)OC)OC)C)C
1556	14.5	Fenbutatin Oxide Fragm 519	(13356-08-6)	13754592	CC(C)(C[Sn+](CC(C)(C)c1ccccc1)CC(C)(C)c2ccccc2)c3ccccc3

CAS Numbers (as listed in PubChem for vast majority of compounds but not all)

SMILES obtained from Chemspider

FORM UPR16

Research Ethics Review Checklist

Please include this completed form as an appendix to your thesis (see the Postgraduate Research Student Handbook for more information

Postgraduate Research Student (PGRS) Information			**Student ID:**	507420
PGRS Name:	Anthony Gravell			
Department:	School of Pharmacy and Biomedical Sciences	**First Supervisor:**	Professor Graham Mills	
Start Date: (or progression date for Prof Doc students)	**February 2010**			

Study Mode and Route:	Part-time ☒	MPhil ☐	MD ☐
	Full-time ☐	PhD ☒	Professional Doctorate ☐

Title of Thesis:	Better tools for investigative monitoring under the Water Framework Directive (WFD)
Thesis Word Count: (excluding ancillary data)	51,085

If you are unsure about any of the following, please contact the local representative on your Faculty Ethics Committee for advice. Please note that it is your responsibility to follow the University's Ethics Policy and any relevant University, academic or professional guidelines in the conduct of your study

Although the Ethics Committee may have given your study a favourable opinion, the final responsibility for the ethical conduct of this work lies with the researcher(s).

UKRIO Finished Research Checklist:
(If you would like to know more about the checklist, please see your Faculty or Departmental Ethics Committee rep or see the online version of the full checklist at: http://www.ukrio.org/what-we-do/code-of-practice-for-research/)

a)	Have all of your research and findings been reported accurately, honestly and within a reasonable time frame?	YES ☒ NO ☐
b)	Have all contributions to knowledge been acknowledged?	YES ☒ NO ☐
c)	Have you complied with all agreements relating to intellectual property, publication and authorship?	YES ☒ NO ☐
d)	Has your research data been retained in a secure and accessible form and will it remain so for the required duration?	YES ☒ NO ☐
e)	Does your research comply with all legal, ethical, and contractual requirements?	YES ☒ NO ☐

Candidate Statement:

I have considered the ethical dimensions of the above named research project, and have successfully obtained the necessary ethical approval(s)

Ethical review number(s) from Faculty Ethics Committee (or from NRES/SCREC):	Not submitted for ethical review

If you have *not* submitted your work for ethical review, and/or you have answered 'No' to one or more of questions a) to e), please explain below why this is so:

The work undertaken in this study involved the statutory sampling and deployment of passive samplers in rivers and waste water treatment works in Wales that is regulated in Wales by Natural Resources Wales. Sampling procedures used were those approved by NRW. This work was a laboratory study that only involved the sampling of water. No humans, animals or any biological materials were involved in the study.		
Signed *(PGRS)*:		**Date:** 2nd June 2017