Identification of micropollutants by combined chromatography and mass spectrometry techniques

by

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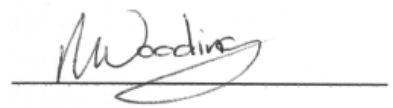
Declaration

I, Madelien Wooding, declare that the dissertation, which I hereby submit for the degree Magister Scientiae at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.



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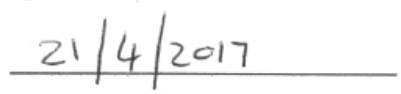

Date

"Whereas there is nothing more necessary for promoting the improvements of Philosophical Matters, than the communication to such, as apply their Studies and Endeavours that way, … , To the end, that such Productions being clearly and truly communicated, desires after solid and useful knowledge may be further entertained, ingenious Endeavours and Undertakings cherished, and those, addicted to and conversant in such matters, may be invited and encouraged to search and try, and find out new things, impart their knowledge to one another, and contribute what they can to the Grand design of improving Natural knowledge and perfecting all Philosophical Arts, and Sciences…"

-Henry Oldenburg. The Introduction. Philosophical Transactions, 1665. 1(1-22): p. 1-2.

Summary

The presence of micropollutants in South African aquatic systems has emerged as an issue of public health concern. Micropollutants, such as endocrine disrupting chemicals (EDCs) and antiretroviral compounds, have previously been detected in surface water of South Africa. Micropollutants are often present in complex environmental matrices at ultra-trace levels, complicating their detection.

In order to address shortcomings with traditional sample preparation methods, an in-house developed cheap, disposable polydimethylsiloxane (PDMS) sorptive sampler was developed. The validity of the PDMS sampler was established by comparison with a commercial stir bar sorptive sampler (SBSE). The sample introduction process into a gas chromatograph (GC) was also simplified by using thermal desorption of a PDMS sampler directly in the inlet liner of a GC. Direct thermal desorption was validated by comparison to time-consuming thermal desorption using an expensive commercial thermal desorption system (TDS).

With the aim of identifying a vast range of micropollutants in aquatic systems comprehensive gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOFMS) was employed. The increased selectivity, sensitivity and larger peak capacity of GC×GC-TOFMS allows the identification of more compounds in complex matrices when compared to conventional GC-MS. An initial screening using sorptive extraction techniques and GC×GC-TOFMS tentatively identified various micropollutants, including EDCs, in surface water samples from the Rietvlei Nature Reserve, Gauteng, South Africa.

Ultra-high pressure liquid chromatography coupled to mass spectrometry (UHPLC-QTOFMS) was used as a complementary analytical technique in conjunction with GC×GC-TOFMS. Solid phase extraction (SPE) and large volume injection (LVI) sample preparation steps preceded analysis by UHPLC-QTOFMS. SPE is more time consuming and uses expensive solvents, however, adds selectivity to the sample preparation step, by reducing possible matrix interferences which can be problematic with LVI.

Matrix matched calibration curves were constructed to identify and quantify target analytes in surface water samples. After validation of the analytical methods using

chemometric approaches, these methods were employed to detect micropollutants in surface water from a metropolitan area (Rietvlei Nature Reserve, Gauteng) and a rural area (Albasini and Nandoni Dams, Limpopo Province) in South Africa. Limits of detection (LOD) for the GC methods ranged from 1 to 98 pg/L for the PDMS loop and 1 to 190 pg/L for SBSE. The LODs for the LC methods ranged from 1.97 to 135 ng/L for LVI and 73 pg/L to 57.3 ng/L for SPE.

The two simplified methods, the in-house developed PDMS loop with GC×GC-TOFMS, and LVI with UHPLC-QTOFMS, were used as complementary methods to detect micropollutants, such as EDCs, in surface water. EDCs such as pharmaceuticals, personal care products and pesticides, as well as the antiretroviral compounds, efavirenz and nevirapine, were detected in surface water from South Africa at concentration levels ranging from 0.16 ng/L to 227 ng/L.

As they have not been reported in literature before, experimental linear retention indices are provided for the target analytes on the proprietary phase Rtx®-CLPesticides II column. Lastly, the variance between different sampling sites was investigated using principal component analysis (PCA). PCA revealed a difference in micropollutant profile between sampling sites in the metropolitan and the rural area.

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Additional outputs based on this work

Published Papers

Madelien Wooding, Egmont R. Rohwer, Yvette Naudé, Determination of endocrine disrupting chemicals and antiretroviral compounds in surface water: A disposable sorptive sampler with comprehensive gas chromatography – Time-of-flight mass spectrometry and large volume injection with ultra-high performance liquid chromatography–tandem mass spectrometry, Journal of Chromatography A, 1496 (2017) 122–132. http://dx.doi.org/10.1016/j.chroma.2017.03.057 (Addendum M)

Madelien Wooding, Egmont R. Rohwer, Yvette Naudé, Comparison of a disposable sorptive sampler with thermal desorption in a gas chromatographic inlet, or in a dedicated thermal desorber, to conventional stir bar sorptive extraction-thermal desorption for the determination of micropollutants in water, Analytica Chimica Acta (2017), *in press,* http://dx.doi.org/10.1016/j.aca.2017.06.030.

Conference Proceeding

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Oral Presentations

Wooding, M., Aneck-Hahn, N., Bornman, R., Rohwer, E. & Naudé, Y. SAAWK Studentesimposium, Bloemfontein (2015). Oral presentation on research entitled: Ontleding van endokrien-ontwrigtende chemikalieë in water met behulp van sorptiewe ekstraksie, GC×GC-TOFMS en UPLC-MS/MS.

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Wooding, M., Aneck-Hahn, N., Bornman, R., Rohwer, E. & Naudé, Y. 40th International Symposium on Capillary Chromatography and 13th GCxGC Symposium, Riva del Garda, Italy (2016). Oral presentation (keynote lecture) on research entitled: Identification of endocrine disrupting chemicals in water by novel sorptive extraction, GC×GC-TOFMS and UPLC-MS/MS.

Poster Presentation

Wooding, M., Rohwer, E. & Naudé, Y. ChromSAAMS 2016, Vanderbijlpark, Gauteng, South Africa (2016). Identification of endocrine disrupting chemicals in water by novel sorptive extraction, GC×GC-TOFMS and UHPLC-MS/MS.

Outline*

*A detailed Table of Contents is included at the beginning of each Chapter

List of Abbreviations

% ME	percentage matrix effects
% PE	percentage process efficiency
^{1}D	first dimension
^{2}D	second dimension
ACN	acetonitrile
APCI	atmospheric pressure chemical ionisation
ARV	antiretroviral
BPA	bisphenol A
BPI	base peak ion
BSTFA	N,O-bis(trimethylsilyl)trifluoroacetamide
CAS	chemical abstracts service
CID	collision induced dissociation
CIS	cooled injection system
DCM	dichloromethane
DDT	dichlorodiphenyltrichloroethane
d_f	film thickness
DIA	data-independent acquisition
EDC	endocrine disrupting chemical
EDP	endocrine disrupting pesticide
EEQ	estradiol equivalent quotient
EI	electron ionisation
ESI	electrospray ionisation
EU	European Union
GC	gas chromatography
GC×GC	comprehensive gas chromatography
GC×GC-TOFMS	comprehensive gas chromatography with time-of-flight mass spectrometry
GC-MS	gas chromatography - mass spectrometry
HRMS	high-resolution mass spectrometry
ID	internal diameter
K_{ow}	octanol-water partitioning coefficient

LC	liquid chromatography
LLE	liquid-liquid extraction
LOD	limit of detection
LOQ	limit of quantification
LRI	linear retention index
LVI	large volume injection
m/z	mass-to-charge ratio
MeOH	methanol
MS	mass spectrometry
MS/MS	tandem mass spectrometry
NIST	National Institute of Standards and Technology
PAH	polycyclic aromatic hydrocarbon
PCA	principal component analysis
PCB	polychlorinated biphenyl
PCP	personal care product
PDMS	polydimethylsiloxane
POP	persistent organic pollutant
ppb	parts per billion
ppm	parts per million
ppq	parts per quadrillion
ppt	parts per trillion
QqQ	triple quadrupole
QTOF	quadrupole time-of-flight
QTOFMS	quadrupole time-of-flight mass spectrometer
RCBA	recombinant yeast screen bio-assays
RIC	reconstructed ion chromatogram
RI	retention index
RSD	relative standard deviation
RT	retention time
S/N	signal to noise ratio
SBSE	stir bar sorptive extraction
SPE	solid phase extraction
SPME	solid phase microextraction

TD	thermal desorption
TDS	thermal desorption system
TIC	total ion chromatogram
TMCS	trimethylsilyl chloride
TMS	trimethylsilyl
TOF	time-of-fight
TOFMS	time-of-fight mass spectrometry
UHPLC	ultra-high pressure liquid chromatography
UHPLC-QTOFMS	ultra-high pressure liquid chromatography with quadrupole time-of-flight mass spectrometer
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation

Preface

Chromatography is a powerful and versatile separation technique that finds applications in all branches of science [1]. In a single step process, closely related compounds of complex matrices can be separated; simultaneously providing qualitative and quantitative assessments of each constituent, aided by a suitable detector [1, 2]. Russian botanist Mikhail Tswett invented and named chromatography in the late 1800s. He separated natural pigments by passing solutions of these compounds through adsorbent packed columns. He named the method after the coloured bands of separated species he saw on the column (Greek *chroma* meaning “colour” and *graphein* meaning “writing”) [1, 2]. In principle all chromatographic separations work by an equilibrium partitioning between two phases. The sample is dissolved in the mobile phase, this can be a gas, a liquid or a supercritical fluid, which is then forced through an immiscible stationary phase. Differences in species’ migration rates are due to the retention, or lack thereof, of the compounds on the stationary phase and consequently, compounds are separated into discrete bands [1].

The field of chromatography has grown significantly over the last century due to the rising need for new and better methods to analyse complex mixtures [1]. Their work on liquid-solid chromatography earned A. J. P. Martin and R. L. M. Synge the 1952 Nobel Prize in Chemistry. In 1954 N. H. Ray inserted a sensing filament into the outlet of a gas chromatographic column to record the first chromatogram with Gaussian-type peaks [2]. In 1955 the first commercial (Janak) gas-liquid chromatography apparatus appeared on the market leading to an exponential growth of chromatography applications [3].

The use of gas and liquid chromatography was heightened by coupling these techniques to mass spectrometers which act not only as a detector but also provide important structural information on the separated compounds. The first studies on mass-to-charge ratios, using a basic mass spectrometer, was done by J. J. Thomson in 1912 in order to separate neon isotopes [4]. Mass spectrometry techniques have undergone numerous improvements and are widely used for structure elucidation and quantification of organic compounds, and more recently are being used for biological applications [1, 4]. In 1958 gas chromatography was coupled to mass spectrometry

enabling the separation, identification and quantification of volatile compounds [2, 4]. The discovery of electrospray ionisation, as an interface, in the late 1980s has greatly facilitated the use of liquid chromatography coupled to mass spectrometry in order to separate, identify and quantify compounds with high molecular masses [2, 4].

The analytical process, however, has several steps, each crucial to obtain correct results [5]. The importance of sample preparation prior to chromatographic analysis should not be underestimated. Analytical samples are usually complex mixtures of species. The matrix is usually not matched with the chromatographic system in terms of volatility, solubility, stability and homogeneity [3].

In spite of the numerous advances in separation and quantification techniques, many sampling and sample preparation methods are based on nineteenth-century technologies, such as Soxhlet extraction [5]. Sample preparation methods are often time-consuming, labour intensive, have multiple steps resulting in analyte loss and use harmful organic solvents. Pawliszyn (1997) estimated that over 80% of all analysis time is spent on sampling and sample preparation [5]. To overcome the shortcomings associated with sample preparation methods, various new (solvent free) sample preparation techniques have been developed. Methods based on polydimethylsiloxane (PDMS) sorption, such as stir bar sorptive extraction (developed by Pat Sandra), has been commercialised and is now widely used to facilitate the analysis process [6].

References

1. Skoog, D.A., Holler, F.J., Crouch, S.R. *Principles of instrumental analysis*. Vol. 6th ed. 2007, Belmont, CA: Thomson Brooks/Cole.
2. Scott, R.P. *Principles and practice of chromatography.* Chrom-Ed Book Series, 2003. **1**.
3. Skoog, D., West, D., Holler, F., Crouch, S. *Fundamentals of analytical chemistry*. 8th ed. 2004, Belmont, USA: Brooks/Cole, Cengage Learning.
4. De Hoffman, E., Stroobant, V. *Mass spectrometry : principles and applications*. 3rd edition ed. 2007: Hoboken, N.J. : Wiley ; Chichester : John Wiley [distributor].
5. Pawliszyn, J. *Solid phase microextraction: theory and practice*. 1997, John Wiley & Sons. p. 1-10.
6. Baltussen, E., Cramers, C., Sandra, P. *Sorptive sample preparation – a review.* Analytical and Bioanalytical Chemistry, 2002. **373**(1): p. 3-22.

Chapter 1

Introduction

Table of Contents

1. Introduction

"There are very few, if any, methods for chemical analysis that are specific to a single chemical species. At best, analytical methods are selective for a few species or class of species. Consequently, the separation of the analyte from potential interferences is quite often a vital step in analytical procedures."

~ Skoog, Holler and Crouch (2007) [1]

The presence of micropollutants in South African aquatic systems has emerged as an issue of public health concern. The purpose of this research is the simplification of sample preparation for the identification of micropollutants, specifically endocrine disrupting chemicals (EDCs) and emerging contaminants, such as antiretroviral drugs, in surface water.

1.1. Micropollutants

Micropollutants, or emerging contaminants, comprise of an ever expanding range of anthropogenic and natural substances [2]. The presence of micropollutants, such as pharmaceuticals (including antiretroviral drugs), personal care products (PCPs), steroid hormones, industrial chemicals, pesticides and endocrine disrupting chemicals (EDCs), in the aquatic environment has become a worldwide environmental concern [2, 3]. Long- and short-term toxicity of drinking water, endocrine disrupting effects and antibiotic resistance of microorganisms are just some examples of the adverse effects associated with micropollutants in the aquatic environment [2].

The diversity of micropollutants and the low concentrations (µg/L to ng/L) at which they occur in the aquatic environment have severely complicated the detection and analysis of these chemicals [2, 3]. Environmental quality standards and contaminant guidelines do not exist for the majority of micropollutants due to the challenges faced with the detection and analysis of these pollutants in aquatic systems [2]. Recent advances in analytical chemistry theory and instruments have enabled the detection of vast ranges of micropollutants at trace level, resulting in increased public awareness and facilitating the legislation process [3].

The main focus of this research project was to detect endocrine disrupting chemicals in surface water due to their emerging concern in the South African environment.

Additionally, the presence of antiretroviral compounds in South African aquatic systems was investigated as these are considered emerging pharmaceutical contaminants.

1.1.1. Endocrine Disrupting Chemicals

Chemical pollutants that negatively impact the health of humans and animals, because of their biological effect, are classified as endocrine disrupting chemicals (EDCs) [4]. An endocrine disrupting chemical (EDC) is defined by the World Health Organization (WHO) (2012) as:

"an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations" [5].

EDCs are a universal problem with exposure occurring at home, in the office, in the air we breathe, the food we eat and the water we drink. It is estimated that of the hundreds of thousands of manufactured chemicals about one thousand might have endocrine disrupting activity [6]. Monitoring of chemicals in body fluids and tissues shows that almost 100% of humans have detectable levels of EDCs in blood, urine, placenta, umbilical cord blood and body tissue [6]. Known EDCs include pesticides such as lindane, atrazine and dichlorodiphenyltrichloroethane (DDT), food packaging materials such as bisphenol A (BPA) and phthalates, antibacterials and personal care products (PCPs) such as triclosan and brominated flame retardants, to name a few. Moreover, there are countless suspected and emerging EDCs that have yet to be tested for endocrine disrupting activity [6].

EDCs interfere with endocrine systems by mimicking or blocking natural hormones causing adverse health effects on humans and animals [5, 6]. EDCs act like hormones and can bind to hormone receptors at very low concentrations. EDCs can either activate the receptor and trigger hormone processes or they can block the receptor and prevent natural hormone processes [6].

The WHO and United Nations Environmental Programme (UNEP) launched a joint report in February 2013 on the state of the science of EDCs [5]. The report highlights the understanding of EDCs, their effect on human health and gives recommendations for improved testing and reducing exposure to EDCs. In 2013 the Berlaymont

Declaration was issued by a group of independent scientists who voiced their concern about EDCs and urged the European Commission to improve regulations regarding these chemicals. The declaration has been signed by almost 100 scientists from 19 countries, including Chile, China, Mexico, South Africa and numerous European Union member states [6].

1.1.1.1. Impacts of EDCs

The first public warning that environmental pollution might be causing reduced bird numbers due to reproductive failure, caused by exposure to DDT and other toxic chemicals, appeared in 1962 in the book *Silent Spring* by Rachel Carson [7]. There has been an exponential increase in the amount of manufactured chemicals leading to an increase in the number of chemicals released (intentionally or not) into the environment since 1940. Irreversible changes to the ecosystem caused by this chemical revolution have had detrimental impacts on human and wildlife health [6].

Hormonal systems ranging from those controlling the development and function of reproductive organs to the tissues and organs regulating metabolism and satiety are all affected by EDCs. Altering the normal functions of these systems can lead to obesity, infertility or lower fertility, learning and memory difficulties, type II diabetes, cardiovascular disease, to name a few [5]. Humans and wildlife are exposed to far more EDCs than merely persistent organic pollutants (POPs), causing a rise in endocrine related diseases and disorders which include [5]:

- Reduced semen quality. Up to 40% of men in some countries are affected.
- Genital malformation incidences, such as non-descending testes and penile malformation, have increased in boys.
- Adverse pregnancy outcomes, such as an increase in preterm births and low birth weights.
- Neurobehavioural disorders linked to thyroid disruption affect a high percentage of children in some countries.
- Rates of endocrine related cancers have increased globally over the past 40 to 50 years.
- Early onset breast development in young girls.
- Occurrences of obesity and type II diabetes have increased drastically worldwide.

1.1.1.2. EDCs in South Africa

Annually, complex mixtures of chemicals are released into the aquatic systems by industry, agriculture and private households [8]. Levels of EDCs in the environment and wildlife in South Africa are similar and even higher in some cases than reported in other countries [9]. Consequently, South Africa has not escaped the threat of environmental oestrogenic pollution on the health of humans and ecosystems including animals and plants. Aquatic systems are particularly vulnerable as EDCs and their breakdown products typically end up in these systems. Oestrogenicity has been reported in water sources in South Africa [9]. However, the high levels of oestrogenic activity could not be explained by chemical analysis. EDC pollution may be more severe and widespread than previously estimated [9].

The exposure of rural communities in South Africa to untreated surface water through lack of water sanitation services, or by accidental contact, have raised concern due to the potential health risks associated with the occurrence of aquatic contaminants. Many rural dwellers and inhabitants of informal settlements are compelled to use untreated water from rivers and dams. There are no current guidelines to monitor the quality of untreated river water in South Africa as it is not considered a source for human consumption [10]. Varying concentrations (ppq to ppt) of steroid hormone EDCs have been found in surface water, such as dams and rivers, and wastewaters in South Africa [8, 9, 11-13].

Aneck-Hahn et al. (2009) reported oestrogenic activity in the drinking water sources of two rural communities in the Waterberg district of the Limpopo Province, South Africa by using a recombinant yeast screen bioassay (RCBA) [11]. DDT is used for malaria vector control in the low altitude parts of the Limpopo Province, Mpumalanga Province and KwaZulu-Natal [12]. Indoor residual spraying of traditional dwellings is allowed under the Stockholm Convention on Persistent Organic Pollutants (POPs) [14, 15]. The main metabolite of DDT is *p,p'*-dichlorodiphenyldichloroethylene (*p,p'*-DDE), a potent anti-androgen. In a study to determine the effects of DDT exposure in men in a malaria area of the Limpopo Province, impaired semen quality due to non-occupational exposure was found [12].

Oestrogenic activity was also reported in water sources at Rietvlei Nature Reserve, Pretoria, South Africa where intersex specimens of the feral sharptooth catfish, *Clarias*

gariepinus, has been reported [8, 16]. Mesenteric fat analysis revealed the presence of alkylphenols, such as nonylphenol, and organochlorine pesticides, such as lindane, aldrin and DDT. These endocrine disrupting compounds could have contributed to the intersex that was observed in the fish [16]. Oestrogenic activity was reported by Aneck-Hahn et al. (2005) in South African laboratory water sources using an RCBA [17]. The occurrence of steroid hormones in effluent and surface water from the wastewater treatment works in Pietermaritzburg, South Africa, was confirmed by Manickum et al. (2014) [18].

The origin and identity of the oestrogenic compounds in many of the above-mentioned studies are not known. Complex mixtures of chemicals with possible endocrine disrupting activity, be it anti-androgenic or anti-oestrogenic, are usually found in water samples and could affect the outcomes of bioassays used to determine oestrogenicity when using estradiol equivalent concentrations (EEQs) [10]. Further studies are needed to identify the chemicals responsible for the oestrogenic activity in water as EEQs only confirms the presence or absence of oestrogenic activity [9, 11].

1.1.1.3. Challenges posed by EDC analysis

EDCs include a range of compounds with very different physicochemical properties [19]. Major sources of EDCs include phthalates, polychlorinated biphenyls, polycyclic aromatic hydrocarbons (PAHs), brominated flame retardants, pesticides, dioxins, hormones, pharmaceuticals and personal care products [20, 21]. The analysis of EDCs is challenging since they represent a diverse group of chemical compounds found at trace levels in complex environmental matrices [22]. Current approaches for EDC extraction and detection require either multiple extractions, large sample volumes, derivatisation or extensive clean up [23]. The majority of methods focus on a specific class of compound (e.g. oestrogen steroids) [19, 23]. There is a need for a comprehensive multi-residue approach for the quantitative determination of EDCs at trace levels in water matrices while minimizing sample preparation time and cost [19].

1.1.2. Antiretroviral Compounds

Antiretroviral (ARV) compounds are pharmaceuticals used for the treatment of influenza and infections such as human immunodeficiency virus (HIV), herpes and hepatitis [24]. ARVs enter the environment through wastewater treatment plant

discharges because, as is the case with other pharmaceuticals, they are not completely metabolised in the patients' system and thus are excreted in the urine or faeces [24]. The major concern with the presence of ARVs in the environment is the possible development of viral resistance [24].

South Africa has one of the largest ARV programmes worldwide [25]. Accordingly, it has been hypothesised that the presence of ARVs in the environment should be greater than reported in other countries [26]. In spite of this, very little research has been done on the presence of these emerging pollutants in the South African environment. Recently, Wood (2015) reported the presence of ARVs (26.5 ng/L to 430 ng/L) in surface water across South Africa using a generic SPE sample preparation method with UHPLC-MS/MS [26].

1.2. Aim and Objectives

The aim of this project was to evaluate cheap, quick and easy complementary sample introduction methods that can detect multiple classes of micropollutants (specifically EDCs and ARVs) at trace level in surface water. To achieve this the following objectives were established:

1. Screening (tentative identification) of South African surface water for micropollutants using comprehensive gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOFMS).
2. Simplification of the sample preparation process by using novel, in-house developed, polydimethylsiloxane (PDMS) samplers with GC×GC-TOFMS and large volume injections (LVI) with ultra-high performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS).
3. Simplification of the GC sample introduction process by thermal desorption (TD) of the in-house developed sampler directly in the GC inlet liner, and the validation of the simplified methods by comparison with TD using a commercial thermal desorption system (TDS).
4. Validation of the in-house developed sampler and LVI by comparing to established sample preparation methods such as solid phase extraction (SPE) and stir bar sorptive extraction (SBSE).

5. Qualification and quantification of selected target analytes (based on the aforementioned screening) in real world surface water samples from two locations in South Africa using GC×GC-TOFMS and UHPLC-MS/MS.
6. Comparison of sampling sites, in terms of micropollutants present at the sites, using principal component analysis (PCA).

1.3. Dissertation Outline

In Chapter 2 a literature review is provided on analytical techniques used to determine micropollutants in water. This includes chromatography, mass spectrometry and sample preparation steps. Emphasis is placed on the detection and analysis of EDCs. Chapter 3 provides a brief overview of the data analysis processes used in order to transform data into meaningful information. The experimental setup used to achieve the aim and objectives of the research project is described in Chapter 4. The results obtained are presented and discussed in Chapter 5. Final conclusions and recommendations for future work are presented in Chapter 6.

References

1. Skoog, D.A., Holler, F.J., Crouch, S.R. *Principles of instrumental analysis*. Vol. 6th ed. 2007, Belmont, CA: Thomson Brooks/Cole.
2. Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S., Wang, X.C. *A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment.* Science of the Total Environment, 2014. **473**: p. 619-641.
3. Jiang, J.-Q., Zhou, Z., Sharma, V.K. *Occurrence, transportation, monitoring and treatment of emerging micro-pollutants in waste water — A review from global views.* Microchemical Journal, 2013. **110**: p. 292-300.
4. Jeannot, R., Sabik, H., Sauvard, E., Dagnac, T., Dohrendorf, K. *Determination of endocrine-disrupting compounds in environmental samples using gas and liquid chromatography with mass spectrometry.* Mass Spectrometry: Innovation and Application. Part II, 2002. **974**(1–2): p. 143-159.
5. *WHO UNEP, State of the Science of Endocrine Disrupting Chemicals 2012, Summary for Decision Makers*. 2012; Available from: http://www.who.int/ceh/publications/endocrine/en/. [Accessed on 16/09/30]
6. Gore, A.C., Crews, D., Doan, L.L., La Merrill, M., Patisaul, H., Zota, A. *Introduction To Endocrine Disrupting Chemicals (EDCs)*. 2014; Available from: http://ipen.org/documents/introduction-endocrine-disrupting-chemicals-edcs. [Accessed on 2016/09/30]
7. Carson, R. *Silent Spring. 1962*. 1994: Houghton Mifflin Harcourt.
8. Aneck-Hahn, N.H., Bornman, M.S., De Jager, C. *Preliminary assessment of oestrogenic activity in water sources in Rietvlei Nature Reserve, Gauteng, South Africa.* African Journal of Aquatic Science, 2008. **33**(3): p. 249-254.
9. Aneck-Hahn, N., De Jager, C., Barnhoorn, I., Bornman, M. *Endocrine disruptors and health: should South Africa be concerned?* 2016; Available from: http://www.ewisa.co.za/literature/files/216%20Aneck_Hahn.pdf. [Accessed on 16/02/15]
10. Genthe, B., Le Roux, W.J., Schachtschneider, K., Oberholster, P.J., Aneck-Hahn, N.H., Chamier, J. *Health risk implications from simultaneous exposure to multiple environmental contaminants.* Ecotoxicology and Environmental Safety, 2013. **93**: p. 171-179.
11. Aneck-Hahn, N.H., Bornman, M., De Jager, C. *Oestrogenic activity in drinking waters from a rural area in the Waterberg District, Limpopo Province, South Africa.* Water SA, 2009. **35**(3): p. 245-251.
12. Aneck-Hahn, N.H., Schulenburg, G.W., Bornman, M.S., Farias, P., De Jager, C. *Impaired semen quality associated with environmental DDT exposure in young men living in a malaria area in the Province, South Africa.* Journal of Andrology, 2007. **28**(3): p. 423-434.
13. De Jager, C., Myburgh, J., Van der Burg, B., Lemmen, J., Bornman, M. *Estrogenic contamination of South African river waters: a pilot study.* American Waterworks Water Association, April, 2002: p. 18-20.

14. Naudé, Y., Rohwer, E.R. *Two multidimensional chromatographic methods for enantiomeric analysis of o, p'-DDT and o, p'-DDD in contaminated soil and air in a malaria area of South Africa.* Analytica Chimica Acta, 2012. **730**: p. 120-126.

15. Naudé, Y., Rohwer, E.R. *Novel method for determining DDT in vapour and particulate phases within contaminated indoor air in a malaria area of South Africa.* Analytica Chimica Acta, 2012. **730**: p. 112-119.

16. Kruger, T., Barnhoorn, I., Jansen van Vuren, J., Bornman, R. *The use of the urogenital papillae of male feral African sharptooth catfish (Clarias gariepinus) as indicator of exposure to estrogenic chemicals in two polluted dams in an urban nature reserve, Gauteng, South Africa.* Ecotoxicology and Environmental Safety, 2013. **87**: p. 98-107.

17. Aneck-Hahn, N.H., De Jager, C., Bornman, M.S., Du Toit, D. *Oestrogenic activity using a recombinant yeast screen assay (RCBA) in South African laboratory water sources.* Water SA, 2005. **31**(2): p. 253-256.

18. Manickum, T., John, W. *Occurrence, fate and environmental risk assessment of endocrine disrupting compounds at the wastewater treatment works in Pietermaritzburg (South Africa).* Science of the Total Environment, 2014. **468**: p. 584-597.

19. Comerton, A.M., Andrews, R.C., Bagley, D.M. *Practical overview of analytical methods for endocrine-disrupting compounds, pharmaceuticals and personal care products in water and wastewater.* Philos Trans A Math Phys Eng Sci, 2009. **367**(1904): p. 3923-3939.

20. Annamalai, J., Namasivayam, V. *Endocrine disrupting chemicals in the atmosphere: Their effects on humans and wildlife.* Environment International, 2015. **76**: p. 78-97.

21. Chang, H.-S., Choo, K.-H., Lee, B., Choi, S.-J. *The methods of identification, analysis, and removal of endocrine disrupting compounds (EDCs) in water.* Journal of Hazardous materials, 2009. **172**(1): p. 1-12.

22. Petrovic, M., Eljarrat, E., López de Alda, M.J., Barceló, D. *Recent advances in the mass spectrometric analysis related to endocrine disrupting compounds in aquatic environmental samples.* Journal of Chromatography A, 2002. **974**(1): p. 23-51.

23. Trenholm, R.A., Vanderford, B.J., Holady, J.C., Rexing, D.J., Snyder, S.A. *Broad range analysis of endocrine disruptors and pharmaceuticals using gas chromatography and liquid chromatography tandem mass spectrometry.* Envir. Chem, 2006. **65**(11): p. 1990-1998.

24. Prasse, C., Schlüsener, M.P., Schulz, R., Ternes, T.A. *Antiviral drugs in wastewater and surface waters: a new pharmaceutical class of environmental relevance?* Environmental Science & Technology, 2010. **44**(5): p. 1728-1735.

25. WHO. *Global update on HIV treatment 2013: results, impact and opportunities.* 2013.

26. Wood, T.P., Duvenage, C.S.J., Rohwer, E.R. *The occurrence of anti-retroviral compounds used for HIV treatment in South African surface water.* Environmental Pollution, 2015. **199**(0): p. 235-243.

Chapter 2

Analytical Techniques

Table of Contents

2. Analytical Techniques

Each step in the analytical process is crucial for obtaining accurate and precise results. In most cases these steps include sampling, sample clean-up and extraction, quantification, statistical evaluation and finally decision making [1] (Figure 2.1). These analytical steps are sequential and the previous step has to be completed before the next one can commence. Consequently, the slowest step will determine the speed of the entire analytical process and all steps need to be optimised for improved outputs [1].

Figure 2.1: Steps in the analytical process (adapted from Pawliszyn (1997) [1]).

Sampling, extraction and separation (and quantification by mass spectrometry) are discussed in detail in this chapter. The focus is on techniques used during this research study. The chapter concludes with a concise literature review on analytical techniques used for the detection of micropollutants, with emphasis on EDCs, in aqueous matrices. Chapter 3: Data Analysis focuses on quantification and statistical evaluation.

2.1. Sample Preparation for Aqueous Samples

Compounds of interest are isolated from the sample matrix during the sample preparation steps. This is necessary as most analytical instruments are not able to analyse the entire matrix directly. Sample preparation usually involves sample clean-up procedures for dirty samples, isolation of the target analytes from the matrix as well as enrichment of analytes to a suitable concentration level for analysis [1].

2.1.1. Sampling

Before sampling, it is useful to have prior information on the sampling site in order to obtain a clear picture of the expected outcomes of the analysis. Information on the

physical location of the sampling site and environmental setting can be used to establish a list of possible contaminants that may be present at the site [2].

Water samples can be from a variety of sources, including surface waters (rivers, lakes, dams, runoff, etc.), groundwater, wastewater (mine drainage, landfill leachate, industrial effluent, etc.), saline waters, estuarine waters, waters from atmospheric precipitation and condensation (rain, snow, fog and dew), drinking water, etc. It is important to note that “water” is a heterogeneous substance and sampling should be conducted at a predetermined depth, area and time. A spatial variation occurs in large water bodies due to layering caused by variations in flow, chemical composition and temperature. Variation with respect to time (temporal) occurs due to heavy precipitation and seasonal changes [2]. After sampling water samples should be stored for a minimal amount of time in the dark at 4 °C in order to preserve organic compounds [2].

2.1.2. Extraction

2.1.2.1. Classical approach

Liquid-liquid extraction (LLE) is classically used for the extraction of compounds from aqueous samples. LLE works by distributing the sample between two immiscible liquids or phases where the analyte and matrix have different solubilities. The two phases are usually aqueous and organic solvents. In principle the more polar hydrophilic compounds prefer the aqueous phase and the more non-polar hydrophobic compounds prefer the organic phase [3]. Discontinuous liquid-liquid extraction using a separating funnel is routinely employed. During discontinuous extraction an equilibrium is established between the two phases opposed to continuous liquid-liquid extraction where equilibrium is not reached [3].

Advantages for LLE extraction include its suitability for a wide range of applications, solvents typically used during LLE are easily available and the apparatus (separating funnel) used for LLE is low in cost [3]. A problem usually encountered with LLE is formation of emulsions. Additionally, for the same analyte, the rate of extraction can vary due to different sample matrices and contamination can be problematic. Consequently, it is crucial to use high-purity organic solvents (which are not only expensive as large solvent volumes are needed but also harmful to the environment)

and to thoroughly wash all glassware before use. Finally, care must be taken to reduce analyte loss due to adsorption on the glass walls of the containers or separating funnel [4].

2.1.2.2. Solid phase extraction

Solid phase extraction (SPE) is commonly used for the isolation, enrichment and/or clean-up of target compounds from aqueous samples [5]. SPE, also known as liquid-solid extraction, works by selective adsorption of analytes onto the surface of a solid phase [4]. A liquid or gaseous sample is brought into contact with the solid phase or sorbent whereby the analytes are adsorbed onto the surface before elution of the target compound from the solid phase [4, 5]. The solid phase is normally packed into small tubes or cartridges (Figure 2.2). Typically, selecting the correct sorbent material should result in retaining the target compound on the sorbent in preference to any other extraneous material present in the sample.

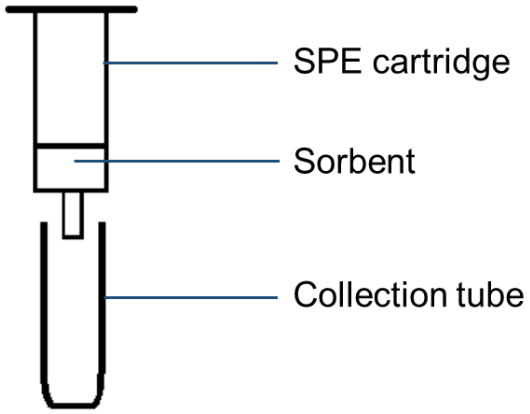

Figure 2.2: Experimental setup for SPE [5]

By selecting appropriate solvents the extraneous material can be washed from the sorbent. After washing, the target compound is eluted from the sorbent by means of suitable solvents. The eluent is collected for analysis. The process can be sped up during the sample loading step by forcing the matrix containing the target analytes through the sorbent by pressure or vacuum [5]. Figure 2.3 depicts the five stages typically involved in SPE.

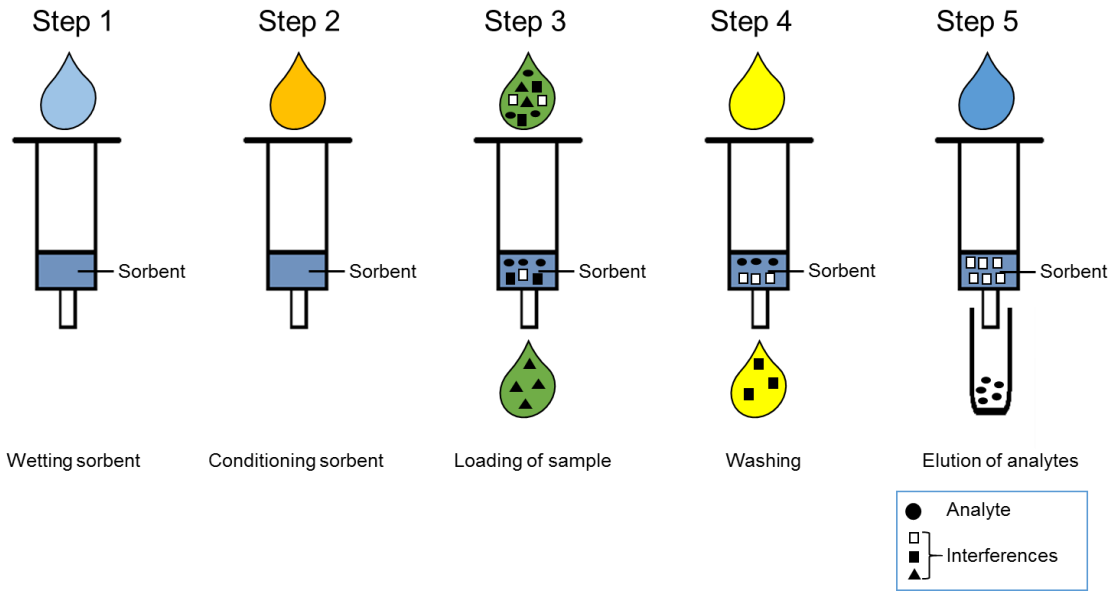

Figure 2.3: The five stages of solid phase extraction. The different colours represent the different solvents used during each stage and sample loading (green) in step 3 (adapted from Dean, 2009) [5].

The choice of the correct sorbent, taking in consideration the type of target analyte, the capacity of the SPE cartridge (the number of active sites on the sorbent cannot be exceeded by the amount of analyte molecules) and the flow rate (too fast flow rates will reduce analyte-sorbent interaction times), should result in the compound of interest being retained in preference to extraneous material present in the sample matrix [4].

The main advantages of SPE are the pre-concentration of the target analyte/s from very large sample volumes to a small extract volume, a high degree of selectivity due to correct sorbent choice and the clean-up of the sample matrix to obtain a particle-free and chromatographically clean extract. The process can also be automated, thus saving time during lengthy method development steps and in application thereof [5]. In SPE, analytes are temporarily stored on the surface of adsorbents (refer to Figure 2.5 (Section 2.1.2.4) for a comparison of absorption vs adsorption). The trapped analytes are normally released by extraction with a small amount of an organic solvent. However, only an aliquot of the extract is injected into the analytical instrument resulting in poor sensitivity as merely a fraction of the sample is analysed [6]. Additional disadvantages of SPE include the use of organic solvents (not a “green” extraction method), time consuming method development process, it is labour intensive (when not automated), cartridges are expensive [4, 5] and cartridges cannot be reused.

2.1.2.3. Large volume injections

Large volume injection (LVI) can be applied as a fast and effective alternative to SPE for aqueous samples with liquid chromatography (LC). LVI involves the direct injection of sample volumes that range from 100 to 5000 µL compared to conventional injection volumes of 10 to 20 µL for LC. The injection of larger sample volumes increases sensitivity and reduces sample handling steps resulting in greater reproducibility [7, 8]. However, matrix effects need to be considered when using LVI as complex environmental samples can interfere with the electrospray ionisation (ESI) process [7, 8]. Good separation and matrix matched standardisation are required for reliable quantification [9]. LVI with ultra-high pressure liquid chromatography (UHPLC) was recently applied by Boix et al. (2015) for the determination of 40 drugs from water [7]; and LVI with liquid chromatography (LC) was applied by Bayen et al. (2014) for the analysis of antibiotics in surface freshwater and seawater [10].

2.1.2.4. Sorptive extraction

New, efficient and inexpensive analytical methods are necessary for environmental monitoring and evaluation [11]. Several research groups have shifted their focus from adsorbents to another class of materials, namely sorption materials [6]. Combining sample extraction, purification and enrichment, using approaches such as solid phase microextraction (SPME) and stir bar sorptive extraction (SBSE), have shifted sample preparation towards a more “green”, i.e. solvent free, approach [12]. Sorption (i.e. dissolution or partitioning) materials are polymers at temperatures above their glass transition point (T_g). T_g is the temperature region where a polymer transitions from a hard, glassy material to a soft, rubbery material. The sorbents are in a gumlike or liquid state in these temperature ranges subsequently behaving similarly to organic solvents. Generally, sorbents are homogenous non-porous materials. The analytes dissolve into the sorbents, therefore, they do not undergo real bonding but are retained by dissolution [6].

Polydimethylsiloxane (PDMS) is currently the most popular sorbent material (Figure 2.4) [6]. It is an apolar 100% methyl substituted siloxane polymer. The popularity of PDMS is due to its: (1) inertness, therefore reducing analyte loss due to irreversible adsorption or catalytic (surface) reactions, (2) retention data for many compounds are widely available, (3) PDMS synthesis is moderately simple leading to reproducible

properties and consistency between manufacturers, and (4) the degradation products are well known and can easily be identified by mass spectrometry [6].

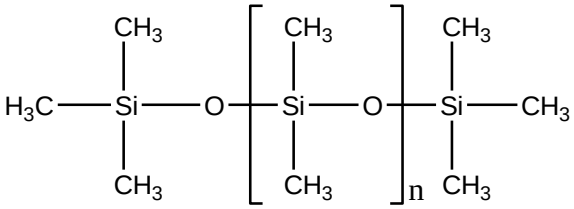

Figure 2.4: Structure of PDMS. Glass transition temperature: -125 °C [6].

Various researchers have employed the advantages of PDMS to develop new samplers. Triñanes et al. (2015) developed disposable silicone disks for the detection of polycyclic aromatic hydrocarbons (PAHs) in water samples [13]. Naudé and co-workers developed a PDMS loop sampler for solvent free extraction of soil [14, 15]. The sampler was recently used by Naudé et al. (2015) as a passive sampler to concentrate pollutants from surface water [16].

When developing customised samplers the use of bulk, low cost PDMS gives the user control over the choice of sorbent volume and creation of application specific sorptive samplers. The low cost of the sorbent material allows the employment of a new sampler for each extraction, thereby avoiding difficulties with carry-over and cross contamination [13]. The hydrophobicity of PDMS enables high recovery of hydrophobic compounds. However, polar compounds show lower recovery. To overcome this limitation Ochiai et al. (2006) developed a sequential salting out extraction procedure for multi-residue analysis using SBSE [17]. This approach provides uniform enrichment over the entire polarity range for organic pollutants at trace levels in water [17].

Widely used commercial sorptive extraction techniques are discussed in the following sections. The focus is placed on SPME and SBSE, both being commercial solvent free sorptive extraction techniques. SBSE was used during this research study; SPME is also discussed in detail as the principles governing its working are applicable to SBSE.

SPME

Solid phase microextraction (SPME) was introduced in 1990 by Arthur and Pawliszyn [18] to address the need for rapid sample preparation in the laboratory and on-site [19]. A small amount of extracting phase, which is coated on a solid support, is

exposed to the sample, for a defined period of time, whereby an organic compound is adsorbed (absorbed when coating with sorptive materials such as PDMS) onto the surface [19, 20]. Convection conditions may or may not be used during the extraction. If convection is not employed a partitioning equilibrium between the sample matrix and extraction phase is reached influencing the amount of analyte extracted. If convection or agitation or both are employed and are constant, then the amount of analyte extracted is related to time and usually results in short-time pre-equilibrium extraction. Time dependent accumulation of analytes onto the coating can then be used for quantification [19]. Adsorption is usually followed by desorption of the organic compounds into a suitable instrument for separation and quantification. Dilute aqueous samples or gas phase samples can thus be pre-concentrated by SPME with the use of a suitable sorbent [20].

As soon as the coated fibre is placed in contact with the sample, analytes are transported from the matrix to the coating. The main difference between solid and liquid coatings is the mechanism of attachment, i.e. adsorption versus absorption (Figure 2.5). Both processes begin with adsorption of the analytes at the extraction phase-matrix interphase followed by diffusion of analytes into the bulk of the extraction phase. Absorption is achieved when the diffusion coefficients of the analytes in the extraction phase are high causing the analyte to fully partition between the two phases. If the diffusion coefficients are low the analyte remains at the interface leading to adsorption. The main disadvantage for adsorption is a highly nonlinear isotherm at higher concentrations due to substantial surface coverage. The advantages of solid sorbents (adsorption) are higher sensitivity and capacity for polar and volatile analytes [19].

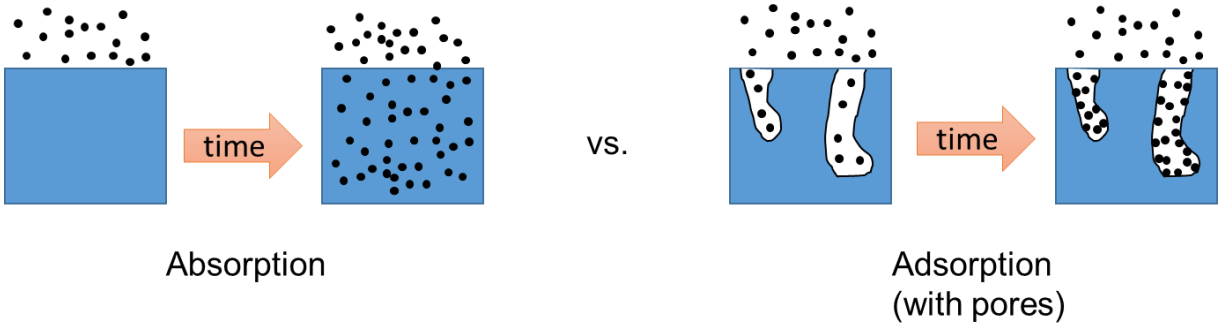

Figure 2.5: Schematic representation of absorption versus adsorption (adapted from Pawliszyn, 2000) [19].

The partitioning of analytes between the sample matrix and the stationary phase is the main principle of operation of SPME [20]. Once equilibrium is reached the extracted amount remains constant and is independent of further increases of extraction time [19]. The equilibrium conditions are described by the following mathematical relationship:

$$n = \frac{K_{fs} V_f V_s C_0}{K_{fs} V_f + V_s}$$

Equation 2-1

Where:
n = mass of analyte extracted by the coating
K_{fs} = fibre coating sample matrix distribution constant
V_f = fibre coating volume
V_s = sample volume
C_0 = initial concentration of a given analyte in the sample [19]

High K values will lead to good pre-concentration of the target analytes and consequently high sensitivity in terms of analysis. It is, however, unlikely that K is large enough for complete extraction, therefore, SPME is an equilibrium technique. Quantification is possible provided the correct calibration strategies are implemented [20]. With very large sample volumes Equation 2-1 can be simplified to:

$$n = K_{fs} V_f C_0$$

Equation 2-2

Equation 2-2 is most useful in field sampling [19].

Sorbents (absorbents) generally have reduced analyte capacity compared to typical adsorbents leading to reduced sensitivity. This in combination with the very small amounts coated onto the SPME fibre, up to 0.5 µl, resulted in the main disadvantage of SPME: lack of sensitivity for liquid samples. SPME is still not as widely accepted as SPE due to this lack of sensitivity despite its numerous advantages including simplicity and cost [19]. Several new SPME coatings have been developed to overcome sensitivity problems. These include PDMS, copolymers of PDMS with divinylbenzene (PDMS-DVB) and Carbowax (PDMS-WAX) and physical mixtures of PDMS with adsorbents such as Carboxen [19].

SBSE

The potential sensitivity drawback of sorptive samplers such as SPME was overcome with the introduction of SBSE (developed by Baltussen and Sandra in 1999) [21]. Sorptive volumes of up to 200 µl for SBSE have allowed for a sensitivity increase of up to 500-fold when compared to SPME enabling quantitative extraction of analytes from aqueous samples [6, 21]. With SBSE, organic compounds are pre-concentrated onto a magnetic stir bar coated with a sorbent, e.g. PDMS, which is placed in an aqueous sample (Figure 2.6). The stir bar is immersed in the sample for a period of time whereafter the organic compounds are either back extracted into a solvent for conventional injection into a gas chromatograph (GC) or liquid chromatograph (LC), or desorbed from the stir bar by a thermal desorption unit connected to a GC [22].

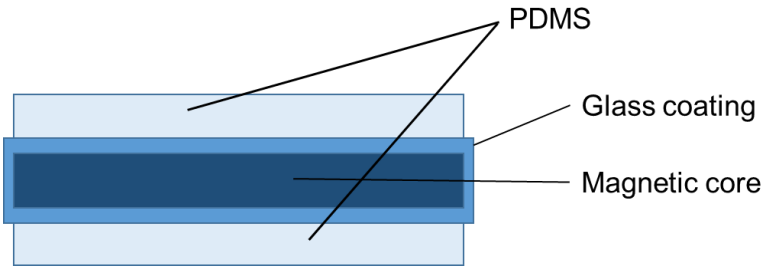

Figure 2.6: Stir bar sorptive extraction [22]

The theory governing SBSE is very similar to that of SPME. Using the approximation that the partitioning coefficients between PDMS and water ($K_{PDMS/W}$) are proportional to octanol-water partitioning coefficients (K_{ow}) the following mathematical relationship applies:

$$K_{OW} \approx K_{\frac{PDMS}{W}} = \frac{C_{SBSE}}{C_W} = \frac{m_{SBSE}}{m_W} \times \frac{V_W}{V_{SBSE}}$$

Equation 2-3

Where:

C_{SBSE} = analyte concentration in the SBSE

C_W = analyte concentration in the water phase

m_{SBSE} = mass of analyte in the SBSE

m_W = mass of analyte in the water phase

V_{SBSE} = volume of the SBSE

V_W = volume of the water phase [21]

Using the phase ratio (β) which equal V_W/V_{SBSE}, Equation 2-3 can be written as:

$$\frac{K_{ow}}{\beta} = \frac{m_{SBSE}}{m_W} = \frac{m_{SBSE}}{m_0 - m_{SBSE}}$$

Equation 2-4

Where:

m_0 = total amount of analyte originally present in the water sample [21]

Lastly, Equation 2-4 can be transformed to give the extraction efficiency or recovery from water:

$$\frac{m_{SBSE}}{m_0} = \frac{\frac{K_{OW}}{\beta}}{1 + \frac{K_{OW}}{\beta}}$$

Equation 2-5

This proves that the only parameter regulating the recovery of an analyte from the sample is the ratio between the partitioning coefficient, K_{ow}, and the phase ratio, β, of the PMDS coated on the stir bar and the water sample [21].

2.1.3. Thermal desorption

In order to overcome sensitivity shortcomings of SPE, sorptive sampling and analytical techniques involving thermal desorption in combination with gas chromatography (GC) are used [6, 9]. Thermal desorption (TD) under an inert gas stream is increasingly being used as an alternative to liquid desorption [6]. During TD volatile and semi-volatile organic compounds are desorbed from the solid support, usually within a stainless-steel or glass tube, by heat followed by direct introduction into the GC injection port via a heated transfer line. The technique is solvent free and can be automated. Cool temperatures (ca. 100 °C) are commonly used to maintain compound integrity during the desorption process of analytes from an adsorbent [23]. However, higher temperatures (ca. 280 °C) are needed for thermal desorption of semi-volatile organic compounds. Different adsorbents used for thermal desorption include inorganic carbon based adsorbent materials that can be heated to 400 – 450 °C without degradation of the material, silica and alumina type adsorbent materials which are thermally stable up to 400 – 600 °C and polymeric adsorbents with maximum temperatures ranging from 150 °C for some Chromosorbs to 350 °C for Tenax [6].

However, slow release of compounds from the adsorbent generally results in broad chromatographic peak shapes. Ideally rapidly desorbing compounds will result in good peak shape. To overcome resolution problems associated with broad peaks the volatiles can be trapped cryogenically onto the GC column prior to the analysis [23]. Using cryogenics permits quantitative transfer of trapped analytes from the adsorbent material to the chromatographic column allowing a substantial increase in sensitivity when compared with liquid desorption, where only a portion of the sample is analysed. However, for the analysis to be successful the analytes undergoing TD must be thermally stable otherwise degradation will occur [6].

Choosing the correct adsorbent material is vital for the TD process. Inorganic adsorbents tend to interact too strongly with the trapped analytes resulting in degradation reactions since high temperatures are needed to remove the analytes from the adsorbent material. Organic adsorbents, on the other hand, tend to give poor blanks due to decomposition of the material itself. Significant catalytic activity is also associated with most adsorbents even at low temperatures. These obstacles led to the development of new sorption material, such as PDMS (refer to Section 2.1.2.4).

2.1.4. Derivatisation with GC

The low volatility of compounds with carboxylic acid (-COOH), hydroxyl (-OH), secondary amine/amide (-NH) and thiol (-SH) functional groups is problematic during GC analysis. The decrease in volatility is attributable to the active hydrogen within the functional group that forms intermolecular hydrogen bonds. Peak broadening is a common occurrence with these compounds as they react with either the stationary phase or fused silica of the GC column [24]. These functional groups need to be protected to facilitate GC analysis.

Micropollutants can be divided into a range of groups based on their functionality and as such different protection strategies are necessary for a multi-residue detection method. The phenolic compounds can readily be acylated using *in situ* derivatisation with acetic acid anhydride [24, 25]. Ethyl carbamate can be used to convert primary amines to their corresponding ethyl carbamates [26]. The polarity of acidic compounds can be reduced by forming less polar ethyl derivatives using ethyl chloroformate in the presence of ethanol [27]. Organotin compounds can be derivatised using sodium tetraethylborate; converting them into ethyl derivatives [28]. Compounds with a log

K_{ow} > 5 (K_{ow} being the octanol-water partition coefficient) require methanol addition to minimise wall adsorption effects (adsorption of analytes on the inner surface of the glass container) [25].

Van Hoeck et al. (2009) adapted the “multi-shot” thermal desorption method developed by Kawaguchi et al. (2006) into a multi-residue method for screening EDCs and pharmaceuticals in aqueous samples [25, 29]. Taking into account the different derivatisation strategies needed to detect compounds within a heterogeneous group, Van Hoeck et al. (2009) completed different *in situ* derivatisation reactions by dividing a single aqueous sample into four aliquots [25]. The derivatisation reactions were carried out in three aliquots using acetic acid anhydride, ethyl chloroformate and sodium tetraethylborate. Methanol was added to the fourth aliquot in order to limit analyte adsorption onto the glass walls. After sampling, the four stir bars were placed in one thermal desorption tube together with a plug of glass wool impregnated with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) for concurrent desorption and analysis in order to increase sensitivity [29]. BSTFA is added to protect active hydrogens of acid, alcohol, thiol, amine and amide functional groups in order to increase compound volatility, temperature stability and detectability [24].

2.2. Chromatography

The separation step in the analytical procedure is typically done with the aid of chromatographic or electrophoretic techniques. During this step, the isolated complex mixture containing the target analytes is separated into its components [1].

2.2.1. Chromatographic principles

Chromatography uses a stationary and mobile phase to separate chemical components in complex mixtures. Separation is based on the differences in migration rates between the mobile phase components (Figure 2.7). The components in the mixture are carried (eluted) by the mobile phase (eluent) through the stationary phase. The resulting difference in rates causes the components of the mixture to separate into bands along the length of the column [30].

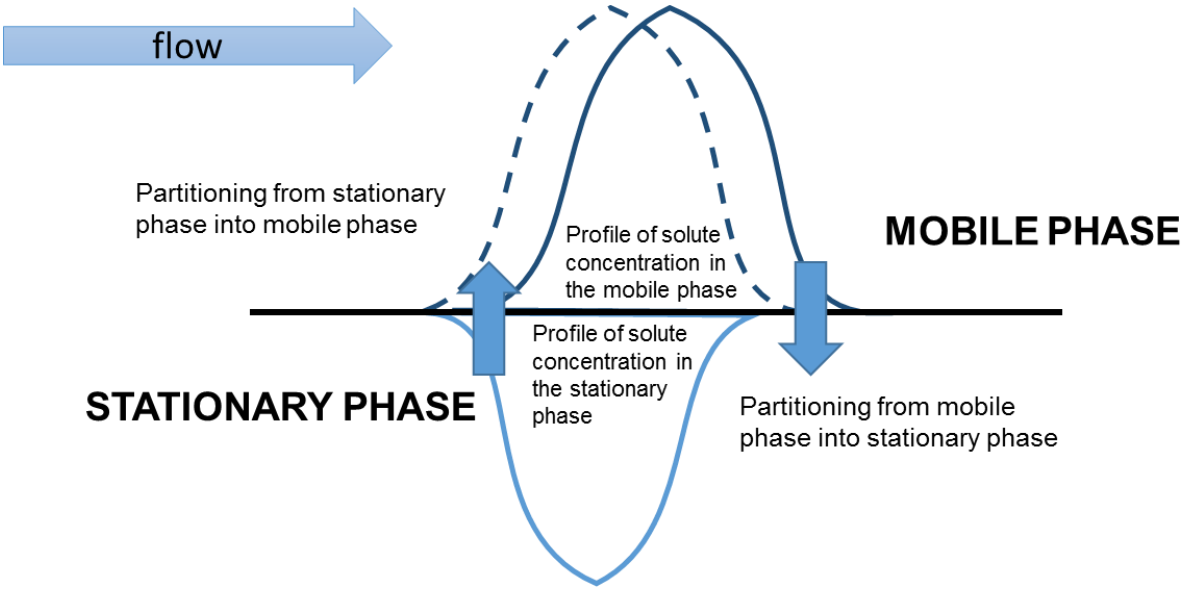

Figure 2.7: The partitioning of a solute between the mobile and stationary phase in accordance with the equilibrium constant (K_c). At time T the concentration of solute in the stationary phase (c_S) is represented by the curve below the horizontal line and the concentration of solute in the mobile phase (c_M) is represented by the dotted curve above the horizontal line. The solute molecules in the mobile phase are carried by the flow and at time T + t, c_M is represented by the solid curve above the horizontal line. K_c has been disturbed in the regions marked by the arrows. The arrows indicate the direction of the movement of the solute molecules in order to re-establish equilibrium. In passing through the column, each solute band continuously evaporates at the rear and is re-established at the front. (Adapted from Jennings et al., 1997) [31].

2.2.1.1. Migration rates of solutes

The distribution of an analyte (A) between the mobile and stationary phase can be described by the following equilibrium equation [30]:

$$A(mobile) \leftrightarrow A\ (stationary)$$

Equation 2-6

The equilibrium constant K_c is called the distribution constant and can be defined as follows:

$$K_c = \frac{c_S}{c_M}$$

Equation 2-7

Where:

c_S = molar analytical concentration of the solute in the stationary phase

c_M = molar analytical concentration of the solute in the mobile phase [30]

The retention time, the time between sample injection and appearance of a peak at the detector, is given by the following equation:

$$t_R = t_S + t_M$$

Equation 2-8

Where:

t_R = retention time

t_S = time analyte spent in the stationary phase

t_M = time analyte spent in the mobile phase [30]

2.2.1.2. The rate theory of chromatography

The shapes and breadths of elution bands based on a random-walk mechanism for the movement of molecules through a column are described by the rate theory of chromatography [32]. Typical Gaussian shaped peaks are obtained when working under linear chromatographic conditions, i.e. K_c is constant. Non-ideal (anti-Langmuir) chromatographic peaks, i.e. peaks that exhibit tailing or fronting, are usually due to a K_c which varies with analyte concentration (Figure 2.8). Fronting can also be caused by a too large amount of sample introduced into the column. Poor separation and non-reproducible elution times result due to non-ideal chromatographic peaks [30, 32].

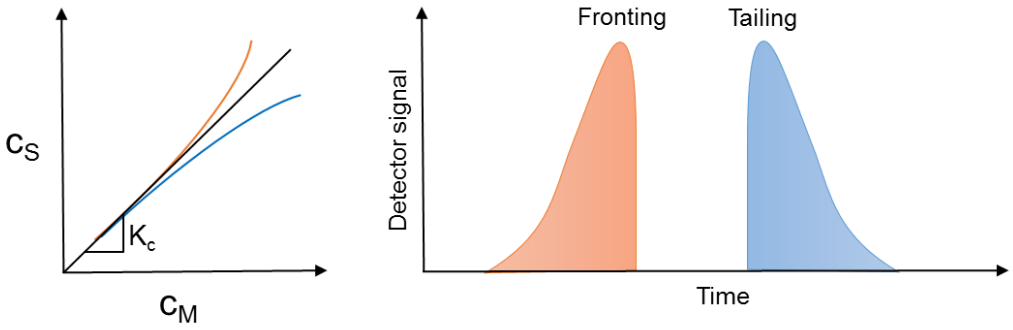

Figure 2.8: The Langmuir isotherm shown in blue (left image) describes the situation where K_c becomes smaller with an increase in analyte concentration; this leads to peak tailing (blue chromatographic peak in the right image). The Langmuir isotherm shown in red (left image) describes the situation where K_c becomes larger with an increase in analyte concentration; this leads to peak fronting (red chromatographic peak in the right image) [33].

2.2.1.3. Column efficiency

The efficiency of chromatographic columns is normally described by plate height (H) and plate count or number of theoretical plates (N). Their relationship is described by the following equation:

$$N = \frac{L}{H}$$

Equation 2-9

Where:
L = length (usually in cm) of the column [30]

The column efficiency consequently increases with higher plate numbers (*N*) and lower plate height (*H*).

The Van Deemter Equation

Peak broadening reflects a loss in column efficiency. In the 1950s Dutch chemical engineers conducted studies on peak broadening which led to the development of the Van Deemter Equation. However, the equation was deemed only appropriate for packed columns at high flow velocities. Today the efficiency of capillary and packed chromatographic columns can be approximated by using the following equation (also refer to Figure 2.9 for optimum efficiency estimation):

$$H = A + \frac{B}{u} + C_S u + C_M u$$

Equation 2-10

Where:
A = eddy diffusion
H = plate height (cm)
u = linear velocity of the mobile phase (cm/s)
B = longitudinal diffusion coefficient
C_S = mass-transfer coefficient for the stationary phase
C_M = mass-transfer coefficient for the mobile phase [30]

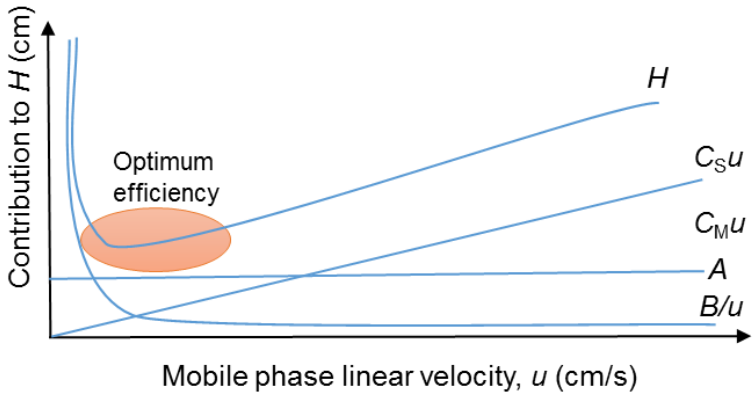

Figure 2.9: Contribution of various mass-transfer terms to plate height, *H* [30].

2.2.1.4. Resolution

The ability of a column to separate two compounds is measured by its resolution, R_S. The resolution measures how far apart two bands are relative to their widths. The resolution for a column is described by the following equation:

$$R_s = \frac{2((t_R)_B - (t_R)_A)}{W_A + W_B}$$

Equation 2-11

Where:

$(t_R)_A$ = retention time for analyte A

$(t_R)_B$ = retention time for analyte B

W_A = peak width at base for analyte A

W_B = peak width at base for analyte B [30]

2.2.1.5. Peak capacity

The resolution of all compounds in very complex samples is rarely possible. This is due to the chromatographic system which has limited resolving power. There is a maximum number of ideally spaced peaks that can be resolved under optimum conditions. This maximum is known as the peak capacity, a concept developed by Giddings (1967) [34]. If the compound peaks are randomly spaced and/or the number of compounds in the sample is more than the peak capacity co-elution will result.

2.2.2. Classes of chromatography

2.2.2.1. Gas chromatography

Gas chromatographic separation starts by vaporising a sample and injecting it into the head of a chromatographic column. The eluent is an inert gas which does not interact with the molecules of the analyte and merely functions as the carrier of the analyte through the column. There are two types of gas chromatography, namely gas-liquid chromatography (GLC), which is usually shortened to gas chromatography (GC), and gas-solid chromatography (GSC). GLC or GC works on the principle that an analyte will partition between a gaseous mobile phase and a liquid phase coated on the surface of an inert solid packing or on the walls of capillary tubing. GSC works by physical adsorption of the analyte on a solid stationary phase. The semi-permanent

retention of active molecules and severe tailing of eluting peaks, due to the non-linearity of the adsorption process, have limited the application of this technique [30].

2.2.2.2. Liquid chromatography

Liquid chromatography (LC) involves the use of a liquid as the mobile phase. There are several types of LC methods which can be classified by either separation mechanism or by the type of stationary phase used. LC methods include partition chromatography, adsorption or liquid-solid chromatography, ion-exchange or ion chromatography, size-exclusion chromatography, affinity chromatography and chiral chromatography. The popularity of LC can be contributed to its high sensitivity, ease of automation, suitability to separate non-volatile species or thermally labile analytes and its widespread application to compounds used in industry and various scientific fields [32].

2.2.3. GC×GC

Comprehensive two-dimensional gas chromatography (GC×GC) was introduced and pioneered by Phillips et al. (1991) [35]. The technique uses fast, continuous heart-cutting (modulation) ensuring the sampling period is less than the width of a first dimension (^{1}D) peak in order to preserve the peak shape in ^{1}D. A multi-dimensional separation is achieved for the entire sample in a single analytical run by using comprehensive techniques. A complete second dimension (^{2}D) chromatogram for each sampled zone (or slice) of the ^{1}D eluent is created using considerably faster separations on the shorter ^{2}D column [36]. Nomenclature and conventions, specifically for comprehensive two dimensional (^{2}D) separations, proposed by Schoenmakers et al. (2003) are used throughout the dissertation [37].

GC×GC is a powerful tool that aids in the determination of vast amounts of compounds in a complex matrix during a single analysis [38]. It realises better resolution (increased selectivity), higher sensitivity and larger peak capacity over conventional 1D GC [39, 40]. The increased resolving power and enhanced sensitivity make GC×GC extremely useful in detecting trace-level components in complex samples [41].

2.2.3.1. GC×GC instrument

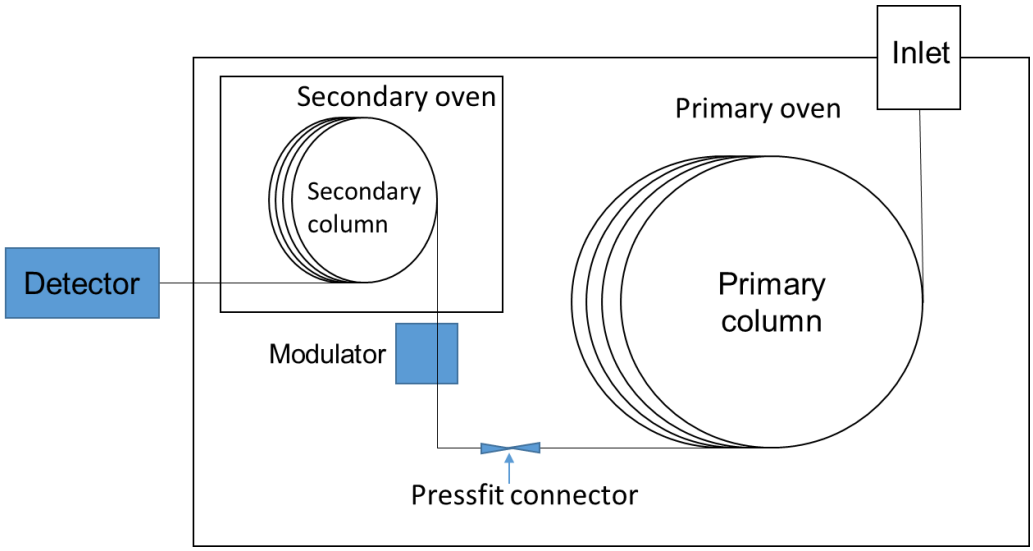

Figure 2.10: Schematic diagram of a GC×GC instrument.

A schematic diagram of a typical GC×GC instrument is given in Figure 2.10. The primary and secondary columns can either be housed in the same oven or, as in Figure 2.10, housed in separate ovens allowing for independent temperature programming. The ^{1}D column is normally a long column (30 to 60 m X 0.25 to 0.32 mm internal diameter (ID) with 0.2 to 0.3 µm stationary phase film thickness (d_f)) with a non-polar stationary phase. A temperature programming rate between 1 and 3 °C/min ensures peak widths between 10 and 20 seconds under optimum conditions [42].

Modulators are used as interfacing or transfer devices which connect the two columns in series. The modulator fractionates, isolates and re-concentrates the ^{1}D column eluent for reinjection onto the ^{2}D column throughout the entire analysis [41]. Peaks are usually modulated between 3 and 4 times in order to preserve the peak shape in ^{1}D [42]. For a peak, 15 seconds wide, to be modulated three times it needs to be recaptured and reinjected onto the ^{2}D column every 5 seconds. This is known as the modulation period [37].

The ^{2}D column typically has a polar stationary phase and is much shorter and narrower (typical dimensions are 1 to 2 m X 0.1 to 0.25 mm ID with 0.1 to 0.25 µm d_f) than the ^{1}D column. The ^{2}D separation is very fast (1 to 10 seconds) resulting in peak widths between 100 and 600 milliseconds at the baseline under optimum conditions. The very fast separations in ^{2}D can consequently be considered to be occurring under isothermal conditions (the temperature usually increases by 2 °C every minute) [42].

2.2.3.2. Modulation

The modulator is considered the heart of the GC×GC system [42]. The modulator samples the eluent from the ^{1}D column and then pulses segments of the analyte onto the ^{2}D column. Several different modulators have been developed either based on temperature, such as the thermal sweeper, or by cryogenic means, such as the longitudinally modulated cryogenic system (LMCS) [36]. The dual-stage jet modulator, which is comprised of two hot air jets and two cold N_2(g) jets (Figure 2.11), is most commonly used today [42].

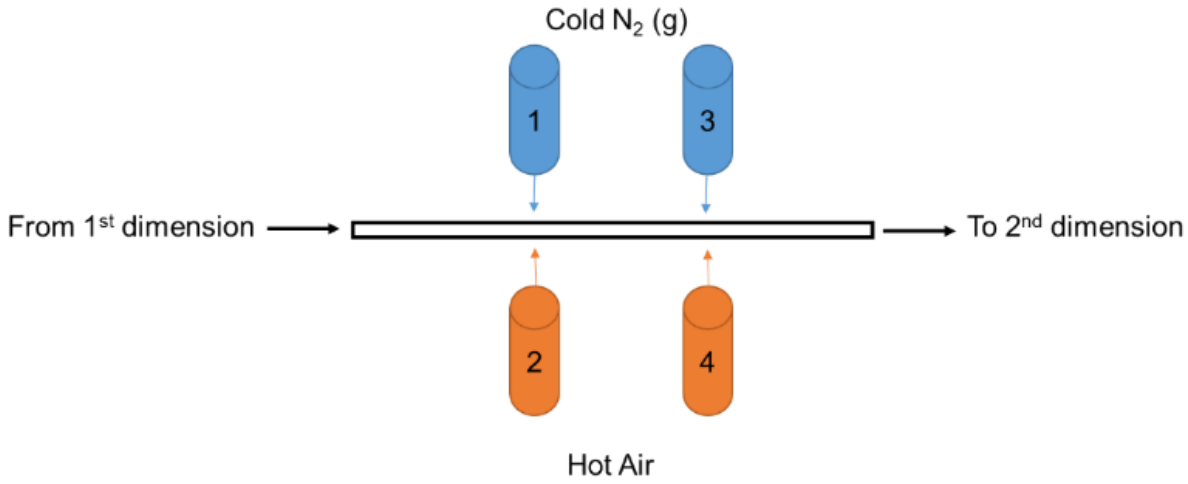

Figure 2.11: Schematic diagram of a dual-stage jet modulator. The first cold jet (1) traps the compounds from the ^{1}D eluent. The first hot jet (2) then releases the trapped fraction. The second cold jet (3) captures and refocuses the fraction which is then reinjected into the ^{2}D column by the second hot air jet (4). While the fraction is being refocused and reinjected in the second step the first cold jet captures the next fraction eluting from ^{1}D [43].

2.2.3.3. Enhanced peak capacity

There is a significant enhancement in peak capacity between 1D GC and 2D GC separations. An ideal comprehensive GC instrument will have a peak capacity equal to the product of the peak capacities in each dimension:

$$n_T = n_{c1} \times n_{c2}$$

Equation 2-12

Where:

n_T = total peak capacity

n_{c1} = ^{1}D peak capacity

n_{c2} = ^{2}D peak capacity [41]

In order to fully exploit the gain in peak capacity, the system has to be orthogonal.

2.2.3.4. Orthogonality

Schoenmakers et al. (2003) [37] states that the definition of orthogonality, relating to analytical chemistry, implies the following:

"…that complete independent retention mechanisms apply in the two dimensions."

Orthogonality is normally achieved by using column sets of different polarity or separation mechanisms and accordingly enhancing peak capacity. The first column is much longer (contains more theoretical plates) than the second column. Consequently, the ^{1}D separation is more efficient than ^{2}D separation [30]. Separation on the ^{1}D non-polar column is essentially based on boiling point whereas separation on the ^{2}D polar column is based on different polarity-based interactions (e.g. H-bonding, dipole-dipole and polarising effects) for isovolatile compounds [41].

2.2.4. UHPLC

The first liquid chromatographic (LC) separations were performed in glass columns with diameters between 10 and 50 mm. The columns, up to 500 cm in length, were packed with solids particles, 150 to 200 µm in size. The particles were coated with an adsorbed liquid functioning as the stationary phase. The process was plagued with extremely slow flow rates which could be increased by applying pressure or vacuum. However, this resulted in lower efficiencies as the higher flow rate tends to move plate heights away from the minimum (optimum) in the plate versus flow rate curve (Van Deemter equation). Scientists realised early on that efficiencies can be improved by using smaller particle sizes. However, technology only enabled the development of stationary phase packing materials with particle diameters of between 3 and 10 µm in the late 1960s. This new technology required sophisticated instruments operating at high pressures. The name high-performance liquid chromatography (HPLC) was consequently given to the new technique in order to differentiate it from the original gravity-flow methods [32].

The trend in decreasing packing material size, $d_{p,}$ in order to increase performance slowly continued until the late 1990s. The more conventional 5 µm phases were replaced with 3 to 3.5 µm particles. However, further reduction of particle size was stunted due to the high pressures needed to operate such columns. In 1997 Jorgenson et al. [44] used high pressure instruments and showed the benefits of using sub 2 µm

phases for fast efficient separations. Since 2005 commercial instruments operating at pressures up to 1 250 bar (ca. 18 400 psi) using a range of columns packed with particles ranging from 1.3 to 1.5 µm have gained widespread acceptance. The technique is now commonly known as ultra-high pressure liquid chromatography (UHPLC) [45].

UHPLC is often used for fast multi-residue screening of organic contaminants in environmental samples [46]. The use of UHPLC coupled with tandem mass spectrometry (MS/MS) such as the triple quadrupole (QqQ) permits the development of faster and more sensitive methods [7]. However, high resolution mass spectrometry (HRMS) analysers such as time-of-flight (TOF) allow for improved identification during broad range screening. The quadrupole time-of-flight (QTOF) instrument provides the user with the option to acquire full scan MS spectra and MS/MS spectra for high confidence identification of compounds [47]. (Refer to Section 2.3.3 Mass analysers). LC-QTOF technology has previously been used by Masiá et al. (2014) and Bueno et al. (2012) for target and non-target screening of contaminants in water [47, 48].

2.2.4.1. Enhanced efficiency

Higher efficiency in UHPLC is the main advantage over conventional HPLC. Smaller particles can significantly reduce the height equivalent of a theoretical plate (HETP) generated during the separation process [44]. Van Deemter curves (Equation 2-13) can be used to describe the variation in plate height (*H*) and the linear velocity of the mobile phase (*u*). The Van Deemter equation:

$$H = A + \frac{B}{u} + Cu$$

Equation 2-13

Where:
H = plate height (cm)
u = linear velocity of the mobile phase (cm/s)
A = multiple path effect coefficient
B = longitudinal diffusion coefficient
C = mass transfer coefficient [30]

According to chromatographic theory, the *A* and *C* terms in the Van Deemter equation will decrease with a particle size reduction. The *A* term, the multiple path effect or eddy

diffusion, describes the various pathways by which an analyte can move through a packed column. In general smaller particles result in smaller differences in the various pathlengths travelled by the analyte. The *C* term results from the resistance to mass transfer of analyte molecules in the stationary, mobile and stagnant mobile phases. Stagnant mobile-phase mass transfer occurs when mobile phase is trapped within the particle pores of a packed column. The stationary phase *C* term is independent of particle size, however, this is not the case with the mobile and stagnant mobile phase *C* terms. As particle size decreases the distance over which analyte molecules must diffuse to reach the particle surface decreases. The two mobile phase *C* terms are proportional to the square of the particle size. Thus, smaller particles will greatly reduce the *C* term. The decrease of the *A* and *C* terms will lower the minimum plate height giving higher efficiency for a given column length. A higher optimal linear velocity is also achieved for smaller particle sizes, as it is related to the flow rate via the column diameter. The higher optimal linear velocity is governed by the Van Deemter equation, thus this effect for smaller particles is due to flattening of the Van Deemter curve in higher flow rate regions for smaller particles due to a lower *C* term, allowing for operation at higher flow rates [30, 44, 45].

2.3. Mass Spectrometry

Mass spectrometers are used not only to identify analytes eluting from a chromatographic column but also in the quantification step of the analytical procedure due to their ability to reduce quantification errors caused by interferences. Quantification involves determining the amount of the identified compound.

2.3.1. Mass spectrometry principles

The production of gaseous ions of a compound is the first step in mass spectrometry (MS). For example, electron ionisation:

$$M + e^- \rightarrow M^{\bullet+} + 2e^-$$

The molecular ion $(M^{\bullet+})$ produced usually undergoes fragmentation. This radical cation has an odd number of electrons and can fragment in two ways: (1) it can form a radical $(R^{\bullet})$ and an ion with an even number of electrons (EE^{+}) or (2) it can form a molecule (N) and a new radical cation $(OE^{\bullet+})$.

Even Ion Radical

$$M^{\bullet+} \rightarrow EE^{+} + R^{\bullet} \ldots\ldots\ldots(1)$$

Odd Ion Molecule

$$M^{\bullet+} \rightarrow OE^{\bullet+} + N \ldots\ldots\ldots(2)$$

The primary product ion can also undergo fragmentation, and the next product ion can undergo fragmentation, and the next (thus resulting in a continual process). Intramolecular rearrangement is also possible due to the fragmentation process. The ions produced are all separated by the mass spectrometer based on their mass-to-charge ratio (*m/z*) and are detected relative to their abundance [49]. The mass-to-charge (*m/z*) ratio of an ion is the unitless ratio of its mass number to the number of fundamental charges, *z,* of the ion [32].

All mass spectrometers are made up of an inlet to introduce the sample, an ionisation source to produce ions from the sample, one or several mass analysers to separate the ions, a detector to count the ions and lastly a data processing system that produces a mass spectrum (Figure 2.12). High vacuum is needed to prevent collisions in the path ions travel to the detector [49].

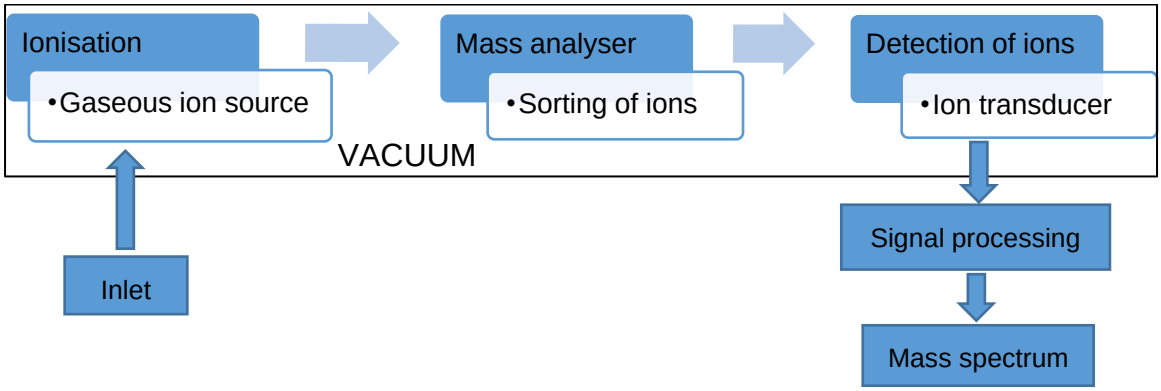

Figure 2.12: Schematic diagram showing the components of a mass spectrometer (adapted from Skoog et al., 2007) [32].

2.3.2. Ion sources

An ion source can either use high energy techniques (hard sources) causing extensive fragmentation or they can be soft (low energy techniques) mostly producing molecular ions only. Volatile and thermally stable molecules can be ionised using gas-phase ionisation techniques such as electron ionisation (EI), chemical ionisation (CI) and field ionisation (FI). Thermally labile compounds or compounds lacking sufficient vapour

pressure must be directly desorbed from the condensed phase to the gas phase [32, 49].

There are two types of direct ion sources, namely liquid-phase ion sources and solid-state ion sources. In the former case, the analyte in solution is introduced into the liquid-phase ion sources. Nebulisation is used to introduce the solution in the form of droplets into the source in order to produce ions at atmospheric pressure. The ions are then focussed into the mass spectrometer through several pumping stages [49]. Liquid-phase ionisation sources include electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI).

In solid-phase ionisation, the analyte is an involatile solid deposit which is irradiated with high energy particles or photons resulting in desorption of ions near the surface. Matrix-assisted laser desorption (MALDI), secondary ion mass spectrometry (SIMS), plasma desorption (PD) and field desorption (FD) sources are examples of solid-state ion sources using involatile solids. Fast atom bombardment (FAB) for an involatile liquid [32, 49].

Ionisation techniques employed in this research study are discussed in the following sections. EI was used in combination with GC-MS, and ESI and APCI were used as interfaces with LC-MS.

2.3.2.1. Electron ionisation

Electron ionisation (EI), formerly known as electron impact ionisation, is widely used in organic mass spectrometry (Figure 2.13). Molecules are bombarded with an energetic electron beam causing ionisation. A wave, with wavelength *λ,* is associated with each electron:

$$\lambda = \frac{h}{mv}$$

Equation 2-14

Where:
h = Planck's constant
m = mass of the electron
v = velocity of the electron

The wave is disturbed if the wavelength is close to the bond length and an energy transfer resulting in electronic excitations can take place. The molecular ions are often not observed due to extensive fragmentation during the ionisation process. The ionisation is also not very efficient, with approximately one ion being formed for every 1 000 molecules entering the source under usual 70 eV conditions [49].

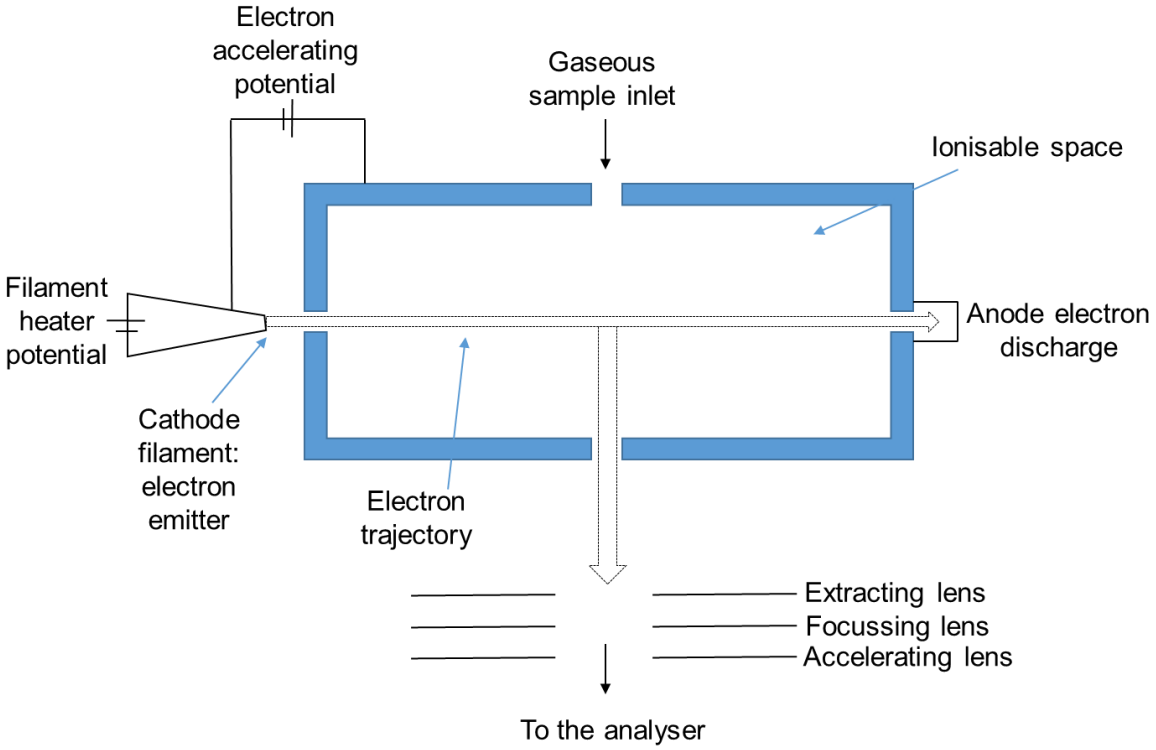

Figure 2.13: Schematic diagram of an electron ionisation source. A heated filament emits electrons which are then accelerated towards an anode by a potential difference of 70 eV. Electrons collide with the gaseous molecules travelling in a perpendicular path. The primary product is a singly charged positive ion. The ions produced are extracted through the slits of the lenses by applying small potential differences (adapted from De Hoffman et al., 2007) [49].

2.3.2.2. Electrospray ionisation

In many mass spectrometry applications the sample to be analysed is in solution or the sample can be the effluent from a liquid chromatography column. Electrospray ionisation (ESI) is performed under atmospheric conditions (Figure 2.14) and removes excess solvent while retaining the dissolved analyte by spraying the sample from a narrow tube (nebulised) into a larger chamber, producing a mist of charged droplets [50]. A strong electric field is applied to a liquid being pumped through a capillary tube with a flow rate of between 1 and 10 µL/min. The liquid at the end of the capillary is consequently charged. The charge accumulation at the surface of the liquid causes the formation of highly charged droplets. The solvent is removed from the droplets by

passing the droplets through a curtain of heated inert gas or a heated capillary. As the droplets decrease in size due to the evaporation of the solvent their charge density increases. The strong electric field in the droplet causes deformation to occur producing a Taylor cone. At this point, the Rayleigh limit, the surface tension can no longer support the charge density resulting in a Coulombic explosion with a release of smaller droplets. The smaller droplets will repeat the process until no solvent is left leaving a single or multiply charged analyte molecule [32, 49].

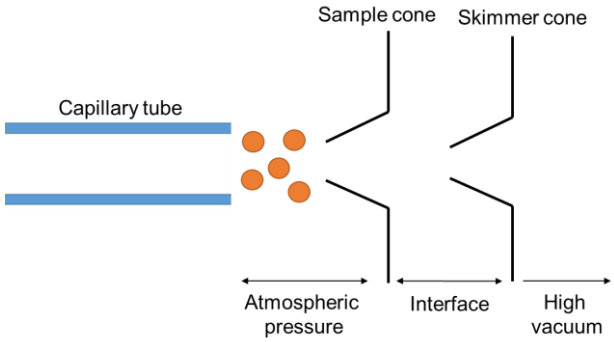

Figure 2.14: Schematic diagram of the electrospray source. Skimmers are used for ion focusing; separating the bulk of the neutral gas phase molecules from the atmospheric pressure ioniser and the produced ions (adapted from Dean, 2009) [2].

ESI results in little fragmentation of large and thermally fragile biomolecules since there is limited energy retained by the analyte upon ionisation. Additionally, the ions formed can be multiply charged (positive and negative charges can form) resulting in *m/z* values low enough to be detected with instruments working in a range of 1 500 *m/z* or less [32]. Molecular ions can be obtained by:

1. Protonation $(M + zH)^{Z+}$ or,
2. Deprotonation $(M - zH)^{Z-}$ where *z* is the number of charges.

ESI can easily be adapted for direct sample introduction from an LC column. Little fragmentation complicates the structural elucidation process, usually making it necessary to employ tandem mass spectrometry in order to obtain fragmentation patterns for identification [32].

2.3.2.3. Atmospheric pressure chemical ionisation

Atmospheric pressure chemical ionisation (APCI) uses gas-phase ion-molecule reactions at atmospheric pressure to ionise analytes. Primary ions are produced by corona discharges on a solvent spray and these can collide with and ionise analytes.

APCI is normally used for polar and relatively non-polar compounds with a molecular weight of up to 1 500 Da and it generally gives singly charged ions [49].

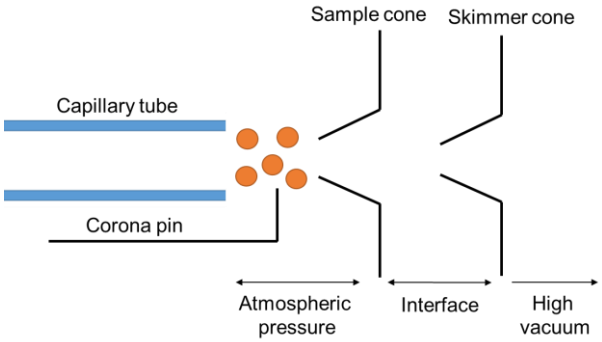

Figure 2.15: Schematic diagram of an APCI source (adapted from Dean, 2009) [2].

In APCI a voltage is applied to a corona pin which is positioned in front of the capillary tube (Figure 2.15). The capillary is heated and surrounded by a coaxial flow of nitrogen gas resulting in the formation of an aerosol. The aerosol then enters the corona discharge producing ions [2]. Ionisation in the positive mode can occur due to proton transfer or adduction of reactant gas ions to produce the ion of the analyte. In the negative mode, ionisation occurs through proton abstraction or adduct formation [49]. Singly charged molecular ions can be obtained by:

1. Protonation $(M+H)^{1+}$ or,
2. Deprotonation $(M-H)^{1-}$

Although APCI is widely used as an ionisation source coupled to LC-MS (<20% of all MS applications) [49], it is not as commonly used as ESI (>80%) [45].

2.3.3. Mass analysers

The time-of-flight mass analyser was used in both GC and LC analyses during this research study. Tandem MS was employed with LC for unequivocal identification of the target analytes. Both techniques are discussed in the following sections.

2.3.3.1. Time-of-flight

Separation in a time-of-flight (TOF) analyser is achieved, after accelerating ions in an electric field, by the linear velocities when ions drift through a field-free region, known as the flight tube. After ions are released from the source they are accelerated toward the flight tube by applying a potential difference between an electrode and extraction grid. At this point all the ions have the same kinetic energy (E_k):

$$E_k = \frac{mv^2}{2} = qV_s = zeV_s = E_{el}$$

Equation 2-15

Where:

E_k = kinetic energy

E_{el} = electric potential energy

m = mass of the ion

v = velocity of the ion

q = total charge = ze

V_s = accelerating potential [49]

After passing through the acceleration area the ions enter a field-free region and are now separated based on their velocities whereafter they reach the detector positioned at the end of the flight tube [49]. The time the ions take to move through the flight tube (field-free region between the source and detector) is used to determine the *m/z*. All the electric potential energy (E_{el}) is converted to kinetic energy (E_k) (Equation 2-15). The pulsed package of ions travel in a straight line at a constant velocity after exiting the acceleration region. The time it takes to reach the detector is given by the following equation:

$$t = \frac{L}{v}$$

Equation 2-16

Where:

t = time

L = distance (length of flight tube)

v = velocity of the ion [49]

The velocity of the ion is given, by rearranging Equation 2-15, as:

$$v = \left(\frac{2zeV_s}{m}\right)^{\frac{1}{2}}$$

Equation 2-17

By substituting Equation 2-17 into Equation 2-16:

$$t^2 = \frac{m}{z}\left(\frac{L^2}{2eV_s}\right)$$

Equation 2-18

Equation 2-18 shows that the *m/z* can be calculated by measuring t^2, provided the terms in the parentheses are constant. Accordingly, the lower the mass of an ion the faster it will travel [49].

There is in principle no upper mass limit for TOF analysers making them ideal for soft ionisation techniques. Their high transmission efficiency gives very high sensitivity and the speed of the TOF enables fast acquisition of mass spectra over a broad range of masses. A major drawback of the first TOF analysers was their poor mass resolution since the mass resolution is proportional to the flight time and flight path. One solution is to increase the length of the flight tube, however, it has to be supplemented with lower voltages to prevent collisions with gas molecules [49].

The delayed pulse and reflectron have greatly improved the resolution shortcomings of the TOF analyser. Delayed pulsed extractions of the ions outside the source correct the energy dispersion of ions with the same *m/z* leaving the source resulting in improved resolution. Reflectrons create a retarding field, which acts as an ion mirror, at the end of the flight tube sending the ions back into the flight tube. Reflectrons also correct the energy dispersion of ions with the same *m/z* leaving the source. Faster ions will spend more time in the reflectron and consequently, they will reach the detector at the same time as the slower ions. Reflectrons, however, increase mass resolution at the expense of sensitivity and introduce a mass range limit [49].

TOF instruments are often used in combination with GC×GC separations because of the instrument's high data acquisition speed, selectivity, reliable deconvolution of overlapping peaks and ability to capture acquisition masses instantaneously over a broad mass range [36].

2.3.3.2. Tandem MS

Tandem mass spectrometry (MS/MS) is any method that uses at least two stages of mass analysis. The method is usually done in combination with a dissociation or chemical process that results in a change of mass or charge of an ion [49].

Normally the first analyser is used to isolate a precursor which then undergoes a fragmentation to produce product ions and neutral fragments:

$$m_p{}^+ \rightarrow m_f{}^+ + m_n$$

Equation 2-19

Where:

m_p = precursor ion

m_f = product ion

m_n = neutral fragment

The second mass spectrometer separates the ions which are then detected by the ion detector [32, 49].

Fragmentations can be produced spontaneously, by reacting with a collision gas or by interacting with an intense laser beam. The ions selected by the first mass analyser are referred to as metastable ions. These are ions with an intermediate lifetime. They are stable enough to be selected by the first analyser and with enough excess energy to fragment before reaching the second analyser. However, the probability of this happening is only about 1% since there are only a small number of these ions and they spend very little time in the reaction region. Collisional activation (CA) can be implemented in order to increase the internal energy of the precursor ion to induce decomposition. Collision-activated decomposition (CAD), also known as collision-induced dissociation (CID), increases the number of precursor ions that can undergo fragmentation and increases the amount of fragmentation pathways. This greatly facilitates the structural elucidation process [49].

Tandem-in-space spectrometers are made up of two independent mass analysers used in two different regions in space. These are the well-known triple quadrupole (QqQ), quadrupole time-of-flight (QTOF) and TOF-TOF spectrometers. Tandem-in-time instruments form ions in a special trapping region and then expel the unwanted ions from that region leaving selected ions to be dissociated and analysed. These instruments include the Fourier transform ion cyclotron resonance (FT-ICR) and quadrupole ion-trap instruments [30].

2.4. Analysis of Micropollutants in Aquatic Systems

The following section highlights relevant points found in literature regarding micropollutant analysis in water matrices. In order to condense the vast information available on general organic contaminants and persistent organic pollutants (POPs), the focus of this literature review was placed on EDCs analysis in aquatic matrices and methods employed during this research study.

2.4.1. Analytical Methods Employed for the Detection of EDCs in the Aquatic Environment

In 2002 Petrovic et al. published a review on mass spectrometric analysis relating to EDCs in the aquatic environment [51]. Their review highlights the complexity of EDCs and the possibility of unforeseen adverse or synergistic effects. The authors furthermore highlighted the challenge of EDC detection due to the diverse properties of EDCs, the complexity of the sample matrix and low detection limits that are required. The advantages of using GC×GC, namely improved peak capacity, lower detection limits and separation of complex samples, in EDC analysis were also discussed [51].

Petrovic et al. (2002) furthermore emphasised the need for comprehensive methods, since the properties of the compounds will greatly influence the selection of the appropriate method particularly for quantification [51]. Apolar (e.g. dioxins and polychlorinated biphenyls (PCBs)) and moderately polar (e.g. alkylphenols and phthalates) compounds are generally analysed using GC while the polar (e.g. alkylphenol carboxylates) compounds are more suited to LC analysis. There are also a range of compounds (e.g. steroid sex hormones and alkylphenolic compounds) that can be analysed using both methods. According to the review article, GC-MS has been the most commonly employed method up to 2002. However, the poor volatility of some compounds leads to the requirement of derivatisation steps which can often result in compound loss [51].

Gabet et al. (2007) published a review specifically focussing on oestrogen analysis in environmental matrices [52]. Oestrogens are a group of steroid hormones which act as endocrine disruptors. The authors concluded that the analysis of oestrogenic compounds in aqueous matrices was well documented which was not the case with the analysis of EDCs in solid samples. LC-MS and GC-MS were stated as being the preferred methods for aqueous sample analysis with limit of detections (LODs) below 1 ng/L for river waters and about 1 ng/L for wastewater [52].

Two major review articles were published in 2009 [53, 54]. Chang et al. (2009) highlighted the importance of sample preparation techniques [53]. The authors concluded that extraction techniques commonly used for EDC analysis included liquid-liquid extraction (LLE), solid phase extraction (SPE), solid phase micro extraction (SPME) and liquid phase micro extraction (LPME) [53]. Comerton et al. (2009)

published a detailed review article giving a practical overview of analytical methods for EDCs, pharmaceuticals and personal care products (PCPs) in water and wastewater [54].

Comerton et al. (2009) stated that at the time of publishing there were no standardised analytical methods available for the analysis of organic micropollutants in environmental matrices. Established analytical procedures focussed on specific classes of compounds with only a few appropriate for a multi-residue analysis [54]. Currently, this is still a major issue of concern. A review by Richardson and Ternes (2014) listed new regulation methods for water analysis developed by the United States Environmental Protection Agency (US EPA) [55]. The majority of the methods pertain to the analysis of a specific compound or class of compounds [55]. Comerton et al. (2009) recommended that a combination of LC-MS/MS and GC-MS techniques appears to be the best approach for a multi-residue analysis because the two complimentary techniques broaden the range of compounds that can be dependably analysed. The authors also underpinned the importance of sample clean-up to minimise matrix interferences [54].

In 2011 LaFleur and Schug identified an increase in the use of SPE and SPME extraction techniques over the conventional LLE [56]. This trend enabled the use of less sample and fewer toxic solvents [56]. The need for lower detection limits was identified because it was found that the concentration of an EDC, or mixture of EDCs, that can affect the endocrine system is very low (less than 1 ng/L in some cases). The authors also highlighted two easy to use, inexpensive and sensitive methods, namely GC-MS using stir bar sorptive extraction with simultaneous derivatisation capable of detecting selected oestrogens, alkyl phenols and phthalates at ng/L level [57] and a HPLC-UV method that detected oestrogens, phenols and phthalates between 0.2 and 1.6 ng/L [58].

Matisová and Hrouzková (2012) found that chromatographic methods coupled to MS provided the best sensitivity and precision for the detection of volatile EDCs and endocrine disrupting pesticides (EDPs) [9]. The authors also noted the QuEChERS (quick, easy, cheap, effective, rugged and safe) approach developed by Anastassiades et al. (2003) was used for the extraction of EDPs [9, 59].

Richardson and Ternes published the major trends in water analysis for 2014 [55]. The trends included (1) using LC tandem MS (LC-MS/MS) and LC with high-resolution MS (LC-HRMS) for structure elucidation of unknown contaminants, (2) using SPE with new sorbent materials such as ion exchange resins, (2) sorptive extraction techniques such as SPME was still being used, (3) ionic liquid extraction was emerging as a "green" extraction technique, and finally (4) UHPLC and GC×GC-TOFMS were used for improved separations [55].

Barreiros et al. (2016) underlined the importance of linking instrumental analysis with biological tools (i.e. bioassays) [60]. Oestrogen analysis with GC-MS requires derivatisation done mainly with pentafluorobenzyl chloride (PFBCI) and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA). The use of LC methods, which are not limited by compound volatility and thermolability, increased for oestrogen and EDCs detection and quantification. GC-MS or GC-MS/MS were performed with EI or chemical ionisation (CI), while the LC methods were performed with ESI, APCI or atmospheric pressure photo ionisation (APPI) modes. The use of SPE for sample clean-up of aqueous matrices was still being widely used [60].

The identification and quantification of micropollutants, such as EDCs, in environmental matrices continue to be a relevant research topic. Identifying the effects these chemicals have on both the environment and humans are crucial for improved human and environmental health. Cost-effective analytical techniques for trace-level EDC analysis can facilitate the establishment of large-scale monitoring networks for monitoring the fate and transport mechanisms of these compounds in the environment and aid in policy making.

References

1. Pawliszyn, J. *Solid phase microextraction: theory and practice*. 1997, John Wiley & Sons. p. 1-10.
2. Dean, J.R. *Pre- and Post-Extraction Considerations*, in *Extraction Techniques in Analytical Sciences*. 2009, John Wiley & Sons, Ltd. p. 1-36.
3. Dean, J.R. *Classical Approaches for Aqueous Extraction*, in *Extraction Techniques in Analytical Sciences*. 2009, John Wiley & Sons, Ltd. p. 37-47.
4. Dean, J.R. *Liquids*, in *Methods for Environmental Trace Analysis*. 2003, John Wiley & Sons, Ltd. p. 141-164.
5. Dean, J.R. *Solid Phase Extraction*, in *Extraction Techniques in Analytical Sciences*. 2009, John Wiley & Sons, Ltd. p. 49-84.
6. Baltussen, E., Cramers, C., Sandra, P. *Sorptive sample preparation – a review.* Analytical and Bioanalytical Chemistry, 2002. **373**(1): p. 3-22.
7. Boix, C., Ibáñez, M., Sancho, J.V., Rambla, J., Aranda, J.L., Ballester, S., Hernández, F. *Fast determination of 40 drugs in water using large volume direct injection liquid chromatography–tandem mass spectrometry.* Talanta, 2015. **131**: p. 719-727.
8. Chiaia, A.C., Banta-Green, C., Field, J. *Eliminating solid phase extraction with large-volume injection LC/MS/MS: Analysis of illicit and legal drugs and human urine indicators in US wastewaters.* Environmental Science and Technology, 2008. **42**(23): p. 8841-8848.
9. Matisová, E., Hrouzková, S. *Analysis of endocrine disrupting pesticides by capillary GC with mass spectrometric detection.* International Journal of Environmental Research and Public Health, 2012. **9**(9): p. 3166-3196.
10. Bayen, S., Yi, X., Segovia, E., Zhou, Z., Kelly, B.C. *Analysis of selected antibiotics in surface freshwater and seawater using direct injection in liquid chromatography electrospray ionization tandem mass spectrometry.* Journal of Chromatography A, 2014. **1338**: p. 38-43.
11. Zhang, Z., Pawliszyn, J. *Headspace solid-phase microextraction.* Analytical Chemistry, 1993. **65**(14): p. 1843-1852.
12. Tranchida, P.Q., Maimone, M., Purcaro, G., Dugo, P., Mondello, L. *The penetration of green sample-preparation techniques in comprehensive two-dimensional gas chromatography.* TrAC - Trends in Analytical Chemistry, 2015. **71**: p. 74-84.
13. Triñanes, S., Pena, M.T., Casais, M.C., Mejuto, M.C. *Development of a new sorptive extraction method based on simultaneous direct and headspace sampling modes for the screening of polycyclic aromatic hydrocarbons in water samples.* Talanta, 2015. **132**(0): p. 433-442.
14. Naudé, Y., Rohwer, E.R. *Two multidimensional chromatographic methods for enantiomeric analysis of o, p'-DDT and o, p'-DDD in contaminated soil and air in a malaria area of South Africa.* Analytica Chimica Acta, 2012. **730**: p. 120-126.

15. Naudé, Y., Van Rooyen, M.W., Rohwer, E.R. *Evidence for a geochemical origin of the mysterious circles in the Pro-Namib desert.* Journal of Arid Environments, 2011. **75**(5): p. 446-456.

16. Naudé, Y., Gorst-Allman, P., Rohwer, E. *A cheap and simple passive sampler using silicone rubber for the analysis of surface water by gas chromatography-time of flight mass spectrometry.* Water SA: WISA 2014-Water Innovations Special Edition, 2015. **41**(2): p. 182-188.

17. Ochiai, N., Sasamoto, K., Hirooki, K., Pfannkoch, E. *A Novel Extraction Procedure for Stir Bar Sorptive Extraction (SBSE): Sequential SBSE for Uniform Enrichment of Organic Pollutants in Water Samples*. 2008; Available from: http://www.gerstel.com/pdf/p-gc-an-2008-12corrected.pdf. [Accessed on 15/11/20]

18. Arthur, C.L., Pawliszyn, J. *Solid phase microextraction with thermal desorption using fused silica optical fibers.* Analytical Chemistry, 1990. **62**(19): p. 2145-2148.

19. Pawliszyn, J. *Theory of solid-phase microextraction.* Journal of Chromatographic Science, 2000. **38**(7): p. 270-278.

20. Dean, J.R. *Solid Phase Microextraction*, in *Extraction Techniques in Analytical Sciences*. 2009, John Wiley & Sons, Ltd. p. 85-116.

21. Baltussen, E., Sandra, P., David, F., Cramers, C. *Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: theory and principles.* Journal of Microcolumn Separations, 1999. **11**(10): p. 737-747.

22. Dean, J.R. *New Developments in Microextraction*, in *Extraction Techniques in Analytical Sciences*. 2009, John Wiley & Sons, Ltd. p. 117-123.

23. Dean, J.R. *Air Sampling*, in *Extraction Techniques in Analytical Sciences*. 2009, John Wiley & Sons, Ltd. p. 211-219.

24. Danielson, N.D., Gallagher, P.A., Bao, J.J. *Chemical reagents and derivatization procedures in drug analysis.* Encyclopedia of Analytical Chemistry, 2000.

25. Van Hoeck, E., Canale, F., Cordero, C., Compernolle, S., Bicchi, C., Sandra, P. *Multiresidue screening of endocrine-disrupting chemicals and pharmaceuticals in aqueous samples by multi-stir bar sorptive extraction–single desorption–capillary gas chromatography/mass spectrometry.* Analytical and Bioanalytical Chemistry, 2009. **393**(3): p. 907-919.

26. Kataoka, H. *Derivatization reactions for the determination of amines by gas chromatography and their applications in environmental analysis.* Journal of Chromatography A, 1996. **733**(1): p. 19-34.

27. Hušek, P. *Chloroformates in gas chromatography as general purpose derivatizing agents.* Journal of Chromatography B: Biomedical Sciences and Applications, 1998. **717**(1): p. 57-91.

28. Devos, C., Vliegen, M., Willaert, B., David, F., Moens, L., Sandra, P. *Automated headspace-solid-phase micro extraction–retention time locked-isotope dilution gas chromatography–mass spectrometry for the analysis of*

organotin compounds in water and sediment samples. Journal of Chromatography A, 2005. **1079**(1): p. 408-414.

29. Kawaguchi, M., Ito, R., Sakui, N., Okanouchi, N., Saito, K., Nakazawa, H. *Dual derivatization–stir bar sorptive extraction–thermal desorption–gas chromatography–mass spectrometry for determination of 17β-estradiol in water sample.* Journal of Chromatography A, 2006. **1105**(1): p. 140-147.
30. Skoog, D., West, D., Holler, F., Crouch, S. *Fundamentals of analytical chemistry*. 8th ed. 2004, Belmont, USA: Brooks/Cole, Cengage Learning.
31. Jennings, W., Mittlefehldt, E., Stremple, P. *Analytical gas chromatography*. 1997: Academic Press.
32. Skoog, D.A., Holler, F.J., Crouch, S.R. *Principles of instrumental analysis*. Vol. 6th ed. 2007, Belmont, CA: Thomson Brooks/Cole.
33. Peters, D.G., Hayes, J.M., Hieftje, G.M. *Chemical separations and measurements: theory and practice of analytical chemistry*. 1974: WB Saunders Company.
34. Giddings, J.C. *Maximum number of components resolvable by gel filtration and other elution chromatographic methods.* Analytical Chemistry, 1967. **39**(8): p. 1027-1028.
35. Liu, Z., Phillips, J.B. *Comprehensive two-dimensional gas chromatography using an on-column thermal modulator interface.* Journal of Chromatographic Science, 1991. **29**(6): p. 227-231.
36. Marriott, P.J., Chin, S.-T., Maikhunthod, B., Schmarr, H.-G., Bieri, S. *Multidimensional gas chromatography.* TrAC - Trends in Analytical Chemistry, 2012. **34**(0): p. 1-21.
37. Schoenmakers, P., Marriott, P., Beens, J. *Nomenclature and conventions in comprehensive multidimensional chromatography.* LCGC Europe, 2003. **25**(5): p. 1-4.
38. Gómez, M.J., Herrera, S., Solé, D., García-Calvo, E., Fernández-Alba, A.R. *Automatic searching and evaluation of priority and emerging contaminants in wastewater and river water by stir bar sorptive extraction followed by comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry.* Analytical Chemistry, 2011. **83**(7): p. 2638-2647.
39. Tranchida, P.Q., Franchina, F.A., Dugo, P., Mondello, L. *Comprehensive two-dimensional gas chromatography-mass spectrometry: Recent evolution and current trends.* Mass Spectrometry Reviews, 2014.
40. Shi, X., Wang, S., Yang, Q., Lu, X., Xu, G. *Comprehensive two-dimensional chromatography for analyzing complex samples: recent new advances.* Analytical Methods, 2014. **6**(18): p. 7112-7123.
41. Mondello, L., Tranchida, P.Q., Dugo, P., Dugo, G. *Comprehensive two-dimensional gas chromatography-mass spectrometry: A review.* Mass Spectrometry Reviews, 2008. **27**(2): p. 101-124.
42. Dallüge, J., Beens, J., Udo, A. *Comprehensive two-dimensional gas chromatography: a powerful and versatile analytical tool.* Journal of Chromatography A, 2003. **1000**(1): p. 69-108.

43. Pursch, M., Eckerle, P., Biel, J., Streck, R., Cortes, H., Sun, K., Winniford, B. *Comprehensive two-dimensional gas chromatography using liquid nitrogen modulation: set-up and applications.* Journal of Chromatography A, 2003. **1019**(1): p. 43-51.

44. MacNair, J.E., Lewis, K.C., Jorgenson, J.W. *Ultrahigh-pressure reversed-phase liquid chromatography in packed capillary columns.* Analytical Chemistry, 1997. **69**(6): p. 983-989.

45. De Villiers, A., Venter, P., Pasch, H. *Recent advances and trends in the liquid-chromatography–mass spectrometry analysis of flavonoids.* Journal of Chromatography A, 2016. **1430**: p. 16-78.

46. Farré, M., Kantiani, L., Petrovic, M., Pérez, S., Barceló, D. *Achievements and future trends in the analysis of emerging organic contaminants in environmental samples by mass spectrometry and bioanalytical techniques.* Journal of Chromatography A, 2012. **1259**: p. 86-99.

47. Masiá, A., Campo, J., Blasco, C., Picó, Y. *Ultra-high performance liquid chromatography–quadrupole time-of-flight mass spectrometry to identify contaminants in water: An insight on environmental forensics.* Journal of Chromatography A, 2014. **1345**: p. 86-97.

48. Bueno, M.M., Ulaszewska, M.M., Gomez, M., Hernando, M., Fernández-Alba, A. *Simultaneous measurement in mass and mass/mass mode for accurate qualitative and quantitative screening analysis of pharmaceuticals in river water.* Journal of Chromatography A, 2012. **1256**: p. 80-88.

49. De Hoffman, E., Stroobant, V. *Mass spectrometry : principles and applications*. 3rd edition ed. 2007: Hoboken, N.J. : Wiley ; Chichester : John Wiley [distributor].

50. Herbert, C.G., Johnstone, R.A. *Mass spectrometry basics*. 2002: CRC press.

51. Petrovic, M., Eljarrat, E., López de Alda, M.J., Barceló, D. *Recent advances in the mass spectrometric analysis related to endocrine disrupting compounds in aquatic environmental samples.* Journal of Chromatography A, 2002. **974**(1): p. 23-51.

52. Gabet, V., Miège, C., Bados, P., Coquery, M. *Analysis of estrogens in environmental matrices.* Emerging contaminants in wastewaters, 2007. **26**(11): p. 1113-1131.

53. Chang, H.-S., Choo, K.-H., Lee, B., Choi, S.-J. *The methods of identification, analysis, and removal of endocrine disrupting compounds (EDCs) in water.* Journal of Hazardous materials, 2009. **172**(1): p. 1-12.

54. Comerton, A.M., Andrews, R.C., Bagley, D.M. *Practical overview of analytical methods for endocrine-disrupting compounds, pharmaceuticals and personal care products in water and wastewater.* Philosophical Transactions. Series A, Mathematical, Physical, and Engineering Sciences, 2009. **367**(1904): p. 3923-3939.

55. Richardson, S.D., Ternes, T.A. *Water analysis: Emerging contaminants and current issues.* Analytical Chemistry, 2014. **86**(6): p. 2813-2848.

56. LaFleur, A.D., Schug, K.A. *A review of separation methods for the determination of estrogens and plastics-derived estrogen mimics from aqueous systems.* Analytica Chimica Acta, 2011. **696**(1–2): p. 6-26.

57. Tan, B.L., Hawker, D.W., Müller, J.F., Tremblay, L.A., Chapman, H.F. *Stir bar sorptive extraction and trace analysis of selected endocrine disruptors in water, biosolids and sludge samples by thermal desorption with gas chromatography–mass spectrometry.* Water Research, 2008. **42**(1): p. 404-412.

58. Zarzycki, P.K., Włodarczyk, E., Baran, M.J. *Determination of endocrine disrupting compounds using temperature-dependent inclusion chromatography: II. Fast screening of free steroids and related low-molecular-mass compounds fraction in the environmental samples derived from surface waters, treated and untreated sewage waters as well as activated sludge material.* Journal of Chromatography A, 2009. **1216**(44): p. 7612-7622.

59. Anastassiades, M., Lehotay, S.J., Štajnbaher, D., Schenck, F.J. *Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce.* Journal of AOAC International, 2003. **86**(2): p. 412-431.

60. Barreiros, L., Queiroz, J.F., Magalhães, L.M., Silva, A.M.T., Segundo, M.A. *Analysis of 17-β-estradiol and 17-α-ethinylestradiol in biological and environmental matrices — A review.* Microchemical Journal, 2016. **126**: p. 243-262.

Chapter 3

Data Analysis

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3. Data Analysis

This chapter provides a brief overview on the use of data analysis in order to provide meaningful and reliable results. A summary on how data is interpreted during a GC×GC analysis is described. The methods used to give meaningful results for quantification and identification, as well as chemometric tools used during this research study, are also outlined. These include matrix matched calibrations, linear retention indices and principal component analysis.

3.1. GC×GC Data

3.1.1. Data interpretation

GC×GC data is normally converted into a three dimensional contour plot. The primary (^{1}D) retention time is displayed on the abscissa (*x*-axis) and the secondary (^{2}D) retention time is displayed on the ordinate (*y*-axis). The peaks appear as spots of varying colours or contours on the chromatogram which correspond to the intensity of the peak (Figure 3.1). The ^{1}D retention time corresponds to a modulation pulse that occurs and it is the same for all peaks that elute between that pulse and the next pulse. The ^{2}D retention time of a peak is calculated by subtracting the ^{1}D retention time from the original (total) one-dimensional retention time [1] (Figure 3.2).

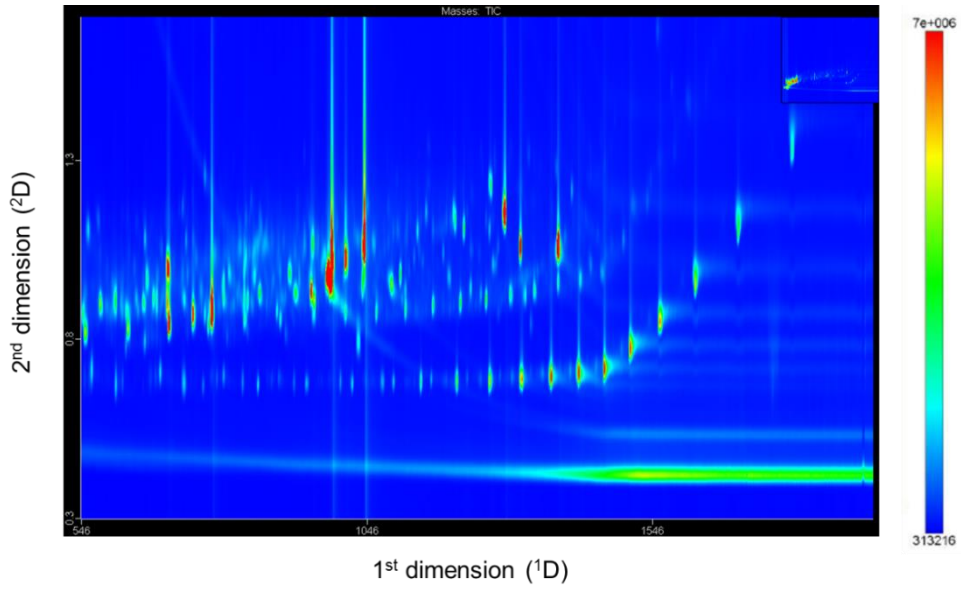


Figure 3.1: An example of a two-dimensional contour plot. The primary (^{1}D) retention time is displayed on the *X*-axis and the secondary (^{2}D) retention time is displayed on the *Y*-axis. The peaks appear as coloured ellipses on the blue background. Blue indicates the base line and red indicate very high intensity.

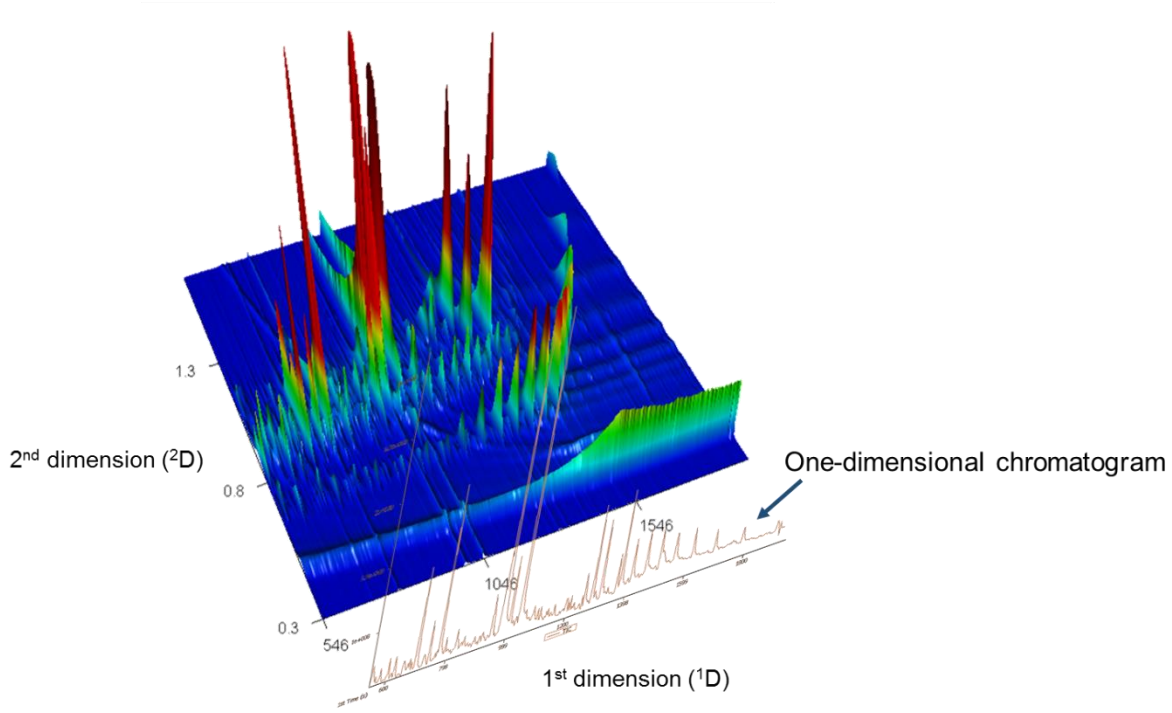


Figure 3.2: One-dimensional chromatograms, obtained during each modulation period, are placed next to each other to form the two-dimensional chromatogram. The intensity of the peaks gives the third dimension. The one-dimensional chromatogram shown indicates the first dimension separation.

A problem encountered during GC×GC data generation is long dead times in the ^{2}D column. The dead time is the time it takes the eluent or unretained compounds to move through a column. If the dead time is long compared to the duration of the modulation period, unretained compounds will appear in the middle of the ^{2}D plane and the remaining compounds might show up in the next modulation period or slice. In order to overcome this problem, the modulation pulse times can be increased with the ^{2}D dead time.

Another problem often encountered is peak wraparound. This is when a peak has a ^{2}D retention time that is longer than the modulation period. This peak will then appear as a broad peak in the next modulation cycle possible resulting in coelution with other peaks of interest [1].

3.1.2. Processing of GC×GC-TOFMS data

3.1.2.1. Targeted analysis

Identification and quantification of analytes with known retention times and mass spectra are usually done in the same manner as one-dimensional GC coupled to the MS. Retention time windows and reference mass spectra are used for identification

and peaks are integrated using algorithms to obtain areas for quantification [2]. The major difference between GC×GC quantification and one-dimensional GC is an increase in the number of peaks per analyte that needs to be integrated. The total response of an analyte is obtained by integrating each ^{2}D peak that corresponds to that analyte and then summing up the areas [1].

A major advantage of GC×GC data is improved identification of analytes. The use of two retention times obtained during the process increases the reliability of identification based on retention parameters [1].

3.1.2.2. Untargeted analysis

In order to understand the composition of a sample, untargeted analysis is usually implemented to gain an overview of the sample components. Normally this involves attempting to identify all peaks above a certain signal-to-noise ratio (*S/N*). Untargeted analysis typically involves peak finding and deconvolution, library searching and further processing [2].

The pure mass spectrum of partly coeluting peaks can be calculated using a deconvolution algorithm. This is achieved with sophisticated computer software. After peak finding and deconvolution, compounds are listed in a peak table containing retention times and library search results, including compound names and mass spectral match factors (similarity). Further processing includes using library match factors to evaluate the reliability of the library identification and using linear retention indices for improved identification [2].

3.2. Matrix Effects

Chromatography has always been afflicted by interfering compounds from within the sample matrix. The earliest problems arose due to coeluting compounds giving similar detector responses when using non-selective detectors (i.e. ultraviolet–visible spectroscopy (UV/VIS) or flame ionisation detectors (FID)). The poor specificity of these early detectors was overcome by coupling chromatography techniques to mass spectrometry (i.e. GC-MS or LC-MS) [3]. Further advances in separation sciences were made with the introduction of multidimensional chromatography significantly reducing the occurrence of coeluting compounds. However, analyte signal

suppression or enhancement by matrix interferences commonly occurs. Quantification errors ensue due to the differences in signal intensities between samples and standards for a specific analyte concentration [4].

GC-MS and LC-MS differ immensely in the matrix effects produced due to the different ionisation mechanisms employed. In GC-MS the ionisation energy (during electron ionisation (EI)) is high enough to overcome competing ionisation processes to eliminate the interference from coeluting compounds which influences the soft ionisation mechanism in the interface of LC-MS. EI occurs in the collisionless low pressure gas, unaffected by other compounds simultaneously present in the ion source, whereas the outcome of atmospheric pressure ionisation in LC-MS depends heavily on the reaction partners present in the ion source when the analyte elutes. In GC-MS matrix compounds thus mostly affect the chromatographic column and the GC inlet but not the ionisation [3]. Matrix compounds block the active sites of the injector or column leading to fewer analytes being adsorbed resulting in a signal enhancement for these analytes (overestimating the concentration). This effect was first described by Erney and co-workers as “matrix induced chromatographic response” [5]. Concurrently the number of new active sites increases due to the gradual accumulation of non-volatile matrix compounds in the GC inlet and front part of the chromatographic column. This leads to a decrease in analyte response (“matrix – induced diminishment effect”) [3].

As stated, matrix effects produced in LC-MS arise during the ionisation step in the mass spectrometer. Not only the efficiency of ionisation, but also the relative contributions of various ionisation methods are dependent on the physicochemical properties of an analyte molecule, the reagent ions in the ion source at that instant, and also on the temperature and pressure conditions in the ionisation interface. A charge competition appears to be happening between the analytes and interfering compounds during electrospray ionisation (ESI). Studies show that atmospheric pressure chemical ionisation (APCI) is less prone to ion suppression than ESI due the differences in the ionisation mechanisms. However, the process is not matrix effect free but the outcome is different from that of ESI and needs to be considered [3].

In conclusion, the effect of all compounds excluding the main analyte on the analytical signal is defined as a matrix effect and is describe as the matrix factor (MF) by the following mathematical equation:

$$MF = \frac{peak\ response\ in\ presence\ of matrix\ ions}{peak\ response\ in\ the\ neat\ solution}$$

Equation 3-1

MF = 1 indicates no matrix effect

MF < 1 indicates ion suppression

MF > 1 indicates ion enhancement [3].

3.3. Linear Retention Index

Linear retention indices (LRIs) provide an additional tool for compound identification. Measured LRIs of chemical compounds can be compared to LRI libraries for compound confirmation [6]. In 1958 Kováts [7] derived a widely used system of retention indices (RIs) that is useful for reporting the retention of a compound on a specific stationary phase under different chromatographic conditions. In 1963 Van den Dool and Kratz [8] extended the system to include linear temperature programming conditions. The index system permits cross usage of results between laboratories [6].

The retention time (RT) of a compound varies with column length, stationary phase type, stationary phase film thickness, temperature conditions and carrier gas flow rate. To compensate for the change in retention times the Kováts system uses a series of *n*-alkanes which is injected using the same chromatographic conditions as the method used to analyse the sample; the adjusted retention times are recorded for all the *n*-alkanes (the adjusted retention time (t'_R) is the actual retention time minus the retention time of an unretained compound). The Kováts RI for each alkane is the number of carbons multiplied by 100. To determine the Kováts RI for a compound on a given phase, the compound is injected using the same phase and conditions as the *n*-alkane mix [9]. An LRI-RT calibration curve is constructed using the LRIs and RTs of the *n*-alkanes and the curve is then used to determine the LRI of a compound from its RT [6]. Linear or non-isothermal indices can also be determined by Equation 3-2.

$$I_x = 100n + 100\frac{(t_x - t_n)}{(t_{n+1} - t_n)}$$

Equation 3-2

Where:

I_x = Kováts retention index of unknown compound *x*,

n = the number of carbon atoms in the smaller n-alkane,
n+1 = the number of carbon atoms in the larger n-alkane,
t_x, t_n, t_{n+1} = retention times [6, 8].

3.4. Principle Component Analysis

GC×GC chromatograms are naturally ordered and have highly reproducible retention coordinates. Even if the identities of components are not known, the detailed pattern provided by GC×GC enables comparison of the origin of the samples. Chemometric tools, such as analysis of variance (ANOVA) with principal component analysis (PCA), have been used in GC×GC separations for pattern matching and recognition. These tools are invaluable for the handling of the large amounts of data acquired by a GC×GC system [1].

The variation in a set of variables can be modelled using a principle component analysis (PCA). This is accomplished by deriving a small number of independent linear combinations (*principal components*) from a set of measured variables that accounts for as much of the variability in the original variables as possible. The arrangement of points across several correlated variables can be visualised by PCA as it shows the most prominent direction of high-dimensional data. Multivariate data is visualised by reducing it to graphable dimensions using as few variables as possible [10].

A linear combination of an eigenvector of the correlation matrix with a variable is used to calculate each principal component. The variance in each component is represented by the eigenvalues. The first principal component is obtained from the standardised original variables; it is a linear combination comprising of the greatest variation in the variables. The succeeding principal components are all linear combinations of the variables that have the greatest possible variance and are uncorrelated with all previously defined components. There are n principal components for n original variables [10].

References

1. Górecki, T., Harynuk, J., Panić, O. *The evolution of comprehensive two-dimensional gas chromatography (GC× GC).* Journal of Separation Science, 2004. **27**(5-6): p. 359-379.
2. Dallüge, J., Beens, J., Udo, A. *Comprehensive two-dimensional gas chromatography: a powerful and versatile analytical tool.* Journal of Chromatography A, 2003. **1000**(1): p. 69-108.
3. Silvestro, L., Tarcomnicu, I., Savu, S.R. *Matrix Effects in Mass Spectrometry Combined with Separation Methods — Comparison HPLC, GC and Discussion on Methods to Control these Effects*. Tandem Mass Spectrometry - Molecular Characterization. 2013.
4. De Hoffman, E., Stroobant, V. *Mass spectrometry : principles and applications*. 3rd edition ed. 2007: Hoboken, N.J. : Wiley ; Chichester : John Wiley [distributor].
5. Erney, D., Gillespie, A., Gilvydis, D., Poole, C. *Explanation of the matrix-induced chromatographic response enhancement of organophosphorus pesticides during open tubular column gas chromatography with splitless or hot on-column injection and flame photometric detection.* Journal of Chromatography A, 1993. **638**(1): p. 57-63.
6. Babushok, V.I. *Chromatographic retention indices in identification of chemical compounds.* TRAC Trends in Analytical Chemistry, 2015. **69**: p. 98-104.
7. Kováts, E. *Gaschromatographische Charakterisierung organischer Verbindungen. Teil 1: Retentionsindices aliphatischer Halogenide, Alkohole, Aldehyde und Ketone.* Helvetica Chimica Acta, 1958. **41**(7): p. 1915.
8. Van den Dool, H., Kratz, P.D. *A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography.* Journal of Chromatography A, 1963. **11**: p. 463-471.
9. Sparkman, O.D., Penton, Z., Kitson, F.G. *Gas Chromatography and Mass Spectrometry: A Practical Guide*. 2011: Elsevier Science.
10. SAS. *Institute Inc. JMP® 12 Multivariate Methods*. 2015, Cary, NC: SAS Institute Inc.

Chapter 4

Experimental

Table of Contents

4. Experimental Setup and Rationalisation

4.1. Overview

The starting point for this research was the collection of surface water samples from Rietvlei Nature Reserve, Gauteng, South Africa. These samples served as the platform upon which the research was initiated and expanded. An initial screening using sorptive extraction techniques and comprehensive gas chromatography with time-of-flight mass spectrometry (GC×GC-TOFMS) tentatively identified various micropollutants, with the focus on endocrine disrupting chemicals (EDCs) and emerging contaminants with potential endocrine disrupting effects in the surface water. Analytical standards for method validation were chosen based on the findings of the initial screening. Matrix matched calibration curves were constructed for qualification and quantification of target analytes using sorptive extraction, GC×GC-TOFMS and ultra-high pressure liquid chromatography-tandem mass spectrometry (UHPLC-QTOFMS) and applied to real world samples. The overview of the work flow is set out in Figure 4.1.

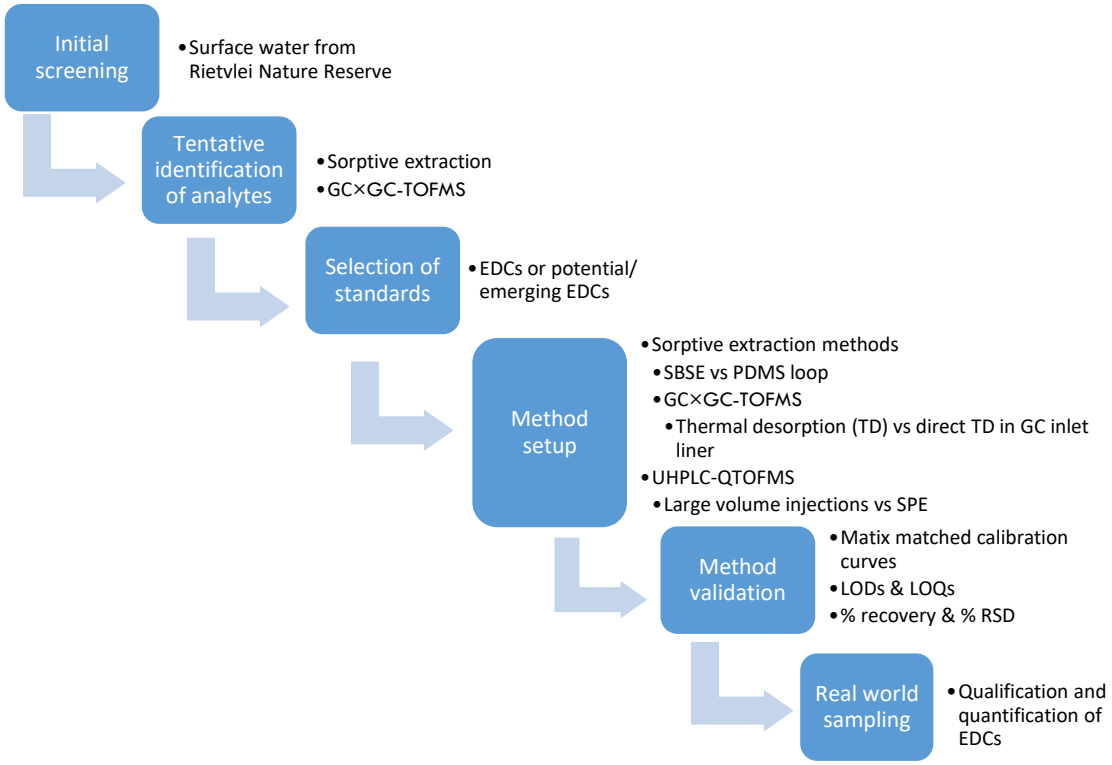


Figure 4.1: Experimental steps followed during the qualification and quantification of EDCs in surface water using novel sorptive extraction, GC×GC-TOFMS and UHPLC-QTOFMS.

4.2. Sampling

4.2.1. Sample sites

4.2.1.1. Rietvlei Nature Reserve

Rietvlei Nature Reserve is situated 20 km south of the central business district of Pretoria, South Africa (Figure 4.2) [1]. The reserve functions as a catchment area for the Rietvlei Water Purification Works. Two large dams are located in the Reserve, namely the Rietvlei Dam and, upstream from it, the Marais Dam. The Marais Dam acts as a sludge dam which receives wastewater from a nearby sewage treatment plant, industries and informal settlements. A peat wetland is found between the two dams. The Rietvlei Dam receives runoff from dolomite springs caused by annual seasonal rain due to the presence of ground water which accumulates in large subterranean dolomite chambers [2]. Water from the Rietvlei Dam is treated by the Rietvlei Water Purification Works which is used as drinking water for Pretoria [2].

Rietvlei Nature Reserve was chosen as the site of the initial screening for endocrine disrupting micropollutants and emerging contaminants as research conducted by Aneck-Hahn et al. (2008) using bioassays, specifically the Recombinant Yeast Screen Bio-Assays (RCBA), showed oestrogenic activity (using estradiol equivalent quotients (EEQs)) in a number of water sources within Rietvlei Nature Reserve[2].

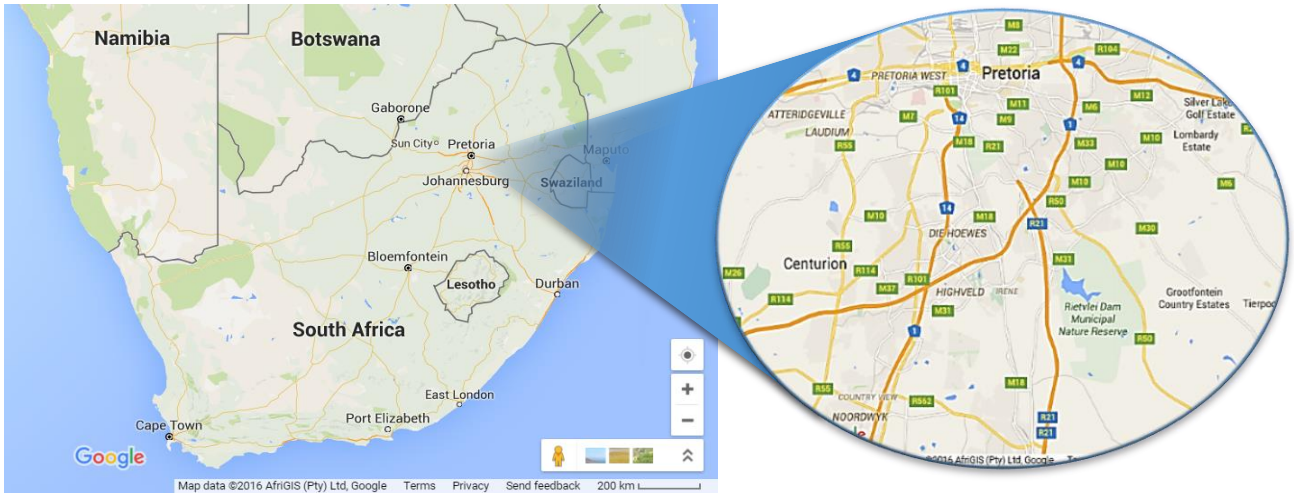


Figure 4.2: Maps showing the location of Rietvlei Nature Reserve, Gauteng, South Africa (25°53'52.7"S 28°17'16.8"E).

4.2.1.2. Albasini Dam and Nandoni Dam

Sampling was conducted at the Albasini and Nandoni Dams, Limpopo Province, South Africa (Figure 4.3) [3]. The dams are located in rural areas of the Limpopo Province where the organochlorine insecticide, DDT (dichlorodiphenyltrichloroethane), is used for malaria vector control. Indoor residual spraying of traditional dwellings is allowed under the Stockholm Convention on Persistent Organic Pollutants (POPs) [4, 5]. The presence of DDT, a classified EDC [6, 7], and research conducted by Aneck-Hahn et al. (2009) which confirmed oestrogenic activity in drinking water samples from the Limpopo Province, with the aid of an RCBA [8], highlight the importance of this sampling site. The compounds responsible for the oestrogenic activity have yet to be identified [8].

Figure 4.3: Maps showing the location of the Albasini (23º6‘24"S 30º6‘13"E) and Nandoni (22º21‘59"S 30º30‘36"E) Dams, Limpopo Province, South Africa and sampling sites (orange dots)

4.2.2. Sample collection

The initial screening water samples, which were known to exhibit endocrine disrupting activity, were provided by Dr. Natalie Aneck-Hahn and Prof. Riana Bornman of the Department of Urology, University of Pretoria, South Africa.

Samples for the initial screening of EDCs were collected at six sites in Rietvlei Nature Reserve on 6/2/2015 by Dr. Natalie Aneck-Hahn (Figure 4.4). At each site, 1 000 mL surface water was collected in glass bottles for extraction in the laboratory.

Rietvlei Nature Reserve sampling sites:

1. STR FAM
2. Otter Bridge
3. Bird Hide Stream
4. Rietvlei Spruit
5. Marais Dam (MD)
6. MD Channel

Figure 4.4: Map showing the six selected sampling sites at Rietvlei Nature Reserve [9]. Sampling site names are reported as were given on the glass sample bottles.

Surface water samples were collected by Prof. Riana Bornman at Albasini Dam (3 000 mL) and Nandoni Dam (1 000 mL); one site per dam (the collection sites are indicated by the orange dots in Figure 4.3). The samples were received on 17/8/2015. Samples were collected during the dry season (anticipated increase in the concentration of analytes).

Follow-up sampling at Rietvlei Nature Reserve was done by the author. The sampling took place on 15/3/2016, approximately one year after the first sampling. 1 000 mL surface water was collected at each of the six sites, as indicated in Figure 4.4, for quantification and qualification of EDCs. Sampling occurred after a period of heavy rainfall and the rivers were in flood (anticipated decrease in concentration of analytes). A picture, GPS coordinates and elevation of each sample site are provided in Figure 4.5.

1. STR FAM

25º53’35’’S 28º17’1’’E
1 500 m Elevation

2. Otter Bridge

25º52’54’’S 28º17’43’’E
1 480 m Elevation

3. Bird Hide Stream

25º52’21’’S 28º16’53’’E
1 470 m Elevation

4. Rietvlei Spruit

25º54’5’’S 28º18’55’’E
1 480 m Elevation

5. Marais Dam

25º54’24’’S 28º18’31’’E
1 500 m Elevation

6. Marais Dam Channel

25º54’40’’S 28º18’25’’E
1 500 m Elevation

Figure 4.5: Picture, coordinates and elevation for six sampling sites at Rietvlei Nature Reserve.

All samples were collected in 1L glass Schott bottles (Duran®, Sigma-Aldrich, South Africa), the opening sealed with foil and then screwed closed. The foil prevented leaching of plasticizers from the lid. Methanol (Merck, South Africa) was added to a final concentration of 5% (v/v) as a preservative to samples not analysed within 5 days of sampling. Samples were stored at 4 ºC prior to analysis.

4.2.3. Sample analysis

Samples were analysed utilising various analytical methods as described in the subsequent sections. Table 4-1 summarises the methods used for the real world samples.

Table 4-1: Summary of analytical methods employed for the six samples collected at Rietvlei Nature Reserve for the initial screening, the six samples collected again at Rietvlei Nature Reserve approximately a year after the first for target analysis, and the samples collected at Albasini and Nandoni Dams in the Limpopo Province.

	Rietvlei Nature Reserve Screening (six samples)	Rietvlei Nature Reserve Target Analysis (six samples)	Albasini Dam Screening and Target Analysis (one sample)	Nandoni Dam* Screening (one sample)
SBSE-TDS-GC×GC-TOFMS	YES	NO	YES	NO
PDMS loop-TDS-GC×GC-TOFMS	YES	NO	YES	YES
PDMS loop-TD GC inlet-GC×GC-TOFMS	NO	YES	YES	NO
LVI-UHPLC-QTOFMS	YES	NO	YES	NO

*Limited amount of sample available.
SBSE: stir bar sorptive extraction
TDS: thermal desorption system
GC×GC-TOFMS: comprehensive gas chromatography – time of flight mass spectrometry
PDMS: polydimethylsiloxane
TD GC inlet: thermal desorption directly in GC inlet liner
LVI: large volume injection
UHPLC-QTOFMS: ultra-high pressure liquid chromatography-tandem mass spectrometry

4.3. Materials and Reagents

4.3.1. Reagents

Methanol (MeOH), *n*-hexane, toluene, de-ionised water, dichloromethane (DCM), acetonitrile (ACN), acetone, ethyl acetate and sodium chloride (NaCl) were all purchased from Merck, South Africa. LC-MS ultra CHROMASOLV® water with 0.1% formic acid and LC-MS CHROMASOLV® acetonitrile with 0.1% formic acid (Fluka® Analytical, Sigma-Aldrich, South Africa) were used for UHPLC-QTOFMS analysis. Glasswool (silane treated) and N,O-Bis(trimethylsilyl)trifluoroacetamide with trimethylsilyl chloride (BSTFA + TMCS) (99:1, Sylon BFT) were purchased from Supelco® Analytical, Sigma-Aldrich, South Africa. Acetic anhydride (Reagent plus®, ≥99%) was purchased from Sigma-Aldrich, South Africa.

4.3.2 Certified reference standards

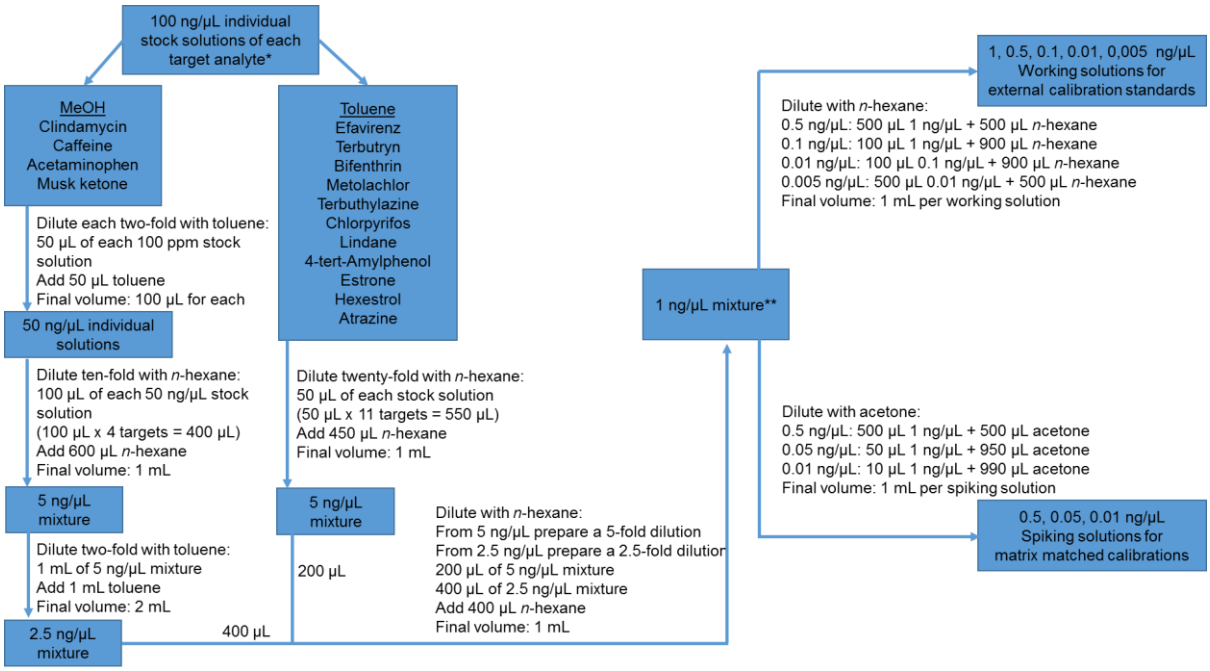
Nevirapine (United States Pharmacopeia (USP) standard), efavirenz (USP standard) and clindamycin (European Pharmacopoeia (Ph. Eur.) standard) were generously supplied by Dr. Tim Wood. Caffeine solution (1.0 mg/mL in MeOH), atrazine (PESTANAL®, analytical standard, purity 98.8%), chlorpyrifos (PESTANAL®, analytical standard, purity 99.7%), musk ketone solution (100 ng/µL in acetonitrile, analytical standard, 95 ng/µL ± 5%), lindane (PESTANAL®, analytical standard, purity 99.8%), metolachlor (PESTANAL®, analytical standard, purity 97.6%), terbuthylazine (PESTANAL®, analytical standard, purity 99.4%) and bifenthrin (PESTANAL®, analytical standard, purity 98.8%) were all purchased from Fluka® Analytical, Sigma-Aldrich, South Africa. Terbutryn (purity 98.1%) was purchased from Supelco® Analytical, Sigma-Aldrich, South Africa and acetaminophen (analytical standard) was purchased from Sigma-Aldrich, South Africa. Hexestrol (purity 99.8%), estrone (purity 99.5%) and 4-tert-amylphenol (purity 99.5%) were purchased from Dr. Ehrenstorfer, Augsburg, Germany. Refer to Addendum A for certificate of analysis for each certified reference standard (CRS).

4.3.3 Stock solutions

Individual stock solutions of 100 ng/µL were prepared by dissolving 5 mg powder in 50 mL (or 2.5 mg powder in 25 mL) of methanol or toluene depending on their

solubility. All standards, except for musk ketone, caffeine, acetaminophen and clindamycin, were dissolved in toluene. A small amount of ethyl acetate and/or methanol was added to clindamycin, terbutryn, metolachlor, terbuthylazine, chlorpyrifos, lindane, 4-tert-amylphenol, estrone and hexestrol to aid in solvation. All the stock solutions were stored in glass vials and kept at 4 ºC.

Figure 4.6 depicts a schematic diagram of the preparation of stock standard solutions, working standards and spiking solutions for GC analysis. Working standard solutions containing a mixture of the target analytes were prepared at five different concentration levels by combining suitable aliquots of each individual stock solution and diluting it with *n*-hexane to a final volume of 1 mL. Stock solutions purchased in MeOH required a four-fold dilution with toluene before being miscible with *n*-hexane. Working solutions for the external calibration were prepared at five different levels of concentration (1, 0.5, 0.1, 0.01 and 0.005 ng/µL) by diluting with *n*-hexane to a final volume of 1 mL. Spiking solutions for the matrix matched calibrations were prepared at three different levels of concentration (0.5, 0.05 and 0.01 ng/µL) by diluting with acetone (miscible with water) to a final volume of 1 mL (Figure 4.6).



*Nevirapine was prepared separately (Figure 4.7)
**1 ng/µL mixture was prepared as needed

Figure 4.6: Schematic diagram showing the preparation of individual stock standard solutions, and working and spiking standard solutions for GC analysis.

A 70 ng/µL stock solution of nevirapine was prepared in toluene (3.5 mg powder in 50 mL). The standard was received after the mixed working and spiking solutions were prepared (Figure 4.6) and consequently it was decided to prepare separate working solutions for nevirapine. Working solutions for the external calibration and matrix matched calibration were prepared as outlined in Figure 4.7.

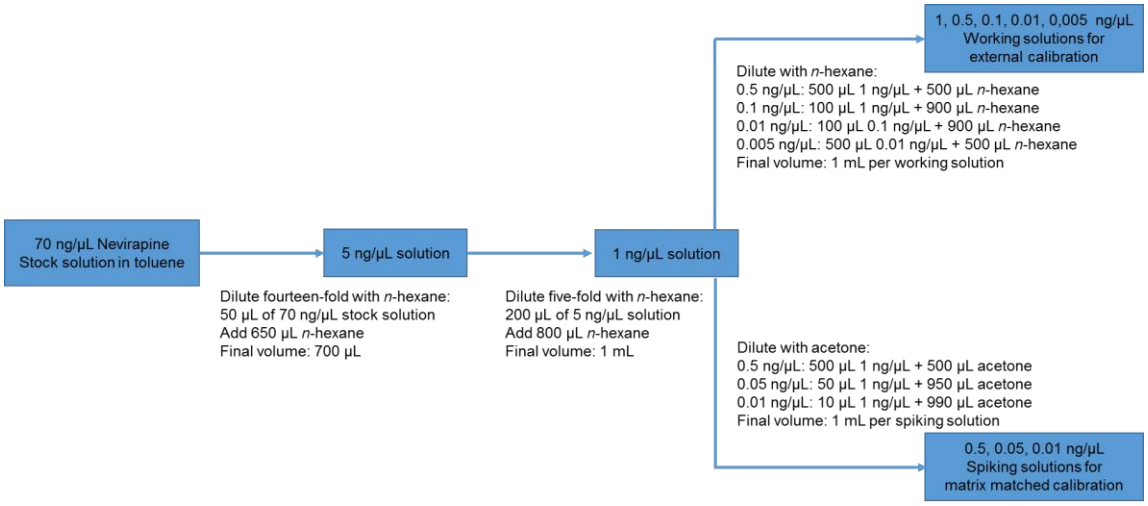


Figure 4.7: Schematic diagram showing the preparation of nevirapine stock standard solutions, and working and spiking standard solutions for GC analysis.

Working solutions for the LC external calibration were prepared at five different levels of concentration (1, 0.5, 0.1, 0.01 and 0.005 ng/µL) by diluting with acetonitrile:water (ACN:H_2O) (1:1, v/v) to a final volume of 1 mL. Spiking standard solutions, for LC analysis, containing a mixture of the target analytes were prepared at 5 and 1 ng/µL by combining suitable aliquots of each individual stock solution and diluting it with acetonitrile to a final volume of 1 mL.

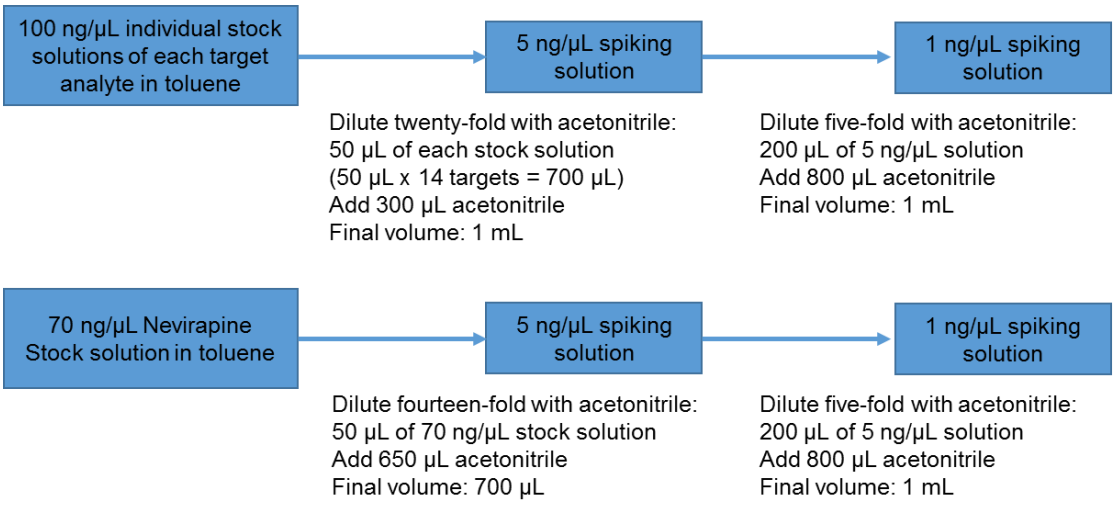


Figure 4.8: Schematic diagram showing the preparation of spiking standard solutions for LC analysis.

4.4. Sample Preparation

4.4.1. Sorptive extraction for GC×GC

A commercial and an in-house developed sorptive sampler were compared to establish the validity of the in-house sampler. Sorptive samplers allow for solvent free analysis by GC×GC-TOFMS.

4.4.1.1. Polydimethylsiloxane loop

A cheap, easy to use, solvent free, disposable sampler was manufactured in-house for sorptive extraction of analytes from surface water. The sampler had been previously developed for solvent free extraction of soil [4, 10] and recently used by Naudé and co-workers as a passive sampler to concentrate pollutants from surface water [11]. The sampler (0.03 ± 0.002 g) was fashioned, as described by Naudé et al. (2015), by forming a loop with a 10.5 cm (0.03 g) length of a silicone elastomer medical grade tubing (0.64 mm OD x 0.3 mm ID, Sil-Tec®, Technical Products, Georgia, USA). The ends were joined by inserting a 1 cm piece of uncoated silica capillary column (250 µm ID) (SGE Analytical Science, Separation Scientific (Pty) Ltd, Roodepoort, South Africa) (Figure 4.9). The loop arrangement keeps water from entering the polydimethylsiloxane (PDMS) tubing and aids ease of handling. The sorption volume of the loop was 26 µL [11].

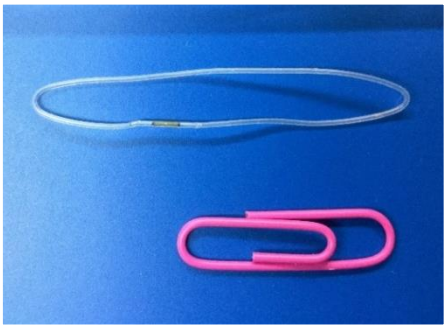


Figure 4.9: PDMS sampling loop; a 10 cm length of a silicone elastomer medical grade tubing joined at the ends by a 1 cm piece of uncoated silica capillary column.

Prior to extraction, the PDMS sampling loops were cleaned using the method outlined by Triñanes et al. (2015) for cleaning silicone sampling disks [12]. The PDMS loops were sonicated three times for 5 minutes each with a MeOH:acetone (1:1, v/v) mixture and then conditioned overnight in a 17.8 cm long glass desorption tube (4 mm ID, 6 mm OD) from Gerstel™ (Chemetrix, Midrand, South Africa) at 250 ºC using a

Gerstel™ tube conditioner with 100 mL/min hydrogen gas flow. After the overnight conditioning, the loops were sonicated three times for 5 minutes each with ACN and stored dry in a glass vial [12].

4.4.1.2. Stir bar sorptive extraction

The in-house developed PDMS sampling loops were compared to stir bar sorptive extraction (SBSE); a commercial solvent free sorptive extraction method. SBSE using Twisters™, magnetic stir bars coated with a volume of PDMS (Gerstel™, Chemetrix, Midrand, South Africa), were employed to extract analytes from surface water (Figure 4.10). The sorption volume of the commercial stir bar was 24 µL (0.5 mm film thickness, 10 mm length).

Figure 4.10: Twisters™; magnetic stir bars coated with a volume of PDMS.

Prior to and between extractions the stir bars were conditioned following the Twister™ reconditioning guidelines set out by Gerstel™. Non-volatile materials were removed by soaking the stir bars in ACN:MeOH (80:20, v/v) for three days. The stir bars were then placed in a clean glass desorption tube and conditioned overnight in a Gerstel tube conditioner at 280 ºC with 100 mL/min hydrogen gas flow. The clean stir bars were stored in a capped glass container.

4.4.1.3. Extraction method

The multi-residue method developed and optimised by Pintado-Herrera et al. (2014) for the extraction of a range of contaminants, including EDCs, fragrances, pesticides, etc., from aqueous matrices using SBSE was adapted as a sorptive extraction method [13]. Optimized conditions were established at an agitation time of 5 hours, the addition of 10% NaCl, 10 mm length stir bars and no methanol addition (organic modifiers like methanol are added to minimize possible adsorption of more hydrophobic compounds onto the glass wall of the container) [13].

Agitation was achieved using magnetic stirrer plates; stirring at a rate of 1 000 rpm and 300 rpm for the stir bars and PDMS loops, respectively. Salt was added sequentially using the method outlined by Ochiai et al. (2008) [14]. Water samples (500 mL) were placed in Schott glass bottles. A sorptive sampler (stir bar or PDMS loop) was added to the sample and the opening of the bottle was sealed with foil and closed with a screw cap. Stirring commenced for 5 hours at room temperature whereafter the sampler was removed with clean stainless steel tweezers, dried with a lint free tissue and placed in a 17.8 cm long glass desorption tube or in a capped glass vial. The desorption tube or glass vial were temporarily stored at 4 ºC. After removal of the sampler, 10% NaCl (w/v) (50 g per 500 mL sample) was dissolved in the sample and a second sampler was placed in the sample.

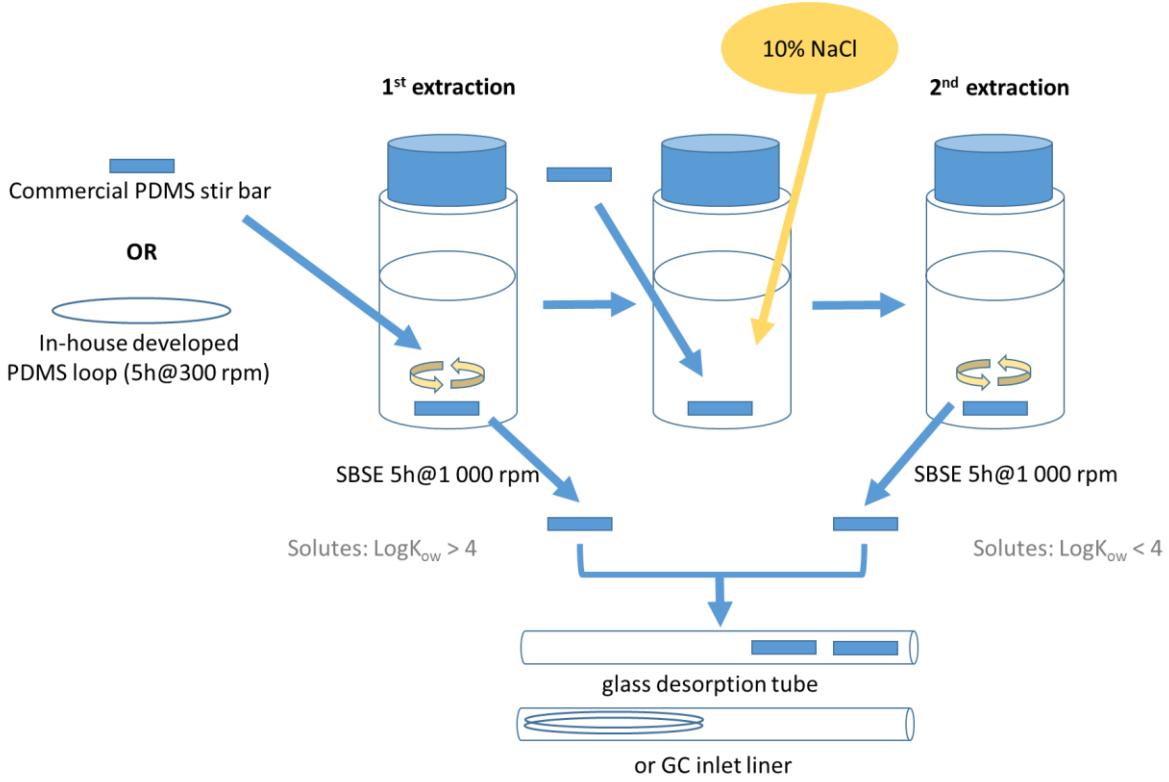


Figure 4.11: Experimental setup of sequential salting out procedure using either SBSE or in-house developed PDMS loop (figure adapted from Ochiai et al. (2015)) [14].

The second extraction was performed under the same conditions as the first extraction. After the second extraction was complete the sampler was removed with clean stainless steel tweezers, dried with a lint free tissue and placed in the glass desorption tube or capped glass vial which contained the first stir bar or loop [14]. Figure 4.11 illustrates the sequential salting out procedure. Samplers placed in the

glass vial were subsequently transferred into the inlet liner of a GC inlet or glass TDS tube for TD.

It is vital that the sampler is submerged in the water matrix and that a vortex is seen during stirring to ensure efficient extraction. The PDMS loop was secured with a stainless steel wire on a glass stirrer bar (Spinbar® Pyrex® magnetic stir bar, size 2.54 cm × 0.9525 cm, Sigma-Aldrich, USA) to immerse it in the water sample (Figure 4.12). A glass stir bar is used for agitation during the PDMS loop extraction to minimize the adsorption of analytes onto the stir bar as would be the case when using a polytetrafluoroethylene (PTFE) stir bar.

Figure 4.12: PDMS loop secured to a glass stir bar using stainless steel wire.

4.4.2. Derivatisation

The multi-shot method of Hoeck et al. (2009) was adapted for this research as follows:

60 mL de-ionised water was spiked with 1 ng of each standard (using the target analytes mixture working solution). The 60 mL was divided into three aliquots of 20 mL each. Each aliquot was treated differently; the first underwent sequential salting out (as described in section 4.4.1.3), 20% methanol was added to the second and 5% acetic acid anhydride, with 5% Na_2CO_3 for pH correction, were added to the third. Figure 4.13 shows the complete multi-shot derivatisation method and Figure 4.14 shows the experimental set-up. After sorptive extraction the four PDMS loops and a glasswool plug containing 2 µl BSTFA + TMCS (99:1) were placed in a glass desorption tube, thermally desorbed (Gerstel™) and analysed using GC×GC-TOFMS.

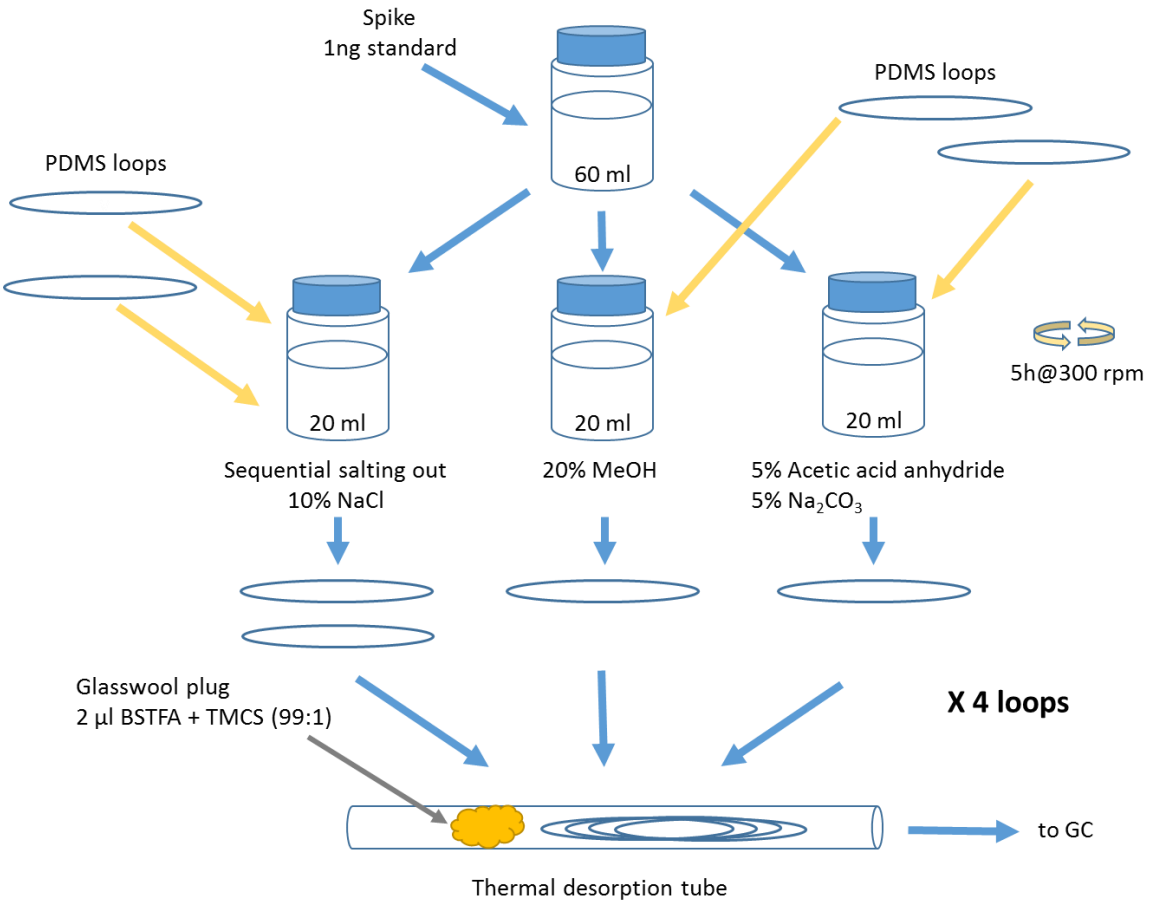


Figure 4.13: Multi-shot derivatisation method.

In situ acetylation was followed by in-tube silylation activated by thermal desorption. The phenolic compounds are derivatised by the acetic acid anhydride in order to increase the recovery and the silylation increases the volatility of the acyl derivative [15, 16]. Van Hoeck et al (2009) found a significant increase in the recoveries of apolar EDCs with methanol addition. The sequential salting out method was employed to cover the entire polarity range (salt addition enhances the extraction of analytes with lower K_{ow} coefficients) [14, 15].

Figure 4.14: Experimental set-up of the thermal desorption tube containing glasswool plug (spiked with 2 µl BSTFA + TMCS (99:1)) and four PDMS loops for the multi-shot derivatisation method.

4.4.3. Sample preparation for UHPLC

Conventional solid phase extraction was compared to direct large volume sample injection of water samples for analysis using UHPLC-QTOFMS.

4.4.3.1. Solid phase extraction

The method used by Lagana et al. (2004) for the determination of EDCs in natural waters was modified [17]. Extraction cartridges (Strata-X 33 µm, Polymeric Sorbent, 500 mg/6 mL, Phenomenex®, USA) were fitted onto a Vacuum Manifold (Waters™). The manifold was connected to a vacuum pump. Sequential conditioning was done with 10 mL DCM:MeOH (50:50, v/v), 5 mL of methanol and 10 mL of water. Each cartridge was loaded with 1 000 mL* of sample and successively washed with 10 mL of de-ionised water. The retained compounds were eluted with 10 mL (using two aliquots of 5 mL each) of DCM:MeOH (50:50, v/v). The extracts were evaporated to dryness under a gentle stream of nitrogen gas. The dried extracts were reconstituted in 200 µL ACN:water (50:50, v/v) [17]. For analysis 5 µL of the final extract was injected into the LC.

*Please note that for the Nandoni Dam 500 mL sample was used during the loading stage due to the limited amount of sample available.

4.4.3.2. Large volume injections

The large volume injection (LVI) method was based on work, done by Bayen et al. (2014) and Boix et al. (2015), employing large volume direct injection liquid chromatography electrospray ionisation tandem mass spectrometry for the rapid determination of drugs in water [18, 19]. The water samples (1 mL each in an Eppendorf vial) were centrifuged at 6 000 rpm; workforce 2 000 g (BG-Qspin™ Hand Centrifuge, Vacutec, South Africa) before injection in order to remove any particulates. 100 µL of the sample was directly injected in the UHPLC for analysis. The UPLC™ system was fitted with a 100 µL sample loop (Figure 4.15) and a 250 µL syringe (both purchased from Waters Inc., Milford, Massachusetts, USA) to accommodate the larger sample volume.

Figure 4.15: UPLC™ system fitted with 100 µL sample loop.

4.5. Instrumentation

4.5.1. Thermal desorption

Direct thermal desorption (TD) of analytes in the inlet liner of a GC inlet was compared to desorption of analytes in a Gerstel™ thermal desorber system (Chemetrix, Midrand, South Africa) using the PDMS loop as sorptive sampler. The isolated analytes were desorbed from the PDMS loops in the inlet liner of a GC inlet and in a Gerstel™ thermal desorber system. Desorption of analytes from the stir bars was only done in the Gerstel™ thermal desorber system.

4.5.1.1. Thermal desorption with cooled injection

The glass desorption tube containing the sorptive samplers (stir bars or PDMS loops), see Figure 4.16, was placed in a Gerstel™ thermal desorber system (TDS) for thermal desorption into a LECO Pegasus® 4D GC×GC-TOFMS (LECO Africa (Pty) Ltd., Kempton Park, South Africa). Compounds concentrated onto the samplers were thermally desorbed by heating the traps in the thermal desorber system from 30 °C (hold 3 minutes) at 60 °C/min to 280 °C (hold 5 minutes) with a desorption flow rate of 100 mL/min at a vent pressure of 10 psi (helium 5.0, Afrox, South Africa). The TDS transfer line temperature was 300 °C. The desorbed analytes were cryogenically focused at –100 °C using liquid nitrogen (Afrox, South Africa) and a cooled injection system (CIS) (Gerstel CIS 4) with an empty baffled deactivated glass liner. After desorption, a splitless injection (purge on at 1.5 minutes, purge flow 30 mL/min, solvent vent mode) was performed by heating the CIS from –100 °C at 10 °C/s to 280 °C and held there for the duration of the GC run (81.07 minutes total run time).

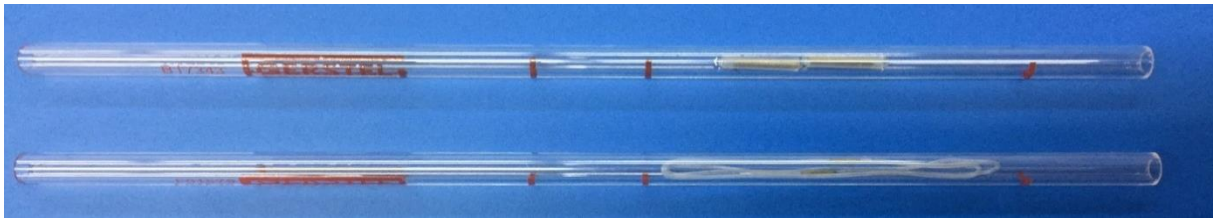


Figure 4.16: Glass desorption tube showing inserted stir bars (top) and PDMS loops (bottom).

4.5.1.2. Thermal desorption in a GC inlet

The PDMS loops were inserted into a splitless glass inlet liner (Agilent Chemetrix, Midrand, South Africa) of a GCxGC-TOFMS (Figure 4.17). The gas flow to the inlet was switched off, and after removing the inlet nut the liner with loops was placed into the GC inlet, the inlet nut and gas flow were restored and the GC run was started. The loops were desorbed at 250 °C with a splitless time of 1 min and an inlet purge gas flow of 20 mL/min. After analysis, the hot inlet liner was manually removed from the GC inlet using a pair of tweezers and the next batch of PDMS loops was inserted for desorption.

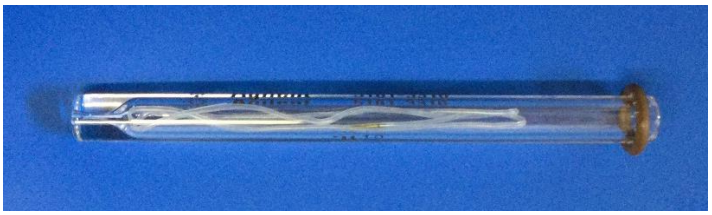


Figure 4.17: GC inlet liner showing inserted PDMS loops.

4.5.2. GC×GC-TOFMS

4.5.2.1. Instrument description

Separation of compounds was performed on a LECO Pegasus® 4D GC×GC-TOFMS system. The system consisted of an Agilent© 7890 GC (LECO Africa (Pty) Ltd., Kempton Park, South Africa) modified to contain a dual stage modulator and secondary oven. Nitrogen gas cooled with liquid nitrogen was used for the cold jets and synthetic air for the hot jets. Gases were of ultra-high purity grade (Afrox, Gauteng, South Africa). The primary column was connected to the secondary column with a presstight column connector (Restek, Bellefonte, PA, USA). ChromaTOF® software (version 4.50.8.0 optimised for Pegasus®, LECO Africa (Pty) Ltd.) was used to operate the instrument and for data capturing and processing. Tentative identification of compounds for the initial screening was based on a comparison of sample mass

spectra to that of the NIST14 library (version 2.2). A spectral match quality of ≥ 70% was reported unless stated otherwise.

4.5.2.2. Column selection

A “normal-phase” column combination set was used for the separation of compounds; the second dimension column phase being more polar than the first dimension column phase.

The column set used for the initial screening (see section 4.1) consisted of a 30 m x 0.25 mm ID x 0.25 µm film thickness Rxi®-1ms (fused silica) (nonpolar phase; Crossbond® dimethyl polysiloxane) as the primary column (^{1}D) joined to a midpolarity Crossbond® phase Rxi®-17Sil MS 1 m x 0.25 mm ID x 0.25 µm film thickness secondary column (^{2}D) (Restek, Bellefonte, PA, USA). To ensure optimum separation of the selected target standards a column set comprising of a proprietary Crossbond® phase Rtx®-CLPesticides II 30 m x 0.25 mm ID x 0.2 µm film thickness (fused silica) as the primary column (^{1}D) joined to a Rxi®-17Sil MS 1 m x 0.25 mm ID x 0.25 µm film thickness secondary column (^{2}D) (Restek, Bellefonte, PA, USA) was used.

4.5.2.3. Operating conditions

The primary oven temperature programme was 70 °C (hold for 1.5 min) at 10 °C/min to 300 °C (hold for 10 min). The GC run time was 34.5 min. The secondary oven was offset by + 10 °C relative to the primary oven. The modulator temperature was offset 15 °C relative to the second oven temperature. The modulation period was 3 seconds with a hot pulse time of 0.75 seconds. The carrier gas (helium 5.0, Afrox, South Africa) flow rate was 1.4 mL/min in the constant flow mode. The MS transfer line temperature was set at 300 °C. The ion source temperature was 230 °C, the electron energy was 70 eV in the electron ionisation mode (EI+), the data acquisition rate was 100 spectra/s, the mass acquisition range was 40–650 Daltons (Da), and the detector voltage was set at 1 570 V.

4.5.3. UHPLC-QTOFMS

In order to effectively analyse polar, non-volatile and thermally unstable compounds LC was employed complementary to GC. The analysis was performed using an ultra-high pressure liquid chromatography (UHPLC) system. The system enables greater

chromatographic resolution using higher pressures and smaller particle size stationary phases. Higher column efficiency, for a given column length, and higher optimal linear velocity is achieved through the use of smaller particle sizes [20]. Analysis of complex environmental matrices is improved due to the enhanced resolving power of the UHPLC system [21].

4.5.3.1. Instrument description

Compound separation and detection were performed using a Waters® Synapt G2 high definition mass spectrometry (HDMS) system (Waters Inc., Milford, Massachusetts, USA). The system comprises of a Waters Acquity Ultra Performance Liquid Chromatography (UPLC®) system hyphenated to a quadrupole-time-of-flight (QTOF) instrument. The system was operated with MassLynx™ (version 4.1) software (Waters Inc., Milford, Massachusetts, USA) for data acquisition and processing. An internal lock mass control standard, 2 pg/µL solution leucine enkephalin (*m/z* 555.2693), was directly infused into the source through a secondary orthogonal electrospray ionisation (ESI) probe allowing intermittent sampling. The internal control was used to compensate for instrumental drift, ensuring good mass accuracy, throughout the duration of the runs. The instrument was calibrated using sodium formate clusters and Intellistart functionality (mass range 112.936 – 1 132.688 Da). Resolution of 20 000 at *m/z* 200 (full width at half maximum (FWHM)) and mass error within 5 mDa were obtained.

4.5.3.2. Operating conditions

Separation was completed using a reverse phase step gradient elution scheme from 95% H_2O (0.1% formic acid) to 100% acetonitrile (0.1% formic acid). Formic acid was added to the solution as buffer (pH correction), preservative and proton source for ionisation. The gradient started with an isocratic flow (hold 0.1 min) followed by a linear increase to 100% ACN (at 13.00 min); subsequently the column was washed for 1 min followed by conditioning and re-establishing of initial conditions to allow for equilibration before the start of the next run (Figure 4.18) for the complete elution scheme. The column temperature was kept constant at 40 ºC and the flow rate was set at 0.3 mL/min for the entire run giving a total run time of 17 min. Injection volumes were set at 5 µL and 100 µL for SPE and LVI, respectively. A Waters UPLC® C_{18} Ethylene Bridged Hybrid (BEH), 1.7 µm particle size (2.1 mm ID x 100 mm length)

column was used. The positive and negative ion mass spectra were collected in separate chromatographic runs (employing the same separation conditions).

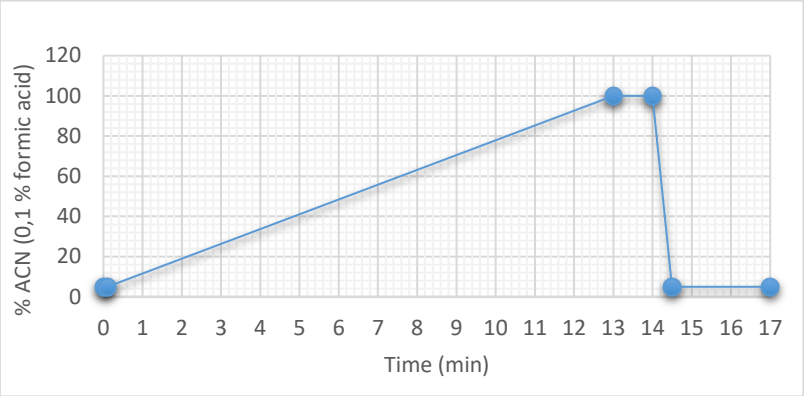


Figure 4.18: Step gradient elution scheme for the UHPLC method.

Table 4-2: Step gradient elution scheme for the UHPLC method.

Time (min)	Flow rate (mL/min)	% H_2O (0.1% formic acid)	% ACN (0.1% formic acid)
0 (initial)	0.3	95	5
0.1	0.3	95	5
13.00	0.3	0	100
14.00	0.3	0	100
14.50	0.3	95	5
17.00	0.3	95	5

The ESCi™ multi-mode ionisation source, which permits quasi-simultaneous analysis alternating between electrospray (ESI) and atmospheric pressure chemical ionisation (APCI), was used during the analysis. The ESI and APCI data were acquired in separate functions and processed separately. The source conditions were as follows: the corona discharge electrode was set at 5 µA for APCI and the capillary voltage for ESI was 2.8 kV and 2.6 kV for positive and negative mode ionisation, respectively. The probe temperature for APCI was set at 550 ºC. The source temperature was set at 110 ºC, the sampling cone voltage at 25 V, extraction cone voltage at 4.0 V and cone gas (nitrogen) flow at 10.0 L/Hr. The desolvation temperature was set at 300 ºC with a gas (nitrogen) flow of 600.0 L/Hr.

Mass spectral scans were collected every 0.3 seconds. The raw data was collected in the form of a continuous profile. Mass to charge ratios (*m/z*) between 50 and 1 200 Da

were recorded. QuanLynx™, an application manager included in the MassLynx software, was used for quantification. Peak integration was done using an automatic peak detection algorithm within the software. Background subtraction was applied prior to integration.

Quantitative data-independent acquisition (DIA) was done using two simultaneous acquisition functions with low and high collision energy (MS^E approach) with a QTOF instrument. The high energy MS scan can be time aligned with the low energy scan in order to predict which fragment ions belong to which precursor ions, consequently the full mass spectrum is acquired. Fragmentation patterns can thus be used for qualitative confirmation. Fragmentation was performed using high energy collision induced dissociation (CID). The fragmentation energy was set at 2 V and 3 V for the trap and collision energy, respectively. The ramping was set from 3 to 4 V and 20 to 40 V for the trap and transfer collision energy, respectively.

4.6. Method Performance

4.6.1. GC×GC-TOFMS

Matrix matched calibration curves of reference compounds were constructed by spiking 50 mL de-ionised water with different volumes of the spiking solutions (using a Hamilton syringe) (Table 4-3). Due to the limited quantities of surface water samples collected de-ionised water was used as a practical alternative to determine % recoveries. The calibration range was from 0 to 40 ng/L. The spiked samples were left to stand for 30 minutes to equilibrate before extraction commenced.

Table 4-3: Spike levels and volumes added to de-ionised water during the preparation of the matrix matched calibration range (0 to 40 ng/L) for GC×GC-TOFMS.

Spike levels (ng)	Volume added to de-ionised water (µL of spiking solutions*)	Final volume (mL)	Final concentration (ng/L)
2	4 µL of 0.5 ng/µL solutions	50	40
1	2 µL of 0.5 ng/µL solutions	50	20
0.5	1 µL of 0.5 ng/µL solutions	50	10
0.25	0.5 µL of 0.5 ng/µL solutions	50	5

Spike levels (ng)	Volume added to de-ionised water (µL of spiking solutions*)	Final volume (mL)	Final concentration (ng/L)
0.1	2 µL of 0.05 ng/µL solutions	50	2
0.075	1.5 µL of 0.05 ng/µL solutions	50	1.5
0.05	1 µL of 0.05 ng/µL solutions	50	1
0.025	0.5 µL of 0.05 ng/µL solutions	50	0.5
0.01	1 µL of 0.01 ng/µL solutions	50	0.2
0.005	1 µL of 0.01 ng/µL solutions	50	0.1
0	Blank	50	0

*Spiking solutions include the mixed spiking solution and nevirapine spiking solution

The sorptive extraction method described in section 4.4.1.3 was followed. The extracts were analysed using GC×GC-TOFMS. Calibration curves for each of the three extraction procedures were obtained: (1) PDMS loop for TD using the Gerstel™ system and (2) PDMS loop for TD in the GC inlet liner and (3) for the SBSE (using only the Gerstel™ TD system) methods. Neat reference standards, for comparison, were injected (injection volume of 1 µL) into the GC to obtain a calibration curve (range 0.005 – 1 ng/µL).

4.6.2. LC-QTOFMS

Matrix matched calibration curves for the reference standards were also constructed using LC-MS analysis. 100 mL de-ionised water was spiked with different volumes of the composite working standard solutions for SPE (Table 4-4). The calibration range was from 0 – 1 000 ng/L. The SPE method set out in section 4.4.2.1 was followed.

Table 4-4: Spike levels and volumes added to de-ionised water during the preparation of the matrix matched calibration range (0 to 1 000 ng/L) for SPE

Spike levels (ng)	Volume added to de-ionised water (µL of spiking solutions)	Final volume (mL)	Final concentration (ng/L)
100	100 µL of 1 ng/µL solutions	100	1 000
50	50 µL of 1 ng/µL solutions	100	500

Spike levels (ng)	Volume added to de-ionised water (µL of spiking solutions)	Final volume (mL)	Final concentration (ng/L)
25	25 µL of 1 ng/µL solutions	100	250
10	10 µL of 1 ng/µL solutions	100	100
5	5 µL of 1 ng/µL solutions	100	50
1	1 µL of 1 ng/µL solutions	100	10
0	Blank	100	0

Calibration curves for LVI were constructed (range 0 – 10 000 ng/L) by spiking 100 or 50 mL de-ionised water with different volumes of the composite working standard solutions and directly injecting 100 µL of the sample into the LC (Table 4-5).

Table 4-5: Spike levels and volumes added to de-ionised water during the preparation of the matrix matched calibration range (0 to 10 000 ng/L) for LVI

Spike levels (ng)	Volume added to de-ionised water (µL of spiking solutions)	Final volume (mL)	Final concentration (ng/L)
1 000	200 µL of 5 ng/µL solutions	100	10 000
500	100 µL of 5 ng/µL solutions	100	5 000
150	30 µL of 5 ng/µL solutions	50	3 000
250	50 µL of 5 ng/µL solutions	100	2 500
75	15 µL of 5 ng/µL solutions	50	1 500
100	20 µL of 5 ng/µL solutions	100	1 000
25	5 µL of 5 ng/µL solutions	50	500
10	2 µL of 5 ng/µL solutions	100	100
1	0.2 µL of 5 ng/µL solutions	100	10
0	Blank	100	0

Solvent and method blanks were done during all runs for quality control purposes. All runs were conducted in positive and negative mode for ESI and APCI (ESCi). The

conditions (i.e. positive mode ESI) giving the best signal for a reference standard were chosen to obtain the calibration curve.

4.6.2.1. Matrix effects

A midpoint (1 000 ng/L) in the calibration range was chosen to calculate the matrix effects (ME) for LVI. Individual neat solutions of acetaminophen, caffeine, atrazine, terbuthylazine, terbutryn, nevirapine, metolachlor and efavirenz were prepared at 1 000 ng/L (0.001 ng/µL) in ACN:H_2O (1:1, v/v) by serial dilutions from the 100 ng/µL stock solutions in toluene. 100 ng/µL was diluted twenty-fold to a 5 ng/µL solution (50 µl of the stock solution and 950 µl ACN; final volume 1 mL). The 5 ng/µL solution was diluted five-fold to a 1 ng/µL solution (200 µl of the 5 ng/µL solution and 800 µl ACN; final volume 1 mL), whereafter the 1 ng/µL solution was diluted twenty-fold to a 0.05 ng/µL solution in ACN:H_2O (50 µl of the1 ng/µL solution and 950 µl ACN:H_2O; final volume 1 mL). Finally, the 0.05 ng/µL solution was diluted fifty-fold to a 0.001 ng/µL solution in ACN:H_2O (20 µl of the 0.05 ng/µL solution and 980 µL ACN:H_2O; final volume 1 mL).

The process efficiency (PE) for SPE was also calculated at a midpoint (100 ng/L) in the calibration range. Prior to extraction 100 mL deionised water was spiked with 10 ng standard. After extraction the analytes were dried and reconstituted in 200 µL ACN:H_2O (1:1, v/v); final concentration 0.05 ng/µL (10 ng per 200 µL).

Matrix effect (ME) and process efficiency (PE) were calculated using the equations defined by Matuszewski et al. (2003):

$$\% ME = \frac{B}{A} \times 100$$

Equation 4-1

$$\% PE = \frac{C}{A} \times 100$$

Equation 4-2

Where:

A = chromatographic peak of the standard in neat solution

B = peak area of the standard in the matrix (after extraction)

C = peak area of the standard spiked before extraction [22].

Equation 4-1 was used to calculate the matrix effect for LVI. Because no extraction was performed for LVI, B is simply the area of the standard spiked directly into the matrix. In order to determine the process efficiency, Equation 4-2 was used for SPE. The concentration and solvent composition of the neat standard must be the same as that of the standard in the reconstituted solution for SPE assuming 100% recovery [23].

4.6.3. Method validation

4.6.3.1. Limit of detection and limit of quantification

Limits of detection (LODs) and limits of quantification (LOQs) were calculated as those concentrations giving a signal to noise ratio (S/N) of 3 and 10, respectively.

$$LOD = \frac{lowest\ concentration\ of\ standard}{instumental\ S/N\ \ for\ lowest\ concentration\ of\ standard} \times 3$$

Equation 4-3

$$LOQ = \frac{lowest\ concentration\ of\ standard}{instumental\ S/N\ \ for\ lowest\ concentration\ of\ standard} \times 10$$

Equation 4-4

4.6.3.2. Accuracy and Precision

Between day analysis utilising three replicates (*n* = 3), one spike per day over three days, was done to determine the method accuracy (% recovery) and precision (% relative standard deviation (RSD)). De-ionised water (50 mL) spiked with 1 ng (1 µL of a 1 ng/µL mixed working standard solution) of each target analyte was used for GC replicates to give a concentration of 20 ng/L for each target analyte. For SPE LC replicates de-ionised water (100 mL) was spiked with 25 ng (25 µL of a 1 ng/µL mixed working standard solution) to give a concentration of 250 ng/L for each target analyte. For LVI LC replicates de-ionised water (100 mL) was spiked with 250 ng (50 µL of a 5 ng/µL mixed working standard solution) to give a concentration of 2 500 ng/L for each target analyte. % Recovery and % RSD of the methods were determined using Equation 4.5 and 4.6, respectively.

$$\%\ Recovery = \frac{experimentally\ determined\ concentration}{true\ spike\ concentration} \times 100$$

Equation 4-5

$$\%\ RSD = \frac{standard\ deviation}{mean} \times 100$$

Equation 4-6

4.6.4. Chemometrics

The Dixon Q-test (Equation 4-7) [24] was used to determine outliers at 95% confidence level.

$$Q_{test} = \frac{|x_q - x_n|}{w}$$

Equation 4-7

Where:
x_q = questionable result
x_n = nearest neighbour
w = spread of the entire set

Analysis of variance (ANOVA F-test) (Equation 4-8) [24] was performed at 95% confidence level for the comparison of means between the three different GC methods, (1) PDMS loop with TDS, (2) PDMS loop TD in GC inlet and (3) SBSE with TDS.

$$F_{test} = \frac{Variation\ between\ group\ means}{Variation\ within\ group\ means}$$

Equation 4-8

A t-test was used to determine the difference between two means (Equation 4-9) at 95% confidence level [24].

$$t_{test} = \frac{(x_{m1} - x_{m2})}{s_{pooled}\sqrt{\frac{N_1 + N_2}{N_1 N_2}}}$$

Equation 4-9

Where:

x_{m1} - x_{m2} = difference between two means

s_{pooled} = pooled standard deviations

N_1 = number of observations in set one

N_2 = number of observations in set two

4.7. Linear Retention Index

Linear retention indices were determined by analysing a mixture of *n*-alkanes (*n*-C_{12} – *n*-C_{28}). Experimental linear retention indices were calculated for non-target compounds according to the method of van Den Dool and Kratz (1963) [25].

4.8. Data Analysis

4.8.1. Principle component analysis

Principle component analysis (PCA) was done using JMP® Pro 12.0.1 a statistical software package from the SAS® Institute Inc. (Cary, North Carolina, USA). PCA was used to determine the difference in the composition of the surface water at the different sampling sites in Rietvlei Nature Reserve and to determine the correlation, if any, between surface water from Rietvlei Nature Reserve (Gauteng Province) and Albasini and Nandoni Dams (Limpopo Province). Peak areas of selected tentatively identified compounds, with a focus on EDCs, were normalised prior to PCA analysis. Replicate samples were not analysed due to the limited availability of samples in addition to the destructive nature of the thermal desorption process.

4.9. Summary

In order to develop a cheap, quick and easy analytical method or methods that detect multiple classes of micropollutants, including EDCs and potential EDCs, at trace level in surface water various extraction and instrumental techniques were investigated. Sorptive extraction techniques, SBSE versus the in-house developed PDMS loop, were compared and GC and LC were used complimentary to compensate for limitations of an analytical technique. The end goal was to find the best method/s for a multi-residue analysis while saving time and cost. A diagram giving the overview of the analytical methods investigated and how they relate is set out in Figure 4.19. The

best-suited methods were used to analyse real world surface water at Rietvlei Nature Reserve and Albasini and Nandoni Dams for EDCs.

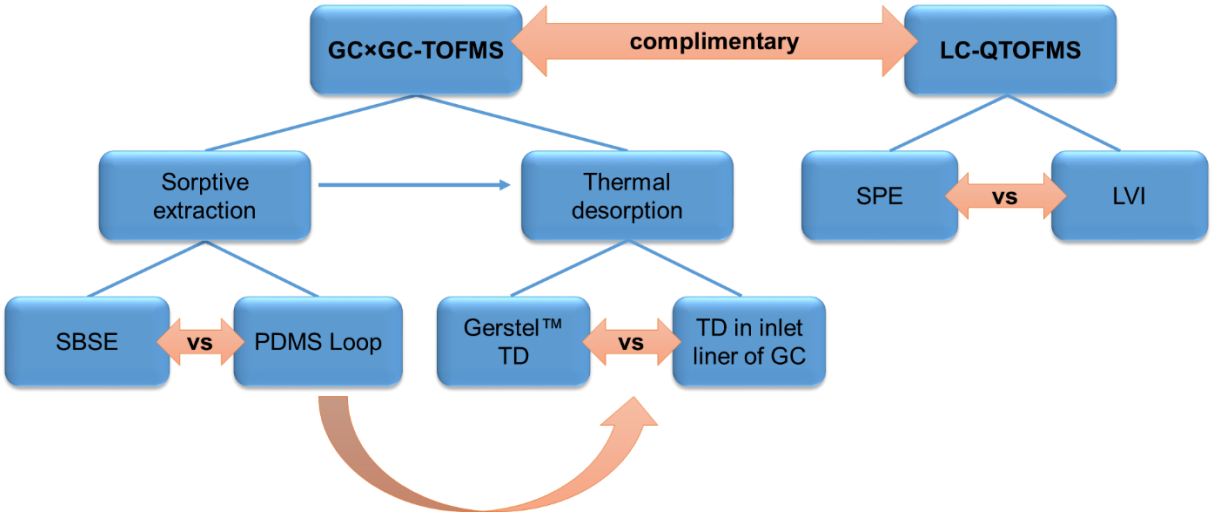


Figure 4.19: Diagram showing analytical methods investigated

References

1. Google. *Google Maps. Rietvlei Dam Municipal Nature Reserve, Pretoria*. 2016; Available from: https://www.google.co.za/maps/place/Rietvlei+Dam+Municipal+Nature+Reserve/@-25.8979996,28.2855543,17z/data=!3m1!4b1!4m2!3m1!1s0x1e9568042114792b:0xac0f71af5f17933a?hl=en. [Accessed on 2016/02/15]
2. Aneck-Hahn, N.H., Bornman, M.S., De Jager, C. *Preliminary assessment of oestrogenic activity in water sources in Rietvlei Nature Reserve, Gauteng, South Africa.* African Journal of Aquatic Science, 2008. **33**(3): p. 249-254.
3. Google. *Google Maps. Louis Trichardt*. 2016; Available from: https://www.google.co.za/maps/place/Louis+Trichardt/@-23.1173619,29.6676988,11z/data=!3m1!4b1!4m2!3m1!1s0x1ec66a27298fc029:0xd538f92bddf1bf1b?hl=en. [Accessed on 2016/02/15]
4. Naudé, Y., Rohwer, E.R. *Two multidimensional chromatographic methods for enantiomeric analysis of o, p'-DDT and o, p'-DDD in contaminated soil and air in a malaria area of South Africa.* Analytica Chimica Acta, 2012. **730**: p. 120-126.
5. Naudé, Y., Rohwer, E.R. *Novel method for determining DDT in vapour and particulate phases within contaminated indoor air in a malaria area of South Africa.* Analytica Chimica Acta, 2012. **730**: p. 112-119.
6. Colborn, T. *TEDX list of potential endocrine disruptors, the endocrine disruptor exchange*. Available from: http://endocrinedisruption.org/endocrine-disruption/tedx-list-of-potential-endocrine-disruptors/chemicalsearch. [Accessed on 2016/02/15]
7. Montes-Grajales, D., Olivero-Verbel, J. *EDCs DataBank: 3D-Structure database of endocrine disrupting chemicals.* Toxicology, 2015. **327**: p. 87-94.
8. Aneck-Hahn, N.H., Bornman, M., De Jager, C. *Oestrogenic activity in drinking waters from a rural area in the Waterberg District, Limpopo Province, South Africa.* Water SA, 2009. **35**(3): p. 245-251.
9. Google. *Google Earth. Rietvlei Dam Municipal Nature Reserve*. 2016; Available from: https://www.google.co.za/maps/place/Rietvlei+Dam+Municipal+Nature+Reserve/@-25.8980044,28.2855543,717m/data=!3m1!1e3!4m2!3m1!1s0x1e9568042114792b:0xac0f71af5f17933a!6m1!1e1?hl=en. [Accessed on 2016/02/15]
10. Naudé, Y., Van Rooyen, M.W., Rohwer, E.R. *Evidence for a geochemical origin of the mysterious circles in the Pro-Namib desert.* Journal of Arid Environments, 2011. **75**(5): p. 446-456.
11. Naudé, Y., Gorst-Allman, P., Rohwer, E. *A cheap and simple passive sampler using silicone rubber for the analysis of surface water by gas chromatography-time of flight mass spectrometry.* Water SA: WISA 2014-Water Innovations Special Edition, 2015. **41**(2): p. 182-188.

12. Triñanes, S., Pena, M.T., Casais, M.C., Mejuto, M.C. *Development of a new sorptive extraction method based on simultaneous direct and headspace sampling modes for the screening of polycyclic aromatic hydrocarbons in water samples.* Talanta, 2015. **132**(0): p. 433-442.

13. Pintado-Herrera, M.G., González-Mazo, E., Lara-Martín, P.A. *Atmospheric pressure gas chromatography–time-of-flight-mass spectrometry (APGC–ToF-MS) for the determination of regulated and emerging contaminants in aqueous samples after stir bar sorptive extraction (SBSE).* Analytica Chimica Acta, 2014. **851**(0): p. 1-13.

14. Ochiai, N., Sasamoto, K., Hirooki, K., Pfannkoch, E. *A Novel Extraction Procedure for Stir Bar Sorptive Extraction (SBSE): Sequential SBSE for Uniform Enrichment of Organic Pollutants in Water Samples*. 2008; Available from: http://www.gerstel.com/pdf/p-gc-an-2008-12corrected.pdf. [Accessed on 15/11/20]

15. Van Hoeck, E., Canale, F., Cordero, C., Compernolle, S., Bicchi, C., Sandra, P. *Multiresidue screening of endocrine-disrupting chemicals and pharmaceuticals in aqueous samples by multi-stir bar sorptive extraction–single desorption–capillary gas chromatography/mass spectrometry.* Analytical and Bioanalytical Chemistry, 2009. **393**(3): p. 907-919.

16. Kawaguchi, M., Ito, R., Sakui, N., Okanouchi, N., Saito, K., Nakazawa, H. *Dual derivatization–stir bar sorptive extraction–thermal desorption–gas chromatography–mass spectrometry for determination of 17β-estradiol in water sample.* Journal of Chromatography A, 2006. **1105**(1): p. 140-147.

17. Laganà, A., Bacaloni, A., De Leva, I., Faberi, A., Fago, G., Marino, A. *Analytical methodologies for determining the occurrence of endocrine disrupting chemicals in sewage treatment plants and natural waters.* Analytica Chimica Acta, 2004. **501**(1): p. 79-88.

18. Bayen, S., Yi, X., Segovia, E., Zhou, Z., Kelly, B.C. *Analysis of selected antibiotics in surface freshwater and seawater using direct injection in liquid chromatography electrospray ionization tandem mass spectrometry.* Journal of Chromatography A, 2014. **1338**: p. 38-43.

19. Boix, C., Ibáñez, M., Sancho, J.V., Rambla, J., Aranda, J.L., Ballester, S., Hernández, F. *Fast determination of 40 drugs in water using large volume direct injection liquid chromatography–tandem mass spectrometry.* Talanta, 2015. **131**: p. 719-727.

20. De Villiers, A., Venter, P., Pasch, H. *Recent advances and trends in the liquid-chromatography–mass spectrometry analysis of flavonoids.* Journal of Chromatography A, 2016. **1430**: p. 16-78.

21. Farré, M., Gros, M., Hernandez, B., Petrovic, M., Hancock, P., Barceló, D. *Analysis of biologically active compounds in water by ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry.* Rapid Communications in Mass Spectrometry, 2008. **22**(1): p. 41-51.

22. Matuszewski, B.K., Constanzer, M.L., Chavez-Eng, C.M. *Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS.* Analytical Chemistry, 2003. **75**(13): p. 3019-3030.

23. Chambers, E., Wagrowski-Diehl, D.M., Lu, Z., Mazzeo, J.R. *Systematic and comprehensive strategy for reducing matrix effects in LC/MS/MS analyses.* Journal of Chromatography B, 2007. **852**(1): p. 22-34.

24. Miller, J.N., Miller, J.C. *Statistics and chemometrics for analytical chemistry*. 2005: Pearson Education.

25. Van den Dool, H., Kratz, P.D. *A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography.* Journal of Chromatography A, 1963. **11**: p. 463-471.

Chapter 5

Results and Discussion

Table of Contents

5. Results and Discussion

5.1. Untargeted Analysis

5.1.1. Screening

The initial untargeted screening of surface water at Rietvlei Nature Reserve, using sorptive sampling (Chapter 4 Section 4.4.1) and GC×GC-TOFMS analysis (Chapter 4 Section 4.5.2), yielded an exhaustive list of emerging and classified micropollutants. Over 3 000 compounds were detected due to the superior resolving power and increased sensitivity of GC×GC-TOFMS. Compounds detected included personal care products (e.g. the EU and the USA banned substance triclosan), pharmaceuticals, sunscreen ingredients, pesticides, hormones and fragrances, with many of these being emerging or classified EDCs.

The compounds described in this chapter have been chosen to show the versatility of the in-house developed PDMS sampler and its ability to concentrate a wide range of listed and emerging micropollutants in the environment. Focus was placed on five main end-use categories, namely pharmaceuticals, sunscreen ingredients, pesticides, hormones and fragrances (Figure 5.1). Compounds within these categories were further investigated for the purposes of method validation and targeted analysis. The reconstructed one-dimensional total ion chromatograms (TICs) and the contour plots of the screening of the six sampling sites can be found in Addendum B.

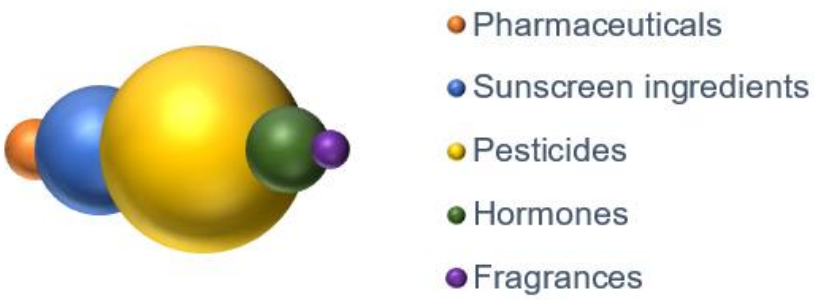


Figure 5.1: Screening: Compound classes detected in surface water at Rietvlei Nature Reserve by GC×GC-TOFMS. Bubble size shows relative amount of the compound classes detected.

5.1.1.1. Compounds tentatively identified

Selected compounds tentatively identified in the surface water at Rietvlei Nature Reserve are set out in Table 5-1. The compounds reported in Table 5-1 demonstrate the trapping efficiency of a wide range of pollutants in the environment into the in-house designed PDMS sampling loop. A comprehensive list of all the tentatively identified compounds, including the mass spectral similarity (library match percentage), for each sample site using both sorptive extraction methods (SBSE and PDMS loop) as well as additional compound information, are given in Addendum C.

Table 5-1: Chemical name, chemical abstracts service (CAS) registry number, molecular formula and weight of tentatively identified compounds (≥70% mass spectral library match) detected in the surface water from Rietvlei Nature Reserve during screening.

Name	CAS	Molecular Formula	Molecular Weight (*m/z*)
Pharmaceuticals- drugs			
Hydantoin	461-72-3	$C_3H_4N_2O_2$	100
Resorcinol*	108-46-3	$C_6H_6O_2$	110
Mequinol	150-76-5	$C_7H_8O_2$	124
Cathinone	71031-15-7	$C_9H_{11}NO$	149
Levomenthol	2216-51-5	$C_{10}H_{20}O$	156
Apocynin	498-02-2	$C_9H_{10}O_3$	166
Phenylephrine	59-42-7	$C_9H_{13}NO_2$	167
Levetiracetam	102767-28-2	$C_8H_{14}N_2O_2$	170
Etilefrine	709-55-7	$C_{10}H_{15}NO_2$	181
Caffeine**	58-08-2	$C_8H_{10}N_4O_2$	194
Methamphetamine, propionyl	0-00-0	$C_{13}H_{19}NO$	205
Diethylpropion	90-84-6	$C_{13}H_{19}NO$	205
Benzyl Benzoate	120-51-4	$C_{14}H_{12}O_2$	212
Cyclizine	82-92-8	$C_{18}H_{22}N_2$	266
Etidocaine	36637-18-0	$C_{17}H_{28}N_2O$	276
Efavirenz	154598-52-4	$C_{14}H_9ClF_3NO_2$	315
Doconexent	6217-54-5	$C_{22}H_{32}O_2$	328
Sulpiride	15676-16-1	$C_{15}H_{23}N_3O_4S$	341
Phenadoxone	467-84-5	$C_{23}H_{29}NO_2$	351
Clindamycin	18323-44-9	$C_{18}H_{33}ClN_2O_5S$	424

Name	CAS	Molecular Formula	Molecular Weight (*m/z*)
WIN 54461	166599-63-9	$C_{23}H_{25}BrN_2O_3$	456
Sunscreen ingredients			
Hydroquinone	123-31-9	$C_6H_6O_2$	110
Benzophenone	119-61-9	$C_{13}H_{10}O$	182
Drometrizole	2440-22-4	$C_{13}H_{11}N_3O$	225
Oxybenzone	131-57-7	$C_{14}H_{12}O_3$	228
Homosalate	118-56-9	$C_{16}H_{22}O_3$	262
Padimate O	21245-02-3	$C_{17}H_{27}NO_2$	277
Octinoxate	5466-77-3	$C_{18}H_{26}O_3$	290
Avobenzone	70356-09-1	$C_{20}H_{22}O_3$	310
Octocrylene	6197-30-4	$C_{24}H_{27}NO_2$	361
Pesticides			
***p*-Chloroaniline**	106-47-8	C_6H_6ClN	127
1-Naphthalenol	90-15-3	$C_{10}H_8O$	144
Phenol, 4-(1,1-dimethylpropyl)- (Amylphenol)	80-46-6	$C_{11}H_{16}O$	164
Dibenzofuran	132-64-9	$C_{12}H_8O$	168
Precocene I	17598-02-6	$C_{12}H_{14}O_2$	190
Diethyltoluamide	134-62-3	$C_{12}H_{17}NO$	191
Xanthone	90-47-1	$C_{13}H_8O_2$	196
Pyrimethanil	53112-28-0	$C_{12}H_{13}N_3$	199
Thiabendazole	148-79-8	$C_{10}H_7N_3S$	201
Chloroneb	2675-77-6	$C_8H_8Cl_2O_2$	206
Atrazine	1912-24-9	$C_8H_{14}ClN_5$	215
Terbuthylazine	5915-41-3	$C_9H_{16}ClN_5$	229
Terbutryn	886-50-0	$C_{10}H_{19}N_5S$	241
Bayer 28,589	728-40-5	$C_{14}H_{21}NO_3$	251
Metolachlor	51218-45-2	$C_{15}H_{22}ClNO_2$	283
Cyclohexane, 1,2,3,4,5,6-hexachloro- (Hexachlor)	608-73-1	$C_6H_6Cl_6$	288
Diazinone	333-41-5	$C_{12}H_{21}N_2O_3PS$	304
Triazophos	24017-47-8	$C_{12}H_{16}N_3O_3PS$	313
Flusilazole	85509-19-9	$C_{16}H_{15}F_2N_3Si$	315
Dimethyl tetrachloroterephthalate (DCPA)	1861-32-1	$C_{10}H_6Cl_4O_4$	330
Piperonyl butoxide	51-03-6	$C_{19}H_{30}O_5$	338
Chlorpyrifos	2921-88-2	$C_9H_{11}Cl_3NO_3PS$	349

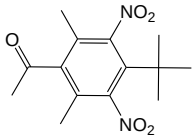
Name	CAS	Molecular Formula	Molecular Weight (*m/z*)
Octicizer	1241-94-7	$C_{20}H_{27}O_4P$	362
Bifenthrin	82657-04-3	$C_{23}H_{22}ClF_3O_2$	422
Hormones/ steroids			
Equilin (match quality > 60%)	474-86-2	$C_{18}H_{20}O_2$	268
Hexestrol (match quality > 60%)	5635-50-7	$C_{18}H_{22}O_2$	270
Estrone (match quality > 60%)	53-16-7	$C_{18}H_{22}O_2$	270
Pregnane	481-26-5	$C_{21}H_{36}$	288
Benzestrol	85-95-0	$C_{20}H_{26}O_2$	298
Norethindrone	68-22-4	$C_{20}H_{26}O_2$	298
Epinephrine, (ß)-, 3TMS derivative	10538-85-9	$C_{18}H_{37}NO_3Si_3$	399
Norepinephrine, (R)-, 4TMS derivative	68595-65-3	$C_{20}H_{43}NO_3Si_4$	457
Fragrances/ musk			
Cashmeran	33704-61-9	$C_{14}H_{22}O$	206
Celestolide	13171-00-1	$C_{17}H_{24}O$	244
Galaxolide	1222-05-5	$C_{18}H_{26}O$	258
Tonalid	21145-77-7	$C_{18}H_{26}O$	258
Musk ketone	81-14-1	$C_{14}H_{18}N_2O_5$	294

*Compounds given in red are classified EDCs (EDCs classified using the EDC databank created by Dr Montes-Grajales and Prof Olivero-Verbel, University of Cartagena [1] and TEDX, The Endocrine Disruption Exchange) [2].
**Caffeine is also found in food products, e.g. tea and coffee.

5.2. Targeted Analysis

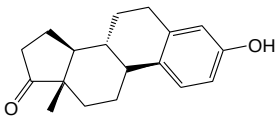
The results from the screening were used to select and purchase 16 analytical reference standards for targeted analysis. The name, structure, molecular weight, exact mass, log K_{ow} and class of each analyte are given in Figure 5.2. The analytes selected represent a broad range of heterogeneous chemical compounds. The goal of selecting these compounds was to simulate the variation in chemical characteristics and classes one would expect when detecting EDCs and emerging contaminants in real world samples. This is essential when developing a multi-residue method. Refer to Addendum D for the chemical characteristics and intended uses of each target analyte.

Fragrances

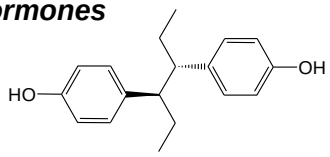


Musk ketone
$C_{14}H_{18}N_2O_5$
Exact Mass: 294,1216
Mol. Wt.: 294,3031
Log K_{ow}: 3.98

Hormones

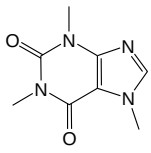


Estrone
$C_{18}H_{22}O_2$
Exact Mass: 270,162
Mol. Wt.: 270,3661
Log K_{ow}: 4.31

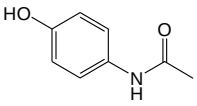


Hexestrol
$C_{18}H_{22}O_2$
Exact Mass: 270,162
Mol. Wt.: 270,3661
Log K_{ow}: 5.37

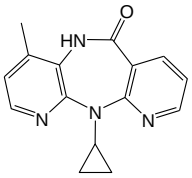
Pharmaceuticals



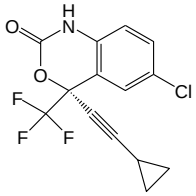
Caffeine
$C_8H_{10}N_4O_2$
Exact Mass: 194,0804
Mol. Wt.: 194,1906
Log K_{ow}: -0.55



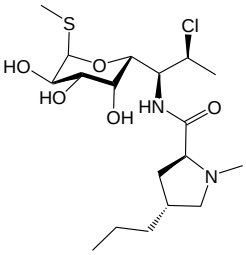
Acetaminophen
$C_8H_9NO_2$
Exact Mass: 151,0633
Mol. Wt.: 151,1626
Log K_{ow}: 0.91



Nevirapine
$C_{15}H_{14}N_4O$
Exact Mass: 266,1168
Mol. Wt.: 266,2979
Log K_{ow}: 2.49

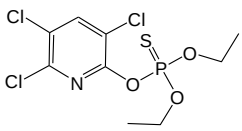


Efavirenz
$C_{14}H_9ClF_3NO_2$
Exact Mass: 315,0274
Mol. Wt.: 315,675
Log K_{ow}: 4.46

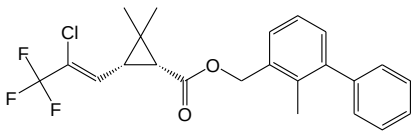


Clindamycin
$C_{18}H_{33}ClN_2O_5S$
Exact Mass: 424,1799
Mol. Wt.: 424,983
Log K_{ow}: 1.04

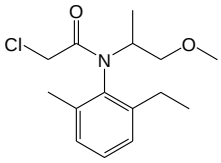
Pesticides



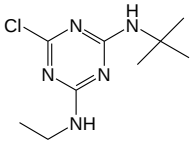
Chlorpyrifos
$C_9H_{11}Cl_3NO_3PS$
Exact Mass: 348,9263
Mol. Wt.: 350,5863
Log K_{ow}: 4.78



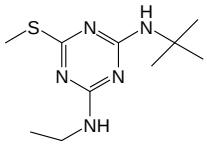
Bifenthrin
$C_{23}H_{22}ClF_3O_2$
Exact Mass: 422,126
Mol. Wt.: 422,8678
Log K_{ow}: 6.59



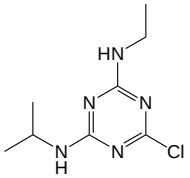
Metolachlor
$C_{15}H_{22}ClNO_2$
Exact Mass: 283,1339
Mol. Wt.: 283,7937
Log K_{ow}: 3.45



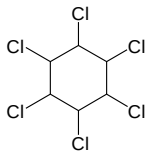
Terbuthylazine
$C_9H_{16}ClN_5$
Exact Mass: 229,1094
Mol. Wt.: 229,7098
Log K_{ow}: 2.48



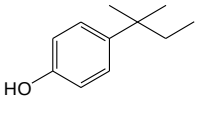
Terbutryn
$C_{10}H_{19}N_5S$
Exact Mass: 241,1361
Mol. Wt.: 241,3564
Log K_{ow}: 2.88



Atrazine
$C_8H_{14}ClN_5$
Exact Mass: 215,0938
Mol. Wt.: 215,6833
Log K_{ow}: 2.20



Lindane
$C_6H_6Cl_6$
Exact Mass: 287,8601
Mol. Wt.: 290,8298
Log K_{ow}: 4.35



4-tert-Amylphenol
$C_{11}H_{16}O$
Exact Mass: 164,1201
Mol. Wt.: 164,2441
Log K_{ow}: 3.66

Figure 5.2: Structures of target analytes and corresponding molecular formulas, exact masses (Da), molecular weights (Mol. Wt.) (Da) and log K_{ow} values.

5.2.1. Method development

5.2.1.1. GC×GC separation of target analytes

A general column combination set of 30 m x 0.25 mm ID x 0.25 µm film thickness Rxi®-1ms (^{1}D) and Rxi®-17Sil MS 1 m x 0.25 mm ID x 0.25 µm film thickness (^{2}D) was used for the initial screening of the water samples. The same column set was also used for analysis of a mixed standard of the target compounds (1 ng/µL in *n*-hexane; Chapter 4 Section 4.3.3). However, unsatisfactory separation of chlorpyrifos and metolachlor was obtained on this particular column set (Figure 5.3). The contour plots show ^{1}D separation on the *X*-axis and ^{2}D separation on the *Y*-axis.

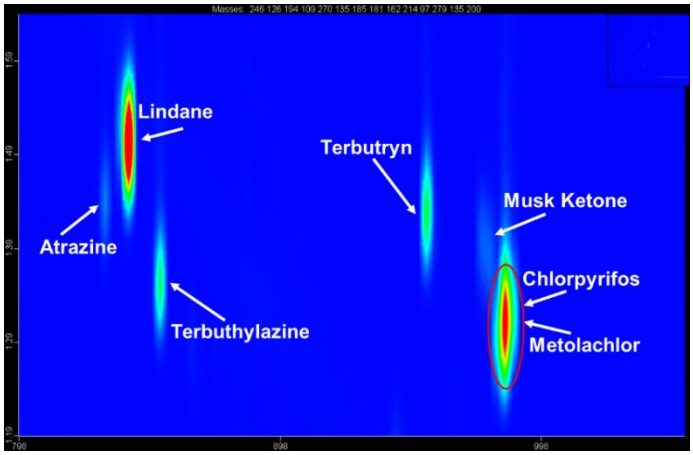


Figure 5.3: Enlarged contour plot of a reconstructed ion chromatogram (246, 126, 194, 109, 270, 135, 181, 185, 162, 214, 97, 279 and 200 *m/z*) showing coelution of chlorpyrifos and metolachlor (red ellipsoid). Working standard mixture (1 ng/µL) in *n*-hexane. Column combination: 30 m x 0.25 mm ID x 0.25 µm film thickness Rxi®-1ms (^{1}D) and Rxi®-17Sil MS 1 m x 0.25 mm ID x 0.25 µm film thickness (^{2}D).

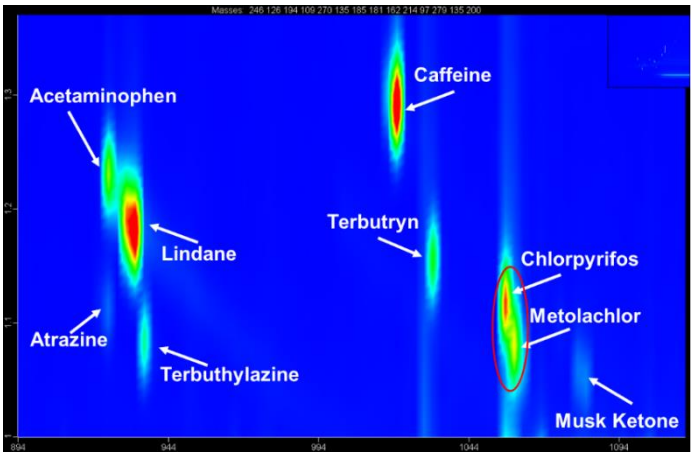


Figure 5.4: Enlarged contour plot of a reconstructed ion chromatogram (246, 126, 194, 109, 270, 135, 181, 185, 162, 214, 97, 279 and 200 *m/z*) showing separation of previously coeluting chlorpyrifos and metolachlor in a standard mixture (red ellipsoid). Working standard mixture (1 ng/µL) in *n*-hexane. Column combination: Rtx®-CLPesticides II 30 m x 0.25 mm ID x 0.2 µm film thickness (^{1}D) and Rxi®-17Sil MS 1 m x 0.25 mm ID x 0.25 µm film thickness (^{2}D).

It was then decided to use a Rtx®-CLPesticides II 30 m x 0.25 mm ID x 0.2 µm film thickness (^{1}D) column instead of the Rxi®-1ms column. Improved separation was achieved for chlorpyrifos and metolachlor (Figure 5.4).

All further GC×GC separations were performed using the Rtx®-CLPesticides II 30 m x 0.25 mm ID x 0.2 µm film thickness (^{1}D) and Rxi®-17Sil MS 1 m x 0.25 mm ID x 0.25 µm film thickness (^{2}D) column combination. The Rtx®-CLPesticides II column proved more effective in separating the chlorinated pesticides compared to the Rxi®-1ms column. This agrees with the findings of Kiridena et al. (2006) that the stationary phase of the Rtx-CLPesticides* column is a poly(dimethylmethyltrifluoatropropylsiloxane) type stationary phase based on the relative magnitude of the system constants [3]. The system constants fully describe all stationary-phase interactions and provide a basis for all stationary-phase classifications. Increased selectivity is achieved with the use of less than 20% methyltrifluoropropylsiloxane monomers [3]. The exact column structure is, however, undisclosed. (*assuming that the Rtx-CLPesticides II column is structurally similar to the Rtx-CLPesticides column)

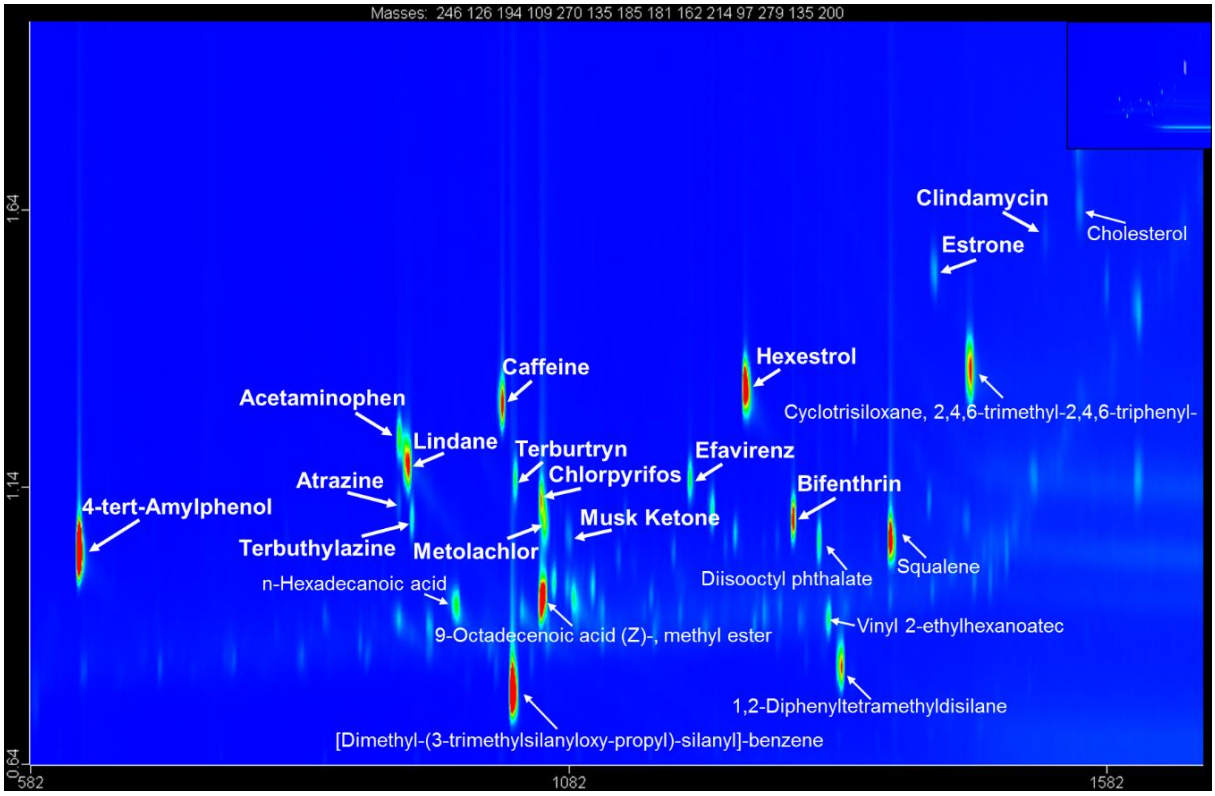


Figure 5.5: Contour plot of a reconstructed ion chromatogram (246, 126, 194, 109, 270, 135, 181, 185, 162, 214, 97, 279 and 200 *m/z*) of a mixed standard solution (1 ppm solution in *n*-hexane with 1 µl injection onto the GC). Column combination: Rtx®-CLPesticides II 30 m x 0.25 mm ID x 0.2 µm film thickness (^{1}D) and Rxi®-17Sil MS 1 m x 0.25 mm ID x 0.25 µm film thickness (^{2}D).

Figure 5.5 shows the contour plot of the separation of the target analytes. The ^{1}D and ^{2}D retentions times of the target analytes are given in Table 5-2.

Table 5-2: ^{1}D and ^{2}D retention times (seconds) of a 1 ng/µL mixed target analyte working solution in *n*-hexane (1 µL injection into the GC).

Target analytes	^{1}D Retention Time (s)	^{2}D Retention Time (s)
4-tert-Amylphenol	627	1.040
Atrazine	924	1.130
Acetaminophen	924	1.240
Lindane	933	1.190
Terbuthylazine	936	1.090
Caffeine	1017	1.310
Terbutryn	1032	1.160
Chlorpyrifos	1056	1.120
Metolachlor	1059	1.090
Musk ketone	1080	1.060
Efavirenz	1194	1.170
Hexestrol	1245	1.330
Bifenthrin	1290	1.100
Estrone	1419	1.550
Clindamycin	1521	1.610

Nevirapine is not included as the standard was received after the 1 ng/µL mixed target analyte working solution was prepared and consequently, it was decided to prepare separate 1 ng/µL and 5 ng/µL working solutions in *n*-hexane for nevirapine (Chapter 4 Section 4.3.3). Nevirapine was not detected by GC×GC-TOFMS analysis at 1 ng/µL (data processing method with a signal-to-noise ratio (S/N) set at 100). It was, however, detected in the 5 ng/µL solution (Figure 5.6). Intermolecular hydrogen bonding between amide (-NH) functional groups on nevirapine can reduce the volatility of the compound and derivatisation may be required for GC analysis at trace levels (Chapter 2 Section 2.1.4).

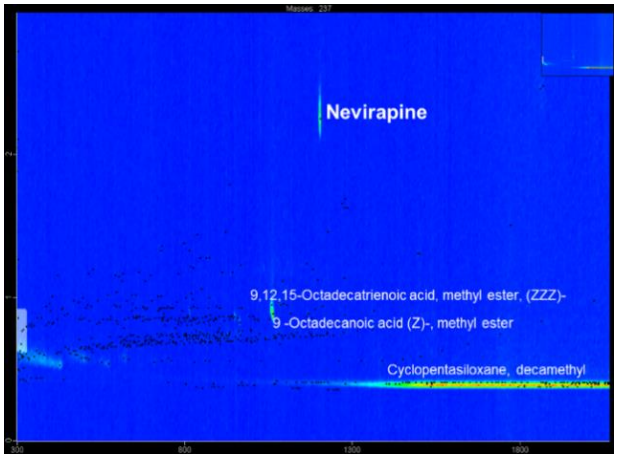


Figure 5.6: Contour plot of a reconstructed ion chromatogram (237 *m/z*) of a 5 ng/µL nevirapine working standard in *n*-hexane (100 S/N). Column combination: Rtx®-CLPesticides II 30 m x 0.25 mm ID x 0.2 µm film thickness (^{1}D) and Rxi®-17Sil MS 1 m x 0.25 mm ID x 0.25 µm film thickness (^{2}D).

5.2.1.2. UHPLC separation of target analytes

5 µL of a 1 ng/µL mixed working solution in ACN:H_2O (1:1, v/v) (Chapter 4 Section 4.3.3) was injected to determine separation using UHPLC (Chapter 4 Section 4.5.3). Adequate separation of target analytes (atrazine, nevirapine, terbuthylazine, terbutryn, clindamycin, caffeine, acetaminophen and metolachlor) was achieved at 0.3 mL/min flow rate operated in APCI positive mode (Figure 5.7). Co-elution of atrazine and terbutryn proved problematic for most flow rates (0.2-0.6 mL/min). Figure 5.7 shows the elution of terbutryn (6.58 minutes) and atrazine (6.54 minutes) at a flow rate of 0.3 mL/min. Their structural similarity (Figure 5.2) might account for the non-baseline separation.

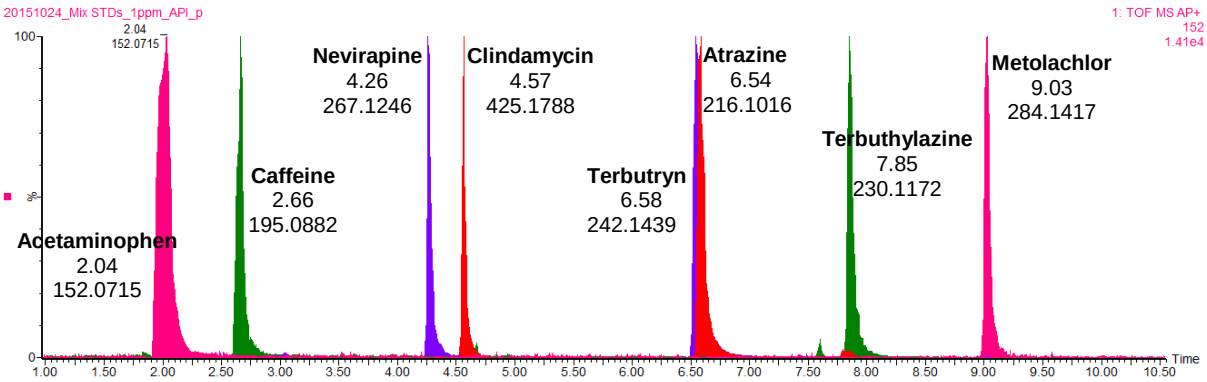


Figure 5.7: Overlaid reconstructed ion chromatograms (RICs) showing UHPLC separation of eight target analytes; 5 µl sample injection; 0.3 mL/min flow rate; 1 ng/µL analyte concentration (APCI+ mode). RICs of 216 (atrazine), 267 (nevirapine), 230 (terbuthylazine), 242 (terbutryn), 425 (clindamycin), 195 (caffeine), 152 (acetaminophen) and 274 (metolachlor) *m/z*. APCI positive mode (TOF-MS). Peak labels show retention time (minutes); $(M+H)^+$ mass (Da).

ESI operated in negative mode showed the best response for efavirenz (Figure 5.8). All further analysis was conducted using APCI positive MS^E mode for atrazine, nevirapine, terbuthylazine, terbutryn, clindamycin, caffeine, acetaminophen and metolachlor and ESI negative MS^E mode for efavirenz. Analysis time was reduced by only doing two runs per sample (APCI positive mode and ESI negative mode using MS^E) as opposed to four runs per sample. ESCi™ multi-mode ionisation cannot be performed in conjunction with MS^E mode, due to instrumental limitations, and consequently four runs would be required for each sample, namely (1) ESI in positive mode with MS^E, (2) ESI in negative mode with MS^E, (3) APCI in positive mode with MS^E and lastly (4) APCI in negative mode with MS^E,

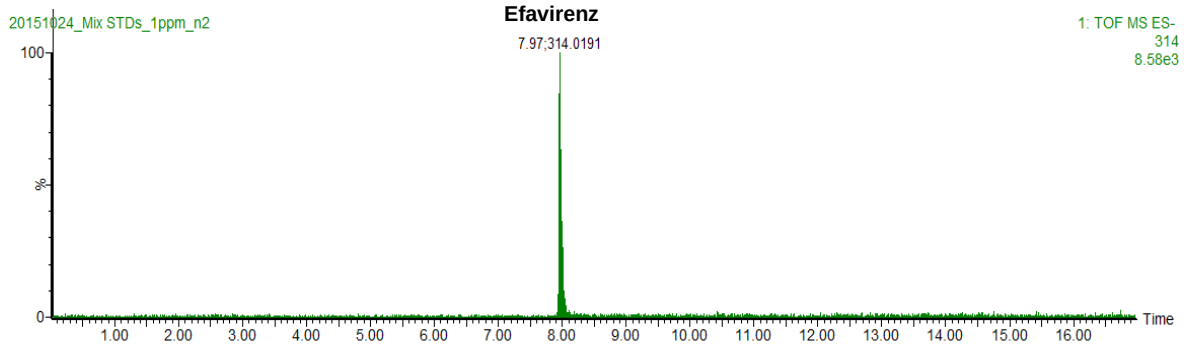


Figure 5.8: RIC of 134 *m/z* showing efavirenz (ESI negative mode (TOF-MS)). 5 µl sample injection; 0.3 mL/min flow rate; 1 ng/µL analyte concentration. Peak labels show retention time (minutes); $(M+H)^+$ mass (Da). (ESI^- mode).

The mass accuracy achieved by the instrument is given in Table 5-3. Accurate mass was used for identification of target analytes. Subsequent analyses used both accurate mass and retention time for quantification; and accurate mass, retention times and fragmentation patterns for unequivocal identification. Refer to Addenda E and F for the ESI- MS^E fragmentation patterns of the target compounds.

Table 5-3: Mass accuracy (mDa) of target analytes using UHPLC-TOFMS.

Target analyte	Monoisotopic mass (Da)*	$(M+H)^+_{lit}$ (Da)	$(M+H)^+_{exp}$ (Da)	Mass accuracy (mDa)
Atrazine	215.0938	216.1016	216.1017	-0.1
Nevirapine	266.1168	267.1246	267.1247	-0.1
Terbuthylazine	229.1094	230.1172	230.1175	-0.3
Terbutryn	241.1361	242.1439	242.1436	0.3
Clindamycin	424.1799	425.1788	425.1866	-7.8

Target analyte	Monoisotopic mass (Da)*	$(M+H)^+_{lit}$ (Da)	$(M+H)^+_{exp}$ (Da)	Mass accuracy (mDa)
Caffeine	194.0804	195.0882	195.0883	-0.1
Acetaminophen	151.0633	152.0712	152.0719	-0.7
Metolachlor	283.1339	284.1417	284.1416	0.1
	Monoisotopic mass (Da)	**$(M-H)^-_{lit}$ (Da)**	**$(M-H)^-_{exp}$ (Da)**	**Mass accuracy (mDa)**
Efavirenz	315.0274	314.0196	314.0180	1.6

*Monoisotopic masses, $(M+H)^+$ and $(M-H)^-$, were determined using MassLynx V4.1 Molecular Mass Calculator.

5.3. Matrix Matched Calibration Curves

In order to get reliable quantitative results enrichment and thorough clean-up of samples by means of various extraction techniques and the use of alternative calibration methods, such as the addition of expensive isotopically labelled internal standards or matrix matched calibration curves are needed [4, 5]. Due to cost and availability considerations with regard to isotopically labelled standards this study employed matrix matched calibration curves to compensate for quantitative errors caused by matrix effects, as well as errors due to extraction techniques, such as enrichment ability, amount of clean-up steps, evaporation, cleanliness of the inlet liner and condition of the column.

5.3.1. GC×GC-TOFMS

The calibration curves obtained for the (1) neat standard, (2) matrix matched PDMS loop TD directly in GC inlet liner, (3) matrix matched PDMS loop TD using the TDS and (4) matrix matched SBSE using TDS are overlaid for each analyte (refer to Section 4.6.1).

Figure 5.9 shows the calibration curves obtained for lindane (0 – 40 ng/L). The complete calibration curve sets for terbuthylazine, caffeine, terbutryn, chlorpyrifos, musk ketone, bifenthrin and atrazine can be found in Addendum G.

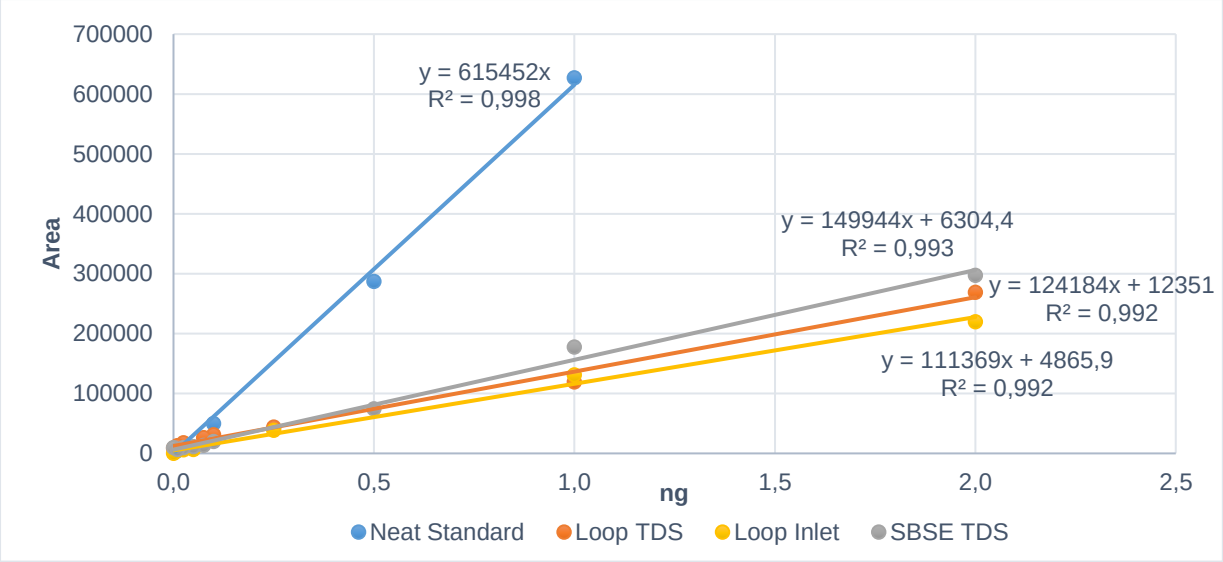


Figure 5.9: GC calibration curves for lindane. Neat solvent standard (external calibration) is the blue trace, PDMS loop using TDS is the orange trace (matrix matched calibration), PDMS loop TD in GC inlet liner (matrix matched calibration) is the yellow trace and SBSE using TDS (matrix matched calibration), is the grey trace. All traces are labelled with the linear regression line and R^2 value. Area plotted is for the quantification ion (181 *m/z*).

The difference in response between the neat standard injections (external calibration) and matrix matched calibration curves highlights the importance of taking the matrix and sample preparation technique into account when generating response curves. The difference is due to GC matrix effects (refer to Chapter 3 Section 3.2 on the difference between GC and LC matrix effects), loss of analytes during sample preparation, adsorption of analytes onto the glass walls of the container, inadequate sorption of analytes into the PDMS, inadequate desorption of analytes from the PDMS, etc. Calibration curves of interest will be highlighted in the succeeding sections. The quantification ion, regression lines and goodness of fit of the calibration data (R^2) are given in Table 5-4.

Table 5-4: Linear regression lines and R^2 values (linearity) for the GC matrix matched calibration curves.

Analyte	Quantification Ion (*m/z*)	Linearity			
		Range (ng/L)	Number of points	Regression Equation*	R^2
PDMS sampling loop TD directly in GC inlet liner					
4-tert-Amylphenol	135	0 - 40	6	y = 14483x + 7650,6	0,970
Atrazine	200	0 - 40	6	y = 22074x + 4667,5	0,970
Lindane	181	0 - 40	8	y = 111369x + 4865,9	0,992
Terbuthylazine	173	0 - 40	5	y = 35518x + 19749	0,956

Analyte	Quantification Ion (*m/z*)	Linearity			
		Range (ng/L)	Number of points	Regression Equation*	R^2
Caffeine	194	0 - 40	5	y = 46921x + 16978	0,970
Terbutryn	226	0 - 40	6	y = 64739x + 1906,5	0,998
Chlorpyrifos	97	0 - 40	6	y = 239299x - 8809,4	0,995
Metolachlor	162	0 - 40	6	y = 183665x + 71499	0,978
Musk ketone	279	0 - 40	8	y = 34561x + 798,07	0,998
Efavirenz	246	5	1	Detected**	n/a**
Bifenthrin	181	0 - 40	6	y = 92871x + 3151,1	0,990
PDMS sampling loop TD using the TDS					
4-tert-Amylphenol	135	0 - 20	5	y = 964938x + 52699	0,993
Atrazine	200	0 - 40	11	y = 12368x + 2953,1	0,969
Lindane	181	0 - 40	8	y = 124184x + 12351	0,992
Terbuthylazine	173	0 - 40	6	y = 25306x + 19080	0,987
Caffeine	194	0 - 20	5	y = 59473x + 12728	0,956
Terbutryn	226	0 - 40	7	y = 52171x + 4323,1	0,993
Chlorpyrifos	97	0 - 40	7	y = 369184x - 6267,9	0,983
Metolachlor	162	0 - 20	5	y = 166612x + 132149	0,991
Musk ketone	279	0 - 40	10	y = 47780x + 50,037	0,961
Efavirenz	246	5	1	Detected	n/a
Bifenthrin	181	0 - 40	6	y = 112296x + 6140,8	0,978
SBSE TD using the TDS					
4-tert-Amylphenol	135	-	-	Detected***	n/a
Atrazine	200	0 - 20	7	y = 24981x + 381,96	0,966
Lindane	181	0 - 40	8	y = 149944x + 6304,4	0,993
Terbuthylazine	173	0 - 20	5	y = 65341x + 4065	0,994
Caffeine	194	0 - 10	6	y = 116730x + 18417	0,988
Terbutryn	226	0 - 20	8	y = 99008x + 650,69	0,966
Chlorpyrifos	97	0 - 40	8	y = 262613x + 6597,3	0,997
Metolachlor	162	0 - 40	5	y = 188125x + 320099	0,946
Musk ketone	279	0 - 40	9	y = 44998x + 465,67	0,999
Efavirenz	246	10	1	Detected	n/a
Bifenthrin	181	0 - 40	6	y = 17994x + 587,09	0,983

*y = peak area of compound; x = concentration of the compound (ng/L)
**Detected, not quantifiable; n/a not applicable; included for semi-quantification purposes.
***Refer to Section 5.3.1.1 on concerns with 4-tert-amylphenol quantification.

Acetaminophen, efavirenz, nevirapine, clindamycin, hexestrol and estrone proved problematic due to poor GC sensitivity in the working range for both the external calibration range (0.005 – 1 ng/µL) and matrix matched calibration (0 – 40 ng/L) (Table 5-5). Intermolecular hydrogen bonding due to hydroxyl functional groups (in estrone, hexestrol, acetaminophen and clindamycin) and amide functional groups (in acetaminophen, nevirapine, efavirenz and clindamycin) can lead to a decrease in the ability to elute these compounds without interactions with the GC column wall, leading to extensive tailing and loss of detectability. Quantification of clindamycin and acetaminophen using PDMS sorptive techniques is further complicated by their low log K_{ow} values (0.91 and 1.04 for acetaminophen and clindamycin, respectively). Low log K_{ow} values will result in less of the analyte partitioning into the PDMS phase. Consequently, derivatisation or UHPLC analysis was investigated as alternative techniques (sections 5.3.2 and 5.5.2).

Table 5-5: Linearity of the GC neat standard (external calibration) calibration curves and matrix matched calibration curves for target analytes showing poor GC sensitivity.

Analyte	Quant. Ion (*m/z*)	Neat standard (external calibration)			PDMS loop TD in GC inlet liner			PDMS loop TDS			SBSE TDS		
		Range (ng/µL)	Number of points	R^2	Range (ng/L)	Number of points	R^2	Range (ng/L)	Number of points	R^2	Range (ng/L)	Number of points	R^2
Acetaminophen	109	0.5 - 1	2	n/a*	n.d*	n.d	n/a	n.d	n.d	n/a	n.d	n.d	n/a
Clindamycin	126	1	1	n/a	n.d	n.d	n/a	n.d	n.d	n/a	n.d	n.d	n/a
Efavirenz	246	0.1 - 1	3	0.985	0.1 - 2	3	0.935**	1.5 - 5	3	0.980	10 - 40	3	0.9892
Nevirapine	237	5	1	n/a	n.d	n.d	n/a	n.d	n.d	n/a	n.d	n.d	n/a
Estrone	270	0.1 - 1	3	0.9753	n.d	n.d	n/a	n.d	n.d	n/a	n.d	n.d	n/a
Hexestrol	135	0.01 - 1	4	0.980	0.2 - 40	3	0.0281	n.d	n.d	n/a	5 - 40	4	0.868

*n.d not detected; n/a not applicable.
**Efavirenz extracted with PDMS loop TD in GC inlet liner gave a calibration curve with negative slope: y = -38733x + 5379.53.

5.3.1.1. Sorptive extraction techniques

Methanol is usually added to water prior to extraction to prevent the adsorption of compounds on the glass walls of sample containers [6]. However, the decision not to use methanol during extraction was founded on experiments showing a decrease in interaction of compounds with the PDMS when methanol addition is high (>20%), as well as work done by Ochiai et al. (2006) showing no noticeable change in recovery of hydrophilic or hydrophobic solutes with methanol addition [7, 8]. Reducing exposure time to the glass wall of the extraction vessel by using smaller sample volumes (i.e. faster equilibration) can alternatively be used to minimise the adsorption effect of the

hydrophobic compounds [8, 9]. The presence of salt (NaCl) enhances the extraction of analytes with low log K_{ow} (log K_{ow} < 4) values; as these are generally more problematic than hydrophobic compounds when considering sorptive extraction using PDMS. Therefore, the addition of salt was deemed more suitable than methanol addition [7, 9]. However, the recovery of hydrophobic solutes (log K_{ow} > 5) decreases with high NaCl concentrations (> 5%). To increase the recovery of more hydrophobic solutes (for multi-residue methods) a sequential method (refer to Chapter 4 Section 4.4.1.3), which provides more uniform enrichment over the entire polarity range, was employed [9].

Comparable linearities were observed for the PDMS loop and SBSE using the sequential salting out method (Table 5-4). The PDMS loop was the preferred extraction method as it is considerably cheaper than the SBSE Twister™ (Table 5-6). Even though the loop is reusable (with proper conditioning in between extractions), its low cost makes it cheap enough to be disposed of after a single extraction and thus ensuring the elimination of analyte carry-over between sample extractions.

Table 5-6: Cost analysis comparing the unit price between the PDMS loop and SBSE sampler.

Sampler	Items bought	Price (ZAR)	Number of units	Price per unit (ZAR)
PDMS loop	0.64 mm OD x 0.3 mm ID silicone tubing (1 524 cm length per packet)	1 471.65 per packet	145 loops (each 10.5 cm)	10.15
Stir bar (SBSE)	GERSTEL Twister™ with PDMS, 0.5 mm film thickness, length 10 mm, package of 10	7 800.88 per package	10	780.09

4-tert-Amylphenol concerns

Quantification of 4-tert-amylphenol proved to be challenging due to background levels from the deionised water. All calibration points, including the blank, for SBSE, gave a similar response, i.e. poor sensitivity (Figure 5.10; red ellipsoid). The benefit of using a disposable PDMS loop for the prevention of analyte carry-over became evident during the quantification of 4-tert-amylphenol (Figure 5.10; green ellipsoid). The reconditioning of the Twister® stir bars in between sample extractions appears not to be adequate for the removal of 4-tert-amylphenol from the stir bars (SBSE blank had a peak area of 858591 vs. PDMS loop blank with a peak area of 4410.6). This could

indicate potential activity of the phenol group with the glass substrate of the Twister® stir bar during reconditioning at 280 °C (refer to Chapter 4 Section 4.4.1.2). There is a possible interaction between 4-tert-amylphenol and the exposed glass surface of the stir bar due to hydrogen bonding between 4-tert-amylphenol (the only acidic compound analysed) and the glass surface (SiOH). This can be tested in future by analysing a range of alcohol, diol, carboxylic acid and phenol compounds. (The PDMS loop using TDS, green trace in Figure 5.10, shows the preferred response. The trace, however, levels off after 1 ng; not shown in Figure 5.10).

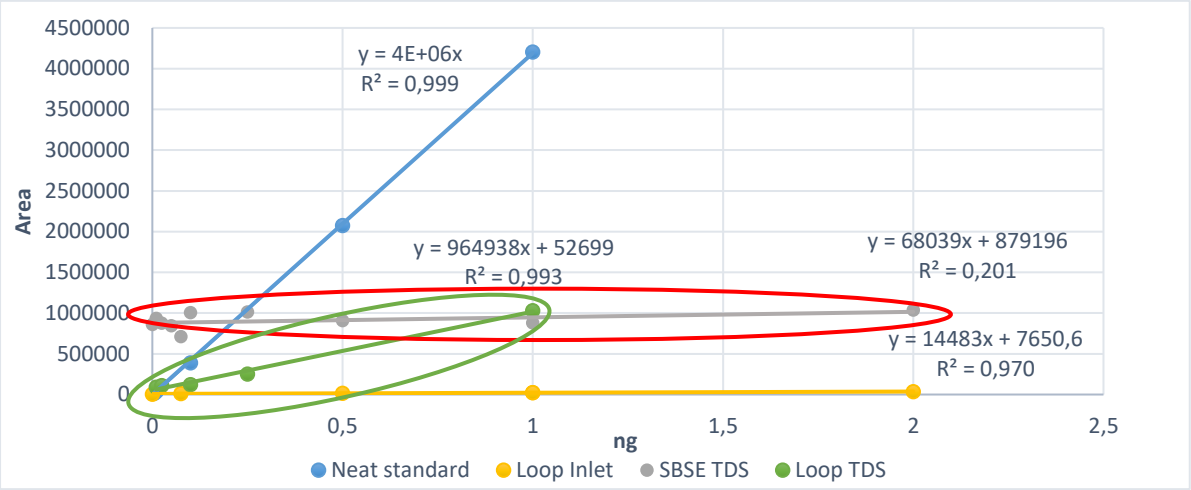


Figure 5.10: GC calibration curves for 4-tert-amylphenol. Neat solvent standard is the blue trace (external calibration), PDMS loop using TDS is the green trace (matrix matched calibration), PDMS loop TD in GC inlet liner is the yellow trace (matrix matched calibration) and SBSE using TDS is the grey trace (matrix matched calibration). All traces are labelled with the linear regression line and R^2 value. Red ellipsoid indicates similar response throughout the working range; green ellipsoid shows preferred response.

It should, however, be noted that the desorption method used for this analyte is also important as TD in the GC inlet liner exhibited poor sensitivity, compared to the PDMS loop desorbed using TDS, across the calibration range (Figure 5.11; yellow trace). The reduced response of 4-tert-amylphenol was likely due to the lower gas flow rate (20 mL/min) in the GC inlet during desorption and the shorter splitless injection time of 60 seconds compared to the TDS which has a high desorption flow rate of 100 mL/min and a longer splitless injection time of 90 seconds. To improve the inlet sensitivity the splitless TD time can be increased and higher gas flow rates in the inlet can be obtained in the pulsed splitless mode (future work). Irrespective of the method used (loops or commercial SBSE) quantification of 4-tert-amylphenol proved challenging, possibly due to an interaction of the phenol group and exposed glass surfaces.

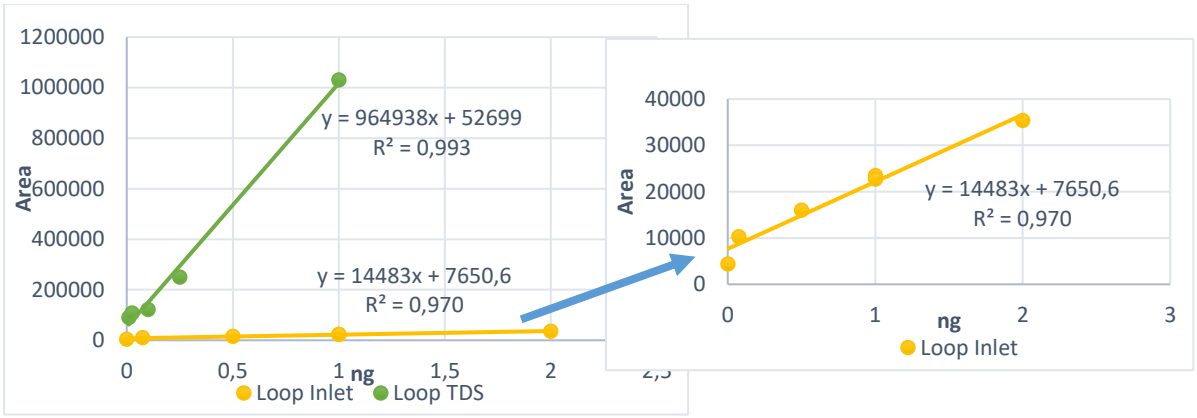


Figure 5.11: GC calibration curves for 4-tert-amylphenol. PDMS loop using TDS is the green trace (matrix matched calibration) and PDMS loop TD in GC inlet liner is the yellow trace (matrix matched calibration). The image on the right shows the positive response of the yellow trace. All traces are labelled with the linear regression line and R^2 value.

Metolachlor concerns

The calibration curves set found for metolachlor showed an increase in the value of the intercept on the *y*-axis of the SBSE calibration curve compared to the PDMS loop calibration curve (Figure 5.12; red arrow). This is likely due to analyte carry-over from the stir bar from analysis to analysis. Reconditioning of the stir bars between extractions proved to be inadequate for the removal of metolachlor from the PDMS, once again showing the advantage of a disposable sampler. Potential analyte carry-over may be evaluated in future by using a new stir bar or by modifying the reconditioning procedure.

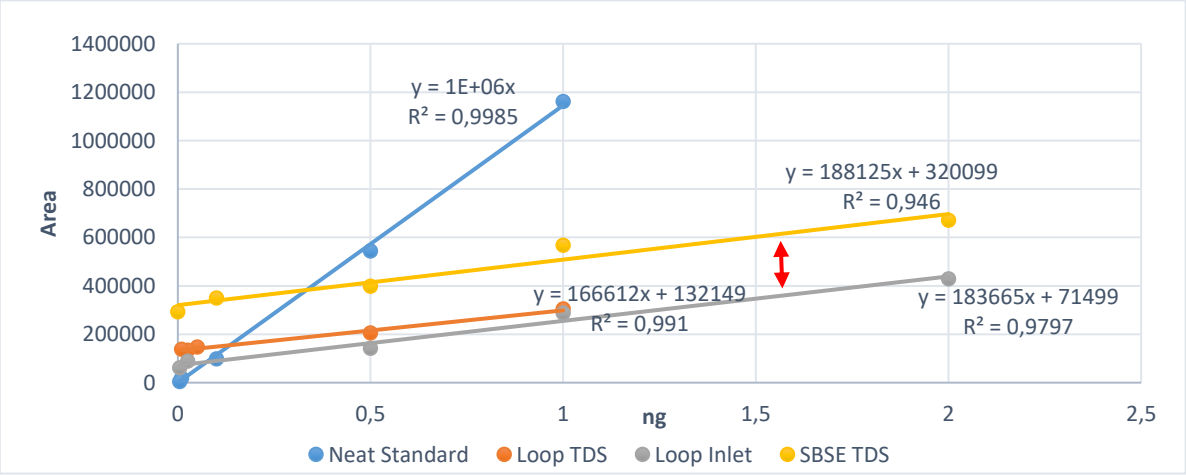


Figure 5.12: GC calibration curves for metolachlor. Neat solvent standard is the blue trace, PDMS loop using TDS is the orange trace (matrix matched calibration), PDMS loop TD in GC inlet liner is the grey trace (matrix matched calibration) and SBSE using TDS is the yellow trace (matrix matched calibration). All traces are labelled with the linear regression line and R^2 value. Red arrow indicates an increase in the intercept on the y-axis for SBSE likely due to carry-over between analysis.

5.3.1.2. Thermal desorption methods

Comparable linearities were observed for the GC inlet TD and the Gerstel™ TDS (Table 5-4) methods. The GC inlet TD was the preferred method as cryo-focussing is not required thereby eliminating the need for consumable liquid nitrogen. Also, there is no additional sample introduction time as is the case when using the Gerstel™ TDS resulting in reduced sample run times (no lengthy desorption step). A further cost saving related to desorbing directly in the inlet of the GC is that less carrier gas is consumed as desorption in the Gerstel™ TDS requires a high desorption flow rate of 100 mL/min carrier gas. Enhanced sensitivity is achieved by thermal desorption compared to liquid injection of a microliter/s fraction of a solvent extract. However, a major drawback of TD is the destructive nature of the process, consequently the reanalysis of samples is not possible.

A notable difference in sensitivity between the two TD methods was highlighted in the previous section (Section 5.3.1.1) for 4-tert-amylphenol. Qualification of 4-tert-amylphenol and metolachlor for screening purposes is still, however, possible using all three extraction techniques.

5.3.2. UHPLC-QTOFMS

Matrix matched calibration curves for SPE (Chapter 4 Section 4.4.3.1) and LVI (Chapter 4 Section 4.4.3.2) are overlaid for each standard and can be found in Addendum H. Figure 5.13 shows an example of the calibration curve set for atrazine. SPE worked best for atrazine, terbuthylazine, terbutryn (triazine pesticides), metolachlor and efavirenz (chlorinated aromatics); i.e. aromatics that are retained on the SPE cartridge. LVI was well suited for acetaminophen, caffeine, atrazine, terbuthylazine, terbutryn, efavirenz, metolachlor and nevirapine, as these compounds were easily detected utilising $APCI^+$ with TOFMS. LVI eliminates the need for extensive sample preparation steps leading to a simplified and cost effective sample preparation procedure.

The difference in response between the two calibration curves is due to pre-concentration during the SPE process. An enrichment factor of 5 000 was obtained for SPE. A lower response is obtained for LVI which is problematic for compounds not easily ionised during ESI or APCI. Charge competition (due to no sample clean-up for

LVI) was taken into consideration by using matrix matched calibration curves for quantification.

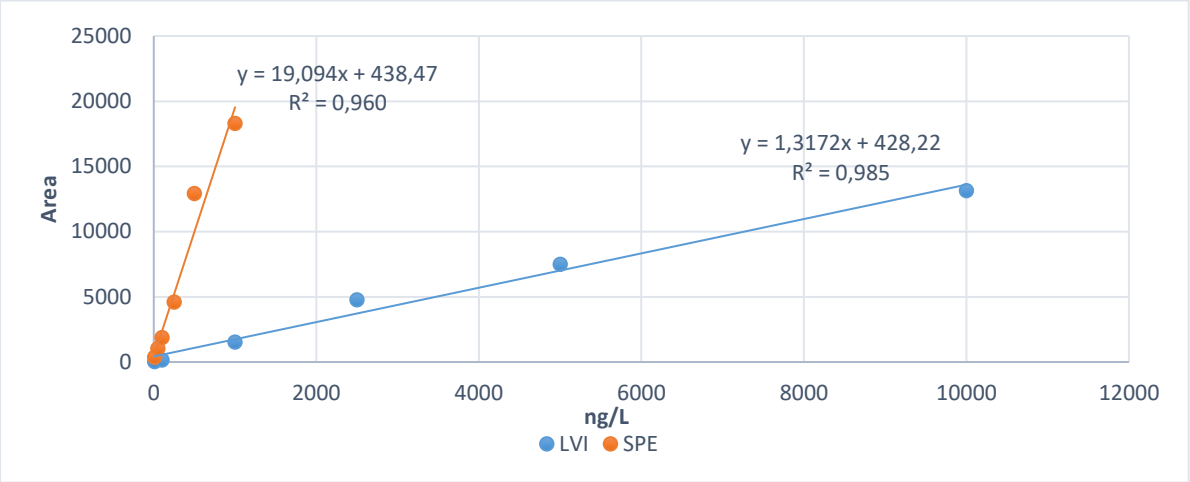


Figure 5.13: LC calibration curves for Atrazine. SPE is the orange trace and LVI is the blue trace. All traces are labelled with the linear regression line and R^2 value.

Clindamycin proved problematic due to low LC sensitivity in the working calibration range. Neat standards (1 ng/µl), SPE extracts (0 – 1 000 ng/L) and LVIs (0 – 10 000 ng/L) of musk ketone, chlorpyrifos, bifenthrin, 4-tert-amylphenol, hexestrol and estrone were not detected using UHPLC-MS (when using the UHPLC-MS method described in Chapter 4 Section 4.53.). The quantification ion, regression lines and goodness of fit of the data (R^2) are given in Table 5-7.

Table 5-7: Regression lines and R^2 values for the LC matrix matched calibration curves.

Analyte	Quantification Ion (*m/z*)	Linearity			
		Range (ng/L)	Number of points	Regression Equation*	R^2
Large volume injection (LVI)					
Acetaminophen	152,071	0 – 10 000	5	y = 0,0848x + 46,156	0,983
Caffeine	195,088	0 – 10 000	5	y = 0,1135x + 26,499	0,989
Atrazine	216,101	0 – 10 000	6	y = 1,3172x + 428,22	0,985
Terbuthylazine	230,117	0 – 5 000	5	y = 1,0353x + 74,004	0,985
Terbutryn	242,144	0 – 10 000	6	y = 1,3946x + 3,1756	0,983
Nevirapine	267,124	0 – 10 000	5	y = 0,1652x + 57,838	0,990
Metolachlor	284,140	0 – 10 000	5	y = 0,0119x + 0,6049	0,982

Analyte	Quantification Ion (*m/z*)	Linearity			
		Range (ng/L)	Number of points	Regression Equation*	R^2
Efavirenz	314,020	0 – 10 000	5	y = 0,0115x + 0,8868	0,994
Solid phase extraction (SPE)					
Acetaminophen	152,071	n/a	n/a	n/a	n/a
Caffeine	195,088	n/a	n/a	n/a	n/a
Atrazine	216,101	0 – 1 000	6	y = 19,094x + 438,47	0,960
Terbuthylazine	230,117	0 – 1 000	5	y = 17,72x + 113,1	0,999
Terbutryn	242,144	0 – 1 000	6	y = 49,397x + 411,68	0,994
Nevirapine	267,124	n/a	n/a	n/a	n/a
Metolachlor	284,140	0 – 1 000	6	y = 1,3507x + 103,62	0,996
Efavirenz	314,020	0 – 1 000	5	y = 1,8455x - 12,4	0,995

**y* = peak area of compound; *x* = concentration of the compound (ng/L)
**n/a not applicable (method not suitable).

Quantification of metolachlor and efavirenz using UHPLC-APCI-QTOF requires pre-concentration and sample clean-up in order to remove matrix interferences. Fewer matrix effects should lead to enhanced signal response. The general purpose C_{18} SPE cartridge used was not suited for the extraction of acetaminophen, caffeine or nevirapine. In future, specialised SPE cartridges (for example the Oasis®HLB cartridges from Waters®) which are more suitable for the extraction of the more polar analytes may be evaluated or the sample loading volume can be lowered in order to reduce analyte breakthrough.

5.3.2.1. Matrix effects

A midpoint (1 000 ng/L) in the calibration range was chosen to calculate the matrix effects (ME) for LVI (Chapter 4 Section 4.6.2.1). The ratio of the area of the spiked standard in the matrix to the area of the 0.001 ng/µL neat standard solution was used to determine the % ME (Table 5-8).

The process efficiency (PE) for SPE was also calculated at a midpoint (100 ng/L) in the calibration range (Chapter 4 Section 4.6.2.1). The ratio of the area of the spiked standard (pre-SPE) to the area of the 0.05 ng/µL neat standard solution (Chapter 4 Section 4.6.2.1) was used to determine the % PE (Table 5-8).

Table 5-8: % Matrix effect (% ME) for LVI of selected analytes at 1 000 ng/L and % process efficiency (% PE) for SPE of selected standards at 100 ng/L.

Analyte	LVI ($APCI^+$ interface)			
	A Area neat standard (1 000 ng/L)	**B Area spiked matrix (1 000 ng/L)**	**B/A x 100 % Matrix effect (% ME)**	**Matrix effect**
Acetaminophen	41,934	68,169	162,6	Ion enhancement
Caffeine	38,248	32,912	86,0	Ion suppression
Atrazine	3572,271	590,874	16,5	Ion suppression
Terbuthylazine	1110,33	327,277	29,5	Ion suppression
Terbutryn	445,079	798,453	179,4	Ion enhancement
Nevirapine	133,883	69,808	52,1	Ion suppression
Metolachlor	76,651	17,040	22,2	Ion suppression
Efavirenz (ESI^-)	274,33	7,270	2,7	Ion suppression
	SPE ($APCI^+$ interface)			
	A Area neat standard (100 ng/L)	**C Area pre-SPE spiked standard (100 ng/L)**	**C/A x 100% Process efficiency (% PE)**	**Process efficiency****
Atrazine	4385,844	1868,364	42,6	< 50% Ion suppression could be a factor
Terbuthylazine	2898,478	1621,737	56,0	> 50%
Terbutryn	10244,935	4872,577	47,6	< 50% Ion suppression could be a factor
Metolachlor	91,827	14,298	15,6	< 50% Ion suppression could be a factor
Efavirenz (ESI^-)	502,025	157,301	31,3	< 50% Ion suppression could be a factor

*Efavirenz was analysed with MS operated in ESI negative mode.
**Process efficiency: %Recovery and matrix effects; matrix effects are reduced due to sample clean up during SPE.

Significant ion enhancement is observed for acetaminophen and terbutryn with LVI. Matuszewski et al. (2003) previously reported significant ion enhancement with the APCI interface (≈130%) [10]. The severe ion suppression observed for efavirenz could be attributed to the ESI interface. APCI interfaces are generally less susceptible to ion suppression than ESI as a result of the different ionisation mechanism involved. During APCI charge transfer occurs from the solvents to the gaseous analytes [5]. The ion

suppression observed for atrazine and the ion enhancement for terbutryn could also be as a result of the non-baseline separation of the two compounds.

Poor process efficiency for SPE could be attributed to poor adsorption of the analytes on the SPE cartridge or/and insufficient elution of the analytes from the cartridge. The equation does not distinguish between %recovery and matrix effects when evaluating process efficiency; i.e. %PE is equal to %recovery and matrix effects. Matrix effects are, however, reduced due to sample clean up during the SPE process.

Matrix effects require special consideration during LVI due to the complexity of the matrix. There is little sample clean-up which could result in charge competition within the matrix leading to a decrease in instrument response. The importance of taking the matrix into consideration during quantification is underpinned by the % matrix effect results (Table 5-8). Both ion suppression and ion enhancement have an effect on the target analytes in this multi-residue LVI method. Matrix matched calibration compensated for these two phenomena.

5.4. Method Validation

The performance of each method was evaluated by plotting multi-level calibration curves using at least five concentration levels of the target analytes. LODs, LOQs, % RSD (method precision) and % recovery (method accuracy) were determined using calibration curves. Good linearity was demonstrated. The goodness of fit (R^2) for all cases was above 0.946 and 0.960 for GC and LC analysis, respectively. However, for most cases, the R^2 was > 0.98 for GC and LC analysis.

5.4.1. Limit of detection and limit of quantification

5.4.1.1. GC×GC-TOFMS

Measurements at ultra-trace levels (ppt to ppq) of the target analytes are realised for the in-house and commercial methods using GC analysis. The LODs and LOQs are significantly lower than the levels required by the EU directive, WHO guidelines and US EPA regulations for drinking water quality (Table 5-9 provides some of the guidelines) [11-13].

Table 5-9: EU directive, WHO guidelines and US EPA regulations for drinking water quality. Values given are the maximum contaminant level goal (the level of a contaminant below which there is no known or expected risk to health) [11-13].

EU* guidelines:	
Pesticides	100 ng/L
Total pesticides	200 ng/L
WHO guidelines:**	
Atrazine:	100 µg/L
Chlorpyrifos	30 µg/L
Lindane	2 µg/L
Metolachlor	10 µg/L
Terbuthylazine	7 µg/L
US EPA regulations***	
Atrazine	0.003 mg/L
Lindane	0.0002 mg/L

*EU: European Union
**WHO: World Health Organisation
***US EPA: United States Environmental Protection Agency

The LODs and LOQs for the different methods used, (1) PDMS loop TD in GC inlet liner, (2) PDMS loop with TDS and (3) SBSE with TDS, for GC analysis are given in Table 5-10. LODs ranged from 0.0010 ng/L for metolachlor (in-house and commercial sorptive extraction methods) to 0.19 ng/L for bifenthrin (SBSE) and LOQs ranged from 0.0030 (metolachlor (in-house and commercial sorptive extraction methods)) to 0.63 ng/L (bifenthrin (SBSE)).

Table 5-10: LODs and LOQs (ng/L) for the respective sorptive extraction and TD methods for GC×GC-TOFMS. The quantification (quant ion) and R^2 value for each analyte are provided.

Analyte	Quant Ion *(m/z)*	PDMS Loop GC Inlet			PDMS Loop TDS			SBSE TDS			Instrumental***		
		R^2	$LOD^*_{loop\ Inlet}$ (ng/L)	$LOQ^{**}_{loop\ Inlet}$ (ng/L)	R^2	$LOD_{loop\ TDS}$ (ng/L)	$LOQ_{loop\ TDS}$ (ng/L)	R^2	LOD_{SBSE} (ng/L)	LOQ_{SBSE} (ng/L)	R^2	LOD Instrumental (pg)	LOQ Instrumental (pg)
4-tert-Amylphenol	135	0,970	0,098	0,33	0,911	0,0010	0,0030	n/a****	n/a	n/a	1,000	1,0	3,3
Caffeine	194	0,930	0,026	0,085	0,916	0,0040	0,013	0,988	0,0069	0,023	0,995	1,0	3,3
Atrazine	200	0,970	0,076	0,25	0,969	0,018	0,059	0,975	0,015	0,049	0,998	1,0	3,3
Lindane	181	0,992	0,0088	0,029	0,992	0,015	0,050	0,994	0,010	0,032	0,998	1,0	3,3
Terbuthylazine	173	0,957	0,0017	0,0055	0,913	0,0043	0,014	0,957	0,0090	0,030	0,998	1,0	3,3
Terbutryn	226	0,998	0,012	0,039	0,993	0,021	0,069	0,989	0,011	0,036	0,995	1,0	3,3
Chlorpyrifos	97	0,995	0,036	0,12	0,983	0,058	0,19	0,997	0,019	0,062	0,998	1,0	3,3
Metolachlor	162	0,947	0,0010	0,0030	0,917	0,0010	0,0030	0,912	0,0010	0,0033	0,999	1,0	3,3
Musk ketone	279	0,998	0,0013	0,0043	0,961	0,018	0,059	0,999	0,014	0,048	0,983	1,0	3,3
Bifenthrin	181	0,990	0,032	0,11	0,978	0,059	0,20	0,983	0,19	0,63	0,994	1,0	3,3

* LOD calculated as the concentration which gives a signal-to-noise ratio (S/N) of 3
** LOQ calculated as the concentration which gives an S/N of 10
***note: instrumental LODs and LOQs are given in pg.
****n/a not applicable (method not suitable).

5.4.1.2. UHPLC-QTOFMS

The LODs and LOQs for the LVI and SPE analysis are given in Table 5-11. LODs and LOQs for LVI ranged from 1.969 (atrazine) to 562.9 ng/L (efavirenz) and 6.56 (atrazine) to 1876 ng/L (efavirenz), respectively. LODs and LOQs for SPE ranged from 0.0734 (atrazine) to 163 ng/L (metolachlor) and 0.245 (atrazine) to 543 ng/L (metolachlor), respectively.

Table 5-11: LODs and LOQs (ng/L) for the respective LVI and SPE methods for UHPLC-QTOFMS. The quantification (quant ion) and R^2 value for each analyte are provided.

Analyte	Quant Ion	LVI			SPE		
		R^2	LOD_{LVI} (ng/L)	LOQ_{LVI} (ng/L)	R^2	LOD_{SPE} (ng/L)	LOQ_{SPE} (ng/L)
Acetaminophen	152,071	0,983	134,6	448,8	n/a*	n/a	n/a
Caffeine	195,088	0,989	53,90	179,7	n/a	n/a	n/a
Atrazine	216,101	0,985	1,969	6,562	0,960	0,0734	0,245
Terbuthylazine	230,117	0,985	3,435	11,45	0,999	0,261	0,871
Terbutryn	242,144	0,983	2,215	7,383	0,994	17,2	57,3
Nevirapine	267,124	0,990	44,40	148,0	n/a	n/a	n/a
Metolachlor	284,140	0,982	359,3	1198	0,996	163	543
Efavirenz	314,020	0,994	562,9	1876	0,995	2,99	10,0

*n/a not applicable (method not suitable).

Measurements at ultra-trace levels (low ppb to ppt) of the target analytes are realised for the SPE and LVI methods using LC analysis. Although the LODs for LVI are higher than for SPE for pesticides the LODs for LVI are nevertheless lower than the levels required by the EU directive and they are also in line with the WHO guidelines for drinking water quality (refer back to Table 5-9 for the guidelines).

A relatively higher LOD and LOQ for metolachlor were obtained even when using a pre-concentration sample clean up step. The LODs for metolachlor (359 and 163 ng/L for LVI and SPE, respectively) were not within the EU guidelines of 100 ng/L maximum pesticide contaminant level, however, the LODs are still well below the guidelines set by the WHO for the maximum contaminant level of metolachlor (10 µg/L).

5.4.2. Accuracy and precision

5.4.2.1. GC×GC-TOFMS

Accuracy (% recovery) and precision (repeatability (%RSD)) for each of the methods were determined using triplicate samples (refer to Chapter 4 Section 4.6.3.2) and the results are presented in Table 5-12. Good accuracy and precision were achieved with % recovery ranging from 85% (musk ketone) to 129% (lindane and bifenthrin) and % RSD ranged from 2.8% (terbuthylazine) to 58% (metolachlor) for GC analysis (omitting caffeine - see succeeding explanation). A precision of less than 30% RSD and a % recovery between 70 and 130% are deemed acceptable for the trace level analysis of water (EPA guideline, 1996a) [14]. The majority of the target analytes had a precision well below 30% and a % recovery well within the guideline limits. A detailed breakdown of % recovery and % RSD calculations for the target analytes using GC analysis can be found in Addendum I.

Table 5-12: % Recovery (accuracy) and % RSD (precision) (n=3) at 1 ng/50 mL (20 ng/L) for the respective sorptive extraction and TD methods for GC×GC-TOFMS.

Analyte	PDMS Loop GC Inlet		PDMS Loop TDS		SBSE TDS	
	$\bar{x}_{\%Rec} \pm S.D._{n=3}^{*}$	% RSD	$\bar{x}_{\%Rec} \pm S.D._{n=3}$	% RSD	$\bar{x}_{\%Rec} \pm S.D._{n=3}$	% RSD
4-tert-Amylphenol	100 ± 11	11	116 ± 13	11	n/a**	n/a
Atrazine	115 ± 8	6,5	101 ± 27	27	114 ± 10	8,7
Lindane	121 ± 10	8,0	129 ± 0.92***	1	128 ± 18	14
Terbuthylazine	109 ± 10	9,1	104 ± 22	21	106 ± 3	2,8
Caffeine	152 ± 84	55	50 ± 38	76	23 ± 3	13
Terbutryn	98 ± 13	14	103 ± 17	17	104 ± 14	14
Chlorpyrifos	93 ± 13	14	92 ± 8	8,8	114 ± 7	6,2
Metolachlor	120 ± 8	6,6	105 ± 22	21	104 ± 60	58
Musk ketone	85 ± 13	15	101 ± 25	25	119 ± 16	13
Bifenthrin	129 ± 33	26	93 ± 28	29	105 ± 23	22

*$\bar{x}_{\%Rec}$ = mean % recovery; S.D. = Standard Deviation
**n/a not applicable (method not suitable).
***n=2 (due to outlier as determined by the Dixon Q test).

The trapping efficiency of the nonpolar PDMS phase of the loop and stir bar for caffeine proved challenging due to the high polarity of caffeine (log K_{ow} = -0.55). However, two unexpected high recovery values (Addendum I) were obtained for caffeine extracted with loops: 95% (TDS) and 245% (inlet desorb) which resulted in higher % RSD

values. This was most likely due to contamination because of the ubiquitous nature of caffeine. Background levels were also responsible for the poor precision of metolachlor occurring in the SBSE method. PDMS is prone to trap any molecule in the surroundings often leading to background contamination. Method blanks confirm the presence of caffeine and metolachlor in the deionised water used as matrix. The Dixon Q-test for outliers (Chapter 4 Section 4.6.4) showed no outliers at 95% confidence level for caffeine and metolachlor (or for any of the target analytes within any of the methods, except for lindane when using the PDMS loop with TDS).

Comparable results were generally obtained for the in-house and commercial sorptive methodology. Analysis of variance (ANOVA F-test) (Chapter 4 Section 4.6.4) was done for the comparison of means (using mean peak areas) between the three different methods, (1) PDMS loop with TDS, (2) PDMS loop TD in GC inlet and (3) SBSE with TDS. ANOVA found no significant difference between the three methods at 95% confidence level for the majority of the target analytes (Table 5-13). Table 5-13 highlights 4-tert-amylphenol and terbuthylazine showing a significant difference between the three methods.

Table 5-13: Statistical analysis (ANOVA) of target analytes: comparison of means between the three different methods; (1) PDMS loop with TDS, (2) PDMS loop TD in GC inlet and (3) SBSE with TDS.

Analyte	F-test (ANOVA) null hypothesis: $mean_1=mean_2=mean_3$		Significantly different at 95% confidence level, reject the null hypothesis*
	F calc	F critical	
4-tert-Amylphenol	227,589	5,143	Yes
Terbuthylazine	36,653	5,143	Yes
Atrazine	0,682	5,143	No
Lindane	1,585	5,143	No
Caffeine	0,007	5,143	No
Terbutryn	3,137	5,143	No
Chlorpyrifos	0,525	5,143	No
Metolachlor	0,694	5,143	No
Musk ketone	0,965	5,143	No
Bifenthrin	0,413	5,143	No

*F calc > F critical, we do not accept the null hypothesis, and the methods differ significantly.

A t-test (Chapter 4 Section 4.6.4), for differences in means (comparing two methods at a time using mean peak areas), was then used to establish between which two methods the difference was occurring (why the null hypothesis was rejected using the F-test) for 4-tert-amylphenol and terbuthylazine at 95% confidence level.

Table 5-14: Statistical analysis (t-test) of target analytes: comparison of means; (1) PDMS loop with TDS vs PDMS loop TD in GC inlet and, (2) PDMS loop with TDS vs SBSE with TDS.

Analyte	t-test (two-tailed test) null hypothesis: $mean_1 = mean_2$			Significantly different at 95% confidence level, reject the null hypothesis*
	t Stat PDMS loop TDS vs GC inlet	t Stat PDMS loop TDS vs SBSE TDS	t critical	
4-tert-Amylphenol	16,527	1,994	±4,303	Yes, significant difference between PDMS loop TDS vs PDMS loop direct GC inlet desorption.
Terbuthylazine	-0,954	-12,268	±4,303	Yes, significant difference between PDMS loop TDS and SBSE TDS.
Atrazine	-1,422	-1,879	±4,303	No
Lindane	1,424	-0,453	±4,303	No
Caffeine	-0,049	-0,221	±4,303	No
Terbutryn	-1,678	-2,693	±4,303	No
Chlorpyrifos	0,657	2,603	±4,303	No
Metolachlor	1,095	-0,895	±4,303	No
Musk ketone	0,312	-3,957	±4,303	No
Bifenthrin	-1,121	1,238	±4,303	No

*If t Stat < - t critical two-tail or t Stat > t critical two-tail, we reject the null hypothesis, and the methods differ significantly.

The difference between methods for 4-tert-amylphenol and terbuthylazine was due to a difference in means between the PDMS loop with TDS and PDMS loop with direct desorption in the GC inlet and, PDMS loop with TDS and SBSE with TDS, respectively (Table 5-14). Problems associated with inlet sensitivity for 4-tert-amylphenol was previously discussed in Section 5.3.1.1 (4-tert-Amylphenol concerns). Terbuthylazine has a higher mean response (peak area) when using SBSE (73 401 ± 1971) compared to the PDMS loop desorbed in a TDS (45 518 ± 5609). A longer desorption time may be used in future to improve the response for terbuthylazine using the PDMS loop with TDS. However, the % recovery for both the SBSE and PDMS loop, 106% and 104% respectively, is similar and the % RSD for both the SBSE and PDMS loop, 2.7 and 21 respectively, is still within acceptable levels for trace analysis.

5.4.2.2. UHPLC-QTOFMS

Accuracy (% recovery) and precision (repeatability (%RSD)) of LVI and SPE were determined using triplicate samples (refer to Chapter 4 Section 4.6.3.2) and the results are presented in Table 5-15. Good accuracy and precision was achieved with

% recovery ranging from 93% (terbuthylazine) to 132% (caffeine) and 39% (terbuthylazine) to 105% (atrazine) for LVI and SPE, respectively and % RSD ranging from 3.5% (atrazine) to 13% (nevirapine) and 13% (terbutryn) to 45% (metolachlor) for LVI and SPE, respectively. Precision (%RSD) for all the target analytes (except for metolachlor) was less than 30% and % recovery (accuracy) was between 70 and 130% for the target analytes (except for terbuthylazine and caffeine). Accordingly, the precision and accuracy of the two methods are acceptable for trace level analysis of water samples (EPA guideline, 1996a) [14]. A detailed breakdown of % recovery and % RSD calculations for the target analytes using LC analysis can be found in Addendum J.

Table 5-15: % Recovery (accuracy) and % RSD (precision) (n=3) for the LVI (2 500 ng/L) and SPE (250 ng/L) methods for UHPLC–QTOFMS.

Analyte	LVI		SPE	
	$\bar{x}_{\%Rec} \pm S.D._{n=3}^{*}$	% RSD	$\bar{x}_{\%Rec} \pm S.D._{n=3}$	% RSD
Atrazine	117 ± 4	3,5	105 ± 26	25
Terbuthylazine	93 ± 4	4,7	39 ± 6,3	16
Caffeine	132 ± 6	4,4	n/a**	n/a
Terbutryn	121 ± 4	3,6	97 ± 13	13
Metolachlor	106 ± 18	17	87 ± 39	45
Efavirenz	106 ± 5	5,0	Not available***	Not available
Acetaminophen	109 ± 6	5,9	n/a	n/a
Nevirapine	112 ± 13	13	n/a	n/a

*$\bar{x}_{\%Rec}$ = mean % recovery; S.D. = Standard Deviation
**n/a not applicable (method not suitable).
***Not available; UHPLC instrument failure during analysis of repeats (complete loss of extract).

The poor precision reported for metolachlor using a general SPE is most likely due to background levels of metolachlor present in the deionised water. Method blanks confirm the presence of metolachlor in the deionised water used as matrix.

Low accuracy (39% recovery) for terbuthylazine using a general SPE method indicates that either the SPE cartridge was not suitable for retaining the compound or an inappropriate solvent or solvent system was used to elute it from the cartridge. High recovery for caffeine (132%) using LVI can be contributed to everyday usage of caffeine-containing products resulting in possible contamination. Method blanks also confirmed the presence of caffeine in the deionised water used as matrix.

5.5. Method Selection

5.5.1. Selecting the best method

LODs, LOQs, accuracy and precision were used as guidelines to select the most appropriate method for micropollutant analysis of surface water. Four different aspects of sample analysis were considered, namely extraction, sample introduction, sample separation and compound detection (including quantification).

5.5.1.1. Extraction

GC×GC-TOFMS

SBSE and sorptive extraction using an in-house developed PDMS loop were investigated. SBSE and PDMS loop extraction were compared, as they are both pre-concentrating sorptive techniques followed by GC×GC-TOFMS analysis. The two extraction techniques were found to be comparable when considering the previously mentioned criteria and ANOVA analysis. The PDMS loop was therefore chosen for all further sample extraction for GC analysis because of its ease of use, low cost and disposability.

UHPLC-QTOFMS

In this study, SPE was considered for LC analysis. Increase in response, due to pre-concentration, is a major advantage of SPE enabling lower detection limits. A major drawback of SPE, when doing multi-residue analysis, is that the cartridge sorbents are compound specific retaining only compatible compounds. This is a major advantage when analysing specific compound classes as it adds an extra degree of separation and it has a pre-concentration effect. However, when analysing heterogeneous chemical compounds such as micropollutants numerous cartridges will be needed adding cost and time to the analysis. Another disadvantage of SPE is the need to use expensive solvents that are often harmful to the environment.

5.5.1.2. Sample introduction

GC×GC-TOFMS

In this study, analytes were removed from the sorptive samplers by thermal desorption (TD) for introduction into a GC×GC-TOFMS. A commercial (Gerstel™) TDS was

compared to TD directly in the inlet liner of a GC. The two techniques give the user versatility in the sample introduction process. Poor sensitivity is problematic for extraction techniques such as SPE as only an aliquot of the extract is injected into the analytical instrument [15]. In order to overcome sensitivity shortcomings of SPE sorptive sampling techniques coupled to thermal desorption in combination with gas chromatography (GC) are preferred [4, 15]. Thermal desorption increases sensitivity, as the entire sample mass is transferred to the GC, and the process is solvent free. The destructive nature of TD is a major disadvantage as once desorbed the sampler no longer retains the analytes. GC inlet TD was selected for all further sample introduction for GC analysis because cryo-focussing is not required, sample introduction time is reduced (no lengthy desorption step) when compared to a commercial TDS and there was no significant difference between TDS and GC inlet TD for the majority of the target analytes. Another advantage for GC inlet TD is that no commercial thermal desorber is required, which saves cost.

UHPLC-QTOFMS

A major drawback for SPE, when compared to TD, is the injection of microliter (5 µl for this study) amounts of a dilute extract. The decrease in sensitivity is usually compensated for by concentrating large volumes of sample (usually 1000 mL of sample is required). LVI can be used as an alternative technique for micropollutant detection and quantification in surface water as the LODs and LOQs were found to be within the regulatory requirements. The major advantage of LVI is minimal sample preparation. This results in reduced analysis time, cost and solvent usage. There is also a reduced risk of compound loss during sample preparation. LVI was selected for all further LC analysis due to its reduction in analysis time and cost.

5.5.1.3. Sample separation

GC×GC-TOFMS

Separation of micropollutants is challenging as these chemicals are found at trace levels in complex environmental matrices and consist of various chemical compounds. Selecting the appropriate column was essential for GC separation (Section 5.2.1.1). Other column combinations might be needed when analysing micropollutants at different locations than those reported in this study, e.g. areas where less pesticide

spraying occurs. See Figure 5.5 for a chromatogram of the separation of the target analytes by GCxGC-TOFMS.

UHPLC-QTOF

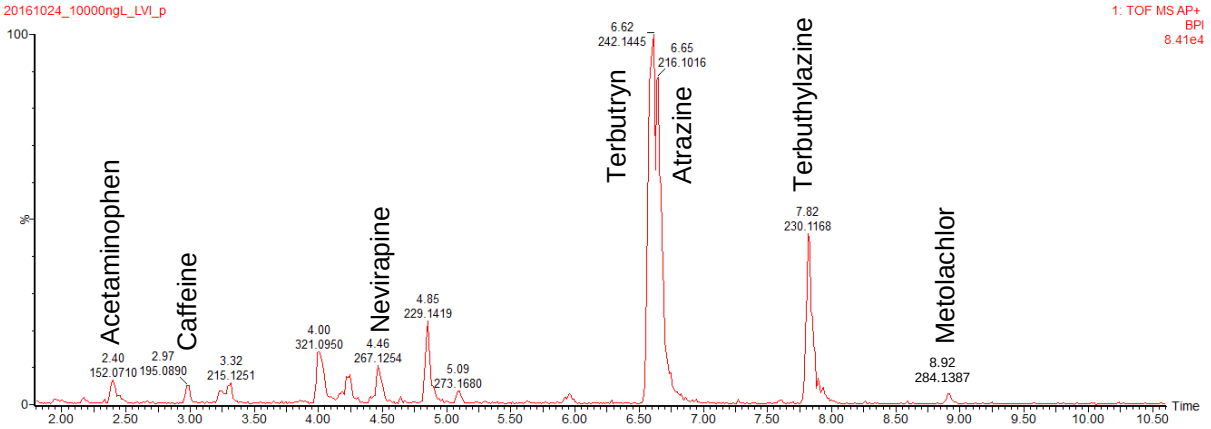


Figure 5.14: BPI chromatogram showing UHPLC separation of target analytes; 100 µl sample injection (LVI); 10 000 ng/L target analyte concentrations. APCI positive mode (TOF-MS). Peak labels show retention time (minutes); $(M+H)^+$ mass (Da).

Satisfactory separation of target analytes was achieved using LVI-UHPLC (Figure 5.14). A general purpose stationary phase was selected for this study. However, separation of the triazine pesticides was problematic due to their structural similarities. Adding an extra dimension of separation using ion mobility or comprehensive two-dimensional liquid chromatography may be considered for future LC separations.

5.5.1.4. Compound detection

Detection and quantification of micropollutants were done using TOFMS (GC) and QTOFMS (LC) detectors. Matrix matched calibration was used to produce reliable quantitative results.

5.5.2. Compound specific methods

The following table highlights the most suitable analytical method for each target analyte (Table 5-16). Please note that suitability is based on analytical parameters used in this study. Parameters used were selected for a multi-residue method and not optimised for individual compounds.

Table 5-16: Summary of the most suitable analytical method for each target analyte.

Analyte	PDMS loop or SBSE	GC inlet TD or Gerstel™ TDS	SPE or LVI	GC or LC	Comments
4-tert-Amylphenol	PDMS loop	Both GC inlet liner low response	n/a*	GC Not detected using LC	Qualification possible by all GC methods Background contamination problematic.
Atrazine	Both	Both	Both	Both	Misidentified by ChromaTOF (GC-TOFMS) software. RT** confirmation crucial for identification.
Acetaminophen	Both Qualitative screening	Both Qualitative screening	LVI	LC Qualification possible using GC	Low response for both GC and LC. LC more suitable.
Lindane	Both	Both	n/a	GC Not detected using LC	None
Terbuthylazine	Both	Both	Both Low accuracy for SPE	Both	None
Caffeine	Both	Both	LVI	Both	Precaution needs to be taken due to the ubiquitous nature of caffeine when quantifying caffeine for GC and LC analysis (background levels).
Terbutryn	Both	Both	Both	Both	Non-baseline separation of triazine pesticides may occur using LC. GC methods suitable.
Chlorpyrifos	Both	Both	n/a	GC Not detected using LC	None

Analyte	PDMS loop or SBSE	GC inlet TD or Gerstel™ TDS	SPE or LVI	GC or LC	Comments
Metolachlor	PDMS loop	Both	SPE Poor precision	GC Low response for LC	Qualification possible by all GC methods. Background contamination problematic, specifically for SBSE due to analyte carry-over.
Musk ketone	Both	Both	n/a	GC Not detected using LC	None
Efavirenz	Both Qualitative screening	Both Qualitative screening	Both	Both Low response for GC	None
Hexestrol	Both Qualitative screening	Both Qualitative screening	n/a	GC low response Not detected using LC	Qualification using GC.
Bifenthrin	Both	Both	n/a	GC Not detected using LC	Misidentified by ChromaTOF (GC-TOFMS) software. RT confirmation crucial for identification.
Estrone	Both Qualitative screening	Both Qualitative screening	n/a	GC low response Not detected using LC	Qualification using GC.
Clindamycin	Both Qualitative screening	Both Qualitative screening	n/a	GC low response Not detected using LC	Qualification using GC.
Nevirapine	Both Qualitative screening	Both Qualitative screening	LVI	LC GC low response	Qualification using GC possible. LC LVI method suitable.

*n/a not applicable (method not suitable).
** RT: retention time.

Qualification of hexestrol and estrone was achieved using GC-MS (without derivatisation). However, quantification proved problematic when following this multi-residue approach. In order to improve sensitivity and selectivity derivatisation is often required for steroid estrogenic hormones, e.g. estrone and hexestrol. This is due to their low molecular weight and low volatility. Different derivatisation protocols have been developed and optimised, however, they are often time consuming and lead to analyte loss [16]. Liquid chromatography is presently more commonly used for oestrogen detection and quantification. Derivatisation with dansyl chloride may be used in future work to improve the ionisation efficiency in positive mode for oestrogen detection by LC.

Quantification of clindamycin proved challenging during this study. The high molecular weight and polarity of the compound indicate analysis using optimised, targeted, LC-MS/MS methods for future research.

5.5.3. Note on derivatisation

The multi-shot derivatisation method used to improve the sensitivity of the GC analysis did not yield the expected results (refer to Chapter 4 Section 4.4.2). Decreased recoveries for the majority of the target compounds were observed (Table 5-17). Sorptive extraction is in principle an equilibrium technique [17]. By dividing the sample into three aliquots (prior to derivatisation with (1) sequential salting out, (2) methanol addition and (3) acetic acid anhydride) equilibrium needs to be established in each aliquot possibly resulting in less total analyte being retained into the PDMS.

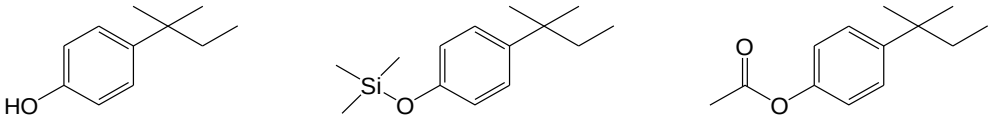
Table 5-17: Peak area of target analytes (1 ng each) without and with multi-shot derivatisation.

Analyte	Peak area without derivatisation (1 ng)	Peak area with multi-shot derivatisation method (1 ng)
4-tert-Amylphenol	1030222	Underivatised fraction: 26097 TMS derivative: 7728,1 Acetate derivative: 156429
Atrazine	12168	not detected
Lindane	119347	44328
Terbuthylazine	40857	21777
Caffeine	68999	94947*
Terbutryn	51169	18994
Chlorpyrifos	300593	30727
Metolachlor	303774	92234
Musk ketone	34747	17090

Analyte	Peak area without derivatisation (1 ng)	Peak area with multi-shot derivatisation method (1 ng)
Efavirenz	7695,1	not detected
Bifenthrin	140914	34347
Estone	not detected	not detected
Hexestrol	not detected	not detected
Acetaminophen	not detected	not detected

*higher response could be due to the ubiquitous nature of caffeine.

Furthermore, analysis of results was greatly complicated by the presence of several derivatives as was seen for 4-tert-amylphenol which yielded underivatised pure standard (from the methanol addition aliquot), the trimethylsilyl (TMS) derivative (from the glasswool plug containing BSTFA in the glass desorption tube) and the acyl derivative (from the acetic acid anhydride aliquot) in a single GC run (Figure 5.15). Van Hoeck et al. (2008), whose method was adapted for this study, did not report on any additional derivatisation products. However, the authors optimised the derivatisation method for specific compound classes, i.e. acetic acid anhydride with phenolics will give acetates, and only quantified the expected derivatised product [6].



4-tert-Amylphenol | 4-tert-Amylphenol, TMS derivative | 4-tert-Amylphenol Acetate

Figure 5.15: 4-tert-Amylphenol and derivatives present in the multi-shot analysis.

Hexestrol, estrone, and acetaminophen, all phenolic compounds, proved challenging for GC analysis and it was therefore anticipated that derivatisation would improve sensitivity. Multi-shot derivatisation did not enhance the detection of these compounds in the calibration working range. This is unexpected as the formation of acyl groups assists the sorption of more polar compounds into the PDMS phase and the silylation increases the volatility. Future work on improving the derivatisation procedure is consequently required.

5.6. Real World Sampling: Targeted Analyses

5.6.1. GC×GC-TOFMS

It was demonstrated that PDMS loop extraction and SBSE gave results that do not differ significantly for the majority of the target analytes, and in the case of 4-tert-amylphenol, the loop sampler performed considerably better than SBSE. Therefore, the PDMS loop may be used as a cost effective alternative to SBSE. In addition, it was demonstrated that the PDMS loop sampler, when desorbed directly in an inlet of a GC, gave results that do not differ significantly from a loop sampler desorbed in a commercial TDS. Hence, the loop sampler with direct desorption in an inlet of a GC was used as a quantitative device for targeted analysis of samples from Rietvlei Nature Reserve and Albasini Dam (there was not sufficient sample from Nandoni Dam for targeted analyses). The results of the target analysis using GC×GC-TOFMS for Rietvlei Nature Reserve (sampling done on 15/3/2016) and Albasini Dam (sampling done on 17/8/2015) are given in Table 5-18.

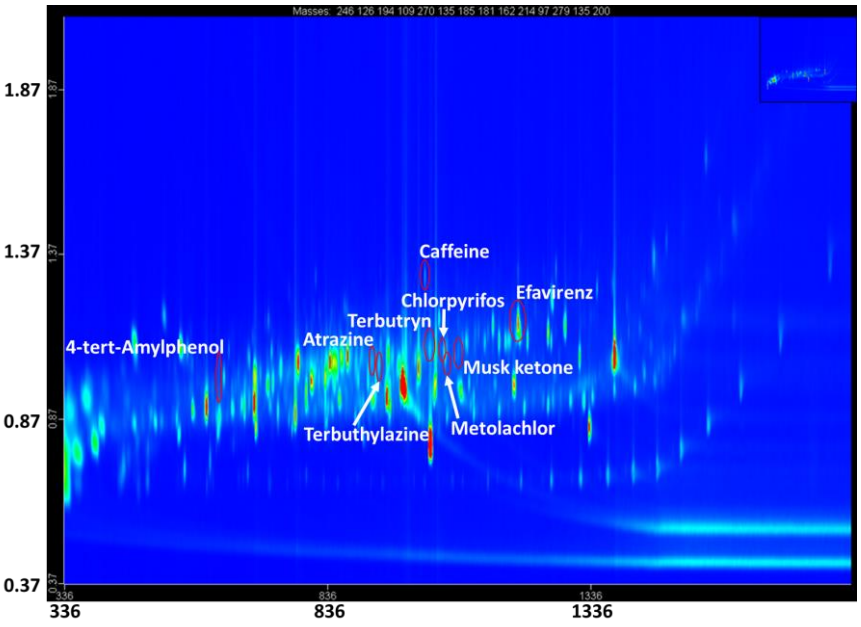


Figure 5.16: Contour plot of a reconstructed ion chromatogram (GC×GC-TOFMS) of surface water collected at site 2 (Otter Bridge) in Rietvlei Nature Reserve. Target analytes detected are indicated. RIC: 246, 126, 194, 109, 270, 135, 181, 185, 162, 214, 97, 279 and 200 *m/z*.

Caffeine, classified as endocrine disrupting compound [1, 2], was detected and quantified at Otter Bridge (0.80 ng/L) (Figure 5.16) and Bird Hide Stream (0.73 ng/L) sample sites at Rietvlei Nature Reserve. Caffeine is an ingredient found in many

beverages, food and medicinal products and can be used as an indicator of human pollution in the aquatic environment as it is excreted in urine [18].

Pesticides are usually found in surface water where the rivers are close to agricultural areas. The presence of lindane (0.38 ng/L) at Otter Bridge sample site, Rietvlei Nature Reserve (Figure 5.16), is cause for concern as production and agricultural use of this organochlorine pesticide was banned under the Stockholm Convention on persistent organic pollutants (POPs) (2009) [19]. The presence of bifenthrin, a pesticide tentatively identified during the untargeted screening, could not be confirmed when a reference standard was analysed. Although a match quality as high as 852 was reported by the ChromsaTOF® software, the RT (696 s in 1st dimension) did not match that of the reference compound (1 290 s in 1st dimension), thereby highlighting the need for reference standards for unequivocal identification of compounds.

Personal care products, such as musk ketone, are usually evident in surface water near residential areas. The presence of musk ketone (as high as 4.0 ng/L at Rietvlei Spruit sampling site) at Rietvlei Nature Reserve is not surprising as the reserve is located only 20 km south of the Pretoria central business district.

The target compounds occur at trace levels in surface water from Rietvlei Nature Reserve as sampling was done during a heavy rainfall period. The higher presence of pesticides and personal care products (PCPs) in the surface water from Rietvlei Nature Reserve located in an urban area compared to surface water from the Albasini Dam in a rural area reflects the human impact on the environment. Of interest is that DDT, which is thought to be a main cause of endocrine disruption in the Limpopo Province [20, 21] and in Rietvlei Nature Reserve [22, 23], was not detected in any of the surface water analyses. Oestrogenic activity was confirmed in drinking water samples from the Limpopo Province [21]. The compounds responsible for this oestrogenic activity have not yet been identified [21]. However, the range of EDCs detected (Table 5-18 and Addendum K) indicates that the reported oestrogenic activity may be due to additive effects, rather than a single chemical. Future monitoring at Rietvlei Nature Reserve and Albasini Dam is thus required as the large range of pollutants detected can increase oestrogenic activity in the water.

Table 5-18: Quantification (ng/L) of target analytes detected in real world samples (six samples from Rietvlei Nature Reserve collected on 15/3/2016 and one sample from the Albasini Dam, Limpopo Province collected on 17/8/2015) using PDMS loop in the inlet liner TD-GC×GC-TOFMS.

Analyte	1 STR Fam (ng/L)	2. Otter Bridge (ng/L)	3. Bird Hide Stream (ng/L)	4. Rietvlei Spruit (ng/L)	5. Marais Dam (ng/L)	6. MD Channel (ng/L)	Albasini Dam (ng/L)
4-tert-Amylphenol	<0,098	<0.098	0,99	6,7	<0,098	<0,098	<0,098
Caffeine	<0,026	0,80	0,73	<0,026	<0,026	<0,026	<0,026
Atrazine	<0,076	0,41	<0,076	<0,076	0,40	<0,076	<0,076
Lindane	<0,0088	0,38	<0,0088	<0,0088	<0,0088	<0,0088	<0,0088
Terbuthylazine	<0,0017	3,8	<0,0017	0,86	0,37	<0,0017	<0,0017
Terbutryn	<0,012	0,17	<0,012	<0,012	0,16	<0,012	<0,012
Chlorpyrifos	<0,036	0,24	<0,036	0,24	<0,036	<0,036	0,14
Metolachlor	<0,0010	0,19	<0,0010	0,54	0,32	0,36	<0,0010
Musk ketone	0,039	2,4	0,066	4,0	3,6	0,43	<0,0013
Efavirenz	n.d.*	148*	n.d.	0,34	76	29	n.d.
Bifenthrin	<0,032	<0,032	<0,032	<0,032	<0,032	<0,032	<0,032

*not detected
**semi-quantitative

5.6.2. UHPLC-QTOFMS

The direct injection of the surface water (100 µL), with minimal prior sample treatment, into the LC, is an attractive approach as it avoids time consuming sample preparation steps making it ideal for fast and high-throughput analysis, and it reduces the amount of solvent used. Therefore, LVI was used in combination with UHPLC-QTOFMS for targeted analysis of samples from Rietvlei Nature Reserve and Albasini Dam (there was not sufficient sample from Nandoni Dam for targeted analyses). The results for the target analysis using LVI-UHPLC-QTOFMS for Rietvlei Nature Reserve (sampling done on 6/2/2015) and Albasini Dam (sampling done on 6/2/2015) are given in Table 5-19. Unequivocal identification of target analytes was attained using retention times, accurate mass and MS^E high energy fragmentation patterns.

The emerging EDC, acetaminophen (paracetamol), was not detected. The levels of atrazine detected during February 2015 at sampling sites Rietvlei Spruit (60.8 ng/L in February and not detected (< 0.076) in March 2016) and Marais Dam (56.7 ng/L in February and 0.40 ng/L in March 2016) were considerably higher than levels detected during the heavy rainfall period of March 2016 (Table 5-18). A decrease in concentration of target analytes was expected as the follow-up sampling in March 2016 for GC-MS analyses was done after a heavy rainfall period which was not the case when sampling in February 2015 for UHPLC-QTOFMS analyses.

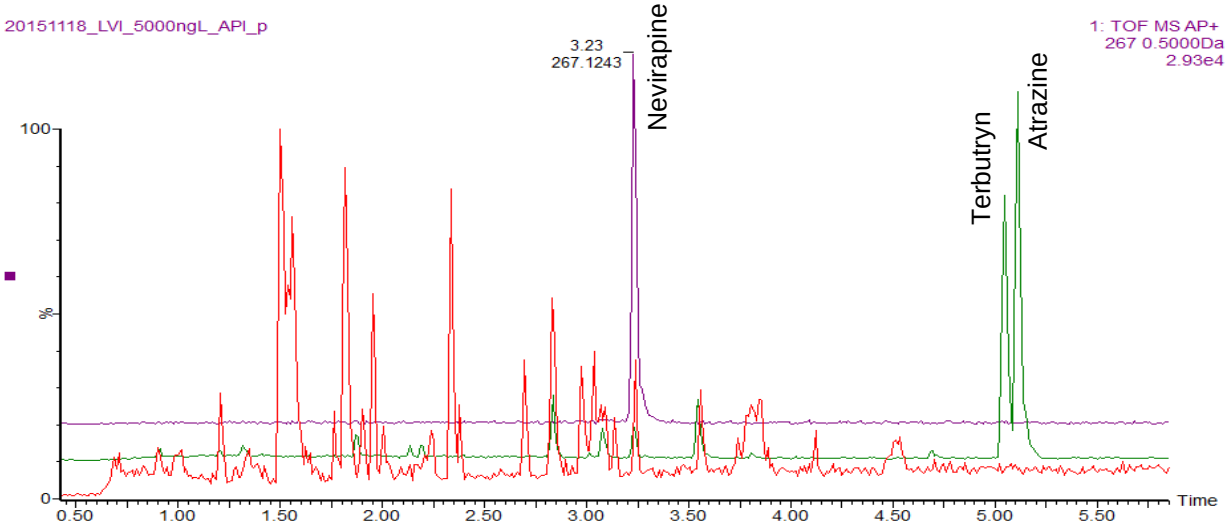


Figure 5.17: LVI UHPLC BPI chromatogram of surface water collected at Rietvlei Spruit in Rietvlei Nature Reserve (bottom red trace); LVI UHPLC BPI chromatogram of a 5 000 ng/L mixed reference standard (middle green trace); LVI UHPLC RIC of 267,124 *m/z* (nevirapine) from Rietvlei Spruit (top purple trace); APCI positive mode (QTOFMS). Peak labels show retention time (minutes); $(M+H)^+$ mass (Da).

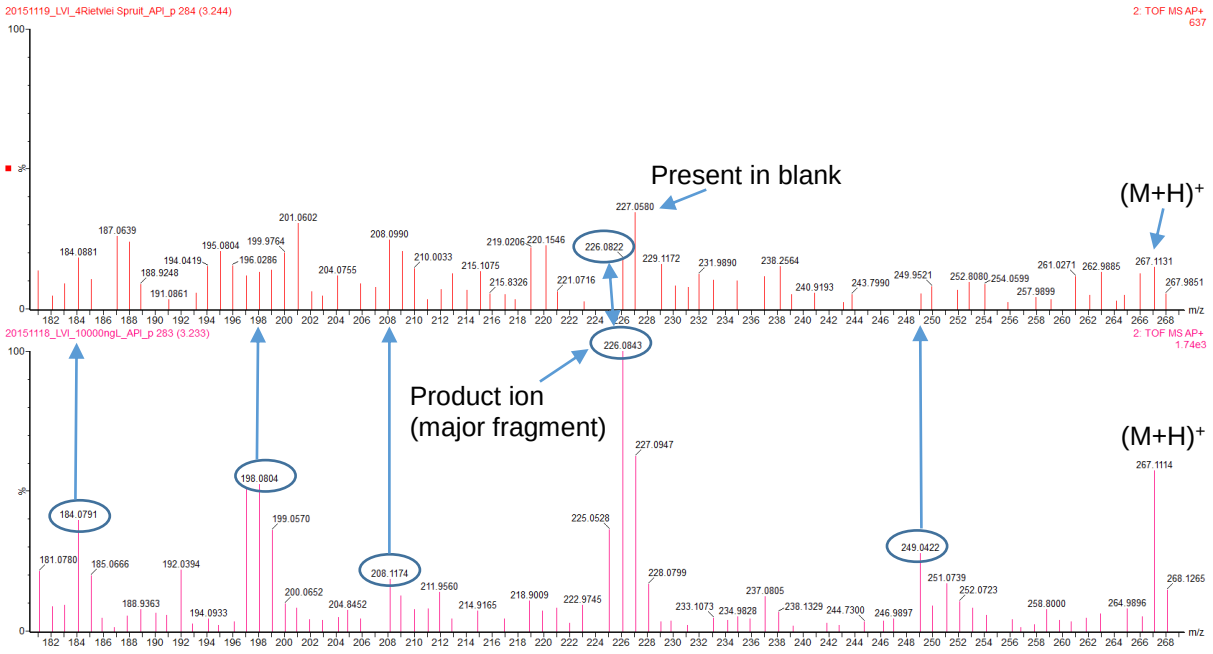


Figure 5.18: MS^E high energy spectrum of a LVI of Rietvlei Spruit (top) compared to a LVI of a reference standard (bottom) for nevirapine 267.11 *m/z.* Peak labels show accurate mass.

LVI-LC was particularly suited for the quantification of nevirapine (Figure 5.17). Accurate mass and MS^E fragmentation patterns for unequivocal identification are shown in Figure 5.18. The presence of efavirenz (Figure 5.16) at four of the Rietvlei Nature Reserve sampling sites and of nevirapine (Figure 5.17) at three of the six Rietvlei Nature Reserve sampling sites (Table 5-19) is cause for concern (compounds were detected below LOQs). Efavirenz and nevirapine are antiretroviral (ARV) compounds used for the treatment of HIV and can be considered emerging pollutants. The concentration levels of the ARVs detected in surface water are higher than that of the EDCs. Considering that inhabitants of informal settlements in South Africa use untreated water from rivers and dams for drinking, cooking and cleaning environmental monitoring of South African surface water should also include ARVs as priority pollutants. The number of target compounds selected was to mainly demonstrate the performance of the two complementary methods. This target list may easily be expanded.

The base peak ion (BPI) chromatograms of the LVI-UHPLC-MS for Rietvlei Nature Reserve (sampling done on 6/2/2015) and Albasini Dam (sampling done on 6/2/2015) can be found in Addendum K.

Table 5-19: Quantification (ng/L) of target analytes detected in real world samples (six samples from Rietvlei Nature Reserve collected on 6/2/2015 and one sample from the Albasini Dam, Limpopo Province collected on 17/8/2015) using LVI-UHPLC-QTOFMS.

Analyte	1 STR Fam (ng/L)	2. Otter Bridge (ng/L)	3. Bird Hide Stream (ng/L)	4. Rietvlei Spruit (ng/L)	5. Marais Dam (ng/L)	6. MD Channel (ng/L)	Albasini Dam (ng/L)
Acetaminophen	<135	<135	<135	<135	<135	<135	<135
Caffeine	<53,9	<53,9	<53,9	<53,9	<53,9	<53,9	<53,9
Atrazine	<1,97	<1,97	<1,97	60,8	56,7	<1,97	<1,97
Terbuthylazine	<3,44	<3,44	<3,44	<3,44	<3,44	<3,44	<3,44
Terbutryn	<2,22	<2,22	<2,22	<2,22	<2,22	<2,22	<2,22
Metolachlor	<359	<359	<359	<359	<359	<359	<359
Efavirenz	<563	<563	<563	<563	<563	<563	<563
Nevirapine	<44,4	<148 (124)	<44,4	<148 (109)	<44,4	227	<44,4

5.7. Linear Retention Index

Knowing the structure or polarity of the column stationary phase used during separation is important for calculating and comparing Kováts retention indices (RIs) as the RIs are phase dependent [24]. In order to identify unknown column stationary phases, the separation characteristics of different columns can be compared using system constants. The system constants contain all the chemical information about stationary phase solvation properties, thus providing an important link between chromatographic selectivity and initial monomer composition for polymeric stationary phases [25]. Using the system constants as a guide for the structure of the Rtx®-CLPesticides II column (and assuming that the Rtx®-CLPesticides II column is structurally similar to the Rtx®-CLPesticide column which has known system constants) it is apparent that the stationary phase used in this study for ^{1}D separation is poly-(dimethylmethyltrifluoropropylsiloxane) (Figure 5.19) with less than 20 - 35% methyl-trifluoro-propyl-siloxane monomer incorporation [25].

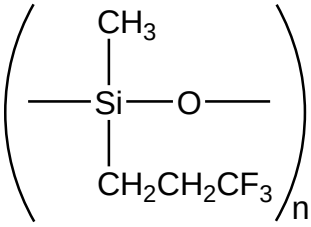


Figure 5.19: Structure of poly(dimethylmethyltrifluoropropylsiloxane).

These types of stationary phases are more dipolar/polarisable and have weaker hydrogen-bond bases than the poly-(dimethylsiloxane) and poly-(dimethyldiphenylsiloxane) stationary phases. The dipolarity is due to the inductive effect of the trifluoropropyl groups. In addition electron, lone pairs on the fluorine atoms will result in repulsive electron lone pair interactions during the separation process [3, 25].

^{1}D retention times were used to determine the RIs (Figure 5.20). The linear retention index – retention time (LRI-RT) calibration curve was constructed using *n*-C_{12} – *n*-C_{28} (Figure 5.21). Table 5-20 shows the experimentally determined RIs and the literature RIs from the NIST14 database for polar and standard non-polar stationary phases. The experimentally determined RIs were not comparable to RIs of a standard non-

polar column. This supports the dipolarity assumption of the Rtx-CLPesticides II column. (Retention indices for the proprietary phase Rtx®-CLPesticides II column could not be found in the literature).

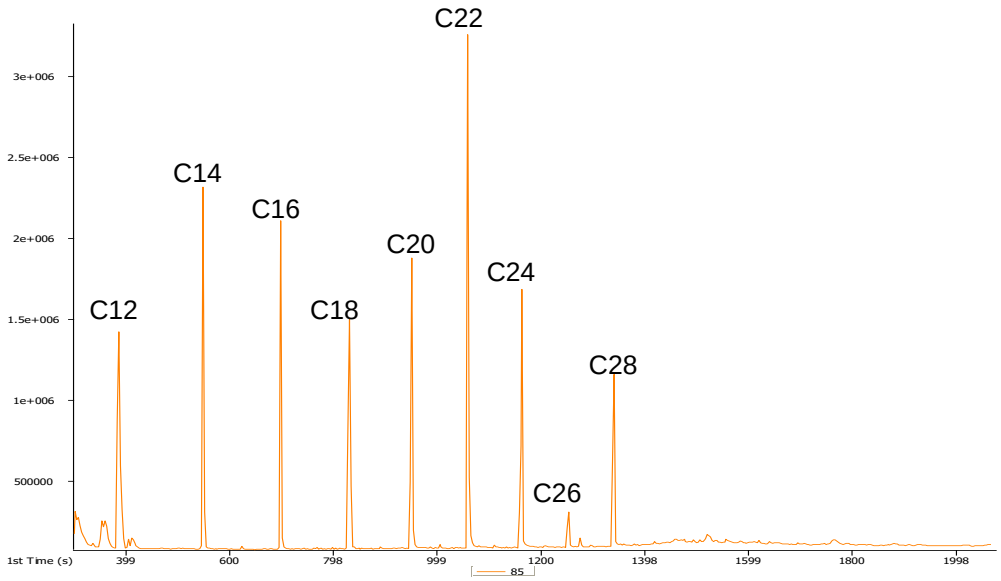


Figure 5.20: First dimension reconstructed ion chromatogram (RIC) (85 *m/z*) of *n*-alkanes by linear temperature programming analysis using Rtx®-CLPesticides II 30 m x 0.25 mm ID x 0.2 µm film thickness (^{1}D) column.

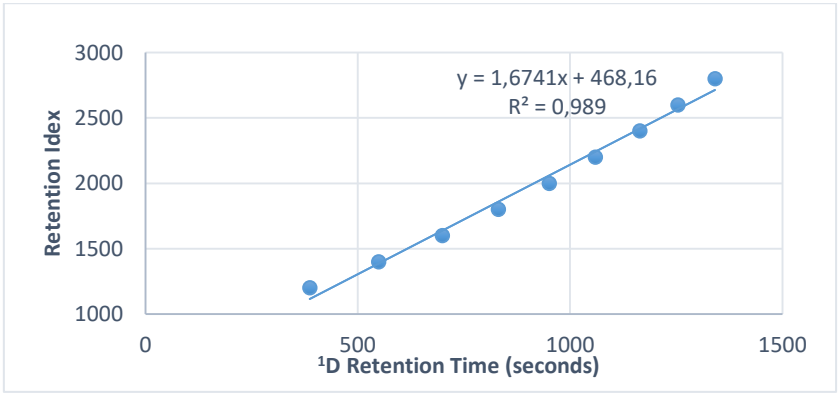


Figure 5.21: LRI-RT calibration curve for *n*-C_{12} – *n*-C_{28}.

Table 5-20: Experimentally determined linear retention indices (LRI) for the target analytes (certified reference standards) using ^{1}D retention times and literature RIs (NIST14) for polar and non-polar stationary phases.

Analyte	^{1}D RT (s)	^{1}D RI_{exp} Rtx®-CLPesticides II	^{1}D RI_{Lit} NIST14	
			Polar	Standard Non-polar
Atrazine	924	2015	2762	1712
Lindane	933	2030	Not available	1728
Terbuthylazine	936	2035	2664	1755
Caffeine	1020	2176	Not available	1800

Analyte	^{1}D RT (s)	^{1}D RI$_{exp}$ Rtx®-CLPesticides II	^{1}D RI$_{Lit}$ NIST14	
			Polar	Standard Non-polar
Terbutryn	1035	2201	2793	1912
Chlorpyrifos	1059	2241	Not available	1957
Metolachlor	1062	2246	Not available	2054
Musk ketone	1083	2281	Not available	1925
Efavirenz	1197	2472	Not available	2103
Bifenthrin	1293	2633	Not available	2551

The experimentally determined LRIs for compounds of interest detected during the Rietvlei Nature Reserve follow-up sampling utilising the PDMS loop - GC inlet liner TD - GC×GC-TOFMS are reported in Addendum L. To our knowledge this is the first report of retention indices for the proprietary phase Rtx®-CLPesticides II column.

5.8. Data Analysis

5.8.1. Principal component analysis

Principal component analysis (PCA) plots were used to visualise the correlation between datasets. Three different plots were used namely, score plots, loading plots and biplots. The score plot shows the variance between datasets (different sampling sites), the loading plot shows the components (compounds) that contributed to the variance and the biplot is an overlay of the two previously mentioned plots. Points near the centre of the PCA loadings plot are considered to be similar, i.e. low variance. The further points are from the origin the higher the variance. Points grouped together in the score plot are considered similar. PCA was done on compounds detected during untargeted screening. Compounds were tentatively identified using the NIST14 library; spectral match quality of ≥ 70%.

The variance between different sampling sites was investigated. Data generated for the six sampling sites at Rietvlei Nature Reserve (samples collected on 6/2/2015) in the Gauteng Province and the Limpopo Province sampling sites (Albasini Dam and Nandoni Dam samples collected on 6/2/2015), utilising the PDMS loop and GC×GC-TOFMS, were used for PCA. The score plot (Figure 5.22; left) shows variance within and between the two provinces. This indicates a difference in the composition of the

surface water at the two geographically different sites. There is a noticeable difference between the surface water at Rietvlei Nature Reserve, Gauteng Province and the Limpopo Province sampling sites. The biplot (Figure 5.23) shows the compounds contributing to the difference at each site.

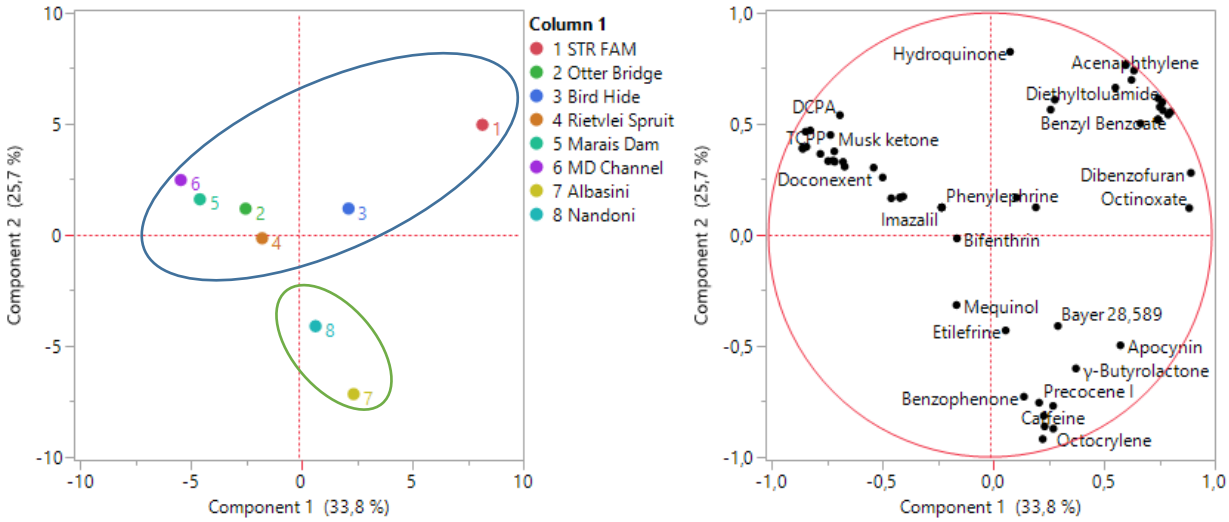


Figure 5.22: Score plot (left) and loading plot (right) showing the variance between Rietvlei Nature Reserve, Gauteng Province (blue ellipsoid) and Limpopo Province sites (green ellipsoid).

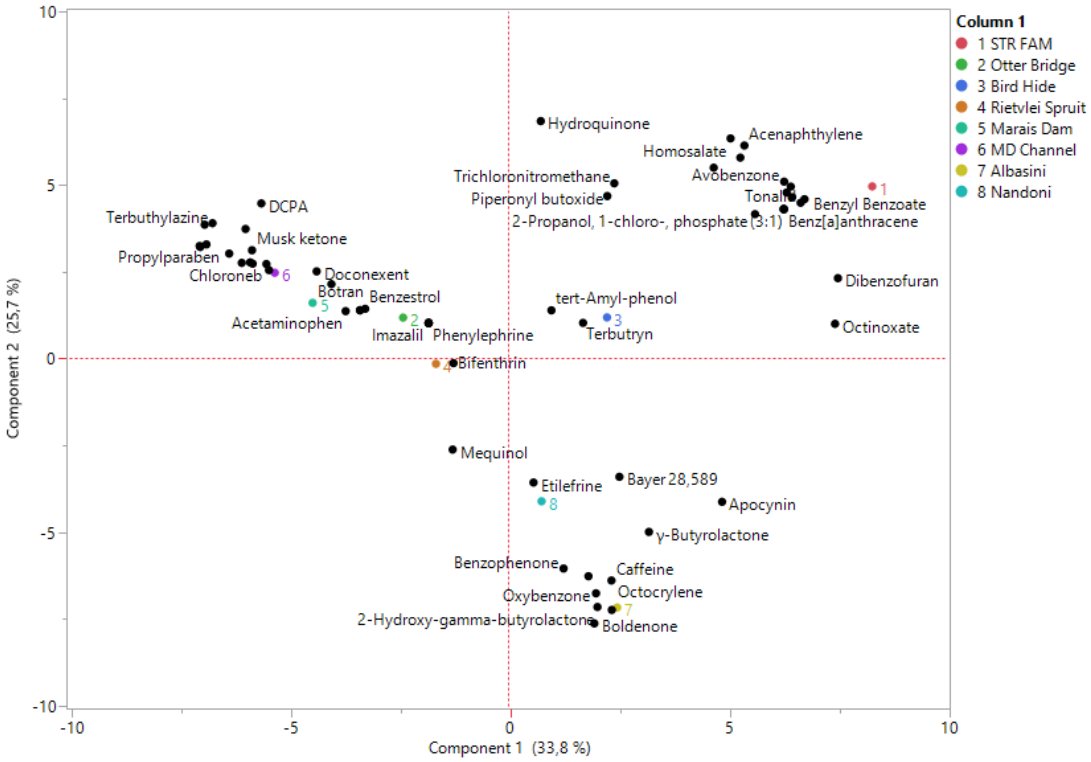


Figure 5.23: Biplot showing the variance and compounds contributing to the variance between the sampling sites.

The variance between different sampling periods was also investigated. Data generated for the six sampling sites at Rietvlei Nature Reserve collected on 6/2/2015 and on 15/3/2016 (approximately a year later), using the PDMS loop and GC×GC-TOFMS, were used for PCA. The score plot (Figure 5.24; left) shows variance within the Rietvlei Nature Reserve sampling sites for both periods and between the two sampling periods. This indicates a difference in the composition of the surface water at the different sites at different periods. The sampling on 15/3/2016 took place after a period of heavy rainfall which accounts for the difference in surface water composition. The biplot (Figure 5.25) shows the compounds contributing to the difference at each site.

The differences within the Rietvlei Nature Reserve sampling event is related to geographical location (refer to Chapter 4 Figure 4.4). For example the Marais Dam site is situated near the MD Channel site (numbers 5 and 6 in Figure 5.22 and Figure 5.24), Otter Bridge and Rietvlei Spruit sampling sites are located in the large stream between Rietvlei Dam and Marais Dam (numbers 2 and 4 in Figure 5.22 and Figure 5.24) and STR FAM and Bird Hide Stream sampling sites are situated in two different smaller streams in the reserve (numbers 1 and 3 in Figure 5.22 and Figure 5.24).

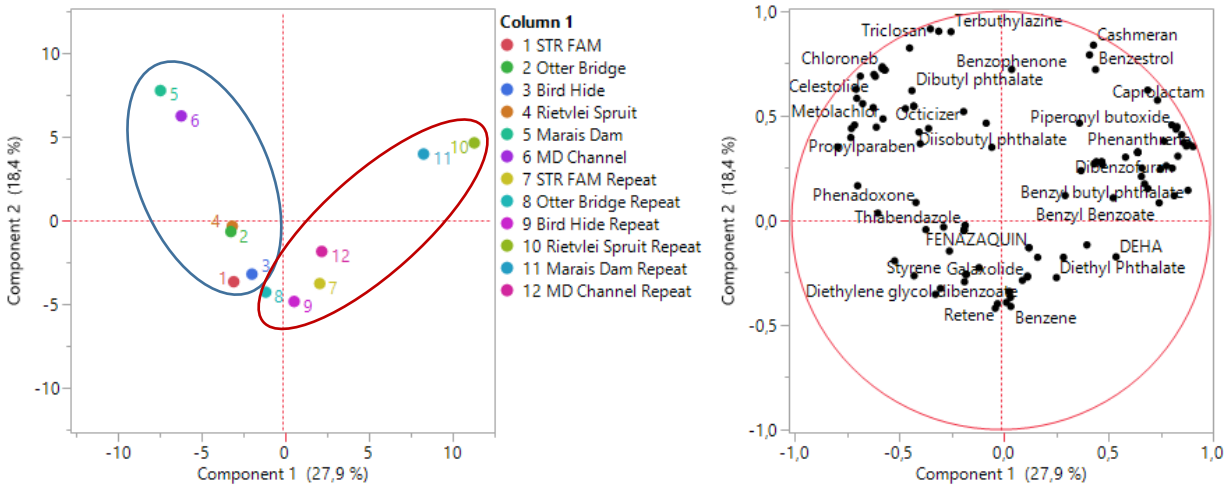


Figure 5.24: Score plot (left) and loading plot (right) showing the variance between two different sampling periods at Rietvlei Nature Reserve on 6/2/2015 (blue ellipsoid) and on 15/3/2016 (red ellipsoid).

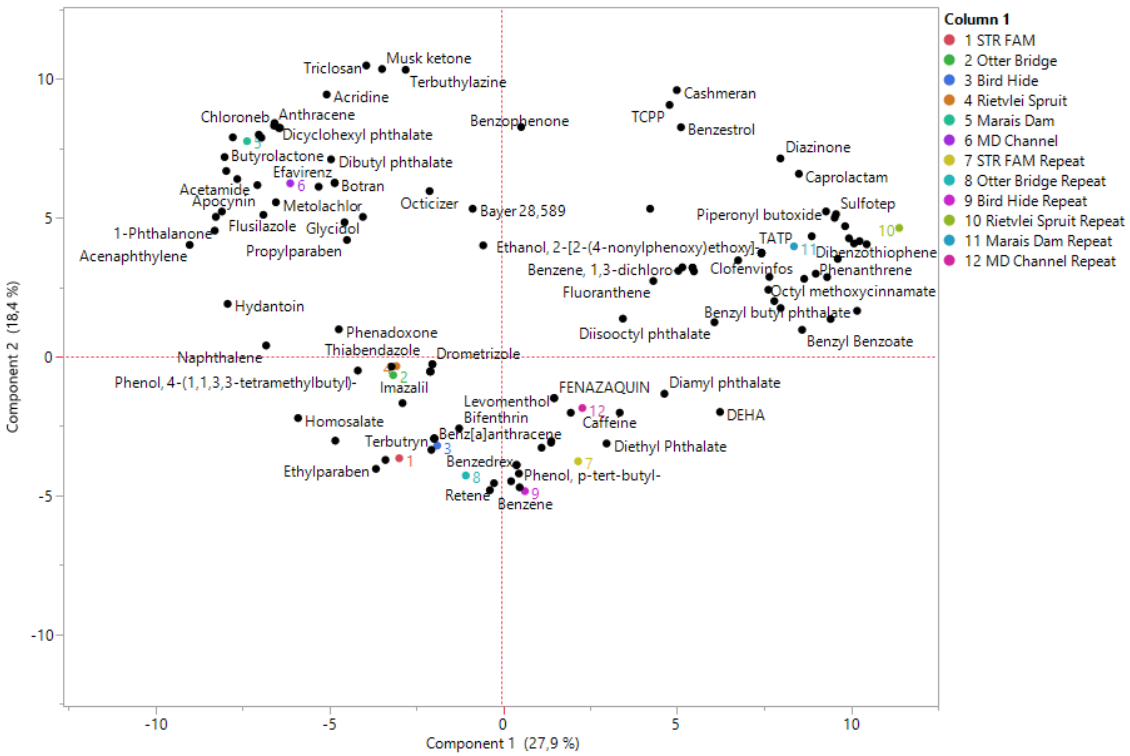


Figure 5.25: Biplot showing the variance and compounds contributing to the variance between the sampling sites and at two different sampling periods.

A broad range of compounds was detected during both sampling periods. Compounds detected included personal care products (e.g. the EU and the USA banned substance Triclosan), pharmaceuticals, ARVs, sunscreen ingredients, pesticides, hormones and fragrances, with many of these emerging or classified EDCs. DDT, the main suspect for endocrine disruption in South Africa [21, 22, 26], was not detected in any of the surface water samples. The broad range of compounds detected confirms that a single chemical is not the primary cause of oestrogenic activity reported in the Limpopo Province [20, 21] and in Rietvlei Nature Reserve [22, 23]. Oestrogenic activity is most likely due to additive effects from a range of chemical compounds. Monitoring of Marais Dam (Rietvlei Nature Reserve), Nandoni Dam and Albasini Dam is of importance due to the potential health risks posed to humans, due to the use of the dams for water sports, fishing and recreation, and to animals, as the dams and surrounding streams are important drinking water sources for wildlife. Sharp tooth catfish (*Clarias gariepinus*) and eland from Rietvlei Nature Reserve showed symptoms associated with chronic oestrogenic exposure [26].

Future monitoring at Rietvlei Nature Reserve and Limpopo Province is crucial in order to better understand the additive effect of a large range of pollutants on the health of humans and animals.

References

1. Montes-Grajales, D., Olivero-Verbel, J. *EDCs DataBank: 3D-Structure database of endocrine disrupting chemicals.* Toxicology, 2015. **327**: p. 87-94.
2. Colborn, T. *TEDX list of potential endocrine disruptors, the endocrine disruptor exchange*. Available from: http://endocrinedisruption.org/endocrine-disruption/tedx-list-of-potential-endocrine-disruptors/chemicalsearch. [Accessed on 2016/02/15]
3. Kiridena, W., DeKay, C., Patchett, C.C., Koziol, W.W., Qian, J., Poole, C.F. *Evaluation of the separation characteristics of application-specific (pesticides and dioxins) open-tubular columns for gas chromatography.* Journal of Chromatography A, 2006. **1128**(1–2): p. 228-235.
4. Matisová, E., Hrouzková, S. *Analysis of endocrine disrupting pesticides by capillary GC with mass spectrometric detection.* International Journal of Environmental Research and Public Health, 2012. **9**(9): p. 3166-3196.
5. Silvestro, L., Tarcomnicu, I., Savu, S.R. *Matrix Effects in Mass Spectrometry Combined with Separation Methods — Comparison HPLC, GC and Discussion on Methods to Control these Effects*. Tandem Mass Spectrometry - Molecular Characterization., ed. Coelho, A.V. 2013.
6. Van Hoeck, E., Canale, F., Cordero, C., Compernolle, S., Bicchi, C., Sandra, P. *Multiresidue screening of endocrine-disrupting chemicals and pharmaceuticals in aqueous samples by multi-stir bar sorptive extraction–single desorption–capillary gas chromatography/mass spectrometry.* Analytical and Bioanalytical Chemistry, 2009. **393**(3): p. 907-919.
7. Pintado-Herrera, M.G., González-Mazo, E., Lara-Martín, P.A. *Atmospheric pressure gas chromatography–time-of-flight-mass spectrometry (APGC–ToF-MS) for the determination of regulated and emerging contaminants in aqueous samples after stir bar sorptive extraction (SBSE).* Analytica Chimica Acta, 2014. **851**(0): p. 1-13.
8. Ochiai, N., Sasamoto, K., Kanda, H., Nakamura, S. *Fast screening of pesticide multiresidues in aqueous samples by dual stir bar sorptive extraction-thermal desorption-low thermal mass gas chromatography–mass spectrometry.* Journal of Chromatography A, 2006. **1130**(1): p. 83-90.
9. Ochiai, N., Sasamoto, K., Hirooki, K., Pfannkoch, E. *A Novel Extraction Procedure for Stir Bar Sorptive Extraction (SBSE): Sequential SBSE for Uniform Enrichment of Organic Pollutants in Water Samples*. 2008; Available from: http://www.gerstel.com/pdf/p-gc-an-2008-12corrected.pdf. [Accessed on 15/11/20]
10. Matuszewski, B.K., Constanzer, M.L., Chavez-Eng, C.M. *Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS.* Analytical Chemistry, 2003. **75**(13): p. 3019-3030.
11. WHO. *Guidelines for drinking-water quality 4th edition*. Geneva, Switzerland 2011; Available from: http://www.who.int/water_sanitation_health/publications/2011/dwq_guidelines/en/. [Accessed on 16/08/05]

12. EU. *Drinking Water Directive (Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption), Brussels, Belgium*. Available from: http://ec.europa.eu/environment/water/water-drink/legislation_en.html. [Accessed on 16/08/05]

13. EPA. *US Environmental Protection Agency: Drinking Water Contaminants*. Available from: https://www.epa.gov/ground-water-and-drinking-water/table-regulated-drinking-water-contaminants. [Accessed on 16/08/04]

14. Popek, E.P. *Sampling and analysis of environmental chemical pollutants: a complete guide*. 2003, San Diego: Academic Press.

15. Baltussen, E., Cramers, C., Sandra, P. *Sorptive sample preparation – a review.* Analytical and Bioanalytical Chemistry, 2002. **373**(1): p. 3-22.

16. Barreiros, L., Queiroz, J.F., Magalhães, L.M., Silva, A.M.T., Segundo, M.A. *Analysis of 17-β-estradiol and 17-α-ethinylestradiol in biological and environmental matrices — A review.* Microchemical Journal, 2016. **126**: p. 243-262.

17. Bicchi, C., Liberto, E., Cordero, C., Sgorbini, B., Rubiolo, P. *Stir-bar sorptive extraction and headspace sorptive extraction: An overview.* LC GC North America, 2009. **27**(5).

18. Ferreira, A.P. *Caffeine as an environmental indicator for assessing urban aquatic ecosystems.* Cadernos de Saúde Publica, 2005. **21**(6): p. 1884-1892.

19. *UNEP, Stockholm Convention on Persistent Organic Pollutants (POPs)*. in *Conference of the Parties at its fourth meeting, Geneva, Switzerland*. 2009.

20. Aneck-Hahn, N.H., Schulenburg, G.W., Bornman, M.S., Farias, P., De Jager, C. *Impaired semen quality associated with environmental DDT exposure in young men living in a malaria area in the Province, South Africa.* Journal of Andrology, 2007. **28**(3): p. 423-434.

21. Aneck-Hahn, N.H., Bornman, M., De Jager, C. *Oestrogenic activity in drinking waters from a rural area in the Waterberg District, Limpopo Province, South Africa.* Water SA, 2009. **35**(3): p. 245-251.

22. Aneck-Hahn, N.H., Bornman, M.S., De Jager, C. *Preliminary assessment of oestrogenic activity in water sources in Rietvlei Nature Reserve, Gauteng, South Africa.* African Journal of Aquatic Science, 2008. **33**(3): p. 249-254.

23. Kruger, T., Barnhoorn, I., Jansen van Vuren, J., Bornman, R. *The use of the urogenital papillae of male feral African sharptooth catfish (Clarias gariepinus) as indicator of exposure to estrogenic chemicals in two polluted dams in an urban nature reserve, Gauteng, South Africa.* Ecotoxicology and Environmental Safety, 2013. **87**: p. 98-107.

24. Kováts, E. *Gaschromatographische Charakterisierung organischer Verbindungen. Teil 1: Retentionsindices aliphatischer Halogenide, Alkohole, Aldehyde und Ketone.* Helvetica Chimica Acta, 1958. **41**(7): p. 1915.

25. Poole, C.F., Poole, S.K. *Separation characteristics of wall-coated open-tubular columns for gas chromatography.* Journal of Chromatography A, 2008. **1184**(1–2): p. 254-280.

26. Aneck-Hahn, N., De Jager, C., Barnhoorn, I., Bornman, M. *Endocrine disruptors and health: should South Africa be concerned?* 2016; Available from: http://www.ewisa.co.za/literature/files/216%20Aneck_Hahn.pdf. [Accessed on 16/02/15]

Chapter 6

Conclusion

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6. Conclusion

6.1. Final Remarks and Conclusion

In order to address environmental concerns due to the presence of micropollutants in South African aquatic systems, a combination of GC×GC-TOFMS and UHPLC-QTOFMS was used with novel (PDMS loop) and simplified (LVI) sample extraction techniques for the multi-residue analysis of surface water from South Africa.

Over 3 000 compounds were detected in South African surface water during an initial untargeted screening of the surface water at Rietvlei Nature Reserve, using sorptive sampling and GC×GCTOFMS analysis. The compounds detected included personal care products pharmaceuticals, sunscreen ingredients, pesticides, hormones and fragrances, with many of these emerging or classified EDCs. The broad range of compounds detected suggests a negative additive effect on the environment and, more seriously, the possibility of synergism enhancing the adverse effect of pollution.

To overcome the problems associated with not only solvent extraction, but also with expensive solvent free samplers, a PDMS sorptive sampling loop was constructed in-house for solvent free extraction of trace analytes from water. The PDMS sampler was shown to be comparable to the more expensive commercial SBSE Twister™ for the analysis of the chosen target analytes. The PDMS sampling loop is cheap (less than R11 each), easy to make and use, is disposable and can therefore eliminate carry-over from previous sampling. However, it should be noted that PDMS is suitable for sampling certain classes of analytes, thus the method is, to some extent, targeted.

The loop was thermally desorbed directly in the inlet liner of the GC, thus reducing sample introduction time and hence cryo-focussing and a commercial desorber were not required. Thermal desorption allows for transfer of the entire sample into the GC resulting in greater sensitivity when compared to injection of microliters amount of a solvent extract. However, the destructive nature of TD allows for a single analysis of a sampler as opposed to multiple injections of a solvent extract. Direct thermal desorption of the PDMS loop in the inlet liner of a GC was found to be a suitable alternative for sample introduction into a GC when compared to desorption with a commercial TDS.

Furthermore, large volume injection was demonstrated to be a suitable alternative to SPE with UHPLC-QTOFMS. The direct injection of surface water (100 µL), with negligible prior sample treatment, into an LC is an attractive approach as it avoids time-consuming sample preparation steps and reduces the amount of solvent used. In order to consider matrix effects when using minimal sample clean-up (when using LVI-UHPLC-QTOFMS) and to compensate for compound loss during sample preparation steps (when using PDMS loop with GC×GC-TOFMS) matrix matched calibrations were critical to obtain accurate quantitative results.

The in-house developed PDMS sorptive sampling loop with direct thermal desorption in the inlet liner of a GC×GC-TOFMS and LVI with UHPLC-QTOFMS were used as complementary methods for the detection of micropollutants, specifically EDCs and antiretroviral compounds, in surface water from real world locations at ultra-trace levels. The simplicity of the two complementary methods makes it highly suitable for implementation in environmental monitoring programmes. LODs for these methods ranged from ppq to ppt levels.

The levels of all quantified pesticides during this study were lower than the levels required by the EU directive, WHO guidelines and US EPA regulations for drinking water quality. However, a broad range of compounds was detected (quantification was not possible for the majority of the compounds as they were detected below LOQs) in the Limpopo Province and in Rietvlei Nature Reserve. PCA revealed a noticeable difference between the micropollutants (and their levels) found in the surface water at the metropolitan area (Rietvlei Nature Reserve, Gauteng Province) and the rural area (Limpopo Province). It should be noted that sampling was done after a heavy rainfall period at Rietvlei Nature Reserve and the concentrations measured might not be representative of average levels. Essentially the results reflect concentrations of grab samples, which are specific for a moment in time at a particular location.

6.2. Future Work

Future work will focus on expanding the range of target compound classes. Firstly, the multi-shot derivatisation method needs to be improved and the shortcomings (refer back to Chapter 5 Section 5.5.3) of the current method need to be addressed.

Derivatisation will greatly broaden the range of compounds that can be analysed by GC.

Secondly, the inlet sensitivity during direct TD can be increased by employing higher gas flow rates using pulsed splitless mode and investigating the time needed for TD. This will improve the response for compounds strongly retained on the PDMS. Loop desorption in the inlet liner can also be automated by e.g. using an automated liner exchange system.

Thirdly, for LC analysis, derivatisation with dansyl chloride may be used to improve the ionisation efficiency in positive mode for oestrogen detection as quantification of these compounds proved problematic with both LC and GC methods.

Lastly, the detection of high molecular weight compounds, such as clindamycin, requires optimised targeted LC-MS/MS methods for future research.

Performing further data mining on existing data should also be considered for future work. GC×GC-TOFMS enabled the tentative identification of hundreds of compounds at each sampling site when using the NIST14 library. These compounds can only be unequivocally identified by comparison with standards – specific focus should be placed on compounds with known adverse health effects such as endocrine disruption – or further identified using linear retention indices. UHPLC-QTOFMS analysis of each sample site also gave an exhaustive list of unknown compounds. Using the MS^E function during all sample analysis gave a large amount of data, specifically fragmentation patterns. The fragmentation patterns of the unknown compounds can be compared to MS/MS libraries or standards for identification.

The list of identified micropollutants in the South African aquatic system may be augmented by using the data generated during this study. Bioassays could be done in future to determine endocrine disrupting effects, or any other adverse health effects, of these compounds and to assess for any additive or synergistic effects of already established EDCs present in the water systems. Future monitoring at Rietvlei Nature Reserve and in the Limpopo Province is required as the large range of pollutants detected can potentially affect humans and the environment in a way not fully described by consideration of compounds tested individually.

Addendum A: Certificates of Analysis for the Certified reference standards

SIGMA-ALDRICH®

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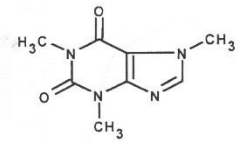
3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Caffeine solution
1.0 mg/mL±5% in methanol
drug standard

Catalog Number **C6035**
Lot Number 029K8740
Storage Temperature 2–8 °C

CAS RN 58-08-2
Synonyms: Coffeinum; Guaranine; Methyltheobromine



Product Description
Molecular Formula: $C_8H_{10}N_4O_2$
Molecular Weight: 194.19

DEA Class: Formulation of a non-controlled substance

Concentration: 0.98 mg of caffeine/mL of GC grade methanol, concentration verified by UV.

Expiration Date: January 2012

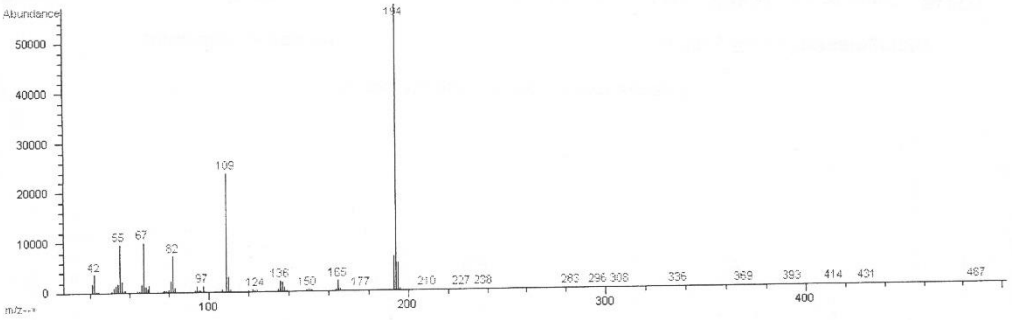
Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
Store the product at 2–8 °C.

If stored at 2–8 °C in an airtight container, protected from light, decomposition of the product is less than 1% in 48 months. After opening, concentration may change due to loss of solvent.

CMW,MAM 03/09-1

70 eV Electron Ionization Mass Spectrum



Hewlett-Packard 5890 Series II Gas Chromatograph
Hewlett-Packard 5971 Mass Selective Detector
Hewlett-Packard G1034C MS ChemStation (HP-DOS series)

SIGMA-ALDRICH

CERTIFICATE OF ANALYSIS

Sigma-Aldrich Laborchemikalien GmbH D-30918 Seelze
Telefon: +49 5137 8238-150

Seelze, 13.11.2012/450618/12/23337

Order-No.:
Customer-No.:

Order-Code:

Quantity:

Production Date: 26.Oct.2012
Expiry Date: 26.Oct.2017

Article/Product: 34314 Batch : SZBC300XV

Bifenthrin PESTANAL®

Reference Material (RM)

1. General Information

Formula: C23H22ClF3O2 Molar mass: 422.87 g/Mole
CAS-No.: [82657-04-3] Recomm. storage temp.: roomtemp.
Usage : Insecticide/Acaricide

The estimated uncertainty of a single measurement of the assay can be expected to be 0.5 % relative (confidence level = 95%, n= 6) whereby the assay measurements are calculated by 100% minus found impurities.

2. Batch Analysis

Identity (NMR)	complying	
Assay (GC)	98.8	area %
Melting range	68.3-69.2	°C
Water (Karl Fischer)	<0.1	%
Date of Analysis	09.Nov.2012	

3. Advice and Remarks

- The expiry date is based on the current knowledge and holds only for proper storage conditions in the originally closed flasks/ packages.
- Whenever the container is opened for removal of aliqout portions of the substance, the person handling the substance must assure, that the integrity of the substance is maintained and proper records of all its handlings are kept. Special care has to be taken to avoid any contamination or adulteration of the substance.
- We herewith confirm that the delivery is effected according to the technical delivery conditions agreed.
- Particular properties of the products or the suitability for a particular area of application are not assured.
- We guarantee a proper quality within our General Conditions of Sales.

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Telefon: +49 5137 8238-150

Seelze, 02.12.2011/278771/11/26451

Order-No.:
Customer-No.:

Order-Code:

Quantity:

Production Date: 17.Nov.2011
Expiry Date: 17.Nov.2016

Article/Product: 45548 Batch : SZBB321XV

Lindane PESTANAL® (gamma-1,2,3,4,5,6-hexachlorocyclohexane)

Reference Material (RM)

1. General Information

Formula: C6H6Cl6
CAS-No.: [58-89-9]
Usage : Insecticide

Molar mass: 290.83 g/Mole
Recomm. storage temp.: roomtemp.

The estimated uncertainty of a single measurement of the assay can be expected to be 0.5 % relative (confidence level = 95%, n= 6) whereby the assay measurements are calculated by 100% minus found impurities.

2. Batch Analysis

Identity (NMR)	complying	
Assay (GC)	99.8	area %
Melting range	112.9-113.7	°C
Water (Karl Fischer)	0.02	%
Date of Analysis	01.Dec.2011	

3. Advice and Remarks

- The minimum shelf life is based on the current knowledge and holds only for proper storage conditions in the originally closed flasks/ packages.
- Whenever the container is opened for removal of aliqout portions of the substance, the person handling the substance must assure, that the integrity of the substance is maintained and proper records of all its handlings are kept. Special care has to be taken to avoid any contamination or adulteration of the substance.
- We herewith confirm that the delivery is effected according to the technical delivery conditions agreed.
- Particular properties of the products or the suitability for a particular area of application are not assured.
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Seelze, 07.03.2014/518743/14/03624

Order-No.:
Customer-No.:

Order-Code:

Quantity:

Production Date: 13.Feb.2014
Expiry Date: 13.Feb.2019

Article/Product: 36163 Batch : SZBE044XV

Metolachlor PESTANAL®

Reference Material (RM)

1. General Information

Formula: C15H22ClNO2 Molar mass: 283.79 g/Mole
CAS-No.: [51218-45-2] Recomm. storage temp.: roomtemp.
Usage : Herbicide

The estimated uncertainty of a single measurement of the assay can be expected to be 1 % relative (confidence level = 95%, n= 6) whereby the assay measurements are calculated by 100% minus found impurities.

2. Batch Analysis

Identity (NMR)	complying	
Assay (HPLC)	97.6	area %
Refractive index (n 25/D)	1.5275	
Water (Karl Fischer)	0.2	%
Date of Analysis	06.Mar.2014	

3. Advice and Remarks

- The expiry date is based on the current knowledge and holds only for proper storage conditions in the originally closed flasks/ packages.
- Whenever the container is opened for removal of aliqout portions of the substance, the person handling the substance must assure, that the integrity of the substance is maintained and proper records of all its handlings are kept. Special care has to be taken to avoid any contamination or adulteration of the substance.
- We herewith confirm that the delivery is effected according to the technical delivery conditions agreed.
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Seelze, 14.05.2013/482614/13/08734

Order-No.:
Customer-No.:

Order-Code:

Quantity:

Production Date: 17.Apr.2013
Expiry Date: 17.Apr.2018

Article/Product: 45678 Batch : SZBD107XV

Terbuthylazine PESTANAL®

Reference Material (RM)

1. General Information

Formula: C9H16ClN5
CAS-No.: [5915-41-3]
Usage : Herbicide

Molar mass: 229.71 g/Mole
Recomm. storage temp.: roomtemp.

The estimated uncertainty of a single measurement of the assay can be expected to be 0.5 % relative (confidence level = 95%, n= 6) whereby the assay measurements are calculated by 100% minus found impurities.

2. Batch Analysis

Identity (NMR)	complying
Assay (GC)	99.4 area %
Melting range	176.2-177.1 °C
Water (Karl Fischer)	0.2 %
Date of Analysis	08.May.2013

3. Advice and Remarks

- The expiry date is based on the current knowledge and holds only for proper storage conditions in the originally closed flasks/ packages.
- Whenever the container is opened for removal of aliqout portions of the substance, the person handling the substance must assure, that the integrity of the substance is maintained and proper records of all its handlings are kept. Special care has to be taken to avoid any contamination or adulteration of the substance.
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Seelze, 08.01.2014/511045/13/26669

Order-No.:
Customer-No.:

Order-Code:

Quantity:

Production Date: 09.Dec.2013
Expiry Date: 09.Dec.2018

Article/Product: 45395 Batch : SZBD343XV

Chlorpyriphos PESTANAL®

Reference Material (RM)

1. General Information

Formula: C9H11Cl3NO3PS
CAS-No.: [2921-88-2]
Usage : Insecticide

Molar mass: 350.59 g/Mole
Recomm. storage temp.: 2-8 °C

The estimated uncertainty of a single measurement of the assay can be expected to be 0.5 % relative (confidence level = 95%, n= 6) whereby the assay measurements are calculated by 100% minus found impurities.

2. Batch Analysis

Identity (NMR)	complying	
Assay (HPLC)	99.7	area %
Melting range	42.1-43.3	°C
Water (Karl Fischer)	<0.1	%
Date of Analysis	06.Jan.2014	

3. Advice and Remarks

- The expiry date is based on the current knowledge and holds only for proper storage conditions in the originally closed flasks/ packages.
- Whenever the container is opened for removal of aliqout portions of the substance, the person handling the substance must assure, that the integrity of the substance is maintained and proper records of all its handlings are kept. Special care has to be taken to avoid any contamination or adulteration of the substance.
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Sigma-Aldrich Laborchemikalien GmbH D-30918 Seelze
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Seelze, 25.06.2013/488046/13/12641

Order-No.:
Customer-No.:

Order-Code:

Quantity:

Production Date: 07.Jun.2013
Expiry Date: 07.Jun.2018

Article/Product: 45330 Batch : SZBD158XV

Atrazine PESTANAL®

Reference Material (RM)

1. General Information

Formula: C8H14ClN5 Molar mass: 215.68 g/Mole
CAS-No.: [1912-24-9] Recomm. storage temp.: roomtemp.
Usage : Herbicide

The estimated uncertainty of a single measurement of the assay can be expected to be 0.5 % relative (confidence level = 95%, n= 6) whereby the assay measurements are calculated by 100% minus found impurities.

2. Batch Analysis

Identity (NMR)	complying	
Assay (HPLC)	99.1	area %
Melting range	175.3-177.6	°C
Water (Karl Fischer)	0.19	%
Date of Analysis	24.Jun.2013	

3. Advice and Remarks

- The expiry date is based on the current knowledge and holds only for proper storage conditions in the originally closed flasks/ packages.
- Whenever the container is opened for removal of aliqout portions of the substance, the person handling the substance must assure, that the integrity of the substance is maintained and proper records of all its handlings are kept. Special care has to be taken to avoid any contamination or adulteration of the substance.
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Seelze, 16.01.2014/512188/13/27413

Order-No.:
Customer-No.:

Order-Code:

Quantity:

Production Date: 18.Dec.2013
Expiry Date: 18.Dec.2018

Article/Product: 46377 Batch : SZBD352XV

Moschus ketone solution 100 ng/µl in acetonitrile, PESTANAL®

Reference Material (RM)

1. General Information

Formula:

Molar mass: g/Mole

CAS-No.:

Recomm. storage temp.: 2-8 °C

Usage :

2. Batch Analysis

concentration	95	ng/µl ± 5 %
purity (HPLC)	98.6	%
Refractive index (n 20/D)	1.3439	
Date of Analysis	15.Jan.2014	

3. Advice and Remarks

- The expiry date is based on the current knowledge and holds only for proper storage conditions in the originally closed flasks/ packages.
- Whenever the container is opened for removal of aliqout portions of the substance, the person handling the substance must assure, that the integrity of the substance is maintained and proper records of all its handlings are kept. Special care has to be taken to avoid any contamination or adulteration of the substance.
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CERTIFICATE OF ANALYSIS

Terbutryne

CATALOG NUMBER	N-13514-1G
LOT NUMBER	56800
DATE CERTIFIED	03/01/12
EXPIRATION DATE	03/01/17
CAS NUMBER	886-50-0
MOLECULAR FORMULA	C10H19N5S
MOLECULAR WEIGHT	241.40
STORAGE	Store in a cool dry place.
HANDLING	See MSDS.
INTENDED USE	For laboratory use only.

Analytical Test	Value
% PURITY (HPLC)	98.1

Chem Service, Inc. guarantees the purity to be +/- 0.5% deviation prior to the expiration date shown on the label and exclusive of any customer contamination.

Certified By:

John Conrad

John Conrad
CSM/TC

COA Form
Revision 1 (01/2013)

ISO 9001
Certificate Number: 31610

ISO/IEC 17025
Accreditation Number: 83829

Certificate of Analysis

Dr. Ehrenstorfer

Reference Materials for Residue Analysis

Product Identification

13213230 Estrone

CA 3-Hydroxy-estra-1,3,5(10)-trien-17-one

IUPAC Delta-1,3,5-estratrien-3-beta-ol-17-one

Formula C18H22O2

Mol.Weight 270.4

CAS No. 53-16-7

Expiry Date 02.04.2018

Lot Number 40228

Store at 20 °C ±4 °C

Please note: The expiry date is valid under recommended storage conditions only.

Toxicological Data

R Code 45-60-61

S Code 53-45

LD50 (Rats female/male in mg/kg) N/A

Physical Data

Phase crystalline solid

Color colourless

Melt.Range dec.255.6 °C

Vapour pressure N/A at °C

Solubility in water N/A g/l at °C

Boiling Range (lit.)

Analytical Data

Detection: HPLC/DAD

Column: ReproSil 100 C18 5µ 250x3

Inj.-Vol.: 10.00 µl

Flow: 1.0 ml/min

Ret.-Time: **1.58 min.**

Method Details:

Acetonitrile:H2O 4:1

Identity: RT, UV

Comment Purity was confirmed by external standard method.

Water Content 0.0 % Determined by Karl-Fischer Titration

Det. Purity 99.5 % Tolerance/Uncertainty +/- 0.5 %

The uncertainty/tolerance of this standard is calculated in accordance with the EURACHEM/CITAC Guide - Quantifying Uncertainty in Analytical Measurement - Second Edition. The uncertainty given is the expanded combined uncertainty and represents an estimated standard deviation equal to the positive square root of the total variance of the uncertainty of components. The expanded uncertainty is U which is Uc(y)*K, where K is the coverage factor at the 95% confidence level (K=2). The expanded uncertainty is based on the combination of uncertainties associated with each individual operation involved in the preparation of this product.

Certified on 02.04.2014

by M. Beck M. Beck

Authorized copy from the original
30. APR. 2015
Sign.:

The Laboratory Labor Dr. Ehrenstorfer-Schäfers is accreditated by DAkkS as indicated by the Accreditation Number D-RM-14174-01 has shown competence based on ISO Guide 34:2009 with relevant parts of DIN EN ISO/IEC 17025:2005 for production of certified reference materials in form of organic pure substances and in form of single and multi-component solutions organic pure substances.

Labor Dr. Ehrenstorfer-Schäfers · Bgm.-Schlosser-Str. 6 A · 86199 Augsburg · Germany
Phone +49 821 906080 · Fax +49 821 9060888 · info@analytical-standards.com

The warranty for this product is limited to the purchasing price of this product.

Certificate of Analysis

Dr. Ehrenstorfer

Reference Materials for Residue Analysis

Product Identification

14202800 Hexestrol

CA meso-3,4-Bis(4-hydroxyphenyl)hexane

IUPAC 4,4'-(1,2-Diethylethylene)diphenol

Formula C18H22O2

Mol.Weight 270.37

CAS No. 84-16-2

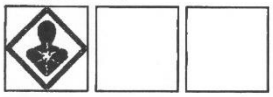
Expiry Date 31.01.2018

Lot Number 31105

Store at 20 °C ±4 °C

Please note: The expiry date is valid under recommended storage conditions only.

Toxicological Data



R Code 40-48

S Code 22-24/25-53

LD50 (Rats female/male in mg/kg) N/A

Physical Data

Phase	crystalline solid	Vapour pressure N/A at °C
Color	colourless	Solubility in water N/A g/l at °C
Melt.Range	dec.183.9 °C	Boiling Range (lit.)

Analytical Data

Detection:	HPLC/DAD	Method Details:
Column:	ReproSil 100 C18 5µ 250x3	Acetonitrile:H2O 4:1
Inj.-Vol.:	10.00 µl	
Flow:	1.0 ml/min	
Ret.-Time:	**1.42 min.**	

Identity: UV, RT

Comment Purity was confirmed by external standard method

Water Content	0.1 %	Determined by Karl-Fischer Titration
Det. Purity	99.8 %	Tolerance/Uncertainty +/- 0.5 %

The uncertainty/tolerance of this standard is calculated in accordance with the EURACHEM/CITAC Guide - Quantifying Uncertainty in Analytical Measurement - Second Edition. The uncertainty given is the expanded combined uncertainty and represents an estimated standard deviation equal to the positive square root of the total variance of the uncertainty of components. The expanded uncertainty is U which is Uc(y)*K, where K is the coverage factor at the 95% confidence level (K=2). The expanded uncertainty is based on the combination of uncertainties associated with each individual operation involved in the preparation of this product.

Certified on 31.01.2014

by P. Feuerriegel

The Laboratory Labor Dr. Ehrenstorfer-Schäfers is accreditated by DAkkS as indicated by the Accreditation Number D-RM-14174-01 has shown competence based on ISO Guide 34:2009 with relevant parts of DIN EN ISO/IEC 17025:2005 for production of certified reference materials in form of organic pure substances and in form of single and multi-component solutions organic pure substances.

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Phone +49 821 906080 · Fax +49 821 9060888 · info@analytical-standards.com

Certificate of Analysis

Dr. Ehrenstorfer

Reference Materials for Residue Analysis

Product Identification

10247000 4-tert-Amylphenol
CA Phenol,4-(1,1-dimethylpropyl)-
IUPAC 4-(1,1-dimethylpropyl)-phenol
Formula C11H16O
Mol.Weight 164.25
CAS No. 80-46-6

Expiry Date 08.04.2019
Lot Number 30325
Store at 20 °C ±4 °C

Please note: The expiry date is valid under recommended storage conditions only.

Toxicological Data

R Code 21-22-34
S Code 25-36-45
LD50 (Rats female/male in mg/kg) 1830

Physical Data

Phase	crystalline solid	Vapour pressure N/A at °C	
Color	yellowish	Solubility in water N/A g/l at °C	
Melt.Range	94.7 °C	Boiling Range (lit.)	

Analytical Data

Detection: GC/MSD
Column: DB-5, 30 m, ID 0.25 mm
Inj.-Vol.: 1.00 µl
Flow: 1.0 ml/min
Ret.-Time: 12.89 min.

Method Details:
Injector: 280° C
Start Temperature: 60° C for 5 min
End Temperature: 280° C for 1 min
Gradient: 15° C/min

Identity: MS, RT
Comment Purity was determined by external standard method.

Water Content 0.1 % Determined by Karl-Fischer Titration
Det. Purity 99.5 % Tolerance/Uncertainty +/- 0.5 %

The uncertainty/tolerance of this standard is calculated in accordance with the EURACHEM/CITAC Guide - Quantifying Uncertainty in Analytical Measurement - Second Edition. The uncertainty given is the expanded combined uncertainty and represents an estimated standard deviation equal to the positive square root of the total variance of the uncertainty of components. The expanded uncertainty is U which is Uc(y)*K, where K is the coverage factor at the 95% confidence level (K=2). The expanded uncertainty is based on the combination of uncertainties associated with each individual operation involved in the preparation of this product.

Certified on 08.04.2013
by A. Storr

The Laboratory Labor Dr. Ehrenstorfer-Schäfers is accreditated by DAkkS as indicated by the Accreditation Number D-RM-14174-01 has shown competence based on ISO Guide 34:2009 with relevant parts of DIN EN ISO/IEC 17025:2005 for production of certified reference materials in form of organic pure substances and in form of single and multi-component solutions organic pure substances.

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The warranty for this product is limited to the purchasing price of this product.

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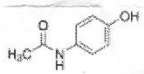
Certificate of Analysis

Product Name:
Acetaminophen - analytical standard



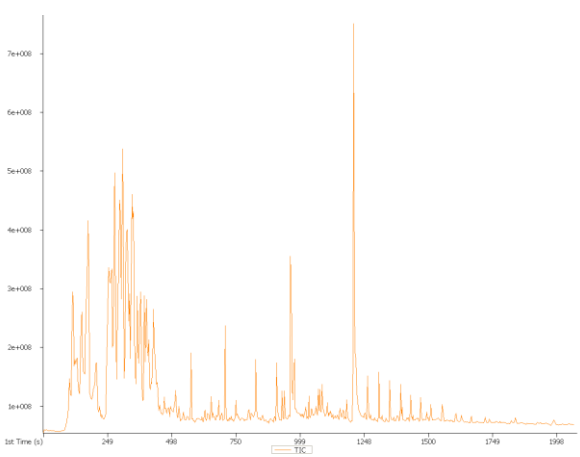
Product Number:	**A3035**
Batch Number:	**SLBK4475V**
Brand:	SIAL
CAS Number:	103-90-2
MDL Number:	MFCD00002328
Formula:	C8H9NO2
Formula Weight:	151.16 g/mol
Quality Release Date:	08 AUG 2014
Recommended Retest Date:	AUG 2019

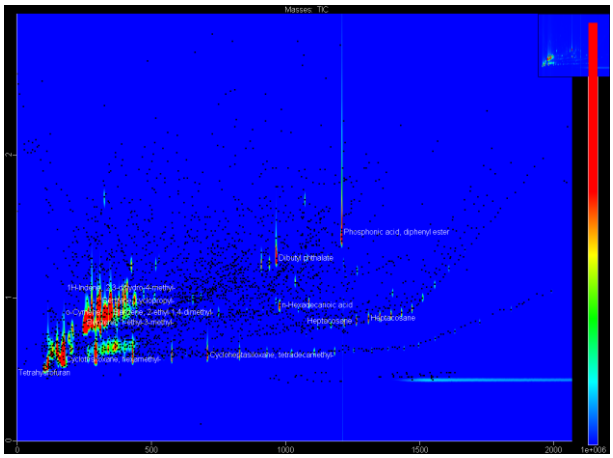
Test	Specification	Result
Infrared Spectrum	Compares to Reference Standard	Conforms
Identification B	Pass	Pass
Loss on Drying	≤ 0.5 %	0.0 %
Residue on Ignition	≤ 0.1 %	0.0 %
Heavy Metals	< = 10 ppm	< = 10 ppm
Free P-Aminophenol	≤ 0.005 %	0.000 %
Organic Impurities	Pass	Pass
Residual Solvents	Meets Requirements	Class 3 Solvents Only, < = 0.5%
Assay Dried basis	98.0 - 102.0 %	100.2 %
Note This product meets USP specifications (current through USP: 37, supplement: 1) and is traceable to USP Reference Standard lot: KOI244	----------	----------

Remarks:
Note: Supplemental spectral image(s) are available upon request.

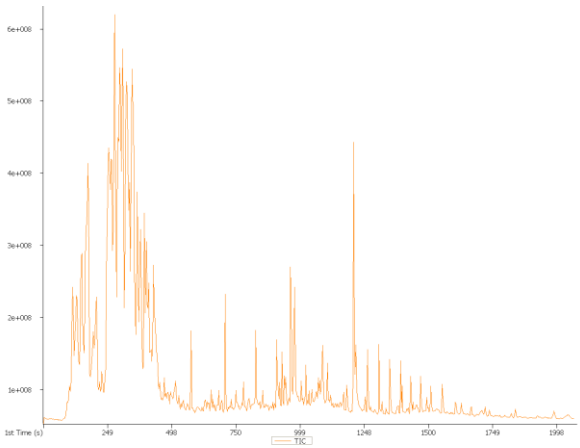
Addendum B: Reconstructed 1st dimension TICs and contour plots of the screening of surface water from the six sampling sites at Rietvlei Nature Reserve, Gauteng and from Albasini and Nandoni Dams, Limpopo Province

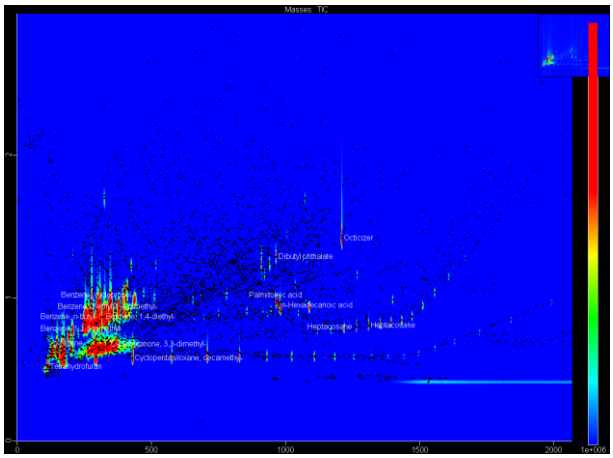
1. STR FAM



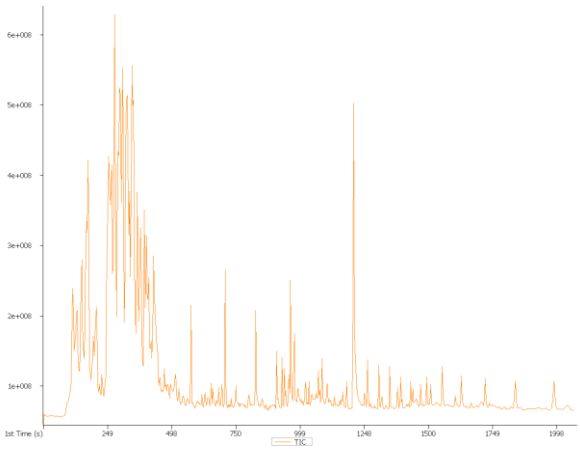


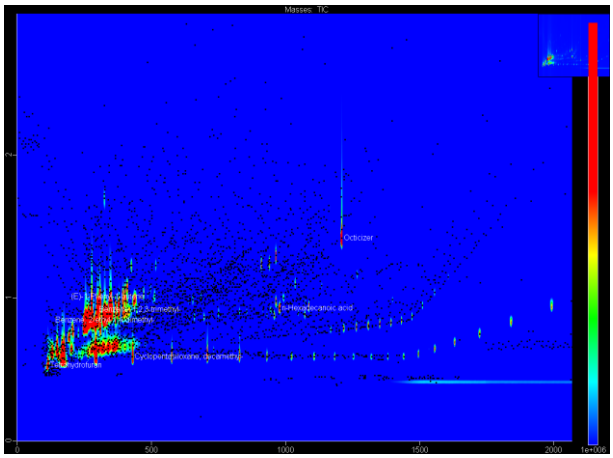
2. Otter Bridge



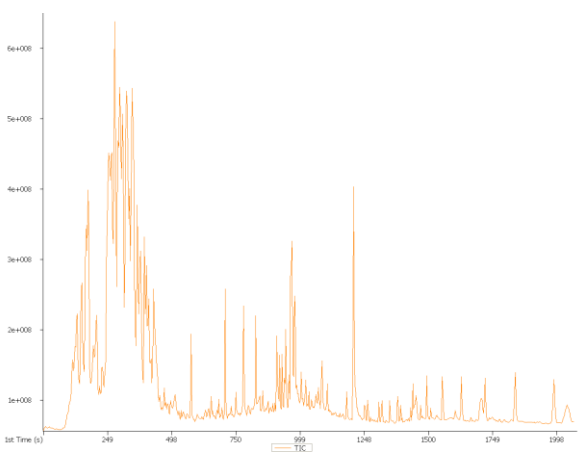


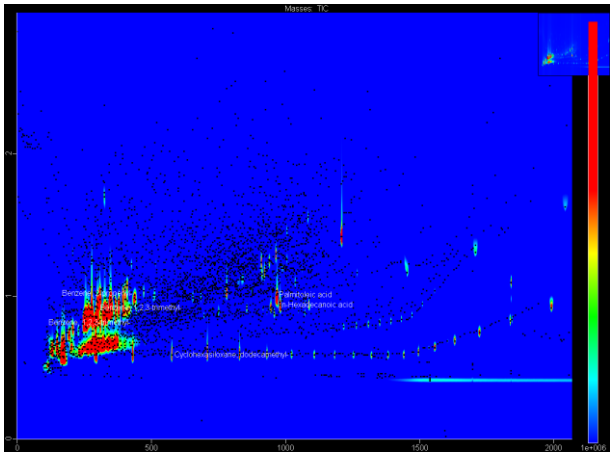
3. Bird Hide Stream



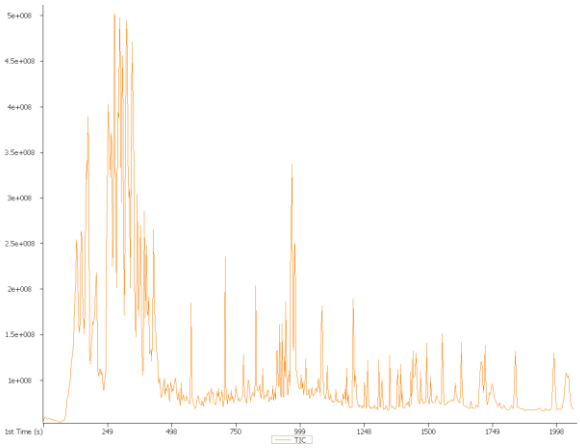


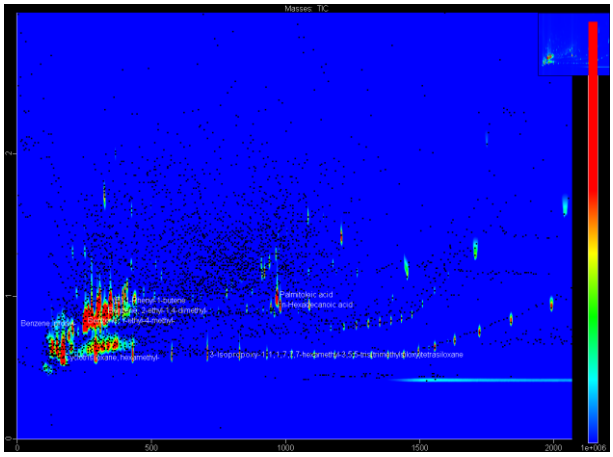
4. Rietvlei Spruit



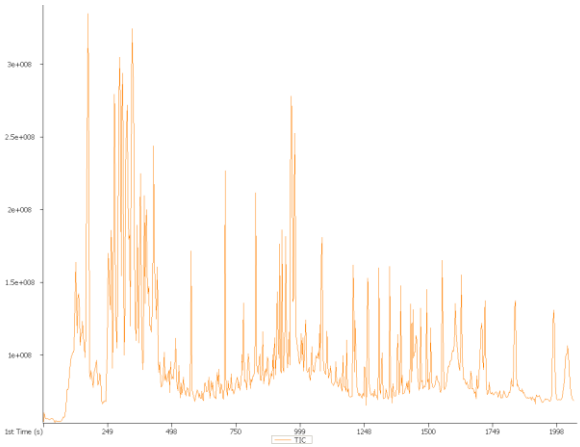


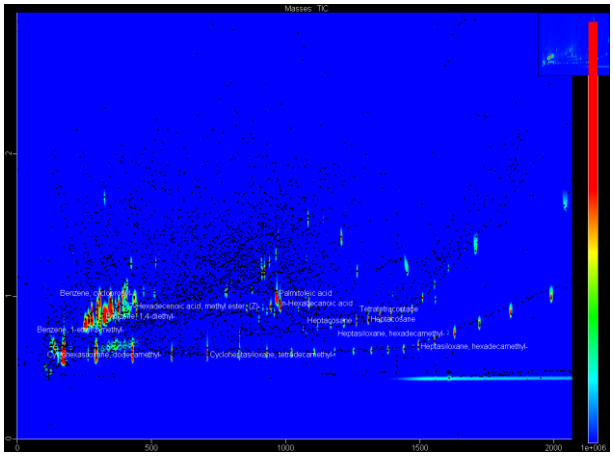
5. Marais Dam (MD)



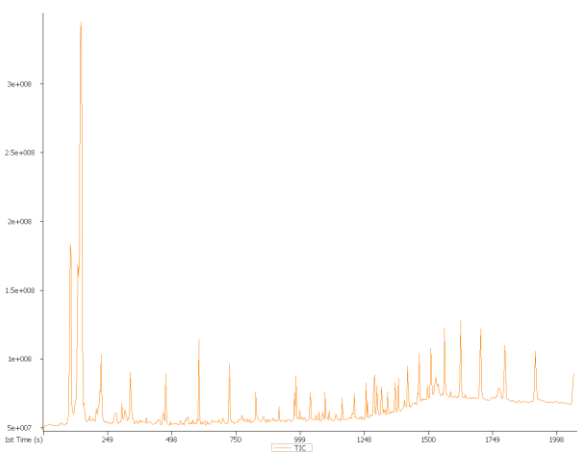


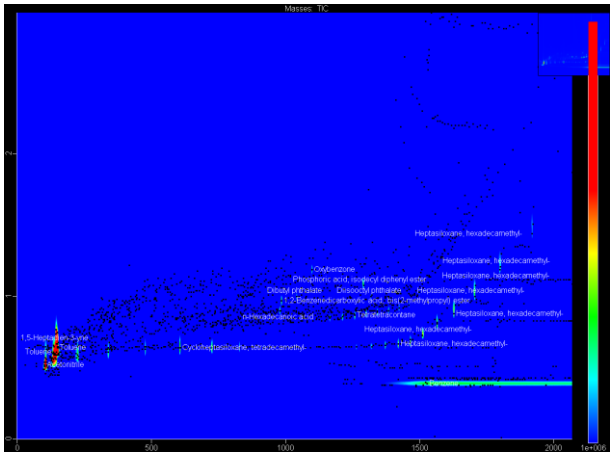
6. MD Channel



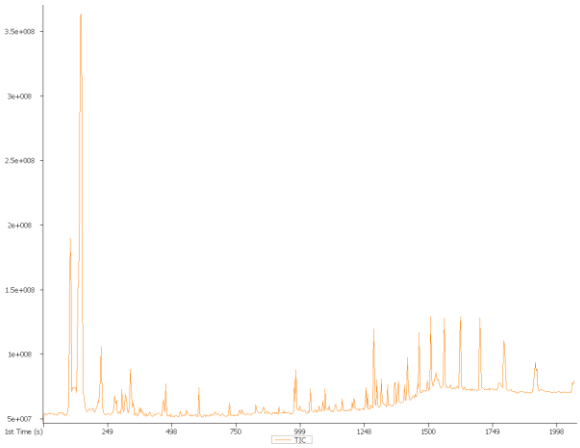


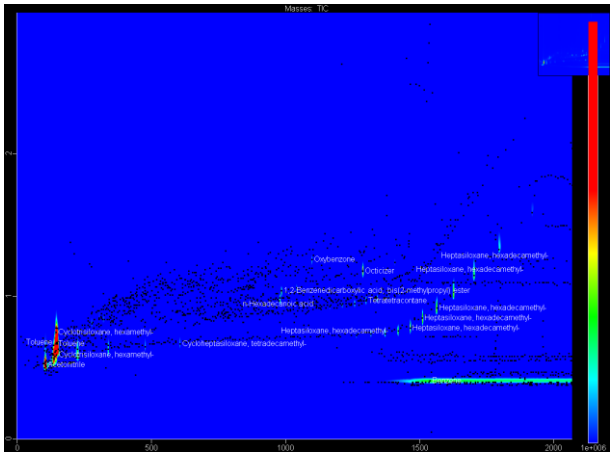
7. Albasini Dam





8. Nandoni Dam





Addendum C: Compounds tentatively identified in surface water from Rietvlei Nature Reserve by sorptive extraction and GCXGC-TOFMS

					Sample name:	20150420C SBSE seq NaCl 1 STR FAM 500mL	20150515A Loop seq NaCl 1 STR FAM TDS 500mL	20150421A SBSE seq NaCl 2 Otter Bridge 500mL	20150515B Loop seq NaCl 2 Otter Bridge 500 mL TDS	20150421B SBSE seq NaCl 3 Bird Hide 500mL	20150515C Loop seq NaCl 3 Bird Hide 500ml TDS	20150421C SBSE seq NaCl 4 Rietvlei Spruit 500mL	20150518A Loop seq NaCl 4 Rietvlei spruit 500mL TDS	20150422A SBSE seq NaCl 5 Marais Dam 500mL	20150518B Loop seq NaCl 5 Marais Dam 500mL TDS	20150423A SBSE seq NaCl 6 MD Channel 500mL	20150521A Loop seq NaCl 6 MD Channel 500mL TDS
Compound name	**Formula**	**Weight**	**CAS**	**Comments/uses**		**Similarity***	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**
1-Naphthalenol**	$C_{10}H_8O$	144	90-15-3	Metabolite of the insecticide carbaryl and naphthalene		n.d.***	n.d.	n.d.	n.d.	764	n.d.	n.d.	n.d.	779	n.d.	n.d.	n.d.
2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	102	19444-84-9	Prodrug for γ-hydroxybutyric acid (GHB)/ solvent		858	846	833	818	835	839	735	813	815	845	849	837
2-Propanol, 1-chloro-, phosphate (3:1)	$C_9H_{18}Cl_3O_4P$	326	13674-84-5	Flame retardant (TCPP)		742	n.d.	894	883	815	791	896	797	896	838	917	878
2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester	$C_{18}H_{26}O_3$	290	5466-77-3	Ultraviolet filter (Octinoxate)		946	942	928	942	937	944	892	916	934	938	923	946
4(1H)-Quinazolinone	$C_8H_6N_2O$	146	491-36-1	Pharmaceutical/ sedative		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	833	n.d.	n.d.	n.d.
4H-Inden-4-one, 1,2,3,5,6,7-hexahydro-1,1,2,3,3-pentamethyl-	$C_{14}H_{22}O$	206	33704-61-9	Synthetic musk fragrance (Cashmeran)		n.d.	n.d.	n.d.	813	n.d.	n.d.	831	794	818	843	848	840
Acenaphthylene	$C_{12}H_8$	152	208-96-8	PAH		805	866	n.d.	811	844	813	771	804	n.d.	759	785	756
Acetaminophen	$C_8H_9NO_2$	151	103-90-2	Pharmaceutical/an algesic		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	769	n.d.	n.d.
Apocynin	$C_9H_{10}O_3$	166	498-02-2	Pharmaceutical		885	829	791	836	828	810	840	n.d.	851	892	899	845
Atrazine	$C_8H_{14}ClN_5$	215	1912-24-9	Herbicide		n.d.	n.d.	733	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Avobenzone	$C_{20}H_{22}O_3$	310	70356-09-1	PCP/sunscreen		n.d.	867	n.d.	794	n.d.	797	n.d.	n.d.	779	n.d.	n.d.	805

Sample name:					20150420C SBSE seq NaCl 1 STR FAM 500mL	20150515A Loop seq NaCl 1 STR FAM TDS 500mL	20150421A SBSE seq NaCl 2 Otter Bridge 500mL	20150515B Loop seq NaCl 2 Otter Bridge 500 mL TDS	20150421B SBSE seq NaCl 3 Bird Hide 500mL	20150515C Loop seq NaCl 3 Bird Hide 500ml TDS	20150421C SBSE seq NaCl 4 Rietvlei Spruit 500mL	20150518A Loop seq NaCl 4 Rietvlei spruit 500mL TDS	20150422A SBSE seq NaCl 5 Marais Dam 500mL	20150518B Loop seq NaCl 5 Marais Dam 500mL TDS	20150423A SBSE seq NaCl 6 MD Channel 500mL	20150521A Loop seq NaCl 6 MD Channel 500mL TDS
Compound name	**Formula**	**Weight**	**CAS**	**Comments/uses**	**Similarity***	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**
Bayer 28,589	$C_{14}H_{21}NO_3$	251	728-40-5	Miticide	841	808	768	n.d.	820	n.d.	n.d.	754	750	774	740	789
Benz[a]anthracene	$C_{18}H_{12}$	228	56-55-3	PAH	800	831	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	816	n.d.
Benzedrex	$C_{10}H_{21}N$	155	101-40-6	Stimulant drug/ pharmaceutical	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	761	n.d.
Benzestrol	$C_{20}H_{26}O_2$	298	85-95-0	Pharmaceutical/ non-steroidal oestrogen antagonist	716	n.d.	754	758	704	711	790	776	790	775	798	712
Benzophenone	$C_{13}H_{10}O$	182	119-61-9	Sunscreen agent	942	948	938	949	939	944	940	949	943	945	947	941
Benzyl Benzoate	$C_{14}H_{12}O_2$	212	120-51-4	Pharmaceutical/ treat lice and scabies infestations	n.d.	903	n.d.	n.d.	870	840	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Bifenthrin	$C_{23}H_{22}ClF_3O_2$	422	82657-04-3	Insecticide	n.d.	n.d.	n.d.	n.d.	n.d.	743	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Botran	$C_6H_4Cl_2N_2O_2$	206	99-30-9	Fungicide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	834	n.d.	n.d.
Caffeine	$C_8H_{10}N_4O_2$	194	58-08-2	Stimulant/ pharmaceutical	893	844	878	803	876	n.d.	834	812	888	n.d.	817	855
Cathinone	$C_9H_{11}NO$	149	71031-15-7	Pharmaceutical/ stimulant	708	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Celestolide	$C_{17}H_{24}O$	244	13171-00-1	Fragrance/musk	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	723	723	779	751	763
Chloroneb	$C_8H_8Cl_2O_2$	206	2675-77-6	Pesticide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	753	n.d.	722	745	742	793
Chlorpyrifos	$C_9H_{11}Cl_3NO_3PS$	349	2921-88-2	Insecticide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	732	n.d.	727	746	798
Clindamycin	$C_{18}H_{33}ClN_2O_5S$	424	18323-44-9	Pharmaceutical/ antibiotic	727	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cyclizine	$C_{18}H_{22}N_2$	266	82-92-8	Pharmaceutical/ antihistamine drug	n.d.	n.d.	n.d.	723	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	733	n.d.
Cyclohexane, 1,2,3,4,5,6-hexachloro-	$C_6H_6Cl_6$	288	608-73-1	Pesticide (Hexachlor)	n.d.	n.d.	747	n.d.	n.d.	n.d.	n.d.	n.d.	792	n.d.	n.d.	n.d.

Sample name:					20150420C SBSE seq NaCl 1 STR FAM 500mL	20150515A Loop seq NaCl 1 STR FAM TDS 500mL	20150421A SBSE seq NaCl 2 Otter Bridge 500mL	20150515B Loop seq NaCl 2 Otter Bridge 500 mL TDS	20150421B SBSE seq NaCl 3 Bird Hide 500mL	20150515C Loop seq NaCl 3 Bird Hide 500ml TDS	20150421C SBSE seq NaCl 4 Rietvlei Spruit 500mL	20150518A Loop seq NaCl 4 Rietvlei spruit 500mL TDS	20150422A SBSE seq NaCl 5 Marais Dam 500mL	20150518B Loop seq NaCl 5 Marais Dam 500mL TDS	20150423A SBSE seq NaCl 6 MD Channel 500mL	20150521A Loop seq NaCl 6 MD Channel 500mL TDS
Compound name	**Formula**	**Weight**	**CAS**	**Comments/uses**	**Similarity***	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**
Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-	$C_{18}H_{26}O$	258	1222-05-5	Fragrance (Galaxolide)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	712	n.d.	n.d.	n.d.	n.d.	n.d.
DCPA	$C_{10}H_6Cl_4O_4$	330	1861-32-1	Herbicide	n.d.	705	807	795	n.d.	n.d.	784	842	823	802	818	850
Diazinone	$C_{12}H_{21}N_2O_3PS$	304	333-41-5	Insecticide	n.d.	n.d.	888	929	n.d.	n.d.	917	911	917	908	915	859
Dibenzofuran	$C_{12}H_8O$	168	132-64-9	Insecticide	899	935	926	896	898	916	906	930	893	875	882	900
Diethylpropion	$C_{13}H_{19}NO$	205	90-84-6	Pharmaceutical/ decreases appetite	n.d.	n.d.	n.d.	n.d.	819	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Diethyltoluamide	$C_{12}H_{17}NO$	191	134-62-3	Insect repellents	804	758	n.d.	797	n.d.	828	824	n.d.	n.d.	n.d.	n.d.	n.d.
Doconexent	$C_{22}H_{32}O_2$	328	6217-54-5	Pharmaceutical	842	739	n.d.	746	832	753	832	838	832	799	n.d.	776
Drometrizole	$C_{13}H_{11}N_3O$	225	2440-22-4	PCP/sunscreen	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	707	n.d.	n.d.	n.d.	n.d.
Efavirenz	$C_{14}H_9ClF_3NO_2$	315	154598-52-4	ARV	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	830	n.d.	929	n.d.	n.d.
Epinephrine, (β)-, 3TMS derivative	$C_{18}H_{37}NO_3Si_3$	399	10538-85-9	Hormone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	722	n.d.	n.d.	n.d.
Ethylparaben	$C_9H_{10}O_3$	166	120-47-8	Antifungal preservative/ pesticide	732	818	745	706	762	785	n.d.	800	n.d.	n.d.	n.d.	n.d.
Etidocaine	$C_{17}H_{28}N_2O$	276	36637-18-0	Pharmaceutical/ local anaesthetic	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	739	n.d.
Etilefrine	$C_{10}H_{15}NO_2$	181	709-55-7	Pharmaceutical/ cardiac stimulant	n.d.	n.d.	706	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Flusilazole	$C_{16}H_{15}F_2N_3Si$	315	85509-19-9	Fungicide	n.d.	n.d.	869	830	794	n.d.	882	799	882	740	798	781
Homosalate	$C_{16}H_{22}O_3$	262	118-56-9	PCP/sunscreen	909	914	887	927	935	928	724	896	872	922	859	894
Hydantoin	$C_3H_4N_2O_2$	100	461-72-3	Pharmaceutical/ anticonvulsants	858	822	913	917	867	875	836	886	915	877	773	792
Hydroquinone	$C_6H_6O_2$	110	123-31-9	Skin whitening	848	861	877	910	863	896	783	888	848	919	886	892
Imazalil	$C_{14}H_{14}Cl_2N_2O$	296	35554-44-0	Fungicide	n.d.	n.d.	n.d.	713	n.d.	n.d.	707	n.d.	707	n.d.	n.d.	n.d.

Sample name:					20150420C SBSE seq NaCl 1 STR FAM 500mL	20150515A Loop seq NaCl 1 STR FAM TDS 500mL	20150421A SBSE seq NaCl 2 Otter Bridge 500mL	20150515B Loop seq NaCl 2 Otter Bridge 500 mL TDS	20150421B SBSE seq NaCl 3 Bird Hide 500mL	20150515C Loop seq NaCl 3 Bird Hide 500ml TDS	20150421C SBSE seq NaCl 4 Rietvlei Spruit 500mL	20150518A Loop seq NaCl 4 Rietvlei spruit 500mL TDS	20150422A SBSE seq NaCl 5 Marais Dam 500mL	20150518B Loop seq NaCl 5 Marais Dam 500mL TDS	20150423A SBSE seq NaCl 6 MD Channel 500mL	20150521A Loop seq NaCl 6 MD Channel 500mL TDS
Compound name	**Formula**	**Weight**	**CAS**	**Comments/uses**	**Similarity***	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**
Levetiracetam	$C_8H_{14}N_2O_2$	170	102767-28-2	Pharmaceutical/ anticonvulsant	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	726	n.d.
Levomenthol	$C_{10}H_{20}O$	156	2216-51-5	Pharmaceutical/ antipruritic agent	n.d.	n.d.	727	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	792	n.d.
Mequinol	$C_7H_8O_2$	124	150-76-5	Pharmaceutical/ skin depigmentation	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	720	799	n.d.	n.d.	780	775
Methamphetamin, propionyl	$C_{13}H_{19}NO$	205	0-00-0	Pyrolysis product of methamphetamine	n.d.	n.d.	746	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Metolachlor	$C_{15}H_{22}ClNO_2$	283	51218-45-2	Herbicide	n.d.	n.d.	881	790	817	798	781	808	874	834	869	773
Musk ketone	$C_{14}H_{18}N_2O_5$	294	81-14-1	PCP/fragrant	n.d.	737	766	785	851	823	946	961	947	966	963	836
Norepinephrine, (R)-, 4TMS derivative	$C_{20}H_{43}NO_3Si_4$	457	68595-65-3	Pharmaceutical	n.d.	n.d.	n.d.	n.d.	739	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Octicizer	$C_{20}H_{27}O_4P$	362	1241-94-7	Pesticide/ insecticide	822	n.d.	n.d.	795	794	844	737	858	737	862	n.d.	862
Octocrylene	$C_{24}H_{27}NO_2$	361	6197-30-4	PCP/sunscreen	899	907	897	919	865	885	840	888	907	908	860	907
Oxybenzone	$C_{14}H_{12}O_3$	228	131-57-7	PCP/Sunscreen	853	901	817	834	871	877	769	809	733	778	726	813
Padimate O	$C_{17}H_{27}NO_2$	277	21245-02-3	PCP/sunscreen	721	n.d.	730	n.d.	757	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
p-Chloroaniline	C_6H_6ClN	127	106-47-8	Production of pesticides, drugs, and dyestuffs	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	895	n.d.	n.d.	n.d.	n.d.	n.d.
Phenadoxone	$C_{23}H_{29}NO_2$	351	467-84-5	Pharmaceutical/ opioid analgesic	n.d.	766	n.d.	n.d.	758	n.d.	n.d.	745	n.d.	731	n.d.	n.d.
Phenol, 4-(1,1-dimethylpropyl)-	$C_{11}H_{16}O$	164	80-46-6	Pesticide (4-tert-amylphenol)	871	709	794	n.d.	815	n.d.	n.d.	872	875	n.d.	864	n.d.
Phenylephrine	$C_9H_{13}NO_2$	167	59-42-7	Pharmaceutical	844	n.d.	n.d.	853	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Piperonyl butoxide	$C_{19}H_{30}O_5$	338	51-03-6	Pesticide	783	757	819	810	n.d.	n.d.	868	816	868	n.d.	n.d.	718
Precocene I	$C_{12}H_{14}O_2$	190	17598-02-6	Pesticide	n.d.	721	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Sample name:					20150420C SBSE seq NaCl 1 STR FAM 500mL	20150515A Loop seq NaCl 1 STR FAM TDS 500mL	20150421A SBSE seq NaCl 2 Otter Bridge 500mL	20150515B Loop seq NaCl 2 Otter Bridge 500 mL TDS	20150421B SBSE seq NaCl 3 Bird Hide 500mL	20150515C Loop seq NaCl 3 Bird Hide 500ml TDS	20150421C SBSE seq NaCl 4 Rietvlei Spruit 500mL	20150518A Loop seq NaCl 4 Rietvlei spruit 500mL TDS	20150422A SBSE seq NaCl 5 Marais Dam 500mL	20150518B Loop seq NaCl 5 Marais Dam 500mL TDS	20150423A SBSE seq NaCl 6 MD Channel 500mL	20150521A Loop seq NaCl 6 MD Channel 500mL TDS
Compound name	**Formula**	**Weight**	**CAS**	**Comments/uses**	**Similarity***	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**	**Similarity**
Preg-4-en-3-one, 17α-hydroxy-17β-cyano-	$C_{20}H_{27}NO_2$	313	0-00-0	Steroid	n.d.	n.d.	n.d.	n.d.	752	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pregn-4-ene-1,20-dione, 16,17-dimethyl-	$C_{23}H_{34}O_2$	342	0-00-0	Steroid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	710	750	713
Pregnane	$C_{21}H_{36}$	288	481-26-5	Steroid derivative	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	816	n.d.	816	n.d.	n.d.	n.d.
Propylparaben	$C_{10}H_{12}O_3$	180	94-13-3	Pesticide	n.d.	818	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	810
Pyrimethanil	$C_{12}H_{13}N_3$	199	53112-28-0	Fungicide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	772	n.d.
Resorcinol	$C_6H_6O_2$	110	108-46-3	Pharmaceutical, etc.	811	n.d.	n.d.	792	n.d.	n.d.	843	n.d.	774	n.d.	n.d.	n.d.
Retene	$C_{18}H_{18}$	234	483-65-8	PAH	871	879	n.d.	n.d.	895	902	758	825	839	n.d.	n.d.	783
Sulpiride	$C_{15}H_{23}N_3O_4S$	341	15676-16-1	Pharmaceutical/ antipsychotic drug	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	727	n.d.	727	n.d.	n.d.	n.d.
TATP	$C_9H_{18}O_6$	222	17088-37-8	Plastic explosive	n.d.	n.d.	709	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Terbuthylazine	$C_9H_{16}ClN_5$	229	5915-41-3	Herbicide	n.d.	n.d.	912	912	870	845	855	881	855	861	891	871
Terbutryn	$C_{10}H_{19}N_5S$	241	886-50-0	Herbicide	n.d.	738	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Thiabendazole	$C_{10}H_7N_3S$	201	148-79-8	Fungicide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	813	n.d.	n.d.	n.d.	n.d.
Tonalid	$C_{18}H_{26}O$	258	21145-77-7	Musk/fragrance	746	780	832	n.d.	775	774	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Triazophos	$C_{12}H_{16}N_3O_3PS$	313	24017-47-8	Pesticide	n.d.	n.d.	n.d.	737	n.d.	n.d.	760	n.d.	760	n.d.	n.d.	n.d.
Triclosan	$C_{12}H_7Cl_3O_2$	288	3380-34-5	Antiseptics/ PCP	n.d.	n.d.	794	817	n.d.	n.d.	842	817	842	854	811	878
WIN 54461	$C_{23}H_{25}BrN_2O_3$	456	166599-63-9	Pharmaceutical/ cannabinoid (CB) mimetic	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	741	n.d.
Xanthone	$C_{13}H_8O_2$	196	90-47-1	Insecticide	n.d.	851	828	728	n.d.	n.d.	828	753	750	786	834	789

* Similarity: spectral match quality (NIST14 library)
** Compounds given in red are classified EDCs (EDCs classified using EU EDC database, EDC databank created by Dr Montes-Grajales and Prof Olivero-Verbel, University of Cartagena and TEDX, The Endocrine Disruption Exchange).
*** n.d. not detected in sample

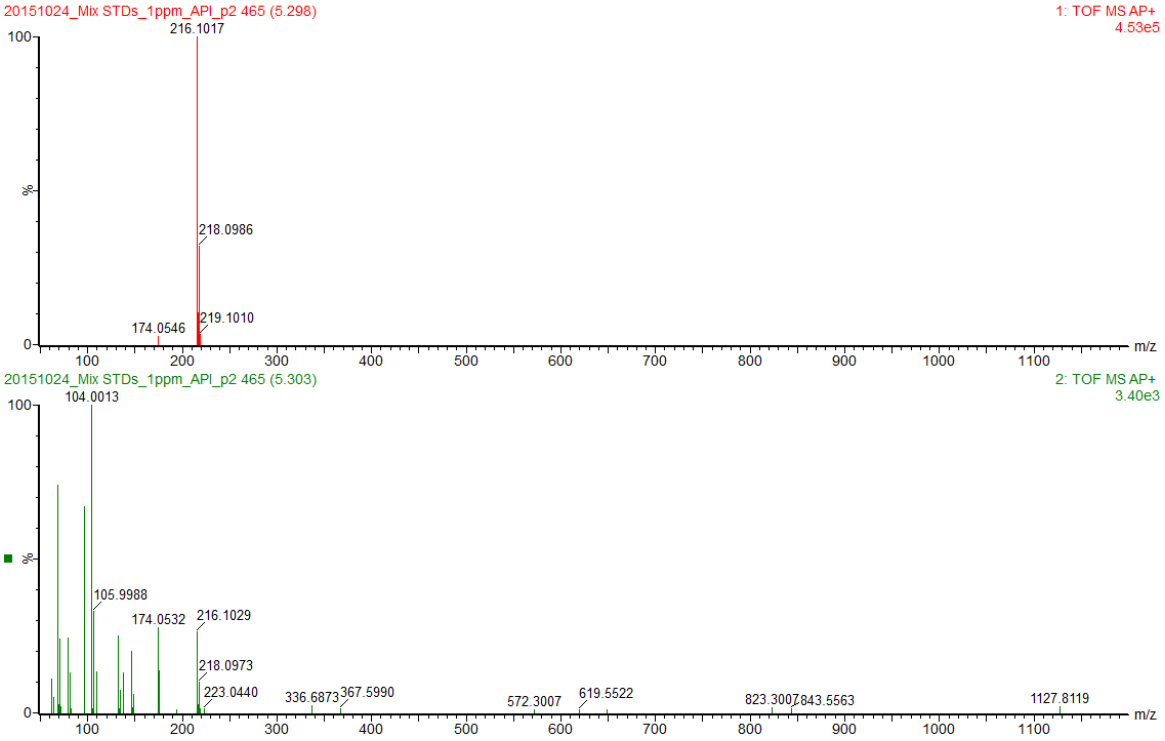
Addendum D: Chemical name, CAS registry number, formula, average and monoisotopic mass, classification and intended use of the 16 target analytes

#	Chemical name	CAS registry number	Formula	Average mass (Da)	Monoisotopic mass (Da)	Class	Chemical Class	Intended use
1	Nevirapine	129618-40-2	$C_{15}H_{14}N_4O$	266.298	266.11676	Pharmaceutical	Non-Nucleoside Analog	ARV
2	Efavirenz	154598-52-4	$C_{14}H_9ClF_3NO_2$	315.675	315.02739	Pharmaceutical	Non-Nucleoside Analog (benzoxamine)	ARV
3	Clindamycin	18323-44-9	$C_{18}H_{33}ClN_2O_5S$	424.983	424.17986	Pharmaceutical	Lincosamide	Antibiotic
4	Caffeine*	58-08-2	$C_8H_{10}N_4O_2$	194.191	194.08037	Pharmaceutical	Alkaloid	Stimulant
5	Terbutryn	886-50-0	$C_{10}H_{19}N_5S$	241.356	241.13611	Pesticide	Triazine	Herbicide
6	Bifenthrin	82657-04-3	$C_{23}H_{22}ClF_3O_2$	422.868	422.12603	Pesticide	Pyrethroid	Insecticide
7	Metolachlor	51218-45-2	$C_{15}H_{22}ClNO_2$	283.794	283.1339	Pesticide	Chloroacetanilide	Herbicide
8	Terbuthylazine	5915-41-3	$C_9H_{16}ClN_5$	229.710	229.10942	Pesticide	Triazine	Algaecide, Herbicide, Microbiocide
9	Acetaminophen	103-90-2	$C_8H_9NO_2$	151.163	151.06333	Pharmaceutical	*p*-aminophenol derivative	Analgesic
10	Chlorpyrifos	2921-88-2	$C_9H_{11}Cl_3NO_3PS$	350.586	348.92628	Pesticide	Organophosphorus	Insecticide, Nematicide
11	Musk ketone	81-14-1	$C_{14}H_{18}N_2O_5$	294.303	294.12157	Fragrances/ musk	Nitro-musk	Aroma-chemical
12	Lindane	608-73-1	$C_6H_6Cl_6$	290.830	287.86007	Pesticide	Organochlorine	Insecticide, Rodenticide
13	4-tert-Amylphenol	80-46-6	$C_{11}H_{16}O$	164.244	164.12011	Pesticide	Phenol	Microbiocide
14	Estrone	53-16-7	$C_{18}H_{22}O_2$	270.366	270.16197	Hormone	Aromatized C_{18} steroid	Natural estrogen
15	Hexestrol	84-16-2	$C_{18}H_{22}O_2$	270.366	270.16198	Hormone	Diphenol	Synthetic estrogen
16	Atrazine	1912-24-9	$C_8H_{14}ClN_5$	215.683	215.09377	Pesticide	Triazine	Herbicide

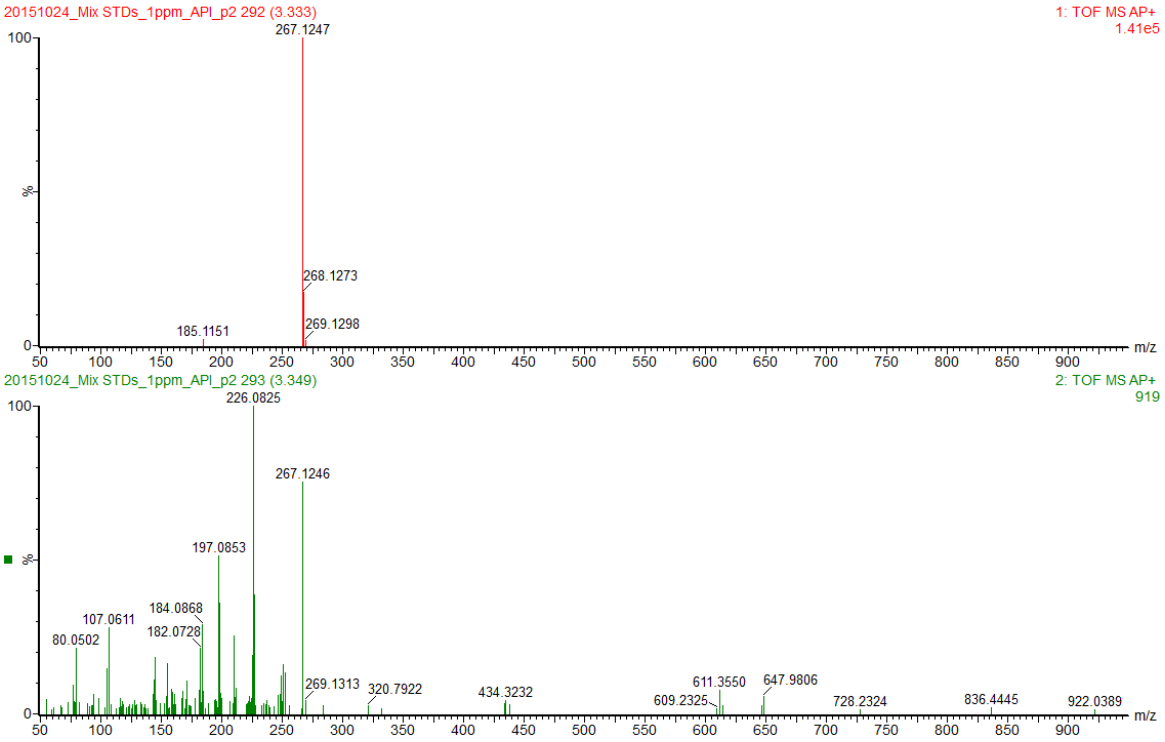
*Compounds given in red are classified EDCs (EDCs classified using EU EDC database, EDC databank created by Dr Montes-Grajales and Prof Olivero-Verbel, University of Cartagena and TEDX, The Endocrine Disruption Exchange).

Addendum E: APCI$^+$ - QTOFMS low energy MS scan (top spectrum) and APCI$^+$ - QTOFMS high energy MS scan (bottom spectrum) spectra of the standards

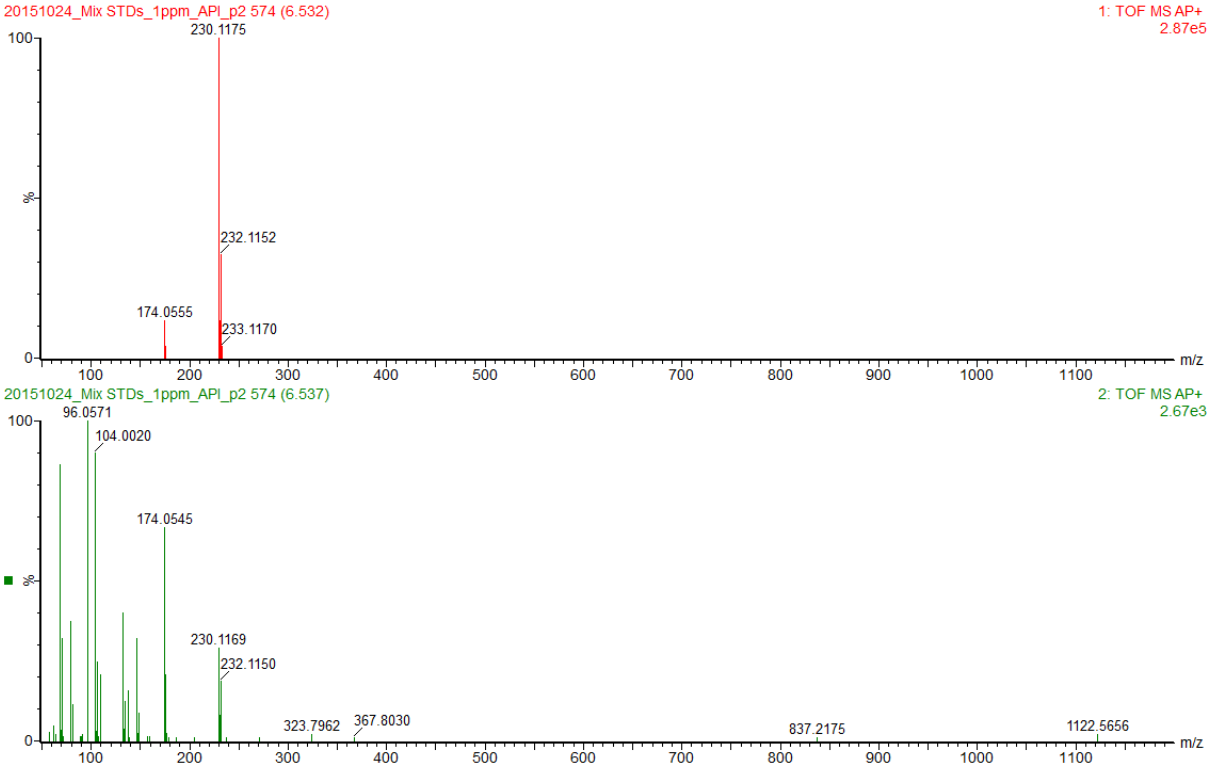
Atrazine (216.1017 *m/z*)



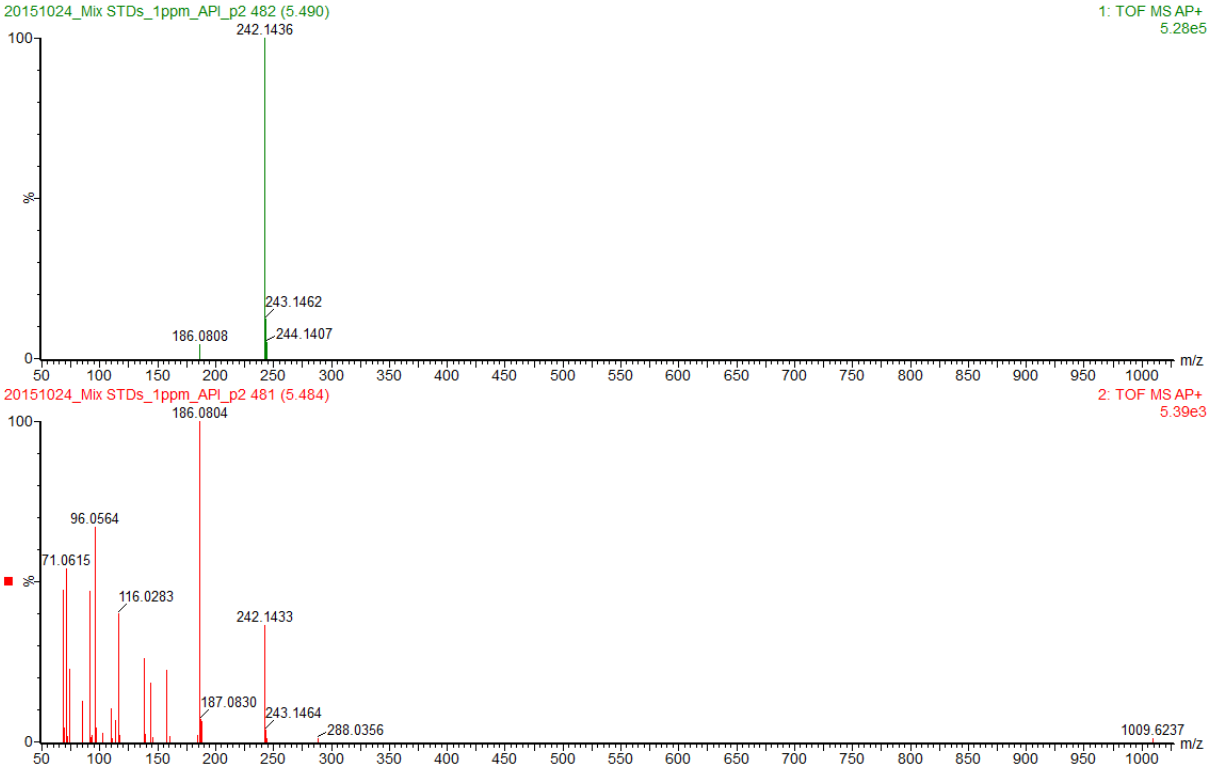
Nevirapine (267.1247 *m/z*)



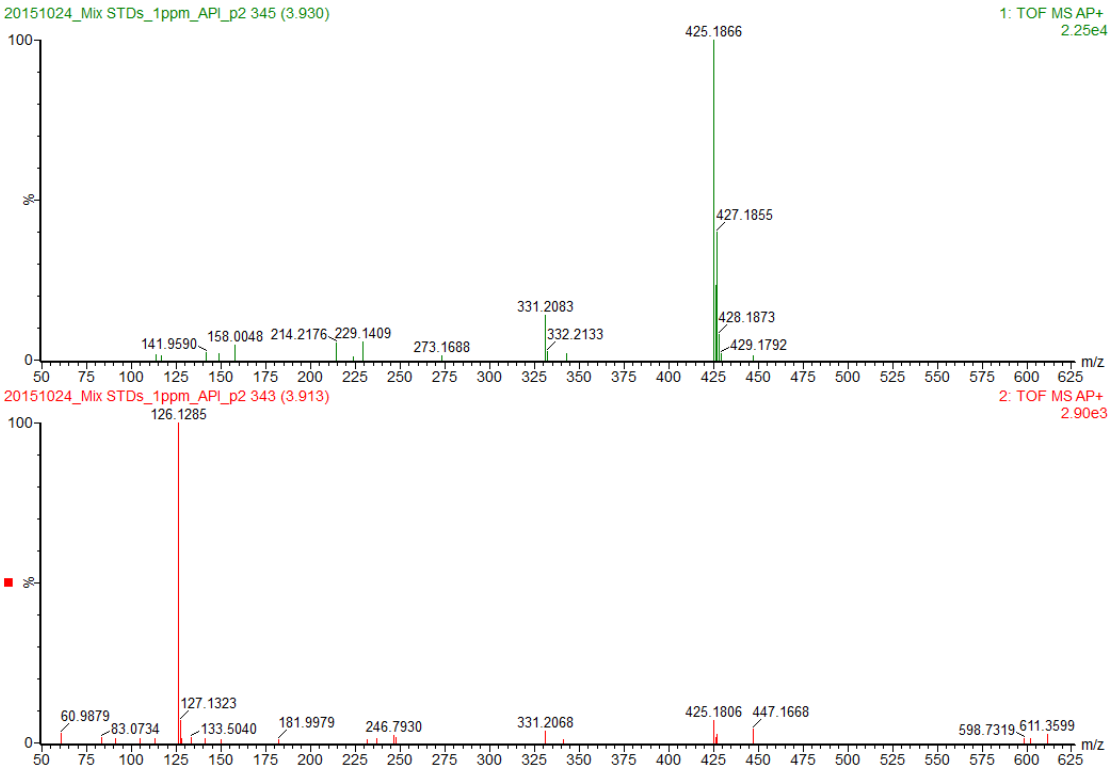
Terbuthylazine (230.1175 *m/z*)



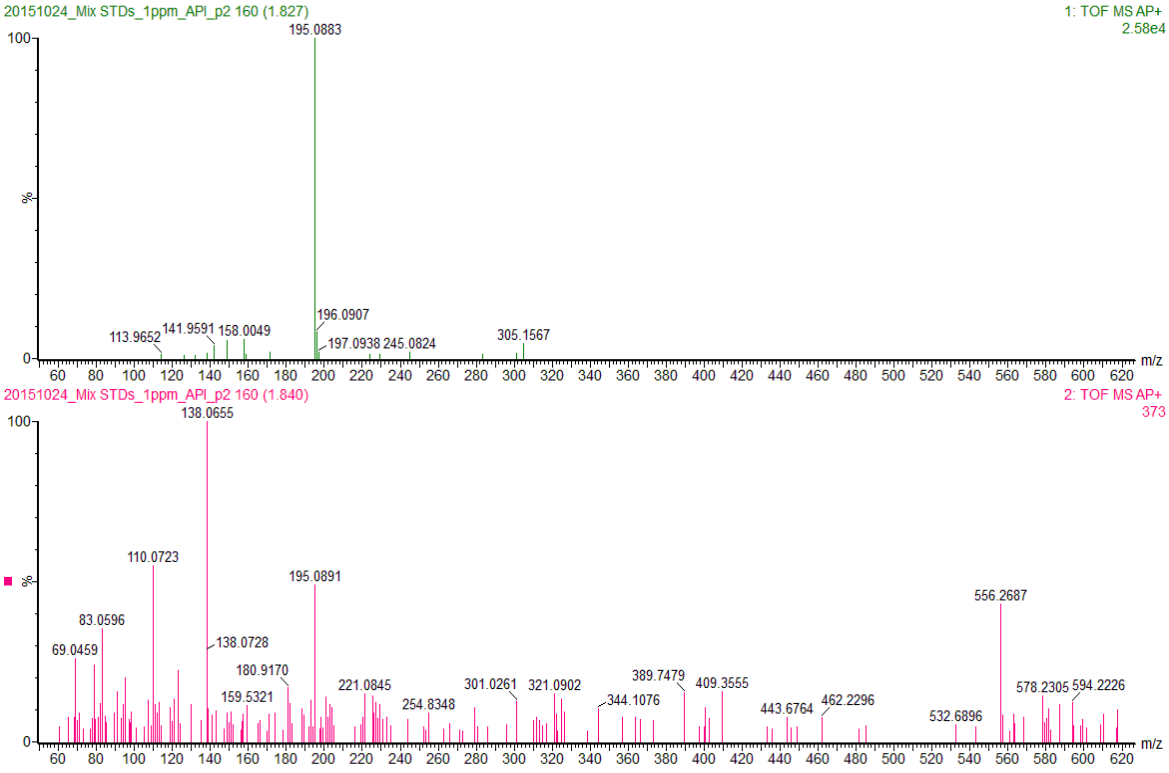
Terbutryn (242.1436 *m/z*)



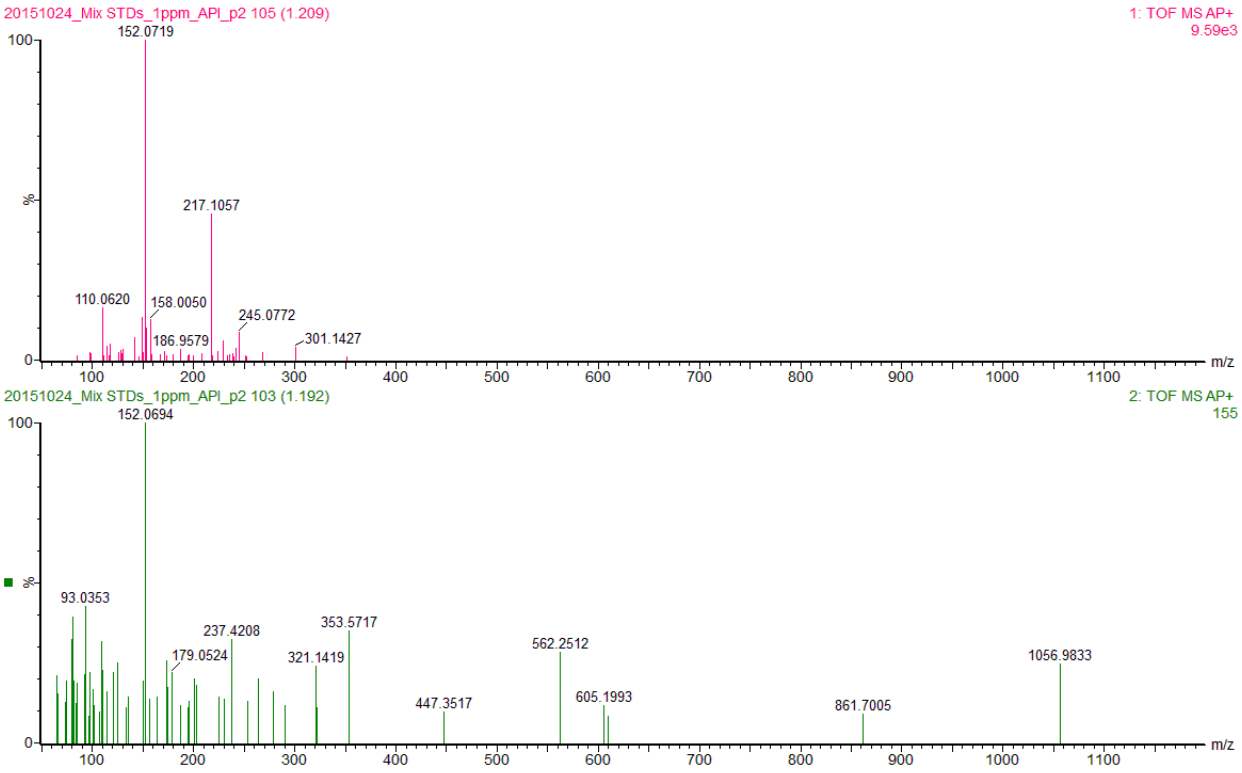
Clindamycin (425.1866 *m/z*)



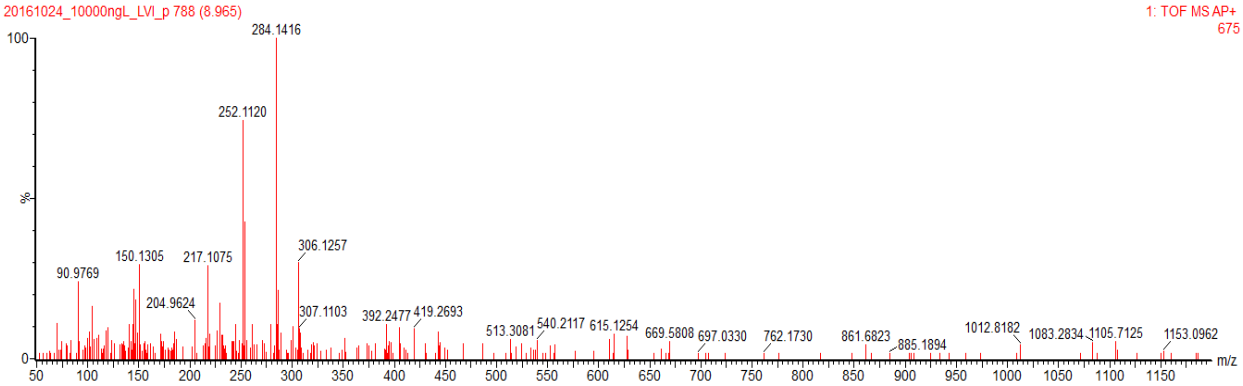
Caffeine (195.0883 *m/z*)



Acetaminophen (152.0719 *m/z*)



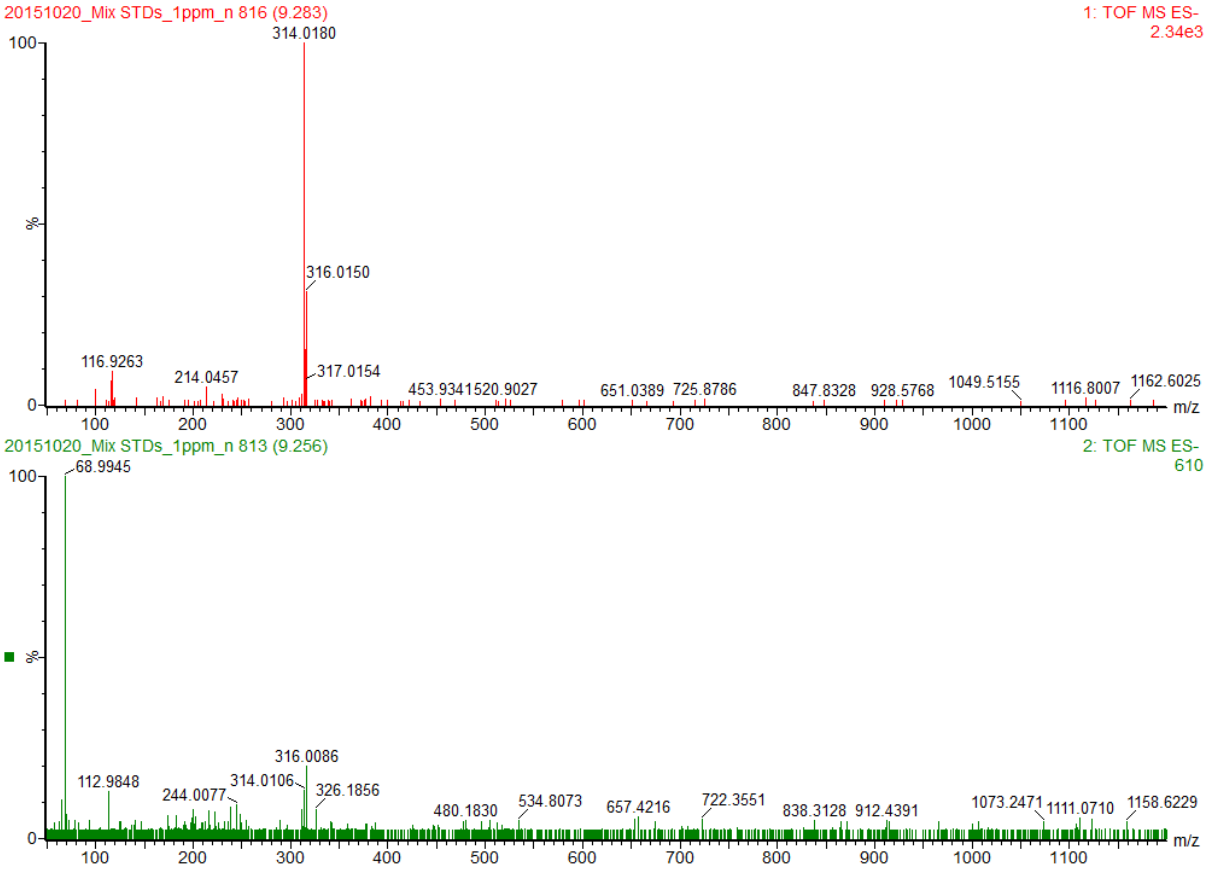
Metolachlor (284.1416 *m/z*)



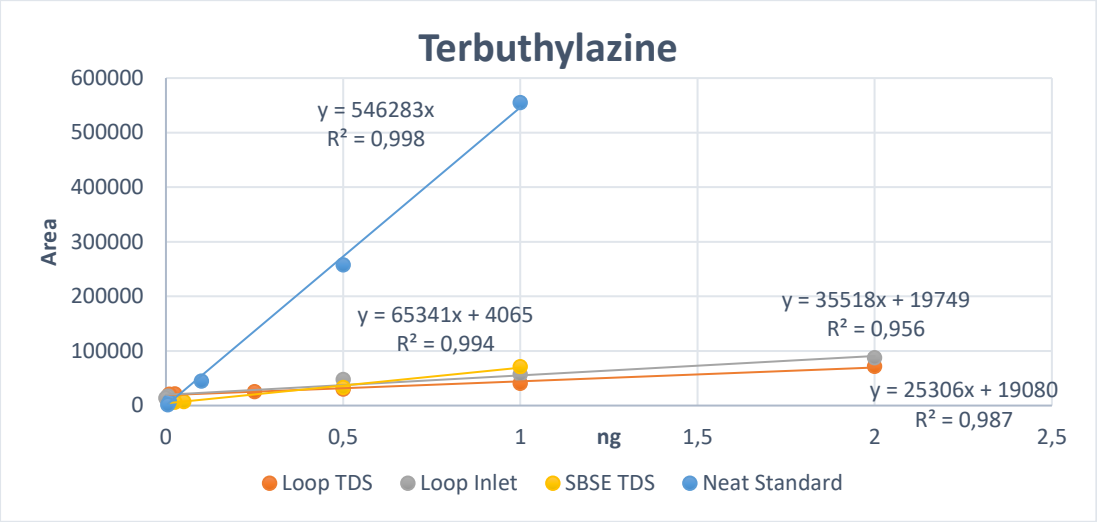
*only low energy MS scan shown; peak not detected in high energy MS scan

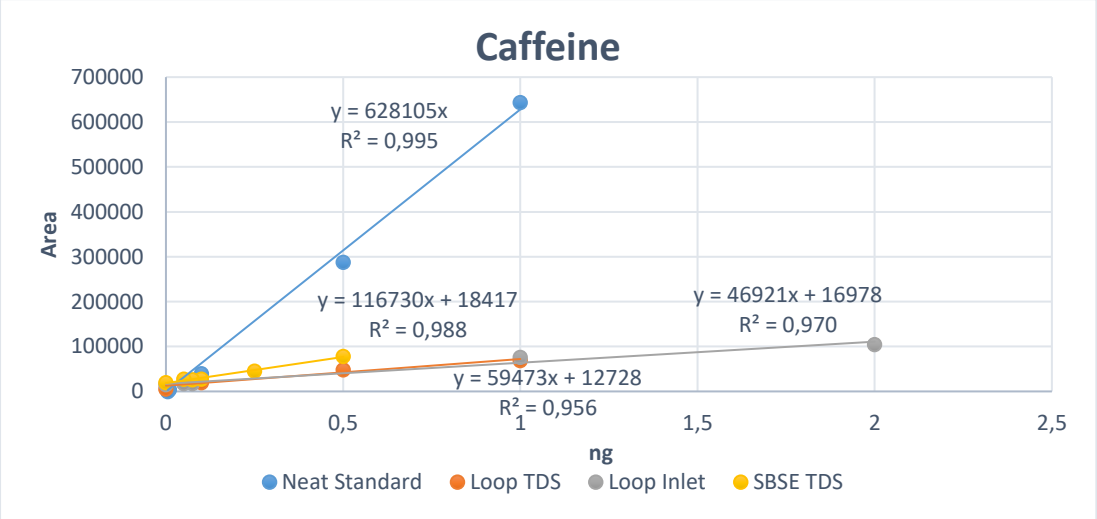
Addendum F: ESI$^-$ - QTOFMS low energy MS scan (top spectrum) and ESI$^-$ QTOFMS high energy MS scan (bottom spectrum) spectra of a standard

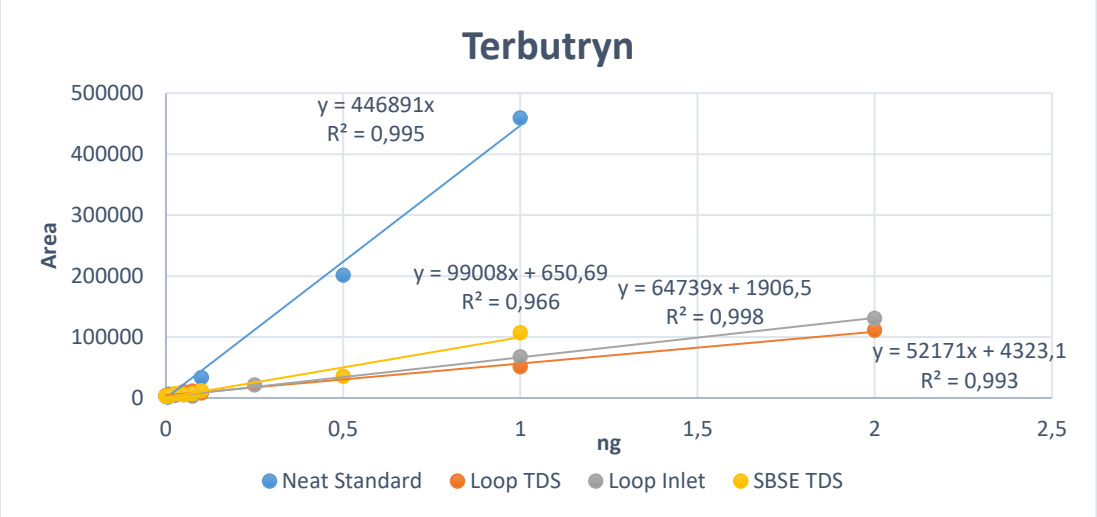
Efavirenz (314.0180 *m/z*)

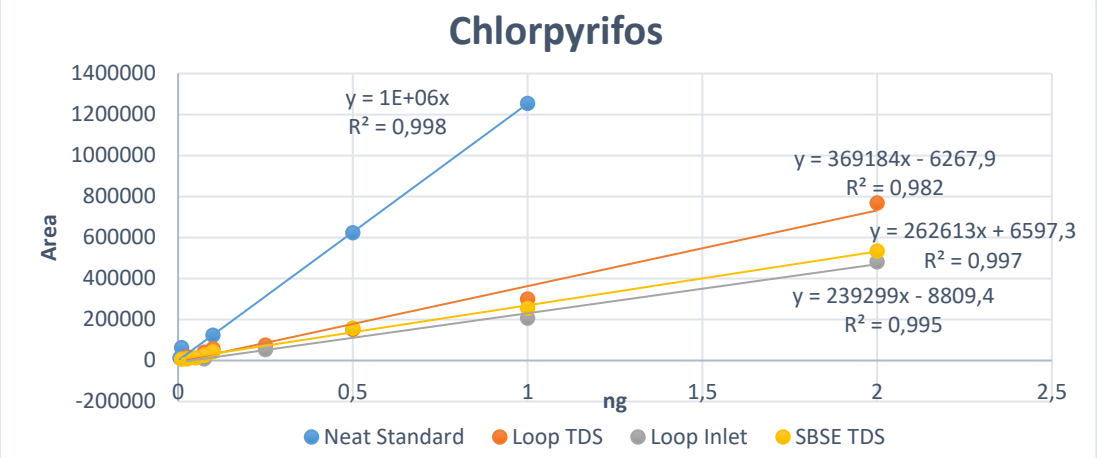


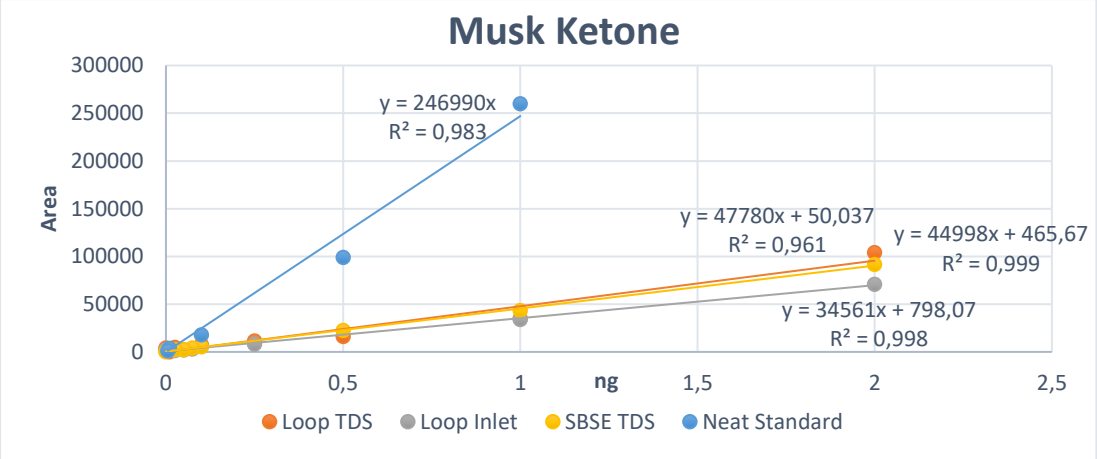
Addendum G: GC×GC-TOFMS calibration curve sets for terbuthylazine, caffeine, terbutryn, chlorpyrifos, musk ketone, bifenthrin and atrazine (Refer to Table 5.4 Chapter 5 Section 5.3.1 for quantification ions)

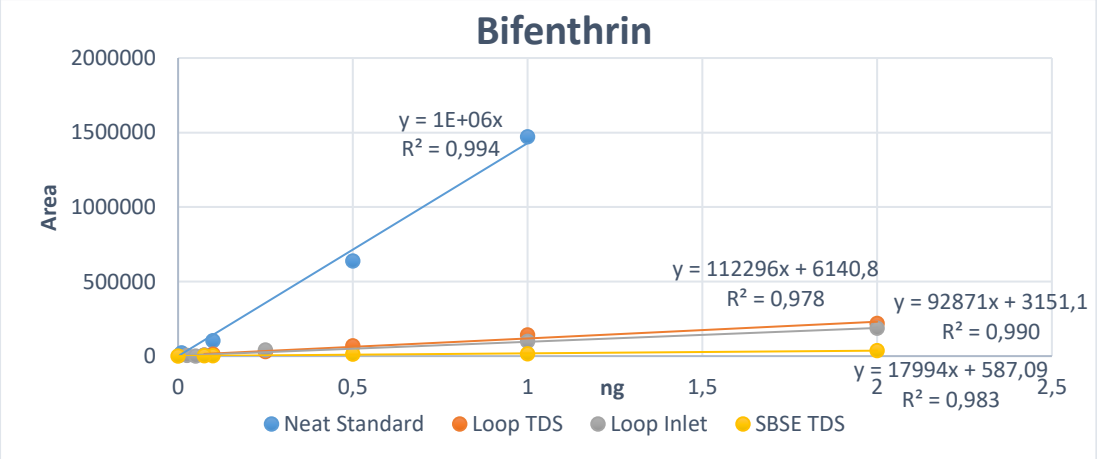


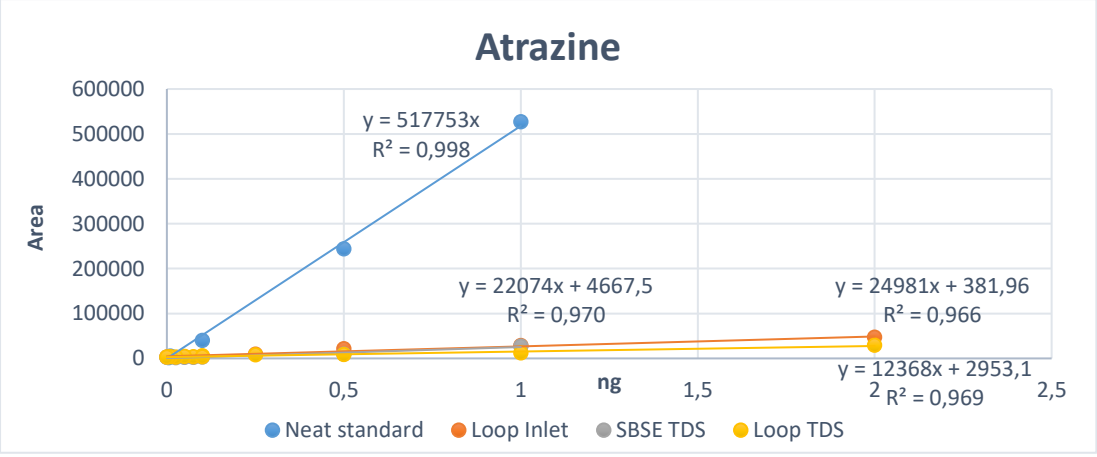




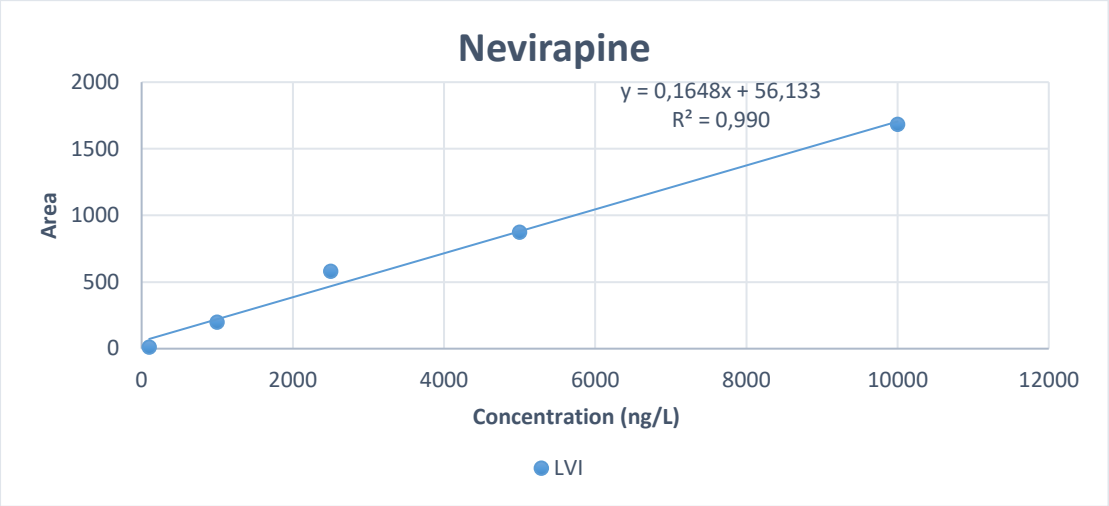


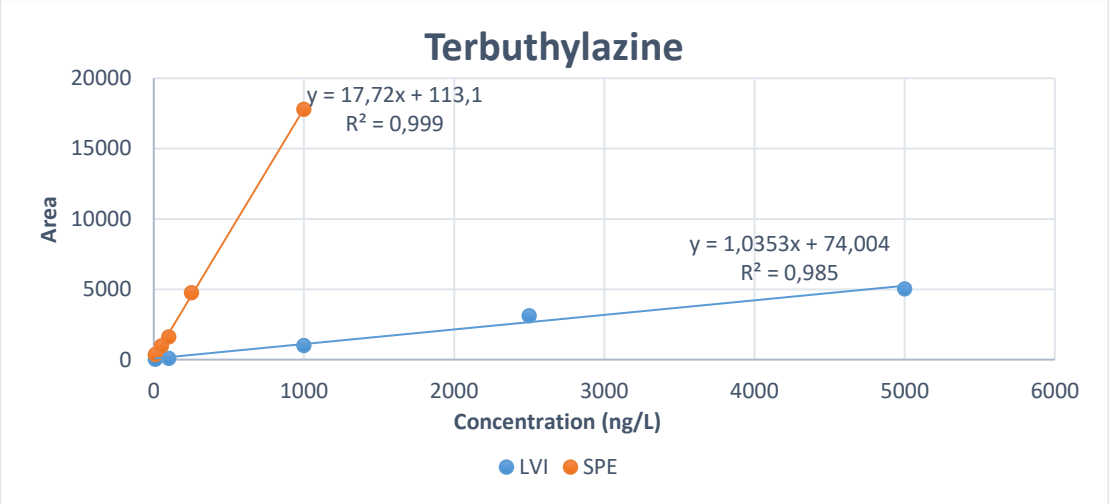


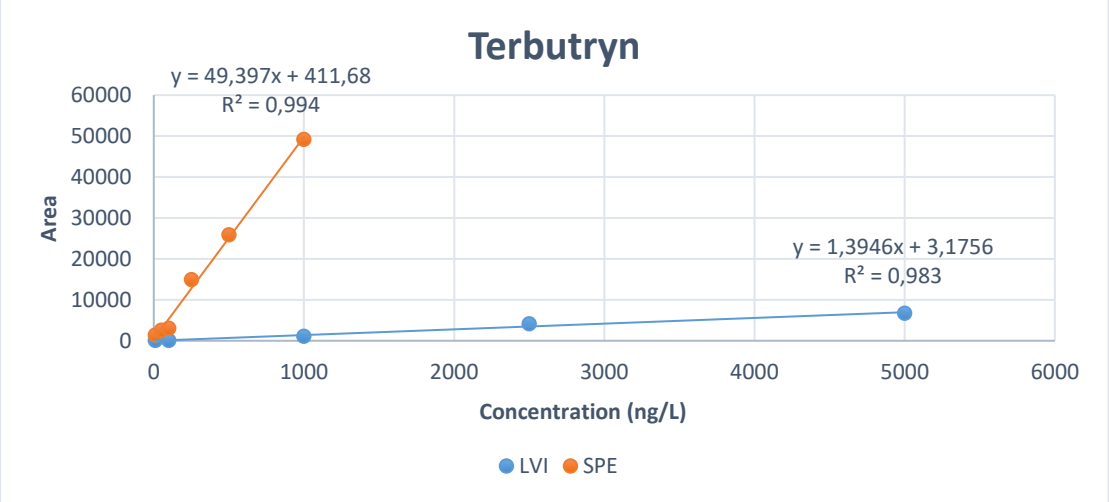


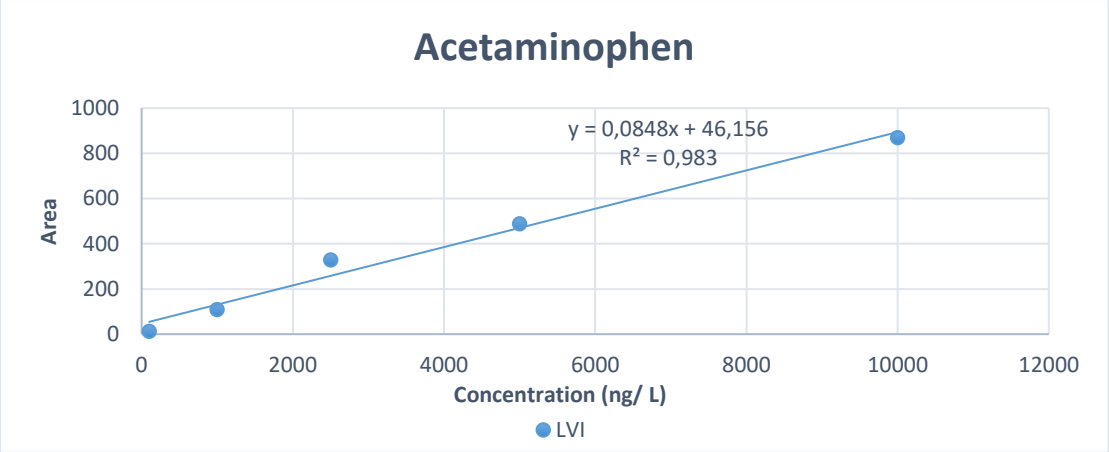


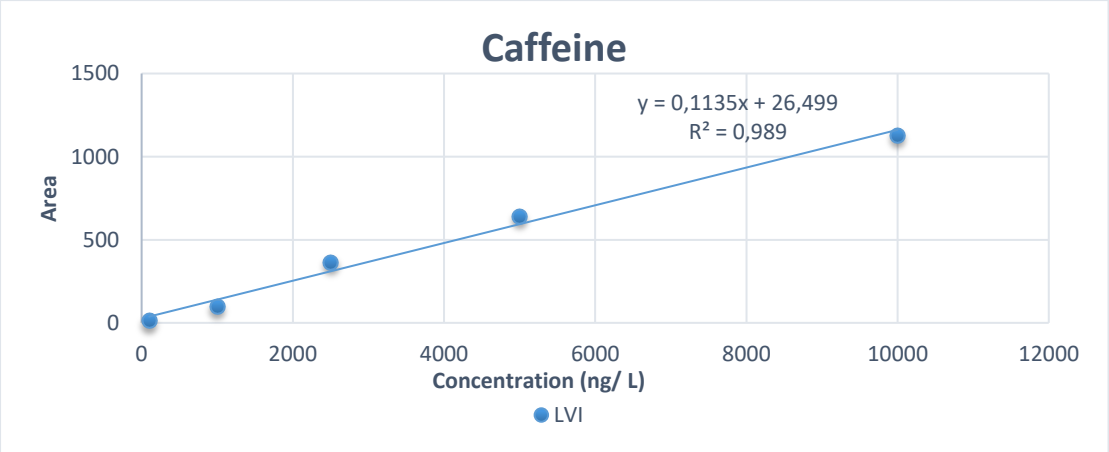
Addendum H: UHPLC-QTOFMS calibration curve sets for nevirapine, terbuthylazine, terbutryn, acetaminophen, caffeine, metolachlor and efavirenz (Refer to Table 5.7 Chapter 5 Section 5.3.2 for quantification ions)

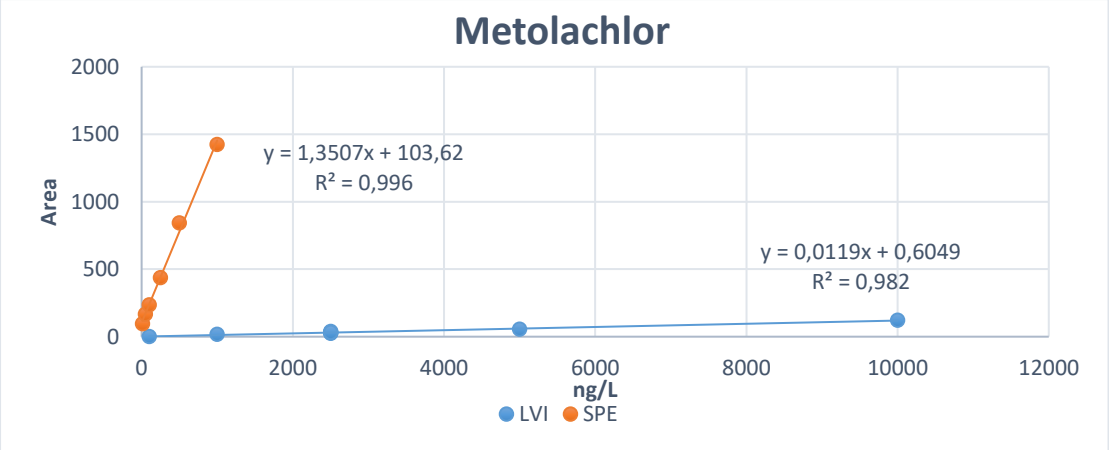


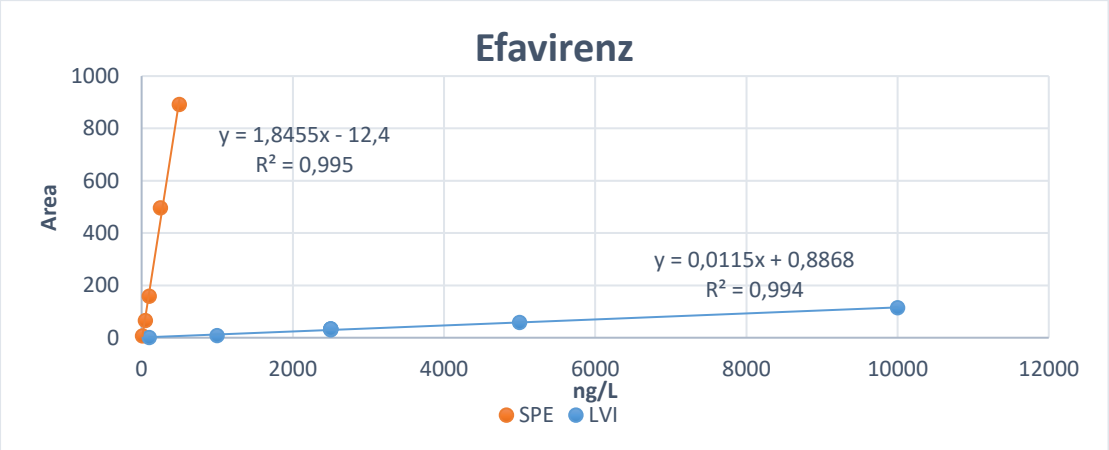












Addendum I: Accuracy (%recovery) and precision (%RSD) (n=3) for GC×GC-TOFMS analysis of target analytes

	Regression line	Peak Area			% Recovery					
		Mean	Std dev*	%RSD	% Rec 1	% Rec 2	% Rec 3	$\bar{x}_{\%Rec\ n=3}$	Std dev	% RSD (Precision)
4-tert-Amylphenol										
Loop Inlet	y = 14483x + 7650,6	22180	1570	7	88	104	109	100	11	11
Loop TDS	y = 964938x + 52699	1168266	121676	10	101	120	125	116	13	11
SBSE TDS	n/a** (Refer to Figure 5.10)	1050519	30176	3	n/a	n/a	n/a	n/a	n/a	n/a
Atrazine										
Loop Inlet	y = 22074x + 4667,5	30078	1657	6	113	109	123	115	8	6,5
Loop TDS	y = 12368x + 2953,1	15404	3345	22	75	99	129	101	27	27
SBSE TDS	y = 24981x + 381,96	28966	2492	9	107	110	126	114	10	8,7
Lindane										
Loop Inlet	y = 111369x + 4865,9	139710	10807	8	113	118	132	121	10	8,0
Loop TDS	y = 124184x + 12351	172777	35933	21	79***	129	130	129	0,92	1
SBSE TDS	y = 149944x + 6304,4	198976	26986	14	108	138	140	128	18	14
Terbuthylazine										
Loop Inlet	y = 35518x + 19749	58610	3527	6	102	105	121	109	10	9,1
Loop TDS	y = 25306x + 19080	45518	5609	12	86	98	129	104	22	21
SBSE TDS	y = 65341x + 4065	73401	1971	3	103	107	109	106	3	2,8
Caffeine										
Loop Inlet	y = 46921x + 16978	88168	39258	45	84	127	245	152	84	55
Loop TDS	y = 59473x + 12728	42750	22758	53	27	30	95	50	38	76
SBSE TDS	y = 116730x + 18417	45217	3450	7,6	21	22	26	23	3	13
Terbutryn										
Loop Inlet	y = 64739x + 1906,5	65674	8690	13	84	101	110	98	13	14
Loop TDS	y = 52171x + 4323,1	58166	8998	15	90	97	123	103	17	17
SBSE TDS	y = 99008x + 650,69	103341	13911	13	88	107	116	104	14	14
Chlorpyrifos										
Loop Inlet	y = 239299x - 8809,4	212572	31540	15	90	107	81	93	13	14
Loop TDS	y = 369184x - 6267,9	335180	30151	9	83	96	98	92	8	8,8
SBSE TDS	y = 262613x + 6597,3	305549	18612	6	106	118	118	114	7	6,2
Metolachlor										
Loop Inlet	y = 183665x + 71499	292551	14699	5,0	114	118	129	120	8	6,6
Loop TDS	y = 166612x + 132149	306368	35852	12	84	103	127	105	22	21
SBSE TDS	y = 188125x + 320099	516137	112738	21,8	35	132	145	104	60	58
Musk ketone										
Loop Inlet	y = 34561x + 798,07	30175	4509	15	89	96	71	85	13	15
Loop TDS	y = 47780x + 50,037	48469	11972	25	124	74	106	101	25	25
SBSE TDS	y = 44998x + 465,67	54053	7208	13	101	123	132	119	16	13
Bifenthrin										
Loop Inlet	y = 92871x + 3151,1	122647	30697	25	103	117	166	129	33	26
Loop TDS	y = 112296x + 6140,8	111107	30892	28	65	95	120	93	28	29
SBSE TDS	y = 17994x + 587,09	19552	5475	28	92	127	84	105	23	22

*Std dev = standard deviation
**n/a not applicable (method not suitable)
***Outlier (according to Dixon's Q test)

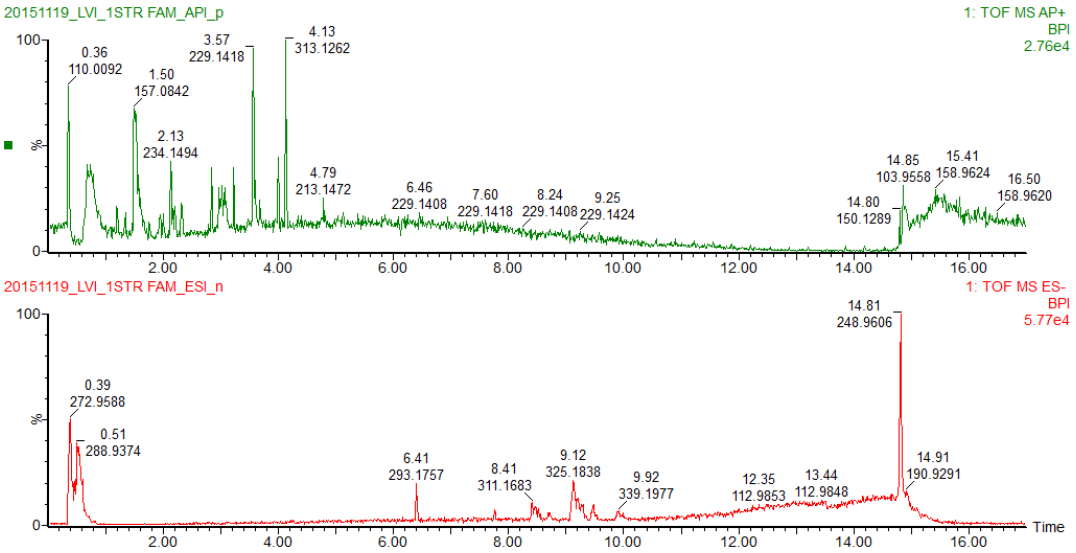
Addendum J: Accuracy (%recovery) and precision (%RSD) (n=3) for UHPLC-QTOFMS analysis of target analytes

	Regression line	R^2	Peak Area			% Recovery					
			Mean	Std dev*	%RSD	% Rec 1	% Rec 2	% Rec 3	$\bar{x}_{\%Rec\ n=3}$	% Rec Std dev	% RSD (Precision)
Atrazine											
LVI	y = 0,5932x + 366,05	0,960	2096	61	3	113	116	121	117	4	3,5
SPE	y = 0,7774x + 107,65	0,984	312	51	16	123	75	117	105	26	25
Terbuthylazine											
LVI	y = 0,4087x - 44,837	0,997	990	44	4	94	95	88	93	4	4,7
SPE	y = 0,7223x + 52,837	0,936	123	11	9,2	38	33	45	39	6,3	16
Caffeine											
LVI	y = 0,0261x + 20,594	0,956	107	4	4	127	130	138	132	6	4,4
SPE	n/a**	-	-	-	-	-	-	-	-	-	-
Terbutryn											
LVI	y = 0,7297x + 506,57	0,955	2717	80	3,0	121	117	126	121	4,4	3,6
SPE	y = 2,4552x + 39,925	0,994	633	79	12	110	84	97	97	13	13
Metolachlor											
LVI	y = 0,0119x + 0,6049	0,982	32	5	17	92	100	127	106	18	17
SPE	y = 0,0327x + 8,6461	0,936	16	3	20	76	55	130	87	39	45
Efavirenz											
LVI	y = 0,0115x + 0,8868	0,994	31	2	5	110	107	100	106	5	5,0
SPE	Not available***	-	-	-	-	-	-	-	-	-	-
Acetaminophen											
LVI	y = 0,0453x + 26,79	0,987	150	7	5	107	116	104	109	6	5,9
SPE	n/a	-	-	-	-	-	-	-	-	-	-
Nevirapine											
LVI	y = 0,0506x + 39,065	0,989	168	17	10	112	87	107	102	13	13
SPE	n/a	-	-	-	-	-	-	-	-	-	-

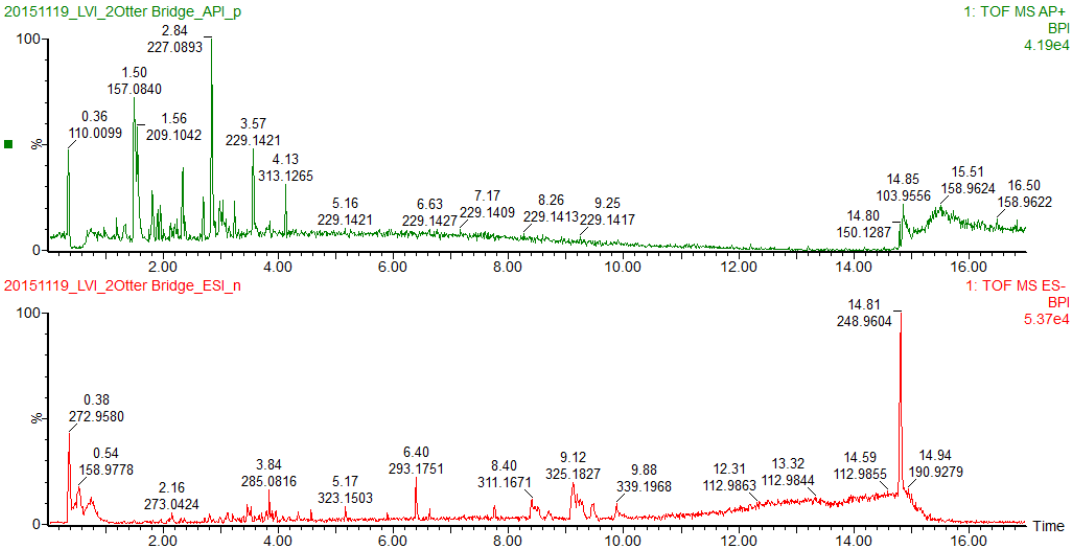
*Std dev = standard deviation
**n/a not applicable (method not suitable)
***no repeats done due to instrumental problems

Addendum K: Base peak ion (BPI) chromatograms (positive mode top and negative mode bottom) of the LVI-UHPLC-MS for surface water from the six sampling sites at Rietvlei Nature Reserve (sampling done on 6/2/2015) and Albasini Dam (sampling done on 17/8/2015)

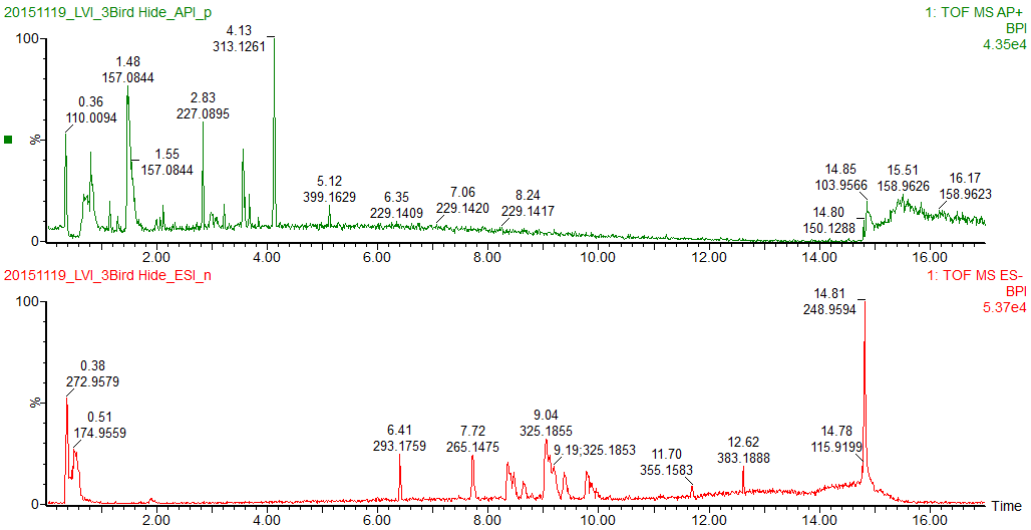
1. STR FAM



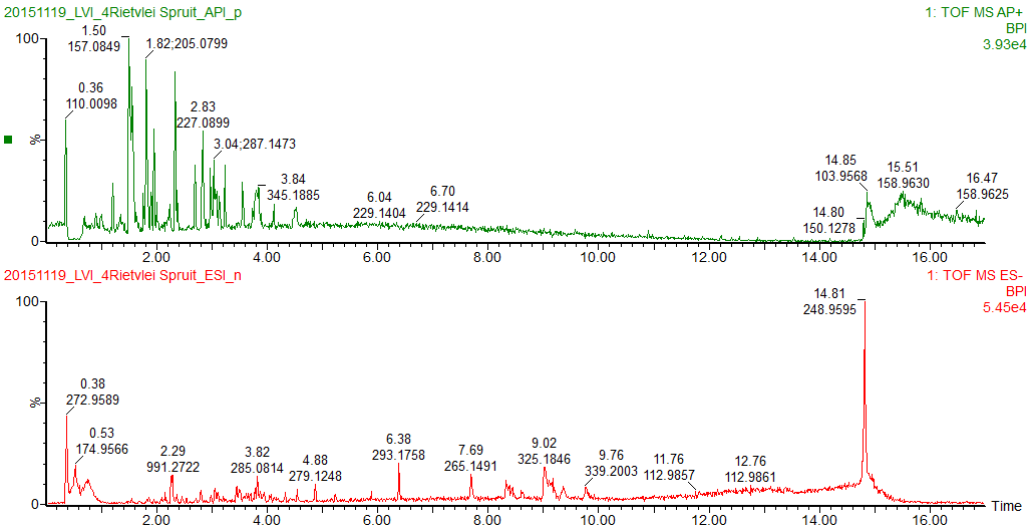
2. Otter Bridge



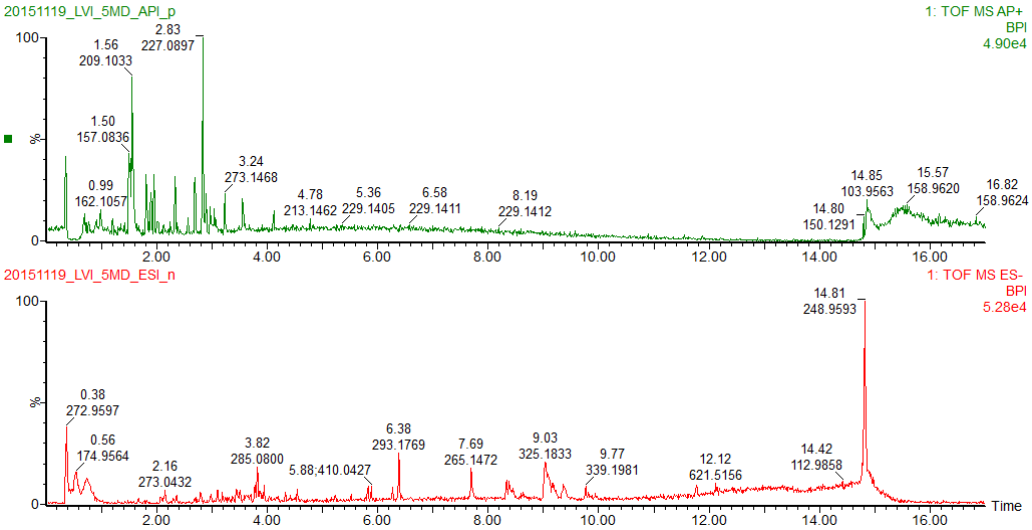
3. Bird Hide Stream



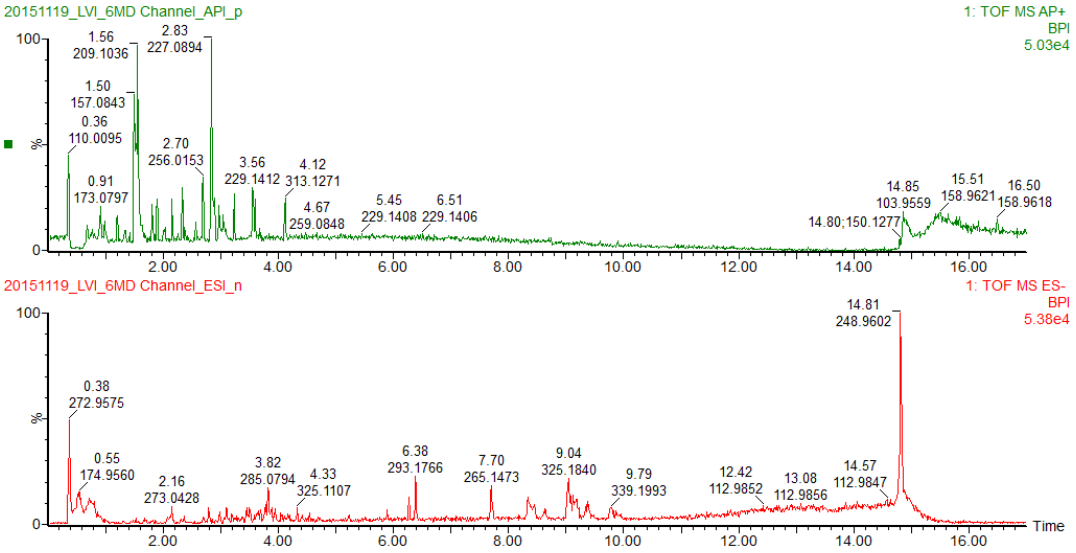
4. Rietvlei Spruit



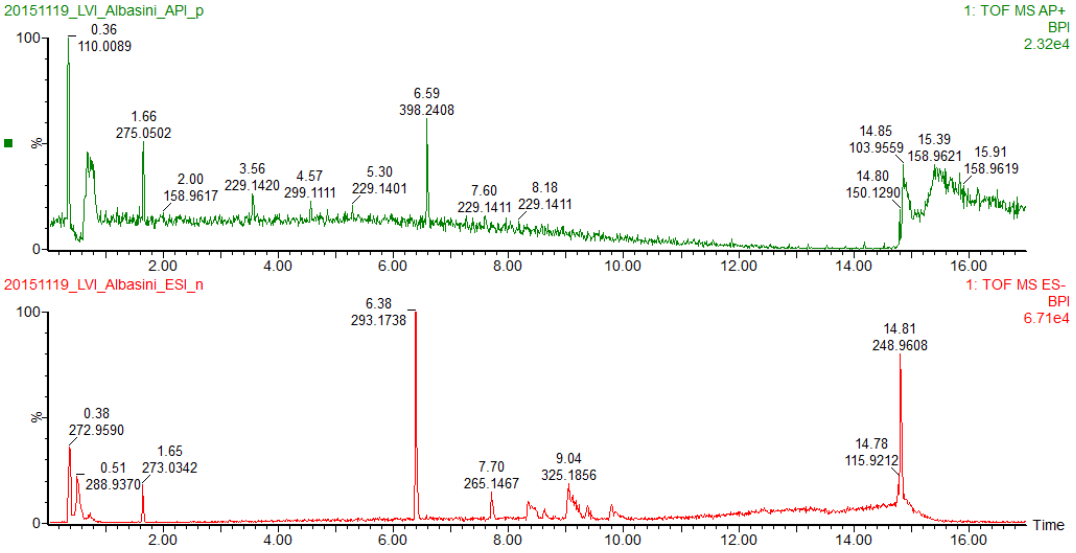
5. Marais Dam (MD)



6. MD Channel



7. Albasini Dam



Addendum L: Uses and experimental 1st dimension linear retention indices (^{1}D-LRIs) for compounds of interest detected during the Rietvlei Nature Reserve follow-up sampling, collected on 15/3/2016, and Albasini and Nandoni Dams, collected on 17/8/2015, using PDMS loop in the inlet liner TD-GC×GC-TOFMS

Compound	Comments/ uses	CAS	Formula	Weight (Da)	1st Dimension Time (s)	2nd Dimension Time (s)	Sample Detected (spectral match quality ≥ 80 %*)	^{1}D RI$_{exp}$ (Rtx®-CLPesticides II column)**
1-Phthalanone	Pesticide	87-41-2	$C_8H_6O_2$	134	684	1,09	2. Otter Bridge 3. Bird Hide Stream	1613
Di-iso-butyl-phthalate***	Phthalate	84-69-5	$C_{16}H_{22}O_4$	278	987	0,92	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam	2120
Di-nonyl-phthalate	Phthalates	84-76-4	$C_{26}H_{42}O_4$	418	1443	1,04	3. Bird Hide Stream	2884
2-Propanol, 1-chloro-, phosphate (3:1)	Flame retardant (TCPP)	13674-84-5	$C_9H_{18}Cl_3O_4P$	326	975	1,02	2. Otter Bridge 4. Rietvlei Spring	2100
Octinoxate	Ultraviolet filter	5466-77-3	$C_{18}H_{26}O_3$	290	1215	1,05	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam	2502
Cashmeran	Synthetic musk fragrance	33704-61-9	$C_{14}H_{22}O$	206	729	0,97	2. Otter Bridge 4. Rietvlei Spring 5. Marais Dam	1689

Compound	Comments/ uses	CAS	Formula	Weight (Da)	1st Dimension Time (s)	2nd Dimension Time (s)	Sample Detected (spectral match quality ≥ 80 %*)	$^{1}D\ RI_{exp}$ (Rtx®-CLPesticides II column)**
Acenaphthene	PAH	83-32-9	$C_{12}H_{10}$	154	2025	2,19	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam Nandoni Dam****	3858
Acetamide	Plastics and as a solvent	60-35-5	C_2H_5NO	59	162	0,8	3. Bird Hide Stream	739
Acetochlor	Pesticide	34256-82-1	$C_{14}H_{20}ClNO_2$	269	1005	1,02	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam	2151
Acetic acid ethenyl ester	Polymer plastics	108-05-4	$C_4H_6O_2$	86	321	0,52	Albasini Dam	1006
Acridine	Dyes	260-94-6	$C_{13}H_9N$	179	960	1,34	2. Otter Bridge 4. Rietvlei Spring 5. Marais Dam	2075
Anthracene	PAH	120-12-7	$C_{14}H_{10}$	178	942	1,31	2. Otter Bridge	2045
Avobenzone	PCP/sunscreen	70356-09-1	$C_{20}H_{22}O_3$	310	1455	1,3	Albasini Dam Nandoni Dam	2904
Benzene	Manufacturing of other chemicals	71-43-2	C_6H_6	78	2052	0,51	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam	3903

Compound	Comments/ uses	CAS	Formula	Weight (Da)	1st Dimension Time (s)	2nd Dimension Time (s)	Sample Detected (spectral match quality ≥ 80 %*)	$^{1}D\ RI_{exp}$ (Rtx®-CLPesticides II column)**
Benzene, 1,2-dichloro-	Antimicrobial/ Pesticide ingredient	95-50-1	$C_6H_4Cl_2$	146	312	2,62	3. Bird Hide Stream 4. Rietvlei Spring 6. MD Channel	990
Benzene, 1,3-dichloro-	Pesticide ingredient	541-73-1	$C_6H_4Cl_2$	146	282	1	1. STR FAM 2. Otter Bridge 4. Rietvlei Spring 5. Marais Dam 6. MD Channel	940
Benzophenone	PCP/sunscreen agent	119-61-9	$C_{13}H_{10}O$	182	843	1,14	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam	1879
Benzyl Benzoate	Pharmaceutical/ treat lice and scabies infestations	120-51-4	$C_{14}H_{12}O_2$	212	924	1,13	1. STR FAM 2. Otter Bridge 4. Rietvlei Spring 5. Marais Dam 6. MD Channel	2015
Benzyl butyl phthalate	Phthalate	85-68-7	$C_{19}H_{20}O_4$	312	1263	1,16	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam	2583
Biphenyl	Organic syntheses, heat transfer fluids, dye carriers, food preservatives, etc.	92-52-4	$C_{12}H_{10}$	154	1674	2,06	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam	3271

Compound	Comments/ uses	CAS	Formula	Weight (Da)	1st Dimension Time (s)	2nd Dimension Time (s)	Sample Detected (spectral match quality ≥ 80 %*)	$^{1}D\ RI_{exp}$ (Rtx®-CLPesticides II column)**
Butanenitrile	Precursor to the poultry drug amprolium	109-74-0	C_4H_7N	69	87	2,12	Albasini Dam	614
Caffeine	Stimulant/ Pharmaceutical	58-08-2	$C_8H_{10}N_4O_2$	194	1020	1,23	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream Albasini Dam Nandoni Dam	2176
Caprolactam	Manufacture of Nylon-6	105-60-2	$C_6H_{11}NO$	113	600	1,1	2. Otter Bridge 5. Marais Dam	1473
Clofenvinfos	Insecticide and acaricide	470-90-6	$C_{12}H_{14}Cl_3O_4P$	358	1122	1,21	5. Marais Dam	2347
Galaxolide	PCP/fragrant	1222-05-5	$C_{18}H_{26}O$	258	951	1,03	1. STR FAM 2. Otter Bridge	2060
Diamyl phthalate	Phthalate	131-18-0	$C_{18}H_{26}O_4$	306	1140	1,01	Albasini Dam	2377
Diazinone	Insecticide	333-41-5	$C_{12}H_{21}N_2O_3PS$	304	933	1,04	2. Otter Bridge 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam	2030
Dibenzofuran	Insecticide	132-64-9	$C_{12}H_8O$	168	741	1,14	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam Nandoni Dam	1709
Dibenzothiophene	Cosmetic ingredient	132-65-0	$C_{12}H_8S$	184	918	1,25	1. STR FAM 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel	2005

Compound	Comments/ uses	CAS	Formula	Weight (Da)	1st Dimension Time (s)	2nd Dimension Time (s)	Sample Detected (spectral match quality ≥ 80 %*)	$^{1}D\ RI_{exp}$ (Rtx®-CLPesticides II column)**
Dibutyl phthalate	Phthalate	84-74-2	$C_{16}H_{22}O_4$	278	1041	0,98	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam	2211
Diethyl Phthalate	Phthalate	84-66-2	$C_{12}H_{14}O_4$	222	810	1,05	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam	1824
Diethylene glycol dibenzoate	Plasticizer	120-55-8	$C_{18}H_{18}O_5$	314	1329	1,25	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam	2693
Diisooctyl phthalate	Phthalate	131-20-4	$C_{24}H_{38}O_4$	390	1314	0,99	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam	2668
Doconexent	Pharmaceutical	6217-54-5	$C_{22}H_{32}O_2$	328	1002	0,96	1. STR FAM	2146
Fenazaquin	Pesticide	120928-09-8	$C_{20}H_{22}N_2O$	306	576	0,96	6. MD Channel	1432

Compound	Comments/ uses	CAS	Formula	Weight (Da)	1st Dimension Time (s)	2nd Dimension Time (s)	Sample Detected (spectral match quality ≥ 80 %*)	^{1}D RI$_{exp}$ (Rtx®-CLPesticides II column)**
Fluoranthene	PAH	206-44-0	$C_{16}H_{10}$	202	1110	1,35	1. STR FAM 2. Otter Bridge 4. Rietvlei Spring 5. Marais Dam Albasini Dam Nandoni Dam	2326
Fluorene	PAH	86-73-7	$C_{13}H_{10}$	166	789	1,18	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam	1789
Glycidol	Organic synthesis/ Polymer plastic	556-52-5	$C_3H_6O_2$	74	195	0,79	Albasini Dam	795
di-(2-ethylhexyl) adipate (DEHA)	Plasticiser	103-23-1	$C_{22}H_{42}O_4$	370	1227	0,88	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam	2522
Metolachlor	Herbicide	1438-62-6	$C_{15}H_{22}ClNO_2$	283	1062	1,09	1. STR FAM	2246
Musk ketone	PCP/fragrant	81-14-1	$C_{14}H_{18}N_2O_5$	294	840	1,07	2. Otter Bridge 4. Rietvlei Spring 5. Marais Dam 6. MD Channel	1874

Compound	Comments/ uses	CAS	Formula	Weight (Da)	1st Dimension Time (s)	2nd Dimension Time (s)	Sample Detected (spectral match quality ≥ 80 %*)	$^{1}D\ RI_{exp}$ (Rtx®-CLPesticides II column)**
Naphthalene	PAH	91-20-3	$C_{10}H_8$	128	468	1,1	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam	1252
Octicizer	Pesticide/ insecticide	1241-94-7	$C_{20}H_{27}O_4P$	362	1263	1,07	2. Otter Bridge 3. Bird Hide Stream 5. Marais Dam Albasini Dam	2583
Octocrylene	PCP/sunscreen	6197-30-4	$C_{24}H_{27}NO_2$	361	1407	1,13	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam Nandoni Dam	2824
Oxybenzone	PCP/Sunscreen	131-57-7	$C_{14}H_{12}O_3$	228	1098	1,2	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam Nandoni Dam	2306
Phenanthrene	PAH	85-01-8	$C_{14}H_{10}$	178	936	1,26	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam Nandoni Dam	2035

Compound	Comments/ uses	CAS	Formula	Weight (Da)	1st Dimension Time (s)	2nd Dimension Time (s)	Sample Detected (spectral match quality ≥ 80 %*)	$^{1}D\ RI_{exp}$ (Rtx®-CLPesticides II column)**
4-tert-Octylphenol	Phthalates/ surfactant	140-66-9	$C_{14}H_{22}O$	206	840	0,97	3. Bird Hide Stream	1874
4-tert-Amylphenol	Pesticide	80-46-6	$C_{11}H_{16}O$	164	852	1	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam Albasini Dam	1894
Bisphenol A	Phthalates/ plasticizer (BPA)	80-05-7	$C_{15}H_{16}O_2$	228	1176	1,34	2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam	2437
Phenylephrine	Pharmaceutical	59-42-7	$C_9H_{13}NO_2$	167	117	0,85	5. Marais Dam	664
Piperonyl butoxide	Pesticide	51-03-6	$C_{19}H_{30}O_5$	338	1257	1,17	4. Rietvlei Spring	2573
Pyrene	PAH	129-00-0	$C_{16}H_{10}$	202	1146	1,4	1. STR FAM 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Nandoni Dam	2387
Retene	PAH	483-65-8	$C_{18}H_{18}$	234	1212	1,27	3. Bird Hide Stream 5. Marais Dam	2497
Spiroxamine	Fungicide	118134-30-8	$C_{18}H_{35}NO_2$	297	942	0,92	4. Rietvlei Spring 6. MD Channel Albasini Dam	2045
Styrene	Plastics and resins	100-42-5	C_8H_8	104	165	0,93	Albasini Dam	744
Sulfotep	Pesticide	3689-24-5	$C_8H_{20}O_5P_2S_2$	322	879	1,03	4. Rietvlei Spring 5. Marais Dam	1940

Compound	Comments/ uses	CAS	Formula	Weight (Da)	1st Dimension Time (s)	2nd Dimension Time (s)	Sample Detected (spectral match quality ≥ 80 %*)	^{1}D RI$_{exp}$ (Rtx®-CLPesticides II column)**
Terbuthylazine	Herbicide	171199-36-3	$C_9H_{16}ClN_5$	229	936	1,1	2. Otter Bridge 5. Marais Dam	2035
Triazophos	Pesticide	24017-47-8	$C_{12}H_{16}N_3O_3PS$	313	1269	1,31	4. Rietvlei Spring 5. Marais Dam	2593
Tributyl phosphate	Extractant and a plasticizer (TBP)	126-73-8	$C_{12}H_{27}O_4P$	266	837	0,85	1. STR FAM 2. Otter Bridge 3. Bird Hide Stream 4. Rietvlei Spring 5. Marais Dam 6. MD Channel Albasini Dam Nandoni Dam	1869
Triclosan	Antiseptics/PCP	3380-34-5	$C_{12}H_7Cl_3O_2$	288	1119	1,25	2. Otter Bridge 4. Rietvlei Spring	2341
Tuaminoheptane	Pharmaceutical/ nasal decongestant drug	123-82-0	$C_7H_{17}N$	115	135	2,93	1. STR FAM	694

*Similarity NIST14 library
**Proprietary Crossbond® phase Rtx®-CLPesticides II 30 m x 0.25 mm ID x 0.2 µm film thickness (fused silica) column
***Compounds given in red are classified EDCs (EDCs classified using EU EDC database, EDC databank created by Dr Montes-Grajales and Prof Olivero-Verbel, University of Cartagena and TEDX, The Endocrine Disruption Exchange).
****Nandoni Dam untargeted screening done using PDMS loop-TDS-GC×GC-TOFMS

Addendum M: Published Paper