



UNIVERSITÀ DEGLI STUDI DI TORINO

This Accepted Author Manuscript (AAM) is copyrighted and published by Elsevier. It is posted here by agreement between Elsevier and the University of Turin. Changes resulting from the publishing process - such as editing, corrections, structural formatting, and other quality control mechanisms - may not be reflected in this version of the text. The definitive version of the text was subsequently published in [*insert name of publication, volume number, issue number, date, and digital object identifier link*].

You may download, copy and otherwise use the AAM for non-commercial purposes provided that your license is limited by the following restrictions:

- (1) You may use this AAM for non-commercial purposes only under the terms of the CC-BY-NC-ND license.
- (2) The integrity of the work and identification of the author, copyright owner, and publisher must be preserved in any copy.
- (3) You must attribute this AAM in the following format: Creative Commons BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/deed.en>), [<http://dx.doi.org/10.1016/j.chroma.2012.09.089>]

**Fast low-pressure microwave assisted extraction and gas chromatographic
determination of polychlorinated biphenyls in soil samples**

M.C. Bruzzoniti^{a*}, R. Maina^b, V. Tumiatti^b, C. Sarzanini^a, L. Rivoira^a, R.M. De Carlo^a

^a Department of Chemistry, University of Torino

^b Sea Marconi Technologies, Collegno (TO)

***Corresponding Author**

Maria Concetta Bruzzoniti

Department of Chemistry

University of Torino

Via P. Giuria 5, 10125 Torino Italy

Ph: +39 011 6705277

Fax: +39 011 6705242

Abstract

A new technology equipment for low-pressure microwave assisted extraction (usually employed for organic chemistry reactions), recently launched in the market, is used for the first time in environmental analysis for the extraction of commercial technical Aroclor mixtures from soil.

Certified reference materials of Aroclor 1260, Aroclor 1254 and Aroclor 1242 in transformer oils were used to contaminate the soil samples and to optimize the extraction method as well as the subsequent gas chromatographic electron capture detection (GC-ECD) analytical method.

The study was performed optimizing the extraction, the purification and the gas chromatographic separation conditions to enhance the resolution of *difficult* pairs of congeners (C28/31 and C141/179). After optimization, the recovery yields were included within the range 79-84%. The detection limits, evaluated for two different commercial polychlorinated biphenyls (PCB) mixtures (Aroclor 1260 and Aroclor 1242) were 0.056 ± 0.001 mg/kg and 0.290 ± 0.006 mg/kg, respectively.

The method, validated with certified soil samples, was used to analyze a soil sample after an event of failure of a pole-mounted transformer which caused the dumping of PCB contaminated oil in soil. Moreover, the method provides simple sample handling, fast extraction with reduced amount of sample and solvents than usually required, simple purification step involving the use of solvent (cyclohexane) volumes as low as 5 ml.

Reliability and reproducibility of extraction conditions are ensured by direct and continuous monitoring of temperature and pressure conditions.

Keywords: low-pressure microwave assisted extraction; controlled extraction conditions; Aroclor; green analytical chemistry; gas chromatography; soil samples.

1 Introduction

Polychlorinated biphenyls (PCBs) are a group of organic pollutants consisting of 209 congeners having biphenyl as the core structural unit and a variable number of chlorine substituents. Basically, PCBs were extensively industrially utilized in 1930s and 1940's in open (as additives to glues, dyes, and construction materials) and in closed systems (coolants and lubricants in transformers, dielectric fluids in capacitors, hydraulic fluids and heat-transfer media) [1].

PCBs are resistant to degradation, have long persistency after their use and immission in the environment, accumulate in sediments and, through sediment dwelling microorganisms living organisms, they tend to enter the food chain. PCBs can be transmitted in breast milk [2] and can cross the placenta. The toxicity of PCB has been well documented [3] and therefore their occurrence poses a risk of causing adverse effects to human health and the environment. PCBs are found in soils, surface waters, sediments, air throughout the world, since they are transported across international boundaries far from their sources, even to regions where they have never been used or produced. PCBs were included in the global Stockholm Convention on POPs, entered into force on 17 May 2004, and are referred to as substances subject to review for possible identification as priority substances or priority hazardous substances according to the Directive 2008/105/EC. Seven PCBs commercial mixtures were placed on the list of priority contaminants under the Clean Water Act of USEPA, namely Aroclor 1242,

Aroclor 1254, Aroclor 1221, Aroclor 1232, Arochlor 1248, Aroclor 1260 and Aroclor 1016.

The classical method for extraction of PCBs from environmental samples is Soxhlet extraction which requires large amounts of solvent and about 20 h to complete the extraction. Ultrasound [4,5] and microwave assisted extractions (MAE) [6,7] have gained wide acceptance due to low temperature requirement, high extraction rate, automation and the possibility of simultaneously extracting different types of samples. Toluene and dichloromethane were recognized among the most efficacious extraction solvents for PCBs allowing extraction yields included between e.g.: 66% (congener 118) and 95% (congener 180) for toluene and 49% (congener 118) and 101% (congener 153) for dichloromethane [8]. More recently, accelerated solvent extraction (ASE) was proposed as alternative promising approach. Under elevated temperature and pressure, an extraction solvent can be used above its boiling point but still remaining in the liquid state, increasing the kinetics of the extraction process. As a result, the extraction time and solvent consumption are significantly decreased. Many ASE applications were proposed for PCBs determination in solid environmental samples [9,10].

As far as MAE approach is concerned, for any heating effect, a polar solvent which is miscible with the nonpolar extraction solvent must be added. Nevertheless, Düring and Gäth [11] used an hydrophobic solvent (n-heptane) as solely applied extraction solvent with heat transformer disks which absorb the microwave energy and allow a rapid convective heating of the non-polar solvent. Anyway, it must be said that microwave assisted extractions are generally performed in a closed-vessel system and have several common features such as: the use of pressurized Teflon extraction vessels that must be carefully rinsed with n-hexane and processed with pure acetone by MAE

before use to avoid cross-contamination; 3-15g of sample; 20-30 mL extraction solvents [7]. The extract should be then subjected to a long purification procedure aimed at the removal of co-extracted interferents, requiring discrete amounts of solvents. A critical appraisal of the analytical protocols, including extraction, for PCBs determination in environmental matrices has been provided by Muir and Sverko [12].

No single, detailed, step-by-step analytical method can be recommended or PCBs determination [12]. A performance-based approach has also been adopted by the US EPA in an effort to introduce flexibility in conducting environmental monitoring. This approach also encourages analytical innovation by allowing the use of new and alternate methods provided that predetermined performance criteria are met [13].

In this study, we show the use of a new, recently marketed, platform for low-pressure microwave digestion technology for the extraction of PCBs from contaminated soils. To the best of our knowledge, this study represents the first use of this equipment for environmental applications.

The study was performed according to the following main analytical steps: optimization of (i) microwave extraction conditions; (ii) purification conditions; (iii) optimization of gas chromatographic separation. The method was then compared with ultrasound assisted extraction and validated by certified samples. The method proposed is characterized by: fast extraction times; the use of very low amounts of sample and solvents, in respect to the already available extraction approaches (included traditional microwave based methods); the use of disposable extraction vessels which avoid contamination problems commonly encountered during routine analysis; easy to use sample holders which do not require additional tools. The low pressures involved do not pose the safety risks (explosion) that can be encountered in the traditional MAE

applications. The method is also characterized by good resolution between *difficult* pairs of congeners and by good reproducibility and accuracy as shown by the validation procedure.

2 Experimental

2.1 Chemicals

All reagents used throughout this work were analytical grade. Acetone (PA-ACS-ISO, 99.5%) was from Panreac, Novachimica (Milan, Italy). Cyclohexane, sulfuric acid, 98%, Na₂SO₄ anhydrous, silica gel, activated granulated Cu, and Al₂O₃ were from Sigma Aldrich (Milan, Italy).

For extract purification, silica gel cartridges were used as such, according to manufacturer indications. Al₂O₃ was activated by heating overnight at 150 °C. Granulated Cu was used as such as recommended by the manufacturer. Before use, Na₂SO₄ was heated overnight at 120 °C.

The internal standard (PCB-Mix 26) is a solution of 300 ng/μL C30 and 100 ng/μL C209 congeners in cyclohexane and was purchased from Dr Ehrenstorfer (Ausburg, Germany).

While both internal standards were used for peak identification (see §2.5), congener C209 is involved in individual congeners quantification (see § 2.6).

Certified reference materials, 50 μg/g each, Aroclor 1260, Aroclor 1254 and Aroclor 1242 solutions in transformer oil matrix were from Ultra Scientific (RI, USA)

Certified solution containing 9 PCBs congeners (C18, C31, C44, C101, C118, C138, C170, C180, C194) and C209 in isooctane all at 10 mg/L (purity 98-99%) were from Dr Ehrenstorfer.

2.2 Instruments

A Discover SP-D (CEM, BG, Italy) microwave digester provided with autosampler was used throughout this work.

For chromatographic measurements, a HP6890 gas chromatograph (HP, SRA, Milan, Italy) provided with a 6890 series split-splitless injector was used. The applied injection conditions were: injector in splitless mode, 1 μ L volume, 340 °C. The column used was a J&W HP-ULTRA2 (5% phenyl-polymethylsiloxane), 50 m, 0.2 mm i.d., 0.33 μ m film thickness (Agilent). The detector was a 7890 Micro-ECD (Agilent). For sample centrifugation, a Thermo Scientific IEC CL10 (Milan, Italy) centrifuge was used. A HB43-S Halogen Moisture Analyzer (Mettler Toledo, Milan, Italy) was used to determine the humidity and dry content in the soil samples.

2.3 Samples and sample preparation

Two certified reference materials (PCBs in soil) of Aroclor 1242 and Aroclor 1254, both from Ultra Scientific, were used.

The real sample was collected at 19/05/2011 in Heillecourt (France), homogenized and stored in a glass holder.

PCB were extracted by microwave assisted extraction according to the procedure optimized throughout this work. Final conditions are summarized in figure 1, where all steps of the protocol are detailed.

2.4 Ultrasound extraction

1 g of soil sample was added to 10 mL isoctane, spiked with the internal standard (C209 and C30) and sonicated for 1h. The sample is decanted and 3 mL of

solvent are added with 2 mL H₂SO₄ and sonicated again for 10 min. After phases separation, the supernatant organic phase is injected into the GC-ECD system and analyzed.

2.5 Congeners identification

The identification of the PCB congeners was performed recognizing the chromatographic peaks through relative retention time (RRT):

$$RRT = \frac{t_{R,i} - t_{R,C30}}{t_{R,C209} - t_{R,C30}} \quad (1)$$

Where $t_{R,i}$ is the retention time of the congener to be identified, $t_{R,C30}$ and $t_{R,C209}$ are the retention times of the congeners C30 and C209, respectively.

Each congener was assigned to signal peak by comparing the elution profile of a mixture of the certified solutions of Aroclor 1242, Aroclor 1254, Aroclor 1260 with reference chromatogram depicted in the Standard IEC 61619 [14].

2.6 Evaluation of PCB concentrations

The PCB concentration was calculated according to the internal standard procedure, through the mass of i -peak (m_i):

$$m_i (mg) = \frac{M_s \cdot A_i}{A_s \cdot RRF_i} \quad (2)$$

Where M_s and A_s are respectively the weight (mg) and the chromatographic peak area of the internal standard congener 209; A_i is the chromatographic peak area of the i -peak and RRF_i is the relative response factor corrected for the i -peak.

Corrected RF_i were calculated as described in Standard IEC 61619, §11.5 [14]. The calculation procedure can be briefly summarized as follows. The 105 peaks that can be separated in the described chromatographic conditions are associated with nine chromatographic segments. For each segment, one congener is used as a reference for quantification by calculating the actual experimental relative response factor ($ERRF$). The nine $ERRF$ values obtained are then compared with the tabulated $ERRF$ values and resulting k ratio is used to calculate all the RRF_{iS} , by assuming that all RRF of peaks belonging to the same segment are in the same ratio with the RF of congener 209.

Total PCB concentration was finally expressed as

$$PCB_{tot} \left(\frac{mg}{kg} \right) = \left(\sum_{i=1}^n m_i \right) \frac{1}{1000 \cdot m_c} \quad (3)$$

Where m_c (g) is the mass of the soil sample.

3 Results and discussion

3.1 Optimization of microwave conditions

The digester used has a focused single-mode cavity designed to maximize the microwave energy input to the sample in a high-density field, giving the system an efficiency that needs only a fraction of the energy required by multimode systems (300 W vs 1400-1600W). A circular cavity, completely encapsulating the disposable pyrex vessel, is designed to focus energy efficiently on the sample, drastically reducing reaction times. For this features, the digester can be considered among the most energy-efficient microwave digestion system available. The use of disposable pyrex vessels is two-fold advantageous. In fact, in this way the cross-contamination among samples is totally avoided and, as a consequence, tedious and solvent consuming washing

procedures of sample vessels are unnecessary. Moreover, since the pyrex sample vessel is transparent to the IR, the system allows a continuous monitoring of temperature and pressure conditions in the extraction ambient, ensuring reliable and reproducible extraction conditions.

Extraction was performed by 5 mL of a 3:2 acetone:cyclohexane mixture. The use of a polar solvent such as acetone allows the absorption of microwave energy and hence a rapid heating of the non-polar solvent, so that temperatures far above the boiling point of cyclohexane (80.7 °C) could be achieved.

The effect of temperature was initially studied. The higher the temperature, the more efficient the extraction yield. Nevertheless, an increase in temperature also leads to increased pressure that can give losses of analytes.

First experiments were performed to study the dependence of pressure (p) on temperature (T). Temperature was varied from 70 °C to 160 °C (ten levels) on blank solutions containing the extraction mixture only, finding a correlation of $p = 1.3T - 95.2$ ($r^2 = 0.9871$). For T as high as 160 °C, pressure values higher than 100 psi and escape of solvent were observed. A T of 130 °C (temperature holding time 20 min), leading to a pressure of 77.5 ± 1.7 psi ($n=4$) was definitively chosen as a compromise of high temperatures and pressures which avoid loss of analytes. In order to achieve the temperature optimized, 300W (ramp time 10 min) was set. The yield of extraction was hence determined on soil samples (0.4 g) contaminated with known amounts of certified solutions Aroclor 1242, Aroclor 1254 and Aroclor 1260 to give a nominal concentration of 2.92 mg/kg PCB. A control soil sample (blank) was run in parallel. Even if with this digester a stirring bar can be used to homogenize the sample inside the solvent mixture, we chose not to use this feature to avoid cross-contamination.

The extracts were purified with sulfuric acid and injected for GC analysis. Since high background noise and overestimates of PCB concentrations (4.25 mg/kg) was obtained, further experiments were aimed to decrease the background signal of the GC analysis, optimizing the purification conditions of the extracts.

3.2 Optimization of purification of extracts

The purification of the extracts is an important step to remove the interferences co-extracted and residual of water. Experiments were performed on a non-contaminated soil sample (blank), added with internal standard solutions and extracted according to the previously optimized conditions. Several purification procedures (listed in table 1) were evaluated. Before each purification procedure, the extracts were centrifuged at 3850 rpm for 5 min. After purification, the resulting solutions were analyzed by GC-ECD at the conditions detailed in table 2 (ramp 1). The results obtained for each purification method are shown in table 1, expressed as sum of peak areas eluted in the time window included between C30 and C209 peaks.

As a first attempt, the extract was injected without any purification (Method A). Results clearly indicate that some co-extracted compounds give rise to signals to ECD detector that can interfere with PCB determination. As a further attempt, a clean-up with silica (Method B), usually recommended for purification of extracts for PCB analysis was attempted. Silica removes co-extracted polar compounds and residual water, by adsorption interactions. Nevertheless, higher background signal was obtained. Two additional clean-up procedures were tested by adding Al₂O₃ (ratio 1:1) (Method C) or activated Cu (ratio 1:1) (Method D) into the silica cartridge. Activated copper [15] was

used to remove elemental sulfur that can be co-extracted from sediment and soil samples, decaying organic material, and from some industrial wastes as well as in oil as residual after desulfurization. As shown in table 1, no appreciable improvements were obtained if compared with no purification of the extract. In the same manner, a combined one step clean-up with silica gel, activated Cu and Al₂O₃ present in equal ratio amounts (Method E) did not improve the signal.

A treatment with Na₂SO₄ directly dispersed into the extraction vial was additionally tested (Method F). Even if the background slightly decreased, this cannot be considered enough. A purification step with concentrated sulfuric acid was also investigated (Method G). This treatment is specifically indicated to remove hydrocarbons and other organic compounds, which are co-extracted with PCB, as well as water, since sulfuric acid is a dehydrating acid. The addition of sulfuric acid caused a strong exothermic reaction that also worsened the background noise. The H₂SO₄ based treatment was repeated after acetone removal, by heating at 60 °C (Method H). The residual was made up to 5 mL with cyclohexane. As shown in table 1, the background noise is very low. Similar background noise can be obtained if acetone removal is performed by sparging with N₂. Nevertheless, we experienced that, in the presence of PCB, the glass sparger is a source of cross-contamination for subsequent samples. Cleaning of the sparger requires an additional rinsing step with ultrasounds and cyclohexane.

The clean up procedure is fast and very simple in respect to other procedures. If compared with usual microwave-assisted extraction, the digester used and the procedure developed requires lower extraction times (25 min vs 45 min [9]), lower amounts of solvent (5 mL vs 25-30 mL [9, 16]) and easier purification procedures not involving

solvent-exchange and/or fractionation on columns which in turn require additional discrete volumes of eluting solvents.

It is worth noting that also the amount of soil extracted (0.4 g) is lower than required by other approaches (5-15 g soil required by ASE [17] or by conventional MAE extractions [7,9,18]). To compare with other environmental matrices, e.g. sediments, minimum amount of 5-10 g must be subjected to extraction [19,20].

It must be also highlighted that the proposed MAE approach can be attractive for those analysis where the availability of the sample is a limiting factor (e.g.: biological, forensic applications).

3.3 Chromatographic separation conditions

In order to test the performance of the separation conditions, a resolution check between the pairs of congeners C28/C31 and C141/C179 was performed, as recommended by the Standard IEC 61619. In fact, separation of the mentioned pairs of congeners is critical in many environmental samples, the order of elution of the isomers 28 and 31 is frequently confused [21] and moreover C28 congener is usually present in the majority of technical mixtures [1].

According to the Standard, the resolution should be at least 0.5 for C28/C31 and 0.8 for C141/C179. At the GC conditions labeled as ramp 1 (table 2), the resolutions between C28/C31 and C141/C179 were respectively 0.8 and 1.3. In figure 2a a typical chromatogram showing the separation between the pair C28/C31 is reported. It can be seen that both the values fulfill the requirement of the Standard. To further improve the resolution of the separation condition, especially for C28/C31 pair, ramp conditions were changed. After several experiments, the ramp giving the best resolution among

peaks was ramp 2 in table 2, that provided a resolution of 1.7 for C28/C31 (see figure 2b) and 1.0 for C141/C179. Ramp 2 was used for further method performance evaluation.

3.4 Method performance

3.4.1. Extraction yield

After the optimization of microwave extraction, purification and chromatographic parameters, the extraction yield was evaluated. A known amount of PCB-free soil was contaminated with a certified solution containing 9 PCB congeners and C209 in isooctane to give a concentration of 1.25 mg/kg for each congener. The certified solution contains, among the others, the congeners C101, C138, C180, which are present in many technical mixtures, and C118 that being a dioxin-like congener [22], belongs to the toxic congener [1].

The PCBs were hence extracted from the soil, and the extract purified as optimized. The procedure was performed in triplicate. The purified extracts were analyzed by the optimized GC-ECD ramp and for each congener, the recoveries were calculated according to the following relationship:

$$R_i (\%) = 100 \cdot \frac{A_{i,soil}}{A_{i,solution}} \quad (4)$$

where $A_{i,soil}$ is the area of the i -peak detected after extraction of the soil, and $A_{i,solution}$ is the area of the i -peak originally present in the certified solution.

The results (table 3) show that recoveries keep constant independently from the congener. In fact, congeners with low and high number of Cl atoms substituents have

statistically the same recovery yield, as well as the internal standard C209. This behavior allows the use of internal standard with a good reliability and accuracy. A typical chromatogram obtained is shown in figure 2.

Recoveries generally depends upon the matrix, the congeners and the extraction method considered. Therefore a direct comparison with literature is not so straightforward. The recovery values obtained can be considered higher than those obtained for e.g. certified reference material river sediment (Soxhlet extraction of congeners 18, 28, 33, 44, 52, 101, 118, 149, 138, 170, 180, 194 and 209) where greater than 65% (rsd from 4.6% to 11.5%) [23] or in agreement with data presented by Pozo et al [20] who reported a recovery for PCB-30, with an average recovery >80% (Soxhlet extraction). As a comparison, for MAE extraction (5 g sample), recovery of 80 ± 9 , 70 ± 11 , 69 ± 4 where obtained by Herbert et al. [18].

To speed up the whole extraction procedure, extraction experiments were repeated at lower temperature holding time (5, 10, and 15 min). Since no significant changes in recovery were observed, further experiments were performed at 5 min temperature holding time.

3.4.2. Detection limit

Detection limit (DL) was evaluated as the concentration related to a signal S_m defined as $S_m = S_b + 3s_b$ (S_b and s_b are the average signal for blank and its standard deviation, respectively).

To evaluate the signals of the blank, a non contaminated soil sample soaked with a PCB-free oil was processed ten folds. The DL were evaluated for two different commercial PCB mixtures, i.e. Aroclor 1260 and Aroclor 1242, and were 0.056 ± 0.001

mg/kg and 0.290 ± 0.006 mg/kg, respectively. The difference in the two DL can be explained by considering the lower ECD sensitivity towards Aroclor 1242 which is mainly composed by congeners with a lower number of Cl atoms.

In order to verify the reliability of measurement in the range of concentrations close to the detection limits, two uncontaminated soil samples spiked with known amounts of certified solution of (i) Aroclor 1260 to provide final concentration of 0.058 mg/kg and (ii) Aroclor 1242 (final concentration 0.303 mg/kg) were analyzed according to the developed procedure. Results obtained (shown in figure 3) were respectively 0.057 ± 0.001 mg/kg for Aroclor 1260 and 0.296 ± 0.043 mg/kg for Aroclor 1242. It should be remarked that a comparison with literature values is rather difficult since values are referred to different congeners (when specified) to those considered in this study. Nevertheless, an agreement of LOQ of units-tenths of $\mu\text{g}/\text{kg}$ in soils is generally found [18,24], even if, differently from our approach, the calculated values are hardly experimentally verified.

3.4.3. Accuracy

The accuracy of the extraction method was evaluated on two certificated soil samples originally contaminated with Aroclor 1254 and Aroclor 1242. Four replicates were performed on each soil sample. As shown in table 4, for both the certified samples the values obtained are in agreement with certified reference value and included inside the prediction intervals. The microwave assisted extraction optimized was compared with an ultrasound extraction (described in §2.4). Since the results obtained were 0.88 ± 0.01 mg/kg for the certified sample Aroclor 1254 and 17.3 ± 0.4 mg/kg for the

certified sample Aroclor 1242, an underestimation of the ultrasound procedure must be assessed.

The microwave assisted procedure was additionally tested on three aliquots of PCB-free soil samples contaminated with certified mixtures of Aroclor 1242, Aroclor 1254 and Aroclor 1260. As shown in table 5, according to the concentration added, deviations were included between 0.3% and 8.2%.

3.5 Real sample analysis

After optimization and validation of the whole procedure, the method was applied to analyze the PCB content in a soil sample collected after an event of failure of a pole-mounted transformer in Heillecourt (France) which caused the dumping of PCBs contaminated oil in soil.

The sample was analyzed in nine replicates. A concentration of PCBs of 3.66 ± 0.28 mg/kg was obtained. Figure 4 shows a typical chromatogram obtained. To the best of our knowledge, a limit for PCBs in soil is not established by a legislative regulation in France. As far Italian legislation is concerned (Legislative Decree 152/2006), a maximum value of 5 mg/kg is set for soil with industrial and commercial uses and 0.06 mg/kg for residential and allotment land uses, as it was the case of the sample analyzed. As a comparison, the sample was also extracted by the ultrasound procedure and analyzed, obtaining a concentration of 1.11 ± 0.08 mg/kg, confirming the underestimation of the ultrasound procedure.

4 Conclusions

In the present study, a novel, fast, sensitive and reproducible low-pressure microwave assisted extraction and purification method was developed, validated, and successfully applied to the determination of PCBs in soil samples by GC-ECD.

The method developed provides short extraction times. The extraction procedure, in combination with the simple clean-up of the extract, reduced drastically the solvent consumption and thus is more environmental friendly. The validated method showed a good performance with regard to, LODs, extraction yields, accuracy and precisions. The method can be applied, as an attractive and very promising approach, to the analysis of PCBs in environmental samples, due to the possibility of automation, easy to use, fast and low cost analysis, when compared to conventional (Soxhlet, MAE, ultrasound) extraction methods. The technology used avoids cross contamination, due to the use of user-friendly disposable vessels, and does not pose safety risks (explosion), due to the low working pressures. Features of the method, including the low sample amount required make it promising for applications where the availability of the sample is a limiting factor (e.g.: forensic).

Acknowledgements

Financial support from MIUR (Ministero dell'Istruzione, dell'Università e della Ricerca, Italy) is gratefully acknowledged.

References

- [1] O.N. Zabelina, V.I. Saloutin, O. N. Chupakhin, *J. Anal. Chem.* 65 (2010) 1098–1108.
- [2] H. Shen, G. Ding, Y. Wu, G. Pan, X. Zhou, J. Han, J. Li, S. Wen, *Environ. Int.* 42 (2012) 84-90.

- [3] S.H. Safe, *Crit. Rev. Toxicol.* 24 (1994) 87-149.
- [4] D. Martens, M. Gfrerer, T. Wenzl, A. Hang, M. Gawlik, K. W. Schramm, E. Lankmayr, A. Kettrup. *Anal. Bioanal. Chem.* 372 (2002) 562–568.
- [5] A.A. Nikonova, A.G. Gorshkov, *Anal. Lett.* 44 (2011) 1290–1300.
- [6] N. Itoh, M. Numata, Y. Aoyagi, T. Yarita, *Anal. Chim. Acta* 612 (2008) 44–52.
- [7] G. Xiong, X. He, Z. Zhang, *Anal. Chim. Acta* 413 (2000) 49–56.
- [8] A. Mechlińska, L. Wolska, J. Namieśnik, *Anal. Lett.* 43 (2010) 1149–1161.
- [9] P. Wang, Q. Zhang, Y. Wang, T. Wang, X. Li, L. Ding, G. Jiang, *Anal. Chim. Acta* 663 (2010) 43–48.
- [10] L. Zhao, H. Hou, Y.Y. Zhou, N.D. Xue, H.Y. Li, F.S. Li, *Chemosphere* 78 (2010) 1285–1293.
- [11] R.A. Düring, St. Gäth, *Fresenius J. Anal. Chem.* 368 (2000) 684–688.
- [12] D. Muir, E. Sverko *Anal. Bioanal. Chem.* 386 (2006) 769–789.
- [13] US EPA (1997) Performance-based measurement system. US Environmental Protection Agency, Washington, DC, pp 52098–52100
- [14] International Electrotechnical Commission, IEC 61619 ed1.0, Insulating liquids - Contamination by polychlorinated biphenyls (PCBs) - Method of determination by capillary column gas chromatography, Geneva, Switzerland (1997)
- [15] A. Mechlińska, L. Wolska, J. Namieśnik, *Trends Anal. Chem.* 31 (2012) 129-133.
- [16] S. Sporning, S. Bøwadt, B. Svensmark, E. Bjorklund, *J. Chromatogr. A*, 1090 (2005) 1–9.
- [17] V. Hodak-Kobasic, M. Picer, N. Picer, T. Kovač1, *Organohalogen Compd.* 66 (2004) 31-37.
- [18] P. Herbert, S. Morais, P. Paiga, A. Alves, L. Santos, *Intern. J. Environ. Anal. Chem.* 86 (2006) 391–400.
- [19] K. Pozo, D. Lazzerini, G. Perra, V. Volpi, S. Corsolini, S. Focardi, *Mar. Pollut. Bull.* 58 (2009) 773-776.

- [20] K. Pozo, R. Urrutia, R. Barra, M. Mariottini, H.C. Treutler, A. Araneda, S. Focardi, *Chemosphere* 66 (2007) 1911–1921.
- [21] P. Konieczka, L. Wolska, J. Namiesnik *Trends Anal. Chem.* Vol. 29 (2010) 706-717.
- [22] <http://www.epa.gov/osw/hazard/tsd/pcbs/pubs/congenertable.pdf>, accessed 13 july 2012
- [23] L. Guzzella, C. Roscioli, A. Binelli, *Chemosphere* 73 (2008) 1684–1691.
- [24] L. Guzzella, G. Poma, A. De Paolis, C. Roscioli, G. Viviano, *Environ. Pollut.* 159 (2011) 2552–2564.

Table 1. Evaluation of the performance of different purification procedure after microwave assisted extraction.

Method	Procedure	Σ noise (sum of peak areas) ^{a)}
A	No purification	380
B	Silica gel	1590
C	Silica gel and Al ₂ O ₃	646
D	Silica gel and activated Cu	1110
E	Silica gel, activated Cu and Al ₂ O ₃	851
F	Na ₂ SO ₄ (in the extraction vessel, directly)	308
G	2 mL H ₂ SO ₄	2250
H	Acetone evaporation by heating and 2 mL H ₂ SO ₄	76

^{a)} Integration parameters: minimum peak width = 0.08 min; minimum area = 1

Table 2. Starting and optimized GC ramp conditions.

Oven temperature (ramp 1)	120 °C for 1 min, 50 °C/min to 200 °C (1 min hold), 5 °C/min to 270 °C, then 20 °C/min to 315 °C (10 min hold). Total run time 30 min
Oven temperature (ramp 2)	90 °C for 3 min, 10 °C/min to 200 °C (3 min hold), 3 °C/min to 290 °C, then 20 °C/min to 315 °C (12 min hold). Total run time 60 min

Table 3. Recovery of PCBs from soil. Soil contaminated with certified solutions of nine PCBs.

Congener number (Descriptor ^a)	PCBs (IUPAC Names)	t _R (min)	Recovery ^{b)} (%)
18	2,2',5-Trichlorobiphenyl	21.8	79.1 ± 2.6
31 (CP1)	2,4',5-Trichlorobiphenyl	24.0	81.0 ± 8.6
44 (4CL_2M)	2,2',3,5'-Tetrachlorobiphenyl	26.9	79.2 ± 2.1
101 (4CL_2M)	2,2',4,5,5'-Pentachlorobiphenyl	30.4	81.2 ± 2.9
118 (CP1_4CL_PP_2M)	2,3',4,4',5-Pentachlorobiphenyl	33.9	80.1±7.8
138 (4CL_PP_2M)	2,2',3,4,4',5'-Hexachlorobiphenyl	36.7	81.2±5.8
170 (4CL_PP_2M)	2,2',3,3',4,4',5-Heptachlorobiphenyl	42.2	81.8±6.6
180 (4CL_PP_2M)	2,2',3,4,4',5,5'-Heptachlorobiphenyl	40.5	81.8±5.6
194 (4CL_PP_2M)	2,2',3,3',4,4',5,5'-Octachlorobiphenyl	45.7	82.0±5.5
209	2,2',3,3',4,4',5,5',6,6'- Decachlorobiphenyl	48.9	83.6±4.9

^{a)} Data taken from EPA [<http://www.epa.gov/osw/hazard/tsd/pcbs/pubs/congenertable.pdf>]

CP0 / CP1. 68 co-planar congeners include 20 with chlorine substitution at none (CP0, non-ortho) and 48 with chlorine substitution at only one (CP1, mono-ortho) of the 2, 2', 6, or 6' positions.

4CL. 169 congeners have a total of four or more chlorine substituents (regardless of position).

PP. 54 congeners have both para positions (4 and 4') chlorinated.

2M. 140 congeners have two or more of the meta positions (3, 3', 5, and 5') chlorinated.

Congeners are those that display all four of the above Descriptors are referred to as "Dioxin-like".

^{b)} n=3

Table 4. Application of the protocol developed to two certified soil samples.

Sample	Concentration found (mg/kg)	Prediction Interval^a (Certified reference value^b) (mg/kg)
Certified soil sample (Aroclor 1254)	1.75±0.03	0.44-2.11 (1.28)
Certified soil sample (Aroclor 1242)	35.6±0.6	0-80.2 (39.4)

^{a)} Measurements should fall within the prediction interval with a confidence level of 95%.

^{b)} Established through interlaboratory testing

Table 5. Application of the protocol developed to two PCB-free soil samples contaminated with certified mixtures (Aroclor 1242, Aroclor 1254 and Aroclor 1260)

Sample	Concentration found (mg/kg)	Nominal value (mg/kg)
Soil contaminated (Aroclor 1254)	2.99	3.00
Soil contaminated (Aroclor 1242)	2.28	2.24
Soil contaminated (Aroclor 1260)	2.65	2.45

Figure Captions.

Figure 1. Microwave assisted extraction and purification protocol optimized.

Figure 2. Separation of C28/C21 congeners with: (a): ramp 1 (table 2) and (b): ramp 2 (table 2).

Figure 3. (a) Typical chromatogram obtained for extraction of PCBs (1.25 mg/kg each) from soil. The soil was contaminated with certified PCBs solution. Extraction conditions as optimized (Figure 1); (b) chromatogram of the certified standard solution.

Figure 4. Analysis of a soil sample contaminated with Aroclor 1242 at LOD levels (0.303 mg/kg), according to the procedure optimized (Figure 1). (a): actual chromatogram. (b): simulated elution profile. Identification of peaks according to eq. (1)

Figure 5. Analysis of PCBs in a contaminated soil sample (Heillecourt, France) after a transformer failure event with the optimized method. (a): actual chromatogram. (b): simulated elution profile. Identification of peaks according to eq. (1)

Soil preparation

Grinding, determination of humidity and dry weight

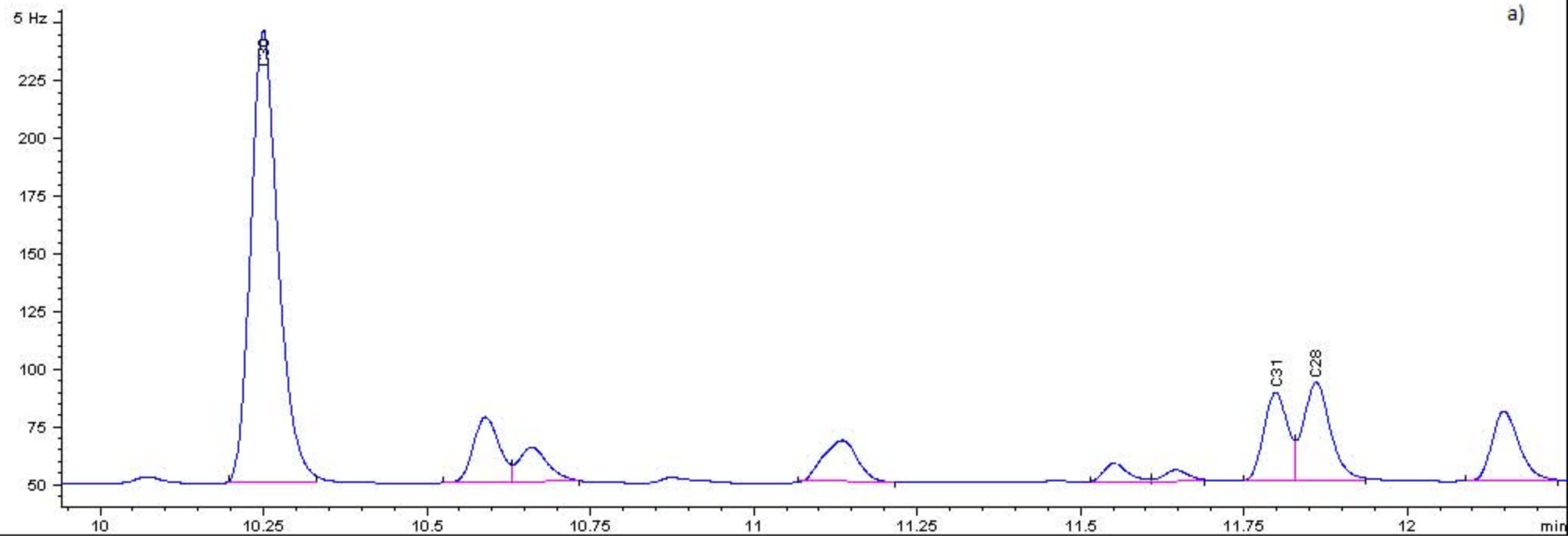
Extraction

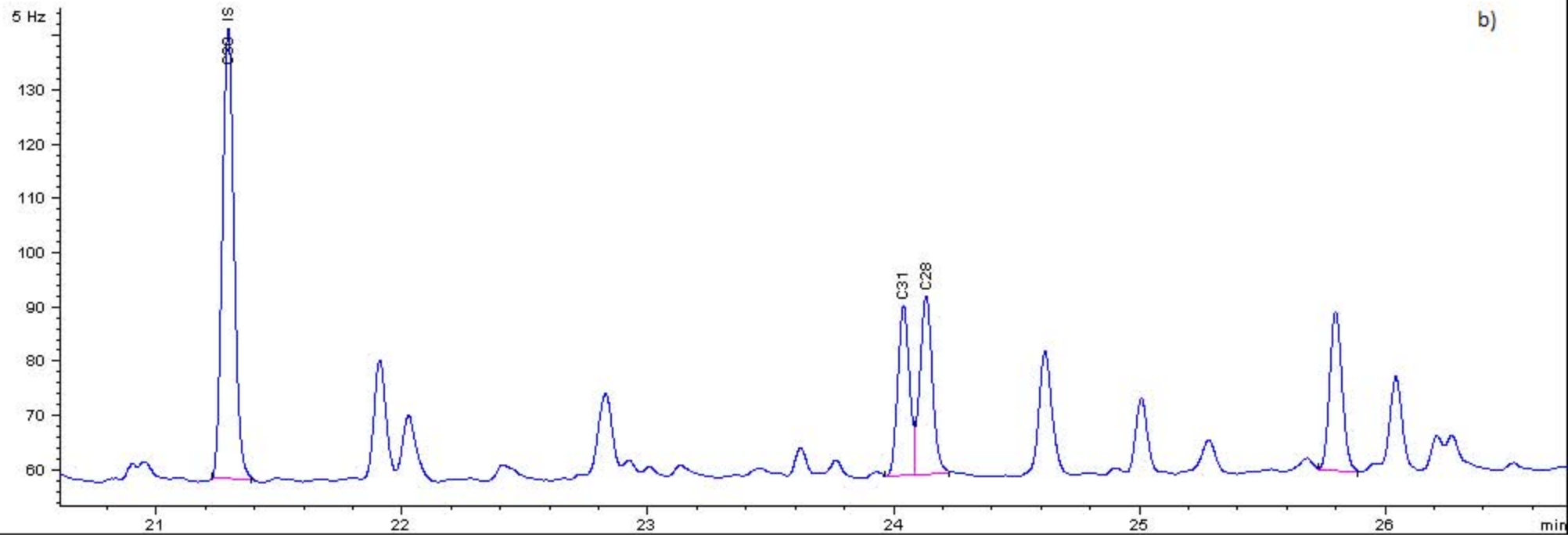
0.4 g soil, 100 μ L i.s., 5 mL acetone:cyclohexane (3:2)
Microwave digestion (max power 300 W):
0-10 min up to 130 $^{\circ}$ C
10-15 min T=130 $^{\circ}$ C
15-25 min T from 130 to 60 $^{\circ}$ C

Purification

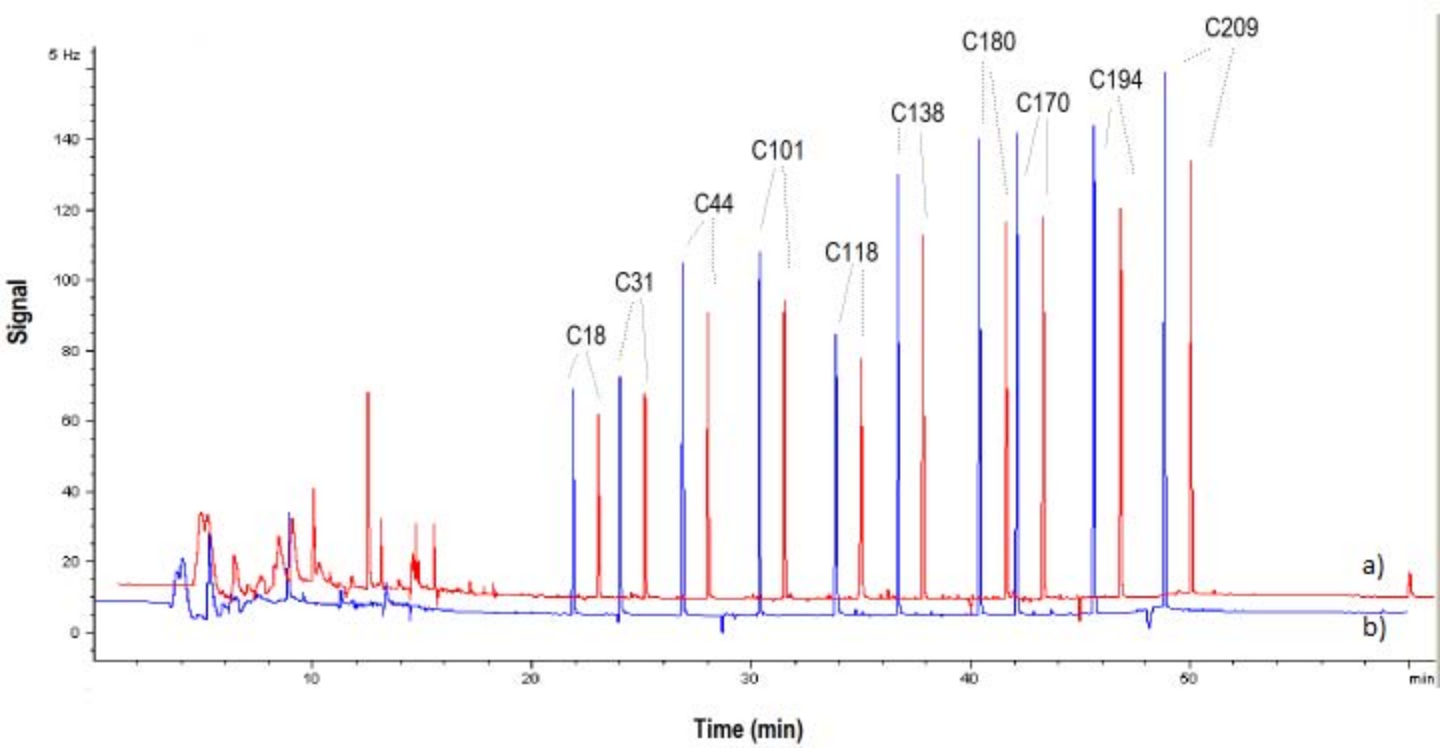
Centrifugation (5 min at 3850 rpm)
 CH_3COCH_3 evaporation by heating
Bring to volume (5 mL) with cyclohexane
Addition of 2 mL H_2SO_4
Decanted for 30 min
Withdrawal of 1 mL of supernatant for further GC
analysis (1 μ L injection, splitless mode)

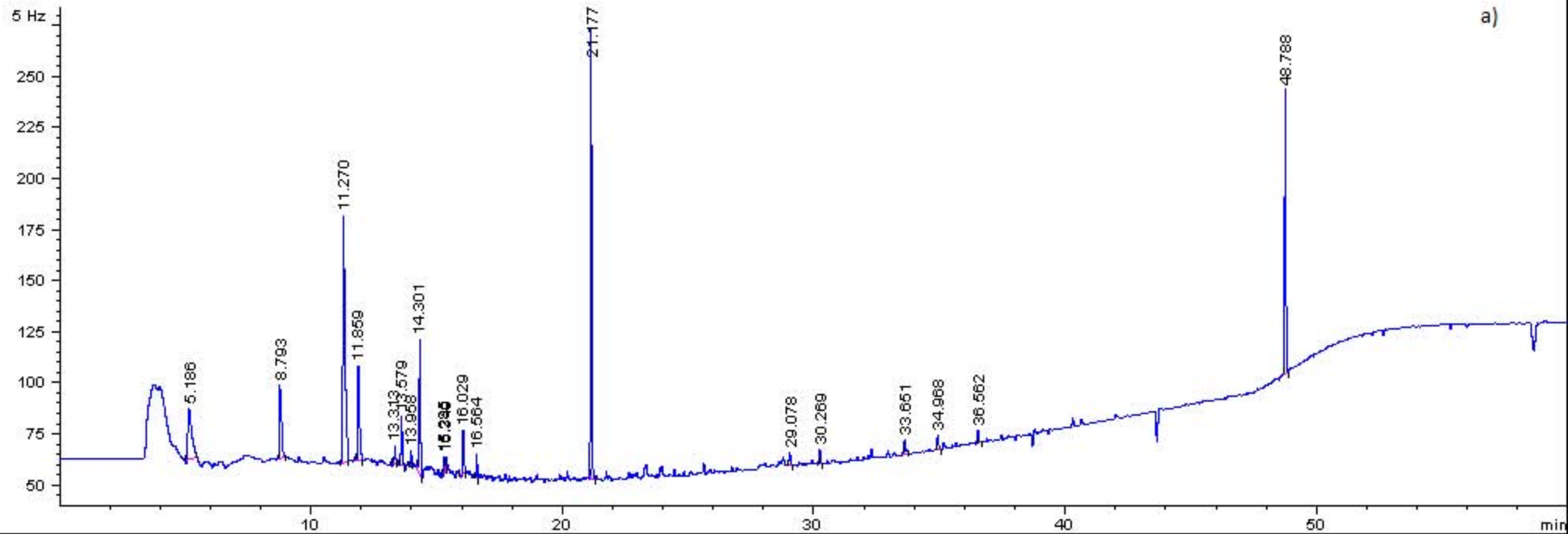
GC-ECD analysis



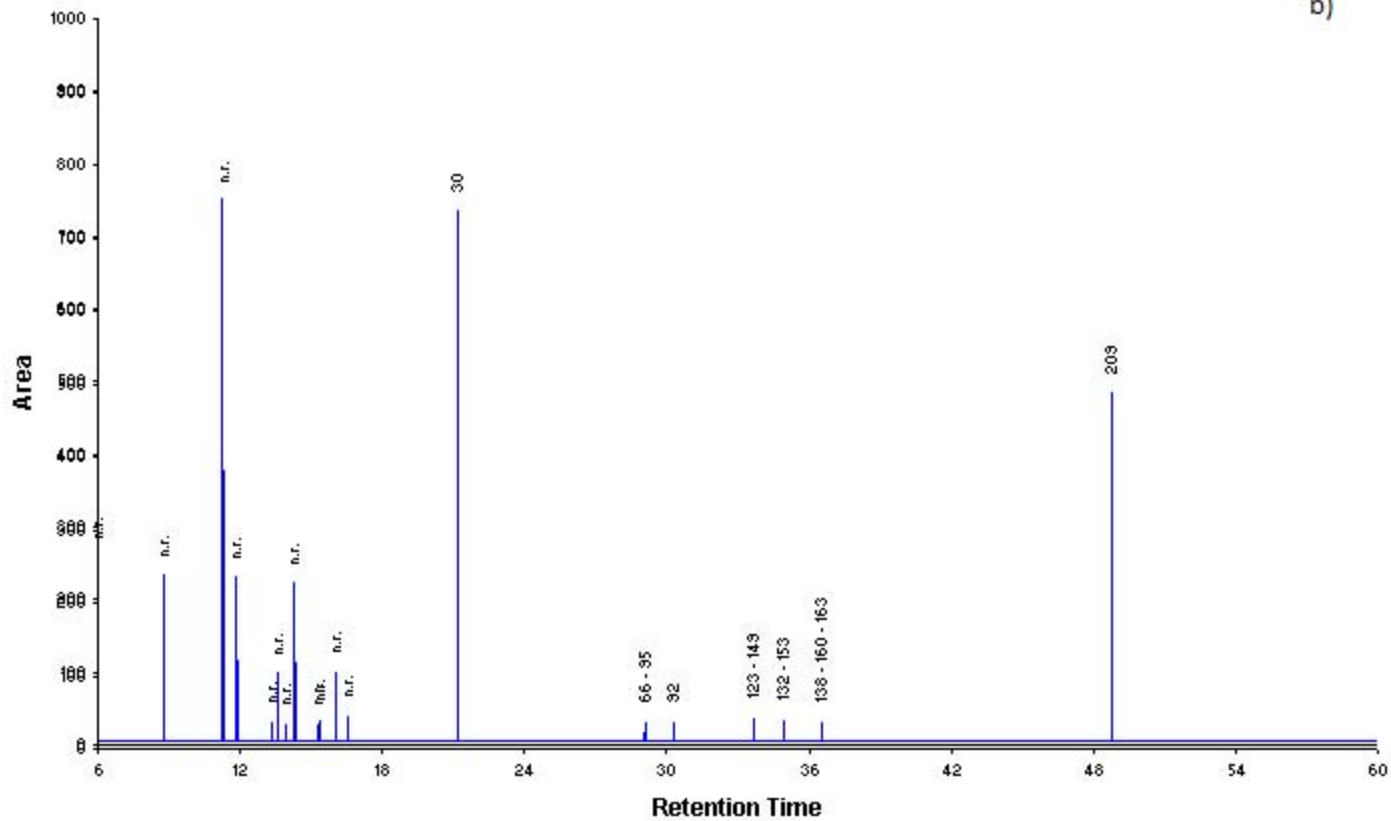


b)





b)



a)

