Rapid and sensitive determination of pyrethroids indoors using active sampling followed by ultrasound-assisted solvent extraction and gas chromatography

Ruth Barro, Carmen Garcia-Jares*, Maria Llompart, Maria Herminia Bollain and Rafael Cela

Departamento de Quimica Analitica, Nutricion y Bromatologia, Facultad de Quimica, Instituto de Investigacion y Analisis Alimentario, Universidad de Santiago de Compostela, E-15782 Santiago de Compostela, Spain.

* Corresponding author:

Tel.: +34 981563100; fax: +34 981595012.

E-mail address: qncgj@usc.es (C. Garcia-Jares).

Abstract

A fast and simple method to analyze pyrethroids as well as other components of frequently used domestic insecticide preparations in indoor air is presented. The proposed method, based on sampling with an adsorbent followed by ultrasound-assisted solvent extraction, was developed with the aim to simplify the traditional extraction methodologies applied up to date to determine pesticides in air. The analytes were retained on a very small amount of adsorbent, which allowed using solely 1 mL of solvent for desorption. The quantification was performed by gas chromatography with micro electron-capture detection (GC-μECD) and gas chromatography coupled to mass spectrometry (GC-MS). The influence of main factors involved in the ultrasound-assisted solvent extraction step (type of adsorbent and type of solvent, solvent volume

and extraction time) was studied using an experimental design approach to account for possible factor interactions. The sampling step was studied for two adsorbents (Tenax TA and Florisil), finding that 1 m^3 air could be sampled without losses of analytes. In this way, the analysis of pyrethroids in air by the proposed method could be carried out within a total time shorter than an hour, including sampling. Linearity was demonstrated in a wide concentration range. Efficiency of the total sampling-extraction process was studied at several concentration levels (2, 10, 100 and 1000 ng/m^3), obtaining quantitative recoveries for all compounds, with good precision (RSD<10%). Method detection limits were below 1 ng/m^3 in air when GC-μECD was employed, and about one order of magnitude higher for GC-MS. In addition, the proposed method was applied to real samples collected in contaminated closed rooms, in which some of the target compounds were determined.

Key words: pyrethroids, ultrasound-assisted solvent extraction, air analysis, factorial design optimization, gas chromatography

1. Introduction

Interest and demand for ambient air analysis have increased as it has the number and diversity of air pollutants of concern. Synthetic pyrethroids are chemicals that have been manufactured since 1950s based upon the structure of natural pyrethrins, which are very unstable in the environment, due to oxidation and UV radiation [1-3]. Since then, pyrethroids are widely applied as insecticides in households and greenhouses, as well as for the protection of crops. Nevertheless, laboratory tests showed that pyrethroids are toxic for fish, aquatic arthropods and honey bees [4,5].

Releases to the air represent the most important emission pathway for pyrethroids. Because of that, inhalation is an important route of exposure for humans, especially just after spraying application in domestic indoors or agricultural close areas. The Occupational Safety and Health Administration (OSHA) has established the occupational exposure limit for an 8-hour workday, 40-hour workweek, at 5 mg of pyrethrins and pyrethroids per cubic meter of workplace air (5 mg/m^3) [1].

Among pyrethroids available today, allethrin, phenothrin, tetramethrin and cyphenothrin are mainly used for household insects, and cypermethrin, deltamethrin, permethrin, λ-cyhalothrin and cyfluthrin are usually utilized for agricultural purposes. Pyrethroids are often commercially combined with synergist compounds like piperonyl butoxide, which enhance their insecticidal activity, or mixed with fungicides as 2-phenylphenol or other pesticides like propoxur (a carbamate pesticide). They can be found in indoor air, sometimes in much higher concentrations than pyrethroids; so, their simultaneous determination in air could be of interest.

Due to the commonly low concentration of pesticides in air, sampling usually consists on collecting high volumes of contaminated air using sampling cartridges filled

with one or more adsorbents where the compounds are retained and then, an appropriate solvent is required, usually at high volumes, to quantitatively elute the analytes. This, in turns, leads to time-consuming steps for concentration and clean up of the organic extracts with the risk of analyte losses. In the particular case of pyrethroids, an additional problem could arise from the possible photodecomposition of certain of these compounds, which has been reported in some multi-pesticide studies [6-8], showing that determination of pyrethroids in air might require performing a rapid and careful trapping-extraction process.

Polyurethane foam (PUF) [6], Empore disks [7], Silicagel [8], Chromosorb 106 [9], Tenax [9,10] and mixtures of PUF and Tenax [11], are adsorbents used to retain certain pyrethroids together with other pesticides in air. Five pyrethroids were retained on Cambridge filter discs after collecting the cigarette smoke [12]. Other adsorbents used to retain pyrethroids from other matrixes than air are graphitised carbon black (GCB) from oils, lipids and fat [13] or Florisil from water [14]. Tenax TA or Florisil have been employed to trap other pesticides different from pyrethroids from air [15,16].

In addition to classical solvent extraction techniques, other extraction techniques have been used to extract some pyrethroids from different matrices, such as supercritical-fluid extraction (SFE) from wool [17] or honey [18], microwave-assisted extraction from soil [19] or matrix solid-phase dispersion (MSPD) from juice samples [20]. The non-exhaustive extraction technique solid-phase microextraction (SPME) is relatively new and could constitute an alternative to solvent-based extraction methodologies [21]. Recently, SPME has been applied for the determination of several pesticides including bioallethrin in confined atmospheres [22]. In addition, the possibilities of SPME to the analysis of pyrethroids in air have been fully studied by the authors and reported elsewhere [23].

Gas chromatography with electron-capture detection (GC-ECD) and GC coupled to mass spectrometry (MS) or tandem MS are the techniques of choice for the analysis of semivolatile pesticides including pyrethroids [6-9,24,25), although the use of liquid chromatography (LC) with fluorescence and MS detection has also been reported [26-28].

The aim of the present study is to develop a fast method for the determination of the components of highly consumed insecticide formulations used for agriculture or indoors, with particular interest in pyrethroid compounds, with enough sensitivity to detect these compounds at their low concentrations in air. For this purpose, 11 pyrethroid insecticides, a fungicide (2-phenylphenol), a carbamate pesticide (propoxur) and an insecticide synergist (piperonyl butoxide) have been selected. The method proposed is based on the use of a very low amount of adsorbent to retain the compounds, and the rapid ultrasound-assisted solvent extraction using a very low volume of solvent. No more exhaustive sample preparation was required to quantitatively extract the target analytes from the adsorbent. The optimization of the methodological parameters was carried out using an experimental design approach to study the main factors and possible factor interactions. Limits of detection lower than 1 ng/m^3 were achieved using GC-μECD. In addition, the proposed method was applied to real samples collected in contaminated closed rooms, in which some of the target compounds have been determined.

2. Experimental

2.1. Reagents

Cypermethrin (mixture of isomers) and deltamethrin were supplied by Supelco (Bellefonte, PA, USA). 2-hydroxybiphenyl (2-phenylphenol), cyphenothrin (mixture of cis and trans isomers), allethrin (mixture of stereo isomers), transfluthrin, empenthrin, cyfluthrin (mixture of isomers), piperonyl butoxide, tetramethrin, permethrin (mixture of cis and trans isomers), phenothrin (mixture of isomers), propoxur and λ-cyhalothrin were of pestanal grade and were purchased to Riedel-de-Haën (Seelze, Germany). All organic solvents (acetone, n-hexane and ethyl acetate) were of pesticide grade and were obtained from Merck (Mollet del Vallés, Barcelona, Spain).

Individual standard stock solutions of 9 000-11 000 µg/mL were prepared in acetone, and a stock mixture solution of all target analytes at a concentration of 100 µg/mL was obtained by appropriate dilution of individual standard solutions in acetone. All working solutions were prepared by convenient dilution of the stock mixture solution in hexane or ethyl acetate. All solutions were stored in amber-colored vials at -20ºC.

2.2. Air sampling and extraction of analytes

The air-sampling device is similar to that previously applied by the authors to determine polychlorinated biphenyls in air [29]. Using a vacuum pump working at 100 L/min (Telstar model S-8, Tarrasa, Spain), a known volume of air was pumped through a glass tube containing 25 mg of an adsorbent. In this study, Tenax TA of mesh size 60-80 (Supelco) and Florisil (activated overnight at 105ºC) of 60-100-mesh size (Aldrich, Steinheim, Germany) have been used as adsorbents. Teflon (PTFE) tubing was used for all connections. The adsorbent was then poured into a 22-mL glass vial and analytes were extracted into an appropriate volume of organic solvent (n-hexane or ethyl acetate) using an ultrasound bath (J.P. Selecta, Barcelona, Spain) for a few minutes. After

filtered through a 0.45 µm Millex HV filter (13 mm diameter) (Millipore, Bedford, USA), the extract (1- to 2-µL) was then injected into the chromatographic injection port.

To study the retention and extraction efficiencies of target compounds, a volume of 100 µL of standard mixtures of the analytes in n-hexane were directly spiked on 25 mg of the adsorbent. The spike was left to homogenize at room temperature for two hours and then, it was treated as described above in this section. Air sample volume ranged from 1 to 10 m^3.

To detect a possible breakthrough of the adsorbent, some experiments required the coupling of a second glass tube filled with 25 mg of the adsorbent (blank) to the first spiked one. Both portions of adsorbent were individually extracted.

2.3. Gas chromatographic analysis

Gas chromatography with micro electron-capture detection (GC-µECD) analysis was performed in an Agilent Technologies 6890N Network GC System, operated by GC Chemstation software and equipped with a split/splitless injector. A HP-5 (30 m x 0.32 mm I.D.. 0.25 µm film thickness) column was used to separate the pyrethroids. Nitrogen was employed as the carrier and make-up gas with a constant pressure of 12 psi (a flow of 2.5 mL/min at 60ºC). The GC oven temperature was: 60ºC hold 2min, first rate 20ºC/min to 230ºC, second rate 5ºC/min to 270ºC hold 5min, third rate 5ºC/min to 290ºC with a total acquisition program of 27.5 min. Detector and injector temperatures were set at 285ºC and 270ºC, respectively. Injector operated in the splitless mode and programmed to return to the split mode after 2 min from the beginning of a run.

The GC-MS analyses were performed on a Varian 3800 gas chromatograph (Varian, Walnut Creek, CA, USA) equipped with an ion trap mass detector Varian

Saturn 2000 (Varian). The system was operated by Saturn GC-MS WorkStation v5.4 software. Analytes were separated on a 25 m length x 0.25 mm I.D., CP-Sil8 CB Low bleed/MS column coated with a 0.25 µm film. Helium (purity 99,999%) was employed as carrier gas, with a constant column flow of 1.2 mL/min. Injector was operated in the splitless mode (2 min). The GC oven temperature program was similar to that used for GC-µECD. Injector temperature was held constant at 270ºC. Trap, manifold and transfer line temperatures were maintained at 250ºC, 120ºC and 300ºC, respectively. The ion-trap mass spectrometer was operated in the electron ionisation mode (70 eV). The mass range was scanned in the full scan mode from 70 to 270 m/z. Experimental parameters for ionisation were, multiplier voltage, 1750 V; filament emission current, 12 µA; axial modulation voltage, 4V; ionisation control, automatic mode; filament/multiplier delay, 8 min. From the total ion current chromatograms, one ion or a group of ions were selected for quantification and are presented in Table 1.

3. Results and discussion

Most of compounds considered in this study showed analytical response in GC-µECD. Nevertheless, some of the target compounds (phenothrin, 2-phenylphenol, propoxur and piperonyl butoxide) did not show measurable signals with this detector. Then, methodology was also developed for GC-MS. In Figures 1 and 2, chromatograms obtained using GC-MS in the fullscan mode and GC-µECD for standard mixtures of compounds at 1000 ng/mL and 10 ng/mL, respectively, are shown. It can be noticed that some of the target pyrethroids, such as cyphenothrin, cyfluthrin and cypermethrin, gave isomeric peak clusters.

Linearity was evaluated for each chromatographic system. Five concentration

levels ranging from 1 to 100 ng/mL for the GC-μECD system (10-100 ng/mL for empenthrin), and six concentration levels ranging from 10 to 5000 ng/mL for GC-MS system (50-5000 ng/mL for cyfluthrin, cypermethrin and deltamethrin) have been considered. In both cases, two replicates have been obtained, and the correlation coefficients were higher than 0.999 for all compounds (see table 1). To validate the regression data, an analysis of variance (ANOVA) with a lack-of-fit test was run [30]. This test allows determining whether the selected model is adequate to describe the observed data, or whether a more complicated model should be used. The test is performed by comparing the variability of the proposed model residuals to the variability between observations (chromatographic response) at replicate values of the independent variable (known concentration of compounds in the standard solutions). Table 1 shows the results of F-ratio and P-values obtained in the calibration range considered. As can be seen, P-values were greater than 0.05 for all compounds and thus, linear regression models were adequate for the obtained data at a confidence level of 95%.

3.1. Optimization of the ultrasound-assisted solvent extraction

Desorption step determines the efficiency of the final method and then, experimental work was initially focused on the optimization of the ultrasound-assisted extraction process using an experimental design approach.

Four factors were selected and studied at two levels: type of adsorbent, type and volume of extracting solvent and ultrasounds application time. The factors selected and their levels are presented in Table 2. Tenax TA and Florisil were the choice for the two levels of factor type of adsorbent. The efficiency of Tenax TA in the retention of some organic pollutants in air, even at such little amounts as 25-mg, was previously reported

[29] and thus, the same amount has been considered in the present study. Regarding Florisil, it has been reported that some pyrethroids were efficiently retained on activated Florisil columns [31] and then, this adsorbent has also been taken into account for optimization. Selection of the two solvents was related to the type of adsorbent we intended to check for optimization. On one hand, a very low polarity solvent such as n-hexane has been selected, while on the other, a medium polarity solvent such as ethyl acetate has been chosen.

A factorial screening 2^4 design, which studied the effects of the four factors in 16 experiments was run. Five degrees of freedom allowed estimating the experimental error [30]. All experiments were performed using adsorbent spiked with 100 ng of each target compound.

Table 3 summarizes the analysis of variance for the four factors studied and their interactions (order two). A main factor or a factor interaction is significant when its p-value is lower than 0.05 at a 95% confidence level. As can be seen in this table, both factors type of solvent and type of adsorbent were significant for the extraction of all pyrethroids. The solvent volume was significant for some compounds while the extraction time was, in general, a not significant factor.

Figure 3 shows the main effects plot for tetramethrin and λ-cyhalothrin, selected as representatives of pyrethroids. For all compounds, main effects plots indicated that the highest responses were achieved using the combination of Tenax and ethyl acetate. As can be seen in this figure, both the solvent volume and the ultrasound application time do not affect the extraction or their influence is much lower than that of other factors.

The interactions solvent-adsorbent (AC) and type of solvent-solvent volume (AB) were significant for most analytes and must be considered in the discussion.

Interaction BC was significant only for thransfluthrin. Other interactions (AD, CD and BD) were not significant for any compound and then, they were not included in Table 3.

Figure 4a shows the combined effect of factors solvent and adsorbent type (interaction AC) for two representative pyrethroids, tetramethrin and λ-cyhalothrin. It can be seen in this figure that both adsorbents (Tenax and Florisil) could be indistinctly used if ethyl acetate was selected as solvent. Using n-hexane or ethyl acetate to extract analytes from Tenax conducted to close responses. On the other hand, if Florisil was selected as adsorbent, the figure clearly points out that, in this case, n-hexane could not be used to extract the pyrethroids.

Figure 4b shows the influence of the solvent volume in the extraction efficiency depending on the type of solvent used (interaction AB) for two selected compounds (tetramethrin and λ-cyhalothrin). As can be seen, for tetramethrin, response was independent of the solvent volume. For λ-cyhalothrin and the other target pyrethroids, no differences in extraction were observed using different volumes of n-hexane, but responses obtained for ethyl acetate extracts were higher using the lowest volume of solvent.

In summary, the experimental conditions that could be selected after the study of the factorial design results involved the use of Tenax, the addition of 1-mL ethyl acetate and sonication of the extraction mixture for 10 minutes. As it has been previously commented, in these extraction conditions, Florisil could be used instead of Tenax.

Under the experimental conditions selected, extraction efficiency was calculated using Tenax spiked at three levels (2, 10 and 100 ng of each compound). GC-μECD was employed for the analysis. As can be seen in Table 4, average recoveries for three replicates ranged from 95 to 118% (%RSD= 1.8-12%). Therefore, the extraction step can be considered quantitative.

3.2. Optimization of the sampling step

Once optimized the extraction process and confirmed that pyrethroids could be quantitatively recovered from Tenax, the sampling step was studied.

To evaluate the possible breakthrough, portions of 25 mg Tenax were spiked in duplicate with 100 and 1000 ng of the analytes and then, different volumes of air ranging from 1 to 10 m^3 were sampled. The portions of adsorbent were individually extracted in the optimized extraction conditions.

Figure 5 shows the results obtained in the experiences using Tenax spiked with 100 ng of each compound, which were similar to those obtained using the highest spike level. In these graphics, responses were normalized to that obtained when sampling 1m^3 air. A clear decrease in response (≥ 20%) was observed after sampling 2.5 m^3 air for empenthrin and phenothrin, whereas for allethrin and cyphenothrin, losses higher than 10% required sampling volumes ≥ 2.5 m^3. For tetramethrin these losses were observed after sampling 5m^3 air. On the other hand, even when 10 m^3 air were sampled, no losses were observed for the remaining pyrethroids: transfluthrin, λ-cyhalothrin, permethrin, cyfluthrin, cypermethrin and deltamethrin. Nevertheless, lost compounds have not been found in the breakthrough cartridge, suggesting that some kind of degradation could occur when high air volumes were sampled [6, 7].

Florisil was also studied, and the obtained results demonstrated that compounds that had shown a decrease in response using Tenax were also lost in similar proportions using Florisil as retention adsorbent.

Thus, a volume of 1 m^3 air was selected to establish a general method for the analysis of pyrethroids in air, although up to 10 m^3 could be sampled if more sensitivity was required for the analysis of the efficiently retained analytes.

As it was commented in the introductory part of this work, some non-pyrethroid compounds could be usually found in commercial formulations, acting as pyrethroid synergists, antifungal, etc, and their determination together with pyrethroids can be of interest [1]. To extend the developed method to the non-pyrethroid target analytes (2-phenylphenol, propoxur and piperonyl butoxide), their retention on Tenax was also studied. In the range of air volumes considered (1-10m^3), nor breakthrough nor degradation was observed for any of these compounds. Since their low or null response in μECD, these non-pyrethroid compounds, as well as phenothrin, were only analyzed using MS detection (see Figure 5).

3.3. Performance of the method

In all validation experiments, results obtained are referred to the sampling of 1 m^3 air. With the aim to assure blank samples, air blanks as well as adsorbent blanks were obtained in a clean room provided with a laminar flow system and analyzed before every set of experiments.

Efficiency of the total sampling-extraction process was evaluated at four concentration levels (2, 10, 100 and 1000 ng/m^3). GC-μECD was used for the first three levels and MS detection for the highest one. Table 5 shows the recoveries obtained for all compounds. For the lowest concentration (2 ng/m^3), recoveries ranged from 81% for allethrin to 110% for deltamethrin. The results obtained at higher concentration levels were even better. Precision of the method can be considered good with RSD values below 10% for all compounds at all concentration levels studied. Using GC-MS, the method allows the determination of phenothrin and the target non-pyrethroid compounds. Then, recovery for phenothrin, 2-phenylphenol, propoxur and piperonyl

butoxide was evaluated, and the obtained results ranged between 97% for phenothrin and 106% for propoxur with RSD values below 5%.

In summary, from the results showed in tables 4 and 5, it can be pointed out that the sampling step does not contribute to increase the variability of the method. In addition, to demonstrate the robustness of the method, inter-day precision was calculated. Analyses were performed at least in duplicate during three non-consecutive days with Tenax as adsorbent using GC-μECD for a concentration level of 100 ng/m^3, and GC-MS for 1000 ng/m^3. Results can be seen in Table 5. The method showed good reproducibility since variability was lower that 16 % for all target analytes.

As it was previously commented, Florisil could also be used for the retention-extraction of pyrethroids. Then, recovery of the method using this adsorbent was also evaluated. In table 6, average results of six replicates obtained at two concentration levels (100 and 1000 ng/m^3) can be seen. After the complete sampling-extraction process, recoveries ranged from 81% for empenthrin and 115% for cyfluthrin, with RSD values lower than 15% in all cases, which can be considered satisfactory and similar to recoveries obtained using Tenax. For phenothrin as well as for the non-pyrethroid compounds 2-phenylphenol, propoxur and piperonyl butoxide, analysed by GC-MS, recoveries from Florisil were also quantitative (81-107%, RSD= 4-9%).

Limits of detection (LOD, S/N= 3) of the proposed method are presented in table 7. LODs lower than 1 ng/m^3 were attained for pyrethroids when the determination is performed using GC-μECD. Empenthrin constitutes an exception since μECD response for this compound is very poor. Generally, higher LOD values, ranging from 1 to 10 ng/m^3, were obtained for pyrethroids using GC-MS. For 2-phenylphenol, propoxur, piperonyl butoxide and phenothrin, GC-MS LODs were also into this range, with values from 1 to 2 ng/m^3. GC-MS LODs obtained (see Table 7) are in good agreement with

those recently reported in literature, while GC-μECD LODs are about one order of magnitude lower. Using GC-MS/MS, Egea et al. [9], found LOD values from 0.42 to 5.63 ng/m^3 for stable pyrethroids (cyhalothrin, permethrin, cyfluthrin, cypermethrin and deltamethrin) after sampling 1440 L air. Similar LOD values have been reported by Elfflein et al. [6] for the same compounds using GC-MS analysis but sampling almost 10-fold higher air volume. Since high air volumes could be sampled without losses of those pyrethroids and some non-pyrethroid compounds, Yoshida et al. [7] sampled 7.2 m^3 air, achieving LOD values of 0.4 and 0.2 ng/m^3 for propoxur and permethrin, respectively, also by GC-MS analysis.

In short, GC-MS and mainly GC-μECD allowed the sensitive analysis of the target compounds in air. Higher sensitivity could be achieved for those compounds for which losses have not been observed by increasing the volume of air sampled.

3.4. Application of the method to real air samples

In addition, the proposed method was applied to several real samples collected in closed rooms that had been treated with aerosols and electrical diffusion units of general domestic use in Spain. Figures 6 and 7 show the chromatograms resulting from the analysis of two different air samples using μECD and MS detection, respectively. As can be seen, some of the target analytes were present in the room air and could be determined. Concentrations of the compounds found in these samples are also reported within the figures.

Conclusions

A very simple and sensitive method to analyze pyrethroids as well as other components of domestic insecticide preparations in indoor air was developed. The method is based on the active retention of the target compounds on a very small amount of Tenax TA and the subsequent desorption by application of ultrasound using only 1-mL ethyl acetate, avoiding for the requirements of extract concentration prior to the chromatographic analysis. Optimization of the extraction step was achieved by an experimental design approach studying four factors (type of adsorbent and type of solvent, solvent volume and extraction time) at two levels. Retention efficiency was studied, finding that no breakthrough occurred for any compound sampling 1 m^3 air. The study of method performance demonstrated its linearity, quantitative recoveries, and good sensitivity, with LODs lower than 1 ng/m^3 using GC-μECD. The use of GC-MS allowed the simultaneous determination of pyrethroid and non-pyrethroid compounds. In addition, the proposed method allows high sample throughput since the total sampling-extraction-analysis process is completed within one hour.

The analysis of several contaminated air samples demonstrated the validity of the proposed method for the analysis of the target compounds in indoors atmospheres.

Acknowledgements

This research was supported by the projects BQU2003-02090 from CICYT, Spanish Commission for Research and Development, (Ministerio de Ciencia y Tecnologia) and PGIDIT04PXIC23701PN and PGIDIT05RAG50302PR from Xunta de Galicia. R. B. would like to acknowledge her Doctoral grant to the Xunta de Galicia.

References

[1] Toxicological Profile for pyrethrins and pyrethroids, US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry (ATSDR), Atlanta, GA, 2003.

[2] International Labour Organisation and World Health Organization (WHO), United Nations Environment Programme, The International Programme on Chemical Safety, Environmental Health Criteria Nos. 64, 82, 87, 94, 96-99 and 142, Geneva, 1986-1992.

[3] Y. L. Chen, J. E. Casida, J. Agr. Food Chem. 17 (1969) 208-215.

[4] J. R. Clark, L. R. Goodman, P. W. Borthwick, J. M. Patrick Jr., G. M. Cripe, P. M. Moody, J. C. Moore, E. M. Lores, Environ. Toxicol. Chem. 8 (1989) 393-401.

[5] J. R. Coats, D. M. Symonik, S. P. Bradbury, S. D. Dyer, L. K. Timson, G. J. Atchison, Environ. Toxicol. Chem. 8 (1989) 671-679.

[6] L. Elflein, E. Berger-Preiss, K. Levsen, G. Wünsch, J. Chromatogr. A 985 (2003) 147-157.

[7] T. Yoshida, I. Matsunaga, H. Oda, J. Chromatogr. A 1023 (2004) 255-269.

[8] T. J. Class, J. Kintrup, Fresenius J. Anal. Chem. 340 (1991) 446-453.

[9] F. J. Egea Gonzalez, A. Mena Granero, C. R. Glass, A. Garrido Frenich, J. L. Martinez Vidal, Rapid Commun. Mass Spectrom. 18 (2004) 537-543.

[10] J. Siebers, P. Mattusch, Chemosphere 33 (1996) 1597-1607.

[11] R. G. Lewis, K. E. MacLeod, Anal. Chem. 54 (1982) 310-315.

[12] J. Cai, B. Liu, X. Zhu, Q. Su, J. Chromatogr. A 964 (2002) 205-211.

[13] A. Ramesh, M. Balasubramanian, Analyst 123 (1998) 1799-1802.

[14] W. R. Barrionuevo, F. M. Lanças, Bull. Environ. Contam. Toxicol. 69 (2002) 123-128.

[15] R. Barro, S. Ares, C. Garcia-Jares, M. Llompart, R. Cela, J. Chromatogr. A 1072 (2005) 99-106.

[16] J. Castro, R. A. Pérez, E. Miguel, C. Sánchez-Brunete, J. L. Tadeo, J. Chromatogr. A 947 (2002) 119-127.

[17] A. M. Nguyen, P. J. Marriot, J. Hughes, J. Biochem. Biophys. Methods 43 (2000) 411-429.

[18] S. R. Rissato, M. S. Galhiane, F. R. N. Knoll, B. M. Apon, J. Chromatogr. A 1048 (2004) 153-159.

[19] F. A. Esteve-Turrillas, C. S. Aman, A. Pastor, M. de la Guardia, Anal. Chim. Acta 522 (2004) 73-78.

[20] B. Albero, C. Sánchez-Brunete, A. Donoso, J. L. Tadeo, J. Chromatogr. A 1043 (2004) 127-133.

[21] J. Pawliszyn, Solid Phase Microextraction, Theory and Practice, Wiley, New York, 1997.

[22] F. Ferrari, A. Sanusi, M. Millet, M. Montury, Anal. Bioanal. Chem. 379 (2004) 476-483.

[23] R. Barro, C. Garcia-Jares, M. Llompart, R. Cela, J. Chromatogr. Sci. (2006) (in press).

[24] E. Berger-Preiss, A. Preiss, K. Sielaff, M. Raabe, B. Ilgen, K. Levsen, Indoor Air 7 (1997) 248-261.

[25] G. Leng, E. Berger-Preiss, K. Levsen, U. Ranft, D. Sugiri, W. Hadnagy, H. Idel, Int. J. Hyg. Environ.-Health 208 (2005) 193-199.

[26] T. López-López, M. D. Gil-Garcia, J. L. Martínez-Vidal, M. Martínez-Galera, Anal. Chim. Acta 447 (2001) 101-111.

[27] C. Ferrer, M. J. Gómez, , J. F. García-Reyes, I. Ferrer, E. M. Thurman, A. R. Fernández-Alba, J. Chromatogr. A 1069 (2005) 183-194.

[28] I.-H. Wang, R. Moorman, J. Burleson, J. Chromatogr. A 983 (2003) 145-152.

[29] R. Barro, S. Ares, C. Garcia-Jares, M. Llompart, R. Cela, Anal. Bioanal. Chem. 381 (2005) 255-260.

[30] Statgraphics-Plus V. 5.1. *Experimental design*, *Appendix C*, Manugistics, Rockville, MD, 2002.

[31] J. You, D. P. Weston, M. J. Lydy, Arch. Environ. Contam. Toxicol. 47 (2004) 141-147.

Figure captions

Figure 1. Total ion current (TIC) chromatogram of a standard solution of the target analytes at 1 µg/mL. 1: 2-phenylphenol, 2: propoxur, 3: empenthrin, 4: transfluthrin, 5: allethrin, 6: piperonyl butoxide, 7: tetramethrin (2 peaks), 8: phenothrin, 9: λ-cyhalothrin (2 peaks), 10: cyphenothrin (3 peaks), 11: permethrin, 12: cyfluthrin (4 peaks), 13: cypermethrin (4 peaks), 14: deltamethrin (2 peaks).

Figure 2. µECD chromatogram of a standard solution of the target analytes at 10 ng/mL. 1: empenthrin, 2: transfluthrin, 3: allethrin, 4: tetramethrin (2 peaks), 5: λ-cyhalothrin (2 peaks), 6: cyphenothrin (3 peaks), 7: permethrin, 8: cyfluthrin (4 peaks), 9: cypermethrin (4 peaks), 10: deltamethrin (2 peaks).

Figure 3. Main effects plots for two selected pyrethroids: tetramethrin and λ-cyhalothrin.

Figure 4. Combined effect of factors type of adsorbent and type of solvent (A) and type of solvent and solvent volume (B), for two selected pyrethroids:
tetramethrin and lambda-cyhalothrin.

Figure 5. Variation of the chromatographic response with the volume of air sampled for the target pyrethroids (GC-µECD), as well as phenothrin and the non-pyrethroid compounds (GC-MS).

Figure 6. GC-μECD chromatogram of a contaminated air sample collected following the proposed method in a closed room sprayed with insecticide aerosols and treated with anti-mosquito electro-evaporators. * Quantified on a diluted sample.

Figure 7. GC-MS analysis of a contaminated air sample collected following the proposed method in a closed room sprayed with insecticide aerosols and treated with anti-mosquito electro-evaporators.

Table 1. Study of linearity using two different detection systems.

	MS detection				μECD detection		
	Quantification ions	Correlation coefficient (R)	F-ratio	P-value	Correlation coefficient (R)	F-ratio	P-value
2-Phenylphenol	169+170	0.9998	0.22	0.9198	n.d.	n.d.	n.d.
Propoxur	110+152	0.9993	0.29	0.8719	n.d.	n.d.	n.d.
Empenthrin	123	0.9991	0.02	0.9992	0.9992	3.43	0.1609
Transfluthrin	163	0.9990	1.19	0.4030	0.9999	0.03	0.9982
Allethrin	123	0.9999	5.63	0.0642	0.9999	0.32	0.8536
Piperonyl butoxide	176	0.9997	0.37	0.8230	n.d.	n.d.	n.d.
Tetramethrin	164	0.9998	0.04	0.9958	1.0000	1.97	0.2188
Phenothrin	123+183	0.9998	0.39	0.8066	n.d.	n.d.	n.d.
λ-Cyhalothrin	181+197	0.9998	1.49	0.3158	0.9998	4.42	0.0526
Cyphenothrin	123	0.9998	0.49	0.7475	0.9999	0.21	0.9261
Permethrin	183	0.9997	0.03	0.9973	0.9997	2.30	0.1733
Cyfluthrin	163	0.9998	6.61	0.0539	0.9998	1.76	0.2557
Cypermethrin	163	0.9998	5.50	0.0711	0.9997	0.13	0.9646
Deltamethrin	253+181	0.9995	10.5	0.0838	0.9996	0.92	0.5085

Table 2. Factors and levels considered in the experimental design.

Factor	Code	Low level	High level	Continuous
Solvent	A	Ethyl acetate	n-hexane	No
Solvent volume	B	1 mL	3 mL	Yes
Adsorbent	C	Tenax	Florisil	No
Extraction time	D	10 min	20 min	Yes

Table 3. Analysis of variance (ANOVA) showing the significance of main effects and their second order interactions.

Factors	A: Solvent		B: Solvent volume		C: Adsorbent		D: Extraction time	
	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
Empenthrin	10.5	0.023	4.54	0.086	53.9	0.001	1.19	0.326
Transfluthrin	393	0.000	32.9	0.002	129	0.000	11.1	0.021
Allethrin	1239	0.000	47.8	0.001	683	0.000	1.77	0.241
Tetramethrin	918	0.000	0.00	0.980	303	0.000	2.41	0.182
λ-Cyhalothrin	857	0.000	62.7	0.000	309	0.000	2.52	0.173
Cyphenothrin	698	0.000	9.16	0.029	277	0.000	1.57	0.266
Permethrin	443	0.000	0.15	0.713	189	0.000	6.02	0.058
Cyfluthrin	535	0.000	11.2	0.020	196	0.000	3.41	0.124
Cypermethrin	386	0.000	9.57	0.027	137	0.000	3.82	0.108
Deltamethrin	269	0.000	10.7	0.022	123	0.000	3.26	0.131

Interactions	AB		AC		BC	
	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
Empenthrin	8.18	0.035	47.4	0.001	5.48	0.066
Transfluthrin	77.2	0.000	145.4	0.000	22.1	0.005
Allethrin	35.1	0.002	674	0.000	3.88	0.106
Tetramethrin	3.42	0.124	361	0.000	3.64	0.115
λ-Cyhalothrin	91.1	0.000	161	0.000	6.49	0.051
Cyphenothrin	37.0	0.002	179	0.000	0.75	0.425
Permethrin	32.4	0.002	103	0.000	3.87	0.106
Cyfluthrin	38.4	0.002	65.1	0.000	0.52	0.505
Cypermethrin	24.9	0.004	38.9	0.002	1.90	0.226
Deltamethrin	24.1	0.004	18.8	0.008	0.15	0.712

Table 4. Extraction efficiency of pyrethroids from Tenax at three spike levels. Analyses were performed by GC-μECD.

	2 ng (n= 3)		10 ng (n= 3)		100 ng (n= 3)	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
Empenthrin	-	-	111	12	97	2.9
Transfluthrin	97	5.6	95	2.5	99	2.5
Allethrin	100	4.1	96	2.0	104	3.2
Tetramethrin	114	4.1	103	5.5	99	7.1
λ-Cyhalothrin	95	5.7	103	8.0	105	7.0
Cyphenothrin	99	4.4	114	4.4	106	7.2
Permethrin	96	5.1	109	3.8	106	6.5
Cyfluthrin	101	6.9	108	1.8	97	8.8
Cypermethrin	101	7.6	103	8.0	102	6.6
Deltamethrin	110	7.5	118	6.7	97	6.4

Table 5. Repeatability, reproducibility and recovery of the total sampling-extraction process using Tenax. Analyses were performed by GC-μECD.

	2 ng/m^3 (n=6)		10 ng/m^3 (n=6)		100 ng/m^3 (n=5)		1000 ng/m^3 (n=3)*		Inter-day precision (R.S.D., %, n= 3 days)	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	100 ng/m^3	1000 ng/m^3*
Empenthrin	-	-	111	7.7	98	6.0	94	6.7	2.6	11.0
Transfluthrin	98	2.9	90	4.5	94	8.5	101	4.5	3.8	5.0
Allethrin	81	3.8	81	6.0	98	9.0	100	4.6	10.5	11.7
Tetramethrin	106	7.3	114	6.4	95	4.2	100	3.9	7.0	9.4
λ-Cyhalothrin	100	4.7	111	6.7	105	4.4	101	4.5	9.0	9.8
Cyphenothrin	82	4.4	98	8.1	100	7.0	97	3.1	8.0	7.1
Permethrin	96	4.4	103	6.3	106	3.0	100	4.4	6.1	10.5
Cyfluthrin	106	4.1	102	5.1	107	8.6	106	3.0	9.2	12.1
Cypermethrin	98	9.8	101	8.0	105	5.0	106	4.7	4.7	9.9
Deltamethrin	110	9.9	113	6.1	98	2.7	105	1.4	3.9	15.5

* Concentration level of 1000 ng/m^3 was analyzed by GC-MS.

Table 6. Repeatability and recovery of the total sampling-extraction process using Florisil (n=6). Analyses were performed by GC-μECD.

	100 ng/m^3		1000 ng/m^3	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
Empenthrin	81	11	84	8.3
Transfluthrin	93	12	102	5.5
Allethrin	89	15	86	9.7
Tetramethrin	89	2.6	93	9.8
λ-Cyhalothrin	97	6.0	100	9.0
Cyphenothrin	87	11	98	12
Permethrin	101	6.6	99	9.2
Cyfluthrin	107	10	115	8.4
Cypermethrin	100	4.5	113	9.4
Deltamethrin	89	5.9	104	11

Table 7. Limits of detection of the proposed method.

	GC-MS (ng/m^3)	GC-μECD (ng/m^3)
Empenthrin	1.7	4.1
Transfluthrin	1.4	0.03
Allethrin	2.7	0.06
Tetramethrin	3.2	0.43
λ-Cyhalothrin	3.0	0.15
Cyphenothrin	7.0	0.52
Permethrin	4.2	0.55
Cyfluthrin	7.1	0.24
Cypermethrin	8.9	0.46
Deltamethrin	9.1	0.30

Figure 1

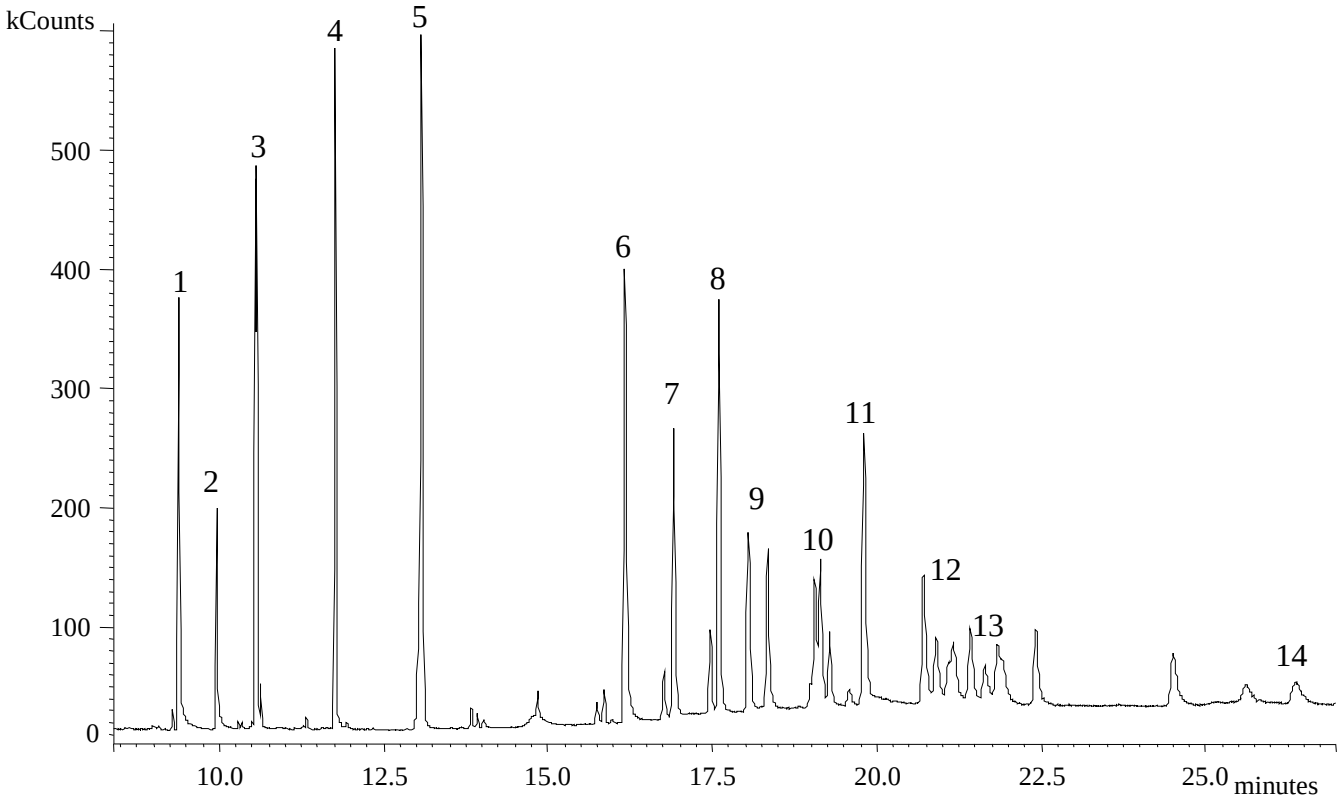

kCounts
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
10.0
12.5
15.0
17.5
20.0
22.5
25.0
minutes

Figure 2

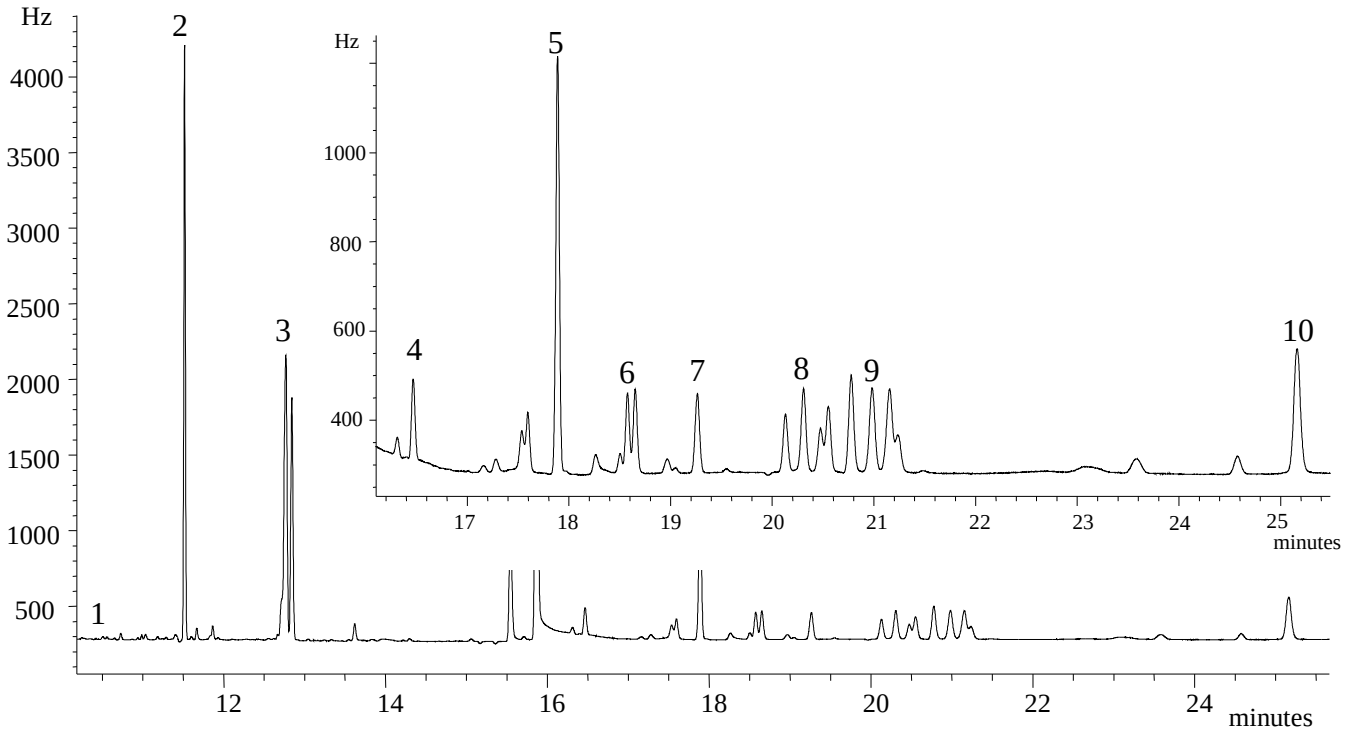

Hz
4000
3500
3000
2500
2000
1500
1000
500
1
2
3
12
14
16
18
20
22
24
minutes
Hz
1000
800
600
400
4
5
6
7
8
9
10
17
18
19
20
21
22
23
24
25
minutes

Figure 3

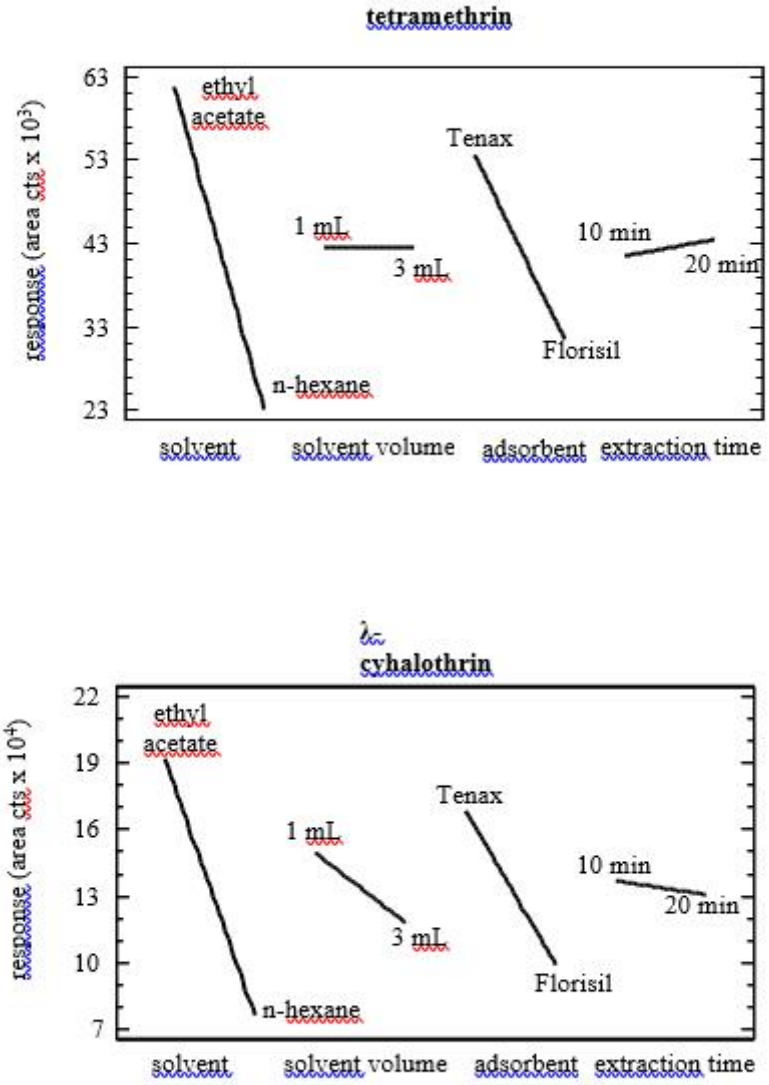
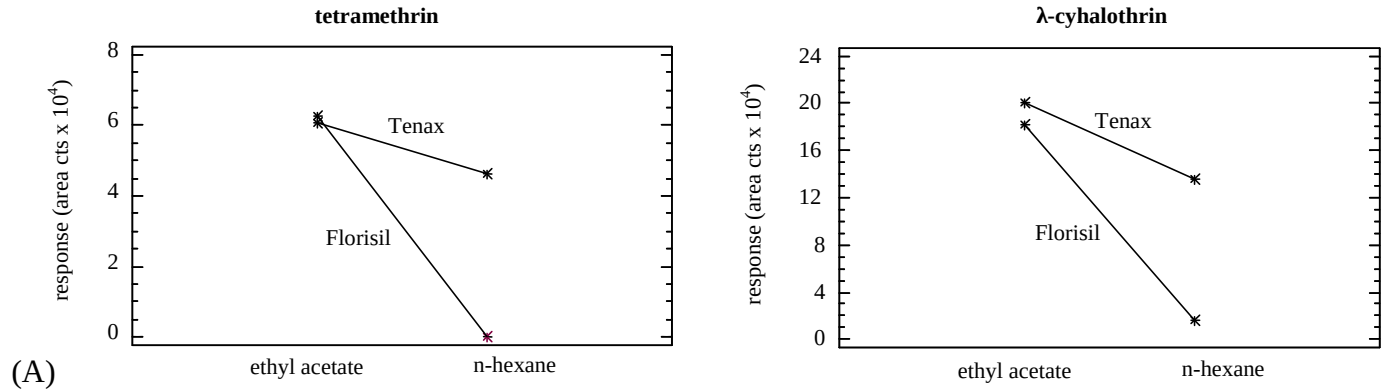

tetramethrin
response (area cts x 10³)
63
53
43
33
23
ethyl acetate
n-hexane
1 mL
3 mL
Tenax
Florisil
10 min
20 min
solvent
solvent volume
adsorbent
extraction time
λ-cyhalothrin
response (area cts x 10⁴)
22
19
16
13
10
7
ethyl acetate
n-hexane
1 mL
3 mL
Tenax
Florisil
10 min
20 min
solvent
solvent volume
adsorbent
extraction time

Figure 4


tetramethrin
λ-cyhalothrin
response (area cts x 10^4)
Tenax
Florisil
ethyl acetate
n-hexane
(A)

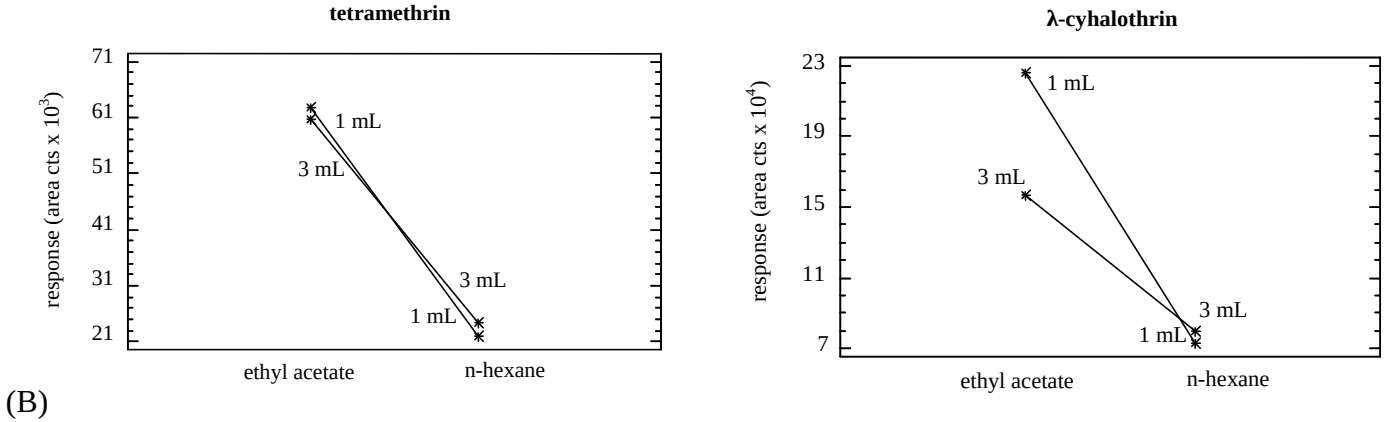

tetramethrin
λ-cyhalothrin
response (area cts x 10^3)
response (area cts x 10^4)
1 mL
3 mL
ethyl acetate
n-hexane
(B)

Figure 5

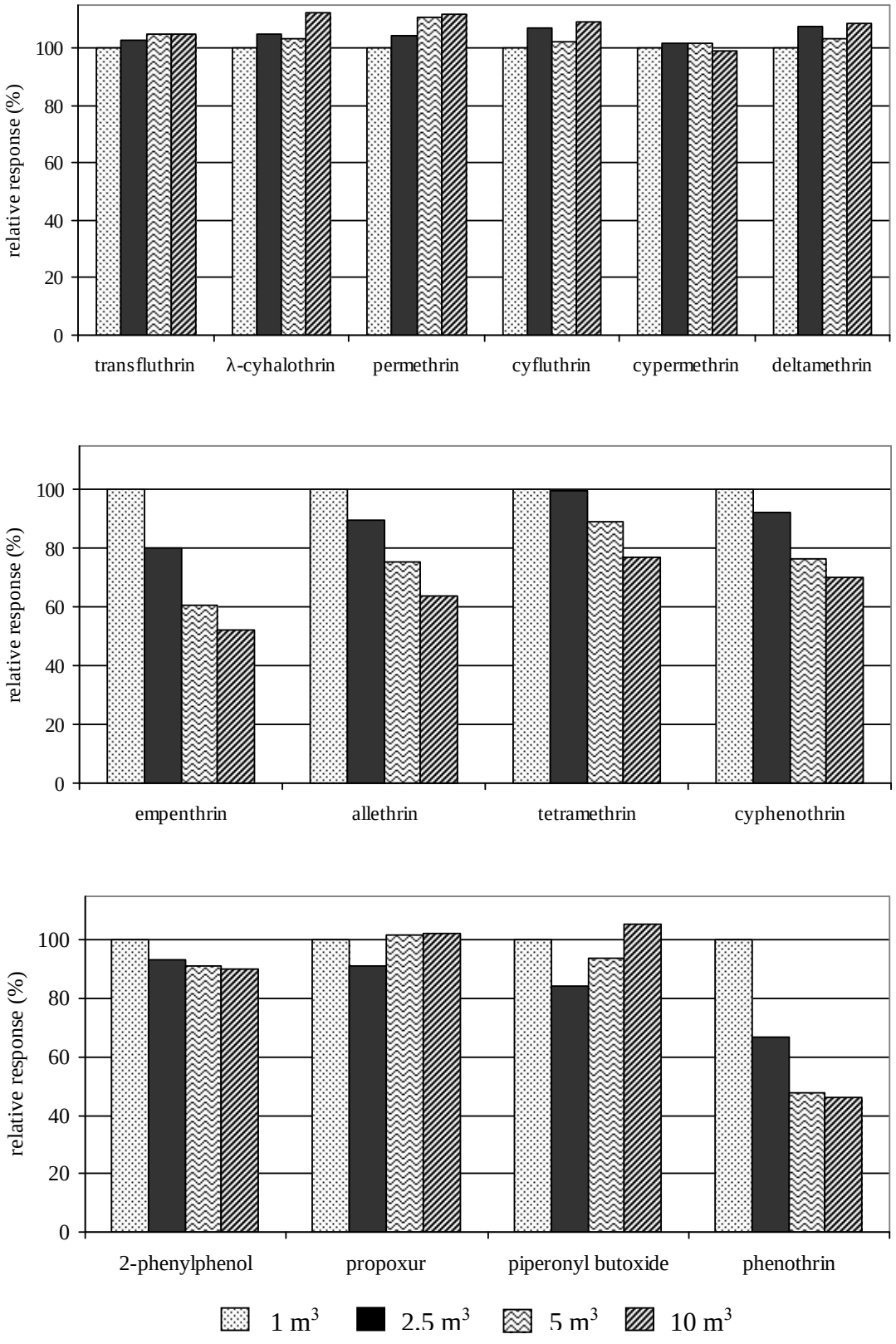

relative response (%)
100
80
60
40
20
0
transfluthrin
λ-cyhalothrin
permethrin
cyfluthrin
cypermethrin
deltamethrin
relative response (%)
100
80
60
40
20
0
empenthrin
allethrin
tetramethrin
cyphenothrin
relative response (%)
100
80
60
40
20
0
2-phenylphenol
propoxur
piperonyl butoxide
phenothrin
1 m3
2.5 m3
5 m3
10 m3

Figure 6

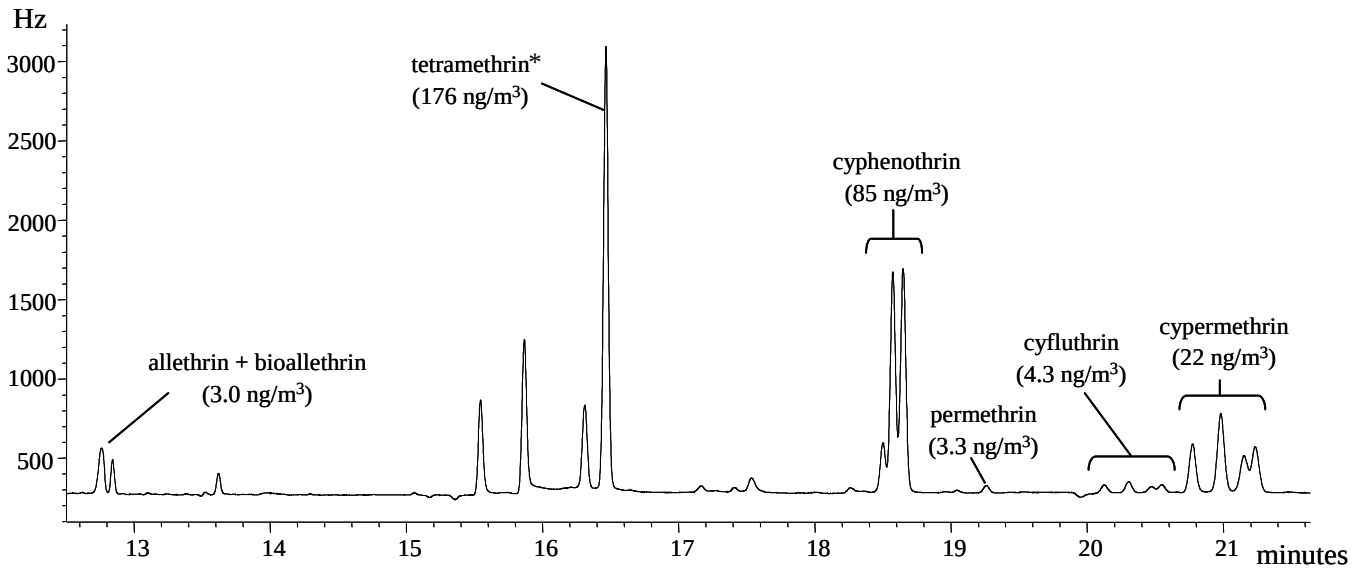
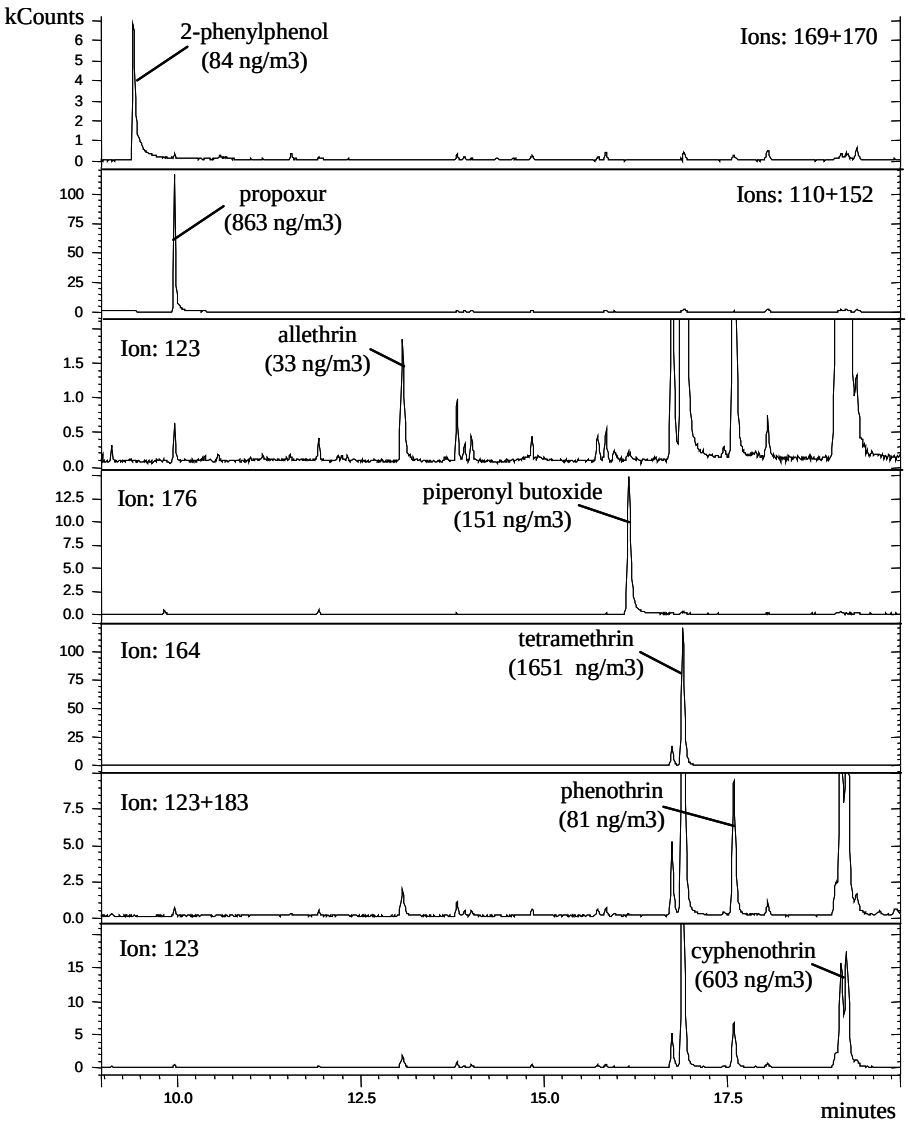

Hz
3000
2500
2000
1500
1000
500
tetramethrin*
(176 ng/m3)
cyphenothrin
(85 ng/m3)
allethrin + bioallethrin
(3.0 ng/m3)
cyfluthrin
(4.3 ng/m3)
cypermethrin
(22 ng/m3)
permethrin
(3.3 ng/m3)
13
14
15
16
17
18
19
20
21
minutes

Figure 7


kCounts
2-phenylphenol
(84 ng/m3)
Ions: 169+170
propoxur
(863 ng/m3)
Ions: 110+152
Ion: 123
allethrin
(33 ng/m3)
Ion: 176
piperonyl butoxide
(151 ng/m3)
Ion: 164
tetramethrin
(1651 ng/m3)
Ion: 123+183
phenothrin
(81 ng/m3)
Ion: 123
cyphenothrin
(603 ng/m3)
10.0
12.5
15.0
17.5
minutes