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Short communication

Electron ionization gas chromatography–mass spectrometric determination of residues of thirteen pyrethroid insecticides in whole blood

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Abstract

A new rapid and sensitive electron ionization gas chromatography–mass spectrometry method in selective ion monitoring mode (SIM) was developed for the determination of l3 synthetic pyrethroid insecticide molecules and their stereo isomers in whole blood. The pyrethroid insecticides investigated are allethrin, bifenthrin, cypermethrin, cyphonothrin, cyfluthrin, lambda-cyhalothrin, deltamethrin, fenvalerate, fenpropathrin, imiprothrin, permethrin, prallethrin and transfluthrin. The residues of pyrethroids are extracted from the whole blood using hexane and acetone mixture (80 + 20%) as solvent. All the pyrethroid residues were separated by using a gas chromatography–mass spectrometry operated in electron ionization mode and quantified in selective ion monitoring mode. The method can detect the residues of different pyrethroids down to the level 0.05–2 ng/ml. Recovery experiments conducted in whole blood samples at the fortification level 1–1000 ng/ml showed 91–103% recovery. The applications of the analytical method for the determination of pyrethroid residues in real samples were tested by analyzing 45 human blood samples collected from the population exposed continuously to different pyrethroid based formulations. The results are confirmed by spiking the known quantity of pyrethroids and subsequently their positive detection.

Keyword: Pyrethroid insecticides

1. Introduction

During the investigations on the chemical structures of natural pyrethrins, a certain number of synthetic pyrethroids were produced with improved physical and chemical properties and greater biological activity. Several of these synthetic pyrethroids were subsequently commercialized, mainly for the control of household insects. Synthetic pyrethroids have been extensively studied for different properties such as mode of action, metabolism, photo stability, mammalian toxicity and environmental fate by different authors [1,2]. The presence of residual concentrations of the pyrethroids in environment [3–7] due to the use of different formulations may possibly contribute to humans exposure either by inhalation or skin resorption. The detection and determination of unchanged insecticide residues in stomach, intestine, liver, kidney, spleen, lungs, brain and other tissues are useful indicators of exposure and helps in accessing adverse health effects [8–10]. The evaluation of residues in the blood [11–16] and in body fluids [17–22] gives an indication about the extent of exposure. Several reports are available in the literature regarding the evaluation of residues of environmental pollutants either in plasma or in serum. However, the reports pertaining to the evaluation of residues in whole blood are scarce. The reason may be due to difficulty in elimination of lipids associated with whole blood samples and subsequently the ease in separation of plasma from a blood sample by simple laboratory technique. In an exposure study, analysis of blood for residues is a difficult task. Even though the serum/plasma represents the part of the blood, separation of serum/plasma involves the collection of large volumes of blood. Further, the analysis of the serum/plasma samples may not authorize the correct indication of the total residual concentrations present in the body compartment due to plausible strong binding at erythrocytes. Thus there is a dire need to analyze and to develop analytical methods

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for the evaluation of residues in whole blood instead of its components like serum or plasma. The evaluation of low quantities of residues in the biological fluids poses always particular analytical problems and likely to question the validity of the data. The validity of the results depends on the suitability of the analytical methods used for the task. EU commission directive 96/46 section 4.2.5 requires analytical methods for the determination of residues in body fluids and tissues for pesticides classified as toxic or very toxic. In view of the greater demand for the sensitive analytical techniques related to monitoring the human health and environment [23–31] and in continuation of our investigations on occupational exposure [32–34], the present studies are aimed at developing a rapid and high sensitive method for the sequential determination of residues of different pyrethroid molecules in whole blood. The applications of the method was investigated in human population exposed to pyrethroid based formulations.

2. Experimental

2.1. Apparatus

A gas chromatograph–mass spectrometer GC–MS QP-5050A with AOC 20i auto injector and Class-5000 software is used. DB-1 capillary column of 30 meters length, 0.25 mm internal diameter and 0.25 μm film thickness supplied by J&W scientific, USA, is used for the quantification purpose. Carrier gas Helium is used at constant flow 1.0 ml/min. The following temperature program is used for the separation, column oven: initial 60 °C for 3 min, increased at 10 °C/min to 200 °C, hold for 5 min, increased at 5 °C/min to 240 °C and hold for 45 min. Injector temperature and Interface temperatures are set at 290 and 300 °C, respectively. Split ratio was kept at 1:3. Perhaps, further studies are necessary to identify the actual fate of the molecules in the intestines.

2.2. Reagents

All the chemicals and reagents used in the studies are organic trace analysis grade. They were purchased from E. Merck, Darmstadt, Germany.

2.3. Reference standards

Reference analytical standards of allethrin (99.0%), cyphonothrin (100.0%), fenpropathrin (99.9%), Imiprothrin (99.5%) and prallethrin (100.00%) are obtained from Sumitomo Chemical Co. Ltd., Osaka, Japan. Bifenthrin (93.5%) is obtained from FMC India Private Ltd., Bangalore, India. Cypermethrin (94.50%) and fenvalerate (purity—94.3%) are obtained from Sharda International, Mumbai, India. Cyfluthrin (95.5%) and lambda-cyhalothrin (98.5%) are obtained from PESTANAL, Riedel de-Haen, seelze, Germany. Deltamethrin (99%) is obtained Isagro (Asia) Ltd., and transfluthrin (95.3%) is obtained from Bayer India limited, Mumbai, India.

2.4. Preparation of stock solutions

Stock solutions of different pyrethroid insecticides are prepared separately using trace analytical grade acetone. Subsequently diluted the stock solutions using hexane and prepared the working standard solutions. An Artic 380 deep freezer supplied by Froilabo, Meyzieue, France with automatic temperature recorder and display facility is used for storing the stock solutions and the samples at -40 ± 2 °C.

2.5. Collection of blood samples

About 5 ml of blood was collected from each donor for the experimental purpose. Informed consent was obtained from all the donors. All the heparinized blood samples are stored at -40 ± 2 °C until analysis.

2.6. Extraction of residues from the whole blood [35,36]

Prepared a mixture of different concentrations of pyrethroids, allethrin, bifenthrin, cypermethrin, cyphonothin, cyfluthrin, lambda-cyhalothrin, deltamethrin, fenvalerate, fenpropathrin, imiprothrin, permethrin, prallethrin and transfluthrin. Controlled blood samples are spiked at different concentration levels to give 1, 10, 50, 100, 200, 400, 600 and 1000 ng/ml of pyrethroids in blood. Quantitatively 1 ml of blood sample was taken and added 20 μl of lindane (γ-BHC or gamma benzene hexachloride) as internal standard. Mixed well and extracted with 5 ml of hexane and acetone (8:2, v/v) mixture by vigorous shaking for 10 min. Allowed for 10 min to settle. Collected the supernatant. The sample was again re extracted. Combined the extracts and centrifuged for 5 min at 5000 rpm. Collected the supernatant in a graduated test tube and concentrated to 0.5 ml at 40 °C under gentle stream of nitrogen in a hood. Final volume was made up to 1 ml using hexane as solvent. Standard and sample are recorded. At the defined conditions injected the representative standard and samples in to the GC–MS and analyzed.

3. Results and discussion

Blood and excreta are the preferred matrices in an exposure analysis. Because of the high lipophilicity of most toxic pesticides and the whole blood being the major body compartment, high concentrations are likely to be expected in erythrocytes. Quantification of residues from the whole blood is a difficult task because of the complexicity of the matrix. The pyrethroids selected in the study are structurally similar with minor changes in their functional groups. The presence of structurally common cyclopropane ring in

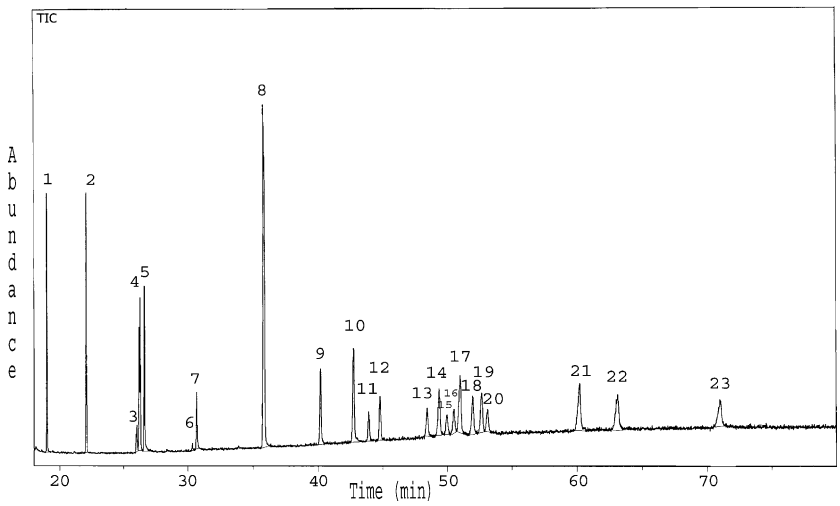

Fig. 1. Electron ionization-mass spectra of different pyrethroids in mode (TIC), 1-lindane (internal standard), 2-transfluthrin, 3,4-*cis* and *trans* isomers of allethrin, 5-prallethrin, 6,7-*cis* and *trans* imiprothrin, 8-bifenthrin/fenpropathrin, 9-*cis* isomers of lambda-cyhalothrin, 10-cyphenothrin, 11,12-*cis* and *trans* isomers of permethrin, 13,14,15,16-*cis* and *trans* isomers of cyfluthrin, 17,18,19,20-*cis* and *trans* isomers of cypermethrin, 21,22-*cis* and *trans* isomers of fenvalerate, 23-deltamethrin.

pyrethroids, that is similar in function to a double bond, different stereo isomers are formed and subsequently eluted during the analysis making the separation complicated. In the present study we have used hexane and acetone as (80 + 20) extraction solvent based on our previous experience on the similar subject. This extraction solvent clearly gave good recoveries of analytes with very less interferences. All the pyrethroid residues were quantified as a mixture of different stereo isomers using GC–MS (Figs. 1 and 2) in SIM mode. From the mass spectra of individual compounds identified two characteristic fragments (Table 1) and used for the quantification purpose. The sensitivity of the method was evaluated by determining the limit of detection (LOD) and the limit of quantification (LOQ). LOD was defined as the concentration with a signal to noise ratio of at least three, while LOQ was the lowest standard with a signal-to-noise ratio of at least 10.

The linearity of the method was investigated by least square method and expressed the correlation coefficient (R^2). Linearity of each of the compounds was determined with at least six concentration levels without blank matrix. The acceptable precision and accuracy (relative standard deviation,

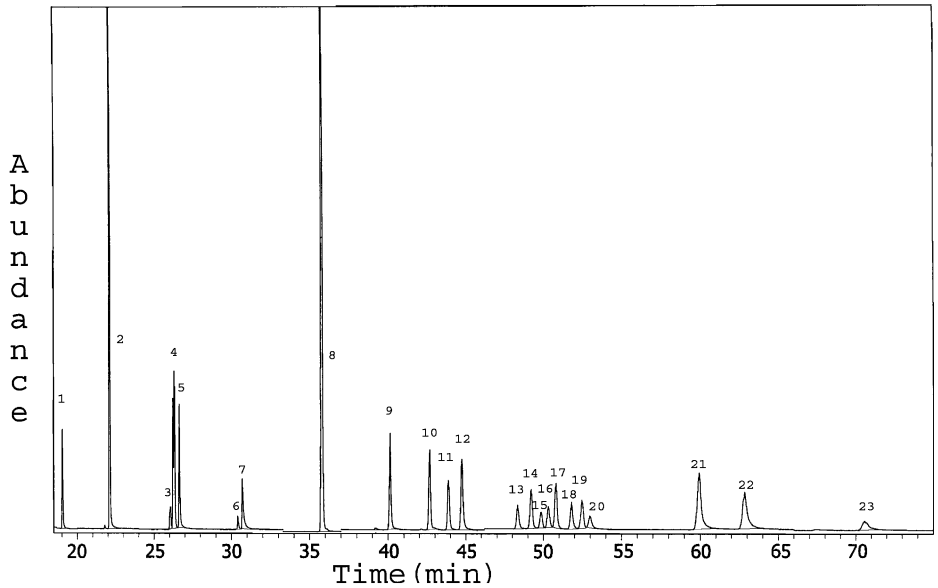

Fig. 2. Electron ionization-mass spectra of different pyrethroids in SIM mode at 100 ppb concentration, 1-lindane (internal standard), 2-transfluthrin, 3,4-*cis* and *trans* isomers of allethrin, 5-prallethrin, 6,7-*cis* and *trans* imiprothrin, 8-bifenthrin/fenpropathrin, 9-*cis* isomers of lambda-cyhalothrin, 10-cyphenothrin, 11,12-*cis* and *trans* isomers of permethrin, 13,14,15,16-*cis* and *trans* isomers of cyfluthrin, 17,18,19,20-*cis* and *trans* isomers of cypermethrin, 21,22-*cis* and *trans* isomers of fenvalerate, 23-deltamethrin.

Table 1
Mass ions used for quantification

Pyrethroid name	Molecular ion (*m*/*z*)	Fragment ions (*m*/*z*)	Retention time (min)
Lindane	290	181, 219	19.10
Transfluthrin	370	127, 163	22.09
Allethrin	302	123, 136	26.04, 26.30
Prallethrin	300	123, 168	26.60
Imiprothrin	318	123, 151	30.35, 30.69
Bifenthrin	422	165, 181	35.30
Fenpropathrin	349	181, 208	35.31
Lambda-cyhalothrin	449	181, 197	40.20
Cyphenothrin	375	123, 181	42.76
Permethrin	391	163, 183	42.93, 44.79
Cyfluthrin	435	163, 206	48.45, 49.38, 49.99, 50.53
Cypermethrin	417	163, 181	51.01, 52.00, 52.67, 53.13
Fenvalerate	419	125, 167	60.19, 63.10
Deltamethrin	505	181, 253	70.97

R.S.D.) parameters were determined empirically by testing a series of concentrations of pyrethroids fortified in blood in multiple replicates. The results obtained are presented in Table 2. The analysis of whole blood samples produced no additional interference peaks that will influence the quantification of individual pyrethroids.

The other common factors which are likely to influence the stability of the analytes during the analysis were studied. Mainly the storage temperature, light and storage time are studied for this purpose. Two series of blood samples are spiked with known quantity of pyrethroids are stored at −10 and −45 °C for a period of 30 days. Samples analyzed thereafter showed the recoveries within 2% deviation. Further no significant difference was observed with respect to samples stored at two different temperatures. Further two different sets of samples were stored under room temperature for a period of 5 days. One set of sample was kept under dark and the other set was kept under day light. The room temperature was maintained at 22 ± 2 °C. The data showed nearly 40–55% dissipation of residues. The reason for the rapid dissipation of residues in the blood stored at room temperature can be attributed due to the plausible influence of bacteria. There is only about 10–15% difference in the dissipation of residues due to sun light exposure.

The applications of the method was investigated in human population continuously exposed to different pyrethroid based mosquito repellents formulations during nights. Collected 45 blood samples from different age groups and tested. All the blood samples show the residues below the LOD (0.5–2 ng/ml) Fig. 3a. The results are confirmed by spiking the known quantity of pyrethroids and subsequently their positive detection Fig. 3b. The non availability of the residues may be due to the rapid excretion of the residues or due to the limitations of the experiments which has to be investigated further.

Table 2
LODs, LOQs, and calibration results

Compound	LODs (ng/ml)	LOQs (ng/ml)	Equation	R^2	Recovery range (%)	Relative standard deviation
Transfluthrin	0.05	0.1	$y = 484.26x - 219.19$	0.9999	95–99	1.51–4.66
Allethrin	0.1	0.5	$y = 13.865x - 0.1727$	1.0000	96–103	1.29–3.40
Prallethrin	0.1	0.5	$y = 4.8662x + 1.4495$	0.9999	96–99	0.95–4.40
Imiprothrin	2	4	$y = 3.1276x + 0.0798$	1.0000	91–95	1.11–4.11
Bifenthrin	0.1	0.5	$y = 29.733x - 5.9919$	1.0000	93–96	1.53–2.74
Fenpropathrin	0.1	0.5	$y = 24.175x + 2.3078$	1.0000	93–97	0.81–2.69
Lambda-cyhalothrin	0.1	0.5	$y = 9.3466x + 7.2418$	0.9996	94–99	1.39–3.73
Cyphenothrin	0.1	1	$y = 9.2208x + 2.935$	0.9999	95–97	0.79–4.28
Permethrin	0.5	2	$y = 15.479x + 2.5213$	1.0000	93–97	1.25–2.61
Cyfluthrin	1	2	$y = 7.3447x - 0.2919$	1.0000	94–96	0.87–2.08
Cypermethrin	1	2	$y = 8.4471x + 0.0451$	1.0000	93–99	1.55–3.32
Fenvalerate	0.5	1	$y = 2.5646x - 0.7341$	0.9999	94–97	1.15–2.55
Deltamethrin	1	4	$y = 3.1276x + 0.0798$	1.0000	93–96	1.58–4.38

Average of six replications, *x*—concentration (ng/ml), *y*—response area.

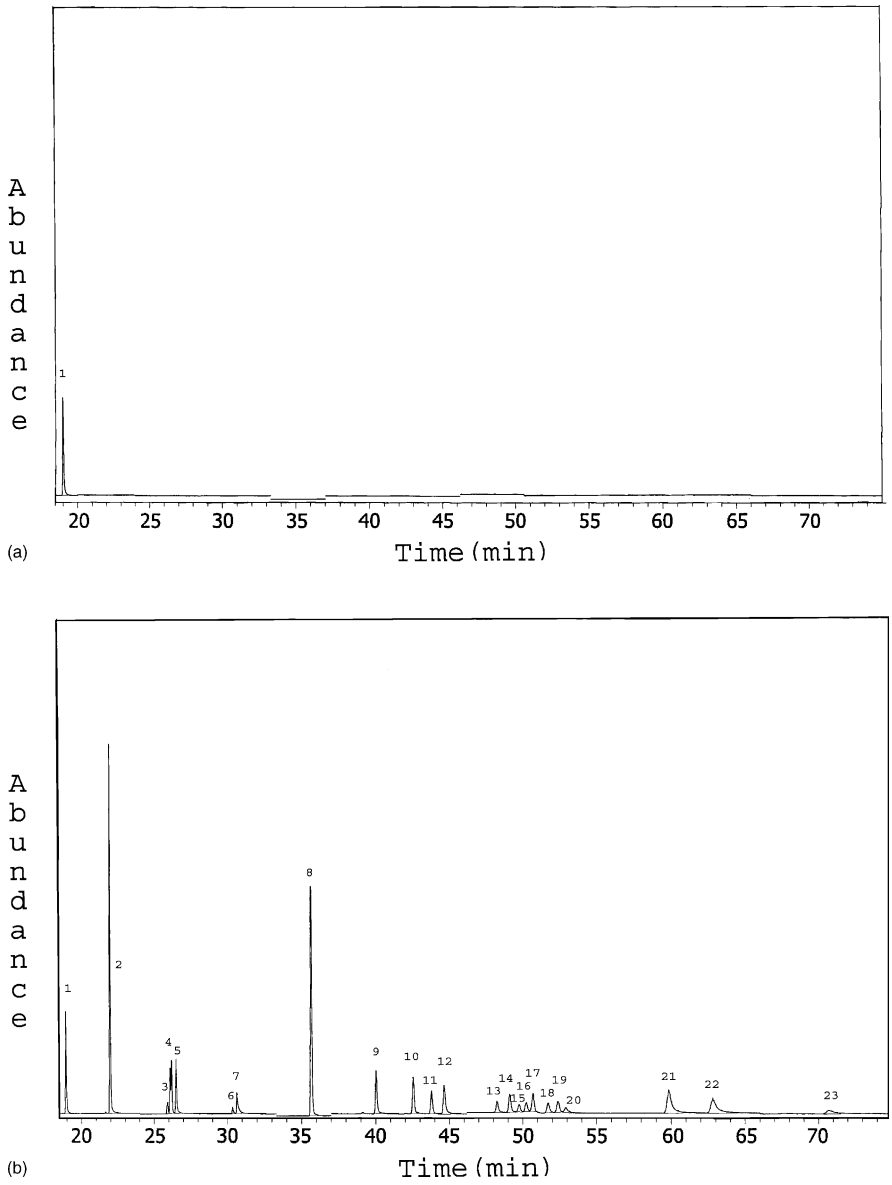

Fig. 3. (a) EI-mass spectra of exposed human blood sample in SIM mode, 1-lindane (internal standard). (b) Electron ionization-mass spectra of human blood sample spiked with different pyrethroids in SIM mode at 50 ppb concentration, 1-lindane (internal standard), 2-transfluthrin, 3,4-*cis* and *trans* isomers of allethrin, 5-prallethrin, 6,7-*cis* and *trans* imiprothrin, 8-bifenthrin/fenpropathrin, 9-*cis* isomers of lambda-cyhalothrin, 10-cyphenothrin, 11,12-*cis* and *trans* isomers of permethrin, 13,14,15,16-*cis* and *trans* isomers of cyfluthrin, 17,18,19,20-*cis* and trans isomers of cypermethrin, 21,22-*cis* and *trans* isomers of fenvalerate, 23-deltamethrin.

4. Conclusion

In an exposure study, analysis of blood for residues is a difficult task. In general the residues in blood are likely to appear at very low concentration because as soon as it enters in to the body most of the chemical may gets excreted. Nevertheless, the ultra low quantities present in the body are enough to create significant impact in deteriorating the health. Problem may be further more serious in case of children due to their labile genetic system. The present method helps in simultaneous detection of 13 different pyrethroids and their isomers in whole blood using a single chromatographic injection. It is very simple, rapid and can be adopted without any matrix interferences and meets the requirement of analysis residues in whole blood. The selective ion monitoring mode provides the detection sensitivity up to 0.05 ng/ml. The method can detect the residues of different pyrethroids down to the level 0.05–2 ng/ml.

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