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A Miniturised Matrix Solid - Phase Dispersion Extraction Method for The Simultaneous Determination of Pyrethroid Insecticides in Biological Samples by GC-MS

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ABSTRACT

A simple and effective extraction method based on matrix solid-phase dispersion (MSPD) was developed to determine thirteen pyrethroid insecticide residues in goat tissues (liver, kidney, muscle and heart) and milk using gas chromatography electron ionization mass spectrometry method (GC-MS). The samples were pre-concentrated using C_{18} packing material (dispersion adsorbent), followed by clean up with multiwall carbon nanotubes (cleanup adsorbent) material. The average extraction recoveries of the pyrethroids from tissues and milk at two concentration levels (spiked at 0.05 and 0.25 μg g^{-1}) were 84.7 – 108.4% and 87.2 – 104.4%, respectively, with relative standard deviations between 1.88 – 8.37% and 1.32 – 7.43%, respectively.

Keywords: Matrix solid-phase dispersion, Pyrethroids, Biological samples, GC-MS

Introduction

Pyrethroids are important insecticides and widely used in agriculture (field-treatment of crops and protection of stored products), public health (hygienic treatments in houses), forestry, horticulture, veterinary (to control ecto- and endo-parasites on animals) and some other aspects due to their high insecticidal potency and relatively few side effects on birds and mammals [1]. The production of these insecticides has increased exponentially. There are a number of ways that these insecticides can affect and contaminate some stock breeding and agricultural production. For example, they can contaminate animal tissues from these possible sources, including foodstuffs containing high levels of pyrethroid residues from post-harvest treatment, food stuffs manufactured from plant material that has been treated during the growing season with insecticides, use of insecticides directly on the animal against disease vectors and use of insecticides against insects in stables and in factories processing animal tissues [2]. Numerous methods have been reported for the determination of pyrethroids by Gas Chromatography Electron Capture Detection (GC-ECD), Gas Chromatography Mass Spectrometry (GC-MS), High Performance Liquid Chromatography Ultra Violet detection (HPLC-UV). The literature clearly shows that most of the monitoring studies are conducted using blood (serum, plasma, whole blood and biological samples) [3-14], vegetables, fruits and its juices, oils [15-22], soil and water [23-28]. The detection and determination of unchanged insecticide residues in tissue samples are useful indicators of exposure and helps in accessing adverse health effects.

Matrix solid-phase dispersion is a sample preparation strategy widely applied to solid, semisolid or viscous samples, including animal tissues and foods with a high lipid content [29-33]. The process consists in blending the matrix onto a solid support, allowing the matrix cell disruption and the subsequent extraction of target analytes by means of a suitable elution solvent. First introduced in 1989 [34], MSPD employment and developments are still growing because of the feasibility and the versatility of the process, as evidenced by the several reviews that have been published since the nineties.

In view of the paucity of information on the quantification of residues of different pyrethroids in milk and animal tissues (liver, kidney, muscle and hart), experiments were conducted to develop a sensitive analytical method based on the application of Multiwall Carbon Nanotubes (MWCNTs) as adsorbent in miniaturized matrix solid - phase dispersion extraction (MSPD) combined with GC-EI-MS.

Experimental

Instrumentation

The configuration of a GC-MS system used includes a GC-17A (Shimadzu, Tokyo, Japan) gas chromatography

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coupled with QP5050A Mass-Selective Detection (MSD) and an AOC 20i auto injector interfaced to a computer for data acquisition supported by the GCMS solution software. HP-1MS (100% dimethyl polysiloxane) capillary column (30 m length, 0.25 mm i.d. and 0.25 µm film thicknesses) was used for the separation of residues of Pyrethroids. Helium gas was used as carrier at 1.0 mL/min, injector temperature was set at 300°C with split ratio 1:5, interface temperature 300°C and column oven temperature was programmed from 100°C for 3 minutes, increased at 10°C to 290°C. The target ions used for the measurement were depicted in **Table 1**. Residues were quantified using the molecular ion peak fragments. At least two molecular ion fragments were used.

Analytical standards and reagents

Analytical reference standards Deltamethrin, Alpha-cypermethrin, Cyfluthrin, Permethrin, Lambda cyhalothrin, d-Phenothrin, Fenpropathrin, Tetramethrin, Imiprothrin, Esbiothrin, Metofluthrin, Transfluthrin were obtained from IIBAT inventory. All standards were at least 98.5% pure. Methanol, acetone, n-hexane, ethyl acetate, dichloromethane and anhydrous sodium sulfate were purchased from Merck Specialities pvt ltd (India). Multiwall carbon nanotubes (diam =110-170 nm, length = 5-9 micron) and C18 materials were purchased from Sigma-Aldrich (USA).

Preparation of standard solutions

A stock solution containing each pyrethroid insecticide at 1000 µg/mL was prepared in methanol. The stock solutions were further diluted with methanol to make a multicomponent stock solution containing thirteen pyrethroid insecticides at 100 µg mL^{-1} and stored at -10°C prior to analysis. Working standard solutions were prepared by diluting the multicomponent stock solution with methanol appropriately. All stock and working solutions were kept at -10°C in the dark and brought to room temperature before use.

Preparation of calibration solutions

The matrix matched calibration standards were prepared in a concentration range of 0.005–2.0 µg/mL by adding the desired amount of working solutions to blank sample extracts (after extraction). The linearity of the method was studied by analyzing standard solutions of at least six different concentrations in the range from 0.005 – 2.0 µg/mL. Aliquots of calibration solutions were injected into the GC-MS system and recorded the peak response. Calibration curves plotted between concentrations injected and peak response.

Collection of milk and tissue samples

Goat milk and tissue samples (liver, muscle, heart and kidney) used for blank and spiked studies were obtained from a local market.

Table 1

Molecular ion fragments of Pyrethroids by GC-EI-MS

Pesticide	Molecular ion peak (m/z)	Fragment ions (m/z)
Transfluthrin	370	127, 163
Metofluthrin	360	109, 207
Esbiothrin	302	123, 136
Prallethrin	300	105, 123
Imiprothrin	318	123, 151
Tetramethrin	331	123, 164
Fenpropathrin	349	181, 208
d-Phenothrin	350	123, 183
Lambda cyhalothrin	449	181, 197
Permethrin	391	163, 183
Cyfluthrin	435	163, 206
Alpha-cypermethrin	416	163, 181
Deltamethrin	505	181, 253

MSPD extraction procedure

The harvested tissue samples (liver, kidney, muscle and hart) were cut into pieces and homogenized using a high speed blender. A 0.5 g of sample (tissue and milk) was transferred into a glass mortar and a suitable amount of multicomponent working standard solution prepared in methanol was added to the sample. Air dried the samples for about 15 minutes to allow the organic solvent to evaporate from the tissue samples before proceeding. After 15 min, 2 g of washed C_{18} packing material (dispersion adsorbent) was mixed with the sample. The sample/packing mixture was allowed to air dry for about 30 minutes. Then the homogeneous mixture was transferred in a syringe – barrel column containing approximately 1.0 g of anhydrous sodium sulfate and 1.0 g of MWCNTs (cleanup adsorbent) packed at the bottom in turn. Approximately 1.0 g of anhydrous sodium sulfate was added at the top of the sample mixture. Then the column was tightly compressed using a soft stick. The column was eluted with a suitable volume of eluent by gravitational flow. Eluting efficiencies were studied using n-hexane, ethyl acetate, n-hexane - ethyl acetate (1:1 v/v), n-hexane-dichloromethane (1:1 v/v), and n-hexane-acetone (7:3 v/v) as eluents. The eluate was evaporated to near dryness using a turbovap evaporator below 40ºC. Then 1.0 mL of n-hexane was added to dissolve the residues. For the analysis of blank and spiked samples, the final volume of n-hexane to dissolve the residues was adjusted to 200 µL. All these solutions were filtered with 0.45 µm membranes and subjected to GC-MS analysis.

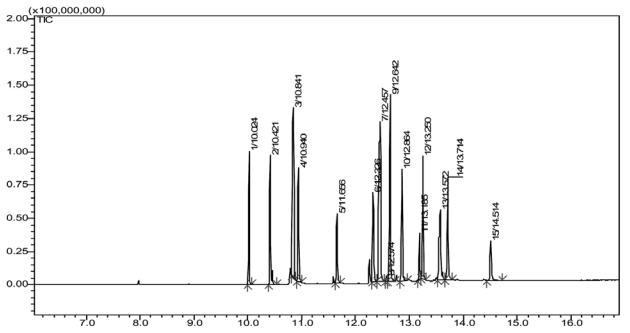

Fig.1: Typical elution pattern of a GC-EI-MS total ion chromatogram of pyrethroid standard mixture on a HP-1MS capillary column

Peak number	Retention time (min.)	Name
1	10.0	Transfluthrin
2	10.4	Metofluthrin
3	10.8	Esbiothrin
4	10.9	Prallethrin
5	11.6	Imiprothrin
6	12.3	Tetramethrin
7	12.4	Fenpropathrin
8	12.5	Cis and trans isomers of d-Phenothrin
9	12.6	
10	12.8	Lambdacyhalothrin
11	13.1	Cis and trans isomers of Permethrin
12	13.2	
13	13.5	Cyfluthrin
14	13.7	Alpha-cypermethrin
15	14.5	Deltamethrin

Method Validation

Specificity

Blank samples were analyzed as described above, and the chromatograms were visually evaluated for the occurrence of substances that might interfere with the peak of interest.

Linearity of calibration curve

The calibration curves were obtained by peak area versus analyte concentration.

Absolute recovery, accuracy and precision

Absolute recovery was calculated from the peak areas of pyrethroids in milk, liver, kidney and heart samples spiked at three concentrations (0.05, 0.25 and 0.5 µg g^{-1}) compared with those of standard solutions. For intraday accuracy and precision, the liver samples (n=3) spiked at concentrations of 0.25 and 0.5 µg g^{-1} were analyzed. The accuracy was expressed as the absolute error percentage and calculated from

$$\text{Absolute error} = \frac{\text{(Mean of measured Conc., - added Conc.,)}}{\text{(Added Conc.,)}} \times 100$$

The precision was expressed as the relative standard deviation (% RSD) and calculated from the standard deviation from the mean of the detected concentration. Interday accuracy and precision were determined in three replicates of the liver samples spiked at concentrations 0.25 and 0.5 µg g^{-1} and performed in three different days.

Results and Discussion

The typical elution pattern of a GC-EI-MS chromatogram of a standard mixture of thirteen pyrethroid insecticides on a HP-1MS capillary column was shown in **Figure 1**. The target and qualifier abundances were determined by injection of individual pesticide standards under the same chromatographic conditions using full scan with mass/charge ratio ranging from *m/z* 50 to 600. Mass analysis was performed with selected ion monitoring (SIM) mode using primary and secondary ions. The selected ion monitoring was performed with one quantitative ion and two or three qualitative ions of pyrethroids are shown in **Table 1**. Analytes were confirmed by their retention times, the identification of target and qualifier ions, and the determination of qualifier-to-target ratios. The developed method was validated with respect to Specificity, linearity, Accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ).

Method Validation

Linearity and repeatability

The criterion used to establish the lower limit in the range is defined as $10S_b/m$, where S_b is the standard deviation of noise (a value of 7 times the standard deviation of the blank); m is the slope of the calibration curve. The calibration curves for milk, liver, kidney and heart showed good linearity over the range from 0.005 – 2 µg mL^{-1}. The relationship between the analyte concentration (X) and peak area of measured signal (Y) are noted as the regression equation for the calibration standards. Calibration curves showed a good linear relationship ($r^2 > 0.999$).

Detection and quantification limits

The limit of detection (LOD) is the lowest concentration of an analyte to produce an analytical signal ~ 3.0 times that can be reliably differentiated from a background level. The limit of quantification (LOQ) is the lowest amount of an analyte that can be quantitatively determined with defined precision and accuracy under the given experimental conditions.

The limit of detection was 0.005 µg g^{-1} for transfluthrin, metofluthrin, esbiothrin, fenpropathrin and d-Phenothrin; 0.01 µg g^{-1} for prallethrin, imiprothrin, tetramethrin, lambdacyhalothrin, permethrin, cyfluthrin, alpha-cypermethrin and deltamethrin.

Absolute recovery, accuracy and precision

As shown in **Table 4**, absolute recoveries of pyrethroids from spiked goat milk and tissue samples were in the range of 84.7 – 108.4% and 87.2 – 104.4%, respectively. Intraday and interday accuracy and precision were determined to evaluate the reliability of the current analytical method. The intraday and interday accuracy and precision were evaluated using 0.25 and 0.5 µg g^{-1} (**Table 2**). **Figure 3,** shows the chromatograms of target analytes for the blank and spiked liver sample. Both intraday and interday accuracy and precision for all the pyrethroids analyzed in goat milk and tissues were between -13.2 – 6.6 (error %) and 1.6-6.7 (%RSD), respectively.

MSPD Extraction

MSPD extraction conditions were carefully selected to achieve the highest recoveries for the pesticides. Elution solvents were studied in order to obtain perfect recoveries of analytes. n-hexane, ethyl acetate, n-hexane-ethyl acetate (1:1), n-hexane- dichloromethane (1:1), and n-hexane-acetone (7:3) were tested. N-hexane, ethyl acetate, acetone and dichloromethane are four kinds of solvents commonly found in pesticide residue analysis. These solvents present dissimilar polarities. Ethyl acetate, acetone and dichloromethane have stronger capabilities for pesticide extraction than n-hexane, which means that a higher number of interferences will be extracted into the eluate. N-hexane is a weak polar solvent and the mixture n-hexane-ethyl acetate, n-hexane-dichloromethane and n-hexane-acetone has intermediate polarities. Results showed that recoveries of pyrethroids were in the acceptable range, when n-hexane-acetone (7:3, v/v) was used as elution solvent (**Figure 2**). A number of interferences were extracted into the eluate using the other four kinds of solvents. To establish the volume of elution solvent required, liver tissue sample containing pyrethroids at 0.25 µg g^{-1} level was prepared. During the extraction of the tissue samples, 10 mL fractions were collected so that the analytes could be determined. The recoveries of pyrethroids increased when the solvent volume increased from 10 mL to 20 mL and then reached a plateau. When the volume of elution solvent increased from 20 mL to 50 mL, no significant increase in recoveries was observed. Hence, the volume of elution solvent was set at 20 mL in further experiments.

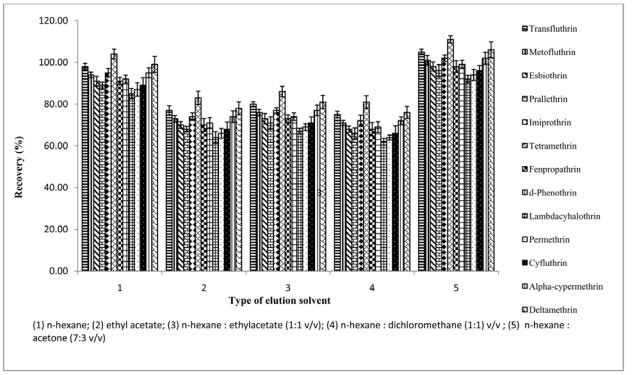

Fig.2 : **Effect of type of solvent on the extraction efficiency of pyrethroids**

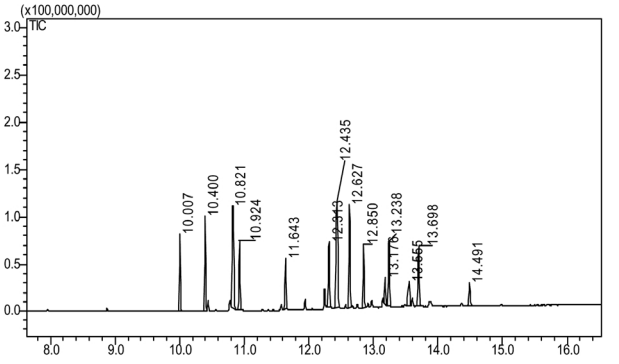

Fig.3 : GC-EI-MS chromatogram of liver spiked 0.05 µg g^{-1}.

Application of method to real samples

Goat tissue and milk samples collected from the local market (Chennai) were analyzed by the present method to evaluate its applicability to analyze real samples. Residues of pyrethroids were found to be below detectable level in all the samples analysed.

Acknowledgement

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Table 2.

The intraday, interday precision and accuracy of pyrethroids analyzed

Pesticide	Conc. added (µg g^{-1})	Intraday			Interday		
		Mean conc. ± S.D.	Accuracy	Precision	Mean conc. ± S.D.	Accuracy	Precision
TRA	0.25	0.26 ±4.2	4.4	4.0	0.23±5.3	-6.8	5.7
	0.50	0.49±2.8	-1.6	2.8	0.49±3.9	-2.8	4.0
MET	0.25	0.24±5.2	-6.0	5.5	0.22±4.8	-10.4	5.4
	0.50	0.52±3.8	3.2	3.7	0.46±4.1	-7.6	4.4

ESB	0.25	0.25±5.3	-1.6	5.4	0.24±6.2	-5.2	6.5
	0.50	0.47±2.1	-5.4	2.2	0.50±5.2	0.4	5.2
PRA	0.25	0.26±5.9	4.8	5.6	0.24±4.6	-3.2	4.8
	0.50	0.53±4.2	6.6	3.9	0.46±3.1	-8.4	3.4
IMI	0.25	0.25±2.2	-1.2	2.2	0.24±5.6	-5.6	5.9
	0.50	0.48±1.5	-3.4	1.6	0.48±2.8	-5.0	2.9
TET	0.25	0.23 ±3.3	-6.4	3.5	0.24±4.8	-2.4	4.9
	0.50	0.51±2.4	2.2	2.3	0.49±2.4	-2.8	2.5
FEN	0.25	0.24±4.3	-2.8	4.4	0.24±3.9	-3.2	4.0
	0.50	0.49±2.3	-2.8	2.4	0.48±1.9	-4.4	2.0
DPH	0.25	0.25±2.9	-0.8	2.9	0.24±6.5	-3.6	6.7
	0.50	0.52±3.2	4.6	3.1	0.48±3.7	-3.8	3.8
LAM	0.25	0.24±3.8	-4.4	4.0	0.24±4.6	-2.4	4.7
	0.50	0.52±2.6	4.4	2.5	0.50±2.9	0.4	2.9
PER	0.25	0.24±5.6	-3.6	5.8	0.24±3.9	-4.8	4.1
	0.50	0.48±3.2	-3.8	3.3	0.49±1.6	-1.4	1.6
CYF	0.25	0.23±4.9	-8.4	5.3	0.25±5.9	-1.6	6.0
	0.50	0.47±3.8	-5.4	4.0	0.49±3.6	-2.6	3.7
ALP	0.25	0.22±3.5	-13.2	4.0	0.23±4.6	-6.4	4.9
	0.50	0.48±2.6	-4.2	2.7	0.46±3.1	-8.2	3.4
DEL	0.25	0.24±4.4	-4.2	4.6	0.24±5.8	-3.9	6.0
	0.50	0.49±2.8	-2.4	2.9	0.47±3.4	-5.6	3.6

Table 3

Absolute recovery (%) of pyrethroids in goat milk and tissues

Pesticide	Conc. added (µg g $^{-1}$)	Absolute Recovery (%) ± Standard Deviation (n=3)				
		Milk	Liver	Kidney	Muscle	Heart
TRA	0.05	104 ±5	92±7	89±6	91±8	96 ±6
	0.25	93±5	95±4	92±5	96±5	98±3
	0.50	98±3	94±3	97±4	105±2	94±3
MET	0.05	88±6	93±5	96±6	103±7	92±5
	0.25	93±4	101±5	94±2	101±2	96±4
	0.50	97±4	96±3	92±3	104±4	99±3
ESB	0.05	98±5	91±6	88±4	101±3	103±6
	0.25	94±3	98±3	89±3	96±2	101±4
	0.50	97±2	103±3	96±4	95±5	95±3
PRA	0.05	88±5	93± 3	86 ±5	99±6	100±4
	0.25	93±4	94±4	93±3	92±4	90±5
	0.50	94±3	96±5	91±2	90±3	93±3
IMI	0.05	104±6	103±4	101±5	87±6	95±4
	0.25	98±4	101±5	95±3	93±5	93±5
	0.50	92±2	100±2	96±2	101±2	98±3

TET	0.05	99±3	96±4	99±5	92±3	96±6
	0.25	92±3	99±2	93±4	96±2	91±5
	0.50	96±1	91±2	91±2	94±3	96±3
FEN	0.05	96±5	90±5	88±6	104±4	94±5
	0.25	90±5	103±4	98±3	101±3	98±3
	0.50	92±3	95±3	89±4	93±2	92±4
DPH	0.05	101±4	93±4	96±5	91±5	90±3
	0.25	93±2	97±5	90±4	90±3	99±5
	0.50	100±2	105±2	91±3	93±3	102±3
LAM	0.05	89±5	105 ±4	92±7	90±6	98±5
	0.25	95±6	93±3	97±5	93±4	93±3
	0.50	99±2	98±5	92±4	91±3	92 ±2
PER	0.05	87±7	84 ±5	103± 4	90 ±6	87± 5
	0.25	92±4	95±4	96±5	96±4	101±5
	0.50	93±3	101±2	93±3	102±2	97±3
CYF	0.05	93±6	102±5	101± 7	96 ±5	100± 6
	0.25	103±3	95±4	97±8	94±4	89±5
	0.50	92±2	98±2	94±2	98±3	92±2
ALP	0.05	88±5	92 ±4	103 ±6	91±4	103 ±6
	0.25	93±6	89±3	106±4	98±3	96±5
	0.50	102±3	90±1	96±5	92±3	92±5
DEL	0.05	93±7	108±3	104± 6	95±5	101±6
	0.25	101±2	95±2	97±4	104±4	97±3
	0.50	103±5	100± 3	102±3	98±4	94±5

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