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A NEW METHOD FOR DETERMINATION OF BIFENTHRIN RESIDUES IN AQUATIC TOX MEDIUM FOLLOWED BY GAS CHROMATOGRAPHY MASS SPECTROMETRY METHOD

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ABSTRACT

A simple and sensitive validated GC-MS-EI analytical method was developed for the determination of bifenthrin residues in different aquatic tox mediums. The tox mediums were those which provide nutrients and help the growth of different aquatic organisms for their survival and multiplication. The constituent of different mediums includes blended water for fish, M4 Medium for Daphnia magna and OECD TG 201 medium for Alga. The method was validated using in aquatic tox samples spiked with bifenthrin sample at different concentration levels (0.5 and 5.0 mg/L). Average recoveries (using each concentration six replicates) ranged 88-96%, with relative standard deviations less than 2%, calibration solutions concentration in the range 0.5-100 mg/L and limit of detection (LOD) and limit of quantification (LOQ) were 0.2 mg/L and 0.5 mg/L, respectively. The proposed method can be applied successfully for the determination of bifenthrin residues in different aquatic solutions.

Key words: Bifenthrin, LOD, LOQ, Aquatic Tox medium, GC-MS-EI method.

INTRODUCTION

Bifenthrin is a member of the synthetic pyrethroid family of pesticides. Like most pyrethroid pesticides, bifenthrin affects the central and peripheral nervous system of insects causing paralysis. Because of their high toxicity to aquatic organisms, bifenthrin products are registered as “restricted use pesticides”, to be sold only to and used by Certified Pesticide Applicators. In addition to Red Imported Fire Ant (RIFA) control, bifenthrin is

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used as a miticide and acaricide in orchards, nurseries, and homes. Bifenthrin is a third-generation synthetic pyrethroid chemical. This group is characterized by greater photostability and greater insecticidal activity than previous pyrethroids. Little research has been done specifically on bifenthrin's mode of action on invertebrates or vertebrates, however, most investigations have found that the pyrethroid families of pesticides demonstrate very similar effects on invertebrate nervous systems. Pyrethroids[1-7] utilize a number of different pathways to cause nervous system damage in invertebrates. Significant among these is interference with sodium channel gating in the nerve cell endings. By acting on the sodium channels to depolarize the pre-synaptic terminals, pyrethroid insecticides effectively paralyze organisms by severely limiting neuro-transmission. This paralysis is often preceded by spastic activity of the organism due to the hyper-activity of nerve endings. The spastic activity is caused by sodium channels repeatedly polarizing and depolarizing, mimicking neuro-transmission where none is actually taking place. Pyrethroids have also been shown to inhibit ATPase enzyme production.

This is of primary importance in understanding why aquatic organisms are much more susceptible to pyrethroid insecticides than terrestrial organisms. Freshwater aquatic organisms must maintain ionic balances and osmoregulation in an extremely dilute environment. Active transport at cellular walls is needed to maintain critical cellular ion levels against a concentration gradient. ATPase enzymes provide the energy needed by cells to maintain this gradient. By inhibiting ATPase enzymes, pyrethroids breakdown the critical concentration gradient, eventually leading to death of the organism. Pyrethroids have the most serious effects on fish and gill breathing aquatic insects because of the large surface area available to de-ionize after ATPase inhibition

EXPERIMENTAL

GC-MS conditions for the determination of Bifenthrin

The configuration of GC–MS system used includes a (Agilent/6890N) gas chromatograph coupled with 5975C Mass-Selective Detection (MSD) and Chemstation software, the detector was set in selective ion monitoring mode (EI) mode. The ions m/z 422.2 and m/z 181 were used as qualifier ions (Fig. 2) and the target ion used for the measurement was the ion at m/z 181. The bifenthrin peak separation was obtained on a DB-1 capillary column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness). The injection system was operated in split mode with a split ratio of 20:1. The injector and the transfer line temperatures were 280ºC and 300ºC, respectively. The oven temperature

program was 150ºC, held constant for 5 min and ramp at 8ºC/min raised the column temperature up to 230ºC, held constant for 10.0 min and ramp at 22ºC/min raised the column temperature up to 270ºC, held constant for 15 min. The carrier gas used was helium (GC grade) at a flow rate of 1.0 mL /min and the sample volume injected onto the column was 2.0 µL. An Agilent Chemstation Software was used for acquisition of data and calculation of peak area. The carrier gas used was helium (GC grade) at a flow rate of 1.0 mL /min and the sample volume injected onto the column was 2.0 µL. An Agilent Chemstation Software was used for acquisition of data and calculation of peak areas. The retention time of bifenthrin was about 22.0 min and the total time of chromatographic analysis was 28 min.

Analytical standards, reagents and solutions

The analytical standard of bifenthrin (Purity 99.2%) was obtained from was purchased from Sigma Aldrich. The hydrochloric acid, ethylenediaminetetraacetic acid disodium salt (EDTA) used were AR grade, n-Hexane HPLC grade were purchased form rankem.

Standard stock solutions

The bifenthrin standard stock solution was individually prepared in n-Hexane at a concentration level 1000 mg/L and stored in a freezer at -18°C. The stock standard solution was used for 3 months. Suitable concentrations of working standards were prepared from the stock solutions by dilution using n-Hexane, immediately prior to sample preparation.

Sample preparation

The samples were allowed to stand at room temperature for one hour, before it was kept at refrigerator condition, until analysis.

Test medium

Test medium is a constitute of different macro nutrients, salts and vitamins. This helps in the survival of different organisms during exposure of different compounds.

Blended water: A mixture of well water and reverse osmosis water in the ratio of 1:1.7 liters. This provides enough nutrients for the survival of fish during test item exposure.

M4 Medium[12,13]: It is a combination of trace elements, marco nutrients and vitamins. The composition was given in Table 1.

Table 1: Preparation of M4 medium nutrients (Daphnia magna)

S. No.	Chemical name	Formula	mg/L
Trace elements			
1.	Boric acid	H_3BO_3	57190
2.	Manganese chloride	$MnCl_2.4H_2O$	7210
3.	Lithium chloride	LiCl	6120
4.	Rubidium chloride	RbCl	1420
5.	Strontium chloride	$SrCl_2.6H_2O$	3040
6.	Sodium bromide	NaBr	320
7.	Sodium molybdate	$Na_2MoO_4.2H_2O$	1230
8.	Cupric chloride	$CuCl_2.2H_2O$	335
9.	Zinc chloride	$ZnCl_2$	260
10.	Cobalt chloride	$CoCl_2.6H_2O$	200
11.	Potassium iodide	KI	65
12.	Sodium selinite	Na_2SeO_3	43.8
13.	Ammonium vanadate	NH_4VO_3	11.5
14.	EDTA*	$Na_2EDTA.2H_2O$	5000
15.	Ferrous sulphate*	$FeSO_4.7H_2O$	1991
Macro nutrients			
16.	Calcium chloride	$CaCl_2.H_2O$	293800
17.	Magnesium sulphate	$MgSO_4.7H_2O$	246600
18.	Potassium chloride	KCl	58000
19.	Sodium hydrogen carbonate	$NaHCO_3$	64800
20.	Sodium silicate	$Na_2SiO_3.9H_2O$	50000
21.	Sodium nitrate	$NaNO_3$	2740
22.	Potassium phosphate monobasic	KH_2PO_4	1430
23.	Potassium phosphate dibasic	K_2HPO_4	1840

Cont…

S. No.	Chemical name	Formula	mg/L
Vitamin stock solutions			
24.	Thiamine hydrochloride	–	750
25.	Cyanocobalamine (B12)	–	10
26.	Biotin	–	7.5

*Both EDTA and Ferrous sulphate solution were prepared separately, Poured together and autoclaved.

OECD TG 201 medium[14,15]

This helps in the growth of green alga as it provides the required nutrients and useful salts, which helps in their growth and multiplication. The composition was given in Table 2.

Table 2: Preparation of OECD TG 201 Medium (Green alga)

S. No.	Composition	mg/L
1.	$NaHCO_3$ (Sodium hydrogen carbonate)	50.0
2.	NH_4Cl (Ammonium chloride)	15.0
3.	$MgCl_2.6H_2O$ (Magnesium chloride)	12.0
4.	$CaCl_2.2H_2O$ (Calcium chloride)	18.0
5.	$MgSO_4.7H_2O$ (Magnesium sulphate)	15.0
6.	KH_2PO_4 (Potassium dihydrogen Phosphate)	1.60
7.	$FeCl_3.6H_2O$ (Ferric chloride)	0.064
8.	$Na_2EDTA.2H_2O$ (E.D.T.A. Disodium salt)	0.100
9.	H_3BO_3 (Boric acid)	0.185
10.	$MnCl_2.4H_2O$ (Manganese (II) chloride)	0.415
11.	$ZnCl_2$ (Zinc chloride)	0.0030
12.	$CoCl_2.6H_2O$ (Cobaltous chloride)	0.0015
13.	$Na_2MoO_4.2H_2O$ (Sodium molybdate)	0.0070
14.	$CuCl_2.2H_2O$ (Copper (II) chloride)	0.00001

Extraction procedure

The 100 mL sample was shaken vigorously and transferred to a separating funnel. To this, 50 mL of n-hexane was added and shaken for 100 times for phase separation. The separated organic layer was collected into a beaker and then again 50 mL of n-Hexane was added to the aqueous layer and shaken for 100 times and the separated organic layer was collected into the same beaker. The organic layer was filtered through sodium sulfate to remove excess moisture. The filtrate was injected into GC-MS.

Method validation

Method validation[8-11] ensures analysis credibility. In this study, the parameters accuracy, precision, linearity and limits of detection (LOD) and quantification (LOQ) were considered. The accuracy of the method was determined by recovery tests, using samples spiked at concentration levels of 0.2 and 0.5 mg/L. Linearity was determined by different known concentrations (0.5, 1.0, 5.0, 10.0, 50.0 and 100.0 mg/L) were prepared by diluting the stock solution. The limit of detection (LOD, mg/L) was determined as the lowest concentration giving a response of 3 times the baseline noise defined from the analysis of control (untreated) sample. The limit of quantification (LOQ, mg/L) was determined as the lowest concentration of a given bifenthrin giving a response of 10 times the baseline noise.

RESULTS AND DISCUSSION

Specificity

Specificity of the method was checked by injecting n- Hexane, standard, and extracts of media control. From the specificity of the method, it was concluded that there was no significant intereference observed, to interfere with the analysis of bifenthrin residues shown in Fig. 2 and 3. Furthermore, the retention time of bifenthrin was about 22.0 min.

Linearity

Different known concentrations of bifenthrin standard (0.5, 1.0, 5.0, 10.0, 50.0 and 100.0 mg/L) were prepared in n-Hexane by diluting the stock solution. Each solution was prepared in triplicate. Injected the standard solutions and measured the peak area. A calibration curve has been plotted of concentration of the standards injected versus area observed and the linearity of method was evaluated by analyzing six solutions. The peak areas obtained from different concentrations of bifenthrin were used to calculate linear regression equation. This was $Y = 2324.34.15\ X + 76.60$, with correlation coefficient of 0.9999. A calibration curve showed in Fig. 1.

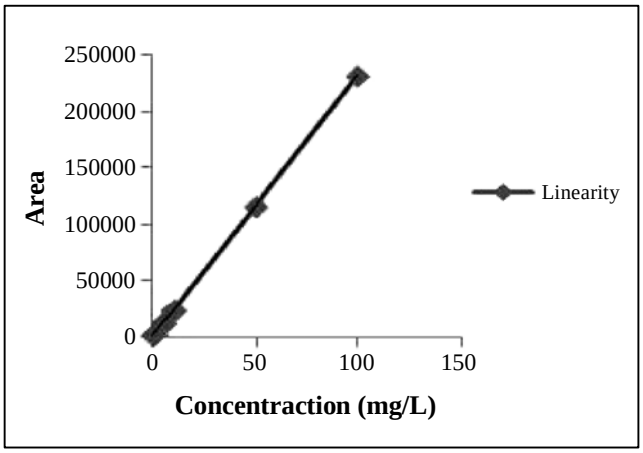

Fig. 1: Representative Calibration curve of Bifenthrin

Accuracy and precision

Recovery studies were carried out at 0.5 and 5.0 mg/L fortification levels for bifenthrin in different tox medium. The recovery data and relative standard deviation values obtained by this method are summarized in Table 3.

Table 3: Recoveries of bifenthrin sample in different mediums

Medium	Fortification level (mg/L)	Bifenthrin	
		*Mean Recovery (%)	% RSD
Blended water	0.5	88.23	1.95
	5.0	93.56	1.23
OECD TG 201	0.5	89.23	1.79
	5.0	95.46	1.12
M4	0.5	89.57	1.85
	5.0	95.74	1.07

*Average of five replications

These numbers were calculated from four (6) replicate analyses of given sample made by a single analyst on one day. The repeatability of method satisfactory (RSDs < 2%).

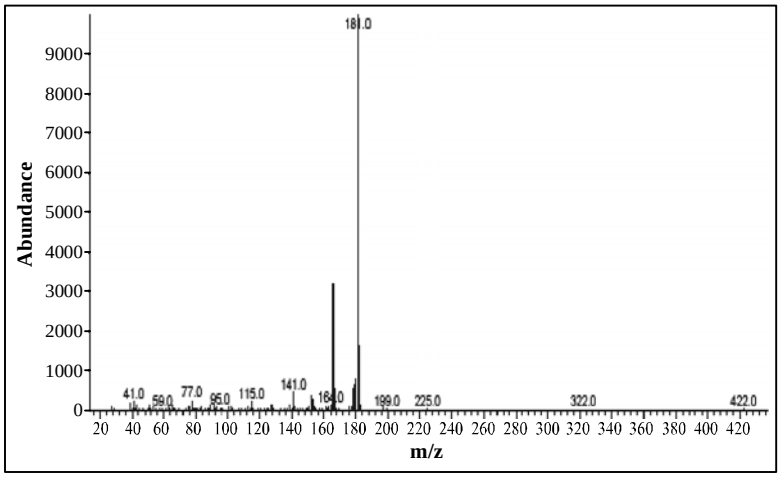

Fig. 2: Representative GC-MS scanned spectrum of bifenthrin tested from fortified medium

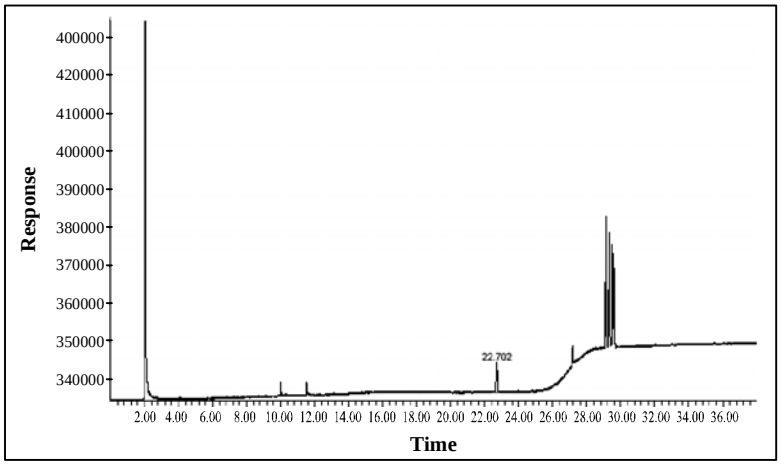

Fig. 3: Representative GC-MS Chromatogram at fortification level of 0.5 mg/L

Detection and quantification limits

The limit of quantification was determined to be 0.5 mg/L. The quantitation limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (88-96%, RSD < 2%) were achieved. This quantitation limit also reflects the fortification level at which an analyte peak is consistently generated at approximately 10 times the baseline noise in the chromatogram. The limit of detection was determined to be 0.2 mg/L at a level of approximately three times the back ground of control injection around the retention time of the peak of interest.

CONCLUSION

Appropriate analytical methodology for the determination of bifenthrin residues in aquatic tox medium (meant for fish, Daphnia and Algae) has been established and validated. Satisfactory validation parameters such as linearity, recovery, precision and very low limits were obtained and according to the SANCO[16] guidelines. The proposed analytical procedure could satisfactorily be useful for regular monitoring of bifenthrin residues in different tox medium samples (meant for fish, Daphnia and Algae).

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