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Research Article

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Determination of pyrethroid pesticide residues in rice by gas chromatography tandem mass spectrometry

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ABSTRACT

A simple and sensitive method for the determination of pyrethroid pesticide residues namely transfluthrin, allethrin, bifenthrin, lambda cyhalothrin, permethrin, cyfluthrin, cypermethrin, ethofenprox, fenvalerate, tauflivalinate & deltamethrin in rice grains by gas chromatography –tandem mass spectrometry (GC-MS/MS) was developed. The pyrethroid residues were extracted from homogenized rice samples initially with water followed by acetonitrile and cleaned up by using dispersive solid phase extraction which is based on QuEChERS (quick, easy, cheap, rugged and safe) method, finally the pyrethroid pesticide residues were separated by DB-5MS gas chromatography capillary column and quantified through tandem mass spectrometry (GC-MS/MS) with electron ionization source in Multiple reaction monitor mode (MRM) for quantification and confirmation. Recoveries were checked at two fortification levels 0.01 and 0.05mg/kg (n=6). The results showed that the mean recoveries for the fortified samples were ranged between 87-117% and %RSD in the range between 5.9 -19.8 for all compounds. Thus the developed analytical method was successfully used to analyze pyrethroid pesticide residues in routine rice samples .

Key words: Rice, Pyrethroids, dispersive solid phase extraction, GC-MS/MS

INTRODUCTION

Pyrethroids are organic synthetic insecticides that are widely used for the protection of crops and food storage against insects and acarids [1]. Pyrethroids are derived from natural pyrethrin, and their use has increased rapidly since the development of the first photostable pyrethroid in 1973 [2]. They have gained worldwide attention because of their effectiveness at low doses, short-term environmental persistence, and relatively low mammalian toxicity. Because pyrethroids are a major group of insecticides widely used in the world, the determination of pyrethroid residues in crops, foods, and environmental matrixes is necessary to monitor and regulate their usage.

For that reason, Food safety Standards Authority of India (FSSAI) under Food Safety and Standards (contaminants, toxins and residues) Regulations, 2011 [3] and European union (EU) under EU Regulation (Ec) No.396/2005 have established maximum residue levels (MRLs) in products of plant origin such as rice [5]. Rice is the most widely consumed staple food for a large part of the world's population. It is one of the important human diets as carbohydrate source obtained from paddy (oryza sativa L). It is the grain with second highest worldwide production after maize (corn) according to data for the year 2010. Pesticides are chemical compounds which are widely used in rice cultivation through different spray schedules and also during storage and transport. This results contamination of paddy of course rice with pesticide residues.

Most of the analytical procedures used in the determination of pyrethroids are based on the use of chromatographic

techniques, mainly gas chromatography (GC) with electron capture detection (ECD). GC/mass spectrometry (MS) with selective-ion monitoring (SIM) or full-scan monitoring has also been used for either confirmation or quantitation [6]. GC/tandem mass spectrometry (MS/MS) with electron impact (EI/MS/MS) and chemical ionization (CI/MS/MS) has also been used successfully to determine some pyrethroids in produce samples [7, 8]. In these methods, because of the complexity of the matrices involved, the extraction step is usually followed by a cleanup procedure before GC analysis. The most frequently used methods for the measurement of pyrethroid residues in vegetable and fruit products include conventional liquid–liquid partitioning or solid-matrix partitioning and/or gel permeation chromatography [9, 10] and/or adsorption chromatography on florisil and silica gel. Supercritical fluid extraction and solid-phase dispersion have also been proposed for these determinations. However, simple extraction methods like QuChERS (quick, easy, cheap, effective, rugged & safe) method [11] for the determination of pyrethroid pesticides in rice have not been reported hitherto. Hence, a simple modified QuChERS extraction method for determination of pyrethroid **(Fig-1)** residues in rice samples using GC-MS has been developed, validated and applied.

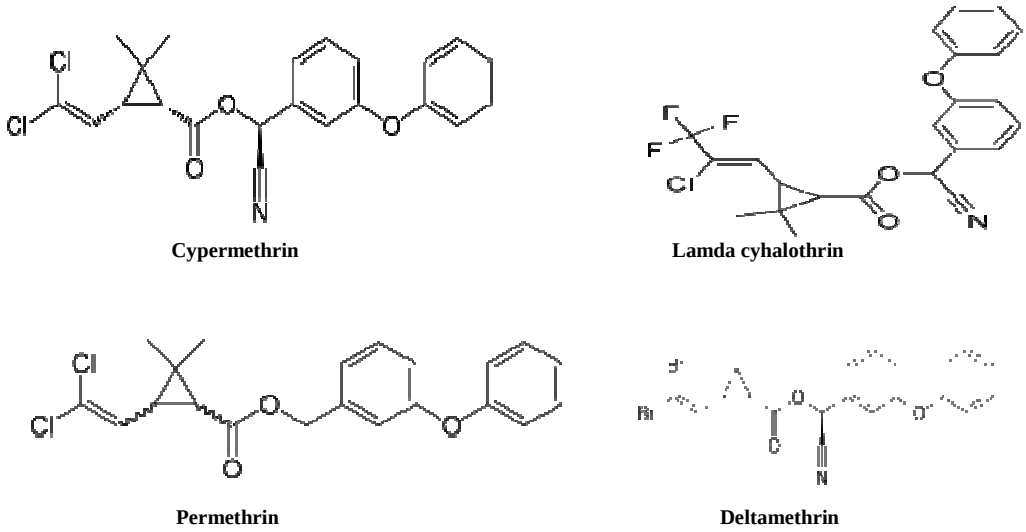

Fig-1 Chemical structures of some of the studied pyrethroids

EXPERIMENTAL SECTION

Instrumentation

A Gas Chromatograph (Agilent Technologies 6890 N) with an auto injector (7683 series), instrument equipped with Quatrro micro tandem (MS/MS) quadrupole mass spectrometer with electron impact (EI) ionization source was used. A capillary column DB-5MS (Film thickness: 0.25 µm, Inner diameter: 0.25 mm an Length: 30 m) was selected for separation of all pyrethroid residues under study.

Chemicals and materials

All the analytical standards of the studied pyrethroid pesticides were of high purity and certified were purchased from Dr.Ehrenstorfer GmbH, Augsburg Germany, with purity ≥99%. The solvents of Acetonitrile(ACN), acetone, methanol, n-hexane were of HPLC-grade from Merck Limited, India. HPLC-grade water was obtained from Merck Limited, India. Magnesium sulfate($MgSO_4$) and Sodium chloride (NaCl) obtained from Merck were used. The rice samples were collected from various supermarkets from Hyderabad city.

Preparation of standard solutions

Stock standard solutions (1000 µg/mL) were prepared individually by dissolving the appropriate quantity of pyrethroid in n-hexane. The pyrethoids, which were not soluble in n-hexane, were first dissolved in acetone and then made up to volume by n-hexane. Working standard solutions as per requirement were prepared from pyrethroid stock solution in hexane. The obtained solutions were stored in a refrigerator at 2-8 °C.

Sample preparation
Rice sample was ground in a mechanical hand grinder and homogenized. 2g of homogenized sample was taken in 50mL centrifuge tube and added 10 mL of HPLC grade water and vortexed 2 minutes and then added 10mL of acetonitrile and further vortexed for 2 minutes. 4 g of Magnesium sulfate and 2 g of sodium chloride were added and vortexed for 5 minutes in a multitube vortexter and the mixture was centrifuged at 4000 rpm for 10 minutes.

5 mL aliquot of ACN layer was transferred to a clean dry ria vial. Placed the ria vial in turbo evaporator and evaporated under nitrogen stream at 45±2 °C. Finally the dried sample residue was reconstituted with 1 mL of ACN into 15 mL tarson tube. Then 1 mL of Hexane and 5 mL of 20% Nacl solution were added, Vortexed for 1 minute and collected the hexane layer and injected into GC-MS/MS. A reagent blank was prepared and processed simultaneously along with samples.

Determination
The pyrethroid residues were determined in accordance with the following GC and MS specific parameters for all the compounds;

GC: Carreir gas :Helium: GC interface temperature: 275° C, Oven : 50° C(1min), 25° C/min to 150° C(0 min), 3° C/ min to 200° C, 8° C/min to 280° C (10min),post run : 320° C (5min); Injection mode:split-less, Injection volume:1µL; Total run time: 40 min.

MS: Quattro Micro Mass (waters),Polarity: EI +, Electron energy (ev): 70; MS temperature: 200° C (Source): Transfer line temperature: 275° C; Trap (µA): 200; Repellor (v):8.0, Solvent delay:4 min.

Tandem quadrupole use two stages of mass analysis-one to pres-selected an ion (the precursor ion) and second to anlayze fragments (product ions) induced by collision with an inert gas in the collision cell. Mulitple Reaction Monitoring (MRM) is the most sensitive, selective and specific techniques for quantification and involved the monitoring of compound specific precusors to product ion transitions, which is essentially advantageous when quantifying low levels of pyrethroid compounds In the presence of a high level of background from sample matrix or co-extractives . It was also highly sensitive, due to elimination of background chemical noise. MassLynnx 4.0 is the latest version software from waters was used for quantification of the data.

Table (1) MRM conditions for the pyrethroid pesticides on GCMS/MS

Name of the pyrethroid	Parent ion	Product ion	Retention time (minutes)	Dwell(secs)	Col.Energy
Transfluthrin	163	91	16.62	0.030	10.0
Allethrin	123	81	19.55	0.030	5.0
Bifenthrin	181	166	25.30	0.030	10.0
Lambda cyhalothrin-I	181	152	26.76	0.030	20.0
Lamda-Cyhalothrin-II	181	152	28.26	0.030	20.0
Permethrin I	183	153	30.02	0.030	12.0
Permethrin II	183	153	30.32	0.030	12.0
Cyfluthrin I	226	206	30.88	0.030	12.0
Cyfluthrin II	226	206	31.08	0.030	12.0
Cyfluthrin III	226	206	31.18	0.030	12.0
Cyfluthrin IV	226	206	31.28	0.030	12.0
Cypermethrin-I	163	127	31.50	0.030	8.0
Cypermethrin-II	163	127	31.72	0.030	8.0
Cypermethrin-II	163	127	31.81	0.030	8.0
Cypermethrin-IV	163	127	31.90	0.030	8.0
Ethofenprox	163	135	32.13	0.030	10.0
Fenvalerate I	167	125	33.33	0.030	8.0
Fenvalerate II	167	125	33.53	0.030	8.0
Taufluvalinate-I	250	55	33.65	0.030	20.0
Taufluvalinate-II	250	55	33.84	0.030	20.0
Deltamethrin	253	93	35.52	0.030	16.0

MRM conditions and retention times for the pyrethorid pesticide residues are given in **Table-1**.

Recovery studies were performed by preparing a representative sample of rice and fortified at 0.01 and 0.05µg/g level with standards of pyrethroids .The fortified samples were processed as per the method described under sample preparation method and residues of pyrethroid were determined by GC-MS/MS using conditions specified above. The chromatograms are acquired using the computer-based Mass lynx 4.1. The concentration of the residue level in sample was calculated from the equation as follows

Pyrethroid residue levels will be estimated in mg/Kg = (Y-C) x V/ M x W,

Where
Y= peak area of the compound
C= y-axis intercept value obtained from regression analysis
M= slope of calibration curve
W= weight of sample taken in g ; V= Final volume of sample made up in mL

RESULTS AND DISCUSSION

In the present study the dispersive SPE was used with some modification in the original QuEchERS method. Cleanup is necessary and recommended when using gas chromatography mass spectrometry as detection technique, because insufficient cleanup of sample causes rapid deterioration of gas chromatographic column, life time of the filament, detector response, thereby precluding reliable results.

The results (not reported) revealed that a mixture of water and acetonitrile as extraction solvent has showed better extraction efficiency over application of only acetonitrile in the determination of pyrethroid residues in rice. Limit of quantification (LOQ) for each pyrethroid was determined by fortification at 0.01 mg/kg concentration, where a signal to noise ratio of 10:1 was achieved with a % RSD of <15. The recoveries at LOQ (0.01 mg/kg) and 0.05 mg/kg for the studied pyrethroid compounds are given in Table-2 & Table 3 respectively. The total run time to separate all the pyrethoid residues was 40 min (approx) and DB -5MS capillary column exhibited good resolution (Fig-2) for all pyrethorid pesticides .

Table (2) Recoveries at LOQ (0.01 mg/kg) for the studied pyrethroid pesticides

Name of Compound	**n =1**	**n =2**	**n = 3**	**n =4**	**n =5**	**n =6**	Average recovery (n=6)	% RSD
Transfluthrin	109	112	114	101	103	118	110	5.9
Allethrin	99	117	109	96	104	108	105	7.1
Bifenthrin	95	117	101	98	118	119	108	10.3
Lambda cyhalothrin*	99	106	92	118	122	118	109	11.2
Permethrin*	86	110	99	74	115	114	100	16.6
Cyfluthrin *	104	110	80	113	123	117	108	14.0
Cypermethrin*	109	109	123	121	124	118	117	5.8
Etofenoprox	94	121	93	88	111	116	104	13.1
Fenvalerate*	94	111	94	82	106	118	101	13.2
Taufluvalinate*	116	78	77	120	105	93	98	19.0
Deltamethrin	96	114	95	119	111	117	109	9.7

Table (3) Recoveries at a level of 0.05 mg/kg for the studied pyrethroid pesticides

Name of Compound	n =1	n =2	n=3	n =4	n =5	n =6	Average recovery (n=6)	% RSD
Transfluthrin	109	115	114	114	125	122	117	5.1
Allethrin	104	114	116	118	106	134	115	9.4
Bifenthrin	106	106	119	117	104	126	113	7.8
Lambda cyhalothrin*	96	100	104	88	87	99	96	7.2
Permethrin *	82	103	101	90	96	119	99	11.0
Cyfluthrin *	80	85	105	115	88	103	102	14.2
Cypermethrin *	108	109	121	115	100	140	115	16.7
Etofenoprox	93	95	105	104	90	114	100	9.1
Fenvalerate *	93	100	117	107	93	120	105	11.0
Taufluvalinate*	77	78	100	113	67	91	87	19.4
Deltamethrin	86	97	118	104	90	130	104	16.3

*Note: * Average recovery and %RSD values for these compounds are calculated by including all other isomer values as applicable*

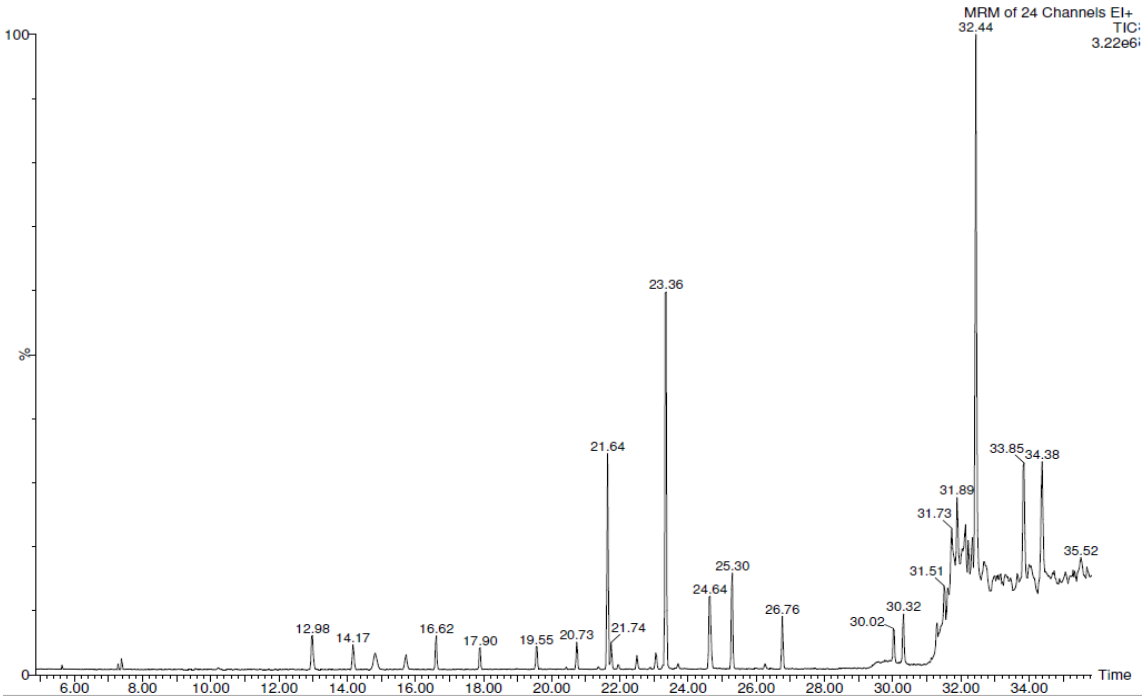

Fig (2) Total Ion Chromatogram (TIC) of pyrethroid pesticides on GCMS

The proposed method was successfully applied for the analysis of pyrethroid pesticides residue namely transfluthrin, allethrin, bifenthrin, lambda cyhalothrin, permethrin, cyfluthrin, cypermethrin, ethofenprox, fenvalerate, tauflivalinate & deltamethrin in rice samples (10 rice samples) and presented in Table 4. From the results it was observed that all of the pyrethroid residues were not detected i.e found to be below the limit of quantification levels (0.01 mg/kg) and the same was confirmed by monitoring MRM transitions through MS method .

Table-4 Results of pyrethroid residues (mg/kg) in rice samples collected from various supermarkets from Hyderabad city. (Mean±SD) (n=6)

Name of Compound	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	S-10
Transfluthrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Allethrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bifenthrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Lambda cyhalothrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Permethrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cyfluthrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cypermethrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Etofenoprox	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fenvalerate	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Taufluvalinate	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Deltamethrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND: not detected

CONCLUSION

A simple and sensitive method for the determination pyrethroid residues by using GC-MS/MS was developed, validated and applied for the analysis of rice samples . The samples were extracted with water and acetonitrile mixture and little matrix effect on MS detection was eliminated by following dispersive SPE clean up. The method was validated to ensure the feasibility of the method for its application in routine analysis. The LOQs achieved through this method were lower than the MRLs established by the FSSAI and EU legislations.

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