Research Article

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

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ABSTRACT

A simple and sensitive method for the determination of organochlorine pesticide residues namely HCH isomers (alpha, beta, gamma (lindane) & delta), DDT isomers (2,4-DDE, 4,4-DDE, 2,4-DDD, 4,4-DDD, 2,4-DDT & 4,4-DDT), endosulfan (alpha and beta), endosulfan sulfate, heptachlor, heptachlor epoxide aldrin, dieldrin, endrin, chlorthalonil, chlordane & dicofol in rice grains by gas chromatography –tandem mass spectrometry (GC-MS/MS) was developed. The organochlorine residues were extracted from homogenized 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 organo chlorine residues were separated by DB-5MS gas chromatography capillary column and quantified through tandem mass spectrometry(GC-MS/MS) with Electron ionization source. Multiple reaction monitor mode (MRM) method was used for quantification and confirmation. Recoveries were checked at two fortification levels of 0.1-0.05mg/kg (n=6). The results showed that the mean recoveries for the fortified samples were in the range of 83-110% and %RSD in the range of 5.7 -14.8 for all compounds. Thus the developed analytical method was successfully used to analyze organochlorine pesticide residues in routine rice samples.

Key words: Rice, Organochlorine, Pesticides, GC-MS/MS.
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INTRODUCTION

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. Food safety is ever increasing concern throughout the world [6]. During the last decades, the increasing demand of food safety has stimulated research with respect to the risks associated with consumption of food toxins. Therefore food rejection or acceptance across international borders is based on the compliance with national and international food regulations. For that reason, Food safety Standards Authority of India (FSSAI) under Food Safety and Standards (contaminants, toxins and residues) Regulations, 2011 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. Rice grains are treated with pesticides including organochlorine, organophosphates, synthetic pyrethroids, carbamates and insect growth regulators in storage premises as well as prior to shipment to other countries [9].

The application of organochlorine pesticides (DDT, HCH, Aldrin, Endrin) in agriculture has been banned in most of the developed countries due to their persistent nature or due to the toxicity of one of their metabolites e.g., endrin-epoxide. Endosulfan is the only organochlorine insecticide still used world wise. In India, although DDT has been banned for use in agriculture, it is still used for control of vectors in public health. In spite of this there are reports of the presence of these insecticide residues in various commodities such as milk, animal feed and vegetables. The

presence of residues of organochlorine insecticides in agricultural products indicates regular use of these compounds in India. In view of the above aspects it is essential to monitor the pesticide residues in food products to ensure the quality of foods. Hence analytical methods for the detection and quantification of pesticide residues should be sensitive enough and validated in order to determine at very low concentrations. Many researchers have develop methods for residue determination in rice using traditional gas chromatographic techniques (GC with ECD, FID) as well as sophisticated instruments (GC/MS) coupling with new extraction techniques. Sharp et al .1988 reviewed on extraction, clean up and chromatographic determination of different insecticides in grain and grain products. The complex matrix of agricultural products affects analysis precision and it is necessary to apply appropriate sample pre-treatment techniques in order to nullify matrix interference thereby increasing the sensitivity of compounds of interest. Some common pre-treatment techniques used for clean-up and extraction of pesticide residues from food matrices are liquid –liquid extraction (LLE) solid-phase extraction (SPE), matrix solid-phase dispersion (MSPD) and solid-phase micro extraction(SPME). In Matrix solid- phase dispersion, samples are blended with inorganic solids (e.g., florisil, silica, alumina) and then analytes are extracted with appropriate solvent. However, simple extraction methods like QuChERS (quick, easy, cheap, effective, rugged & safe) method [1] for the determination of organochlorine pesticides in rice have not been reported hitherto. Hence, a simple modified QuChERS extraction method for determination of organochlorine residues **(Fig-1)** in rice samples using GC-MS has been developed, validated and applied.
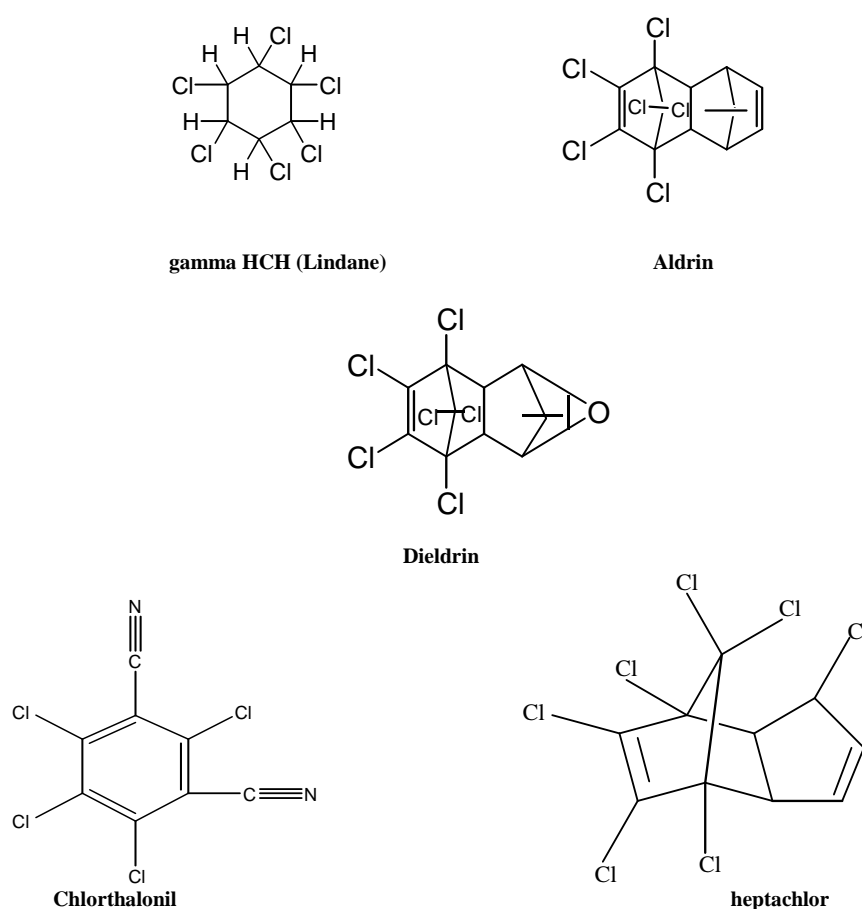

gamma HCH (Lindane)   Aldrin

Dieldrin

Chlorthalonil   heptachlor

Fig (1) Chemical structures of some of the studied pesticides

EXPERIMETNAL SECTION

Instrumentation
A Gas Chromatograph (Agilent Technologies 6890 N) with an auto injector (7683 series), instrument equipped with Quattro 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 organochlorine compounds under study.

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Chemicals and materials
All the analytical standards of the studied 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 pesticide in n-hexane. The pesticides, 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 pesticide 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 10mL of acetonitrile was added and further vortexed for 2 minutes. Then 4 g of Magnesium sulfate and 2 g of sodium chloride were added and vortexed for 5 minutes in a multitube vortexer. Then the mixture was centrifuged at 4000 rpm for 10 minutes. Then 5 mL of 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. 1 mL of Hexane and 5 mL of 20% NaCl solution were added, Vortexed for 1 minute and then collected the hexane layer and injected into GC-MS/MS. A reagent blank was prepared and processed simultaneously along with samples.

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

GC: Carrier 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 pre-selected an ion (the precursor ion) and second to analyze fragments (product ions) induced by collision with an inert gas in the collision cell. Multiple Reaction Monitoring (MRM) is the most sensitive, selective and specific techniques for quantification and involved the monitoring of compound specific precursors to product ion transitions, which is essentially advantageous when quantifying low levels of organochlorine 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. Mass Lynx 4.0 is the latest version software from waters was used for quantification of the data. MRM conditions for the organochlorine compounds 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 organochlorine pesticides .The fortified samples were processed as per the method described under sample preparation method and residues of organochlorine 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

Pesticide 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

Table (1) MRM conditions for the organochlorine compounds on GCMS/MS

Name of compounds	Parent Ion	Product Ion	Dwell (secs)	Col.Energy (ev)
Alpha-HCH	219	183	0.03	8
Beta-HCH	219	183	0.03	8
Lindane	219	183	0.03	8
Chlorothalonil	266	133	0.03	25
Delta HCH	219	183	0.03	8
Heptachlor	272	237	0.03	10
Aldrin	263	193	0.03	22
Heptachlor epoxide	353	263	0.03	22
Alpha-Endosulfan	241	206	0.03	10
Trans-Chlordane	373	266	0.03	15
Cis-Chlordane	373	266	0.03	15
2,4-DDE	246	176	0.03	20
4,4-DDE	246	176	0.03	15
Dieldrin	345	263	0.03	10
Endrin	263	193	0.03	22
Beta-Endosulfan	241	206	0.03	15
2,4-DDD	235	165	0.03	15
4,4-DDD	235	165	0.03	15
2,4-DDT	235	165	0.03	15
Endosulfan sulfate	272	237	0.03	10
4,4-DDT	235	165	0.03	15
Dicofol	251	139	0.03	10

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 organochlorine residues in rice. Limit of quantification (LOQ) for each organochlorine 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 organochlorine compounds are given in Table-2 & Table 3 respectively. The total run time to separate all the organochlorine pesticides was 40 min (approx) and DB -5MS capillary column exhibited good resolution (Fig-2) for all pesticides except beta HCH which overlapped somewhat on gamma (lindane).

Table (2) Recoveries at LOQ (0.01 mg/kg) for the studied organochlorine compounds

Name of Compound	n=I	n=II	n=III	n=IV	n=V	n=VI	Average	%RSD
Alpha-HCH	115	110	90	80	84	102	96.8	14.8
Beta-HCH	83	99	84	80	78	107	88.5	13.2
Lindane	103	108	103	84	91	110	99.8	10.2
Chlorothalonil	97	109	108	90	87	104	99.2	9.4
Delta HCH	87	94	87	92	110	102	95.3	9.5
Heptachlor	103	90	85	94	102	78	92.0	10.6
Aldrin	113	102	96	76	115	117	103.2	15.1
Heptachlor epoxide	93	84	104	82	97	83	90.5	9.9
Alpha-Endosulfan	102	94	78	95	110	112	98.5	12.7
Trans-Chlordane	89	113	93	112	86	117	101.7	13.6
Cis-Chlordane	118	112	95	116	109	109	109.8	7.4
2,4-DDE	118	124	98	96	118	124	113.0	11.2
4,4-DDE	107	123	106	94	120	135	114.2	12.8
Dieldrin	80	110	113	91	119	116	104.8	14.9
Endrin	116	119	107	105	94	119	110.0	9.0
Beta-Endosulfan	97	91	105	109	84	92	96.3	9.7
2,4-DDD	110	112	99	91	113	124	108.2	10.7
4,4-DDD	104	118	98	94	113	122	108.2	10.4
2,4-DDT	103	119	97	92	113	130	109.0	13.1
Endosulfan sulfate	101	82	76	105	80	100	90.7	14.0
4,4-DDT	76	102	86	73	91	105	88.8	14.8
Dicofol-1	109	116	98	104	113	119	109.8	7.1

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

| Name of Compound	n= I	n= II	n= III	n= IV	n= V	n=VI	Average	%RSD
Alpha-HCH	92	93	109	105	96	115	101.7	9.3
Beta-HCH	96	94	109	118	100	118	105.8	10.1
Lindane	80	91	95	111	95	119	98.5	14.4
Chlorothalonil	89	87	92	84	80	102	89.0	8.5
Delta HCH	93	99	108	106	91	118	102.5	9.9
Heptachlor	87	113	93	115	117	103	104.7	11.9
Aldrin	100	101	108	115	89	115	104.7	9.6
Heptachlor epoxide	81	78	106	111	107	92	95.8	14.8
Alpha-Endosulfan	109	87	117	107	118	104	107.0	10.5
Trans-Chlordane	87	96	109	115	104	114	104.2	10.5
Cis-Chlordane	108	114	98	113	100	117	108.3	7.2
2,4-DDE	100	100	109	103	90	115	102.8	8.3
4,4-DDE	97	102	109	105	95	114	103.7	6.9
Dieldrin	111	118	93	118	117	107	110.7	8.8
Endrin	104	68	106	78	90	84	102.0	14.5
Beta-Endosulfan	102	104	108	115	119	115	110.5	6.2
2,4-DDD	101	100	114	101	96	117	104.8	8.1
4,4-DDD	90	97	101	97	85	108	96.3	8.4
2,4-DDT	90	96	101	96	85	107	95.8	8.1
Endosulfan sulfate	113	103	116	98	114	109	108.8	6.5
4,4-DDT	72	92	81	95	68	90	83.0	13.5
Dicofol	103	118	112	102	114	111	110.0	5.7
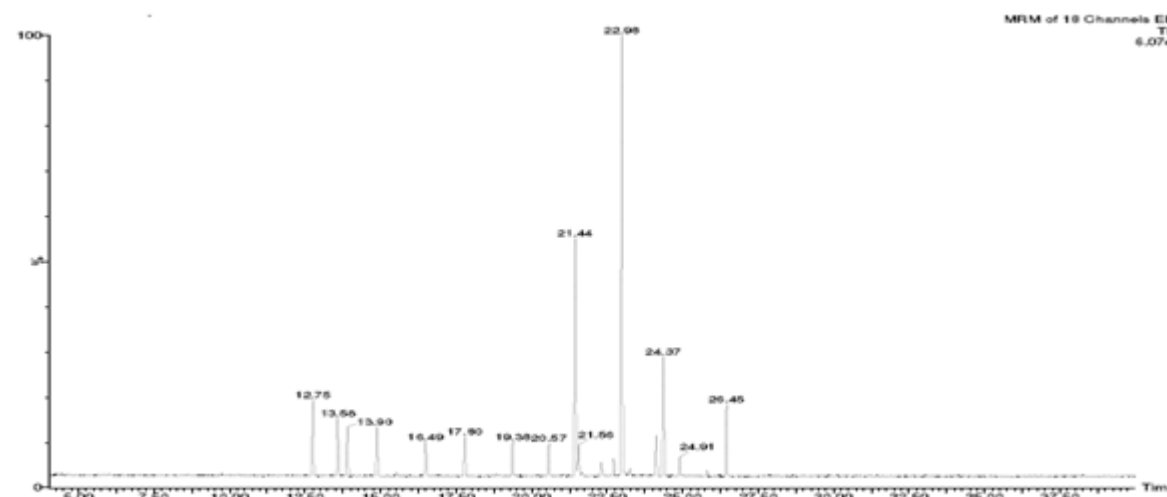
Fig (2) Total Ion Chromatogram (TIC) of organochlorine pesticide residues

The proposed method was successfully applied for the analysis of organochlorine pesticides residue namely HCH isomers (alpha, beta, gamma (lindane) & delta), DDT isomers (2,4-DDE, 4,4-DDE, 2,4-DDD, 4,4-DDD, 2,4-DDT & 4,4-DDT), endosulfan (alpha and beta), endosulfan sulfate, heptachlor, heptachlor epoxide aldrin, dieldrin, endrin, chlorothalonil, chlordane & dicofol in rice samples (10 rice samples) and presented in Table 4. From the results it was observed that only the residues of HCH alpha and dicofol were found in samples at a level of 0.01 mg/kg, which was below the permissible limit. The rest of the organochlorine pesticides residues were not detected in any of the samples analyzed. The identified endosulfan and dicofol were confirmed by the presence of both MRM transitions for each with accepted relative ion intensity ratios.

Table (4) Concentration of organochlorine 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
Alpha-HCH	ND	ND	ND	ND	ND	ND	0.011 ± 0.002	ND	ND	ND
Beta-HCH	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Lindane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Chlorothalonil	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Delta HCH	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Heptachlor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Aldrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Heptachlor epoxide	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Alpha-Endosulfan	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Trans-Chlordane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cis-Chlordane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DDE	ND	ND	ND	ND	ND	ND		ND	ND	ND
4,4-DDE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dieldrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Endrin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Beta-Endosulfan	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DDD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4,4-DDD	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
2,4-DDT	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Endosulfan sulfate	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
4,4-DDT	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dicofol	0.010 ± 0.003	ND	ND	ND	ND	ND	0.011 ± 0.003	ND	ND	ND

ND: not detected

CONCLUSION

A simple and sensitive method for the determination organochlorine 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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