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Determination of Pesticide Residues in Rice Grain by Solvent Extraction, Column Cleanup, and Gas Chromatography-Electron Capture Detection

Riaz Uddin · Sajid Iqbal · Muhammad Farhanullah Khan · Zahida Parveen · Mubarik Ahmed · Muhammad Abbas

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Abstract A simple, easy, cheap and efficient analytical method for determination of multiple pesticide residues including organochlorine, organophosphorus, synthetic pyrethroids and herbicides in rice grain by capillary gas chromatography is developed. The quantification of residues was done by capillary gas chromatography with a μ-ECD detector and a HP-5MS capillary column. Known amounts of a mixture of pesticides were added to grain prior to extraction, cleanup and GC-determination. Recoveries were checked at two fortification levels; 0.1 and 0.5 µg/g. Qualitative and quantitative analysis were carried out based on the retention time and peak area basis. The results show that the average recovery of the analytical method for the fortified rice samples was in the range of 74%-111% and %RSD in the range of 2.41–12.42. The analytical method was used to analyze commercial rice grain samples.

Keywords GC-ECD · Multiple pesticide residues · Rice grain

Generally rice grain are contaminated by the pesticides through two principal sources, pesticide residues resulting from field spraying and the residues accumulated as a result of pesticides treatment during storage (Jamil et al. 2005; Iqbal and Ali. 2006). Food safety is a major public concern throughout the world. During the last decades, the increasing demand of food safety has stimulated research regarding the risk associated with consumption of food toxins. Thus, food rejection or acceptance across international borders is based on the compliance with international food regulations. Rice grain are treated with pesticides including organophosphates, carbamates, synthetic pyrethroids and insect growth regulators in storage premises as well as prior to shipment to other countries (Khan et al. 2007a). For this reason, monitoring programs are implemented for determinations of residues of pesticides in food. Traditionally, gas chromatography has been the analytical technique of choice. Many research papers have been published on method development for residue determination in rice grain using traditional gas chromatographic techniques (GC with ECD, FID) as well as sophisticated instruments (GC/MS) with coupling of new extraction techniques. Haroldo and Ledjane (2004) developed a method using GC-ECD for malathion, parathion-methyl and endosulphon. Bottomley and Baker (1984) developed a multi-residue method for determination of organochlorine, pyrethroids and organophosphorus pesticides in grain using Gas Chromatography. Sharp et al. (1988) described a brief review paper on extraction; cleanup and chromatographic determination of different insecticides in grain and grain products. Zhang et al. (2006) developed a multi-residue method for determination of 109 pesticides with different properties in unpolished rice using gas chromatography/mass spectrometry. Dong et al. (2008) described a multi-residue method for the analysis of 203 pesticides in rice paddies by GC/MS. Guo-Fang et al. (2006) developed a multi-residue method for simultaneous determination of 405 pesticides residues in grain using GC/MS and LC–MS-MS. Aldana-madrid et al. (2008) reported insecticides

R. Uddin (✉) · S. Iqbal · Z. Parveen · M. Ahmed · M. Abbas
Grain Quality Testing Laboratory, Southern Zone Agricultural Research Centre, Pakistan Agricultural Research Council, Karachi University Campus, Karachi 75270, Pakistan
e-mail: riaz_1969@yahoo.com

M. F. Khan
Department of Zoology, University of Karachi, Karachi 75270, Pakistan

residues in stored grain. Ping et al. (2007) developed a multi-residue determination of the thermolabile *N*-methylcarbamate and organophosphorus insecticides in cereal products by GC/MS. Xiuli et al. (2008) developed a rapid analysis method for determination of 13 pesticides residues in rice by GC/MS.

In the method described here, samples of rice grain can be screened for residues of five organochlorine pesticides, two organophosphorus pesticides, five synthetic pyrethroids and three herbicides using gas chromatography with electron capture detection (ECD). The present study was restricted to those pesticides which are internationally used and usually detected. These pesticides are also used in Pakistan. Some of them such as BHC is banned worldwide but still used by the farmers in some countries. Therefore, the objective of this study was to develop easy, simple and inexpensive technique for the analysis of multi pesticide residues in rice grain. The second objective of this study was to develop a method which could be readily used in the laboratories that could not access current extraction techniques, automated equipment and gas chromatographs interfaced to mass spectrometers.

Materials and Methods

Acetone, n-hexane, methanol, dichloromethane, anhydrous sodium sulphate and activated graphitized charcoal were of analytical grade and were purchased from Merck (Germany). Acidic aluminum oxide pH 4.5 ± 0.5, Brockmann activity was purchased from Fluka (Switzerland). Pesticides standards were purchased from AccuStandard, Inc (USA). All standards were at least 99.9% pure. Aluminum oxide was activated at 500°C for 3 h while charcoal was activated at 300°C for 3 h and anhydrous sodium sulphate was dried at 500°C. Cotton wool washed with a mixture of acetone and hexane (1 + 1). Stock standard solution of the pesticides was prepared in n-hexane and those pesticides, which were not soluble in n-hexane, were first dissolved in acetone and then mark up to volume by n-hexane. Working standard solutions were prepared as per requirement from pesticide stock solution in hexane. These solutions were all stable for at least 1 month if stored in the dark at 4°C. Filter papers of Whatman # 542, chromatography glass column of 58 cm length and 2.4 cm id, centrifuge machine Jouan, Inc (USA) Model # CR412 and a rotary evaporator Model BUCHI V-512, with chiller were used.

A Gas Chromatograph (Agilent Technologies 6890 N, USA), instrument equipped with an auto injector (7683 series), Ni^{63} electron capture detector, and a 30 m × 0.25 mm capillary column coated with a 0.25 μm film HP-5MS, was used. Column temperature was maintained at 80°C for 1 min then programmed at 30°C min^{-1} to 180°C then at 3°C min^{-1} to 205°C hold for 4 min and finally at 20°C min^{-1} to 290°C, which was hold for 5 min. The injection port temperature was 280°C and detector temperature 320°C. Nitrogen was used both as a carrier gas at a flow rate of 1 mL min^{-1} and the make-up gas with total flow rate of 60 mL/min.

A control rice grain sample (free from pesticides) was ground on a mechanical hand grinder and fortified with known quantities of each studied pesticide by adding known volumes of mixed standard solutions of the pesticides. The fortified rice grain samples were left at room temperature for at least 24 h before extraction in order for the pesticides to be absorbed on to the grain and correspond more closely to commercially treated grain. These fortified samples along with a blank and a control sample (free from any pesticide) were then processed in triplicate through the following procedure and finally analyzed for percent recovery by gas chromatography.

Transferred 4 grams of ground rice grain into a 50 mL Teflon centrifuge tube, having a screw cap. Added to it 40 mL of acetone-methanol mixture (1:1). Caped the tube and thoroughly mixed the contents by hand shaking for minimum 2 min. Centrifuged the contents at 2,500 rpm for 3 min and decanted the supernatant solvent through the filter funnel into a 500 mL separating funnel. Added a further 35 mL of acetone-methanol mixture (1:1) to the centrifuge tube and homogenized it by hand shaking for two min. Centrifuge the contents of the tube for 3 min and decanted the solvent through the filter funnel into the same

Table 1 Limit of quantification and retention time of pesticides under studies

Peak	Name of pesticide	Limit of determination (μg/g)	Retention time (min)
a	Trifluralin	0.02	8.02
b	Alpha-BHC	0.002	8.63
c	Beta-BHC	0.003	9.42
d	Lindane	0.005	9.46
e	Delta-BHC	0.004	10.22
f	Malathion	0.05	12.57
g	Chlorpyrifos	0.05	13.02
h	Pendimethalin	0.05	14.39
i (1,2)	Fipronil	0.01	14.65, 15.09
J	Alpha-endosulfan	0.05	16.21
K	Oxadiazone	0.10	17.74
L	Bifenthrin	0.05	20.73
m	Cyfluthrin	0.05	23.02
n (1,2,3)	Cypermethrin	0.05	23.18, 23.29, 23.39
o	Fenvalerate	0.05	24.42, 24.71
p	Deltamethrin	0.05	25.51

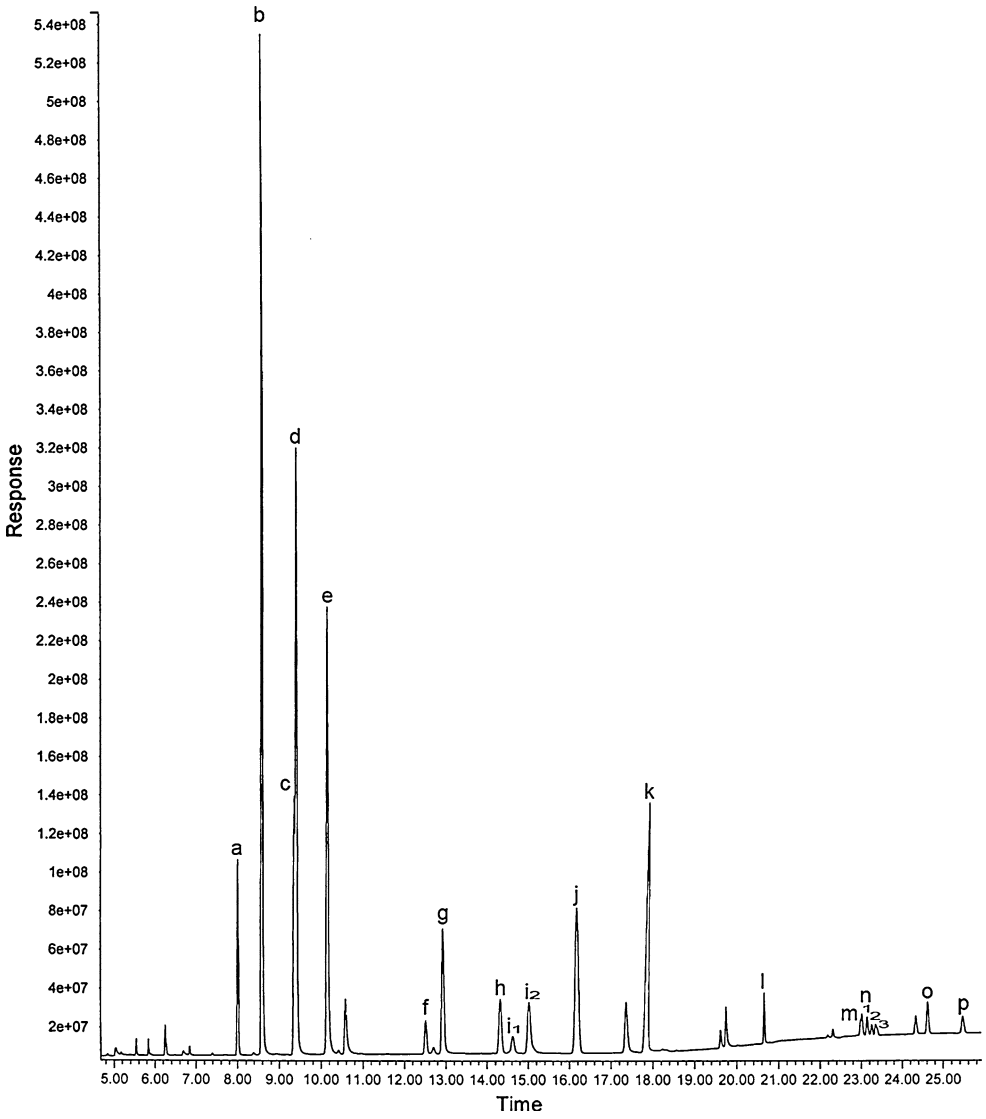

Fig. 1 GC-ECD, Separation of 13 pesticides and 3 herbicides (1 ng each) on a 30 m HP-5MS capillary column. *a* Trifluralin, *b* α-BHC, *c* β-BHC, *d* Lindane, *e* δ-BHC, *f* Malathion, *g* Chlorpyrifos, *h* Pendimethalin, *i* (1,2) isomers of Fipronil, *j* α-Endosulphon, *k* Oxadiazone, *l* Bifenthrin, *m* Cyfluthrin, *n* (1,2,3) isomers of Cypermethrin, *o* Fenvalerate, *p* Deltamethrin

separating funnel. Added 200 mL of sodium sulphate solution (2.5 g/100 mL) to the separating funnel followed by 25 mL of dichloromethane and shake vigorously for 2 min. Allow the phases to separate and passed the lower dichloromethane phase through 25 g of anhydrous sodium sulphate in a 58 cm × 2.4 cm id glass column. Collect the moisture free extract into a 300 mL conical flask. Repeated the partitioning of aqueous extract with two further 25 mL of dichloromethane and finally wash the sodium sulphate column with 10 mL of dichloromethane. Concentrated the combined extract and washing to approximately 1 mL to 2 mL on a rotary evaporator maximum at 40°C.

Transfer the concentrated dichloromethane extract onto a 16 g mixture of aluminum oxide and charcoal (15:1) glass column that has been slurry packed with dichloromethane with pasture pipette. Allow the extract to pass through the column until it reached the top of the column material. Rinsed rotary evaporator flask twice with two 5 mL portions of dichloromethane and transfer the washing to the column. The column was eluted with 160 mL of dichloromethane at the rate of 1 mL min^{-1}. The combined washings and elute was concentrated to dryness on a rotary evaporator and dissolved the residue in 2 mL of acetone. Injected 1 µL through auto-injector and compared peak areas with those, obtained from similar injection of the standards.

Results and Discussion

In the present study the cleanup step was used with some modifications in the method of Bottomley and Baker (1984) and Khan et al. (2007a). Cleanup step is necessary and recommended when using ECD as the detection

Table 2 Percent recoveries and percent RSD at 0.1 μg/g fortification level

Peak	Name of pesticides	Percent recovery			Average	% RSD
		I	II	III		
a	Trifluralin	74	95	87	85.33	12.42
b	Alpha-BHC	91	105	87	94.33	10.02
c	Beta-BHC	102	89	91	94.00	7.45
d	Lindane	97	89	85	90.33	6.76
e	Delta-BHC	92	99	102	97.67	5.25
f	Malathion	105	97	89	97.00	8.25
g	Chlorpyrifos	91	104	98	97.67	6.66
h	Pendimethalin	75	83	86	81.33	6.99
i (1,2)	Fipronil	88	84	80	84.00	4.76
j	Alpha-endosulfan	82	68	72	74.00	9.74
k	Oxadiazone	108	96	90	98.00	9.35
l	Bifenthrin	100	96	85	93.67	8.29
m	Cyfluthrin	106	99	109	104.67	4.90
n (1,2,3)	Cypermethrin	94	95	109	99.33	8.44
o	Fenvalerate	96	102	84	94.00	9.75
p	Deltamethrin	72	87	84	81.00	9.80

Table 3 Percent recoveries and percent RSD at 0.5 μg/g fortification level

S#	Name of pesticides	Percent recovery			Average	% RSD
		I	II	III		
a	Trifluralin	96	81	90	89	8.48
b	Alpha-BHC	110	105	116	110.33	4.99
c	Beta-BHC	100	107	95	100.67	5.99
d	Lindane	110	97	104	103.67	6.28
e	Delta-BHC	96	111	106	104.33	7.32
f	Malathion	112	110	100	107.33	5.99
g	Chlorpyrifos	105	113	101	106.33	5.75
h	Pendimethalin	94	103	99	98.67	4.57
i (1,2)	Fipronil	106	100	103	103	2.91
j	Alpha-endosulfan	112	99	116	109	8.15
k	Oxadiazone	97	110	93	100	8.89
l	Bifenthrin	114	98	109	107	7.65
m	Cyfluthrin	109	113	108	110	2.41
n (1,2,3)	Cypermethrin	99	108	114	107	7.06
o	Fenvalerate	97	101	92	96.67	4.66
p	Deltamethrin	100	115	119	111.33	9

system, because insufficient cleanup of sample causes rapid deterioration of gas chromatographic system especially electron capture detector, thereby precludes reliable results.

For the determination of residues, electron capture detector was used with ramped temperature program to get good separation for the studied pesticides. A mixture of acetone and methanol (1:1) as extraction solvent was proved to be best. Rice grain contain oil and other oil soluble materials. Acidic alumina was the best in its capacity to retain oils while charcoal retained coloring co-extractives.

Limit of quantification (LOQ) for each pesticide was determined by fortification. The lower fortification concentration giving a signal to noise ratio $\geq$10 was fixed as LOQ of the method. The LOQ of the studied pesticide are given in Table 1. A typical Chromatogram of the studied

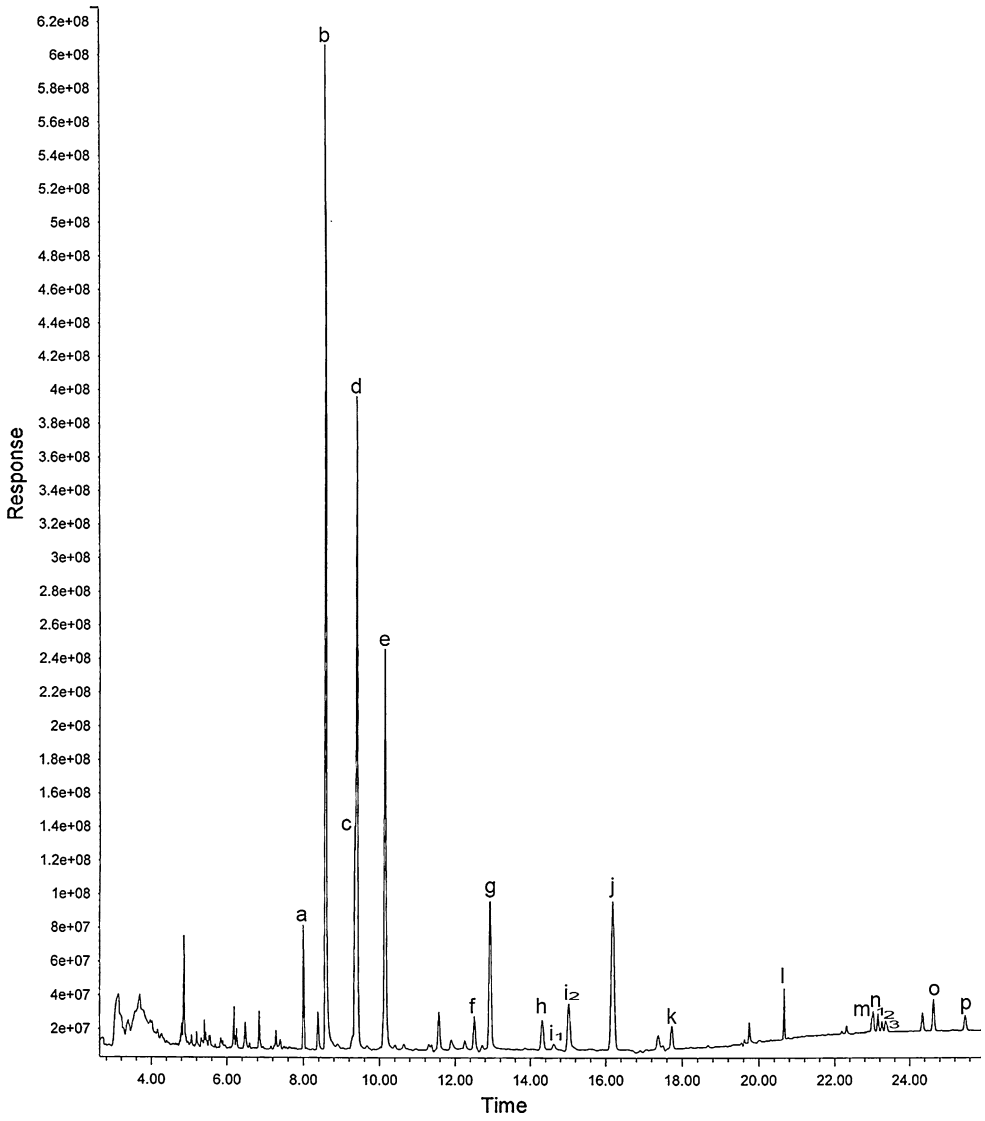

Fig. 2 GC-ECD, Separation of 13 pesticides and 3 herbicides in rice grain fortified at 0.5 µg/g. *a* Trifluralin, *b* α-BHC, *c* β-BHC, *d* Lindane, *e* δ-BHC, *f* Malathion, *g* Chlorpyrifos, *h* Pendimethalin, *i* (1,2) isomers of Fipronil, *j* α-Endosulfan, *k* Oxadiazone, *l* Bifenthrin, *m* Cyfluthrin, *n* (1,2,3) isomers of Cypermethrin, *o* Fenvalerate, *p* Deltamethrin

pesticides and herbicides standards (having concentration of 1 ηg each) is shown in Fig. 1. Recoveries of the 16 different pesticides including organochlorine, organophosporus, synthetic pyrethroid pesticides and herbicides from rice grain were checked at two fortification levels of 0.1 and 0.5 µg/g by adding known volumes of mixed standard solutions to 4 g portion of ground grain shown not to contain residues of the pesticides. The samples were then treated as mentioned under "Materials and Methods". The results obtained are shown in Tables 2 and 3.

Typical chromatograms obtained from rice grain are shown in Figs. 2 and 3. Results showed that the average range of recovery for 0.1 and 0.5 µg/g fortification level of the method fell within 74%–104.67% with %RSD in the range of 4.90–12.42 and within 89%–110% with %RSD in the range of 2.41–9.00 respectively. Total run time to separate all the sixteen pesticides was only 26 min (approx) to screen all the sixteen pesticides and HP-5MS capillary column gives good resolution for all pesticides except beta BHC which overlapped somewhat on gamma (Lindane) BHC. Minimum determination limits and retention times are shown in Table 1. Pesticide residue analytical method should be so sensitive that at least the lower permissible levels can be quantitatively determined (Khan et al. 2007b). The present results are in full agreement with this recommendation. Based on these results the method has been proven to be efficient and thus suitable for routine monitoring of pesticide residues in rice. The proposed method was applied to the analysis of 100 rice grain samples, received from exporter/Trading Corporation of Pakistan. Only three samples were found contaminated with α-endosulfan (0.1 µg/g), Deltamethrin (0.067 µg/g) and Fipronil (0.01 µg/g) respectively. All these results were much below the permissible limit.

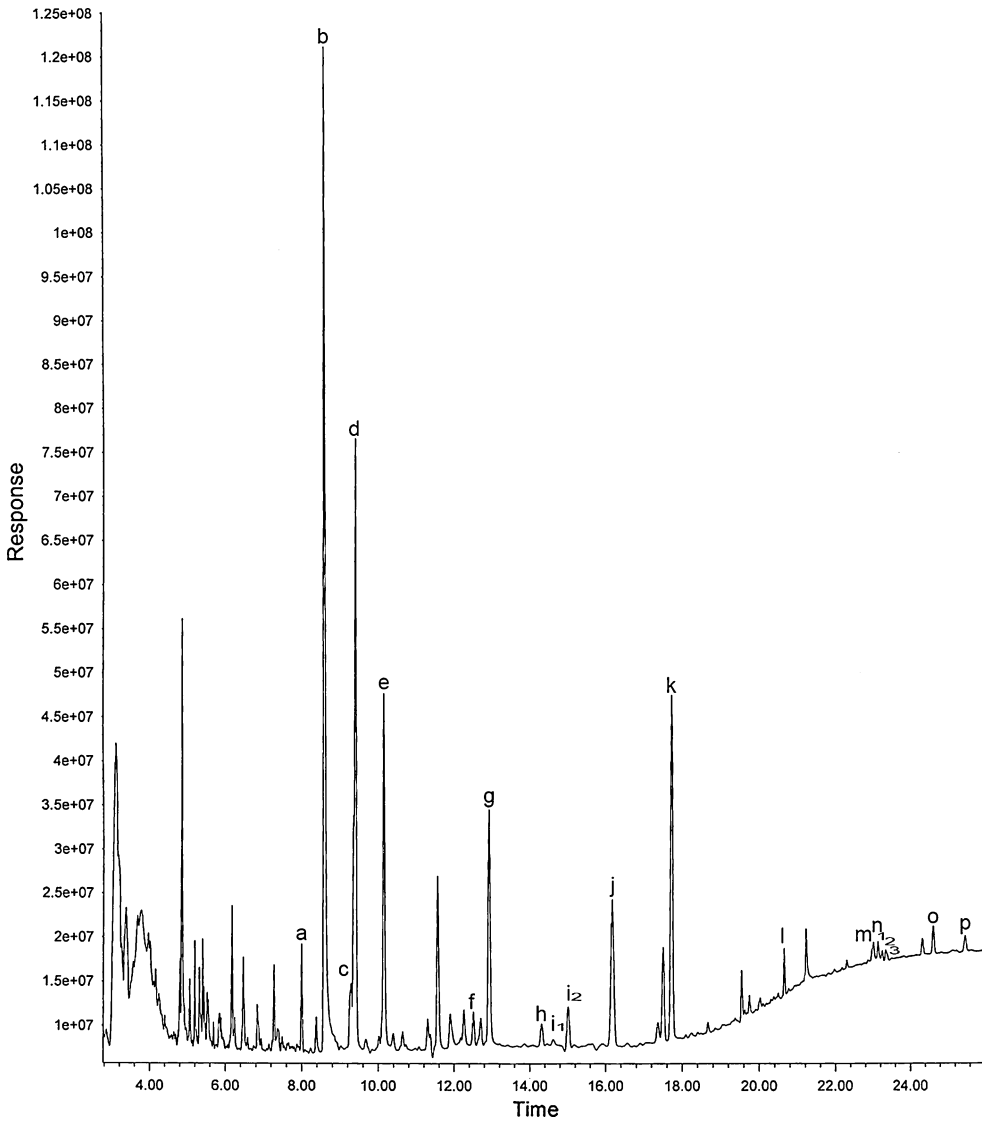

Fig. 3 GC-ECD, Separation of 13 pesticides and 3 herbicides in rice grain fortified at 0.1 µg/g. *a* Trifluralin, *b* α-BHC, *c* β-BHC, *d* Lindane, *e* δ-BHC, *f* Malathion, *g* Chlorpyrifos, *h* Pendimethalin, *i* (1,2) isomers of Fipronil, *j* α-Endosulfan, *k* Oxadiazone, *l* Bifenthrin, *m* Cyfluthrin, *n* (1,2,3) isomers of Cypermethrin, *o* Fenvalerate, *p* Deltamethrin

This method has three advantages over our previous method (Khan et al. 2007a).

- The additional mini-column cleanup step after glass column cleanup step is not required.
- All the studied pesticides were analyzed in the same injection run with best separation as compare with the previous method in which two separate temperature program for organophosphorus pesticides and synthetic pyrethroids pesticides were used.
- In this method sixteen pesticides were studied as compared to the previous method (Khan et al. 2007a) in which only nine pesticides were described.

The present study has resulted in development of a sample, easy, efficient and inexpensive method for determination of multiple pesticide residues in rice grain for those laboratories that have no access to current extraction techniques and sophisticated instruments such as GC/MS. This method has been successfully applied for routine analysis of pesticide residues in rice grain samples. There are so many new highly sophisticated techniques and methods for extraction as well as automated sample cleanup systems that have been worldwide introduced and used in developed countries but those are very expensive and difficult to approach in developing countries. This method can be readily used in those laboratories that could not access current extraction techniques, automated equipment and gas chromatographs interfaced to mass spectrometers.

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