Pragney Deme[1]
Tirupathi Azmeera[2]
Prabhavathi Devi L.A. Bethala[2]
Padmaja R. Jonnalagadda[3]
Prasad B.N. Rachapudi[2]
Vijaya Sarathi V.R. Upadhyayula[1]

[1]National Centre for Mass Spectrometry, Indian Institute of Chemical Technology, Tarnaka, Hyderabad, Andhra Pradesh, India
[2]Center for Lipid Research, Indian Institute of Chemical Technology, Tarnaka, Hyderabad, Andhra Pradesh, India
[3]Food and Drug Toxicology Research Center, National Institute of Nutrition, Tarnaka, Hyderabad, Andhra Pradesh, India

Received May 3, 2013
Revised September 25, 2013
Accepted October 4, 2013

Research Article

Effect of background derivatization on the signal enhancement of pesticide residues extracted from edible oils

The effect of background derivatization on the signal enhancement of pesticide residues extracted from edible oil samples was studied by GC with negative chemical ionization MS. The analytes were extracted by a solvent extraction process, and the extract was subjected to rapid low-temperature fat precipitation. The residual fatty acids were silylated by derivatization with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide. The chromatograms obtained from the derivatized samples showed higher signal intensity and lower detection levels when compared to the direct analysis without derivatization. The sensitivity levels of the method are either better or comparable to that of previously reported methodologies. The LODs of the analyzed organochlorine, organophosphorus, and synthetic pyrethroid residues in sunflower, rice bran, and ground oil samples were in the range of 0.02–0.5 ng/g, and the LOQs were in the range of 0.1–2 ng/g. The intraday and interday accuracies were in the range of 81–116% with RSDs less than 14%. The recoveries obtained were in the range of 53–89% with the RSD values less than 13% for all the studied pesticide residues.

Keywords: Derivatization / Edible oils / Gas chromatography / Negative chemical ionization / Pesticide residues
DOI 10.1002/jssc.201300454

1 Introduction

The quantitative analysis of pesticide residues from fatty matrices is a challenging task since extraction of hydrophobic molecules from a hydrophobic matrix is difficult. The major hurdle in the sample preparation of such matrices is the coextraction of a significant amount of fatty acids and other polar glycerides. The extracted matrix is often a great problem in the analysis as it interferes in chromatographic elution. Hence, several efficient sample preparation steps such as solvent extraction (SE) and low-temperature fat precipitation (LTFP) followed by SPE, dispersive solid-phase extraction (DSPE), QuEChERS (quick, easy, cheap, effective, rugged, and safe) and matrix solid-phase dispersion (MSPD) have been developed. The major developments in the sample clean-up techniques have been reviewed recently [1–3]. Among the described extraction methods, SE followed by LTFP is still the most preferred method for its simplicity and low cost [4–6]. The application of QuEChERS procedures [7–12] after SE followed by LTFP showed highly promising results when compared to direct analysis. Although methods like SPE, MSPD, headspace solid-phase microextraction, and DSPE were reported, they have some important limitations. MSPD and DSPE methods are not applicable for large volumes of the matrix as it proportionately needs a large amount of extraction phase and saturation of the extraction phase with fat. Headspace solid-phase microextraction methods are useful for only volatile pesticides and the recovery of analytes is very low. The fibers are easily saturated by oil vapors and their stability is decreased over time.

Another alternate method for reducing the interference of the background in GC is derivatization. This is generally used for increasing the volatility and sensitivity of polar molecules. Derivatization is a proven methodology for trace-level quantification of polar analytes present in very complex matrices. Various derivatization methods with variety of derivatizing agents are reported in some recent articles [13–23]. Apart from analyte derivatization, it can also be used to derivatize the background matrix and reduce the matrix interaction with the target nonpolar analytes. Hence, the sensitivity of detection for the analytes can be increased.

In the present work, we have explored the effect of background derivatization for the specific GC–MS/MS

Correspondence: U.V.R. Vijaya Sarathi, Senior Scientist, National Centre for Mass Spectrometry, Indian Institute of Chemical Technology, Uppal Road, Tarnaka, Hyderabad 500007, Andhra Pradesh, India
E-mail: saradhi@iict.res.in
Fax: +91-40-27193156

Abbreviations: **BSTFA**, *N*,*O*-bis(trimethylsilyl)trifluoroacetamide; **DSPE**, dispersive solid-phase extraction; **HCH**, hexachlorocyclohexane; **HEE**, heptachlor exo epoxide; **IS**, internal standard; **LTFP**, low-temperature fat precipitation; **MRM**, multiple-reaction monitoring; **MSPD**, matrix solid-phase dispersion; **NCI**, negative chemical ionization; **OC**, organochlorine; **OP**, organophosphorus; ***p,p′*-DDD**, *p,p′*-dichlorodiphenyldichloroethane; ***p,p′*-DDT**, *p,p′*-dichlorodiphenyltrichloroethane; **PCB**, pentachlorobenzene; **RLTFP**, rapid low-temperature fat precipitation; **SE**, solvent extraction; **SP**, synthetic pyrethroid

quantification of pesticide multiresidues in negative chemical ionization (NCI) from edible oil samples. The developed method was applied for the analysis of some crude and refined edible oils and the obtained results were compared to that of previously reported sensitive methods.

2 Materials and methods

2.1 Chemicals and apparatus

Reference standards of organochlorine (OC) pesticides: α-, β-, γ-, and δ-hexachlorocyclohexane (HCH) isomers, α- and β-endosulfan isomers, α- and γ-chlordane isomers, heptachlor, heptachlor exo epoxide (HEE), *p,p′*-dichlorodiphenyltrichloroethane (*p,p′*-DDT), *p,p′*-dichlorodiphenyldichloroethane (*p,p′*-DDD), *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE), aldrin, endrin, dieldrin, endrin aldehyde, endrin ketone; organophosphorus (OP) pesticides: phorate, diazinon, fenitrothion, malathion, chlorpyrifos, parathion ethyl, profenofos, and imidan; synthetic pyrethroids (SP): allethrin, α-cypermethrin, β-cyfluthrin, and deltamethrin isomers; internal standard (IS) pentachlorobenzene (PCB), *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), were obtained from Sigma–Aldrich. Optima LC–MS-grade acetonitrile, methanol, and ethyl acetate were purchased from Thermo Fisher Scientific (Fair Lawn, NJ, USA). Analytical reagent grade acetone was obtained from E-Merck (Mumbai, Maharashtra, India). The CI reagent gases methane, isobutane, and ammonia were purchased from Bharuka gases (Bengaluru, Karnataka, India).

2.2 Preparation of stock solutions and working standards

Individual standard stock solutions were prepared at 1 mg/mL by dissolving 10 mg of each pesticide in 10 mL of acetonitrile and were stored at −20°C until use. Working standard solutions at different concentration levels were prepared in ethyl acetate.

2.3 Fortification and extraction procedure

A total of 5 g of homogeneous blank edible oil (sunflower, rice bran, and groundnut) samples were each weighed and transferred into 50 mL centrifuge tubes. The samples were fortified with the appropriate amount of working standard solutions at different concentrations to produce final concentrations equal to 200, 100, 50, 20, 10, 5, 2, 1, 0.5, 0.2, 0.1, 0.05, and 0.02 ng/g. PCB at a concentration 10 ng/g was used as IS. The fortified samples were mixed thoroughly by vortex mixer for 3 min and equilibrated at room temperature for 1 h. The analytes from the fortified samples were extracted with 10 mL of acetonitrile by shaking them manually for two minutes and mixed vigorously by the vortex mixer for 5 min. The samples were centrifuged at 10 000 rpm for 5 min and were placed in the dry ice bath containing acetone for 10 min for rapid precipitation of fat. The supernatant acetonitrile layer was collected immediately into 20 mL glass test tubes. The solvent was evaporated to dryness under gentle flow of nitrogen gas using N-EVAP nitrogen evaporator equipment (Organomation Associates, Berlin, MA, USA).

2.4 Derivatization by BSTFA

The residues were reconstituted in 200 μL of ethyl acetate and silylated by adding 200 μL of BSTFA and heated at 60°C for 30 min in sealed glass vials. One microliter of the silylated sample solution was injected into the GC–NCI-MS/MS system for quantitative analysis of the recovered pesticide residues.

2.5 GC–NCI-MS/MS analysis

The GC–NCI-MS/MS analysis was carried out on a Agilent 7000 B mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) coupled to 7890 A GC that consisted of an autosampler (model 7683 B) and split/splitless injector. A total of 1 μL of sample solution was injected using autosampler in pulsed splitless mode (pulse pressure was 25 psi until 0.75 min, purge flow to split vent 30 mL/min at 1 min) into the GC–MS/MS system. The injector and the transfer line temperatures were set to 250°C. Analytes were separated on a ZB-5MS fused-silica capillary column of 30 m length, 0.25-mm internal diameter, and 0.25 μm film thickness (Phenomenex, Torrance, CA, USA). Helium (99.9999 pure) was used as a carrier gas at a flow rate of 1 mL/min. The oven temperature was programed from an initial temperature 100°C held for 2 min and increased to 200°C at 15°C/min, and then to 260°C at 5°C/min followed by a final ramp at 25°C/min to 320°C with a final temperature hold up time of 6 min. The temperatures of the ion source and quadruple analyzer were set to 150°C. Helium was used as a quench gas and nitrogen was used as a collision gas in mass analyzer for all MS/MS experiments with the flow rates of 2.35 and 1.5 mL/min, respectively. The mass spectrometer was operated in NCI mode using ammonia as reagent gas at a flow rate of 40 units. The data were acquired in multiple-reaction monitoring (MRM) mode by selecting a target ion and two qualifier and quantifier ions. The MRM parameters used in the current method are presented in Table 1.

3 Results and discussion

The multiresidue analysis was conducted for three types of commonly used pesticides: OP, OC, and SP pesticides from three commonly used edible oils (sunflower, groundnut, and rice bran oils). The quantification of the analytes was carried out by GC–MS/MS in the NCI mode.

Table 1. List of pesticides, along with their time segments, retention times, quantifier, and qualifier ion transitions along with collision energies (eV), percentage quantifier, and qualifier ion ratios and reproducibility of the transitions represented as CV for five replicate injections of the analytes at LOQ levels

S. No.	Pesticides	Time segments (min)	RT (min)	MW	(NCI) Quantifier transition (Q)	(NCI) Qualifier transition (q)	Q/q ratio (%)	% CV
IS	PCB	3.0–8.70 (1)	7.93	248	250 → 35 (10)	–		
1	Phorate	8.7–9.6 (2)	9.27	260	185 → 111 (20)	185 → 157 (10)	60	8.2
2	α-HCH	8.7–9.6 (2)	9.37	288	71 → 35 (10)	73 → 37 (10)	33	2.3
3	β-HCH	9.6–10.8 (3)	9.81	288	71 → 35 (10)	73 → 37 (10)	33	1.7
4	γ-HCH	9.6–10.8 (3)	9.92	288	71 → 35 (10)	73 → 37 (10)	33	4.6
5	Diazinon	9.6–10.8 (3)	10.14	304	169 → 95 (20)	169 → 141 (10)	45	6.3
6	δ-HCH	9.6–10.8 (3)	10.33	288	71 → 35 (10)	73 → 37 (10)	33	2.8
7	Heptachlor	10.8–11.4 (4)	11.26	370	300 → 35 (35)	266 → 35 (30)	60	4.3
8	Fenitrothion	11.4–12.6 (5)	11.62	277	277 → 168 (4)	277 → 157 (8)	70	2.2
9	Malathion	11.4–12.6 (5)	11.82	330	157 → 142 (25)	172 → 84 (5)	65	4.1
10	Aldrin	11.4–12.6 (5)	11.96	362	237 → 35 (43)	330 → 35 (30)	80	3.7
11	Chlorpyrifos	11.4–12.6 (5)	12.06	349	313 → 189 (10)	313 → 95 (25)	67	2.3
12	Parathion ethyl	11.4–12.6 (5)	12.10	291	291 → 154 (5)	291 → 169 (10)	55	3.7
13	HEE	12.6–13.6 (6)	12.86	386	318 → 35 (10)	282→ 35 (20)	62	2.4
14	Allethrin	12.6–13.6 (6)	13.00	302	167 → 41 (30)	167 → 111 (20)	70	1.9
15	γ-Chlordane	12.6–13.6 (6)	13.44	406	410 → 35 (10)	266 → 35 (15)	65	6.7
16	α-Endosulfan	13.6–14.7 (7)	13.75	404	406 → 35 (5)	242 → 35 (15)	80	7.2
17	α-Chlordane	13.6–14.7 (7)	13.82	406	410 → 35 (10)	266 → 35 (15)	70	5.4
18	*p,p′*-DDE	13.6–14.7 (7)	13.94	316	316 → 35 (5)	318 → 37(5)	33	2.6
19	Dieldrin	13.6–14.7 (7)	14.21	378	380 → 35 (5)	346 → 35 (5)	60	4.6
20	Endrin	14.7–16.3 (8)	15.01	378	380 → 35 (5)	346 → 35 (5)	60	7.2
21	β-Endosulfan	14.7–16.3 (8)	15.24	404	406 → 35 (5)	242 → 35 (15)	80	4.5
22	*p,p′*-DDD	14.7–16.3 (8)	15.48	318	71 → 35 (10)	73 → 37 (10)	33	1.8
23	Endrin aldehyde	14.7–16.3 (8)	15.79	378	380 → 35 (5)	272 → 35 (15)	45	6.9
24	Endrin ketone	14.7–16.3 (8)	16.12	378	380 → 35 (10)	272 → 35 (15)	45	4.6
25	*p,p′*-DDT	16.3–18.4 (9)	16.53	352	71 → 35 (10)	73 → 37 (10)	33	3.7
26	Profenofos	16.3–18.4 (9)	17.78	372	310 → 274 (15)	310 → 272 (10)	98	2.1
27	Imidan	16.3–18.4 (9)	18.01	317	157 → 112 (25)	157 → 79 (35)	65	8.2
28	β-Cyfluthrin	18.4–21.4 (10)	20.18	433	406 → 257 (15)	207 → 35 (10)	37	5.8
29	α-Cypermethrin	18.4–21.4 (10)	20.86	415	388 → 239 (15)	388 → 211 (25)	55	3.8
30	Delta methrin	21.4–26.0 (11)	23.3, 23.5	503	297 → 79 (15)	299 → 81 (15)	98	2.7

CV, coefficient of variance; RT, retention time; MW, molecular weight.

3.1 Optimization of MS conditions

NCI was reported to be more sensitive method for OC and SP pesticides [24–27]. The OP pesticides gave signals in both positive chemical ionization (PCI) and NCI modes; however, the response is more in PCI mode. Hence, in order to maintain the uniformity of methodology and analysis in a single method, we have adopted the NCI-MS/MS technique for the analysis of all OP, OC, and SP pesticide residues. Two optimal MRM transitions quantifier (Q) and qualifier (q) (precursor to product ion) for each pesticide were determined by collision-induced dissociation experiments using dynamic MRM method development software features. As more number of MRM transitions in a single segment reduces the number of data points and thereby decrease the sensitivity of detection, the analytes were grouped into 11 segments according to their retention times. The time segments of each analyte, their retention times, and MRM transitions are shown in Table 1.

3.2 Optimization of sample preparation and extraction procedure

Sample preparation is the most crucial step of the total method as it is very challenging to extract the hydrophobic OC pesticides from the hydrophobic fatty matter. We have attempted the SPE procedure on normal phase silica, florisil, and cyano cartridges. Each time the final aliquots after elution of the analytes and removal of the solvent showed the residual oils. The low-temperature precipitation of the obtained aliquots has reduced the total residual oil content. But the obtained recoveries and sensitivities did not match the requirement of the maximum residue levels (MRL) values defined in various regulatory norms [http://www.codexalimentarius.net/pestres/data/commodities/details.html?id=247, http://ec.europa.eu/sanco_pesticides/public/index.cfm?event=substance.selection]. Hence, we have adopted the acetonitrile SE procedure for extraction of pesticide residues. The fat precipitation by overnight cooling

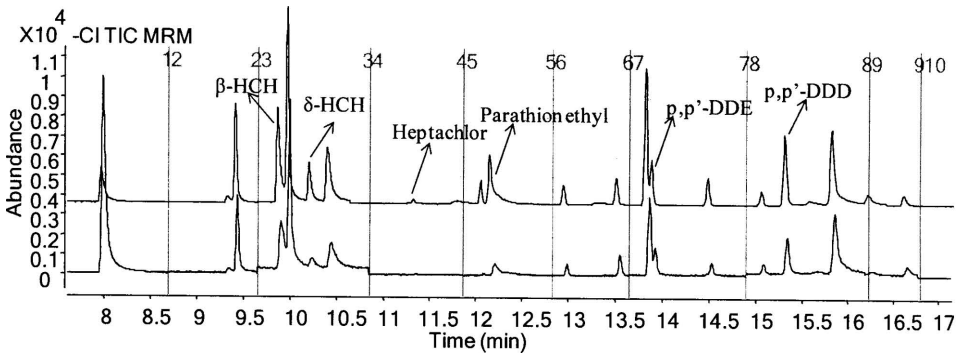

Figure 1. Extracted GC–NCI-MS/MS chromatograms showing the depleted analytes after derivatization (top) compared to that of underivatized sample (bottom).

of the acetonitrile fraction at −20°C was time consuming, and hence a rapid low-temperature fat precipitation (RLTFP) procedure was adopted by placing the aliquot in dry ice bath containing acetone (the temperature of which is typically maintained at −40 to −60°C) for 5 min. This reduced the total time of analysis and reduction in the total fat content compared to that of previous reports [4,6]. The acetonitrile fraction was carefully decanted after rapid centrifugation at 5000 rpm for 1 min (at −10°C), and the solvent was evaporated under gentle stream of nitrogen gas. The residue showed a very thin layer of oil around the sides of the test tube. The residue was reconstituted in 400 μL of ethyl acetate and analyzed directly by GC–MS operated in electron ionization mode. The results showed the staggered peaks of fatty acids that were observed to be interfering with target analytes. The GC–NCI-MS/MS analysis did not show any significant fatty acid peaks. However, some of the analytes were found to be depleted by the background. In order to reduce the background effects, we have chosen to derivatize the sample so that the tailing of the fatty acid background is reduced. Hence, after evaporation of the solvent, the residue was reconstituted in 200 μL of ethyl acetate and 200 μL of BSTFA was added for derivatization of the fatty acid background. After derivatization, the EI analysis showed significant reduction of the interference of fatty acids with that of the analytes and sharp silylated peaks of fatty acids were observed. The NCI-MS/MS chromatogram also showed the missing peaks that are free from other interfering peaks. The ion chromatogram obtained from the GC–NCI-MS/MS analysis of the pesticide residues extracted from rice bran oil before and after derivatization is shown in Fig. 1. The significant differences in the peaks that were detected before and after derivatization are also clearly shown.

3.3 Method validation

The analytical method validation was performed according to the European Union guidelines document SANCO/12495/2011 [http://ec.europa.eu/food/plant/protection/pesticides/docs/qualcontrol_en.pdf]. The LOD of the method for each pesticide was measured at S/N of 3:1. The LOD values of the analytes varied from 0.02 to 0.5 ng/g. The LOQ values were measured at S/N of 10:1 and were in the range of 0.1–2 ng/g, which were much higher that the method conducted without derivatization whose LOQ values were in the range of 10–20 ng/g. The linearity of the method was tested over the concentration range of 0.02–200 ng/g by spiking working standard solutions along with a fixed concentration of IS at 10 ng/g into blank edible oil samples. The calibration curves plotted against the ratio of the peak areas (analyte/IS) to the concentration of the analytes. The obtained results showed good linearity with regression coefficients (r^2) greater than 0.997 for all the studied pesticides. The intraday accuracies were determined by preparing quality control samples at three concentration levels LOQ (0.1–2), 20, and 50 μg/kg in six replicates ($n = 6$) and analyzed along with the calibration samples within day. Interday accuracies were determined by preparing quality control samples freshly in each day at three concentration levels LOQ (0.1–2.0), 20, and 50 μg/kg in six replicates ($n = 6$) and analyzed along with the calibration samples in three consecutive days. The intraday and interday accuracies were in the range of 81–116%. The calculated intraday and interday precision values evaluated in terms of % RSDs were less than 14%. The validation parameters are depicted in Table 2.

The sensitivities of the current method are either better or comparable to that of the previously reported sample preparation methods. The LOQ values of the method are much lower than the required limits by SANCO and WHO guidelines, which are typically in the range of 0.2–2.0 mg/kg. Although the background fatty acids are not completely eliminated, the interference of fatty acids could be significantly reduced by derivatizing the fatty acids. The linear range of the current method, the LOD, LOQ values, and the values reported in previous reports are presented in Table 3.

3.4 Matrix effect and recovery

The matrix effect and recovery of the method was quantitatively measured at three levels LOQ range (0.1–2), 20, and 50 ng/g in six replicates ($n = 6$). Matrix effect [28, 29] was evaluated by extracting 5 g of blank edible oils by SE followed by RLTFP and reconstituted with 200 μL ethyl acetate standard solution of pesticide mixture containing LOQ range (0.1–2), 20, and 50 ng/g residues and silylated with 200 μL of BSTFA. IS was added to the solution at 10 ng/g. The samples

Table 2. The validation parameters for all the pesticide residues in six replicates at LOQ (0.1 to 2), 20, and 50 ng/g

Pesticide	Intraday validation			Interday validation			Recoveries								
							Sunflower (SF) oil			Rice bran (RB) oil			Groundnut (GN) oil		
	SF	RB	GN	SF	RB	GN	LOQ	20 ng/g	50 ng/g	LOQ	20 ng/g	50ng/g	LOQ	20 ng/g	50 ng/g
Phorate	84 (9.3)	91 (5.5)	93 (5.6)	95 (8.3)	87 (8.5)	85 (2.3)	73 (8.8)	79 (5.3)	81 (7.0)	69 (5.5)	65 (5.4)	72 (8.2)	67 (4.7)	76 (8.2)	73 (3.8)
α-HCH	103 (5.8)	94 (4.4)	89 (8.3)	91 (7.1)	94 (5.7)	101 (5.5)	89 (4.8)	82 (5.4)	85 (3.7)	64 (4.8)	70 (6.7)	67 (9.4)	71 (9.1)	67 (3.8)	70 (6.0)
β-HCH	97 (4.4)	89 (10.9)	107 (7.3)	86 (8.8)	89 (3.8)	94 (3.9)	75 (6.8)	70 (6.7)	77 (5.1)	62 (2.9)	69 (5.2)	72 (11)	67 (6.5)	71 (5.9)	73 (11)
γ-HCH	96 (5.4)	94 (6.2)	93 (7.1)	105 (5.7)	103 (4.7)	93 (9.4)	72 (5.3)	69 (5.2)	70 (4.6)	55 (5.4)	68 (8.8)	64 (7.2)	66 (7.6)	72 (6.9)	71 (8.3)
Diazinon	91 (3.5)	97 (7.1)	84 (9.7)	96 (5.9)	88 (5.4)	93 (8.7)	71 (8.2)	78 (8.8)	81 (3.9)	61 (5.4)	73 (7.4)	70 (7.8)	61 (5.5)	67 (4.6)	65 (8.1)
δ-HCH	93 (9.2)	107 (7.1)	89 (11.7)	108 (10)	105 (3.9)	110 (6.3)	64 (7.8)	73 (7.4)	71 (6.1)	59 (6.3)	64 (4.0)	66 (8.3)	62 (6.2)	64 (6.9)	67 (4.8)
Heptachlor	101 (4.9)	97 (8.7)	103 (2.2)	95 (9.5)	95 (2.3)	106 (7.2)	81 (5.9)	71 (6.8)	80 (5.6)	68 (6.4)	67 (3.2)	72 (5.8)	77 (7.4)	70 (8.0)	80 (3.8)
Fenitrothion	98 (2.3)	85 (9.9)	96 (6.1)	88 (8.6)	111 (3)	97 (6.2)	73 (7.3)	65 (3.2)	73 (6.2)	53 (10)	58 (7.3)	61 (9.0)	61 (5.3)	67 (3.3)	64 (12)
Malathion	98 (7.9)	85 (6.0)	82 (9.4)	96 (4.0)	105 (6.8)	97 (7.5)	64 (8.8)	73 (7.3)	71 (5.5)	59 (4.8)	64 (8.8)	66 (7.6)	72 (13)	64 (9.1)	70 (8.3)
Aldrin	84 (3.7)	93 (10.6)	85 (8.2)	105 (6.3)	96 (11.4)	98 (10.3)	79 (5.2)	64 (8.8)	70 (3.9)	58 (4.4)	65 (6.2)	67 (8.4)	77 (5.3)	71 (7.2)	74 (9.3)
Chlorpyrifos	103 (9.4)	107 (7.6)	103 (3.7)	96 (2.6)	106 (8.7)	93 (2.1)	82 (5.3)	75 (6.2)	84 (4.2)	68 (5.5)	78 (3.8)	74 (5.9)	76 (6.5)	81 (8.6)	83 (4.8)
Parathion ethyl	98 (3.9)	93 (2.4)	84 (11.3)	95 (4.5)	90 (7.1)	110 (4.5)	68 (7.3)	73 (3.8)	75 (9.2)	60 (6.3)	67 (9.9)	71 (7.6)	61 (5.7)	69 (5.4)	72 (9.2)
HEE	93 (2.8)	89 (7.3)	107 (3.7)	94 (2.3)	102 (9.5)	105 (3.6)	81 (4.3)	73 (9.9)	77 (6.2)	77 (4.4)	64 (7.8)	73 (9.5)	72 (6.6)	67 (7.8)	70 (12)
Allethrin	98 (4.5)	88 (5.0)	99 (5.3)	81 (7.9)	103 (4.7)	109 (3.9)	69 (4.9)	84 (7.8)	75 (7.1)	63 (3.8)	72 (5.2)	75 (4.7)	62 (7.3)	69 (4.6)	67 (8.3)
γ-Chlordane	103 (11)	89 (9.4)	105 (8.6)	89 (13.1)	103 (6.2)	96 (8.3)	64 (6.2)	78 (9.6)	81 (7.0)	60 (6.2)	71 (8.1)	68 (10)	67 (8.9)	75 (5.5)	70 (4.8)
α-Endosulfan	109 (6.4)	89 (11.7)	99 (6.5)	86 (6.7)	103 (7.7)	93 (4.1)	73 (7.2)	84 (8.1)	88 (5.1)	68 (6.2)	64 (4.1)	70 (4.8)	63 (4.7)	75 (4.3)	73 (9.4)
α-Chlordane	90 (2.5)	90 (8.4)	116 (4.6)	94 (5.7)	87 (9.8)	91 (5.5)	62 (5.2)	74 (4.1)	73 (8.1)	69 (5.9)	73 (8.4)	71 (5.4)	77 (9.7)	75 (8.6)	70 (5.2)
p,p′-DDE	109 (7.5)	97 (10.3)	89 (11.5)	113 (4.5)	106 (10)	102 (5.8)	78 (4.9)	73 (8.4)	81 (3.7)	64 (5.9)	63 (4.2)	67 (12)	71 (6.0)	66 (5.6)	74 (3.8)
Dieldrin	103 (10)	94 (3.8)	95 (3.6)	112 (5.4)	97 (8.5)	97 (4.5)	81 (6.3)	63 (4.2)	77 (6.2)	71 (5.4)	66 (5.4	74 (7.2)	67 (4.7)	71 (2.9)	73 (6.2)
Endrin	92 (9.7)	101 (8.6)	81 (6.8)	99 (11.8)	95 (8.8)	96 (6.2)	73 (2.3)	66 (5.4)	70 (4.1)	54 (5.8)	63 (5.2)	65 (6.4)	64 (5.3)	72 (7.2)	77 (3.6)
β-Endosulfan	103 (5.8)	98 (4.9)	114 (6.5)	93 (11.1)	93 (9.1)	110 (9.5)	85 (4.2)	73 (5.2)	75 (6.3)	69 (4.3)	73 (2.2)	70 (8.2)	79 (7.1)	73 (8.7)	72 (4.9)
p,p′-DDD	105 (6.6)	97 (9.4)	99 (7.7)	108 (3.7)	99 (11.4)	92 (3.6)	72 (9.3)	73 (2.2)	80 (6.9)	67 (8.3)	63 (7.1)	65 (10)	68 (4.4)	74 (5.8)	70 (7.3)
Endrin aldehyde	92 (11.2)	86 (8.2)	89 (6.7)	84 (13)	107 (7)	89 (7.7)	76 (4.5)	63 (7.1)	72 (5.3)	64 (4.7)	74 (6.1)	77 (6.9)	60 (8.5)	65 (5.3)	68 (7.6)
Endrin ketone	108 (9.3)	111 (7)	94 (3.7)	114 (13)	102 (5.6)	95 (3.8)	78 (5.7)	68 (6.3)	73 (7.8)	65 (5.3)	73 (5.7)	75 (8.2)	65 (5.5)	71 (4.5)	76 (4.9)
p,p′-DDT	92 (4.9)	94 (10.6)	85 (5.5)	84 (5.7)	104 (5)	113 (3.2)	62 (5.3)	73 (5.7)	76 (6.3)	63 (3.7)	68 (6.5)	71 (4.4)	71 (9.4)	69 (5.5)	73 (7.0)
Profenofos	95 (4.8)	101 (8.8)	111 (12)	92 (6.3)	104 (13)	90 (2.8)	58 (7.9)	68 (6.5)	65 (9.1)	62 (5.8)	65 (4.3)	73 (8.2)	67 (7.8)	71 (6.5)	68 (3.2)
Imidan	89 (5.6)	81 (5.2)	93 (11.8)	108 (8.7)	86 (3.3)	116 (14)	55 (9.6)	63 (4.3)	62 (6.0)	53 (5.5)	55 (6.7)	59 (7.3)	53 (4.5)	57 (13)	60 (7.2)
β-Cyfluthrin	94 (6.4)	84 (5.7)	108 (2.6)	111 (4.9)	106 (3.9)	93 (4.4)	66 (3.7)	72 (5.8)	70 (3.8)	59 (5.2)	65 (6.9)	66 (5.2)	66 (4.5)	63 (5.5)	69 (5.8)
α-Cypermethrin	83 (2.8)	91 (5.6)	91 (8.1)	86 (6.8)	91 (6.1)	82 (5.2)	67 (5.2)	78 (6.9)	82 (5.2)	62 (4.1)	58 (3.8)	60 (7.2)	56 (6.6)	69 (7.5)	64 (3.8)
Deltamethrin	87 (4.3)	89 (6.4)	92 (5.1)	93 (3.9)	91 (6.4)	89 (6.4)	68 (5.8)	74 (6.1)	79 (4.8)	67 (4.9)	74 (4.3)	69 (8.2)	67 (7.7)	79 (6.5)	77 (6.1)

The values in the parentheses were precision values (% RSD).

Table 3. Linear range of pesticide residues along with LOD and LOQ values (calculated in six replicates) and the comparative literature values for the same

S. No.	Pesticides	Linear range (μg/kg)	Current method		Reported methods		References
			LOD (μg/kg)	LOQ (μg/kg)	LOD (μg/kg)	LOQ (μg/kg)	
1	Phorate	0.05–100	0.05	0.2	2	7	[7]
2	α-HCH	0.02–50	0.02	0.1	2	7	[7]
3	β-HCH	0.02–50	0.02	0.1	3	10	[7]
4	γ-HCH	0.02–50	0.02	0.1	3	10	[7]
5	Diazinon	0.02–50	0.02	0.1	0.06	0.2	[10]
6	δ-HCH	0.02–50	0.02	0.1	3	10	[7]
7	Heptachlor	0.05–100	0.05	0.2	–	4	[6]
8	Fenitrothion	0.02–50	0.02	0.1	–	20	[7]
9	Malathion	0.02–50	0.02	0.1	5	9	[8]
10	Aldrin	0.1–200	0.1	0.5	0.75	2.5	[6]
11	Chlorpyrifos	0.02–50	0.02	0.1	–	6	[6]
12	Parathion ethyl	0.02–50	0.02	0.1	3	10	[7]
13	HEE	0.1–200	0.1	0.5	0.12	0.5	[6]
14	Allethrin	0.02–50	0.02	0.1	–	–	–
15	γ-Chlordane	0.02–50	0.02	0.1	–	–	–
16	α-Endosulfan	0.02–50	0.02	0.1	–	40	[6]
17	α-Chlordane	0.02–50	0.02	0.1	–	–	–
18	*p,p′*-DDE	0.1–200	0.1	0.5	1	3	[7]
19	Dieldrin	0.1–200	0.1	0.5	–	4	[6]
20	Endrin	0.05–100	0.05	0.2	–	8	[6]
21	β-Endosulfan	0.02–50	0.02	0.1	–	40	[6]
22	*p,p′*-DDD	0.1–200	0.1	0.5	2	7	[7]
23	Endrin aldehyde	0.02–50	0.02	0.1	–	–	–
24	Endrin ketone	0.05–100	0.05	0.2	–	–	–
25	*p,p′*-DDT	0.02–50	0.02	0.1	3	10	[6]
26	Profenofos	0.5–200	0.5	2	–	20	[6]
27	Imidan	0.02–50	0.02	0.1	–	20	[6]
28	β-Cyfluthrin	0.05–100	0.05	0.2	–	10	[6]
29	α-Cypermethrin	0.1–200	0.1	0.5	8	27	[7]
30	Deltamethrin	0.02–50	0.02	0.1	–	50	[6]

were analyzed along with the standard solutions prepared in ethyl acetate at same concentration levels. The relative detector responses were measured by comparing the relative slopes obtained from the matrix and neat solvent standards. The matrix effect observed for most of the pesticide residues was below 35%. The obtained recoveries in derivatization procedure were in the range of 53–89%, and the results are presented in Table 2.

3.5 Application to real-sample analysis

The developed method was applied successfully to the 65 edible oil samples (40 refined and 25 crude) collected from various places in Hyderabad for monitoring multiresidue analysis. Oil was extracted from seeds using the AOCS official method Aa 4–38 [http://lipidlibrary.aocs.org/processing/solventextract/index.htm]. Refined edible oil samples did not show any residues above the detection limits of the method. Among the extracted crude oil samples, some of them were showed the presence of endosulfan isomers in the range of 1.35 ng/g (5.7), α-cypermethrin 1.52 ng/g (8.3), chlorpyrifos 0.56 ng/g (3.7), diazinon 1.27 ng/g (6.5), and *p,p′*-DDD 2.14 ng/g (4.8), the values in the parentheses represent % RSDs calculated in six replicates ($n = 6$). Based on the obtained results, it can be concluded that the refining process, which consists degumming, neutralization, bleaching, and deodorization may have contributed to the elimination of pesticide residues from the oil samples. The determined pesticides peaks were confirmed based on GC–NCI-MS/MS retention time, qualifier, and quantifier MRM transitions with acceptable deviations reported previously [9]. The representative chromatograms showing the pesticide peaks are presented in Fig. 2.

4 Concluding remarks

The derivatization of fatty acid matrix that is coextracted during the SE process after the RLTFP reduces the interference of the acidic substances with pesticide residues when analyzed by GC–NCI-MS/MS. The method could reach the required

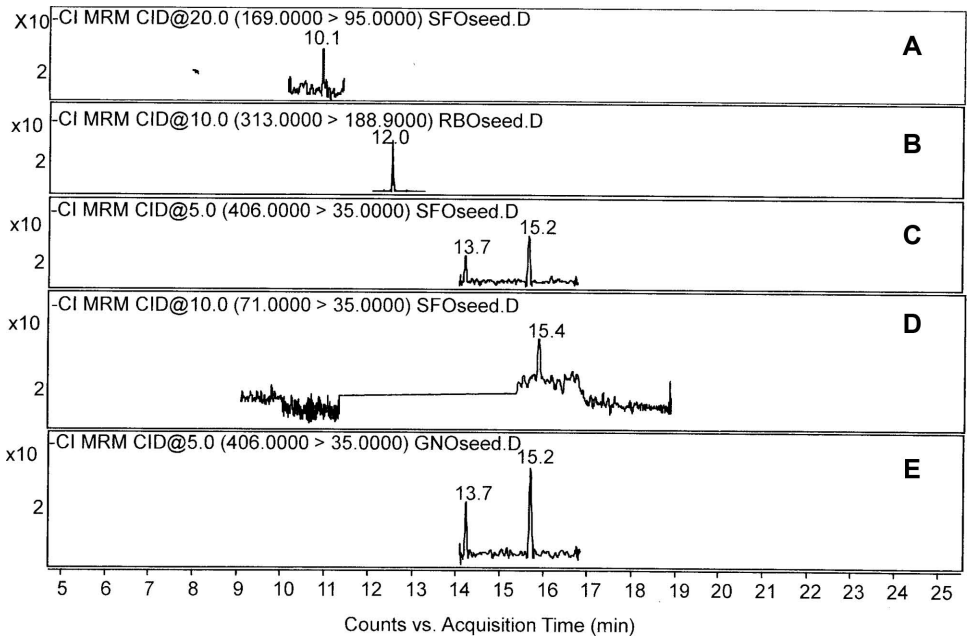

Figure 2. GC–NCI-MRM extracted ion chromatograms of pesticides found in crude oil samples extracted from oil seeds. (A) Diazinon, (B) chlorpyrifos, (C) endosulfan isomers, (D) *p,p′*-DDD, and (E) endosulfan isomers.

MRL value limits described in SANCO and WHO guidelines. The method was applied to some crude and refined edible oils. The refined oils did not show any pesticide residues and the crude oils show some pesticide residues whose MRL values are within the required limit described in SANCO guidelines. The matrix effect of the method was found to be in acceptable range due to high selection of NCI-MS/MS method. Hence, the proposed method is adequate to perform multiresidue screening in different types of edible oils and oil seeds.

The authors thank Council of Scientific and Industrial Research for sponsoring the projects AARF (CSC0406) and PEOPLEHOPE (CSC0112) for carrying out this research work.

The authors have declared no conflict of interest.

5 References

[1] Chung, S. W. C., Chen, B. L. S., *J. Chromatogr. A* 2011, *1218*, 5555–5567.

[2] García-Reyes, J. F., Ferrer, C., Gómez-Ramos, M. J., Fernández-Alba, A. R., García-Reyes, J. F., Molina-Díaz, A., *Trends Anal. Chem.* 2007, *26*, 239–251.

[3] Gilbert-López, B., García-Reyes, J. F., Molina-Díaz, A., *Talanta* 2009, *79*, 109–128.

[4] Muhamad, H., Zainudin, B. H., Abu Bakar, N. K., *Food Chem.* 2012, *134*, 2489–2496.

[5] Nguyen, T. D., Lee, M. H., Lee, G. H., *Microchem. J.* 2010, *95*, 113–119.

[6] Li, L., Zhang, H., Pan, C., Zhou, Z., Jiang, S., Liu, F., *J. Sep. Sci.* 2007, *30*, 2097–2104.

[7] Sobhanzadeh, E., Bakar, N. K. A., Abas, M. R. B., Nemati, K., *Environ. Monit. Assess.* 2012, *184*, 5821–5828.

[8] Sack, C., Smoker, M., Chamkasem, N., Thompson, R., Satterfield, G., Masse, C., Mercer, G., Neuhaus, B., Cassias, I., Chang, E., Lin, Y., MacMahon, S., Wong, J., Zhang, K., Smith, R. E., *J. Agric. Food Chem.* 2011, *59*, 6383–6411.

[9] Gilbert-López, B., García-Reyes, J. F., Lozano, A., Fernández-Alba, A. R., Molina-Díaz, A., *J. Chromatogr. A* 2010, *1217*, 6022–6035.

[10] Huskova, R., Matisova, E., Hrouzkova, S., Svorc, L., *J. Chromatogr. A* 2009, *1216*, 6326–6334.

[11] Cunha, S. C., Lehotay, S. J., Mastovska, K., Fernandes, J. O., Beatriz, M., Oliveira, P. P., *J. Sep. Sci.* 2007, *30*, 620–632.

[12] Li, M., Liu, X., Dong, F., Xu, J., Qin, D., Zheng, Y., *J. Sep. Sci.* 2012, *35*, 2743–2749.

[13] Iwasaki, Y., Nakano, Y., Mochizuki, K., Nomoto, M., Takahashi, Y., Ito, R., Saito, K., Nakazawa, H., *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* 2011, *879*, 1159–1165.

[14] Zaikin, V. G., Halket, J. M., *Eur. J. Mass Spectrom.* 2006, *12*, 79–115.

[15] Zaikin, V. G., Halket, J. M., *Eur. J. Mass Spectrom.* 2004, *10*, 555–568.

[16] Zaikin, V. G., Halket, J. M., *Eur. J. Mass Spectrom.* 2003, *9*, 421–434.

[17] Zaikin, V. G., Halket, J. M., *Eur. J. Mass Spectrom.* 2005, *11*, 611–636.

[18] Halket, J. M., Zaikin, V. G., *Eur. J. Mass Spectrom.* 2006, *12*, 1–13.

[19] Halket, J. M., Zaikin, V. G., *Eur. J. Mass Spectrom.* 2005, *11*, 127–160.

[20] Halket, J. M., Zaikin, V. G., *Eur. J. Mass Spectrom.* 2004, *10*, 1–19.

[21] Halket, J. M., Zaikin, V. G., *Eur. J. Mass Spectrom.* 2003, *9*, 1–21.

[22] Stashenko, E. E., Martínez, J. R., *TrAC, Trends Anal. Chem.* 2004, *23*, 553–561.

[23] Tao, L., Chen, M., Collins, E., Lu, C., *J. Sep. Sci.* 2013, *36*, 773–780.

[24] Shen, C. Y., Cao, X. W., Shen, W. J., Jiang, Y., Zhao, Z. Y., Wu, B., Yu, K. Y., Liu, H., Lian, H. Z., *Talanta* 2011, *84*, 141–147.

[25] Chaler, R., Vilanova, R., Santiago-Silva, M., Fernandez, P., Grimalt, J. O., *J. Chromatogr. A* 1998, *823*, 73–79.

[26] Feo, M. L., Eljarrat, E., Barcelo, D., *Rapid Commun. Mass Spectrom.* 2011, *25*, 869–876.

[27] Louter, A. J. H., Slobodník, J., Vreuls, R. J. J., Brinkman, U. A. T., *Analyst* 1997, *122*, 1497–1503.

[28] Walorczyk, S., Drożdżyński, D., Gnusowski, B., *Talanta* 2011, *85*, 1856–1870.

[29] Garrido Frenich, A., Martínez Vidal, J. L., Fernández Moreno, J. L., Romero-González, R., *J. Chromatogr. A* 2009, *1216*, 4798–4808.

