Development and Validation of an Analytical Method for Pesticide Residues Analysis in Crude Cottonseed Oil

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Summary: This study reports the development and validation of a fast, efficient, and cost effective multiresidue method for determination of 14 lipophilic and analytically problematic pesticides in crude cottonseed oil. Crude cottonseed oil contains high amount of saturated fatty acid and pigments that are problematic in pesticide residues analysis. Modified liquid-liquid partitioning with acetonitrile and n-hexane in 10:1 (v/v) ratio was used to extract pesticides. For clean-up, different combinations of sorbents were used and optimum recovery and minimal matrix effect were obtained with the combination of activated charcoal and primary secondary amine for the selected pesticides. For majority of the analyzed pesticides, the method validation parameters i.e. percent recovery (71.6-140.0%), precision (%RSD 9.7 to 33.0), LOD (0.041 to 0.096 µg/g), LOQ (0.125-0.264 µg/g), linearity (0.998-0.999) and matrix effect (±27%) were in acceptable range as prescribed by EU SANTE guidelines. Two-way Analysis of variance of inter-labs comparison study revealed non-significant interaction effects for most of studied pesticides indicating that the current method can be confidently used in labs for monitoring of these pesticides in crude cottonseed oil.

Keywords: Crude Cottonseed oil; Pesticide residues; Fats; Pigments; Extraction; Cleanup.

Introduction

Cotton is grown in 70 countries around the world and cottonseed oil contributes more than 15% to world's vegetable oil [1, 2]. Cottonseed oil has unsaturated to saturated fatty acids in a ratio of 2:1. It generally consists of 65-70% unsaturated fatty acids, 17-24% mono-unsaturated, 40-52% poly-unsaturated and 25-35% saturated fatty acids. Cottonseed oil contains pigments gassypurpurin, gossycaerulin, gossyfulivin, gossyverdurin and toxic gossypol. The gossypol pigments are greater in raw cottonseed oil and create enormous problems of seed processing and utilization of cottonseed oil. [3]. Refined cottonseed oil is mainly used for edible purpose as frying oil, salad oil and in the manufacturing of margarine, shortenings, potato chips and other snack food [4, 5]. Cotton crop is susceptible to a range of insect pests and diseases. A number of pesticides are applied at various stages of its cultivation to provide protection against insect pests [6, 7]. Pesticides are persistent and can easily last till the final stage of harvesting [8].

Cotton is heavily sprayed and cottonseed has been found contaminated with pesticides. Among 250 samples of cottonseed from Punjab, Pakistan, 73% samples were found contaminated with different pesticides of which 40% samples were exceeding FAO Codex Alimentarius prescribed MRLs [9]. These hazardous pesticides can potentially contaminate the cottonseed oil [10].

Dispersive, single drop, air-assisted liquid–liquid microextraction and solid-phase microextraction methods for less lipophilic triazole, pyrethroids and organophosphorus pesticides has been developed in different edible oils [11-15]. Most of the traditional analytical methods that are available in literature are specific for pesticide residues present in olive oil [8, 16-18]. Up to our limited knowledge only two methods are available for pesticide residues analysis in cottonseed oil [19, 20]. In one of these methods lipophilic pesticides have been completely overlooked [20] while in other method in spite of using expensive clean up sorbents low recoveries of lipophilic pesticides have been observed [19]. Moreover, in these analytical methods no attention has been paid to remove the pigments from cottonseed oil that affects recovery and increases matrix effect. For the removal of pigments, Graphitized Carbon Black (GCB) has been used in a number of methods for cleanup of fatty samples but using GCB reduces the recovery of planar pesticides [21]. Activated charcoal has been suggested as a promising sorbent for extraction of pesticide residues from edible oil but has never been studied [22]. Due to higher number of pigments and low recoveries of lipophilic pesticides from cottonseed oil there is a dire need to develop a method that can remove these bottlenecks. Hence, the present study was designed to develop a simple, rapid and cost-effective analytical method for 14 analytically problematic lipophilic pesticides in crude

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cottonseed oil. The suitability of activated charcoal as an alternate sorbent instead of GCB for cleanup of fatty pigmented sample was also evaluated.

Experimental

Pesticides free crude cottonseed oil was provided by PARC Research & Training Station, Multan, Pakistan. Fourteen pesticides were selected for method development and validation from the three major groups including organophosphates, pyrethroids and analytically challenging organochlorines (OCPs).

Chemicals & Consumable

GC/ HPLC grade Acetonitrile (ACN), n-hexane, ethyl acetate and dichloromethane (DCM) were procured from Merck. 14 Pesticide standards (α-HCH, Heptachlor, Chlorpyrifos, Methidathion, α-Endosulfan, 4,4-DDE, 2,4-DDD, Endrin, β-Endosulfan, 2,4-DDT, 4,4-DDT, λ-Cyhalothrin, α-Cypermethrin and Deltamethrin) of PESTANAL grade (purity ≥ 98.0%) and sorbents (PSA, C_{18} and Florisil) were purchased from Sigma-Aldrich. Activated charcoal and anhydrous sodium sulphite was purchased from Merck.

Samples Pre-treatment and Fortification

Prior to extraction, the suspended particles in crude cottonseed oil were filtered out by using the Whatman Filter Paper No.1. Miscibility of cottonseed oil with different solvents like acetonitrile, ethyl acetate and dichloromethane were evaluated to find out the best solvent for extraction. For partitioning of pesticide residues and non-polar impurities acetonitrile and n-hexane were evaluated in 10:4, 10:3 and 10:1 ratio. For evaluation and fitness of extraction and cleanup, recovery experiments were conducted. The samples were fortified with 14 selected pesticides at three spiking levels i.e. 0.25µg/g, 0.5µg/g and 2.5 µg/g in triplicates.

Extraction

One-gram filtered cottonseed oil was accurately weighed in 50 mL falcon tube. Oil was dissolved in one mL of n-hexane and then 10 mL acetonitrile was added in falcon tube. The tubes were vortexed at 12000 rpm for 3 minutes using VELP Scientific vortex mixer. The well mixed samples inside tube were placed in refrigerator for 30 minutes. The cooled samples were subjected to centrifugation at 3000 rpm for 5 minutes. Five mL of the supernatant acetonitrile layer was collected and subjected to further cleanup.

Cleanup

The SPE columns were prepared in 10mL disposable syringe. The barrel end of each syringe was plugged with glass wool and then ~1.5g of anhydrous sodium sulphite. On top of this following three sorbents combinations were evaluated for optimum cleanup combination.

i. Activated charcoal (500 mg) pre-heated at 250 ^{o}C + Florisil (300 mg)
ii. Activated charcoal (500 mg) pre-heated at 250 ^{o}C + C_{18} bonded silica (300 mg)
iii. Activated charcoal (500 mg) pre-heated at 250 ^{o}C + PSA (300 mg)

A PTFE syringe filter of 0.45 µm pore size was fixed at the end of the syringe and then mounted on vacuum manifold SPE assembly. The pressure inside the assembly was adjusted to maintain a constant flow of 2 drops/ second. The SPE columns were first conditioned with 5mL of dichloromethane and ethyl acetate mixture in a ratio of 7:3. Five mL of extracts were loaded on SPE column and the pesticides were eluted with 5mL of DCM: EA mixture. The eluate was collected in 50mL round bottom flask and evaporated to dryness at 40 ^{0}C under vacuum on rotary evaporator. The schematic procedure of cleanup is described in Fig. 1.

Analysis/Instrumentation

The samples were analyzed on Gas Chromatograph equipped with Ni^{63} Electron Capture Detector (Model 7890B, Agilent Technologies, USA), HP-5 (30m x 320um x 0.25um) capillary column and ChemStation® software (Hewlett–Packard, Palo Alto, CA, USA). The carrier gas was N_2 with 99.99% purity. A constant flow of 2 mL/min was maintained with variable pressure. Analytical parameters on GC were: Injector temperature 250 ^{o}C with splitless injection mode, injection volume 1 µL, detector temperature 300 ^{o}C; oven program was as follows: oven initial temperature was held at 70 ^{o}C for 1.0 min, 50 ^{o}C /min ramp to 150 ^{o}C withhold time of 0 minutes, then 6 ^{o}C /min to 225^{o}C withhold time of 0 minutes. Then 16 ^{o}C/min to final temperature 295 ^{o}C held for 10 min. The total run time was 29.5 minutes. Pesticides were identified on the basis of respective retention times and quantified on the basis of peak areas.

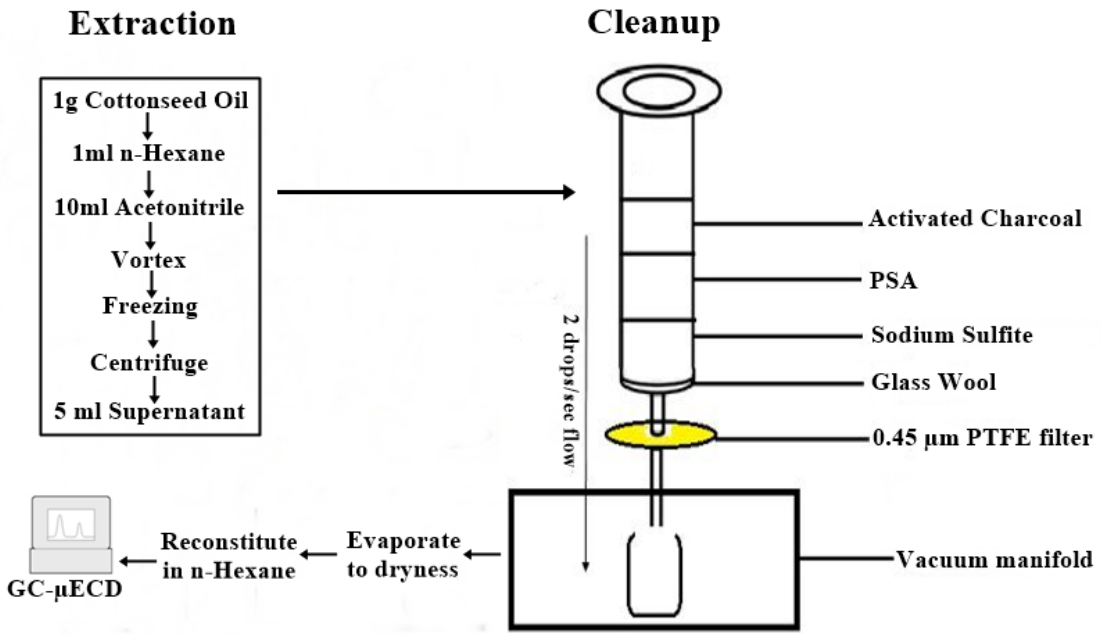

Fig. 1: Graphical representation of experimental steps

For inter-laboratory experiment, the same method was repeated on Agilent GC Model 6890N under the same parameters in Food Science Research Institute (FSRI), NARC. The results obtained in ERP and FSRI were compared using two-way ANOVA (Statistix 8.1). Analytical method was validated in term of accuracy (% recovery), precision (inter laboratory repeatability), limit of detection (LOD), limit of quantification (LOQ) and linearity. These parameters were calculated as an average of three spiked samples at 0.25 µg/g, 0.5 µg/g and 2.5 µg/g using the formulae given in Table 3.

Results and Discussion

Optimization of Extraction Procedure:

Among the three solvents tested, ethyl acetate was found to be completely miscible, dichloromethane was partially miscible and acetonitrile was immiscible with crude cottonseed oil. Thus, only acetonitrile was used in subsequent method optimization experiments. Similarly, among the three solvent combination ratios, 10 mL acetonitrile and one mL n-hexane were found best. The cottonseed oil gets dissolved and concentrated in one mL n-hexane, so density of n-hexane increases and it moves to the bottom of the falcon tube leaving acetonitrile as upper layer. While using two and three mL n-hexane in extraction, the acetonitrile layer occupied lower position and hence caused difficulties in separation of acetonitrile layer. Miscibility of cottonseed oil with acetonitrile, dichloromethane, ethyl acetate and effect of increasing n-hexane ratio are presented in supplementary data (Fig i and ii respectively).

Optimization of Cleanup

Recovery from Sorbent Combination-i (activated charcoal & florisil)

The average recoveries of 14 pesticides cleaned with activated charcoal & florisil are shown in Fig. 2(a). Activated charcoal is well known sorbents for the removal of pigments from colored samples [23]. The acidic and basic functional groups on the surface play an important role in the removal of pigments from cottonseed oil. The hydrophobic surface of activated charcoal may also retain fats to some extent due to hydrophobic interactions [22, 24]. Florisil magnesium silicates activated is a polar sorbent. In some earlier studies [17, 25] this sorbent has been recommended for cleanup of fatty samples. Combination of activated charcoal and florisil gave highly variable average % recoveries (40-290%) at three spiking levels (0.5, 1.0 and 2.5µg/g) for all pesticides. This shows that florisil was less effective as it was not able to retain matrix compounds. These highly variable recoveries make the florisil un-suitable sorbent in cottonseed oil analysis. The %RSD for all pesticides was also greater than 20%.

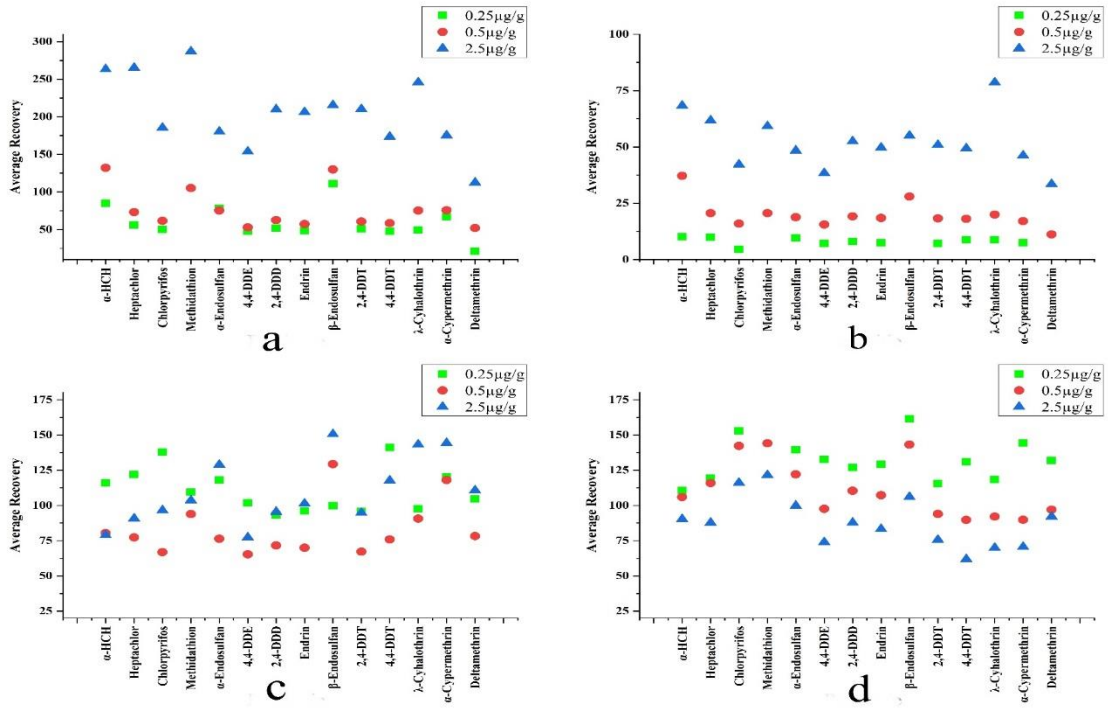

Fig. 2: Average recovery of 14 pesticides obtained after cleanup with (a) Activated charcoal & Florisil (b) Activated charcoal & C18 (c) Activated charcoal & PSA (Lab 1 ERP) (d) Activated charcoal & PSA (Lab 2, FSRI).

Recovery from Sorbent Combination-ii (activated charcoal & C18)

The average recoveries of 14 pesticides cleaned with activated charcoal & C18 are shown in Fig. 2(b). C18 is non-polar sorbent that contains octadecyl chain bonded to silica surface that makes this sorbent hydrophobic. Nonpolar-nonpolar interaction are the main binding forces that retain fatty acid from oil extract. Due to this interaction C18 is widely used and recommended for the cleanup of fatty foods/ oil extracts [16, 19, 26]. But the combination of activated charcoal with C18 gave very low recoveries for all of the 14 selected pesticides. Even methidathion, β-endosulfan and deltamethrin were not detected at lowest (0.5µg/g) spiking level. However, %RSD values among triplicates were lower than sorbent combination 1 containing activated charcoal and florisil. These low recovery of pesticides from C18 SPE columns have been reported in earlier studies [8, 19]. Due to high lipophilic nature of selected pesticides, these pesticides strongly bind with C18 sorbent that ultimately gives low recoveries.

Recovery from Sorbent Combination-iii (activated charcoal & PSA)

The average recoveries of 14 pesticides cleaned with activated charcoal & PSA are shown in Fig. 2(c). PSA consist of ethylenediamine-N-propyl group bonded to silica. This sorbent has been used in many studies for the retention of non-polar compounds in SPE or d-SPE [16, 19, 26, 27]. The combination of activated charcoal and PSA gave average recovery within the acceptable range for most of studied pesticides. However, chlorpyrifos and 4,4-DDT showed slightly high recoveries i.e. 137% & 141%, respectively. % RSD value among triplicates were also within acceptable range of less than 20% for most of the studied pesticides. However, α-HCH (33%), β-endosulfan (32%) and 4,4-DDT (31.7%) showed high % RSD in lower and higher spiking level

For confirmation of the findings obtained with activated charcoal and PSA, an inter-lab repeatability experiment was performed in Food Science Research Institute (FSRI), NARC. The average recoveries obtained in two laboratories are presented in Table-1 (Fig. 2 (c) and 2 (d). AT FSRI Lab, just like ERP lab, high recoveries were observed at lower spiking level especially for chlorpyrifos, β-endosulfan and α-cypermethrin. But in higher spiking level acceptable recoveries were obtained for all the studied pesticides. These results show that activated charcoal with PSA can be an excellent sorbent combination for cleanup of different classes of pesticides including the challenging organochlorine pesticides in crude cottonseed oil that

give low recoveries with other sorbents. The recovery of triplicates and overlay chromatograms after three cleanup columns are given in Tables (1-4) and chromatograms. To further verify the results of this cleanup combination i.e. activated charcoal and PSA different method validation parameters were performed.

Methidathion was not detected in lower spiking level from all the sorbents combination. This is probably due to the low response of methidathion on GC-µECD and strong interaction with activated charcoal while using ethyl acetate and dichloromethane mixture as an eluting solvent.

Inter-lab Precision

For Intra-lab precision, the results of spiked samples analyzed in the laboratories of ERP, NARC and FSRI, NARC were compared. The %RSD values of both laboratories are presented in Table-1. The calculated %RSD for intra-lab repeatability were generally below than 20% for most of pesticides that can fulfill the requirement of European SANTE/12682/2019 guidance document [28]. The values of % RSD in ERP lab for α-HCH (33.1), methidathion (26.2) and 2, 4-DDT (24.5) were a little high at some spiking levels but never exceeded more than 35%. However, both endosulfan isomers showed %RSD higher than 40%. Similarly, in FSRI lab high % RSD higher than 37 were obtained for chlorpyrifos and β-endosulfan, but these values never exceeded than 40%.

For inter-laboratory repeatability the results (Table-4) obtained in ERP and FSRI were compared using two-way ANOVA at $P< 0.05$ (Statistix 8.1). Interaction effects (concentration × labs) were found non-significant for all the studied pesticides at $p<0.05$. Moreover, at different spiking levels 11 out of 14 pesticides were recorded having non-significant differences. Only Chlorpyrifos, 4,4-DDE and 2,4-DDD were having significant differences in inter-lab comparison.

LOD & LOQ

LOD and LOQ were calculated from the calibration curve of matrix matched standards. The values of LOD and LOQ for each pesticide together with their FAO Codex Alimentarius Maximum Residues Limits (MRLs) in cottonseed oil are given in Table-2.

Table-1: Percent recovery at three spiking levels using activated charcoal and PSA in cleanup in ERP & FSRI Laboratory.

	ERP Laboratory						FSRI Laboratory					
Pesticides	**0.25µg/g***	**%RSD**	**0.5µg/g***	**%RSD**	**2.5µg/g***	**%RSD**	**0.25µg/g***	**%RSD**	**0.5µg/g***	**%RSD**	**2.5µg/g***	**%RSD**
α-HCH	116	33.1	80.4	15.9	78.9	27.3	110.5	18.1	105.9	18.6	90.3	28.9
Heptachlor	121.9	21.2	77.3	11.5	90.7	6.7	119.3	22.1	115.9	18.3	87.7	27.3
Chlorpyrifos	137.8	16.5	66.9	33.6	96.5	18.9	152.9	36.8	142.2	19.7	116.1	30
Methidathion	109.4	9.7	93.8	26.2	103.5	7.1	218.2	ND	144.1	26.7	121.5	29.2
α-Endosulfan	118	17.5	76.4	16.5	128.9	74.6	139.4	21.6	122.1	18.9	99.8	28.1
4,4,DDE	101.9	17.6	65.3	14	77.3	16.3	132.6	19.2	97.6	17	73.9	25.6
2,4,DDD	93.2	15.6	71.6	21.4	95.5	9.8	126.9	15.6	110.4	21.4	87.8	31.6
Endrin	96.1	19.7	70	24.5	101.3	18.3	129.2	16.7	107.3	20.7	83.3	31.7
β-Endosulfan	99.8	32.1	129.3	18.8	150.6	62.5	161.5	24.5	143.2	22.9	106	36.4
2,4,DDT	95.8	20.5	67.2	24.5	94.7	11.9	115.5	6.5	94	21.2	75.5	33.1
4,4,DDT	141	31.7	75.9	14.9	117.7	38.7	130.9	9	89.7	18.6	61.9	31.7
λ-Cyhalothrin	97.6	13.5	90.6	10.5	143.2	15.1	118.3	4.4	92.1	20.3	69.9	33.1
α-Cypermethrin	120.3	9.2	118	11.6	144.3	3.8	144.3	11.3	89.8	19.2	70.6	30.1
Deltamethrin	104.6	10.7	78.2	27	110.6	3.3	132	5.7	96.9	19	92	26.6

***Average recovery of triplicates**

Table-2: List of LOD, LOQ, FAO Codex Alimentarius reference MRLs, Linearity and Matrix effect of the method for cottonseed oil.

S.No.	RT (min)	Pesticide	LOD (mg/kg)	LOQ (mg/kg)	MRLs (mg/kg)	Linearity (R^2)	ME (%)
1	**8.231**	**α-HCH**	**0.041**	**0.125**		**0.991**	**-10**
2	**10.828**	**Heptachlor**	**0.049**	**0.149**	**0.2**	**0.994**	**-11**
3	**12.002**	**Chlorpyrifos**	**0.071**	**0.215**	**0.3**	**0.993**	**-14**
4	**13.615**	**Methidathion**	**0.161**	**0.49**		**0.988**	**-8**
5	**13.847**	**α-Endosulfan**	**0.044**	**0.133**	**0.3**	**0.992**	**-22**
6	**14.587**	**4,4-DDE**	**0.045**	**0.138**		**0.993**	**-19**
7	**14.823**	**2,4-DDD**	**0.047**	**0.141**		**0.995**	**-12**
8	**15.176**	**Endrin**	**0.051**	**0.153**		**0.994**	**-11**
9	**15.442**	**β-Endosulfan**	**0.051**	**0.155**	**0.3**	**0.992**	**-26**
10	**15.718**	**2,4-DDT**	**0.087**	**0.264**		**0.992**	**-11**
11	**16.542**	**4,4-DDT**	**0.075**	**0.228**		**0.992**	**-7**
12	**18.486**	**λ-Cyhalothrin**	**0.061**	**0.184**		**0.992**	**16**
13	**19.863**	**α-Cypermethrin**	**0.067**	**0.204**	**0.5**	**0.992**	**29**
14	**21.197**	**Deltmethrin**	**0.096**	**0.29**		**0.999**	**29**

Table-3: Formulae used for different method validation parameters

%Recovery	$\%Recovery = \frac{Amount\ observed}{Amount\ added} x\ 100$
Repeatability	**In term of %RSD**
LOD	$LOD = \left(\frac{\text{S. D. of regression line}}{\text{slope}}\right) \text{x } 3.3$
LOQ	$LOQ = \left(\frac{\text{S. D. of regression line}}{\text{slope}}\right) \text{x } 10$
Matrix effect	$ME(\%) = \left(\frac{Slope\ of\ standard\ in\ Matrix}{Slope\ of\ standard\ in\ hexane} - 1\right) x\ 100$

Table-4: Comparison of results obtained in ERP and FSRI.

Concentration x Labs	α-HCH	Heptachlor	Chlorpyrifos	Methidathion	α-Endosulfan	4,4-DDE	2,4-DDD
0.25µg/g x ERP	116.03 A	121.90 A	137.77 A	96.23 A	117.93 A	101.90 AB	93.20 AB
0.5µg/g x ERP	80.43 A	77.27 A	66.90 A	93.83 A	76.40 A	65.23 B	71.57 B
2.5µg/g x ERP	75.97 A	90.67 A	96.50 A	103.50 A	128.93 A	77.27 B	95.53 AB
0.25µg/g x FSRI	110.47 A	119.23 A	152.97 A	119.40 A	139.47 A	132.60 A	126.93 A
0.5µg/g x FSRI	105.93 A	115.90 A	142.23 A	144.13 A	122.13 A	97.60 AB	110.43 AB
2.5µg/g x FSRI	90.27 A	87.70 A	116.10 A	121.53 A	99.77 A	73.97 B	87.80 AB
LABS	NS	NS	S	NS	NS	S	S
CONCENTRATIONS	NS	NS	NS	NS	NS	NS	NS
Conc. ˣLabs	NS	NS	NS	NS	NS	NS	NS

Concentration x Labs	Endrin	β-Endosulfan	2,4-DDT	4,4-DDT	λ-Cyhalothrin	α-Cypermethrin	Deltamethrin
0.25µg/g x ERP	96.13 AB	99.83 A	95.83 A	140.97 A	97.60 A	120.27 AB	104.60 AB
0.5µg/g x ERP	70.00 B	129.33 A	67.23 A	75.90 A	90.60 A	118.03 AB	78.17 B
2.5µg/g x ERP	101.27 AB	150.63 A	94.67 A	117.70 A	118.77 A	119.53 AB	97.07 AB
0.25µg/g x FSRI	129.17 A	161.50 A	115.47 A	130.83 A	118.27 A	144.33 A	132.00 A
0.5µg/g x FSRI	107.23 AB	143.23 A	93.97 A	89.70 A	92.07 A	89.77 AB	96.90 AB
2.5µg/g x FSRI	83.27 AB	106.00 A	75.47 A	61.87 A	69.93 A	70.63 B	92.00 AB
LABS	NS	NS	NS	NS	NS	NS	NS
CONCENTRATIONS	NS	NS	NS	NS	NS	NS	NS
Conc. ˣLabs	NS	NS	NS	NS	NS	NS	NS

The LOD range was 0.041 to 0.096 µg/g and LOQ range was 0.125-0.264 µg/g for 14 analyzed pesticides. As shown in Table-2, LOQ values for all pesticides are lower than the reference MRLs given by FAO codex alimentarius. So, this method can detect the residues of these pesticides below FAO Codex Alimentarius MRLs.

Linearity

The calibration curve of matrix matched standards at 0.05, 0.1, 0.25, 0.5, 1.0 and 2.5 µg/g, showed a satisfactory linearity and a strong correlation for 13 pesticides $R^2 \geq$ (0.991 to 0.999). Only Methidathion showed slightly less linearity of 0.9882. The values of linearity (R^2) are given in Table-2.

Matrix Effect

The values of percent matrix effect calculated from the calibration curve of standards in n-hexane and in matrix are given in Table-2. For organochlorine and organophosphates, slight signal suppression and for pyrethroids slight signal enhancement was observed. The values of matrix effect were less than ±20% for α-HCH, heptachlor, chlorpyrifos, methidathion, 4,4-DDE, 2,4-DDD, endrin, 2,4-DDT, 4,4-DDT and λ-cyhalothrin. Only four pesticides (α-endosulfan, β-endosulfan, α-cypermethrin and deltamethrin) showed high matrix effect value. But even these high values never exceeded from ±27. These values are mostly similar or better than values obtained for different pesticides in olive oil using expensive sorbents such as EMR-Lipids and Z-Sep+ [26, 27].

Conclusion and Recommendation

This study reports on development and validation of a fast, efficient and cost-effective method for the determination of 14 analytically difficult pesticide residues in crude cottonseed oil. Extraction with acetonitrile and n-hexane in 10:1 ratio and cleanup with activated charcoal and PSA were found optimum for the analysis of lipophilic pesticides in crude cottonseed oil. Freezing step is recommended to reduce the solubility of fatty compounds in acetonitrile extract. All the method validation parameters including percent recovery (70-120%), precision (%RSD less than 20), LOD (0.041 to 0.096 µg/g), LOQ (0.125-0.264 µg/g), linearity (0.998-0.999) and matrix effect (less than ±20) were in acceptable range

for most of the analyzed pesticides. Planar α-HCH show good average recovery (116% and 78%) with precision values (%RSD 27-33 %) at all spiking level. The same recovery of α-HCH with good precision was observed in inter-lab comparison study. The two-way ANOVA of inter-labs comparison shows non-significant difference for most of the studied pesticides at $p<0.05$. Thus, this method can be confidently used in labs for monitoring of lipophilic pesticide residues in crude cottonseed oil. To detect pesticide at stringent Maximum Residues Limits (MRLs) and to lower the Limit of Detection (LOD) of the method, it is recommended to optimize this method on GC-MS/MS.

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References

1. M. El-Hamidi, F.A. Zaher, *Production of vegetable oils in the world and in Egypt*, an overview, *Bull. Natl. Res. Cent*., **42** (2018).
2. F. Gunstone, *Vegetable oils in food technology: composition, properties and uses*, John Wiley & Sons. p.333. (2011).
3. D.K. Agarwal, P. Singh, M. Chakrabarty, A. Shaikh, S. Gayal, Cotton seed oil quality, utilization and processing, *CICR Tech. Bull* **25** (2003).
4. N.V. Yanishlieva, E.M. Marinova, Stabilisation of edible oils with natural antioxidants, *Eur. J. Lipid Sci. Technol*, **103,** 752 (2001).
5. S.C. Sekhar, B. Rao, Cottonseed oil as health oil, Pertanika *J. Trop. Agric. Sci*, **34,** 17 (2011).
6. M. Khan, H.Z. Mahmood, C.A. Damalas, Pesticide use and risk perceptions among farmers in the cotton belt of Punjab, Pakistan, *Crop Protection*, **67**, 184 (2015).
7. M.A. Khan, M. Iqbal, I. Ahmad, M.H. Soomro, M.A. Chaudhary, Economic evaluation of pesticide use externalities in the cotton zones of Punjab, Pakistan, *The Pakistan development review*, 683 (2002).
8. J.F. García-Reyes, C. Ferrer, M.J. Gómez-Ramos, A.R. Fernández-Alba, A. Molina-Díaz, Determination of pesticide residues in olive oil and olives, *Trends Anal. Chem.* **26,** 239 (2007)
9. Z. Parveen, PhD. Thesis, *Studies on monitoring and persistence of cotton/grain protectant pesticides under environmental/simulated conditions and their effects on food constituents*, University of Karachi, (1993).
10. E.J. Foundation, The deadly chemicals in cotton, Environmental Justice Foundation in collaboration with Pesticide Action Network UK, London, (2007).
11. M.A. Farajzadeh, B. Feriduni, M.R.A. Mogaddam, Determination of triazole pesticide residues in edible oils using air-assisted liquid–liquid microextraction followed by gas chromatography with flame ionization detection, *J. Sep. Sci.* **38,** 1002 (2015).
12. M.A. Farajzadeh, L. Khoshmaram, A.A.A. Nabil, Determination of pyrethroid pesticides residues in vegetable oils using liquid–liquid extraction and dispersive liquid–liquid microextraction followed by gas chromatography–flame ionization detection, *J. Food Compost. Anal.,* **34,** 128 (2014).
13. E. Zahiri, J. Khandaghi, M.A. Farajzadeh, M.R.A. Mogaddam, Combination of dispersive solid phase extraction with solidification organic drop–dispersive liquid–liquid microextraction based on deep eutectic solvent for extraction of organophosphorous pesticides from edible oil samples, *J. Chromatogr. A*, **1627,** 461390 (2020).
14. C. Tsoutsi, I. Konstantinou, D. Hela, T. Albanis, Screening method for organophosphorus insecticides and their metabolites in olive oil samples based on headspace solid-phase microextraction coupled with gas chromatography, *Anal. Chim. Acta.,* **573**, 216 (2006).
15. X. Mao, A. Yan, Y. Wan, D. Luo, H. Yang, Dispersive solid-phase extraction using microporous sorbent UiO-66 coupled to gas chromatography–tandem mass spectrometry: A QuEChERS-type method for the determination of organophosphorus pesticide residues in edible vegetable oils without matrix interference, *J. Agri. Food chem.* **67,** 1760 (2019).
16. N. Chamkasem, T. Harmon, Analysis of Pesticides in Olive Oil Using a Modified QuEChERS Method with LC-MS/MS and GC-MS/MS, *J. Regul. Sci.* **3**, 16 (2015).
17. E.G. Amvrazi, T.A. Albanis, Multiresidue method for determination of 35 pesticides in virgin olive oil by using liquid− liquid extraction techniques coupled with solid-phase extraction clean up and gas chromatography with nitrogen phosphorus detection and electron capture detection, *J. Agric. Food Chem.* **54**, 9642 (2006).
18. P. Cabras, A. Angioni, M. Melis, E.V. Minelli, F.M. Pirisi, Simplified multiresidue method for the determination of organophosphorus

insecticides in olive oil, *J. Chromatogr. A*, **761**, 327 (1997).

19. S. Chawla, H.K. Patel, K.M. Vaghela, F.K. Pathan, H.N. Gor, A.R. Patel, P.G. Shah, Development and validation of multiresidue analytical method in cotton and groundnut oil for 87 pesticides using low temperature and dispersive cleanup on gas chromatography and liquid chromatography-tandem mass spectrometry, *Anal. Bioanal. Chem.*, **408**, 983 (2016).
20. T.N. Rao, K. Apparao, M. Babu, M. Rao, Determination of four neonicotinoid insecticide residues in cotton seed oil using matrix solid-phase dispersion coupled to high-performance liquid chromatography with ultraviolet detection, *Int. j. pharm. chem. biol. sci.*, **2**, 447 (2012).
21. M. Anastassiades, S.J. Lehotay, D. Štajnbaher, F.J. Schenck, Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce, *J. AOAC. Int.*, **86**, 412 (2003).
22. R. Muhammad, K. Ahad, F. Mehboob, Extraction techniques for pesticide residues analysis in edible oils and role of sorbents in cleanup, *Sep. Sci. Plus*, **3**, 51 (2020).
23. B.I. Kaipper, L.A. Madureira, H.X. Corseuil, Use of activated charcoal in a solid-phase extraction technique for analysis of pesticide residues in tomatoes, *J. Braz. Chem. Soc.*, **12,** 514 (2001).
24. R. T. Yang, Adsorbents: fundamentals and applications, *John Wiley & Sons*, (2003).
25. C. Ferrer, M.J. Gómez, J.F. García-Reyes, I. Ferrer, E.M. Thurman, A.R. Fernández-Alba, Determination of pesticide residues in olives and olive oil by matrix solid-phase dispersion followed by gas chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry, *J. Chromatogr. A.*, **1069**, 183 (2005).
26. D. Moreno-González, J.F. Huertas-Pérez, A.M. García-Campaña, L. Gámiz-Gracia, Determination of carbamates in edible vegetable oils by ultra-high performance liquid chromatography–tandem mass spectrometry using a new clean-up based on zirconia for QuEChERS methodology, *Talanta*, **128**, 299, (2014).
27. R. Lopez-Blanco, R. Nortes-Mendez, J. Robles-Molina, D. Moreno-Gonzalez, B. Gilbert-Lopez, J.F. Garcia-Reyes, A. Molina-Diaz, Evaluation of different cleanup sorbents for multiresidue pesticide analysis in fatty vegetable matrices by liquid chromatography tandem mass spectrometry, *J. Chromatogr. A.*, **1456**, 89 (2016).
28. SANTE/12682/2019. Method validation and quality control procedures for pesticide residues analysis in food and feed. (2020)