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Original article

Gas chromatography–mass spectrometry based sensitive analytical approach to detect and quantify non-polar pesticides accumulated in the fat tissues of domestic animals

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ABSTRACT

A fast and simple technique is proposed for the detection and quantitative determination of six non-polar pesticides including pyrethroids (cypermethrin, deltamethrin), organochlorines (hexachlorobenzene, α-hexachlorocyclohexane) and organophosphorus (chlorpyrifos, fenitrothion) accumulated in fat tissues of local cattle, sheep and goats. Gas chromatography coupled to mass spectrometry detection (GC–MS) adapted to cleanup procedures based on solid-phase extraction from QuEChERS method was adopted. The work was performed for quantitative affirmation of most customarily used pesticides in Sulaymaniyah, Kurdistan Region of Iraq and also the impact of boiling (100 °C, 30 min) and broiling (176 °C, 20 min) on chosen pesticides was evaluated. Among the results of 150 fat samples presented, the dominant compound in cattle samples was hexachlorobenzene (0.236 mg kg^{-1}); while, in sheep and goats it was deltamethrin (0.248 and 0.122 mg kg^{-1} respectively). Boiling reduced pesticide concentration significantly ($P < 0.05$) and the most reduced group was pyrethroids in both techniques. Good responses for the six analytes were obtained at validation level of 0.01–0.1 mg kg^{-1}. The linear coefficient was between 0.9997 and 0.9999 and limits of detection (LOD) and quantification (LOQ) ranged 0.0052–0.014 mg kg^{-1} and 0.015–0.044 mg kg^{-1} respectively. Acceptable recoveries (81.5–98.6%) and relative standard deviation (0.3–9.3%) were obtained in different spiked levels. The validation results confirmed that the proposed GC–MS technique can be utilized as a dependable screening apparatus for the quantitative screening of studied pesticides in fat tissues with accuracy and sensitivity, if deployed along with solid-phase extraction based QuEChERS method.

1. Introduction

Pesticides are chemicals applied for preventing, destroying or controlling pests considered as vectors of animal, plant and human diseases (Solomon et al., 2014) including herbicides, insecticides, rodenticides and fungicides (Smalling et al., 2013). In veterinary medicine, they are utilized for treatment of external parasites (Pan et al., 2014). The same pesticides in agriculture are used for treatment of crops before and after harvests to control diseases; as well as, used for indoor/outdoor pest control (Burns and Pastoor, 2018). Nevertheless, the inconsiderate use of pesticides has resulted in a widespread distribution of residues on ground, in soil and water, crops, seasonal grasses and animal feeds. Thus, a part of pesticide could be stored in animals tissues when they feed on fodder contaminated with these pesticides (Gullick et al., 2016).

These contamination cycles can prompt bioaccumulation of persistent pesticides in foods of animal source, such as meat, fat and milk, lastly transferred to humans via the food chain (La Rocca and Mantovani, 2006). Non-polar pesticides are more hazardous than polar pesticides because of their inordinate inclination to get stored in muscles to fat tissues and little amounts being excreted through kidneys (Lainsbury, 2018). The ingestion of tiny doses daily or weekly causes many chronic health issues including life-long illnesses, deformities in newborn, neurological

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problems, early onset cancers, anemia and cardiovascular illnesses (Kaonga et al., 2015).

Pesticides are classified into five main groups: the pyrethroids (PYRs), organochlorines (OCs), organophosphates (OPs), carbamates (CARs), and phenylpyrazoles (PPs) (IRAC, 2017), but, pyrethroids, organochlorines, organophosphorus and carbamates are the main pesticides groups. Fat tissues are complex matrices which require accurate extraction techniques and proficient detection strategies before quantification (Zidane et al., 2019). Most detection techniques are tedious and utilize huge amounts of solvents to extract analytes and are excessive in cost. However, chromatographic procedures are still considered as the most appropriate, sensitive and dependable techniques to distinguish and evaluate pesticide residues in animal tissues (Neelam et al., 2017).

Among chromatography techniques, gas chromatography is one of the most applicable ones. But, much trouble shooting is required when it is used for detection of multi analytes (Han et al., 2016). Recently, researchers used GC–MS for detection of multi analytes in complex matrixes but faced some troubles shooting up during analysis as well as, consumes time and needs large quantities of organic solvents (Niu et al., 2017). This could be due to the method used for detection of several residues in a complex matrix by a single injection (Stefanelli et al., 2009) especially if the matrices are meat and fat tissues (Kiranmayi et al., 2016), like liver and kidney (Letta and Attah, 2013).

Now, researchers believes that the use of MS may overcome the problems arising from chromatographic interference that occurs with ECD and can provide better sensitivity especially for the determination of OCPs (Han et al., 2016). On the other hand, it needs modified extraction and clean up procedures depending on matrix and analyte types and is difficult to optimize a reliable screening method for several groups of pesticides in a complex tissue (Cao et al., 2015).

Methods of cooking have different effects on pesticide reduction (Yun-Sang et al., 2016). Recently, (Kiranmayi et al., 2016) used boiling process in bovine muscle; found that the technique has less effect to reduce pesticides concentrations compared to pressure cooking process. This result is different if different cooking types used such as in (Muthukumar et al., 2010) study, who found that the reduction rate of OCs in beef is less with boiling compared broiling. The reduction rate depends on kind of tissues in and/or animals' species and pesticides class (MacBean, 2015). Yet, there is hardly any study performed to assess the effect of studied boiling (100 °C, 30 min) and broiling (176 °C, 20 min) temperature on the studied pesticides in fat tissues.

The center of motivation behind this investigation is to obtain a reliable and sensitive screening method of GC–MS and adapt it to a modified QuEChERS for detection three different groups of pesticides in fat tissues by optimization of experimental conditions in both chromatographic and sample preparation through acceptable validation procedures. In addition, the aim was to assess the impact of the most two customarily utilized cooking technique on pesticides residual levels.

2. Materials and methods

2.1. Sample collection

Fat samples (n = 150) were randomly collected from adult cattle (n = 50), sheep (n = 50), and goat (n = 50) carcasses at slaughterhouse in Sulaimaniyah city/Kurdistan region of Iraq. Each sample was sliced into three equivalent portions (50 g × 3 = 150 g). The first 50 g was directly prepared for extraction and analysis. The second 50 g and third 50 g samples were boiled (100 °C, 30 min) and broiled (176 °C, 20 min) respectively. Around three blank fat samples were acquired from animals free from all pesticides. The blank samples were tested to confirm that they were free from the studied pesticides. Then aliquot of blank samples was spiked for selectivity study, the rest portion was used for recovery, matrix matched standards calibration, and sensitivity studies.

2.2. Chemicals and apparatus

All solvents were of pesticide-residue grade. Pesticides standards of cypermethrin (CMT)(94%), deltamethrin (DMT)(99%), hexachlorobenzene (HCB)(98%), α-hexachlorocyclohexane (α-HCH) (98%), chlorpyrifos (CPS)(96%) and fenitrothion (FTN)(95.5%) were obtained from Dr. Ehrenerstorfer™ (Augsberg, Germany). Acetonitrile (ACN) (99.5%), acetic acid (99.9%), primary secondary amine (PSA) 40 μm particle size, octadecylsilane (C18, 50 μm), hexane, sodium chloride (NaCl), and anhydrous magnesium sulphate ($MgSO_4$) were obtained from Merck Ltd. Syringe filters (0.25, 0.45 μm), and capillary columns, 30 m DB-5, with an internal diameter of 0.25 mm and thickness of 0.1 μm were purchased from Supelco Analytical Co., UK.

2.3. GC–MS system

The pesticides' concentrations were detected by gas chromatography, along with mass spectrometry. This was performed using a QP GC–MS gas chromatograph from Shimadzu (Kyoto, Japan), equipped with a mass-selective detector and capillary column of a 30 m DB-5, with 0.25 mm internal diameter and 0.1 μm film thicknesses. The injector, interface, and ion source temperatures were 250 °C, and splitless injection (1.0 min) was performed using helium as the carrier gas with a flow rate of 0.75 mL/min. The oven temperature was set to increase at a pace of 4 °C/min from 120 °C to 190 °C. Next, the temperature was increased from 32 °C/min to 270 °C, and held for 4 min. The mass spectrometer was operated on scan mode, between m/z 45 and m/z 475 Daltons, which can detect analytes in a solvent to a limit of 1.0 mg/kg. The injection volume was 50 μL with a splitless injection mode.

2.4. Heat treatment

Samples were made into small patties of about 1.5 cm thickness. Fat samples (n = 150) were placed separately into low-density water-impermeable polyethylene bags and cooked in boiling water (100 °C, 30 min) using a water bath (Memmert W200, Germany). Similarly, the other 150 samples were put in a glass bowl and broiled in a preheated air oven (Memmert 93/42 EEC, Germany), at 176 °C for 20 min, being turned over every 5 min. During the experiment, the water bath and oven temperatures were monitored with a thermometer and an oven thermometer gauge respectively.

2.5. Method of validation

Validation method for this study was carried out according to the internationally accepted SANTE/11813/2017 criteria, i.e., selectivity, recovery percentages, precision, linearity, and sensitivity. Method selectivity was tested by injection of five independent fat sample extracts into GC–MS. The absence of interfering peaks above a signal-to-noise ratio of 3 at the retention time window of interest was checked for each analyte. The target retention times of the analytes were identified by separately injecting analytical standards (10 mg/L) into the GC–MS apparatus.

Multi-standard solutions were injected in to the GC–MS to check maximum retention time tolerance range (±0.2 min), and improve analytical validation. Recovery was determined by comparing the obtained concentrations with the same concentrations

of the pesticides prepared in the dissolvent. The inter-day precisions (3 replicates in 3 successive days) were determined by analyzing all spiked levels through the injection of multi-standard solutions, containing six analytes, at concentrations of 0.010, 0.020, 0.050, and 0.100 mg/kg for control matrices. Since a different maximum residue limit (MRL) has been established for each analyte in fat, 0.01–0.1 mg/kg of standard solutions were spiked into blank matrices to obtain the highest method reliability during screening.

The linearity test in fat was carried out by injecting six matrix-matched standards for calibration studies. Limits of detection (LOD) and quantitation (LOQ) were determined based on a signal-to-noise ratio, and concentrations showing peak intensity of signal-to-noise ratio of 3 and 10 were designated as LOD and LOQ, respectively.

2.6. Matrix-matched calibration (MMC)

According to SANTE/11813/2017 criteria, matrix constituents negatively influence the quantitation of target analyses in GC–MS analyses and may increase or decrease the analytical signals. Hence, matrix-matching (standards added to blank extracts) is performed mainly to minimize matrix effects. For the preparation of analytical MMC curves, individual stock solutions of HCB, α-HCH, FTN, CPS, CMT, and DMT were prepared in acetonitrile in Pyrex glass vials at a concentration of 100 mg/L and stored at −20 °C in dark amber bottles. Working standard solutions (WSS) was prepared at a concentration of 50 mg/L by diluting stock solution in acetonitrile. Matrix-matched calibration standards was prepared just before injection by diluting the working solutions and spiked into extracted blank samples to obtain concentrations of 0.010, 0.020, 0.050, 0.100, 0.200 and 0.500 mg/kg. The MMC curves for each compound were built, and coefficients (r^2) of calibration curves were used to assess linearity.

2.7. Preparation of fat samples using QuEChERS method

Extraction and cleanup procedures used were adapted with the original developed QuEChERS method (Mastovska and Lehotay, 2006, and Lehotay et al., 2005, with some modifications. The samples were thawed at 4 °C overnight and blended prior to use. Blended samples (2 g) were transferred to a 50 mL Falcon tube. For the first step of extraction, double - phase extraction was performed by adding 5 mL hexane to the mixture and agitated in a vortex mixer for 1 min, next, 10 mL ACN (containing 1% (v/v) of A.A) was added and mixed by vortex mixer for 1 min. Then, 1.6 g anhydrous $MgSO_4$, and 0.5 g NaCl were added and the mixture was homogenized again in a vortex for 1 min. The mixture was centrifuged at 3000 rpm for 3 min to separate the phases (liquid-liquid partition). The upper phase, corresponding to the organic solvent hexane, was drawn off with the aid of a pipette and discarded. The next phase, corresponding to the organic solvent ACN, was transferred to a tube containing 70 mg of the adsorbent PSA and 150 mg $MgSO_4$. The tube was shaken by hand for half a min and centrifuged at 3000 rpm for 1 min. The supernatant was filtered by syringe filters (0.45 and 0.20 μm successively). The filtered supernatant of each sample was subjected to evaporation under a stream of nitrogen at 45 °C to remain 1 mL and stored at 4 °C. Subsequently, Samples were transferred for analysis with GC–MS.

2.8. Data processing and statistical analysis

Matrix-matched standards data were subjected analysis using MS Excel (Analysis Tool Pak, Regression) for sensitivity. The obtained real samples' data, concentration data for each pesticide and animal species plus heat treatment data were subjected to the Analysis of Variance (One-way ANOVA, Post Hoc = Duncan, using SPSS software (Version 18.0) and multiple ranges were used to significantly compare means ($p < 0.05$).

3. Results and discussion

3.1. Sample cleanup optimization and chromatographic separation conditions

In this work, the extraction of pesticides in fat samples was effectively performed by a fast cleanup methodology comprising an extraction step with organic solvent, then consecutive SPE steps. In the first test, unacceptable recovery was obtained due to little ACN, PSA volume and two steps of filtration in our extraction method. The utilization of acetonitrile prompted high recovery values of pesticides in fat samples; as well as, the chromatographic partition (GC–MS), generally provides extraordinary sensitivity to numerous of pesticides compounds.

The efficiency of the extraction process was evaluated by taking in to account the recovery and relative standard deviation (RSD) values; therefore, optimization of the extraction process was carried out considering their results. Many parameters affecting the extraction ability were optimized to accommodate the best result including: types of solvent, polarity and cleanup sorbents involving ACN and PSA (Fernandez-Alvarez et al., 2008).

The QuEChERS method used for extraction was carried out with some modifications such as high amounts of ACN, PSA and double filtration; hence, acceptable recovery obtained, as these two sorbents remove fatty acids from other extracted components which considered the main co-extract to the non-polar pesticides in fat samples and double filtration steps prompted cleanup in prepared solution before injection (Harshit et al., 2017). Hence, QuEChERS method is considered as advisable due to benefits including speed, ease of use, less cost, and; good performance aspects; as well as, applicability to complex matrices and analytes which is not available in other methods used for extraction of pesticide residues in foods.

The CMT, DMT, HCB, α-HCH, CPS, and FTN presented peaks area, with no enantiomeric pairs and isomers in the standards. The retention times of the analytes were: 2.93 min HCB, 3.83 min α-HCH, 5.46 min FTN, 7.44 min CPS, 8.52 min CMT and 9.75 min DMT (Fig. 1, [1]).

3.2. Calibration curves and limits of detection and quantitation

The analytical standards for the six analytes were injected in to the GC–MS, with the equipment in SCAN mode. Then, the retention time of each analyte was found, and by the mass spectra, required ions were selected to be analyzed in SIM mode. The improvement of the analytical validation and extraction methods were carried out in SIM mode also (Fig. 1, [1]). Method selectivity was tested by the analysis of blank preparations of fat samples with no interferes presented in the monitoring window (Fig. 1, [3]).

Linearity performed through spiking matrix matched standards to blank samples after extraction (Fig. 1, [2]). An acceptable linearity was found for all target analytes in the range 0.01–0.5 mg kg^{-1} with correlation coefficients $\geq$ 0.9997. The goodness-of-fit of the data to the calibration curve was assessed through the response factor distribution, by calculating the signal-to-concentration ratio (y/x) for each test point. Consequently, the xi/yi ratios were tested to confirm that their distribution from the average value of signal-to-concentration ratio was less than ± 10%. The acceptability of regression model was also confirmed by using all the calibration datasets (6 calibration points, 3 replicates at each calibration

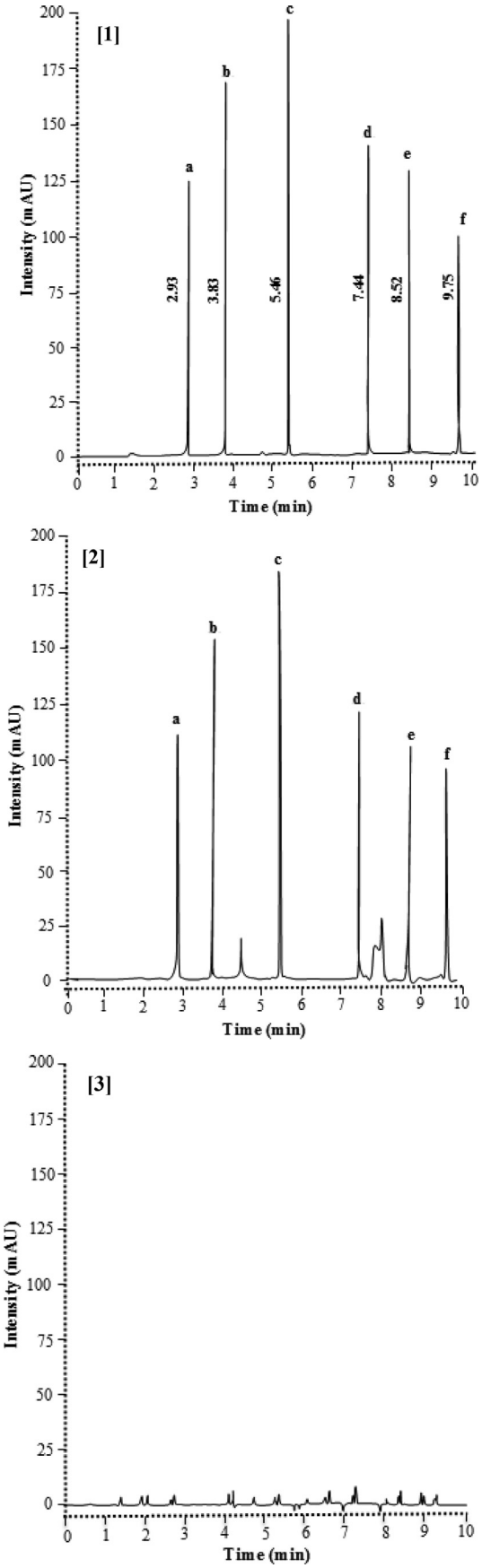

Fig. 1. Ion Chromatograms of QuEChERS method. 1. Multi-standard solutions 10 mg L^{-1}; 2. Spiked blank sample after extraction 0.1 mg kg^{-1}; 3. Blank fat samples. A. Hexachlorobenzene; B. α-Hexachlorocyclohexane; C, Fenitrothion; D. Chlorpyrifos; E. Cypermethrin; F. Deltamethrin.

point). Linear regression curves all around fitted the experimental data whose calibration parameters, evaluated for each pesticide presented in Table 1. The pesticides matrix matched standard solutions' smallest calibration level in the chromatograms. The limits of detection (LOD) and quantitation (LOQ) were predicted at a signal-to-noise ratio of 3 and 10, conjointly.

In this study, the limits of detection (LOD) and quantification (LOQ) values ranged from 0.0052–0.014 and 0.015–0.044 mg kg^{-1} respectively. The detection limits (LOD) of all studied pesticides were below their respective pesticides' MRLs (Table 1), which inferred that the applied method validated to detect the studied pesticides are at adequately low level. The LOD values in our study could be better than those obtained by analytical method designed for the analysis of mentioned analytes by GC and MS (Khay et al., 2009) and ECD (Fan et al., 2013; Wu et al., 2016; Liu et al., 2016). Because lower LOD values mean lower probability of false negative results and this feature is the key-factor for determination in analytical methods.

3.3. Precision and recovery

The recovery for each analyte, was verified by calculating the average recovery which was in compliance with the recovery range of 70–120%, set in the formal documents of SANTE/11813/ 2017. The recovery achieved at four analyte concentrations in the fat, preferred recovery values were obtained at concentrations of (0.01, 0.020, 0.05, 0.1 mg kg^{-1}). The recovery percentages were in the range of 81.5–98.6% (Table 2). This result demonstrates a good accuracy of the method, which can be considered as a useful tool for the screening of studied pesticides in fat tissues. Related Standard Deviation arranged from (RSD) 0.3–9.3%, meets the SANTE/11813/ 2017 criteria (see Table 3).

The regression was statistically analyzed by the coefficient of determination (r^2), and the homoscedasticity assumption was evaluated by plotting residue versus concentration the determination coefficients were $\geq$ 0.9997 for the studied analytes of fat samples (Table 2). The residual plot displayed that the errors were haphazardly distributed around the concentration axis.

3.4. Pesticides concentration

In this work, deltamethrin (DMT) in sheep samples presented the highest concentration (0.248 ± 0.010 mg kg^{-1}); followed by, hexachlorobenzene (HCB) in cattle samples (0.236 ± 0.009 mg kg^{-1}); while, fenitrothion (FTN) presented the lowest residual concentrations in all samples of goats (0.029 ± 0.003 mg kg^{-1}), sheep (0.089 ± 0.004 mg kg^{-1}) and cattle (0.077 ± 0.003 mg kg^{-1}) (Table 2).

Existence of high level of deltamethrin and cypermethrin in sheep and goat fat tissues have also been found in sheep and goats near Sulaimaniyah by Abdurrahman in 2016, and same year Khashan found small amount in cattle tissues also. Same results were also found in cow and ewes milk (Al-Zahra, 2017). The presence of high level of deltamethrin and cypermethrin could be attributed due to the regular use of these pesticides by almost all farmers in Iraq to control ticks, flies, fleas, lice, mites and dipping animals on pyrethroids pools or directly spray on small animal fields, or treat of seasonal crops, due to their rapid effects, low cost and less toxicity compared with other pesticides (Lainsbury, 2018).

High levels of Hexachlorobenzene (HCB) and Hexachlorocyclohexane (α-HCH) residual concentrations have been detected in cattle samples and vice versa in sheep and goat samples (Table 2) which is attributed to the fact that those pesticides are applied in agriculture in large scale, such as HCB to treat wheat and control the fungal diseases and α-HCH to control mosquito that spread malaria and as an antifouling agent, (MacBean, 2015), which may explain the higher concentrations of this compound in cattle used

Table 1
Determination of linearity range, regression equation for analytical curves, coefficients (r^2), Limit of Detection (LOD) and Quantification (LOQ) for studied analytes.

Analytes	Linearity range	Linearity equation	r^2	LOD (mg kg^{-1})	LOQ (mg kg^{-1})
HCB	0.01–0.5	y = 1139.7x + 1142	0.9998	0.0059	0.017
α-HCH	0.01–0.5	y = 1572.2x + 414.92	0.9999	0.0054	0.016
FTN	0.01–0.5	y = 1860.5x − 2091.8	0.9999	0.0064	0.019
CPS	0.01–0.5	y = 1266.3x − 385.31	0.9999	0.0052	0.015
CMT	0.01–0.5	y = 1082.7x + 3710.9	0.9997	0.0148	0.044
DMT	0.01–0.5	y = 908.83x − 1953.9	0.9998	0.0111	0.033

r^2, coefficient; LOD, Limit of Detection; LOQ, Limit of Quantification; CMT, Cypermethrin; DMT, Deltamethrin; HCB, Hexachlorobenzene; α-HCH, alpha-Hexachlorocyclohexane; CPS, Chlorpyrifos; FTN, Fenitrothion.

Table 2
Recovery, relative standard deviations (RSD %), in spiked fat samples.

Matrices		Recovery ± RDS (%)				
Con. (mg kg^{-1})	HCB	α-HCH	CMT	CPS	DMT	FTN
0.01	87.7 ± 5.3	83.2 ± 7.6	91.2 ± 4.9	82.5 ± 7	88.7 ± 4.5	81.5 ± 5
0.020	85.9 ± 3.8	86.8 ± 6.9	88.4 ± 2.3	87.6 ± 9.3	86.3 ± 6.8	84.5 ± 3.4
0.05	91.7 ± 5	90 ± 5.2	93.5 ± 2.1	95.6 ± 3.3	92.5 ± 2.8	90.8 ± 2.2
0.10	98.3 ± 0.4	98.2 ± 0.3	98.6 ± 0.3	97.9 ± 1.2	98.3 ± 0.67	97.6 ± 0.4

Table 3
Mean residual levels of analyzed pesticides concentrations in cattle, sheep and goat fat samples.

Pesticides types	Fresh(Cattle) fat (mg kg^{-1} ± RSE)	Fresh(Sheep) fat (mg kg^{-1} ± RSE)	Fresh(Goats) fat (mg kg^{-1} ± RSE)
CMT	$0.075^{a,v}$ ± 0.005	$0.169^{c,wx}$ ± 0.005	$0.087^{b,w}$ ± 0.002
DMT	$0.104^{ab,w}$ ± 0.007	$0.248^{c,z}$ ± 0.010	$0.122^{b,y}$ ± 0.006
HCB	$0.236^{c,y}$ ± 0.009	$0.185^{b,xy}$ ± 0.006	$0.114^{a,xy}$ ± 0.004
α-HCH	$0.194^{b,x}$ ± 0.008	$0.191^{b,y}$ ± 0.005	$0.109^{a,x}$ ± 0.005
CPS	$0.192^{c,x}$ ± 0.006	$0.156^{b,w}$ ± 0.004	$0.084^{a,w}$ ± 0.004
FTN	$0.077^{b,v}$ ± 0.003	$0.089^{c,v}$ ± 0.004	$0.029^{a,v}$ ± 0.003

$^{a,b,c.}$ Different superscript letters denote significant differences within row ($p < 0.05$).
^{v,w,x,y,z} Different superscript letters denote significant differences within column ($p < 0.05$).

in this study. Same results were observed in several other studies of such as Kiranmayi et al. (2016).

The chlorpyrifos (CPS) and fenitrothion (FTN) pesticides were found to be in the lowest level in this study, which is in consistence with the results of Muhammad et al. (2010). A contrary result have also been reported by Kumar et al. (2011) in animals grazing outdoor such as cattle and buffalo which could be due to less use of these pesticides by our farmers compared with DMT, CMT, HCB and α-HCH. Recently, CPS is used to control of pests in a wide range of crops, including cereals, some fruit and vegetables; hence, found in high concentration above maximum residue limits in vegetables (Harshit et al., 2017).

3.5. Heat treatment

In this study, boiling process (100 °C, 30 min) reduced pesticides concentrations significantly ($p < 0.005$) in cattle, sheep and goat fat tissues (Table 4). The most reduced group was pyrethroids (34.43–39.05%) including CMT and DMT. Followed by Organochlorines including HCB and α-HCH which were reduced by 33.33–37.11%.While, the least reduction level (21.09–29. 69%) was found in OP pesticides including CPS and FTN (see Table 5).

On the other hand, broiling (176 °C, 20 min) could not reduce pesticides concentration significantly in which the most reduced

Table 4
Effects of cooking methods on the levels of pesticides concentrations (mg kg^{-1}) in cattle, sheep and goats fat samples.

Pesticide	Species	Raw fat (mg kg^{-1} ± RSE)	Boiled at 100 °C for 30 min	Broiled at 175 °C for 20 min
CMT	Cattle	0.075^{bv} ± 0.005	0.046^{av} ± 0.003	0.064^{bv} ± 0.004
	Sheep	0.169^{cwx} ± 0.005	0.103^{aw} ± 0.003	0.138^{bw} ± 0.004
	Goat	0.087^{cw} ± 0.002	0.056^{aw} ± 0.002	0.073^{bw} ± 0.001
DMT	Cattle	0.104^{cw} ± 0.007	0.066^{aw} ± 0.004	0.086^{bw} ± 0.006
	Sheep	0.248^{cz} ± 0.010	0.158^{az} ± 0.006	0.207^{by} ± 0.008
	Goat	0.122^{cy} ± 0.006	0.080^{axy} ± 0.004	0.105^{by} ± 0.005
HCB	Cattle	0.236^{cy} ± 0.009	0.150^{az} ± 0.006	0.202^{by} ± 0.008
	Sheep	0.185^{cxy} ± 0.006	0.120^{ay} ± 0.004	0.168^{bx} ± 0.006
	Goat	0.114^{cxy} ± 0.004	0.076^{ax} ± 0.003	0.101^{by} ± 0.004
α-HCH	Cattle	0.194^{cx} ± 0.008	0.122^{ax} ± 0.005	0.168^{bx} ± 0.007
	Sheep	0.191^{cy} ± 0.005	0.125^{ay} ± 0.003	0.168^{bx} ± 0.004
	Goat	0.109^{bx} ± 0.005	0.072^{ax} ± 0.003	0.094^{ax} ± 0.008
CPS	Cattle	0.192^{cx} ± 0.006	0.135^{ay} ± 0.004	0.170^{bx} ± 0.006
	Sheep	0.156^{cw} ± 0.004	0.115^{ax} ± 0.003	0.140^{bw} ± 0.004
	Goat	0.093^{aw} ± 0.004	0.068^{ax} ± 0.006	0.083^{ax} ± 0.008
FTN	Cattle	0.077^{cv} ± 0.003	0.059^{aw} ± 0.002	0.069^{bv} ± 0.003
	Sheep	0.089^{bv} ± 0.004	0.069^{av} ± 0.003	0.081^{bv} ± 0.004
	Goat	0.029^{av} ± 0.003	0.022^{av} ± 0.002	0.026^{av} ± 0.003

"Raw fat" values represent mean concentration (mg kg^{-1} ± RSE). Values of the "boiled and broiled" columns represent the percentage of reduction after treatment. ^{a,b,c}: Different superscript letters denote significant differences within row ($p < 0.05$). ^{v,w,x,y,z}: Different superscript letters denote significant differences within each species, between each pesticide ($p < 0.05$).

Table 5
Effects of cooking methods, on the levels of pesticides with reduction percentages (RD %) of concentrations (mg kg^{-1}) in fat of cattle, sheep and goats.

Pesticide	Species	Raw fat (μg/g ± RSE)	RD% in boiled samples	RD% in broiled samples
CMT	Cattle	0.075 ± 0.005	−38.67	−14.33
	Sheep	0.169 ± 0.005	−39.05	−18.34
	Goat	0.087 ± 0.002	−35.63	−16.09
DMT	Cattle	0.104 ± 0.007	−36.54	−17.31
	Sheep	0.248 ± 0.010	−36.29	−16.53
	Goat	0.122 ± 0.006	−34.43	−13.93
HCB	Cattle	0.236 ± 0.009	−36.44	−12.41
	Sheep	0.185 ± 0.006	−35.14	−12.05
	Goat	0.114 ± 0.004	−33.33	−11.40
α-HCH	Cattle	0.194 ± 0.008	−37.11	−13.40
	Sheep	0.191 ± 0.005	−34.55	−12.04
	Goat	0.109 ± 0.005	−33.94	−13.74
CPS	Cattle	0.192 ± 0.006	−29.69	−11.46
	Sheep	0.156 ± 0.004	−26.28	−10.26
	Goat	0.084 ± 0.004	−26.88	−10.75
FTN	Cattle	0.077 ± 0.003	−23.38	−10.39
	Sheep	0.089 ± 0.004	−21.09	−8.99
	Goat	0.029[a] ± 0.003	−24.14	−10.34

concentration was achieved in pyrethroids (18.34–13.93%) referring CMT and DMT; followed by, organochlorines (13.74–11.40%) involving α-HCH and HCB in cattle, sheep and goats samples. The least reduction percentages was presented in organophophorus pesticides including FTN 8.99–10.39% in cattle, sheep and goats samples.

Generally, the maximum reduction was noticed in pyrethroids and the minimum reduction noticed was in organophosphorus pesticides. This might be due to chemical and physical properties of these pesticides and variation in susceptibility to heat among different chemical compounds in pesticides that are effected by boiling and broiling (Muthukumar et al., 2010).

The reduction in levels of pyrethoid and organochlorines are almost identical to each other, but far from organophosphorus, however there is no strong correlation between a single physiochemical property of pyrethoid, organochlorines and organophosphorus pesticides. Since various parameters involving molecular weight, volatility (vapor pressure), hydrolysis rate and water solubility impacts reduction rate, no critical statement can be reported about the degree that each parameter contribute to the amount loss of pesticides during boiling or broiling. Hence, a study could be performed for a larger group of pyrethroids, organochlorines and organophosphorus to find out more about mechanisms involved in this process.

There were also non-significant differences between animal species in terms of pesticides reduction level by boiling and broiling process but only tiny reduction presented in those pesticides which had been found in low concentrations such as those found in goat samples; while, high reduction levels were found in cattle and sheep samples because relatively high pesticide concentration were found in their tissues (Table 4).

All samples showed high reduction level in boiling at 100 °C (30 min) compared with broiling at 176 °C (20 min). Additional boiling time may have vital effect in reducing concentrations to more than one third. Coincidently, it dissolves and washes away water-soluble vitamins and 60–70% of minerals (Yun-Sang et al., 2016). On the other hand, boiling is preferred to obtain maximum nutrition value without sacrificing flavor (Singh, 2015), but besides its poor ability to destroy pesticides, broiling also converts fatty acids to carcinogenic substances such as advanced glycation end products (AGEs) (Nguyen and Katta, 2015).

4. Conclusion

In this study, a sensitive and reliable analytical method was used for detection and quantification of six pesticides in cattle, sheep and goat fats tissue. The method was validated through the evaluation of linearity, detection and quantitation limits, selectivity, recovery and precision. While optimizing the chromatographic conditions, minimal sample preparation cleanup was applied to ensure good results in terms of rapidity, response sensitivity, and separation efficiency. The results of the method validation, performed according to the European Commission directives, demonstrated that the proposed method is well suited to satisfy to accurate screening of pyrethroids (deltamethrin, cypermethrin), organochlorines (hexachlorobenzene and α-hexachlorocyclohexane) organophosphorus(chlorpyrifos and Fenitrothion) in fat tissues. The use of GC–MS for detection, solid phase extraction, and QuEChERS preparation method could be an inexpensive, fast, and easy method and can be successfully employed for the accurate detection and quantification of the studied pesticides in fat tissues. The technique also enables to provide effective sample cleanup, demonstrated by the fact that the recovery and precision values obtained were within the acceptable ranges according to international standards.

All of the six commonly used pesticides were found in cattle, sheep and goats samples as residues and their levels of contamination were exceeding and higher than MRLs specified by EC. Effects of boiling at 100 °C for 30 min were higher than broiling at 176 °C for 29 min and may solve issues of pesticide residues in meat if contaminated in limited low levels.

Declaration of Competing Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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