



Research Article

Validation study of quantification of some pyrethroid residues in sheep meat and milk by gas chromatography–mass spectrometry, employing the modified QuEChERS method

Ahmad Yaseen Hamadamin^{a,*}

^aCollege of Veterinary Medicine, Department of Microbiology, Sulaimani University, Sulaimaniyah, Old campus, Sulaimani, 46001, Iraq

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ABSTRACT

The wide spectrum properties and benefits of pyrethroid (PYR) compounds have led to their extensive usage in agriculture and livestock, and this has caused accumulation in animal tissues and, in turn, animal products, causing negative health effects on consumers. This study developed a method to measure three pesticide residues in two different matrices, including sheep meat and milk. The study was conducted using gas chromatography–mass spectrometry (GC–MS) combined with the “quick easy cheap effective rugged safe” (QuEChERS) method for sample extraction. The validation was also conducted by firstly, accuracy including recovery, which ranged between 80.6% to 96.85%, and 91.5% to 103% for meat and milk samples, respectively, giving an overall recovery standard range of 70.0–120%. Secondly, precision, including reproducibility of the measurements, was between 0.4 to 9.2% for sheep meat, and 0.7 to 12.3% for milk, respectively, which were in a typical precision level ($\leq 19\%$) set by SANTE/11813/2017 criteria. Monitoring was carried out on livestock products randomly collected from local markets in Sulaimaniyah. The detected pesticide residue levels in meat samples were found to be below the established maximum residue limits (MRLs), whereas residues in milk samples exceeded the permissible thresholds. Owing to its high analytical efficiency, reproducibility, and straightforward operational procedure, the developed method is well-suited for routine surveillance of multiple PYR residues in livestock products with varying fat contents, including both high-fat (meat) and low-fat (milk) matrices.

1. Introduction

Pesticides are chemical compounds aimed at killing pests, insects, rodents, plant fungi, and other microorganisms. These pesticides are highly toxic to animals, humans, and nontargeted organisms and affect the environment. The term “pesticide” denotes a plant protection product intended to protect plants. They have several functions, such as protecting plants from microbial or pest diseases, preventing the growth of pests, and completely destroying undesirable weedy plants. Pesticides or their residues often contaminate foods, especially stored foods, and there is an urgent need to prevent this contamination. Presently, farmers use synthetic pesticides to control pests, bacteria, and fungi. The continuous application of highly toxic pesticides affects agricultural fields and water systems. The residual pesticides enter the ruminant body and human tissues through the food web, and special attention has recently been given to reducing the accumulation of pesticides and residues. Since not all nations in the world produce agricultural products and several countries import agricultural products, serious concerns about pesticide residues in imported products, especially food products from developing countries, cause serious concern. Synthetic pesticides are produced by several countries, and there is no unique regulation for the use of these chemicals. The farmers apply these pesticides in all seasons during the crop growing

period. Environmental conditions such as humidity, high temperature, intensive solar radiation, and precipitation significantly influence pesticides in particular environments (Singh *et al.*, 2022).

To enhance agricultural productivity, the use of pesticides, with pyrethroids (PYRs) being particularly dominant, has become widespread. However, their misuse is an escalating concern, as pesticide residues can contaminate livestock products through direct application, dermal contact, inhalation, or ingestion. China is one of the largest producers of synthetic pesticides, which are consumed in significant amounts and contribute to major pesticide pollution in East Asia. Among these pesticides, PYRs have been detected in water systems and in the natural environment. Recently, China has been reported to pose a serious risk to PYR pesticides. Pesticides such as lambda-cyhalothrin (CHN), pendimethalin, procymidone, cypermethrin, and isocarbophos have been detected in agricultural lands. In countries such as India, the use of pesticides varies from region to region. The presence of organophosphates, synthetic PYRs, and carbamates has been detected in agricultural fields. These pesticides accumulate in vegetables, cereals, and some fruits. However, the presence of these pesticides varies with season, crop, and environmental conditions. Intensive agricultural practices and the use of pesticides result in the contamination of pesticides such as chlorpropham and chlorpyrifos with vegetables and fruits. The presence of pesticides in animal feed

*Corresponding author:

E-mail address: ahmad.hamadamin@univsul.edu.iq (A Hamadamin)

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has been reported, and accumulated pesticides or derivatives have been detected in animal foods or products. Moreover, the amount of pesticides in meat and meat products varies depending on the basis of the type of pollutants and environmental conditions. Herbicides are detected in freshwater environments and can be found in livestock feed and food. Glyphosate is a common herbicide that has been detected in animal droppings, intestines, liver, kidney, spleen, muscles, and urine (Kongmee *et al.*, 2019; Sehrawat *et al.*, 2021).

PYRs are synthetic analogs of pyrethrin, a natural insecticide derived from chrysanthemum flowers (*Chrysanthemum cinerariaefolium* and *C. coccineum*), which have been chemically modified for greater environmental persistence (Gammon *et al.*, 2019). Their insecticidal action primarily involves altering the dynamics of voltage-gated sodium channels in nerve cell membranes. This disruption prevents the channels from closing properly, leading to a prolonged sodium current that impairs the nervous system in both insects and vertebrates. Pesticide residues were also detected in the eggs, and the level was higher than the acceptable limits. The application of disinfectants, pesticides, feed additives, disinfectants, and insecticides has been widespread over the past few decades in agriculture as growth promoters or to control the growth of weedy plants, algal species, and bacterial infections. Pesticide-contaminated soil further reaches the freshwater system, followed by lakes and seas, resulting in the accumulation and biomagnification of pesticides. The accumulation of pesticides poses a serious threat to human beings and affects export quality. Pesticide residues and chemical contaminants have been detected, and fungicides have been detected in fish species (Farag *et al.*, 2021).

These compounds are deployed across multiple domains. In veterinary medicine, they control ectoparasites like lice, fleas, and ticks, as well as disease vectors including *Aedes aegypti* and *Anopheles gambiae* (Kongmee *et al.*, 2019). In agriculture, they target pests such as aphids and cutworms in fields and greenhouses, while household applications manage insects like ants, cockroaches, and bed bugs. The manufacture and application of organochlorine pesticides have been banned in various countries because of their toxic effects on nontargeted organisms, including animals and humans. Most synthetic pesticides have increased lipophilicity and are found in the adipose tissue of humans and pesticide-exposed animals. Notably, these compounds are resistant to biotransformation by living beings, and their presence is relatively long-lasting in the body. Polychlorinated biphenyls are widely used for the preparation of synthetic pesticides. They are used as vehicles for inorganic pesticides, and the wide application of synthetic pesticides has led to the presence of polychlorinated biphenyls in the environment (Sands *et al.*, 2018). A wide range of PYRs, including allethrin, bifenthrin, cyfluthrin (CFN), CHN, cycloprothrin (CPN), cypermethrin, and deltamethrin, share this mode of action and are employed in these settings (Anadón *et al.*, 2025). Despite their stability, PYRs are susceptible to degradation through photolysis and evaporation shortly after application. The major source of organochlorine pesticide entry to humans is *via* fish and cattle. Prolonged exposure to these pesticides through food leads to the development of skin diseases, liver malfunction, reproductive disorders, neurological dysfunction, endocrine abnormalities, immune failure, chronic diseases, and the development of various types of cancer (Zhu *et al.*, 2020).

Given their extensive use in livestock for parasite control, comprehensive monitoring of PYR residues in animal products is crucial, necessitating robust analytical methods. The amount of pesticide in the environment has been tested via gas chromatography or liquid chromatography–mass spectrometry (LCMS), and these methods are highly sensitive, selective, reliable, and robust. LCMS analysis is popular because it is more sensitive than gas chromatography. Robust, sensitive, and highly selective analytical procedures are essential for the determination of pesticides from the environment and food sources. To achieve this goal, various methods of extraction have been proposed for vegetables, fruits, and meat. The accumulated pesticides can be extracted from tissues and other sources via solvents. Moreover, the selection of solvents is based on the type of pesticide, and solvents such as ethyl acetate, acetonitrile (ACN), methanol, and dichloromethane are widely used for the extraction of pesticides or their derivatives. The major criterion for the selection of a solvent should be its low solubility, so that it does not react with the targeted pesticide, and

that it does not alter the chemical structure of the pesticide. Currently, a unified analytical technique for the concurrent determination of PYRs and veterinary drugs in both meat and milk is lacking. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method is considered a promising approach for multi-residue analysis in such diverse matrices (Verdini and Pecorelli, 2022). This sample preparation technique typically employs ACN for extraction, a solvent suitable for both liquid and gas chromatography (Tran-Lam *et al.*, 2021). For cleanup, various dispersive solid-phase extraction (d-SPE) sorbents can be applied to different food matrices (Hamadamin and Hassan, 2020). While several studies have analyzed pesticides in livestock products (Kang *et al.*, 2020), challenges remain due to intricate procedures and the efficient extraction of the fatty compartment.

To address this, the present study aims to develop a simultaneous analysis for multi-component pesticides in high-fat, high-protein livestock products. Specifically, we report the development and validation of a combined QuEChERS and GC-MS/MS method for the simultaneous determination of three PYRs, CPN, CFN, and CHN. Gas Chromatography-tandem mass spectrometry (GC-MS/MS) is utilized for its high selectivity and sensitivity, which helps exclude matrix interferences and results in a significantly lower limit of quantification (LOQ) (Smriti *et al.*, 2023; Romniou *et al.*, 2022). The primary objective is to establish a simple and efficient quantitative method that complies with maximum residue limits (MRLs) and European regulations for monitoring chemical residues in food of animal origin.

2. Materials and Methods

2.1 Sample collection

Meat samples (n=25) were collected from carcasses of local animals raised in Sulaimaniyah, and fresh milk (n = 25) samples were collected in markets in Sulaimaniyah city/Iraq. All meat and milk samples were transferred to the laboratory and packed in polythene zip lock bags in a cold box with respective labels in order to avoid any contamination and kept at – 18°C. Samples of meat (100g) and milk (100 ml) were straightforwardly prepared for extraction and analysis by GC-MS. All samples were collected in the spring season from Sulaimaniyah city.

2.2 Chemicals and reagents

CPN ($\geq 98.0\%$), CFN ($\geq 95.0\%$), CHN ($\geq 98.0\%$), were purchased from AccuStandard (New Haven, CT, USA), ACN (MeCN), hexane, anhydrous magnesium sulfate $MgSO_4$, sodium chloride (NaCl), octadecylsilane (C18), primary and secondary Amine (PSA), formic acid (F.A) were purchased from Toronto Research Chemicals (North York, ON, Canada). All reagents were high performance LC (HPLC) grade. Standard solutions were prepared by dilution of the stock solutions with ACN and stored in amber bottles at 4°C.

2.3 GC-MS system

Analysis was performed using a Gas Chromatograph system coupled with a tandem mass spectrometric (GC-MS) detector. The method described by Sartarelli *et al.* (2012) was followed with modifications. Separation was achieved using a DB-5 capillary column (30 m length, 0.25 mm internal diameter, 0.1 μm film thickness). The injector, interface, and ion source temperatures were all set at 250°C. A splitless injection mode (1.0 min) was used, with helium as the carrier gas at a constant flow rate of 0.75 mL/min. The oven temperature program began at 120°C, increased at a rate of 4°C/min to 190°C, then ramped sharply at 32°C/min to 270°C, holding for 4 min. The mass spectrometer operated in scan mode across a mass range of 45–475 Da. For lower concentrations, enhanced sensitivity was achieved by using single ion monitoring (SIM) mode for the specific target compounds.

2.4 Standards preparation

Individual stock solutions for each PYR (CPN, CFN, CHN) were prepared in ACN within Pyrex glass vials at a concentration of 100 mg/L. These stock solutions were stored in dark amber bottles at

-18°C to ensure stability. A working standard solution (WSS) of 50 mg/L was then prepared by diluting the stock with ACN. A suite of multi-standards was created from the WSS for the purposes of spiking, recovery assessment, linearity testing, and sensitivity studies. All standard solutions were protected from light using aluminum foil and maintained at -4°C.

2.5 Control samples

Blank meat (100 g) and milk (100 g) samples, verified to be free of the target pesticides, were acquired from cattle. These control samples served multiple purposes: an aliquot was used for the selectivity test, another was spiked for recovery studies, and the remainder was dedicated to preparing matrix-matched calibration (MMC) standards and conducting sensitivity studies. The absence of the studied pesticides in these blanks was confirmed prior to their use in spiking experiments.

2.6 Method validation

The method's applicability was verified through a validation process based on the SANTE/11813/2017 guideline. Parameters determined included linearity, the limit of detection (LOD), LOQ, recovery (accuracy), and reproducibility (precision). Selectivity was confirmed by examining chromatograms from blank solutions and matrix samples for interfering peaks. Linearity was assessed via correlation coefficients (R^2) from calibration curves at concentrations of 5, 10, 20, 30, and 50 µg/kg. The LOD and LOQ were established at signal-to-noise ratios of 3:1 and 10:1, respectively. Recovery was determined by fortifying blank meat and milk matrices at three levels (5, 10, and 20 µg/kg), each in triplicate. Precision was expressed as the relative standard deviation (RSD) of these recovery results.

2.7 Matrix-matched calibration

The accurate quantification of target analytes in complex samples like meat and milk is often compromised by the "matrix effect," a phenomenon where co-extracted constituents can suppress or enhance the analyte signal in GC-MS analysis. To correct for this and ensure accurate results, the use of MMC is mandated by the SANTE/11813/2017 guidelines.

The foundation of the MMC involved preparing calibration standards within a blank matrix extract, thereby replicating the chemical environment of the actual samples. The process began with the preparation of individual stock solutions of CHN, CPN, and CFN, at a concentration of 100 mg/L in ACN, using Pyrex glass vials. These stock solutions were stored in dark amber bottles at -20°C to prevent photodegradation and thermal decomposition. A WSS with a concentration of 50 mg/L was then prepared by diluting the stock solutions with ACN.

Immediately prior to injection into the GC-MS system, the MMC standards were freshly prepared. This was done by fortifying (or "spiking") extracts of the verified blank meat and milk matrices with appropriate volumes of the WSS. This procedure yielded a series of calibration standards at specific concentrations: 0.010, 0.020, 0.050, 0.100, 0.200, 0.300, and 0.500 mg/kg (Table 1). A calibration curve for each of the three PYRs was constructed by plotting the peak area against the corresponding concentration. The linearity of the detector response for each compound was then rigorously evaluated by calculating the correlation coefficients (r^2) from the regression equations of these curves, ensuring precise and reliable quantification across the specified range.

2.8 Sample extraction and purifications

The sample preparation was founded on the QuEChERS methodology for extraction (Anastassiades *et al.*, 2003) and cleanup (Lehotay *et al.*, 2005), with specific modifications for each matrix.

Meat Samples: A 2 g portion of homogenized meat was placed in a 50 mL Falcon tube. A single-phase extraction was performed by adding 4 mL of ACN (containing 1% formic acid). Then, 1.6 g of anhydrous MgSO₄ and 0.4 g of NaCl were added, and the mixture

was vortexed for 1 min. The mixture was centrifuged at 3000 rpm for 3 min at room temperature to achieve liquid-liquid partitioning (LLP). For the dispersive solid-phase extraction (d-SPE) cleanup, the supernatant (organic MeCN phase) was transferred to a tube containing 70 mg of PSA, 70 mg of C18, and 150 mg of anhydrous MgSO₄. After hand-shaking for 30 s and centrifuging at 3000 rpm for 1 min, the supernatant was filtered through a 0.45 µm syringe filter. The filtrate was evaporated under a gentle nitrogen stream to a volume of 1 mL and stored at 4°C until analysis by GC-MS.

Milk Samples: The procedure for milk was adapted from the QuEChERS method for pesticide extraction (Mastovska and Lehotay, 2006) and cleanup (Lehotay *et al.*, 2005a). After thawing and homogenization, a 2 mL milk sample was transferred to a 50 mL Falcon tube. First, 5 mL of hexane was added and vortexed for 1 min. Then, 4 mL of MeCN with 1% (v/v) acetic acid was added, and the tube was vortexed again. Subsequently, 1 g of anhydrous MgSO₄ and 0.2 g of NaCl were added, followed by vortexing and centrifugation at 3000 rpm for 3 min. The upper hexane layer was discarded. The lower MeCN phase was cleaned up using 35 mg of PSA and 75 mg of MgSO₄. After hand-shaking and centrifugation, the extract was filtered, concentrated under nitrogen to 1 mL, and stored at 4°C for GC-MS analysis.

2.9 Data processing and statistical analysis

Data from the MMC standards were analyzed using MS Excel (Analysis Tool Pak, Regression) to determine sensitivity. The concentrations of the target pesticides obtained from the sample analysis were subjected to statistical evaluation. A one-way Analysis of Variance (ANOVA) was performed using SPSS software (Version 18.0), with the Duncan post-hoc test applied for multiple mean comparisons. A probability value of $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1 Verification of the GC-MS/MS conditions

Food safety is a serious challenge worldwide because of the increasing global population and the urgent need to meet the global demand. The use of pesticides in agricultural fields protects against pests and thus increases crop production. However, the use of pesticides leads to the accumulation of pesticides in the environment and pesticide residues. The increased pesticide residues in agriculture pose a serious threat to animals. Moreover, the use of toxic pesticides in agricultural fields can cause environmental pollution, resulting in teratogenic, immunosuppressive, and carcinogenic effects. The GC-MS operating conditions for determining pesticide residues across the tested matrices were optimized using a separation column. Extraction efficiency was assessed based on recovery percentages and RSD values; accordingly, the extraction procedure was refined to achieve the most favorable results. Several parameters influencing extraction performance were systematically optimized, including solvent type, polarity, and cleanup sorbents such as ACN and primary-secondary amine (PSA) (Fernández-Calviño *et al.*, 2008). The QuEChERS extraction technique was applied with slight modifications, namely, increased volumes of ACN and PSA, and an additional filtration step. These adjustments yielded satisfactory recovery rates, as the selected sorbents effectively removed fatty acids and other co-extractive interferences commonly associated with non-polar pesticides in meat matrices, while the extra filtration step enhanced the purity of the final extracts prior to injection (Harshit *et al.*, 2017).

The QuEChERS approach remains highly recommended due to its numerous advantages, including simplicity, rapid processing, low operational cost, and strong performance, as well as its broad applicability to complex food matrices and diverse analytes—capabilities often absent in alternative extraction methods for pesticide residue analysis.

Chromatographic analysis of CPN, CFN, and CHN showed distinct peak areas without evidence of enantiomeric or isomeric interference in the standards. The total ion chromatograms (Figs. 1 and 2) of the three pesticides were obtained using standard solutions of 10 mg/L in ACN, corresponding to 0.1 mg/kg in meat and 0.1 µL/L in milk. The observed

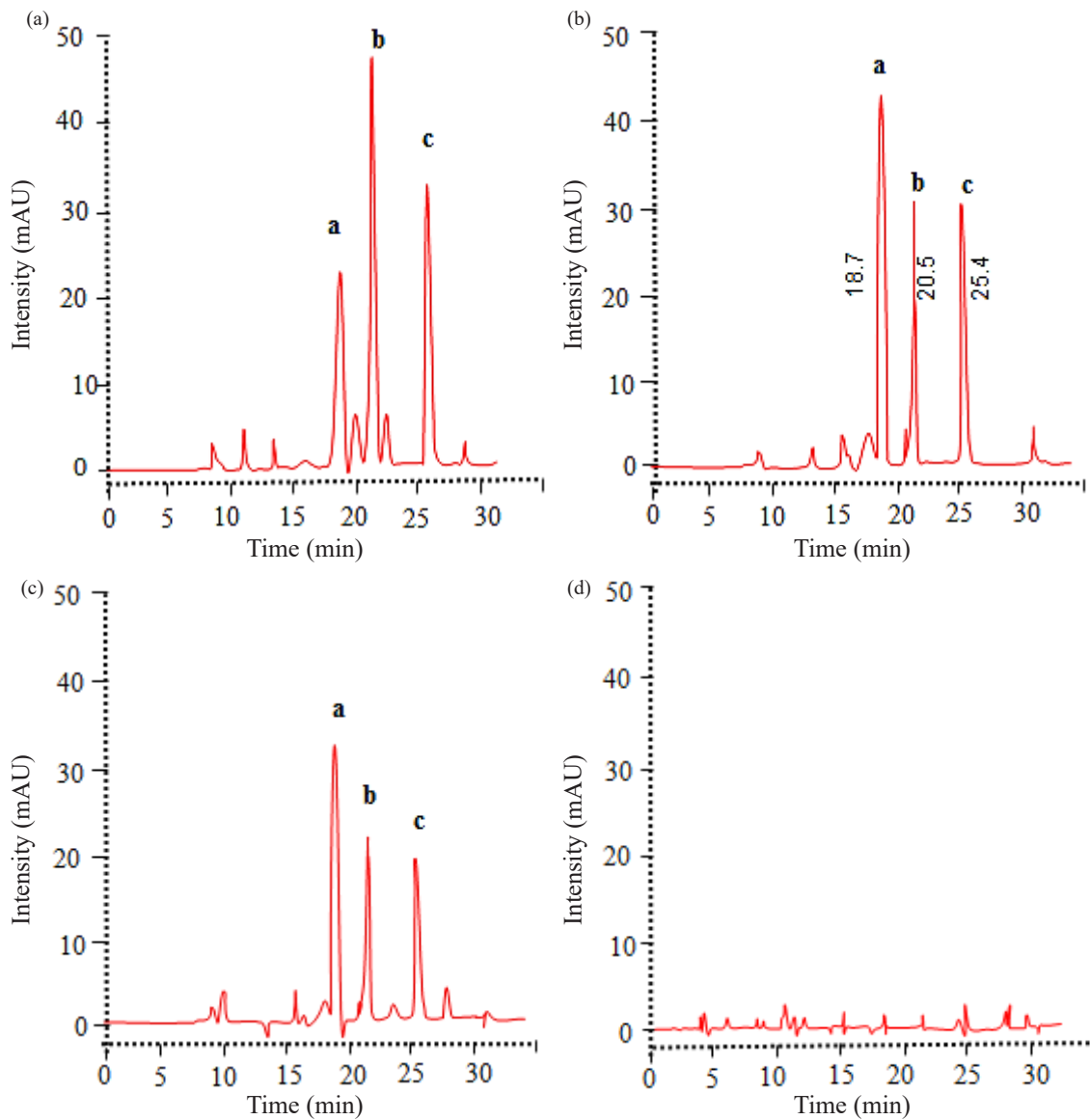


Fig. 1. Ion Chromatograms of QuEChERS method. (a) Tested samples [a. CHN; b. CFN; c. CPN]. (b). Multi-standard solutions 10 mg/L. [a. CHN; b. CFN; c. CPN]. (c) Spiked meat sample before extraction 0.1 mg/kg. [a. CHN; b. CFN; c. CPN]. (d) Blank meat samples.

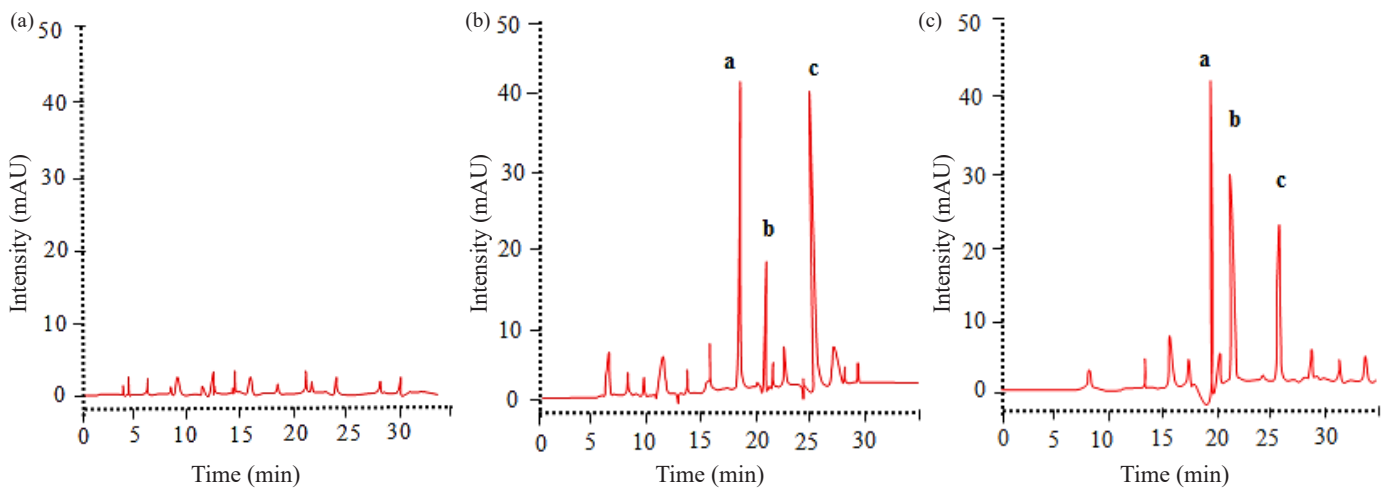


Fig. 2. Ion Chromatograms of QuEChERS method. (a) Blank milk samples. (b) Spiked milk sample before extraction 0.1 mg/L. [a. CHN; b. CFN; c. CPN]. (c) Multi-standard solutions 10 mg/L. [a. CHN; b. CFN; c. CPN].

retention times for the analyte peaks ranged between 18.6 and 25.6 minutes. Table 2 summarizes the peak numbers, associated retention times, precursor ions (m/z), product ions (m/z), and collision energies for both quantifier and qualifier transitions of the analyzed pesticides.

3.2 Method of validations

3.2.1 Selectivity and specificity

The analytical standards of the three target compounds were initially injected into the GC-MS system operating in SCAN mode. Retention times for each analyte were determined, and characteristic ions identified from the mass spectra were subsequently selected for quantitative analysis under SIM mode. Both extraction optimization and analytical validation procedures were also performed using the SIM configuration (Figs. 1 and 2).

Selectivity of the developed method was evaluated by analyzing blank meat and milk matrices to confirm the absence of interfering peaks within the monitored ion windows (Figs. 1 and 2). Linearity was assessed by spiking matrix-matched standards into blank control samples following extraction. Excellent linearity was achieved for all analytes within the range of 0.01–0.5 mg/kg, with correlation coefficients (R^2) of 0.9997. Statistical validation of the regression models was carried out through the determination coefficient and evaluation of homoscedasticity by plotting residuals against concentration. The data showed strong goodness-of-fit, as indicated by consistent response factor

Table 1. Standard preparations and spiking levels in meat and milk samples for studying accuracy.

WSS	Used Vol (μL) of single standard	Conc. of analytes (μg/20 μL)	Used Vol (μL) multi standards	Total conc. of 3 analytes (μg/120 μL)	Matrices (meat and milk) weight (g, mL)	Conc. of each analyte in 2g, mL matrix (mg/kg, μL/L)
Preparation for meat samples						
1mg/L	20	0.02	60	0.06	2	0.01
2.5 mg/L	20	0.05	60	0.15	2	0.025
5 mg/L	20	0.1	60	0.3	2	0.05
10 mg/L	20	0.2	60	0.6	2	0.1
20 mg/L	20	0.4	60	1.2	2	0.2
Preparation for milk samples						
1 mg/L	20	0.02	60	0.06	2	0.01
2 mg/L	20	0.04	60	0.15	2	0.020
5 mg/L	20	0.1	60	0.3	2	0.050
10 mg/L	20	0.2	60	0.6	2	0.1
20 mg/L	20	0.4	60	1.2	2	0.2

Table 2. GC-MS condition for PYRs insecticides.

Analytes	M.Mass (g/mol)	RT (min)	AS spiked	AS MMC	AS Std
Meat					
CHN	449.9	18.6- 18.8	34601	39601	43312
CFN	461.1	20.4- 20.5	22979	25990	32234
CPN	482.4	25.4-25.6	21504	23997	30834
Milk					
CHN	449.9	18.6- 18.8	41453	59834	63897
CFN	461.1	20.4- 20.5	30258	32456	46723
CPN	482.4	25.4-25.6	24504	27989	35674

Values are referring concentration of 10 mg/L standards at spiking level of 0.1 mg/kg, mL/L. R.t = retention time, AS Spiked = Areas of spiked samples, AS std = Areas of standards

distributions (signal-to-concentration ratios, y/x) across the calibration levels. The linear regression models were further confirmed by analysis of five calibration levels, each prepared in triplicate, demonstrating stable and reproducible calibration parameters for all compounds.

The lowest calibration points for the matrix-matched standards were clearly distinguished in the chromatograms. The limits of detection (LOD) and quantification (LOQ) were established at signal-to-noise ratios of 3 and 10, respectively. To verify the method's specificity, both retention times and selected ion transitions were examined, ensuring that only the designated precursor and product ions were detected in blank, spiked, and mixed standard samples. The lowest LOD values obtained were 0.008 μg/kg for CFN in sheep meat and 0.006 μg/kg for CPN in milk samples, whereas the lowest LOQs were 0.027 μg/kg for CHN in meat and 0.020 μg/kg for CPN in milk. All detected LODs were well below the corresponding MRLs, confirming the suitability of the developed method for trace-level quantification of these pesticides. The sensitivity achieved in this study was superior to that reported in previous GC-MS-based analytical approaches for similar analytes (Harischandra *et al.*, 2021; Ferreira *et al.*, 2023). Lower LOD values directly contribute to minimizing false-negative outcomes, which is a critical factor in ensuring the reliability of analytical determinations.

3.3 Accuracy and precision

The recovery efficiency of each analyte was confirmed by calculating the mean recovery values, which fell within the acceptable range of 70–120% established by the SANTE/11813/2017 guidelines. Recovery tests were performed at three concentration levels (0.010, 0.050, and 0.200 mg/kg) within the matrix, and satisfactory results were obtained. The recoveries ranged from $80.6 \pm 9.6\%$ to $96.8 \pm 3.5\%$ for sheep meat and from $91.5 \pm 12.3\%$ to $103 \pm 4.3\%$ for milk samples, corresponding to an overall recovery range within the recommended limits of 70–120% (Table 3). These results confirm the high accuracy of the developed method, indicating that it can serve as a dependable analytical tool for monitoring the selected pesticides in meat and milk samples.

Table 3. Residual levels (n=25) of pesticides in sheep meat and milk analyzed by GC-MS

Pis	Concentrations (mg/kg)	
	Meat	Milk
CPN	0.063 ^{Ay} ± 0.05	0.108 ^{By} ± 0.073
CFN	0.103 ^{Az} ± 0.10	0.034 ^{Bx} ± 0.041
CHN	0.026 ^{Bx} ± 0.012	0.105 ^{Ay} ± 0.049

A,B Different superscript letters denote significant differences within row ($p < 0.05$). x,y,z: Different superscript letters denote significant differences within column ($p < 0.05$). Values refer mean of 25 samples ± Standard error of the mean (StD).

Table 4. Recovery percentages (n = 3), specificity, and linearity of pyrethroids-spiked meat and milk using modified QuEChERS and GC-MS/MS (mg/kg).

Insecticide	Spiking level (mg/kg)	Recovery ± (RDS %)		Validation in the spiking level of 0.1 mg/kg, mL/L.			
		Meat	Milk	LOD		LOQ	
				Meat	Milk	Meat	Milk
CPN	0.05	98.3±9.2	96.0±8.6	0.012	0.006	0.039	0.020
	0.01	94.6±4.8	91.5±12.3				
	0.2	99.1±2.3	96.9±5.6				
CFN	0.05	96.8±3.5	95.9±5.7	0.008	0.008	0.027	0.025
	0.01	99.2±2.8	103±4.3				
	0.2	99.6±0.4	99.5±0.7				
CHN	0.05	80.6±9.6	92.6±8.06	0.009	0.008	0.025	0.024
	0.01	82.1±4	95.4±7.4				
	0.2	95.4±2.9	98.1±1.8				

The precision of the method was evaluated by calculating the RSD of the recoveries obtained from both matrices. The RSD values ranged from 0.4–9.2% for sheep meat and 0.7–12.3% for milk samples. All values met the acceptance criteria set by the SANTE/11813/2017 document (RSD < 20%) for the tested concentrations. The data in Table 2 show that all pesticides exhibited consistent recovery behavior across the studied samples. The improved Peak areas is attributed to the addition of 1% formic acid in the ACN extraction solvent, which enhanced extraction performance by facilitating the release of analytes from the matrix (Bakanov *et al.*, 2023).

3.4 Matrix-matched calibration

To ensure quantitative analysis where the instrument's response is directly proportional to the pesticide concentration in a sample, demonstrating linearity is essential. This can be achieved through methods such as standard addition or the use of an internal standard. In the present work, a MMC approach was employed specifically to compensate for the matrix effect. The linearity was evaluated using a GC–MS derived calibration curve across a concentration series of 5, 10, 20, 30, and 50 mg/L, with each level analyzed in triplicate. By utilizing the MS intensity data for specific ions captured in the chromatograms at each concentration, the calibration curves were constructed. The correlation coefficients (R^2) for the three analytes fell within a satisfactory range of 0.990–0.995 for meat and 0.995–0.997 for milk, thereby meeting the requirement of $R^2 \geq 0.99$ stipulated by the SANTE/11813/2017 criteria. Consequently, these results validate the developed method as appropriate for accurately quantifying the residual amounts of the target pesticides in the studied samples over the designated concentration range.

3.5 Tested samples

In the GC analysis, the kind of pesticide found in the highest concentration was CPN in sheep milk samples (0.108 mg/kg) (Table 4), followed by CHN in milk samples (0.105mg/kg). In addition, the lowest concentration of pyrethroid type found was CHN in meat samples (0.026 mg/kg) and followed by CFN found in milk samples of cattle (0.034 mg/kg).

Results in this project showed that all of the studied pesticides (CPN, CFN, CHN) residual levels in the meat samples were lower than international MRLs (CPN 0.2 mg/kg, CFN 0.02 mg/kg, CHN 0.02 mg/kg, respectively). This result was also true for milk samples in which the residual level of CPN, CFN, and CHN found in the milk samples was lower than international MRLs (CPN 0.2 mg/kg, CFN 0.1 mg/kg, and CHN 0.5 mg/kg, respectively) set by (WHO., FAO, 2024). Statistically, there was a significant difference between pesticide residual levels of CPN, CFN, and CHN in meat samples ($P < 0.05$); while there was no difference between CPN and CHN in milk samples ($P > 0.05$). There were also significant differences in all residual levels between meat and milk samples ($P > 0.05$). However, most of PYRs used in agriculture, animal husbandry have a specific affinity for animal products and tissue; these ultimately lead to their accumulation in livestock products, including milk, meat, and internal organs; hence, they are the key items for pesticide accumulation (Atta *et al.*, 2022). While finding low levels of studied pesticides attributes to some processes that happen to PYRs after using, i.e., decomposition, evaporation, and degradation, etc., which may vary with the chemical nature of each type of PYRs (Meng *et al.*, 2022). Besides, 80–90% of an applied PYRs can be volatilized within a few days of application; the rest remains and is saved in tissues (Ali *et al.*, 2021).

4. Conclusions

Through conducting comparative experiments aimed at optimizing the QuEChERS method, the most appropriate GC–MS method for PIs was established. The concurrent examination of pesticides discussed in this research demonstrated the method's capability for the swift assessment of residual pesticides in livestock products adapted to meat (high-fat) and milk (low-fat) matrices, which is very useful for researchers, owing to its brief duration, cost-effectiveness, straightforward process, high

efficiency, and use for a wide range of matrices. The developed method demonstrated high sensitivity and met all performance requirements outlined in the SANTE/11945/2017 guidelines. In addition, it offers an environmentally sustainable alternative to conventional procedures, as it requires smaller volumes of solvent and generates less chemical waste. The findings of this research contribute to the establishment of a continuous, accurate, and dependable monitoring system for pesticide residues in livestock-derived products, allowing for rapid and efficient analysis. Given that PYRs are widespread contaminants frequently detected in animal products, future work will aim to further enhance and expand the simultaneous determination of these pesticide residues in various livestock matrices.

CRedit authorship contribution statement

Ahmad Hamadamin: Conceptualization; experimentation; analysis; validation; formal analysis; resources; writing—original draft preparation; writing—review and editing; funding acquisition. He also implemented validation; formal analysis; review and editing, software, writing – review and final editing.

Declaration of competing interest

The author declare that they have no competing financial interests or personal relationships that could have influenced the work presented in this paper.

Declaration of Generative AI and AI-assisted technologies in the writing process

The author confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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