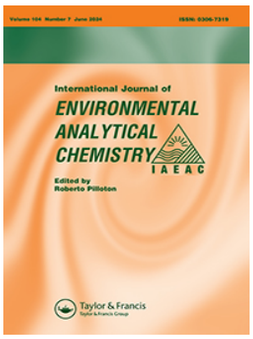

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Analysing multi-class pesticide residues in tilapia fish: development and validation of LC-MS/MS and GC-MS/MS methods

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ABSTRACT

Pesticides are an integral part of increasing food production and have remarkable agricultural benefits such as an increase in crop yield. However, they have been identified as some of the most hazardous substances in food, particularly in fish, posing a risk to both ecosystems and human health. Therefore, this study focuses on the development and optimisation of an analytical technique to detect multiple pesticide residues in fish products. The method involves liquid-liquid extraction, followed by analysis using both liquid and gas chromatography coupled with mass spectrometry. A total of 411 pesticides were selected (e.g.: insecticides, fungicides, and herbicides) and the concentrations of their residues in tilapia (Oreochromis niloticus) were determined. The samples were processed according to the optimised QuEChERS (quick, easy, cheap, effective, rugged, and safe) method, including extraction and clean-up procedures. Lipid co-extracts were successfully minimised before instrumental analysis using optimised cleanup. Matrix-matched calibrations were performed at two different levels (0.05,0.01 mg/kg) in tilapia fish to compensate for signal enhancement/suppression. The validation procedure was performed to assess accuracy, precision, limit of quantification (LOQ), and uncertainty according to the SANTE guidelines. The LOQs of this method were 0.01 mg/kg for all selected pesticides except Cyazofamid, Dodine, Fenpyrazamine, Methomyl, Oxadiargyl, Parathion ethyl, Parathion methyl, Prothioconazole Desthio, and Tolylfluanid were 0.05 mg/kg. The suggested method was implemented to investigate the levels of pesticide residues in fresh fish samples ($n = 15$) procured from local markets in Egypt. Only two samples had residues of the targeted analytes. In light of our findings, the devised methodology was effectively utilised for the detecting and measurement of pesticide residues present in fish samples.

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1. Introduction

Pesticides are extensively employed in agriculture to enhance both the quality and quantity of food production [1]. In 2021, total pesticide use in agriculture was

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3.5 million tonnes of active ingredients and total pesticide exports in 2021 reached 7.1 million tonnes of formulated products [2]. Despite their benefits, pesticides have been identified as some of the most toxic substances in food, particularly fish, and pose a risk to ecosystems and human health [3]. Pesticides have the potential to infiltrate the aquatic ecosystem via various natural pathways, leading to contamination of food, the environment, and living organisms [4]. They are acknowledged to spread from treated agricultural regions into the broader environment, exerting effects on non-target organisms [3].

Fish serve as a significant protein source in the human diet, and the assessment of pesticide levels in fish muscle establishes a crucial aim within the realms of environmental and health sciences [5]. While a wide range of pesticides could potentially transfer into the muscle of fish such as tilapia (Oreochromis niloticus) [6].

Tilapia represents the most prevalent freshwater fish in Egypt and is extensively consumed owing to its accessibility and cost-effectiveness. Nonetheless, tilapia often inhabits turbid and murky waters, rendering it more susceptible to a diverse array of environmental contaminants compared to other fish species [7]. Because of the comparatively elevated fat content in tilapia meat, fat-soluble environmental contaminants like organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are more prone to be present in tilapia [8]. In addition, organophosphate pesticides (OPs) may contaminate tilapia fish and catfish meat from recent agricultural applications [9]. Therefore, the consumption of fish presents a potential avenue for the accumulation of pesticides in humans.

The accumulation of these pollutants in fish reduces their quality within the hatchery and compromises their existence post-release, causing financial losses for aquaculture operations. Hence, adopting multi residue methodologies with stringent quantification limits serves as a swift methodological solution for analysing pesticides in fish, aiming to enhance the quality of such analyses, although only a limited number of studies have explored this approach [10].

For complex matrices, the development of an appropriate extraction phase is typically necessary. Numerous techniques have been devised to extract compounds from tilapia fish, encompassing solid-liquid extraction, microwave-assisted extraction, and pressurised liquid extraction [11]. Nevertheless, the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method is presently recognised as the predominant approach for extracting pesticides from complex matrices, offering high-quality outcomes with minimal procedural steps [12]. Fish inherently possess elevated lipid content, which frequently results in the coextraction of lipid components during sample preparation, thereby impeding the detection and quantification of target analytes by gas chromatography tandem mass spectrometry (GC-MS/MS) and liquid chromatography tandem mass spectrometry (LC-MS /MS) [13]. A previous survey study using QuEChERS on tilapia fish samples from Edko Lake in Egypt has shown the presence of heptachlor epoxide, p,p-DDE, dieldrin, p,p-DDD, and endrin were detected in muscles of fish [7]. Another investigation assessed the levels of pesticides in Nile tilapia and African catfish sourced from the Kitchener Drain in Egypt. The research revealed that the elevated fat content of the fish facilitates the accumulation of soluble lipid-bound organochlorine and organophosphorus compounds. Furthermore, the study identified instances, where the concentrations of endosulfan, heptachlor, dicofol, and p,p'-DDT in fish surpassed the acceptable daily intake (ADI), limits for

pesticides in fish [10]. Hence, a recent QuEChERS methodology was devised to mitigate lipid co-extraction in fish samples before the analysis of pesticides using GC-MS/MS and LC-MS/MS. However, further complementary investigations are warranted to ascertain the analysis of a broad spectrum of pesticides in matrices with higher fat content.

Therefore, the objectives of the current study were: Firstly, it introduces a novel multi-residue method capable of concurrently analysing a wide range of 411 multi-class pesticides in tilapia fish, employing both LC-MS/MS and GC-MS/MS techniques. Secondly, the validation procedure rigorously complies with the analytical quality control and method validation criteria specified in the SANTE/11312/2021v2 international guidelines for analysing pesticide residues in food and feed. Lastly, the study surveys some tilapia fish sourced from the Egyptian market utilising the newly developed method. This real-world application of the method provides valuable insights into the prevalence and levels of pesticide residues in commercially available tilapia fish, contributing to the broader understanding of food safety and consumer health in the region.

2. Materials and methods

2.1. Chemicals and reagents

The pesticide reference standards analysed, boasting purities exceeding 96% and certified for pesticide residue analysis, were procured from Dr. Ehrenstorfer in Germany. Additionally, the acetonitrile, methanol, acetone, and ethyl acetate pesticide residue grades were purchased from JT Baker in the USA. The purchase of ammonium formate, methanol, and formic acid was made by Sigma-Aldrich in Canada. Furthermore, n-hexane, dichloromethane, and sodium sulphate were purchased from Merck in Germany. The QuEChERS buffer kits including (4 g magnesium sulphate anhydrous ($MgSo_4$), 1 g sodium chloride, 1 g sodium citrate tribasic dihydrate, and 0.5 g sodium citrate dibasic sesquihydrate) and dispersive solid-phase reagent containing (900 mg $MgSo_4$ and 150 mg primary secondary amine (PSA)) were purchased from Agilent in the USA. Graphitized carbon black (GCB) was purchased from Sigma-Aldrich in Canada. Octadecyl-modified silica (C18) was supplied by DIKMA Technologies in Beijing, China. Cellulose Extraction Thimbles was purchased from CHMLAB Group in Spain. The Milli-Q water purification device (USA) was used to obtain water of high purity.

2.2. Standard preparation

Stock solutions of 1000 µg/ml were individually prepared for each pesticide in a suitable solvent, following the specifications outlined in Table S1 of the supplementary material. Afterwards, a mixture of LC pesticides and another mixture of GC pesticides, both at a concentration of 10 µg/ml, were prepared in toluene to create intermediate standard solutions. This process was then followed by a separate dilution of each mixture to produce a 2.5 µg/ml spiking mixture in toluene. The calibration levels for LC-MS/MS were prepared in methanol at concentrations 0.001,0.002, 0.01, 0.05, 0.1 and 0.5 µg/ml. On the other hand, for GC-MS/MS analysis, an internal injection standard (IS) was prepared by creating a standard solution of aldrin (100 µg/ml) in n-hexane. The calibration levels for GC-MS/MS analysis were 0.002, 0.01, 0.05, 0.1, and 0.5 µg/ml in green bean extract devoid

of pesticides. These extracts were obtained using the QuEChERS method with a solvent mixture of n-hexane and acetone (9:1), and each calibration level included 0.1 µg/ml aldrin as an analyte protectant (Ap). The stock, intermediate standard, and IS were kept at −20°C until analysis, while the calibration and spiking mixtures were stored at 4°C. For further details on pesticides, such as molecular weight, chemical class, formula, and CAS number, please consult Table S1 in the Supplementary Material.

2.3. Apparatus

Samples were homogenised employing the Knife Mill Grindomix GM 300 manufactured by Retsch, headquartered in Germany. The volumetric flasks (5, 10, and 20 ml), graduated glass pipettes (5 ml), bottle top dispenser (5–50 ml), and micropipettes (with variable capacities of 2–20 µl, 10–100 µl, and 100–1000 µl) utilised in this investigation were sourced from Hirschman in Germany. Furthermore, the analytical balance, pH metre, and precision balance were obtained from Mettler-Toledo in Switzerland. The centrifuge used was a Z32 HK manufactured by Hermle in Germany, and the rotary evaporator employed was a Hei-VAP produced by Heidolph, also headquartered in Germany. The polytetrafluoroethylene (PTFE) acrodisc with a pore size of 0.45 µm and the glass injection vials with Teflon-coated caps were obtained from Agilent Technologies. Additionally, the Geno-Grinder shaker was provided by SPEXR® SamplePrep in the UK. ultra-turrax was provided from France. Soxhlet/Dean Stark Extractor was manufactured in Spain.

2.4. Instrumentation and conditions

2.4.1. LC-MS/Ms

LC-MS/MS analysis was conducted utilising a Shimadzu high-performance liquid chromatography (HPLC) system (Exion LC) coupled with an API 6500^{+} QTRAP mass spectrometer manufactured by (AB SCIEX). Soft electrospray ionisation (ESI) was utilised as the ionisation mode for the analysis of pesticides. Chromatographic separation was performed employing an Agilent Poroshell 120 C_{18} solvent-saver LC column, characterised by dimensions of 3 mm × 50 mm × 2.7 µm particle size. The mobile phase consisted of solvent (A), comprising a mixture of Milli-Q water and methanol in a ratio of 9:1 (v:v), adjusted to pH 4 using ammonium formate, and solvent (B), consisting solely of methanol. The examination of the designated pesticides in fish was conducted during a 16-minute runtime, with the mobile phase flowing at a rate of 0.3 mL/min, as per the solvent gradient programme outlined in Table 1. The temperature of the thermostatic column oven was set to 40°C, and the injection volume was standardised to 2.0 µL. Electrospray ionisation (ESI) functioned in positive mode, with the following parameters: source temperature (TEM) maintained at 450°C, ion spray voltage (IS) set at 5500 V, collision gas (CAD) optimised to medium, curtain gas pressure (CUR) maintained at 25 psi, atomising air pressure set to 45 psi, auxiliary gas pressure adjusted to 45 psi, and the input potential calibrated to 10 V [14]. A Multiple Reaction Monitoring (MRM) separation and detection system employing positive ionisation confirmation ions was utilised to facilitate quantification. The method acquisition, instrument control, and data processing were facilitated by Analyst version 1.6.3 software. Table S2 describes the target pesticides under study along with their respective (MRM) transitions and LC conditions.

Table 1. Gradient elution programme for LC-MS/MS.

Total time(min)	Flow Rate (mL/min)	A (%)	B (%)
0	0.3	60	40
1	0.3	60	40
11.5	0.3	10	90
12	0.3	0	100
13	0.3	0	100
14	0.3	60	40
16	0.3	60	40

Where solvent A comprising a mixture of water and methanol in a ratio of 9:1 (v:v), adjusted to pH 4 using ammonium formate while solvent B consisting solely of methanol.

Table 2. GC parameters for the acquisition method.

	Rate (°C/min)	Value (°C)	Hold time (min)	Run time (min)
(Initial)		60	1	1
Ramp 1	40	170	0	3.75
Ramp 2	10	310	3	20.75

2.4.2. GC-MS/Ms

GC-MS/MS analysis was conducted employing an Agilent 8890 gas chromatograph equipped with a multi-mode inlet system, which was coupled to an Agilent 7010B triple quadrupole mass spectrometer. Chromatographic separations were conducted utilising coupled columns of the HP-5 MS Ultra inert capillary column (composed 5% phenyl – methylpolysiloxane), each with dimensions of 15 metres in length, 0.25 millimetres in inner diameter, and a film thickness of 0.25 µm, procured from Agilent Technologies. The midpoint backflush occurred during the analysis.

The analysis of GC parameters can be summarised in Table 2. The entire run lasted 20.75 minutes with a constant flow rate of 0.917 ml/min. Post-column backflush was initiated for 3 min at a pressure of 50 psi to expedite analysis time and reduce maintenance needs. The inlet temperature was set at 280°C, and a 1 µL injection volume was used in splitless mode via a multi-mode inlet. High purity helium (99.999%) served as the carrier gas, while nitrogen (99.9999%) was utilised as the collision gas. Ionisation was conducted in the electron ionisation (EI) mode with an ionisation energy of 70 eV. The ion source temperature was set at 280°C, while the quadrupole temperature was maintained at 150°C [15]. Dynamic (MRM) was utilised to enhance sensitivity compared to traditional time segments. The analysis employed MassHunter version 10.0 software for instrument control, method acquisition, quantitation, and data processing. Table S3 detailed the target pesticides investigated, along with their respective (MRM) transitions and GC conditions.

2.5. Collection of tilapia samples

All tilapia samples were collected from the Egyptian commercial market. Each sample, weighing approximately 500 g, was minced and homogenised to a particle size below 300 µm using an electric mill at room temperature. Before extraction, all samples were tested as blanks to confirm the absence of the residues under study. Subsequently, the samples were stored at −20°C until the extraction process.

The extraction of fat content in a fish sample was done using the Soxhlet instrument [16]. The process involved several steps, weighing 25 g of the fish sample and mixing it with 50 g of sodium sulphate, adding 20 ml of methanol, and placing the sample in Cellulose Extraction Thimbles, which were then put in the Soxhlet instrument. Subsequently, a mixture of 400 ml n-hexane and dichloromethane (1:1) was added, and the sample was left in the instrument for 24 hours. The fat percentage in the tilapia fish was determined to be 1.7% using the formula:

$$\text{Fat percentage\%} = \frac{\text{fat weight}}{\text{sample weight}} \times 100 \quad (1)$$

2.6. Sample preparation

The basic method used in this study was the citrate buffered QuEChERS method [17]. Further optimisation of the QuEChERS method was carried out to make it more suitable for the nature of the raw material studied. The optimised conditions were as follows: 2 g of fish sample was weighed into a 50 mL polypropylene centrifuge tube, followed by the addition of 10 mL acetonitrile: acetone (9:1). The sample was then ground at 300 rpm for 1 min using Ultra-Turrax. The sample underwent shaking for 1 min at 500 rpm using a shaker. Subsequently, the QuChERS buffer kit was added, followed by another round of shaking for 1 min at 500 rpm. The tube was then centrifuged for 5 min at 4500 rpm at 4°C. A 2 mL portion of the acetonitrile layer underwent filtration through a 0.45 mm syringe filter before being transferred to an autosampler vial for subsequent injection into the LC-MS/MS system. A further 5 mL of acetonitrile: acetone extract was transferred to a 15 mL tube containing dispersive solid phase extraction (D-SPE) reagent (150 mg PSA, 150 mg GCB, and 900 mg $MgSo_4$ per 5 mL of extract). The tube was vortexed by hand for 30 s and centrifuged at 4500 rpm for 2 min at 4°C. After the centrifugation step, 2 mL of the supernatant was transferred to a 50 ml glass conical flask. Subsequently, the solution was desiccated to complete dryness utilising a rotary evaporator set at 300 rpm and maintained at 40°C. The resulting residue was then dissolved in 2 mL of hexane: acetone (9:1) solution supplemented with aldrin at a concentration of 0.1 µg/mL. This mixture was subjected to ultrasonication for 30 seconds. The solution was then filtered through a 0.45 µm syringe filter and injected into the GC/MS-MS system as shown in Figure 1.

2.7. Method validation

The validation of the developed protocol followed the method validation criteria outlined in document SANTE 11,312/2021v2 [18,19]. The parameters assessed included sensitivity/linearity of the calibration to verify linearity across calibration levels. Different spiking levels were carried out on the tilapia fish to calculate four key parameters: Trueness, represented by the mean recovery at each concentration level, precision indicated by the relative standard deviation (RSD) at each level, accuracy derived from the combined assessment of trueness and precision, and the limit of quantification (LOQ), indicating the lowest validated spike level meeting criteria for trueness and precision, were evaluated. Linearity was assessed based on the correlation coefficient (R^2) and the deviation between the back-calculated and actual concentrations. The assessment of the matrix

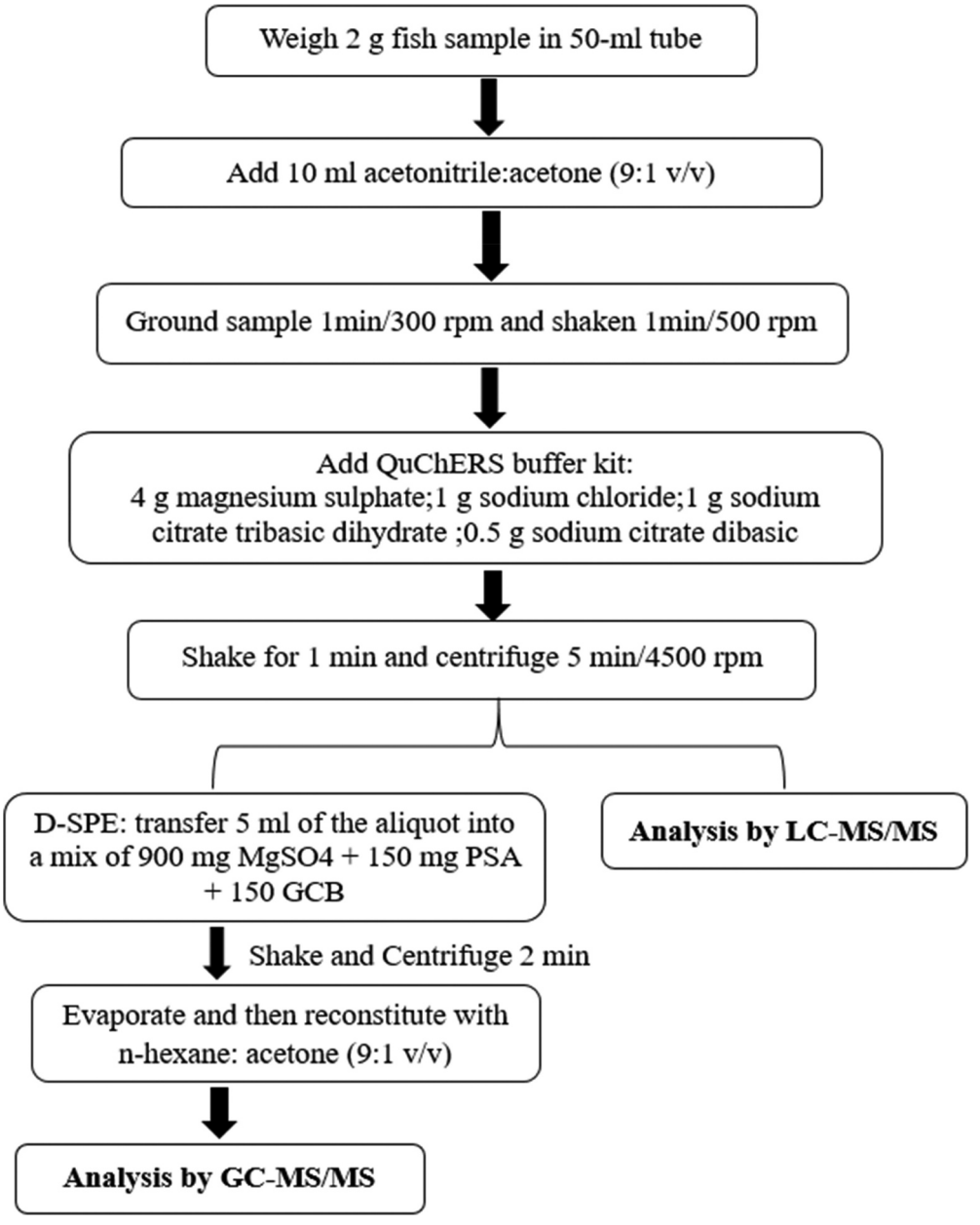

Figure 1. Analytical flow the QuEChERS development procedure used to determination of pesticides in fish.

effect involved comparing a two-point matrix-matched solution with the calibration curve. To balance any enhancement or suppression caused by the matrix effect on the results, a two-level matrix-matched standard was employed. The calculations were conducted utilising the following formula:

$$\text{Matrix Effect\%} = \frac{\text{peak area standard in matrix}}{\text{peak area standard in solvent}} \times 100 \quad (2)$$

2.8. Real samples apllication

The developed method was practical for determining of multi-class pesticides in 15 samples of tilapia fish (Oreochromis niloticus). The tilapia fish were purchased from 3 local markets (Dokki, Imbaba, and Hawamdya) in Giza, Egypt during the spring of 2023 (March-May). The samples were thoroughly homogenised using the procedure Grindomix Knife Mill and stored at −4°C until used.

3. Result and discussion

3.1. Optimization of the extraction and clean-up procedure

Sample preparation plays a crucial role in multi-residue pesticide analysis, significantly impacting quantification and detection limits. The efficiency of extraction is heavily influenced by the choice of organic solvents, sample characteristics, and the chemical properties of pesticide residues [20]. Fatty animal matrices, such as fish tissues, comprise numerous matrix constituents possessing characteristics akin to the target pesticides. Consequently, conventional solvent extraction methods fail to adequately differentiate between these matrix chemicals and the analytes of interest [21,22]. Therefore, different experimental conditions were evaluated to eliminate co-extractives, including different solvent extraction methods, different ratios of optimal solvent extraction with acetone, and different clean-up procedures. Different solvents such as acetonitrile, methanol, and acetone were used to minimise or eliminate lipid co-extraction [23,24]. The highest recovery was observed with acetonitrile, therefore different ratios of acetonitrile to acetone were tested. The mixture of (9:1 v/v) acetonitrile with acetone proved to be the most effective extraction solvent, indicating minimal partitioning of lipids into the acetonitrile and acetone phases. In addition, acetone can efficiently eliminate salts and highly polar matrix constituents from the extract.

The modified QuEChERS approach was applied to all 411 targeted pesticides, and recovery ranged from 70 to 120 with no matrix influence as shown in Table S4. Except for 14 pesticides, which showed high recovery and were influenced by matrix enhancement in GC-MS/MS. To address the issues arising from matrix enhancement in GC-MS/MS, we employed PSA (150 mg) combined with MgSO4 (900 mg), a blend of PSA (150 mg) and MgSO4 (900 mg), octadecylsilane (C18) (150 mg), and a mixture of PSA (150 mg), MgSO4 (900 mg), and GCB (150 mg) for sample purification. These cleanup agents were chosen for their effectiveness in eliminating interfering substances and highly pigmented components commonly encountered in food and environmental samples, as documented [25] as shown in Table 3.

For challenging pesticides affected by the matrix enhancement in GC-MS/MS, the mixture between PSA and GCB sorbent demonstrated satisfactory recovery rates (70–120%) and significantly reduced matrix effects during (d-SPE) treatment. The LOQs of these compounds were 0.01 mg/kg. A comparison of the selected cleanup reagent is illustrated in Table S5.

Table 3. Comparison between pesticides using different clean up method (PSA, mix of PSA and GCB, and mix of PSA and C18) in GC- MS/MS. Recoveries at 0.01 mg/Kg for selected pesticides that are not within the range according to SANTE.

pesticides	Main Rec % PSA	RSD % PSA	Main Rec % PSA+C18	RSD % PSA+C18	Main Rec % PSA+GCB	RSD % PSA+GCB
Chlorpyrifos	137	10	135	5	117	2
Difenoconazole	146	12	121	11	102	7
Ethion	124	7	113	13	101	11
Etoxazole	142	9	112	6	110	5
Flucythrinate	124	5	113	1	108	5
Fluvalinate-tau	141	13	109	1	104	4
Iprodione	131	14	103	17	101	12
Methiocarb	125	10	106	7	103	9
PCBs 118	124	5	131	6	111	6
PCBs 138	128	4	136	9	120	7
PCBs 52	123	6	124	7	119	5
Phenthoate	125	14	100	8	89	6
Tetramethrin	235	5	145	10	120	9
Triadimenol	163	6	131	6	120	11

3.2. Method validation

3.2.1. Selectivity

A selectivity study was assessed to evaluate the ability of the developed method's capability to detect multiclass pesticides without interference from sample composition. Using the developed protocol, three blank replicate samples were analysed from the tested tilapia samples. In addition, the LC-MS/MS and GC-MS/MS were directly injected with blank acetonitrile and acetonitrile/acetone (9/1). No peaks were seen at the same retention time of 411 pesticides. This confirmed the selectivity of the method.

3.2.2. Trueness and precision

The accuracy and precision of the method were assessed through the injection of six replicate samples of fish at spiking concentrations of 0.01 and 0.05 mg/kg. Mean recoveries were used to evaluate the method's trueness, with an acceptable range set at 70–120%. Precision was determined by calculating the (RSD), with values considered acceptable if ≤ 20% as shown in Table S4. The method demonstrates satisfactory performance for tilapia fish, with average recovery falling within the range of 71 to 120%, except for Bispyribac, Cinidon Ethyl, Dichlofluanid, and Thiodicarb which showed a recovery above 120 or lowest 70, as shown in table S4. For all selected analytes, the RSD% was in the range of 1–20. The repeatability of the method was evaluated via intra-day precision by analysing six different spiked samples into tilapia matrices at two spiking levels of 0.01 mg/kg and 0.05 mg/kg. The chosen spiking concentrations were aimed at aligning with the Maximum Residue Limits (MRL) established for pesticide residues in the fish. To evaluate the method's reproducibility at the lowest spiking level, the inter-day precision test was conducted, involving ten replicates performed over five consecutive days by five distinct analysts. This evaluation aimed to determine the consistency and reproducibility of the method under varying conditions and across different analysts. The developed method demonstrated satisfactory precision, with average recoveries ranging from 1 to 20% for intra-day and 2 to 19% for inter-day analyses, as indicated by the RSD values. Moreover,

employing the specified LC-MS/MS and GC-MS/MS parameters, the retention time for the chosen pesticides remained stable, exhibiting variances of less than 0.1 minutes ($n = 20$).

3.2.3. Linearity of calibration

The concept of linearity in a methodology concerns the range of analyte concentrations within which the method is applicable. The linearity of the developed method in LC-MS/MS was assessed by injecting six calibration levels (0.001, 0.002, 0.005, 0.01, 0.05, and 0.01 µg/ml) in both the solvent (acetonitrile) and blank tilapia samples. Also, the evaluation of GC-MS/MS involved the injection of five calibration levels (0.002, 0.01, 0.05, 0.01, and 0.05 µg/ml), which were prepared in the solvent (Hexane: Acetone 9:1) and in blank tilapia samples. Linear responses were attained for the chosen analytes in both solvent and matrix-matched calibrations throughout the investigated concentration ranges. The deviation between the back-calculated concentrations and the true values fell ≤ ± 20% and all examined fish samples demonstrated notably high correlation coefficients ($R^2 > 0.998$) as shown in Table S4, indicating exceptional linearity across a wide concentration range.

3.2.4. Limit of quantification

As per the SANTE/11312/2021v2 guidelines, the (LOQ) signifies the minimum validated concentration that satisfies the mean recovery and (RSD) criteria outlined by the analytical method employed. In this study, the LOQ of the developed method for all selected pesticides was determined to be 0.01 mg/kg except,(Cyazofamid- Dodine- Fenpyrazamine- Methomyl- Oxadiargyl- Parathion ethyl-Parathion methyl-Prothioconazole Desthio -Tolylfluanid) which were 0.05 mg/kg. Chromatograms of selected pesticides at the LOQ level for tilapia in the matrix were presented in Figures 2 and 3.

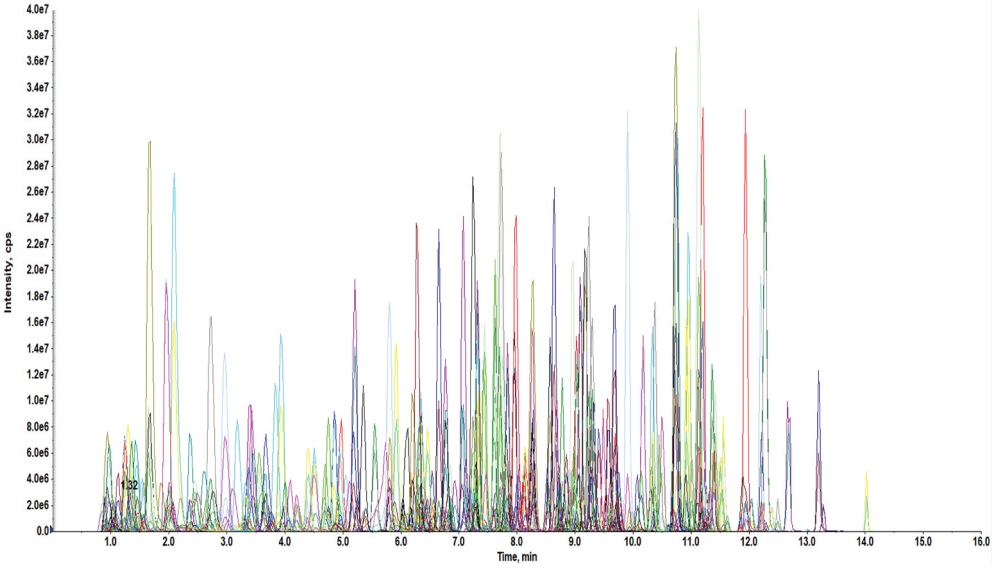

Figure 2. Injecting a fish sample into the LC-MS/MS system to generate an extracted Ion Chromatogram for all Multiple Reaction Monitoring (MRM) separation and detection systems, with a concentration of 0.01 µg/ml for pesticides.

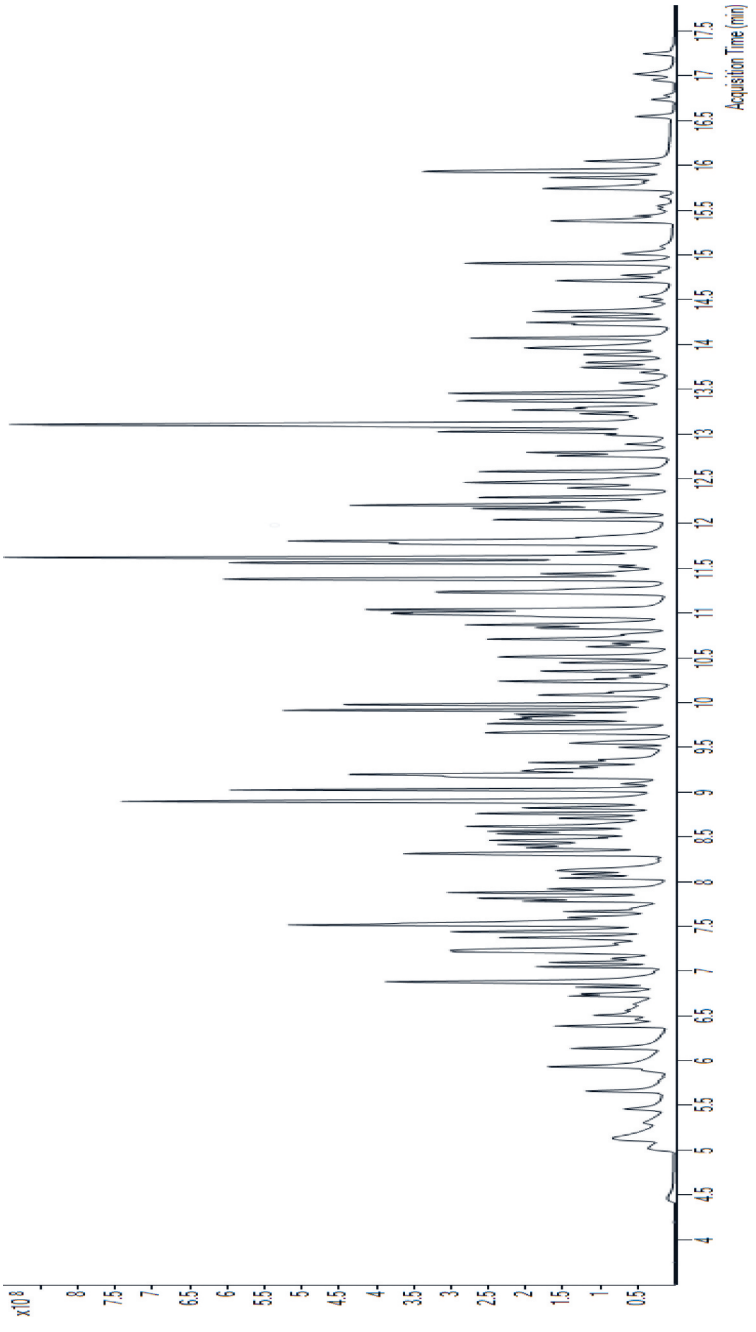

Figure 3. Injecting a fish sample into the GC-MS/MS system to generate an extracted Ion Chromatogram for all Multiple Reaction Monitoring (MRM) separation and detection systems, with a concentration of 0.01 µg/ml for pesticides.

3.2.5. Matrix effect

The phenomenon where the signal of a target analyte can be enhanced or suppressed owing to the presence of interfering components in the sample matrix is referred to as the Matrix Effect (ME). This phenomenon is influenced by variables including the chemical attributes of the target analyte, the matrix type, and the proportion of matrix to analyte concentration [26,27]. To assess the (ME), the ratio of the slope derived from the pure solvent and the slope derived from the matrix-matched calibration prepared in the blank sample extract (tilapia samples) was computed using Equation (2). Based on the resulting value, the nature of the (ME) can be discerned. A slope ratio exceeding 1 signifies signal enhancement, whereas a slope ratio below 1 indicates signal suppression of the target analyte, attributable to the presence of interfering components within the sample matrix.

As is often the case in GC-MS/MS, the analyte is subject to enhancement because the matrix components enhance the ionisation of the analyte, increasing the analyte signal [28]. Accordingly, as shown in Table S5, matrix-matched calibration solutions were used to quantify this matrix-influenced analyte.

The developed GC-MS/MS method exhibited matrix effect and recovery for most analytes within the specified range according to SANTI, except for some pesticides. Chlorpyrifos, difenoconazole, ethion, etoxazole, flucythrinate, fluvalinate-tau, iprodione, methiocarb, PCBs 118, PCBs 138, PCBs 52, phenthoate, tetramethrin, and triadimenol showed matrix effect and recovery outside the limited range in both PSA and a mixture of PSA and C18. However, after employing a clean-up reagent with a mixture of PSA and GCB, the matrix effect and recovery for these pesticides fell within the acceptable range as per the guidelines.

3.3. Method application and monitoring of real samples

The effectiveness of the established method was confirmed through its utilisation for the analysis of specific pesticide residues in 15 authentic samples [29]. The tilapia were bought from (Dokki, Imbaba, and Hawamdya) in Giza, Egypt, between March and May 2023, three (acetamiprid, chlorpyrifos, and pp′- DDE) out of 411 pesticides were detected in tilapia fish products. This represents a detection rate of 13.3% (2 out of 15 samples) with concentrations ranging from < LOQ to 0.07 mg/kg as shown in Table 4. According to Eissa et al. [30], pesticide residues were detected in tilapia fish from the Nile River's Rosetta Branch, representing some pesticides investigated in this study.

In contrast, the concentration of acetamiprid was above the (LOQ). This raises concerns about its presence, despite being detected at minimal levels, as acetamiprid, an insecticide, has been associated with various effects on fish. In a study in zebrafish, long-term exposure to acetamiprid at environmentally relevant concentrations disrupted endocrine function, bioaccumulated, feminised, and resulted in transgenerational effects. Apart from

Table 4. Levels of pesticide residues detected in the analysed fish samples using LC-MS/MS and GC-MS/MS.

Pesticides	concentration (mg/kg)	Instrument
Acetamiprid	0.066 and 0.04	LC-MS/MS
Chlorpyrifos	0.01	LC-MS/MS & GC-MS/MS
pp′ - DDE	< LOQ	GC-MS/MS

influencing hormone levels, it induced feminisation and reproductive dysfunction in zebrafish, impacting the development and production of their offspring [31]. These findings underscore the potential impact of acetamiprid on fish and the environment, underscoring the necessity for further research and stringent regulation of its usage. Regular and comprehensive monitoring of this contaminant is essential to ensure compliance with regulatory standards and mitigate potential risks to aquatic ecosystems [32].

Finally, the decision was made to include data for a huge number of pesticides (411) to facilitate a comprehensive analysis of a broad range of fish samples, aiding in meeting export requirements.

4. Conclusion

In this study, an analytical method for monitoring pesticide residues in fish products was developed, with a focus on the quantification of 411 pesticide residues in fish products using LC-MS/MS and GC-MS/MS. The method was optimised using the modified QuEChERS method and demonstrated satisfactory accuracy and precision. EGYPT Samples of tilapia (Oreochromis niloticus) were analysed to determine the levels of target pesticide residues. The findings of this study offer insights into assessing the levels of pesticide residues in fishery products. Subsequent studies are warranted to appraise the health implications linked to the consumption of fish contaminated with pesticide residues.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Credit authorship contribution statement

Mohamed Mamdouh: Writing – original Draft, Visualisation, Validation, Methodology Investigation, Formal analysis, Data curation.

Mohamed E. Amer: Conceptualisation, Writing – review & editing, Supervision, Resources, Formal analysis.

Ahmed A. Omran: Conceptualisation, Writing – review & editing, Supervision.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

Informed Consent was obtained from all individual participants included in the study.

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