Assessment of Pesticide Contamination in Vegetables Using QuEChERS and LC-MS/MS Techniques.

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Abstract:

Food safety and regulatory compliance depend on detecting pesticide residues in vegetables. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is a sophisticated analytical technique that offers superior sensitivity and specificity for this purpose. This paper provides a comprehensive method for extracting, cleaning, and quantifying various pesticide residues in vegetable samples using LC-MS/MS. Before extracting the vegetable samples, the QuEChERS method homogenises them with acetonitrile, followed by a salting-out step to achieve efficient partitioning. Matrix interferences are then removed from the extract using dispersive solid-phase extraction (d-SPE). I took some vegetables, including cauliflower, cabbage, and okra, from the Sardar market in Surat. The homogenized extract was then created. Tandem mass spectrometry provides comprehensive mass analysis by using precursor ion fragmentation and product ion detection. In contrast, LC-MS/MS uses liquid chromatography to extract the pesticides from the cleaned extract. Accurate measurements are made possible by calibration curves constructed from recognised pesticide standards. The robustness and dependability required for food safety assessments are ensured by this technique's excellent performance in detecting trace levels of multiple pesticides simultaneously. By ensuring compliance with stringent regulatory requirements, LC-MS/MS pesticide residue analysis protects consumer health.

1. Introduction:

To ensure food safety and consumer health, it is essential to detect pesticide residues in vegetables. Vegetable tissues and surfaces can retain pesticides, which could be harmful to human health if ingested. Pesticides are widely used in agricultural practices to reduce pests and boost crop yields. Global regulatory authorities have established maximum residue limits (MRLs) for specific pesticides in food products to reduce these risks. To monitor pesticide levels and ensure compliance with these regulations, we require accurate and reliable analytical techniques. For the analysis of pesticide residues in complex matrices such as vegetables, the combination of liquid chromatography and tandem mass spectrometry (LC-MS/MS) has shown promise. LC-MS/MS is created by combining the separation power of liquid chromatography with the high sensitivity and specificity of mass spectrometry. Because it allows simultaneous detection and quantification of multiple pesticides at trace levels, this approach is very useful for routine monitoring and enforcement of food-safety regulations. Important LC-MS/MS procedures include sample preparation, extraction, cleanup, chromatographic separation, and mass spectrometric detection. A typical step in sample preparation that ensures consistency is homogenization. Because of its ease of use and efficiency, we regularly employ the

QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method for extraction. To remove co-extractives that can obstruct the analytical process, we use cleaning methods such as dispersive solid-phase extraction (d-SPE). The chromatographic phase uses a suitable LC column, typically a reversed-phase C18 column, to separate pesticides based on their chemical properties. After separation, the chemicals are ionized and analyzed by the mass spectrometer. By identifying specific precursor-product ion transitions, tandem mass spectrometry (MS/MS) increases selectivity and enables precise pesticide identification and quantification [1].

Apart from these technological problems, the development of targeted, risk-oriented sampling and analysis techniques is becoming increasingly important. These tactics involve gathering information from several laboratories and organizing its interchange. The Pesticides Online information exchange service, which already has over 750 users from 65 countries, is a helpful resource in this regard. QuEChERS is a novel sample preparation technique for pesticide multi-residue analysis that was developed between 2000 and 2002 and first published in 2003 [2]. Even though it is a relatively new technology, pesticide residue experts worldwide have already widely accepted it, and it has been covered in many publications, both in its original form and in modified forms [3].

Acetonitrile is first extracted during the QuEChERS procedure, followed by the addition of a salt mixture and another extraction or partitioning step. The next step is to use dispersive solid-phase extraction (D-SPE) to clean an aliquot of the raw extract. The resulting acetonitrile extract can be immediately subjected to determinative analysis using LC or GC. The QuEChERS method efficiently extracts highly polar pesticides as well as those that are very basic and acidic. Other advantages include the method's high sample throughput and low solvent, glassware, and bench area. The method can be used to analyze commodities with intermediate or high fat concentrations, provided particular considerations are made, even though it was primarily designed for low-fat commodities [4].

Several pesticides found in food extracts can be precisely identified and quantified when LC and GC are used in conjunction with MS/MS detection. These methods have been successfully used in several recent papers to analyze pesticides in fruits and vegetables. Simple extraction methods with minimal washing are used because MS/MS detection offers such great selectivity. A GC-MS/MS and LC-MS/MS multi-residue technique for 130 multiclass pesticides after ethyl acetate extraction [5].

After extraction with acetone, partitioning with dichloromethane, and SPE cleanup, we employed a combination of GC-MS and LC-MS/MS techniques to assess 450 pesticides in wine, honey, and fruit juice, and 446 pesticides in fruits and vegetables. A method for analyzing

405 pesticides in grain samples following SPE cleanup and acetonitrile-based accelerated solvent extraction (ASE) was reported by the same team. After ASE, it analyzed several pesticides using GC-MS/MS and LC-MS/MS [6].

Compared with powdered pesticide preparations, liquid pesticides sprayed on plants contaminate them more. Because OCP insecticides can build up in the waxy covering that coats the rind of many fruits, especially citrus, the structure of the plant is also important. Pesticide residues in fruits and vegetables can increase the human risk of a number of diseases; we need to keep a close eye on them. Determining the level of pesticide contamination in fruits and vegetables requires an understanding of MRLs. The Maximum Residue Limits (MRLs) for pesticides found in fruits and vegetables are established by the EU. Acephate, aldrin, dichlorvos, and fenthion are among the pesticides with a limit of detection (LOD) of 0.01–0.05 mg/kg. Simazine's LOD for cherries is 0.25 mg/kg, which is 0.1 mg/kg more. Grape samples can contain up to 5 mg/kg of malathion, whereas tomato and tangerine samples can contain up to 7 mg/kg [7].

2. Materials and Methods

2.1 Reagents and Chemicals:

- Acetonitrile (HPLC grade)
- Methanol (HPLC grade)
- Water (HPLC grade)
- Formic acid
- Magnesium sulphate ($MgSO_4$)
- Sodium chloride (NaCl)
- Primary Secondary Amine (PSA)
- C18 sorbent
- Pesticide standards (certified reference materials)

Equipment:

- Homogenizer
- Centrifuge
- Vortex mixer
- Analytical balance
- Liquid Chromatography system
- Tandem Mass Spectrometer (MS/MS)
- Solid-Phase Extraction (SPE) cartridges
- Pipettes and micropipettes

2.2 Methods

a) Collection and Identification of Samples

The target vegetables, such as okra, cabbage, and cauliflower, for pesticide analysis were collected from a market sample in Surat. The different fresh samples were stored at room temperature. Once homogenized, the samples were stored in the refrigerator. The authorized person identified the collected samples.

b) Sample Preparation and Extraction

Weigh 1kg of vegetable samples (e.g., okra, cauliflower, and cabbage), then homogenize using a homogenizer to ensure a uniform sample matrix. Weigh 15 g of crushed sample in a 50 mL Centrifuge tube. Add 15 mL of 1% acetic acid in acetonitrile, vortex for 30 sec., and add 6 g anhydrous $MgSO_4$, 1.5 g of anhydrous sodium acetate, and 75 µL I. S. solution. Shake vigorously for 1 min. Centrifuge at 4000 rpm for 5 min. Transfer 1mL supernatant into a 15 mL centrifuge tube containing 50mg PSA & 150mg anhydrous $MgSO_4$ and shake for 30 s. Centrifuge at 4000 rpm for 5 min. Carefully transfer the cleaned extract to an LC vial for analysis [8].

The methods and phases of sample preparation may affect the results. Therefore, whether the analysis provides the necessary information depends on how well the sample is prepared. For analysis, we require a homogeneous and representative sample of the substance. The chemical composition of a representative sample is intended to be as close as feasible to the average composition of all the material being studied. The sample should be kept cool and dark. The sample preparation procedure typically consists of several stages. Washing veggies in distilled water to remove surface impurities is one such method. The sample is then dried at room temperature, with a desiccant, or both. The sample is next broken up and crushed, or pounded in a mill or with a mortar and pestle, before being homogenized. The preparation procedure will differ depending on the kind of material being studied, and any combination of these processes could result in analyte loss or further contamination of the sample [7].

c) The LC-MS/MS device list was as follows, carried out at IIT Mumbai.

- Binary pump, autosampler, column, MS-Q-TOF (G6550A), DAD.

Mobile Phase:

- Solvent A: Water with 0.1% formic acid
- Solvent B: Methanol with 0.1% acetonitrile

Gradient Elution:

- Start with 95% A and 5% B.
- Gradually change to 5% A and 95% B over 20 minutes.

- Maintain at 5% A and 95% B for 5 minutes.
- Return to initial conditions and re-equilibrate for 5 minutes.
- Flow Rate: 0.3 mL/min
- Injection Volume: 3 µL

Mass Spectrometry:

- Ionization Source: Electrospray ionization (ESI) in positive and negative modes, depending on the pesticide.
- MS/MS Detection: Use multiple reaction monitoring (MRM) mode to detect specific precursors and product ion transitions for each pesticide [9].

Instrument Settings:

a) Source temperature: 250°C

b) Desolation temperature: 300°C

For the LC gradient to function, the two eluent components listed below were required: A: 5 mM water and ammonium formate; B: 5 mM methanol and ammonium formate (%). At injection time, it was operated at 300 µL min−1, with 100% component A initially. We gradually changed it to 60% A (40% B) over three minutes, and 22 minutes later, to 10% A (90% B). We maintained this eluent composition for 8 minutes, after which it returned to its initial state (100 % component A) in 0.1 minutes, where it remained until 38 minutes after injection. The injection volume was 5 µL, and the column temperature was 40 °C. We performed MS/MS detection in multiple reaction monitoring (MRM) mode using an ESI interface in positive ion mode. The nebuliser gas was synthetic air at 60 psi, the curtain gas was nitrogen at 30 psi, and the ionisation voltage was 5500 V. A drying gas (heated synthetic air at 420 °C/50 psi) helped the solvent evaporate at the source. Through a series of investigations using solutions of particular analytes, we discovered declining potentials, collision energies, and optimal MRM transitions for each molecule. We constantly injected the standard solutions into the apparatus using a syringe. An Applied Biosystems API 3200 Qtrap device, which we initially used to set the compound acquisition parameters, proved inappropriate for the investigation. The acquisition files were converted using software from the same manufacturer to fit the API 4000 Qtrap model [10].

3. Results and Discussion

1 kilogram of each vegetable was purchased from a Sardar market in Surat. The geographical location was 21.1935470 Lat and 72.851120 Long. They were stored in polyethylene bags and labelled correctly. The sample was identified by the authorised person from B.K.M. Science College, Valsad, and their voucher no 2026 for Brassica oleracea var. capitata (cabbage) from the Brassicaceae family. The 2027 voucher no for Brassica oleracea

var botrytis (cauliflower) is also from the Brassicaceae family. The 2028 voucher no for Abelmoschus esculentus (okra) from the Malvaceae family.

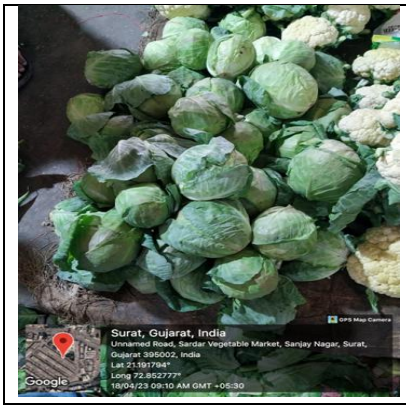
		
Cabbage	Cauliflower	Okra

Figure :1 Collection of samples

The analysis found pesticide residues in many vegetable samples, including okra, cabbage, and cauliflower. We found varying amounts of pesticides; some vegetables had residues below the limit of quantification (LOQ), while others had detectable levels of many pesticides.

The results include the chromatogram.

Okra: Detected pesticides included **Imidacloprid, Acetamiprid, and chlorpyrifos**.

Cabbage: Detected pesticides included **chlorpyrifos**.

Cauliflower: Detected pesticides included **Imidacloprid, Acetamiprid, and chlorpyrifos**.

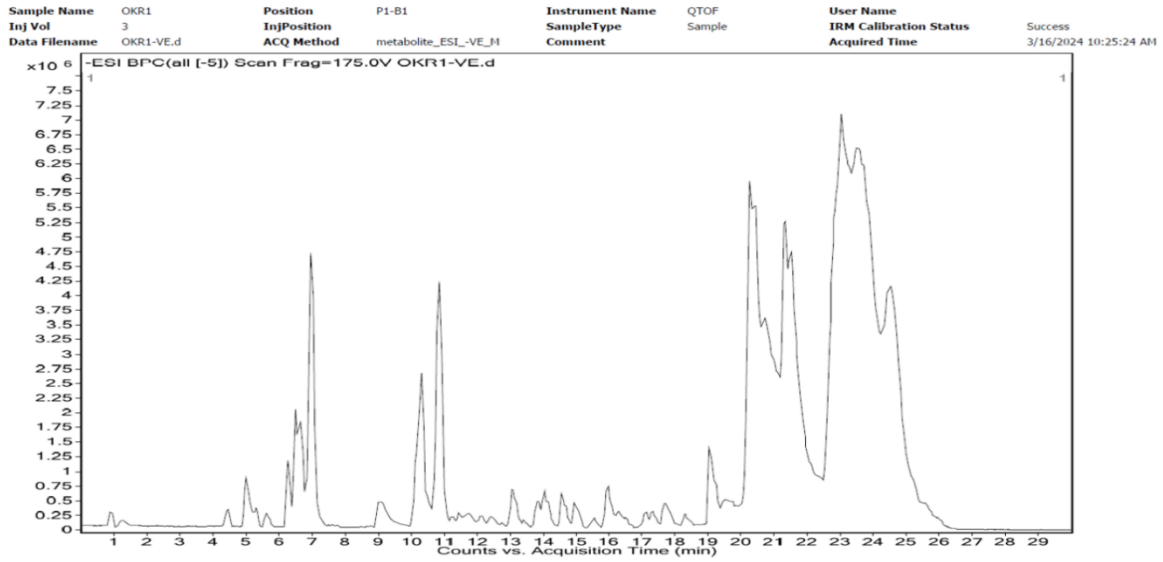
Vegetable samples met food safety criteria because all detected pesticide residues were below the maximum residue limits (MRLs) established by regulatory bodies. Next, the LC-MS/MS approach demonstrated remarkable success in simultaneously detecting and quantifying various pesticide residues in vegetable samples. The high selectivity and sensitivity of the MRM mode in MS/MS enabled accurate identification and quantification of pesticides at trace levels. When paired with dispersive SPE cleanup, the QuEChERS technique was effective at removing pesticide residues from the complex vegetable matrix. The precision data and recovery rates demonstrated the robustness and reproducibility of the sample preparation process.

The most common pesticides in the samples examined were organophosphates, followed by carbamates, pyrethroids, and organochlorines. Similar results were reported in Ghana, where synthetic pyrethroid pesticides, organochlorines, and organophosphates were detected in fresh fruit and vegetable samples. These findings show that nearly all pesticides used in production remain as residues after harvest. Chemicals not allowed for use as pesticides were found in some samples. Despite detecting measurable levels, Tanzania has outlawed the

use of organochlorine pesticides in agriculture. The US and Ghana both released similar results [11].

Vegetables Name	Retention time (Min)	Chemical Formula	Compound Identification	Base Peak	M/Z Ratio
Okra	6.646	C9 H10 Cl N5 O2	Imidacloprid	175.0964	256.0584
	17.701	C9 H11 Cl3 N O3 P S	Chlorpyrifos	197.9255	349.9313
	7.259	C10 H11 Cl N4	Acetamiprid	126.0093	223.073
Cabbage	20.831	C9 H11 Cl3 N O3 P S	Chlorpyrifos	124.086	351.9273
Cauliflower	6.59	C9 H10 Cl N5 O2	Imidacloprid	175.0961	256.0586
	17.654	C9 H11 Cl3 N O3 P S	Chlorpyrifos	197.9255	349.9309
	7.357	C10 H11 Cl N4	Acetamiprid	126.0097	223.0736

Table 1: Pesticide compound identification from vegetable samples.



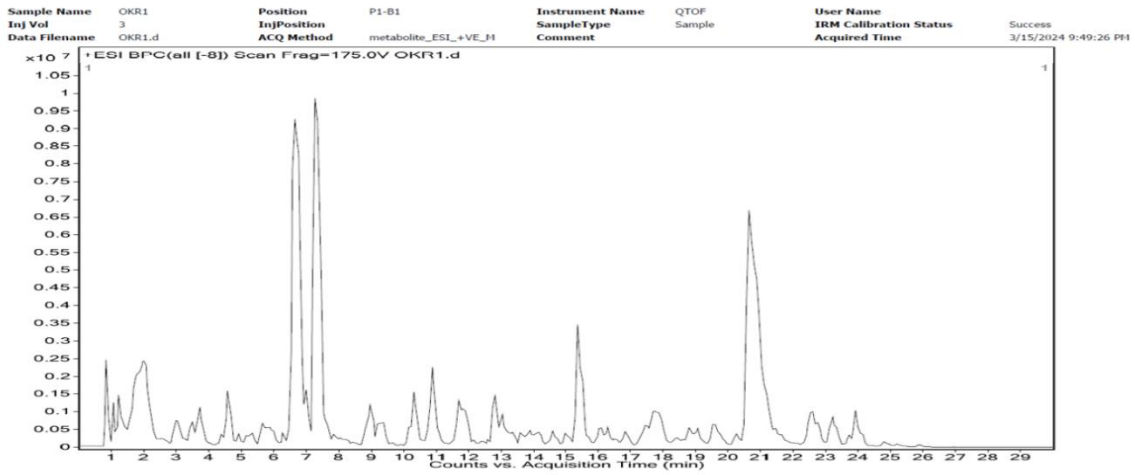


Figure 2: Chromatogram of okra.

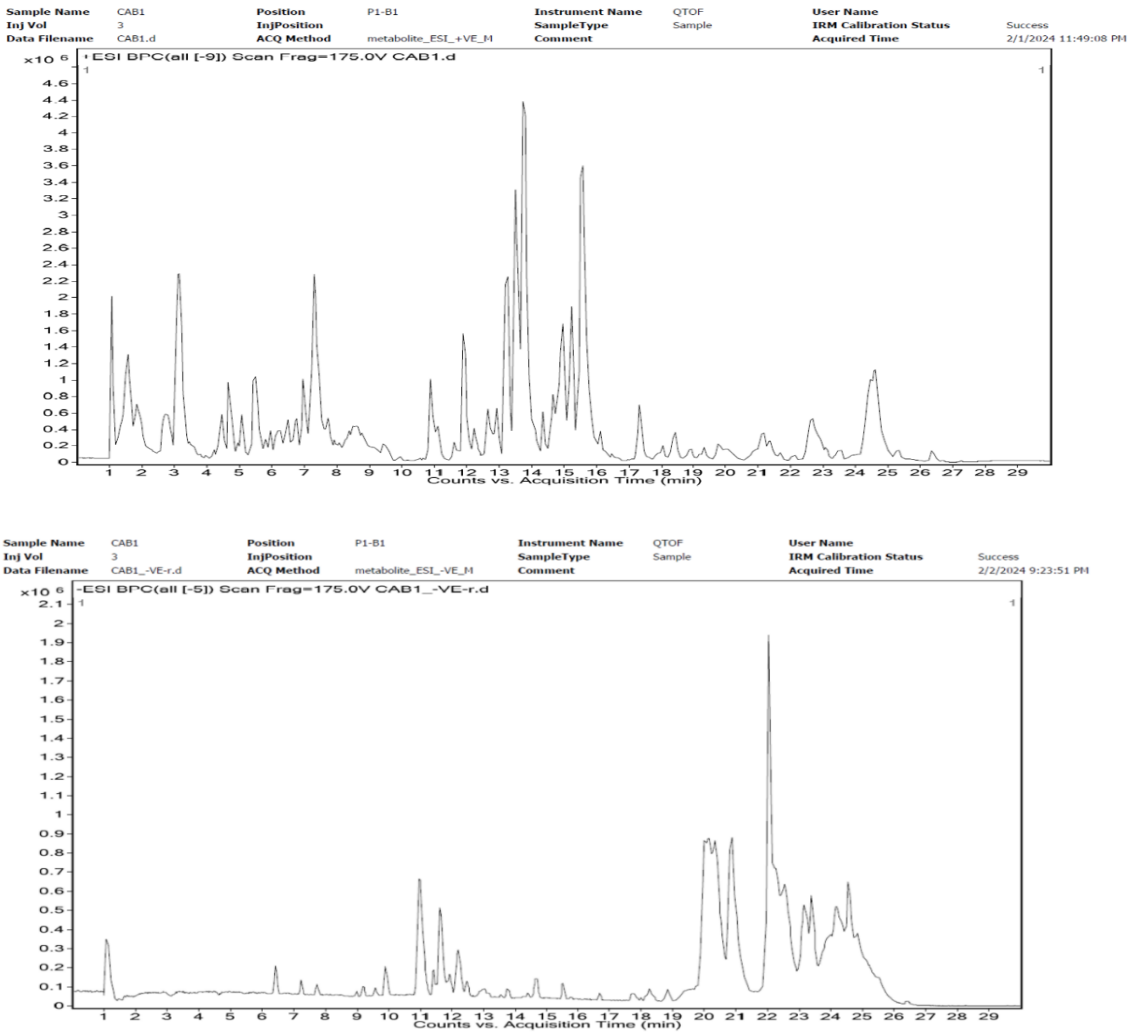


Figure 3: Chromatogram of cabbage.

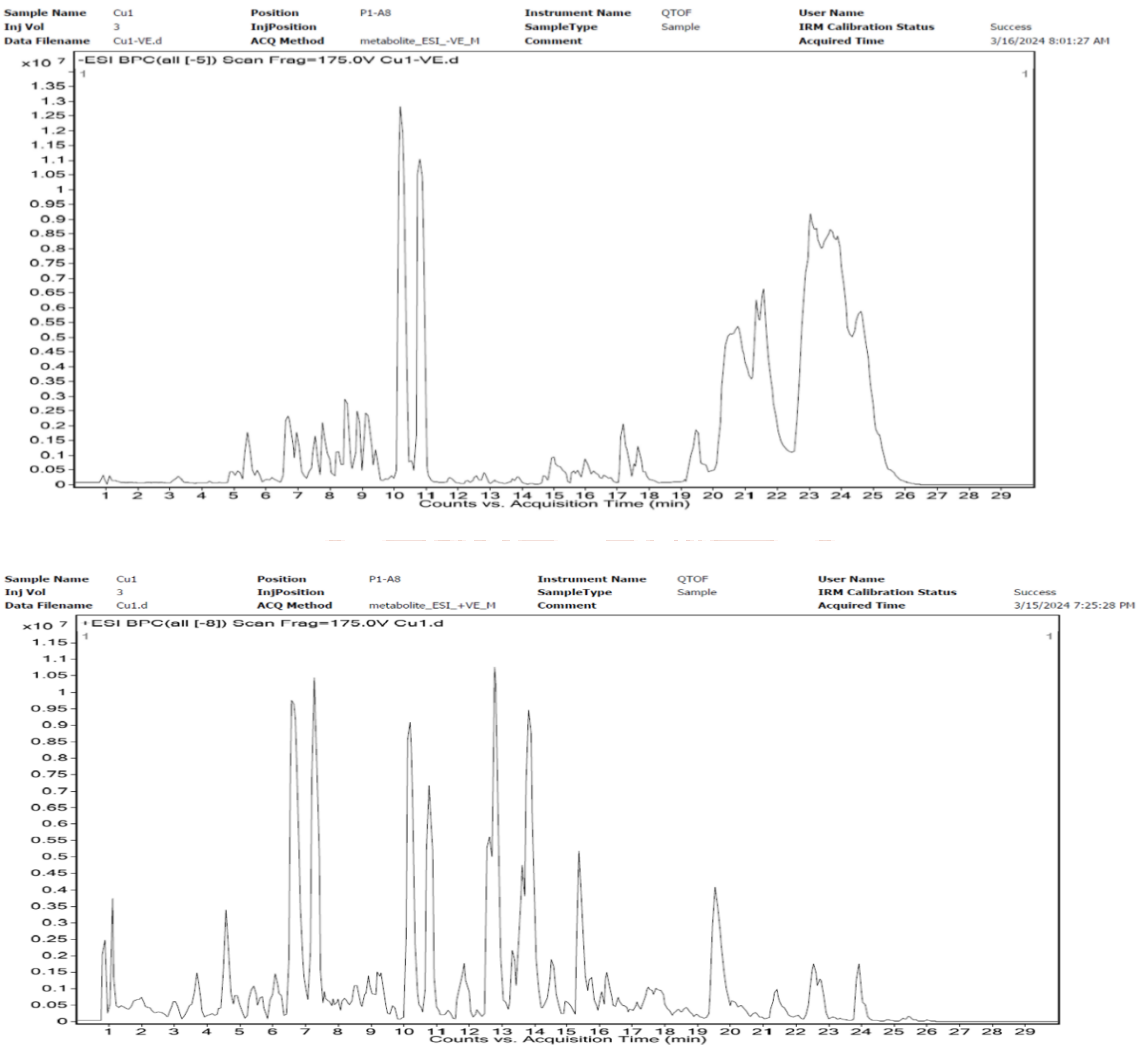


Figure 4: Chromatogram of cauliflower.

Customers run the risk of consuming pesticide residues in their food, given the quantities discovered in vegetable samples. Changes in haematological parameters, liver and renal failure, and reduced acetylcholinesterase activity have all been linked to exposure to organophosphate insecticides [12]. Samples from high routes tended to contain higher levels of pesticides than those from farms and marketplaces, suggesting that farmers applied higher doses of pesticides before harvesting. Farmers may occasionally give extra treatment to vegetables at collection hubs and overspray crops in an attempt to extend their shelf life and attract more consumers. Another possible explanation is the negligent use of pesticides and the disregard of pre-harvest intervals [13]. 95.2% of all pesticide residues found were organophosphates, the most common chemical family of pesticides used in horticultural production, as shown by this study and others [14]. Recovery experiments were conducted at multiple levels on several representative agricultural product samples to evaluate the technique's performance using the adjusted LC-MS/MS parameters and the optimal extraction

procedure. Recovery experiments were conducted on grapes and green beans in six replicates at dosages of 0.01, 0.05, and 0.1 mg/kg [15]. Due to the non-symmetrical peak morphologies caused by injecting 25 µL of acetonitrile into the LC system, the acetonitrile was evaporated, and the sample was redissolved in a methanol-water solution. As previously mentioned, this procedure reduced the matrix impact caused by the precipitation of several insoluble compounds and enhanced the peak shapes of the pesticides. The recovery rates of seven pesticides (chlorfluazuron 24±17, 30±5, 24±16 at concentrations of 0.01, 0.05, and 0.5 mg/kg, L-cyhalothrin, deltamethrin, diafenthiuron, flufenoxuron, lufenuron, and pymetrozine) were less than 60% because of the evaporation of acetonitrile and re-dissolution in water solution [16].

4. Conclusion

The study developed and verified an efficient, reliable LC-MS/MS approach for the detection and quantification of pesticide residues in vegetables. This method demonstrated excellent sensitivity, specificity, and accuracy, making it a useful tool for regular monitoring and legal compliance. The QuEChERS extraction and dispersive SPE cleanup techniques effectively removed pesticide residues from complex vegetable matrices, reducing matrix effects and ensuring reliable analysis. The safety of the vegetables under examination was demonstrated by the absence of pesticide residues exceeding the maximum residue limits (MRLs) established by regulatory organisations. Pesticide compounds like imidacloprid, acetamiprid, and chlorpyrifos were found in several vegetable samples. A thorough approach to tracking pesticide residues in vegetables is enabled by the method's ability to detect multiple pesticides simultaneously at low concentrations. Regular application of this method can guarantee food safety, protect consumer health, and encourage compliance with stringent regulations. The chemical's toxicity can then be ascertained. Although we are all aware that pesticides are harmful, farmers still use them, and we consume them daily through the fruits we eat. To determine the toxicity of ingested substances and their effects on human bodies.

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Conflict of Interest

The authors have no conflicts of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

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