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Analysis of Pesticide Residue in Fruit Using the QuEChERS Method Coupled With LC-MS/MS Detection

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Abstract

Identifying pesticide residues in fruits is essential to both regulatory compliance and food safety. For this use, liquid chromatography combined with tandem mass spectrometry, or liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS), is an advanced analytical method that provides excellent sensitivity and specificity. This work offers a thorough procedure for employing LC-MS/MS to extract, clean up, and quantify various pesticide residues in fruit samples. The QuEChERS technique, which uses acetonitrile and a salting-out step for effective partitioning, first homogenizes the fruit samples before extracting them. Dispersive solid-phase extraction (d-SPE) then eliminates matrix interferences from the extract. I went to the Sardar market in Surat and picked some fruits, such as watermelons, oranges, and grapes. Next, the homogenised extract was made. LC-MS/MS uses liquid chromatography to separate the pesticides from the cleaned extract, while tandem mass spectrometry uses precursor ion fragmentation and product ion detection to provide thorough mass analysis. Calibration curves built using established pesticide standards enable accurate quantification. This technique shows great performance in simultaneously detecting trace amounts of many pesticides, ensuring the robustness and dependability required for food safety evaluations. Pesticide residue analysis using LC-MS/MS guarantees adherence to strict regulatory limitations, protecting consumer health in the process.

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Introduction

Detecting pesticide residues in fruits is critical to ensuring food safety and consumer health. Pesticides can linger on fruit surfaces and in tissues, suggesting health hazards if consumed.[1] Agricultural methods frequently employ pesticides to control pests and increase crop yields. To reduce these dangers, regulatory bodies from all over the

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world have set maximum residue limits (MRLs) for certain pesticides in food products. Therefore, we need precise and trustworthy analytical procedures to monitor pesticide levels and ensure compliance with these rules. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has shown to be a useful technique for investigating pesticide residues in complex materials such as fruits. Mass spectrometry's high sensitivity and specificity combine with liquid chromatography's separation powers to create LC-MS/MS. This technique enables the simultaneous detection and quantification of several pesticides at trace levels, making it very helpful for routine monitoring and enforcing food safety laws. Sample preparation, extraction, cleanup, chromatographic separation, and mass spectrometric detection are some of the crucial processes in the LC-MS/MS technique. Homogenization is a common step in sample preparation that guarantees uniformity. Because of its simplicity and efficacy, we commonly employ the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method of extraction. We use cleaning methods such as dispersive solid-phase extraction (d-SPE) to get rid of any co-extractive contaminants that can interfere with the analysis. The chromatographic phase separates pesticides according to their chemical characteristics using an appropriate LC column, typically a reversed-phase C18 column. The mass spectrometer breaks up and ionizes the compounds after their separation. The tandem mass spectrometry (MS/MS) method improves selectivity and makes it possible to accurately identify and measure pesticides by identifying certain precursor and product ion transitions.[2]

Apart from these technical issues, there is an increasing focus on developing intelligent methods for sampling and analysis that are targeted and risk-oriented. These strategies entail coordinating information exchange and collecting it across laboratories. The Pesticides Online information exchange portal is a helpful resource in this area; it now has 750 users from 65 different countries. QuEChERS is a novel sample preparation technology for pesticide multi-residue analysis that was created between 2000 and 2002 and first released in 2003.[3] Despite being a relatively new technique, it has already gained widespread acceptance among pesticide residue experts worldwide, and numerous publications have already covered it, either in its original form or in modifications.[4]

During the QuEChERS process, acetonitrile is initially extracted, then a salt mixture is added, and another extraction or partitioning step is performed. The next step involves cleaning an aliquot of raw extract using the dispersive solid-phase extraction. Determinative analysis using LC and/or GC is immediately applicable to the final extract in acetonitrile. Pesticides that are very polar as well as extremely acidic and basic are all successfully covered by the QuEChERS technique. The method's high sample throughput and minimal requirements for solvent, glassware, and bench space are other benefits. Although the approach was mainly developed for low-fat commodities, it can also be used to analyse commodities with intermediate or high-fat concentrations if specific considerations are made.[5]

When combined with MS/MS detection, LC and GC provide precise identification and quantification of several pesticides present in food extracts. Several recently published studies have successfully analysed pesticides in fruits and vegetables using these approaches. Because MS/MS detection offers such great selectivity, straightforward extraction methods with minimal cleaning are used. Using GC-MS/MS after ethyl acetate extraction, Martínez-Vidal *et al.* developed a multi-residue approach for 130 multiclass pesticides.[6]

Following acetone extraction, dichloromethane partitioning, and SPE cleanup, we employed a combination of GC-MS and LC-MS/MS techniques to analyse 446 pesticides in fruits and vegetables and 450 pesticides in honey, fruit juice, and wine. A method for assessing 405 pesticides in grain samples following acetonitrile-based accelerated solvent extraction (ASE) and SPE cleanup was published by the same scientists. It analysed a range of pesticides using GC-MS/MS and LC-MS/MS after ASE.[7]

Spray-applied liquid pesticides contaminate plants more than powdered pesticide preparations. The plant's structure is also significant because, for instance, OCP insecticides can build up in the waxy coating that covers the rind of many fruits, particularly citrus fruits. Since pesticide residues in fruits and vegetables can raise the risk of a number of diseases in people, we need to keep a constant eye on their levels. Any evaluation of pesticide contamination in fruits and vegetables must take MRLs into consideration. Maximum Residue Limits (MRLs) for pesticides found in fruits and vegetables are established by the EU. The limit of detection (LOD) of acephate, aldrin, dichlorvos, and fenthion is 0.01–0.05 mg/kg. For cherries, simazine has an LOD of 0.25 mg/kg, which is 0.1 mg/kg higher. Grape samples allow a limit of 5 mg/kg of malathion, while tomato and tangerine samples allow a limit of 7 mg/kg.[8]

Materials and Methods

Sample Preparation and Extraction

Weigh 10 grams of fruit samples like grapes oranges and watermelon then homogenize using a homogenizer to ensure a uniform sample matrix. Transfer the different homogenized samples into a 50 mL centrifuge tube for each sample. Fill each tube with 10 mL of acetonitrile. To guarantee complete mixing, vortex the mixture for one minute. To the entire tube, add 1 gm of sodium chloride and 4 gm of magnesium sulphate. For one minute, give the tube a vigorous shake to help in phase separation. For five minutes, centrifuge the tube at 4000 rpm. Pour 1 millilitre of the supernatant into a sterile centrifuge tube together with 150 mg of MgSO4 and 25 mg of PSA. For thirty seconds, vortex the mixture. Repeatedly centrifuge the tube for five minutes at 4000 rpm. The cleaned extract should be carefully transferred to an LC vial for analysis.[9]

The procedures and stage of sample preparation can have an impact on the outcome. As a result, the appropriate, accurate preparation of the sample is critical to whether the analysis yields the needed information about it. We need a representative and homogeneous sample of the material for analysis. A representative sample's chemical makeup aims to approximate the average composition of all the material under study as precisely as possible. Store the sample cold and darkly. In general, there are multiple phases involved in the sample preparation process. One such procedure for fruit and vegetables is to wash the samples in distilled water to remove any surface contaminants. Next, we dry the sample using a desiccant, at room temperature, or both. Next, we break up and crush the sample, or pound it with a pestle and mortar or in a mill, before homogenizing it. Depending on the type of material under investigation, the preparation process will vary, and any combination of steps in this process has the potential to lose analytes and/or further contaminate the sample.[10]

For LC-MS/MS device list were as,

Binary pump, Hip Sampler, Column, MS-Q-TOF (G6550A), DAD

Mobile Phase

a. Solvent A: Water with 0.1% formic acid
b. Solvent B: Methanol with 0.1% Acetonitrile

Gradient Elution

a. Start with 95% A and 5% B
b. Gradually change to 5% A and 95% B over 20 minutes
c. Maintain at 5% A and 95% B for 5 minutes
d. Return to initial conditions and re-equilibrate for 5 minutes
e. Flow Rate: 0.3 mL/min
f. Injection Volume: 3 µL

Mass Spectrometry

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

Instrument Settings

a) Source temperature: 250°C
b) Desolation temperature: 300°C.[11]

The LC gradient needed the two eluent components mentioned below in order to work: A: Ammonium formate and water at 5 mM; B: Ammonium formate and methanol at 5 mM (%). It was initially run at 300 µL min^{-1} with 100% component A at injection time. Over the course of three minutes, we adjusted it progressively to 60% A (40% B), and then 22 minutes later, we changed it to 10% A (90% B).

We kept the eluent composition at this level for 8 minutes. After that, it recovered to its initial state (100 percent component A) in 0.1 minutes, staying there for 38 minutes following injection. The temperature of the column was 40°C, and the injection volume was 5 µL. Using an ESI interface in the positive ion mode, we performed MS/MS detection in the multiple reaction monitoring (MRM) mode. Synthetic air at 60 psi served as the nebulizer gas, nitrogen at 30 psi served as the curtain gas, and 5500 V was used as the ionisation voltage. The solvent was assisted in evaporating at the source by a drying gas, which was heated synthetic air at 420 °C/50 psi. We found declusing potentials, collision energies, and ideal MRM transitions for each molecule through a series of studies using solutions of specific analytes. We used a syringe to continuously inject the standard solutions into the device for this purpose. We first created the compound acquisition parameter sets on an Applied Biosystems API 3200 Qtrap instrument, which proved unsuitable for the study. We modified the capture files using software so that they would match the same manufacturer's API 4000 Qtrap model.[10]

Results and Discussion

The analysis found pesticide residues in many fruit samples, including watermelons, oranges, and grapes. We found varying amounts of pesticides; some fruits had residues below the limit of quantification (LOQ), while others had detectable levels of many pesticides.

The Results Include the Chromatogram

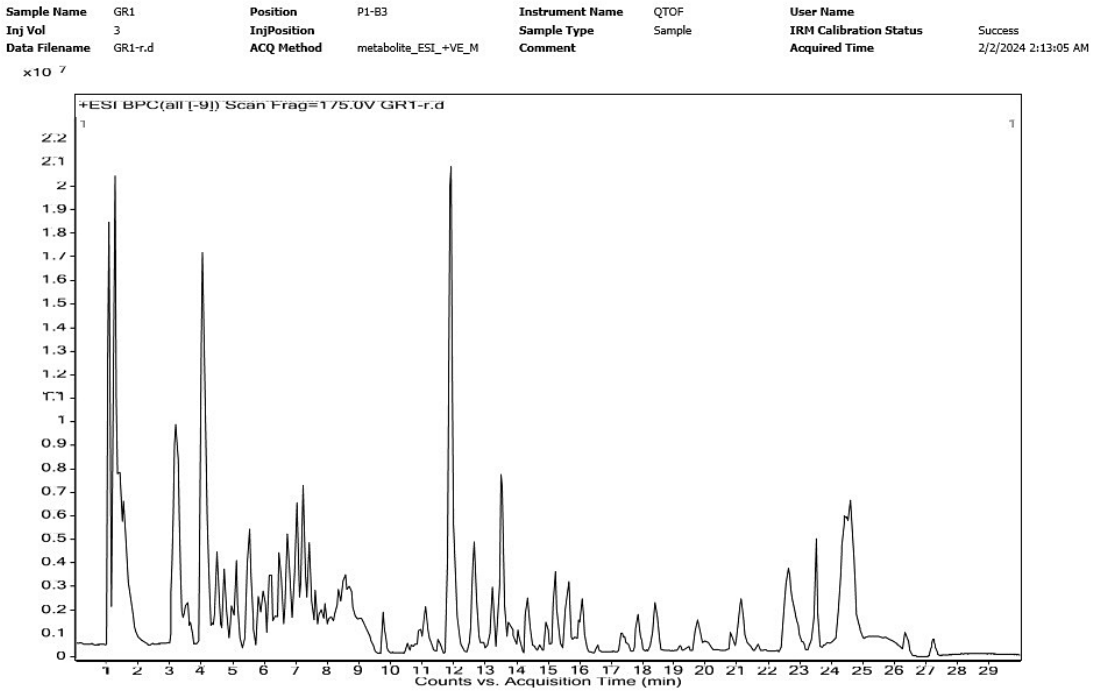
Grapes: Detected pesticides included chlorpyrifos.
Oranges: Detected pesticides included chlorpyrifos.
Watermelon: Detected pesticides included Imidacloprid, Acetamiprid and Chlorpyrifos.

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

In most of the samples analysed, organophosphorus was the most prevalent pesticide, followed by carbamates, pyrethroids, and organochlorines. Ghana reported similar findings, showing that the fresh fruit and vegetable samples examined included organochlorine, organophosphorus, and synthetic pyrethroid insecticides.[12] Given the quantities found in vegetable samples, consumers run the risk of ingesting pesticide residues in their meals. Exposure to organophosphate insecticides has been associated with changes in haematological parameters, liver and renal failure, and a decrease in acetylcholinesterase activity.[13] Samples from high routes tended to contain more pesticides than samples from farms and marketplaces, indicating that farmers sprayed higher doses of pesticides before harvesting. Farmers may occasionally give extra treatment to fruits 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 disdain for pre-harvest intervals.[14] 95.2% of all pesticide residues found were organophosphorus, which is the most frequent chemical family of pesticides used in horticultural production, as shown by this and other studies.[15] These results indicate that almost all the herbicides sprayed during production end up as residues following harvest. Some samples contained chemicals prohibited from use as pesticides. Tanzania has banned the use of organochlorine pesticides in agriculture, despite the discovery of quantifiable levels. Ghana and the US both published results that were comparable.[12] Pesticides frequently spray mango trees one month before harvesting and at pre-mature stages. Therefore, we examined mangoes from all three stages. We analysed three distinct mango types (deshehari, langra, and safeda) in triplicate for the presence of pesticide residues, and provided the results. We found only two pesticides malathion and chlorpyrifos out of seventeen in three different mango cultivars.[16]

Table 1: Pesticides compound identification from fruit samples

Fruits Name	Retention time (Min)	Chemical Formula	Compound Identification	Base Peak	M/Z Ratio
Watermelon	6.523	$C_9 H_{10} C_l N_5 O_2$	Imidacloprid	175.0959	256.0581
	17.658	$C_9 H_{11} C_{l3} N O_3 P S$	Chlorpyrifos	197.9252	349.9312
	7.272	$C_{10} H_{11} C_l N_4$	Acetamiprid	126.0094	223.073
Oranges	20.846	$C_9 H_{11} C_{l3} N O_3 P S$	Chlorpyrifos	124.0861	351.9275
Grapes	20.793	$C_9 H_{11} C_{l3} N O_3 P S$	Chlorpyrifos	124.086	351.9271



Sample Name	GR1	**Position**	P1-B3	**Instrument Name**	QTOF	**User Name**	
Inj Vol	3	**InjPosition**		**Sample Type**	Sample	**IRM Calibration Status**	Success
Data Filename	GR1-r.d	**ACQ Method**	metabolite_ESI_+VE_M	**Comment**		**Acquired Time**	2/2/2024 2:13:05 AM

Fig. 1: Chromatogram of Oranges

Sample Name	OR1	**Position**	P1-B2	**Instrument Name**	QTOF	**User Name**	
Inj Vol	3	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	OR1.d	**ACQ Method**	metabolite_ESI_+VE_M	**Comment**		**Acquired Time**	2/2/2024 1:01:05 AM

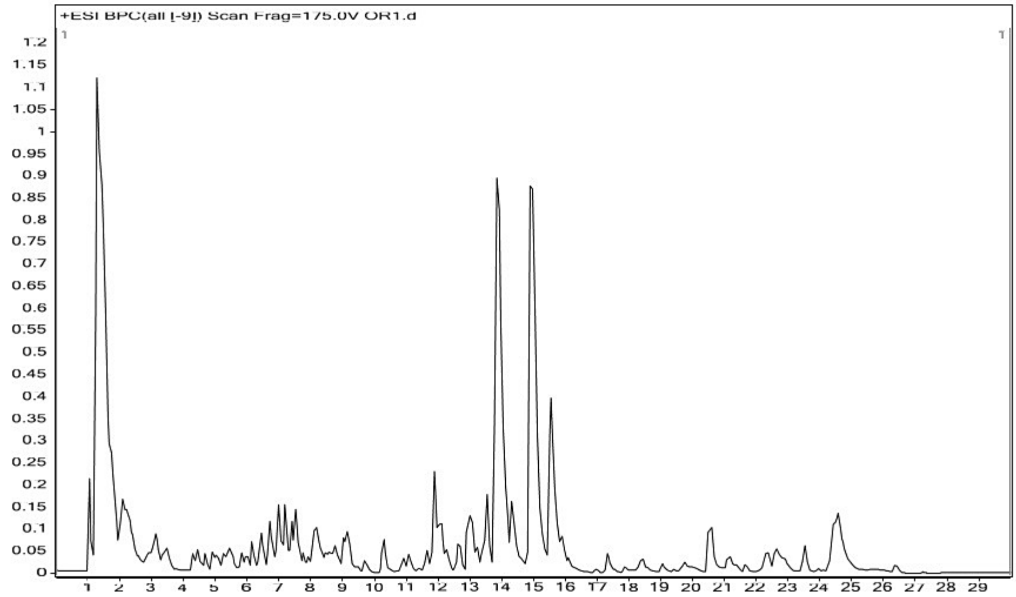


Fig. 2: Chromatogram of Grapes

Sample Name	Wat1	**Position**	P1-A9	**Instrument Name**	QTOF	**User Name**	
Inj Vol	3	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Success
Data Filename	Wat1.d	**ACQ Method**	metabolite_ESI_+VE_M	**Comment**		**Acquired Time**	3/15/2024 8:37:26 PM

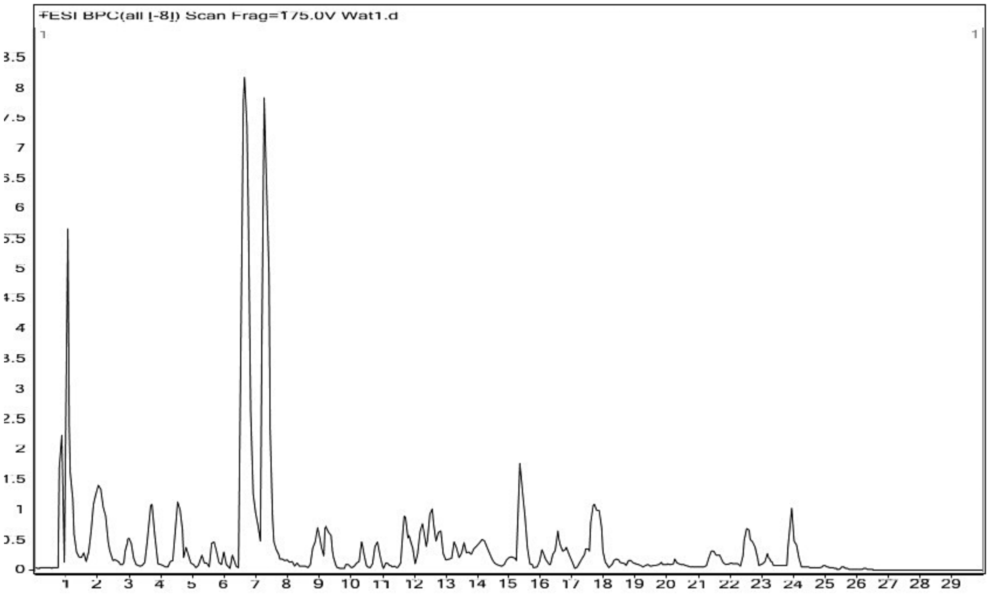


Fig. 3: Chromatogram of watermelons.

Conclusion

The investigation developed and validated an effective and robust LC-MS/MS technique for the identification and quantification of pesticide residues in fruits. This approach showed excellent sensitivity, specificity, and accuracy, which made it a perfect instrument for regular observation and legal compliance. To keep matrix effects to a minimum and make sure the analysis was correct, the QuEChERS extraction and dispersive SPE cleanup methods were able to remove and clean up pesticide residues from complex fruit matrices. The fact that the pesticide residues found in none of the evaluated fruit samples exceeded the maximum residue limits (MRLs) set by regulatory bodies confirmed the safety of the examined fruits. Different fruit samples contained pesticide chemicals such as imidacloprid, acetamiprid, and chlorpyrifos. The method's ability to simultaneously detect numerous pesticides at low levels enables a comprehensive strategy for monitoring pesticide residues in fruits. Frequent use of this technique can promote adherence to strict regulatory requirements, safeguard consumer health, and ensure food safety. After that, we can find out the toxicity of the compound. we all know that pesticide compounds are toxic but farmers use these toxic compounds and we eat every day this absorbed compound from fruits. so we can find out the toxicity of absorbed compounds and how to affect our bodies.

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Conflict of Interest

The authors do not have any conflict 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

Authors' Contribution

- Experimental work was done by Nirali Patel.
- Samples collected by Dr. Dhale Datta and
- Manuscript Prepared by Dr. Mayuri C. Rathod (Corresponding Author/Guide)

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