MSc. THESIS

AMINA ABDULLAHI MAALIM

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Hungarian University of Agriculture and Life Sciences

Budai Campus

Institute of Food Science and Technology

Department of Food Chemistry and Analysis

MSc THESIS

Determination of Pyrethroid Pesticides by UHPLC-MS/MS. Can it be a real alternative technique to GC-MS/MS?

Insider consultant: Marczika Andrásné Dr. Sörös Csilla
Insider consultant's institute/ department:

Food chemistry and Analysis Department

Created by: Amina Abdullahi Maalim

Food Chemistry and Analysis Department

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1. Introduction

Pesticides are important to modern-day agriculture by managing pests, weeds and diseases to support crop production and more importantly, food agriculture for the global population (Ruijten, 2020). Without its utilization, crop production losses reach up to 78% for fruits, 54% for vegetables and 32% for cereals (Bolognesi and Holland, 2019). Insecticides, herbicides, fungicides and other chemicals are all classified as pesticides since they are made to target particular pests.

There are significant health and environmental risks associated with the widespread use of pesticides. Pesticide residues when not controlled can cause degradation of water bodies and loss of biodiversity and affect the soil. Harmful pesticide residues negatively affect beneficial insects, birds and soil pests (A. Sharma et al., 2019). Pesticides can also cause devastating effects on human population, by exposing consumers and agricultural workers to harmful pesticides that can have a long-lasting effect, including lung disease, cancer and general neurological disorders (Hernández, 2023).

Pesticide regulation is crucial yet controversial, different nations employing varying degrees of control to strike a balance between agricultural requirements and environmental and health concerns (Mussali-Galante et al., 2023). For instance, as a key player in the global and agrochemical market, the European Union and China have different regulatory frameworks (Zolin et al., 2018). Despite regulations, farmers' improper handling and disposal of pesticides continues to be major issue that contributes to environmental contamination and health risks (Oyebamiji et al., 2023).

Efforts to mitigate the negative impacts of pesticides include promoting sustainable agricultural practices, developing biopesticides, and improving farmer education on safe pesticide use (Olguín-Hernández et al., 2024). Novel approaches like bio beds, which break down pesticide residues using biological systems, are viable low- cost substitutes for lowering environmental pollution (A. K. Sharma et al., 2020).

For food safety, monitoring pesticide residue id crucial especially for pyrethroids**,** which are frequently used in fruits and vegetables due to their great efficacy.

Historically, gas chromatography coupled with mass spectrometry (GC–MS/MS) has been the main technique, however it has drawbacks such as thermal degradation of pyrethroids, complex sample preparation (Hirano et al 2023).

Ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) is another technique however it faces challenges such as matrix effect (ME), which describes ion suppression or enhancement resulting from co-extracted substances present in complex samples. This typically arises in the ionization source commonly electrospray ionization (ESI), where matrix constituents compete with analytes during ion formation (Kruve et al., 2015).

MEs are generally less problematic in GC–MS/MS because only volatile compounds are injected into the system lowering the possibility of non-volatile interferences therefore evaluating and minimizing matrix effects is particularly important in UHPLC–MS/MS-based pyrethroid residue analysis.

With relatively few studies offering systematic comparisons of matrix effects, linearity and limit of detection in various food matrices, UHPLC-MS/MS is still less established than GC-MS/MS for pyrethroid analysis, despite UHPLC-MS/MS benefits including compatibility with versatile extraction techniques like QuEChERS, lower operating temperatures and robustness in handling thermally liable compounds (Kittlaus et al., 2017).

2. Objective

The aim of this study is:

- Optimize a reliable ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method for the determination of selected pyrethroid pesticides
- To systematically evaluate the most important analytical performance characteristics of the UHPLC-MS/MS method such as Limit of Detection (LOD), linearity and matrix Effect (ME) in various sample matrices.
- To critically compare these performance parameters with those reported in the literature for GC-MS/MS methods therefore assessing the probability of UHPLC-MS/MS as an Alternative for Pyrethroid Residue Analysis.

3. Literature Review

3.1. History and advantages of ultra-high-performance Liquid Chromatography (UHPLC-MS/MS)

UHPLC-MS/MS's journey can be documented along with the advancements in the discipline of analytical chemistry especially within the analytical chemistry of pharmaceuticals, the environmental and food. Due to the advancements in technologies within chromatographic techniques, the evolution of HPLC to UHPLC brought about the much-praised speed, resolution and sensitivity especially the use of 1,000 bars of pressure and the ability to use high pressure liquid pumps. This evolution to UHPLC was, in large part, to the demand for rapid and effective techniques to analyze highly complex matrices (Dong, 2017).

The turning point in the commercialization of the technology came in the early 2000s, when customers were able to access the technology that remarkably minimized the analysis time and waste of solvents. This improvement was economically and environmentally favorable to customers. The ability to offer and use sub sub-2-µm particle technology was an important leap, since it was now possible to achieve high resolution and quicker separations. The resolution of separations to UHPLC was unprecedented due to the improved low-dispersion systems that were designed with the equipment (Arroyo-Manzanares et al., 2015).

Further improving the capabilities of UHPLC is the coupling of UHPLC with mass spectrometry (MS) specifically tandem mass spectrometry (MS/MS). Highly selective and sensitive detection of analytes makes it an excellent tool for both targeted and non-targeted analysis.

The integration of UHPLC system with triple quadrupole and high-resolution MS has been particularly beneficial for the analysis of mixtures particularly complex mixtures providing high-throughput and robust analytical solutions (Bhattacharya et al., 2022).

The past decade has seen the widespread adoption of UHPLC-MS/MS across various disciplines such as pharmaceutical bioanalysis, environmental monitoring, and food safety testing. This has become the standard analytical technique in most laboratories owing to the rapid high-resolution separations and precise quantification it offers.

Improvements in methodologies and instrumentation have tackled the initial problems. These include high operating pressures and potential problems with the durability of the column, providing reliable and reproducible results (Dong, 2013a).

Compared to simple HPLC-MS, ultra-high-performance liquid chromatography combined with tandem mass spectrometry (UHPLC-MS/MS) breaks through more barriers. Ironically, even time-based analyses, where fast determinations are a critical factor, receive more attention. This attention pivots from HPLC, which is structurally limited to UHPLC-MS/MS systems. They achieve faster time results due to higher pressure, which allows for more accelerated separation of analytes (Alanazi, 2025).

Another significant benefit is the excellent sensitivity of UHPLC-MS/MS. The advanced instrumentation and sophisticated working principles of UHPLC-MS/MS enable the detection of chemical substances that have very low concentrations, which is crucial for applications involving exact quantification, such as pharmacokinetics, forensics, and clinical diagnostics. This high sensitivity is complemented by the improved resolution provided by UHPLC, which allows for better separation of complex mixtures and more accurate identification of compounds (Dong., 2023).

The robustness and reliability of UHPLC-MS/MS systems are also noteworthy. These systems are intended to handle an extensive selection of sample types or conditions, making them versatile tools in various fields. The robustness includes enhanced software handling capabilities, extended dynamic range and prolonged column lifetime which contribute to the overall reliability of the method. Additionally, UHPLC-MS/MS systems are known for their high selectivity which is essential for distinguishing target analytes from complex biological matrices or environmental samples (Dong, 2013b).

Furthermore, UHPLC-MS/MS offers high-throughput capabilities making it an ideal choice for laboratories that need to process many samples quickly and efficiently. This is particularly important in clinical settings where timely results can impact patient care. The method's ability to perform both targeted and non-targeted analyses adds to its versatility, allowing for comprehensive profiling of samples (López-Ruiz et al., 2019).

3.2. Pyrethrin

Pyrethrin are a group of naturally occurring organic insecticides derived from the flowers of *Chrysanthemum cinerariaefolium* and *C. coccineum* (Siddiqui et al., 2020a).

Figure 1:Chrysanthemum cinersriaefolium *(Borden et al., 2018)*

According to Nichol et al., (2023) Natural pyrethrin are a group of six esters, pyrethrin 1 and 11, jasmolin 1 and 11, cinerin 1 and 11 formed from the combination of an alcohol and acid. Two types of acids and three types of alcohols combine to form six distinct pyrethrin compounds. Depending on the type of acid utilized, they fall into either Category I (with pyrethric acid) or Category II with (chrysanthemic acid)

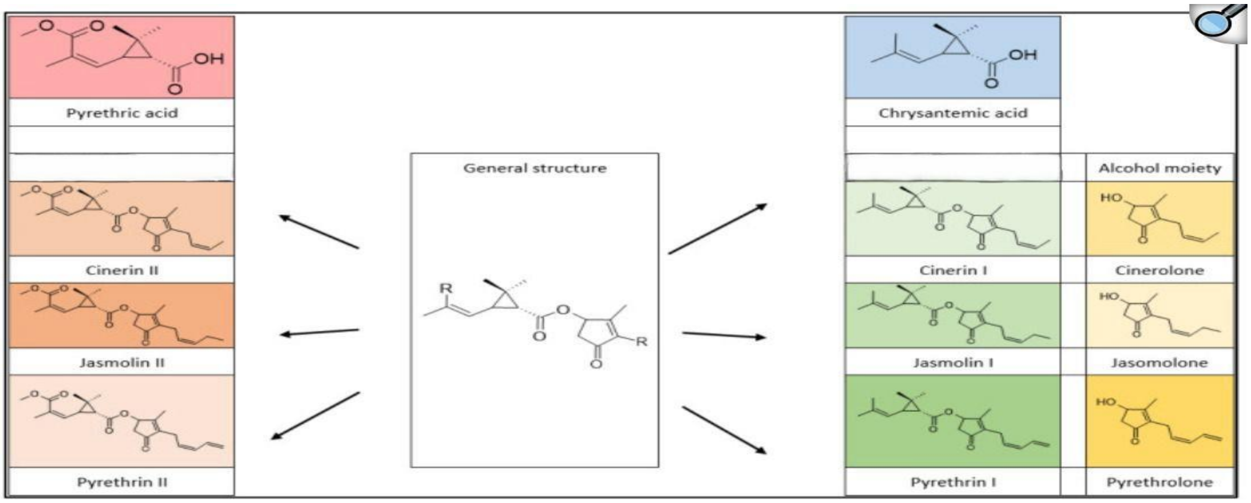

Figure 2:Chemical structures of pyrethrin (Hodoșan et al., 2023)

With regard to their effectiveness against a broad range of pests and their relative lack of environmental persistence, pyrethrin are utilized even though they have relatively poor stability when exposed to environmental conditions like heat and sunlight (Weiner et al., 2009).

Due to their efficiency against a wide spectrum of insects and pests, their application is evident in agriculture, horticulture, public health, veterinary and agricultural applications (Pfeil, 2013).

3.3. Pyrethroid Pesticides

3.3.1 Classification

Pyrethroids are synthetically derived from pyrethrins, which are organic insecticides extracted from the flowers of *Chrysanthemum cinerariaefolium* and *C. coccineum* (Siddiqui et al., 2020b).

Pyrethroids are classified into two main categories depending on their toxicity as well as chemical structure: Type I and Type II. Type I pyrethroids include compounds such as permethrin, allethrin, tetramethrin and permethrin do not have a cyano group at the phenyl benzyl alcohol position. These pyrethroids typically cause tremors (T syndrome) in affected organisms. Type II pyrethroids on the other hand, possess an α-cyano group, which enhances their insecticidal effectiveness and alters their toxicological profile, frequently leading to choreoathetosis with salivation (CS syndrome). Cypermethrin, lambda cyhalothrin and deltamethrin are a few types of type II pyrethroids (Aznar-Alemany et al, 2020).

Structurally, pyrethroids consist of two main components: an acid compound and an alcohol compound which are joined by an ester group (Bhardwaj et al., 2020). Acid moiety often contains a cyclopropane carboxylate group, while the alcohol moiety can differ, contributing to the diversity and specificity of different pyrethroid compounds (Du et al., 2009). In type II pyrethroids, the occurrence of α-cyano group significantly affects their interaction with voltage-gated sodium channels in the nervous system, prompting prolonged channel opening and increased neurotoxicity (Gajendiran et al.,2018).

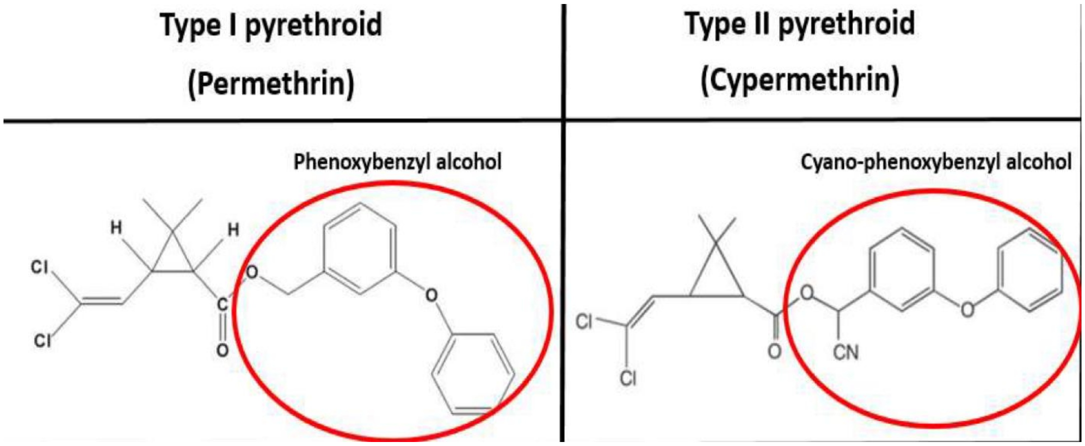

Figure 3:Chemical Structure of pyrethroid (Molnar & Rakosy-Tican, 2021)

3.3.2. Mechanism and function

Pyrethroids are highly lipophilic, meaning they tend to accumulate in fatty tissues and can be absorbed through the gills of aquatic organisms causing significant environmental concerns. Despite their low persistence in the environment, their high photostability and strong binding to soil and sediment particles can result in prolonged exposure risks (Matsuo., 2019).
agriculture, horticulture, public health, veterinary and agricultural applications (Pfeil, 2013).

At high dosages, pyrethroids and pyrethrin can cause symptoms like excitation and convulsions by altering sodium channels in neuronal membranes interfering with electrical signals in the nervous system. Due to their persistence nature and build up in soil, water and even food, they pose a severe environmental threat despite not being harmful to humans over a long term and having mild toxicity. Pyrethroids are structurally modified to function as pyrethrin and possess enhanced stability which allows them to resist deterioration by environmental factors for prolonged periods of time (Jin et al., 2017).

3.3.3. Toxicity to human, environment and aquatic

Pyrethroids are frequently used in traditional crop protection because they are substantially more stable than pyrethrin and are less expensive to produce. Although pyrethrin is used in organic farming, the costly price prevents its widespread use. The widespread usage of pyrethroids has expressed concerns about their impact on the environment especially when it comes to contaminating soil and water bodies which can harm aquatic life and damage soil fertility (Ahamad et al.,2023).

There are several ways through which pyrethroid pesticides enter aquatic life which include leaching into agricultural farms, run off from farmlands during rains and industrial effluents (Hodoșan et al.,2023).

Pyrethroids have been reported to be harmful to lobster and salmon whom are vulnerable to cis-permethrin, cypermethrin and deltamethrin. Some studies suggest that pyrethroids are harmful to fish throughout their early phase of development and reproduction. Due to the lipophilic nature of fish, pyrethroids are readily absorbed through their gills leading to toxic effects. They are also unable to hydrolytically detoxify synthetic pyrethroids in comparison to humans as they lack hydrolase (Yang et al., 2020).

In mammals, pyrethroid absorption happens through the skin by ingestion or inhalation. However, because they do not bind well to mammalian sodium channels, their intake rate through the mammalian liver is low, making them less hazardous to human than to pests (Akelma et al., 2019). Although it is unclear how pyrethroid exposure affects human reproductive health, reports have indicated that elevated urinary 3-phenoxybenzoic acid concentrations in men increase serum follicle stimulating hormone and luteinizing hormone while decreasing sperm quality (Radwan et al., 2014).

3.4. QuEChERS Method for Sample Preparation and Clean Up

Matrix complexity has been appropriately addressed by QuEChERS (quick, easy, cheap, effective, rugged and safe) approach in multi-residue analysis. It consists of an extraction step that uses partitioning via salting-out to balance an aqueous and organic layer. A dispersive solid phase extraction (dSPE) step follows which uses MgSO4 in combination with different sorbents, like graphitized carbon black (GCB), Octadecylsilane (ODS), or primary secondary amines (PSA), to further clean up and eliminate interfering substances. Complex samples like fruits and vegetables can be well cleaned with it, and it also makes it possible to use a smaller amount of organic solvent. Having all of these advantages, the method emerged more popular becoming a suggested technique determining pesticide residues and is adopted by European Committee for Standardization, CEN, 2008; (Schenck et al 2017).

3.5. Analytical determination in fruits and vegetables.

Pyrethroids which are classified as non-polar, hydrophobic compounds with relatively high molecular weights and varying degree of thermal stability, traditionally, GC-MS/MS has been the primary choice for pyrethroid analysis thanks to its high separation efficiency and volatility of many pyrethroids. GC-MS/MS uses electron ionization (EI) which provides characteristic fragmentation patterns useful for identification and quantification. However, some challenges related to pyrethroids analysis with GC-MS/MS such as isomerization and degradation of pyrethroids in the hot injection port particularly epimerization at the cyano-substituted carbon which can lead to quantification errors if not addressed by calibration and method optimization (Michlig et al., 2024).

UHPLC-MS/MS may be considered as an alternative technique specifically suited for thermally liable pyrethroids. It uses ESI in a positive mode. It overcomes thermal degradation and isomerization issues as identified with GC-MS/MS. This method also provides high sensitivity and selectivity through MRM (multiple reaction monitoring) of distinctive precursor and product ion transitions (Liapis et al., 2022).

Due to co-eluting matrix components, UHPLC-MS/MS is prone to ME, such as ion suppression or ion enhancement, therefore rigorous sample cleanup and matrix-matched calibration (Zhang et al., 2023). With this comparison, recent studies have demonstrated that UHPLC-MS/MS can achieve lower limit of quantification (LOQ) and can be better suitable for a wide spectrum of pyrethroids in complex matrices such as vegetables (Wahab et al., 2022; yuan et al., 2023).

3.6. Essential performance Characteristic Parameters

3.6.1. Matrix Effect

ME can be explained by the difference in the mass spectral signal intensities of a target compound at the same concentration when the sample is injected and when the solvent is injected. In a single run, in a multi-residue analysis, tens or hundreds of analytes are analyzed and each analyte's ME varies significantly (Zhang et al.,2023; Michlig et al., 2021).

Assessment of matrix effect seeks to evaluate how the strength of these effects influences method performance following the approach previously described by Raposo et al., (2021).

To compensate for ME, two primary correction strategies are widely used. Matrix-matched (MM) calibration and use of isotopically labelled internal standards (ILIS). In MM calibration, standard solutions are made in blank extracts of a similar sample matrix. This ensures that both standards and unknown samples experience comparable ion suppression or enhancements effects during ionization. This approach is simple and works with a variety of matrices.

ILIS uses stable isotopically labelled compounds such as deuterated or ^{13}C-labelled compounds of the target analytes which co-elute and ionize under identical conditions. As a result, it directly compensates for MEs and instrumental variability. Although ILIS calibration guarantees great accuracy and precision, its use is restricted by cost and availability. MM calibration was developed to preserve analytical reliability while enhancing efficiency across a range of matrices (Besil et al., 2017).

MEs have an impact on important technique parameters such as LOQ, LOD, linearity and accuracy. MEs may not be totally eliminated but can be lowered by modifying original QuEChERS method and chromatography (Stahnke et al., 2012; Kasperkiewicz et al., 2022).

According to European commission's directorate-general for health and food safety (SANTE, 2019), food commodities are classified into representative matrix groups:

1. Hight water content (apricot, lettuce, eggplant, lettuce, leek, cabbage, cherries, pear, tomato, potatoes, apples and carrots)
2. High water and acid content (grapes, lemon, orange, strawberry, blueberry, raspberries)
3. High sugar and low water content (apricots and honey)
4. Hight oil content and very low water content (walnuts, sunflower and hazel nuts, seed rape, cottonseed, soybeans, peanuts, sesame)
5. High oil content and intermediate water content (olive, pastes, avocado)
6. High starch and protein and low water and fat content (wheat, rice Wheat, barley and oat grains; maize, whole meal bread, white bread, crackers, breakfast cereals, and maize) Bulaić Nevistić and Kovač Tomas (2023) have highlighted these differences by investigating the ME for over 200 pesticide residues and apples(high water content), grapes(high water and acid content), spelt kernels(high protein and starch and low water and fat content) and sunflower(high oil content and very low water content) and demonstrated how the commodity classification groups influenced the extent of MEs in GC-MS/MS.

According to Bulaić Nevistić & Kovač Tomas (2023), the following formula can be utilized to determine ME through comparison of the slopes of solvent curves to the slopes of matrix-matched calibration curves:

$$ME_s\% = \left(\frac{Slope\ matrix{-}matched\ calibaration\ curve}{Slope\ solvent\ calibration\ curve} - 1\right) x100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots[1]$$

ME_s represents the matrix effect calculated using the slope of the calibration curves.

As recommended by SANTE,2019,The difference in detector response between the pesticide residue standard in sample matrix extract (matrix-matched standard) and standard in pure solvent(acetonitrile) at the same concentration can also be used to calculate the matrix effect.

$$ME_A\% = \left(\frac{Area\ standard\ in\ matrix}{Area\ standard\ in\ solvent} - 1\right) x100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..[2]$$

ME_A represents the ME computed using analyte response (area).

Table 1*:* Matrix effects (%) for pyrethroids determined using LC–MS/MS in various food matrices

Reference	**Analytical Technique**	**Matrix**	**Analyte and ME (%)**	**Compensation Used**
Prodhan et al., 2016	LC-MS/MS	cabbage	Deltamethrin (+97%) Cypermethrin (+49%)	Matrix-matched calibration
Wan et al 2021	LC-ESI-MS/MS LC-MS/MS	tea and orange	Cypermethrin (-2%) Cyhalothrin (+8%) Deltamethrin (-3%) Fluvalinate (-23%)	Matrix-matched calibration
Zhuang et al 2022	HPLC-MS/MS	tea, cucumber and tomato	λ-Cyhalothrin in tea (+412%), λ-Cyhalothrin in cucumber (+170%) λ-Cyhalothrin in tomato (+265%)	Matrix-matched calibration

			Deltamethrin in tomato (+271%), Deltamethrin in cucumber (+221%), deltamethrin in tea (+320%)	
Yuan et al 2024	UHPLC-MS/MS	eggplant, leek, grape, orange, tomato	Cinerin 1 in leek (-62%) Cinerin 2 in leek (-62%) Cyhalothrin in leek (–66%) Cypermethrin in leek (-77%) Deltamethrin in leek (+27%) Jasmolin 1 in leek (-71%) Jasmolin 2 in leek (-75%) Tau-fluvalinate in leek (-67%) Pyrethrin 1 in leek (-60%) Pyrethrin 2 in leek (-65%)	Matrix-matched calibration

*ME was calculated by slope-based method in all work, „+” means signal enhancement, „-” means signal suppression.

In table 1, all the reviewed studies employed MM calibration which compensates for matrix-induced signal fluctuations. In two studies, moderate to strong ion enhancement was observed with MEs ranging from +49 to +412 for pyrethroids such as deltamethrin and λ-Cyhalothrin (Prodhan et al., 2016; Zhuang et al., 2022). Two other studies Yuan et al., (2023) and Wan et al., (2021) show ion suppression effects especially for cypermethrin (-77%)

Table 2: Matrix effects (%) for pyrethroid determined by GC–MS/MS in various matrices

Reference	Analytical technique	Matrix	Analyte	ME(%)	Compensation Used
Arena et al 2023	GC-MS/MS	Hemp Seed	Deltamethrin cypermethrin	-98 to +184%	Matrix-matched calibration
Tian et al 2020	GC-MS/MS	Edible Mushroom	Deltamethrin cyhalothrin	+47 to +177%	Matrix-matched calibration

Ye at al 2020	GC-MS/MS	Tomato	Cypermethrin Cyhalothrin	+6 to +32%	Matrix-matched calibration
Lin et al 2018	GC-MS/MS	Fruits and vegetables (cucumber, tomato, pear, waxberry, cowpea)	Cypermethrin, cyhalothrin, deltamethrin and tau-fluvalinate	Fruits +5% to +145% Vegetables (-9 to +297)	Matrix-matched calibration
Shendy et al 2018	GC-MS/MS	Honey	λ-Cyhalothrin, Cypermethrin, Deltamethrin tau-fluvalinate	−58% to +44%	Matrix-matched calibration

ME was calculated by slope-based method in all work "+" means signal enhancement, "-" means signal suppression.

Table 2 summarizes reported ME for pyrethroid pesticides using GC-MS/MS across various food matrices. The values depend on the matrix type and compound. Arena et al., (2023) and Lin et al., (2018) reported strong signal suppression and enhancement ranging from -98% to +297% while ye et al., (2020) reported relatively low matrix suppression in tomato.

ME data in both GC-MS/MS and LC-MS/MS reveal that both techniques face matrix effect challenges however the magnitude vary. In GC-MS/MS strong ion enhancement and suppression are exhibited by most compounds (-98% to +297%) with mostly ion enhancement dominating while in LC-MS/MS ion suppression is dominant however extreme enhancement (+412%) was reported from one study by λ-Cyhalothrin in tea.

3.6.2 Limit of detection (LOD) and Limit of quantification (LOQ)

LOD is the lowest concentration of an analyte that can be systematically identified from the background noise of an analytical system, which is not always quantified with sufficient precision or accuracy. In practice, LOD is the lowest concentration that produces a signal which can be recognized as originating from the analyte rather than an instrumental background or noise (Goh et al.,2024).

There are several ways of calculating LODs, but the two most common ones are detailed below;

1. Signal to noise ration;

In this approach, the LOD is the concentration that gives a signal three times the measured baseline noise (S/N= 3).

The baseline noise is measured in a blank and the concentration that yields the required S/N is determined.

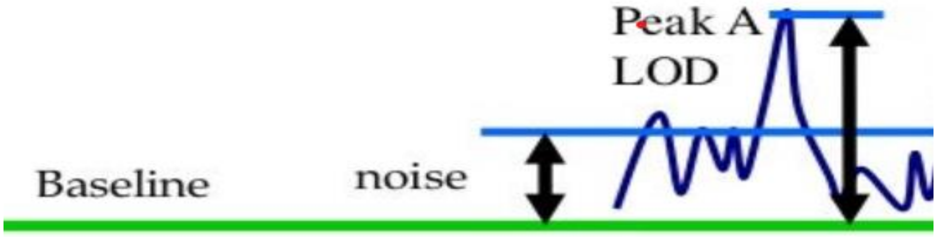

Figure 4: Illustration of LOD in analytical measurement (GMP Insiders Expert Team, 2023).

1. Standard deviation of the response and slope of calibration curve:

LOD= 3.3 x (σ /S)

σ = standard deviation of the response (usually y-intercept of regression line) and

S = slope of calibration curve (SANTE,2019).

LOQ is the lowest amount of a compound which be reliably measured quantitatively with specified levels of repeatability and trueness under certain experimental conditions. Since LOQ ensures that the given result is both statistically and analytically correct, it is higher than the Limit of Detection (LOD), which just reflects detectability (Franco et al.,2024).

3.6.3. Linearity

Linearity is an important performance characteristic in an analytical method. Response factors are plotted against concentration to create linearity plots. Linearity is the process of obtaining a linear response from a set of calibration data by adjusting a linear function and expecting that the points are dispersed randomly along the resulting line (Jurado et al., 2017).

Numerous approaches for linearity testing are reported, which are divided broadly into two categories namely the graphical and numerical method. Plotting the response against concentration on a calibration curve allows one to determine whether a response arises proportionately with the concentration. In numerical testing, numeric factors like correlation coefficient(R^2), standard deviation(SD) of slope and SD of regression line are considerably evaluated. In this method statistical tools and significant tests to model parameter (Ismail et al., 2014).

Numerous literatures have reported linearity, LODs and LOQs, For example:
The study by Peruga et al., (2013) came up due to the lack of LC-MS/MS methodology for pyrethrin detection in fruits and vegetables. The method was based on Acetone or Acetone/ water extraction Electrospray Ionization (ESI, positive mode) and C18 column with gradient elution (water + acetonitrile) and MRM detection. The q transition for Cinerin 1 was interfered by matrix in Pistachio sample which called for the use of APCI (atmospheric pressure chemical ionization) which showed lower sensitivity than ESI but minimal matrix interference The LOQs ranged from 2-5ug/kg while LODs were between 0.05-1.6 ug/kg.

A method developed by Li et al., (2016) using Quenchers method and salting out with SPE Cartridges to measure Pyrethroids and their degrades using LC-MS/MS in 5 matrices and was able to obtain LOQs <500 ng/Kg and LODs <150ng/kg. The LC-MS/MS used for this study was unable to alternate between positive and negative ionization modes within a single analytical run, therefore separate positive and negative runs for cyhalothrin were required. Furthermore, Linearity and Matrix effects were not evaluated.

Comprehensive direct comparison of analytical performance characteristics such as LOQ, LOD and Matrix effects using standard conditions are still limited however Yuan at al., (2023) showed that UHPLC/MS/MS achieved suitable LOQ, LOD, Matrix effect and Linearity compared to GC-MS/MS for selected 32 pyrethroids in fruits and vegetables with five selected matrices by QUECHERS method. The linearity was assessed using matrixed-matched and solvent based and were >0.99 while MEs Ranged from -50% to 80% which was corrected by MM calibration. Full Scan mode TOF MS (100-1000m/z and dependent Product ion scan MS/MS(50-1000 m/z) were used. The GC-MS/MS used could only identify Cinerin1 and 11, Jasmolin 1 and Pyrethrin 1.

A study done by Wan et al., (2021) where he compered GC-EI-MS/MS in the analysis of tea and orange samples to LC-ESI-QQQ-MS and LC-ESI-Q-TOF and found out while some pyrethroids like Fluvalinate and Deltamethrin shared the same MRM Transitions of 181>152 in GC-EI-MS/MS, his method was able to determine Cyhalothrin, Deltamethrin and Fenvalerate by their own quantification and confirmation ions with lower LODs compared to GC-EI-MS/MS. For his method, Medium Matrix effect of 120-150% occurred for Deltamethrin which prompted Matrix matched calibration. Good linearity of R^2 0.9914-0.9993.

A study done by Zhuang et al., (2022) using UHPLC-MS/MS for pyrethroids such as λ-Cyhalothrin and deltamethrin in Cucumber, Tea and Tomato samples where previous similar GC-MS/MS methods were time consuming, costly and needed derivatization, used UHPLC-MS/MS to overcome these challenges. Triple quadrupole was used with MRM mode. The precursor ions were selected and monitored in the range of 300-500m/z and the most abundant ion monitored. The LOD and LOQ were in the range of 0.007-1.875 and 0.025-6.250 and R^2 >0.99. ME ranged from 46% to 775% hence the need for Matrix-matched Calibration. These literatures are summarized in the table below.

Table 3:Linearity, LODs And LOQs for Pyrethrin and pyrethroid compounds in Various Matrices (LC-MS/MS)

Reference	**Compound**	**Matrix**	**Analytical method**	**Mass Transition**	**LOD ug/kg**	**LOQ ug/kg**	**R2**
Zhuang et al 2022	λ-Cyhalothrin, deltamethrin	tea, Tomato and cucumber	UHPLC-MS/MS	ESI+ MRM CYH m/z 467>224 DEL m/z 522.9>280.7	0.007-1.875	0.025-6.250	R2>0.99
Wan et al 2021	cyhalothrin, deltamethrin, cypermethrin	tea and Peeled Orange	LC-ESI-QQQ-MS/MS and LC-MS/MS	MRM CYH467 > 225, 469 > 227 DEL523 > 281, 525 > 283	0.07-0.29ug	-	R2=0.9914-0.9993

Li et al 2016	cypermethrin, deltamethrin, cyhalothrin	lettuce, tomato, apple, grape, banana peel	LC-MS/MS	ESI+, ESI- MRM	0.001-0.094	0.004-0.168ug/kg	-
Yuan et al 2024	cypermethrin, deltamethrin, tau-fluvalinate, cyhalothrin, cenirin 1 and 2, pyrethrin 1 and 2, jasmolin 1 and 2	orange, leek, grape, tomato, orange and eggplant	UHPLC-MS/MS		-	2-5ug/kg	R2>0.99
Peruga et al 2013	pyrethrin 1 and 2, jasmolin 1 and 2, cinerin 1 and 2	potato, rice, pistachio, pepper, tomato, lettuce, strawberry and potato	LC-MS/MS	ESI+, MRM	0.05-1.6 ug/kg	2-5ug/kg	R2>0.998

According to studies by Torbati et al., (2018) and Nemati et al., (2022) which determined various pesticide pyrethroids residues in samples of fruit juices like apricot, sour cherry, apples, mangoes, grape, peach and orange using GC-MS method. The LOQs, LODs and Linearity values were closer in both methods. The LODs for Cyhalothrin and Cypermethrin ranged from 13-47ng/ml and 12-59ng/ml and R2>0.995. Both methods used different techniques of liquid-liquid extraction. The MEs of the samples were not reported by the literature. In Both studies, both instruments were operated in full scan mode of all across the range of m/z 55-400.

A recent study by Zhang et al., (2023) investigated presence of cyhalothrin with other pyrethroid insecticides specifically Bifenthrin, Permethrin, in a variety of fruit juices including apple, orange, and strawberry. GC-MS was employed which involved Selected Ion Monitoring (SIM) for precise quantification. Key ions used for quantification for cyhalothrin were 197 and 208 m/z. The LODs obtained were in the range of 0.8 to 1.5 ng/g, indicating that even very low concentrations of these insecticides could be detected. The LOQ**s** ranged from 2.9 to 4.6 ng/g R^2 values between 0.9961 and 0.9998

According to studies conducted by Liu et al., (2015), Tian et al., (2020) and Shendy et al., (2016) which employed determining pesticide residues in Maize, Honey and edible mushroom samples applying GC-MS/MS in MRM mode had close findings where the LODs ranged from 3ng/ml for Honey, 1.5ug/L for Maize and 1.67ug/kg for Mushroom. LOQs ranged from 10ng/ml for Honey, 5ug/L for Maize and <100ug/kg for Mushroom. The Linearity for the methods was found to be R2>0.990 and ME of -19.91% to +16.7%.

Lin et al., (2018) investigated pesticide residue in Chinese fruits and vegetables like pear, wax berry, tomato, cucumber and cowpea. Pyrethroid pesticide residues such as Tau-fluvalinate, cypermethrin, deltamethrin and Cyhalothrin were quantified using MRM mode by GC-MS/MS through quenchers extraction. The LODs and LOQs ranged from 0.3-4.9ug/kg and 10-15ug/kg. the linearity R2>0.990 and ME was found higher in vegetables than the fruits (0.91-3.97). The peaks for the isomers of fenvalerate and tau-fluvalinate overlapped in the total ion chromatogram (TIC) mode which they were able to resolve.

Tankiewicz and Berg (2022) further improved detection strategies through optimization of QuEChERS extraction method and used GC–MS/MS. The fruits and vegetables investigated included tomato, apple, carrot, pear, cauli flower, broccoli and parsley and pyrethroids were λ-Cyhalothrin, deltamethrin and cypermethrin. The LODs and LOQs ranged from 0.21-13ug/kg and <10ug/kg respectively and R^2=0.9570-0.9992 while the ME was found to be -20% to +20%. The LOQs seem to be much less in this method than the previous one done by Lin et al., (2018)

Ye et al., (2020) investigated the determination of seven pyrethroids including cypermethrin, cyhalothrin and fenvelarate in tomatoes using GC-MS/MS in MRM mode and obtained the LOQs which were in the range of 0.3-20ug/kg and Linearity with correlation coefficient of R2>0.994. However, the ME ranged from 74% to 232% in all the seven pyrethroids. Analyte Protectant was used which eliminated the ME and reduced to 6% to 32%.

Table 4: LOQs, LODs and Linearity in various matrices (GC-MS/MS)

Reference	**Compounds**	**Sample Matrix**	**Methods**	**LOD/LOQ**	**Mass Transition (m/z)**	**Linearity**	**Parameter**
Tankiewicz & Berg, 2022	Cypermethrin, λ-Cyhalothrin, Cypermethrin	Fruits and Vegetables	GC-MS/MS	LOD= 0.00021-0.013 LOQ<0.01	Full Scan(45-450m/z) MRM λ-Cyhalothrin 181.05>127.10(24) Cyp 163>127.10(9) DEL 181.05>152(24	R2= 0.9570-0.9992	QUECHERS
Liu et al., 2015	Deltamethrin	Maize	GC-MS/MS	LOD= 5ug/kg LOQ= 40-100ug/kg	MRM Deltamethrin253>174(10) 253>172(5)	R2>0.992	QUECHERS dSPE (C18, Mgso4, PSA)
Zhang et al., 2023	Cyhalothrin	Apple, W. melon, Pear, Guava, Orange, Strawberry	GC-MS	LOD 0.8-1.5ng/g LOQ 2.9-4.6ng/g	181 197,208(Quant) Full scan(50-500m/z) SIM	0.9961-0.9998	QUECHERS
Torbati et al., 2018	Deltamethrin, Cypermethrin, Cyhalothrin,	Apricot, Sour cherry, Peach, Orange, Fresh Apple and Strawberry	GC-MS	LOD=0.06-0.038ng/ml LOQ=0.023-0.134ng/ml	Full Scan 55-350 m/z Cyhalothrin 141.181,197 Cypermethrin163,165,181 Deltamethrin 181,244,253	R2= 0.994-0.999	QUECHERS
Lin et al., 2018	Cypermethrin, Cyhalothrin, Deltamethrin, Tau-fluvalinate	Cucumber, Tomato, Waxberry, Cowpea, Pear	GC-MS/MS	LOD= 0.3-4.9ug/kg LOQ= 10-15ug/kg	MRM Cyhalothrin 181>152(30) Cypermethrin 163>91(25) Tau-fluvalinate 250>55(15) Deltamethrin253>93(20)	R2>0.990	QUECHERS
Nemati et al., 2022	Cyhalothrin, Cypermethrin,	Apple, Pomegranate, Grape, Orange, Sour Cherry Juices	GC-MS	LOD=9-12ng/L LOQ= 31-69 ng/L	SIM	R2≥ 0.995	Dispersive Liquid-Liquid Microextraction
Ye et al., 2020	Cyhalothrin, cypermethrin	Tomato	GC-MS/MS	LOQ=0.3-20.0ug/kg	MRM	R2≥0.994	QUECHERS

4. Materials and Methods

4.1. Materials

4.1.1. Chemicals

The analysis procedure used chemicals such as Acetonitrile 99.9% (ACN), water, ammonium acetate, 0.1% Acetic Acid, methanol (MeOH) which were purchased from the same company as was written in the diploma thesis of Majercsik, 2020. Stock solution of the analytes (Pyrethrin, Cyhalothrin, Cypermethrin, Deltamethrin and Tau-fluvalinate were provided by the department of food chemistry and Analytics, Hungarian university of agriculture and life sciences

Table 5: different analytes and their chemical formula

Compound Name	**Abbreviation**	**Chemical Formula**
Deltamethrin	DEL	$C_{22}H_{19}Br_2NO_3$
Cypermethrin	CYP	$C_{22}H_{19}Cl_2NO_3$
λ-cyhalothrin	λ-CYH	$C_{23}H_{19}ClF_3NO_3$
Tau-fluvalinate	TFL	$C_{26}H_{22}ClF_3N_2O_3$
Pyrethrin I	PYR I	$C_{21}H_{28}O_3$
Pyrethrin II	PYR II	$C_{22}H_{28}O_5$
Cinerin I	CIN I	$C_{20}H_{28}O_3$
Cinerin II	CIN II	$C_{21}H_{28}O_5$
Jasmolin I	JAS I	$C_{21}H_{28}O_3$
Jasmolin II	JAS II	$C_{22}H_28O_5$

4.1.2. Apparatus

Apparatus including fisher microcentrifuge tubes, beakers, 200ml volumetric flask, 50ml centrifuge tube, 4ml black vials, refrigerator, HPLC syringe filter, syringes, analytical balance, trays, vortex mixer, UHPLC-MS/MS instrument, zorbax eclipse plus C18 RRHD chromatographic column with particle size of 1.8µm and with a dimension of 2.1 x 50mm were purchased from the same company as was written in the diploma thesis of Majercsik, 2020.

4.2 Method

The method comprised of injection parameters and instrument settings, a gradient elution profile using methanol and ammonium acetate, optimized ion source parameters, MRM transitions specific to each analyte and the sample preparation.

Within the European union, QuEChERS-based approaches are widely regulated to ensure food safety, protect public health and safeguard the environment. The control of pesticide residues analyzed using this technique is governed by several EU frameworks, notably Regulation (EC) No 396/2005, which establishes maximum residue limit (MRL) for pesticides in food and feed of plant and animal origin. QuEChERS plays a vital role in stabilizing samples and minimizing unwanted reactions during analysis. The procedure involves sequential steps such as sample collection, homogenization, extraction and concentration.

Table 6:injection parameters and setting

Parameter	**Value**	**Unit**
Injection Volume	4.00	µL
Needle Wash Mode	Flush Port	-
Needle Wash Time	3	S
Needle Wash Repeat	3	-
Draw Speed	100	µL/min
Eject Speed	400	µL/min
Sample Flush-Out Factor	5.0	X injection
Overlapped injection	Off	-

Table 7: Gradient Elution Profile

Time	**Eluent A(5mM Ammonium acetate in water, 0.1% A.A)**	**Methanol**
0 minute	70%	30%
1 minute	50%	50%
4 minutes	5%	95%
6 minutes	0%	100%

Table 8: Ion Source Parameters

Parameter	Value/setting	Units
Ion source	ESI	
Stop time limit	Off/ no limit	-
Start time	0	Min
Type	MRM	-
Gas temperature	250 (set), 290	°C
Gas flow	10 (set), 10	L/min
Nebulizer pressure	30, (set), 30	Psi
Capillary voltage	3000	V
Capillary current	4805	nA
Chamber current	0.13	µA

Table 9:MRM transitions for the compounds

Pesticide	Precursor mass(m/z)	Product mass(m/z)	Dwell time (ms)	Fragmentor(V)	Collision energy(V)	Polarity
Deltamethrin	523.0	181.0	3	110	10	Positive
Deltamethrin	523.0	280.9	30	110	10	Positive
Cypermethrin	433.1	416.0	50	110	4	Positive
Cypermethrin	433.1	190.8	5	110	20	Positive
λ-cyhalothrin	467.1	141.0	3	110	20	Positive
λ-cyhalothrin	467.1	225.1	30	110	20	Positive
Tau-fluvalinate	520.0	208.0	30	110	20	Positive
Tau-fluvalinate	520.0	181.0	3	110	20	Positive
Cinerin 1	317.2	149.1	30	110	20	Positive
Cinerin 1	317.2	107.0	3	110	20	Positive
Cinerin 2	361.2	149.0	30	110	20	Positive
Cinerin 2	361.2	107.0	3	110	20	Positive
Jasmolin 1	331.2	163.1	30	110	20	Positive
Jasmolin 1	331.2	107.0	3	110	20	Positive

Jasmolin 2	375.2	163.1	30	110	20	Positive
Jasmolin 2	375.2	107.0	3	110	20	Positive
Pyrethrin 1	329.2	143.0	3	110	15	Positive
Pyrethrin 1	329.2	161.1	30	110	15	Positive
Pyrethrin2	373.2	161.1	30	110	20	Positive
Pyrethrin 2	373.2	133.1	3	110	20	Positive

4.3. Sample

Sample extracts of lettuce, lemon and cherry were provided. These samples were selected because they represent different matrix groups. Lettuce as a high-water content leafy vegetable, cherry as a high-water content and less sugar fruit with pigment-rich matrix, and lemon as a high-acid citrus fruit. Such diversity allows for a broader evaluation of matrix effects, which are critical in pesticide analysis.

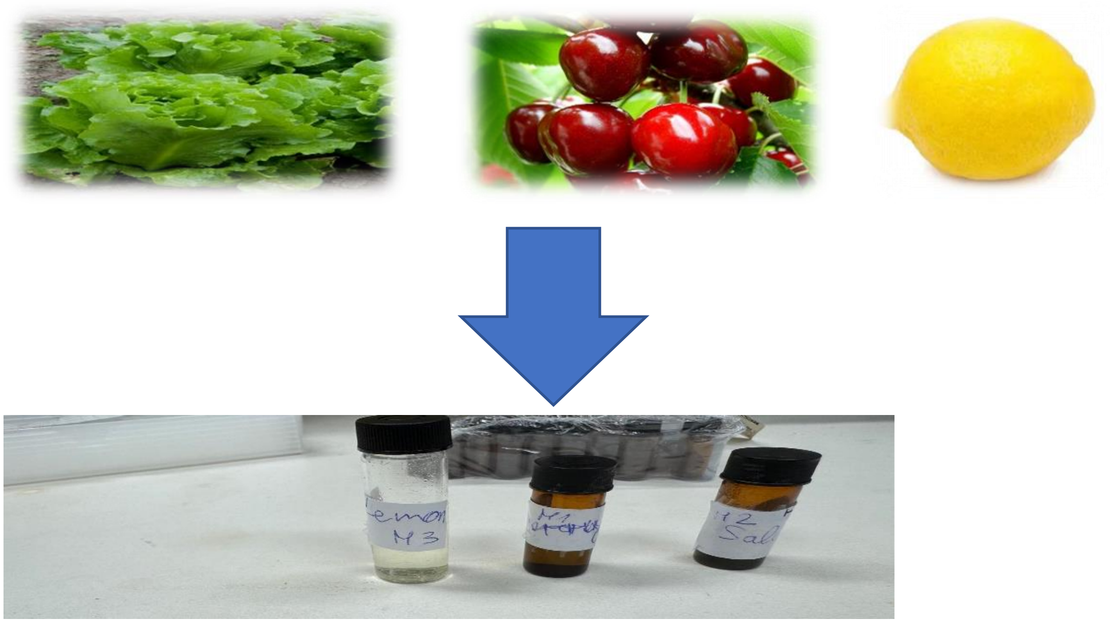

Figure 5: Sample extracts of cherry, lemon and lettuce

4.4. Preparation and Dilution stock solutions and creation of mix working solutions of the pesticides

Table 10: Concentration of selected compounds

Compound	Concentrations (mg/L)
Deltamethrin	645.04
Cypermethrin	3190.20
Pyrethrin	97600.0
Lambda- cyhalothrin	4057.04
Tau-fluvalinate	3067.70

The above prepared stock solutions were diluted with ACN to prepare Mix working solutions of the pesticides to cover the desired concentration range. The stock solution was diluted using a method referred to as primary dilution. Working solutions with the proper concentration for sample analysis and calibration could be created by means of primary dilution. A mix working solution of 100 mg/L was prepared from appropriate measurements of the stock solutions and diluted to create different concentrations of mix working solutions of 10mg/L,1mg/L and 0.1mg/L as follows.

Table 11: Dilution of stock solutions to create mix working solutions of the pesticide compounds

		100mg/L	**10mg/L**	**1mg/L**	**0.1mg/L**	**0.1mg/L**
	original conc.(mg/L)	**Volume to pipette(µl)**				
deltamethrin	645.04	155	100µl	100µl	100µl	100 µl
lambda-cyhalothrin	4057.04	25				
pyrethrin	97600.0	1				
cypermethrin	3154.90	32				
tau-fluvalinate	3067.70	33				
	ACN:	755µl	900µl	900µl	900µl	900µl

4.5. Preparation of calibration solutions

Solvent-based standards were used to establish external calibration curve and to assess linearity, and detection limits under ideal conditions. The external calibration curve with 10 points series was prepared by diluting the standard working solutions appropriately in the range of 1ng/ml to 1000ng/ml as follows:

Table 12: Preparation of Calibration standards for the pesticide compounds using different concentrations of working solutions

Calibration points (ng/ml)	**Volume(**µl) **to pipette from 10(ng/ml)**	**Volume(**µl) **to pipette from 1(ng/ml)**	**Volume(**µl) **to pipette from 0.1(ng/ml)**	**ACN(**µl	**Water(**µl)
1	0	0	10	490	500
5	0	0	50	450	
10	0	0	100	400	
25	0	0	250	250	
50	0	50	0	450	
100	0	100	0	400	
250	0	250	0	250	
500	0	500	0	0	
750	75	0	0	425	
1000	100	0	0	400	

4.6. Preparation of matched calibration solutions

To account for the matrix effects, 200µl of blank extracts of representative sample matrices(M) which consisted of cherry(M1), lettuce(M2) and lemon(M3) extracts prepared by QuEChERS were taken from the deep freezer, diluted with water, ACN and spiked with known concentration of analyte into each matrix to obtain calibration levels as shown in the table below.

Table 13:Preparation of matrix matched calibration using different concentration of working solution

Calibration points(ng/ml)	**Volume(**µl) **to pipette from 10(ng/ml)**	**Volume(**µl) **to pipette from 1(ng/ml)**	**Volume(**µl) **to pipette from 10(ng/ml)**	**Matrix(**µl)	**ACN(**µl)	**Water(**µl)
1	0	0	5		45.0	
5	0	0	25		25.0	
10	0	0	50		0	
25	0	12.5	0		37.5	

50	0	25.0	0		25.0	
100	0	50.0	0	200	0	250
250	12.5	0	0		37.5	
500	25.0	0	0		25.0	
750	37.5	0	0		12.5	
1000	50.0	0	0		0	

The samples were filtered into autosampler vials using 0.22 μm PTFE syringe filter and the analysis was done using UHPLC-MS/MS

4.7. Preparation of mobile phase

The mobile phase comprised of Eluent A (5mM ammonium acetate in water containing 0.1% acetic acid and eluent B(methanol). It was prepared by diluting the stock eluent (100mM filtered stock ammonium acetate) with water. It was prepared by putting 10ml of stock eluent into 200ml volumetric flask and adding 200µl of Acetic Acid and the line filled until 200ml and was transferred to eluent bottle A. eluent bottle B comprised of Methanol (HPLC grade).

4.8. Measurement using UHPLC/MS/MS

Analysis was performed using UHPLC instrument which composed of a pump, autosampler with a temperature control module. Eluent A and B were used as mobile phase. A C18 column(Agilent Zorbax eclipse plus (C18 RRHD) was used for the chromatographic separation. Detection was done by mass spectrometer using triple quadruple(QQQ) in multiple reaction monitoring (MRM) mode. The method used was previously developed and validated for pesticide residue analysis at the Department of Food Chemistry and Analytics (Majercsik,2020).

5. Results and discussion

In this study, evaluating the LOD, linearity and ME utilizing the optimized UHPLC-MS/MS method was the main focus. In order to evaluate ME and linearity, the external calibration curve and matrix-matched calibration curves were constructed for each analyte. The impact of ME were calculated by comparing the slope ratios of the MM calibration curves to those of external calibration curves using the equation recommended in SANTE/12682/2019 which allowed for evaluation of ion suppression and enhancement in the three sample matrices of lemon, cherry and lettuce and hence validation of the method's reliability compared to GC-MS/MS.

Table 14: Regression equations, MEs and R2 for both external and MM calibration

Compound	Matrix	Regression Equation	R2	Matrix Effect*(%)
Cinerin 1	Acetonitrile	y = 60.017x + 177.36	0.9992	-
	Lemon	y = 21.863x -18.692	0.9988	-64
	Cherry	y = 22.609x + 272.32	0.9945	-62
	Lettuce	y = 26.706x + 2223.77	0.9927	-56
Pyrethrin 1	Acetonitrile	y = 219.23x - 1085.9	0.9986	-
	Lemon	y = 63.834x – 180.31	0.9991	-71
	Cherry	y = 61.587x + 14.826	0.9958	-71
	Lettuce	y = 81.07x + 22.71	0.9945	-63
Jasmolin 1	Acetonitrile	y =35.441x – 117.52	0.9978	-
	Lemon	y = 16.43x + 51.345	0.9969	-54
	Cherry	y = 11.047x + 26.032	0.9938	-69
	Lettuce	y = 10.597x + 25.214	0.9949	-70
Cinerin 2	Acetonitrile	y= 30.721x + 39.098	0.9994	-
	Lemon	y = 18.87x + 52.821	0.9975	-39
	Cherry	y = 17.968x – 62.535	0.9999	-42
	Lettuce	y = 12.234x – 18.067	0.9959	-60
Pyrethrin 2	Acetonitrile	y = 62.339x - 35.596	0.999	-
	Lemon	y = 37.613x - 15.367	0.9987	-40
	Cherry	y = 33.657x + 58.992	0.9975	-46

	Lettuce	y = 24.308x + 60.806	0.9963	-61
Jasmolin 2	Acetonitrile	y = 15.383x + 36.971	0.999	-
	Lemon	y = 6. 1459x + 45.359	0.9985	-60
	Cherry	y = 7.0975x - 33.639	0.9957	-54
	Lettuce	y = 6.5927x – 45.359	0.9988	-57
Cypermethrin	Acetonitrile	y = 72.87x + 472.39	0.9992	-
	Lemon	y = 22.938x + 54.616	0.999	-69
	Cherry	y = 11.226x + 47.083	0.9965	-85
	Lettuce	y = 4.4413x + 104.8	0.9925	-94
λ-cyhalothrin	Acetonitrile	y = 22.783x	0.9959	-
	Lemon	y = 15.218x + 60.246	0.9982	-33
	Cherry	y = 9.9505 + 40.108	0.9960	-35
	Lettuce	y = 12.766x + 190.15	0.9901	-44
tau-fluvalinate	Acetonitrile	y = 6.4662x + 19.033	0.9913	-
	Lemon	y = 3.6327x + 46.369	0.9957	-44
	Cherry	y = 2.2116x + 12.661	0.9955	-66
	Lettuce	y = 2.418x + 21.124	0.9971	-63
Deltamethrin	Acetonitrile	y = 61.761x +381.67	0.9984	-
	Lemon	y = 38.073x – 209.54	0.9996	-38
	Cherry	y = 14.74x + 103.45	0.9943	-76
	Lettuce	y = 21.432x + 203.12	0.9903	-65

5.1. Matrix effect Determination in Cherry, Lettuce and Lemon samples

All the compounds experienced ion suppression ranging from mild to stronger depending on the compound. MEs were calculated, a negative value indicates ion suppression while a positive value indicates ion enhancement.

5.1.1. Lemon matrix

In lemon matrix, ME ranged from -33% to -71. Across the pyrethroid compounds, the lowest suppression was observed for λ-cyhalothrin with ME value of -33% followed by cinerin 2 (-39%), pyrethrin 2 (-40%) and tau-fluvalinate (-44%) which showed relatively low ion suppression. On the other hand, cinerin 1 and pyrethrin 1 and cypermethrin (-69%) experienced higher suppression.

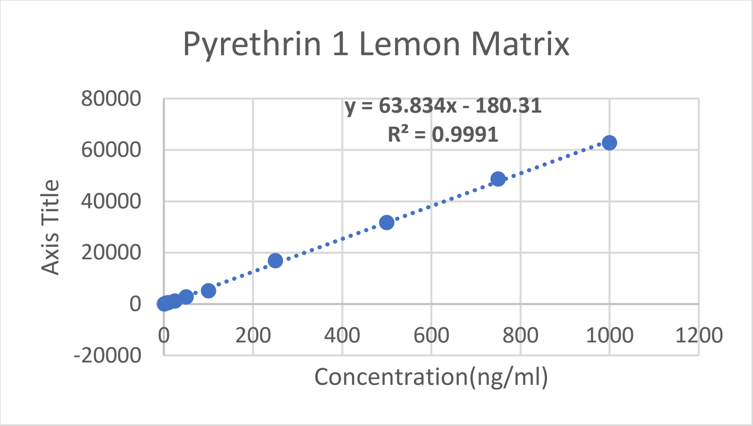

Figure 6: MM calibration curve for pyrethrin 1 in lemon showing highest ME%

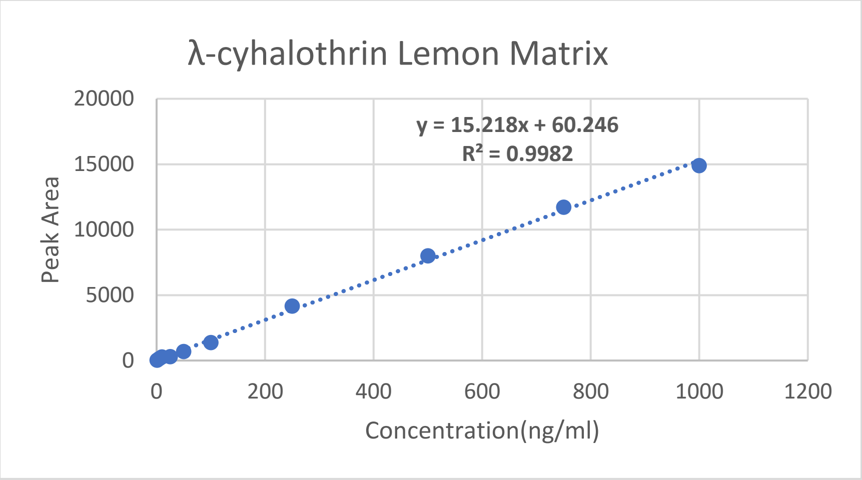

Figure 7:MM calibration Curve for cyhalothrin in lemon showing lowest ME%

5.1.2. Cherry Matrix

In cherry matrix, ME ranged from -35% in λ-cyhalothrin to -85% in cypermethrin. Like the lemon matrix, cypermethrin (-85), deltamethrin (-76%) and pyrethrin 1 (-71) showed the highest ion suppression followed by jasmolin 1(-69%) and tau-fluvalinate (-66%). λ-cyhalothrin and cinerin 2 (-42%) were least affected by ion suppression.

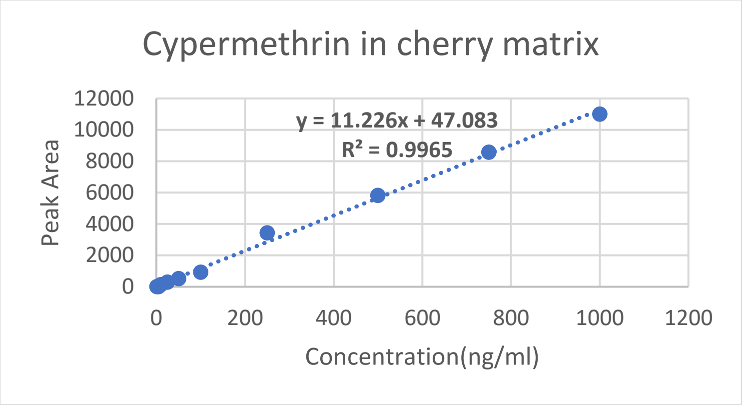

Figure 8:Compound with the highest ME (-94%) in Cherry Matrix

5.1.3. Lettuce Matrix

Among all the matrices, lettuce exhibited the strongest ion suppression, with ME ranging from -44% to -96%. The highest ion suppression was experienced by cypermethrin followed by jasmolin 1 (-70%), pyrethrin 2(-61%) and jasmolin 2 (-57%) while like in all matrices, λ-cyhalothrin was least affected.

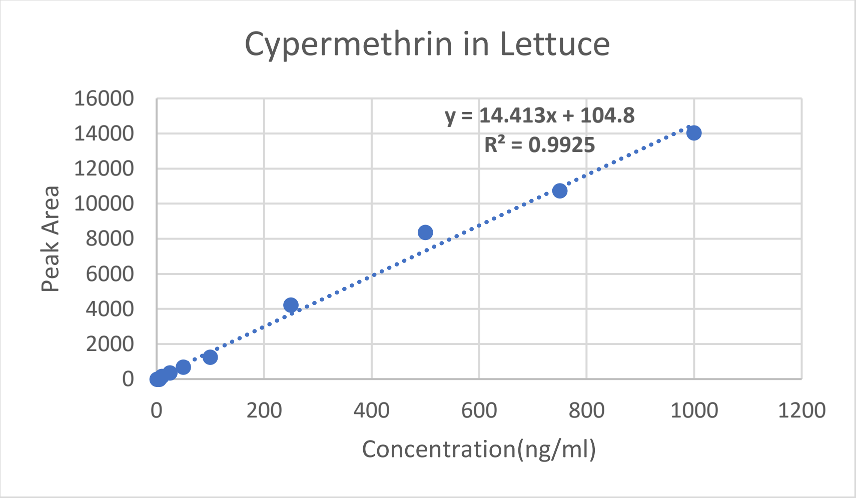

Figure 9:Compound with the highest ME% in Lettuce Matrix

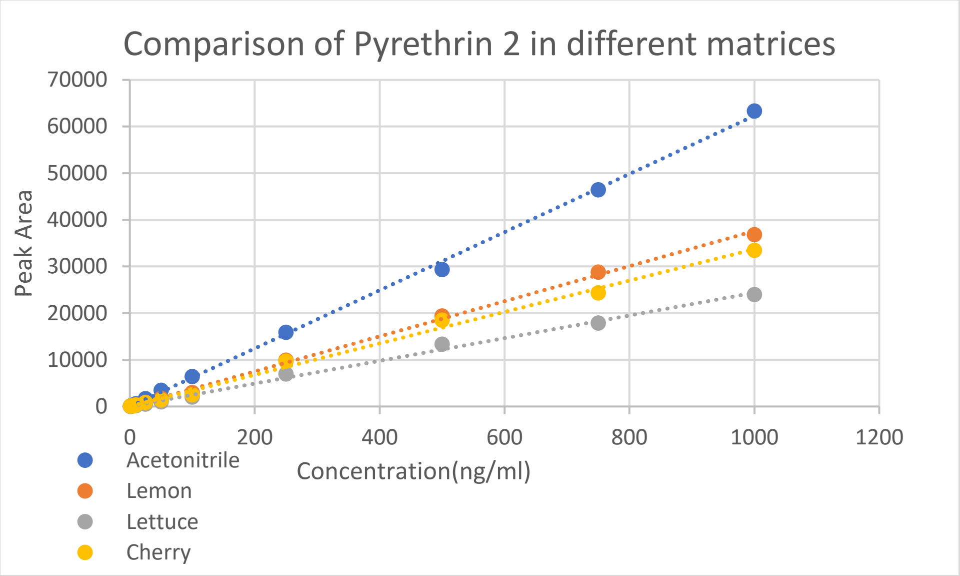

Figure 10:ME of pyrethrin 2 in different matrices

In all the matrices ion suppression was strongest in lettuce followed by cherry and finally lemon. Exceptional case was the Cinerin 1 and jasmolin 1 where both showed slightly higher MEs in cherry than lettuce. This pronounced ion suppression in lettuce sample despite the QUEChERS sample preparation could be due to the richness of lettuce in chlorophyll, organic acids and lipids which are known to interfere with ionization.

Cherry matrix exhibited moderate MEs than lettuce but higher than lemon. Cherry matrix is rich in compounds like anthocyanin, polyphenolic compounds and moderate sugars which competed with ionization of the compounds.

Lemon matrix which contains high water and acid was the lowest MEs. This suggests that lemon’s matrix is less complex compared to the other two.

Across all matrices, λ-cyhalothrin exhibited lowest overall suppression (-33% to -44%) suggesting more stable ionization behavior and less matrix interference. While cypermethrin (-69% to -94%) is more sensitive to matrix components and is greatly affected by co-eluting interference.

Table 15:Comparison of ME to other studies

	Matrices and ME in this study			**Yuan et al 2023**			**Wan et al 2021**
Compound	Lettuce	Cherry	lemon	leek	orange	Egg-plant	Orange
Cypermethrin	-94	-85	-69	-77	-76	-74	-2
Tau-fluvalinate	-66	-66	-63	-67	-67	-63	-23
λ-cyhalothrin	-44	-35	-33	-66	-58	-57	8
Deltamethrin	-65	-76	-38	27	48	50	-3
Cinerin 1	-56	-62	-64	-62	-66	-61	-
Cinerin 2	-60	-42	-39	-62	-61	-55	-
Jasmolim 1	-70	-69	-54	-71	-69	-66	-
Jasmolin 2	-60	-54	-60	-75	-71	-69	-
Pyrethrin 1	-63	-71	-71	-69	-68	-71	-
Pyrethrin 2	-61	-46	-40	-60	-63	-60	-

This study results are in consistent with the study done by Yuan et al (2023) which utilized UHPLC-MS/MS on different matrices such as leek, tomato, eggplant, grape and orange obtaining ion suppression for all the pyrethroid compounds (-27 to -77). Leek matrix had the showed the highest suppression which agree with our study findings as lettuce and leek are in the same matrix group.

Yuan et al (2023) similarly reported that cypermethrin had experienced the highest ion suppression (-77%) in leek while λ-cyhalothrin had the lowest ion suppression.

Furthermore, Wan et al (2021) quantified 19 pyrethroids employing LC-MS/MS and observed ion suppression for most of the pyrethroid compounds including cypermethrin, tau-fluvalinate and deltamethrin (-2 to -23).

On the contrary, studies of prodhan et al (2016) employed LC-MS/MS on cabbage matrix and observed significant ion enhancement with ME values of +97 in deltamethrin and +49% in cypermethrin. Similarly, Zhuang et al (2022) have observed strong ion enhancement employing HPLC-MS/MS to determine presence of pyrethroid residues in matrices such as tea, tomato and cucumber where cyhalothrin in tea exhibited ME value of 412%.

Linearity was evaluated for both external solvent-based calibration curves and matrix-matched calibration curves for all the ten pyrethroids. Calibration was performed across the concentration range of (1-1000ng/ml). it was assessed based on the correlation coefficient(R^2) obtained from the regression equation.

The external calibration curve showed excellent linearity with R^2 ranging from 0.9913 to 0.9994 signaling strong correlation proportionality between the signal and concentration in the solvent system. The slopes of the regression line varied widely depending on the compound's detector response. For example, pyrethrin 1 and cypermethrin showed the highest slope (219.23 and 72.87) while tau-fluvalinate (6.4662) showed the smallest. This reflects different ionization efficiencies.

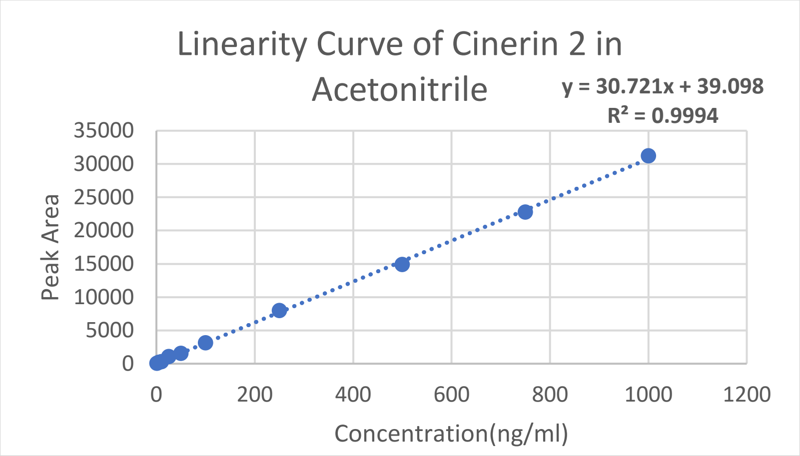

Figure 11:Linearity curve of cinerin 2 in Acetonitrile

As we know from the ME calculation lettuce being the most complex matrix, the R^2 values ranged from 0.9901 to 0.9988 which is still excellent. Wan et al (2021) reported that proper sample clean-up and matrix-matched calibration allow accurate quantification of pesticide residues in leafy vegetables despite the presence of matrix interference.

In cherry, R^2 values ranged from 0.9938 to 0.9975 maintaining excellent linearity despite the matrix interference from sugars and anthocyanins present in the cherry. Among the compounds jasmolin 1 had the lowest R^2 value (0.9938) while pyrethrin 2 and cinerin 2 had the highest R^2 value (0.9975).

In lemon, R^2 values ranged from 0.9957 to 0.9991. The highest R^2 was observed for pyrethrin 1 0.9991 despite the strong ion suppression of (-71%). This indicates that the linear response is independent of ion suppression encountered by the compounds and affects the signal intensity.

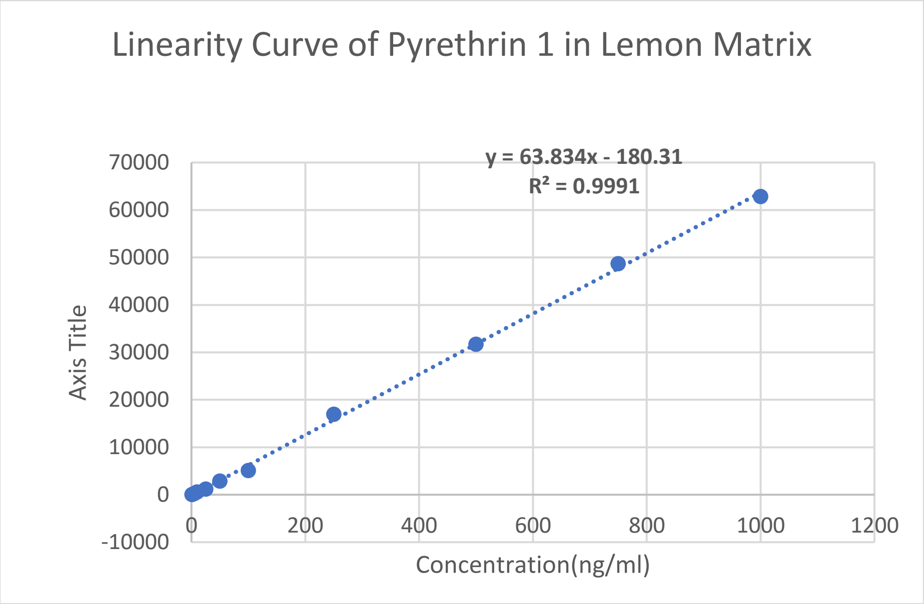

Figure 12:Linearity curve of pyrethrin 1 in lemon matrix

Overall, this study produced R^2 of 0.9901- 0.9994. All the compounds met the minimum threshold of $R^2 \geq 0.98$ according to European Commission (SANTE,2019).

5.2. Detection limit evaluation

The analysis was performed within 7 minutes and the LODs of each analyte were determined using a S/N of 3 measured from the chromatograms of quantitative MRM transitions in both External and MM calibration curves. Compounds elute at different retention times.

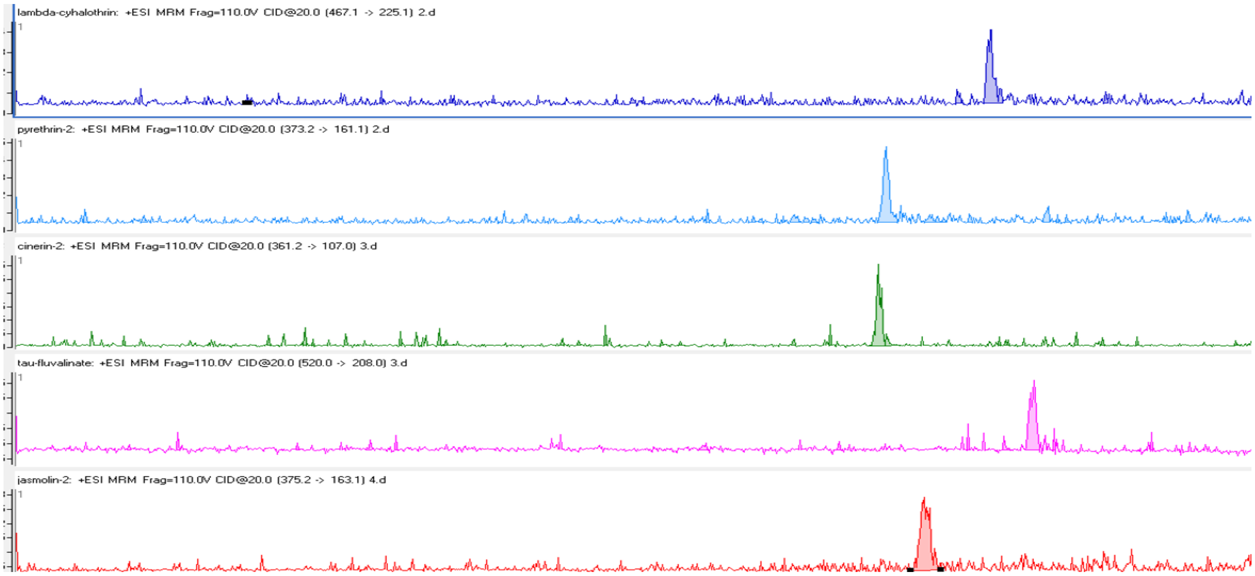

Figure 13::Extracted ion chromatograms in acetonitrile using MRM mode

The figure above represents extracted ion chromatograms in acetonitrile for different pyrethroid compounds. The purple corresponds to λ-Cyhalothrin detected at 1ng/ml, the blue one for pyrethrin 2 at 1ppb, the green one for cinerin 2 at 5ppb, the pink one is for tau-fluvalinate at 5ppb, the red one for jasmolin 2 at 10ppb showing the capability and the sensitivity to detect at low concentration.

Table 16: Analyte's retention times and LODs in Acetonitrile and different Matrices

Pyrethroid Compound	**Retention Time(min)**	**LOD(ng/ml) External calibration curve**	**LOD(ng/ml) Lemon**	**LOD(ng/ml) Cherry**	**LOD(ng/ml) Lettuce**
Deltamethrin	4.9	1	5	5	5
Cypermethrin	5.0	10	10	25	25
λ-Cyhalothrin	4.8	1	25	25	25
Pyrethrin 1	4.6	1	5	5	5
Pyrethrin 2	4.1	1	5	10	25
Jasmolin 1	5.0	1	25	50	50
Jasmolin 2	4.5	10	25	50	100
Cinerin 1	4.6	5	10	25	10
Cinerin 2	4.1	5	10	10	25
Tau-Fluvalinate	4.8	5	10	10	25

Higher LODs up to 100 ng/ml were observed across all the matrices in the matrix-matched calibration, indicating the impact of matrix effects, whereas the LODs for the external curve ranged from 1 to 10 ng/ml.

Deltamethrin and pyrethrin 1 exhibited the lowest LODs of 5ng/ml in all matrices. This suggests minimal matrix interference in both and good detectability. Jasmolin 1, jasmolin 2 and cypermethrin showed the highest LODs up to 100ng/ml in the lettuce matrix which may be due to strong ion suppression or complexity of co-extracted compounds in leafy matrices.

Lettuce matrix presented the highest LODs followed by cherry and lemon. This pattern can be explained by the higher pigment differences among the matrices, which affect extraction efficiency and ionization.

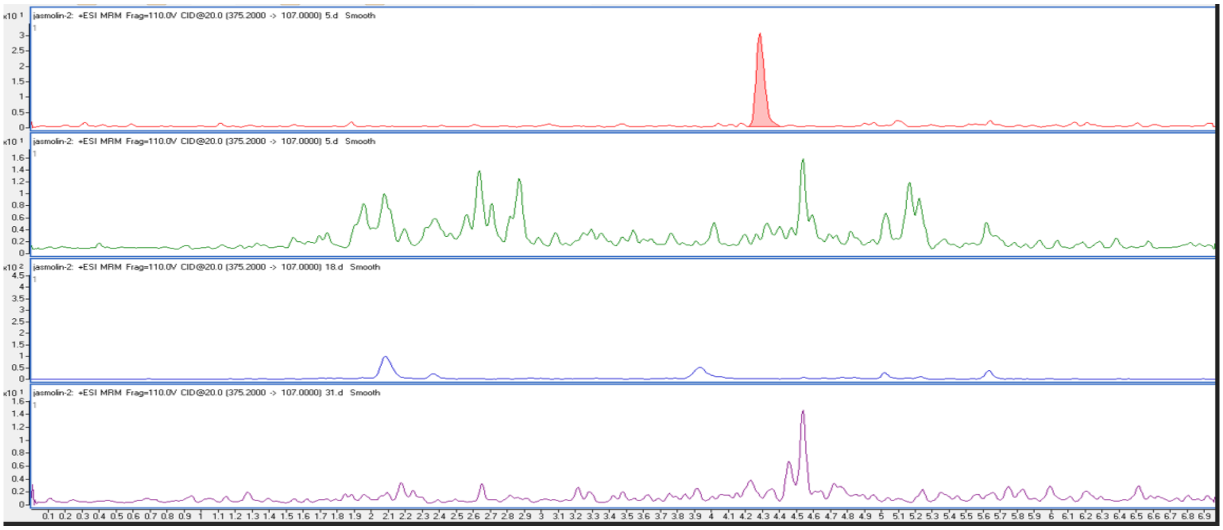

Figure 14:Jasmolin at 25ppb in Acetonitrile, cherry, lettuce and lemon matrix respectively

As demonstrated in the figure above, jasmolin 2 peak is detected hence its peak is observed in acetonitrile at 25ng/ml however no peak is observed in the three tested matrices of cherry, lemon and lettuce at the same concentration. This is due to ion suppression (ME) which reduces signal intensity of the compounds thereby increasing LODs and reducing the overall sensitivity of the method.

Table 17:Comparison of LODs with reported literature

Compound	**LODs(ng/ml) in our study(UHPLC-MS/MS)**			**LODs in (ng/ml) LC-MS/MS Li et al 2016**			**LODs(ng/ml) GC-MS/MS Lin et al 2018**
	Lemon	Cherry	Lettuce	Lettuce	Tomato	Grape	Chinese fruits/ vegetables
Deltamethrin	5	5	5	0.2	0.2	0.5	3.0
Cypermethrin	10	25	25	0.09	0.9	0.5	4.9
λ-Cyhalothrin	25	25	25	0.1	0.1	0.04	3.0
Tau-fluvalinate	5	5	5	-	-	-	1.5
Pyrethrin 1	5	10	25	-	-	-	-
Pyrethrin2	25	50	50	-	-	-	-
Cinerin 1	25	50	100	-	-	-	-
Cinerin 2	10	25	10	-	-	-	-
Jasmolin 1	10	10	25	-	-	-	-
Jasmolin 2	10	10	25	-	-	-	-

Table 10 represents comparison of LODs using different techniques and different matrices. The method presented by Li et al., (2018) exhibited two orders of magnitude lower than those achieved in this study. This is because two additional SPE procedures were used hence lower matrix effects and lower LODs. Similarly, for Lin et al., (2018) using GC-MS/MS, LODs were also lower than this study highlighting the impact of MEs on the LODs.

6. Conclusion

Pyrethroids have been routinely determined by GC-MS/MS however, in this study, the detection of pyrethroid residues and evaluation of the three analytical performance parameters in various food matrices, the UHPLC-MS/MS method showed excellent sensitivity and reliability.

Linearity was achieved for all the target analytes with $R^2 \geq 0.98$, which meets the SANTE, 2019 performance criteria. The limit of detection obtained ranged from 1-10 ng/ml in solvent and were generally higher in matrices such as lemon, cherry and lettuce up to 100ng/ml `reflecting the influence of matrix effect in UHPLC-MS/MS.

Matrix effect revealed variability among the compounds and matrices. All the analytes showed ion suppression. The resultant ME ranged from (-33% to -94%). The highest ion suppression was seen in lettuce matrix (-44% to -94%) hence the highest ME among the three matrices. Cherry matrix followed with ME value ranging from (-35% to -85%), while for lemon ME ranged from (-33% to -71%). The pattern illustrates the differing matrix complexity and co-interferences in each matrix from pigments, chlorophyll, organic acids and sugars that compete during the ionization process. In every matrix, cypermethrin exhibited the strongest ion suppression while λ-cyhalothrin exhibited the least suggesting differential ionization behavior based on compound structure.

These values were either equal or close to other reported UHPLC-MS/MS and GC-MS/MS techniques.

6.1 Recommendations and Future Directions

Matrix effects are a major challenge in UHPLC-MS/MS analysis of complex food samples as co-extracted compounds can suppress or enhance the ionization of target analytes. To minimize these interferences and improve quantification accuracy, matrix-matched calibration is recommended which involves preparing calibration curves in extracts of blank matrix samples that have undergone the same extraction and clean up procedures as the test samples. By matching the chemical environment of the analyte in both calibration and sample solution, matrix-matched calibration effectively compensates for ion suppression or enhancement leading to more reliable quantification across all matrices.

QuEChERS method provides an efficient approach for extracting pesticides from food samples, however matrix components such as lipids, pigments, sugars and organic acids still remains in the extract and contribute to ion suppression. To improve clean-up efficiency, more focus should be put on optimizing the (dSPE) step by evaluating different sorbents such as the ODS, and GCB. These could reduce MEs and enhance overall sensitivity and reliability of pyrethroid detection.

7. Summary

Pesticide residues continue to pose a serious threat to food safety especially in fruits and vegetables which are consumed fresh. This increasing concern has prompted reliable analytical techniques for detecting pyrethroid compounds in fruits and vegetables. This study aimed to optimize and evaluate a sensitive UHPLC-MS/MS method for detecting ten pesticide compounds in lemon, cherry and lettuce. Important analytical parameters were assessed such as linearity, limit of detection and matrix effects.

Results showed excellent linearity with ($R^2 \geq 0.98$), low LODS, ranging from 1-10ng/ml in solvents and ≥ 100ng/ml in matrices although matrix effects were higher up to -94% and significant for certain compounds such as cypermethrin. The findings in the study suggest that UHPLC-MS/MS method provides reliable quantification of pyrethroid pesticides despite matrix-induced suppression remaining a challenge.

To improve analytical reliability and lower matrix interferences in complex food samples, further improvement employing matrix-matched calibration and optimization of clean up sorbents is recommended.

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9. List of figures

10. List of tables

11. Acknowledgement

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I am incredibly thankful for all these blessings.

MATE Organizational and Operational Regulations

Ill. Requirements for Students

111.1. Study and Examination Regulations

Appendix 6.13: The MATE Uniform Thesis /thesis / final thesis / portfolio guidelines

Annex 4.2: Declaration of public access and authenticity of the thesis/thesis/dissertation/portfolio

DECLARATION

the public access and authenticity of the thesis/dissertation/portfolio

Student's name:	Amina Abdullahi Maalim
Student's Neptun code:	RXGHJZ
Title of thesis:	Determination of Pyrethroid Pesticide by UHPC-MS/MS,

Can it a real alternative to GC-MS/MS?

Year of publication: 2025

Name of the consultant's institute: Hungarian University of Agriculture and Life Sciences

Name of consultant's department: Department of Chemistry and Analysis

I declare that the final thesis/thesis/dissertation/portfoli02 submitted by me is an individual, original work of my own intellectual creation. I have clearly indicated the parts of my thesis or dissertation which I have taken from other authors' work and have included them in the bibliography. Furthermore, I declare that the artificial intelligence tools (e.g. text generation, linguistic correction, translation, data analysis) used during the preparation of the thesis did not substitute my own research and creative work; their use was indicated either in the list of sources or in the methodology section, and I acted in accordance with professional and ethical expectations.

If the above statement is untrue, I understand that I will be disqualified from the final examination by the final examination board and that I will have to take the final examination after writing a new thesis.

I do not allow editing of the submitted thesis, but I allow the viewing and printing, which is a PDF document.

I acknowledge that the use and exploitation of my thesis as an intellectual work is governed by the intellectual property management regulations of the Hungarian University of Agricultural and Life Sciences.

I acknowledge that the electronic version of my thesis will be uploaded to the library repository of the Hungarian University of Agricultural and Life Sciences. I acknowledge that the defended and - not confidential thesis after the defence - confidential thesis 5 years after the submission will be available publicly and can be searched in the repository system of the University.

Date: 2025,November 3rd

Student's signature

[1] While keeping the appropriate thesis type, all other types are to be removed.

[2] While keeping the appropriate thesis type, all other types are to be removed.

DECLARATION

Amina Abdullahi Maalim student (Neptun code: RXGHJZ) as a consultant, I declare that I have reviewed the final thesis/thesis/dissertation/portfoliol and that I have informed the student of the requirements, legal and ethical rules for the correct handling of literary sources.

[✓ recommend] / do not recommend[2] the final thesis / dissertation / portfolio to be defended in the final examination.

The thesis contains a state or official secret: yes [✓ no]

Date: 2025 november, 3rd

Insider consultant

The other types should be deleted while retaining the corresponding thesis type.
The appropriate one should be underlined. [3] The
appropriate one should be underlined.

Declaration of Students and Doctoral Candidates on the Use of Artificial Intelligence (Al)"

1. general information:

Name of the student:	Amina Abdullahi Maalim
Neptun ID:	RXGHJZ
Level of program (mark with X):	BSc/BA X MSc/MA Doctoral (PhD) School C] Other --------------------
Name and code of the subject*:	
Title of the work:	Determination of pyrethroid pesticide using UHPC-MS/MS. Can it be alternative to GC-MS/MS

* Not required to be completed in the case of a doctoral dissertation.

2. Declaration on the Use of Al I, the undersigned, fully aware of my ethical responsibility, make the following declaration:

(Please choose one of the options below!)

O A) I have not used any artificial intelligence system or service.

(If you selected this option, completing the subsequent tables is not required.)

VB) I have used an artificial intelligence system or service.

(Please fill in the relevant tables!)

3. Details of Artificial Intelligence Usage

TABLE I: Assistant or Minor Usage (e.g., translation, language proofreading, brainstorming, etc.)

(For these uses, attaching the specific prompts and responses is not required.)

Purpose of Use	Name and Version of the Al Tool Used	Affected Section (if not applicable to the entire text)

I used AI for Brain Storming to understand some concepts better and grammer check	Chat (GPT-5, open AI)	(only in wording)

TABLE II: Significant Content Contribution (e.g., generating an entire figure or a longer text section)

(In these cases, documenting the key prompts used and the raw responses provided by the AI, and attaching them as an appendix to the work, is required.)

Purpose of Use	Name, Version, and Access Information of the AI Tool Used	Exact Number of the Affected Chapter / Figure / Table	Entry Number of the Appendix Containing the Prompt Log

3/A. Additional Rules Prescribed by the Lecturer (if any)

If the instructor or supervisor of the course has established specific rules or expectations regarding the use of AI tools, please summarize them in the field below:

For example: prohibition of AI use for certain types of tasks; only specific tools are permitted; different citation requirements; documentation format, etc.

Rules Prescribed by the Lecturer or Supervisor

4. Declaration Applicable to All Students:

I declare that I have critically reviewed, edited, and incorporated any content potentially generated by AI in all cases. I take full responsibility for every element of the submitted work, including its originality and scientific validity. I acknowledge that the Hungarian University of Agriculture and Life Sciences may check the submitted work with an artificial intelligence detector and may initiate proceedings if my declaration is found to be false or incomplete.

Place and Date: Budapest 2025, November 3rd

Signature of the Student

Signature of the Advisor/Supervisor