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LC/MS – Food Safety – Polar Pesticides

A Non-derivatized Quantitative Method for Anionic Polar Pesticides in Mango using LC-MS/MS with IonFocus Feature

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Abstract

Food safety testing with a hazard-based approach regulation being enforced leads to increased use of polar pesticides, which exhibit lower persistence and health toxicity. Glyphosate and ethephon are a few examples that are widely used as plant protection agents with maximum residue limits (MRLs) set at 0.1 mg/kg and 0.05 mg/kg in the EU regulation, respectively [1,2]. However, analyzing these polar pesticides has been challenging as conventional reversed-phase chemistry is ineffective due to poor retention and possible surface interaction. In this study, a simple and fast analytical method was developed without compound derivatization to quantify anionic pesticides in fruit matrix using LC-MS/MS. A small injection volume of 5 µL demonstrates good sensitivity achieved with a quantitation limit of 0.5 ppb for both glyphosate and ethephon in the matrix.

Analysis of polar pesticides in mango using LC-MS/MS

Keywords:
LCMS, anionic polar pesticides, glyphosate, fruits, non-derivatization

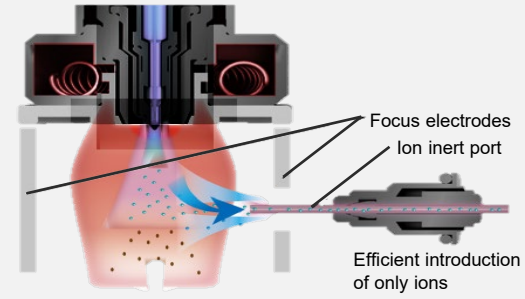
Highlights

- *A simple and fast analytical method of only 16 min is developed for analysis without the need for derivatization.*
- *Shimadzu LCMS-8060NX enables a direct yet sensitive detection and quantitation of glyphosate and ethephon in fruit matrix.*
- *The use of the IonFocus feature effectively reduces the introduction of matrix interference into LCMS system, thereby ensuring accurate quantitation.*

Technologies Featured

LCMS-8060NX

IonFocus Feature

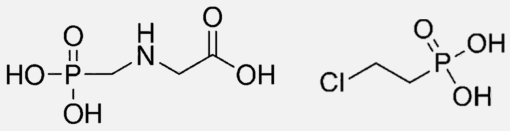


1. INTRODUCTION

The use of pesticides in the environment are constantly under review, as pesticide residues on crops meant for consumption and feed production can pose health risks. Hence, regulations are put in place to regulate the use of pesticides and monitor the maximum residue levels allowable in food and crop produce. The use of hazard-based approach in food safety regulation has led to an increased use of polar pesticides, which exhibit lower persistence and toxicity.

Food safety testing is mandatory to comply with maximum residue levels (MRLs) and assess consumption exposure. Additionally, it is required to check for the use of non-permitted pesticides. A robust method that provides fast and accurate results for a broad spectrum of chemicals in diverse food samples is essential in enforcing pesticide limits.

However, due to the physicochemical properties of polar anionic pesticides such as glyphosate and ethephon (Figure 1), the standard reversed-phase analytical method is not applicable due to the poor retention of the analytes. The presence of multiple pKa values due to multivalency further complicates the analysis with possible surface interaction. Consequently, multiple single residue methods are often adopted to analyze these groups of compounds, in some cases including the use of pre- or post-column derivatization or ion-pairing. Thus, there is a need to reduce the number of separation methods and simplify the sample treatment.



Glyphosate Ethephon

The aim of this study was to develop a simple and fast yet sensitive analytical method for highly polar pesticides that eliminates the need for multiple single residue methods, derivatization and ion-pairing. Several different columns and mobile phases were evaluated. Initial data was collected in fruit matrix using a triple quadrupole mass spectrometer in MRM mode.

2. EXPERIMENT

2.1 Standard and Sample Preparation

The standard stock solutions were prepared by dissolving the solid standard bought commercially from Sigma-Aldrich in LCMS grade methanol. Mango was purchased from local market as matrix for method development and performance evaluation. Firstly, the mango was blended, weighed out and extracted with acidified methanol. It was then centrifuged and filtered to be used as matrix blank. A matrix-matched calibration curve was established with eight standard calibration levels diluted serially.

2.2 Analytical Setup and Experimental Conditions

The calibration standard solutions and mango extract were then analyzed using Shimadzu LCMS-8060NX triple quadrupole MS. The analytical conditions for UHPLC and LCMS are described in Table 1 and Table 2, respectively. Polypropylene vials (P/N 226-54302-01) was used for injections and the steel tubing connections were changed to PEEK materials to reduce the possibility of surface adsorption of the anionic pesticides.

Table 1**. Analytical LC conditions for detection of pesticides in mango extract on **Nexera X3 LC-40

Column	Imtakt Intrada column (100 mm x 2 mm, 2.7 µm)
Mobile phase	A : Ammonium formate in water B : Formic acid in aqueous methanol
Gradient program	16 min elution gradient program
Flow rate	0.3 mL/min
Oven temperature	37˚C
Injection volume	5 µL

***Table 2**. MS conditions for detection of compounds in mango extract on LCMS-8060NX*

Interface	Heated ESI with ion focus
Acquisition Mode	MRM, negative mode
Heat block temperature	400˚C
DL temperature	300˚C
Interface temperature	400˚C
Nebulising gas	N_2, 3 L/min
Drying gas	N_2, 10 L/min
Heating gas	Zero air, 10 L/min

***Table 3**. MRM transitions for glyphosate and ethephon*

Compound	Mode	MRM Transitions	
		Quantifier	Reference
Glyphosate	-	168.05>63.00	168.05>79.00
Ethephon	-	143.00>107.05	145.00>107.00

3. RESULTS AND DISCUSSION

3.1. Optimization of LC-MS/MS

Various analytical columns with appropriate mobile phases have been investigated for the retention and separation of glyphosate and ethephon. In this study, an Imtakt Intrada multi-mode column was used on optimized LC conditions to obtain proper retention and peak shape, in addition to minimizing surface adsorption in fruit matrix. Figure 2 shows the MRM chromatogram of mixed standard at 5 ppb in mango matrix with 5 µL injection.

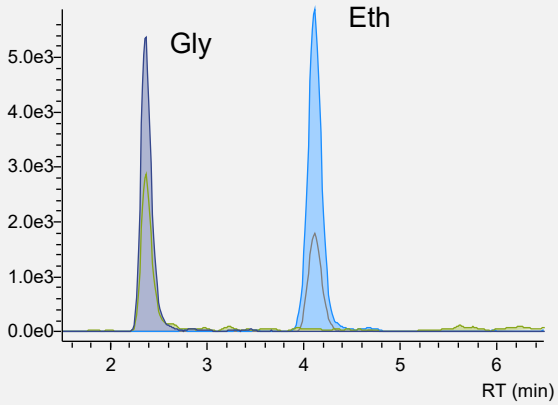


***Figure 2**. MRM chromatogram (negative mode) of mixed standard at 5 ppb in matrix*

3.2 Linearity and Sensitivity

The calibration curves were established with mixed standards in mango matrix from 0.5 ppb to 400 ppb (Figure 3). Good linearity was obtained with R^2 of more than 0.998 for both glyphosate and ethephon. The sensitivity for this matrix-matched method is estimated to be 0.3 ppb for detection limit (LOD) and 0.5 ppb for quantitation limit (LOQ) based on the MRM peaks measured, which correspond to approximately 0.03 mg/kg and 0.05 mg/kg, respectively.

3.3 Accuracy and Precision

The blank mango matrix was spiked with glyphosate and ethephon at 1 ppb and analyzed in consecutive replicates (intra-day repeatability of n = 6) for 2 days to measure precision. The percent accuracy results for both compounds are shown in Table 4 which is in accordance with the guideline within ± 20%. Precision was determined based on investigation of concentration measurement and expressed in terms of % RSD as shown in Table 5.

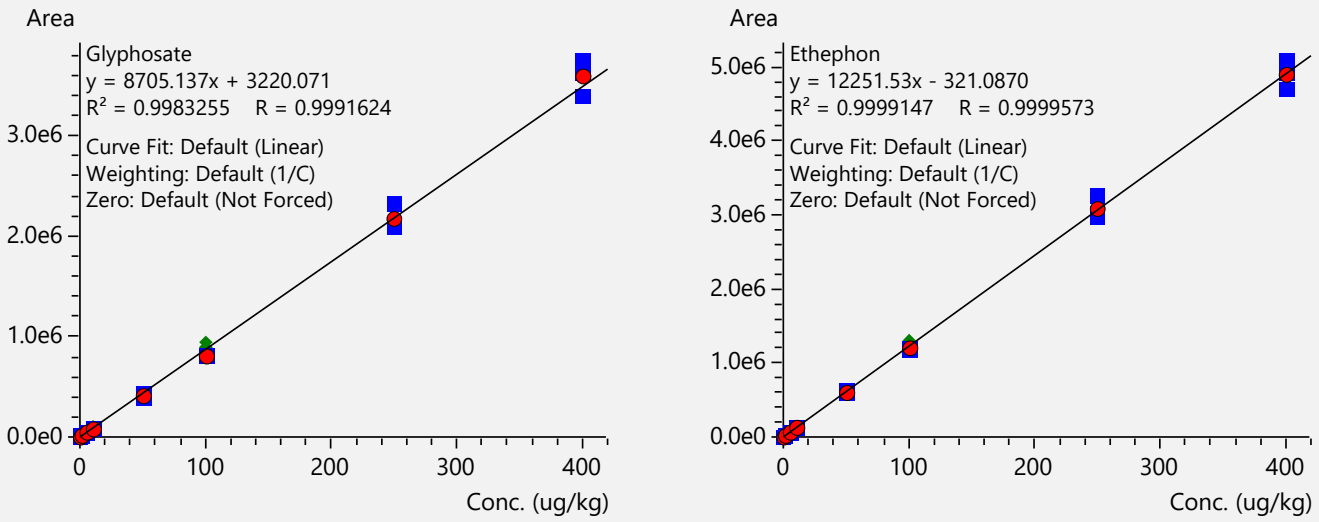


***Figure 3**. Matrix-matched MRM calibration curve of glyphosate and ethephon for the range 0.5 ppb – 400 ppb.*

***Table 4**. Concentration and accuracy results for glyphosate and ethephon on LCMS-8060NX*

Calibrant Level	Glyphosate		Ethephon	
	Average conc. (ppb)	Acc. (%)	Average conc. (ppb)	Acc. (%)
1	0.58	115.4	0.51	102.5
2	1.09	109.2	0.97	97.3
3	4.63	92.64	4.78	95.58
4	9.71	97.1	10.34	103.4
5	46.29	92.57	50.04	100.1
6	92.09	92.1	98.10	98.1
7	249.74	99.9	251.61	100.6
8	412.39	103.1	400.13	100.0

***Table 5.** Precision (% RSD) results for glyphosate and ethephon for two consecutive days (n = 6)*

Calibrant Level	% RSD			
	Glyphosate		Ethephon	
	D1	D2	D1	D2
1	1.28	2.68	1.33	5.95
2	6.89	3.03	0.14	4.59
3	0.49	1.04	0.62	2.76
4	0.03	2.85	0.67	0.94
5	2.45	4.30	1.51	2.43
6	0.11	0.24	2.04	0.69
7	0.67	5.84	0.85	5.00
8	1.04	4.76	0.71	3.75

Figure 4 shows the overlay MRM chromatograms of each compound in six consecutive injections.

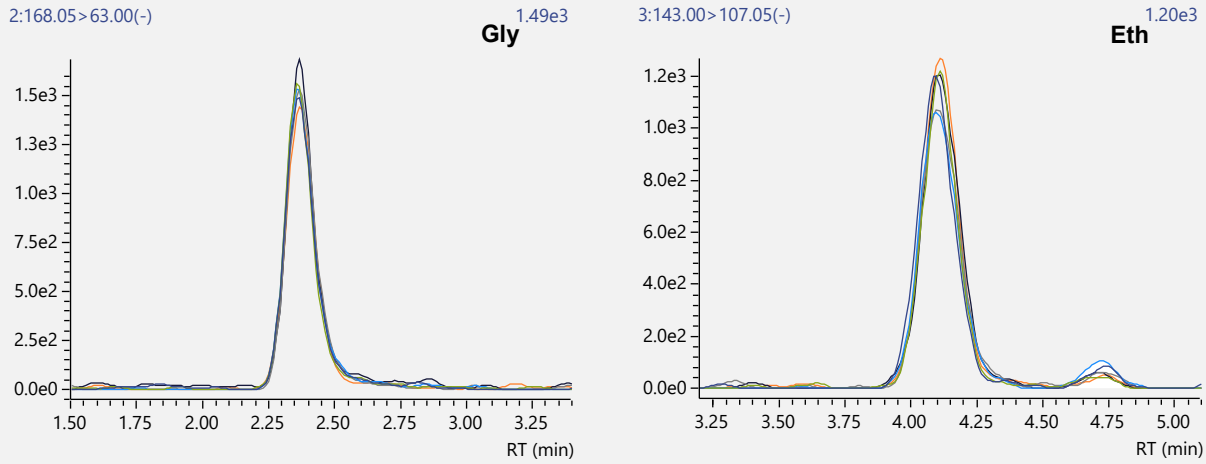


***Figure 4**. Overlaid MRM chromatograms for glyphosate (left) and ethephon (right) at 1 ppb in mango matrix (n = 6)*

Two unknown mango matrices were analyzed to determine the amount of residual pesticides with five consecutive injections (n = 5) for repeatability and stability in matrix, as shown in Table 6 and Figure 5.

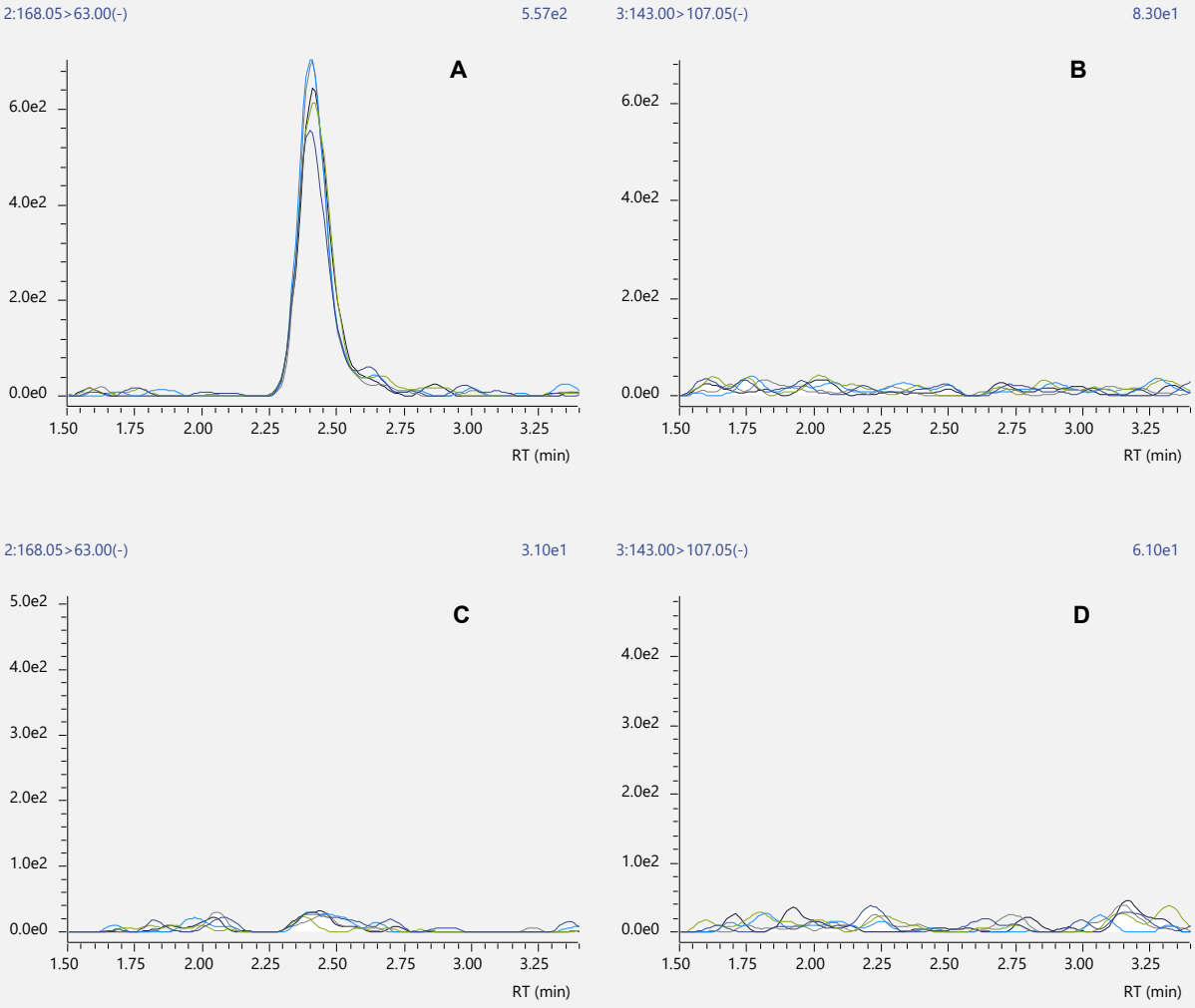


***Figure 5**. Overlaid MRM chromatograms for glyphosate (A) and ethephon (B) in Sample 1 and glyphosate (C) and ethephon (D) in Sample 2.*

There is a small amount of glyphosate estimated at 0.20 ppb found in Sample 1 while no ethephon found in the same sample. On the other hand, there were no pesticides found in Sample 2 as shown in Table 6.

Table 6. *Results on glyphosate and ethephon determined for Sample 1 and Sample 2 (n = 5)*

Compound	Sample 1		Sample 2	
	Ave Conc.	% RSD	Ave Conc.	% RSD
Glyphosate	0.20 (< LOQ)	2.48	N.D.	-
Ethephon	N.D.	-	N.D.	-

** Concentration was estimated with the extrapolation of calibration curve (values < LOQ)*
N.D. denotes not detected

4. CONCLUSION

The use of a hazard-based approach in food safety regulation has led to increased use of polar pesticides, which exhibit lower persistence and toxicity.

However, there are several challenges in analyzing polar anionic pesticides, such as poor retention on reversed-phase columns and possible surface interactions, which can complicate the analysis.

Herein, a direct LC-MS/MS method is developed for quantifying anionic pesticides, glyphosate and ethephon, without derivatization in the fruit matrix. Using the new IonFocus feature on a heated electrospray ion source, the possibility of introducing matrix interference into the MS is minimized, thereby achieving better sensitivity with a lower background.

The LOQ of the method in the matrix achieved is 0.5 ppb which meets the MRLs for glyphosate and ethephon set by EU regulation. The method has met the performance evaluation criteria in terms of stability and repeatability, thereby making it a suitable method for food safety testing.

5. REFERENCE

[1] Commission Regulation (EU) No 293/2013 of 20 March 2013 https://eur-lex.europa.eu/eli/reg/2013/293/oj (Accessed on 8 July 2022)

[2] Commission Regulation (EU) 2017/1777 of 29 September 2017 https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32017R1777 (Accessed on 8 July 2022)

[3] M. Kawashima, Shimadzu Appl. News C181 (2018)

[4] M. Kawashima, Shimadzu Appl. News C210A (2021)

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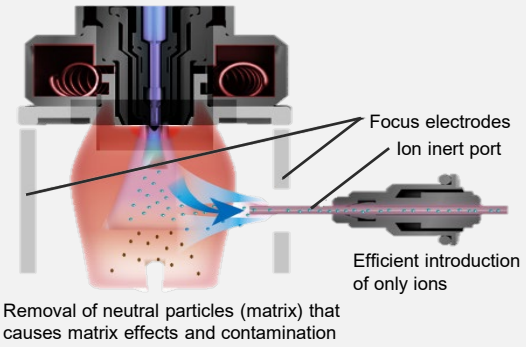

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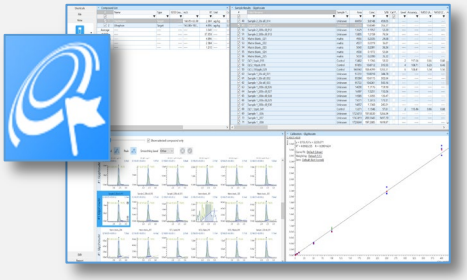

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