A Comparison of IC-MS/MS and LC-MS/MS Techniques for the Multi-Residue Analysis of Polar Pesticides and Metabolites in Food

Ed George, Cristina Jacob, Thermo Fisher Scientific, 355 River Oaks Pkwy, San Jose, CA 95134

ABSTRACT

Purpose: To compare results obtained from IC-MS/MS and LC-MS/MS analytical methods for common polar pesticides and their metabolites in a variety of food matrices.

Methods: Extracts from food samples based on a modification of the Quick Polar Pesticide (QuPPe) method[1] are analyzed by a) LC-MS/MS with a porous graphitic carbon column (PGC, or Hypercarb™) b) LC-MS/MS with a column containing a hybrid stationary phase with a mixed-mode between hydrophilic interaction liquid chromatography (HILIC) and ion exchange interactions and c) An IC-MS/MS system equipped with a high-capacity ion exchange column and post column eluent suppression.

Results: Overall, IC-MS/MS was able to obtain the best precision and accuracy for polar anions spiked into the various matrices with excellent retention time stability and peak shape. LC-MS/MS using the mixed mode column had good performance for most compounds studied, however the inability to analyze a critical metabolite, n-acetyl glyphosate, was observed. Finally, LC-MS/MS using the PCG column required extensive pre-conditioning with sample extract. Once conditioned, good precision, accuracy, and peak shape is observed.

INTRODUCTION

One of the most challenging groups of pesticides are the polar pesticides, such as glyphosate, perchlorate, chlorate, and the like, which often occur as residues in food, but are not always included in pesticide monitoring programs. Polar anionic pesticides are commonly retained and separated using a hydrophilic interaction liquid chromatography (HILIC) column that provides strong retention of polar pesticides that are unretained under conventional reversed phase conditions. Another common approach is to use porous graphitic carbon, which has unique properties as a stationary phase to retain polar analytes. Finally, IC-MS/MS based workflows have been implemented recently in many labs to achieve excellent sensitivity and reliable determination of multi-residue polar anionic pesticides and metabolites at low µg/kg levels in a single run.

In this study, a modified (QuPPe) extraction procedure using a cartridge solid phase clean-up is applied to a wide range of matrices, including leek, fruit-based baby food, and turmeric powder. All three analytical techniques are compared in terms of recovery, precision, accuracy, peak shape, and retention time stability for polar pesticides spiked at 10 and 50 ng/g.

MATERIALS AND METHODS

Sample Preparation

Briefly, spiked samples of leek (10g), fruit-based baby food (10g), and turmeric powder (2g), were added to 50 mL falcon tubes with fixed amounts of water and were extracted with methanol on a mechanical shaker for 10 minutes. The sample extracts were placed in a freezer at -20 C for 15 minutes and then centrifuged (4200 rpm, 10 minutes). A 10-fold diluted aliquot of the supernatant was passed through a Thermo Scientific™ Dionex™ OnGuard™ II RP 2.5 mL sample pretreatment cartridge. Finally, the extracts were filtered using a Thermo Scientific™ Nalgene™ 25 mm Syringe Filter, PES, 0.2 µm and placed into a PTFE autosampler vial ready for determination. For turmeric extracts, additional aliquots were diluted 1:7 with water and taken through an alternative cleanup method in order to evaluate a Thermo Scientific™ Hypercarb™ PGC extraction cartridge.

Test Methods

A Thermo Scientific™ Vanquish Flex™ Binary UHPLC system was used for evaluation of both **LC column phases** in this study. A description of the gradients are shown in below figures:

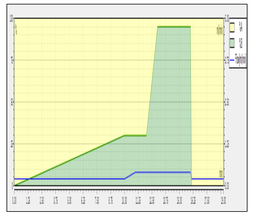


Figure 1. LC gradient profile for the PGC column. Mobile phase A: 1% Acetic acid + 5 % MeOH in Water, Mobile phase B: 1% Acetic acid + 2% Water in MeOH with a 5uL injection, column temperature of 40 C.

Time	Flow (ml/min)	% B
0.0	0.200	0.0
10.0	0.200	30
11.0	0.400	30
12.0	0.400	30
13.0	0.400	95
16.0	0.400	95
16.1	0.200	0.0
19.0	0.200	0.0

Table 1. Gradient table. Column: Thermo Scientific™ Hypercarb, 100 × 2.1 mm, 5 µm (PGC column)

MATERIALS AND METHODS- Cont.

Test Methods- Cont.

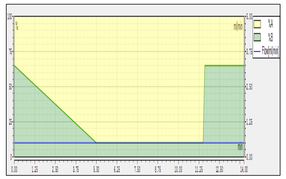


Figure 2. LC gradient profile for the mixed-mode (MM) column. Mobile phase A: 0.5% formic acid in water, Mobile phase B: 0.5% formic acid in acetonitrile with a 10uL injection, column temperature of 35 C

Time	Flow (ml/min)	% B
0.0	0.500	65
5.0	0.500	10
11.5	0.500	10
11.6	0.500	65
14.0	0.500	65

Table 2. Gradient table. Restek Raptor X, 30mm × 2.1 mm, 2.7 µm

Ion Chromatography: The system configuration included a Thermo Scientific™ Dionex™ Integrion™ HPIC™ system, fitted with an electrolytic eluent generator and conductivity cell coupled to a Thermo Scientific™ Dionex™ AS-AP Autosampler. A schematic of the system is shown in Figure 3. Separation was achieved using a Thermo Scientific™ Dionex™ IonPac™ AS19-4µm Guard column (2 × 50 mm) coupled to a Thermo Scientific™ Dionex™ IonPac™ AS19-4µm Analytical column (2 × 250 mm) with elution of polar anionic analytes using a potassium hydroxide gradient. Injection volume was 25 uL with a total run time of 20 minutes.

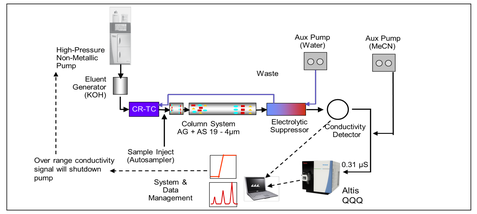


Figure 3. Schematic of the IC-MS/MS with suppressed conductivity

Mass Spectrometry: A Thermo Scientific™ TSQ Altis™ Triple Quadrupole Mass Spectrometer was used for both the LC and IC; data acquisition was performed by selected reaction monitoring (SRM) in the negative mode. The parameters for best response for each precursor to product ion transition were individually optimised by infusing standards.

System Suitability: A system suitability test (SST) sample was analyzed by all three methods to check for calibration linearity, sensitivity, retention time stability and peak shape. Calibration solutions were prepared at 1, 2, 5, 10, and 20 ppb in pure water. The 1 ppb standard was injected 8 times to check for %RSD. Analytes include Glyphosate, Glufosinate, Fosetyl-Al, N-acetyl-glyphosate, and perchlorate.

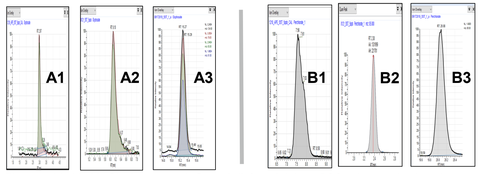


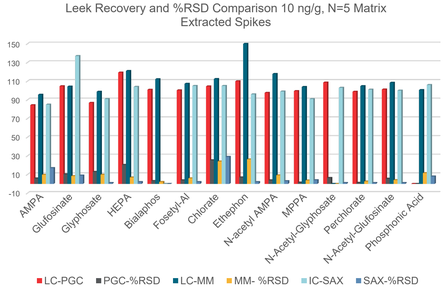
Figure 4. A1, A2, A3: Glyphosate peak @ 5 ppb (left to right) on PGC column, mixed-mode (MM) column, and IC SAX column in the SST solution; B1, B2, B3: Perchlorate @ 5 ppb on PGC column, mixed-mode column, and IC SAX column in the SST respectively.

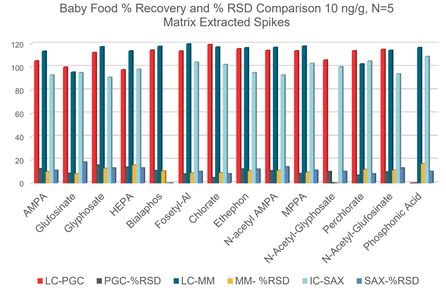
RESULTS

	PGC LC Column			Mixed-mode LC Column			SAX IC Column		
Compound	R^2	% RSD @ 1ppb	RT % RSD	R^2	% RSD @ 1ppb	RT %RSD	R^2	% RSD @ 1ppb	RT %RSD
Glufosinate	0.9998	3.7	0.54	0.9985	4.46	0.32	0.9997	1.63	0.15
Glyphosate	0.9974	9.8	0.4	0.995	9.28	0.47	0.9997	2.68	0.06
Fosetyl-Al	0.9986	2.8	0.22	0.9979	1.58	0.22	0.9997	1.21	0.00
N-Acetyl-Glyphosate	0.9998	5.7	1.42	ND	ND	ND	0.9996	3.10	0.06
Perchlorate	0.9997	3.6	3.03	0.9973	0.72	0.28	0.9999	0.82	0.00

Table 3. SST sample comparison of calibration linearity, % RSD of peak area response at 1 ppb, and retention time (RT) %RSD across the 3 method types. Poor % RSD was observed for the RT on the PGC column; the mixed-mode column was not able to retain N-acetyl-glyphosate.

Absolute recovery (no internal standard correction) and %RSD data for the analysis of leek, fruit-based baby food, and turmeric powder for matrix extracted spike (MES) samples at 10 ng/g (N= 5 replicates) are summarized in Figure 5, for all three techniques.





RESULTS- Cont.

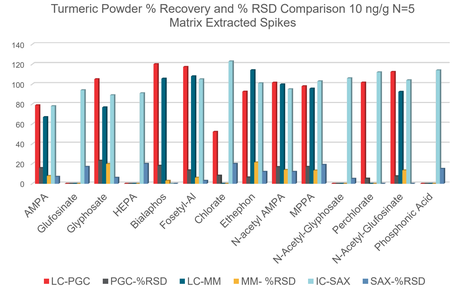


Figure 5. Recovery and RSD summary for the three techniques. Cyanuric acid and maleic hydrazine were based on 50 ng/g spikes and were poorly recovered overall by any method. Leek and baby food were based on a 10g sample; Turmeric based on 2g starting material.

In general, phosphonic acid was not detected at 10 ng/g in all three matrices on the PGC column. N-acetyl glyphosate was not detected on the LC mixed mode column, and it was also difficult to detect it in the turmeric matrix on the PGC column. Overall, the IC-MS/MS technique had the best performance for all the matrices studied, with the exception of bialaphos (it was later corrected when a new suppressor was installed on the instrument).

Matrix effects were further studied in turmeric and ginger extracts. As can be seen in Figure 6, ion suppression for most analytes is observed. Therefore, more study into improved sample cleanup for these very complex botanical extracts is necessary to obtain better sensitivity--this will be a benefit to all 3 analytical techniques.

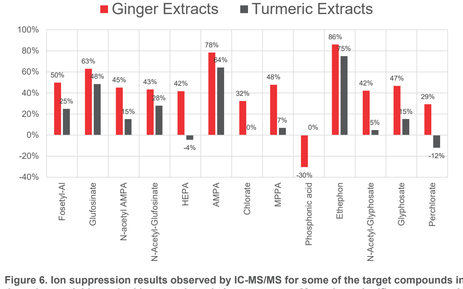


Figure 6. Ion suppression results observed by IC-MS/MS for some of the target compounds in the polar pesticide method in turmeric and ginger extracts. Most show significant suppression with the exception of phosphonic acid. Further work on sample preparation cleanup to reduce matrix co-extractives is crucial to improve detection levels.

RESULTS- Cont.

Method Attribute	LC-MS/MS PGC	LC-MS/MS Mixed-Mode	IC-MS/MS SAX
Sensitivity	Yellow	Yellow	Green
RT Precision	Red	Yellow	Green
Overall Peak Shapes	Red	Yellow	Green
Recovery	Red	Yellow	Green
Precision	Red	Yellow	Green
All anions detected	Yellow	Red	Green
Cations detected	Green	Red	Red
Complex Botannicals	Red	Yellow	Green
Overall Robustness	Red	Yellow	Green
System Conditioning	Red	Yellow	Green
Analytical Run Time	Yellow	Green	Red

Table 4. Summary table of overall relative method performance when compared to key method performance characteristics. Colors (red-yellow-green) indicate poor, medium, and excellent performance, respectively. Some polar cationic pesticides were evaluated in terms of retention and peak shape. As expected, they were not retained on the column chemistries used in the MM and SAX columns. However, IC-MS/MS has been used routinely for cations with the correct column chemistry and mobile phase conditions. The PGC column has a universal advantage to retain both polarities under the same conditions and give reasonably good peak shapes.

CONCLUSIONS

- Overall, the IC-MS/MS technique had the best peak shapes and sensitivity for the target analytes, especially in difficult matrices such as turmeric, which requires less starting material (2g).
- The PGC column required extensive conditioning in order to obtain acceptable peak shapes and stable retention times. It is therefore the least robust of the three methods.
- The mixed mode column was more robust as it did not require passivation with matrix and had very stable retention times with good peak shapes. N-acetyl-glyphosate is not retained by the column and is a big disadvantage since this analyte is required to be monitored by some residue definitions.
- More work is required to improve the cleanup of complex botanical matrices, in order to reduce co-extractives and ion suppression. IC-MS/MS had superior performance in terms of detection limit and reproducibly.
- For all methods, internal standard calibration is recommended which will improve performance in with all the techniques.

REFERENCES

1. Quick Polar Pesticides Method. https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=887&LabID=200&Lang=EN

TRADEMARKS/LICENSING

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