The Analysis of 16 Polar Anionic Pesticides and Contaminants in Food by a Robust and Sensitive IC-MS/MS Workflow

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ABSTRACT

Purpose: This presentation describes the development of a new workflow for robust and sensitive analysis of 16 polar anionic pesticides and contaminants in food.

Methods: The workflow is based on a modified QuPPe method and followed by quantification anionic pesticides using a high capacity ion exchange column in ion chromatography coupled to a high sensitivity triple quadrupole mass spectrometer(IC-MS/MS).

Results: The results show that this IC-MS/MS workflow can overcome many issues experienced with previous methods for anionic pesticides.

INTRODUCTION

Due to environment pollution and the residue of pesticide or herbicide, many kinds of anionic pesticides and contaminants commonly appear in food, such as glyphosate, glufosinate, ethephon, chlorate and perchlorate. But it was lack of a good method to monitor and analyze. These high polar pesticides are not easy to analyze directly by LC-MS, it demands derivatization or other different columns and conditions. So, the workflow of IC-MS/MS could overcome these issues and set up a simple, robust and sensitive system for polar anionic pesticides.

MATERIALS AND METHODS

Sample Preparation

Wheat flour and leek were bought from supermarket in Beijing, China. Leek samples were homogenized by a blender. Samples were extracted by methanol and centrifuged sample solution in 8000 rpm, 8 minutes. Then diluted supernatant in 10-fold by ultrapure water and passed through a Thermo Scientific™ Dionex™ OnGuard™ II RP cartridge and a syringe filter (Thermo Scientific™ Nalgene™ 25 mm Syringe Filters, PES, 0.2 μm) in series, collected 1.5 mL sample solution for IC-MS/MS determination.

Test Method(s)

The system configuration included; a Thermo Scientific™ Dionex™ Integrion™ HPIC™ system, fitted with an electrolytic eluent generator and conductivity cell was coupled to a Thermo Scientific™ Dionex™ AS-AP Autosampler and Thermo Scientific™ TSQ Altis™ Triple Quadrupole Mass Spectrometer. Separation was achieved using a Thermo Scientific™ Dionex™ IonPac™ AG19-4μm Guard, 2 × 50 mm coupled to a Thermo Scientific™ Dionex™ IonPac™ AS19-4μm Analytical, 2 × 250 mm column with elution of polar anionic analytes using a potassium hydroxide gradient. A Thermo Scientific™ Dionex™ ADRS 600 Anion Dynamically Regenerated Suppressor (2 mm) installed after the column converted the KOH to water before the eluent flow entered a conductivity detector and mass spectrometer which were connected in series. Acetonitrile solvent modifier delivered by an auxiliary pump, via a T junction between the conductivity cell and mass spectrometer, assisted more efficient desolvation and typically increased the response of most analytes by 3-4 fold. 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. The injection volume of cleaned-up extract was 25 μL. The system control, data acquisition and data processing was done using Thermo Scientific™ Chromeleon™ Chromatography Data System.

Figure 1. IC-MS/MS configuration
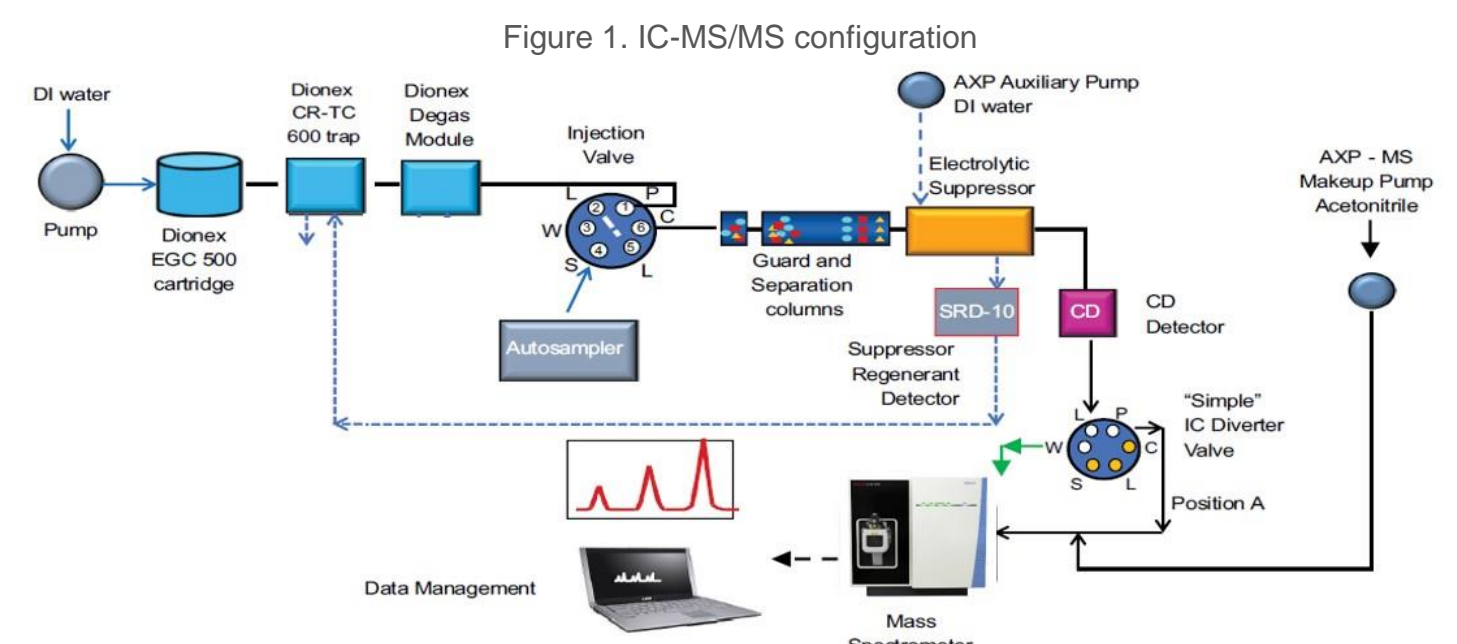
Data Analysis

Chromeleon 7.2.9 software was used for identification and quantitation of target compounds.
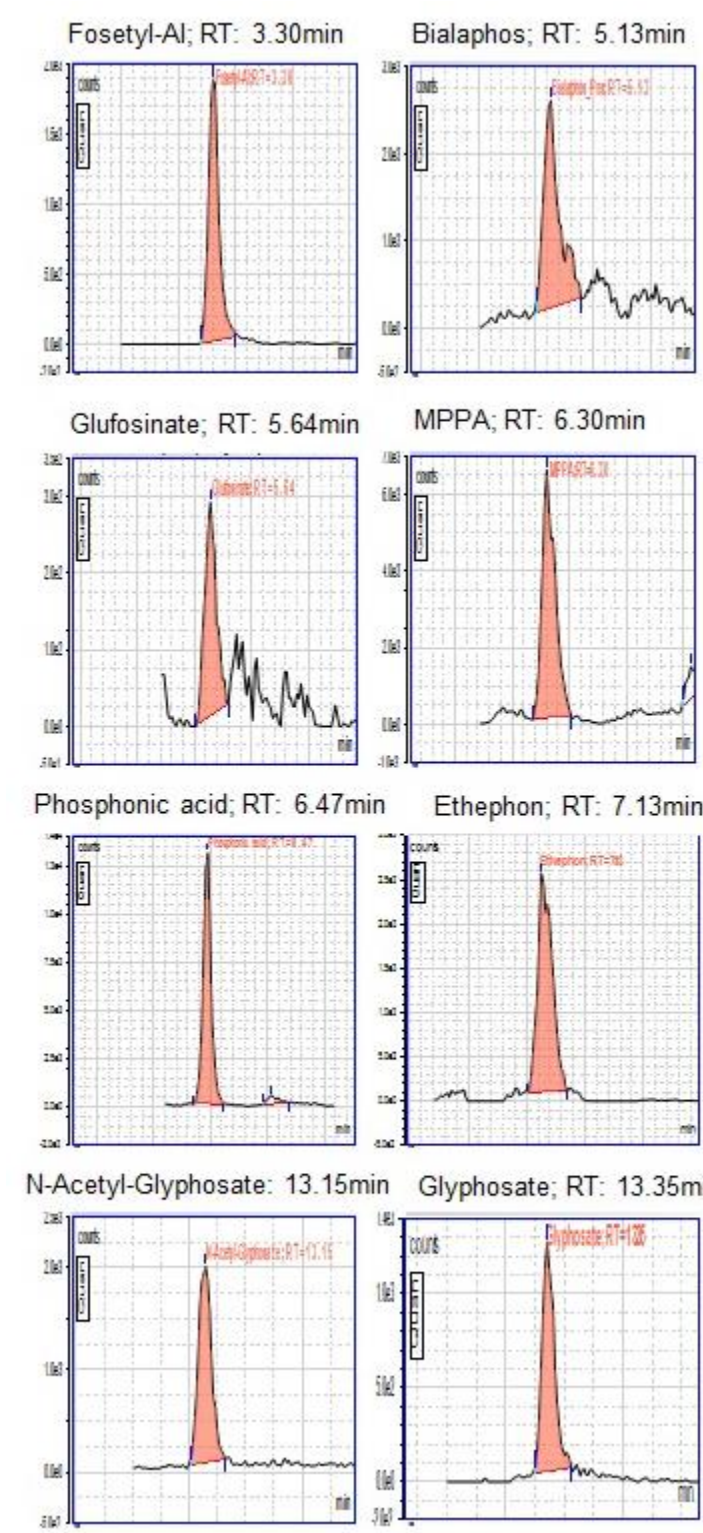
RESULTS

Chromatographic separation and MS/MS response for polar anionic pesticides

It could get good resolution and sensitivity for the 16 anionic analytes (pesticides, relevant metabolites, chlorate and perchlorate) in leek (see Figure 2 and Figure 3) and wheat flour matrix (not shown). MS peaks and calibration curves of eight analytes in leek matrix are shown in Figure 3 and Figure 4.

Figure 2. Total chromatogram of 16 anionic pesticides and contaminants in leek matrix at the lowest calibration level (15 anionic pesticides are equivalent to 4 ng/g, maleic hydrazide is equivalent to 50 ng/g).
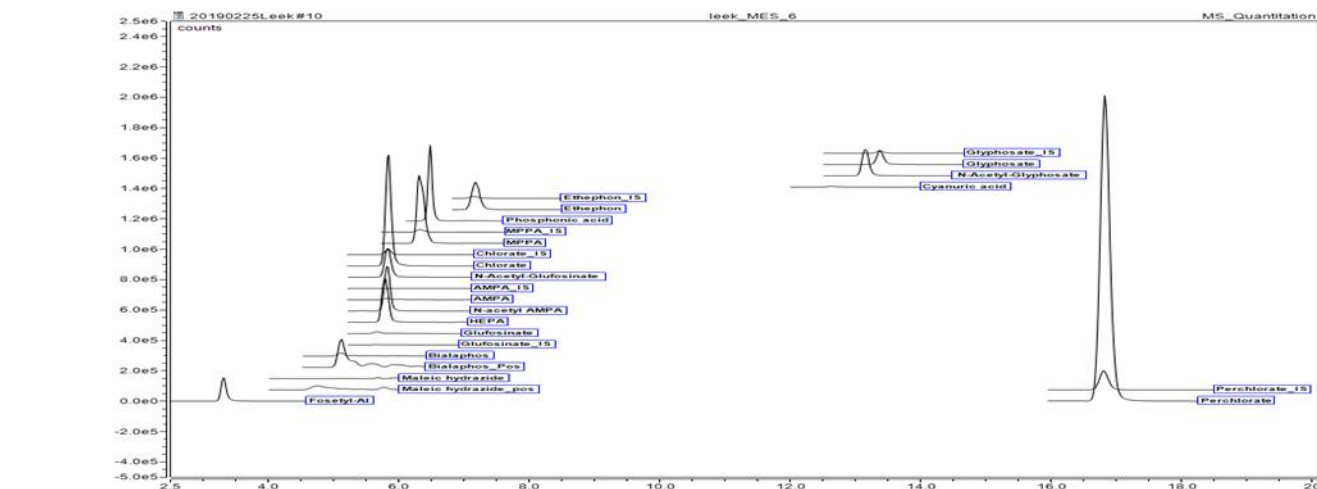
Figure 3. Response at the lowest calibration level (equivalent to 4 ng/g) in leek matrix.

Figure 4. Calibration curves of some compounds from 4 ng/g to 200 ng/g in leek matrix.
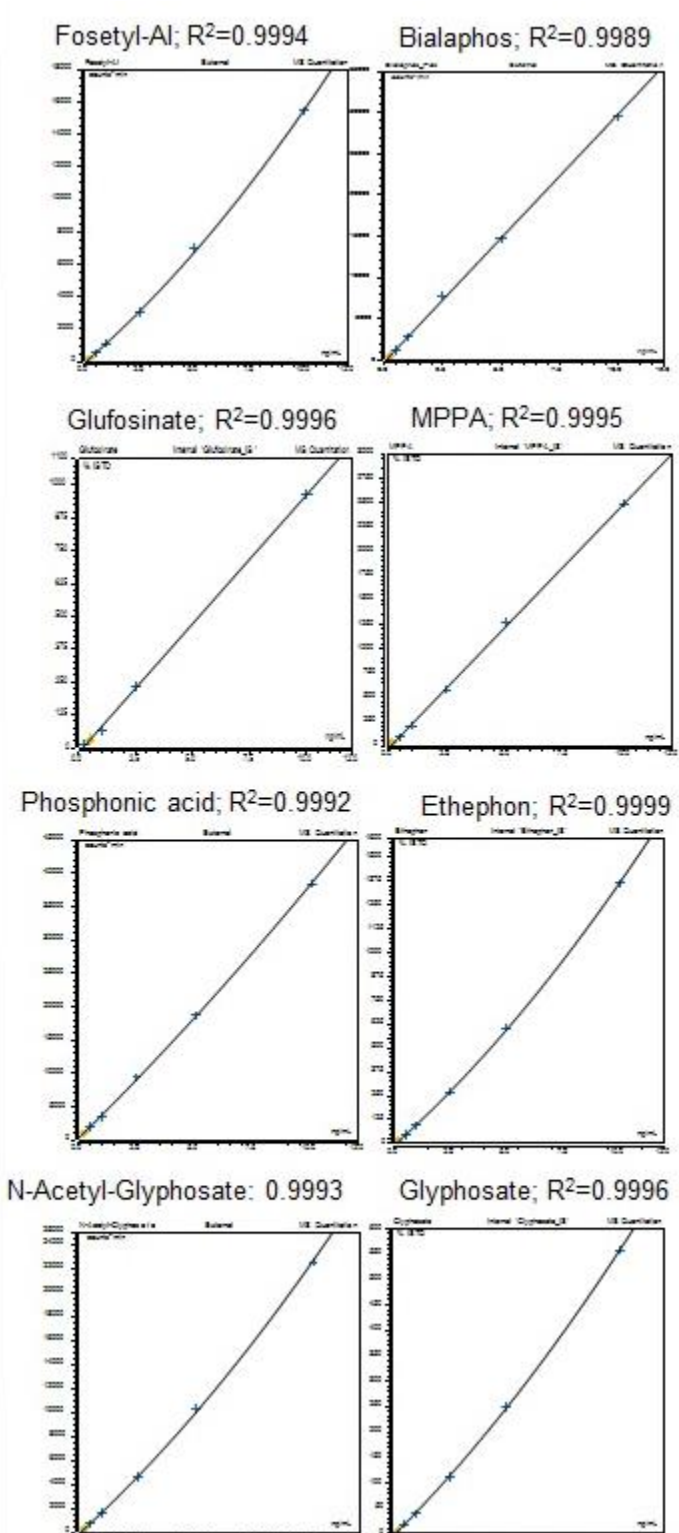
Result of anionic pesticides and contaminants in leek matrix calibrated by matrix extracted standards

Leek is a representative sample type of vegetables due to it is high moisture content and high natural pigment. 7 kinds of isotopically labelled internal standards (ILIS- AMPA, chlorate, perchlorate, ethephon, glyphosate, glufosinate and MPPA) were added to matrix extracted standards and samples before extraction to correct for matrix effects. A good recovery result was achieved by adding ILIS. But ILIS always costs a lot for a lab, so here shows the comparison of internal standard method result and external result by matrix extracted standards. Matrix extracted standards (MES) means samples spiked with known concentrations of pesticides before extraction and then make the result as calibration curves. Table 1 shows the result of recoveries and RSD in leek matrix by MES with or without ILIS.

Table 1. The result of recovery and RSD in leek matrix at two spiked levels. In spiked level 1, the concentration for 15 anionic pesticides is equivalent 10 ng/g, maleic hydrazide is 100 ng/g. In spiked level 2, the concentration for 15 anionic pesticides is equivalent 50 ng/g, maleic hydrazide is 400 ng/g.

Analytes	Spiked level 1 (n=5)				Spiked level 2 (n=5)			
	With ILIS		(Without ILIS) External (MES)		With ILIS		(Without ILIS) External (MES)	
	RSD%	Rec.%	RSD%	Rec.%	RSD%	Rec.%	RSD%	Rec.%
AMPA	9.62	94.3	8.39	96.3	6.14	97.6	1.49	98.1
Chlorate	1.80	104.8	2.55	86.1	1.52	96.4	2.25	88.5
Ethephon	2.76	102.1	2.67	97.0	1.96	98.5	0.43	99.0
Glufosinate	8.03	119.0	7.49	95.5	4.79	111.1	6.00	95.1
Glyphosate	1.56	93.8	1.62	95.0	2.03	100.5	2.20	99.7
MPPA	1.81	92.4	1.90	96.0	2.00	100.5	2.28	101.2
Perchlorate	0.42	97.8	0.60	95.4	0.27	100.0	0.64	100.7
Cyanuric acid	-	-	4.36	103.9	-	-	5.01	86.6
Bialaphos	-	-	8.96	81.8	-	-	9.46	75.9
Fosetyl-Al	-	-	0.82	92.7	-	-	1.08	94.6
HEPA	-	-	6.13	86.9	-	-	2.45	78.7
Maleic hydrazide	-	-	4.96	87.9	-	-	6.59	86.1
N-acetyl-AMPA	-	-	0.74	99.6	-	-	0.97	89.3
N-Acetyl-Glufosinate	-	-	1.64	88.2	-	-	2.37	82.5
N-Acetyl-Glyphosate	-	-	0.80	102.7	-	-	1.24	98.4
Phosphonic acid	-	-	8.05	85.4	-	-	3.53	84.7

By using a same matrix extracted standard (MES) of leek, it could quantitate these 16 analytes in other leeks. The result shows good recovery and RSD value, so this method for leek matrix could be used to other same kind of leeks. See Figure 5.

Figure 5. Recovery obtain at 10 ng/g of 15 anionic pesticides and 100 ng/g maleic hydrazide (n=5 per sample, RSD% below 10%) by MES calibration without ILIS. Full compliance with EU SANTE guideline criteria.
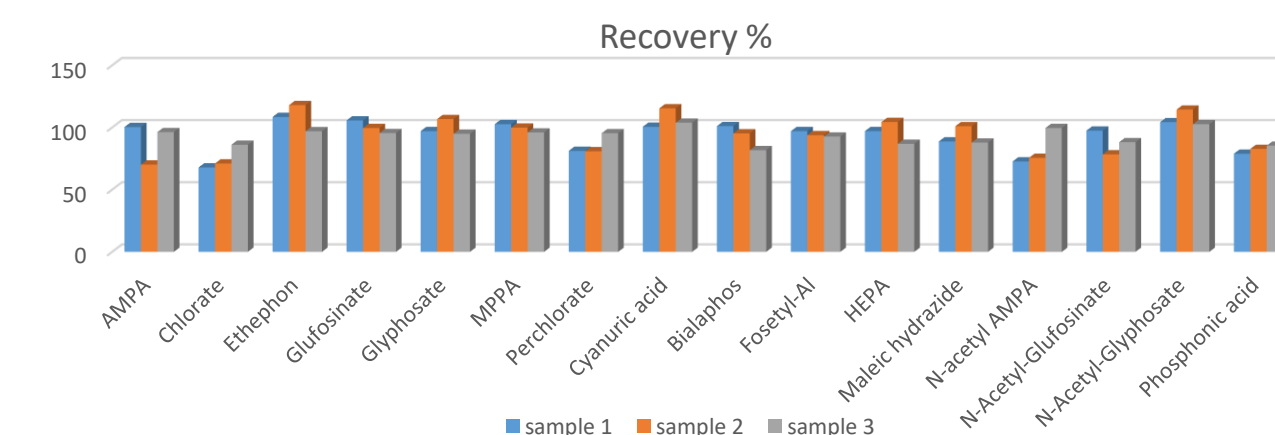
Result of anionic pesticides and contaminants in wheat flour matrix calibrated by matrix extracted standards

Wheat flour is a representative sample matrix for dry commodities, like cereals. The result also shows a good recovery and RSD value by MES with or without ILIS (see Table 2).

Table 2. The result of recovery and RSD in wheat flour matrix at two spiked levels. In spiked level 1, the concentration for 15 anionic pesticides is equivalent 10 ng/g, maleic hydrazide is 100 ng/g. In spiked level 2, the concentration for 15 anionic pesticides is equivalent 50 ng/g, maleic hydrazide is 400 ng/g.

Analytes	Spiked level 1 (n=5)				Spiked level 2 (n=5)			
	With ILIS		(Without ILIS) External (MES)		With ILIS		(Without ILIS) External (MES)	
	RSD%	Rec.%	RSD%	Rec.%	RSD%	Rec.%	RSD%	Rec.%
AMPA	6.51	107.8	6.47	103.6	9.09	106.1	4.86	96.9
Chlorate	1.70	97.7	2.29	99.9	0.71	93.4	2.11	100.1
Ethephon	2.16	97.2	1.43	99.8	0.28	95.0	0.84	98.3
Glufosinate	8.69	98.2	12.41	87.2	3.01	102.1	4.50	82.0
Glyphosate	2.37	101.5	5.42	103.5	1.57	97.8	2.09	97.9
MPPA	1.43	95.7	1.05	95.4	1.91	94.7	2.60	93.0
Perchlorate	1.00	86.3	5.22	90.0	0.75	94.0	3.13	99.3
Cyanuric acid	-	-	12.22	95.4	-	-	1.78	[1]100.4
Bialaphos	-	-	5.66	95.4	-	-	3.16	82.9
Fosetyl-Al	-	-	2.67	96.2	-	-	1.90	94.3
HEPA	-	-	2.61	95.5	-	-	0.67	93.7
Maleic hydrazide	-	-	4.06	101.6	-	-	4.42	92.7
N-acetyl-AMPA	-	-	1.14	97.5	-	-	0.58	95.6
N-Acetyl-Glufosinate	-	-	2.81	87.0	-	-	3.04	95.2
N-Acetyl-Glyphosate	-	-	3.02	99.7	-	-	1.77	94.5
Phosphonic acid	-	-	13.79	84.2	-	-	3.12	96.2

By using the same MES standard curves to quantitate other brands of wheat flour samples, most of the 16 analytes could get a good recovery and compliance with EU SANTE. But Bialaphos, N-Acetyl-glyphosate, N-Acetyl-glufosinate and Fosetyl-Al which didn't have corresponding ILIS couldn't get a good recovery between 70%-120% at 10 ng/g, so in wheat flour matrix, different sample may have different effect on final result. The best method to analyze other different wheat flour should be find a suitable ILIS for these four analytes.

Robustness of system

After about 80 injections of wheat flour extracts, the peak shapes and retention time didn't change, the analyte responses remained stable and the mass spectrometer source remained clean demonstrating the within-batch robustness of the system. In Figure 6 left is the peak shapes and retention time of Fosetyl-Al and chlorate. The biggest change of retention time for the 16 analytes is about 0.09 min. And after 230 injections of leek matrix, we could observed the same robust result, see Figure 6 right part.

Figure 6. Stability of response, peak shape and retention time after a sequence of 89 injections of wheat flour extracts and 230 injections of leek extracts (wheat flour and leek are in different batches; first row of mass spectrum is Fosetyl-Al and second row is Chlorate).
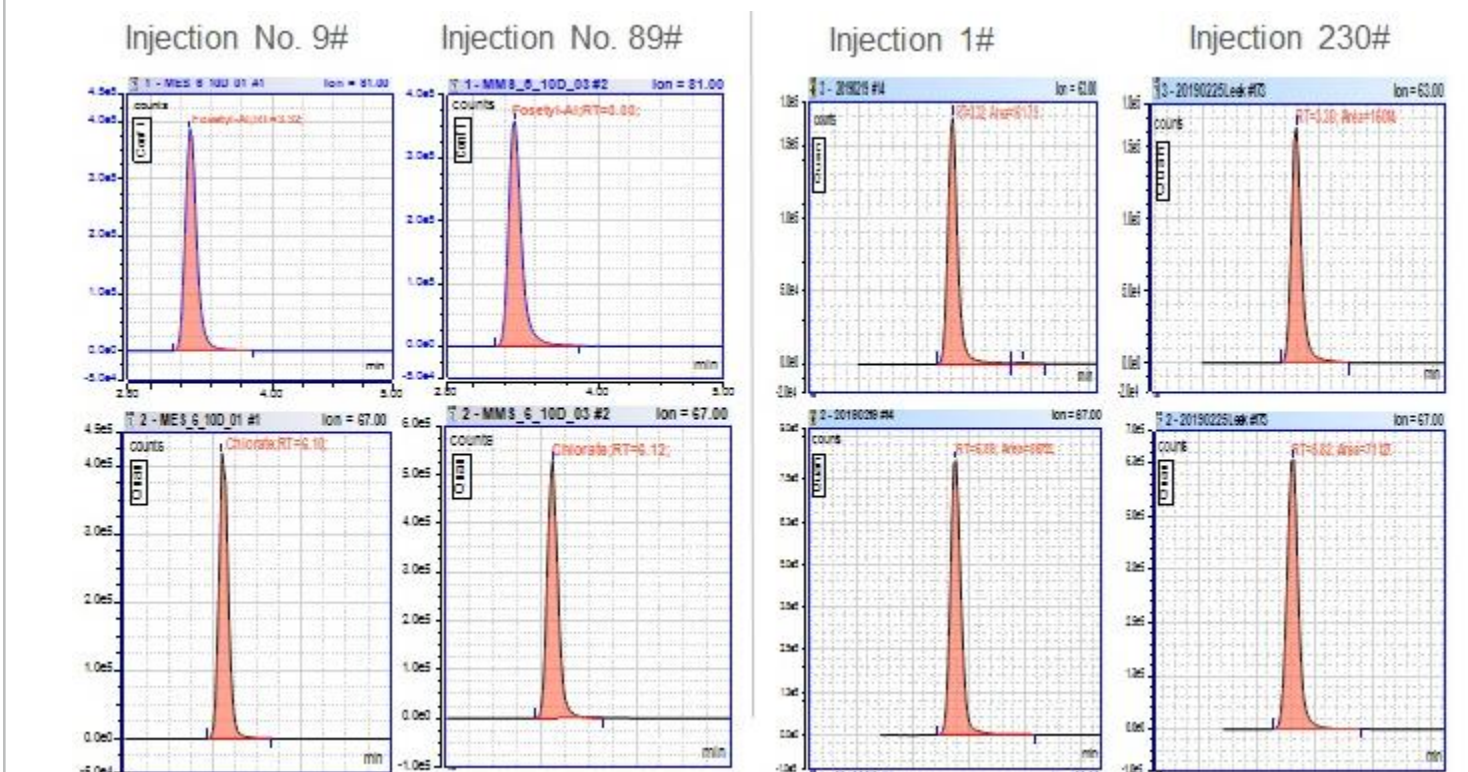
CONCLUSIONS

The IC-MS/MS integrated workflow provides robust, reproducible and reliable quantitation and identification of multi-residue polar anionic pesticides in compliance with EU SANTE method performance criteria and in a single analysis . Further work is ongoing to develop extract clean-up procedures for more kinds of food and to define the most appropriate calibration procedures to overcome matrix effects.

REFERENCES

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Reference 2.
SANTE/11813/2017, Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed; https://ec.europa.eu/food/sites/food/files/plant/docs/pesticides_mrl_guidelines_wrkdoc_2017-11813.pdf (accessed Mar 12, 2019).

TRADEMARKS/LICENSING

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