

An Executive
Summary

Ion Chromatography Tandem Mass Spectrometry- A Perfect Marriage for Polar Pesticides?



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Introduction

Polar ionic pesticides such as glyphosate, glufosinate, and chlorate are not usually amenable to common multi-residue methods so are typically extracted using the Quick Polar Pesticides Extraction (QuPPE) method¹. To obtain sufficient chromatographic retention and acceptable peak shapes for all of the polar pesticides listed in the method, it is necessary to analyse an individual extract multiple times using different chromatographic columns and conditions. This increases the total sample analysis times and the overall cost. By contrast, the multi-residue capability of suppressed ion chromatography coupled to tandem quadrupole mass spectrometry can provide significant time and cost savings for these types of analyses. This manuscript will describe the challenges and successes in the development, validation, and implementation of this approach for routine analysis.

IC-MS/MS Analysis of Polar Ionic Pesticides

The Quick Polar Pesticide Extraction (QuPPE) method is often used for the analysis of polar ionic pesticides such as glyphosate, ethephon, chlorate, perchlorate, glufosinate, fosetyl aluminum, relevant metabolites and alike. Glyphosate is of particular interest due to a number of factors, including the differences between the regulations in Europe and the US. The MRLs in Europe, set by the European Commission, are lower than tolerance values set by the Environmental Protection Agency (EPA) in the US. Also, in 2015 the International Agency for Research on Cancer (IARC), that informs the World Health Organization (WHO) on cancer risk factors, classified glyphosate as a 'probable carcinogen'. As a result of the ongoing controversy glyphosate continues to feature in the news. In 2016, residues of glyphosate had been reported in beer and many popular breakfast foods.

Historically glyphosate analysis required time-consuming derivatization prior to detection and quantification. More recently ion chromatography has been used for the direct analysis of glyphosate and its metabolites without the need for derivatization. One advantage of using ion chromatography-tandem mass spectrometry (IC-MS/MS) is the capability to use in-line concentration cartridges, which can help with the removal of matrix components as well as concentrating the ionic analytes of interest.

In 2007, this approach was employed at Fera for the analysis of glyphosate residues in sugars which are used extensively in the manufacture of food products. The challenging target concentrations were 1 µg/kg for glyphosate and 5 µg/kg for glufosinate. At that time a Dionex ICS 3000 (ion chromatography system) was coupled to a mass spectrometer with relatively low sensitivity by today's standards. To compensate for low MS sensitivity large volume injections of several milliliters of sample were required. Although this approach enabled the target

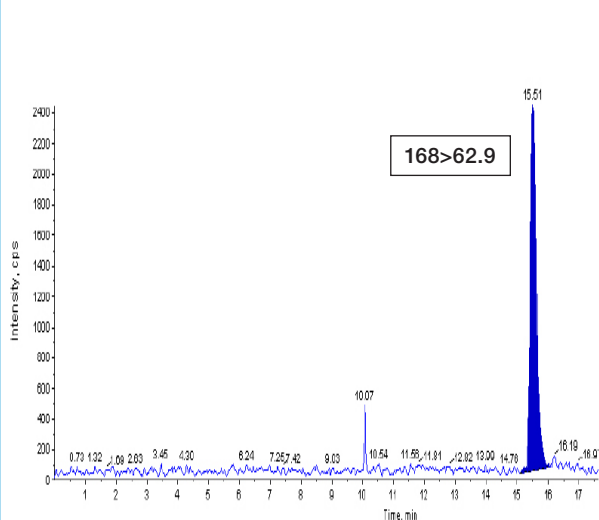
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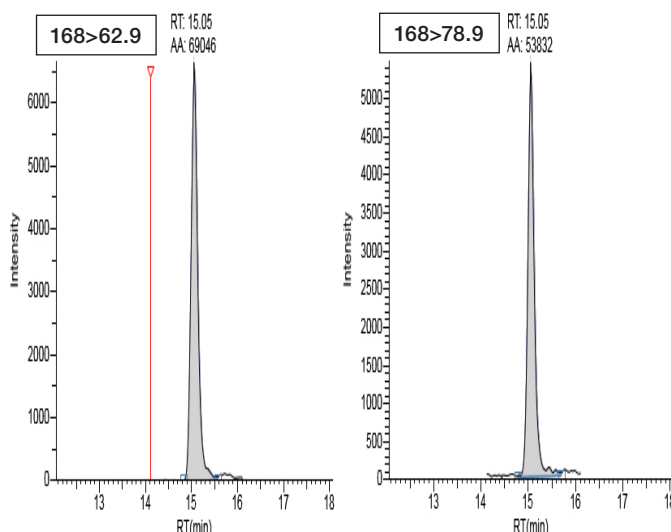
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Figure 1: IC-MS/MS Then and Now—Glyphosate

2007: Glyphosate @ 100 µg/kg in cereals with 2.5 mL injection online concentration



2016: Glyphosate @ 100 µg/kg in cereals with 1/10 extraction dilution of QuPE extracts, 100 µL loop injection



concentrations of 1 µg/kg and 5 µg/kg to be achieved, such high volumes of complex sample extracts resulted in relatively rapid contamination of the analytical column, the MS-system (after 100 injections), and longer term contamination of the post column suppressor (loss of peak shape and response after 4000 injections).

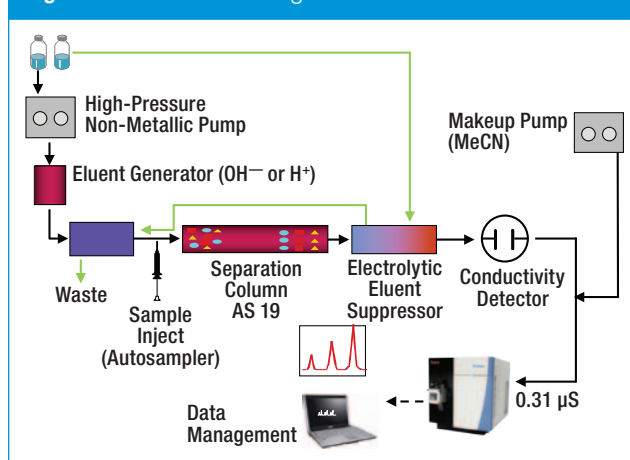
Fera are currently evaluating the performance of the latest IC-MS/MS system; the Thermo Scientific™ Dionex™ ICS-5000 HPIC™ system coupled to the Thermo Scientific™ TSQ™ Quantiva™ Triple Quadrupole MS. One of the advantages of using the Quantiva is a decrease in instrument downtime due to the ability to change the ion transfer tube whilst the system is still under vacuum. The Thermo Scientific™ TraceFinder™ 3.2 software, makes the system significantly more user friendly as it eliminates the need for two control software packages, required previously. Only one sequence, not two, is needed so that the risk of errors is reduced. Also, Fera are evaluating the new 4µm particle size IC columns which have the potential to provide better peak shapes. In combination with the high sensitivity Quantiva MS (which is has been optimized for low mass), the overall increase in sensitivity allows the use of smaller injection volumes (5–100 µL).

Where Are We Now?

Figure 1 shows that the new system with only a 100 µL injection (equivalent to 10 µL extract) can provide a better response compared to the system using 2500 µL in 2007.

The new ion chromatography system configuration is shown in **Figure 2**. Compared to a standard LC-MS/MS configura-

tion there are some subtle and unique differences including a high pressure non-metallic pump, an eluent generator, and an electrolytic eluent suppressor. The eluent generator only requires the addition of water to produce the mobile phase in situ resulting in very reproducible eluent gradients and retention times. The suppressor which electrolyzes the KOH mobile phase to water at the exit of the column, and before the detector, is critical to enable the coupling to a mass spectrometer. It is important to monitor the backpressure on the suppressor at the start of each run, and to maintain the pressure at 100–150 psi to avoid damaging it. Both the MS system and the acetonitrile makeup flow will add system

Figure 2: IC-MS/MS Configuration

backpressure. In the unlikely event the suppressor fails the conductivity signal will increase, and a feedback loop will shut down the system automatically to ensure that the MS isn't exposed to hydroxide eluent. The post suppressor addition of acetonitrile, to assist desolvation of water in the mass spectrometer, provides an average increase in signal response up to a four-fold without any distortion of peak shapes. Details of the chromatographic conditions used are shown in **Figure 3**. Since the presence of formic acid in the QuPPE extracts can affect the peak shape, the extracts were diluted 10-fold before injection of 100 μL . This dilution provided better peak shapes compared to direct injection of 10 μL of crude non-diluted extract. So what is the QuPPE method?

The QuPPE Method

The QuPPE method was developed to consolidate several single residue methods into one generic extraction method. Version 1 was published in January 2009 and the current version (V9) was released in March 2016.

The method continues to evolve to include new compounds of interest and different liquid chromatography conditions to cover the analysis of some of the most challenging polar pesticides.

Figure 3: Multi Residue Separation Program

Column: AS19 250 \times 2mm, 4 μm particle size

- Column flow: 0.35 mL/min
- Column temp: 40°C

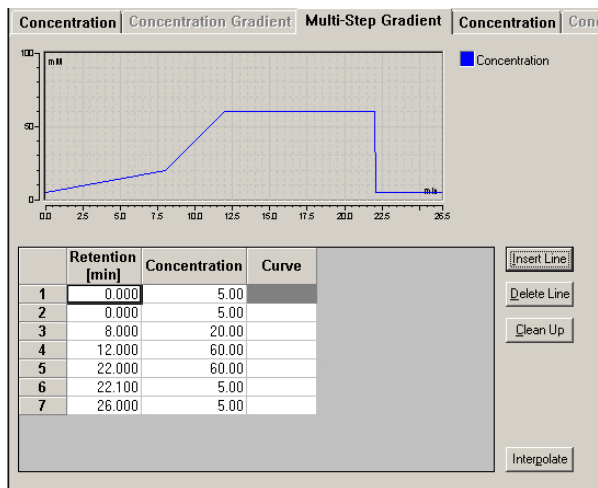
Suppressor: AERS 500 2mm (used in external water mode)

- External water flow: 0.5 mL/min
- Suppressor current: 52 mA

Eluent: KOH gradient with EGC 500

Injection volume: 100 μL

Post column MeCN flow (make-up): 0.2 mL/min



To perform the extraction, internal standards, if available, are added to the sample (10 g). Acidified methanol (10 mL) is added, and then the extract is shaken using a mechanical shaker for 20 minutes. The extract is centrifuged, filtered into a plastic LC-MS vial, and analyzed using the appropriate method conditions. This generic extraction approach is not without its challenges. There is no liquid-liquid partitioning stage so a large number of co-extractives can end up in the final extract. Also, there is no single clean-up method applicable due to the diversity of chemical properties of the analytes. This can lead to co-eluting interferences and make it more challenging to meet the method performance criteria specified in the SANTE/11945/2015 document².

Method Validation (QuPPE -IC-MS/MS)

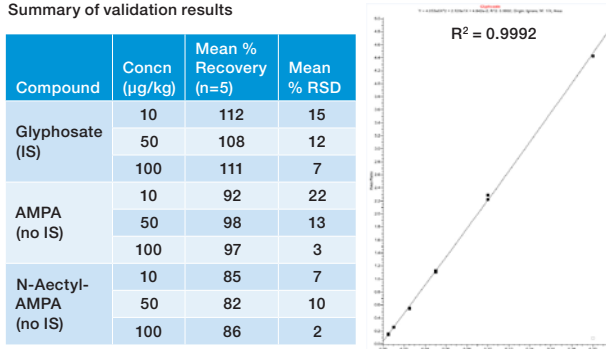
The QuPPE -IC-MS/MS method developed was validated for cereal and grape matrices, according to the SANTE validation criteria. These criteria include the recovery which must be within 70 to 120%; the associated %RSD which should be $\leq 20\%$; a sensitivity/linearity check (residuals $< \pm 20\%$); and retention time (at least two-times the void volume of the column and within ± 0.1 minute of the retention time of a calibration standard). If available, isotopically labelled internal standards were used during validation experiments.

The validation results for the analysis of glyphosate and two of its transformation products, AMPA, and N-acetyl-AMPA,

Figure 4: Glyphosate—Cereals (Flour)

Summary of validation results

Compound	Concn ($\mu\text{g}/\text{kg}$)	Mean % Recovery (n=5)	Mean % RSD
Glyphosate (IS)	10	112	15
	50	108	12
	100	111	7
AMPA (no IS)	10	92	22
	50	98	13
	100	97	3
N-Acetyl-AMPA (no IS)	10	85	7
	50	82	10
	100	86	2



in cereals (flour) are presented in **Figure 4**. At 10, 50 and 100 µg/kg (ppb) the results for % recovery (in the range 82–112), % RSD (2–22), linearity (R² values > 0.99), and retention time stability (<0.1 min) were all, apart from 1 exception, within acceptable values for all analyte-concentration combinations. The one exception was the %RSD for AMPA at 10 µg/kg (not internally standardized), at 22%, fractionally outside the limit of 20%. The IC-MS/MS method is multi-residue and excellent recovery and precision data, compliant with SANTE criteria, were achieved for glufosinate, 3-MPPA, N-acetyl glufosinate, perchlorate, chlorate, and ethephon. Excellent data were obtained for Fosetyl-AL and phosphonic acid at 200, 1000 and 2000 µg/kg (ppb). These compounds

were spiked at higher concentrations to reflect their higher EU MRLs. Clopyralid displayed poor sensitivity at 10 ppb, but provided good data at 50 and 100 ppb. Meanwhile, cyanuric acid gave insufficient response at 10 ppb and slightly variable results at 50 ppb.

The results for grapes were even more impressive. All of the compounds, at the same concentrations as in cereals, were compliant with the SANTE validation criteria, with exception of cyanuric acid which gave poor response at 10 ppb. The validation results for the analysis of ethephon at 10–100 µg/kg in grape matrix are summarized in **Figure 5**.

Figure 5: Analysis of Ethephon in Grapes

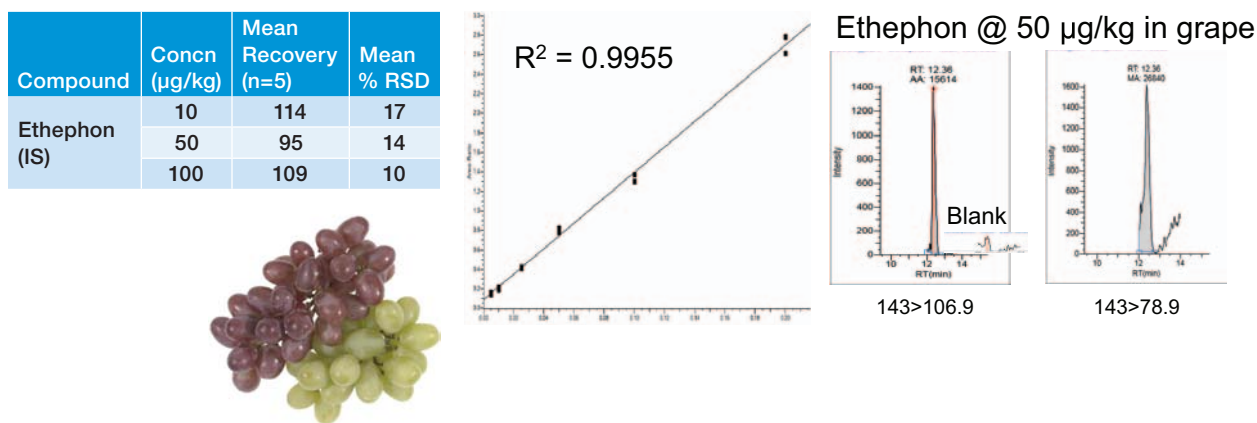
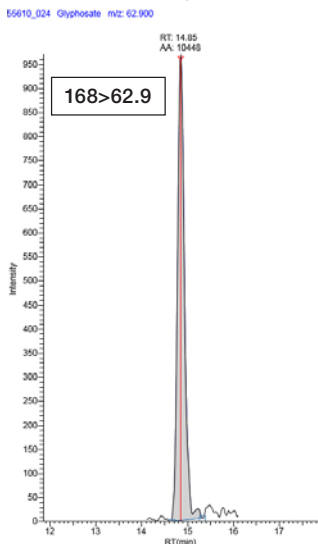
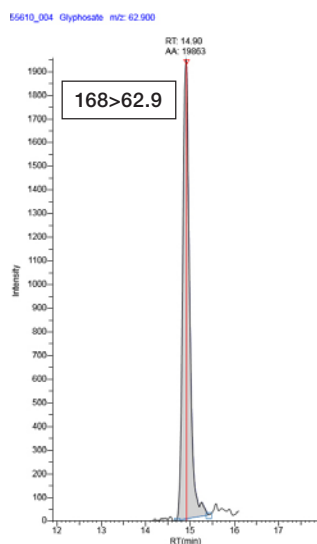


Figure 6: Glyphosate in Beer—No Extraction Required!

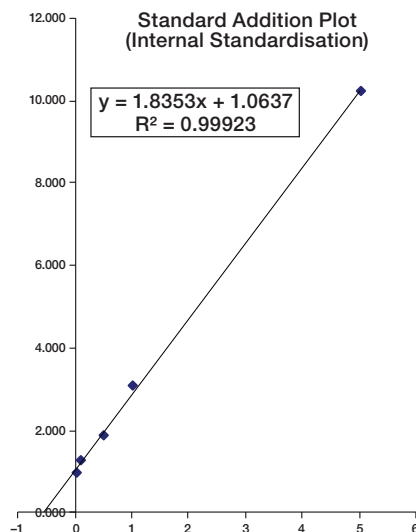
- Glyphosate incurred residue @ 0.58 µg/L



- Glyphosate spike @ 0.5 µg/L



- Calibration plot 0.1–5 µg/L spikes



- 1/10 dilution with water and internal standard added

Other Application Examples

Another application example, the analysis of glyphosate in beer is illustrated in **Figure 6**. Sample preparation in this case involved degassing the sample, then diluting 1/10 in water (no extraction required), followed by addition of an internal standard. Excellent stability of retention time was obtained as seen in **Figure 7**. The retention time of glyphosate drifted

Figure 7: Glyphosate in Beer—Retention Time Stability

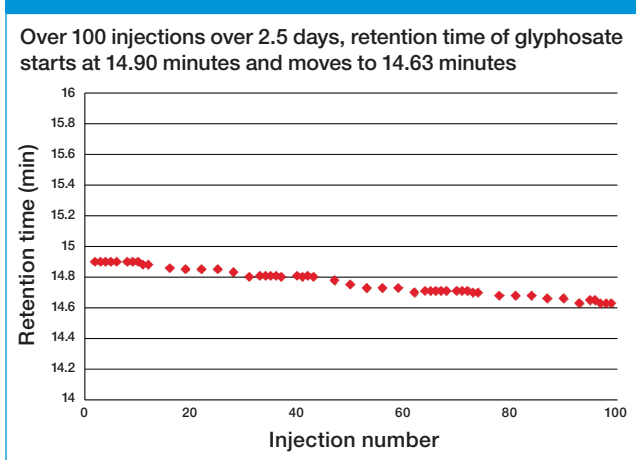
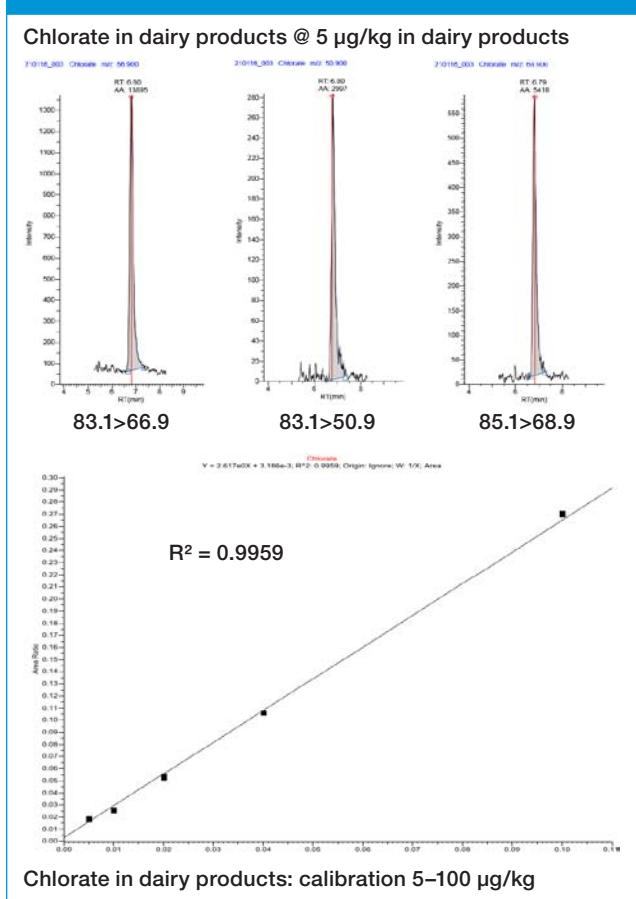


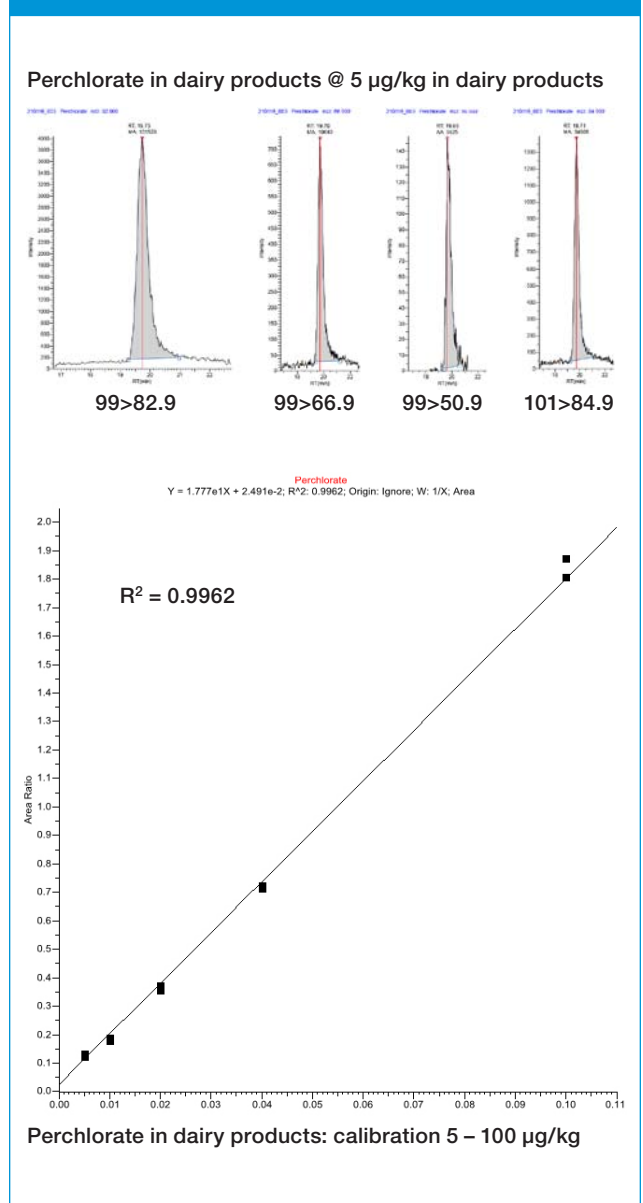
Figure 8: Chlorate in Dairy Products



by only about 0.3 minutes for 100 injections spread out over 2.5 days.

At the start of this decade chlorate was identified as a potential problem and its presence in food was banned in the EU in 2010. It was determined that the presence of chlorate in food most likely occurred as a result of a disinfection by-product in the water used in food preparation facilities, and in disinfection products used to clean equipment and work surfaces. The current default MRL of 10 µg/kg is applied. Because of ongoing concerns the European Food Safety Authority called for more data on the presence of chlorate residues in foods. In response the IC-MS/MS approach has been used to successfully conduct several chlorate surveys in the UK. Using IC-MS/MS chlorate can be detected at 5 µg/kg in dairy products, as shown

Figure 9: Perchlorate in Dairy Products



in **Figure 8**, with good linearity over a calibration range of 5 to 100 µg/kg.

Equally good results were obtained for the analysis of perchlorate in dairy products as illustrated in **Figure 9**.

Conclusion

The IC-MS method developed was successfully validated for 13 anionic pesticides in two commodity groups, displaying excellent sensitivity and selectivity. Simplified sample preparation, smaller particle size columns, a more sensitive MS instrument and unified control software have made systems easier to use. The high system sensitivity and lower injection volumes have resulted in decreased system downtime (due to lesser contamination) and decreased analysis costs while improving sample throughput. In the future it is easy to see this technology being extended to the analysis of other applications such as halo acetic acids, metals speciation, organic acids, carbohydrates, and other types of cations and

amines. Dr. Stuart Adams concluded the webinar by stating that in his opinion “Yes, IC-MS is an essential tool for modern food and environmental analytical laboratories; and it is a very good, if a not yet perfect marriage.”

References

1. 9th Version of the QuPPE-PO method. European Reference Laboratories for Residues of Pesticides (EURL-SRM.) http://www.crl-pesticides.eu/docs/public/tmpl_article.asp?CntID=887&LabID=200&Lang=EN
2. European Commission Directorate-General for Health and Food Safety (SANTE)/11945/2015. Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed. http://ec.europa.eu/food/plant/docs/plant_pesticides_mrl_guidelines_wrkdoc_11945_en.pdf

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