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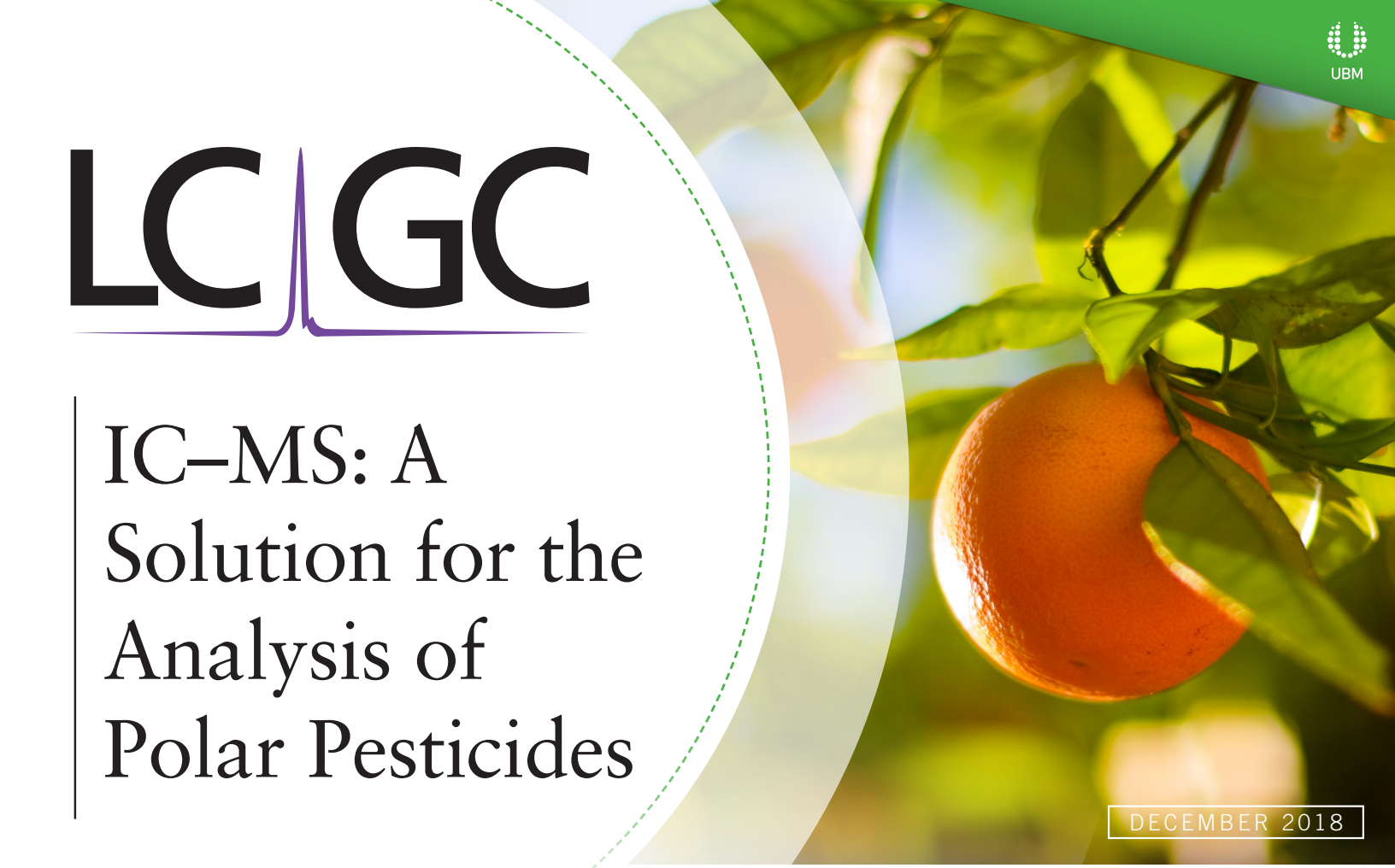

IC–MS: A Solution for the Analysis of Polar Pesticides

DECEMBER 2018

Advances in Stationary Phases for Ion Chromatography
Interview with Chris Pohl

The Benefits of Ion Chromatography–Mass Spectrometry for Polar Pesticides Analysis
Interview with Richard Fussell

Improve Your Ability to Detect and Analyze Polar Pesticides using IC–MS/MS
Jonathan Beck and John Madden

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INTRODUCTION

Polar pesticides such as glyphosate are widely used in agriculture, but the analysis of their residues is challenging. *LCGC*'s new ebook on *IC–MS: A Solution for the Analysis of Polar Pesticides* (sponsored by Thermo Fisher Scientific) explains why ion chromatography–mass spectrometry (IC–MS) systems provide advantages for laboratories tasked with detecting and characterizing polar pesticides in diverse samples.

First, Chris Pohl, Vice President of Chromatography Chemistry at Thermo Fisher Scientific, offers his thoughts on some important trends in ion chromatography. He focuses on how new developments in stationary phases are helping analytical scientists respond to changing needs such as making methods more environmentally friendly and better able to characterize smaller particles. He states, "In ion chromatography, the chemistry and architecture of the stationary phases have changed rather dramatically over the last 40 years. So, I expect you'll see continued improvements in column design based on novel architectures."

Also, we hear from Richard Fussell, Vertical Marketing Manager, Food & Beverage at Thermo Fisher Scientific, who explains how analysts can use IC–MS for detecting and quantifying polar pesticide residues in food and environmental samples. For instance, Fussell explains how versatile IC–MS systems can analyze several analytes (e.g., glufosinate and its metabolites, ethephon, fosetyl, chlorate, and perchlorate) together at low µg/kg concentrations in a single analysis. He adds, "Two of the main advantages of using suppressed IC–MS systems in the laboratory are the productivity gains resulting from the aggregation of multiple chromatographic methods into a single analysis while maintaining compliance with residue definitions and regulatory levels worldwide."

Last, learn how the Thermo Scientific™ Dionex™ line of ion chromatographs and auxiliary hardware, coupled with their triple-quadrupole mass spectrometers, offers a straightforward workflow for both anionic and cationic pesticide analyses.

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Advances in Stationary Phases for Ion Chromatography

Interview with Chris Pohl

Ion chromatography coupled with mass spectrometry is a useful technique that offers higher sensitivity, accurate quantitation, and increased efficiency for ion analysis. Here, Chris Pohl, Vice President of Chromatography Chemistry at Thermo Fisher Scientific, tells *LCGC* about current trends in ion chromatography and where the field is heading in the future.

LCGC: What are some current trends in ion chromatography stationary phases?

Pohl: One trend is the development of more hydrophilic stationary phases to allow the analysis of a greater diversity of ions without organic solvents. Previously, organic solvents were routinely used in the mobile phase to overcome problems in ion chromatography. It is much more environmentally friendly to use water as a mobile phase, so we must make the stationary phase more hydrophilic to prevent excessive retention. We can do this with hydrophobic molecules as well as molecules like hexafluorophosphate that don't sound very hydrophobic, but are not very hydrated. Anything that is not hydrated essentially offers very high retention time in hydrophobic materials.

Another ongoing trend among all types of chromatography is smaller particle sizes. One challenge with ion chromatography is that the entire flow path should be free from any metals. So, we've pretty much been limited to using polyether ether ketone (PEEK) polymer for the flow path, which limits the pressure we can go to more than any other single factor. Unlike HPLC, where the average particle sizes are in the 3 µm range, in ion chromatography, particle sizes are often in the 5–6-µm range. We are seeing movement toward even smaller particles in the 4 µm size range, but we can't go much beyond that without making big changes in instrument design.

A third trend in ion chromatography stationary phases is the movement to higher capacity columns. Historically, ion chromatography was always surprisingly

low capacity—single-digit micro-equivalents per column, whereas a typical liquid chromatography column today can have up to 20–50 times higher capacity. Higher capacity columns can handle a much wider range of samples without overloading.

LCGC: Can you expand on the special requirements for stationary phases in IC–MS?

Pohl: Because we are typically using nonmetallic hardware, grounding is a special consideration. PEEK tubing is usually a good ground, but not in this case. Usually, you need some sort of a ground between the electrospray source and the detector. Otherwise, you can have high voltage applied to the detector which will damage the detector electronics.

Another consideration is that with ion exchange, most mobile phases are not a volatile eluent, yet most mass spectrometry processes require a mobile phase where every component is volatile enough for removal with the vacuum system. So, in ion chromatography, the prevalent mobile phase is methanesulfonic acid for cation analysis and potassium hydroxide for anion analysis. That represents approximately 60–70% of all the mobile phases used in ion chromatography and neither one is very mass spectrometry friendly.

There are really two approaches to addressing this issue. One is to work on making phases that work with different eluents. The other approach is to use a suppressor for reducing mobile phase conductivity. It essentially eliminates the problem of eluent ions being incompatible with the mass spectrometer, because it converts methanesulfonic acid or KOH to water.

A second consideration, which is true for both ion chromatography and HPLC, is that you need stationary phases that have low bleed, because the mass spectrometer can be very sensitive to impurities coming out of the column. We routinely consider this factor when we design our phases. One advantage of ion chromatography, though, is that the phases are typically extremely stable, chemically, because they are continuously exposed to strong acid or a strong base for 12 months or more, so they typically have very low bleeds just by the nature of the phases.

LCGC: Can you compare and contrast ion chromatography–mass spectrometry (IC–MS) and capillary electrophoresis–mass spectrometry (CE–MS) techniques?

Pohl: Both techniques are very powerful in their own right. In some ways, it's actually an impressive feat to couple capillary electrophoresis to a mass spectrometer, given the scale of the dimensions of the capillary and the electrical requirements of the mass spectrometer and the CE instrument.

CE has amazingly efficient separations and much higher efficiencies in terms of plates per column than HPLC or ion chromatography. On the other hand, one shortcoming of CE–MS is precision of the measurement in terms of migration time;

in addition, response factors are not as good as chromatography. So, there's a tendency to use electrophoresis where you need really high resolution and not high precision.

Meanwhile, IC–MS is obviously a lower resolution technique, but it is a lot more robust from an analytical standpoint. It's easier to couple an ion chromatograph to a mass spectrometer, because you can use more or less the same kind of equipment that has already been developed for LC–MS. With CE, you have to make a special etched capillary chip to get the capillary effluent into the mass spectrometer. It's easier to implement and it's more robust, but of course it's not as high resolution. In some cases, like in really complex environmental samples, CE–MS might be the best option. For a routine lab, IC–MS is easier to implement and is more robust, especially in the hands of a less-trained operator.

"For a routine lab, IC–MS is easier to implement and is more robust, especially in the hands of a less-trained operator."

LCGC: How do you think the field of IC–MS may grow and evolve in the near future?

Pohl: Today, most MS instruments used for IC–MS or LC–MS analysis are as big as the HPLC instrument or the ion chromatograph. In the future, we might see smaller MS tools and detectors.

Certainly, we can expect to see smaller particle size columns. Some research groups are already using 3 µm and 2.5 µm particles in ion chromatography. And, in our own work, we have produced prototype phases in those size ranges and demonstrated reduced plate heights, which is very comparable to the best reduced plate heights you see in HPLC columns.

You'll continue to see expansion of column capacity to even higher capacities. In fact, part of what limits the capacity in ion chromatography is the amount of electrolytes that the suppressor can tolerate. I expect that you'll see some improvements in the performance of suppressors that will allow even higher eluent concentrations.

Another trend that will continue is miniaturization. Researchers at the University of Texas at Arlington are doing a lot of work on open tubular capillary and ion chromatography, which might be useful for portable instrumentation because the operating pressure on tubular columns is fairly low. A few companies already make "portable" ion chromatographs, but they often are not small and light weight, so there is room for improvement.

One thing about ion chromatography is that there's been a surprisingly long history of chemistry improvements of the stationary phase. If you look at HPLC, by far and away the most common column is the same one that's been most common for 40 years: the C18 phase.

Meanwhile, in ion chromatography, the chemistry and architecture of the stationary phases have changed rather dramatically over the last 40 years. So, I expect you'll see continued improvements in column design based on novel architectures. With monolithic columns, for instance, we hope one day we can make a long capillary, cut it into pieces, and have every piece be the same in terms of performance. If you could do that, then monolithic columns would really take off.

Overall, I think in the future you may find that we are designing different phases for IC–MS that specifically optimize the mobile phases for that mode of operation, not just the electrolyte but also the solvent system.

LCGC: Can you suggest reference materials for someone new to ion chromatography?

Pohl: Three good references are:

- H. Small, *Ion Chromatography,* Plenum Press, 1989
- J.S. Fritz and D. Gjerde, *Ion Chromatography*, 4th edition, Wiley-VCH, 2009
- J. Weiss, *Handbook of Ion Chromatography, 4th edition,* Wiley-VCH, 2016

"In ion chromatography, the chemistry and architecture of the stationary phases have changed rather dramatically over the last 40 years. So, I expect you'll see continued improvements in column design based on novel architectures."

The book by Hamish Small has an excellent introduction to ion chromatography theory. The Fritz and Gjerde text has the most comprehensive coverage of nonsuppressed ion chromatography, although this approach has largely fallen out of favor. More than 85% of all practitioners these days are using suppressed ion chromatography. The most comprehensive and up-to-date reference is that by Joachim Weiss. This four-volume set is 1,576 pages in length, covering not only the theory and practice of ion chromatography, but also ion chromatography applications.

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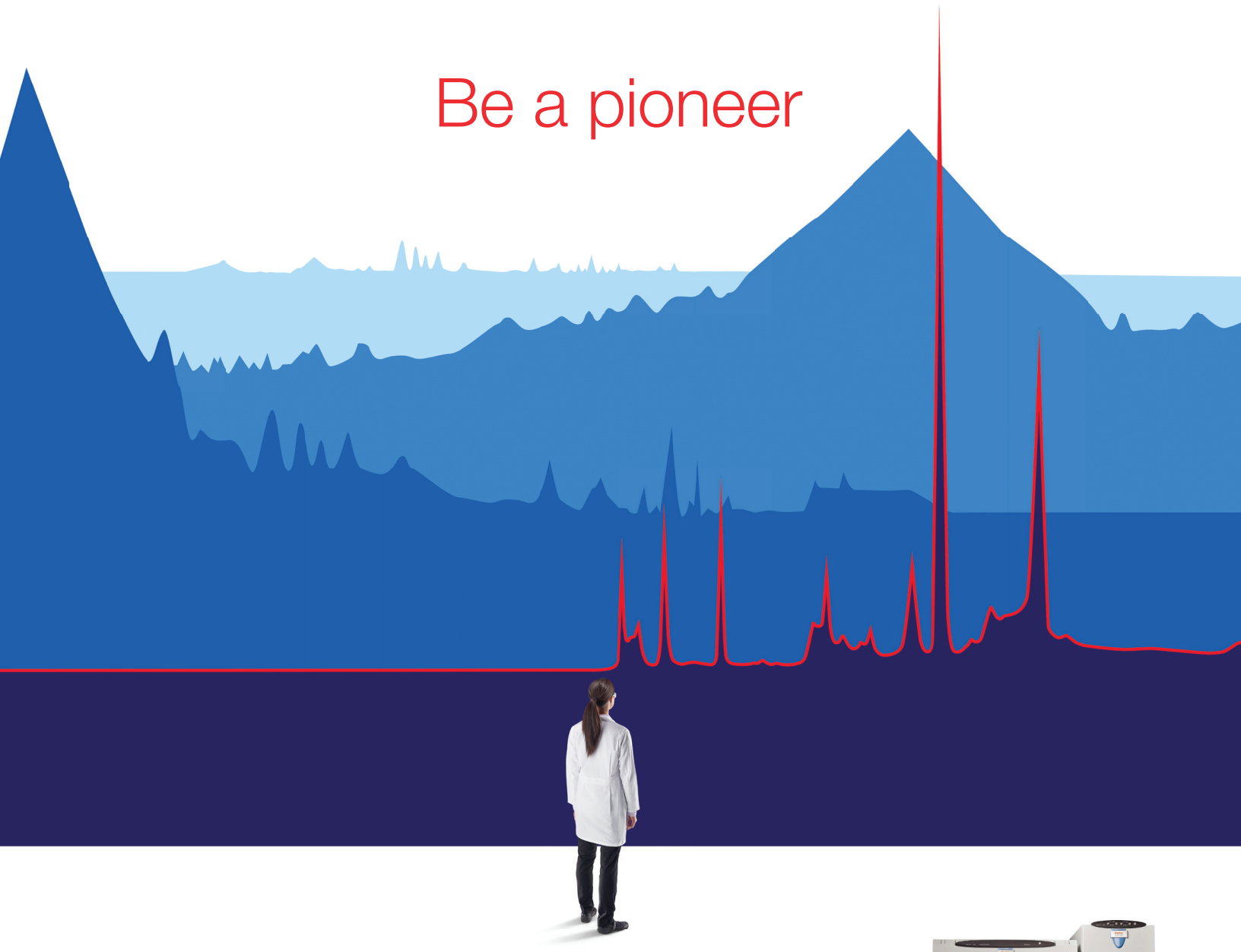

Sometimes, there are more questions than answers when taking on ion analysis challenges. A highly flexible ion chromatography system, like the new Thermo Scientific™ Dionex™ ICS-6000 HPIC™ system, provides you with the freedom to develop, explore and run different methods, one at a time or simultaneously.

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The Benefits of Ion Chromatography–Mass Spectrometry for Polar Pesticides Analysis

Interview with Richard Fussell

When it comes to the analysis of polar pesticide residues, analytical laboratories face several challenges. Here, Richard Fussell, Vertical Marketing Manager, Food & Beverage at Thermo Fisher Scientific, explains why ion chromatography–mass spectrometry (IC–MS) may be an especially helpful technique for detecting and quantifying polar pesticide residues in a variety of food and environmental samples.

LCGC: When did you first use ion chromatography and which analysis did you cover?

Fussell: My initial experience with ion chromatography was in the early 1980s using low-capacity ion exchange columns with conductivity and UV detectors. Because there was no suppressor capability, the method was restricted to the use of phthalic acid as a low conductance, low UV-absorbing mobile phase. Hence, our analyses were limited to anions in drinking water and nitrate/nitrites in soil and vegetables.

At that time, the big concerns were acid rain and eutrophication of rivers and waterways from agricultural run-off. Nevertheless, the outputs from these early crude experiments highlighted the potential of ion chromatography and made the case for us to upgrade to an integrated and automated Dionex ion chromatography system with a hollow fiber membrane suppressor.

This was a huge leap forward as suppression enabled the use of higher capacity columns because higher strength eluents (bicarbonate at that time) could be converted to water—after the suppressor and before the detector. The benefits of high-capacity columns were a higher sample loading providing greater sensitivity and more effective chromatographic resolution of components in the samples. Incidentally, the same argument holds true today. Not only was the system very robust in routine use and the quality of the data

improved, but the scope of analysis was also increased to other matrix–analyte combinations—even the determination of sulphate in pig slurry.

Over the past 40 years, researchers behind the scenes at Dionex (now part of Thermo Fisher Scientific) developed new column technologies, electrolytic eluent generation and electrolytic membrane suppressor technology, ensuring that the Dionex brand remained at the forefront of ion-chromatography. The beauty of ion chromatography is that columns can be designed to solve specific analytical challenges: it is amenable to inline trapping of analytes (concentration), inline clean-up (removal of unwanted matrix components), two-dimensional IC (IC–IC), and coupling to range of detectors including mass spectrometers.

The addition of the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software for instrument control and data processing provides an incredibly versatile one-vendor solution for a wide range of applications, including anions, cations, carbohydrates, metals speciation (e.g., arsenic speciation in rice using ICP–IC-MS), amino acids, organic acids, and more.

The relatively recent hyphenation with mass spectrometers (both triple quadrupole and high-resolution accurate-mass (HRAM) has further increased the potential and scope of application. For example, ion chromatography–mass spectrometry is now used worldwide for the analysis of haloacetic acids and polar ionic pesticides in food and environmental applications.

“The benefits [of ion chromatography] include increasing the scope of applications because of the higher selectivity of MS compared with semi-selective detectors and the fact that certain application areas (e.g., pesticides) require mass spectrometric information for unequivocal identification of the analytes of interest.”

LCGC: What are the benefits and challenges of coupling ion chromatography to mass spectrometers?

Fussell: The benefits include increasing the scope of applications because of the higher selectivity of MS compared with semi-selective detectors and the fact that certain application areas (e.g., pesticides) require mass spectrometric information for unequivocal identification of the analytes of interest. Coupling of suppressed ion chromatography to a mass spectrometer is sometimes perceived to be problematic, but perhaps by analysts with limited or no experience

"Polar anionic pesticides are widely used, but monitored infrequently, primarily, because they are difficult, and thus costly, to analyze."

with IC instruments. Using potassium hydroxide (KOH) as eluent is completely alien to them, so additional training may be needed.

The post-column suppressor device will convert the KOH eluent to water before it enters the mass spectrometer. Should the suppressor fail unexpectedly, the signal from the inline conductivity cell will immediately shut down the IC pump to protect the MS instrument. There is an ever-increasing number of IC–MS users and, to my knowledge, no serious problems have been reported. As with any system, the user must play a part in the monitoring and maintenance to sustain optimum performance. The addition of organic modifier (using an auxillary pump and a T-piece) after the suppressor aids desolvation in the MS source and improves the response by three- to four-fold in some cases. The system is fully integrated and automated, so, once set up, the analyst must only add water for the eluent generation, top-up the suppressor regenerant (typically water) and organic modifier as needed, and empty the waste.

LCGC: Why is there so much interest in the analysis of polar pesticides today?

Fussell: The main driver is regulation to protect the health of consumers and the environment.

Polar anionic pesticides are widely used, but monitored infrequently, primarily, because they are difficult, and thus costly, to analyze. Perhaps not surprisingly, there is suspicion of misuse of some polar pesticides because of the current inadequacies in monitoring.

Another driver is the high interest in glyphosate. It is not only the most widely used herbicide worldwide, but it is also one of the most controversial pesticides because of reported concerns over toxicity and effects on human health. 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" in March 2015, whereas the European Food Safety Authority (EFSA) concluded glyphosate unlikely to cause cancer. What is not in doubt is the fact that the use of glyphosate as a weed killer on genetically modified crops (soybean, wheat) tolerant to glyphosate, and as a desiccant on cereal crops, results in a high frequency of residues in cereal-based products such as bread and breakfast cereals; and the permitted levels are typically higher than for other pesticides.

The EFSA recently published a Reasoned Opinion that indicated that the analysis of glyphosate for risk

assessment purposes should also include the metabolites: AMPA, N-acetyl AMPA, and N-acetyl glyphosate. Typically for these studies, the expectation is to reach residue concentrations as low as practically possible. These requirements amplify an already difficult challenge. In the US, a report by the Government Audit Office criticized the responsible government agencies (EPA, FDA, and USDA) for the lack of testing for glyphosate residues in food.

Similarly, there is interest in glufosinate and its metabolites (n-acetyl glufosinate, 3MPPA), ethephon (the metabolite HEPA), fosetyl, (phosphonic acid), and chlorate and perchlorate (technically a contaminant). It is an advantage that all these analytes can be analyzed together at low mg/kg concentrations in a single analysis using IC–MS equipment. The system can be easily and quickly switched to the analysis of cationic polar pesticides and metabolites such as chlormequat, mepiquat, paraquat, diquat, aminoglycosides, and others, which are also receiving a lot of interest. All that is necessary is to exchange the eluent generator, column and suppressor from anionic to the cationic forms or vice versa. Alternatively there is a dual-channel system that can be configured for anions in one channel and cations in the other—so the switch is quick, easy and can even be automated. Since the anionic method is a little more developed and the same questions and benefits essentially apply to both, then it is probably best to focus on the anionic compounds for the sake of simplicity and time.

LCGC: What are the advantages of ion chromatography over other approaches for the analysis of polar anionic pesticides?

Fussell: The results published in the recent peer-reviewed literature—using Thermo Scientific™ Dionex™ IC systems coupled to either high-resolution accurate-mass (HRAM) Thermo Scientific™ Orbitrap™ mass analyzer technology[1] or triple-quadrupole MS^2—clearly show the benefits of ion chromatography over other approaches. The results obtained were in compliance with EU SANTE/11813/2017 guidelines for method validation, the EU pesticide residue definitions, and the EU maximum residue levels (MRLs).

The extraction method was based on the Quick Polar Pesticides Extraction (QuPPe) Method developed by the European Reference Laboratory for single-residue methods. This generic extraction method for anionic polar pesticides is used by the vast majority of laboratories. One disadvantage of the published QuPPe approach is that several different chromatographic separations using different non-suppressed IC and hydrophilic interaction liquid chromatography (HILIC) columns are required to cover all the anionic pesticides of interest. These approaches have limitations in chromatographic column sample capacity. This is important because the QuPPe method is based on

extraction with methanol water, with no partition and no effective clean-up for removal of co-extractives (except for fat removal in the case of products of animal origin). Several clean-up options have been evaluated, but with limited success, typically due to low recovery for some analytes. Without effective clean-up, the extracts can be relatively "dirty" so the most common strategy is to dilute the extracts as much as possible, to minimize matrix effects, but accepting the compromise that the 0.01 mg/kg target reporting level cannot be reached for all of the analytes in the more difficult matrices, such as cereals and soybean. The higher column capacity provided by Dionex suppressed ion chromatography enables excellent chromatographic retention and resolution, and generally stable retention times within the ±0.1-minute tolerance specified in the EU SANTE guidelines, even at higher sample /matrix loading. The outcome is improved reporting limits, especially for complex matrices, compared to other approaches.

Again, there is an exception even with suppressed ion chromatography–mass spectrometry, which simply highlights the challenging nature of the analysis of polar analytes in diverse matrices. It has been reported that high levels of organic acids in some types of samples (e.g., citric in lemons) will cause the retention time to decrease slightly for some analytes. This is not a substantial issue in the few cases where this is known to occur, because it is easily rectified by using exact matrix-matched calibration standards or isotopically labelled standards, both common procedures in most pesticides laboratories.

There have been some suggestions that electrolytic suppressors are not reliable, but these comments are misplaced as they do not consider all of the facts. It is the case that suppressors, like columns, can become contaminated with continuous analysis of samples containing high levels of matrix co-extractives. Again, any such issues can be easily resolved by recommended cleaning procedures to sustain optimum performance. It is also the case that many labs have been running IC–MS systems for many years satisfactorily, and it is evident that these experienced users encounter fewer issues than analysts new to IC instruments. Again, a situation can easily be resolved by implementation of adequate training, clear guidance on interpreting diagnostic monitoring (built into the system), and appropriate maintenance protocols. The benefits of electrolytic suppression far outweigh the minimal time for maintenance. It is also worth noting that the flow-through electrolytic suppressor is unobstructed, so there is no dispersion and band broadening that is sometimes observed with packed-bed suppression systems.

In addition to column capacity issues, some HILIC separations can suffer from metal contaminants that leach from using conventional metal-based ultrahigh performance liquid chromatography systems (UHPLC). Vendors recommend flushing of the systems with EDTA

to minimize the interactions between analytes and metals. The Thermo Scientific™ Dionex™ Integrion™ HPIC™ system and ICS-6000 ion chromatograph systems are metal free and do not suffer from these interactions.

Apart from the benefits of suppressed ion chromatography, we should also consider mass spectrometry. Several laboratories are now using IC instruments coupled to either a Thermo Scientific™ TSQ Altis™ Triple Quadrupole Mass Spectrometer or a Thermo Scientific™ Q Exactive™ Focus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer. Compared to other HRAM systems, the Q Exactive Focus MS system provides the best mass accuracy and stability, and the highest mass resolving power and hence selectivity in the low mass range, which encapsulates the molecular ions and fragments for polar pesticides. The TSQ Altis Triple Quadrupole MS instrument has many new features to provide extreme sensitivity, speed and robustness for targeted quantification, and identification of polar pesticides, especially the anionic pesticides in the negative ion mode. Both MS systems are equally effective for the analysis of polar pesticides, and the choice of the customer is typically based on other MS-based applications of interest.

The results published in the literature clearly indicate that ion-exchange chromatography with electrolytic ion suppression coupled to mass spectrometry is an excellent technique for the accurate, precise, and reproducible analysis of anionic polar pesticides at low concentrations in a diverse range of matrices. Two of the main advantages of using suppressed IC–MS systems in the laboratory are the productivity gains resulting from the aggregation of multiple chromatographic methods into a single analysis while maintaining compliance with residue definitions and regulatory levels worldwide.

LCGC: Can we expect to see any further improvements and expansion of the applications? You mentioned anionic pesticides, but are there any developments for cations?

Fussell: There have been substantial developments in the analysis of polar pesticides, demonstrated by the number of laboratories undertaking the analysis and reporting residues in a wide range of sample types. Nevertheless, there are undoubtedly more improvements necessary at all steps in the workflows for both anionic and cationic polar pesticides.

We often see low recovery of analytes that have been spiked onto the sample. This is evident for low-moisture samples such as cereals, and the current solution is to correct the results using isotopically labelled standards. This corrects only for procedural losses of spiked analytes and does not take into account the extraction efficiency of incurred residues, which could lead to an underestimation of residue levels. At some point, we should explore the use of pressurized

solvent extraction (e.g., accelerated solvent extraction) to see if it is possible to improve extraction efficiency—or at least to "validate" the QuPPe extraction method.

I suspect and hope that new sample clean-up options, either offline or inline, will be developed to reduce maintenance, lower costs, and further improve productivity for the routine laboratories. In the case of IC–MS systems, we will see further development of suppressors and columns in terms of resilience to matrix to sustain excellent peak shapes for all analyte-matrix combinations. Retention times tend to be longer compared with conventional reversed-phase LC–MS separations, so some laboratories have requested shorter columns for faster cycle times, even though we have shown the analysis of 18 anionic polar pesticides and metabolites in around 18 minutes.

Similarly, we have shown the separation for up to 16 of the cationic polar pesticides and metabolites listed in the QuPPe method, but further optimization is required to expand the scope and improve the quantification and identification limits for some compounds.

Also, we have seen possible applications in the analysis of food contact materials and in the analysis of residues of ionic veterinary medicines as customers explore the capabilities of IC–MS analysis, especially as IC–MS systems fill the gap for the direct analysis of substances that cannot easily be analyzed by GC–MS or LC–MS systems.

In the case of IC–MS analysis, we are generally working on the further optimization and standardization of the workflows. The improvements that can be achieved by implementing relatively small changes is surprising. Thermo Fisher Scientific recognizes that this application is very important to the laboratories, so standardization and robustness for routine analysis is a key objective, as it will enable improved customer support and enable laboratories to more easily compare and exchange information for mutual benefit.

At first glance, the IC–MS systems that have more accessory components may appear more complicated than conventional LC reversed-phase–MS systems, but the Thermo Scientific Dionex IC-MS systems are fully integrated and automated and can be controlled by a single software—the only option for a complete workflow from a single vendor.

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Improve Your Ability to Detect and Analyze Polar Pesticides using IC–MS/MS

A straightforward workflow from start to finish

Jonathan Beck and John Madden

Introduction

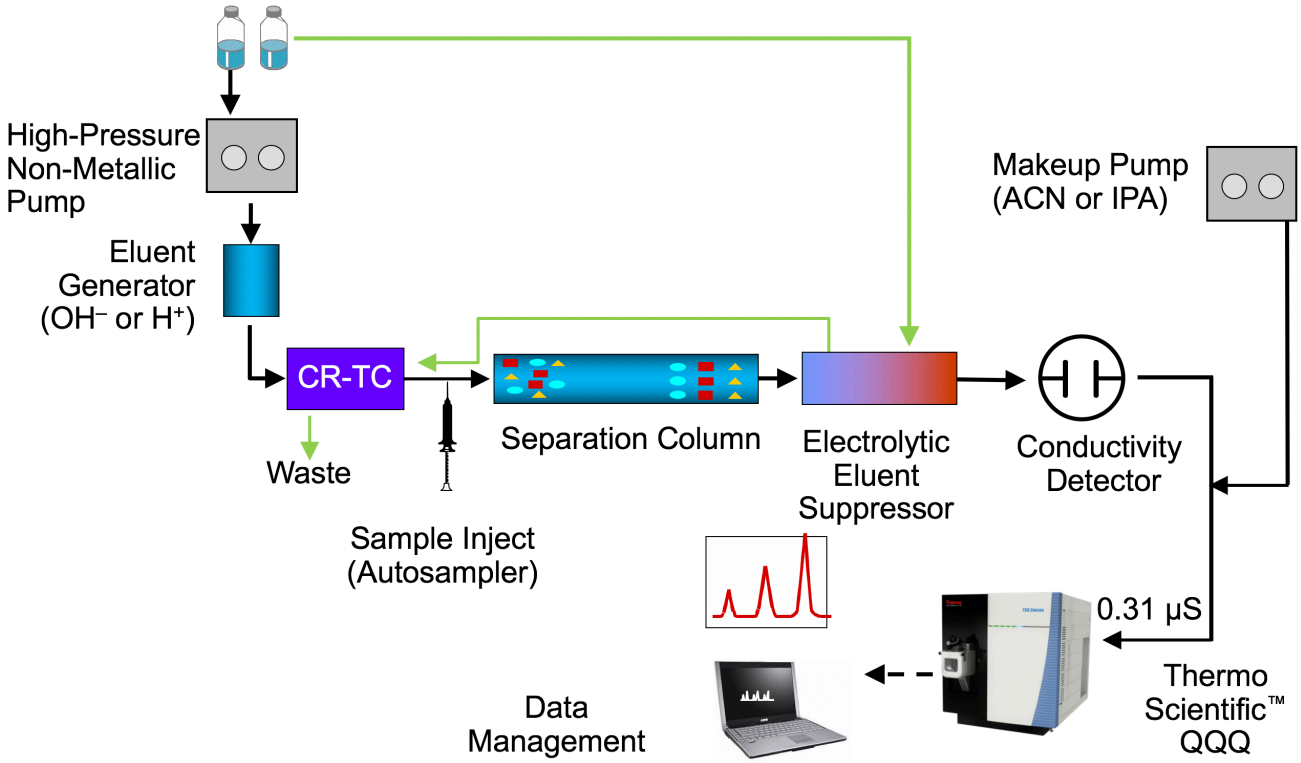
Glyphosate (N-[phosphonomethyl]glycine) has been controversial in recent years due to concerns surrounding its detection in popular foods and beverages. Expedient and reliable methods for measuring the herbicide in various matrices would be beneficial for understanding the complex issues in which it is involved. However, analysis of glyphosate and many polar pesticides using traditional techniques can be difficult for several reasons. In addition to being zwitterionic, these pesticides also have low volatility and are highly soluble in water. They generate low product ion masses in tandem mass spectrometry and generally lack characteristic large chemical groups that would aid with detection.

LC–MS/MS analysis of these polar molecules typically includes several different methods that employ various columns. Trace level analysis may also require derivatization with 9-fluorenylmethoxycarbonyl chloride (FMOC) or other cumbersome preparation protocols. In contrast, IC–MS/MS offers direct analysis of many polar pesticides simultaneously.

IC–MS/MS of Anionic Polar Pesticides in Drinking Water

A workflow diagram for anionic polar pesticide analysis is shown in **Figure 1**. The schematic illustrates the interior components of the Thermo Scientific™ Dionex™ Integrion™ HPIC™ system, which begins with a pump that delivers deionized water to the Eluent Generator. The pump is non-metallic so that unwanted metal ions will not be added to the mobile phase. The Thermo Scientific™ Dionex™ EGC 500 KOH Eluent Generator Cartridge conveniently generates the concentration of buffer that is specified by the software. No manual preparation of solvents is necessary; deionized water is simply added to the HPIC system via the solvent bottles on top and the Eluent Generator prepares the mobile phase automatically. The eluent stream flows

Figure 1: IC–MS flow diagram.



through a continuously regenerated trap column and is then ready for sample introduction. Once an anionic polar pesticide is injected into the flow by the Thermo Scientific™ Dionex™ AS-AP Autosampler, it is separated on a Thermo Scientific™ Dionex™ IonPac™ AS24 column. The potassium is then removed by a continually regenerated membrane in an electrolytic eluent suppressor so that the eluent stream is amenable to the triple-quadrupole mass spectrometer.

Using this workflow with a Thermo Scientific™ TSQ Quantiva™ triple-stage quadrupole mass spectrometer, five polar pesticides were spiked into and analyzed in drinking water: fosetyl aluminum, clopyralid, glyphosate and its metabolite aminomethylphosphonic acid (AMPA) and glufosinate. The potassium hydroxide concentration was changed throughout the 20-minute method. The concentration gradient is analogous to a reversed-phase high performance liquid chromatography gradient where the percentage of organics is increased throughout the separation. As the concentration of potassium hydroxide was increased in the eluent stream, compounds were pushed off the column in a similar way to organic compounds eluting from a C18 column. Suppressor current was increased with higher concentrations of potassium hydroxide in order to maximize performance.

The calibration for each analyte showed good linearity and a broad dynamic range. Comparable results were obtained in bottled water, which has higher mineral content. Limits of detection (LOD) and quantitation (LOQ), with relative standard deviation (RSD), are tabulated in **Table 1**. Single-digit LODs and LOQs were obtained for six separate replicates of most analytes with low RSDs, demonstrating excellent sensitivity and reproducibility. The results for glyphosate in **Figure 2** exhibited favorable signal-to-noise even at 5 ppt. Although peak tailing was observed for this analyte, its reproducibility allowed it to be reliably integrated for quantification.

Table 1: Bottled water performance.

Name	LOD [ppt]	LOQ [ppt]	RSD % (10 ppt) level
Fosetyl-Al	1	2.5	5
Clopyralid	10	50	9
AMPA	2	5	9
Glyphosate	5	10	15
Glufosinate	2	5	4

Figure 2: Bottled water—glyphosate.

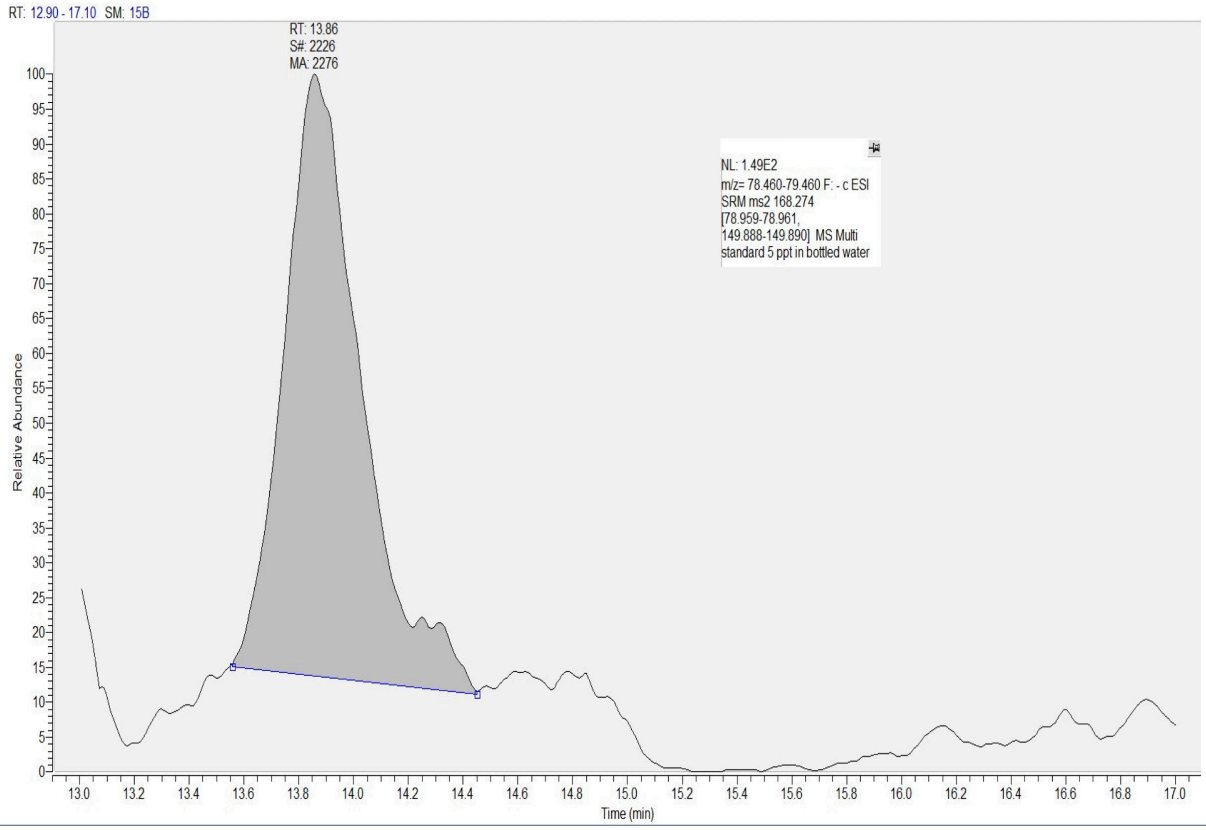


Table 2: LODs and LOQs for 11 analytes.

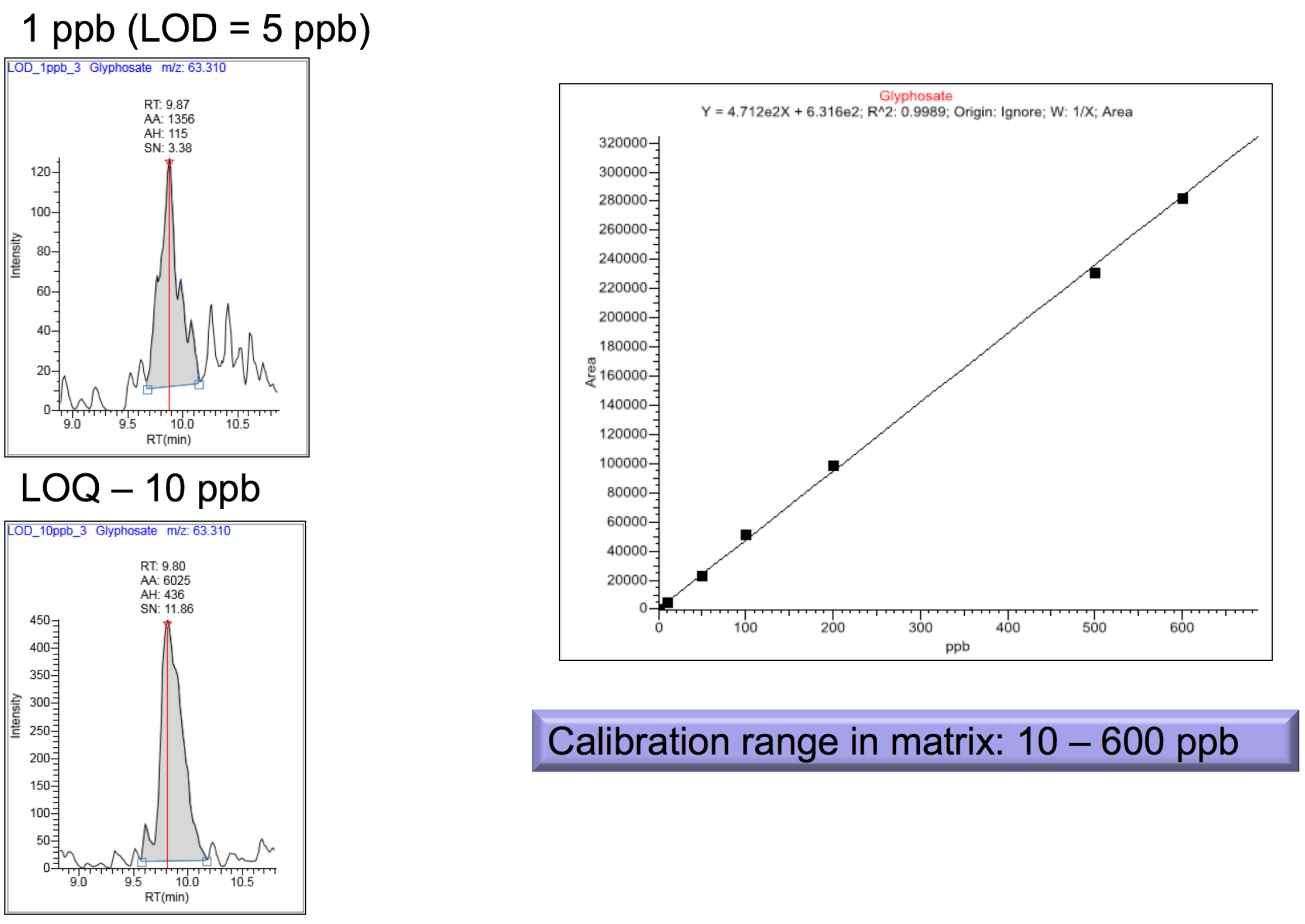
Analyte	LOD (µg/kg)	LOQ (µg/kg)	LOD (pg on column)	LOQ (pg on column)
AMPA	10	20	100	200
Ethephon	10	20	100	200
Fosetyl-Al	10	20	100	200
Glufosinate	1	10	10	100
Glyphosate	5	10	50	100
HEPA	10	20	100	200
MPPA	1	10	10	100
N-acetyl-AMPA	1	10	10	100
N-acetyl-glufosinate	3	10	30	100
Phosphonic acid	1	10	10	100

IC–MS/MS of Anionic Polar Pesticides in Food

Analysis of anionic polar pesticides in lettuce by IC–MS/MS began with the preparation of analyte solutions using the Quick Polar Pesticides Method (QuPPe). The simple steps of QuPPe include adding water to a homogenized sample, shaking, adding cold methanol, shaking again, centrifuging and filtering. These steps were all performed using plastic materials to avoid adsorption of the compounds onto glass surfaces.

Eleven common anionic pesticides were analyzed using the workflow with a Thermo Scientific™ Dionex™ ICS-2100 IC system coupled to a TSQ Endura MS instrument. The suppressor, which facilitates the IC-MS interface by removing potassium, was the Thermo Scientific™ Dionex™ AERS 500 Anion Electrolytically Regenerated Suppressor. The potassium hydroxide eluent source was a Thermo Scientific™ Dionex™ EGC III KOH Eluent Generator Cartridge and samples were injected using a Thermo Scientific™ Dionex™ WPS-3000 TRS-AS Autosampler. Six repetitions of 50, 200, and 500 ppb solutions of the analytes yielded excellent recovery and repeatability. **Table 2** shows the LODs and LOQs for the 11 analytes. Ten of the 11 pesticides had LODs ranging from 1 to 10 µg/kg, while maleic hydrazide was the again the outlier. Linearity and response results for glyphosate are included

Figure 3: Quan details for glyphosate.



in **Figure 3** in which the calibration curve ranges from 10 to 600 ppb, and the R^2 coefficient of determination for the liner regression is nearly 1.

IC–MS/MS of Cationic Polar Pesticides in Food

Green bean and prune samples were spiked with cationic pesticides and extracted using the QuPPe method and separated using a methane sulfonic acid (MSA) gradient with a Thermo Scientific™ Dionex™ IonPac™ CS17 column. The MSA concentration was ramped from 0.5 mM to 60 mM during the 18-minute run. In this positive ion analysis, the suppressor removed anions from the eluent before the stream flowed into the mass spectrometer. In addition, the green bean and prune samples contained high levels of magnesium and potassium that were diverted to waste to protect the H-ESI probe. The mass spectrometer was used in selected reaction monitoring (SRM) mode for enhanced sensitivity. The data presented in **Table 3** show that the ma-

Table 3: IC analysis—quantitative analysis results (µg/L).

Analyte	SANTE LOQ	LOQ	LOD
Ethylene Thiourea			
Diethanolamine	< 0.5 (8.7%)	0.68	0.22
N,N-Dimethylhydrazine			
Triethanolamine	< 0.5 (2.9%)	0.26	0.086
Morpholine	< 0.5 (7.9%)	0.37	0.12
Trimethylsulfonium	< 0.5 (8.0%)	0.37	0.12
Melamine	< 0.5 (3.6%)	0.26	0.086
Chlormequat	< 0.5 (4.5%)	0.20	0.065
Mepiquat	< 0.5 (2.0%)	0.094	0.031
Nereistoxin	< 0.5 (8.6%)	0.52	0.17
Cyromazine	> 0.5 (21.8%)	0.82	0.27
Streptomycin			
Diquat	> 0.5 (20.8%)	1.08	0.36
Paraquat	< 10 (3.6%)	4.74	1.56
Propamocarb	< 0.5 (1.9%)	0.088	0.029

jority of analytes had LOQs in compliance with the European Commission's SANTE guidelines that mandate 0.5 ppt level of quantitation. According to the guidelines, LOQs are established by identifying the lowest level that has a relative standard deviation of less than 20%.

Chlormequat and mepiquat generated calibration curves with excellent linearity and easily passed the SANTE limit, demonstrating that the method is extremely sensitive for these quaternary pesticides even in relatively complicated sample matrices. Note that ethylene thiourea was not retained on the column, and N,N-dimethylhydrazine coeluted with matrix. Furthermore, the divalent pesticides, paraquat and diquat, coeluted on the Dionex IonPac CS17 column, but could be separated using the CS14 column and were prone to ion suppression in the H-ESI source. The Thermo Scientific™ Dionex™ IonPac™

CS14 column separates paraquat and diquat. Quadrupole based mass spectrometers lack sufficient resolution to separate these two compounds by their masses, which are only 2 Da apart, but fragments are isobaric and the fragmentation can be variable, so even with HRAM, resolution is important. A new Thermo Scientific™ Dionex™ IonPac™ column is currently under development to resolve the four major quaternary amine pesticides (paraquat, diquat, chlormequat, and mepiquat).

Conclusion

IC–MS/MS analysis enables determination of polar pesticides in both food and environmental samples. The Dionex line of ion chromatographs and auxiliary hardware, coupled with their triple-quadrupole mass spectrometers, offers a straightforward workflow for both anionic and cationic pesticide analyses. A Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap™ mass spectrometer is a propitious alternative for complex matrices and sensitivity. Linear calibrations over a broad range of concentrations were obtained for numerous polar pesticides, with excellent LODs and LOQs. With high sensitivity, straightforward sample preparation and fast separation, IC-MS/MS analysis is the preferred technique for polar pesticide analysis.

“The Dionex line of ion chromatographs and auxiliary hardware, coupled with their triple-quadrupole mass spectrometers, offers a straightforward workflow for both anionic and cationic pesticide analyses.”

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