Reliable, accurate and rapid quantitation of polar pesticides in honey

Eurofins Food Integrity Control Services, an industry-leading laboratory offering honey testing services, decided to introduce a more sensitive and fit-for-purpose methodology — ion chromatography — to reliably perform target analysis of polar pesticides. Working closely with Thermo Fisher Scientific on system installation and staff training, the site now benefits from:

- Better analytical separation and reliable analyte measurement
- Shorter sample preparation protocol
- Faster turnaround time
- Reduced system maintenance
- Cost- and time-effective operations
- Higher sample throughput rates

Introduction

Based in Ritterhude, Germany, Eurofins Food Integrity Control Services (EFICS) is an independent business unit of the Eurofins Food and Feed Testing Network, specializing in food authenticity and integrity verification. Dr. Kurt-Peter Raezke, Senior Technical Director at EFICS, developed an LC-IRMS (Liquid Chromatography coupled with Stabile Isotope Ratio Mass Spectrometry) method to detect foreign sugar additions in honey. As an ISO accredited method, it is nowadays part of routine testing of honey.

Known for its long-standing excellence in honey analysis, the Eurofins Ritterhude site offers a range of honey testing services, such as confirming authenticity, determining botanical and geographical origins, assessing product quality, and measuring contaminants. These services are tailored to the needs of clients spanning across the entire supply chain of the global honey industry.

Processing around 50-100 samples each day, this busy facility carries a suite of analytical methods on-site. Of these, liquid chromatography (LC) and gas chromatography (GC), coupled with mass spectrometry (MS), are most commonly used for honey analysis. Recently, however, due to increasing concerns over the sensitivity of LC-MS/MS methods in quantifying traces of polar pesticides, the team decided to switch to a more analytically sensitive and better-suited method — ion chromatography (IC) coupled with tandem MS (IC-MS/MS).

Here, we outline why an upgrade was necessary, how IC-MS/MS was successfully implemented at Eurofins Ritterhude and what benefits have been achieved ever since.

The need to accurately measure traces of polar pesticides in honey

Honey samples can contain traces of polar pesticide components, such as glyphosate, aminomethylphosphonic acid (AMPA) and glufosinate. When collecting nectar, bees ingest or pick up these components from crops treated with polar pesticides. In recent times, the development of genetically modified crops, that exhibit high tolerance levels towards glyphosate and glufosinate to allow broad-spectrum herbicide usage, has further increased the possibility of cross-contamination in honey. As such, to maintain quality and safety standards, the EU has set the maximum residue level for these analytes at 0.05 mg/kg with the limit of quantitation (LOQ) at 0.002 mg/kg in honey samples.

Typically, LC-MS/MS has been widely used to detect glyphosate and other polar metabolites in honey. However, in at least two instances, glyphosate-contaminated honey samples were reported by consumer organizations despite having been analyzed with the recommended LC-MS/MS method. Although glyphosate isn't currently categorized as a toxin or a carcinogen, adhering to the tolerance levels set by regulatory authorities — and doing so reliably and accurately — is a mandatory requirement for food manufacturers.

Cases of inadvertent oversight related to polar pesticide traces in honey can be directly linked to unsatisfactory analyte separation due to their higher polarity. The chromatographic separation of polar analytes can be challenging when using the conventional reversed-phase liquid chromatography (RPLC) technique. Often, unique RPLC separation columns may be required or an alternative method, hydrophilic interaction liquid chromatography (HILIC), may be used. Even so, experimental limitations are frequently reported due to inadequate column robustness, resulting in problems with retention time, peak resolution and, ultimately, quantitation.

Moreover, the higher carbohydrate content in honey (~600-700 g/kg) can cause resinification of the mass spectrometer, contaminating its inlet cone. Restoring the system's performance requires technical maintenance, resulting in frequent instrument downtime. To limit resinification and minimize system repair, a separate matrix-specific workflow may be adopted. This would, however, add more time and resources to the site's existing operations.

Additionally, the sample preparation protocol for honey analysis with LC-MS/MS involves a lengthy derivatization step, making the process tedious and time-consuming. For service-providing laboratories, long turnaround times can have a direct impact on the revenue potential.

To continue being industry-leading experts in honey analysis, the Eurofins Ritterhude site needed to resolve the current limitations associated with LC-MS/MS. In an effort to boost operational efficiency and turnaround times, the team decided to trial IC-MS/MS as an improved alternative.

IC-MS/MS as a preferred method for polar pesticide quantitation in honey

As the analytes — glyphosate, AMPA and glufosinate — are anionic at high pH, separation using anion-exchange chromatography increases the chances of achieving a better separation. The IC protocol can then be coupled with MS using electrolytic eluent suppressors to perform analyte detection and quantitation. Using the IC-MS/MS workflow for target analysis of these polar components is more robust compared to LC-MS/MS, and the results, more reliable. In fact, even lower injection volumes can yield reliable results.

Moreover, the IC method doesn't require derivatization, making the sample preparatory steps simpler and shorter. This means, switching to IC-MS/MS for polar pesticide analysis would not only provide improved analyte separation, but also has the potential to reduce workflow time.

In preliminary testing experiments, the Eurofins Ritterhude team made a direct comparison between LC-MS/MS and IC-MS/MS, measuring both sensitivity and accuracy in quantifying polar pesticides in honey samples. Upon examining the results, IC-MS/MS clearly emerged as the more sensitive and reliable option, prompting the team to move ahead with the full implementation.

Onboarding IC-MS/MS at Eurofins food integrity control services

After initial testing and validation, the Eurofins Ritterhude team introduced IC-MS/MS into their service portfolio by installing Thermo Scientific IC and MS systems, along with the corresponding software, Chromeleon Chromatography Data System (CDS). During this implementation phase, the team collaborated with Thermo Fisher's technical support staff to optimize methods and get staff trained.

"The Thermo Fisher application support with setting up the IC-MS/MS instrument, along with the technical support from the Chromeleon team, helped us significantly in improving our method and getting it ready for implementation. We also received assistance in training our routine staff to use these methods appropriately."

— Dr. Kurt-Peter Raezke,
Senior Technical Director at Eurofins Food Integrity Control Services

With system installations and training completed, the site successfully updated its polar pesticide analysis to include IC-MS/MS, and immediately reaped the benefits both analytically and operationally.

Benefits of IC-MS/MS: Better separation, reliable results and faster workflows

Introducing IC-MS/MS at Eurofins Ritterhude has brought tangible benefits to (1) the technical staff in terms of ease and efficiency, (2) the strategic team, by way of cost- and time-effectiveness, and, ultimately, (3) the customers, who receive reliable and accurate quantitation of polar analytes.

Better separation and improved sensitivity

The immediate advantage of using IC-MS/MS at Eurofins Ritterhude was obtaining better separation of the polar analytes, namely, glyphosate, glufosinate and AMPA. Team members performed proficiency tests that further confirmed repeatability and reproducibility across multiple batches. As the method could be reliably replicated, fewer repeats were necessary for result confirmation requests. Even when challenging matrices were introduced — where honey samples were diluted with cheaper sugars — the regulatory standards for the polar pesticides were reliably met. This demonstrated that method sensitivity could be maintained over a wide range of unknown samples.

Most notably, no matrix effects were observed despite analyzing high-carbohydrate content. This was due to the addition of a timed second switching valve that directs the high-sugar effluent into waste instead of the mass spectrometer, completely circumventing the risk of resinification. Eliminating matrix effects, in turn, increased the team's confidence in the observed test results.

Easier and faster workflows

With IC, sample preparation is straightforward and quick. The 'dilute and shoot' method used by the site involves two simple steps: diluting honey samples with the extraction solvent followed by 5-minute centrifugation. This method took only a quarter of the time previously taken for sample preparation, saving valuable hours at a busy facility, and creating room for higher sample throughput.

Additionally, as MS source resinification is prevented due to inline matrix elimination, weekly instrument maintenance was reduced to monthly, minimizing maintenance costs and workflow interruptions. The collective time gains resulting from easy sample preparation as well as minimal downtime have helped improve sample turnaround times and boost productivity in the facility.

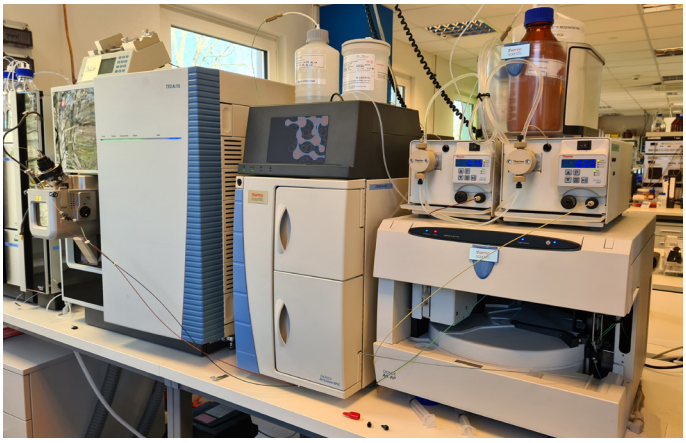
Benefits of implementing IC-MS/MS for honey analysis

- 50% cost savings
- Sample preparation is 4 times faster
- Faster turnaround times
- Higher sample throughput
- Reduced system maintenance

“Overall, our collaboration with Thermo Scientific was very professional and fruitful. Upon successful implementation of IC-MS/MS, we were able to improve turnaround times and save costs,” says Raezke. “We look forward to future projects where we can introduce innovative methods to our workflows.”

Cost-effective operations

The updated IC-MS/MS protocols are shorter, requiring less time to perform, and simpler, requiring fewer chemicals per analysis. These changes have amounted to cost savings of at least 50%, potentially doubling the revenues. Due to the inherent robustness of the IC column, it can handle over 1000 sample injections without performance loss. This means the column needs to be replaced less frequently, reducing expenses for the site. Moreover, extended service intervals for the MS systems minimize the associated maintenance costs and site downtime, further boosting cost-effectiveness.



Continued improvements in honey analysis

Staying up-to-date with new, improved techniques – and being able to acknowledge the analytical limitations of existing ones — is key to gaining a competitive advantage in the fast-paced food industry. The decision to include IC-MS/MS into its existing analytical portfolio upon identifying the limitations of LC-MS/MS for reliably detecting polar pesticide traces in honey has set Eurofins Ritterhude up for continued success, maintaining its position as the top provider of honey analysis. The site also projects increased revenue in the near future, given its higher throughput rates and cost-saving measures.

In the coming years, to continue making improvements to their honey analysis services, the Eurofins Food Integrity Control Services will turn its focus to analyzing multi-class compounds, integrating the site’s data with information management systems, and incorporating automation, where possible.

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