NEW Food

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Food Integrity Collection 2017

TRUST your foods are all they should be.

TRUST YOUR FOODS ARE ALL THEY SHOULD BE.

Food adulteration has become an increasing problem globally, raising the urgency of testing olive oil, honey, spices, and more for purity, authenticity, and label claims.

Thermo Scientific's advanced instrumentation streamlines determination of both known and unknown components. The world's top ten food and beverage companies trust them to help keep products safe, authentic, and unadulterated.

Contents

EXPERT VIEW

New Food asks Khalil Divan, Senior Director Global Marketing, Food & Beverage, Thermo Fisher Scientific for his opinions on food fraud.

Food fraud is a concerning issue for many companies – not only from a business perspective but also that of the consumer. With regards to adulteration, what is your experience of dealing with instances such as this?

With a complex global food supply chain and many players involved between production and consumption it can be quite easy to conduct fraud without being detected. Adulteration is carried out to increase volume; mask inferior quality; and replace the authentic substances for the seller's economic gain. Food adulteration has become increasingly sophisticated, often being specifically designed to avoid detection through routine analysis. There are a number of different terms used in this area such as, adulteration, economic adulteration, authenticity, food fraud, deliberate mislabelling, origin mislabelling, misbranding and counterfeiting.

The fraud could be the addition of a single chemical such as Sudan I dye aimed at improving the colour of spices to give the appearance of a higher quality. Alternatively, it could be the simple blending of a premium product, such as virgin olive oil with a low-value vegetable oil, at levels that cannot be readily detected by taste. The same result can also be achieved by deliberate

mislabelling of products like olive oil, wine or honey in terms of geographical or botanical origin without any adulteration taking place. Counterfeiting refers to the deliberate manufacture of a food or beverage and using imitation packaging and/or labelling to pass-off as a recognised premium brand. All these deceptions are about misleading the consumer into believing a product is of a higher quality than it actually is and therefore paying a higher price for an inferior product. Is it always clear if a contamination has been deliberate?

Proving whether contamination of a product was deliberate is challenging. To address this there are 'targeted' and 'untargeted' methods of analysis. Targeted methods are used to detect and quantify a known substance used for adulteration. Untargeted methods can be used initially to screen for possible adulteration, leading to identification of the substance responsible and then subsequent targeted analysis is performed. Untargeted methods are primarily used to 'fingerprint' foods, by measuring a number of different variables and looking for characteristic patterns or identifying peaks that are not present in an authentic sample.

Data is highlighted as the best way to combat food fraud – in your opinion, is there currently sufficient good data that is specific enough to aid food fraud?

There is a wealth of good quality and specific data out there in the community with multiple technologies used to address this challenging area. For targeted analysis the approach is the same as that used for the analysis of contaminants in foods.

For origin testing, Isotope Ratio Mass Spectrometry is the gold standard and recent technology introductions, such as high resolution mass spectrometry with Orbitrap technology, has revolutionised the way screening of products is performed.

The key challenge with the data being generated by a multitude of technologies is how to consolidate, harmonise and mobilise this data so it can be used by industry. We need novel ways to reduce the current barriers to data-sharing and utilisation that is crucial to combating food fraud by supplying methods and tools that will address both enforcement and industry needs.

What advancements do you see in the future?

The practices of adulterating food and misrepresenting its authenticity have strong economic incentives, making it unlikely to decline in the future. As scandals are exposed, the perpetrators switch to new fraudulent practices, which are difficult to identify.

Chromatography, mass spectrometry, elemental analysis, molecular spectroscopy and stable isotopic ratio are all powerful tools employed to combat food fraud and will continue to be used in the future. Untargeted food analysis has developed rapidly in recent years, particularly with the improved ability to generate very specific 'fingerprints' of foods. The use of high resolution mass spectrometry with Orbitrap technology will rise in the future.

However, although there are many powerful statistical tools that are employed for multivariate analysis of complex datasets, applying these requires considerable experience and this is not always available in a typical food laboratory. In the future this gap needs to be filled by means of 'user-friendly' software packages becoming available and perhaps being supplied with the instruments being used. Additionally, large databases of varied parameters for authentic foods need to be generated and shared by users. However, this can only happen when some standardisation is agreed, both in terms of measurement and data storage. The Food Integrity Work Package 18, INTELLItrace Project, is underway and is charged with finding a way to address these large data sets and its findings will be beneficial for the future.

David Psomiadis on food authenticity testing

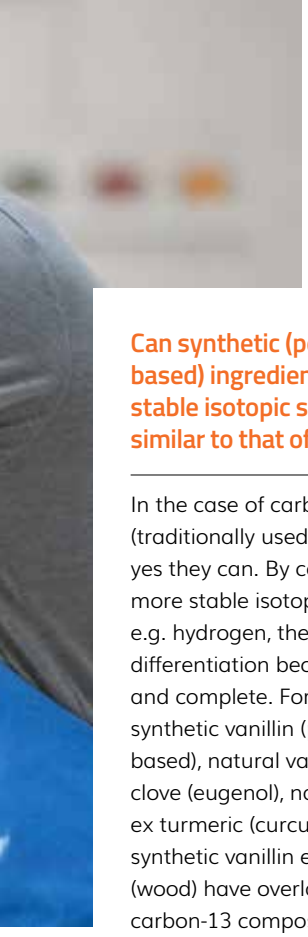
Dr. David Psomiadis, Laboratory Manager, Imprint Analytics GmbH, was the speaker in the latest webinar sponsored by Thermo Fisher Scientific: 'Use of Stable Isotope Analysis in Commercial Food Authenticity Testing'. In this article David selects a few of his favourite questions from the audience, and provides his answers.

Why are big databases not the most suitable tool for the geographic origin testing of food?

The main disadvantage is that a database is only a snapshot of a particular time. Experience shows that the possibility of the database providing erroneous predictions is high, especially when the geographic question refers to neighbouring areas. The databases require continuous updating, evaluation and processing. Even then, there is no direct comparison to the authentic primary product on the field, which is necessary for cases with a high demand of strong evidence. Seasonal and annual differences of the conditions in a given area will also distort the test. Furthermore, one should consider the time and costs needed to develop a new database before testing a certain product and the complexity of getting multiple authentic reference samples.

Why is 2D-isotope fingerprinting of flavour substances necessary for the proof of naturalness?

The use of single isotope testing limits the detection capabilities of the technique. It is impossible to distinguish all sources of nature-identical flavours using only one isotope ratio. It has also been reported that manipulation of the carbon isotope ratios was already feasible in the 1980s, making it possible to produce synthetic vanillin with similar carbon isotope composition to natural vanilla. Other techniques are also limited in respect to the sample matrix, excluding processed or consumer products from the range of tested materials. The combination of GC with the IRMS technology can offer this analysis, with limited effort in sample preparation.



Can synthetic (petroleum based) ingredients have a stable isotopic signature similar to that of a plant?

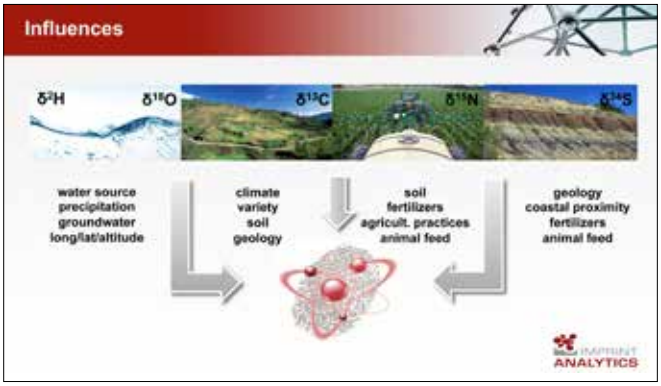
In the case of carbon-13 (traditionally used for this), yes they can. By combining more stable isotope ratios, e.g. hydrogen, the differentiation becomes clear and complete. For example, synthetic vanillin (petroleum based), natural vanillin ex clove (eugenol), natural vanillin ex turmeric (curcumin) and synthetic vanillin ex lignin (wood) have overlapping carbon-13 compositions. Similarly, all natural vanillins (including vanilla) have similar carbon-14 composition, due to their modern source material. The combination of two isotope ratios stretches the differing clusters of each type of the compound, making it possible to distinguish all source materials, natural and synthetic.

Are there currently other isotopes for similar use being considered, rather than those mentioned?

Yes, actually the sixth to mention for geographic origin testing would be Sr isotopes. Also, other heavier elements have been used in research. However, one should consider the costs of routine analysis of such heavy elements' isotope ratios. IRMS cannot be used for the determination of the stable isotopes of the heavy elements. MC-ICP-MS and TIMS are appropriate technologies. The cost of this equipment is much higher and the sample preparation, standards and materials are much more expensive today for such analysis. However, the instrumentation is available, modern and very reliable. It is a matter of having commercially available services that can provide this type of testing, which in some cases combined with the stable isotope ratios of the light elements can provide further information.

Do you think we will reach the point where one equipment configuration will serve for all products – C3, C4 Plants, fruit juices, flavour ingredients, food additives, wine etc.?

In principle, no. The basic detecting instrument is the IRMS (Isotope Ratio Mass Spectrometer). The sample introduction takes place through different peripherals, depending on the type of analysis, the type of sample, and the target element. In general, one can list the configurations of the IRMS coupled with one of the following: a. Elementar Analyser, b. Thermal Conversion oven, c. Equilibration unit, d. Dual inlet, e. Gas chromatograph, f. Liquid chromatograph. Different interfaces also need their respective coupling devices to optimise the gas flows and the connections between the peripherals and the IRMS. Also, different autosamplers can be used for liquid injection or for encapsulated solid samples.



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ON DEMAND

FOOD FOR THOUGHT

Origin testing

New Food interviews ***Dr Christopher Brodie***, Product Specialist Isotope Ratio MS, Thermo Fisher Scientific

Authentication of geographical origin is compulsory on products with a protected designation of origin (PDO). How can manufacturers and retailers ensure that their products comply with labelling regulations?

Products with Protected Designation of Origin (PDO) include meat, cheese, wine, beer, salt, fruit and vegetables and even products like wool. There is an increase in retailer and consumer demand to see proof that their product is what it says it is, including origin and authenticity. Compliance with labelling regulations is enforced through legislation globally, either at the country level or cross-country level, such as in the European Union. Complexities in the food supply chain between production site and consumer have presented significant opportunity for economically motivated fraudulent activities to occur and be undetected, this includes mislabelling of geographical origin.

In order to trace fraudulent activities, manufacturers and retailers must have a foundation from which reliable information on product origin, whether it is the overall finished product or the amount of its constituent ingredients, can be obtained and verified against the product label. With this information, they can then supply and prove throughout the supply chain that products are what they say they are and they come from where they claim: in the end, it verifies the product label.

This is where analytical testing has an important role to play: to help manufacturers and retailers ensure products comply with labelling regulations. Each food product has a fingerprint, like your fingerprint. To visualise this fingerprint, we need analytical tools because the food fingerprint is chemical: in Isotope Ratio Mass Spectrometry (IRMS) we call this the isotope fingerprint. When IRMS testing is used to examine product origin and authenticity label claims, documentation can be passed through the supply chain giving confidence to manufacturers, retailers and ultimately consumers.

How does IRMS work and what are its key benefits over other techniques?

IRMS works by detecting the 'isotope fingerprint' of a sample, a unique chemical signature that changes from sample to sample. With regards to food, the isotope fingerprint is region or process specific, which means that you can differentiate food products based on geographical region (cheese, coffee, sugar, fish and animal feeding areas are just some examples). Botanical processes (beans, seeds, olive oil, vanilla), soil and fertilisation processes (fruits and vegetables) and other fraudulent practices (sugar addition to honey, watering of wines and spirits), are further examples, but by no means all! By tracing the isotope fingerprints, analysts get access to unique information which enables them to have conclusive answers on origin, authenticity and adulteration questions.

There are a number of approaches to preparing food samples before detecting their isotope fingerprints, however, the fundamental process for IRMS is the conversion of a solid or liquid sample to a gas under high temperature. Carbon, nitrogen, sulphur, hydrogen and oxygen are produced, separated from one another using gas chromatography and then transferred in a continuous gas flow to a detector that measures the isotope fingerprint of the sample. Compared to some other techniques, the system is easy to use and automated, which means that operators simply introduce their samples for analysis and the result at the end is easily interpreted. Other significant benefits include its high throughput, the relatively low cost of analysis and ownership, ensuring a quick ROI. All these factors contribute to the main benefit of IRMS which is: reliable data that provides conclusive answers to questions of product origin.

Is it just origin testing or can the technique assist with other claims?

Origin testing of products is one aspect in food fraud that IRMS can provide reliable answers for. IRMS is also used to identify adulterated products to determine if products have been mislabeled with regards to ingredient information. Adulteration can relate to increasing product volume by adding ingredients of lesser quality, simply extending the real product in order to increase sales revenue. This is also linked to product mislabeling, where the label states the origin and ingredients of the original, authentic product but the product inside has been changed. IRMS has successfully been used for testing of high value commodities that are always economically motivated frauds, these include: watering of wines and spirits, sugar addition to honey, diluting olive oil with cheaper vegetable oil, and differentiating botanically grown vanilla from synthetically produced vanilla.

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Reach higher peaks in EA-IRMS

Detecting origin and authenticity with the Thermo Scientific EA IsoLink IRMS System by using temperature ramped gas chromatography

The origin, correct labeling and adulteration of food and beverage products affect producer and consumer value and food safety and laboratories need an analytical technique providing unique answers. Analyzing food and beverage products for carbon, nitrogen, sulfur, oxygen and hydrogen isotopes allows laboratories, tracing food fraud activities, to determine origin and authenticity of samples.

By using a combination of temperature ramped gas chromatography and very low helium consumption per sample, the automated, high throughput and cost effective Thermo Scientific™ EA IsoLink™ IRMS System provides precise data that allows you to determine if the label states the truthful origin and if food or beverages were adulterated. The EA IsoLink IRMS System enables you to answer questions on food and beverage fraud and to protect producer revenue and food markets, while delivering consumer confidence.

Find out more at **thermofisher.com/EAIsoLink**

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Can we perform gelatin speciation and adulteration using bioinformatics, proteomics and high resolution mass spectrometry?

Gelatin is composed of highly processed proteins, which is widely used as a gelling and thickening agent in a variety of food products, including meat, confectionery products and water-based desserts. It is also widely used in the pharmaceutical industry. Gelatin is obtained by hydrolysis of collagen, which is extracted from materials such as bone, hide and skin from animal slaughterhouses. Nearly 80% of gelatin is produced from pig by-products.

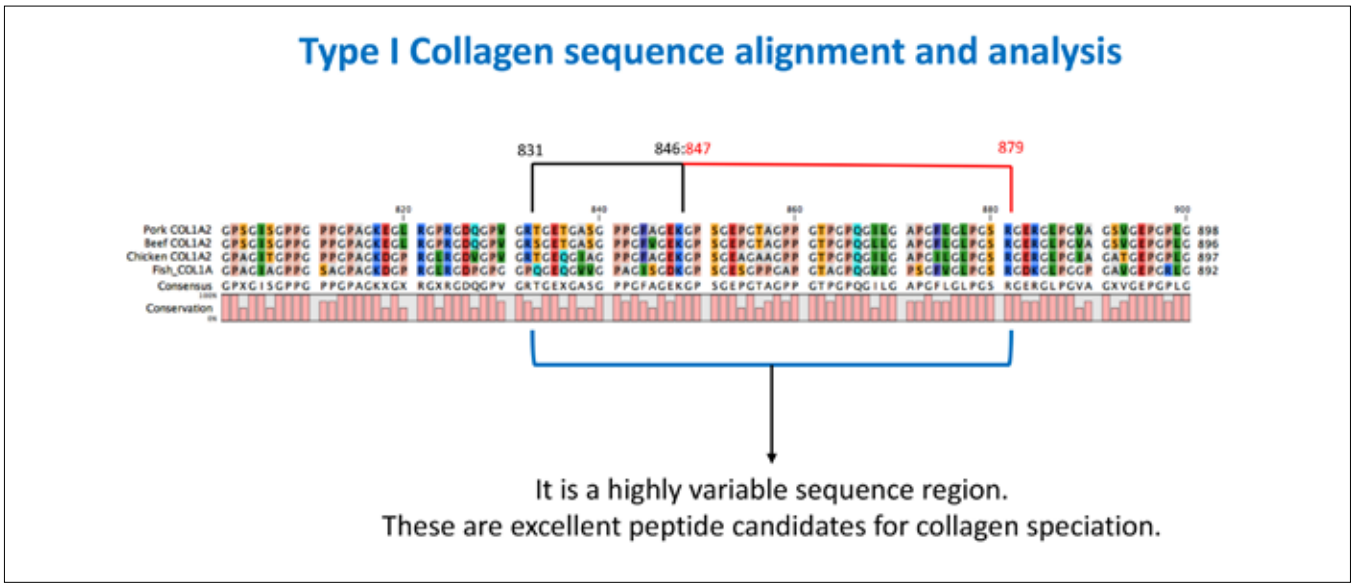
THE webinar, sponsored by Thermo Fisher Scientific, presented a strategy to detect specific peptide biomarkers in the digested gelatin, and food samples using HPLC–Orbitrap. Dr. Francis Beaudry, University of Montreal, also demonstrated that this method can be an effective strategy to detect gelatin adulteration. Here, Francis answers some questions raised in the Q&A portion of the webinar.

According to your sample preparation, it seems you would need a very large amount of trypsin to digest the proteins well

Typically, we homogenize 100mg (0.1 g) of gelatin in an ammonium bicarbonate buffer (pH 8.5) in a ratio of 1:5. The digestion can be performed directly with trypsin at this point but we do obtain better results when we perform an ethanol precipitation and re-dissolve the protein pellets in 100mM ammonium bicarbonate (pH 8.5). We then add 2µg of proteomic-grade trypsin and carry the digestion for 16-24h at 40˚C. After monitoring specific tryptic peptide formation, the digestion appears to be completed after 12h. We have tested 1, 2, 5 and 10µg of trypsin and there was no benefit in adding more than 2 or 5µg if the reaction was performed for more than 12h at 40˚C.

Could that explain the low sequence coverage?

The sequence coverage was 23, 16 and 35% for collagen type I, II and III respectively from gelatin extract from meat samples. We were able to obtain 45% sequence coverage with Sigma purified collagen for all tested species. Gelatin is a hydrolysed form of collagens and it is highly processed resulting in variable denaturation and degradation.



Moreover, gelatin purity in meat samples is inconsistent, leading to more inherent variability. The sequence coverage of 23, 16 and 35% for collagen type I, II and III is therefore relatively high and targeted peptide biomarkers can be effectively used for analysis.

The survey scan may be less sensitive than a targeted MS/MS analysis. Could you miss some peptides from other species only with the XIC?

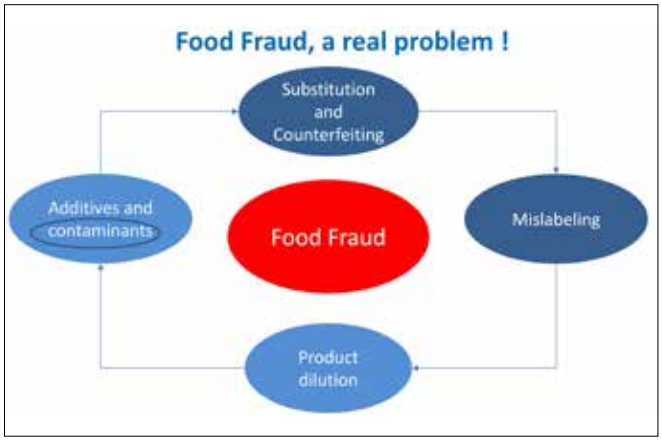
A hybrid Orbitrap, as well as other ion traps, is generally more sensitive in full scan MS or MS/MS compared to a targeted approach similar to SRM or MRM performed on triple quadrupole instruments. The Q Exactive Orbitrap instrument use for this study has the benefit of high mass accuracy and resolution. It is certainly an asset when analysing adulteration since it provides unparalleled strength on the MS and MS/MS levels for animal species identification. However, the method can also be adapted for triple quadrupole instrument working in SRM or MRM. The assay will gain sensitivity but will lose the possibility of performing data mining.

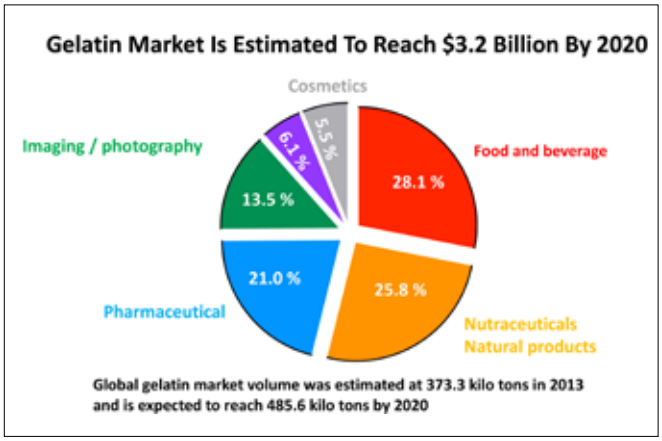
What is the LOD for species detection in gelatin?

Currently, the LOD is 0.1% w/w but can be improved by using more gelatin samples and use a SPE protocol for tryptic peptide clean-up and concentration prior to analysis. Moreover, if sensitivity is your ultimate goal, you could adapt the method on a triple quadrupole instrument using a SRM or MRM strategy.

Which fish species did you use, or did you use a mixture of several species?

It is gelatin derived from cold water fish skins but species were not specified in the certificate of analysis obtained from Sigma. We have also tested fish gelatin from food additive providers but fish species were not identified and very limited information was available. However, the results we have obtained from fish gelatin was relatively constant.





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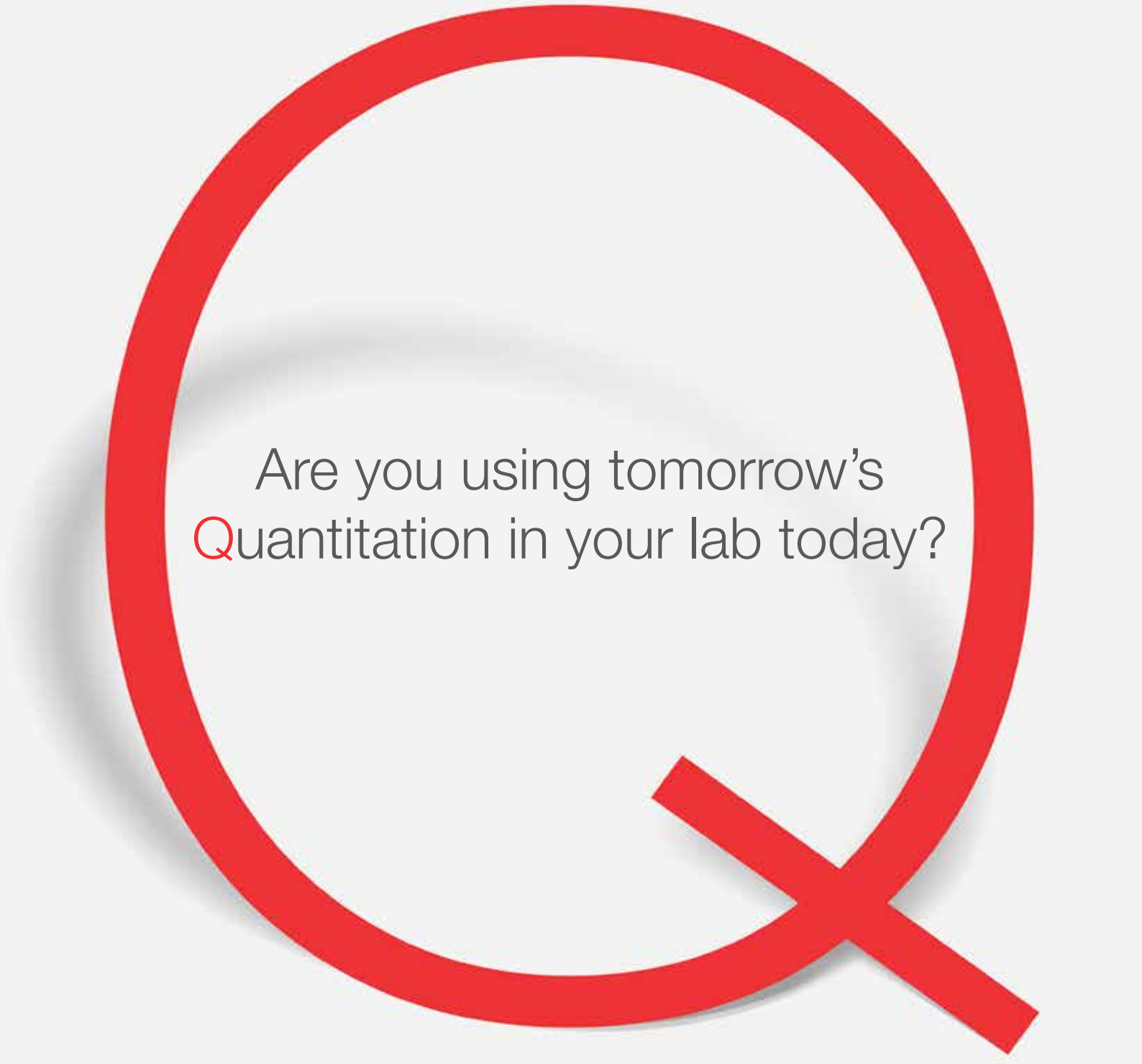

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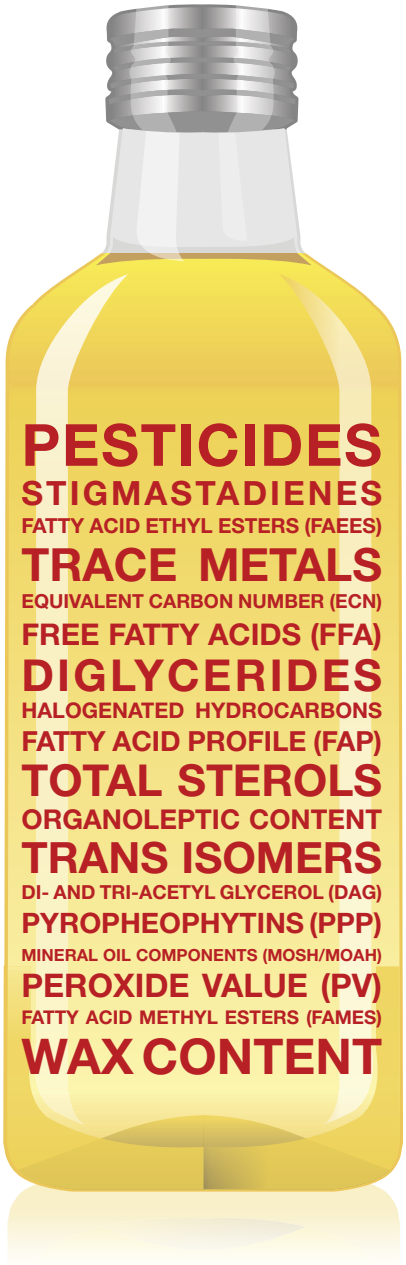
Olive oil should ONLY be olive oil

The olive oil industry faces increased pressure to prove that its products live up to the quality and origin on the bottle. Consumers are now more aware than ever, that olive oils may not always be what is claimed or advertised. Our separation and detection technologies provide ideal solutions to address these challenges the olive oil industry faces today.

WHAT'S POTENTIALLY IN MY BOTTLE?



KEY AREAS IN OLIVE OIL TESTING

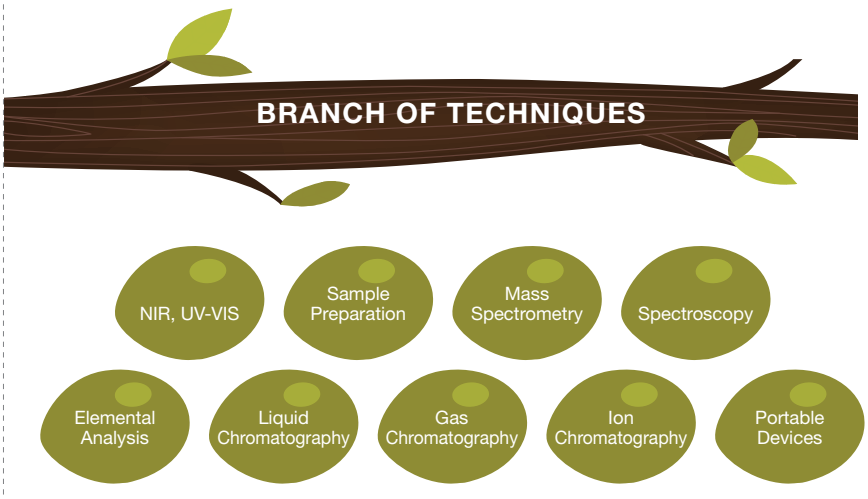
NUTRITION FACTS

Nutritional value per 100 g (3.5 oz)		
Energy	3,699 kJ (884 kcal)	
Carbohydrates	0 g	
Fat	100 g	
Saturated	14 g	
Monounsaturated	73 g	
Polyunsaturated	11 g	
omega-3	0.8 g	
omega-6	9.8 g	
Protein	0 g	
Vitamins		
Vitamin E	14 mg	(93%)
Vitamin K	60 µg	(57%)
Minerals		
Iron	0.56 mg	(4%)

Units
µg = micrograms • mg = milligrams
IU = International units

Percentages are roughly approximated using US recommendations for adults.
Source: USDA Nutrient Database

BRANCH OF TECHNIQUES



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Charged aerosol detection for LC analysis of triglycerides: a novel tool for extra-virgin olive oil characterisation

Extra-virgin olive oil has long been a favourite object of fraudsters. In fact, this oil, which represents an essential part of the healthy Mediterranean diet, is the edible oil with the highest nutritional and sensory quality and consequently the most expensive among them. Within this context, continuous efforts have been devoted to the development of new and improved analytical methods able to detect emerging and sophisticated frauds.

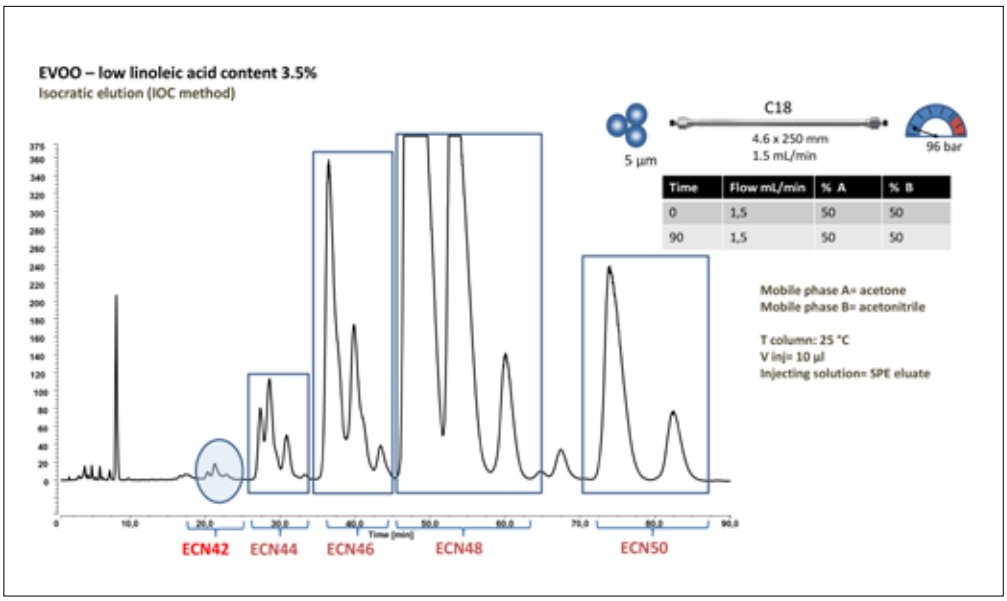
THE webinar, sponsored by Thermo Fisher Scientific, offered Lanfranco Conte, Dept.of Agri-Food, Environmental and Animal Sciences, University of Udine and Paolo Lucci, Dept.of Agri-Food, Environmental and Animal Sciences, University of Udine, a great opportunity to discuss this issue. In this article they provide their responses to some questions on the topic.

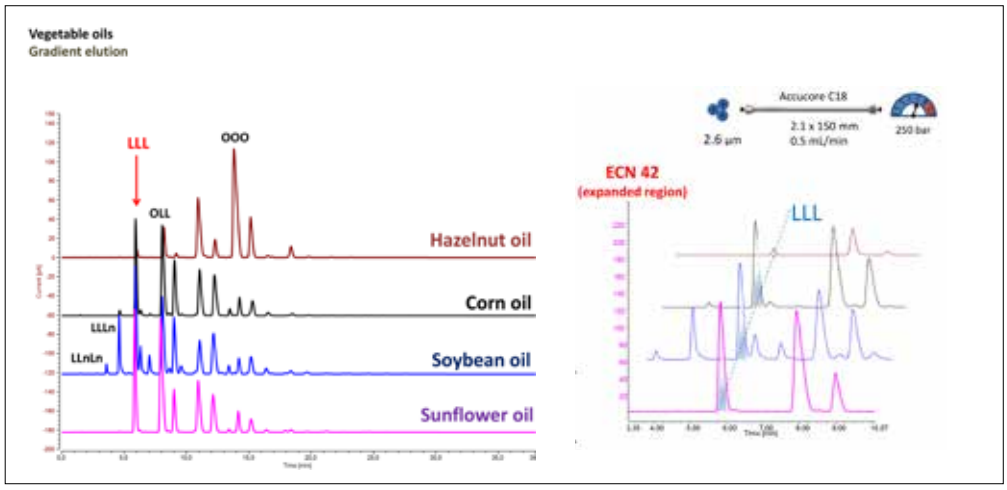
Is it possible to avoid SPE sample treatment procedure when using your UHPLC-CAD method?

CAD can accommodate gradients. Therefore, it is possible to start with a more polar mobile phase composition (i.e., higher percentage of acetonitrile) to elute oxidation products as well as mono- or diacylglycerols just at the beginning of the trace and before the elution of low ECN TAGs such as trilinolein. In this way, it can be possible to eliminate a sample preparation step. By the way, it obviously depends on the nature of the oil to be analysed.

Can the method be used for detecting extraneous seed oils in extra-virgin olive oil?

The proposed UHPLC-CAD method allows to obtain a baseline separation of trilinolein (LLL) and therefore to correctly quantify this TAG. In our opinion, the quantification of trilinolein could be useful for detecting the presence of extraneous seed oils in olive oils. However, before doing that, an evaluation of the real range of trilinolein content in a large amount of pure olive oils with different amount of linoleic acids is mandatory.





DETERMINATION OF THE DIFFERENCE BETWEEN ACTUAL AND THEORETICAL CONTENT OF TRIACYGLYCEROLS WITH ECN 42

(PURE OILS)

SAMPLE	% ECN 42 experimental	% ECN 42 theoric	Δ ECN 42	Δ ECN 42 (limit ≤ |0,2|	% LLL experimental	% LLL experimental	% LLL theoric	Δ LLL	Δ(LLL-ECN 42)
EVOO	0.0732	0.1302	0.0500	0,1	0.0156	0,02	0.0053	0.0103	0.1146
PALM OLEIN	0.5133	0.3603	0.1530	0,2	0.6392	0,64	0.1747	0.4645	0.2789
HOSFO	0.2181	0.0016	0.2165	0,2	0.3016	0,03	0.0014	0.3002	0.3000
MIX 50-50	0.4833	0.1223	0.3610	0,4	0.3254	0,33	0.0385	0.2867	0.2031

Can the method be used in a conventional HPLC system?

The method has been developed for being applied also to a common conventional HPLC system. The column used is an Accucore C18 150 x 2.1 x 2.6um (Thermo). Under the chromatographic conditions described in the webinar the backpressure will not exceed 250 bar. So, the method can be used on both HPLC and UHPLC systems.

Is the method suitable to check the authenticity of other vegetable oils than olive?

The method had been developed for olive oils authenticity control. It can be used to analyse the composition of TAGs mixtures, that's to say virtually every vegetable oil, however, peak separation needs to be carefully optimised. Care must be taken when short medium fatty acids triacylglycerols are present because of their vapour pressure that could give a different behavior in the nebulisation step in the CAD. Nevertheless, a good separation of TAGs has been obtained with sunflower oil, hazelnut oil, grapeseed oil, corn oil, palm olein, pomace oil, and pistachio oil, among others.

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ON DEMAND

Improved method for authenticity control of olive oils using charged aerosol detection

Extra virgin olive oil has long been a target for fraudsters. It is the edible oil with the highest nutritional and sensory quality and, as a result, the most expensive. There has been a drive towards the development of new and improved analytical methods capable of reliable and accurate determinations of olive oil constituents, which enable scientists to detect emerging and sophisticated frauds.

THE ability to distinguish seed oils such as hazelnut oil, corn oil, soybean oil and sunflower oil, from extra virgin olive oil would be ideal in maintaining the integrity of the product.

Triacylglycerol (TAG) analysis using parameter ΔECN42 is one of the methods available for detection of small amount of seed oil mixed with olive oil. However, from an analytical point of view, the use of a refractive index detector for triglycerides analysis, stated in some methods, prevents gradient elution separations, thus leading to incomplete TAGs resolution and overlapping peaks.

Thermo Scientific Vanquish charged aerosol detectors represent an evolutionary refinement in liquid chromatographic detection design, based on the widely adopted charged aerosol detection (CAD) technology. The charged aerosol detector generates a signal in direct proportion to the quantity of analyte present and does not require a chromophore, which lipids typically do not possess. It can be used with gradient elution, thus representing a potential alternative to refractive index detectors.

These factors, coupled with sensitivity and precision, enhanced linear dynamic range and a consistent response makes the charged aerosol detector ideal for the successful analysis of olive oils.

Research presented by Professor Lanfranco Conte and Dr Paulo Lucci, from Department of Agri-Food, Animal and Environmental Sciences, University of Udine, Italy, has demonstrated CAD has excellent linearity for trilinolein over a wide range, from 50 to 10,000ng injected on-column.

They also proved when coupling to a UHPLC that CAD gives good separation and quantification of trilinolein and is capable of separating TAGs in a reduced time and with a much lower consumption of mobile phases when compared to the official method. Their proposed method is able to detect small increases in trilinolein content in extra virgin olive oil adulterated with 2, 4, 6, 8 and 10% of high oleic sunflower oil, palm olein and a mix of both.

For more detailed information on the research download their poster: https://tools.thermofisher.com/content/sfs/posters/PO-72387-LC-CAD-Triglycerides-Olive-Oils-HPLC2017-PO72387-EN.pdf and watch their educational webinar: www.newfoodmagazine.com/webinar/34555/charged-aerosol-detection-lc-analysis-triglycerides-novel-tool-extra-virgin-olive-oil-characterisation/

A Vanquish charged aerosol detector has the flexibility and performance for analytical R&D and the simplicity and reproducibility needed for QA/QC. To learn more please visit: thermofisher.com/cad

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HPAE-PAD determination of carbohydrates in honey to evaluate samples for quality and adulteration

Authors: Manali Aggrawal and Jeffrey Rohrer, Thermo Fisher Scientific

FRUCTOSE and glucose are the major components in honey and account for 85-95% of the honey sugars. The concentrations of fructose and glucose, as well as their ratios, are useful parameters for the classification of monofloral honeys. The remaining carbohydrates are a mixture of at least 11 disaccharides, 11 trisaccharides, and several larger oligosaccharides. Minor honey sugars may be useful for the determination of floral origin and may act as a 'fingerprint' for a sample's floral source. Besides the reducing sugars (glucose and fructose), the amount of sucrose is a very important indicator for evaluating honey quality. High levels of sucrose may indicate a variety of adulterations, such as adding cheap sweeteners, like cane sugar or refined beet sugar, during early harvest. Due to these factors, various regulations require a minimum amount of reducing sugars and a maximum amount of sucrose among other honey quality parameters. The Codex Alimentarius Committee on Sugars (2001) specified a maximum value of 5g of sucrose in 100g of floral honey (Codex Standard for Honey, 2001). Therefore, carbohydrate analysis is important as a honey quality parameter and for floral origin determinations.

High Performance Anion Exchange chromatography coupled with Pulsed Amperometric Detection (HPAE-PAD) is one of the most useful techniques for carbohydrate determinations. This application note demonstrates a method to assay fructose and glucose, plus the entire profile of di- and trisaccharides in honey.

Results and discussion

The honey sugars were separated into 15 sugar standards in a single run. Of the 15 sugars, two are monosaccharides (glucose and fructose), nine are disaccharides (trehalose, sucrose, kojibiose, gentiobiose, turanose, palatinose, nigerose, isomaltose and maltose) and four are trisaccharides (melezitose, raffinose, 1-kestose, and erlose). For this study 12 commercial honey samples (***Table 1***) were analysed using HPAE-PAD. This method offers several benefits over previous methods. First, the method uses a Dionex CarboPac PA210-Fast-4µm column. Its smaller resin particles (4µm) provide fast, high-resolution separations and this method does not require a sodium acetate eluent and therefore it can use eluent generation. This eliminates eluent preparation errors and the need to handle sodium hydroxide and sodium acetate as required for the preparation of eluents for previous HPAE-PAD honey applications. Eluent generation allows chromatographers to run a full range of gradient and isocratic separations more reliably than manually prepared eluents.

Conclusion

An HPAE-PAD method was successfully developed and validated for the sugar analysis of 12 commercial honey samples using the Dionex CarboPac PA210-4µm column. This column allows the separation of 15 sugars in honey with minimal sample preparation and an overall cycle time of 45min. PAD is sensitive, thus allowing the determination of low concentration carbohydrates in honey, while at the same time detecting the high concentrations of the major components, glucose and fructose. The method showed good precision and accuracy with recovery range of 80-120%. This method enabled us to detect the addition of industrial sugar syrups (adulteration) to honey samples.

TABLE 1 List of commerical honey samples

HONEY SAMPLE	FLORAL SOURCE
HS1	Clover
HS2	Clover, sunflower and alfalfa
HS3*	Wildflowers
HS4	Manuka tree
HS5	Clover
HS6	Mixed
HS7	Manuka tree
HS8	Mixed
HS9	Clover
HS10*	Wildflowers
HS11*	Blackberry blossoms
HS12*	Mixed

*Local beekeeper honey

To download the full application note visit our website: thermofisher.com/foodintegrity

Is your honey *really* honey?

Food fraud is a growing problem that can take many forms, but is nearly always motivated by economic gain. The typical targets are highly valued products. This webinar discussed how ion chromatography (IC) can be used to detect certain types of food fraud with examples of honey, coffee, and fruit juice adulterations. Here are the pick of the questions asked during the webinar, along with expert answers.

How do you detect adulteration in honey – is the instrument hand held?

The instrument is a bench-top instrument. An IC system is similar to an HPLC but it has a non-metallic pathway that is necessary for chromatography. The IC can detect adulteration of honey with sugars. Essentially the IC separates and detects the sugars present in honey, and from the amounts and types of sugars present it can detect likely adulteration.

If you were going to use one IC for testing in many different sectors / matrices would there need to be any major changes to the instrument? For instance, if you wanted to analyse for carbohydrates, amines, and anions for food adulteration testing as well as anions and cations in waters/effluents?

The same instrument could be used for all those tests as long as you had both a conductivity and an electrochemical detector. Switching between applications will require rinsing the system (if the mobile phase changes), replacing the column with the column for your new application, installing the new eluents (i.e. mobile phases) or new eluent generation cartridges to produce the required mobile phase, possibly switching the detector and detector cell, and if changing from anions to cations, or visa versa, changing the suppressor type.

For these various methods of fraud detection, is the sensitivity change using a conductivity detector instead of a pulsed amperometric detector too significant? Are these methods transferable between detectors?

The answer depends on the analyte. For many analytes the sensitivity of the two detectors is similar. For some analytes only one detector will work (e.g. glucose can be detected

electrochemically but not by suppressed conductivity). For analytes that can be detected by both with similar sensitivity we need to then look at what other compounds are in the sample. Conductivity is less selective than pulsed amperometric detection (PAD), so if there are other ionic species that are not detected by PAD, PAD could be the better detection choice.

What if I buy organic honey, should I still test it?

Buying organic honey does not ensure that is has not been adulterated. Actually, it does not even ensure that it is organic. When we buy something that is labelled honey or labelled organic honey, we trust that it is in fact honey or in fact organic honey. Unfortunately, the parties that dilute honey with other sweeteners or take honey that was not produced organically and label it as organic, violate our trust to make more money.

You have shown analysis of smaller carbohydrates, can I analyse for larger carbohydrates with the methods you have shown?

You certainly can. High performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD) can be used to analyse for nearly all types of carbohydrates. HPAE-PAD has been used to separate maltodextrins with degrees of polymerisation as high as 80. Other HPAE-PAD applications include the separation of the prebiotics, polyfructans, fructooligosaccharides, and trans-galactose oligosaccharides.

Can I use a mass spectrometry (MS) detector with the methods you have shown?

Yes, all the anion, cation, and carbohydrate methods I showed can be interfaced with a mass spectrometer using electrospray ionisation. This requires an anion suppressor for anion and carbohydrate analysis and a cation suppressor for cation analysis. For anions and carbohydrates the suppressor converts the potassium or sodium hydroxide eluent to water before it enters the electrospray interface. When larger carbohydrates are separated with both sodium hydroxide and sodium acetate eluents, the suppressor converts the eluent to weak acetic acid before it enters the electrospray interface.

This webinar is the fourth in a series of educational webinars, providing you with the opportunity to learn from experts and in-house specialists from Thermo Fisher Scientific, on the use of differentiated technology in the field of food integrity.

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Scanning GC Orbitrap

Thermo Fisher Scientific's multi-award-winning Orbitrap GC-MS technology has allowed scientists to break new ground to gain a broader and deeper understanding of their samples through the use of high-resolution, accurate-mass (HRAM) analysis. In a recent webinar the full potential of the technology was explored and *Dominic Roberts* answers the pick of the questions asked.

When using GC-Orbitrap, what resolving power would you recommend for a profiling study?

The experiments shown in this webinar were run at 60,000 resolution (FWHM at m/z 200) and this is the mode that we would use in routine as it provides sub 1ppm mass accuracy with a high number of data points across a peak for robust deconvolution. At this resolution mode the scan speed is around 7 spectra/second, so across a typical GC peak of around four seconds we have 28 points across the peak. The resolution is important for two reasons; firstly, it provides resolution of target ions from matrix ions against a complex chemical background. The high resolution becomes extremely powerful when the sample is complex, which is often the case with profiling studies where the sample preparation is minimal to preserve as many compounds as possible. Secondly, it provides us with very small mass errors regardless of peak intensity, so that when we detect a known or unknown compound we can be confident that it is the correct result. This is particularly valuable for unknowns as it reduces the number of possible elemental compositions that need to be evaluated. For example you may have 20 possible formulae at 5ppm, but perhaps only 1 or 2 to evaluate at < 1ppm. In addition

to mass accuracy, the isotopic pattern can also be matched against the theoretical pattern to provide a further point of identification. The individual isotopes would also have good mass accuracy which results in quicker identification and with higher confidence.

Can I develop and use my own spectral library for GC-Orbitrap?

Yes, this can be done and by having a high resolution Orbitrap library the confidence in matched compounds will be very high and the number of possible matches often reduced to one suggestion. A custom library can be curated within the NIST MS search interface and operated in the usual way as you would a nominal mass library. The advantage with a high resolution accurate mass library is that the spectra will have a high search index result because the spectra were acquired on the same instrument, and secondly the spectra are accurate mass so there are very few suggested matches compared with those using nominal mass. The process to build a library is simple; the deconvoluted spectra, as seen in the webinar, can be exported to NIST MS Search from TraceFinder and added to any library either existing or a new one created. I would also like to mention that we have developed a HRMS contaminants library containing accurate mass spectra for over 700 compounds, including 500 pesticides. There is also a GC-Orbitrap Metabolomics library available containing over 850 metabolite spectra. For each entry in the library the theoretical exact mass of major ions and spectra, determined from curated Orbitrap GC-MS data, is included.

What is the scan speed on the GC-Orbitrap and does it impact on the sensitivity?

On the QExactive GC the scan speed (spectra/second) is directly related to the resolution level selected. There are four resolution levels available on the QExactive GC-15, 30, 60 and 120K. At 120K the scan speed is around 3.5 spectra/second, 7 spectra/second at 60K and increasing down to 15K. Simply put, the longer ions spend in the Orbitrap the higher the resolution, but slower the scan speed. But most importantly, the sensitivity does not change significantly whether you are using 15K or 120K mass resolution. So we have the ideal situation of having access to the highest resolution levels whilst maintaining maximum system sensitivity. At each resolution level the scan speeds are still fast enough for GC, so it's possible to have well defined deconvoluted peaks.

What kind of mass calibration should be used to achieve sub 1ppm mass accuracy?

This is where the QExactive GC is very easy to operate and it ensures that, regardless of a users technical background, the same high quality, sensitive data is generated. The QExactive GC can be tuned, calibrated and leak checked in under five minutes. The system is calibrated prior to analysis and this is valid for 25 hours, after which the software will usefully indicate that this has expired. In reality the mass accuracy is maintained for many days afterwards without any further calibration. On the QExactive GC perfluorotributylamine (FC43), is diffused into the source and the system calibrated to the masses in this spectrum (ions 69, 100, 131, 364, 515, 502). These masses are ideal for a typical full scan acquisition range of 50-600. It is also possible to do a higher mass calibration (>600) should that be needed.

Can I acquire in SIM and full scan simultaneously on the QExactive GC?

In this case SIM stands for "selected ion monitoring" and this is where it is possible to isolate a particular mass in the quadrupole. This experiment could be done either to perform MS/MS on a particular mass for structural elucidation, or for a targeted analysis where a particular ion is selected in the quadrupole, so that marix ions are excluded from the C-Trap. In answer to the question, yes, it is possible to run SIM at the same time as a full scan experiment. This is performed as two functions – one function is full scan and the other SIM, where an ion or ions are selected in the quadrupole. This is very easy to set up in the QExactive method editor, simply drag and drop the experiment type and define the masses you want to select in the quadrupole and the full scan mass range to measure.

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Application Summary Compendium

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Beer should only be beer

Application summary compendium

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Current trends in food and beverage

This e-book examines some of the current trends in food and beverage analysis from experienced industry experts and in-house specialists from Thermo Fisher Scientific. Read interviews with key industry experts discussing current trends in food and beverage analysis, with topics ranging from rice to beer and each article features a different analytical technique.

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