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magazine

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Analytics of EtO and 2-CE in sesame seeds

Trees call birds for help against leaf-feeding insects

VOCs in plant defense

Shining a light on clean energy

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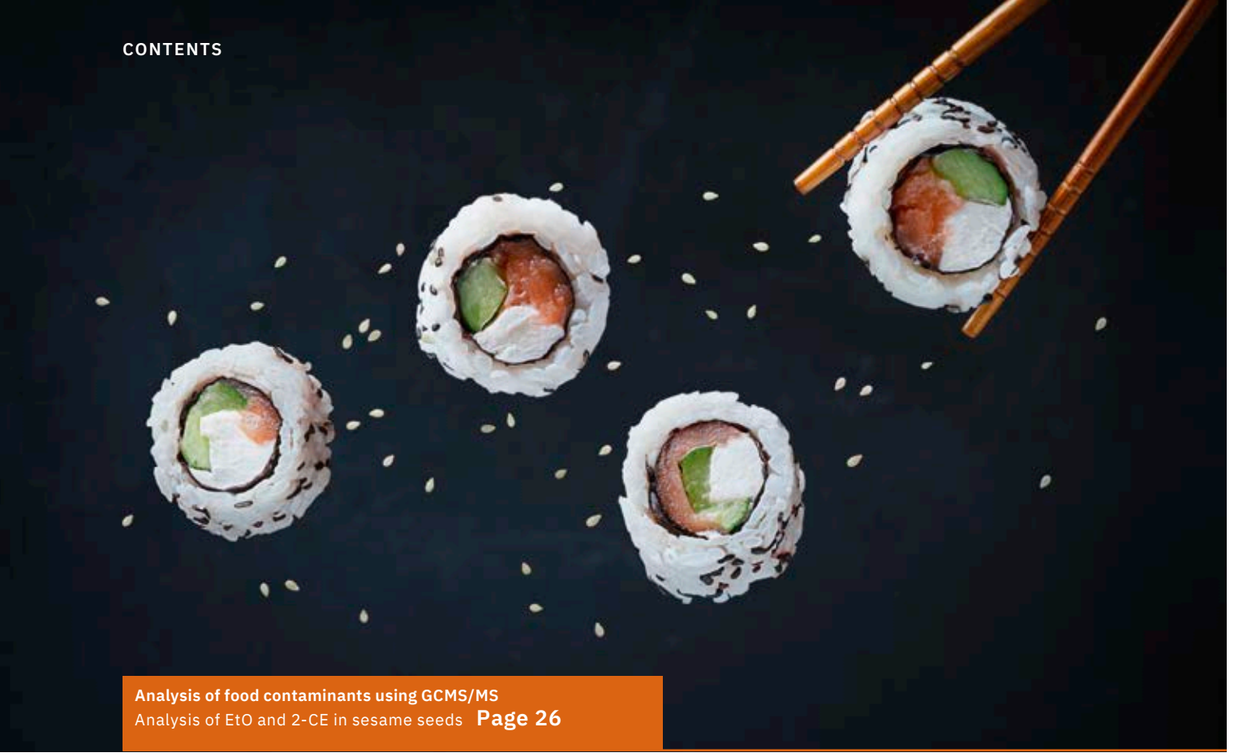

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Cyanobacteria: omni-present organisms with great research potential

SWITCH ON

Necessity of chromatography in the study of secondary metabolites of cyanobacteria

Dr. Ariel Kamiński, Saravana Selvaraj, Jagiellonian University, Faculty of Biochemistry, Biophysics and Biotechnology, Laboratory of Metabolomics

Michał Adamski, W. Szafer Institute of Botany, Polish Academy of Sciences

Cyanobacteria can be found in virtually all habitats worldwide. Their amazing success in adapting to such diverse habitats can be attributed to a very long-standing evolutionary history and ability to synthesize various secondary metabolites. These fascinating microorganisms still keep their secrets from us, which we try to discover in the Laboratory of Metabolomics at Jagiellonian University in Krakow. →

Cyanobacteria or the unwelcomed side effects of human civilisation

Especially in summer and autumn, when people take a vacation by the water, massive phyto-plankton, also known as blue-green algae, grows in freshwater affecting aquatic life. This phenomenon is becoming more and more common all over the world and is directly related to human activity. Agriculture, industry and urban agglomerations release additional compounds into the water such as nitrogen, phosphorous, organic biomass. Such a nutrient-rich environment causes the growth of autotrophic microorganisms like cyanobacteria, which are very often the dominant organism in phytoplankton at that time.

Cyanobacteria are a unique and very interesting group of prokaryotes. They are one of the oldest organisms known on Earth – almost 3.5 billion years old – and known as the first organisms to have produced oxygen. Currently, the cyanobacteria cluster consists of 150 genera and over 3,000 species. They are capable of releasing oxygen in the photosynthesis process, and some of their representatives, as one of the few known organisms, have the ability to fix atmospheric nitrogen, which allows them to grow in environments poor in nitrogen sources. Although cyanobacteria appear massively in water, they can be found in virtually all habitats worldwide – in the icy deserts of the Arctic and Antarctic, hot springs, the Sahara Desert and generally in many aquatic and terrestrial environments. Their amazing success in adapting to such diverse habitats can be attributed to a very long-standing evolutionary history and ability to synthesize various secondary metabolites (Mishra et al., 2019).

You may not know cyanobacteria, but you do know their ecological effects

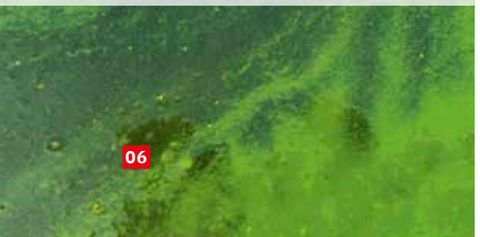
Today, cyanobacteria are widely used as model organisms for research photosynthetic pathways, nitrogen fixation, secondary metabolites and biofuel synthesis. They are an important source of cheap primary and secondary metabolites, including certain types of pharmaceutical compounds, cyanotoxins, biopesticides and plant growth factors. So why are many people afraid of cyanobacteria? This is probably related to the ecological effects of cyanobacterial blooms. It can cause water clouding up to a depth of 2 m, sunlight intensity reduction in water, which in turn leads to anoxia formation (an absence of oxygen in water), anaerobic decomposition of organic matter and finally decrease in aesthetic values (smell and taste of water). Additionally, during cell lysis a release of bioactive secondary metabolites including cyanotoxins can be observed.

A wide range of toxicity

Usually, in the cyanobacterial algal blooms there is a mixture of toxic and nontoxic cyanobacterial species. The most frequent freshwater toxic cyanobacteria known worldwide are *Microcystis, Dolichospermum* (former *Anabaena*), *Aphanizomenon* and *Raphidopsis* (former *Cylindrospermopsis*). These cyanobacteria genera can synthesize health-affecting cyanotoxins such as: neurotoxic anatoxins (ATXs), which affect the neuromuscular junction, neurotoxic saxitoxins (STXs), reversible voltage-gated sodium channel blockers, cytotoxic cylindrospermopsin (CYN), inducing inhibition of protein synthesis, proliferation of membranes, lipid accumulation within cells and eventually cell death, or hepatotoxic microcystins (MCs), modifying the action of proteins in the cytoplasm of liver cells. Contamination of water by cyanotoxins restricts its usage as drinking water, aquaculture, irrigation or re-creation. This is one of the reasons why our team undertook detailed research on cyanobacteria, in particular cyanobacterial toxins. We work closely with scientists from domestic and foreign research centers, incl. Prof. Linda Lawton and Prof. Christine Edwards from Environmental Engineering Research Group, Robert Gordon University, Scotland, and Ph.D. Spyros Gkelis, Head of CyanoLab at the School of Biology, Aristotle University of Thessaloniki, Greece. →

Figure 1: A. Kamiński in the Laboratory of Metabolomics

Figure 2: M. Adamski in the laboratory of the Institute of Botany



Material and methods: liquid chromatography

One of the fastest and relatively cheap ways to locate cyanotoxins in water samples and to determine their concentration is to use high-performance liquid chromatography. In our team at Laboratory of Metabolomics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University in Krakow, we use a Shimadzu Nexera-i LC-2040C 3D Plus High-Performance Liquid Chromatograph (HPLC) equipment for this. Within 15 minutes, we are able to initially confirm or exclude the presence of anatoxin-a (ATX-a), CYN and microcystin-LR (MC-LR). The toxins' determination is performed accordingly to the method of Kucała et al. (Kucała et al., 2021). Briefly, the gradient mobile phase consists of MilliQ water/acetonitrile (both acidified with 0.05% trifluoroacetic acid), where the organic phase increased from 2% to 90% over 15 minutes at a flow rate of 0,75 ml min^{-1}. Samples are separated on a Dr. Maisch ReproSil Gold Column (120 Å, 3.0 µm, 150 mm x 4.6 mm) maintained at 40 °C. Autosampler cooler temperature is 4 °C and PDA cell temperature is 40 °C. Cyanotoxins are identified by comparing the retention time and UV-spectra determined for commercial standards and quantified by absorbance at 227, 239, 261 nm for ATX-a, MC-LR, CYN, respectively.

Our HPLC exceeds all our basic requirements. By conducting research on cyanobacteria, we not only determine the concentration of cyanotoxins in environmental samples, but also find answers to research questions (Nowicka-Krawczyk et al., 2022). Once the cyanotoxins are present in the water, it is important to check how the selected physicochemical factors used in the water treatment plants affect their stability and degradation. HPLC not only allows us to determine the degree of toxin degradation, but sometimes also to detect the products of degradation. These studies were the first ones undertaken by our team. Thanks to the use of HPLC, we were able to determine the stability of ANTX and CYN molecules under various pH range conditions and the influence of other physical factors, such as high temperature or UV radiation.

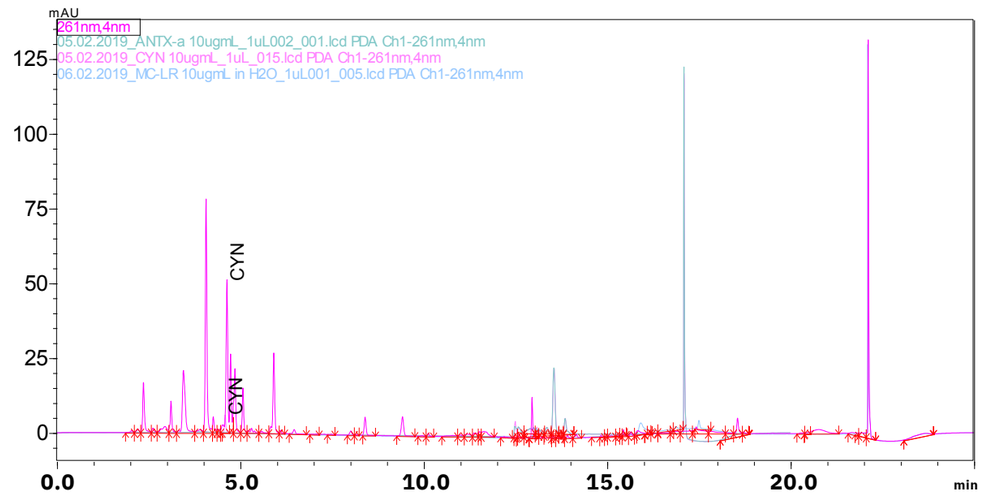


Figure 3: Analysis of a sample containing CYN along with the standards of the three cyanotoxins

Ambivalent effects of cyanotoxins on aquatic plants

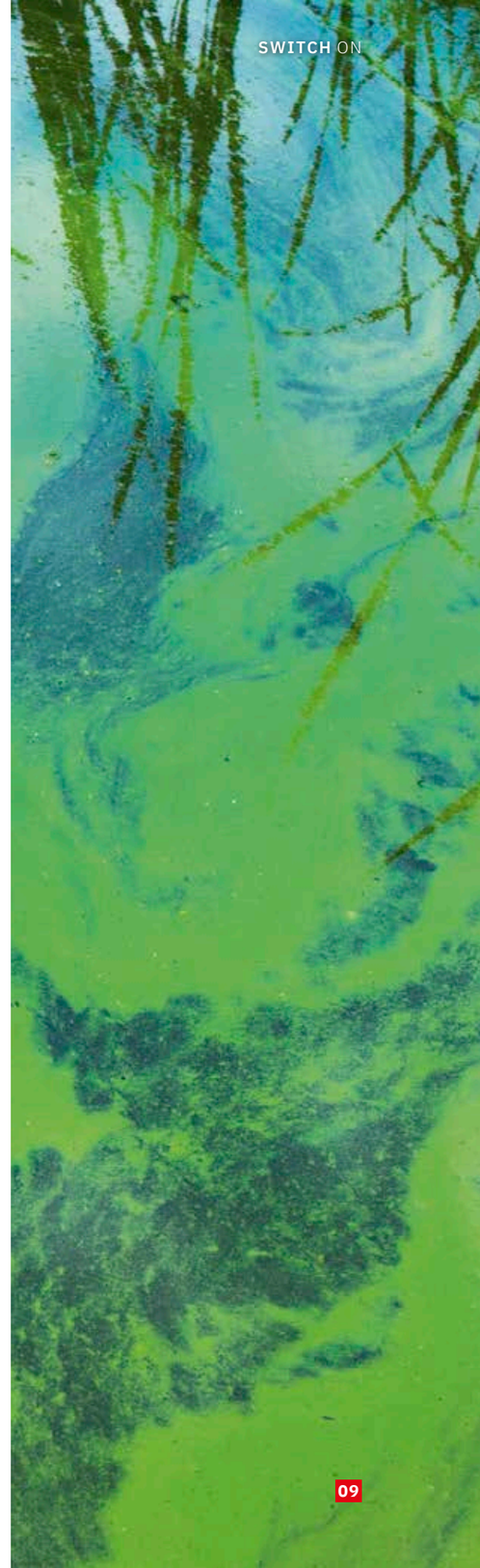
Our second major research topic is the effect of cyanotoxins on aquatic plants. As plants form the basis of most trophic pyramids, it seems extremely important to determine the influence of the secondary metabolites of cyanobacteria on the basic physiological parameters of plants, such as the intensity of photosynthesis and respiration as well as the synthesis of photosynthetic pigments and stress induction (Adamski and Kaminski, 2022). Some of the metabolites of cyanobacteria have a negative effect, while others have a positive effect on plant growth and development. Many studies have also shown the potential for cyanotoxin bioaccumulation in aquatic and terrestrial plants such as lettuce. Therefore, it seems crucial to monitor surface waters with cyanobacteria blooms, which are also used for irrigation of agricultural fields. Our equipment also allows us to confirm or exclude the presence of secondary metabolites of cyanobacteria in plant samples (Kucała et al., 2021).

Plans for the future

In the future, we plan to further develop our study on cyanobacteria and lichens, including in particular the search for new secondary metabolites with biotechnological applications and check the influence of selected physicochemical conditions or interaction with other organisms on the synthesis of selected metabolites. In the case of obtaining financing, our priority is also to expand the equipment with a compatible mass spectrometer, which will allow us to conduct comprehensive studies.

Note

For more information and references, please refer to the digital version of this edition.



Quick … not dirty!

Highly efficient sample preparation for dioxin and PCB analysis

Martin Meyer, Shimadzu Europa GmbH

Dioxins and polychlorinated biphenyls (PCBs) are extremely toxic substances that occur everywhere despite their danger. Therefore, the ability to reliably determine even the smallest quantities is particularly important. The Japanese company Miura has developed the GO-EHT system, which simplifies sample preparation for dioxins and PCBs considerably. In addition, the system fulfills the increasing number of legal requirements.

Scandals, attacks, accidents: Dioxins have gained notoriety as toxic substances. For example, the use of the dioxin TCDD – better known as Agent Orange – in the Vietnam War or the accident in a chemical plant in Seveso in 1976, in which almost 200 people contracted chloracne. More recent events that prompted public interest were the poisoning of the Ukrainian president Viktor Yushchenko in 2004 and the dioxin scandal in Germany in 2011, when dioxin levels in meat and eggs were 77 times higher than permitted levels.[1]

Dioxins and polychlorinated biphenyls (PCBs), as seen in Figure 1, are produced in a variety of thermal processes in the presence of chlorine, such as the manufacture of chemicals and waste incineration. In recent decades, the emission of these substances has been greatly reduced through filters and process optimization, however illegal waste incineration remains a large emission source of dioxin contamination.

Due to their chemical properties, dioxins and PCBs are very persistent. In animals, they are predominantly stored in the liver and adipose tissue, where they accumulate. Humans mainly ingest the substances in food of animal origin.[2] Given their high toxicity and prevalence, it is important for these substances to be detected even in small quantities. To do this, they must be isolated from the respective foodstuff so that the samples can then be analyzed using GC-MS/MS.

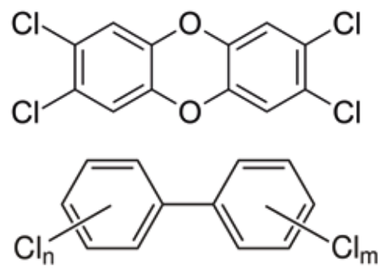


Figure 1: The dioxin TCDD (above) and the general structure of polychlorinated biphenyls (PCBs) (below)

Sample preparation for dioxins

Before the analysis, the test materials must undergo an extensive sample preparation procedure. Most samples must first be homogenized and freeze-dried. Then either solid-liquid extraction or liquid-liquid extraction takes place. The samples are then cleaned using chromatography columns. Up to three different manually packed columns must be used. This purification process removes many of the matrix components from the sample, such as fats and fatty acids. After the purification, the samples are usually concentrated by evaporating the solvent in order to improve the detection limit. In short: This type of sample preparation is very complex, and it may take several days before a measurement can be performed.

Due to ever stricter legal regulations stipulating lower and lower limit values, the requirements for dioxin analytics are increasingly high. It was decided only recently to tighten the dioxin limit values for waste material in the EU.[3] This also means that more and more measurements are needed. →

The Miura system is the solution

The Miura GO-EHT fully-automated sample preparation system reduces the preparation time for dioxin analysis to just over an hour. Moreover, the system is less labor-intensive, reduces labor costs and enables a higher throughput of samples. Specially prepared columns are used that are already packed and ready for purification. In addition to the 6-module design (Figure 2), the system is also available with 2 and 4 modules so that up to 6 samples can be managed at the same time via the user-friendly control unit.

Figure 2: Miura GO-EHT system

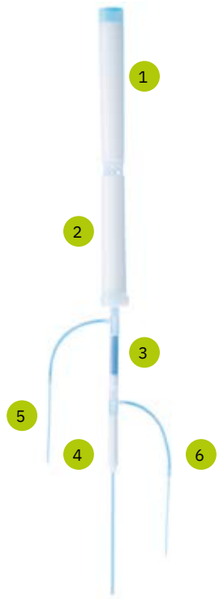


Figure 3: Columns for the Miura system
1) $AgNO_3$ silica gel column
2) H_2SO_4 silica gel column
3) Carbon column
4) Aluminum column
5) Dioxin fraction
6) PCB fraction

Sample processing with the Miura system is as follows:

First, the fat is extracted from the food manually or by using an extraction system (e.g. Büchi). The food extract is dissolved in hexane and placed on the Miura column. The columns are inserted into the system and the program is started. The purification, concentration and fractionation of dioxins and PCBs is completely automated. The first two columns 1 and 2 (Figure 3) serve to capture matrix components of the food samples so that only the dioxins and PCBs can proceed.

Column 3 contains special carbon that retains the dioxins through structural effects. The PCBs continue to column 4, where they are captured through polar interactions with aluminum oxide; the remaining matrix components are separated. Through the targeted use of toluene, dioxins and PCBs are extracted from the columns and collected in separate ampules. This fractionation means that dioxins and PCBs can subsequently be analyzed separately. Depending on the sample being tested, there are differences in the composition of the material, the fat content and the question of whether dioxins should be examined alone or in combination with PCBs. Thus, the columns are available in different dimensions (Table 1).

Full automation minimizes human error and always delivers reliable measurements. Heating the system improves the purification and accelerates the separation from other components. The columns designed for the system attach with a click and because they are used once, the columns do not need to be washed, which prevents cross-contamination. One of the greatest advantages is the significant reduction in solvent consumption. While other methods use up to 1,000 ml hexane, the GO-EHT system only requires around 100 ml. Moreover, the solvent dichloromethane (DCM) that is commonly used in other systems can be completely dispensed with when using the Miura system. This is another advantage because DCM is very toxic and heavily pollutes the environment.

Sample type	Environmental samples		
		Food samples	
Type	DXNmini 18 Ø	Standard 20 Ø	Mini 18 Ø
Sample	Environmental - EPA -	Food 3 g fat Environmental	Food 1 g fat Environmental
Purification (1)	$AgNO_3$ silica gel	$AgNO_3$ silica gel	$AgNO_3$ silica gel
Purification (2)	H_2SO_4 silica gel	H_2SO_4 silica gel	H_2SO_4 silica gel
Concentration (3)	Carbon	Carbon	Carbon
Concentration (4)	-	Aluminum oxide	Aluminum oxide
Solvent consumption (hexane)	85 ml	90 ml	85 ml
Run time	73 min	80 min	78 min

Table 1: Column parameters for GO-EHT

Analysis with the Shimadzu GC-MS/MS analysis package

The subsequent analysis can be performed in several ways. Historically, the market for dioxin analysis has long been dominated by gas chromatography (GC) with a high-resolution mass spectrometer (HRMS). Due to new regulations, it has also become possible to use triple quadrupole MS. For this reason, a GC-MS/MS method package was developed by Shimadzu to facilitate the analysis and subsequent reporting. The validity of the Shimadzu method has been confirmed by several independent laboratories.

Note
For more information and references, please refer to the digital version of this edition.

Conclusion

The Miura GO-EHT system offers many attractive advantages for sample preparation compared to the standard method. The combination of Miura preparation and Shimadzu GC-MS/MS provides ideal conditions for dioxin and PCB analysis.

VOICES

Custom GC helps researchers tackle the energy crisis

Shimadzu's custom gas systems enable research into alternative energy sources

Dr. Anna Cooper, Shimadzu UK

Despite the versatility of the gas chromatography (GC) systems and columns available off-the-shelf, the GC analysis of permanent gases remains a specialist field requiring a more bespoke approach. We talk to Peter Klawitter, who for nearly 20 years has been the in-house expert on these custom GC instruments at Shimadzu UK. As well as hearing from him what's involved in designing these systems, we discover why these bespoke GC systems are proving so useful and popular in the rapidly growing research field of "new energy".

Hello Peter! Can you start by describing what you do in the custom GC group at Shimadzu?
In our group, we devise, build and sell bespoke gas chromatography (GC) systems to the UK market. These are mostly for analysis of commonly encountered permanent gases such as hydrogen, oxygen, nitrogen, methane, carbon dioxide and carbon monoxide, but we also deal with some of the more obscure gases such as ammonia and nitrous oxide.

Figure 1: Custom GC expert Peter Klawitter with one of the team's bespoke instruments for the analysis of permanent gases, based on a Shimadzu GC-2030

Why are custom GC systems needed? Isn't it possible to devise a "one-size-fits-all" solution for gas analysis?
That's a fair point. Unlike the VOCs (volatile organic compounds) and SVOCs (semivolatile organic compounds) analysed by standard GC systems, permanent gases have a much greater variety of physical and chemical characteristics – what we call 'chemistries'. These really complicate separation and detection and limit the types of columns we can use.

So, for example, CO_2 poisons the commonly used molecular sieve columns, while FID (flame ionization detector) detection won't work for anything without a hydrocarbon bond.

Ammonia is a tricky one as well as it's pretty aggressive and so needs to be split off into its own detection and analysis line. And in the most challenging situations involving sulfur species, we have to refit the internals of the GC with specially treated alloys to stop the samples 'sticking' to the components!

As a result of all this, you typically need multiple columns and detectors for gas analysis as well as valving to separate out the target species, and carefully considered valve flows and timings to ensure optimum performance.

Essentially, this means that each time a customer comes to us with an application request, we need to create a customized GC system for them. The 'chemistries' just don't allow a 'one-size-fits-all' solution. ⟶

Apart from analyte chemistry, what other considerations are there in designing a new system? A major factor is the analyte level we're looking at. So, if the customer just wants to detect at levels of say 0.1% and above, then a thermal conductivity detector (TCD) is usually fine. But even then, we might have to think about the carrier gas because TCDs can't distinguish between hydrogen and helium. And, of course, we need to consider whether the detector might be overloaded by high-abundance compounds and so require the flow to be split. On the other hand, when the customer needs low detection limits, which is happening quite a lot now especially with our customers in academia, we'll need a more sensitive detector.

But ultimately, we make our system designs as simple as we can and deal with the necessary complexity as far as possible through the method. That way, our customers get the best system robustness, performance and flexibility.

When you build the custom GCs, do you start from a standard GC system? Yes, the core frame for the majority of our builds is the Shimadzu Nexis GC-2030. Its design allows all our additional valving to be fitted on top of the instrument. This frees up the left-hand side of the GC for coupling to an MS (mass spectrometry) detector, which is very handy for applications involving isotope work.

And as for detection, I've already mentioned TCD, but there's also FID for hydrocarbons and ECD (electron capture detector) for nitrous oxide. Plus, we have our own patented barrier ionization detector or BID – released back in 2014 – which offers much lower detection limits for the permanent gases.

That sounds like a really useful development for custom GC. How does it work? Yes, it's been a game-changer for us really. At the heart of the BID is a quartz dielectric chamber, which generates a helium plasma that ionizes pretty much everything that elutes from the GC. What

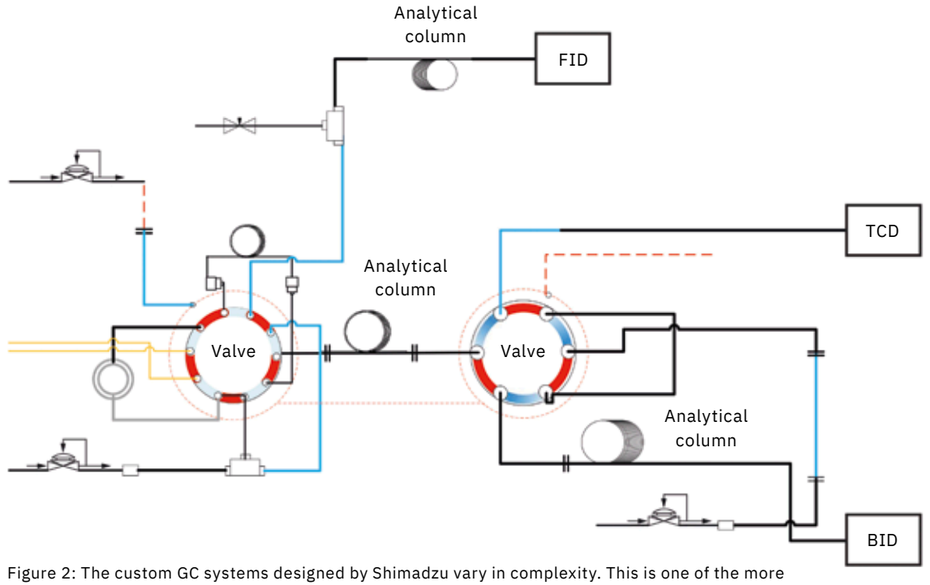


Figure 2: The custom GC systems designed by Shimadzu vary in complexity. This is one of the more intricate designs, involving two valves, three columns and three detectors.

makes the BID different from previous helium ionization detectors is that the electrodes have a dielectric coating, preventing the plasma from eating away at them and giving the detector much higher precision and excellent long-term stability.

But the main benefit for the analyst is the lower detection limits: the BID is 100 to 200 times as sensitive as TCD! When specifying systems for customers, I usually quote down to about 30 ppm for most of the permanent gases, but you can fairly easily get lower than that by optimizing the method once the system's up and running. We can also equip the Nexis GC-2030 with two BID detectors to get more out of each analysis.

To find out more about BID, visit https://www.shimadzu.eu/gc/nexis_technologies/bid →

◄ ► Figure 3: Close-up of the custom GC valving and flowpaths in place on top of the GC. An MS detector can be added, if needed.

What are the main applications for custom GC? Now, about 80% of the systems we're selling are for 'new energy' applications, with our primary customers being in academia. So, for example, we're delivering systems to analyze methane and other gases generated from catalytic or light-mediated reduction of CO_2 or for generating hydrogen from hydrocarbons for use in fuel cells. So, the thrust of the research is all about diversifying our energy sources and becoming less carbon-intensive in how we generate energy.

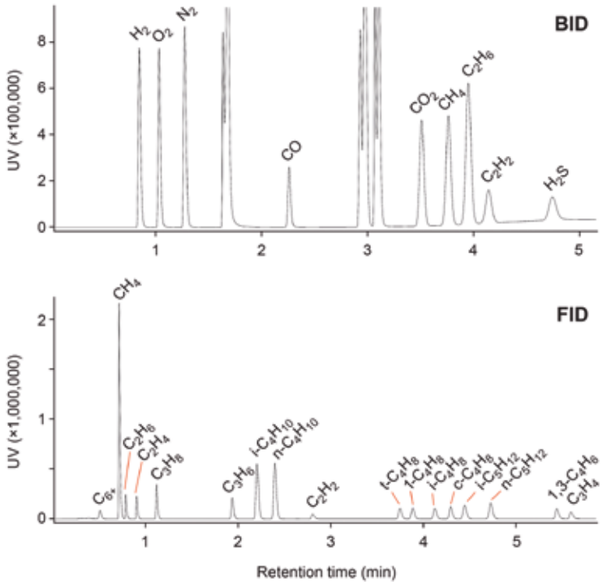


Figure 4: Example of an analysis conducted using a custom GC system with three sample loops. For full details please see Application Data Sheet No. 126 (see digital version).

What are the main challenges presented by such applications? A common theme through much of this research into 'new energy' is quantifying gases at a wide range of levels in one sample. So, for example, as well as bulk amounts of the gas you're synthesizing, you may be looking for trace levels of impurities. Determining these values is vital for optimizing a process and making the end product as pure as possible – which is obviously vital if it's going to be used on an industrial scale.

Another related application that has the same challenge is the analysis of greenhouse gases such as CO_2, methane and nitrous oxide – again requiring determination of levels from low percent down to parts per billion, all in one analysis. Having the BID detector has really opened up these applications to us because it allows us to get down to the really low levels that researchers want.

How does this compare to other systems on the market? Well, it's a different ball game, as they say. For example, many people are still using packed columns for gas analysis, which are fine for some purposes, but we've largely moved over to capillary columns now. The obvious benefits are better separation with faster runs, but they also work with smaller samples, which of course is perfect for lab-scale research. If customers have had previous experience with packed columns for gas analysis, then they're usually pretty impressed by what can be achieved with capillary systems, especially in conjunction with the BID detector.

And in terms of the overall service that you offer, what makes customers come back to you? I think it's really the bespoke service and the expertise we have – you don't get that with every supplier. We've been doing custom GC at Shimadzu for nearly 20 years now, so we've accumulated a lot of knowledge about how to design systems for pretty much every situation, and customers really appreciate that. But there are always new challenges, and what's great about many of our customers is that they're always looking to push the boundaries of what's possible, which keeps us on our toes! Looking to the future, we've built up a great team here now.

Like me, they're basically engineers with an analytical chemistry background, so they understand the customers' requirements and design systems the best way and test them in our demo lab. And we pre-define the methods for our customers too, so once the system is installed and the customer has received training in the software, they can be up and running very quickly.

To wrap things up, what do you love most about working for Shimadzu? That's a good question! I think it's really the ability to help people and the satisfaction you get from successfully carrying out a challenging project. I'm a problem-solver at heart and I love getting stuck into a new challenge and seeing it through to completion.

For example, an application we worked on recently was particularly tricky because the customer wanted to detect low-ppm levels of permanent gases produced by a very small reaction cell. It took a while to design and optimize the system, but they're very happy now. And that's rewarding in itself, but of course it's even better because we know we're ultimately helping research into technologies that will provide a greener and more secure energy supply for future generations.

Interested in speaking to the custom GC team about your application needs?
Get in touch at GC@shimadzu.eu!

Note
For more information and references, please refer to the digital version of this edition.

Award-winning innovation: the new iMScope QT

Perfect interaction for mass spectrometry imaging

Mass spectrometry and microscopy in perfect combination: With the new iMScope QT, Shimadzu is placing the only instrument in the world on the market that can provide mass spectra in conjunction with morphological information. Together with the sample preparation devices and software specially developed for mass spectrometry imaging, the instrument also impresses thanks to its optimal workflow and sophisticated design.

The iMScope QT combines a mass spectrometer with a built-in optical microscope and it is characterized by the best analysis speed and imaging capabilities. In addition to numerous other functions, the iMScope QT, in conjunction with the iMLayer, iMLayer AERO and IMAGEREVEAL, covers the entire workflow from sample preparation through to simple sample analysis and data analysis. This makes Shimadzu the only vendor in the world to offer this type of all-in-one solution.

The integrated microscope enables the iMScope to directly overlay the morphological image with the result of the MALDI-MS imaging. In this way, the distribution and concentration of molecules in tissue or organ sections can be discerned. Furthermore, by means of these results, conclusions can be drawn about the biological function in the tissue or morphological changes.

It can therefore be used in a meaningful way for medical and pharmaceutical applications as well as in agriculture and food science. For example, in cancer therapy, the iMScope QT can be used to verify whether a drug specifically accumulates in the tumor.

Perfectly adaptable to individual requirements

Users can easily connect or disconnect the mass spectrometer and MALDI microscope unit. If the microscope part is removed and a liquid chromatograph is installed, the system can be used as an LC-Q-TOF system.

In 2021, the new iMScope QT was awarded two of the most prestigious design prizes in the world: a Red Dot Design Award and an iF Design Award.

Note
For more information and references, please refer to the digital version of this edition.

The iMScope QT combines mass spectrometry imaging and microscopy

Newfound clarity for biopolymers

The new Nexera XS inert improves the analysis of biopharmaceuticals

The new bio-inert, biocompatible UHPLC system – developed together with Kyoto University – provides better reliability, robustness and expandability. The corrosion-resistant system is best suited to the analysis of biopolymers thanks to its metal-free sample flow path.

The new Nexera XS inert is a bio-inert and biocompatible ultra-high performance liquid chromatography (UHPLC) system. Shimadzu specifically developed the system to solve the most common problems in biopharmaceutical analysis, such as the adsorption of target molecules on metal surfaces and corrosion due to mobile phases with high salinity and extreme pH values. The development of the Nexera XS inert is based on the results of joint research with Professor Yasushi Ishihama of the Graduate School of Pharmaceutical Sciences at Kyoto University.

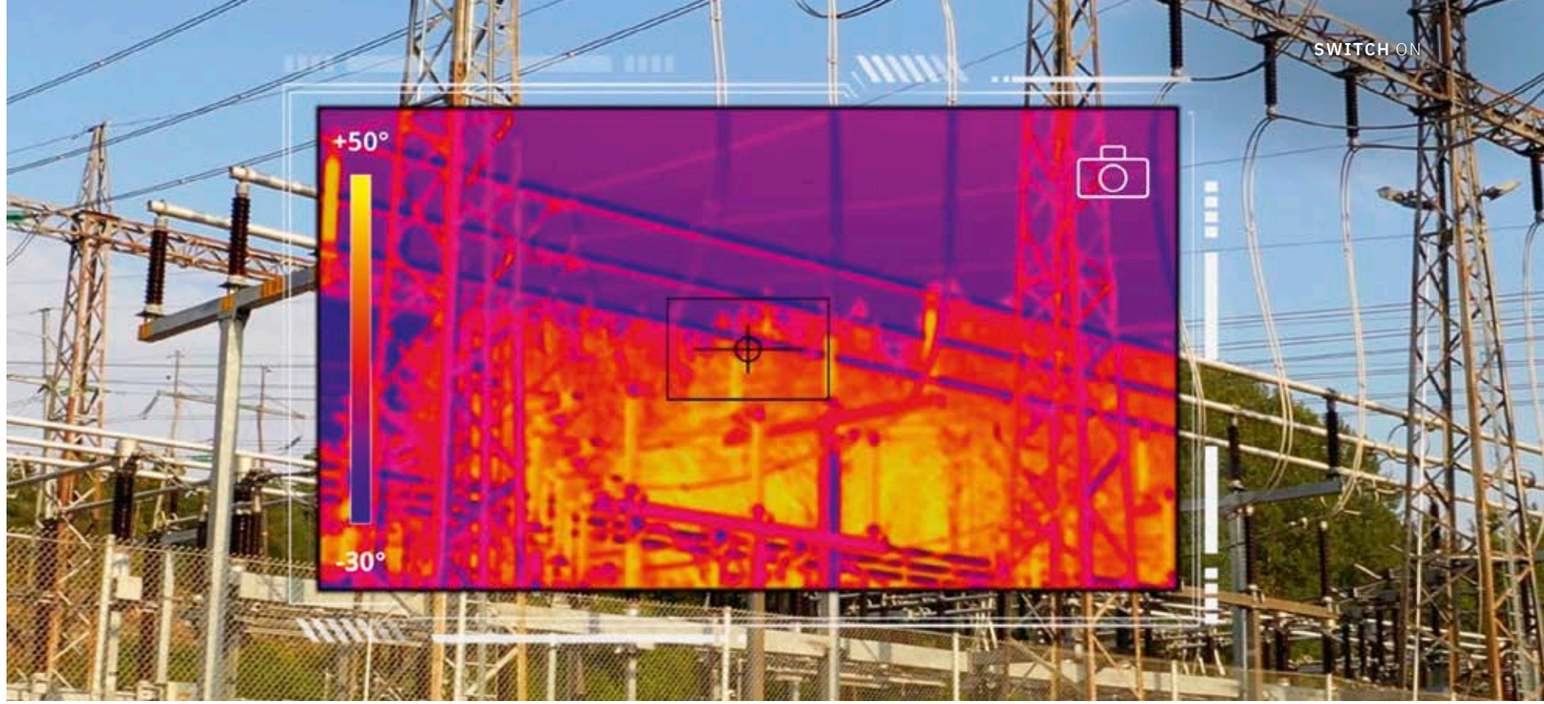
The system has a metal-free sample flow path that prevents unwanted interactions with biopolymers, which tend to be adsorbed at exposed metal sites. Therefore, the Nexera XS inert achieves reduced sample loss and excellent peak shape for reproducibly high sensitivity and high-quality, reliable data. In addition, all the contact surfaces are made from corrosion-resistant materials, which makes them stable against mobile phases that contain high concentrations of salts or acids.

There are also other new and advanced features such as bio-inert column switching, UHPLC connections that can be tightened by hand without additional tools (“finger tight”) and real-time pH monitoring.

Nexera XS inert provides the same outstanding reliability, robustness and expandability as the other UHPLC systems of the Nexera series. It is especially suited to the analysis of biopolymers such as antibodies, peptides and nucleic acid medicines.

Note
For more information and references, please refer to the digital version of this edition.

Bio-inert and biocompatible UHPLC: the Nexera XS inert



SWITCH ON

Safety first: rapid evaluation of insulating oil in electrical equipment

Shimadzu develops optimized UHPLC method for degradation products in insulating liquid

Dr. Gesa J. Schad, Shimadzu Europa GmbH

BTA, TTAA and furanic compounds in insulating oil are indicators of degradation of electrical equipment such as transformers. They are periodically monitored according to standard procedures described in BS148:2009 and ASTM D5837-15. This article describes a proof-of-concept study of the optimization of the two separate standard methods. The resulting new method allows for a time-saving simultaneous analysis using a single assay. Additional time can be saved by the simple transfer of the 30 min HPLC gradient method to a 5 min run using UHPLC conditions.

Protecting the energy grid

In electrical transformers and condensers, insulating oil and paper are used to insulate conductors and cool the interior of electrical devices. Long-term operation or exposure to heat can result in degradation of these insulators, which can ultimately lead to device failure. Therefore, periodic inspection to monitor any signs of degradation should be standard practice. Insulating paper is fixed to the device and difficult to remove, but insulating oil is easily collected and can be analyzed to verify device status.

Testing for degradation in transformers and condensers

Two substances are common additives in insulating oil, where they act as passivators (metal deactivators): BTA – 1,2,3-benzotriazole – and TTAA – tolutriazole derivative N-bis[2-ethylhexyl]-aminomethyltolutriazol. As both substances can also lead to sulfidation corrosion, quantification of BTA and TTAA is required as specified in the British Standards BS148:2009 [1], which describe an HPLC method for this purpose. Insulating paper used as a coating for windings in transformers and condensers is made of cellulose, which decomposes at high temperatures and under contact with water or oxygen. It then dissolves into furanic compounds. Hence, the concentration of furanic compounds in the insulating oil is an indicator of degradation of electrical equipment. ASTM D5837-15 [2] specifies an HPLC method for this analysis.

Optimizing the standard testing methods

This article introduces a proof-of-concept optimization of the methods described by ASTM and BS to offer simultaneous analysis of both passivators and furanic compounds in one assay [3]. As both methods were originally designed for a conventional HPLC system, the usual runtime is 30 min. By simple transfer to a UHPLC system, analysis time can be drastically reduced to approximately 5 min [4].

Analysis of the standard solutions

Figure 1 shows the chemical structures of BTA, TTAA and five furanic compounds. Standard solutions were prepared according to ASTM D5837-15. Furanic compounds, BTA and TTAA were weighed individually, dissolved in acetonitrile and then diluted with water. As there is no analytical standard TTAA commercially available, Irgamet® 39 (manufactured by BASF) was used instead, as noted in BS148:2009. Table 1 and Figure 2 respectively show the analytical conditions and the HPLC chromatogram of the standard solutions. As Irgamet® 39 is a mixture of two →

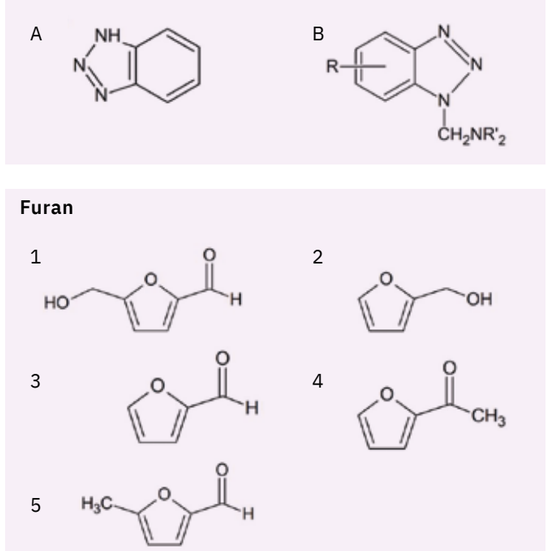


Figure 1: Structures of A: BTA, B: TTAA and 1: 5-hydroxylmethyl-2-furaldehyde (5HMF), 2: furfuryl alcohol (2FOL), 3: 2-furaldehyde (2FAL), 4: 2-acetylfuran (2ACF), 5: 5-methyl-2-furaldehyde (5MEF)

TTAA isomers, it elutes as two partly overlapping peaks. In this analysis, in accordance with BS148:2009, the combined value of peak areas of these two isomers was used for quantification.

System	Shimadzu i-Series (LC-2050)
Column	Shim-pack VP-ODS (250 mm × 4.6 mm I.D., 5 µm)
Flow rate	1.0 ml/min
Mobile phase	A) water B) acetonitrile
Time program	15% B (0 min) → 45% B (10 min) → 100% B (20 min) → 15% B (30 min)
Column temp.	40 ˚C
Injection volume	15 µL
Detection (PDA)	λ = 220, 260 and 280 nm

Table 1: Analytical conditions of the HPLC analysis of BTA, TTAA and furanic compounds

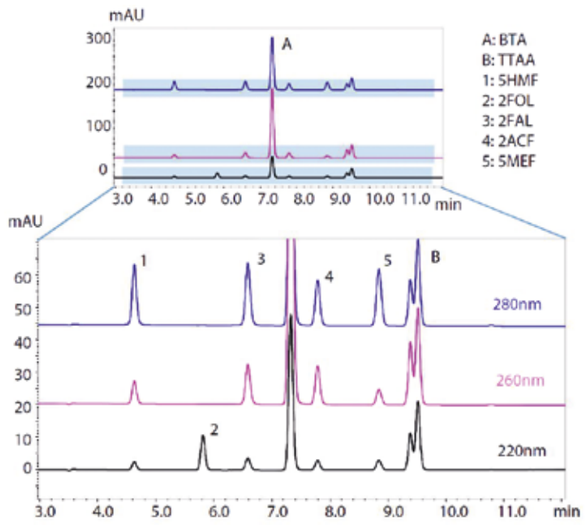


Figure 2: HPLC chromatogram of a standard solution of 20 mg/L (A) BTA and (B) TTAA and 1 mg/L of 1: 5HMF; 2: 2FOL; 3: 2FAL; 4: 2ACF and 5: 5MEF

5-point calibration curves for all compounds of interest were prepared using the standard solutions for BTA and TTAA in the concentration range of 0.1–20 mg/L and for the five furanic compounds in the concentration range of 0.005–1 mg/L. Good linearity with a regression coefficient r2 > 0.9999 was obtained for the determination of all components.

A recovery test was carried out by spiking a standard mixture of all compounds dissolved in toluene into white oil, followed by extraction using the pretreatment method described in ASTM D5837-15, as shown in Figure 3. The dilute supernatant was analyzed by HPLC. Acceptable recovery (≥ 79% for passivators and ≥ 106% for furanic compounds) and good reproducibility (%RSD ≤ 1.9) were achieved using this method.

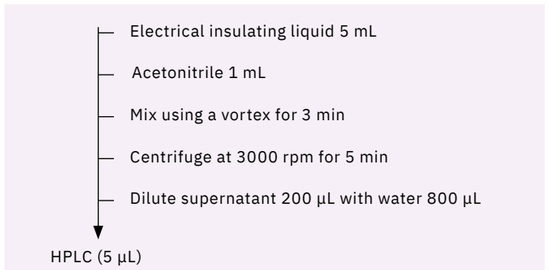


Figure 3: Sample pretreatment protocol according to ASTM D5837-15

Further optimization

After it was demonstrated that the passivators and furanic compounds in insulating oil can be analyzed simultaneously by optimizing the HPLC test method for furanic compounds specified in the ASTM D5837-15, further optimization was investigated by method transfer to UHPLC conditions.

Analytical conditions and standard chromatograms of the UHPLC analysis can be found in Table 2 and Figure 4 respectively. Baseline separation of the seven compounds of interest was achieved in only 1 min in a 5 min gradient run. Analytical conditions were adjusted for TTAA isomers to elute in one peak.

System	Shimadzu Nexera X3 UHPLC
Column	Shim-pack XR-ODS III (75 mm × 2.0 mm I.D., 1.6 µm)
Flow rate	0.7 ml/min
Mobile phase	A) water B) acetonitrile
Time program	20% B (0-0.3 min) → 90% B (1 min) → 100% B (3 min) → 20% B (5 min)
Column temp.	50 ˚C
Injection volume	5 µL
Detection (PDA)	λ = 220, 260 and 280 nm

Table 2: Analytical conditions of the UHPLC analysis of BTA, TTAA and furanic compounds

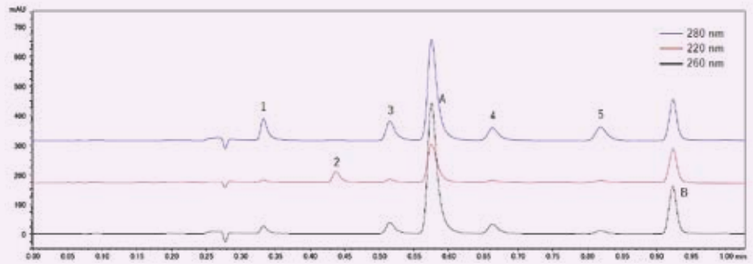


Figure 4: UHPLC chromatogram of a standard solution of 20 mg/L (A) BTA and (B) TTAA and 1 mg/L of 1: 5HMF; 2: 2FOL; 3: 2FAL; 4: 2ACF and 5: 5MEF

Linearity and repeatability

Calibration curves were prepared from standard solutions within the concentration range of 0.2, 1, 5, 10 and 20 mg/L for BTA and TTAA and in the range of 0.01, 0.05, 0.25, 0.50 and 1 mg/L for the furanic compounds. Repeatability was determined at the highest concentration for each analyte. With r2 > 0.9999 and %RSD ≤ 0.25% for all compounds under investigation, good linearity and repeatability could be proven.

Sample pretreatment and determination of recovery

All samples were pretreated following ASTM D5837-15, as shown in Figure 3. To ensure good recovery using this method, BTA, TTAA and furanic compound standards dissolved in toluene were spiked into white oil and extracted using the proposed protocol. The dilute supernatant was analyzed by UHPLC. As shown in Table 3, high recovery (≥ 86% for passivators and ≥ 97% for furanic compounds) and good reproducibility (%RSD ≤ 1.4) were achieved using the described pretreatment method.

Compound	Recovery (%)	Reproducibility (%RSD)
BTA (A)	89	0.85
TTAA (B)	86	0.84
5HMF (1)	98	0.78
2FOL (2)	102	0.71
2FAL (3)	99	1.2
2ACF (4)	99	0.85
5MEF (5)	97	1.4

Table 3: Recovery (%) and reproducibility (%RSD) (n = 3) of the extraction of BTA, TTAA and furanic compounds from white oil following ASTM D5837-15

Conclusion

Proof of concept was successful. First, a fast and simple HPLC method for simultaneous analysis of passivators and furanic compounds in insulating oil was developed by optimizing the test method for furanic compounds specified in ASTM D5837-15. Even though ASTM D5837-15 and BS148:2009 specify separate analytical methods for quantification of BTA, TTAA and furanic compounds, separation of the seven analytes from each other and impurities in insulating oil could be readily achieved [1]. And second, a method transfer to UHPLC conditions showed a further reduction in the time needed for analysis. Using UHPLC, quantification of all analytes of interest could be done in one 5 min gradient assay, using the proposed conditions.

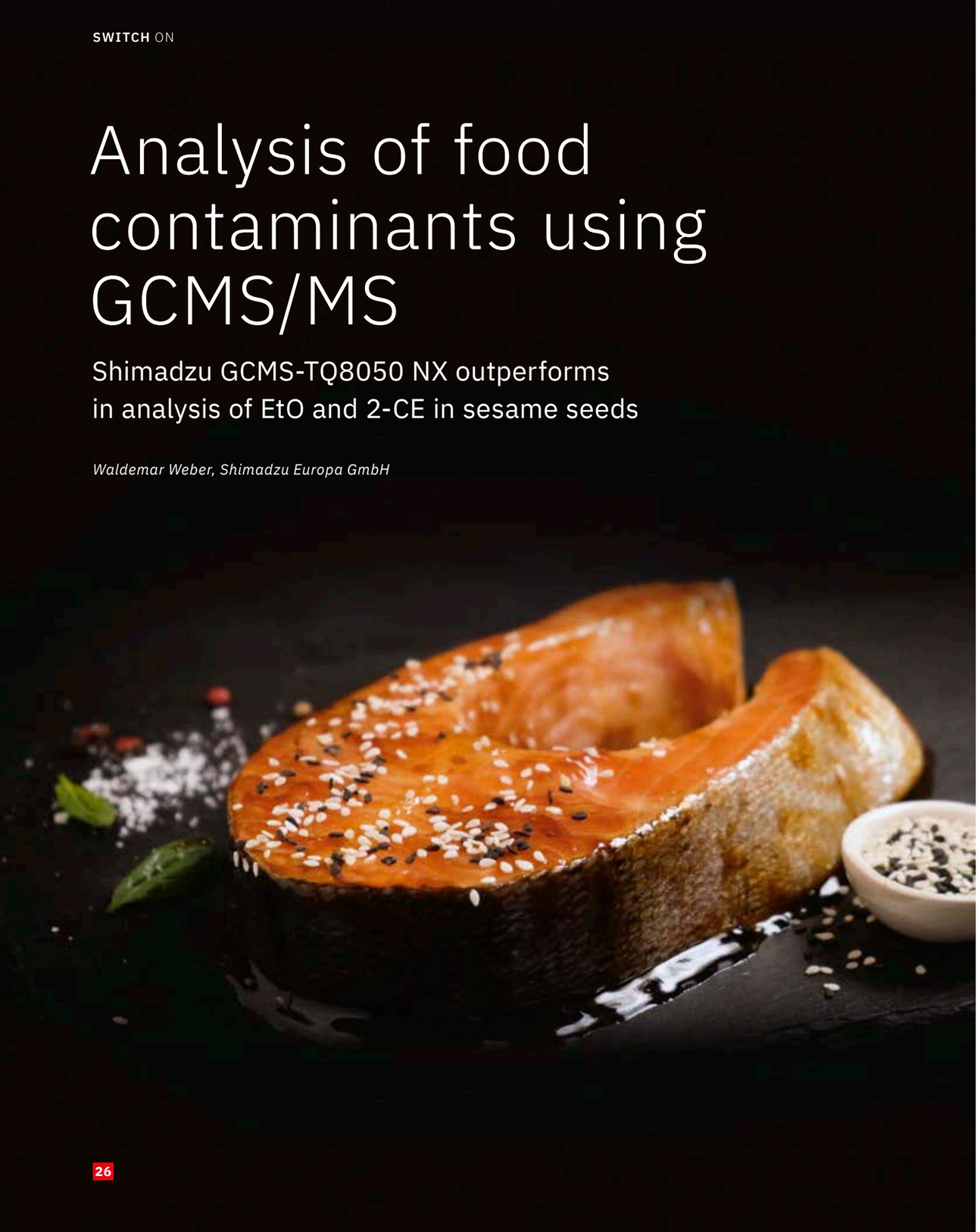
Sometimes relatively small steps can lead to large advances. In this case, keeping electrical transformers and condensers working well is now a lot easier to do.

Note
For more information and references, please refer to the digital version of this edition.

Analysis of food contaminants using GCMS/MS

Shimadzu GCMS-TQ8050 NX outperforms in analysis of EtO and 2-CE in sesame seeds

Waldemar Weber, Shimadzu Europa GmbH



Ethylene oxide (EtO) is heavily used in the food industry because of its high diffusivity and strong penetrating properties, making it very effective in the disinfestation and/or disinfection of dry food commodities. However, EtO is highly dangerous to people, and it is essential to quantify the amount of EtO in food matrices. Tests of EtO and 2-CE trace levels in sesame seeds using the Shimadzu GCMS-TQ8050 NX with AOC-20i liquid autosampler and HS-20 NX dynamic headspace sampler outperformed both liquid injection techniques and current regulatory limits.

Ethylene oxide: a very useful substance …

Ethylene Oxide (EtO, C_2H_4O) is a colorless, flammable gaseous cyclic ether with a slightly sweet smell. It is heavily used in the food industry due to its very strong antibacterial properties. Its high diffusivity and strong penetration make it very effective in the disinfection of dry food, almost 10 times more effective than methyl bromide and phosphine.

… and a very toxic and heavily regulated substance

However, EtO is also highly carcinogenic, mutagenic and genotoxic for living beings. As a result, it is heavily regulated. For instance, the EU's European Chemicals Agency (ECHA) classifies EtO in category 1B and it lists EtO in Health Hazard category 3 regarding its acute toxicity. For these reasons, it is essential to exactly quantify the amount of EtO in food matrices.

EtO fumigation of sesame seeds

Commodities such as spices, oilseeds and nuts are especially susceptible to EtO/2-CE post-fumigation and combine high lipid content with low water content. One example is sesame seeds, where EtO fumigation is used to reduce contamination with salmonella and other fecal bacteria.

After fumigation with EtO, evaporation and reactions with matrix constituents are the main dissipation pathways of EtO in food. EtO undergoes various reactions within the matrix and generates a number of reaction products, including ethylene glycol, diethylene glycol, 1,4-dioxane, 2-bromoethanol and ethylene chlorohydrin (2-CE). 2-CE is the most prominent reaction product of EtO and is itself an extremely hazardous substance.

Naturally, EtO fumigation requires strict quality control. Neglect can result in cases such as the 2020 discovery in India where EtO was still above the regulated limits in treated sesame seeds.

Improving analytical methods through experimentation

With EtO so useful yet so toxic to people, it becomes imperative to ensure the trace amounts left in foods are well within safe guidelines for human consumption. Traditionally, laboratories testing for traces of EtO and 2-CE have employed two time-consuming, manual extraction methods: A) the QuEChERS method (EN15662) and B) the QuOil method (CEN/TS 17062:2019 modified). Both methods require extraction procedures with the only difference in the used materials.

The experiment – Extracted solutions using these methods were analyzed using GCMS/MS equipped with a liquid sampler. Some matrices require clean-up reagent optimization, which can have a variable effect on extraction efficiency. To overcome these difficulties, three different dynamic headspace methods were developed and optimized using a GCMS-TQ8050 NX system with a HS-20 NX headspace sampler (Figure 1). That provided a more precise determination of the analytes present.

A mixture of EtO and 2-CE standards (2 ppm) was analyzed using scan-mode for identification. Steps such as precursor ion selection and Multiple Reaction Monitoring (MRM) optimization at different Collision Energies (CE) were performed. A method with segmented MRM and optimum CE energies was thereby created. The optimized MRM transitions of EtO and 2-CE standards are shown in Table 1.

Analytical conditions – The description of analytical conditions for liquid injection and three headspace methods are collected in Table 2 and Table 3. ⟶

Figure 1: GCMS-TQ8050 NX system

Method of sample preparations – The sample preparations for the four methods of analysis are slightly different and include up to six steps. Table 4 illustrates the steps used for EtO and 2-CE extraction from the sample.

The result – Trace level quantification of EtO and 2-CE impurities in sesame seeds was successfully performed using the Shimadzu GCMS-TQ8050 NX with AOC-20i liquid autosampler and HS-20 NX headspace sampler (dynamic). The extraction method used allowed for a good recovery of 73–102% for both analytes with an RSD of 2–9%. The lowest possible quantification limit (LOQ) is dependent upon the specific sample preparation method used, but was in every case in the range 0.5–10 ppb. The quantitative values calculated for the sesame seed samples are shown in Table 5.

MRM transitions						
Analyte	MRM-1	CE	MRM-2	CE	MRM-3	CE
EtO	44 > 29	6	44 > 28	6	44 > 14	18
2-CE	80 > 31	6	80 > 44	5	82 > 31	6

▲ Table 1: MRM transitions of EtO and 2-CE

Method 1 (liquid)	EtO and 2-CE
Method 2 (HS)	EtO and 2-CE
Method 3 (HS)	2-CE only
Method 4 (HS)	EtO only

▲ Table 2: Analytical conditions: injection methods developed

▶ Table 3: Analytical conditions for HS GCMS/MS system

System configuration	
GCMS system	GCMS-TQ8050 NX
Liquid sampler	AOC-20i / AOC-20s
Headspace sampler	HS-20 NX (Dynamic headspace)
Gas chromatography parameters	
Capillary column	RTX-VMS (60 m x 0.45 mm ID x 2.55 µm df)
Injection mode	Split
Flow control mode	Column flow
Carrier gas	Helium
Carrier gas	3 mL/min
Split ratio	1:5 for liquid injection
Temp. program	35 °C for 5 min, 20 °C/min to 235 °C, 235 °C for 5 min
MS Parameter	
Ionization mode	EI
Ion source temp.	230 °C
Interface temp.	230 °C
Mode	MRM

Headspace parameters & split ratio	Method 2	Method 3	Method 4
Oven temperature	115 °C	110 °C	115 °C
Sample line temperature	120 °C	120 °C	120 °C
Transfer line temperature	130 °C	130 °C	130 °C
Trap cooling temperature	–10 °C	–10 °C	–10 °C
Trap desorb temperature	280 °C	260 °C	280 °C
Equilibrating time (min)	15	15	15
Pressurizing time (min)	0.5	0.5	0.5
Split ratio	20	5	20
Total flow (mL)	66	21	66

▼ Table 4: Sample preparation for extraction of EtO & 2-CE from sesame

Method	Liquid injection 1 EtO and 2-CE	Headspace method 2 EtO and 2-CE	Headspace method 3 2-CE only	Headspace method 4 EtO only
1	5,000 mg sesame seeds + 10,000 µL acetonitrile mixed for 15 min	1,000 mg sesame seeds + 1,000 µL acetonitrile mixed for 15 min	100 mg sesame seeds + 1,000 µL acetonitrile mixed for 15 min	5,000 mg sesame seeds + 5,000 µL acetonitrile mixed for 15 min
2	Centrifuge at 5,000 rpm for 5 min at 10 °C			
3	5,000 mL of supernatant transferred into 15 mL Tarson tube	Removed 100 uL solution, transferred into 20 mL HS vial		Removed 1,000 uL solution, transferred into 20 mL HS vial
4	Add QuEChERS and vortex for 5 min	Not required		
5	Centrifuge for 5 min at 10 °C	Not required		
6	Removed supernatant can be used for GCMS analysis			

▼▼ Table 5: Summary of comparison data

Method	Liquid injection		Headspace injection			
	Method 1		Method 2		Method 3	Method 4
	EtO	2-CE	EtO	2-CE	2-CE	EtO
LOQ level conc. (on column)	5 ppb	5 ppb	10 ppb	10 ppb	0.5 ppb	6 ppb
LOQ level conc. (w.r.t. sample)	10 ppb	10 ppb	10 ppb	10 ppb	5 ppb	6 ppb
%RSD (n = 6)	7.7	9.4	2.1	4.9	9.1	1.7
R2	0.99889	0.99917	0.99950	0.99785	0.99974	0.99906
Sample preparation time	35–40 min		20–25 min			
Costs	QuEChERS required		QuEChERS not required			

Conclusion: outperforming current regulatory limits

A simpler, faster, more robust (and, by extension: cheaper) method of quantitative analysis of EtO and 2-CE in sesame seeds could be developed. Using a dynamic headspace-based mode, the achieved results outperformed current regulatory limits and offered advantages over liquid injection techniques, including easier sample preparation, less matrix interference and more precise quantitation. In addition, the Shimadzu GCMS-TQ8050 NX features a new, highly efficient detector and superior noise reduction technology to enhance sensitivity and enable quantitation of EtO and 2-CE – even at trace levels.

Note
For more information and references, please refer to the digital version of this edition.

MOVE ON

Discovering how our ancestors used to live

Using HPLC-MS and GC-MS approaches in archaeology to reveal ancient lipid species

Valentina Chiaia, PhD Danilo Donnarumma, PhD Giuseppe Micalizzi, PhD Anna Irto, Prof. Clemente Bretti, Prof. Paola Cardiano, Prof. Luigi Mondello, University of Messina

Lipids are the main chemical class of biomolecules studied in ancient pottery, due to their stability over time compared to other organic compounds such as carbohydrates and proteins. However, alteration processes such as thermal decomposition, oxidation and hydrolysis may occur. An artificial ageing approach has been performed to simulate the degradation processes of lipids occurring in archaeological pottery. Lipid species were separated and identified by using HPLC-MS and GC-MS methodologies.

Reviving ancient daily life by tracing lipids in pottery

The analyses of organic residues in archaeological pottery are fundamental to unveiling crucial hints about the daily life of ancient societies. Pottery vessels absorb organic material and preserve it totally or partially during burial over millennia, hereby providing valuable insight about the use of various natural resources. Analytical investigations for traces of organic matter, visible organic residues, surface deposits or encrustations can establish not only their origin but also the function and the use of the pottery vessels. The attention of analysts is particularly focused on lipid compounds due to their greater stability over time compared to other organic compounds such as carbohydrates and proteins [1]. The hydrophobic character of lipids, in fact, limits their percolation over the centuries and lets them stay in the original site. Although the hydrophobic character of lipids could make them excellent candidates for “archaeological biomarkers” [3], the presence of reactive functional groups along the molecular structures may lead to their alteration. In addition, the preservation of lipid matter strongly depends on the environmental condition and on the material in which they are trapped.

A peek in the pots of ancient cuisine – distinguishing marine and terrestrial products

The simulation of thermal decomposition, oxidation or hydrolysis of lipids can provide key elements to better interpret the origin of animal fats and vegetable oils partially or totally altered over time [3]. In the past, accelerated ageing studies have already been carried out in order to simulate lipids’ natural alteration occurring in the archaeological site. For instance, Colombini et al [6] established that main oxidation products of monounsaturated fatty acids (MUFAs) as gondoic acid (C20:1n9) and erucic acid (C22:1n9), particularly abundant in Brassica juncea seed oil, were (α,ω)-undecanedioic and (α,ω) tridecanedioic acids. This means that the molecular structure of oxidation products strongly depends on the location of the double bond along the carbon chain of the native fatty acid.

Combining GC and HPLC with MS for the separation of aged lipid species

Ageing studies generally involve the use of chromatographic techniques with a high resolving power coupled to mass spectrometry (MS). The alteration/degradation products are initially separated based on their interaction with mobile and stationary phases and then detected in the MS dimension. The relative retention time (linear retention index, LRI) and fragmentation pattern can therefore be used in order to unambiguously reveal the identity of unknown lipid compounds. The main aim of this research study is the development of analytical methodologies as gas chromatography (GC) and high-performance liquid chromatography (HPLC) coupled to mass spectrometry (MS) for the separation and identification of aged lipid species. In-lab thermal-oxidative treatments have been carried out in order to simulate the natural degradation of olive oil, traditionally used in Mediterranean ancient societies. ⟶

Materials and methods

Tristearin (SSS), triolein (OOO) and trilinolein (LLL) standards (10 mg) were kept in an oven at 120 °C for three weeks. The thermo-oxidative treatment was also performed on 10 mg of extra-virgin olive oil.

Aged intact lipids were initially diluted in 2-propanol (1,000 mg L^{-1}) and 1 µL was directly injected into an Ultra High-Performance Liquid Chromatograph (UHPLC) Nexera X2 system, including two LC-30 AD dual-plunger parallel-flow pumps, a DGU-20A5R degasser, a CTO-20AC column oven and a SIL-30AC autosampler. The UHPLC system was coupled to a LCMS-8060 triple quadrupole mass spectrometer equipped with APCI interface. The separation was achieved by Non-Aqueous Reversed Phase High-Performance Liquid Chromatography (NARP-HPLC) using 2 Ascentis Express C18 columns (100 mm L × 2.1 mm ID, 2.7 µm particle size). The mobile phase consisted of: (A) acetonitrile and (B) 2-propanol, with a flow rate of 0.4 mL min^{-1}. Chromatographic separation was performed according to the following gradient elution: 0–105 min, 0–50% B, held 20 min. MS parameters were as follows: *m/z* range 250–1200; event time 0.2 s; nebulizing gas (N_2) flow rate 3 L min^{-1}; drying gas (N_2) flow rate 5 L min^{-1}; interface temperature 350 °C; DL temperature 200 °C; heat block temperature 200 °C. Data were collected and processed using the LabSolution software. For LRI calculation, the odd carbon number triacylglycerol (TG) mixture from C9C9C9 to C17C17C17 was used as reference homologue series [9].

GC analyses

Aged lipids were initially converted into trimethylsilyl (TMS) derivatives in order to increase volatility and reduce polarity. In this respect, all samples were derivatized with 200 µL of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) reagent containing 1% of trimethylchlorosilane (TMCS) and 500 µL of dichloromethane (CH_2Cl_2). Then, the reaction mixture was heated for 20 min at 80 °C. Separation and identification of fatty acids were performed on a GCMS-QP2020NX system. The injection of the samples was carried out in an automatic way by using an AOC-20i autosampler. A split/splitless injector was installed on the GCMS instrument. The separation of TMS derivatives was achieved by using a non-polar capillary column, namely SLB-5ms (equivalent in polarity to poly-5% diphenyl/95% dimethyl siloxane-phase) 30 m x 0.25 mm ID x 0.25 µm df. The temperature program was: 50 °C to 360 °C at 3 °C min^{-1}. Injection volume and split ratio were: 0.5 µL in splitless mode. Helium was used as carrier gas with a constant linear velocity of 30 cm sec^{-1}. MS parameters were: electronic ionization (70 eV); ion source temperature: 250 °C; mass range: *m/z* 40–650; interface temperature: 250 °C. A C7–C40 saturated alkanes standard mixture was used for LRI calculation. The GCMS solution software was used for both data acquisition and processing.

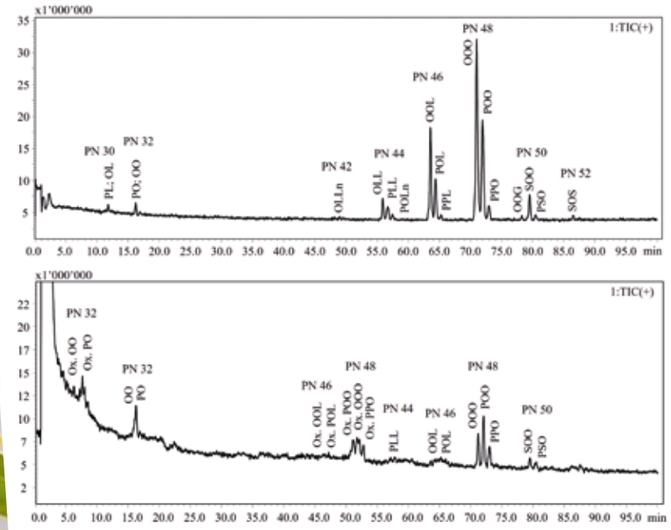
Results and discussion

In the present work, a NARP-HPLC method coupled to MS via APCI interface was applied for the analysis of intact lipids in TG standards and extra-virgin olive oil subjected to thermal-oxidative treatments. Figure 1 shows the HPLC-MS chromatograms of the intact lipids in not aged and aged olive oils. Lipid species were identified according to Oteri et al. [9], in which two different identification criteria, MS fragment patterns and LRIs, were used. In Table 1 are listed the identified lipids and retention times (RT), detected ions, experimental and reference LRIs for each species. A lab-constructed database containing LRI values was utilized to evaluate the correspondence of experimental LRIs.

NARP-HPLC-APCI-MS analysis revealed the formation of oxidized lipid species, characterized by a mass increasing of 16 Dalton (molecular weight of an oxygen atom) as reported in Table 1. ⟶

RT	$[M-H_2O+H]^+$	$[M+H]^+$	$[M+NH_4]^+$	Diagnostic fragments	Class	CN:DB	Compound	PN	LRI_{exp}	LRI_{ref}	Not aged	Aged
7.92	593.5	611.5		313.3 355.3	Ox. DG	34:1	Ox. PO	32	2,844	-	nd	x
8.28	619.5	637.5		339.3 355.3	Ox. DG	36:2	Ox. OO	32	2,865	-	nd	x
12.13	575.5			313.3 337.3	DG	34:2	PL	30	3,089	-	x	nd
12.13	601.5			337.3 339.3	DG	36:3	OL	30	3,089	-	x	nd
16.72	577.5			313.3 339.3	DG	34:1	PO	32	3,327	-	x	x
16.72	603.5			339.3	DG	36:2	OO	32	3,327	-	x	x
47.84		899.7	916.7	617.5 619.5	Ox. TG	54:4	Ox. OOL	46	4,144	-	nd	x
49.31		873.7	890.7	591.5 593.5 617.5	Ox. TG	52:3	Ox. POL	46	4,178	-	nd	x
49.68		879.7	896.7	597.5 599.5 601.5	TG	54:6	OLLn	42	4,187	4,192	x	nd
52.38		875.7		593.5 619.5	Ox. TG	52:2	Ox. POO	48	4,249	-	nd	x
53.07		901.7	918.7	619.5	Ox. TG	54:3	Ox. OOO	48	4,265	-	nd	x
54.05		849.7		593.5	Ox. TG	50:1	Ox. PPO	48	4,288	-	nd	x
56.65		881.7	898.7	599.5 601.5	TG	54:5	OLL	44	4,348	4,342	x	nd
57.44		855.7	872.7	575.5 599.5	TG	52:4	PLL	44	4,366	4,358	x	x
58.25		855.7	872.7	573.5 577.5 599.5	TG	52:4	POLn	44	4,385	4,383	x	nd
64.28		883.7	900.7	601.5 603.5	TG	54:4	OOL	46	4,528	4,516	x	x
65.16		857.7	874.7	575.5 577.5 601.5	TG	52:3	POL	46	4,551	4,539	x	x
66.06		831.7	848.7	551.4 575.4	TG	50:2	PPL	46	4,574	4,571	x	nd
71.69		885.7	902.7	603.5	TG	54:3	OOO	48	4,722	4,729	x	x
72.73		859.7	876.7	603.5 577.5	TG	52:2	POO	48	4,749	4,756	x	x
73.81		833.7	850.7	551.5 577.5	TG	50:1	PPO	48	4,777	4,776	x	x
78.91		913.8	930.8	603.5 631.5	TG	56:3	OOG	50	4,911	4,905	x	nd
80.23		887.7	904.7	603.5 605.5	TG	54:2	SOO	50	4,945	4,948	x	x

Table 1: List of lipid species identified in not aged and aged olive oils (x: detected; nd: not detected)
Note: Ox. DG: oxidated diacylglycerol; DG: diacylglycerol; Ox. TG: oxidated triacylglycerol; TG: triacylglycerol.
Abbreviations: RT: retention time; $[M-H_2O+H]^+$, $[M+H]^+$, $[M+NH_4]^+$ and diagnostic fragments: detected ions; Class: lipid class; CN : DB: carbon number: double bond; PN: partition number; LRI_{exp}: experimental LRI; LRI_{ref}: reference LRI.



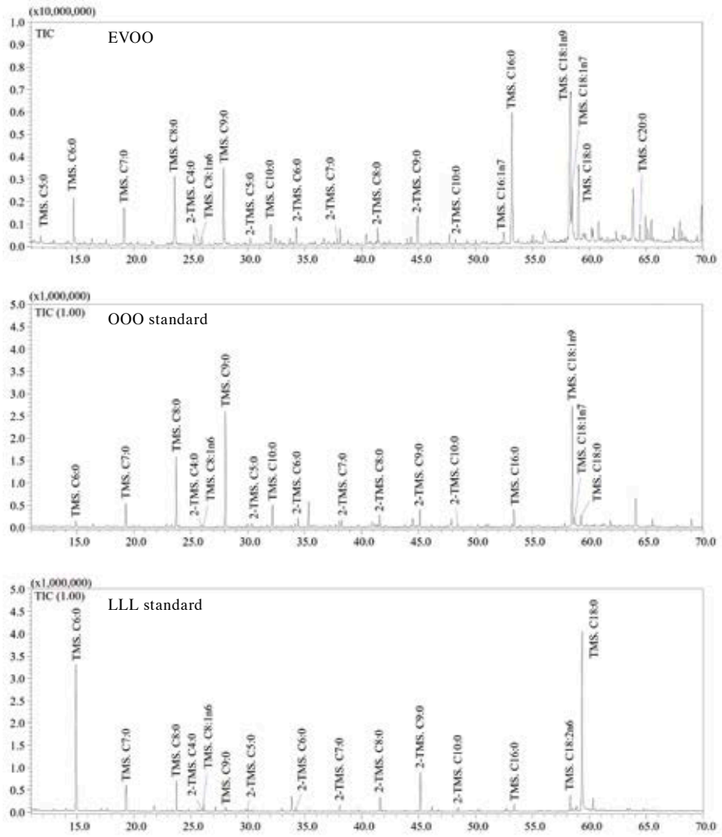


Figure 1: HPLC-MS chromatograms of intact lipid detected in not aged (upper) and aged (bottom) extra-virgin olive oils

Note: Ox. DG: oxidated diacylglycerol; DG: diacylglycerol; Ox. TG: oxidated triacylglycerol; TG: triacylglycerol

Abbreviations: P: palmitic acid; S: stearic acid; O: oleic acid; L: linoleic acid; Ln: linolenic acid; A: arachidic acid; G: gondoic acid

A significant loss of the most abundant TGs, such as triolein (OOO), was observed, probably related to the formation of free fatty acids as confirmed by the GC analyses.

GC-MS analyses confirmed that thermo-oxidative treatments caused the formation of high levels of short-chain fatty acids (SCFAs) containing a number of carbon atoms ranging from 5 to 9 units. Hexanoic (C6:0), heptanoic (C7:0), octanoic (C8:0) and nonanoic (C9:0) acids were the most abundant SCFAs in aged olive oil as shown in the GC-MS chromatograms illustrated in Figure 2. The thermo-oxidative treatment implied the inclusion of an oxygen atom in the carbon chain, the scission of the double bond and formation of lower molecular weight species, as also reported by Colombini et al. [6]. For instance, the detection of C9:0 in aged olive oil was due to the scission of the double bond in the C9-position of the oleic acid (C18:1n9), while the high amount of C6:0 was due to the scission of the double bond in the C6-position along the carbon chain of the linoleic acid (C18:2n6) (Figure 2).

Figure 2: GC-MS chromatograms of aged extra-virgin olive oil, triolein and trilinolein standards

With regards to tristearin, the GC-MS chromatogram (not reported) revealed the high stability of the compound due to absence of double bonds along the carbon chain. Therefore, fatty acids containing at least one double bond are more susceptible to the oxidative stress. Finally, (α,ω)-dicarboxylic acids such as adipic, pimelic, suberic, azelaic and sebacic acids were also detected in aged samples (Figure 2), according to literature data [6]. Considering their lower volatility compared to SCFAs, (α,ω)-dicarboxylic acids may be considered as potential archaeological biomarkers, although further investigations will be needed to find characteristic biomarkers for olive oil. In Table 2 the list of fatty acids (as TMS derivatives) detected in analyzed samples is reported.

Uncovering the secrets of ancient life left behind

Both HPLC-MS and GC-MS analytical methodologies were used for the elucidation of the main oxidation and degradation products in extra-virgin olive oil subject to the thermo-oxidative treatment. Two different identification criteria, MS fragment patterns and LRI, were used to reveal the exact identity of chromatographic peaks. Such strategy will allow to determine putative archaeological biomarkers, useful to better interpret the origin of animal fats and vegetable oils used by ancient societies that are partially or totally altered over time. In particular, the HPLC-MS approach allowed to evaluate the oxidation at intact lipids level, while the GC-MS methodology was pivotal for the identification of species at low molecular weight such as SCFAs and (α,ω)-dicarboxylic acids.

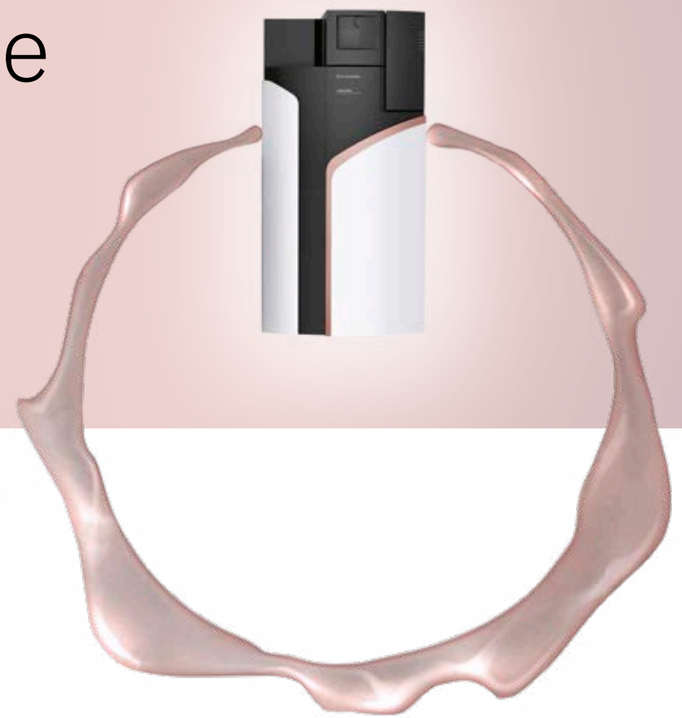
Note

For more information and references, please refer to the digital version of this edition.

Compound	MS similarity	LRI_{exp}	LRI_{ref}
Pentanoic acid; TMS. C5:0	89	980	974
Hexanoic acid; TMS. C6:0	93	1,072	1,069
Heptanoic acid; TMS. C7:0	93	1,167	1,166
Octanoic acid; TMS. C8:0	92	1,262	1,262
Succinic acid; 2-TMS. C4:0	92	1,312	1,310
2-Octenoic acid; TMS. C8:1n6	93	1,316	-
Nonanoic acid; TMS. C9:0	89	1,359	1,358
Pentanedioic acid; 2-TMS. C5:0	90	1,404	1,403
Decanoic acid; TMS. C10:0	90	1,456	1,456
Adipic acid; 2-TMS. C6:0	88	1,504	1,503
Pimelic acid; 2-TMS. C7:0	90	1,602	1,601
Suberic Acid; 2-TMS. C8:0	88	1,696	1,694
Azelaic Acid; 2-TMS. C9:0	91	1,795	1,792
Sebacic acid; 2-TMS. C10:0	90	1,891	1,889
Palmitelaidic acid; TMS. C16:1n7	96	2,023	-
Palmitic acid; TMS. C16:0	89	2,045	2,043
Linoleic acid; TMS. C18:2n6	96	2,207	-
Stearic acid; C18:0	93	2,168	-
Oleic acid; TMS. C18:1n9	93	2,215	2,207
Cis-vaccenic acid; TMS. C18:1n7	95	2,222	2,226
Stearic acid; TMS. C18:0	97	2,243	2,237
Arachidic acid; TMS. C20:0	96	2,440	2,437

Table 2: List of fatty acid compounds detected as TMS derivatives in aged samples
Abbreviations: MS similarity: mass spectral similarity; LRI_{exp}: experimental LRI; LRI_{ref}: reference LRI

More time for real research



LCMS-9050: the fastest product on the market for polarity switching

The new LCMS-9050 Q-TOF: high mass accuracy thanks to high stability and low-maintenance design

The quadrupole Time-of-Flight mass spectrometer LCMS-9050 has very high mass accuracy with a high-precision temperature control system that prevents even minimal mass differences caused by external factors. This allows mass ranges to be measured precisely without the need for recalibration. Thanks to its ease of use, the LCMS-9050 also makes working with the acquired data all the more productive.

The innovative LCMS-9050 Q-TOF sets new records in four categories: High Mass Accuracy, High Stability, High Speed and Highest Sensitivity. Even minimal mass differences influenced by external causes are now a thing of the past. The system measures with high accuracy over long periods of time without repeated mass calibration. The highly efficient high-speed polarity switching technology enables the simultaneous analysis of positive and negative ions, thus contributing to increased analysis efficiency. Identification and structural analysis are less likely to be ambiguous.

The new LCMS-9050 delivers maximum reliability and automation. The focus is clearly on data analysis, as very little time is required for calibration and maintenance. In addition, the LCMS-9050 delivers faster results than other MS/MS devices thanks to its top speed.

Of course, the LCMS-9050 can be combined with other well-known and high-quality accessories from Shimadzu and is also available as an upgrade for the LCMS-9030. In this way, the full potential of analytical possibilities can be utilized, and the volume of data can be significantly expanded.

Note
For more information and references, please refer to the digital version of this edition.

SUXESS

The IRXross FTIR spectrophotometer – the new reference

Increased sensitivity, resolution and analysis speed – for high-quality data in less time

Shimadzu is celebrating a new FTIR spectrophotometer: The IRXross is at the top of its class in terms of sensitivity, resolution and analysis. Users benefit from high-quality data, shorter analysis times and simple operation.

Higher sensitivity, better resolution and easy analysis: The new IRXross FTIR spectrophotometer is impressive on several levels. The FTIR model in the mid-range performance class surpasses its predecessor, the IRAffinity-1S. When it comes to sensitivity, the IRXross is best in class with an improved signal-to-noise ratio of 55,000:1, enabling high-quality data to be obtained in less time. The higher resolution makes it easier to separate adjacent peaks. With the optional Rapid Scan software, up to 20 infrared spectra can be recorded per second for the investigation of chemical reaction processes.

Quick-click starting: analysis at the push of a button

The included IR Pilot analysis software facilitates everything from measuring to printing out measurement results with just one click. Even users unfamiliar with FTIR spectroscopy can easily analyze samples by selecting the analysis method. The device and accessories are recognized automatically, and the parameters are loaded. Several samples can be analyzed to save time. Shimadzu's own algorithms enable the identification of substances and/or the analysis of impurities.

Compliance with applicable regulations

Password length and period of validity can be configured for user accounts, enabling operation at a high level of security. All analysis data and information are reliably managed. This prevents files from being deleted. It goes without saying that the IRXross meets the European Pharmacopoeia requirements for the device in terms of resolution, signal-to-noise ratio and reproducibility.

The user-friendly IRXross impresses in terms of sensitivity, resolution and analysis

Note
For more information and references, please refer to the digital version of this edition.

VOICES

Trees call birds for help against leaf-feeding insects

The vital role of VOCs in plant defense in the canopy of a floodplain forest

Dr. Alexander Weinhold, Molecular Interaction Ecology – German Centre for integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig

Volatile organic compounds (VOCs) are important mediators of interactions of plants with their environment for instance through their involvement in pollination, host choice and defense against herbivores. We studied these interactions within the canopy of a floodplain forest by measuring plant volatiles trapped on custom-made silicone tubes and analyzing them by TD-GC-MS. We could show that plant volatiles change with herbivory and that those changes are recognized by predators of the herbivores, therefore confirming the role of those volatiles in indirect plant defenses.

How trees use home-made perfume as defense

VOCs play an important role in the defense of plants against attacking herbivores. They act as repellents and keep insects away from the precious leaf tissues. Unfortunately, this line of defense is often not enough and if it falls, VOCs can also act as an indirect defense. Right after the herbivore starts feeding, pools of VOCs are emitted by the attacked plants or via De novo synthesis calling in the "cavalry": natural enemies of the attacking herbivores. They are attracted by the odor cues of the attacked plant and the feeding insect. This phenomenon is widely referred to as the "cry for help" of plants. Natural enemies can be parasitoids and other insects or even birds who follow this cry for help and feed directly on the attacking insects. ⟶

Figure 1: Pieces of PDMS are attached on a wire to the branches

A field test in the forest

Now researchers at the German Centre for Integrative Biodiversity Research in Leipzig (iDiv) tested this in adult trees for the first time. They chose oak trees known to produce a wide array of chemical defenses from phenolic compounds and tannins but also including volatiles. Oak trees also react to insect attacks by enhancing the production of those defensive chemicals.

The experiment took place 40 meters above the ground in the tree branches within the canopy crane in the Leipziger Auwald. The scientists used methyl jasmonate, a plant hormone, to elucidate the defensive response of oak branches on different trees and compared them to untreated branches on control trees. The volatile bouquet of the oak trees consisting mainly of monoterpenes, sesquiterpenes, some phenolic compounds and some short-chain fatty acid-derived esters, was collected in plastic oven bags and trapped

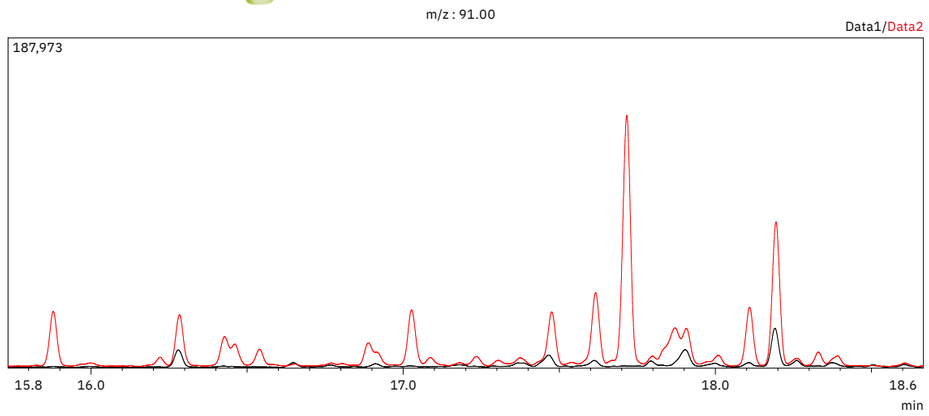


Figure 3: Comparison of two chromatograms from oak tree branches treated with methyl jasmonate (red) and control branches (black). MIC is depicted for a 91 m/z characteristic for sesquiterpenes. The induction of VOC production by methyl jasmonate is clear visible in the bigger peaks in the red chromatogram.

Figure 4: PDMS tube on wire

Figure 5: PDMS tube on wire

Figure 6: Removing tube from wire

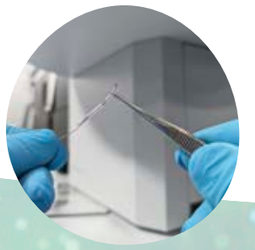


Figure 7: Transfer to TD tube

on short cuttings of polydimethylsiloxane tubing. The volatiles were sampled over a period of 24h and analyzed via thermodesorption gas chromatography mass spectrometry (TD-GCMS). In addition, dummy caterpillars were placed on treated and control branches, then checked regularly for bite and peck marks as proof for predator presence. The amount of real caterpillars was also checked regularly.

Treated branches saw an increase in the VOC production which in return led to more predators. At the same time, lower numbers of caterpillars were found on treated leaves. The increased volatile production of the oak trees after herbivore attack attracted more predators which led to a lower number of caterpillars. The cry for help of the oak tree was heard and responded to.

Enabling the application of biological controls instead of insecticides

This is an example of integrative biodiversity research using different platforms and techniques to study the interaction of plants and their environment. The lab of the molecular interaction ecology group at iDiv uses several analytical techniques to study biotic interactions and the chemicals mediating them. HPLC-UV is used for the routine analysis of glucosinolates in cabbage and LC-MS is used for the metabolomics analysis of secondary metabolites of various plant species.

An integral part is the Shimadzu GCMS-QP2020NX coupled to a TD-30R that is not only used to study trees but also other plants of agricultural importance. Tomato plants do also release VOCs after being attacked, attracting predatory bugs to feed on the caterpillars thereby protecting the plant. Identifying the volatiles involved in the process can help to improve the application of biological controls in greenhouses and reduce the use of insecticides. Another example is the collection of volatiles of winter wheat after aphid infection. Here the aim is to identify VOCs that could be used to enhance plant protection in the greenhouse or in the

Figure 2: Oak trees wrapped in plastic oven bags to catch VOCs on PDMS

field. The Shimadzu TD-30R unit with a large enough sampler is perfect for both high throughput and high sample numbers. The easy-to-handle sample setup combined with very cheap PDMS tubes allowed 50 to 250 sample numbers in one experiment alone. In addition, the resampling option provided a means to analyze precious field samples again with different chromatographic conditions, for instance.

The research questions at iDiv are diverse, and this makes a very valuable and versatile instrument for the biodiversity research.

Note

For more information and references, please refer to the digital version of this edition.

Shining a light on clean energy

The UV-3600i Plus as a research tool for organic photovoltaic materials

Frederik Kiel, Prof. Cemal Esen, Ruhr-Universität Bochum

Photovoltaics is an important pillar of the energy revolution and is more topical than ever before as a research field. Classic rigid silicon solar cells have long been part of everyday life. In the future, flexible or even transparent solar cells made from organic materials could enable an even wider range of applications. The development of high-performance UV-Vis spectrometers is valuable for identifying and testing materials or cell prototypes and paves the way to technically sophisticated products.

The current and future challenges facing humankind in terms of climate protection and energy supply require a broad approach to energy conversion, storage and recovery. Photovoltaics is already an integral part of the current energy mix, and this will steadily increase in the future. The continued development of organic photovoltaics (OPV) and its production processes also plays an important role here. The aim is to have flexible solar cells that can be manufactured locally and can reduce dependence on fossil fuels.

OPV cells can be wet-chemically applied to flexible substrates such as polyethylene terephthalate (PET). The resulting dry layers have layer thicknesses in the 10–100nm nanometer range. This enables many applications which are not possible with established photovoltaics (silicon), additionally leading to reduced material requirements and a cost-effective production process.

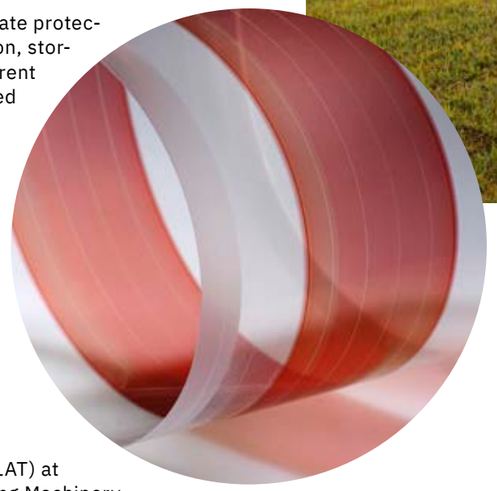


Figure 1: Production sample of a flexible photovoltaic material

Basic research in “EffiLayers” project

In its “EffiLayers” project, the Chair of Applied Laser Technologies (LAT) at Ruhr-University Bochum is working with the partners Coatema Coating Machinery GmbH, LIMO Lissotschenko Mikrooptik GmbH, Ortmann Digitaltechnik GmbH and Fraunhofer Institute for Laser Technology on the process optimization of the roll-to-roll (R2R) production of innovative, highly efficient organic photovoltaic cells. This involves the selection of materials and laboratory-scale evaluation for the subsequent R2R process, the evaluation of processes for improved processability (e.g. surface treatment) and the substitution of conventional process technologies with photonic technologies (e.g. laser drying).

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For each of these tasks, the acquisition of the transmission/absorption spectra using the UV-3600i Plus is an essential part of the subsequent evaluation. In addition, the spectra make it possible to evaluate numerous parameter variations in a single layer in advance without the laborious process of manufacturing complete cells.

Checking the electron transport layer including anti-reflective function

Starting with a transparent substrate (PET) with a coated, transparent indium tin oxide (ITO) electrode, the first layer to be applied is the electron transport layer (ETL). By adjusting the energy levels, negative charge carriers can be transported, while positive charge carriers are blocked to avoid recombination. Just like the substrate and the transparent electrode, this layer should have a high transmission rate so that as many photons as possible can reach the photoactive layer for generating charge carriers. In OPV, zinc oxide (ZnO) is a commonly used material for wet-chemical coating. →

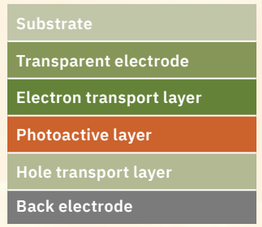


Figure 2: Typical layer structure of an organic photovoltaic cell

Figure 3 shows the transmission spectra of the base substrate PET/ITO and ZnO layers of dispersions from different providers applied to this, measured with a Shimadzu UV-3600i Plus with an ISR-603 integrating sphere. The application of the ETL layer improves transmission regardless of the provider.

Further investigations with the integrating sphere show that this is due to a reduction in reflection by the electron transport layer (Figure 4). Zinc oxide has a lower refractive index than indium tin oxide and thus forms an anti-reflective layer. However, this refractive index varies greatly, especially in the case of wet-chemically produced thin films, due to factors such as solvent, particle size, post-treatment and the resulting homogeneity and crystallization of the dry layer.

Analysis of the impact of heat treatment

Another way to reduce production and system costs is to dry the wet-chemically applied layers via laser drying.[2] For the photoactive layer Poly(3-hexylthiophene-2,5-diyl): Phenyl-C61-butyric acid methyl ester (P3HT:PCBM) the heat treatment serves not only to dry the solvent but also to ensure crystallization of the P3HT. This results in a red shift in the absorption spectrum so that even lower-energy photons can be absorbed improving the efficiency of the OPV cell.

Measurements showed that laser drying improves crystallization and, based on efficiency measurements, also ensures sufficient drying, but it is not sufficient on its own to achieve optimum cell performance. However, the additional process step known as “solvent annealing” leads to a comparable spectrum of the layer and enables a similar level of cell efficiency to be obtained. In this process, the layer

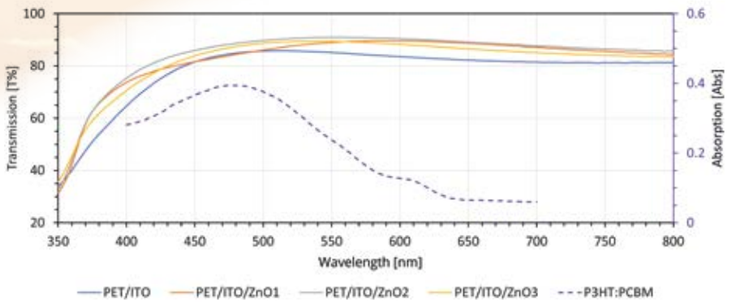


Figure 3: Transmission spectra of various zinc oxide layers on PET/ITO substrate and absorption spectrum of the photoactive layer P3HT:PCBM

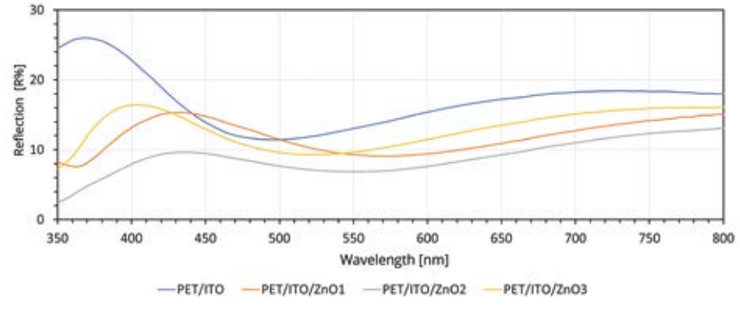


Figure 4: Reflection spectra of different zinc oxide layers on PET/ITO substrate

is exposed to a solvent-containing atmosphere for a short period of around 10 seconds, which facilitates crystallization because the molecules remain in a mobile phase for longer.

Impact of surface treatment – surprising findings

A difficulty in coating the hole transport layer of poly (3,4-ethylene dioxythiophene) with polystyrene sulfonate (PEDOT:PSS) results from the relatively high contact angle of the PEDOT:PSS dispersion on the photoactive layer. Contact angles of less than 25° are required for effective wetting of the fluid on the substrate. Larger contact angles lead to inhomogeneous coating and even to completely uncoated areas (Figure 5).

By diluting the dispersion with less polar solvents, the contact angle can be reduced to less than 30°. However, all coating parameters change due to the reduced solids concentration, while the drying effort is considerably increased as a result of the greater wet layer thickness.

Another way to improve the contact angle is to increase the surface energy on the substrate. This can be achieved by means of irradiation with a UV excimer lamp. The photons of the lamp used are highly energetic (172 nm or 7.2 eV) and can break up the molecules of a variety of organic bonds or oxides.

However, since the photoactive layer is also organic material, further investigations are needed to identify and evaluate any further effects of UV radiation on the material. Evaluation of the absorption spectra (Figure 7) reveals a decrease in absorption for all treated layers and a blue shift in the main absorption peak from 505 nm to 500 nm at a distance of 20 mm and to 495 nm at a distance of 10 mm.

A comparison of the electrical parameters shows that although the wettability of the photoactive layer has been improved by UV radiation, it does not outweigh the deterioration of the optical properties. The efficiency of the cell prepared at a distance of 25 mm is already reduced by 25% compared to the untreated cell. This also applies to the short-circuit current which is related to the absorption height (level of absorption). The cell prepared at 10 mm demonstrates a 75% reduction in efficiency. In addition to a reduced short-circuit current of 50%, there is also a reduction in the open circuit voltage of 15%. This is also related to the wavelength of the main absorption peak.

Conclusion

In organic photovoltaics, spectroscopy plays a key role in comprehensive sample and material characterization. Thanks to this, it is often possible to evaluate the theoretical suitability of materials, individual layers or processes in advance without the need to construct entire cells. In addition, it provides information that enables a better understanding of cell performance based on a complex interaction of optical, electrical and interface-dominant properties.

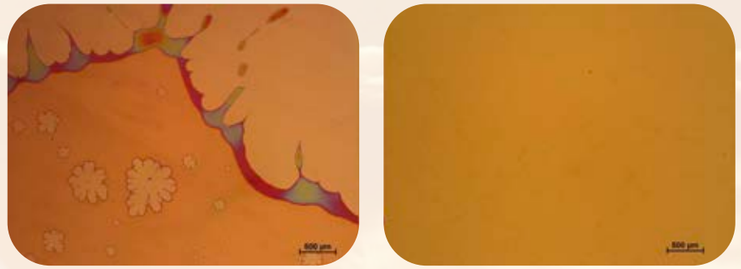


Figure 5: Wetted surface without (left) and with (right) additional surface treatment to reduce the contact angle. In the picture on the left, there are clear defects in the coating.

Figure 6: Light microscopic images of a PEDOT:PSS dispersion droplet on the photoactive layer P3HT:PCBM; left: without pretreatment; right: after excimer treatment

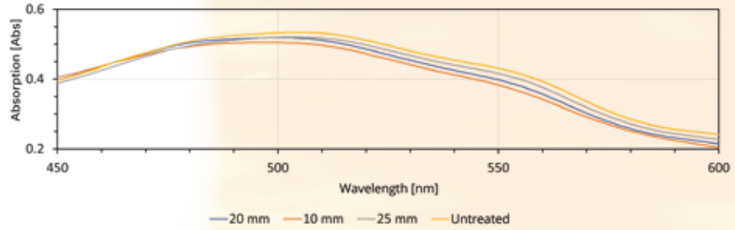


Figure 7: Absorption spectra of the photoactive layer P3HT:PCBM after irradiation for 4 minutes at different intervals

Note

For more information and references, please refer to the digital version of this edition.

The story of a market leader

Shimadzu celebrates 50 years of innovation in Total Organic Carbon instruments

Markus Janssen,
Shimadzu Europa GmbH

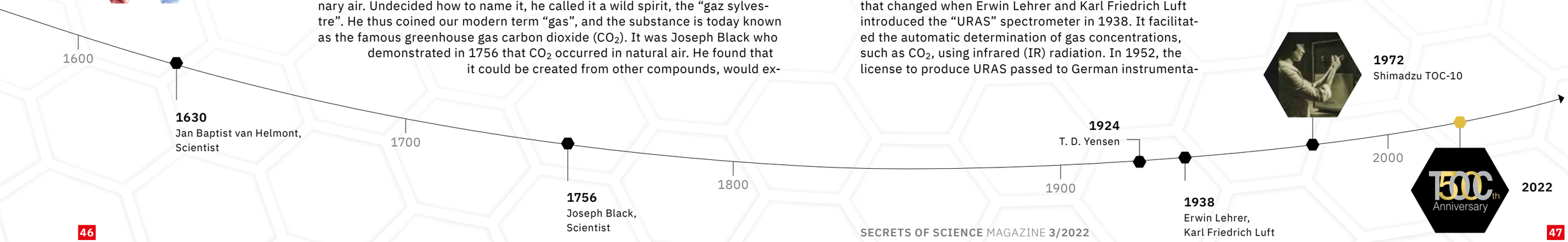
Water is an essential resource in both our ecosystem and economy. Total Organic Carbon (TOC) is one of the most important parameters in the assessment of water quality and thus essential for the protection of the valuable liquid. 2022 marks the 50th anniversary since Shimadzu first released a TOC analyzer. Today, Shimadzu's market leading TOC analyzers are used in a wide variety of fields, ranging from laboratory water quality control to ultrapure water management in pharmaceutical production and online TOC monitoring in industrial wastewater treatment.

From the Age of Discovery to modern day analysis

Shimadzu has been instrumental in shaping TOC analysis over the past decades. However, the basic principles were established much earlier by inventors and scientists. During the Age of Discovery, around 1630, Flemish scientist Jan Baptist van Helmont recognized that when both wood and coal were burned, a vapor was produced whose properties differed from those of ordinary air. Undecided how to name it, he called it a wild spirit, the "gaz sylvestre". He thus coined our modern term "gas", and the substance is today known as the famous greenhouse gas carbon dioxide (CO_2). It was Joseph Black who demonstrated in 1756 that CO_2 occurred in natural air. He found that it could be created from other compounds, would extinguish a flame and was exhaled by humans. While doing research on magnesium carbonates, Black invented the analytical balance and promptly used it to measure carbon dioxide by loss on ignition (LOI). The LOI method, in which samples are heated and the resulting reduction in mass is measured, is the first quantitative test for organic matter.

Initiating the successful TOC product line

The invention of the necessary measurement technology began in 1924 when T. D. Yensen of the Westinghouse Electric and Manufacturing Company patented a measuring device that placed steel samples in a horizontal 1,000 °C furnace, combusted carbon in an oxygen carrier gas and collected carbon dioxide cryogenically. The quantification of CO_2 was still rather complicated, but that changed when Erwin Lehrer and Karl Friedrich Luft introduced the "URAS" spectrometer in 1938. It facilitated the automatic determination of gas concentrations, such as CO_2, using infrared (IR) radiation. In 1952, the license to produce URAS passed to German instrumentation manufacturer Hartmann & Braun, with whom Shimadzu established a technical partnership starting 1965. In 1967, the National Industrial Research Institute of Nagoya asked Shimadzu to develop a TOC analyzer and thus work began on the first Shimadzu instrument "TOC-1", an internal prototype. The same year, James Teal at Dow Chemical Company patented a "Method and Apparatus for Determination of Total Carbon Content in Aqueous Systems". This system was a combustion analyzer similar to Yensen's device and manually injected aqueous samples directly, using a syringe, into a stream of oxygen flowing through a 700–900 °C furnace measuring the CO_2 generated by infrared absorbance. Teal's device appears to be the first combustion TOC analyzer for water. →

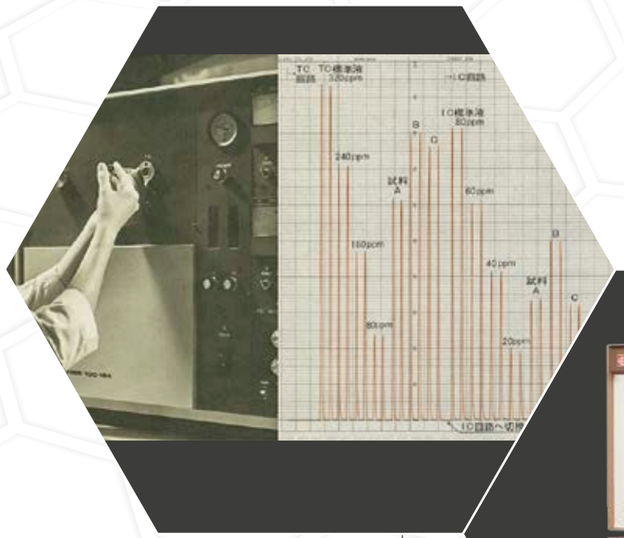


Advancing environmental protection through research

In the wake of Japan's rapid economic growth, air and water pollution became a social problem. For this reason, the Japan Environmental Protection Agency was established in 1971. TOC became an important environmental parameter within "JIS K 0102 – Test Methods for Industrial Wastewater". The Shimadzu Corporation reacted by establishing an Environmental Instrument Department to strengthen the commercialization of environmental measurement instruments. In the following year 1972 – 50 years ago –, Shimadzu introduced its first TOC analyzer products in Japan: the TOC-100 automatic water quality monitoring system and the TOC-10 Total Organic Carbon analyzer for laboratory use. TOC-100 was used for continuous monitoring of water quality and pollutants in public waters based on Japan's Water Pollution Control Law. TOC-100 was rapidly improved so that the successor model TOC-100A was released the same year.

The systems were optimized and ideas for improvements were generated at a fast pace, shaping Shimadzu's unique TOC analyzers. As a result, the continuous TOC analyzers TOC-401 and TOC-402 could be released as successors of TOC-100 as well as the laboratory successor model TOC-10A. TOC-10A allowed manual injection of water samples into a 950 °C hot furnace under oxygen atmosphere using a micro syringe. CO_2 originating from the combustion of organic carbon, was quantified, using a URA-3B infrared gas analyzer by Hartmann & Braun.

Back then, the measured CO_2 signals, so-called peaks, were not integrated but printed on chart paper and compared with a prior calibration by peak height using a ruler. It was thus important to obtain consistently sharp peaks for all substances to be able to measure their height with as little inaccuracy as possible. That is why very high temperatures of around 1,000 °C had to be used. This would turn out to be a problem to solve for Shimadzu.



1973
Manual injection being performed on a TOC-10A. On the right a peak printout with measurement scale.

1975
TOC-10B

International expansion

The initial TOC analyzer models were quite successful in Japan, having made Shimadzu become a major player for TOC instruments in their domestic market by the end of the 1970s. However, since the market in Japan was still relatively small and the company wanted to expand its business, it was decided to introduce the devices overseas as well. The first overseas debut of Shimadzu TOC analyzers took place at an international exhibition in Tianjin, China in 1980, where the TOC-10B was exhibited as one of Shimadzu's various analytical instruments.

Establishing the 680 °C combustion method still in use today

A breakthrough for Shimadzu TOC instruments was the TOC-500 completed in 1983. The analyzer layout had been significantly improved with this product. It was the birth of the 680 °C combustion method, still used with great success today. In TOC analysis, where 100% recovery of all organic matter is desirable, a higher combustion temperature was considered safer and produced sharper peaks for the measurement of peak height. However, the 950 °C combustion was at a disadvantage when samples contained salt. The salt melted at these high temperatures and would wear out the combustion tube and catalyst, significantly shortening its maintenance life. Since salt is contained not only in seawater but also in many wastewater samples, its effect on analysis robustness was significant. Shimadzu solved this problem by lowering the furnace temperature, while at the same time using a high-performance platinum catalyst. The new high temperature catalytic oxidation (HTCO) method was designed and evaluated to meet the required performance even at the lower temperature of 680 °C. In 1985, the method was submitted to Japanese standard committee JIS and approved. In addition, the catalyst replacement method, which had been the biggest drawback of TOC analyzers up to that time, had been significantly simplified, which attracted attention worldwide.

The first global launch in 1987: the easy-to-use TOC-500

Data processing had been revised so that peaks could be integrated automatically. The system would compare the peak area instead of height to calibration, in turn becoming much more selective for carbon, regardless of how easy or hard a compound was to oxidize. TOC-500 could be programmed for various methods via keyboard, and after analysis a report could be printed on thermal paper, including result and statistical parameters necessary for quality control. The effort for the operator to evaluate data could be significantly reduced that way. TOC-500 was the first Shimadzu TOC instrument to be launched globally, and in 1987 it was well received with major chemical and pharmaceutical manufacturers in Europe and petrochemical production in the southern United States. It made a name for itself through its ease of use in both analysis and maintenance on the Pittcon exhibition in 1987. →

1983
Brochure cover of TOC analysator TOC-500

1970 — 1980 — 1990

Becoming market leader with the TOC-5000

The TOC-500 was well received, but it was the 1989 successor model, the TOC-5000, that established Shimadzu as market leader in TOC analyzers. It was equipped with a unique automatic sample injection mechanism, employing a syringe pump with 4-port valve and sample slide injector. The analyzer had a large display and control keyboard simplifying operation. The TOC-5000 was fully compatible with autosamplers, eliminating the need for manual injection by the analyst, saving labor and improving measurement accuracy.

Together the improvements and additions resulted in a broader TOC application range suitable for the pharmaceutical, environmental and chemical market ranges. The TOC-5000(A), the upgrade of the TOC-5000, had been released in 1994. While many details had been improved, the biggest advance was certainly the release of the first Shimadzu PC software for TOC analyzers, “TOC Control”, developed originally in Europe.

The TOC-4000 – the first modern online TOC

Process Analyzer Technology (PAT) continued to develop as well. With the TOC-4000, a first modern online TOC instrument came onto the market, which combined the advantages and robustness of the 680 °C combustion method with the sled injection technique. In order to perform automatic dilution, the 4-port valves were soon replaced by 8-port valves.

In addition, larger syringes were used, which offered the possibility of purging the inorganic carbon in the syringe barrel, a technique that has proven itself to this day and is still in use. In 1998, with the development of the TN module, the determination of TN_b was made possible in the TOC-4100 series.

2003
TOC-4110 online
TOC / TN_b analyzer

1989
TOC-5000 with
ASI-5000 autosampler

from **1994**
The first PC-controlled
TOC analyzer TOC-5000A

Enabling the analysis of saline samples with long service life

In 2000, the proven technology of the online systems was integrated into the TOC-V laboratory instrument series. The automatic dilution function now became available for laboratory users. A new infrared detector was introduced for these systems with an electronic auto-zero function. In 2002, the TOC-V series was completed with an instrument that uses wet chemical UV oxidation to determine TOC. Among numerous other improvements, options and modules, the High Salt Kit was developed for the TOC-4110 series online systems. This unique catalyst enables the analysis of saline samples with long service life.

This kit for saline samples can now also be used in the current TOC-L series. With different catalysts and options, the TOC-L instruments have a wide range of applications, from ultrapure water to wastewater or even to the measurement of soil suspensions. The same is true for the current online system TOC-4200. For highly sensitive TOC analysis required in online monitoring of ultrapure water, for example in the pharmaceutical sector or for electronics manufacturers, Shimadzu has introduced the TOC-1000e in 2021. It is the first TOC analyzer to use environmentally friendly excimer technology for oxidation.

Note
For more information and references, please refer to the digital version of this edition.

Current product portfolio:
TOC-L, TOC-4200, eTOC series

1980
1990
2000
2010
2020
2022

LAB-SUPPLY
Münster, Germany
Nov. 09, 2022

Food Safety Live
Virtual
Nov. 16, 2022

EBF
Barcelona, Spain
Nov. 16–18, 2022

EWCPS
Ljubljana, Slovenia
Jan. 29–Feb. 03, 2023

Bioprocessing
Barcelona, Spain
Mar. 14–16, 2023

ANAKON
Vienna, Austria
Apr. 11–14, 2023

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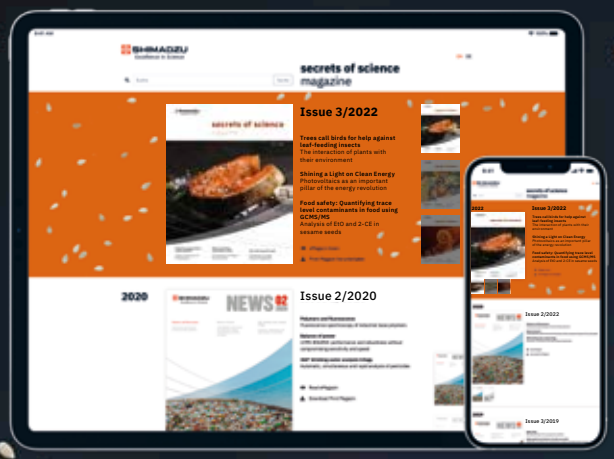

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