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Speeding development of electric hypercars

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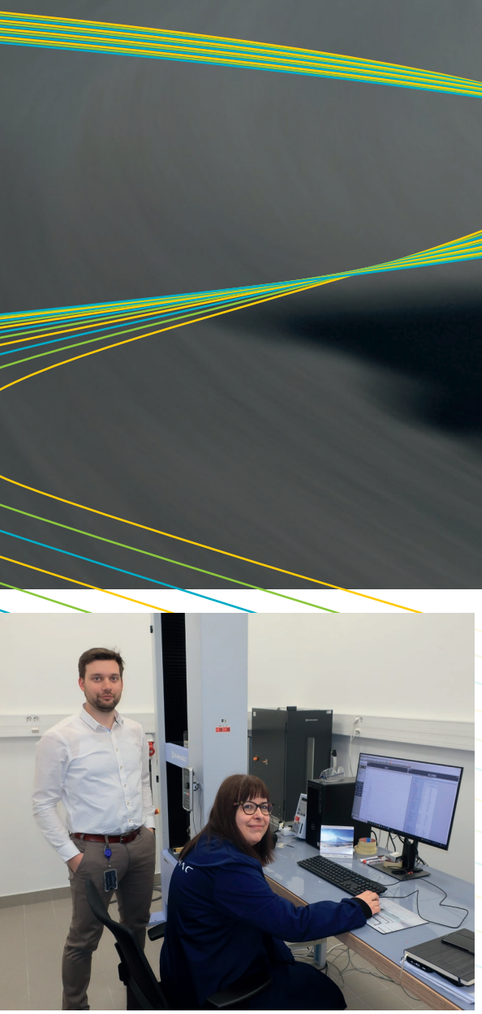
VOICES

Speeding development of electric hypercars

Rimac Technology steers simulation data with Shimadzu's Autograph AGS-X



Gordana Ribarić, Dr. Primož Štefane, Rimac Technology

Rimac Technology designs and manufactures high-end electric hypercar components. Based in Zagreb, Croatia, the company has a well-established reputation for the engineering, development and production of high-performance battery systems, electric drive units, electronic systems and user interface components. As part of Rimac's drive to refine its competitive edge in this exceptionally demanding field, the company recently acquired advanced Shimadzu material-testing machines. Two company experts tell us why.

Figure 1: Simulation engineers Gordana Ribarić and Primož Štefane

Leaders of the pack

Hypercars are the highest-performing consumer sports cars in the world. In recent years, these exceptional vehicles have begun to adopt electrification, and completely electric vehicles (EV) are now increasingly common. Within this highly competitive niche, a number of companies continuously strive to create even greater performance – by testing the limits of what is possible.

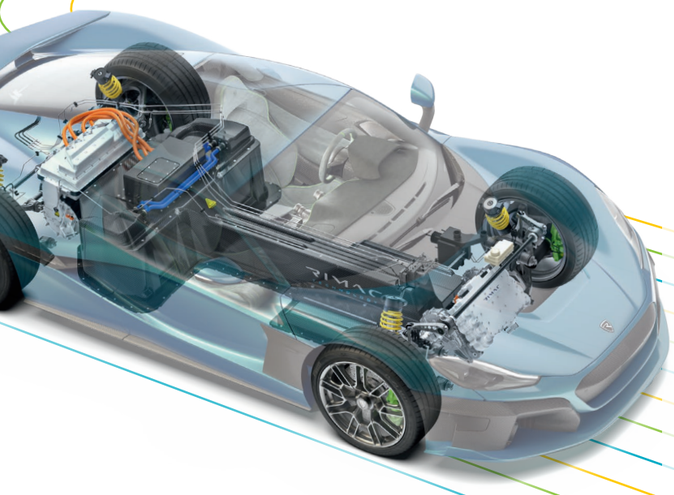
One of these fast-moving companies is Rimac Technology, which designs, engineers and manufactures electric hypercar components.

Staying ahead of the curve

As Rimac Technology has grown the company's desire to better utilize the potential of mechanical-properties testing for research and development has also increased.

After thoroughly investigating the matter, they decided to purchase a universal testing machine (UTM) from Shimadzu. Rimac Technology simulation engineers Gordana Ribarić and Primož Štefane explain why:

How did you become familiar with Shimadzu? Shimadzu is very well-known for its cutting-edge products, and many facilities in Croatia use their equipment. ⟶



What were your reasons for choosing Shimadzu? After a detailed analysis of several options, we chose Shimadzu's Autograph AGS-X 100kN device, which we determined would provide us with high-precision measurement results at an appropriate price.

How are you utilizing the equipment? We use the UTM to test materials and components for our automotive components. We need to precisely measure the mechanical properties of any given material because the accuracy of simulation results is dependent on the accuracy of the input data.

Simple & safe

While the original purpose of the AGS-X 100kN was quality control, an increasing number of companies are now also using more advanced R&D applications. The AGS-X 100kN allows users full control over all the test parameters, and they can run as many tests as they like – at any time that's convenient for them. Gordana Ribarić and Primož Štefane tell us more:

Which features of the equipment do you consider to be most valuable? The most valuable feature is the possibility to create our own methods for testing materials and components through the Trapezium X software.

Has the AGS-X 100kN met your expectations? Yes. For us, the most significant characteristic of the machine is its ability to allow us to create customized methods for testing materials. We are also very pleased with the overall high safety level for the machine operators. There are several levels of protection.

Was it easy to learn how to use the new equipment? The AGS-X 100kN – together with its software Trapezium X – is very intuitive to use. In addition, Shimadzu application engineers are always available to us for consultation and assistance.

Figure 2: Rimac simulation engineers working on Shimadzu testing machine

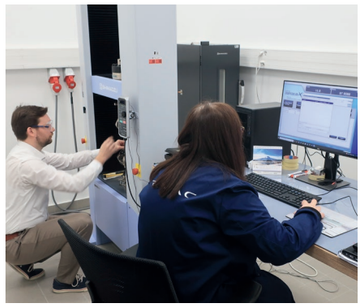
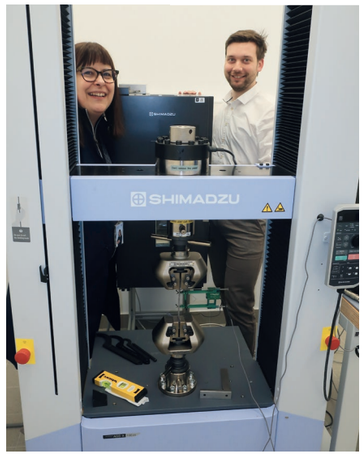


Figure 3: Working on AGS-X 100kN



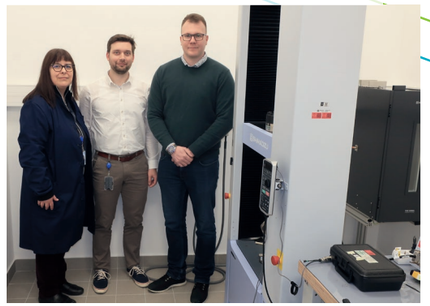


Figure 4: Rimac Technology simulation engineers with Shimadzu representative Matej Tkalčić

When your destination is success

Dedicated expert support was another reason why Rimac Technology chose Shimadzu. Even the best hardware and software sometimes need expert assistance, and Shimadzu is able to supply that to Rimac through its well-developed support network in Croatia. As Gordana Ribarić and Primož Štefane put it:

Are you satisfied with the support you have received from Shimadzu? We are extremely satisfied with the service and support of the Shimadzu team and with their level of understanding of our needs and the speed with which they have responded to our requirements.

How satisfied are you with the reliability and durability of the AGS-X 100kN? We are extremely satisfied with the reliability and durability of the equipment.

And how would you describe Shimadzu's impact on your R&D process? Our new UTM machine has had an extremely significant and positive effect. It has accelerated the process of testing materials and glued and welded joints and helps us to optimize the process parameters in bonding and welding technologies as well as in selecting the proper materials for our requirements.

Success in this case also has ramifications for environmental sustainability. The data gathered by Shimadzu's AGS-X 100kN helps improve simulations run by Gordana Ribarić, Primož Štefane and their colleagues. This, in turn, helps them and Rimac Technology create lighter, stronger automotive components for electric vehicles.

Sustainable satisfaction

From battery and vehicle systems to torque vectoring, e-axles, advanced driver-assistance systems, infotainment, connectivity and UX/UI – Rimac Technology is a full technology solutions provider to global OEMs. Shimadzu is an established provider of high-quality precision lab equipment and expert support. It is particularly pleasing when such companies can work together in the creation of high-quality products – especially when those products are part of the growing movement away from fossil fuels and toward a more sustainable future for all.

Note

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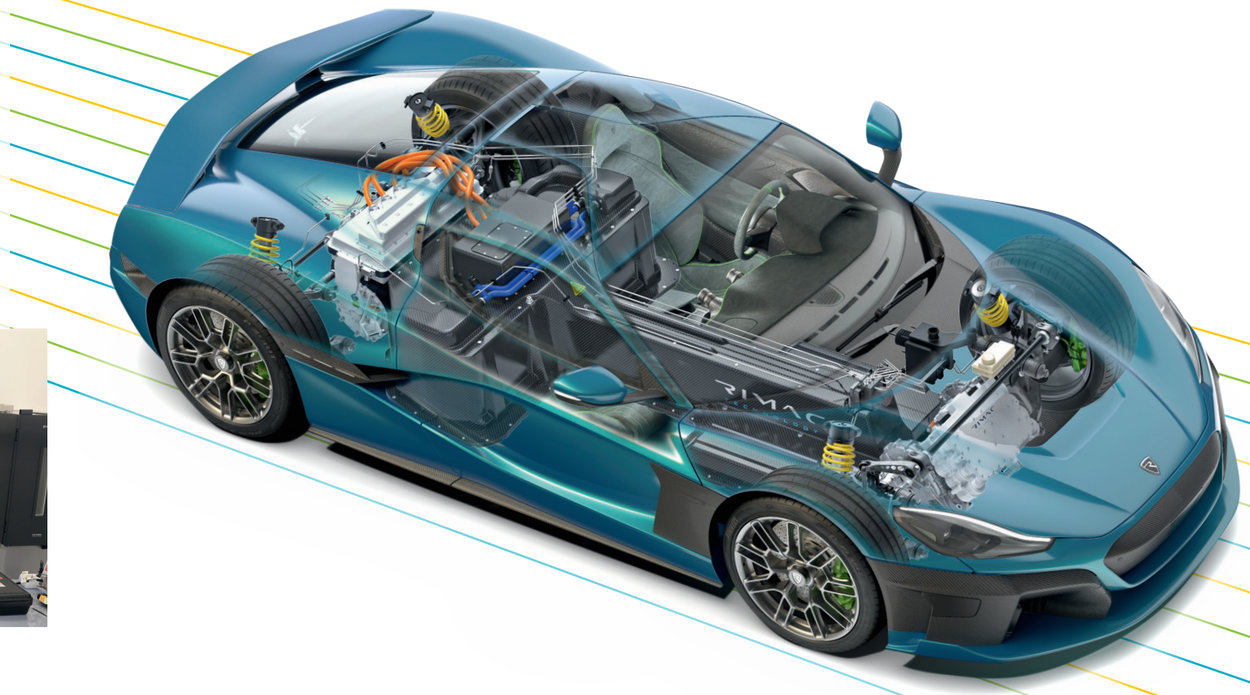


MOVE ON

A closer look into mineral oils in food

Project develops new method for detecting DNA-reactive mineral oil fractions

Sanja Savić, Elisa Mayrhofer, Austrian Research Institute for Chemistry and Technology (OFI), Microbiology & Cell Culture

Andrea Hochegger, Erich Leitner, University of Technology Graz

Mineral oil contamination is common nowadays and is often in the news, from unsafe sunflower oil in 2008 and the foodwatch International report on hazardous infant formula in 2019 to the annual alerts about tainted Christmas chocolate products.[1, 2, 11] Food contaminated with mineral oil residue has long been an issue of concern and has prompted reports and risk evaluations by the European Food Safety Authority (EFSA) for many years. But how do we assess levels of contamination, and can we do it better? An Austrian government-funded research project has produced a helpful answer.

Mineral oil contamination

In 2012, the European Food Safety Authority (EFSA) published a scientific opinion on the health effects of mineral oil contamination in food.[3] That report identified food packaging, different packaging additives and lubricants as the main sources of contamination. Today, it's known that contamination can occur throughout the entire production cycle, not simply through packaging. A distinction is also made between actual food contamination and the permitted use of certain mineral oil products as food additives, e.g. microcrystalline waxes (E905). In either case, the presence of mineral oil hydrocarbons (MOH) in food means that they will be metabolized by the body during digestion.[3, 4, 5]

Separating MOSH from MOAH

MOH are complex mixtures of hydrocarbons that originate in crude mineral oil. What ends up in our food are certain subfractions, each having varying compositions of thousands of compounds. This complexity makes a clear substance-based risk evaluation difficult. During laboratory analyses, an initial separation into fractions of Mineral Oil Saturated Hydrocarbons (MOSH) and Mineral Oil Aromatic Hydrocarbons (MOAH) is possible due to their chemical structure. MOSH consist of branched and unbranched open-chain hydrocarbons and saturated cyclic hydrocarbons. Although MOSH accumulate in the human body, they are not associated with major negative effects. MOAH, on the other hand, are made up of highly alkylated aromatic substances and are considered to be potentially mutagenic and carcinogenic due to the possible presence of 3-7 ring polycyclic aromatic compounds (PACs). In crude mineral oil, this MOAH fraction makes up 15–30 %.[3, 6]

Methods in search of improvement

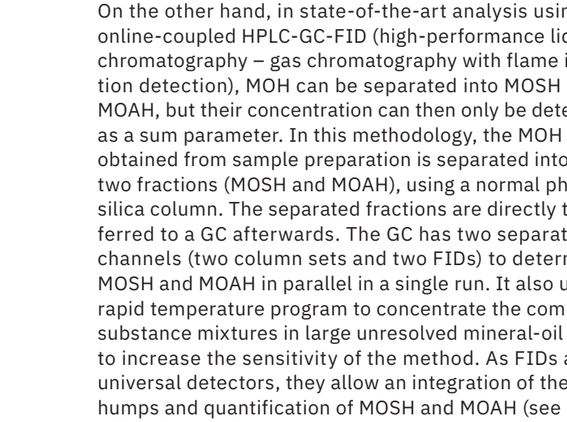
To ensure product quality and safety for consumers, it is important to routinely monitor, identify and eliminate MOH contamination in food. Although standardized methods are continuously improving (e.g. Update of EN16995 in 2022, JRC method for MOAH in infant formula), analysis remains challenging.[4, 15, 16] On the one hand, sample preparation is difficult, mainly due to the highly varying food matrix and the related fat content. In addition, exceptional clean-up, often manually done, is necessary, e.g. by saponification to remove fat; by Al_2O_3 clean-up to remove naturally occurring n-alkanes from the MOSH fraction (Alox); or by epoxidation to remove olefins from the MOAH fraction (Epox), followed by an enrichment through solvent evaporation. ⟶

On the other hand, in state-of-the-art analysis using online-coupled HPLC-GC-FID (high-performance liquid chromatography – gas chromatography with flame ionization detection), MOH can be separated into MOSH and MOAH, but their concentration can then only be determined as a sum parameter. In this methodology, the MOH extract obtained from sample preparation is separated into the two fractions (MOSH and MOAH), using a normal phase silica column. The separated fractions are directly transferred to a GC afterwards. The GC has two separated channels (two column sets and two FIDs) to determine MOSH and MOAH in parallel in a single run. It also uses a rapid temperature program to concentrate the complex substance mixtures in large unresolved mineral-oil humps to increase the sensitivity of the method. As FIDs are universal detectors, they allow an integration of the total humps and quantification of MOSH and MOAH (see Figure 1).[7, 8] However, the many automation options available for the system offer a significant reduction in the workload of routine analysis: most of the required clean-up steps needed for state-of-art analysis according to the standardized methods, such as Alox and Epox to remove natural interferences, can be fully automated. For fats and oils, a fully automated workflow, including saponification, has also recently been introduced.[9]

Setting new limits

In December 2021, foodwatch International – a food-consumer-interest organization – released their findings on MOAH contamination in food. In April 2022, the Standing Committee on Plants, Animals, Food and Feed of the European Commission published a report in response to this.[10, 11] They agreed to recall products from the market across the entire European Union if they tested above a certain limit of quantification (LOQ) for MOAH. These LOQs are based on current analytical limitations of the LC-GC-FID method and depend on the fat content of the food. For food having < 4 % fat, the LOQ is 0.5 mg/kg. For food with a fat content of 4–50 %, it is set at 1 mg/kg, and for food with > 50 % fat, the limit is 2 mg MOAH/kg.[10]

It is also possible to establish limits better related to the real toxicological health effects, using a Threshold of Toxicological Concern (TTC) concept. Since MOAH is considered to be DNA-reactive, a much lower daily exposure than the LOQs is indicated: 0.15 µg/day or 0.15 µg/kg food for a person with a body weight of 60 kg.[14] This limit could be increased, but only if there were definitive proof that certain substances or sub-fractions of MOAH are not DNA-reactive. To do that, though, a more substance-based risk assessment would be needed to replace the simple determination of a sum parameter as is done by using LC-GC-FID. In 2019, EFSA recommended using additional analytical methods for samples contaminated with MOAH to identify if 3-7 ring PACs are present, as these are believed to be responsible for the DNA-reactive character of MOAH.[5] The method of choice for this characterization is 2D-comprehensive GC×GC. However, since thousands of substances may be detected with this method, risk evaluation of every single substance identified using GC×GC is not feasible. As an alternative, the entire MOAH mixture – or individual, isolated subfractions of known composition – could also be tested for their DNA-reactive effects using bioassays.



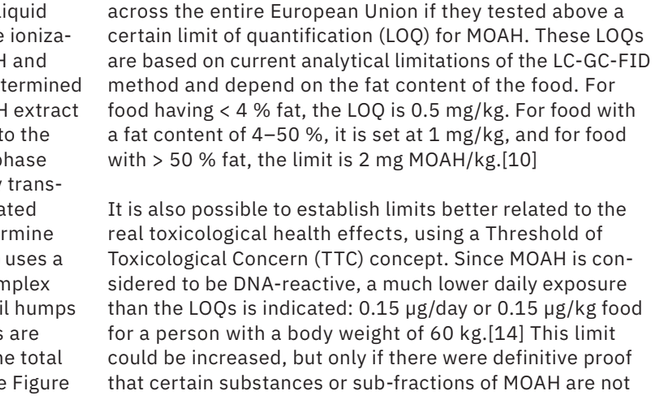
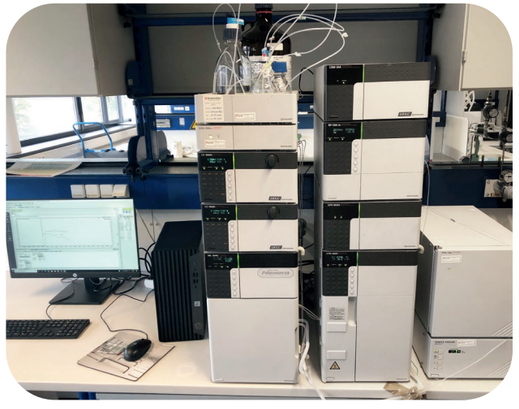


Figure 1: Online-coupled HPLC-GC-FID (left) and chromatogram of typical mineral oil (right, black = MOSH, pink = MOAH)

Project MOSH MOAH combines methods

To overcome the challenges inherent in current methods, a collective research project was established under the coordination of GLi Austria (Gemeinnützige Lebensmittelinitiative für Österreich) and funded by the Austrian Research Promotion Agency (FFG). During the project – "MOSH MOAH – Reduction of Mineral Oil in Food" – research partners at the University of Technology Graz (TU Graz) and the Austrian Research Institute for Chemistry and Technology (OFI) worked on developing a new assessment approach which combined state-of-the-art instrumental analysis of MOSH/MOAH with a toxicological assessment using *in vitro* bioassays to enable a more substance-based risk assessment of MOAH.[12]

One of the main challenges facing the researchers was to separate MOAH according to ring numbers into mono- and diaromatic substances and three- and higher ring aromatics as well as to isolate those subfractions for further characterization. Koch et. al (2020) proposed a donor-acceptor complex chromatography for the group-type separation of MOAH.[13] →



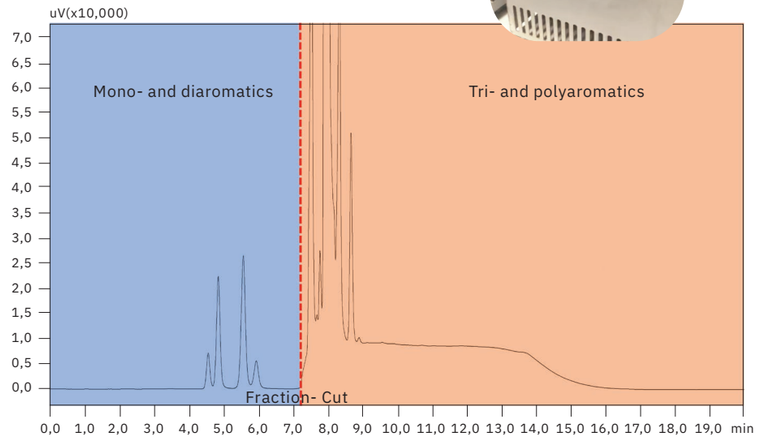


Figure 2: Shimadzu Nexera LC-20AD with Prominence RF-20Axs fluorescence detector (FLD), SPD-M20A diode array detector (DAD) and fraction collector for isolating mono- and diaromatic substances and tri- and polyaromatics from MOAH[13]

This was implemented in the project using a Shimadzu Nexera LC-20AD (Figure 2) equipped with a Nucleosil Chiral-2, 5-µm column (250 × 4 mm, MACHEREY-NAGEL GmbH & Co. KG) and a Shimadzu Prominence RF-20Axs fluorescence detector (FLD) as well as a Shimadzu SPD-M20A diode array detector (DAD). In addition, the system was equipped with a fraction collector to collect the mono- and diaromatic substances and tri- and polyaromatics isolated. The fractions were enriched and further analyzed using 2D-comprehensive GC×GC to evaluate the composition of MOAH of different origin, with a focus on the presence (or absence) of 3-7 ring PACs.

To evaluate the DNA reactivity of the isolated fractions, a miniaturized Ames test was applied. The assay used histidine auxotrophic *Salmonella* strains, which revert to prototrophy after contact with DNA-reactive substances, triggering growth in minimal media. Figure 3 shows the final experimental approach of the developed method that was successfully implemented in the "MOSH MOAH" project.[12]

Proof of concept

In a proof-of-concept study following the initial development of the new method, a reference mineral oil with high MOAH content was investigated.[12] The results of the instrumental characterization using GC×GC clearly showed the different composition of the isolated mono- and diaromatic fraction and tri- and polyaromatic fraction and that the separation according to the ring number had been successful. Even more interesting were the results of the bioassay: The mineral oil as such was positive in the Ames test, which meant that it showed DNA reactivity. Further, the isolated MOSH scored negative, while the isolated total MOAH was positive. A further sub-fractionation of MOAH showed that the isolated mono- and diaromatic substances were negative in the Ames test, while the tri- and polyaromatic fraction scored positive. Figure 4 shows a summary of the results.

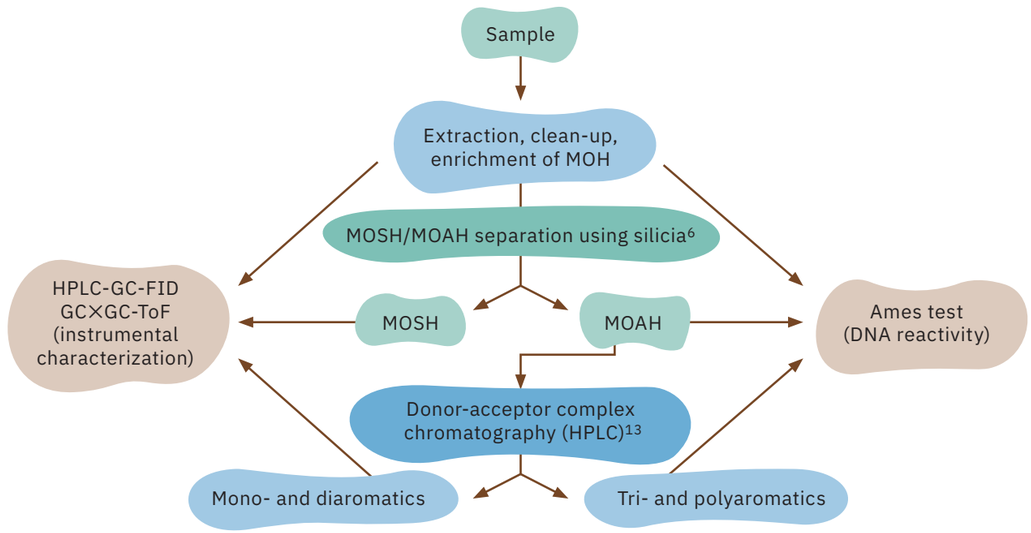


Figure 3: Experimental overview of method to determine DNA reactivity developed in the "MOSH MOAH" research project[12]

Following the study, the developed method was applied to additional samples to collect more information on MOAH composition and DNA reactivity using different food-grade- and non-food-grade lubricants, as well as food packaging materials. The correlation observed in the proof-of-concept study was confirmed: In MOAH fractions showing DNA reactivity in the Ames test, tri- and polyaromatic substances could be detected using GC×GC, while they were absent in non-DNA-reactive MOAH fractions. The conclusion is gratifying: Using this combined approach – and the knowledge it generates – offers a clearly better way to identify hazards and assess risks in food samples contaminated with MOAH.

Dedication to purity

In the "MOSH MOAH – Reduction of Mineral Oil in Food" project, researchers were able to actively contribute to overcoming a significant challenge in the monitoring of mineral oil residue in food. Existing methods were improved upon and new strategies developed to make identification of contamination sources easier as well as to reduce contamination and to assess the related risk using a more substance-based approach.

The knowledge generated by this project will be particularly useful to the food packaging and food industries in their constant quest to improve the quality of food and ensure consumer safety by reducing mineral oil contamination. Shimadzu is proud that its precision instruments could be of use to the dedicated scientists who are helping us all.

Note

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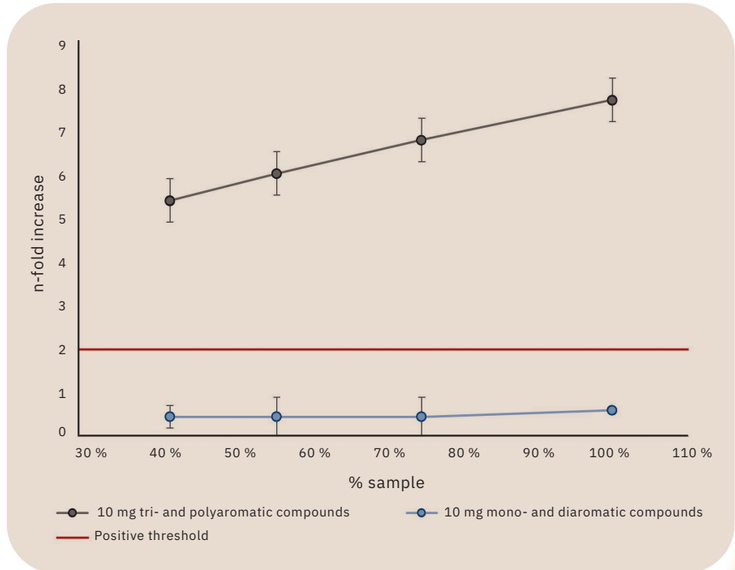


Figure 4: Results of Ames test: mono- and diaromatic substances isolated from a reference mineral oil scored negative (non-DNA-reactive) while tri- and polyaromatics scored positive[12]

An integrated systems approach to medicinal gas

Improving UV spectroscopy for analysis of medicinal nitric oxide mixtures

Riccardo Nava, Massimiliano Cattaneo, Simone Baccaro, SOL GROUP

The medical applications of nitric oxide (NO) are significant and growing. A recent collaboration between Shimadzu and the SOL Group – who produce both an active ingredient and a finished medicinal product based on nitric oxide – has led to a new system-integrated solution that streamlines analytic performance in the quality control of an NO-based medical gas. This article presents the results of that cooperation – which make the process of drug batch release faster, more accurate and more reliable – and hints at its potential to improve other analytical applications for gaseous samples.

Developing medical gases

The SOL Group, established in 1927, is a multinational company headquartered in Italy that focuses on the production and marketing of technical and medical gases. At its Specialty Gases Production Facility in Monza (near Milan), ultrapure gases and high-accuracy scientific mixtures are produced for customers such as research centers, universities, laboratories and the pharmaceutical industry. The Monza facility is authorized for the manufacture of gaseous active drug substances and drug products by the European Medicines Agency (EMA) and thereby meets the criteria of their Good Manufacturing Practice (GMP).

It was in Monza that a new medicinal gas using nitric oxide as an active ingredient was developed and is today produced (Figure 1). That product is registered with a marketing authorization in Europe under the name Neophyr and is widely used in European hospitals. Indeed, the market demand for nitric-oxide-based medicinal products in general is growing, due to the increasing spread of therapeutic applications aiming to improve the health of patients.

Ensuring parts-per-million quality

In the production cycle of a gaseous drug mixture in which the active ingredient is diluted on a parts-per-million scale in an (inactive) excipient – such as pure nitrogen – it is vital to comply with all GMP requirements to avoid any risk of contamination of the final product. It is also essential at all stages to ensure the most stringent quality assurance as well as the highest production quality.

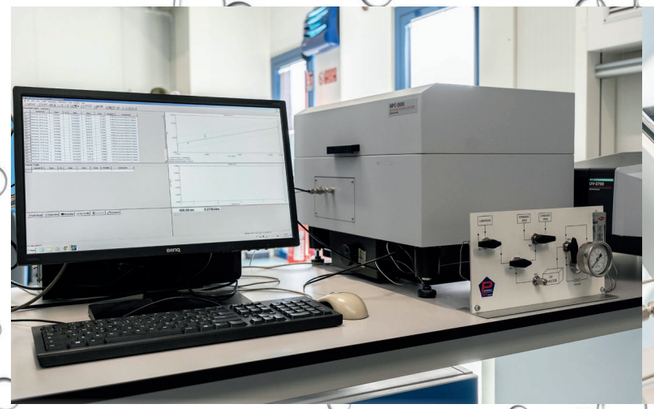
In particular, to guarantee the final quality of each product, the SOL Laboratory in Monza (Figure 2) uses instruments that can estimate, with absolute precision, values of impurities in a parts-per-million order through the use of detection systems based on multiple techniques.

In order to obtain an active pharmaceutical ingredient and intermediate product in full compliance with GMP requirements – and especially regarding the Nitrogen Dioxide (NO_2) impurity content – the Monza lab concluded that they needed to upgrade to the next level of technologically advanced instrumentation. Reference was made to the European Pharmacopoeia [Ph. EU monograph of the NO: EU Ph. 01/2008:1550], in which a UV-Vis spectrophotometer reading at a wavelength of 400 nm is specified as a reference technique for the analysis of Nitrogen Dioxide impurity. ⟶

Figure 1: Nitric oxide active principle production

Figure 2: The SOL laboratory in Monza

Figure 3: The project team



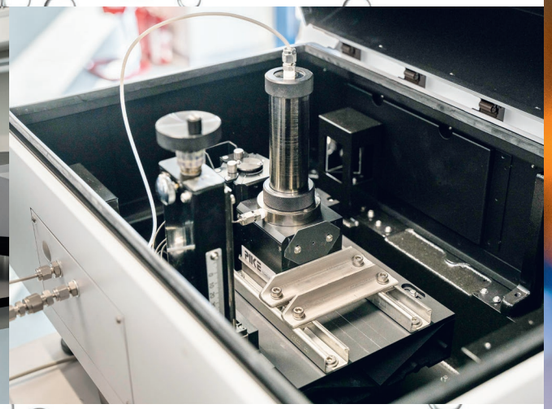


Figure 4: The UV-2700 coupled with an MPC-2600 module

Working toward a new SOLution

In view of the gaseous nature of the samples to be subjected to analysis and the need to have precise levels of accuracy for the various NO_2 concentrations to be analyzed, the Monza lab needed an instrument equipped with a specific gas cell in which the optical path was several meters in length and which was capable of reflecting electromagnetic radiation a certain number of times thanks to a system of mirrors inside the cell.

The SOL lab team at Monza identified one such instrument of special interest: Shimadzu's UV-2700 spectrophotometer, in which the 2.4 m long PIKE gas cell could be installed with an MPC-2600 module. Just as interesting was Shimadzu's interest in working with the lab to adapt and fully utilize the instrumentation for the lab's specific needs.

Together with technical support personnel from Pentatec and Selas Lab (Figure 3), experts from Shimadzu Italia and the SOL Monza team worked closely to optimize the analysis system in terms of gas cell fastening and alignment. By employing a quartz window inside the cell and a specially designed sampling piping, the instrument was also made ready for use for the analysis of pressurized samples, thereby lowering the analytical detection threshold. The system integration solutions adopted (Figure 4) gave positive results on the analyses, especially in terms of measurement repeatability and instrumental detection limits.

This tailor-made solution also revealed potential enhancements that could be made to other analytical applications used on gaseous samples and for which analytical techniques such as gas chromatography or infrared spectrophotometry – but lacking the advantages of a system-integrated approach – are currently more common.

Collaboration to improve the state of the art

Today, the collaboration between SOL and Shimadzu is making the quality assurance process of an important drug even faster, more accurate and more reliable. This firmly supports the continued expansion of helpful medical applications of nitric oxide as well as offering an intriguing possibility for improving other analytical applications for gaseous samples.

Note

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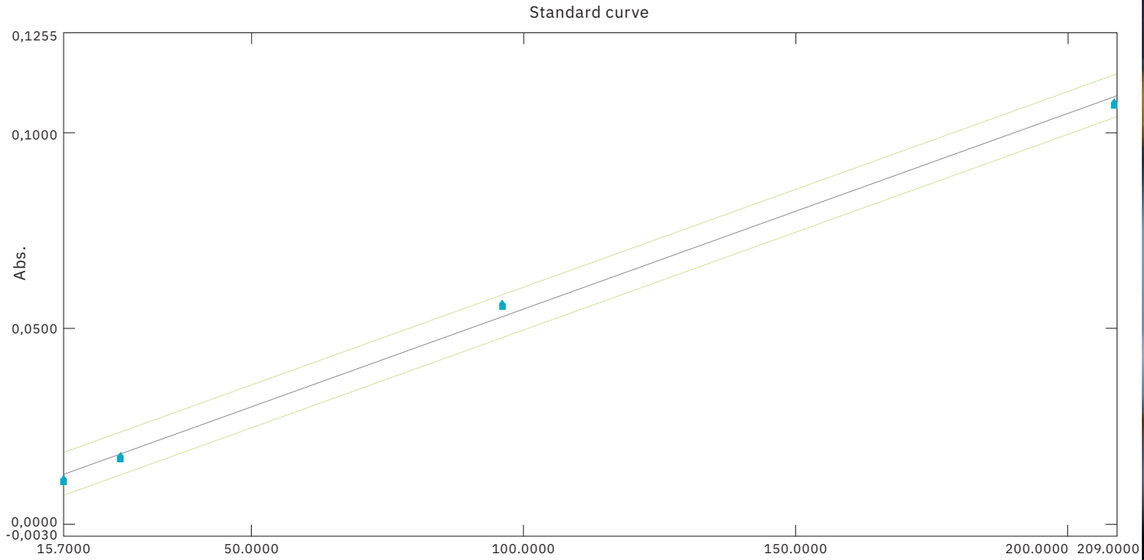
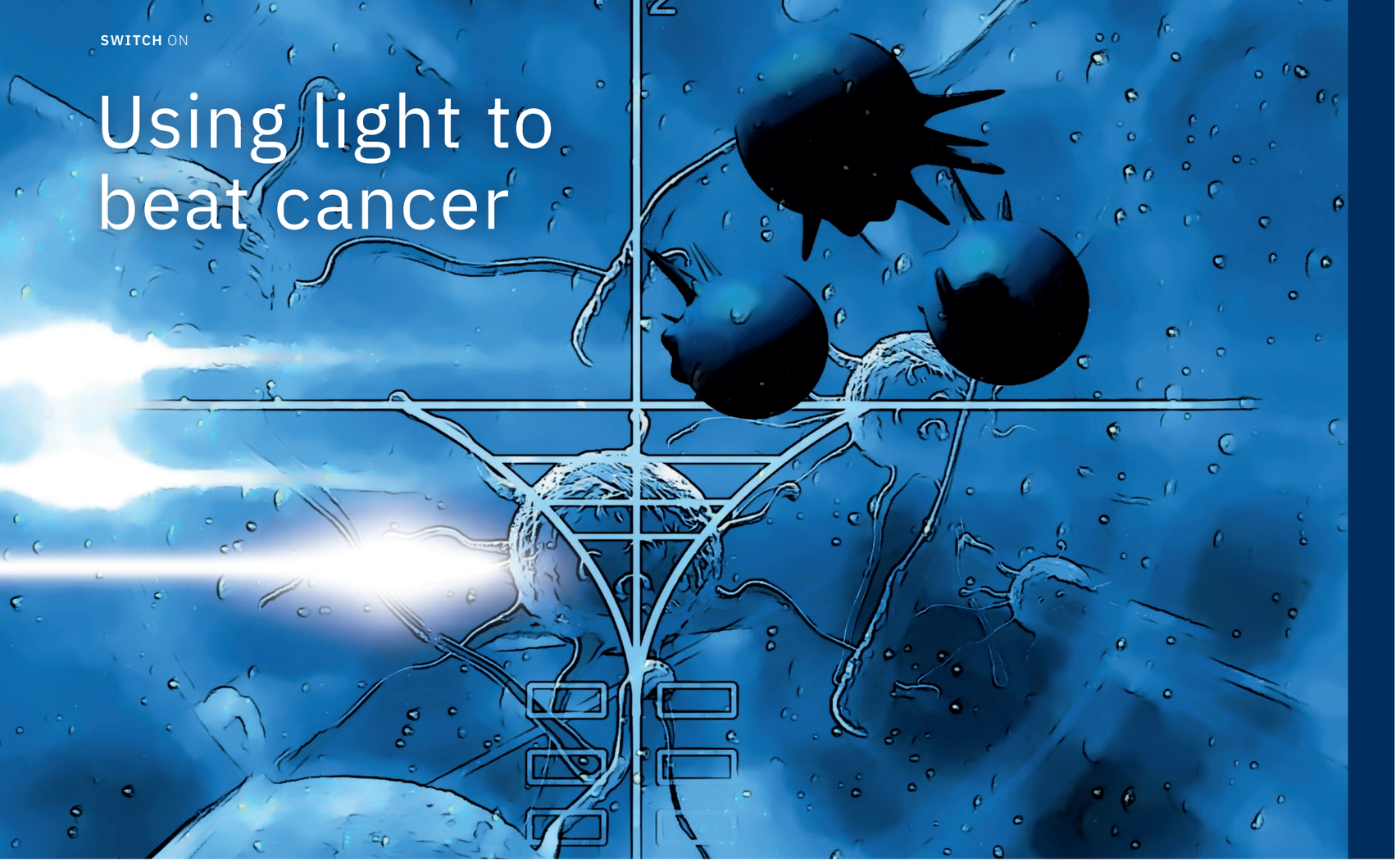


$y = 4.99511^{e-004} x + 0,00509268$
Correlation coefficient $r^2 = 0.99786$
Residual standard deviation = 0.00183

Figure 5: Straight line acquired in the concentration range from 15 to 200 ppm of NO_2

Using light to beat cancer



New approaches in photodynamic therapy

Dr. Daniel Obitz, Ruhr-Universität Bochum

With an aging population and increasing environmental pollution, cancer is on the rise. Mutations lead to uncontrolled cell growth and the formation of tumors. Traditional cancer therapies include the surgical removal of the affected tissue or killing it by ionizing radiation. A novel approach is photodynamic therapy, which is being studied at Germany's Ruhr University Bochum.

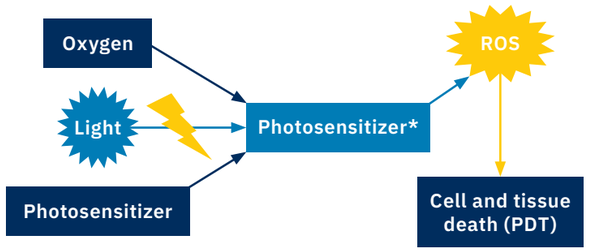
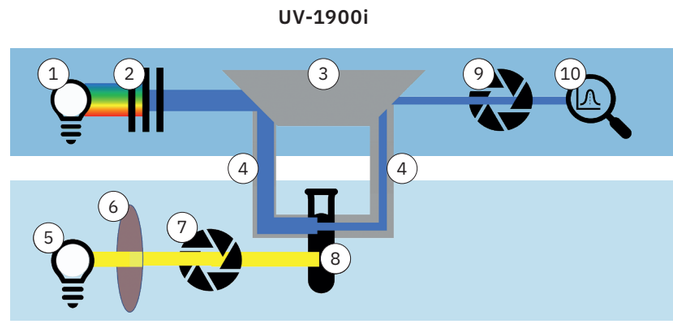


Figure 1: Schematic mechanism of photodynamic therapy

Figure 2: Schematic diagram of the experiment, consisting of UV-Vis spectrometer and exposure chamber.
1: D2 and WI lamp
2: Monochromator
3: Coupling optics for optical fiber
4: Optical fiber
5: Xe lamp
6: Longpass filter
7: Lamp shutter
8: Sample (formation of ROS)
9: Detector shutter
10: Photodiode

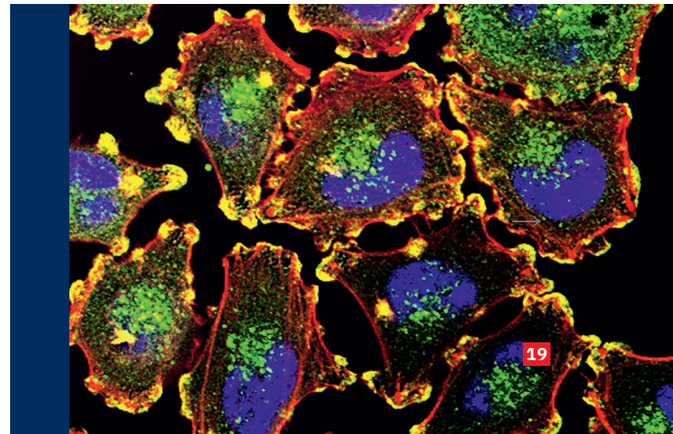


Can you destroy cancer cells with the help of light? Photodynamic therapy (PDT) is thought to be a promising alternative to traditional approaches in cancer therapy. A photosensitizer is used for this: It's as nontoxic as possible without exposure to light. The photosensitizer is excited – and therefore activated – using light of a specific wavelength. The activated photosensitizer reacts with the oxygen in the tumor tissue to form reactive oxygen species (ROS). This can lead to cell or tissue death in the tumor tissue and in this way fight the tumor (Figure 1). The aim of the research led by Prof. Dr. Nils Metzler-Nolte at Ruhr University Bochum is the development of novel photosensitizers for PDT.

Searching for the right compound

To preselect promising compounds, tests of various candidate compounds in solution are performed prior to the elaborate cell culture experiments (Figure 2):

A Shimadzu UV-1900i UV-Vis spectrometer is used to measure the absorption spectra. The light from a deuterium or halogen lamp (1) is split into monochromatic radiation by a monochromator (2) and coupled into an optical waveguide (4) using special optics (3). ⟶



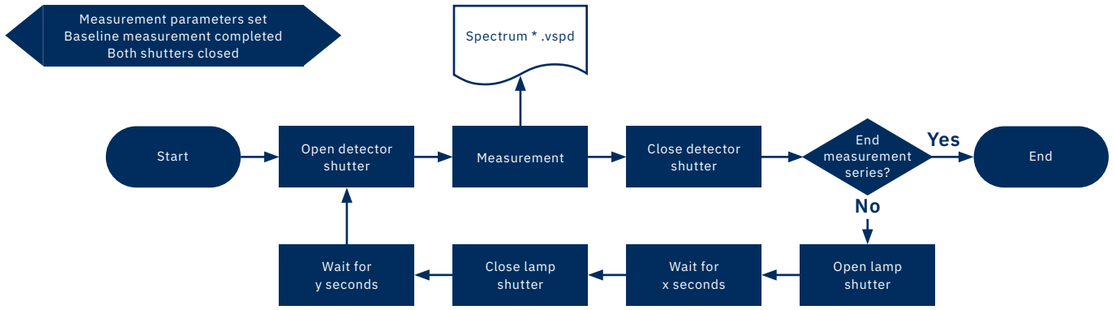


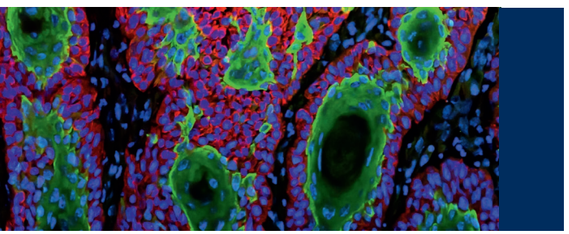
Figure 3: Schematic diagram of a sequence consisting of exposure of the sample and measurement of the absorption spectrum. Before the starting point, the spectrometer and apertures have already been initialized and the baseline recorded.

The compound being studied is dissolved together with a substance that reacts with reactive oxygen species (ROS) (8). The solution is then illuminated using a xenon gas discharge lamp (5) with a specific longpass filter (6). The exposure time is controlled by an electronic shutter (7).

After exposure is completed, the lamp shutter is closed and the absorption spectrum is measured. Another shutter (9) protects the sensitive photodiode (10) on the spectrometer from damage due to any intense stray light during exposure of the sample and is only opened during measurement.

Control of the shutter and measurement software is synchronized via a LabSolutions UV-Vis Automatic-Control macro. The experiment sequence is shown schematically in Figure 3. The start point in this diagram does not describe the program start but the start of the measuring sequence after the baseline measurement.

The setup is used to measure ROS production by the decay of the sample substance using UV-Vis spectroscopy (Figure 4). Various sample substances can be used here, in this case 1.3-diphenylisobenzofuran (DPBF).



In addition, the photostability of the photosensitizer can be shown by recording whole spectra. The setup is capable of performing high temporal resolution measurements at one-minute intervals, thanks to the very small time requirement of the UV 1900i. This very high scan rate makes it possible to observe the stability of substances by measuring entire spectra. This also allows for a more detailed look at fast reaction kinetics, which were previously inaccessible. The total illumination time is usually up to 60 minutes with illumination intervals of 2 to 5 minutes. Figure 5 shows the course of decomposition of 1.3-diphenylisobenzofuran by reactive oxygen species generated by a holmium complex over 45 minutes, with an illumination interval of 3 minutes.

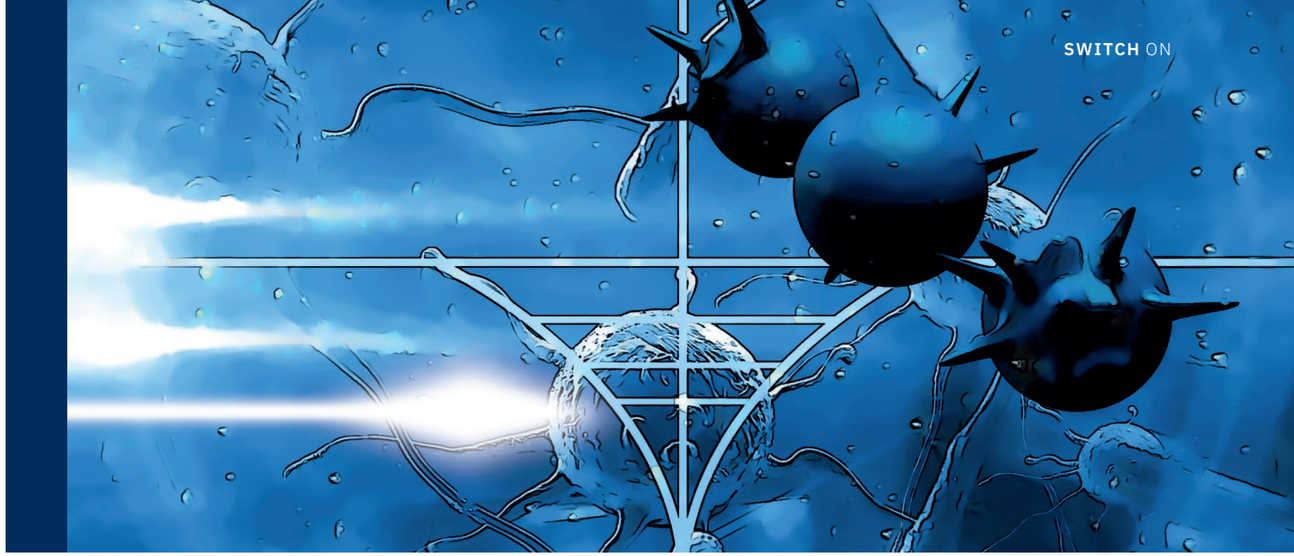
The decomposition of the sample can be clearly seen by the decreasing intensity of the absorption peak at 410 nm, while the absorption of the solution remains constant in time in the range from 500 to 700 nm.

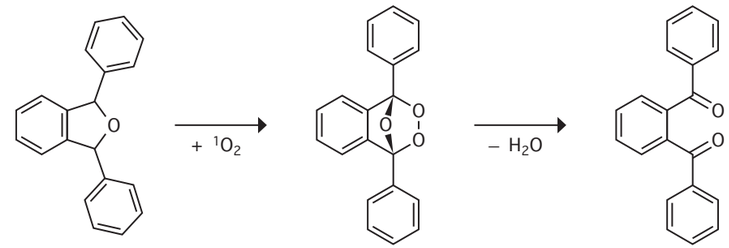
Specific pre-selection thanks to fast analysis

The automated measurement sequence and short measurement time of Shimadzu's UV-1900i allow photosensitizers to be analyzed quickly. This enables a qualified preselection of suitable molecules that are promising for more elaborate studies. The hope is that these studies will ultimately lead to a new and better way (photodynamic therapy) to treat cancer.

Note

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





yellow **colorless**

Figure 4: Decomposition of DPBF by reactive oxygen species

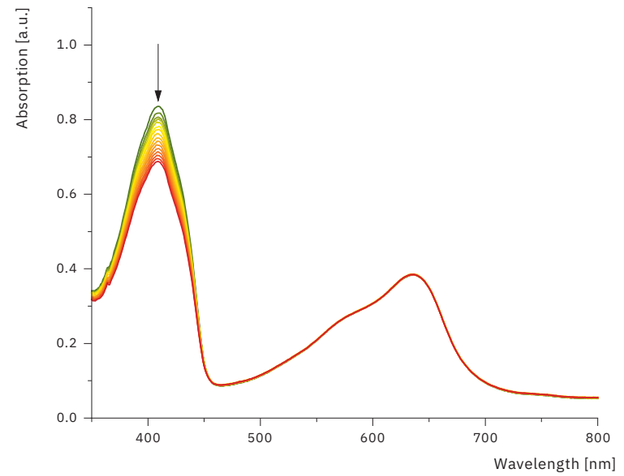
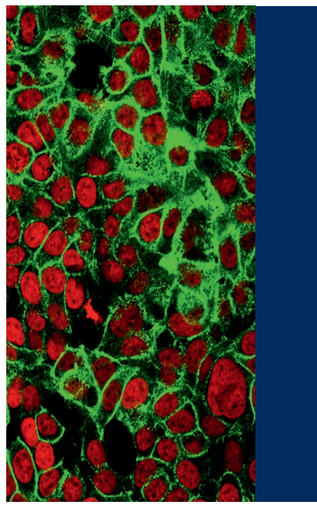


Figure 5: UV-Vis absorption spectra of 1.3-diphenylisobenzofuran during decomposition

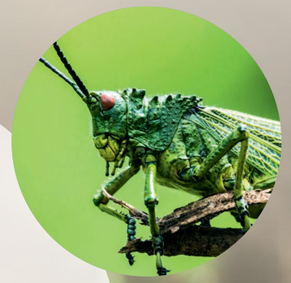


VOICES

Pure performance: Saving time with ultra-fast preparative LC

How Syngenta is using UFPLC to extract and purify gram-scale by-products for regulatory tests

Louise Bacon, Cyprien Bone, Lorraine Ezra, Syngenta



Chemicals such as herbicides, fungicides, insecticides and seed treatments are widely used to maximize crop yields worldwide. Ensuring the efficacy and safety of products requires studying the formulations in detail, including even the most minor of components. In this article, we talk to Syngenta about how they're using Shimadzu's UFPLC systems to speed up the extraction and purification of gram quantities of by-products that are present in technical-grade active-ingredient material at levels below 1 %, so providing the material needed for regulatory tests and toxicity studies.

Isolating by-products – a major challenge

In the world of agriculture, there are increasing pressures to maintain or increase crop yields in the face of growing threats – whether from pests, diseases, competitive weed species or the changing climate. To minimize the effect of these threats, farmers need to deploy a range of methods to promote strong and healthy crop growth. Crop protection products such as herbicides, fungicides, insecticides and seed treatments are an essential part of this effort, and Syngenta works with farmers, researchers and agriculture experts to understand the challenges faced and to develop new or improved treatments.

As a heavily-regulated industry, crop protection products must meet numerous criteria before they can be registered for use, which includes rigorous tests to assess their safety for workers, the environment, crops and consumers.

Some of these tests must be carried out on all components of technical-grade material of an active ingredient at, or close to, the 0.1 % weight level. However, obtaining these "by-products" in sufficient quantity and purity, is a big challenge. →

Developing analytical solutions at Syngenta

Isolating these by-products in usable quantities is where separation scientists such as Louise Bacon come in. Louise works at Syngenta as the Team Lead for the Preparative and Isolation Chemistry Team, which sits within the company's Analytical Solutions Group at Jealott's Hill International Research Centre in Bracknell, UK. Her team assists their colleagues around the world with the toughest of separation challenges with the help of preparative-scale liquid chromatography.

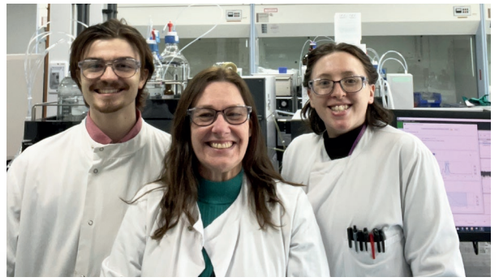


Figure 1: Louise Bacon (center) with her colleagues Cyprien Bone (left) and Lorraine Ezra (right) in the Preparative and Isolation Chemistry Team at Syngenta

Louise has had several roles at Syngenta over the last 30 years and has been working full-time on the preparative side since 2016. She describes the sorts of projects that her team receives: "Most of our projects come from our 'global analytical champions', who are responsible for an active ingredient and all the associated method validation and regulatory work. As part of that, they may need our help to extract and purify milligram levels of an unknown by-product for identification. But more often they will need larger quantities of a by-product for use as analytical standards, extending up to the gram levels required for toxicity testing. For example, it's not uncommon to have a by-product present at 0.5 % by weight in a product, and that requires a toxicity test on the pure chemical in order for the active ingredient as a whole to be registered".

It's this need for larger quantities, she explains, that presents the biggest challenge. "We'll liaise closely with our chemistry colleagues to work out the best route to these by-products. The preferred route is synthesis because it's quicker – but not everything can be synthesized, and even if it can, it usually results in an impure mixture. So we're often asked to extract the pure by-product from these mixtures or from extracts or washes obtained from the original synthesis of the active ingredient".

Figure 2: ▶

(A) The 5 liters of acetic acid washes that provided the starting material for the required acid isomers, containing just 0.3–0.4 % of the target material

(B) The final product as a white powder (1.5 g, 93 % purity)

(C) Chromatogram obtained from the mixture at the final stage of method optimization, using Shimadzu UFPLC with a 100-mm pentafluorophenyl reverse-phase column and a run-time of just 13 minutes. The analysis shows a sharp peak of the target compound, well-separated from a nearby "hump" of unwanted material.

The value of ultra-fast preparative LC

Louise and her team usually like to tackle these purifications through a two-stage approach: "We've found it works best if we do a 'rough and ready' large-scale purification of the supplied material, for example to enrich it from 0.1 % up to 5 %, perhaps up to 20 % if we're lucky. We then follow this with a 'polishing' step to bring it up to the required purity, which is usually 90 % or higher".

This first step is the most challenging, Louise explains, because it involves dealing with such large volumes of material. "To take one example, we were working with an insecticide by-product that was a highly volatile acid, supplied at 0.3–0.4 % in acetic acid solution that also contained aliphatic components and salts. The request was for 800 mg, which we knew at the outset was going to be tough", she says.

Their initial approach was to run the washings through an HPLC and collect/combine all the fractions. This was far from ideal, requiring 1,000 injections with a 22-minute run time, giving liters of extracts to deal with. Not only that, but subsequent processing was also difficult. Lyophilization didn't work because the target compound was too volatile, while the alternative – extraction of the acetonitrile/water fractions from HPLC with dichloromethane (DCM) – gave mixtures that were difficult to evaporate down without losing material. Louise explains the upshot: "All this consumed 5 months of the team's time and, to add insult to injury, the final material was only 55 % pure!".

So when the request for a further 4 grams of the target compound came in, Louise knew they needed another, more automated approach: "The HPLC route was scarcely feasible at the smaller scale, and so obtaining more material the same way was clearly going to be impossible. But fortunately at about this time, in the middle of 2021, an opportunity came up to acquire a Shimadzu UFPLC instrument. We immediately saw how it could help us and jumped at the opportunity!".

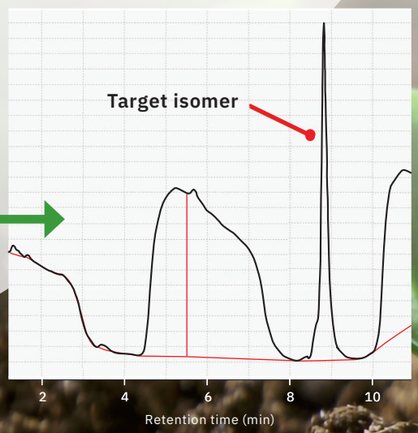
Shimadzu's Ultra-Fast Preparative LC (UFPLC) works by automatically heart-cutting the fraction containing the target compound and trapping it on a short column, rather than leaving it to elute. Louise's team realized that this would enable them to backflush off their target compound in acetonitrile, dispensing with the need for DCM extraction and leaving them with a solution from which the acetonitrile could be gently removed without problems.

Following some work to optimize the column to avoid coelution of the analyte with a "hump" of unwanted material, the team finally found success, says Louise: "From 5 liters of washings, we obtained well over a gram of material, and the purity of the product was better than we'd achieved before. But more than that, we'd avoided all the manual handling, eliminated the problems with drying-down and reduced the processing time to a matter of weeks". →

A

B

C



Automated fraction collection for faster turnaround

In fact, the efficiency savings with the first system were so good that Louise used it as an example to request the purchase of a second system – which was set up by the beginning of this year. So what will the team be using their new equipment for?

Louise says: “We see the greatest value of UFPLC in cases where we have large volumes of impure material requiring hundreds or even thousands of injections. Using HPLC would generate multi-liter quantities of fractions, which would slow us down because of the backlog of samples awaiting evaporation, especially when we’re being asked to collect multiple products. But with the Shimadzu system we can automate the collection of up to five fractions with no manual intervention”.

This, she says, makes it ideal for the initial clean-up and enrichment stage of a complex mixture. “We don’t even have to do a full separation – in a recent project on a herbicide by-product, we used a 50-mm column with a 5-minute run time, simply to remove the majority of the unwanted material and provide a small volume of a greatly enriched extract, which we could then ‘polish’ on one of our other preparative systems”.

She adds that the chromatographic reproducibility of UFPLC is critical to the ability to automate the collection of fractions – because the team are looking at products that are scarcely off the baseline, they have to rely on known retention times rather than gradient information. Another benefit is the small fraction volumes: “In the herbicide project, the target material wasn’t particularly stable. It was therefore really useful that we were able to get it into small volumes so that subsequent processing steps were as quick as possible”.

Reducing solvent usage

An important consequence of the team’s new equipment is not only product quality and turnaround time but reducing the environmental impact of their methods. This is a result of being able to use short columns and short run times, with a consequent reduction in the amount of solvent they’re using.

“Reducing our solvent usage is a major benefit of using UFPLC, and one we’re going to be looking at more”, says Louise. “On a prep HPLC method, you might be running thousands of injections on a 20-minute method at 50 mL per minute – which generates a vast amount of solvent to evaporate and ultimately dispose of. But with UFPLC, we reduce that need for solvents and the associated energy costs in evaporating them, as well as being able to use more environmentally benign solvents”.

In fact, this mission to reduce solvent usage is also a priority for Syngenta because it makes a significant contribution to the company’s sustainability goals. Supporting this, Louise’s team have a student on an industrial placement from Imperial College London, who will be using the new UFPLC systems to identify where the biggest solvent reductions can be made.

Making life easier

In conclusion, says Louise, with the help of the Shimadzu UFPLC, they’ve been able to achieve what was previously very difficult – the extraction of sub-percent levels of by-products from very complex mixtures, all while keeping solvent usage down, minimizing degradation of the target compounds and reducing manual processing to the bare minimum. As she concludes, “It’s been brilliant, absolutely brilliant, and saved us so much time!”.

Figure 3: One of the two Shimadzu UFPLC systems in the lab of the Preparative and Isolation Chemistry Team at Syngenta. The system uses trapping cartridges to collect fractions, each of which can hold ca. 100 mg of material, and can be flushed to change solvent, pH, remove buffers – or in the case of the acid isomers – backflushed with solvent to elute the target material. The team uses a second UV detector during this backflushing step to minimize theeluant volume, which was kept to just 5 mL in this case.

Note

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

Plastics in North Sea fulmars

Dissection, stomach analysis and plastic identification with infrared spectroscopy

Dr. Susanne Kühn, Wageningen Marine Research, Netherlands

The northern fulmar – a type of petrel – is widespread throughout the North Sea region. The bird eats practically everything it finds on the sea surface – including a lot of plastic waste. This makes it interesting for research. Most countries along the North Sea report annually on developments in the amount and composition of plastic in fulmar stomachs and exchange information at regular workshops. At a workshop in October 2022 at the Wageningen Marine Research Institute in the Netherlands, infrared spectroscopy was used to analyze the polymer composition found in fulmar stomachs.

In October 2022, an international group of experts met at the Wageningen Marine Research Institute in the Nether-lands for another in a series of “Fulmar workshops”. There, they shared the current research being carried out by the various working groups present.

They also dissected a large number of fulmars found dead in or along the North Sea. The reason was to examine the amount and composition of plastic that had been swallowed by the animals. Infrared spectroscopy as a method was presented and carried out during the workshop, and the polymer composition of many of the plastics from the fulmar stomachs was identified.

The organizer of this year’s Fulmar workshop, Dr. Susanne Kühn, is an expert in the field of environmental pollution and its impact on marine wildlife, with a focus on fulmars. Shimadzu has been participating in the Fulmar workshops since 2014.

The fulmar – “trash can” of the North Sea

The fulmar is a true high-sea bird. Outside the breeding season, this bird species spends all its time at sea. Fulmars are not picky about their food: Everything that floats on the water surface is on the menu. This diverse diet was a successful strategy of the birds for a long time. In the last century, their population spread from Iceland and Scotland across the entire North Atlantic.

However, industrial production and use of plastic has also increased greatly during the same period. One consequence of this is that plastic waste pollution is increasing in the oceans. The indiscriminate diet of fulmars ensures that these animals also regularly swallow plastic that floats on the sea surface. This is why the EU and all North Sea countries use the fulmar as an indicator species, and fulmars that wash up dead on North Sea beaches are used to measure marine plastic pollution. →

▸ Figure 1: The fulmar is the only seabird that comes to land to breed. In the image, Dr. Susanne Kühn takes measurements from a fulmar during the workshop.

▸▸ Figure 2: Stomach contents of a fulmar: foam, thread and industrial granules (left); styrofoam cells and various fragments of unclear origin (Jan Van Franeker, WMR) (right). The ruler at the bottom of the image shows mm scaling.

Figure 3: Workshop atmosphere during the analysis of plastic from fulmar stomachs with the Shimadzu IRSpirit infrared spectrometer

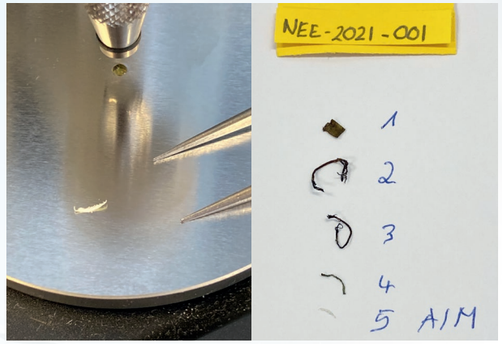


Figure 4: Sample NEE-2021-001 number 5 for size comparison on the measuring station of a QATR-S. The round diamond window is smaller than 2 mm in diameter (left). The sample is from a bird stomach from north-east England (right).

Workshop – a place for learning

In addition to the other activities, standardized methods were taught and calibrated during the workshop. Age and sex characteristics as well as the condition of the plumage and possible causes of death of the fulmars were determined. The stomach contents were rinsed out over a 1-millimeter sieve, and all plastic parts were sorted under the microscope to be dried and weighed later.

Microplastic analysis

The sieving yields fragments in cm, mm and also µm. The smaller the fragments, the more interesting the question: What can be analyzed and how? An infrared device and/or infrared microscope can be used for quick screening to identify the polymer. If the question concerns additives that are present in minute quantities in the plastics – and whether these harm the birds or other animals that ingest the particles – then such ingredients can be extracted, selectively visualized and traced by chemically processing the polymers.

The analytical instrumentation techniques for this are gas chromatography – mass spectrometry (GC-MS) couplings with pyrolysis unit and elemental analysis (EDX, AAS, ICP, ICP-MS) used for characterization. Research is striving to analytically follow fractal decay into microparticles and nanoparticles.

Infrared spectroscopy

During the workshop, the polymer composition of plastics from fulmar stomachs could be determined through rapid screening with infrared spectroscopy (FTIR, Shimadzu IRSpirit).

The infrared spectroscopy used here is FTIR with ATR technique. The samples can be placed on a measuring window and analyzed without wet chemical preparation, i.e. nondestructively. Somewhat more complex, sample preparation is required for chromatographic and elemental analysis. In addition, the equipment required for this is stationary. An IRSpirit, on the other hand, can also be brought into the dissecting room to perform a quicker analysis. Impure plastic samples from stomachs that are still contaminated with residue of organic material can also be analyzed.

Infrared spectroscopy uses thermal radiation from a wavelength of 2,500 to 25,000 nm (FTIR usually 4,000–400 cm^{-1}) depending on the accessories used. A substance under heat absorbs energy, which causes the molecules and molecular frameworks of the substance to move Each polymer has its own infrared spectrum. If these are collected in a spectra library, one can quickly identify unknown substances.

Rapid screening via ATR, the attenuated total reflection technique, makes analysis very effective. Handling them under ATR conditions takes about 1.5 minutes per sample measurement:

1. Place the sample on the measuring window.
2. Press the sample against the measuring window with the pressing unit.
3. Measure (accumulate for 1 minute).
4. Remove sample and clean window.

Infrared spectroscopy is increasingly being used to analyze plastic composition in stomach samples. The analysis can distinguish plastic particles from organic material. The polymer type can also provide information about the origin of the ingested plastic. Finally, analyzing the polymer can provide an initial assessment of how much additive can be expected in the plastic.

Results contribute to the protection of the seas and their inhabitants

The preliminary results of the FTIR measurements confirm previous findings: The plastics most commonly ingested by fulmars are mostly polyethylene and polypropylene, two plastics that are among the ones most widely produced in the world and which mostly float on the surface of the oceans.

These results can also serve policymakers, for example, to make regulations that further restrict the influx of certain types of plastic into the environment. Everyone can contribute to this: By conscientiously handling plastic (waste), the amount of marine litter can be reduced, and thus the fulmar can be protected. A study from the Netherlands shows that the amount of plastic in birds' stomachs decreased significantly between 1979 and 2021. The decrease is probably related to the widespread political and social attention that plastic waste has received in recent years. Education and stricter regulations contribute to the fact that, at least within the North Sea, plastic waste is slowly decreasing. Nevertheless, virtually every fulmar in the North Sea (over 90 %) still has pieces of polymer in its stomach.

Note

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

Faster and automated analysis of aroma compounds in cosmetics

MOVE ON

Aromas have a major effect on consumers. Personal care brands and products are often judged by how they smell. In product testing labs, fragrances are typically evaluated by highly trained experts, and evaluations are quite time-consuming. In recent years, this has led to increasing interest in the use of instrumental analysis to speed up productivity. Gas chromatography can be used, but sample complexity makes it difficult to do this as quickly and accurately as desired. This article describes a fast and precise technique for analyzing aromas in cosmetics using Shimadzu's Smart Aroma Database.

Aroma analysis is an important part of ensuring that brands and products – especially in the food and personal care sectors – appeal to consumers. Traditionally, such analyses were done manually by highly trained experts. This method is very time-consuming, and industry has sought to supplement this with instrumental analyses. Yet even using gas chromatography can be challenging due to the large number of analytes and the requirement for very experienced operators capable of using sophisticated technical solutions.

Clearly, labs and companies working in the field of aroma analysis could use a more speedy and accurate method, which regular lab technicians can easily operate. This article describes a new method that meets those criteria.

Analysis using the Smart Aroma Database

The key to simplifying this complex work is the Smart Aroma Database, which contains analytical information for 500 types of important compounds relevant to fragrances. When using the Smart Aroma Database, the creation of analytical methods only requires an adjustment of retention times using a standard n-alkane mixture before sample analysis. The results can then be used to automatically and accurately identify the relevant target aroma compounds. ⟶

Digital precision of the Smart Aroma Database simplifies formerly complex task

Dr. Waldemar Weber,
Shimadzu Europa GmbH

Figure 1: GCMS-TQ8040 NX system + AOC-6000 Plus

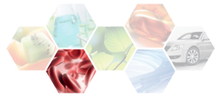


Figure 2: Analysis process flow using the Smart Aroma Database

MOVE ON

Samples and analytical conditions

A 20-mg sample of a commercial lip gloss was weighed and sealed in a screw vial for solid phase microextraction (SPME) analysis. First, the compounds contained in the sample were identified by scan-mode analysis using analytical conditions registered in the Smart Aroma Database. Next, SIM and MRM methods were automatically created for the identified compounds, and the sample was analyzed in SIM and MRM modes (Table 1).

Using the Smart Aroma Database

Using the Smart Aroma Database, 31 aroma compounds were detected by scan-mode analysis. These compounds and the corresponding library search similarity scores are listed in Table 2. The Smart Aroma Database narrows down the list of target compounds based on retention times and ion ratios as well as on the similarity scores. This results in a more precise and efficient targeted analysis. In addition, as the sensory information is registered, the aroma characteristics of compounds can be checked simultaneously when identification results are obtained (Figure 3). Evaluating product aromas requires determining how the respective compounds affect the product fragrance. With the Smart Aroma Database, identification results and sensory information can be checked at the same time.

SIM- and MRM-mode analysis

Next, the 31 compounds identified by scan mode were analyzed using the SIM and MRM methods automatically created by the Smart Aroma Database. Because fragrance is determined by the balance between respective aroma compounds, an accurate quantitative analysis is important. However, cosmetics and other personal care products often contain fragrances or ingredients that are extracted from plants or other sources. Such compounds are typically

Name	RT	Area	Comment
		> 0	
Limonene	8.652	199705.00	lemon, orange
Benzyl alcohol	8.740	2494982.00	sweet, flower
Diethyl malonate	9.467	299970.00	apple

Figure 3: LabSolutions Insight data analysis window

System	
GCMS model	GCMS-TQ8040 NX
Autosampler	AOC-6000 plus
Column	SH-I-5Sil MS (30 m x 0.25 m ID x 0.25 µm)
AOC-6000 conditions	
SPME arrow	DVB/Carbon WR/PDMS
Conditioning temp.	270 °C
Sample extract time	30 min
Sample desorb. time	1 min
GC conditions	
Injection mode	Split
Split ratio	5
Carrier gas	He
Carrier gas control	Pressure (83.5 kPa)
Column temperature	50 °C (5 min) → 10 °C/min → 250 °C (10 min)
MS conditions	
Ion source temp.	200 °C
Interface temp.	250 °C
Data acquisition mode	SCAN, SIM, MRM
Scan range	*m/z* 35–400

Table 1: Analytical conditions

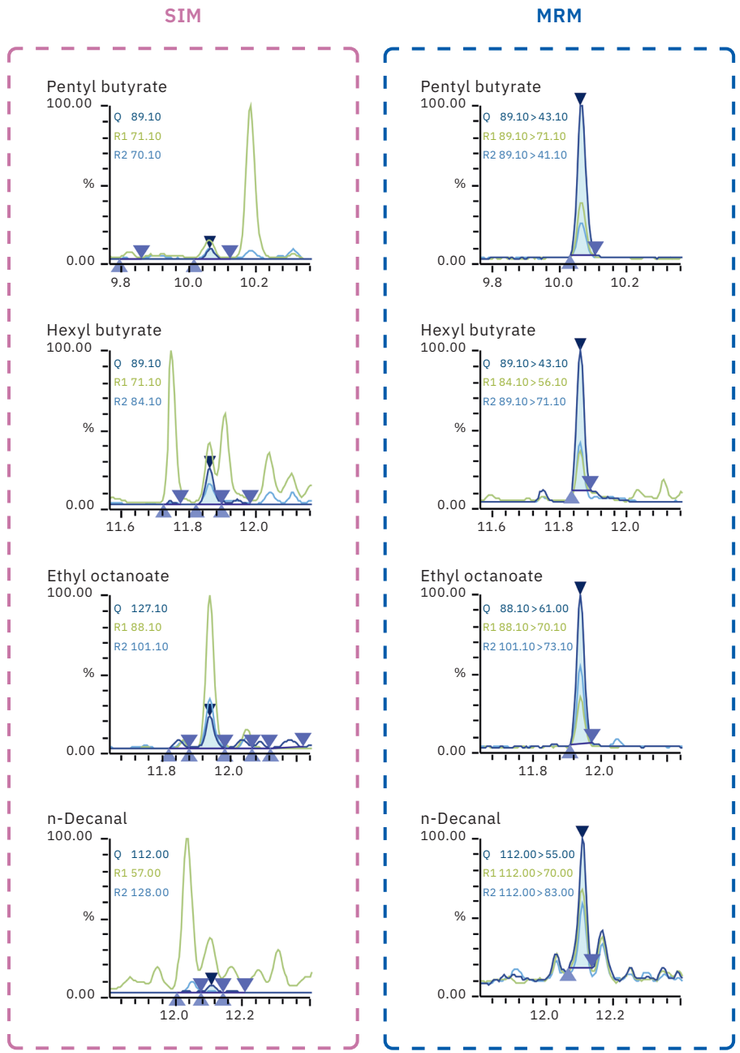


Figure 4: Comparison of SIM- and MRM-mode analysis

detected along with multiple interfering peaks, which can make it difficult to accurately quantify target compounds due to coelutions.

In particular, when comparing multiple analytes, contaminants can vary depending on the sample. When analyzing data from SIM-mode analysis, it may be necessary to make appropriate adjustments to quantitation ions or incorrectly identified peaks in accordance with the sample. In such cases, the higher selectivity of MRM-mode analysis can help minimize the effects so that target compounds can be quantitated more accurately.

Typically, determining MRM-mode analytical conditions is a difficult, time-consuming process. However, the Smart Aroma Database can create MRM methods for the selected compounds automatically, thus ensuring that advanced MRM-mode analyses can be performed easily without special experience or time-consuming steps. Figure 4 is a comparison of SIM- and MRM-mode analysis results, showing that a large number of contaminant peaks are included near target compounds in SIM mode, but that those targets are detected with greater selectivity in MRM mode. Thus, for cosmetics and other samples with complex matrices, MRM mode can be an effective way to suppress the effects of contaminants and achieve more accurate quantification and data analysis – with less effort. →

Smart databases make a difference

Shimazu's Smart Aroma Database enabled 31 aroma compounds emitted from lip gloss to be quickly identified. This helped to increase the accuracy and efficiency of qualitative analysis of aroma compounds by using mass spectral similarity scores calculated based on the aroma compound library. The library included within the database can narrow down the list of candidates – rather than using only mass chromatograms and ion comparisons for identification.

In addition, the Smart Aroma Database can be used to easily create SIM and MRM methods for more sophisticated analyses of aroma compounds in cosmetics or other samples with complex matrices. And all of that makes a big difference.

Compound	Similarity score	Compound	Similarity score
1-butanol	95	Limonene	96
Methyl butanoate	96	Benzyl alcohol	94
Ethyl isobutyrate	92	Diethyl malonate	93
Ethyl butanoate	96	(E)-linalool oxide	84
Ethyl lactate	92	Pentyl butyrate	92
Butyl acetate	97	Linalool	96
Ethyl 2-methylbutyrate	96	Nonanal	94
cis-3-Hexen-1-ol	80	Benzyl acetate	94
Isoamyl acetate	97	(Z)-3-hexenyl butyrate	96
Methyl hexanoate	96	Hexyl butyrate	85
Benzaldehyde	94	Ethyl octanoate	92
Ethyl hexanoate	94	n-Decanal	95
Octanal	88	Benzyl butyrate	81
(3Z)-3-hexenyl acetate	95	Methyl cinnamate	86
Hexyl acetate	98	gamma-Decalactone	93
		gamma-Undecalactone	93

Table 2: Summary of identification results

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

Determining nutritional value and detecting fraud

Combining GC-2030 and GCMS-QP2020NX increases information from edible-oil analysis

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Gas chromatography (GC) is a well-established technique for identifying and quantifying fatty acids in edible oils, especially in combination with a flame ionization detector (GC-FID). Quantification of fatty acids in edible oils is useful in determining their nutritional value and in detecting fraud. However, GC-FID has certain limitations, which researchers were able to overcome by also using GC-MS in the analysis of six edible oils. The combined technique was then used to identify squalene in three samples.

Ensuring purity in edible oils

Edible oils with high concentrations of monounsaturated (MUFA) and poly-unsaturated (PUFA) fatty acids have rich nutritional value.[2]

Discovering the truth

So, it is important for consumers to know what they are buying. Ensuring that they do, is usually the responsibility of the governmental authorities. And because government regulators are frequently faced with deliberate fraud in the case of edible oils – in which higher-value oils are diluted with lower-value oils – proper testing is often the only way to find out the truth.

Gas chromatography (GC) is a well-established technique for identifying and quantifying fatty acids in edible oils, especially in combination with a flame ionization detector (GC-FID). However, GC-FID has certain limitations. First, the results obtained pertain to a limited group of fatty acids, excluding the vast majority of compounds contained in the samples. Second, GC-FID provides no information on the identity of the obtained peaks, so it is obligatory for the operator to inject reference standards for confirmation.

Researchers decided to compare this commonly used method with a new method using gas chromatography and a mass spectrometric detector (GC-MS). To assist them, they turned to Shimadzu, who produce all of the high-quality scientific instrumentation necessary for this test. Five different types of edible oils were then studied and analyzed using both GC-FID and GC-MS techniques.

Preparing to test the tests

Gas chromatography requires fatty acids to be derivatized in order to become sufficiently volatile to be eluted at lower temperatures without thermal decomposition. Methyl esters are commonly studied derivatives which are produced by methylation. According to the method used, the ester bonds are hydrolyzed to release free fatty acids, which are transmethylated to form fatty acid methyl esters (FAMEs). The resulting FAME profile determined by GC is referred to as the fatty acid composition.

GC-FID analytical conditions

Gas chromatograph:
Shimadzu GC-2030 equipped with AOC-20i+s, Split/Splitless injector, FID detector

Capillary column:
MEGA-10 (30 m × 0.25 mm ID, 0.25 µm film thickness)

Part of GC	Values
Split injector temperature	245 °C
Split ratio	200
FID temperature	245 °C
FID air flow	200 mL/min
FID hydrogen flow	32 mL/min
FID make-up flow	24 mL/min
Carrier gas	He
Linear velocity	31.3 cm/sec
Column flow	1.1 mL/min
Oven temperature program	150 °C → 12 min 1 °C → 175 °C → 5 min 3 °C → 200 °C → 1 min
Column	MEGA-10 (30 m * 0.25 mm, 0.25 µm)

Table 1: FAME method parameters of GC-FID analysis

GC-MS analytical conditions

Gas chromatography-mass spectrometry:
Shimadzu Nexis GCMS-QP2020NX equipped with AOC-30i+s, Split/Splitless injector

Capillary column:
MEGA-WAX_MS (30 m × 0.25 mm ID, 0.25 µm film thickness) →

Part of GC	Values
Split injector temperature	240 °C
Split ratio	200
Carrier gas	He
Linear velocity	31.3 cm/sec
Column flow	1.2 mL/min
Acq. mode	Scan (40–500 *m/z*)
Ion source temperature	220 °C
Interface temperature	240 °C
Oven temperature program	150 °C → 5 min 2 °C → 175 °C → 5 min 12 °C → 200 °C → 5 min 10 °C → 240 °C → 10 min
Column	MEGA-WAX_MS (30 m * 0.25 mm, 0.25 µm)

Table 2: FAME method parameters of GC-MS analysis

The results

The (%) area of FAMEs was calculated separately for each oil using both the GC-FID and GC-MS techniques. Ratios of monounsaturated/polyunsaturated acids were also determined. Oleic acid is the main monounsaturated acid for edible oils, while linoleic and linolenic acids are the basic polyunsaturated acids. Tables 3 and 4 show the overall results from the area percentage of fatty acids in five different edible oils.

	Olive oil		Pomace oil		Corn oil	
FAMES	**GC area (%)**	**GC-MS area (% TIC)**	**GC area (%)**	**GC-MS area (% TIC)**	**GC area (%)**	**GC-MS area (% TIC)**
C14:0	0.013	–	–	0.02	0.012	0.04
C16:0	**11.060**	**11.72**	**12.604**	**13.22**	**11.286**	**13.64**
C16:1	0.938	0.98	1.049	1.12	0.136	0.16
C17:0	0.041	0.04	0.066	0.06	0.064	0.07
C17:1	0.073	0.08	0.091	0.10	0.036	0.03
C18:0	2.429	2.68	2.625	2.97	1.969	2.36
C18:1	**77.291**	**76.74**	**69.477**	**68.76**	**31.808**	**34.92**
C18:2	**6.470**	**6.00**	**12.207**	**11.65**	**52.594**	**46.40**
C18:3	**0.764**	**0.59**	**0.733**	**0.69**	**1.011**	**0.92**
C20:0	0.424	0.50	0.483	0.58	0.425	0.55
C20:1	0.313	0.35	0.363	0.45	0.338	0.44
C22:0	0.125	0.21	0.199	0.25	0.147	0.22
C24:0	0.058	0.11	0.103	0.13	0.174	0.25
	100.000	100.000	100.000	100.000	100.000	100.000

Table 3: (%) area of fatty acids in olive oil, pomace oil, corn oil

	Soybean oil		Cannabis oil	
FAMES	**GC area (%)**	**GC-MS area (% TIC)**	**GC area (%)**	**GC-MS area (% TIC)**
C14:0	0.024	0.11	–	0.05
C16:0	**10.644**	**12.29**	**6.126**	**7.33**
C16:1	0.087	0.11	0.166	0.18
C17:0	0.080	0.10	0.054	0.06
C17:1	0.053	0.05	0.022	0.03
C18:0	3.871	4.73	2.254	2.78
C18:1	**25.117**	**28.66**	**22.374**	**25.67**
C18:2	**52.955**	**46.87**	**49.416**	**45.28**
C18:3	**5.894**	**5.38**	**17.534**	**15.95**
C20:0	0.448	0.48	0.836	1.10
C20:1	0.189	0.33	0.651	0.77
C22:0	0.479	0.68	0.400	0.59
C24:0	0.159	0.22	0.168	0.21
	100.000	100.000	100.000	100.000

Table 4: (%) area of fatty acids in soybean oil, cannabis oil

Notice that the results obtained from GC-FID are not directly comparable with those achieved by the GC-MS for all samples. We can see that the (%) area of the SFA in GC-MS was higher than the one in GC-FID. Meanwhile, PUFAs (e.g. linoleic, linolenic acids) were determined in higher ratios in the GC-FID analysis. We conclude that GC-MS analysis of FAMEs leads to an overestimation of SFA, regardless of sample type.

MUFA/PUFA ratio

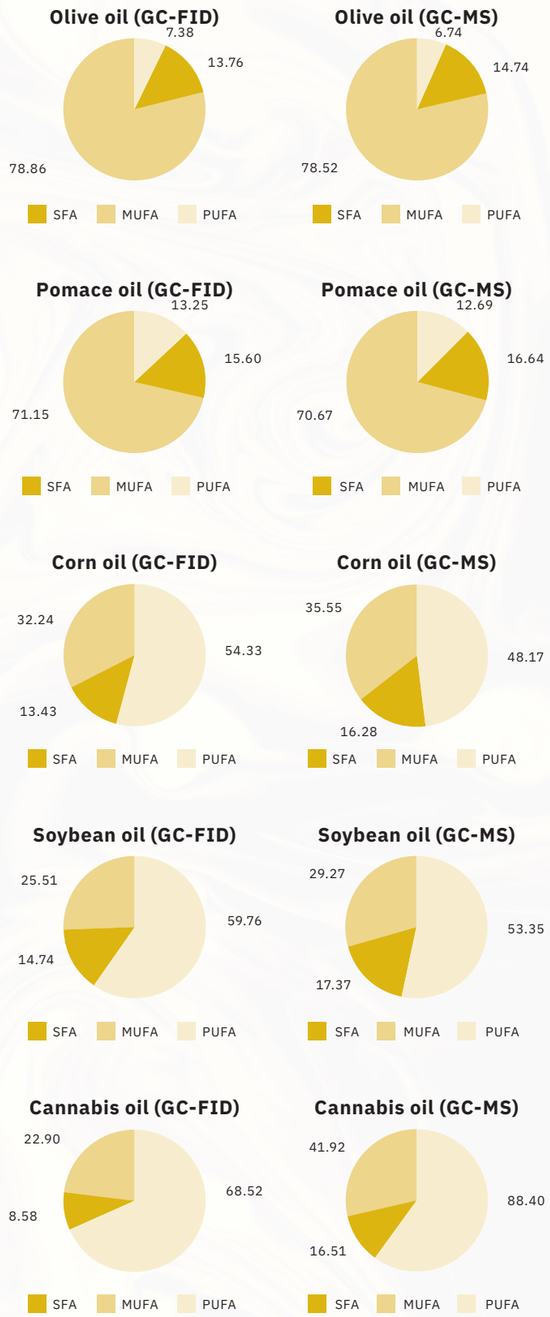
The ratios between oleic acid/linoleic acid and between oleic acid/linolenic acid were examined in the five edible oils (Table 5). The ratios obtained between MUFA (oleic acid) and PUFA (linoleic and linolenic acid) differ significantly between the two techniques. The deviations in the peak area were also reflected in the ratios of fatty acids. This is due to an underestimation in the GC-MS analysis of the PUFAs (linoleic, linolenic), which are in the denominator of the above fractions.

	Oleic acid/ linoleic acid		Oleic acid/ linolenic acid	
EDIBLE OILS	**GC ratio**	**GC-MS ratio**	**GC ratio**	**GC-MS ratio**
Olive oil	**11.95**	**12.79**	101.30	130.09
Pomace oil	**5.69**	**5.89**	94.75	99.89
Corn oil	0.60	0.75	31.65	38.09
Soybean oil	0.47	0.61	4.26	5.33
Cannabis oil	0.45	0.57	1.28	1.61

Table 5: Oleic/linoleic and oleic/linolenic ratios in edible oils

► Figure 1: Percentage (%) of SFA, MUFA, PUFA in olive oil and pomace oil

► Figure 2: Percentage (%) of SFA, MUFA, PUFA in corn oil, soybean oil and pomace oil



TSFA – MUFA – PUFA

The percentages of SFA, MUFA and PUFA were compared in the five edible oils. Peaks of palmitic and stearic acids were chosen for SFA, a peak of oleic acid for MUFA and the sum area of linoleic and linolenic acids for PUFA.

The percentage of MUFA in olive oil and pomace oil showed the same values in both the GC-FID and the GC-MS techniques. The abundance of oleic acid in these samples (> 65 %) eliminated the deviations in SFA and PUFA levels. This was different from the rest of the samples as the percentages showed significant deviations. SFA (palmitic and stearic acid) always showed higher values using GC-MS.

Figure 3: GC-2030

The meaning of the results

In this study, the evidence obtained showed conclusively why GC-FID remains the benchmark method in the quantitative analysis of fatty acids, despite the development of newer instrumentation.

Nonetheless, the GC-MS technique was shown to clearly add value to the analysis of edible oils. In particular by combining the robustness of the GC-2030 for targeted analysis with the high sensitivity of the GCMS-QP2020NX to obtain structural information for a large number of compounds in each sample, researchers were able to show how much more information could be obtained.

To summarize: While GC-FID clearly answers the question of “how much” for known peaks, GC-MS sensitively answers the question of “what else exists” for unknown peaks, thus enabling a much more thorough screening of the samples.

Figure 4: GCMS-QP2020NX

Demonstrating what GC-MS adds

To demonstrate this conclusion, the researchers used GC-MS data to identify squalene in three samples.

Squalene’s mass spectrum was acquired by mass-to-charge *(m/z)* range 40–500. The single ion monitoring method (SIM) was determined after the identification of the most abundant and characteristic ions in SCAN conditions.

Three of edible oils (olive oil, pomace oil, corn oil) were shown to contain squalene in different concentrations. GC-MS determination of squalene was accomplished by retention time and, for confirmation, by referencing the NIST-20 and Wiley-7 libraries for structural identification. The library search for this target compound had a 90 % similarity index. Also, the presence of *m/z*:69 as the target ion and *m/z*:81, *m/z*:41 as ref. ions at the same retention time confirmed the squalene detection.

By complementing GC-FID with GC-MS – apart from the determination of fatty acids – it was confirmed that olive oil is the most important source of squalene among edible oils. Pomace oil showed lower levels, while corn oil had even lower levels.

Working together to achieve more

In this study, researchers showed that GC-FID in the analysis of fatty acids in edible oils is, in fact, the most appropriate technique. In contrast, the integration of Total Ion Chromatograms (TIC) showed that this did not give reliable results. Yet even if GC-FID performs very well in quantitative analysis, it still only screens a very narrow group of compounds. This study showed that, by complementing the use of GC-FID with that of GC-MS, a far broader spectrum of compounds could be identified, which offers important information for determining the nutritional value of edible oils.

Note

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

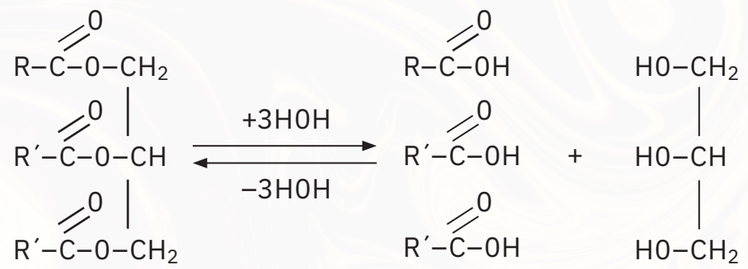


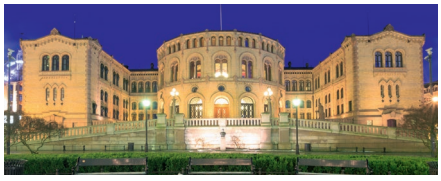
Figure 5: Triglycerides broken down by hydrolysis into fatty acids and glycerol

AAIC
Amsterdam, Netherlands
July 16–20, 2023

TIAFT
Rome, Italy
August 27–31, 2023

Dioxin
Maastricht, Netherlands
September 10–14, 2023

ISEO
Messina, Italy
September 13–16, 2023



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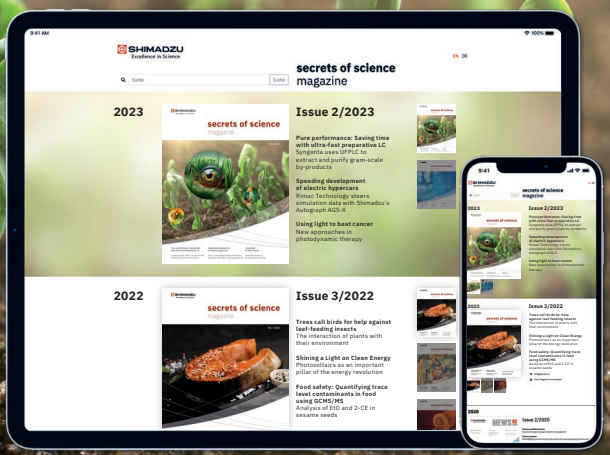

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