SHIMADZU
Excellence in Science

secrets of science
magazine

02/2022

Analyzing the red thread of historical tapestries

Using smaller sample quantities for textile analysis

Short on helium? Consider the carrier-gas alternatives

Advantages of hydrogen and nitrogen for gas chromatography

Faster. Higher. Further.

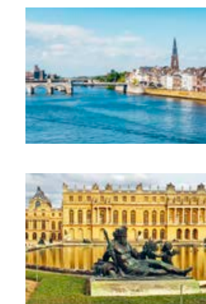
A reunion at analytica 2022

Analyzing the red thread of historical tapestries
Using smaller sample quantities for textile analysis Page 28

CONTENTS

Short on helium? Consider the carrier-gas alternatives
Advantages of hydrogen and nitrogen for gas chromatography
Page 46

Faster. Higher. Further.
A reunion at analytica 2022
Page 26



Events

Page 56

The five categories in the Secrets of Science

SWITCH ON
Discover more about our products and applications as well as current topics of interest.

MOVE ON
Explore the frontiers of science: new applications and fields of use for our systems and new configurations for applications.

ON SHOW
Accompany Shimadzu in action, with reports on events, exhibitions and seminars.

VOICES
Hear what our customers have to say about their work in interviews and guest-written articles and commentaries.

HANDS-ON
Learn more about tips and tricks for getting the most out of our devices (functions, maintenance, etc.) as well as service topics.

The bees are on the loose

Working for the environment and nature conservation with beeswe.love

04

Emerging chemical contaminants: Water analysis moves up a gear

Rapid ppt-level monitoring of little-understood water-borne chemicals using LCMS

06

Quick Column Analysis for priority pollutants

The new Shim-C18-PAH column provides fast and excellent results in PAH analysis

12

Simplifying the potency-testing of CBD gummies for labs

Flexible new method streamlines accurate assessment of cannabidiol content in gummy-based sweets

14

Food fat analysis: quickly and cleanly

Spread and measure: time-saving analysis of spreadable fats using molecular spectroscopy

21

The forensic fight against fake pharmaceuticals

Using UHPLC-MS/MS to streamline identification of illegal PDE-5 inhibitors

32

Testing sunscreens to save your skin

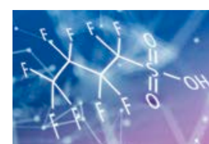
More sensitive analysis using Shimadzu UV-2600i, integrating sphere and LabSolutions UV-Vis

38

Ambitious young lab takes on hazardous waste recycling

Premifab needed top-level instruments to meet its goals

42



Detecting PFAS compounds in food packaging

Better assessing the hidden threat of “forever chemicals”

52

Digital version

More details and insights are available in the digital edition, including additional chromatograms, evaluations and content.

There are almost 600 different bee species in Germany. About half of them are now on the brink of extinction. Pesticides, vermin and lack of food are just some of the reasons why. With beeswe.love, a beekeeping project, Shimadzu actively supports species protection.

The bees are on the loose

Working for the environment and nature conservation with beeswe.love

Insects form the basis of a functioning ecosystem. They pollinate more than 85% of all plant species, including many that are essential for humans as food suppliers. Without insects, flowers would have to be pollinated by hand and the ecosystem, on which humans and animals depend, would gradually collapse.

Of bees and blossoms

Bees are among the world‘s most important pollinators and are therefore a critical factor in agriculture. With their pollination activity, their economic significance amounts to about $265 billion per year. According to the World Bee Day organization, crops that rely on pollination have tripled in the last 50 years. These include plant species that produce fruits, vegetables, seeds, nuts and oilseeds. Bees and their pollination not only increase the quantity of agricultural products, but also improve their quality and resistance to pests. And with more bee and insect colonies, the Earth‘s biodiversity increases. However, monocultures, the use of pesticides, diseases and parasites endanger the diversity of bee colonies. Half of all bee species are facing extinction.

Working together for a healthy ecosystem

Shimadzu is actively committed to protecting biodiversity, a responsibility that each individual can take on. In a joint beekeeping project with beeswe.love, the company has adopted a bee colony. The goal of beeswe.love is to make sustainability efforts possible for everyone – whether for individuals, small businesses or medium-sized companies – and thus limit wildlife extinction.

Above: Glass with Shimadzu honey
Right: The Shimadzu bees
Below: Beehive with Shimadzu logo

With the Shimadzu bees, almost 100 sqm of bee pasture is created. This natural meadow provides living space for a variety of animals, insects and plants. And on top, the busy bees also produce Shimadzu honey.

The bee colony is set up by beekeepers, who tend to and care for the bees. In the bee pasture, different plant species such as snowdrops, sunflowers, mallows or phacelia, also give other insects a new home. The flowers attract both bees and bumblebees, which diligently collect nectar. The resulting honey is harvested, centrifuged and filled into jars. In 2020, it was possible to collect both early and summer harvests. In the winter months, the Shimadzu bees went into their well-deserved hibernation.

The bee initiative in France

To mark #WorldBeesDay, not only Shimadzu in Germany but also the French subsidiary Alsachim took in a total of three bee colonies on their own premises. Together with the local beekeepers‘ association Asapistra, the company‘s employees take care of the bees and their hives. Asapistra was founded in 2008 by the initiative of young beekeepers in Strasbourg with a large number of local members. The organization’s goal is to draw attention to the preservation of biodiversity through targeted actions to ensure a healthy ecosystem.

The beekeeping projects with beeswe.love and Asapistra are examples of Shimadzu‘s philosophy of responsibility towards the environment and people. The company‘s technologies are used in a wide range of sectors. Especially in the food industry and agriculture, they serve consumer and environmental protection as well as product safety.

Because: Together we can #beemore

Check out the latest information and recent developments of the Shimadzu bees on the Shimadzu websites. Get tips and tricks for a bee-friendly garden in the digital edition of this text.

Dr Leon Barron, Imperial College London
Dr Helena Rapp Wright, Imperial College London

SWITCH ON

The issue of environmental pollutants has recently been in the spotlight and is set to receive more attention as the hazards that some chemicals pose to human and environmental health become clearer. We talk to Dr. Leon Barron and Dr. Helena Rapp Wright at Imperial College London about how they're using Shimadzu's LCMS-8060 and LCMS-9030 to conduct large-scale, rapid analyses of wastewater and river water, and how the insights they gain are contributing to a better understanding of the thousands of unregulated chemicals about which little is known – so-called "emerging chemical contaminants".

Emerging chemical contaminants: Water analysis moves up a gear

Rapid ppt-level monitoring of little-understood water-borne chemicals using LCMS

Understanding the environmental burden of chemical pollutants

Dr. Leon Barron has had a lifelong passion for analytical chemistry: "I guess my enthusiasm for the subject was kindled when I was still at school and got a work placement in a lab, analyzing fertilizers. And although the equipment I'm using now is vastly superior to what I was using then, it's essentially the same process – using analytical tools to get information from physical substances."

And in his current role at Imperial College London, that information is playing a valuable role in helping to understand the effect that humans are having on the environment. "Using the equipment we've got, we're looking to chemically profile water in unprecedented detail – including not just regulated chemicals on target lists, but to get a handle on everything that's in the water, especially unregulated chemicals and those we know very little about," he says.

Dr. Barron leads the Emerging Chemical Contaminants team at Imperial, which is one of nine teams forming the Environmental Research Group, itself part of the School of Public Health. This is a leading provider of air and water quality information in the UK and uses a range of approaches to determine the impacts of pollution, as well as supporting actions to mitigate those effects. ⟶

At a site on the River Thames in London, Dr. Barron demonstrates the simple but effective equipment used by the team for water collection.

One of his staff members is Dr. Helena Rapp Wright, who is one of the main users of the group's Shimadzu LCMS equipment for analysis of organic chemicals in water.

The work that's done using the Shimadzu kit is focused on so-called "chemicals of emerging concern". Dr. Rapp Wright explains what these are: "New chemicals are constantly being registered for use, but in addition to these, there are tens or even hundreds of thousands of unregulated metabolites and transformation products that have been detected in the environment. The problem is that we simply don't know very much about these chemicals – and because of the sheer number of them, we need really powerful tools to separate, identify, and quantify them."

Maximizing sensitivity, robustness, and speed

This is where the Shimadzu instrumentation comes in, says Dr. Barron: "We have two Shimadzu LCMS-8060 systems, which we use for our routine target analyses and target research, and also we've recently acquired an LCMS-9030, which we're using largely for suspect screening."
He goes on to say how their systems deal with the challenges posed by their samples: "To achieve our research goals, we need sensitivity, robustness, and speed. And what's great for us is that our 8060 and 9030 systems do brilliantly on all three."

"On the sensitivity front, on both the 8060 and 9030, we can routinely get down to low nanograms per liter levels, equivalent to low ppt, which is vital for picking up on those really trace-level substances. This means we now routinely directly analyze only 10 µL of a filtered sample. Incidentally, that in turn has an effect on sample sizes required and in many cases does away with the need to keep liters-worth of samples stored frozen – we can fit thousands of samples in a single double-door freezer."

"Robustness is another big issue for us, because we wanted to inject raw municipal wastewater, without any form of cleanup other than filtration, he explains. Most analysts would be horrified by that – but thanks to Shimadzu's help with method setup, we've been doing it for nearly three years now ... and we still have the same instruments!"

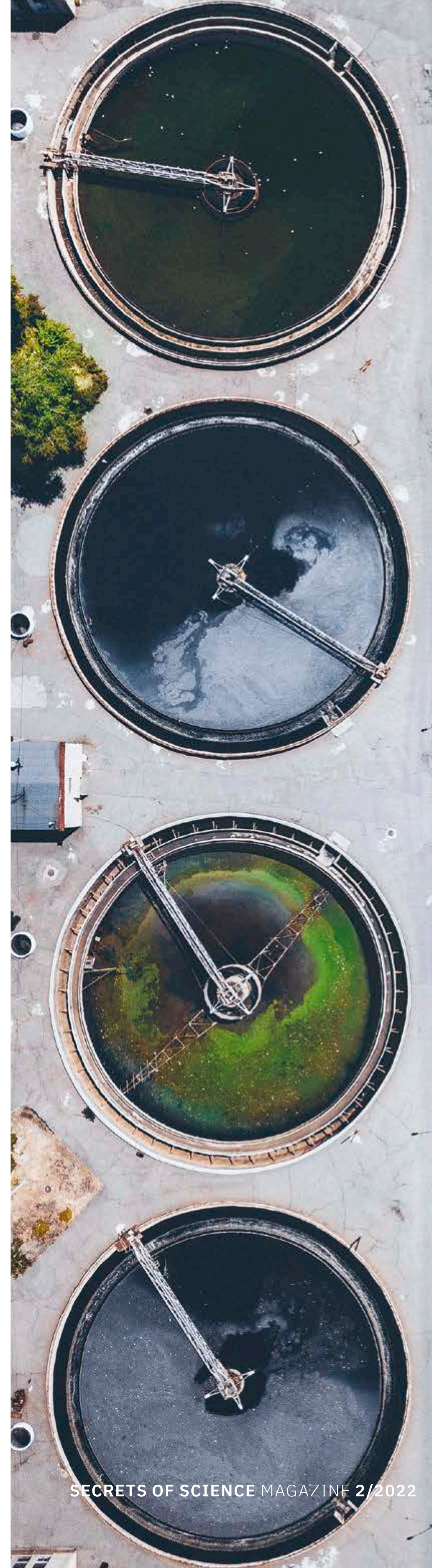
"But I guess the most important thing for our research capability is speed. On the 8060, for example, we're using run times of just over 5 minutes, enabling us to run about 260 injections per day. Once you've factored-in quantitation in triplicate for 200 chemicals, plus matrix-matched calibration lines, that means we can deal with between 30 and 60 samples a day. This allows us to take lots of samples across a geographical area or a time period and so get really fine-grained information on the distribution of the chemicals we're seeing."

The team's 9030 system has been running since October 2021 and has allowed them to extend the number of chemicals that they can detect."Because it's a QTOF (Quadrupole time-of-flight), it's got the high-resolution mass capability needed for suspect screening," explains Dr. Barron. "Currently we're looking at about 1,200 compounds – and in a run time of about 17 minutes!"

Easy-to-use software for streamlined workflows

All this gives the team a vast quantity of information, so ease of data processing is really important for them too. Dr. Rapp Wright uses the systems daily and gives us her perspective: "We have so much data to get through, it could easily be the limiting step in analytical throughput. But the Shimadzu software really streamlines everything – the graphs are easy to understand and tell you exactly what you need to know. And as for the data explorer, I love the flagging system used in the library: I can set a signal-to-noise level at a particular threshold value, and the color-coding lets me know immediately if any samples don't meet the detection criteria."

Dr. Rapp Wright with the Shimadzu LCMS-9030 system used for suspect screening of emerging chemical contaminants in river water and wastewater.



The library of compounds that comes with the 9030 is vital for them, says Dr. Rapp Wright: "Shimadzu were great in creating for us an amazing library of MS data focused on the pharmaceuticals and pesticides that we're primarily interested in," she says. "Of course, as the scope of our projects continues to grow, we'll want to expand this library even more, so it's good to know that Shimadzu will be there to help us do that," she points out.

And not only that, but having a seamless data flow between the Shimadzu instruments is vital for their work, she says: "They're actually really interdependent on each other – so, for example, we often use the 8060 as a quick way to identify a point source of pollution or ingress of a chemical into an aquatic system. And then we run the 9030 to find out what else is there, and then some of those chemicals might be flagged up for quantitative monitoring back on the 8060. So it's a constant feedback loop between these methods, allowing us to refine and improve the level of detail in our analyses."

Reliable technical support

And through the whole process – from lab configuration to method design and ongoing support – the Shimadzu team have been there, ready to help them.

Dr. Rapp Wright found the instrument on-boarding process for the 9030 very thorough: "As not everyone in the lab had used a QTOF before, we found it really useful that the team at Shimadzu went through the principles from the beginning. And when it came to the software, they processed some real-life samples for us, which was great because it gave us a clear example of what to expect in our day-to-day work." Not only that, but when the training was over, they definitely weren't left on their own: "If we had any questions, we'd get responses really quickly – whether by email, phone or Teams, we had answers within the hour."

The same is true more generally, says Dr. Barron: "In our research, we've got to deliver a large number of sample analyses, so we've got to keep our instruments running pretty much 24/7. Where I've been particularly impressed with Shimadzu is the quality of their support, even during the pandemic. The overall responsiveness of the team all the way through has been brilliant." →

SWITCH ON

Reimagining achievable results with LCMS

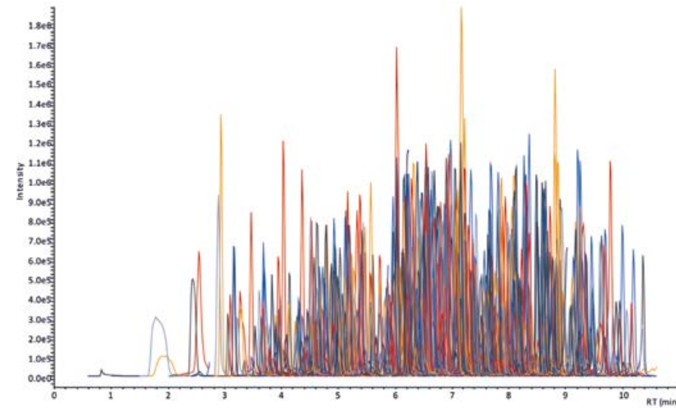
So what about their results? Dr. Barron is keen to highlight the significance of what they've done: "Thanks to Shimadzu, we've absolutely slashed the run time and sample volume for this sort of analysis, which has allowed us to achieve the sample throughput needed for large-scale monitoring projects. For example, in some recent work, we monitored over 100 sampling sites on the River Thames, for over 200 chemicals of emerging concern every time, with all the samples and data analyzed on the 8060 to provide a risk assessment within a month. If you'd asked me a few years ago, I'd have said this wasn't possible."

Putting their work in perspective, he adds: "I think it's the first time anyone anywhere has taken an analytical method for hundreds of chemicals in raw wastewater and transitioned it from a research environment into being a tool for high-throughput monitoring, while keeping the injected sample volumes required in the low tens of microliters."

And the data is great quality, too, says Dr. Rapp Wright: "Because we're injecting the raw sample, we're not losing analytes from our samples during the extraction process – so our calibration lines on our 8060 quant work are perfectly straight in most cases, with R2 values that are absolutely spot-on – and this is on a log–log plot across three orders of magnitude!"

Pushing the boundaries of suspect screening

And what of the future? Dr. Barron is enthusiastic: "One thing we've already done on the 8060 is to discriminate riverine or wastewater catchments from each other, based on multivariate analysis of their chemical content. But we haven't extended it to high-resolution MS data yet, so I'd love to try that on the 9030 and see what extra insights we can gain Currently we can do about 1,200 chemicals for our suspect screening, but ideally we'd like to add a zero or two to that number to take account of transformation products, metabolites, and so on – and our Shimadzu equipment in conjunction with machine learning might just enable us to do that".



This analysis of 652 pesticides on a Shimadzu LCMS-8060 in just over 10 minutes is a good example of the performance that can be routinely achieved in the Barron lab. The method used (see Barron & Loftus, 2019) is also applicable to suspect screening on the LCMS-9030.

LCMS: An essential tool for tackling emerging chemical contaminants

Taking a broader perspective on the team's work, it's clear that demand for this combination of subject expertise and analytical performance will continue to increase.
Dr. Barron summarizes the state of play: "The issue of emerging chemical contaminants isn't going away – in fact, polyfluorinated alkylated substances [PFAS] have been in the news very recently. So I think we'll see a growing need for highly-resolved watercourse monitoring, working out routes of exposure, understanding the role of synergetic effects between chemicals, and much more besides."

Until very recently, such capabilities would have seemed impossible to achieve, but Dr. Barron makes it clear that this has changed: "Our Shimadzu 8060 and 9030 systems are now an essential part of our toolkit for achieving high-speed, high-sensitivity analysis. In this way, we're helping to obtain a fuller picture of chemical pollutants and so helping to reduce humanity's footprint on the environment".

And throughout all their work, they've been able to rely on Shimadzu's support, says Dr. Barron: "Shimadzu have always been keen to understand emerging challenges, refine instrument setups and develop new methods; and for me, that's what sets them apart from other manufacturers. We wouldn't have achieved the results we've got without their enthusiasm and hard work, that's for sure!"

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

Quick Column Analysis for Priority Pollutants

The new Shim-C18-PAH column provides fast and excellent results in PAH analysis

Dr. Carola Thiering, Shimadzu Europa GmbH

What do rubber ducks, barbecued meat and car tires all have in common? All of them are things we come across on a daily basis and they all may contain polycyclic aromatic hydrocarbons (PAHs). PAHs can be harmful to health where the concentration is too high. For this reason, strict compliance with limit values is particularly important. The new Shim-C18-PAH column enables rapid and precise analysis in finding PAH residues, even at low concentrations.

PAHs pose a risk to human health. Many of them are carcinogenic, alter genetic material and can even have a dangerous effect on fertility. In addition, they are virtually impossible to break down in the environment. [1] The U.S. Environmental Protection Agency (EPA) has classified a total of 16 PAHs as priority pollutants. [2] And this is why PAH analyses are of immense importance.

Because of this importance, the Shim-C18-PAH column – a recent addition to the Shimadzu LC column portfolio – has been especially designed to rapidly and reliably measure polycyclic aromatic hydrocarbons (PAH).

Exposure is unavoidable

PAHs are found practically everywhere. They are produced during any combustion process – mainly in industry, but also in such cases as campfires – and can therefore be consumed through ordinary respiration. But that's not all: PAHs also enter the water and soil, where they continue to disperse. In everyday life, we consume them when eating smoked and barbecued foods. What's more, PAHs are often found in rubber and plastic or refinery products, such as car tires, road surfacing, and even children's toys like rubber ducks [1] Since we can scarcely escape exposure, PAH analysis is critical.

Useful for analysis: special PAH columns

The Shim-C18-PAH column is specifically designed for demanding PAH analysis and therefore offers several advantages over conventional C18 columns, such as the previously recommended Shim-pack GIS C18-P.

Shim-C18-PAH enables faster analysis while maintaining a very good resolution. In a nutshell, it saves time. This is an important factor, especially for users performing routine PAH analyses.

Figure 1: Shim-C18-PAH P/N 961-18002; 3 µm; 100 x 4.0 mm ID.

How the Shim-C18-PAH performs in a quality test

To demonstrate the separation qualities of the Shim-C18-PAH, a standard PAH mixture was selected using the 16 PAHs classified as "primary pollutants" with 2 additional critical PAHs (Fig. 2). The analysis of these 18 PAHs on the Shim-C18-PAH was carried out with two different column dimensions using a Shimadzu LC-40-X3 system.

P/N	Description	Particle size	Length	I.D.
961-18002	Shim-C18-PAH	3 µm	100 mm	4.0 mm
961-18001	Shim-C18-PAH	3 µm	150 mm	2.0 mm

The Shim-C18-PAH portfolio includes two column dimensions.

The concentration of all PAHs in the samples detected by the photodiode array (PDA) detector was 2 µg/ml and that of PAHs in the sample for fluorescence detection was 10 µg/l.

Short analysis times

PAHs were also measured by fluorescence detection, as this is much more sensitive than detection with PDA. The samples used for this measurement had a concentration of 10 ppb. This low concentration is important for many PAH analysis guidelines and regulations.

The Shim-C18-PAH columns demonstrate excellent performance for PAH analysis both with PDA and fluorescence detection when using different column dimensions. In the performance test described, analysis times were short and all 18 PAHs were well separated.

Note

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

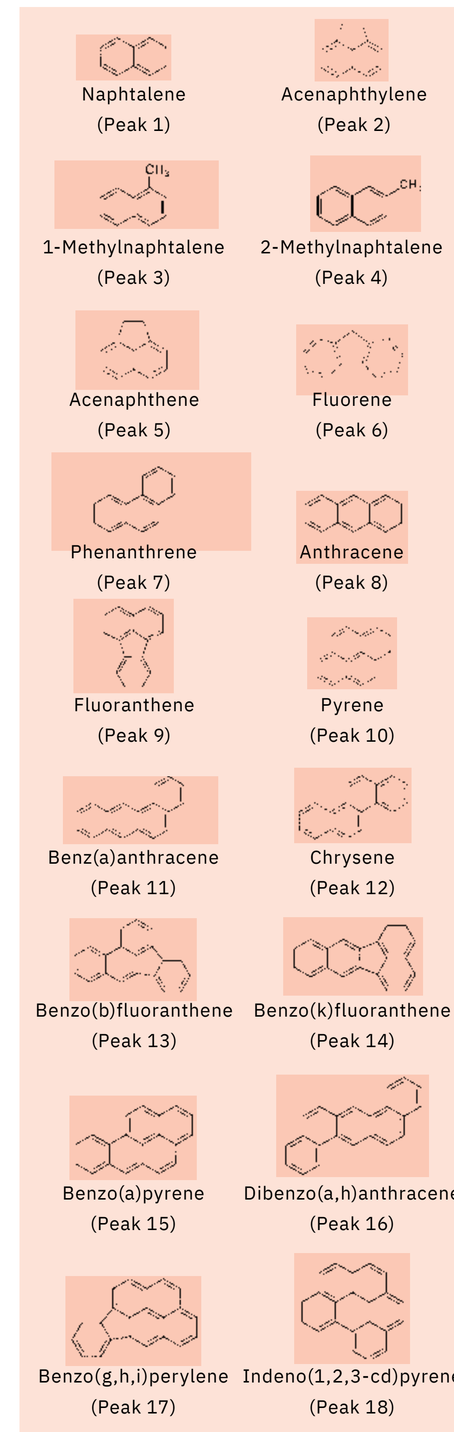


Figure 2: Structures with names and peak numbering of the investigated 18 PAHs.

Simplifying the potency-testing of CBD gummies for labs

Flexible new method streamlines accurate assessment of cannabidiol content in gummy-based sweets

Angela Jein, Shimadzu UK

The recent and rapid growth of the global cannabinoid industry has included the commercial introduction of a wide range of nutraceutical products. Among the most popular of these are gummy-based candies. Shimadzu conducted a series of experiments to assess the ability of currently available equipment to measure cannabidiol (CBD) potency in gummy-based candy. The tests clearly showed that simple solvent extraction, followed by HPLC analysis on the i-Series LC-2060 with photodiode array (PDA) detector provided a sensitive, robust method for accurately extracting and measuring CBD content, while using minimal additional laboratory equipment.

In recent years, the cannabis plant has been receiving renewed attention, and much of that has been of a positive nature. While its use for medicinal or recreational purposes is still subject to much debate, products containing < 0.2% of the psychoactive compound Δ9-tetrahydrocannabinol (THC) have long been legal in many countries.

As a result, the market for edibles containing cannabidiol (CBD) has expanded dramatically. This has increased the burden on manufacturers for accurate quantification of cannabinoids in their products. Especially with products that resemble children's candies – such as CBD-containing gummy bears – it is essential to guarantee the absence of any potentially intoxicating ingredients. Angela Jein, a liquid chromatography technical specialist with Shimadzu in the UK, had become increasingly aware that both manufacturers and laboratories were facing challenges in quantifying the CBD content of their confectionary products. Eventually, Angela was able to initiate a six-month-long study of how CBD extraction processes could be improved to better meet market needs.

Goal No. 1: Testing a new method for better performance

According to Angela Jein, the first goal of the study was to test and "provide a simple, yet reliable method for extraction and analysis of cannabinoids (in CBD nutraceuticals) to make it easy and straightforward for manufacturers and control labs to ensure their quality."

Analysis of active ingredients in edibles in general is always challenging, as food samples contain a large variety of substances that can interfere with the assay. In gummy-based candies (Figure 1), the high sugar and gelatine content are such challenges.

Figure 1: Gummy-based candy samples

Goal No. 2: Creating an economical method

The second goal of the study focused on the bottom-line practicalities of testing. Again, according to Angela Jein, the few CBD extraction methods available for food, cosmetic and nutraceutical products "usually feature a large amount of high-tech equipment, such as cryogenic sample preparation, automated shaking equipment, centrifuges and more." Therefore, the scope of this test included the goal of constructing a robust method whereby the extraction procedure could be satisfactorily achieved with minimal additional laboratory equipment.

Materials and methods

Analysis of all samples was carried out using Shimadzu's high sensitivity method for potency testing [1], run on the LC-2060 with photodiode array (PDA) detector, an integrated i-Series ultra-high-performance liquid chromatgraphy (UHPLC) system. →

Reference standards were prepared from individual cannabinoid standards across a concentration range of 0.5 to 90.9 ppm for 11 cannabinoids (Figure 2): cannabidivarin (CBDV); cannabidiolic acid (CBDA); cannabigerolic acid (CBGA); cannabigerol (CBG); cannabidiol (CBD); tetrahydrocannabivarin (THCV); cannabinol (CBN); Δ9-tetrahydrocannabinol (Δ9-THC); Δ8-tetrahydrocannabinol (Δ8-THC); cannabichromene (CBC); Δ9-tetrahydrocannabinolic acid (THCA).

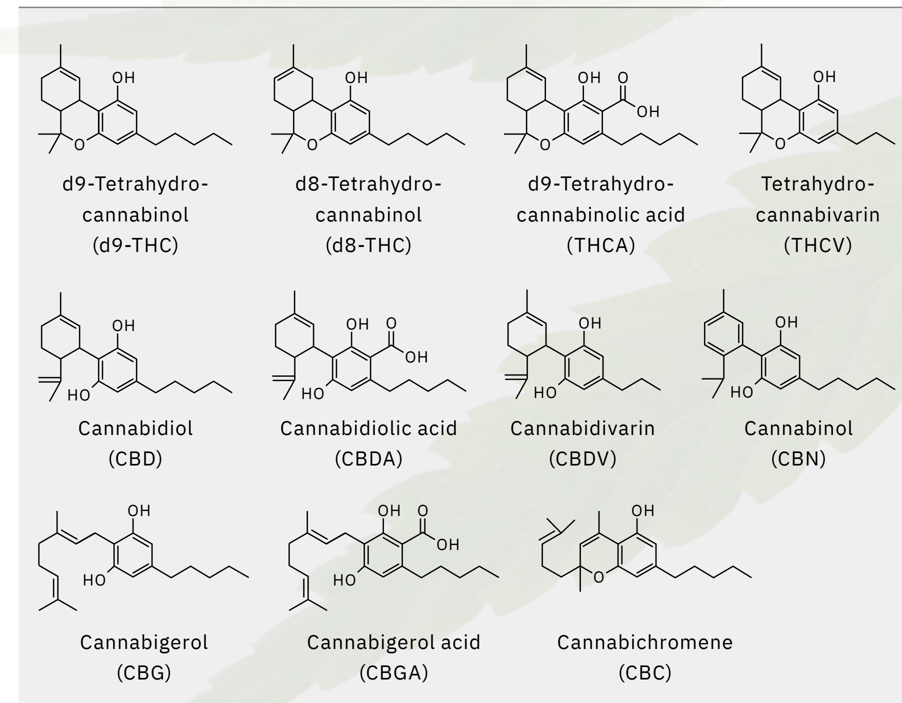


Figure 2: Cannabinoids analyzed by high sensitivity method for potency testing

Sample preparation

The spiking solution (A) for CBD-only was prepared at approximately 100 mg/L using an isolate of CBD with known purity in methanol. The spiking solution (B) for all cannabinoids was prepared by placing a known volume of each cannabinoid directly into the sample.

Control gummy confectionary with no cannabinoid content were cut into small pieces and thoroughly mixed to form a representative sample.

Spiking with specific quantities of spiking solution A or B was carried out and allowed to absorb into the gummy before proceeding with the extraction method depicted in Figure 3.

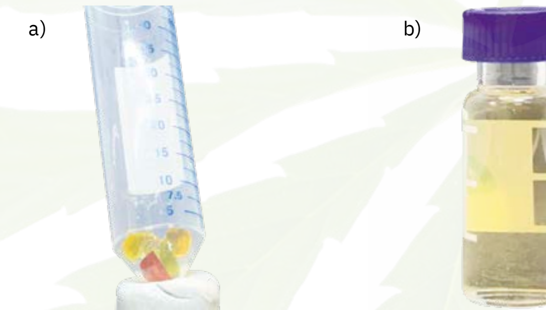


Figure 3: Extraction flow path

To evaluate the precision of the extraction procedure, six replicate samples were prepared by addition of spiking solution A for a final CBD concentration of 20 ppm. In addition, two samples were spiked with solution (B) for duplicate analysis of all 11 cannabinoids after extraction.

Commercially available CBD edibles are sold in a wide range of sizes and concentrations, so accuracy testing was carried out in duplicate at 5 ppm, 20 ppm and 40 ppm.

To establish selectivity of the assay against unknown peaks present in the gummy matrix – and which could potentially interfere with the detection of cannabinoids – the following reference samples were tested:

1. Clear (only) gummy / no spike
2. Red (only) gummy / no spike
3. Orange (only) gummy / no spike
4. Yellow (only) gummy / no spike
5. Green (only) gummy / no spike
6. Gummy of all colors / no spike
7. Randomly colored gummy / no spike
8. No gummy present / spike
9. No gummy present

For real-life samples, three commercially available CBD gummies (vegan and non-vegan), from two different manufacturers were analyzed using the method described. Sub-samples from each type were taken and prepared by cutting them into small pieces (< 1/16 sizes). Multiple aliquots (8) were tested for each sample to look at the robustness of the samples themselves.

Sample 1 – non-vegan – Manufacturer A [multicolored]
Sample 2 – vegan – Manufacturer A [multicolored]
Sample 3 – vegan – Manufacturer B [green only]

Results and discussion

Table 1 lists the results obtained for the precision, accuracy and selectivity of the extraction and quantification of CBD in the reference samples. →

Identification / Number		CBD content (ppm)	% nominal
Spiking solution A	---	107.2 mg/ml	---
Precision	1	19.886	98.9
	2	19.905	99.0
	3	20.187	100.4
	4	20.254	100.8
	5	19.862	98.8
	6	19.933	99.2
Accuracy	1 (25% precision)	4.984	99.2
	2 (25% precision)	4.930	98.1
	3 (100% precision)	20.109	100.1
	4 (100% precision)	19.839	98.7
	5 (200% precision)	39.821	99.1
	6 (200% precision)	40.194	100.0
Selectivity	1 – Clear / No spike	---	---
	2 – Red / No spike	---	---
	3 – Orange / No spike	---	---
	4 – Yellow / No spike	---	---
	5 – Green / No spike	---	---
	6 – All / No spike	---	---
	7 – Mixed / No spike	---	---
	8 – Mixed with spike	19.848	98.8
	9 – No gummy / No spike	---	---

Table 1: Extraction testing results (CBD)

Identification / Statistical Aspect		CBD content (ppm)	% nominal
Precision	Average	20.005	99.5
	Standard Deviation	0.170	99.5
	%RSD	0.851%	0.851%
Accuracy	Average 25%	4.957	98.7
	Standard Deviation	0.038	0.008
	%RSD	0.770%	0.770%
	Average 100%	19.974	99.4
	Standard Deviation	0.191	0.009
	%RSD	0.956%	0.956%
	Average 200%	40.008	99.5
	Standard Deviation	0.264	0.007
	%RSD	0.659%	0.659%

Table 2: Statistical analysis of the extraction testing results (CBD)

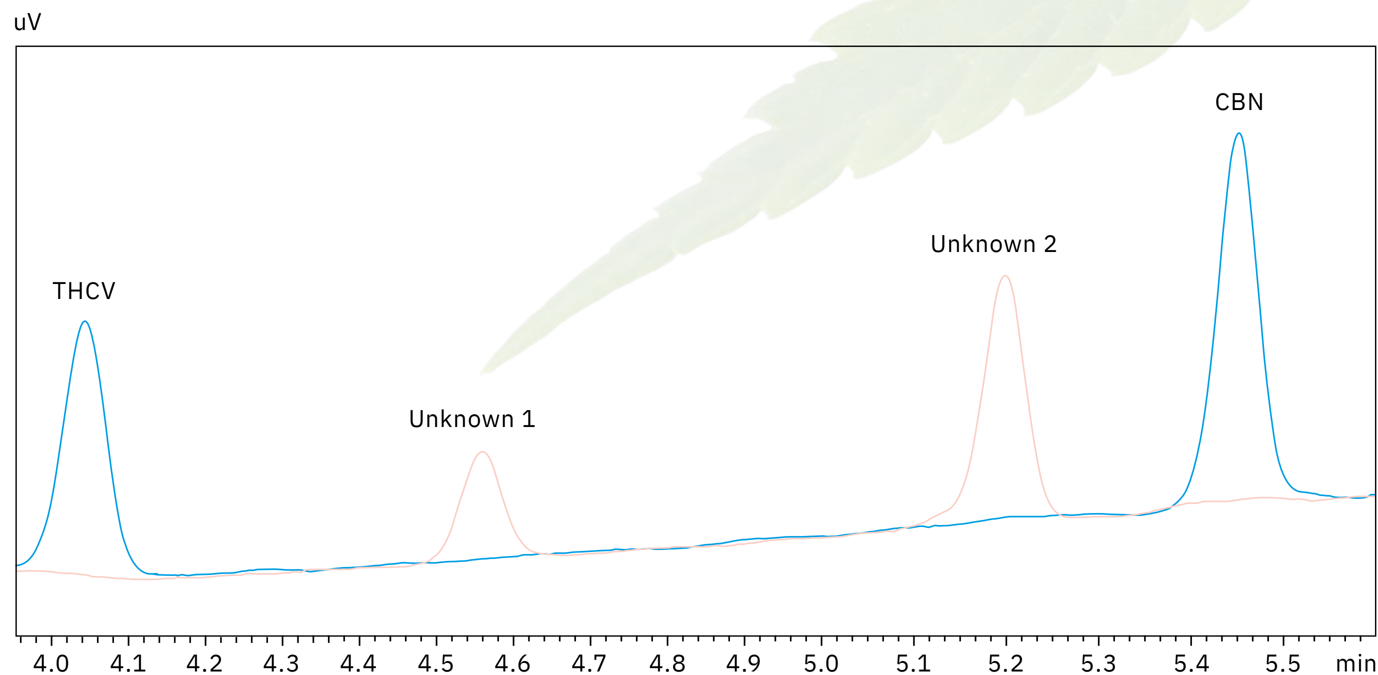


Figure 4: Overlay of chromatograms from reference sample 4 (pink) and 0.5 ppm standard solution (blue) (3.9–5.6 min)

MOVE ON

To establish selectivity of the assay, all gummy samples were reviewed for any unknown peaks outside of the 11 cannabinoids. A signal at a retention time (tR) of 3.88 min was seen in all chromatograms at varying concentrations. It was found to represent CBD at a level of approximately 0.05 ppm, significantly below the lowest standard.

Gummy samples 4, 6, 7 and 8 also showed peaks at 4.52 and 5.15 minutes, most likely due to the yellow colorant in the product, which was the commonality in the four extracts. However, as these two signals – located between peaks of THCV and CBN – were well separated from any other compound of interest, they would not cause any misinterpretation or integration issues. Unknown peaks at tR = 4.52 and 5.15 min are present in reference sample 4, but not in a 0.5 ppm standard solution, as can be seen in the overlay of the two chromatograms (Figure 4).

Additional precision samples, spiked with solution (B) to contain all 11 cannabinoids, were also analyzed using the high sensitivity method. Due to the lower concentration of minor cannabinoids within samples they did not require the additional dilution step after extraction (Figure 3).

Table 3 displays the average recovery results and obtained %RSD for extraction of the two samples to evaluate precision of the procedure for all 11 cannabinoids.

The three selected commercially available products were extracted and analyzed for all 11 cannabinoids. In sample 1 and 2, the 10 minor cannabinoids were quantified using the non-diluted extract (A), while CBD was determined in the dilution (4 x as described in Figure 3). As sample 3 showed a lower amount of CBD, all cannabinoids were quantified using the non-diluted extract (A). Results of the quantification of cannabinoid content in real-life samples are displayed in Tables 4–6.

As CBD was the only peak found in all three samples within the concentration range, statistical analysis of CBD alone was performed on the eight extractions. The results are shown in Table 7. ⟶

Cannabinoid	Mean content (ppm)	% nominal	%RSD
CBDV	1.976	98.8	0.465
CBDA	2.032	101.6	1.427
CBGA	2.030	101.5	0,627
CBG	1.997	99.9	0.637
CBD	2.033	101.7	0.835
THCV	1.983	99.1	1.177
CBN	1.969	98.4	1.401
D9-THC	2.025	101.2	0.873
D8-THC	1.967	98.3	1.043
CBC	1.971	98.6	0.359
THCA	2.008	100.4	0.493

Table 3: Statistical analysis of extraction testing results for 11 cannabinoids

	Result (mg/g)							
	1	2	3	4	5	6	7	8
CBDV	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
CBDA	---	---	---	---	---	---	---	---
CBGA	---	---	---	---	---	---	---	---
CBG	---	---	---	---	---	---	---	---
CBD	4.490	4.923	4.812	4.783	4.839	4.959	4.614	4.681
THCV	---	---	---	---	---	---	---	---
CBN	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
D9-THC	---	---	---	---	---	---	---	---
D8-THC	---	---	---	---	---	---	---	---
CBC	---	---	---	---	---	---	---	---
D9-THCA	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5

Table 4: Results of the quantification of cannabinoid content in commercial sample 1

	Result (mg/g)							
	1	2	3	4	5	6	7	8
CBDV	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
CBDA	---	---	---	---	---	---	---	---
CBGA	---	---	---	---	---	---	---	---
CBG	---	---	---	---	---	---	---	---
CBD	4.541	4.463	4.294	4.548	4.550	4.124	4.595	4.520
THCV	---	---	---	---	---	---	---	---
CBN	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
D9-THC	---	---	---	---	---	---	---	---
D8-THC	---	---	---	---	---	---	---	---
CBC	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
THCA	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5

Table 5: Results of the quantification of cannabinoid content in commercial sample 2

	Result (mg/g)							
	1	2	3	4	5	6	7	8
CBDV	---	---	---	---	---	---	---	---
CBDA	---	---	---	---	---	---	---	---
CBGA	---	---	---	---	---	---	---	---
CBG	---	---	---	---	---	---	---	---
CBD	0.903	0.608	0.733	0.677	0.726	0.713	0.968	0.637
THCV	---	---	---	---	---	---	---	---
CBN	---	---	---	---	---	---	---	---
D9-THC	---	---	---	---	---	---	---	---
D8-THC	---	---	---	---	---	---	---	---
CBC	---	---	---	---	---	---	---	---
THCA	---	---	---	---	---	---	---	---

Table 6: Results of the quantification of cannabinoid content in commercial sample 3

Sample#	Average	Standard Deviation	% RSD	mg/gummy	% nominal
1	4.762	0.159	3.33	9.51	95.1
2	4.454	0.162	3.64	18.86	94.3
3	0.746	0.126	16.91	2.41	48.2

Table 7: Statistical analysis of the quantification of CBD in commercial sample extracts

Additional information for the gummies analyzed was also considered. The concentration displayed on commercial products is typically represented by mg/gummy or mg/bag and the number of gummies reported. Accordingly, the analysis was carried out by weighing 10 pieces from each sample to obtain an average weight of the gummies.

Sample 1 – 19.9713 g/10 gummies [10 mg/gummy]
Sample 2 – 42.3488 g/10 gummies [20 mg/gummy]
Sample 3 – 32.2951 g/10 gummies [5 mg/gummy]

Results shown in Table 7 also include the mg/gummy results and the % nominal results as stated on the packaging.

CONCLUSION

The study concluded that Shimadzu's high sensitivity method for potency testing using the i-Series LC-2060 with photodiode array (PDA) detector provided a highly robust way to accurately measure CBD content and a simple solvent extraction procedure limiting the overall amount of additional laboratory equipment required for sample pretreatment.

Evaluations of extractions of spiked gummy bears provided accurate data with good recoveries (greater than 98%, < 1.5% RSD for all cannabinoids).

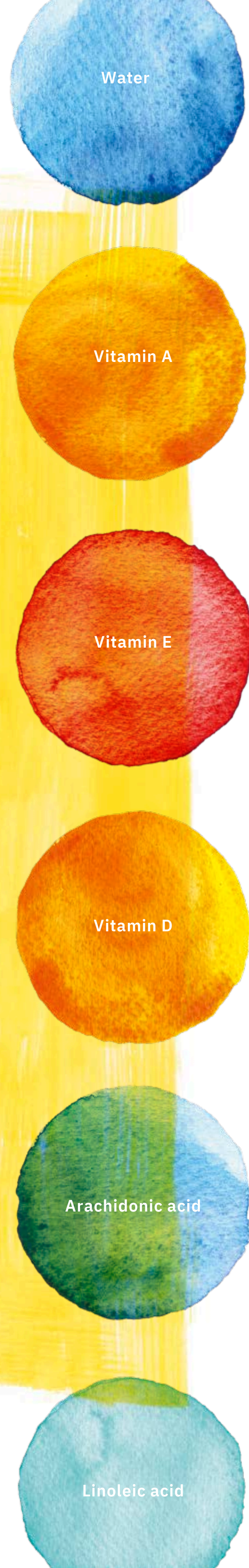
Note
For more information and references, please refer to the digital version of this edition.

Food fat analysis: quickly and cleanly

Spread and measure: time-saving analysis of spreadable fats using molecular spectroscopy

Marion Egelkraut-Holtus, Shimadzu Europa GmbH

Real butter, clarified butter, and margarine are all indispensable to fine cuisine. The EU strictly regulates what can be included in each type of spreadable fat. In food analysis, molecular spectroscopy can be used to quickly determine whether the food contains what it should according to the regulations – and without prior chemical analysis preparation. ⟶



Golden yellow, with a delicate flavor, and therefore used frequently: butter, clarified butter, and margarine. But what are the differences between the fats?

Fat is not just fat

Each type of spreadable fat has a different ratio of fat to water. According to EU legislation, butter must have a milk fat content of at least 80% and no more than 90%. It must also contain no more than 16% water. Butter mostly consists of saturated fatty acids, is rather hard when chilled, and only becomes spreadable through the addition of oils.

Clarified butter, on the other hand, is butter that is heated slowly to remove almost all the water, milk protein, and lactose. As a result, it has a fat content of 99.8% and is thus ideal for heating. The healthy aspect is meant to be a high vitamin A content, but this needs to be verified.

Finally, margarine is an emulsion of vegetable and/or animal fats and water or skimmed milk. It has a fat content of between 39% and 90%, so it normally contains a lot less fat. Margarine is also considered healthy because it is rich in vitamins such as A, D, and E. But these are added during the production process.

Well-being at a glance

The debate over which of these fat products is the healthiest or highest quality is a perennial one. In the past, margarine was considered a “cheap substitute” for “real butter”, but today it is well-established among the ranks of spreads and even considered healthier than its animal-fat-based competitors.

In order to verify the information on the packaging, as well as the safety and the nutritional benefits of the fats used, the analysis of spreadable fats and oils using infrared spectroscopy is a common method in food inspections. Manufacturers of spreadable fats and oils want to know the precise composition of saturated and unsaturated fatty acids in their products because high blood-cholesterol levels are responsible for heart disease and other conditions. The nutritional physiology represented by the cis/trans value of the unsaturated fatty acids is also of interest. Indeed, some unsaturated fatty acids are essential for life, such as linoleic acid and arachidonic acid.

Any chemical analysis should (in theory) include a qualitative and quantitative determination of each fatty acid/each fatty acid ester, but this type of analysis is very complex and time-consuming. Therefore, the industry uses analysis figures (fat parameters) to characterize and identify fats. These include the acid value, saponification value, ester value, and iodine value. The cis and trans fatty acids can be very accurately determined using infrared spectroscopy.

Quick quality control

Using infrared spectroscopy, it is possible to determine to subject fats to a quick quality control and to determine their water content without having to prepare the fat for the analysis.

In the test, five commercially available fats from the supermarket were examined and the results were compared with the information on the packaging. Infrared spectroscopy analysis was used to demonstrate the differences in water content. Subsequently, fluorescence spectroscopy was used to verify the presence of vitamin A. The fats were measured at room temperature in a solid state.

Since the focus of the analysis was on the speed and ease with which information about the composition could be obtained, the objective for both techniques was to carry out the analysis with simple sample preparation and without chemical pretreatment of the fats. [1], [2], [3]

Simple application, quick cleaning: sample preparation and analysis

The sample preparation is simple for both measuring techniques: For Fourier transform infrared spectroscopy (FTIR), the fat is applied bubble-free to the measurement window using a spatula. For the fluorescence spectroscopy, the so-called “front-face” analysis is used. For this, a sample holder of the solid sample holder is filled with the fat up to the rim and covered with a quartz plate so that the quartz is in contact with the fat. This is placed in the solid sample holder in the sample compartment.

For the infrared spectroscopy, a Shimadzu IRSpirit-T FTIR laboratory instrument fitted with a diamond-based ATR accessory was used for a measurement in single-reflection ATR. The diamond is robust and easy to maintain. The measurement window – only 2 mm in size – is wiped with paper until it is subjectively free from fat and is then cleaned again with a fat-dissolving cleaner until it is completely free of any fat. In total, the cleaning takes less than one minute and the accessory is then ready to be used for the next sample.

The Shimadzu RF-6000 fluorescence spectrometer was used for the fluorescence spectroscopy to detect naturally occurring or added vitamins. For example, vitamin A, which is dissolved in the fat, can be measured and determined using fluorescence without chemical pretreatments. Paper is used for cleaning in this case as well, and the sample container and quartz plate are wiped until they are clearly fat-free, and then everything is cleaned again with a fat-dissolving agent. The entire cleaning takes less than five minutes.

Various cooking fats and spreads

Type	Description
Ghee/butter fat from cow´s milk	Clarified butter with a fat content of 99.8 g per 100 g.
Butter	Butter from cow's milk with a fat content of 82.5 g on 100 g, water content ~17.5 g
Butter plus	Butter from cow´s milk plus rapeseed oil, a mixture of 63 % butter and 13 % rapeseed oil, with a fat content of 65 g per 100 g, water content ? g
Margarine	Soft margarine with a fat content of 60 g per 100 g and 800 µg vitamin A, water content ~40 g
Margarine	Baking and cooking margarine with fat content of 70 g per 100 g, water content ~30 g

Table 1: Various cooking fats and spreads of known brands from the supermarket, list of five commercially available spreadable and cooking fats and their ingredients (water, vitamin A) according to the description on the packaging ⟶

A first look – infrared spectroscopy results

The mid-infrared spectra show a strong signal at 966 cm^{-1} for the low water content fat spectra. This is a clear indication of trans-substituted fatty acids – double bonds – which are responsible for the softness or spreading quality of the fat. Hence, lots of trans-fatty acids are found in oils, but proportionally less in butter and margarine. These so-called "good" fats are added to the latter synthetically.

One of the types of butter selected for the analysis is mixed with a rapeseed oil to increase the proportion of trans-fatty acids.

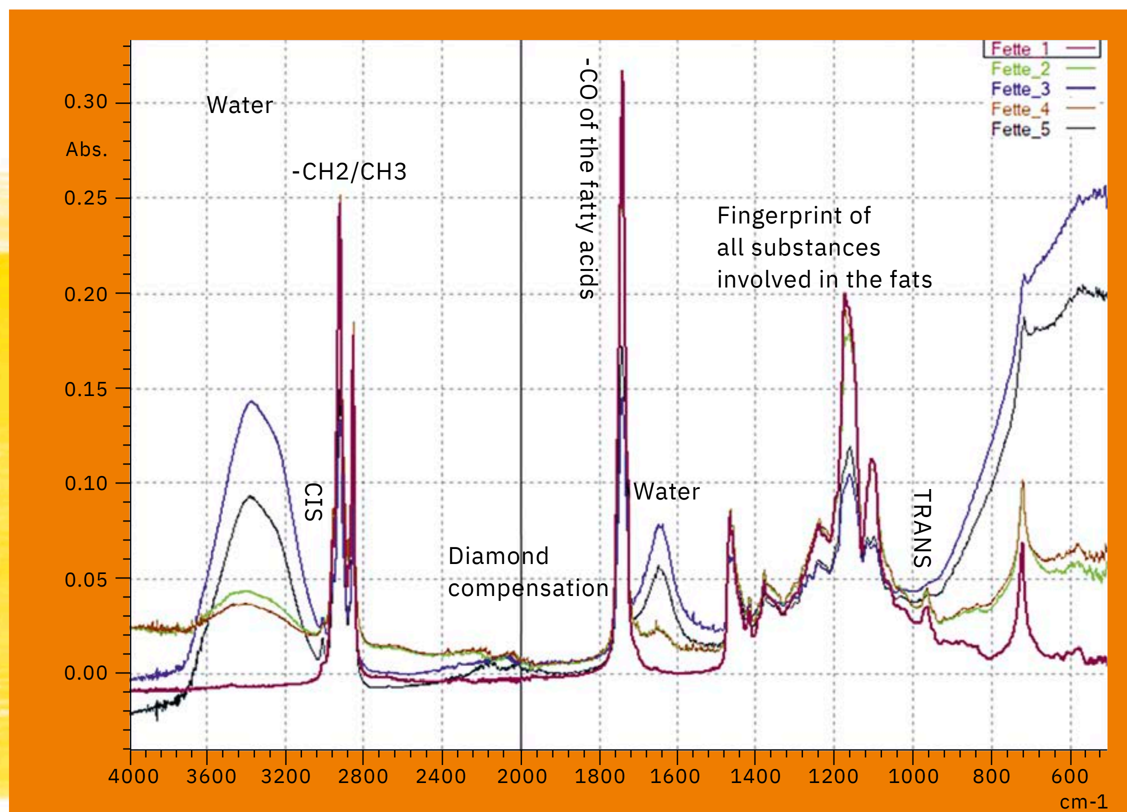


Fig. 1: Infrared spectra of five different butter and margarine samples: ghee/clarified butter (fat 1), butter and oil mix (fat 2), soft margarine (fat 3), butter (fat 4), margarine for baking (fat 5)

The analysis range can be expanded to include additional ingredients. This is helpful if there are a large number of products. The water content is easy to determine. The created calibration could be used for the water-content determination of other industrially produced spreadable fats.

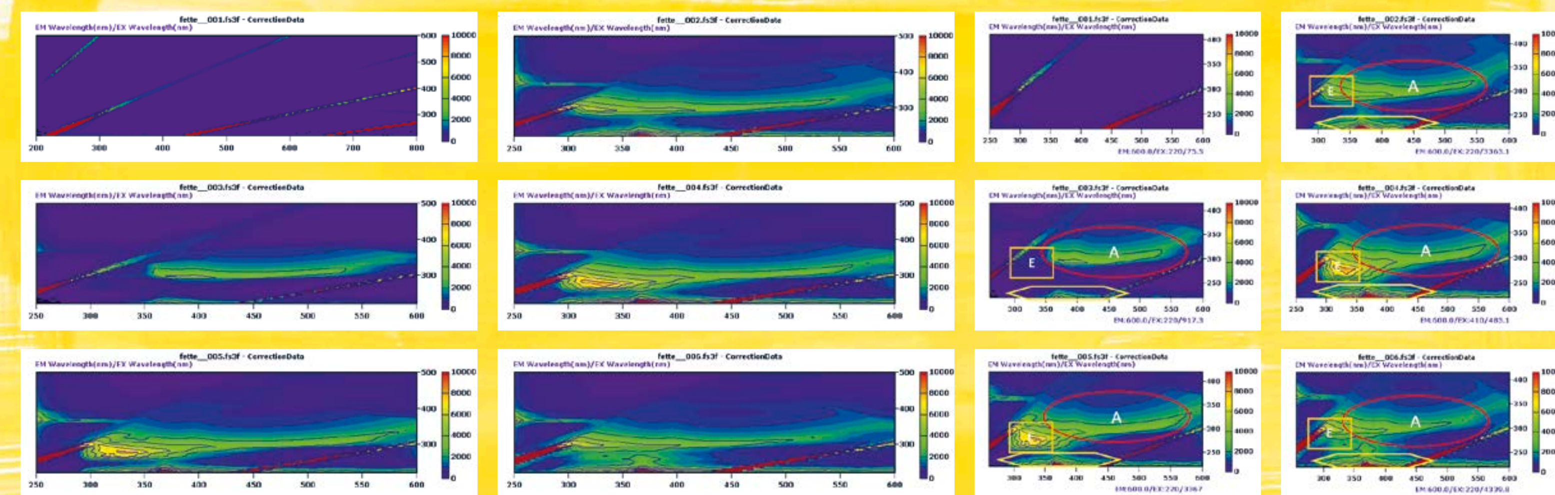
MOVE ON

A second look – fluorescence spectroscopy results

The same fats were measured using fluorescence spectroscopy with the implementation of an EEM matrix. For the analysis, the excitation wavelength was gradually increased against the emission spectrum. When presented as a graph, the wavelengths of the emission spectra (x axis) were plotted against the excitation wavelengths (y axis). These area diagrams feature fluorescence-active areas. Areas, intensity clouds, or hot spots arise due to individual substances or families (such as vitamins) to be classified. In this example, they are retinol (vitamin A, region A), tocopherol (vitamin E, region E), and UV-active ingredients as a matrix (region M).

The EEM matrix of the clarified butter clearly showed a vitamin A-induced spectrum, whereas the butter and margarine demonstrated other emission areas. These can be assigned to the remaining ingredients of butter and margarine, such as vitamins, proteins, and water.

Fig. 3: Fluorescence EEMs of the quartz plate and holder and five different fat samples, region A shows the vitamin A area (red oval), region E shows vitamin E (orange rectangle), region M is the area of the UV-active matrix of the fat in the holder (yellow diamond)



Time is money

The test confirms that the fat spectra can be easily and quickly identified, the content of saturated and unsaturated fatty acids (cis/trans) verified, and the water content determined by using ATR infrared spectroscopy.

Fluorescence spectroscopy demonstrates another perspective on food. Chemically untreated, solid fats can be classified. A fat can easily be measured in comparison with a reference material, and thus quality control can be carried out in relation to vitamins.

For both systems, only a short length of time is required for the measurement, compared to the time needed to clean the measurement accessories. For both device systems together, an analysis can take less than ten minutes.

Note

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

FASTER. HIGHER. FURTHER.

A reunion at analytica 2022 in Munich

Dr. Isabelle Spenner,
Shimadzu Deutschland GmbH

The wait is over! After two long years during which numerous trade fairs could only take place virtually – or even had to be canceled – due to COVID-19, Shimadzu's trade fair calendar is now completely full again. A big highlight on that calendar is analytica 2022 in Munich, one of the largest leading trade fairs in the world for innovative laboratory technology and pioneering biotechnology. Several Shimadzu product innovations will celebrate their European premieres at analytica 2022.

For over 50 years, the analytica trade fair in Munich has been a fixture in the diaries of people interested in analytics. At the last live event in 2018, the trade fair broke all records, with nearly 1,200 exhibitors and over 35,000 visitors. After a purely virtual analytica in 2020, it's that time again: Visitors can finally breathe in that trade-fair air of inspiration from June 21 until June 24. For Shimadzu, analytica is an absolute highlight of the trade fair schedule. This year, the theme permeating over 190 square meters of exhibition space will be: Faster. Higher. Further.

Innovations and product premieres

The urge to discover something new and push beyond previous boundaries is what drives both science in general and instrumental analytics. This is reflected in the exhibited products. Visitors can learn about new highlights and current systems in chromatography, spectroscopy, mass spectroscopy, TOC and material testing, which includes several European premieres as well as systems never before exhibited in Germany.

At the Shimadzu press conference on June 21, the highlights and new products will be presented to the European public for the first time.

Four new products for improved performance in the laboratory

After a long period of absence, Shimadzu is once again looking forward to presenting new products and software live at analytica. We are particularly excited about the presentation of these four innovations:

LCMS-2050: High-end performance in a compact form

With the LCMS-2050, Shimadzu introduces a new single quadrupole LCMS detector that is impressive not only due to its compact size, but also because of its long service life and ease of use – without compromising on key specifications such as sensitivity, flexibility or speed. The LCMS-2050 combines high-performance liquid chromatography (LC) with the qualitative possibilities of mass spectrometry (MS). With an extensive range of features such as a dual ion source (HESI/APCI), high sensitivity, and the MASS-IT function, it provides reliable analysis with high-end results.

LabSolutions MD – new software

The new LabSolutions MD software provides additional support for the established "Method Scouting" functionality for automated column and solvent screening using "Analytical Quality by Design" (AQbD). Thanks to a multifactorial design, only a reduced number of experiments are thus required to capture a set analytical space. Computer simulations of the retention behavior of the substances being analyzed within this space enable fast, statistically sound decisions about suitable, robust separation conditions, while also reducing the risk of error.

MALDI-8030 – sophisticated technology for the laboratory bench

The MALDI-8030 is the latest in a long line of MALDI-TOF products from Shimadzu. Compared with the MALDI-8020, the performance data of the MALDI-8030 have been extended to capture compounds that are best suited to analysis in the negative ion mode. This benchtop, linear MALDI-TOF mass spectrometer with dual-polarity provides outstanding performance in a compact housing, and is thus the ideal choice for today's increasingly sophisticated laboratories.

TOC-1000e – analysis in miniature

TOC-1000e is the first instrument of the eTOC series of online TOC analyzers designed for pure water applications. TOC-1000e provides highly-sensitive detection in the smallest and lightest housing on the market. This makes it ideal for fields requiring high-purity water applications, such as precision manufacturing, pharmaceuticals and semiconductors. Thanks to its minimal space requirements of less than an A4 piece of paper, the TOC-1000e can be installed flexibly – either as a benchtop, wall-mounted, or pole-mounted unit.

Larger team – optimized trade fair stand

It's not just the array of products that has grown significantly during the break from in-person trade fairs: The Shimadzu Deutschland team is bigger, too. Many Shimadzu colleagues are looking forward to visiting their very first analytica.

Shimadzu has optimized its trade fair stand this year to better present product highlights and innovations. The safety of customers and the trade fair team is at the forefront of all considerations – from product presentations to the giving out of the much-loved Giant Microbes. So that people can visit the stand and get in-person consultations in compliance with our new hygiene approach, visitors may obtain the much-sought-after microbe plush toys by presenting their invitation card at the trade fair stand, or by using the QR code from the newsletter. The popular daily draw for the extra-large microbes will only occur digitally. "It makes us happy to offer solutions to our customers that make life in the laboratory more comfortable and convenient for them," says Michael Lahme, Sales Manager Shimadzu Deutschland. "To do this, we provide personal and qualified consultations, whether directly on site at analytica in Munich or digitally."

From **June 21 until 24, 2022** you will find Shimadzu at analytica at **stand 502** in **hall A1** of Munich Trade Fair Center.

We look forward to seeing you again!

Shimadzu trade fair team 2018

Analyzing the red thread of historical tapestries

HPLC-PDA-ESI-MS methodology reduces sample sizes required in textile analysis

Dr. Anna Baroni, Dr. Valeria Comite, Prof. Vittoria Guglielmi, Dr. Mattia Casanova, Dr. Paolo Redegalli, Prof. Paola Fermo

This study presents the results of using HPLC-PDA-ESI-MS methodology to identify (specifically red) natural dyes used in 17th–19th century tapestries. Analyses were performed at the University of Milan using a Shimadzu LCMS-8045 equipped with a Nexera series UHPLC with photodiode array (PDA) detector, electrospray ionization (ESI) and triple quadrupole. The results showed excellent and fast identification of various species of anthraquinones in different types of dyes using only small sample sizes, even where the concentration of coloring matter had been reduced over time.

Preserving historical tapestries for posterity

Antique and ancient tapestries are an important part of humanity's heritage. In olden times, they served as important signifiers of wealth and position. In addition, they also conveyed a cultural story accessible to both the literate and the illiterate. In that way, they provided pictorial art similar to paintings, stone statuary, and stained glass.

Unlike some of those forms, tapestries are easily transportable. That meant that an important story of cultural continuity – of a famous victory or a foundational religious event could – be reproduced wherever there was enough space to hang the tapestries.

But like all forms of pictorial art, tapestries are vulnerable to degradation over time, and most will eventually need the services of professional restorers.

Their job is to safeguard the artefact by targeted interventions and/or to restore it to its original glory. In either case, they need to use historically accurate materials and techniques.

The challenge, however, has long been the sample size required. No one wants to cut out a large chunk of an historical tapestry simply to analyze it. As a result of this obstacle, there has not been nearly enough diagnostic analysis of historical dyes and materials. →

Weaving science into cultural preservation

The reason why samples have needed to be so large is that tapestries are frequently subject to degradation phenomena. These alter the dyes and reduce the amount to be found in the tapestry. In addition, the presence of chromophores with similar structures can make the identification of the coloring matter difficult. [1]

Researchers at the Department of Chemistry at the University of Milan wondered if there might be a better way to analyze the dyes. They knew that the best technique in terms of selectivity and efficiency is high performance liquid chromatography coupled with mass spectrometry (HPLC-MS) that also allows the resolution of complex mixtures of dyes. [2] They also knew that the development of tandem mass spectrometers with the possibility to investigate the fragmentation pattern of a specific molecule represented an improvement in the capability of recognizing different compounds, particularly isomers. [3]

Working with other experts specializing in the restoration of textile artefacts, they decided to use these methods to test whether they could use smaller sample sizes to correctly identify the dyes used in tapestries produced between 17th and 19th century. Specifically, they decided to focus on the red yarns.

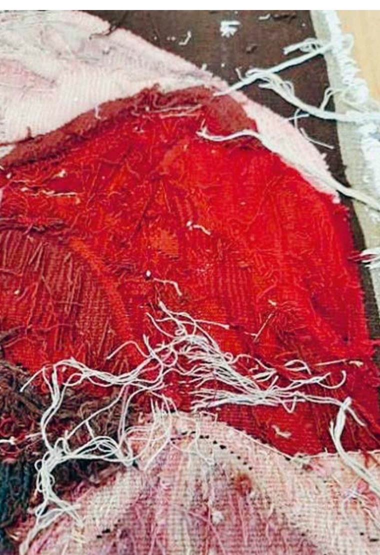


Figure 1

Figure 2

Using LC-MS/MS to identify natural dyes in historical tapestries

The analyses were performed using a Shimadzu LCMS-8045. The instrument was equipped with a Nexera series UHPLC with photodiode array (PDA) detector, electrospray ionization (ESI) and triple quadrupole, which operated both in scan and product ion scan mode. A biphenyl column was used (2.1 x 100 mm, 2.7 µm) and the mobile phase selected was gradient of water (eluent A) and methanol supplemented with formic acid (eluent B); the gradient used is reported in Table 1.

Time (min)	A (% conc.)	B (% conc.)
0	95.0	5.0
1.00	90.0	10.0
3.00	45.0	55.0
10.5	0.0	100.0
12.00	0.0	100.0
12.01	95.0	5.0
0	95.0	5.0

Table 1: Gradient used

Shimadzu equipment was chosen for two reasons. First, the equipment is highly regarded in the scientific community for its advanced technology and overall sensitivity. Second, Shimadzu Italia S.r.l. offered dedicated expert assistance in optimizing the ability of the researchers to exploit the full potential of their equipment.

The red dyes were extracted from the textile fibers and prepared accordingly to a methodology previously set up [4, 5] with an acid-methanolic mixture. Samples were treated with 3 mL of CH_3OH and 100 µL of HCl. The solution was kept in a water bath at 70 °C for one hour. Then, the solution was dried under N_2 gentle stream. The residual matter was finally dissolved in 120 µL of CH_3OH before injection. [5, 6]

The red yarns belonging to tapestries of different historical periods show hues ranging from pink to violet (Figure 1). Samples were collected and investigated in order to understand the type of dyes involved and their chemical nature.

Initially, the focus was on fibers from a tapestry dated to the 17th century; the use of madder as the main dye was shown in all samples tested. Specifically, the presence of the anthraquinone compounds alizarin 239 m/z [M-H$^-$] and purpurin 255 m/z [M-H$^-$] together with other compounds was studied (Table 2). All of the identified anthraquinones were compared both with the data-base previously created and the literature data. [7, 8, 9]

Analyses performed on yarns collected from tapestries from the 19th century (Figure 2) highlighted the use of a mixture of madder and cochineal in all the samples; the second of these was identified by the presence of carminic acid 491 m/z [M-H$^-$], kermesic acid 329 m/z [M-H$^-$] and flavokermesic acid 313 m/z [M-H$^-$] (Table 3). All the identified compounds were compared both with the database previously created and with the literature data. [4, 10, 11]

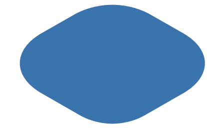
Based on their work, the researchers concluded that the HPLC-PDA-ESI-MS technique was eminently suitable for the identification of various species of anthraquinones in different types of dyes, even in historical artefacts where concentration of the coloring matter had been reduced over time or where degradation processes had occurred. In addition, observations of the samples from the 19th century revealed that it is possible to highlight the use of a mixture of dyes. In particular, the possibility of collecting the fragment ions spectra was fundamental to confirming the attributions.

Doing more with less

What the researchers at the University of Milan discovered was that new techniques and technology now made it possible to increase the speed of data acquisition. They also concluded that it is possible to identify various species of anthraquinones in different types of dyes using only very small samples, even in artefacts where the concentration of the coloring substance has decreased over time.

This is extremely important news for the preservation and restoration of all historically significant textiles. Not only does the HPLC-PDA-ESI-MS method provide the information necessary for the correct restoration of an artefact; it does so with a minimally invasive approach that respects the integrity of the object under examination.

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



The forensic fight against fake pharmaceuticals

Using UHPLC-MS/MS to streamline identification of illegal PDE-5 inhibitors

Phosphodiesterase-5 (PDE-5) inhibitors such as Viagra® are approved and widely used in the treatment of erectile dysfunction (ED). But a number of unapproved synthetic PDE-5 inhibitors are also widely available, especially in online marketplaces. Many of these substances are dangerous and have caused damage to health or even death. Successful criminal prosecution of the providers of these products relies heavily on forensic toxicology, and researchers in Poland have now developed a streamlined method to detect illegal PDE-5 substances using UHPLC-MS/MS equipment from Shimadzu.

Kaja Tusiewicz, BSc
Olga Wachełko, MSc
Agnieszka Chłopaś-Konowałek, PhD
Paweł Szpot, PhD
Marcin Zawadzki, PhD
Institute of Toxicology Research

Illegally imitating success

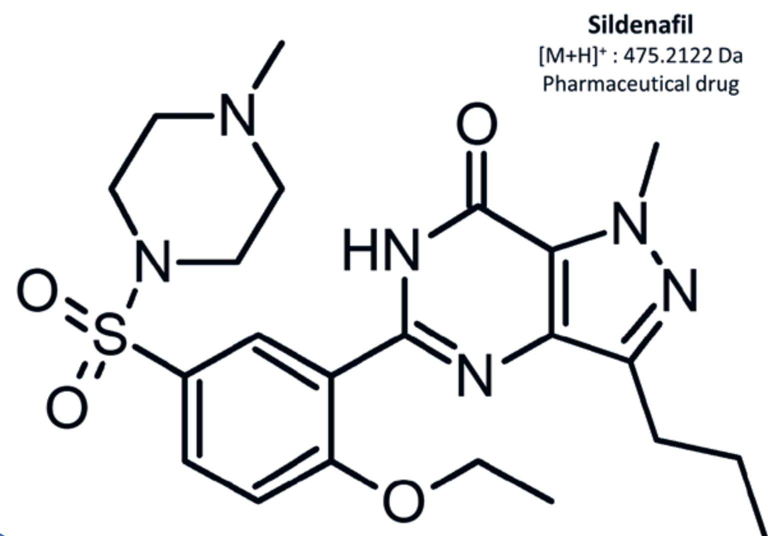
Medications for erectile dysfunction (ED) have proved to be wildly popular and extremely lucrative. This has led to the rise of counterfeit and other unapproved medications promising the same results as tested and approved medications such as Viagra®.

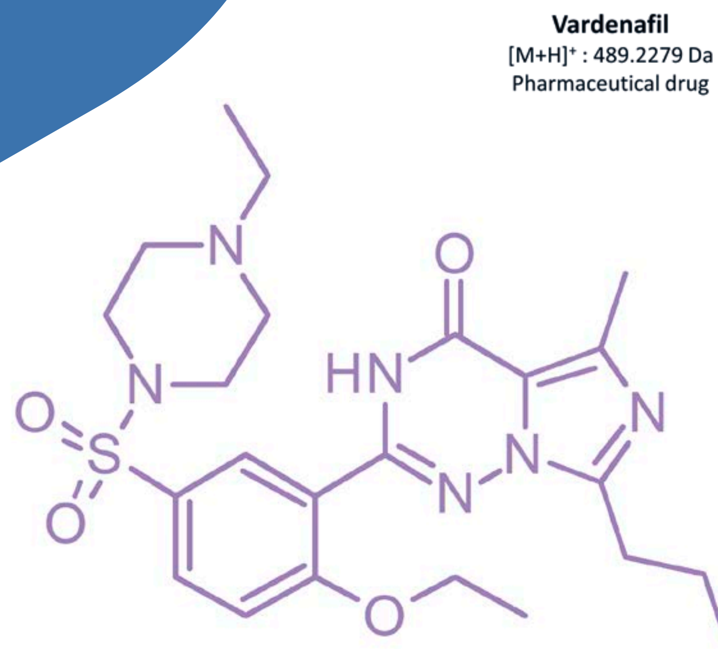
Both legal and illegal PDE-5 inhibitors can be dangerous – even fatal – to human beings.

Addressing the problem of erectile dysfunction

Erectile dysfunction (ED) is the constant inability to get and/or keep an erection, which complicates sexual intercourse. [1] It is estimated that more than 150 million men globally are affected by this condition. [2] First-line medications in ED therapy are oral drugs containing selective and reversable phosphodiesterase-5 (PDE-5) inhibitors. The most frequently used medicinal substances are sildenafil and its analog vardenafil. ⟶

MOVE ON





Sildenafil was the first oral PDE-5 inhibitor approved for ED treatment. In 1996, Pfizer Inc. received approval from the US Food and Drug Administration (FDA) to produce sildenafil under the trade name Viagra®. [16] The product was launched in 1998, and became so popular that, in its first six months on the market, six million prescriptions were written. [18] Sildenafil is now an approved drug in more than 120 countries around the world. [2]

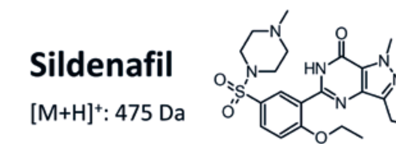
As an alternative for ED treatment, vardenafil was introduced onto the market in 2003. [6] Both sildenafil and vardenafil exhibit a similar mechanism of action, efficacy, and treatment indications. Other erectile dysfunction drugs containing different phosphodiesterase-5 inhibitors have also been introduced, such as tadalafil and avanafil. [19]

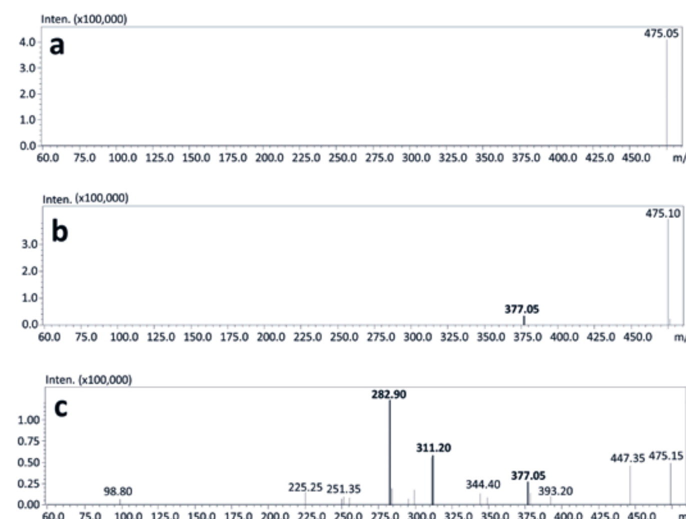
Unapproved ED medications – a dangerous market

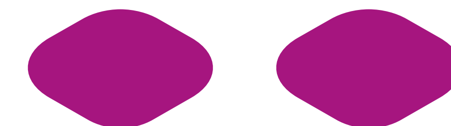
Before Viagra® lost its patent in 2013 [19], competing products which had not been approved for medical use nor investigated for consumer safety were starting to appear on the market – including synthetic analogs of sildenafil. From 2002 to 2014, 39 illegal compounds were identified [16], most frequently: homosildenafil; hydroxyhomosildenafil; hongdenafil; hydroxyhongdenafil; aminotadalafil; piperidenafil; and methiosildenafil. [20]

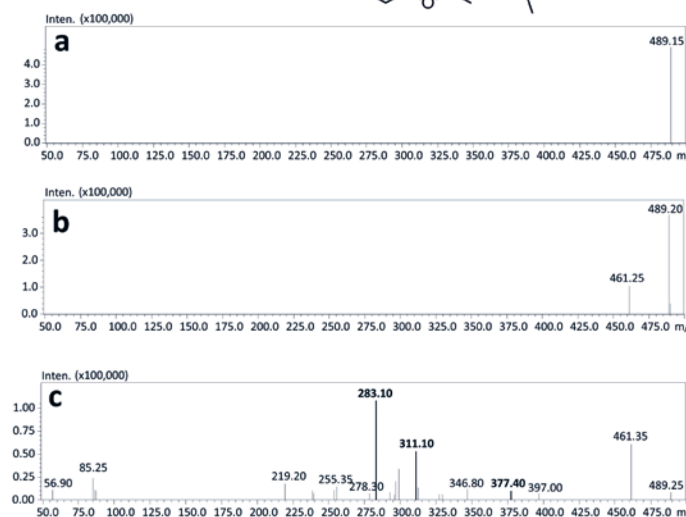
Another sildenafil analog – isosildenafil – has been very little studied, and has not been approved for medical use. [19]

The truth is that counterfeit and other illegal substances masquerading as legitimate approved medications carry a significant health risk. Impurities found in illegal ED medicines include glibenclamide, talcum powder, amphetamines, and dyes. Both illegal and legal pharmaceutical PDE-5 inhibitors usage can lead to headaches, muscle pain, nausea, vomiting, hot flashes and vision problems. And, given unregulated concentration levels, hypotension and similar effects may occur. This is especially dangerous when taken together with other vasodilating agents such as allyl nitrites, a.k.a. "poppers" – and can even lead to death. ⟶





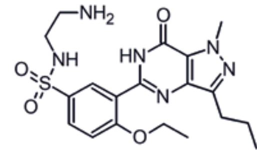


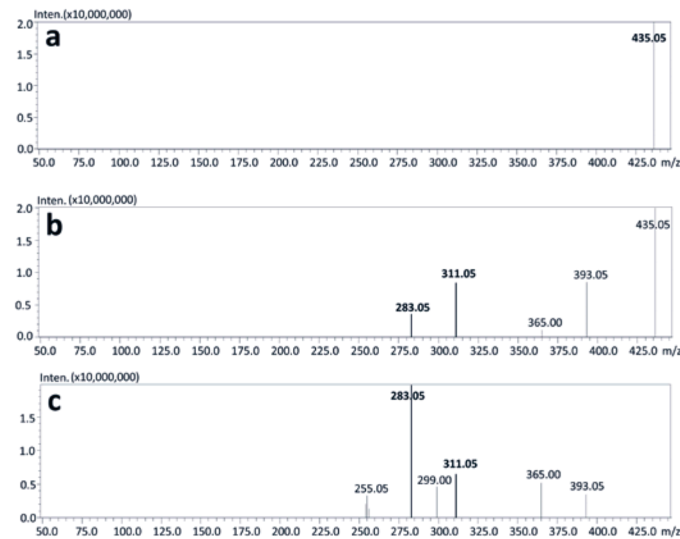


MOVE ON

UK-331, 849

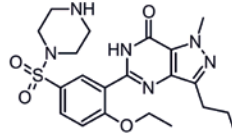
[M+H]$^+$: 435 Da

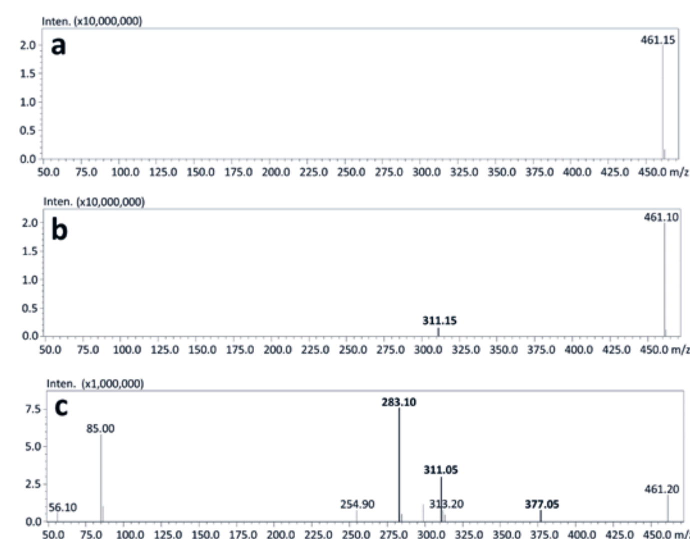




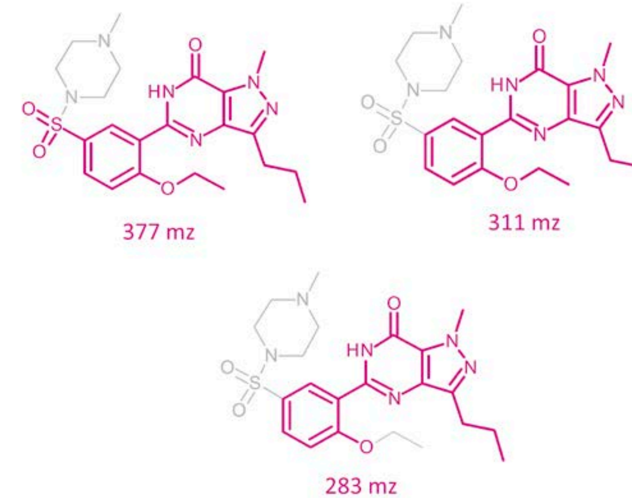
N-desmethyl sildenafil

[M+H]$^+$: 461 Da





Characteristic fragments



Applying UHPLC-MS/MS to forensic investigations of fake pharma

For a court case resulting from fatal intoxication, judicial authorities asked the Institute of Toxicology Research in Borowa, Poland, for assistance in a toxicological analysis of a suspected counterfeit compound. The Institute's researchers did this, and simultaneously developed a useful new method for use in identifying illegal ED medications.

Here's a brief look at how they did that. The Institute had previously developed a screening method for the simultaneous determination of many groups of substances, including PDE-5 inhibitors and their metabolites. This method was developed and fully validated with the use of spiked blood samples and a quantification method using an internal standard.

Using that method, the sample preparation procedure was based on quick and simple liquid-liquid extraction (pH 9 with ethyl acetate). The identification of unknown substances was achieved with the use of an ultra-high-performance liquid chromatograph (UHPLC) Shimadzu Nexera X2 coupled with a tandem mass spectrometry (QqQ) Shimadzu LCMS-8050.

Due to the high sensitivity of this equipment, the sample volume could be reduced to only 200 µL. For the screening analysis of the court-provided blood sample, the Q3 scan mode was applied, while confirmation of the chemical compounds was done with the use of product ion scan experiments. Precursors were fragmentated at three collision energies (10, 20 and 35 V).

The results of this toxicological analysis with detailed chromatographic and mass spectrometry conditions as well as characteristic fragments are presented in figures. Five analogs belonging to the phosphodiesterase inhibitors group (including two metabolites) were identified by the aforementioned method. The substances detected were sildenafil and its metabolites (N-desmethylsildenafil and UK-331, 849), as well as two synthetic analogs of PDE-5 inhibitors (isosildenafil and homosildenafil), whose presence in the blood may indicate that the medication was manufactured by and/or purchased from illegal sources.

The danger associated with sildenafil analogs is the lack of data regarding toxicity and safety. This unpredictability and the fact that sildenafil, other phosphodiesterase-5 inhibitors, and their analogs may be present in a number of widely available products, creates a great demand for quick, simple, and reliable methods for substance identification.

Note

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

Conclusion

By adapting a previously developed method for biological analysis, researchers were able to identify and quantify the presence of hazardous substances – PDE-5 inhibitors – in unregulated medications for the treatment of ED.

The method they developed provides a solidly replicable approach of interest to toxicologists and clinical or analytical chemists wishing to detect and identify new drugs emerging on the illicit pharmaceutical market. The method highlights the high sensitivity and selectivity of the Shimadzu Nexera X2, as well as how its high-speed MS/MS detection makes it possible to operate the Q1/Q3 in a scanning mode: something unique for this type of detector. In addition, the method minimizes the use of biological sample material, resulting in the need for much lower organic solvent volumes in the sample preparation process.

Testing sunscreens to save your skin

Dr. Benjamin Thomas, Shimadzu Europa GmbH

More sensitive analysis using Shimadzu UV-2600i, integrating sphere and LabSolutions UV-Vis

It's finally summertime: The sun is blazing from a blue sky and the beach is calling. But first it is time to hit the drug store and get some sunscreen! The most important criterium: The sun protection factor, which is advertised in big letters on every product. Since sunscreen protects against ultraviolet and visible light, UV-Vis spectroscopy is the ideal tool to test this protection. And the analysis is even easier with the LabSolutions UV-Vis Automated Control function.

Sunscreen products protect our skin against damage caused by ultraviolet and intense visible radiation: everything from annoying sunburn to dangerous skin cancer. But choosing the right one can be difficult. There are just so many different types of cream, lotion and spray, all colorfully calling out to us and promising protection from the sun.

The most important criterium when choosing a sunscreen is the sun protection factor (SPF) – often 30 or 50. Multiply this number by the safe unprotected exposure time for your skin type and you know how much exposure time is safe for you after correctly applying the sunscreen.

Figure 1: A large selection of sunscreen – the agony of choice

Type	Skin color	Tanning	Sunburn	Skin cancer	Self-protection [min]
I	Very light	Freckles only	Frequent	High risk	3 – 10
II	Light	Minimal, slow	Frequent	High risk	10 – 20
III	Medium	Slow	Sometimes	Risk present	20 – 30
IV	Brownish	Fast	Rarely	Low risk	> 45
V	Dark	Fast	Very rarely	Low risk	> 60
VI	Dark to black	Not relevant	Never	Low risk	> 90

Table 1: Skin types and unprotected exposure time

All of these products are skin-compatible emulsions containing at least one screening agent. This agent accumulates in the corneal layer of the skin and protects it against damage from sunlight – either until the screening agent is destroyed by the absorbed radiation or washed out. Sunscreens can be roughly grouped into oil-in-water emulsions ("sun milk") and water-in-oil emulsions ("sun lotion").

Measurement in vitro

The sun protection factor and other parameters are tested on volunteer test persons in vivo or on living cells outside of the body in vitro.[1] There are two main reasons to use in vitro techniques. The testable parameters of in vivo studies are limited, since too long exposure or exposure at very high light intensities can harm the test person. The other reason is that a defined or even automated sample preparation is required for reproducible results.

The in vitro test procedures for the sun protection factor were first defined by the industry interest group Cosmetics Europe (then known as COLIPA) in the 1990s and were issued as open access guideline until 2011, since no harmonized standard was available at that time.[2] Based on this guideline, ISO standard 24443 was developed and finally revised in 2021.[3] As in the original COLIPA guideline, the sample is applied on a roughened PMMA plate and rubbed into this well-defined surface to simulate massaging sunscreen into human skin. →

Figure 2: PMMA plate according to COLIPA before rubbing in sunscreen (left) and ready to measure, mounted at the integrating sphere (right)

The samples prepared by this procedure are measured with an integrating sphere to collect all light scattered on the rough surface. The inner surface of this hollow sphere is coated with highly reflective material. All light transmitted and scattered through the sample is reflected on the inner sphere walls until it hits the detector. The sun protection factor (SPF) is calculated from this spectrum with equation 1 – ideally automated by the instrument software.[3]

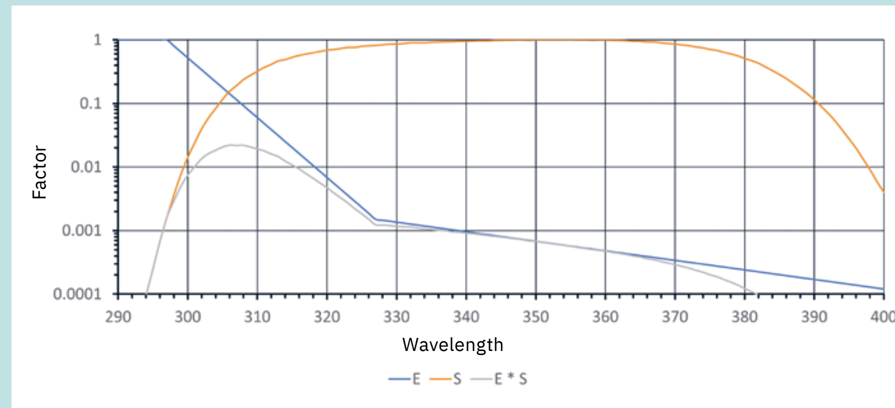


Figure 3: Plot of factors E (blue line), S (orange line) and the product E*S (grey line) from equation 1 as defined by Webb at al [4]

The wavelength range from 315 to 380 (UV-A) is especially relevant for skin reddening (Fig. 3). At wavelengths above 380 nm, the product from the erythemal action spectrum E(λ) (Erythema = reddened skin) and standard sunlight S(λ) is near zero. That is the reason for the limits of the integrals in equation 1 and the distinction between the UVA (315–380 nm) and UVB (280–315 nm) ranges. Many products are tested and certified explicitly to offer UVA and UVB protection.

The scientific community has also agreed on other parameters based on the SPF from the COLIPA guideline. The erythemal action spectrum can be substituted for a curve describing the individual sensitivity of a patient or skin type, e.g., to test a product specifically for people with sun poisoning. In this case, the factor obtained is called photosensitivity protection factor (PPF).[5] Standard sunlight curve S can also be replaced by a curve more relevant for conditions of the target market, e.g., summer sun in Australia or a sun bed.

Macro-automated analysis

These calculations are implemented into a macro for the LabSolutions UV-Vis control software, which automates the workflow from measurement to reporting. (Fig. 4)

In this macro, custom curves can be loaded for factors E and S. The ISO 24443 standard curves and CIE standard illuminants [6] (Fig. 3) are already implemented. The sample names and file names of each curve are tracked to trace the final report back to the correct raw data set. The absorbance values are automatically averaged after each measurement.

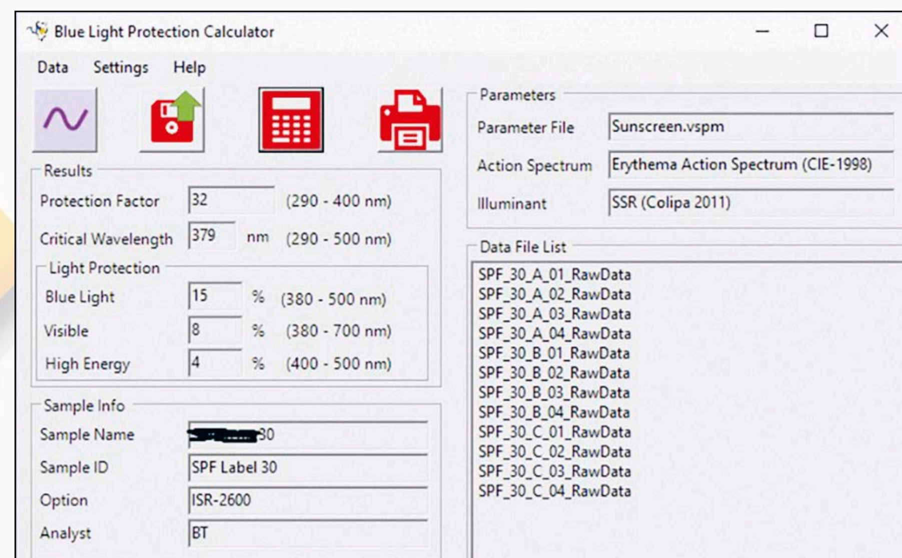


Figure 4: User interface of the LabSolutions UV-Vis sun protection macro. On the left side, the calculated parameters and the sample description are shown. On the right side, a list of the used curves and raw data names is shown for reference.

For example, in one recent analysis, three plates were prepared with a sunscreen with a sun protection factor of 30, and measured at four different positions to compensate for any inconstancy during sample preparation. A Shimadzu UV-2600i spectrometer outfitted with an ISR-2600 integrating sphere was used.

Other applications

The focus of all these parameters is protection against light in or close to the ultraviolet range, as these can cause erythema, sunburn or – in the worst case – skin cancer. But this analysis can also be applied to infrared light of up to 2500 nm. Infrared light – also known as heat radiation – is usually perceived positively and technically used as a beneficial heat source. But excessive exposure to high intensity infrared light can accelerate skin ageing, which is why sunscreens should also offer some protection against light in the near infrared wavelength range.[8]

The macro was therefore designed to let the user adjust the wavelength ranges depending on the specific application. With a Shimadzu UV-3600i Plus and the integrating sphere ISR-603, measurement is possible in the wavelength range from 220 to 2500 nm. Additionally, the UV protection factor of fabric can be calculated with the LabSolutions UV-Vis UPF software. Using this combination of technology, the entire range of sun protection topics can be examined and reveal much more information than does the sun protection factor printed on the sunscreen packaging.

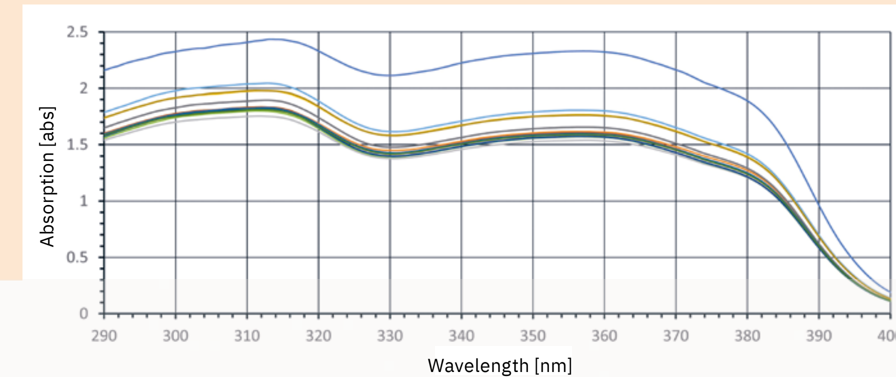


Figure 5: Absorption spectra of different samples of a sunscreen with SPF 30

Apart from the protection factor SPF or PPF, the macro calculates the critical wavelength and the specific protection factor for up to three wavelength ranges, which are also relevant during product development.

In addition to SPF and PPF, three other protection factors are relevant. These are defined for the wavelength range from 290 to 400 nm and the critical wavelength:

- Blue light at 380–500 nm
- Visible light at 380–700 nm
- High-energy visibly (HEV) light at 400–500 nm

Protection against blue light is especially relevant for people with photodermatitis (“sun poisoning”), since light in this wavelength range can induce photochemical reactions which cause the symptoms. HEV light as a sub-range of the blue light range can accelerate skin ageing or cause irregular pigmentation.[7]

Note

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

SWITCH ON

Ambitious young lab takes on hazardous waste recycling

Premifab needed top-level instruments to meet its goals

Silvija Petković, Premifab

Premifab is a Croatian company that is ambitiously pursuing a specialized goal of providing high-tech solutions in the regeneration of organic solvents and material recovery of other hazardous waste. As part of an EU-funded joint R&D project with the University of Zagreb, Premifab recently acquired advanced Shimadzu instrumentation for gas chromatography (GC) and UV-Vis spectrophotometry.

Silvija Petković, Premifab lab manager, explains why the lab chose Shimadzu.

European environmental perfomance

The European Union (EU) is at the forefront of global efforts to protect the environment. Among the many rules and tools produced by the EU to improve sustainable consumption and production is the Eco-Management and Audit Scheme, or EMAS.

Meet Premifab

Located on the outskirts of Zagreb, Premifab is the third company in Croatia to have received EMAS registration. The company is young, growing and energetic, especially about environmental protection. It has to be, as it has chosen to work in a very challenging area: hazardous waste recycling.

Specifically, Premifab works with the regeneration of organic solvents and material recovery of other hazardous waste. It is the first company in Croatia to work primarily in this area. The goal is to recover and recycle as much material as possible and return it to market as a usable product. This reduces the amount of unusable waste and expands the frontiers of the circular economy. It currently recovers over 1,300 tonnes of industrial solvents every year.

Like all EMAS-registered organizations, Premifab must meet a number of stringent requirements, including adherence to strict criteria for legal compliance and transparency. In addition, EMAS demands an exceptionally high level of environmental performance measurement and improvement. This makes the choice of the tools used in measurement and analysis more important than ever.

Innovative and reliable solutions

Specifically, Premifab chose to obtain a number of Shimadzu precision instruments, including the Nexis GC-2030 with FID detector, the Nexis GC-2030 with AOC-6000 Plus multifunctional autosampler, and the UV-1900i spectrophotometer. →

Figure 1:
Premifab lab with Shimadzu equipment

Figure 2:
Shimadzu UV-1900i spectrophotometer

Figure 3:
Analysis on a Nexis GC-2030 gas chromatograph

Shimadzu equipment in practice
Customer satisfaction is of vital importance for Shimadzu, so we asked Premifab's Silvija Petković to tell us a bit about the company's experience with Shimadzu equipment.

How did you become familiar with Shimadzu equipment?

Everyone in our company – in fact, everyone I know in the field of chemical technology – has known about Shimadzu since their university studies.

What is Premifab's main field of research?

As part of the chemical industry and with an accredited internal laboratory for organic solvents, we need to assure the quality of all the materials in our processes – raw materials, regenerates, and finished goods. All these materials are based on organic solvents and have various applications. That's why we decided to purchase Shimadzu equipment.

Could you be more specific about your reasons for choosing Shimadzu?

Apart from instruments' reliability and user-friendliness, the decisive factor was the readiness of the Shimadzu team in Croatia to support us and provide expert guidance in the process of choosing instruments appropriate to our needs. It also helped that many of our colleagues had previous experience working with Shimadzu equipment.

Did the instruments you chose meet your expectations?

Without a doubt! From a technical point of view, the instruments' software offers everything we require, and the hardware is easy to operate. I have to add that the devices are very easy on the eye as well. They really look quite elegant.

How are you currently applying gas chromatography (GC) to your work, and what are you hoping to do in the future?

We are currently working on a joint project with the Geotechnical Faculty of the University of Zagreb. The goal of the project is to find additional uses for non-recoverable distillation residues, i.e. sludge. We also hope to find new ways to work with residual solvents in solid and semi-solid samples from various industries. The Nexis GC-2030 with AOC-6000 Plus multifunctional autosampler device makes it possible for us to identify and measure volatile organic compounds in both liquid and headspace phases.

How are you currently applying UV-Vis spectroscopy (UV-Vis) to your work, and what are you hoping to do in the future?

The UV-Vis assists us in determining coloration in our finished goods. Such information is critical to advancing our R&D efforts to fully explore the potentially useful properties of sludge. In addition, its ability to determine color in our finished goods in accordance with different color scales provides us with increased flexibility to meet future demands.

Which equipment feature(s) did your lab find most useful?

That would probably be the headspace injection of the gas chromatography. And the AOC-6000 Plus robotic arm on the GC-FID, which could be described as the "cherry on top of the cake".

Figure 4:
Easy to use UV-1900i interface

Figure 5:
Multifunctional autosampler improves GCMS analysis productivity

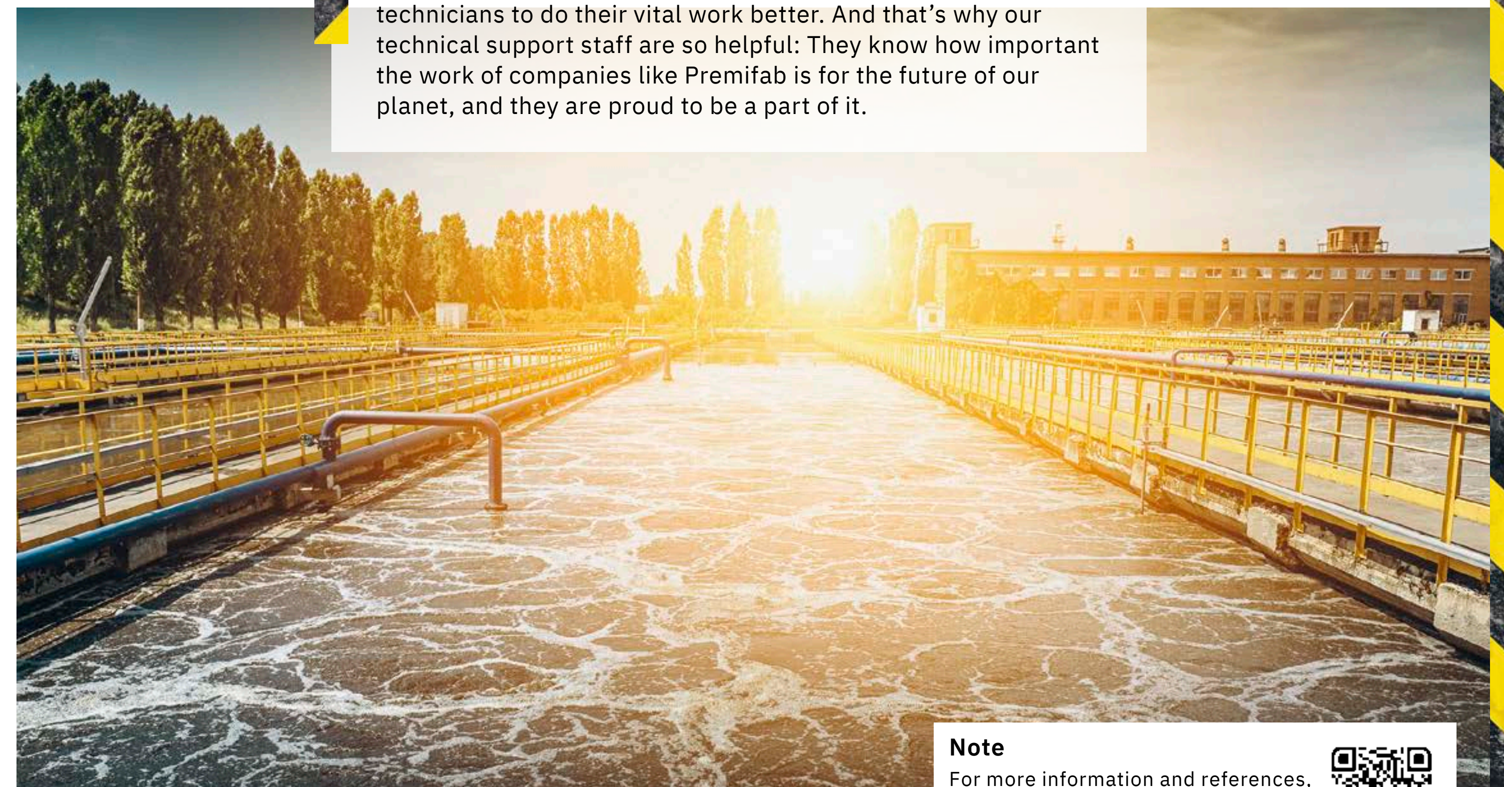
Figure 6:
Head of Spectro group at Shimadzu Croatia, Mirna Markusi Tomljanović (right), with Premifab's Quality Control and Laboratory Manager, Silvija Petković (left)

Conclusion

Environmental protection is a global responsibility. Small companies, large companies, governments, NGOs, civil society and every individual consumer must work together to solve our common problems if we are to achieve a desirable common future.

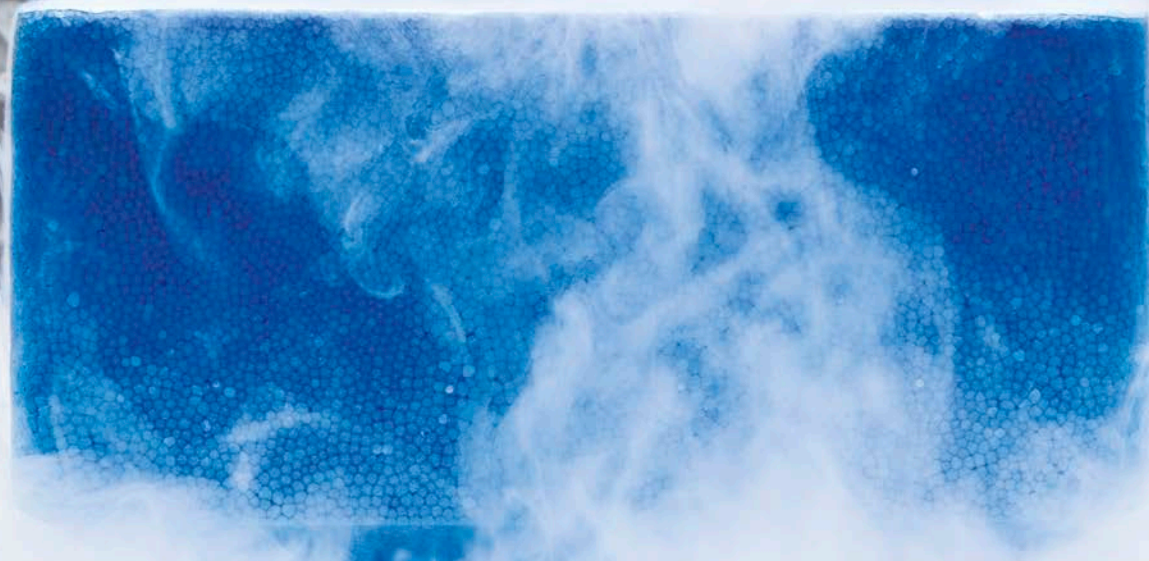
Premifab is working hard in the regeneration of organic solvents and hazardous waste management through the recycling of waste. By combining hard science with market forces, the company seeks to find attractive ways to reduce adverse environmental impacts and risks to human health.

Shimadzu's role in these efforts is a humble yet critical one. The company creates exceptionally accurate and easy-to-use high-tech tools that allow and inspire frontline scientists and technicians to do their vital work better. And that's why our technical support staff are so helpful: They know how important the work of companies like Premifab is for the future of our planet, and they are proud to be a part of it.



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

Short on helium? Consider the carrier-gas alternatives



The advantages of using hydrogen and nitrogen in gas chromatographic measurement

Dr. Rebecca Kelting, Dr. Franz Kramp, Shimadzu Europa GmbH

Helium is a preferred carrier gas in gas chromatography because of its high inertness and abilities supporting chromatographic separation. Demand for helium has been increasing, but limited availability and current events have exacerbated a pre-existing helium shortage. Prices therefore continue to rise, forcing users to look for alternatives. Nitrogen and hydrogen offer great potential to GC users. Both have drawbacks, but together with flexible gas-type selection and reduction of consumption they offer the key to success.

The role of helium in gas chromatography

In gas chromatography (GC), the carrier gas transports the sample from the injection system via the separation column to the detector. Helium is a preferred carrier gas due to its high inertness and good separation abilities. However, it is a limited natural resource, and demand has been rapidly increasing for use in ever more applications in the medical, scientific, and industrial fields. Current circumstances have restricted supplies and lengthened delivery times, resulting in a helium shortage. This has led to rising prices, and some labs have been having a hard time getting any helium at all.

What are the choices?

Historically, inert gases were commonly used for gas chromatographic systems as they are easy and safe to handle. Helium and nitrogen were the most popular choices available among the inert gases.

Hydrogen is the best alternative to helium with respect to gas chromatographic separation. It is a highly efficient carrier gas that maintains its separation efficiency across a wide linear velocity range. In addition, hydrogen has no supply limitations, as it can be mass-produced by gas generators.

That said, hydrogen is reactive and bears an explosion risk if the concentration rises to 4% in the air. So strict precautions for safe operations with the gas must be taken. In addition, hydrogen is not always an appropriate carrier gas, in particular in combination with some detection systems or advanced techniques used in gas chromatography. →

HEPT – [u] curve for C17 at 175°C with k = 4.95
Capillary column: OV-101 25 m, 0.25 mm ID. 0.4 µm film

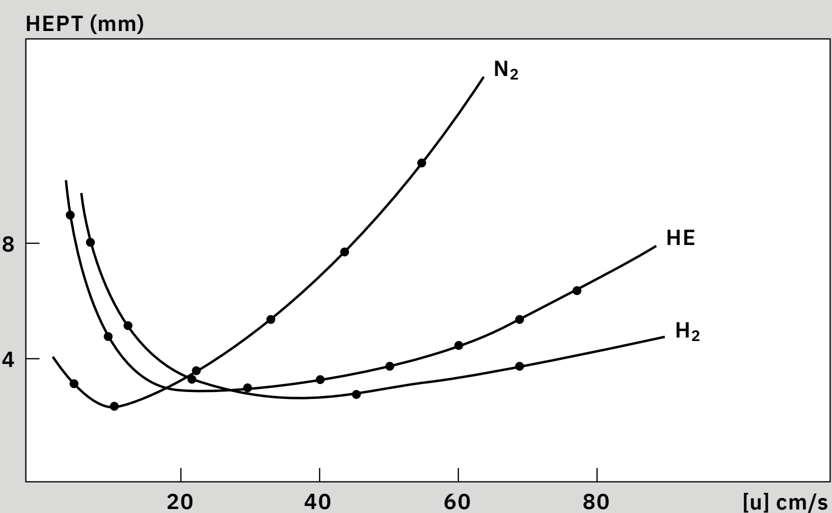


Figure 1: Van Deemter curves measured using different carrier gases (Shimadzu Corporation, Kyoto, Japan)

Using nitrogen without compromising performance

Typical, conventional GC analysis times using helium as a carrier gas range from 15 to 40 min, depending on the number of compounds, the separation power needed, and the matrix bakeout to prevent carryover. An application example at the higher end of this time range is the quantification of polychlorinated biphenyls (PCBs) in oil according to DIN EN 12766-1 and DIN EN 12766-2. A method using helium carrier gas and an SH-I-5 MS column (60 m, 0.25 mm ID, 0.25 µm df) that is sufficient for compound identification and separation has a runtime of 40 min at a linear velocity of 23 cm/s. Direct method translation to nitrogen carrier gas can be achieved, keeping compound separation and chromatographic runtime constant (Fig. 2).

Runtime benefits using hydrogen

Another example with a runtime in the range of conventional gas chromatography using helium is the analysis of higher alcohols and ethyl acetate in alcoholic beverages. The alcoholic beverages are extracted using dichloromethane and the extracts then injected into the gas chromatograph. The last eluting target compound is pentanol, showing a retention time of 14.6 min on an SH-I-624 Sil MS column (30 m, 0.25 mm ID, 1.4 µm df). Yet, despite extraction, the remaining matrix elongates the runtime to around 24 min, limiting sample throughput to two per hour (Fig. 3).

When using hydrogen as the carrier gas however, analysis runtime can be drastically reduced due to a higher linear velocity and an adjusted oven temperature program. Pentanol now shows a retention time of only 9.1 min, giving a time saving of 38%, which allows three samples per hour to be processed (Fig. 4). Separation efficiency is not compromised by this shorter chromatographic runtime, as demonstrated by the resolution between acetaldehyde and methanol: With helium carrier gas it is calculated to be 1.4, whereas hydrogen shows a value of 1.2, still ensuring reliable results (Fig. 5). In summary, switching from helium to hydrogen as carrier gas can provide significant time benefits, even for already short analysis times.

The analysis of mineral oil content in water according to EN ISO 9377-2 involves the monitoring of all compounds in the boiling point range of 175 °C to 525 °C. This describes all peaks found between the retention time markers n-decane and n-tetracontane. As it is not required to assign individual substances due to the complexity of the hydrocarbon mixtures, the analysis can be performed with a high linear velocity (60 cm/s). Using an SH-MetalX-1 column (15 m, 0.25 mm ID, 0.1 µm df), runtimes around 9 min can be achieved with helium as the carrier gas. However, switching to hydrogen allows an even higher linear velocity (80 cm/s) which, in combination with an adjusted oven temperature program, leads to a chromatographic runtime below 6 min. This results in a decrease in retention time for n-tetracontane of 30%, from 7.8 min to just 5.4 min (Fig. 6), allowing injection cycle times of 10 min.

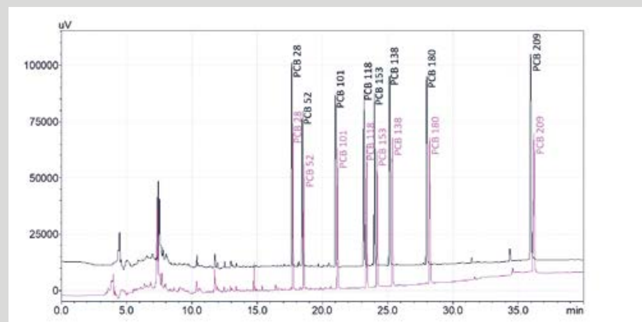


Figure 2: Chromatogram of a PCB standard mix with helium (black) and nitrogen (pink) as carrier gas, chromatograms base-shifted for better visibility

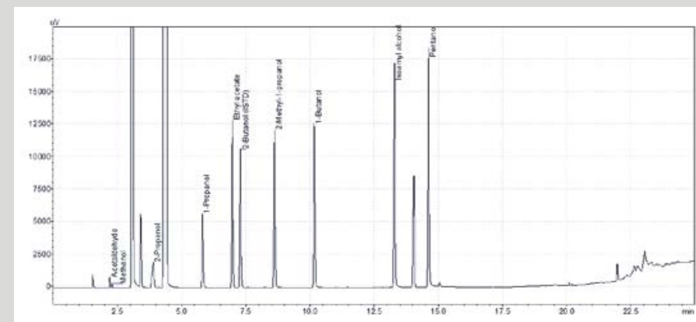


Figure 3: Chromatogram of a standard mix for the determination of higher alcohols in alcoholic beverages with helium as carrier gas

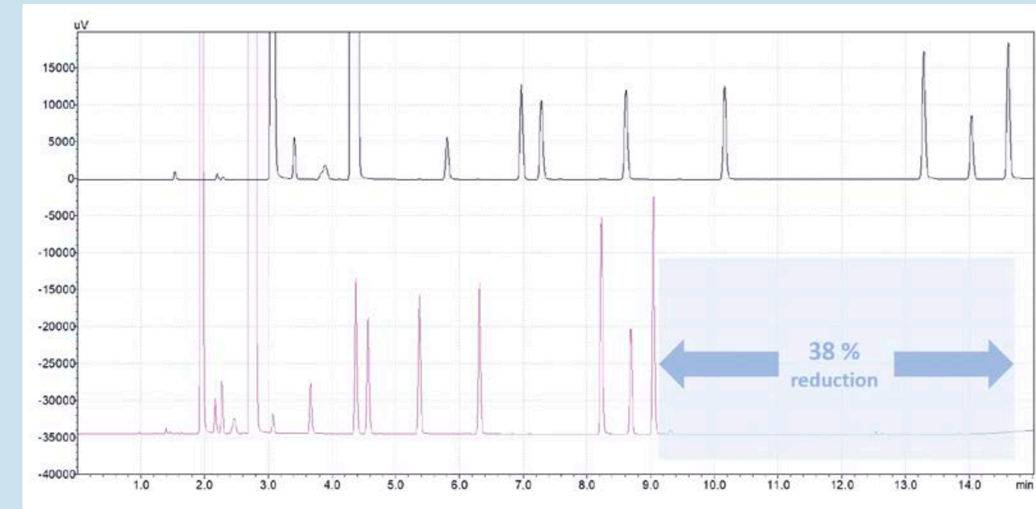


Figure 4: Chromatogram of a standard mix for the determination of higher alcohols in alcoholic beverages with helium (black) and hydrogen (pink) as carrier gas

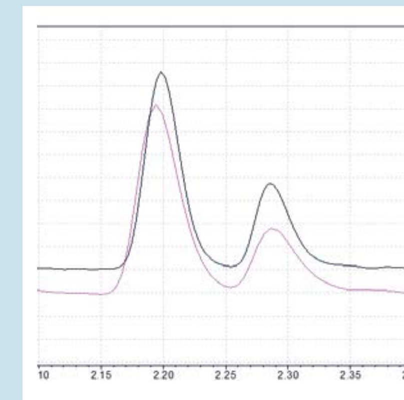
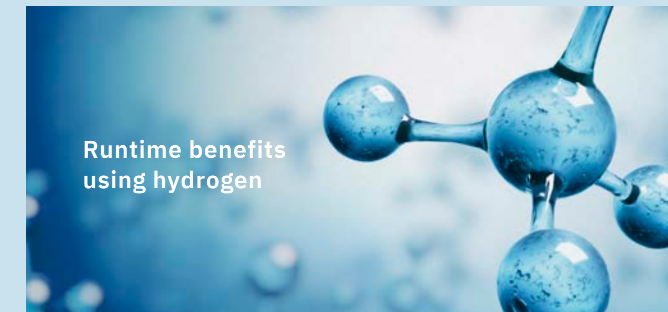


Figure 5: Resolution between acetaldehyde and methanol with helium (black) and hydrogen (pink) as carrier gas, hydrogen chromatogram rescaled to fit the helium scale



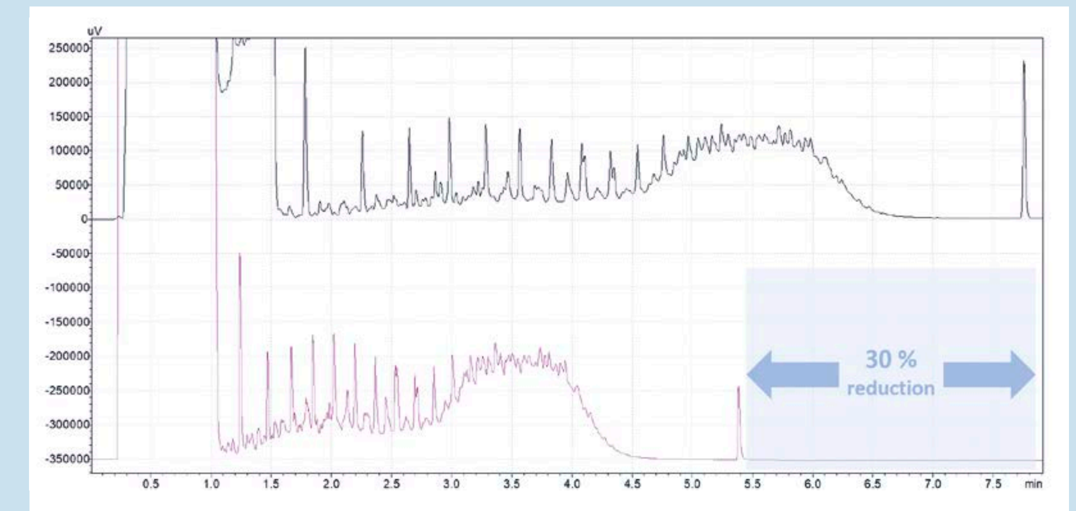


Figure 6: Chromatogram of a mineral oil standard with helium (black) and hydrogen (pink) as carrier gas →

Using hydrogen safely in GC

Despite its efficiency as a carrier gas, hydrogen remained less popular than helium for decades due to its lack of inertness. Hydrogen is a flammable gas that can potentially explode in air in a concentration range from 4% to 75%.

To ensure that this does not occur, the Shimadzu Nexis GC-2030 offers a three-pillar safety concept that supports the safe use of hydrogen as a carrier gas. A preventative, automatic leak-check function helps the operator to check for possible leakages after any kind of column maintenance. In the case of severe carrier-gas leakage occurring during standby or runtimes, the fast-responding advanced flow controller (AFC) immediately shuts off the carrier-gas supply.

To cope with the risks resulting from less severe leakages or breakages of the capillary column during operation, independent hydrogen sensors for checking the air inside the column oven are available (Fig. 7). The basic sensor shuts off the carrier gas control once a level of 1% of hydrogen in air is reached. The advanced sensor model monitors the hydrogen level constantly and switches the gas control to an inert gas once the 1% concentration level limit is reached.

Figure 7: Schematic of Nexis GC-2030 with hydrogen sensor

Cutting costs by reducing helium consumption

Helium is expensive, so users interested in efficient gas usage and instrument utilization need to consider both the active analysis times of a gas chromatograph and instrument standby times. To help reduce costs, the Nexis GC-2030 supports an automated gas selector for convenient gas switching between analyses and for standby times, facilitating the use of different gas types on the same instrument. This can be used for carrier and/or make-up gas selection and offers the choice between two gases that are simultaneously connected to the selector inlets (Fig. 8).

Automatic switching to nitrogen carrier gas for standby times after a sample sequence with helium significantly reduces helium consumption, even for applications where helium use is inevitable (Fig. 9). In combination with the carrier gas-saver functionality of the Nexis GC-2030 – which reduces the gas flow during analyses after injection and during brief standby periods – the overall gas savings can reach up to 90%, depending on standby times and analytical conditions.

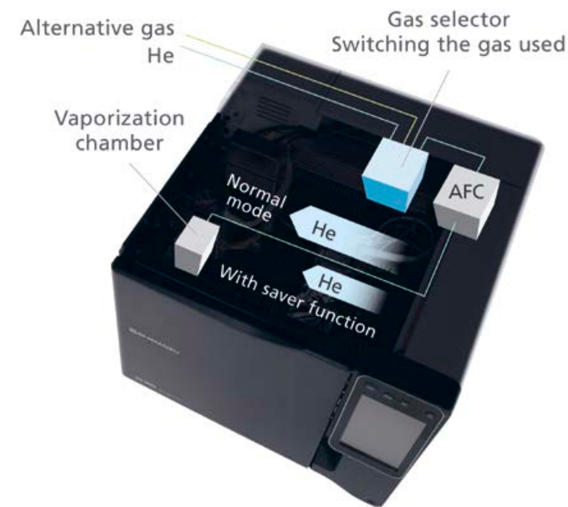


Figure 8: Schematic of Nexis GC-2030 with gas selector

Optimising workflow with smart automatic features

Independent of carrier gas choice, the Nexis GC-2030 provides additional measures to further decrease overall gas consumption. Carrier gas-saver functionality reduces gas flow during analysis and the gas selector can help to save helium during brief standby times. For longer standby periods, the available automatic start/stop functions reduce running costs. An automatic stop function stops GC temperature and gas control after the last analysis of a sequence or after a sequence queue has been completed (Fig. 10).

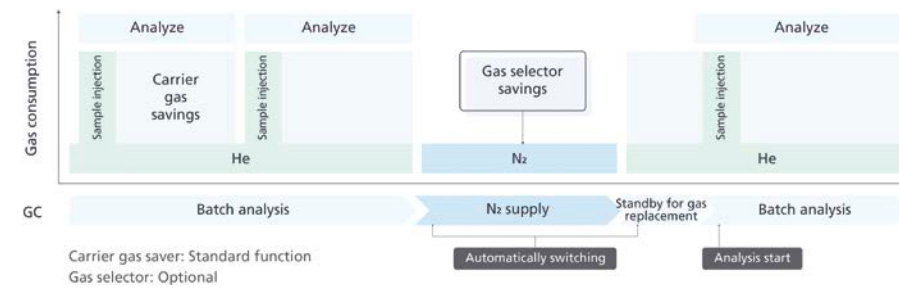


Figure 9: Automated gas saver and gas selector functionalities of Nexis GC-2030

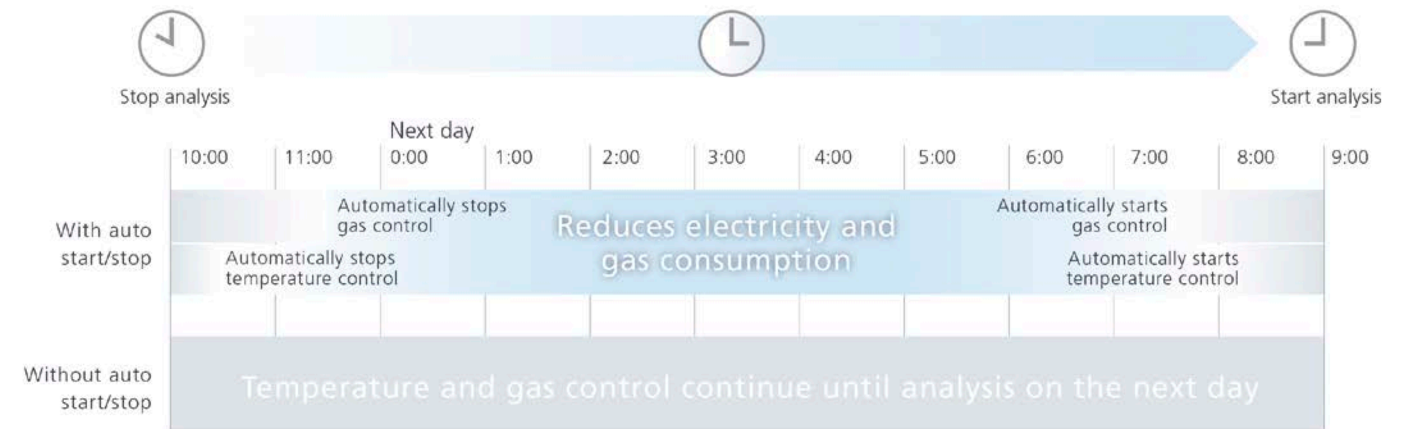


Figure 10: Smart automatic start/stop functionalities of Nexis GC-2030

Safety. Value for money. Flexibility.

To reduce over-reliance on increasingly unavailable and progressively expensive helium, the Shimadzu Nexis GC-2030 supports cost-efficient gas chromatographic measurements with nitrogen and hydrogen as lower-cost carrier gas alternatives to helium. Nitrogen can be used as a carrier gas in conventional GC without compromising chromatographic performance and can substitute for helium as a make-up and pressurizing gas. Hydrogen allows a significant reduction of chromatographic runtimes, increasing sample throughput without compromising reliability of the results. And the Nexis GC-2030 reduces the total amount of whatever carrier gas is used.

In addition, an automatic gas selector enables convenient gas switching between different analyses and during standby periods. Smart automatic features help to optimize the GC's daily working time and gas consumption, further reducing running costs.

Finally, the Shimadzu Nexis GC-2030 ensures lab safety for users and their equipment and offers the most economical safety option for using hydrogen as a carrier gas. The Nexis GC-2030 comes equipped with automatic leak-check functionality and hydrogen sensor solutions with automatic safety features to ensure a safe working place.

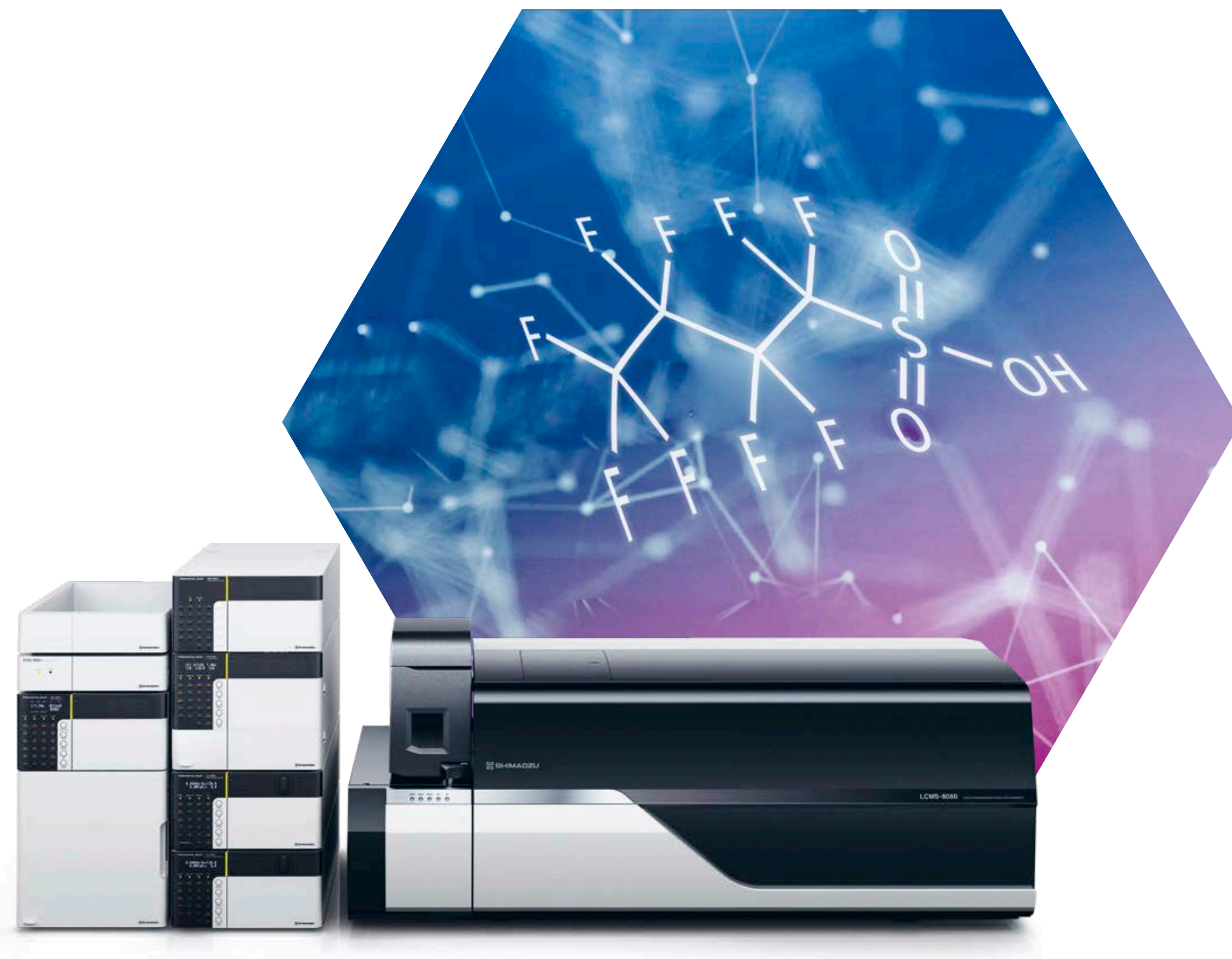
Shimadzu understands the concerns and bottom-line challenges of gas chromatography users. The Nexis GC-2030 is designed to help labs reduce costs by reducing gas-carrier amounts and by safely and flexibly utilising the full potential of lower-cost alternates to helium.

Note

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

Detecting PFAS compounds in food packaging

Better assessing the hidden threat of “forever chemicals”



SWITCH ON

Milica Jovanovic, BSc, MSc
Univ. Prof. DI Dr. Erich Leitner
Institute of Analytical Chemistry and Food Chemistry,
Graz University of Technology, Austria

Poly- and perfluoroalkyl substances (PFAS) – a.k.a. “forever chemicals” – are widely used in everyday applications, and there are approximately 5,000 known PFAS structures. This article presents a targeted approach for detecting and quantifying 24 PFAS commonly found in paper and board matrices. Using accelerated solvent extraction to extract PFAS, identification and quantification was done using high-performance liquid chromatography coupled with triple quadrupole mass spectrometry. Apparent recovery values were in the 84–94 % range, with method detection limit values in the 0.1–0.5 ng/g range.

Forever chemicals

Poly- and perfluoroalkyl substances – PFAS – are man-made chemicals which are widely used in a range of applications, from water-resistant clothing to food-packaging materials. Often called “forever chemicals”, these compounds have a special chemical structure: multiple fluorine atoms attached to an alkyl chain.

The bond between carbon (C) and fluorine (F) is one of the strongest chemical bonds known. Additionally, the size of the fluorine atom is just right to pack closely around a carbon chain and shield it from interaction with other atoms. [1]

This is both good news and bad news. Because of their properties, PFAS repel water, fat and dirt. This makes them very useful for various applications.

But because PFAS are highly resistant towards chemical and physical strains, the bad news is that they are long-lasting, bio-accumulative and unfortunately toxic.

In recent years, PFAS have been increasingly detected in water, soil, air, as well as in wildlife and human beings. For instance, in Belgium in 2021, the Flemish government issued a warning to residents to stop eating products from their own gardens because of the high levels of PFAS in their water, soil and food.

PFAS have also been found in packaging products from many fast-food chains. [2]

As a result, some PFAS have recently begun to be regulated or even phased out. But so far only a few of the substances have been assessed for risk by the European Food Safety Authority (EFSA) or the Environmental Protection Agency (EPA). [3, 4]

Assessing the threat of PFAS in food packaging

PFAS can enter the human body through various sources, but one of the major pathways is food consumption. [5] As a result, in recent years much focus has been placed on PFAS found in packaging materials which are in direct contact with food.

There are several different analytical methods used for detecting and quantifying PFAS in food-packaging materials, depending on the desired type of analysis. →

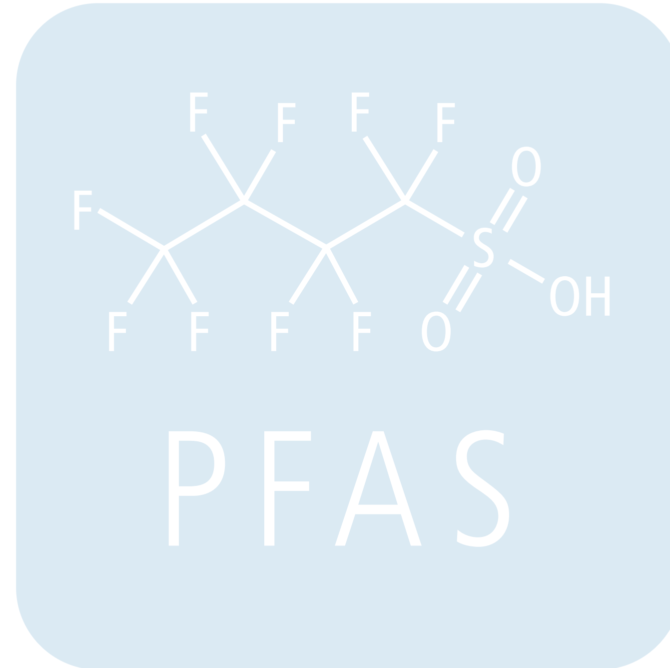
SWITCH ON

However, the most selective and sensitive method is liquid chromatography coupled with triple quadrupole mass spectrometry – LC-MS/MS.

Recently, researchers at the Institute of Analytical Chemistry and Food Chemistry at the Graz University of Technology in Austria tested a simple method for extracting PFAS from paper and board matrices using accelerated solvent extraction (ASE), followed by quantification using LC-MS/MS.

Question: What did you do?

We analyzed 24 PFAS substances most commonly found in paper food-contact materials. A mixture of these, with a concentration of 2,000 ng/mL, was purchased from Wellington Laboratories (Guelph, Ontario). For each substance, we identified the precursor ion and product ion, as well as the retention time.



Two series of calibration solutions were prepared by dilution with 50:50 % (v/v) methanol:water. For high-concentration calibration, we prepared a series of 9 calibration solutions at concentrations of 100, 50, 25, 20, 15, 10, 5, 2.5 and 1.25 pg/µL. For low-concentration calibration, 10 calibration solutions at concentrations of 20, 10, 5, 2.5, 1, 0.5, 0.25, 0.1, 0.05 and 0.01 pg/µL were prepared.

As a sample we used unprinted recycled paper (70 g/m^2), which had previously been analyzed for the presence of PFAS. The paper was cut into small pieces, and 2 grams were used for experiments. The paper was spiked with a PFAS mixture with different concentrations of our standard solution – 100, 50, 20, 10, 5, 2.5, 1, 0.5 and 0.1 ng/g. After drying, the samples were extracted with methanol using accelerated solvent extraction (ASE). The collected extracts were placed in a nitrogen evaporator and evaporated to dryness under a gentle stream of nitrogen and then reconstituted in methanol:water (50:50) % (v/v).

Finally, the solutions were filtered using 0.22 µm regenerated cellulose filters and transferred into polypropylene vials for LC-MS/MS measurement.

What kind of equipment did you use?

We used an extraction method in combination with a chromatographic separation process and mass-selective detection. Specifically, the PFAS analysis was carried out by injecting 5 µL of the prepared solutions into a Shimadzu LCMS-8050 system with the parameters detailed in Table 2 and Table 3. The analytes were chromatographically separated using a Restek Raptor C18 column. To separate PFAS which could potentially leach out from the instrument upstream of the injector, we used a Restek Delay column installed between the mixer and autoinjector.

Question: What were your results?

The calibration solutions were analyzed at five injections for each concentration. Both at high-range and low-range concentration solutions, the regression coefficient (R2) was above 0.99 for most analytes. However, the standard deviation between the five measurements at low-range concentrations was for some analytes – especially higher PFAS – somewhat higher.

All the samples were measured five times, and linearity was determined from five measurements for each analyte. Recovery of the analyte was calculated using the calibration curves from previous experiments. Even after extraction the regression coefficient was above 0.99 for the majority of analytes, except for higher PFAS. It is evident that PFAS with 10 carbon-atom chains have lower linearity, which is decreasing with the length of the carbon chain. The average recovery between all the analytes is 88.8 %, which is within the required criteria (80–120 % of the true value) set by the EU Reference Laboratory for Halogenated POPs. [6]

Conclusion

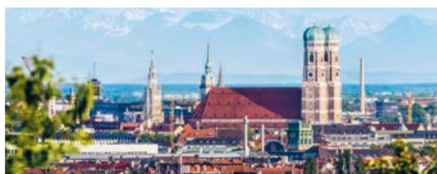
Accurately recording contamination

Despite their many benefits, PFAS currently pose a risk worth measuring.

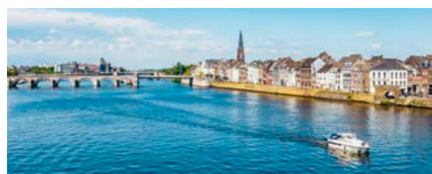
The researchers in Graz succeeded in developing a method to specifically identify and precisely quantify PFAS in food packaging. They also concluded that the Shimadzu LCMS-8050 reliably measures PFAS concentration ranges up to 0.01 pg/µL and that its combination of high sensitivity with outstanding speed parameters makes it well-suited for high-throughput multi-component analysis. This means that the LCMS-8050 can be beneficially used as an integral part of a simple and efficient method for the monitoring and quantification of PFAS in paper-based food-packaging materials.

Note

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



analytica
Munich, Germany
June 21–24, 2022



IMSC
Maastricht, The Netherlands
Aug. 27–Sept. 02, 2022

TIAFT
Versailles, France
Sept. 05–08, 2022

RAFA
Prague, Czech Republic
Sept. 06–09, 2022

ISC
Budapest, Hungary
Sept. 18–22, 2022

EPRW
Bologna, Italy
Sept. 19–23, 2022

secrets of science magazine Customer Magazine of Shimadzu Europa GmbH, Duisburg

Publisher Shimadzu Europa GmbH
Albert-Hahn-Str. 6–10
D-47269 Duisburg
Tel.: +49(0)203 7687-0
shimadzu@shimadzu.eu
www.shimadzu.eu

Editorial Team Uta Steeger, Sonja Wischnewsky, Andrea Wagner-Neumann, Christopher Brünn

Design Bartenbach AG
An der Fahrt 8
D-55124 Mainz

Circulation German: 4.450
English: 4.430

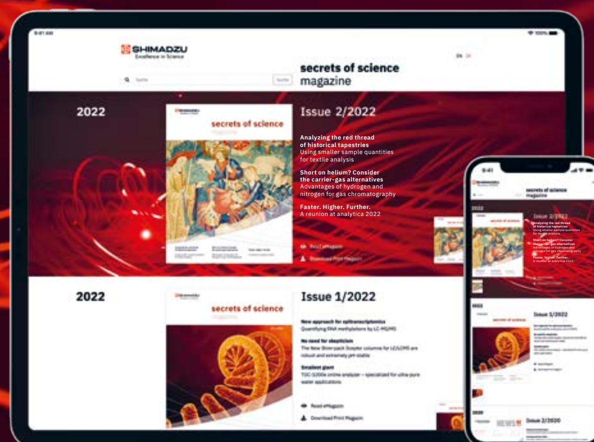
©Copyright Shimadzu Europa GmbH, Duisburg, June 2022. Reprint, also in extracts, only with the permission of the editors.

@ShimadzuEurope

Even more insight

Dive deeper into the topics covered in “Secrets of Science” magazine! The digital version offers even more information, including additional chromatograms, tables and content details.
Go for it: and get to the bottom of these secrets of science.

Digital Version
shimadzu-webapp.eu



Printed on 100% recycled paper