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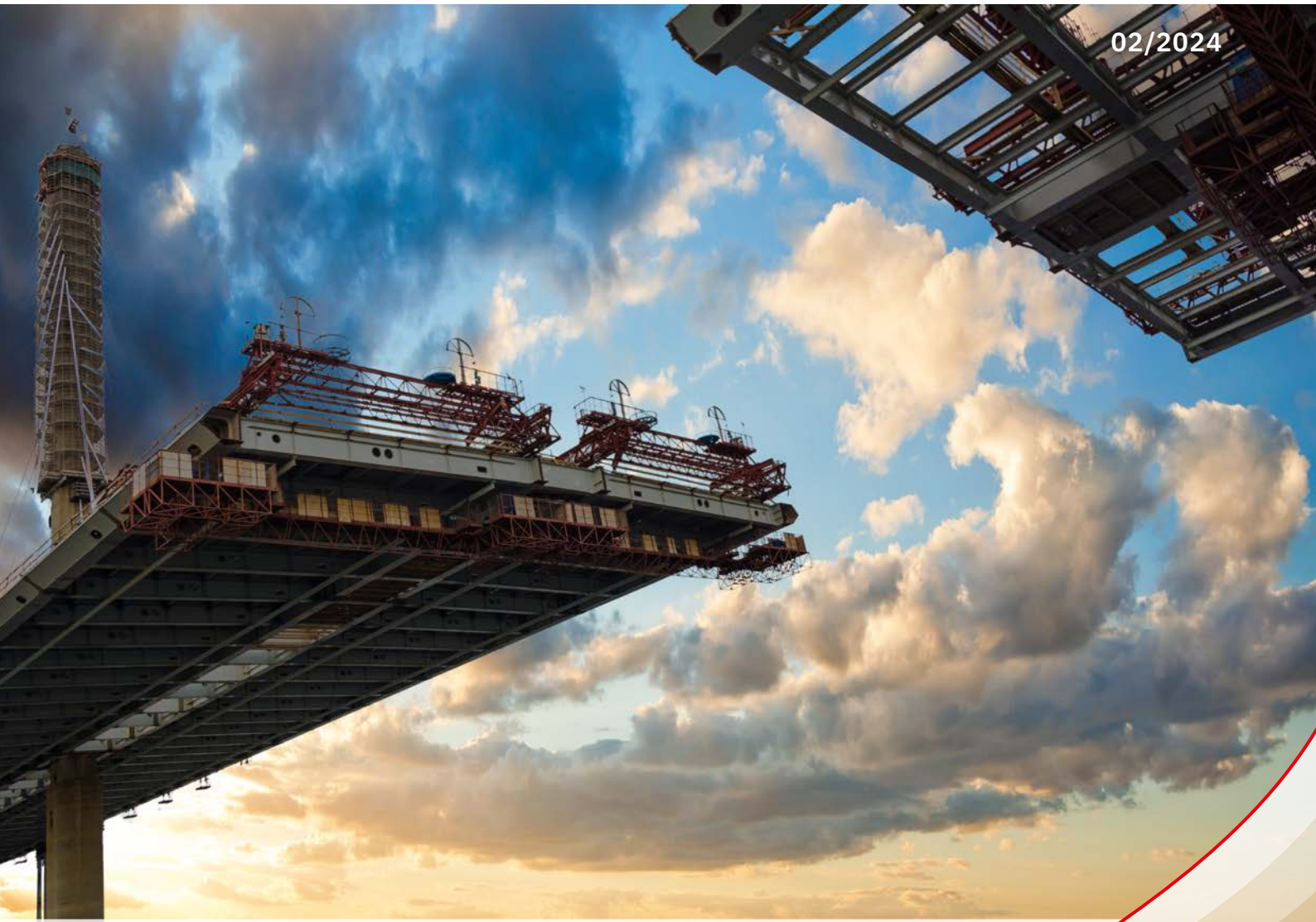

02/2024

How to create a nervous system for infrastructural buildings
Design and testing of distributed optical sensors and other diagnostic solutions

Accelerating towards the future of drug discovery
Using AI-led design and automated synthesis to speed up discovery of potential new leads

Simultaneous analysis of pesticides in water using AI peak integration
Peakintelligence™ for GCMS

How to create a nervous system for infrastructural buildings
Design and testing of distributed optical sensors and other diagnostic solutions **Page 22**

The five categories in the Secrets of Science

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

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

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

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Simultaneous analysis of pesticides in water using AI peak integration

Peakintelligence™ for GCMS

04

Accelerating towards the future of drug discovery

Using AI-led design and automated synthesis to speed up discovery of potential new leads

12

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Assessment criteria for plastics and other organic materials in contact with drinking water (KTW-BWGL) as a pioneer of the EU Drinking Water Directive

May wine – done right!

Quantitative HPLC analysis of coumarin in alcoholic woodruff extracts

Harnessing the power of light

The mighty role of tiny optics in the discovery of our world

The complete solution for analyzing cannabis

From sample preparation to the analytical result

Urban mining – sustainable and safe

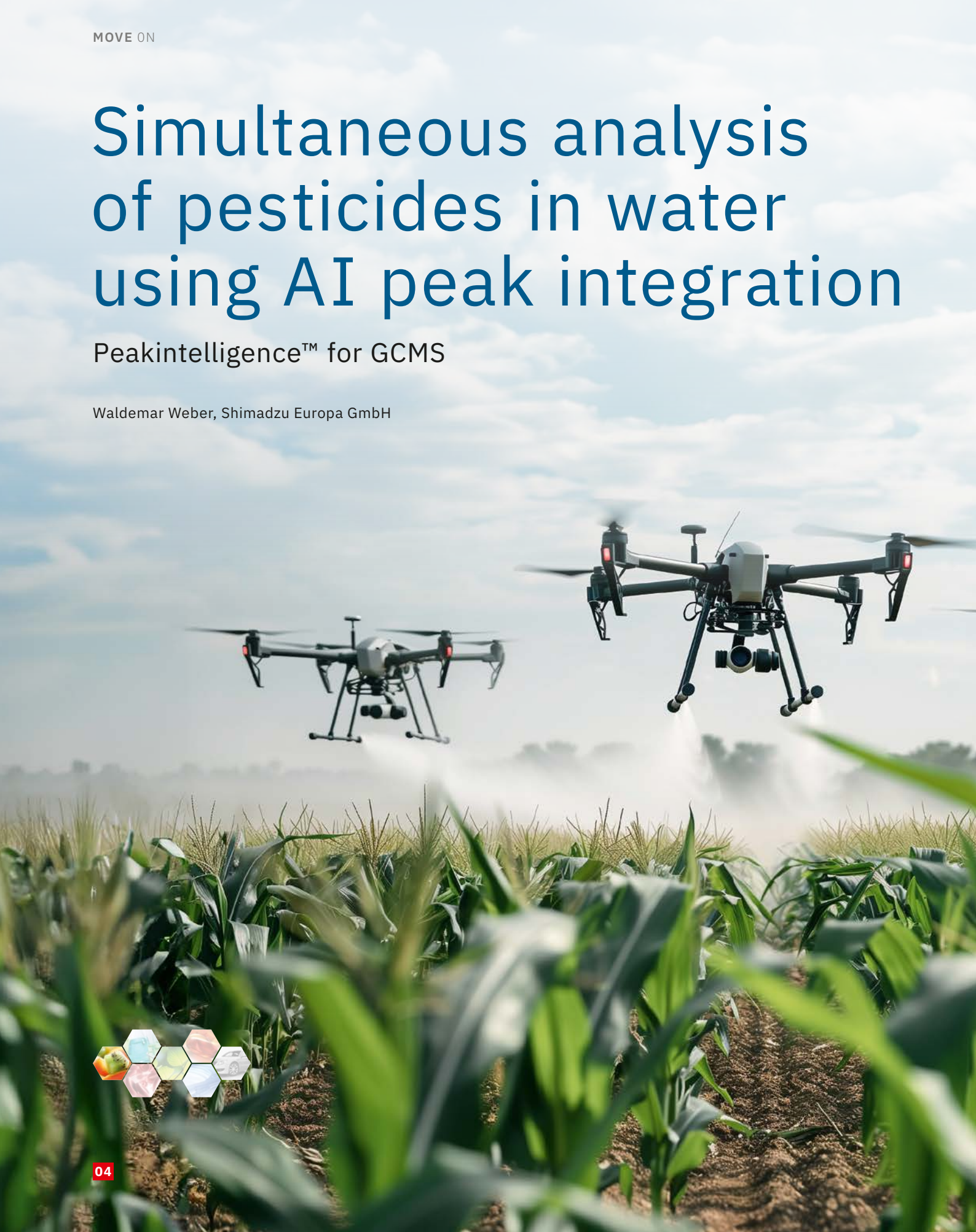
Germany's new Substitute Building Materials Ordinance is forcing laboratories to rethink

Events

Simultaneous analysis of pesticides in water using AI peak integration

Peakintelligence™ for GCMS

Waldemar Weber, Shimadzu Europa GmbH



The global problem of water pollution is jeopardizing the collective health. It occurs when harmful substances like chemicals or microorganisms contaminate oceans, lakes, streams, rivers or any other body of water, degrade water quality and render it toxic for humans, animals or the environment. In order to measure the multitude of pesticides and toxins in water, the multi-component simultaneous analysis of GC-MS is used. Among the tasks for chromatographers, peak integration is the one that has some difficulty to fit in.

Most of the public methods or official analytical documents rarely define a strict rule for peak integration. Therefore, peak integration may be a process that has a risk for missing sufficient analytical objectivity. Peakintelligence™ for GCMS is an option software for LabSolutions Insight™ GCMS, which is trying to resemble the way of peak integration by professionals. This is totally independent of person, because there are no parameters for peak integration.

Protecting human health as a number one priority

The regulation of chemical substances in environmental water and drinking water is of global importance in order to protect human health and the health of animals and plants. A particular focus lies on agricultural chemicals or agrochemicals, referring to biocides (pesticides including insecticides, herbicides, fungicides and nematicides) and synthetic fertilizers. These agrochemicals are widely used worldwide to control weeds and insect pests and have the risk of eluting into the soil and water resources. GC-MS, thanks to its superior performance in multi-component simultaneous analysis, is utilized to measure the wide array of pesticides in water. Its new, higher efficiency adds to its performance and takes the shortage of operators and reduction of training costs into account making it the new state-of-the-art commodity in the modern business environment. The GCMS-QP2050 represents a new generation of GCMS with a completely new ion optical system and boasts high sensitivity, quantitative analysis performance and durability. High productivity and reliability support the work of operators. Below, an example of an analysis of agricultural chemicals in water utilizing the GCMS-QP2050 entry model is described, which offers excellent cost performance and AI peak integration. →

Samples and analysis conditions

Mixed standard solutions with concentrations of 0.003, 0.005, 0.01, 0.025, 0.05, 0.1 and 0.5 mg/L were prepared by diluting standard agricultural chemical samples containing 140 types of agricultural chemicals in water. The internal standard samples used at this time were anthracene-d10, 9-bromoanthracene and chrysene-d12. Repeated verification of analytical accuracy was carried out at a concentration of 0.005 mg/L. A GCMS-QP2050 entry model was used with an AOC-30i as autoinjector (Figure 1). Table 1 shows the conditions used in this analysis.

Figure 1: Appearance of instruments (GCMS-QP2050, AOC-30i/20s U)

	System
GCMS model	GCMS-QP2050 entry model
Autosampler	AOC-30i
Column	SH-I-5Sil MS (30 m x 0.25 mm ID x 0.25 µm); P/N 221-75940-30
Insert	Topaz Liner Splitless Single Taper; P/N 227-35008-01
	GC conditions
Injection mode	Splitless
Injection volume	2 µL
Carrier gas	He
Carrier gas control	Constant velosity (44.5 cm/s)
Column temperature	80 °C (2 min) → 20 °C/min → 180 °C → 5 °C/min → 300 °C (3 min)
	MS conditions
Ion source temp.	230 °C
Interface temp.	250 °C
Data acquisition mode	SIM

Table 1: Analysis conditions

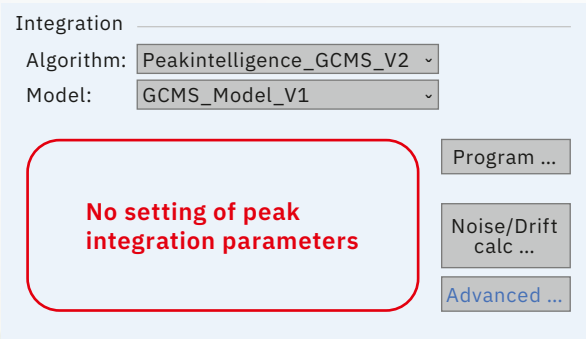


Figure 2: Labsolutions Insight software integration with Peakintelligence for GCMS

Peakintelligence for GCMS

The multi-analyte quantitation software LabSolutions Insight was used in the data analysis, and Peakintelligence for GCMS was used as the algorithm for peak waveform processing. Peakintelligence is a new AI peak integration algorithm that uses machine learning to simulate the peak integration of experience operators. Peak integration using Peakintelligence does not require parameter setting by the operator and can achieve peak integration results equal to that of experienced operators (Figure 2).

Results of quantitative analysis

Figure 3 shows the SIM chromatograms at 0.005 mg/L and calibration curves of representative pesticides. Even at this low concentration, sensitivity with a sufficient margin of performance and satisfactory linearity of the calibration curves were achieved by using the GCMS-QP2050. ⟶

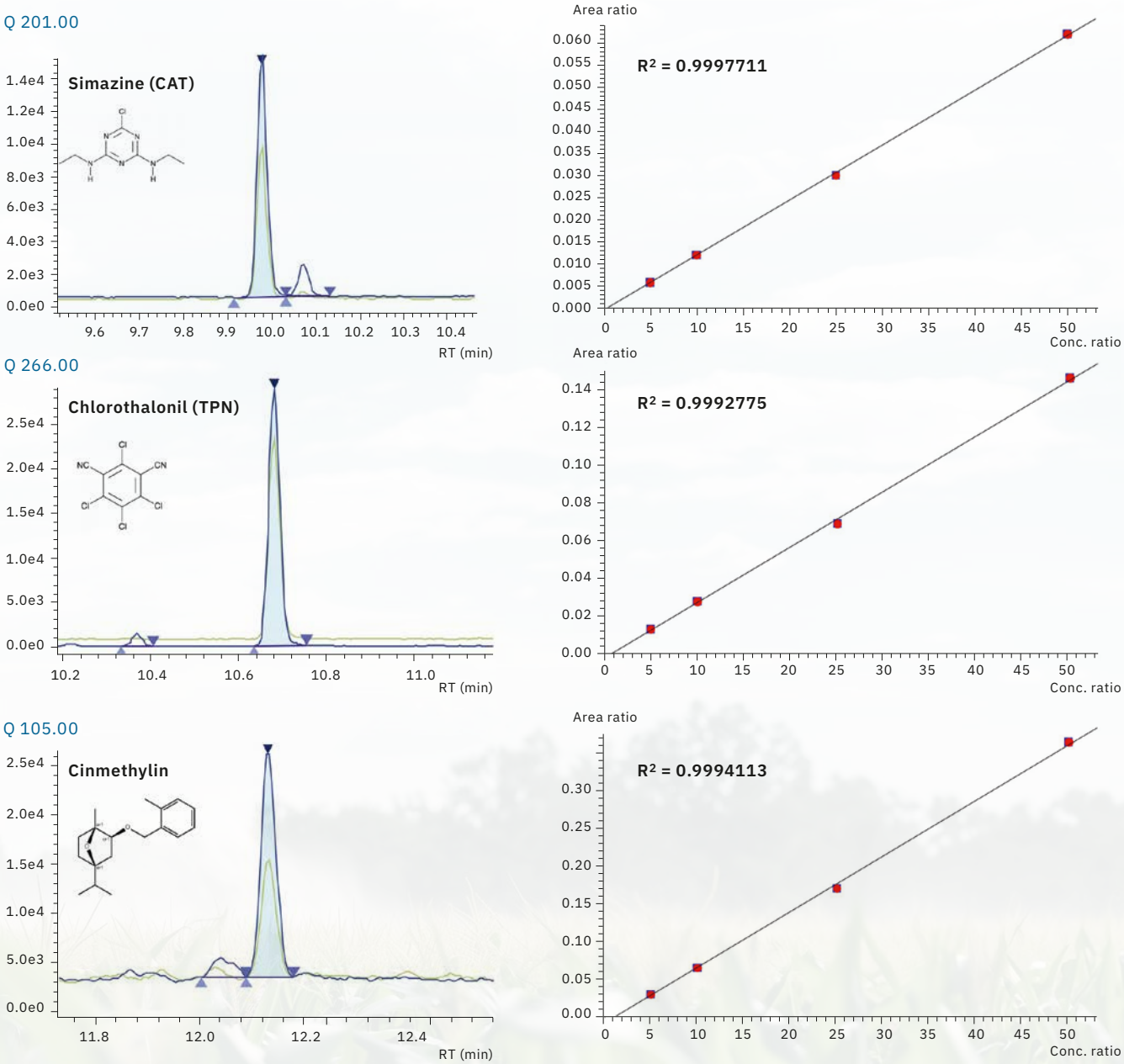


Figure 3: SIM chromatograms (0.005 mg/L) and calibration curves of agricultural chemicals in water

Peak integration by Peakintelligence for GCMS

The results of peak integration by the AI-based software Peakintelligence for GCMS and conventional peak integration by Shimadzu Chromatopac were compared (Figure 4). In conventional peak integration, there were cases in which integration was incorrect, for example, in the low concentration region and when small neighboring peaks exist. In contrast, proper peak integration was possible with Peakintelligence even with these chromatograms. Thus, peak integration by Peakintelligence not only reduces the time required for correction of peak integration but also makes it possible to obtain highly reliable quantitative analysis results by eliminating individual differences between operators.

Immaculate results thanks to excellence sensitivity and Peakintelligence

The GCMS-QP2050 entry model achieved excellent sensitivity and quantitative analysis accuracy in a multi-component simultaneous analysis of pesticides in water. In peak integration, highly accurate results were obtained, and processing time was substantially reduced by using the AI-based software Peakintelligence for GCMS. It may also be noted that the GCMS-QP2050 demonstrates high performance when hydrogen is used as the carrier. In that case, selection of a turbomolecular pump (TMP), which enables a higher evacuation rate, is recommended. The GCMS-QP2050 with its superior performance in multi-component simultaneous analysis and higher efficiency hereby contributes to identifying pesticides in water and creates the basis for purification and a cleaner and safer environment.

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

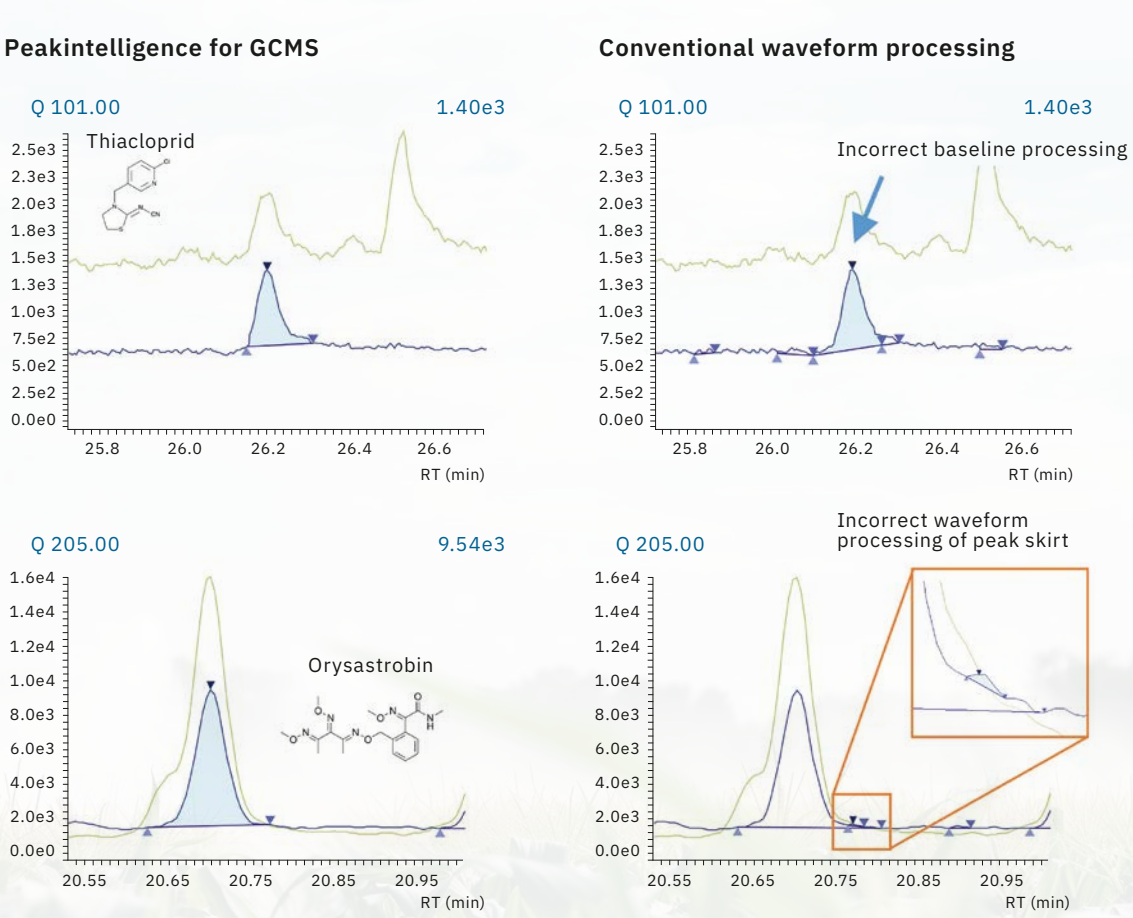


Figure 4: Comparison of waveform processing results by Peakintelligence for GCMS and conventional waveform processing

Safe plastics for hygienic drinking water

Assessment criteria for plastics and other organic materials in contact with drinking water (KTW-BWGL) as a pioneer of the EU Drinking Water Directive

Sascha Hupach, Shimadzu Deutschland GmbH

Markus Janssen, Shimadzu Europa GmbH

Clean drinking water is an important commodity that thankfully we get reliably here in Europe. But the quality is of crucial importance. Manufacturers who make products such as pipes, hoses and other plastic parts that come into contact with drinking water face the challenge of meeting the stringent requirements of the EU Drinking Water Directive. To ensure that these products do not pose a risk to human health, they undergo extensive laboratory testing, including TOC analysis to detect unwanted organic components. →

The public is becoming increasingly aware of harmful substances that can migrate from plastics into water, such as plasticizers. Manufacturers are therefore obliged to ensure that products that come into contact with drinking water are technically and hygienically suitable in order to guarantee consumer safety. Article 11 of the EU Drinking Water Directive [1] was consequently introduced for Europe and must be implemented in all member states as of this year.

Fortunately, testing the hygienic suitability of plastic materials and products is nothing new in Europe. Even before the EU Drinking Water Directive came into force, four EU member states, including Germany, had joined forces in what's known as the "4 Member States (4MS) Initiative" and assumed a pioneering role.[2] Until 2021, Germany adhered to the KTW (plastics/drinking water) Guideline. To implement the EU directives, this was then replaced by the KTW-BWGL (assessment criteria for plastics and other organic materials in contact with drinking water).[3] This guideline helps to assess the hygienic suitability of organic materials for contact with drinking water and outlines the requirements that plastics must meet. The quality of the drinking water must not be impaired through contact with the materials from the systems or through contamination with extractable substances.

The requirements apply not only to plastics but also to organic coatings and lubricants. Aside from pipes or hoses, this also affects many different products or components, such as seals, taps, meters, supply lines, membranes for expansion vessels and much more.

These tests are designed to achieve a "confirmation of conformity of the drinking water hygiene suitability of products", which are carried out by accredited institutions.

Testing plastics with migration waters

The suitability of plastics for use in drinking water is tested using what's known as migration waters, which are produced in the laboratory. In accordance with the applicable specifications, eluates are prepared in a certain ratio of water to the surface of the product. These are then analyzed for odor, turbidity, coloration, foaming and TOC.

To produce the migration waters, the laboratory pretreats each specimen. This includes a rinsing phase and a stagnation phase. Drinking water is then added to the specimen being tested for at least three migration phases to generate the migration water. These migration periods can be carried out with cold water (72 hours at 23 °C) or hot water (24 hours at 60–85 °C), depending on the intended use of the component or material.

The procedure for a hose used in cold water applications is as follows:

For pretreatment, the specimen being tested (a hose section) is rinsed with cold water for 1 hour. The hose, filled with water, is then shut off and the water is left to stagnate for 24 hours. The hose is then prewashed again for 1 hour.

Criteria	Organic materials	Metallic materials	Cementitious materials	Enamels and ceramic materials
Organoleptic tests				
Odor and flavor	X		X	
Color and turbidity	X		X	
General hygiene assessments				
Leaching of total organic carbon (TOC)	X		X	
Surface residues (metals)		X		
Migration testing				
DWD-relevant parameters	X	X	X	X
MTC_{tap} of positive list substances	X		X[1]	
Unexpected substances (GCMS)	X		X[1]	
Compliance with composition lists		X		X
Enhancement of microbial growth	X		X[1]	

Table 1: Extract from the EU Drinking Water Directive (EU) 2020/2184, Article 11 – Testing in relation to the type of material

X[1]: Depending on the existence of organic substances in the material

Producing the migration water: After prewashing, the hose is filled with water and sealed. The water is left to stagnate over a migration period (in this case 72 hours at 23 °C). The migration water (analysis sample) is then removed and the tube is refilled – the next migration period begins. It is important here that the migration periods take place one after the other without being interrupted.

Once the migration waters have been produced, the required analyses are promptly carried out in accordance with the relevant standards.

The sum parameter TOC

The total organic carbon (TOC) is an important sum parameter. It indicates the sum of all organic components that have migrated into the water in a concentration value.

The laboratory uses the established method of determining the TOC using what's called the direct or NPOC method. The water sample is mixed with a mineral acid for sample preparation, and any carbonates or bicarbonates present are converted to carbon dioxide. The CO_2 is then removed from the sample using a purge gas. An aliquot of the prepared sample is then injected onto a hot platinum catalyst in an oxygen-containing atmosphere. The organic compounds are oxidized to carbon dioxide and transported through a carrier gas to a CO_2-selective detector (NDIR). The area of the resulting peak is the equivalent of the TOC concentration.

Modern TOC analyzers, such as the TOC-L series from Shimadzu, handle the entire sample preparation (acidification and degassing) fully automatically – either in the autosampler or in the analyzer itself. They oxidize the organic compounds at a temperature of 680 °C on a highly effective platinum catalyst. The TOC-L systems have an automatic dilution function for samples as well as for standard solutions to create multi-point calibrations, even at equidistant concentration intervals. This allows 10-point calibrations to be created automatically from a stock solution, for example, saving the user a lot of time. The system can also extend the measurement range by automatically diluting the sample.

Guaranteeing the safety of our drinking water

Laboratories that perform product tests are a crucial part of guaranteeing the safety of our drinking water. They ensure that only materials and products that have passed stringent tests come into contact with our drinking water. This prevents substances from these materials leaching into our drinking water and contaminating it or making it undrinkable. For this purpose, migration waters are produced and analyzed. In addition to the sum parameter TOC, odor/taste, turbidity and coloration are also determined to ensure that the quality of our drinking water is not impaired. The EU Drinking Water Directive along with the KTW-BWGL provide the necessary guidelines that laboratories then put into practice to protect our water quality.

Note

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

Accelerating towards the future of drug discovery

Using AI-led design and automated synthesis to speed up discovery of potential new leads

Craig White, Paul Cox and Andrew Ledgard, Exscientia

How can you accelerate the development of therapeutic drug candidates while also reducing their cost? The global AI-driven precision medicine company Exscientia in Oxford, UK, might have the answer. By integrating AI and machine-learning in its discovery process, Exscientia has been able to generate new molecules far faster than with conventional methods and has already delivered compounds into clinical development. Exscientia's scientists explain this new approach and how their recent collaboration with Shimadzu helped further automate the discovery process to synthesize and purify new drug candidates in superfast time.

Changing the status quo in drug discovery

Conventional drug development is a slow, high-risk process, resulting in few new drugs reaching the market at an expensive cost. For example, traditional drug discovery commonly requires between 2,500–5,000 molecules to be synthesized in order to generate just a single compound suitable for clinical development, in a process that often takes five years or more.

However, Exscientia is on a mission to transform how the biopharma industry designs and develops medical therapies by using an AI-led and increasingly automated approach to make drug discovery much more efficient and productive. The company wants to "turn drugs from a sparse resource into an abundant resource" and so ultimately help patients live better and healthier lives.

Founded in 2012, Exscientia has already made waves with its transformative approach, with the first AI-designed drug candidate to enter clinical trials announced in early 2020, developed for Sumitomo Pharma. Following this initial groundbreaking success and five other compounds that have since entered into clinical development, the company is typically able to deliver development candidates in around 12–15 months, with 150–250 molecules being synthesized in the process – a marked contrast with traditional industry averages.

As well as revolutionizing the status quo in drug discovery, the company itself has also undergone a dramatic transformation, employing a substantial number of tech specialists who work hand in hand with savvy "drug hunters" to integrate the best science with the most advanced technologies. Some of those staff members are based at Milton Park in the south of Oxford, UK, where the company has recently completed a state-of-the-art automation facility (about which more later).

Figure 1: Part of Exscientia's new building at Milton Park, south of Oxford, showing its state-of-the-art facility for robot-enabled synthesis and testing of pharmaceutical drug leads

Encoding information and the role of AI

But first, let's examine why Exscientia's ideas have become such a compelling proposition. To get an insight, we first talked to **Dr. Paul Cox**, Director of Chemistry Automation at Exscientia, who knows first-hand the pitfalls of conventional drug discovery. He explains what makes Exscientia different: *"It all started with our founders' deep frustration with how long it takes to discover new medicines and the cost of getting them to patients. Of course, they understood very well that many processes in the drug discovery pipeline center on handling complex sets of information – for example, about the binding affinities of molecular structures or the yields of synthetic processes."* But one of the difficulties, Dr. Cox continues, has always been deciding what data needs to be looked at. *"The sheer quantity and variety of data involved means that scientists spend a vast amount of time sifting through it to home in on the insights that may lead to new medicines."*

And even if you're not concerned about timescales, he says, there is another problem.

"Conventional drug discovery processes may exhibit human bias. Consider the role of molecular structure where medicinal chemists, drawing from their expertise, may lean towards a specific type of molecule for targeting a particular receptor. In their pursuit, they may synthesize many variants in search of the optimal candidate. However, what if this approach merely leads to a 'local minimum', overlooking a potentially superior alternative structure? Striking the right balance between exploring diverse options and leveraging existing data becomes crucial in this context."

The big insight at Exscientia, Dr. Cox explains was that if you could *encode* all available information on biological targets and molecular structures, the analytical power of AI (or, strictly speaking, machine learning) could be used to process it and come up with a small set of diverse molecules that most effectively test the widest range of hypotheses. As a result, to get one compound into clinical trials, you would only need to test a few hundred rather than thousands or tens of thousands. And, importantly, because computer processing would be taking care of the time-consuming work, you would also dispense with much of the tedious trial-and-error aspect of drug discovery. But the team at Exscientia is looking at every aspect of the drug discovery and early development processes and how they could be improved, says Dr. Cox.

→

In many tasks they apply AI to, Exscientia staff use a paradigm they call *"model-driven adaptive design"*, involving repeated rounds of feedback to refine the models. Dr. Cox says: *"Of course, the AI models need ongoing improvement, and by feeding back all the test data and every single human decision, we're constantly making them better. So, for compound discovery, we'll typically go through about 10–15 'design cycles', each involving perhaps 25 compounds to evolve the compound set and answer a project's hypothesis."*

Streamlining synthesis with robotics

Although Exscientia has attracted most attention for its use of AI, the company is also pushing forward on another labour- and time-saving innovation: laboratory automation. Dr. Cox explains the thinking behind this: *"Traditional drug discovery is not just held back by the speed of information processing, hypothesis testing and decision-making but also by the practical aspects of making, purifying and testing drug candidates. So, we want to address that, too."*

So once again, conventional thinking needs to be reassessed. He explains that automation in chemistry has typically focused on "parallel synthesis" – basically, making lots of rather similar molecules. However, this often isn't the most effective way of testing hypotheses. That's why Exscientia is increasingly looking at more bespoke workflows that enable a wider range of molecules to be synthesized. To move towards this goal, the team designed and commissioned a series of automated platforms geared towards enabling the synthesis of a more diverse range of chemical structures through a wider array of reaction classes and workflows.

As Dr. Cox outlines, true automation of these syntheses will require more than just hardware – reaction protocols, whether generated in-house or taken from the literature, must also be encoded to turn them into automation-friendly procedures that can be reliably executed. However, experimental descriptions can vary substantially between chemists. Therefore, a big challenge is standardizing this information to make it machine-interpretable. *"That's why the company is developing its own reaction datasets in-house – we absolutely have to ensure that the data we feed our AI models is properly structured, unbiased and reliable,"* he adds.

Andrew Ledgard, Senior Automation Chemist at Exscientia, describes the basic workflow: *"Our AI-driven process will come up with a target molecule and a number of synthetic routes to it. It's then our job to select the ones that are most likely to work, set up the dosing sequences ready for automated synthesis, and ultimately ensure that we get a sample of pure product ready for testing."*

Versatile instruments for novel compounds

Ledgard's colleague **Craig White**, who leads Analysis and Purification, describes the four "analytical touchpoints" across the whole process. These involve the application of Shimadzu's analytical and preparative liquid chromatography (LC) and supercritical fluid chromatography (SFC) coupled with mass spectrometry (MS). *"The first stage is reaction monitoring, for which we use an analytical LCMS configured with a flow-through vial. The next stage is solid-phase extraction (SPE), followed by purification using either preparative LC or SFC. And, finally, we need to perform quality control prior to biological screening,"* he explains. →

Figure 2: Some of the Shimadzu equipment used at Exscientia for reaction monitoring: the analytical LCMS (top) and the flow-through vial configuration (bottom), which physically connects to the chemistry platform

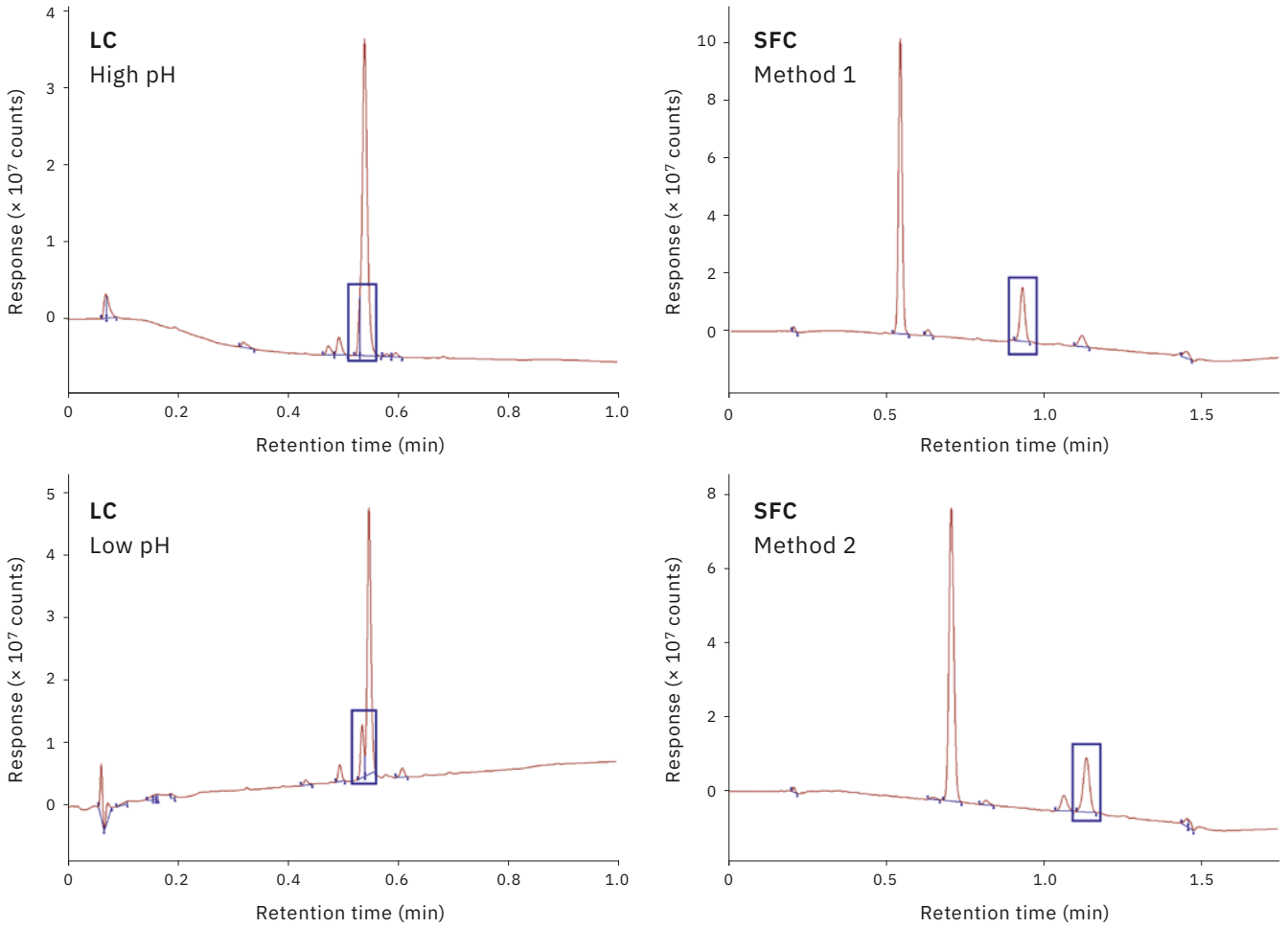


Figure 3: Example of exploratory purification work carried out at Exscientia on an impure sample, showing two LC separations at different pH (left) and two SFC separations using different methods (right). Both SFC methods easily separate a significant impurity that is only partially resolved or completely unresolved using LC.

The entire analytical landscape is completely different from what would be encountered in conventional drug discovery, says White, because of the need to stream intermediates and final compounds with diverse polarity, across different automated platforms. It was therefore important to consider LC and SFC 'side by side' as complementary techniques, to successfully isolate pure material.

"And when we're dealing with multi-sample and multi-gram purifications overnight, there are big implications for solvent handling," he adds. *"Specifically, we needed to design novel solutions for solvent delivery and solvent waste removal – which are all now operational and integrated with the Shimadzu instruments."*

Figure 4: Shimadzu's ultrafast preparative LC system used at Exscientia, showing the complete setup (left) and the fraction-trapping capability (right) that was vital for avoiding lengthy dry-down procedures

Innovation, collaboration, integration

Ledgard and White have been closely involved in system design at Exscientia's automation lab throughout its development, and they're not exaggerating when they say that this is just a small sample of the challenges they have faced.

However, through it all, Shimadzu's help with the analytical setup has been invaluable, says Ledgard: *"We initially contacted Shimadzu in late 2021 because we were interested in the cutting-edge fraction-trapping capability on their ultra-fast preparative LC. This was vital because it enabled us to eliminate the lengthy dry-down that is otherwise unavoidable when isolating a target compound from an aqueous mobile phase."*

But then they realized that other considerations also made Shimadzu a strong partner. For instance, the company offered both the LC and SFC techniques, simplifying the process of creating the integrated platform that Exscientia was looking to design. *"At that time, not many vendors offered that combination of assets,"* White says.

Another factor, as he explains, was reliability. *"Any 24/7 operation will need a robust platform, so the reported reliability of Shimadzu equipment and software was a big draw for us. It's also been great to integrate their LabSolutions Sync software into our setup, as it provides flexibility to orchestrate sample submissions outside the environment of the Shimadzu chromatography data system, using our in-house informatics platform."*

And finally, there's the collaborative mindset, he says. *"Our project here in Oxfordshire has never been about simply optimizing individual instruments. We've been on a mission to put together a completely novel, integrated setup, so we needed bespoke configurations and bespoke software. Therefore, it was really important that we had an instrument vendor who really understood the need for collaboration and was prepared to innovate quickly to provide solutions."*

A new vision for drug discovery

Although the new facility will need time to build up to peak performance, a lot of progress has been achieved in a short period. *"From the analytical and purification angle, we still have a lot to do,"* says White, *"especially in automating the movement of physical samples and the flow of information. However, we installed the complete lab infrastructure in just two years, and as part of that timeframe, it took only six months to get our analytical and purification platforms operational. We also have an internal team of engineers and software developers working on automating the input/output of purification tools to further streamline the entire workflow."*

The joined-up approach to compound preparation, says Ledgard, is becoming more important, because vendors are beginning to realize that their customers don't just want standalone instruments. *"They want to be able to connect compound workup to purification, and purification to evaporation. Developing solutions for connectivity is mostly our responsibility currently, but through collaboration with vendors, I think we'll see that become more mainstream in the near future."*

Figure 5: Craig White, Paul Cox and Andrew Ledgard (left to right) in the lab at Exscientia

That sentiment – automating and linking up the process so it runs seamlessly from start to finish – fits neatly into the wider picture of drug discovery at Exscientia, concludes Paul Cox. *"Our ultimate aim is to create a drug discovery pipeline that's human-led in terms of overall strategy, target setting and other key decision-making, yet could operate more or less without human interference from concept to reaction execution to delivery of the product for testing – and in a matter of weeks rather than years. It kind of blows my mind when I think that this might be possible, let alone that Exscientia seems well on track to achieving it!"* he states.

Note

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

May wine – done right!

Quantitative HPLC analysis of coumarin in alcoholic woodruff extracts

Dr. Brigitte Bollig,
Shimadzu Europa GmbH

When scouring the internet for recipes for May wine, you're sure to come across very different, sometimes contradictory information on how long you should leave the woodruff to infuse in the wine. What you don't want is to extract too much of the potentially toxic coumarin contained in the plant into the wine. But how long should you perform an extraction of woodruff leaves so that you don't end up with a harmful amount of coumarin but still get the characteristic flavor of the finished May wine? This question was investigated using HPLC analysis and photodiode array detection.

Shimadzu LC Product Manager, Dr. Gesa Schad, recently recalled how the use of woodruff in May wine is a popular spring tradition in Germany. When she first started looking for May wine recipes, she was aware that woodruff contains the natural flavoring coumarin, which can be harmful to health in high doses. But when she was researching recipes, she realized: There are huge differences in the recommended "extraction time", meaning how long the dried woodruff can be left in the wine without running the risk of reaching the permitted limits for coumarin. On one site, the maximum permissible "infusion time" was 45 minutes, while another stated that the plant should be left to infuse in the wine "for 1–3 hours, depending on the desired flavor intensity".[1–4] So what should you do if you want to make a wine that has a nice woodruff flavor but without a dangerous coumarin content? Dr. Schad's curiosity as a researcher was sparked! In order to be able to make a scientifically sound recommendation, it was first necessary to analyze the coumarin content in woodruff extracts with various extraction times. Her colleague at the time, Robert Ludwig, carried out the test series. The results of his quantitative determination are analyzed in more detail in this article in light of the established limits for coumarin in foodstuffs. The results are explained at the end, along with the recommendation on how to safely prepare May wine.

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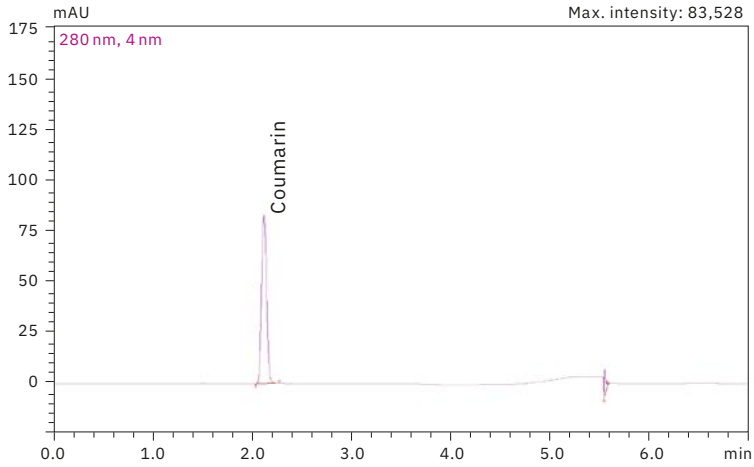


Figure 1: Chromatogram of the 5-mg/L standard

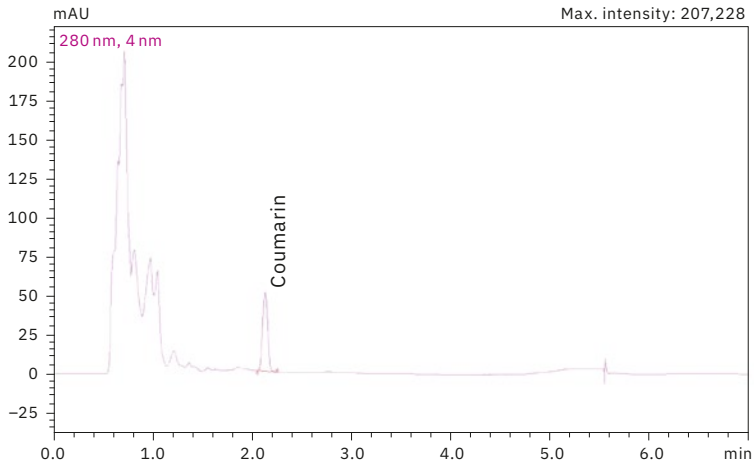


Figure 2: Chromatogram of the woodruff extract after 60 minutes

Extraction of coumarin from woodruff leaves

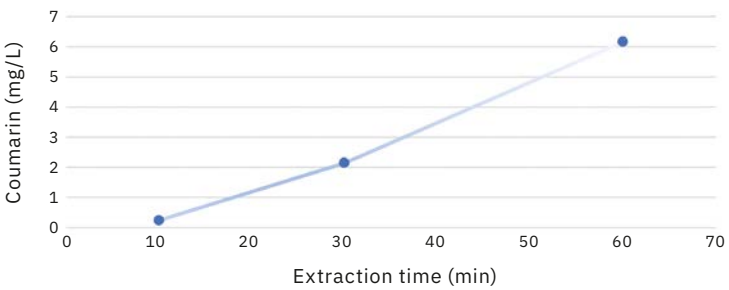


Figure 3: Extraction of coumarin from woodruff leaves in periods of 10 to 60 minutes

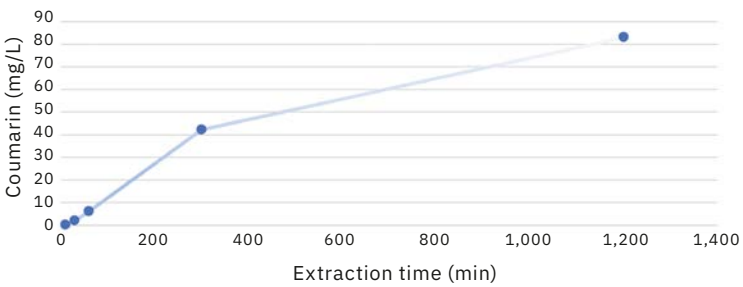


Figure 4: Extraction of coumarin from woodruff leaves in periods of 10 minutes to 20 hours

There is no defined limit value for beverages, however, the tolerable daily intake (TDI) value can be taken into account to assess the results described below [6], where a daily intake of 0.1 mg coumarin per kilogram of body weight is considered harmless.

Gesa Schad harvested woodruff from a commercially available plant and dried the parts of the plant overnight. Once dried, she tied together 3 g of the cut stems with a string and hung them upside down in 90 mL of white wine for extraction (a ratio of woodruff to wine commonly used for extraction in recipes), without immersing the cut ends in the liquid. Samples were taken at different times, which were filtered only through a 0.45-µm filter before injection into the high-performance liquid chromatography (HPLC). Robert Ludwig carried out the HPLC method on a Nexera XS system with binary pumps and a PDA detector.

A chromatogram of the extract after 60 minutes in Figure 2 shows a very good separation of the coumarin from the earlier eluting matrices, which were extracted from the woodruff leaves as well as the coumarin and so cannot be seen in the standard measurements (Figure 1).

The two graphs in Figures 3 and 4 were created to illustrate the coumarin content determined as a result of the different extraction times of 10, 30 and 60 minutes as well as 5 and 20 hours (300 and 1,200 minutes). This clearly shows that the concentration of coumarin in the sample increases continuously over the entire duration of the experiment.

Coumarin | $C_9H_6O_2$

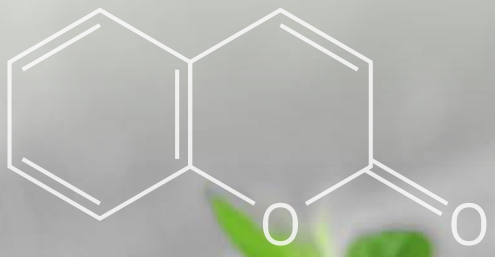


Table 1 shows these results of the determination of the coumarin content of 3 g woodruff in 90 mL white wine as a result of the extraction time (columns 2 and 3). For a whole bottle of wine, i.e. 750 mL, you would use about 25 g of dried woodruff (column 4 shows the coumarin content). This one bottle of white wine is then diluted with a second bottle of white wine and a bottle of sparkling wine to make the finished product (2.25 L). The last column shows the coumarin content in a glass of May wine (250 mL).

Extraction time (min)	Coumarin (mg) in the sample (converted to 1 L)	Coumarin (mg) in the sample (converted to 1 mL)	Coumarin (mg) in 0.75 L white wine (also applies to 2.25 L of finished May wine)	Coumarin (mg) in a 250-mL glass of the finished May wine
10	0.238	0.000238	0.18	0.02
30	2.139	0.002139	1.60	0.18
60	6.163	0.006163	4.62	0.51
300	42.229	0.042229	31.67	3.52
1,200	82.966	0.082966	62.23	6.91

Table 1: Results of the quantitative analysis of coumarin in alcoholic woodruff extract and extrapolation to a typical quantity of finished May wine

This means that thinking a harmful amount of coumarin could be contained in the May wine if the infusion time is too long is quite justified at first glance. However, if you take a closer look at the absolute values of the coumarin content in the finished May wine (column 5), you will notice that a quantity of 6.9 mg was only obtained in a glass of wine after 20 hours of extraction. Based on an average adult with a body weight of 60–80 kg, the limit value of pure coumarin that can still be consumed without any issue is 6–8 mg.

Fans of May wine can breathe a sigh of relief

HPLC was used as a very reliable analytical method to determine the final concentration of coumarin in alcoholic woodruff extracts. The results of the analysis show a significant increase in the coumarin concentration in the white wine used for the extraction over several hours. However, the concentration in a glass of wine only reaches harmful levels for an adult after at least 20 hours. If you keep the extraction time under 5 hours and don't drink the 2 liters of wine alone, no harmful side effects of coumarin are to be expected. That means the taste and not the potentially toxic effect of woodruff should determine the length of exposure time. Please note: If the woodruff hangs in the wine for too long, the end product becomes bitter. Just try it for yourself!

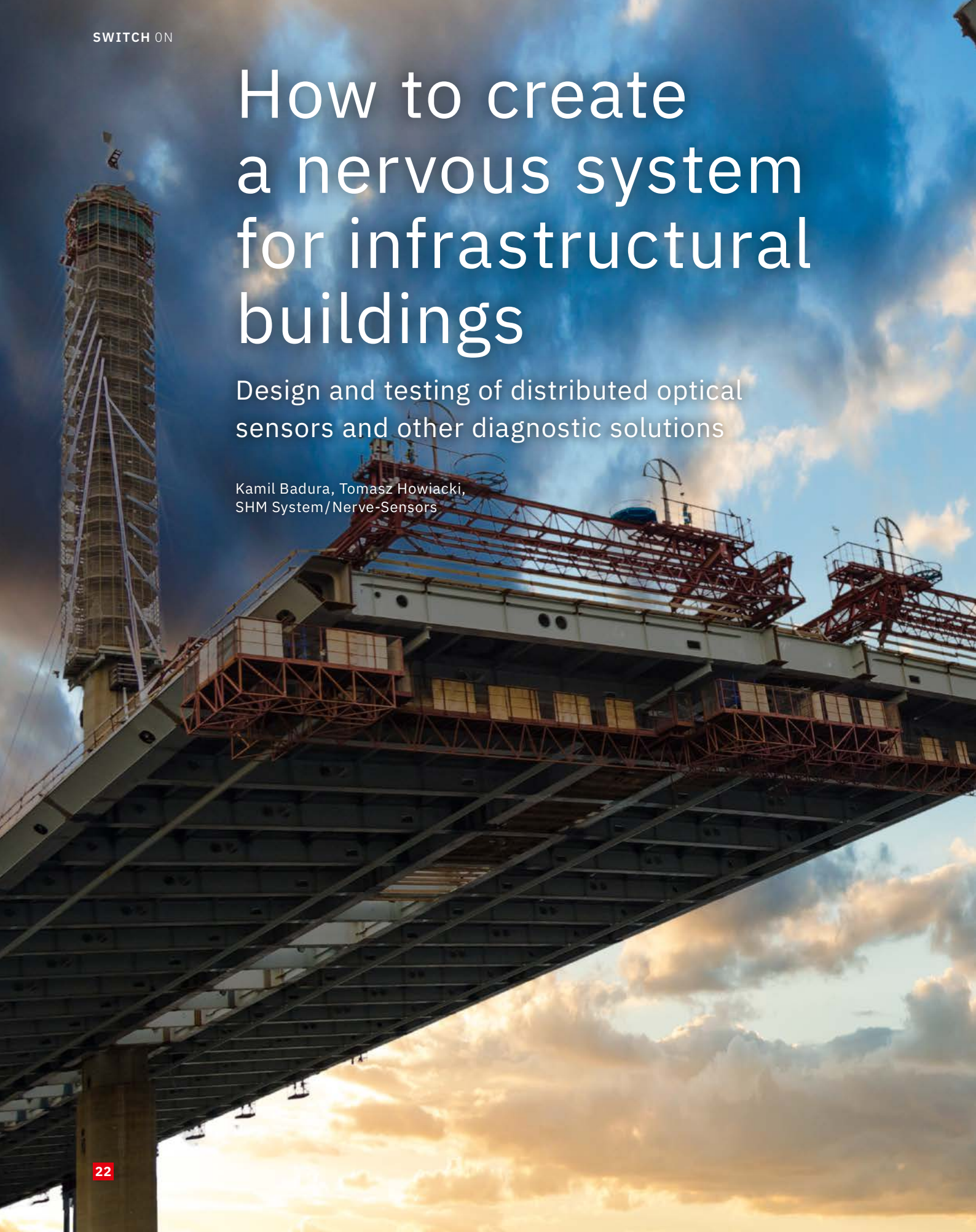
Note

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

How to create a nervous system for infrastructural buildings

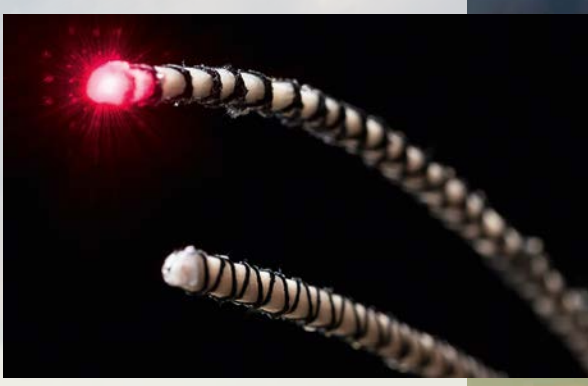
Design and testing of distributed optical sensors and other diagnostic solutions

Kamil Badura, Tomasz Howiacki,
SHM System/Nerve-Sensors



Today, societies require an intelligent infrastructure capable of detecting threats and equipped with early warning systems. Though built to last, collapsing buildings and infrastructure is a common occurrence throughout the world. Diagnosing civil engineering and geotechnical facilities is therefore absolutely mandatory in order to avoid damage and save human lives. One of the most promising approaches is the use of monolithic fiber optic sensors that are fully integrated into a given structure, providing strain, displacement, temperature or vibration monitoring continuously over its entire length – from millimeter to kilometer range!

The collapse of the Ponte Morandi highway bridge in Genoa in 2018 made big news and seemed like a singular event, but the collapse of infrastructural buildings is not that seldom. Many buildings and bridges break down every year, many due to structural failure from ageing infrastructure. And not all buildings are built alike. Modern infrastructure is usually designed with non-standard geometry, using new materials and advanced structural solutions. On the other hand, ageing infrastructure requires appropriate maintenance to ensure an adequate level of safety. Therefore, knowledge of the mechanical and thermal behavior of the structure under normal operating conditions is essential for assessing its technical condition and thus for optimal decision-making.



Responsibility for the surrounding infrastructure leads to the search for effective solutions that can benefit society as a whole. Firstly, proper monitoring can help prevent the failure or catastrophe of safety-critical engineering structures, which directly translates into the protection of human health and life. Secondly, it leads to economic, environmental and social benefits. →

Monolithic fiber optic sensors – a nervous system for structures

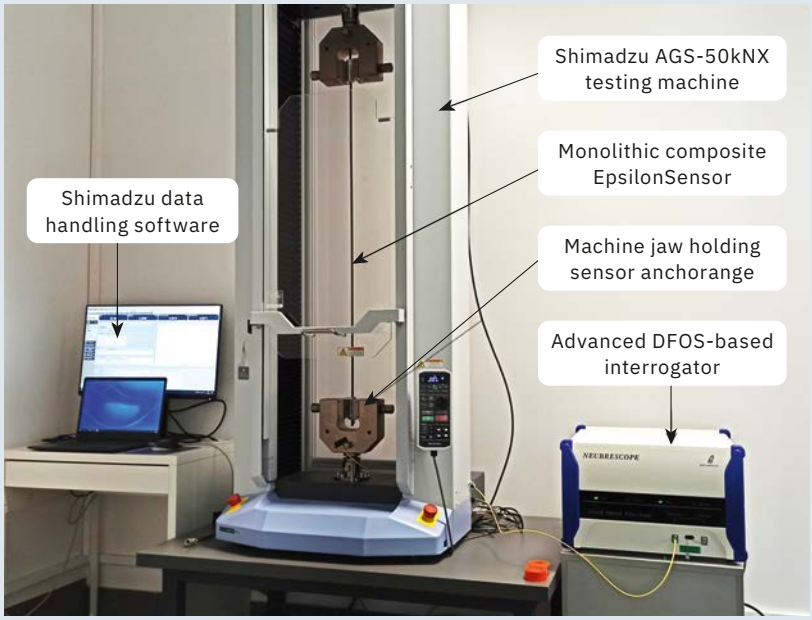
Among the many measurement techniques available on the market, there is a constant search for the optimum solution in terms of diagnosing civil engineering and geotechnical facilities. One of the most promising approaches is the use of monolithic fiber optic sensors that are fully integrated into a given structure, providing strain, displacement [1], temperature or vibration monitoring continuously over its entire length [2] – from millimeter to kilometer range! This means that instead of a single number obtained at the location of the gauge, we get the continuous profile of the measured quantities, allowing us to detect anomalies or local events such as cracks [3], stress concentrations or other damage.

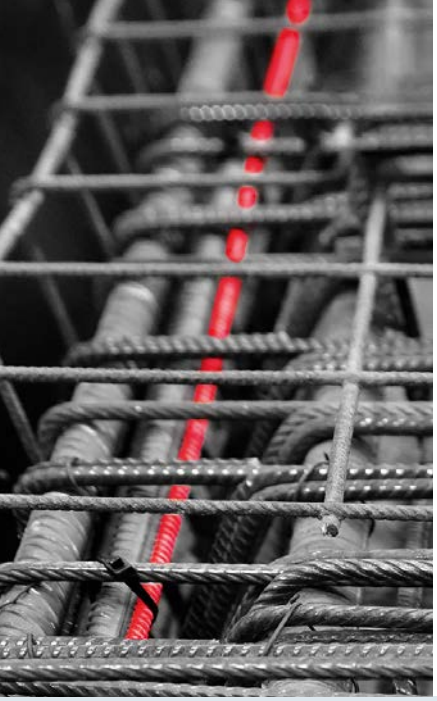
Despite the undeniable benefits of distributed fiber optic sensing (DFOS) technology, it is not a "plug and play" solution. There are several aspects that need to be carefully considered and planned prior to measurement. These include the type of the sensors and their parameters like diameter, elastic modulus, external surface or internal design. Nerve-Sensors as a department within SHM System is engaged in the design, development and research of worldwide unique monolithic sensors for DFOS.

Initial market research in 2015 showed that there were no alternatives for layered sensing cables used in field applications. This solution was well-known from telecommunications, where optical sensing fibers are protected by a set of multiple layers. However, from a measurement point of view, they were limited by several drawbacks. Firstly, the use of plastic and steel components with low elastic range meant that the cables were destroyed very quickly. Secondly, the intermediate layers disturbed the strain transfer mechanism due to the slippage effect. Finally, the smooth outer surface did not provide adequate adhesion to the surrounding material.

A pioneering innovation, inspired by human design

There was no solution on the market designed directly for civil engineering and geotechnical monitoring. To fill this gap, the Nerve-Sensors family has been developed to overcome the existing limitations. The new sensors consist of a monolithic core without layers, which ensures the most accurate strain measurements. Plastic and steel components have been replaced with highly elastic composite materials. External braiding improves the bonding quality between the sensor and the surrounding material, e.g. concrete or soil. Monolithic sensors are designed to be integrated into a structure similar to the human nervous system [4], and this was the main idea behind the proposed name for the family.





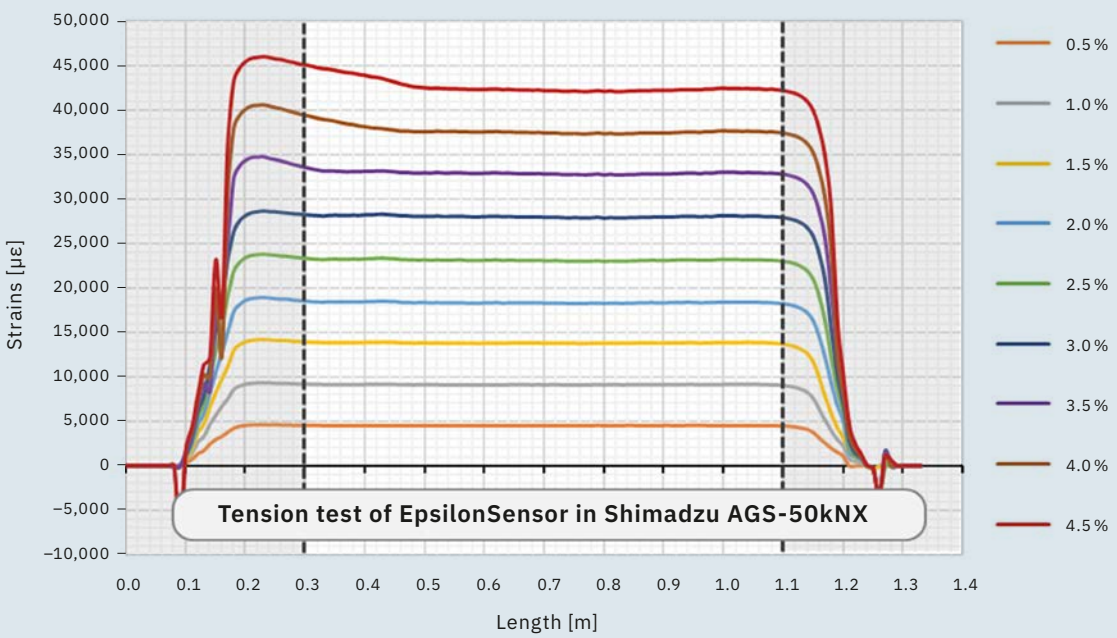
Calibrating for effectiveness and accuracy with Shimadzu testing machines

Monolithic sensors are taking structural health monitoring (SHM) to a new level, but their effectiveness and accuracy had to be verified in precise and rigorous laboratory tests to remove any doubt about their behavior. That is why SHM System's laboratory was equipped with two Shimadzu testing machines: AGS-50kNX and AGX-V-300kN (Figure 1). Both are used for precise mechanical testing of different types of monolithic sensors, for example stiff and robust EpsilonRebar or flexible and versatile EpsilonSensor (Figure 2). The main specifications that need to be analyzed include the strain accuracy and maximum strain range. Thanks to the special composite core, EpsilonSensor has the widest measuring range on the market at up to 4 % (Figure 3), which is as much as four times the typical range of layered cables. This opens up new possibilities for analyzing the condition of structures, including the measurement of local cracks and fractures that cannot be reached by competing solutions. →

◄ ◄ Figure 1: A general view of one of SHM's laboratory rooms with two Shimadzu machines: AGS-50kNX and AGX-V-300kN

◄ Figure 2: The monolithic composite EpsilonSensor during the tension test in Shimadzu's AGS-50kNX

► Figure 3: Example results of strain profiles measured by the EpsilonSensor during tensile testing in Shimadzu's AGS-50kNX



Providing old structures with new sensory intelligence

In practice, the distributed sensing approach is often complemented by spot diagnostic solutions. These are also developed and tested in the SHM System laboratory. Two of these are highlighted hereafter. The first solution is called the plastic deformation sensor (PDS) and is the world's first and only sensor capable of detecting steel yielding without any knowledge of material properties, initial stress-strain state and actual technical condition. It is not only suitable for diagnosing steel structures but also for detecting the near-to-failure state of other materials such as concrete. The sensor can be made using different measurement technologies like optical fibers (Figure 4), foil strain gauges (Figure 5) or vibrating wire sensors (Figure 6). It can therefore be easily integrated into existing monitoring systems. Thanks to the special

► Figure 4: Close-up of the DFOS-based plastic deformation sensor installed on a steel specimen tested in the Shimadzu machine

▼ Figure 5: View of Shimadzu machine testing plastic deformation sensor based on foil strain gauges

calculation algorithm, this is an excellent tool for both new and existing ageing infrastructure with unknown deformation state and lack of documentation.

The second unique solution relates to fiber bragg gratings (FBGs), one of the most commonly used fiber optic spot gauges. Unfortunately, standard FBGs have a limited strain range and are only suitable for measuring uniform materials, usually within their elastic behavior. Large deformations in inhomogeneous materials such as concrete (local effect of aggregate or cracks), composite textiles (disturbance by perpendicular fibers) or simply steel working in a plasticized state cause standard FBGs to split the spectrum, and further measurement is no longer possible. The new FBG sensor developed by the specialists at SHM System overcomes these limitations and allows the measurement range to be significantly extended, thus increasing the range of possible applications.

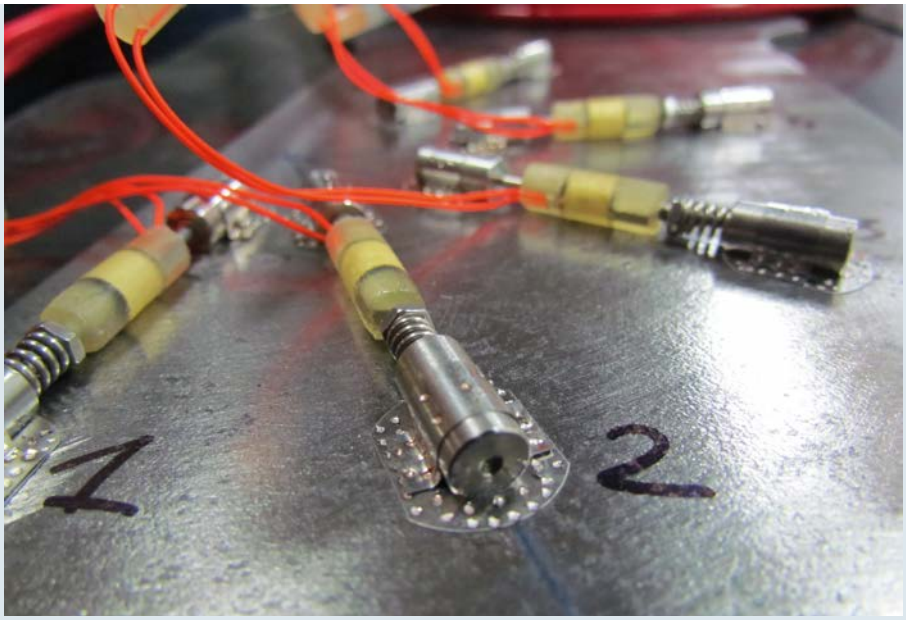
A dream only a few years ago, today a life-saving innovation

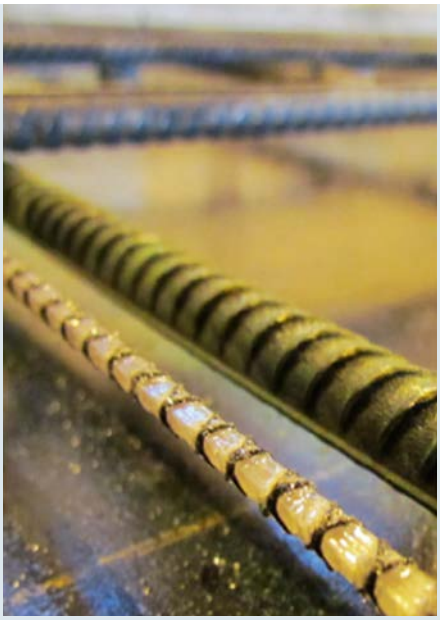
It must be emphasized once again that the failure consequences of civil engineering and geotechnical facilities include not only financial, social and environmental losses but also loss of human health or life. In order to preserve human life, the team from SHM System & Nerve-Sensors went full circle and took inspiration from human life: the human nervous system, perfect in terms of hazard identification and early warning. It served as an unsurpassed model to develop a diagnostic system for engineering and geotechnical structures. The linear composite sensors, fully integrated into the structure, are designed as the nerves responsible for detecting threats, while the data logger is the brain that processes, visualizes and interprets the data. This bold vision was only a dream a few years ago but is now a reality on hundreds of bridges [5] and other engineering facilities around the world saving structures and most importantly – human lives.

Note

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

▼ Figure 6: Close-up of the plastic deformation sensor based on vibrating wire sensors





Harnessing the power of light

The mighty role of tiny optics in the discovery of our world

Tomofumi Seto, Shimadzu Europa GmbH

The universe, a vast expanse of celestial wonder, has captured the imagination of mankind for centuries. But how do we unravel the mysteries hidden within this cosmic fabric? One of the answers is light. By analyzing the light emitted or absorbed by celestial objects, scientists can reveal a great deal about their composition, temperature, motion and even their distant history. Through the spectroscopy, we can explore the universe in ways never imagined before. This article takes a journey into the field of the spectroscopic elements, "diffraction gratings" offered by Shimadzu and their key role in exploring our world.

The origins of spectroscopy

In the 17th century, British physicist Isaac Newton, who made great research achievements in various fields, discovered a secret of light. Using polished transparent devices called prisms, he observed white light split into a band of different colors like a rainbow. His experiments revealed that the white light is composed of different colors, or wavelengths. This means that the sunlight or fluorescent light we usually see includes all wavelengths of light, such as red, green, blue and many more.

A band of different wavelengths discovered by Newton is known as a spectrum. By analyzing the interaction of a material with the spectrum, valuable information about its properties can be obtained. This principle is the basis of modern spectroscopy. →

Figure 1: Prism

Key device for spectroscopy

The first step in performing spectroscopy is to use an optical element to split the light into different wavelengths. There are basically two typical spectroscopic elements, prisms and diffraction gratings (Figure 2).

Prisms work on the principle of refraction – they are typically triangular solids made of quartz or glass that bend the angle of light depending on its wavelength due to the different speed of propagation in different media, this property being called the refractive index.

Diffraction gratings use a different principle – diffraction based on the Huygens' principle, where light is bent around the corners of an obstacle. Therefore, diffraction gratings have countless very small grooves (obstacles) on a substrate surface. Again, the bending angle depends on the wavelength, and this is how different colors can be separated. This effect is what makes the back of a CD or DVD reflect many different colors when viewed from certain angles.

Nowadays, diffraction gratings are the standard for spectroscopic applications because of their optical advantages. The wavelength resolution accuracy is high, and this wavelength accuracy is more stable with temperature changes compared to prisms. Therefore, diffraction gratings are often a more practical spectroscopic choice.

Figure 2: Principles of prism and diffraction grating

History of diffraction grating

Until the middle of the 20th century, a prism was the main element used in spectroscopy. Diffraction gratings also existed but weren't widely used because they are difficult to produce. Their grooves should be precisely and uniformly on the surface, but the fabrication technology was not advanced, and therefore their performance was inferior to that of prisms at that time. Additionally, the production number of gratings was limited, and the availability of gratings was inferior in terms of price and time.

However, the manufacturing technology of diffraction gratings was steadily improving. Eventually, with the establishment of high-performance and large-scale production technology of diffraction gratings, they became increasingly popular in spectroscopic instruments in place of prisms.

Shimadzu's contribution to manufacturing technology of diffraction gratings

There are typically two types of diffraction gratings. One is a ruled grating, where the grooves are physically engraved by machine. The other is a holographic grating, which is produced by a holographic exposure method using the two-flux interference of a laser.

The holographic grating has the advantage of low stray light. Stray light is generally undesired light or wavelengths that are generated unexpectedly. Especially in the field of spectroscopic analysis, stray light has a negative impact on analysis accuracy. The grooves of a holographic grating are formed more precisely than those of ruled grating so that stray light is lower.

On the other hand, holographic gratings have the disadvantage that the intensity of diffracted light is lower. One way to circumvent this is to use "blazed grooves", which are saw-shaped grooves that increase the light intensity of desired wavelengths, but in the past these groove shapes could only be manufactured by the ruled method. At that time, it was considered impossible to produce blaze grooves by using the holographic method.

However, Shimadzu was striving to develop a blazed holographic grating which realizes low stray light and the same level of diffraction light intensity as ruled gratings. After many years of joint research with RIKEN (Institute of Physical and Chemical Research), the development of blazing grooves for holographic gratings succeeded and the world's first manufacturing technology for blazed holographic gratings was established.

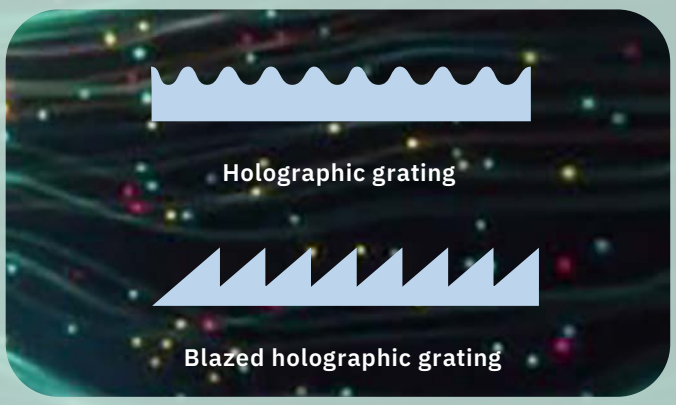


Figure 3: Groove shape of diffraction grating

Current state of Shimadzu's diffraction grating

Nowadays, diffraction gratings have been widely used in several fields with its manufacturing technology and the extensive lineup/custom-made capability according to each application.

In analysis and inspection fields, diffraction gratings contribute to in-house products and external customers as a key device of equipment. Furthermore, the use has been spreading in areas such as food, semiconductor manufacturing and blood testing.

The use of diffraction gratings is not only for analysis purposes but also to support optical communications as a part of wavelength selective switches (WSS). WSS control the wavelength of optical signals to realize efficient transmission of information at optical communications. →

Shimadzu's contribution is not limited to the earth but extends even into space. The spectroscopic satellite "Hisaki" (SPRINT-A) was successfully launched in Japan in 2013. The company's diffraction grating is used as the heart of the satellite. This satellite is the world's first space telescope for remote observation of the planets such as Venus, Mars and Jupiter.
It analyzes the atmosphere of planets by observing extreme ultraviolet light, which cannot be detected on Earth, and contributes to the elucidation of mysteries in space.

Suitable for each customer's needs

Shimadzu's diffraction gratings have been supporting spectroscopic technology in very diverse industrial fields and research institutes and have accumulated technological expertise over many years. Today there are several types of diffraction gratings that are suitable for each customer's needs and applications, with the advantage of low stray light and high diffraction efficiency. The company's relentless challenge to meet the diversifying needs of spectroscopy will continue, just like the discovery of the universe.

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

The complete solution for analyzing cannabis

From sample preparation to the analytical result

Uwe Oppermann, Shimadzu Europa GmbH

Dr. Tanja Butt, Retsch GmbH

Ulf Sengutta, CEM GmbH

The advantages and disadvantages of cannabis use are once again being hotly debated in Germany since home cultivation was legalized in 2024. Medical prescriptions for medicinal cannabis (flowers and extracts) have been permitted for a few years now, as has the sale of foodstuffs made from so-called industrial hemp or foodstuffs containing industrial hemp ingredients: hemp seeds, protein powder, oils, teas, drinks or even CBD chewing gum and gummy bears. Reliably analyzing the hemp plants and/or the products made from them is required in order to rule out damage to health caused by contaminants such as pesticides, mycotoxins and heavy metals and to prevent batches with excessive THC or CBD content from coming onto the market. Companies that grow cannabis, the food industry, pharmaceutical companies – they all either have their own laboratories or commission experts to analyze the cannabis and carry out quality control. Together with its partner companies Retsch and CEM, Shimadzu now offers solution packages to laboratories that cover the entire testing process, including grinding, preparation and analysis. →

It's late summer – the beginning of the hemp flowering season. For farmers who grow "Western Cherry", "Marina", "Felice" or other hemp varieties, it will soon be time to have some of the flowers and leaves tested in the laboratory. After all, their customers, i.e. manufacturers of hemp protein powders, hemp oils, hemp teas or CBD gummy bears and gels, want a natural product of the highest quality. Nobody wants to ingest pesticides, fungi and heavy metals when chewing on a piece of CBD gum. Cannabis laboratories not only determine the amount of contaminants but also the amount of active ingredients. The content of CBD and THC, for example, varies from year to year and from field to field and must be measured on a random basis, as the various end products have different legally permitted maximum levels for active ingredients from the cannabis plant.

When farmers Smith or Miller send in their selected hemp plants, they are analyzed in detail in a workflow that is not entirely straightforward. However, Shimadzu, Retsch and CEM now offer a complete solution package that can also be customized according to customer requirements. This article explains the individual steps of what is known as potency testing, from homogenization and extraction to analysis, and also looks at heavy metal and pesticide analysis, among other things.

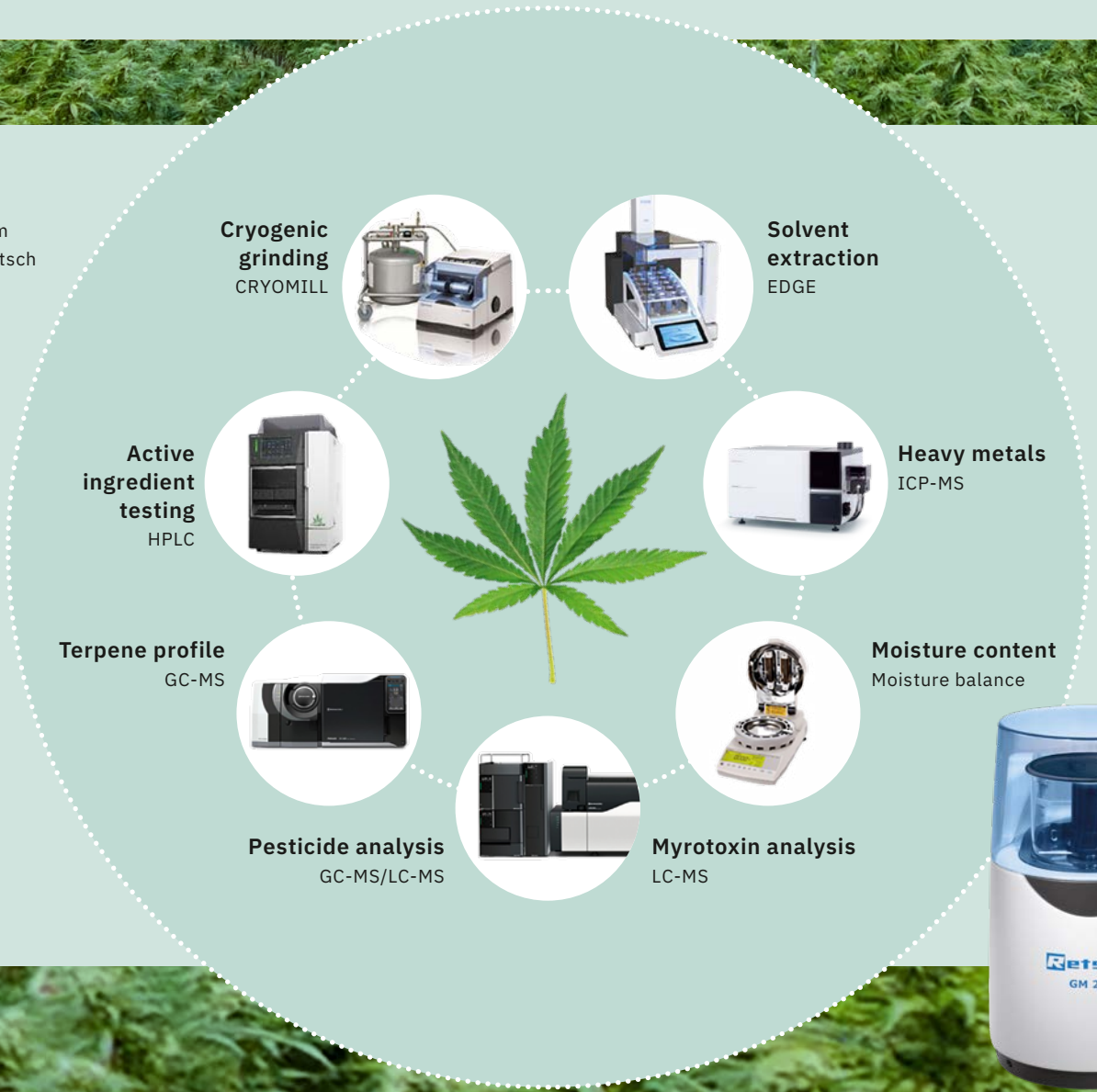


Figure 1: Solutions from Shimadzu, Retsch and CEM for cannabis analysis

Figure 3: The EDGE from CEM (left). Filling the homogenized sample into the Q-Cup before extraction (right).

Potency testing (analysis of active ingredients): CBD, CBDA, THC and THCA

The quality control of cannabinoids is essential for correctly labeling cannabis products, both in medical and food applications. The potency of cannabis (hemp) is usually determined by the content of a few selected major cannabinoids such as THCA, THC, CBD and CBN, although the plant contains more than 500 unique compounds, including over 80 chemical alkaloids.

Step 1: Homogenization. Before being analyzed, samples must be sufficiently homogenized so that the subsequent analysis of a sample subset is representative of the initial sample and reproducible. However, grinding plant parts can pose numerous challenges for the user, especially when the parts are oily and difficult to homogenize, as in the case of cannabis. Retsch's many years of expertise are available to you and provide advice: Selecting the optimum laboratory grinder, accessories and the entire process mainly depends on the sample quantity to be ground and the subsequent analysis. The sample quantity to be ground should be large enough to represent the entire sample. For example, a sample of only a few grams, i.e. a few petals, could not sufficiently represent a heterogeneous sample such as cannabis flowers.

The Vibrating Mill MM 400 and a special adapter (Figure 2) can be used for potency testing, where up to eight samples can be homogenized in conical centrifugal tubes per grinding pass. For this purpose, the dried flowers are deep-frozen at –20 °C, and then 4 g are used per tube. After adding 2 x 15 mm steel grinding balls, homogenization can take place at 30 Hz for 3 minutes, which achieves a fineness of 1 to 2 mm. This method leads to highly reproducible analysis values with minimized sample loss for CBD, CBDA, THC and THCA

Figure 2: Vibrating Mill MM 400 with adapter for centrifugal tubes for simultaneous grinding of up to eight samples, e.g. as sample preparation for potency testing

and also saves time thanks to the high sample throughput, the short grinding time and the fact that disposable vessels don't need to be cleaned. This method is also suitable for grinding samples for pesticide analysis. After grinding, a partial quantity of 500 mg can be used for the subsequent steps.

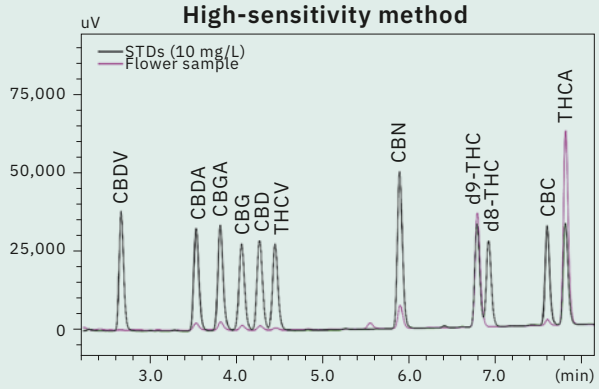
Step 2: Extraction. In classic Soxhlet extraction, samples containing CBD and THC are typically extracted over many hours, usually up to 24 hours, under reflux with solvent volumes of 250 to 500 mL. This straightforward work step takes a lot of time and incurs high costs since large quantities of solvent are used and thrown away. Soxhlet appliances also require immense space. Complete fume cupboards are needed and, in terms of sustainability, it is important to remember that hundreds of liters of drinking water are used for the reflux cooling of the Soxhlet equipment. On the other hand, analysis methods that save time and costs and are more sustainable are becoming increasingly important in both research and routine analysis. With the EDGE solvent extraction system, solvent extractions are carried out quickly, easily, safely and cost-effectively.

CEM's EDGE is an excellent choice for laboratories that need to extract cannabinoids (and pesticides) from cannabis and its products with high recovery rates and repeatable results (Figure 3). The EDGE is a simple automated extraction system that uses solvents to extract samples quickly and effectively. Extractions are carried out in the EDGE under pressure at defined temperatures, which leads to a significant acceleration of the reaction kinetics.

Accelerated solvent extraction is considerably faster than Soxhlet, ultrasound, classic ASE, QuEChERS or other traditional extraction methods and requires much less solvent and considerably less work. The EDGE only takes up around one DIN A3 sheet of space, meaning very small, and can be set up practically anywhere, even outside a fume cupboard. ⟶

Step 3: Analytics. HPLC has become the gold standard for fast and simple cannabinoid analysis since this method can be used to separate and detect all cannabinoids. Furthermore, the HPLC-UV method offers good linearity, a low detection limit and high precision of retention time and peak area for the cannabinoids of interest (Figure 4).

The Cannabis Analyzer from Shimadzu (Figure 5) can be used to analyze all eleven important cannabinoids. The basis is the i-Series Plus compact system with UV detection. The predefined methods make it very easy to get started with cannabis active substance testing. The i-Series Plus automates sample preparation, such as sample dilution or the addition of reagents. This reduces the risk of measurement errors caused by carrying out the steps manually and ensures highly reproducible and reliable data, not only when analyzing cannabis, pharmaceutical products and foodstuffs. The i-Series Plus meets the data integrity requirements of the pharmaceutical industry with special functions.



◄ ◄ Figure 4: Chromatogram of an extract from hemp flowers compared to a standard solution

◄ Figure 5: Shimadzu Cannabis Analyzer based on the i-Series Plus compact system with UV detector

Terpene analysis

Terpenes are organic aromatic substances that are formed in the trichomes (in which THC is produced) and influence the overall homeopathic effect as essential medicinal hydrocarbon components. A very important aspect when grinding samples for terpene analysis is avoiding heating. This can cause the terpenes to be lost as volatile sample components, which would distort the results of the subsequent analysis. It was demonstrated that closed grinding systems such as ball mills are particularly suitable for analyzing terpenes (Figure 6).

They can also be used cryogenically, which prevents the loss of volatile components and at the same time improves the fracture properties of oily samples so that good homogenization is achieved. A maximum of 1 x 20-mL sample or 2 x 40-mL samples can be first made brittle in the CryoMill or in the MM 500 control and then ground with liquid nitrogen within a few minutes (Figure 7). Both systems are particularly safe and convenient, as there are no open liquid nitrogen baths that the user could come into contact with. Cooling happens automatically. Programmable cooling pauses should be long enough to effectively prevent heating. In ball mills, samples such as dried cannabis flowers can be crushed to 0.1 mm. If larger

Terpenes in hemp tea (ground differently)

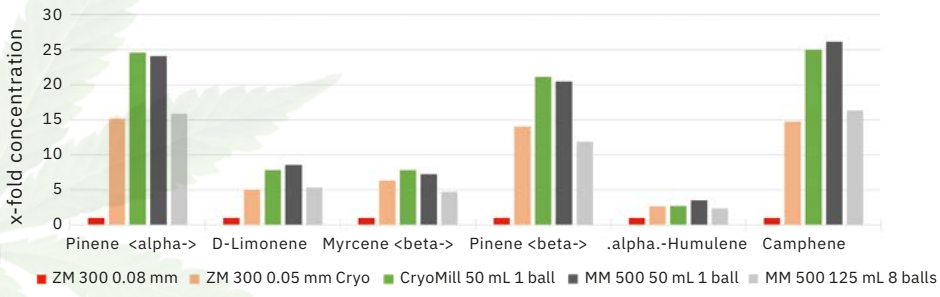


Figure 6: If the sample gets too warm (provoked in the ZM 300 with a 0.08-mm sieve), the terpenes evaporate. If a sieve with larger apertures is selected and the sample is processed cryogenically, loss of the volatile components can be minimized. The best results are achieved in closed ball mills (CryoMill, MM 500 control) with cryogenic grinding.

▼ Figure 7: Vibratory Mill MM 500 control and CryoMill for cryogenic sample preparation of small sample volumes, Ultra Centrifugal Mill ZM 300 for sample preparation of sample volumes up to 4 liters (from left to right)

sample quantities of up to approx. 4 liters need to be homogenized, this can also be done in the Ultra Centrifugal Mill ZM 300 (Figure 8). Here, an optional cyclone generates an air flow to cool the sample. In order to keep heating to a minimum, ring sieves of < 0.5 mm should not be used; a final fineness of approx. 300 µm is standard and sufficient to provide good follow-up analysis (Figure 6).

The ground samples can be analyzed directly. Terpenes and their synergistic effect with cannabinoids can be easily characterized thanks to gas chromatography (GC) systems from Shimadzu and range from pinewood aromas to fresh citrus scents.

The Shimadzu GCMS-TQ8050 NX (Figure 9) with the HS-20 Headspace Sampler and the NIST spectral library can identify more than 3,000 flavors and fragrances, fulfilling an important requirement for terpene profiling, which is one of the quality criteria for cannabis plants. The same system configuration can be used for analyzing residual solvents, while an additional liquid autosampler is used for pesticide analysis.

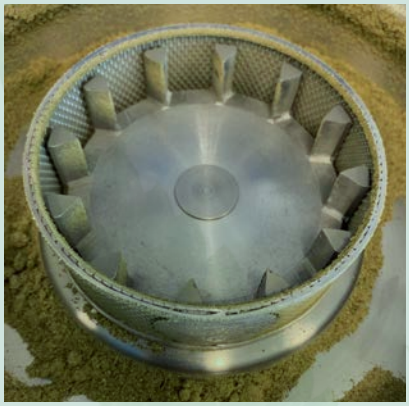


Figure 8: Cannabis flowers before grinding and after cryogenic grinding in a ball mill (total of 4 min for 20 g sample) or the ZM 300 (10 min for 500 g sample)

Figure 9: GCMS-TQ8050 NX for terpene analysis

Pesticide analysis

Pesticides used in the commercial cultivation of cannabis can be both carcinogenic and mutagenic and cause serious health issues, especially in immunocompromised users of medicinal cannabis products. Due to the risk of pesticide exposure from inhaled and consumed cannabis products, pesticides in the plant material and in products made from it must be monitored. Once again, the mills already mentioned in the previous sections are suitable for grinding and homogenization. In principle, finer ground samples can be extracted more easily, which is why ball mills are advantageous here.

Shimadzu offers the most sensitive and comprehensive pesticide analysis in the European market with liquid chromatography-mass spectrometry systems (LC-MS/MS). For example, a highly sensitive LC-MS/MS analysis of 211 pesticides in dried cannabis products is possible in less than 12 minutes using a Shimadzu LCMS-8060NX triple quadrupole mass spectrometer (Figure 10). →

Figure 10: LCMS-8060NX for detecting pesticides and mycotoxins

Standard deviation from 5-fold detection

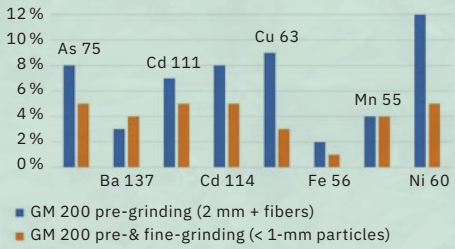


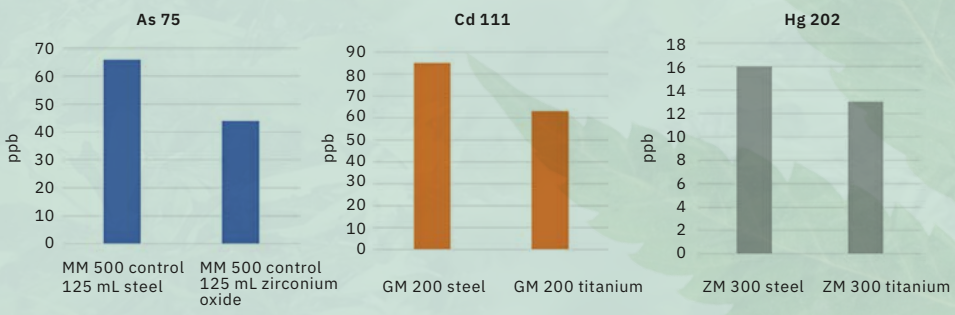
Figure 11: Particle size effects on the reproducibility of heavy metal analysis in cannabis flowers. After 10 s of grinding (interval at 4,000 rpm) in the GM 200 knife mill, fibers remain; the corresponding standard deviations in the heavy metal analysis are higher than for samples ground for 20 s with final finenesses of < 1 mm (10-s interval, 4,000 rpm + 10 s at 10,000 rpm).

Mycotoxins

Since cannabis has a high moisture content, long-term storage can lead to fungal growth known as mold, where the mycotoxins are toxic secondary metabolites. Aflatoxins, for example, are a subgroup of mycotoxins that mostly occur in soil and decaying vegetation. In Europe, the EU Commission has set out strict guidelines for the sampling and analysis methods used to test for mycotoxins.

Heavy metal analysis

As cannabis plants grow, they can absorb toxic heavy metals such as lead, cadmium, arsenic and mercury from the soil. If samples are to be analyzed for heavy metals, the knife mill GM 200, which is particularly easy to handle and homogenizes samples up to 200 mL in one go, is also suitable alongside the ball mills and the ZM 300, which have already been mentioned. It has been demonstrated that a particle size of < 1-mm particles is sufficient to achieve very good reproducibility. The standard deviation was below 5 % for all analyzed elements. If a shorter grinding time is selected, resulting in coarser particles (2 mm), standard deviations of up to 12 % can be expected (Figure 11). As a result, it pays off to carry out homogenization carefully in order to minimize these kinds of particle size effects.



◀ Figure 12: Effects of the correct grinding tool on analysis results. The abrasion from steel tools leads to higher heavy metal values, which distorts the analysis. This can be avoided by using zirconium oxide or titanium tools.

There are various methods for determining trace metals in plant material such as cannabis or in edibles (foodstuffs mixed with hemp). All require digestion with mineral acid to destroy the organic matrix, dissolve metal traces and so obtain a liquid sample. The MARS 6 from CEM is suitable for this (Figure 13). It is the only system that uses integrated sensor technology to recognize the type of vessel and sample number to create a user-defined algorithm that ensures the highest quality extract.

Heavy metals such as arsenic, cadmium, chromium and lead are natural components of the earth's crust and typically occur in our environment, meaning they are present in water and soil in varying concentrations. The concentration of heavy metals in plants intended for human consumption must be carefully controlled due to their potentially dangerous toxicity. Cannabis in particular is a plant that extracts heavy metals from the soil, which then accumulate in the plant.

Figure 13: MARS 6 microwave digestion system from CEM

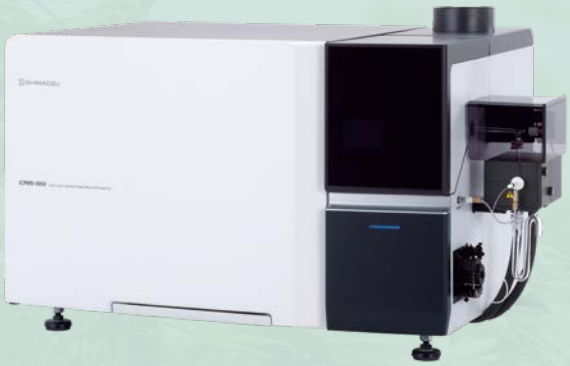


Figure 14: ICPMS-2050 for detecting heavy metals

The Shimadzu ICPMS-2050 mass spectrometer with inductively coupled plasma (Figure 14) and its newly developed collision cell to minimize interference provides the sensitivity required to measure the lowest concentrations of these toxic metals with a high degree of accuracy.

All quality control tools

Three partners, one complete solution: The latest analytical toolbox now includes various system configurations, starting with the indispensable analysis-oriented sample preparation with different laboratory mills from Retsch (ball, rotor, knife or cutting mills), sample digestion with microwave laboratory equipment and microwave-accelerated solvent extraction systems from CEM (MARS 6 and EDGE), through to instrumental analysis. Shimadzu is offering the HPLC Cannabis Analyzer for active ingredient analysis, the LCMS-8060NX triple quadrupole mass spectrometer for pesticide and mycotoxin analysis, the triple quadrupole mass spectrometer TQ-8050 NX for terpene analysis and the ICPMS-2050 for measuring heavy metals. With this toolbox, cannabis laboratories and manufacturers with their own laboratory can work efficiently and in full compliance with international regulations.

To guarantee a representative and reproducible analysis, appropriate care should be taken in all three areas covered by the above-mentioned companies. If the test results are positive, nothing stands in the way of farmers Smith or Miller obtaining a certificate – the industrial hemp can then be further processed and may soon end up in your local supermarket in the form of oil or tea.

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

Urban mining – sustainable and safe

Germany's new Substitute Building Materials Ordinance is forcing laboratories to rethink

Dr. Johannes Hesper, Shimadzu Europa GmbH

Nico Gilles, Shimadzu Deutschland GmbH

Growing environmental awareness and a shortage of raw materials are causing the construction industry to rethink. More and more secondary raw materials are being used in construction and redevelopment projects. The new Substitute Building Materials Ordinance (Ersatzbaustoffverordnung or EVB) [1] lays down the legal framework to ensure that these materials do not pose a risk to people or the environment. Part of this also includes lowering the limits for many heavy metals. This has consequences for test laboratories, since they will have to use new methods and equipment in the future in order to stay ahead of the competition.

Versatile, sustainable and can be used in many different ways: That's what substitute building materials are. They are made from secondary raw materials and are used in the construction industry as a replacement for primary raw materials. These include materials such as ash/slag, waste sand, excavated soil and recycled materials. Using these reduces the demand for primary raw materials, lessens the amount of waste and minimizes the environmental impact.

Substitute building materials are typically used for road and building construction as well as redeveloping landfill sites and mining areas.

In order to further promote the use of these materials and, in doing so, strengthen the circular economy, Germany developed the Substitute Building Materials Ordinance (EBV), which came into force on August 1, 2023. It introduces significant changes in how substitute building materials should be used but also ensures greater legal certainty for users and recycling companies.

But it's not just Germany, in fact, many countries throughout Europe are pursuing regulations and standards for substitute building materials with similar goals to the German EBV.

All aim to promote the use of substitute building materials while ensuring high standards of safety and environmental compatibility. They highlight the fact that striving for sustainability and environmental protection in the construction industry is a high priority for many countries throughout Europe.

New methods, new devices

Although this makes things safer and easier for recyclers and construction companies, it means new procedures and maybe even necessary updates for many testing laboratories. This is because one important change concerns the limit values for the soil material and dredged material group. The new ordinance sets stricter limits for a range of pollutants, including heavy metals such as lead, cadmium, thallium and mercury.

The lower limit values that have been introduced, especially for cadmium and thallium, require many laboratories to rethink their analytical methods, as the frequently used inductively coupled plasma optical emission spectrometry (ICP-OES) technique does not meet the required limit values. In fact, the proven atomic absorption spectrometry (AAS) is only intended for determining mercury in the new regulation.

This means that two analysis techniques that were frequently used before have to be replaced. The inductively coupled plasma mass spectrometry (ICP-MS) technique is particularly good here, as it offers very high sensitivity and accuracy. It can detect even the smallest amounts of heavy metals present in a sample (ppb and smaller). →

Figure 1: The new ICPMS-2050

Nico Gilles, Product Specialist for Elemental Spectroscopy at Shimadzu Germany, has developed a new method that allows testing laboratories to easily achieve robust and stable mea surement results using ICP-MS despite difficult sample matrices. Testing was carried out on the Shimadzu ICPMS. The samples had to pass all the required tests for long-term stability, recovery rate and detection limit.

The optimized torch design of the new ICPMS-2040 and -2050 series also ensures low argon consumption and increased sample throughput.

Method development

When developing the method, all samples were taken and processed as specified in the EBV. The required aqueous eluate was prepared according to DIN 19529, and the aqua regia digestion was carried out according to DIN 13657.

In ICP-MS, an internal standard is normally added to the previously dissolved sample to compensate for possible sources of error during the analysis. This internal reference is therefore an important part of quality assurance, which increases the reliability of the measurement results.

The liquid sample is transformed into a fine aerosol via a cooled spray chamber, which is then ionized in hot argon plasma. The resulting ions are then separated in a mass spectrometer according to their mass-to-charge ratio and determined by the detector within a specified integration time. In this way, very low concentrations of the elements under investigation can be determined precisely. The elements relevant for the analysis of soil material and dredged material are arsenic, lead, cadmium, chromium, copper, nickel, mercury, thallium and zinc in the solid material as well as antimony, molybdenum and vanadium in the eluate.

Verifying the long-term stability using CCV

It is possible to continuously verify the method by regularly measuring calibration standards during the analysis in what is known as “continuous calibration verification” (CCV). The results of this long-term observation are shown in Figures 2 and 3 for calibration standards Cal 2 and Cal 4. The limits shown (red lines) are 70 % and 130 % in accordance with DIN EN 16171:2017 and were not exceeded in any case.

Verification: CRM recovery rate

An aqua regia digestion of the certified reference material (CRM) BAM-U115 was carried out in order to be able to externally verify the measurement results, reviewing the method at the same time. As with the CCV, measurements were taken every hour and compared with each other. The results were all within the tolerance range.

Aside from the long-term stability and recovery rate, of course it is also necessary to comply with the detection and quantification limits in order to meet the EBV. As already mentioned at the beginning, the new Substitute Building Materials Ordinance with its very low limit values, especially for cadmium and thallium, places demands on the measurement technology that can no longer be implemented with ICP-OES.

Detection limits

In accordance with the requirements of the EBV, the detection limit of the method may not exceed one third of the specified limit value of the corresponding element. As a result, the respective limit values of the EBV were used as the limit of determination. Thanks to the ICP-MS method developed by Shimadzu, the result was that the required limit values, i.e. limit of detection (LoD) and limit of quantification (LoQ), could be reliably met with maximum robustness and long-term stability at the same time (Table 1).

The introduction of the new Substitute Building Materials Ordinance for soil material and dredged material in Germany on August 1, 2023 has placed new demands on testing laboratories. Although purchasing a new analyzer may indeed be necessary, Shimadzu’s development work has provided laboratories with a method of reliably complying with the specified limit values using ICP-MS. This means they can continue to fulfill their important task of ensuring the safety of substitute building materials.

Continuous calibration verification – CCV Cal 2

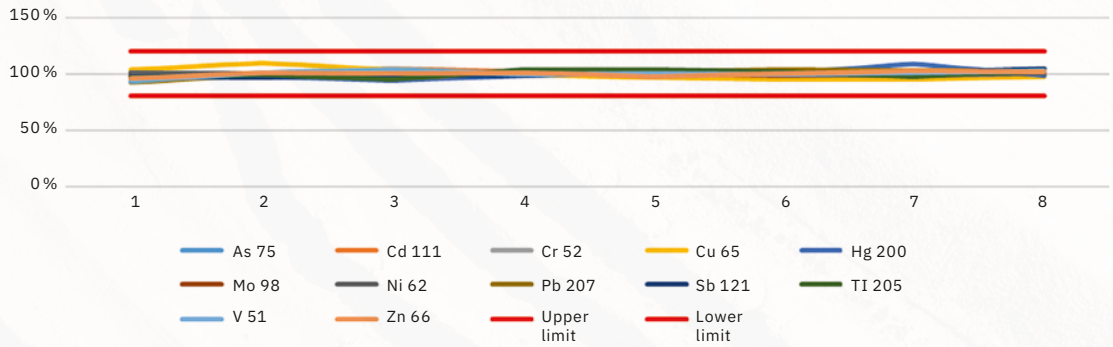


Figure 2: CCV of Cal 2

Continuous calibration verification – CCV Cal 4

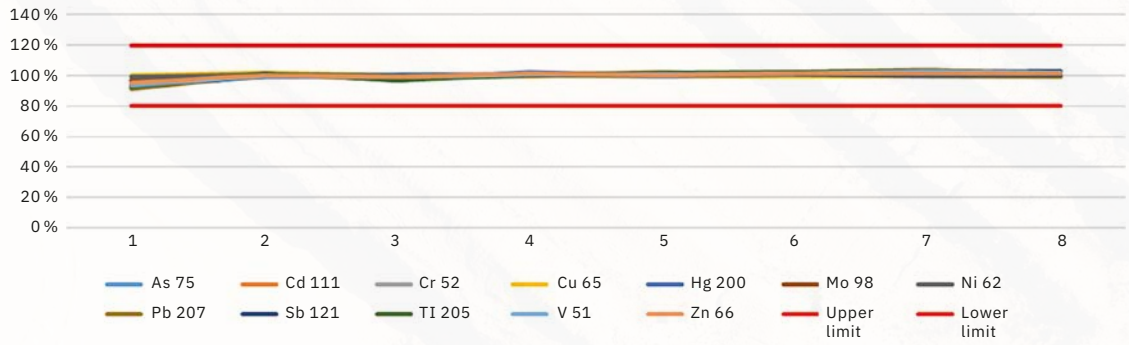


Figure 3: CCV of Cal 4

	ILoD [µg/L]	ILoQ [µg/L]	MLoQ [µg/kg]	MLoQ [mg/kg]	EBV-LoD [mg/kg]	EBV-LoQ [mg/kg]	
As	0.04	0.15	0.04	0.15	3.33	10.00	✔
Cd	0.02	0.06	0.02	0.06	0.13	0.40	✔
Cr	0.11	0.38	0.11	0.38	10.00	30.00	✔
Cu	0.97	3.25	0.97	3.25	6.67	20.00	✔
Hg	0.00	0.00	0.00	0.00	0.07	0.20	✔
Ni	0.23	0.75	0.08	0.27	5.00	15.00	✔
Pb	0.08	0.27	0.01	0.02	13.33	40.00	✔
TI	0.01	0.02	0.00	0.01	0.17	0.50	✔
Zn	0.04	0.13	0.10	0.33	20.00	60.00	✔

Table 1: Detection and quantification limits ILoD & ILoQ: Instrumental limit of detection/quantification; EBV-LoD: 1/3 of the EBV-LoQ (limit of quantification of EBV) for the measurement of aqua regia digestions

Note

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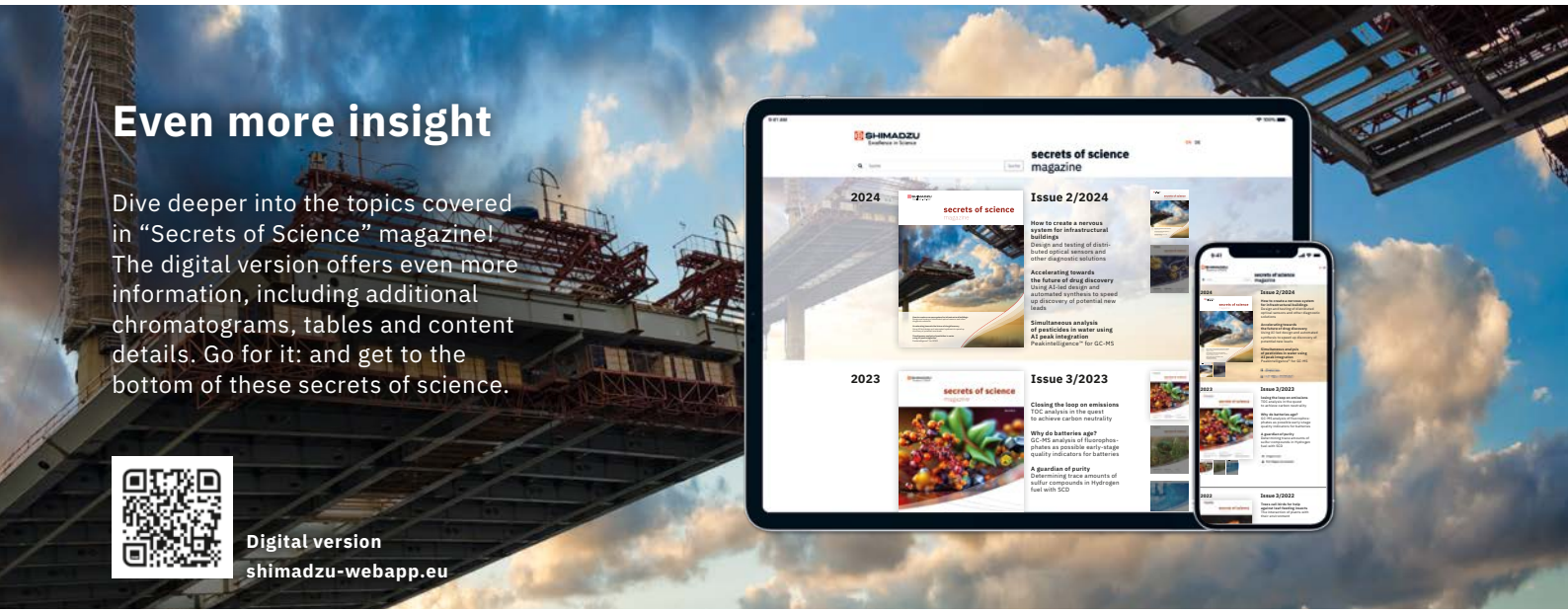

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