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Developing successful methods for complex sample analysis

Hard to break but easy to degrade?
Bioplastics: How do they measure up?
Setting new standards for accurately testing bioplastics' real-world biodegradability

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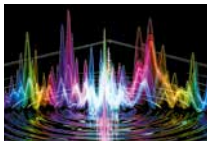

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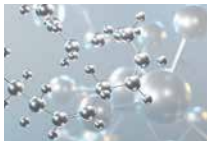

Revealing the hidden structural diversity of lipids

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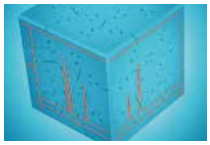

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Hard to break but easy to degrade? Bioplastics: How do they measure up?

Setting new standards for accurately testing bioplastics' real-world biodegradability

Dr. Harry Lerner, KU Leuven (Belgium)

Prof. Dr. David Schleheck, University of Konstanz (Germany)

Sascha Hupach, Shimadzu Deutschland GmbH

Markus Janssen, Shimadzu Europa GmbH

That yogurt cup after breakfast, the ice cream wrapper by the pool, the empty crisp bag after movie night – plastic packaging is everywhere. Versatile and convenient, it comes with a downside: Slow to degrade, it leaves behind microplastics that burden ecosystems and the food chain. Bioplastics made from renewable resources are considered a sustainable alternative. But do they actually decompose as rapidly outside the laboratory as promised? A research team at the University of Konstanz has developed a method to accurately measure biodegradability under real-world conditions – setting new standards for sustainable materials.

Everyday plastic – hidden risks

Plastic is present in almost every corner of our lives. Its strengths, such as durability and toughness, become harmful to the environment if it doesn't fully degrade. Instead, tiny micro- and nanoplastics are released, threatening delicate ecosystems and our health. Microplastics have already been detected in human tissues, including the placenta and the brain. The full impact on health remains unknown. On top of that, traditional fossil-based plastic production has a heavy carbon footprint.

Bioplastics: Claims vs facts

Bioplastics made from plant oils or biomass offer a sustainable alternative: They claim lower environmental impact and higher biodegradability.
Yet outside ideal conditions, many of these plastics fail to degrade fully. Accurately judging their biodegradability requires new, precise testing methods.

Getting an accurate measurement is the goal

Researchers are working to precisely measure how bioplastics break down under real-world conditions. The goal is to generate reproducible results that can be applied reliably both in the lab and directly in natural ecosystems.

Current methods, such as gravimetric techniques or respirometers, aren't well suited for in-depth analysis of biological decomposition. While enabling parallel measurements, these methods often need large samples (sometimes over 100 g), bulky equipment and plenty of space for incubators and other equipment. They also often fail to capture a complete carbon balance.

The research team at the University of Konstanz has tackled these challenges and devised a scalable, compact method. It works with much smaller samples while delivering more precise, reproducible results. →

Using mineralization to measure sustainable degradation

Mineralization refers to how microorganisms biologically break down plastics. In this process, some of the carbon is converted into inorganic substances such as CO_2, water and minerals, while the rest is incorporated into biomass. This makes mineralization a key metric for fully characterizing the biological decomposition of a plastic.

Where gravimetric methods only measure leftover plastic, mineralization provides precise data to reveal the decomposition process and the resulting end products. This makes it a crucial tool for assessing the sustainability of a material under real environmental conditions.

Experiment setup: Compact, efficient, precise

Central to the method are custom-made reaction vessels made from 15-mL culture tubes (Figure 1). Each tube holds roughly 50 mg of bioplastic along with 1 g of biological material, such as soil samples or bacterial cultures. In the tube is a small glass vial containing a sodium hydroxide (NaOH) solution, which chemically captures the CO_2 produced during the degradation process as carbonate.

Each tube is tightly sealed using a modified rubber stopper. The stopper has two cannulas: One is used for adding and removing the sodium hydroxide solution, while the other allows controlled gas exchange to prevent oxygen depletion. Thanks to its compact design, up to 60 tubes can fit in a standard tube rack – making it perfect for high-throughput testing.

During the degradation process, the sodium hydroxide solution is regularly removed at set intervals and replaced with fresh solution. The removed solution, containing the bound CO_2, is transferred to the Shimadzu TOC-L for TIC (Total Inorganic Carbon) analysis.

Analysis with the Shimadzu TOC-L

TIC analysis enables precise measurement of the CO_2 released during the degradation process. A diluted portion of the removed sample is acidified with phosphoric acid in the Shimadzu TOC-L. This converts the previously chemically bound carbonate back into gaseous CO_2, which is then measured with a non-dispersive infrared detector (NDIR).

This method provides a complete carbon balance: It not only captures the released CO_2 but also documents the remaining plastic mass and the formation of biomass. This approach offers a more comprehensive picture of how plastic degrades compared to traditional techniques.

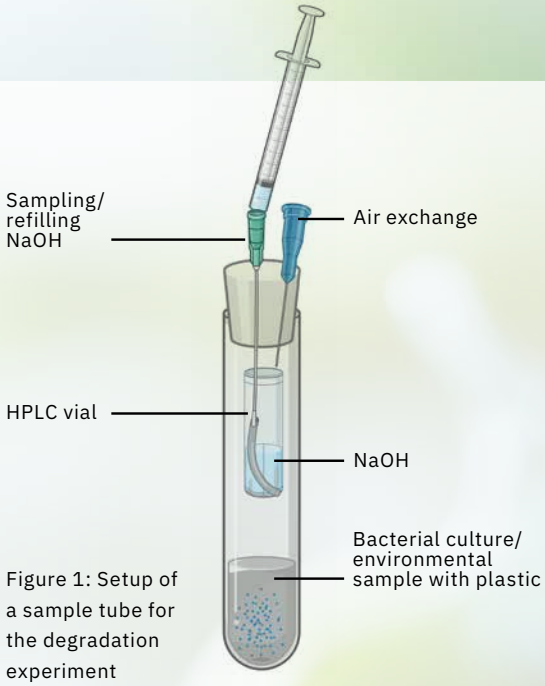


Figure 1: Setup of a sample tube for the degradation experiment

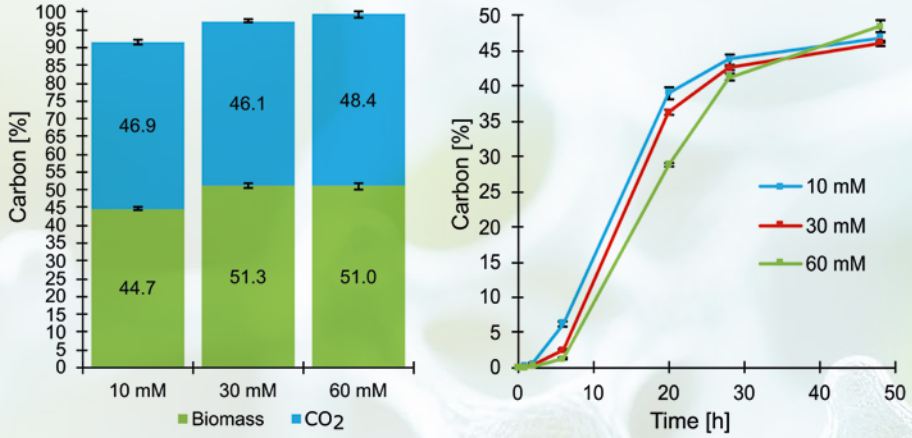


Figure 2: Percentage of carbon from glucose converted by bacteria into biomass and CO_2 after 48 hours (left). Progress of the percentage of carbon converted to CO_2 (right).

Validating the method: Glucose test

To verify the accuracy of the method, the team conducted initial experiments using glucose as the carbon source. Glucose is often applied as a reference material because its degradation products are well researched. Under ideal conditions, roughly half of the carbon should be released as CO_2, while the other half remains bound in biomass.

The experiments confirmed this prediction: The measured carbon balance matched the theoretical values, proving that the method works reliably (Figure 2).

Testing PHBV bioplastic in practice

After successful validation, the team tested the method on the bioplastic polyhydroxybutyrate-co-valerate (PHBV), which is commonly used in food packaging and medical applications.

The experiments showed that PHBV could be completely decomposed under optimal conditions. Achromobacter fully degraded the plastic, while Pseudomonas left a residual fraction of less than 10 % (Figure 3). The method precisely documented the degradation process and provided valuable insights into how different bioplastics respond under specific conditions.

Precision meets sustainability

Researchers at the University of Konstanz have taken an existing method to the next level, enhancing its precision. By combining a compact reaction system with the Shimadzu TOC-L, the biodegradability of bioplastics can be measured comprehensively, reproducibly and under realistic conditions.

This research offers a proven method for systematically and thoroughly analyzing bioplastics in the future. It lays the groundwork for reliably assessing their environmental friendliness and sustainability – a crucial step to ensure that the resource cycle doesn't end in the trash but can be effectively closed.

Note

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

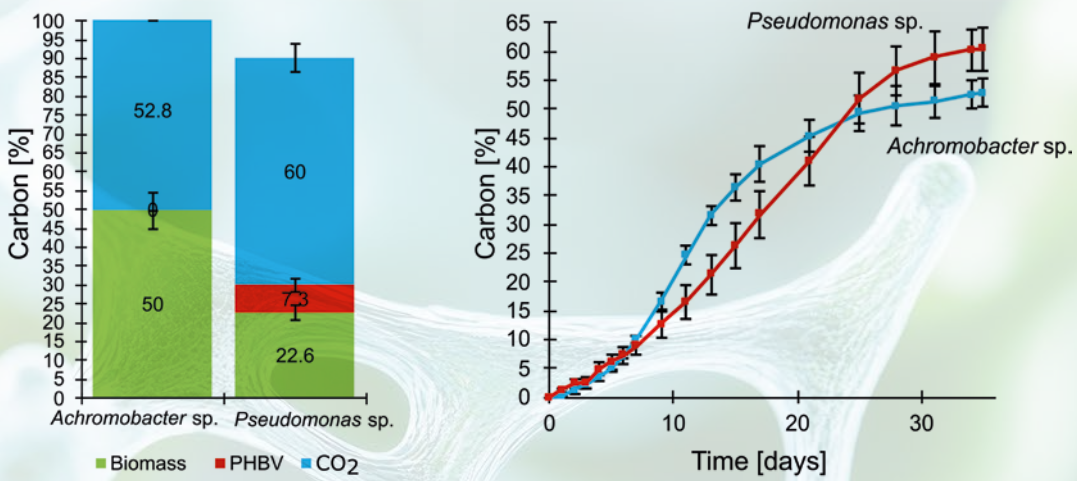


Figure 3: Percentage of residual plastic and carbon from PHBV converted by the bacterial cultures Achromobacter and Pseudomonas into biomass and CO_2 over 35 days (left). Progress of the percentage of carbon converted to CO_2 (right).

Expert troubleshooting: Tips for achieving sharper HPLC peaks

Lab users well know that troubleshooting is a regular part of everyday lab work. And they often reach out to the experts at Shimadzu for advice. This article marks the beginning of a new Secrets of Science series on instrumentation troubleshooting. Specifically, this article explores the prevention and cure of peak shape problems when using high-performance liquid chromatography (HPLC).

Troubleshooting HPLC peak shape issues

Achieving good peak shape is essential for reliable chromatography. Any deviation from expected peak profiles can indicate issues with the system, method or column performance, ultimately affecting resolution, quantitation and data integrity. Because peak shape is influenced by many interconnected factors, troubleshooting requires a systematic approach that considers both hardware and method-related causes. The goal is to ensure sharper peaks – and better data quality.

Are your peaks revealing data – or concealing it?

Dr. Anna Cooper, Shimadzu UK

Common causes of peak shape problems

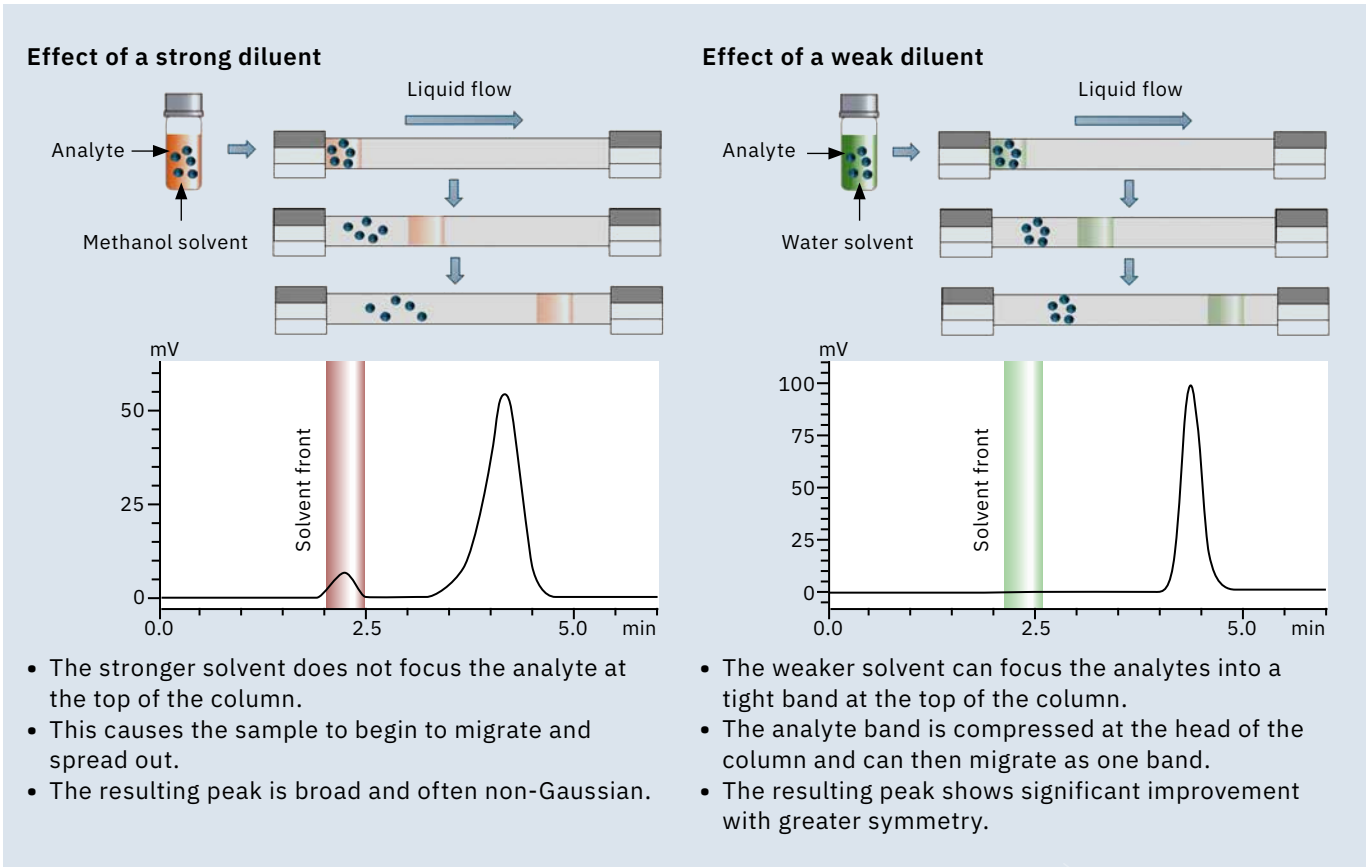
Sample solvent strength

Sample solvent composition plays a significant role in peak shape. Ideally, it should closely match the starting conditions of the chromatographic method to minimize disturbances.

- Weaker solvents (e.g. water in reversed-phase HPLC) can enhance peak sharpness by concentrating analytes at the column head.
- Strong solvents (e.g. 100 % methanol or acetonitrile) risk peak broadening or even splitting, as analytes may be carried along by the solvent rather than being retained effectively. This is particularly problematic for early eluting peaks which experience detrimental peak shape with a strong diluent (Figure 1, next page).

Solutions

- Match the sample solvent to the initial mobile phase wherever possible.
- If the sample cannot be dissolved in a more favorable solvent, a coinjection with water – known as a “sandwich” injection – can be employed. This is where the autosampler is programmed to aspirate a volume of water, then sample, then water again to create a focused sample at the head of the column, thereby negating the adverse band-broadening effects. ⟶



- The stronger solvent does not focus the analyte at the top of the column.
- This causes the sample to begin to migrate and spread out.
- The resulting peak is broad and often non-Gaussian.

- The weaker solvent can focus the analytes into a tight band at the top of the column.
- The analyte band is compressed at the head of the column and can then migrate as one band.
- The resulting peak shows significant improvement with greater symmetry.

Figure 1: Effect of sample solvent on a peak shape

Excess injection volume or solvent overload

Even with an appropriate solvent, excessive injection volumes distort peaks and reduce efficiency. If too much sample is injected, all active sites at the column head are occupied, and the remaining sample flows past the occupied sites with reduced interaction. Overloading saturates the stationary phase's active sites – particularly at the column inlet – resulting in peak fronting or tailing, depending on the analyte.

Additionally, column overloading can shift retention times and broaden peaks. This is particularly critical during scale-up: A compound that is well-resolved under analytical conditions may shift and broaden under overloaded preparative conditions, potentially causing smaller impurity peaks to coelute and remain undetected.

If high injection volumes are unavoidable, consider using a column with a larger inner diameter or higher loading capacity to minimize these effects (Figure 2).

Solutions

- Reduce injection volume if sensitivity permits.
- Concentrate samples to allow smaller injection volumes without compromising detection.
- Use columns with higher loading capacities or larger internal diameters if high-volume injections are unavoidable.

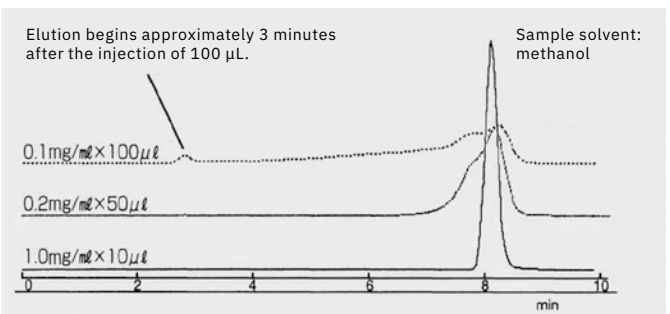
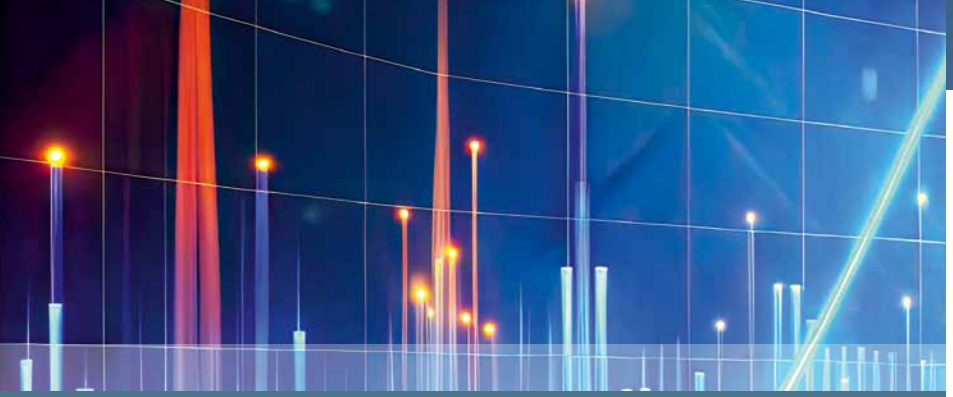


Figure 2: Effect of injection volume on peak shape: Increasing injection volume can distort peaks despite constant analyte load



Data acquisition rates and detector settings

A frequently overlooked factor in chromatography is the data acquisition rate, which significantly impacts the chromatogram quality. It is essential to adjust the acquisition rate to suit the specific chromatography method. This adjustment affects not only the appearance of the chromatogram but also the accuracy of the data.

Peaks measured with a low data acquisition rate are inadequately described due to an insufficient number of data points. This can result in significant fluctuations in peak area, as slight variations may prevent any data point from capturing the true peak maximum. Conversely, an excessively high data acquisition rate can also have drawbacks, such as increased noise levels, which may obscure smaller peaks or reduce overall signal clarity, as well as result in significantly large data files.

At first glance, the peaks in the image on the top may appear nearly identical, despite being measured with different data acquisition rates. However, when the chromatograms are overlaid and the section is enlarged, as shown in the image bewlow, the differences become evident (Figure 3).

Another parameter is the detector's response setting. If the response is set too high, peaks are artificially broadened, reducing resolution. On the other hand, setting the response too low narrows the peaks but amplifies noise (Figure 4).

Solutions

Use the fastest acquisition rate and shortest response time that still maintains an acceptable signal-to-noise ratio. Optimize these settings during method development rather than relying on default instrument parameters. UHPLC methods require a higher acquisition rate in order to describe the peaks accurately. This is generally above 12.5 Hz. ⟶

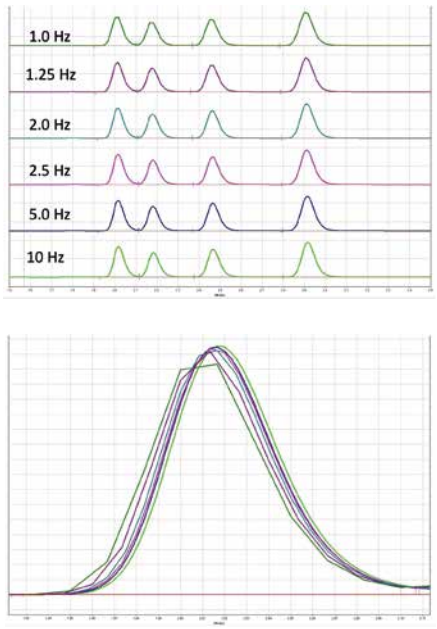


Figure 3: Effect of acquisition rates on peak shape

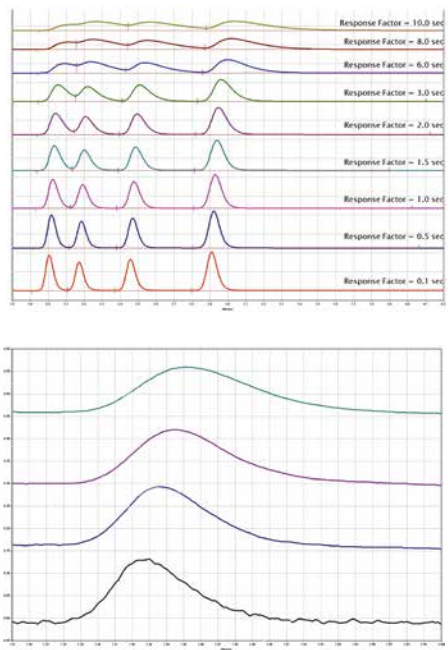


Figure 4: Effect of detector response factor settings on peak shape

Practical solutions for specific peak shape problems

Peak tailing

One of the most common peak shape changes is tailing, where the back half of the peak is broader than the front, and trails and elongates the peak. This is often caused by the analyte having more than one mechanism of retention on the column.

In reversed-phase chromatography, the main mode of analyte retention is through hydrophobic interactions, however, so there are often synergistic mechanisms within the column. For example, for ionized basic compounds, the positive charge can interact with the free silanol groups on the silica, which can lead to tailing. As silanol groups are acidic, it's possible to minimize interactions with the analyte by lowering the pH of the chromatographic conditions. If that isn't possible, try replacing the column with a deactivated column such as a polymer column, one with good endcapping or a column with a slightly positive character surface. Some column manufacturers (e.g. Shimadzu) offer special columns for the analysis of strongly basic substances.

A damaged column or incorrect analysis conditions, such as an incorrect pH value of the mobile phase, can also lead to tailing (Figure 5).

Fixes

- Lower the mobile phase pH to suppress silanol activity.
- Switch to endcapped or polymer-based columns designed to minimize silanol interactions.
- Check that pH and mobile phase conditions fall within column specifications.
- Increase the ionic strength of the buffer to reduce silanophilic interactions.

Peak fronting

Fronting refers to the opposite asymmetry of the peak found in tailing, where the leading edge is broader than the tail, and looks similar to a shark fin. If fronting gradually increases, in most cases a defective column is the cause. The stationary phase can be damaged due to normal aging or operating the column outside the specifications (e.g. of temperature or pH). In this case, only replacing the column and adjusting the chromatographic conditions will help.

However, as discussed earlier, column overloading can also cause fronting. Reducing injection volume or using a higher-capacity column are key fixes.

Another cause of peak fronting can be an incompatible sample solvent or poor sample solubility with the mobile phase. In this case, only changing the sample solvent will help. A too low column temperature can also cause fronting (Figure 6).

Fixes

- Reduce sample load.
- Use a higher-capacity column, if possible.
- Ensure good solvent compatibility.
- Verify temperature control.
- If fronting worsens gradually, column aging may be to blame: Replacement is usually the only remedy.

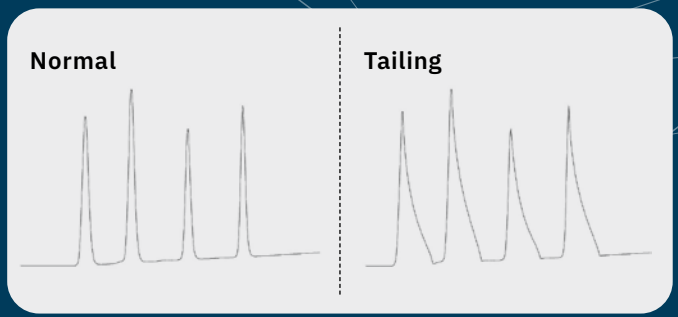


Figure 5: Normal peaks vs tailing peaks

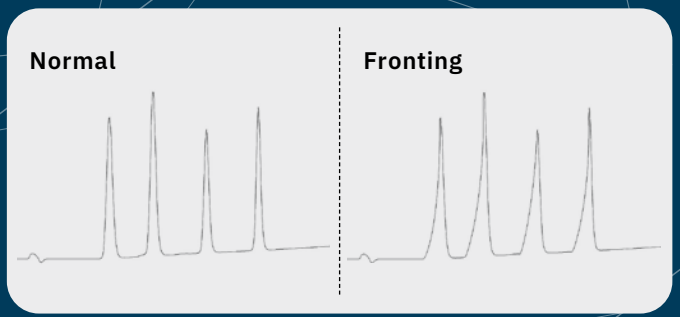


Figure 6: Normal peaks vs fronting peaks

Peak broadening

In isocratic separations, it is expected that as the analyte band migrates down the column bed, the band will broaden as a function of resident time in the column. This results in broader peaks for later eluting compounds. This is one of the driving factors for ensuring the analyte has sufficient interaction with the stationary phase but elutes within a reasonable time frame to prevent the detrimental effects of band broadening.

In gradient chromatography, the peaks should all have the same peak width, as the changing composition of the mobile phase compresses the band. If there is an unexpected change in peak width in gradient chromatography, this is often the result of a degrading column which needs to be replaced.

Broad peaks compromise resolution and sensitivity, especially for late-eluting compounds in isocratic separations. As already mentioned, a detector response that's set too high can contribute to peak broadening. It's also important to remember that normal column aging is a common cause.

If peak broadening occurs due to dispersion in the injection valve, a "sandwich injection" with small air bubbles can help reduce this effect. Another cause of dispersion can be due to the use of capillaries with an excessively large diameter, or the unintentional introduction of dead volume due to improperly tightened connections.

Look what happens in the chromatograph on the right when a wider, 0.5-mm ID capillary is used instead of a 0.125-mm ID: Baseline separation is lost, and the peaks have broadened. However, also note that narrower capillaries can generate higher back pressures in the system. Therefore, adjust the dimensions of the capillaries to the chromatographic system so that the limits of the permissible pressure maxima are not reached (Figure 7). ⟶

0.5-mm ID (black) capillary instead of 0.125-mm ID (red) between column and detector

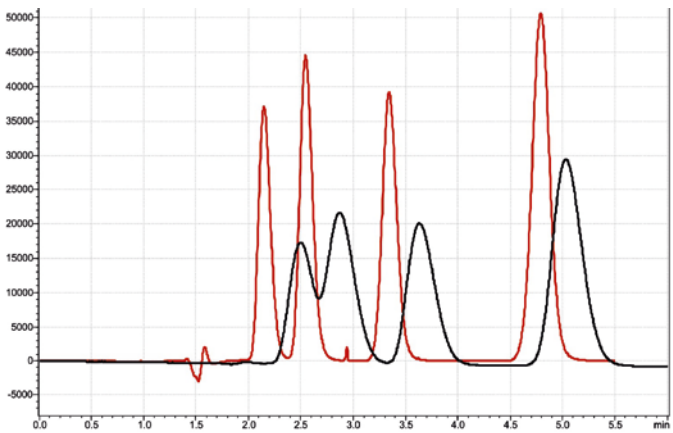


Figure 7: Effect of capillary ID on peak shape: Wider tubing (0.5 mm) increases system volume, causing peak broadening and loss of separation (example of isocratic run)

Fixes

- Inspect and retighten fittings and minimize dead volume.
- Use capillaries appropriate to the flow rates (avoid unnecessarily wide IDs).
- Preheat the mobile phase and use a column oven to maintain uniform temperature.
- Switch to gradient elution to reduce natural diffusion effects for late peaks.

Troubleshooting together

Effective troubleshooting depends on observation, comparison with reference data and methodical elimination of potential causes – ultimately restoring sharp, symmetrical peaks and reliable chromatographic performance.

It also helps to share experiences, challenges and solutions, and that's exactly what this new series of articles is all about. When lab users ask, Shimadzu listens. Because while lab work may seem solitary at times, it is also part of a vast community of inquiring minds who every day create fertile new fields of beneficial knowledge. Troubleshooting is just part of the journey, and it is good to remember that no one needs to travel alone.

Note

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

What makes a gin special?

GC-MS analysis of aroma-relevant volatiles in gins

Waldemar Weber, Shimadzu Europa GmbH

This article marks the third and final part of a series on gin, a famous old spirit that has lately been experiencing a great resurgence of popularity. The appeal of any specific gin is often tied to its aroma and flavor – which are chemicals, after all – and gin makers need good tools to ensure their gin appeals to customers. So how should gin makers analyze their gin to better understand what works? →

Figure 1: GCMS-QP2020 NX with AOC-6000 sampler

G1	27		G10	12	
G2	20	Alcohol-free	G11	26	
G3	20		G12	70	Saffron-infused
G4	68		G13	61	
G5	40		G14	61	
G6	54		G15	67	
G7	39	Alcohol-free	G16	67	
G8	26	Alcohol-free	G17	67	
G9	26		G18	70	Oak barrel-aged

Table 1: Overview of analyzed gin samples and their corresponding market prices

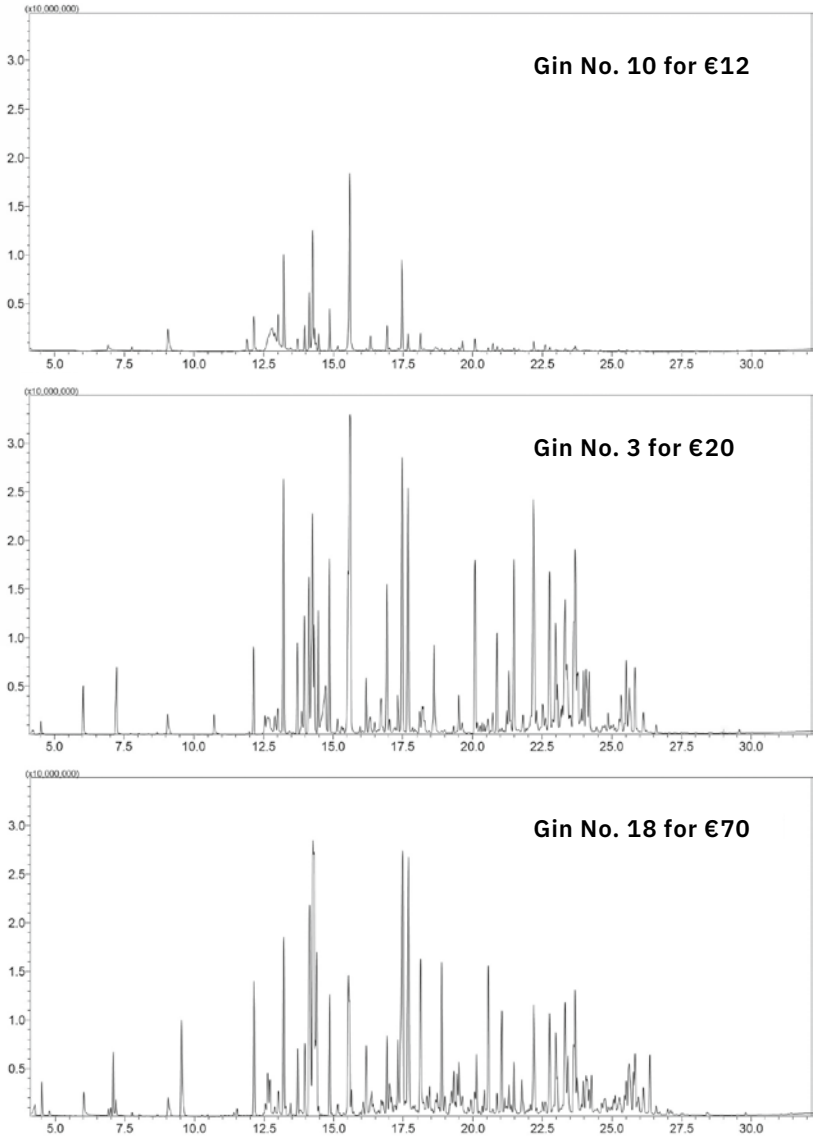


Figure 2: GC-MS chromatograms for three different gin samples

Gin price vs measured content of volatiles

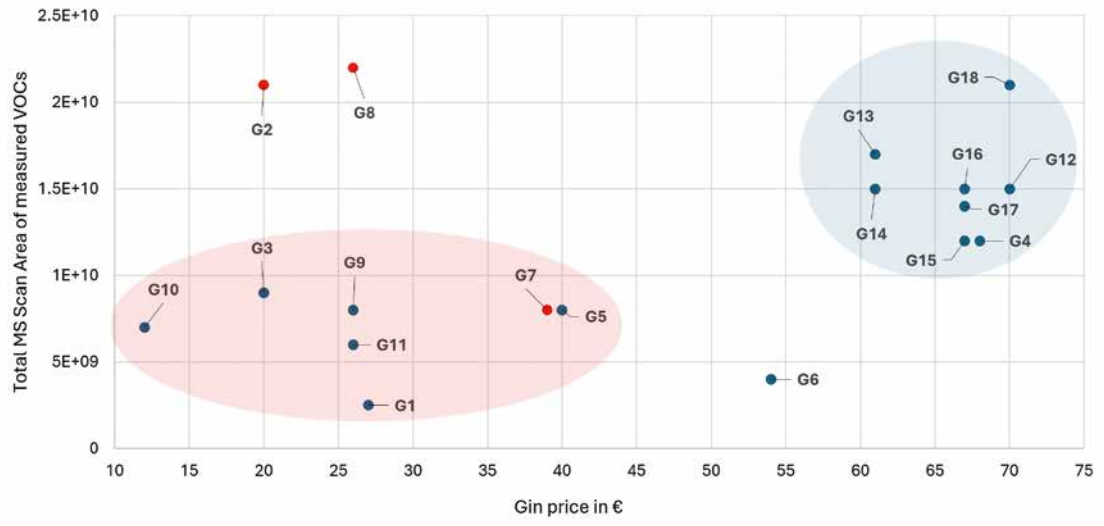


Figure 3: Correlation between the gin price and the content of VOCs

The global gin market has experienced robust growth in recent years, driven by a rising interest in premium and craft brands, enticing new recipes for mixed drinks and the seemingly unquenchable curiosity of consumers and producers alike for exciting new gin flavors.

Of course, you can flavor your own gin any way you wish at home. But most consumers prefer that someone knowledgeable has made certain that the gin they are drinking tastes as good – or better – than they expect. Producers in the highly competitive world of premium gin know that their success depends on delivering a flavorful gin that breathes originality, nuance, distinction and quality. Yes, flavor is the key ingredient in premium gins. But how do you ensure that you get it?

The role of terpenes

A big part of the answer is terpenes. Terpenes are volatile compounds, meaning that they evaporate easily and can be detected by smell. Among the many roles that terpenes play is their significant contribution to characteristic smells. Sesquiterpenes, for example, are a subgroup of terpenes that are often used to create earthy, woody and spicy aromatic notes.

So how do producers know which terpenes, in which amounts and in which combinations deliver that specific aroma and flavor that attracts customers to their gin?

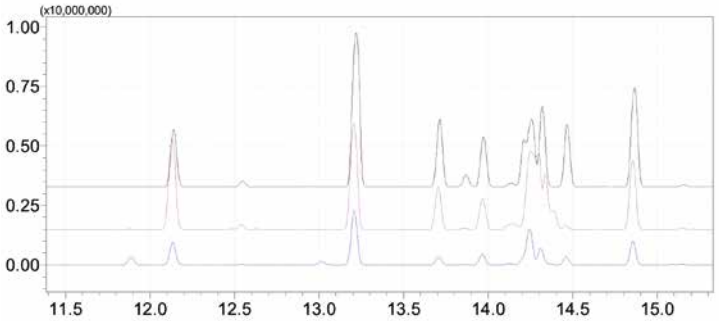
Exploring gin aromas with GC-MS

This question prompted a gin-curious researcher to wonder whether gas chromatography-mass spectrometry (GC-MS) could be used to better identify and analyze the terpenes used in flavoring gins. He reasoned that finding a simple and precise method to do this would help producers in their arduous task of creating gins that continued to quench the thirst of discerning consumers.

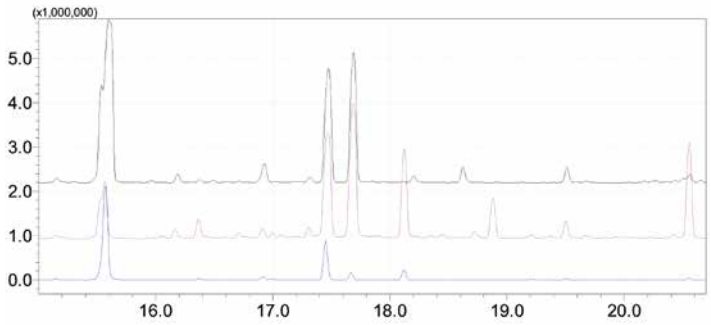
He opted for a straightforward approach: 18 gin samples were collected from the UK, Austria, Germany and Japan. The selection encompassed a variety of styles, including alcohol-free products, saffron-infused gins and oak barrel-aged varieties. This diversity allowed for a comprehensive analysis of the different flavor profiles and production techniques employed across these regions.

Table 1 shows a summary of the analyzed gin samples. For a comparison of volatile compounds detected in measured samples, three gins of different price levels were compared. G10 is a low-priced gin, G3 is a medium-priced gin and G18 is a high-priced premium gin. The chromatograms of these gins are shown in Figure 2, displaying that both higher-quality gins (G3 and G18) contain significantly more volatile components than the cheapest gin (G10). Figure 3 gives a relative overview of all gin samples analyzed, illustrating a correlation between the price and the peak area of detected volatile compounds. This representation more or less indicates a direct relationship between a gin's market value (i.e. its flavor and aroma) and its price. →

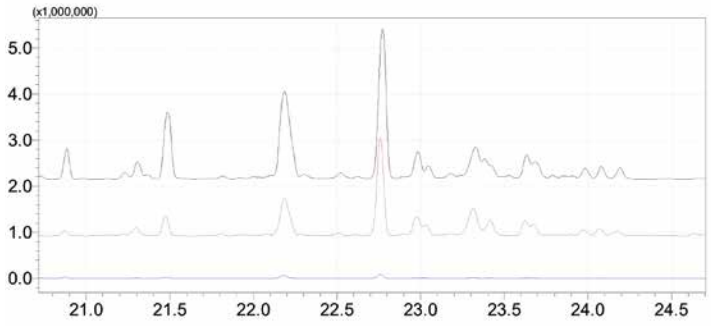
Monoterpenes



Oxygenated monoterpenes



Sequiterpenes



Oxygenated sequiterpenes

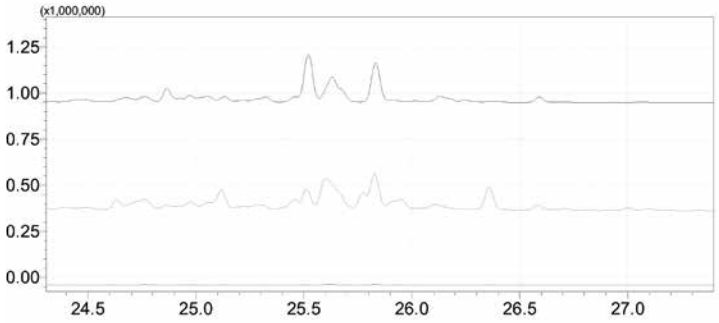


Figure 4: Selective GC-MS chromatograms for *m/z* 93, representative of different terpene groups. Blue: gin for €10, red: gin for €20, black: gin for €70.

Premium gins scientifically have more taste

The study shows that approximately two groups of gins can be distinguished from the examined samples (colored in red and blue).

GC-MS technology allows for even more precise analysis through the use of multidimensional GCxGC chromatography, which offers significantly better chromatographic resolution. Nevertheless, a simple, one-dimensional GC-MS analysis also provides very insightful results.

The comparison of the terpene content in the three gin varieties is shown in Figure 4. Here, it is also evident that the amount of terpenes – so crucial to the aroma of gin – is significantly lower in the cheaper gin. Figure 5 summarizes the integrated areas of the terpene groups. The terpene content in the more affordable gin is lower; for sesquiterpenes, only trace amounts have been detected, which naturally has a strong impact on the aroma profile.

To sum it all up: In a study comparing 18 gin samples from different countries and price ranges, GC-MS revealed that higher-quality gins contain significantly more volatile compounds that are key to their aroma, especially terpenes like monoterpenes and sesquiterpenes. Cheaper gins tend to have fewer of these compounds, resulting in a less complex scent profile.

Tools to create the tastes of tomorrow

The aroma of gin depends heavily on its chemical composition. Chemical research has begun to identify and better understand how various elements contribute to a gin's flavoring. GC-MS is a powerful and practical tool that can be easily used to analyze the volatile compounds in gin responsible for its aroma and flavor. Overall, GC-MS helps us better understand the connections among a complex mixture of flavors and aromas, production quality, price and the attractive taste profile of the gin in your drink.

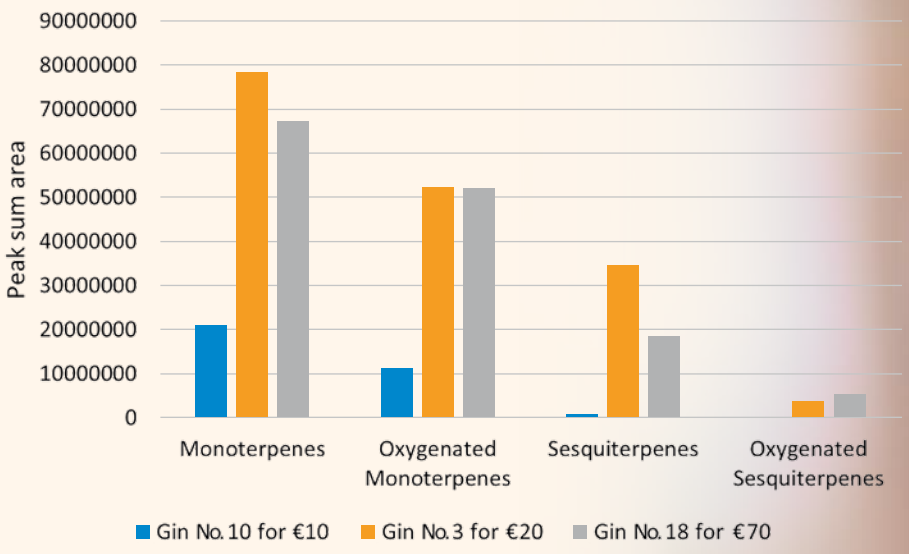


Figure 5: Integrated GC-MS chromatogram area of different terpene groups at *m/z* 93

These findings are of interest to scientists and the beverage industry, as they help improve their understanding of the specific chemical components that contribute to a gin's flavor. GC-MS can also enable producers to more efficiently develop new recipes and to refine their existing products for greater customer appeal.

Note

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

Like putting on spectral glasses to see hidden colors

Solving co-elution challenges for beta-blocker separation with i-PDeA II

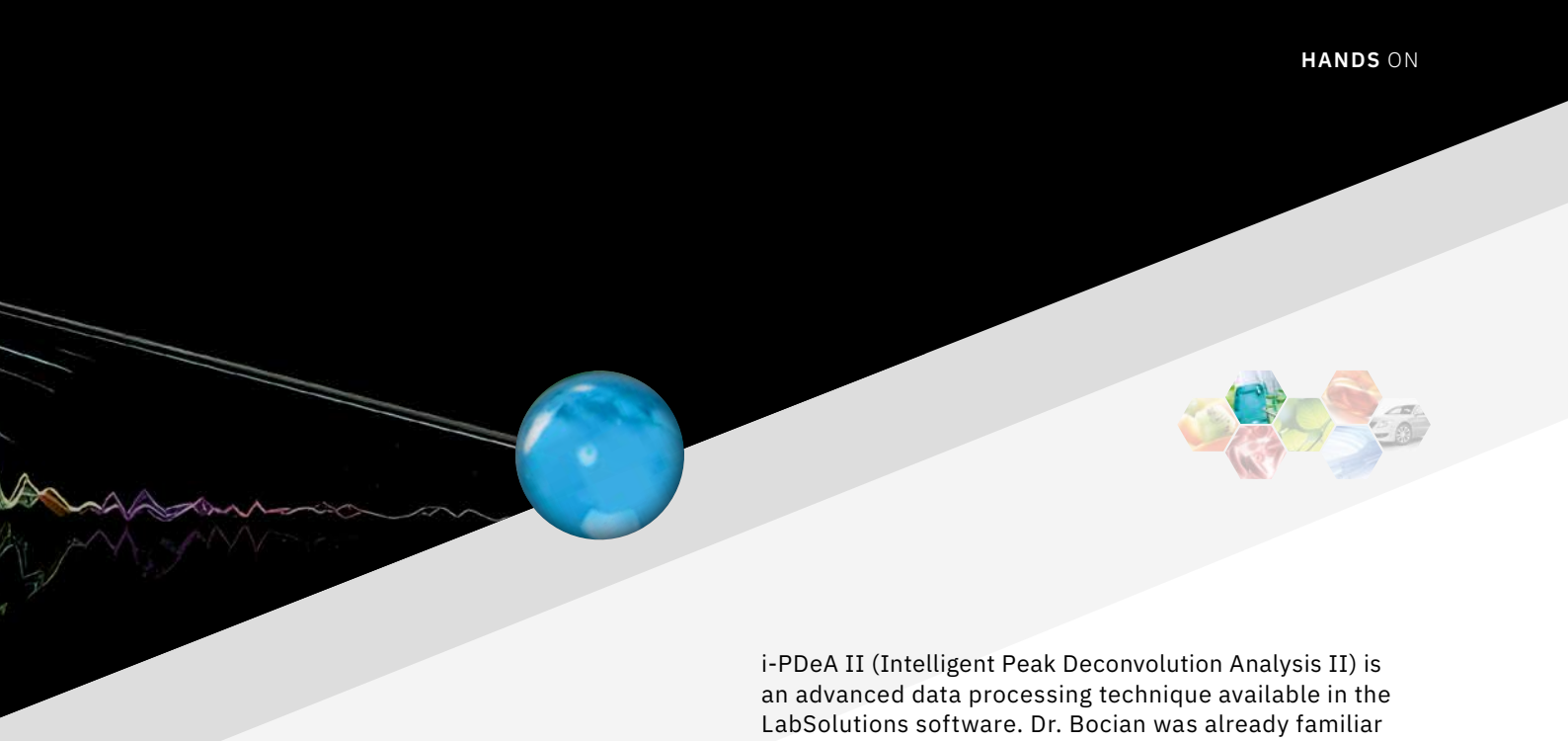
Oktawia Kalisz and DSc Szymon Bocian, Nicolaus Copernicus University, Toruń (Poland)

In liquid chromatography, separating structurally similar compounds like beta-blockers poses a challenge due to frequent co-elution. This article describes an attempt to identify eight beta-blockers within a complex mixture without laboriously injecting each compound separately. The specific goal was to find a faster, more efficient method to resolve overlapping peaks using digital tools.

In the beginning was the challenge ...

Here's the beginning of a story any researcher can relate to: A PhD student in Poland encounters a practical research challenge ... In this case, the challenge was how to identify individual beta-blockers within a complex mixture in a simple and efficient way.

Clearly, she was working in the realm of high-performance liquid chromatography (HPLC), one of the most powerful analytical techniques for separating many complex mixtures of compounds. However, even with optimized chromatographic conditions, analysts frequently encounter the challenge of co-elution, where two or more compounds elute simultaneously, making their individual detection and quantification difficult.



This is particularly true for structurally similar compounds, such as beta-blockers, which exhibit similar retention behaviors.

In the PhD student's case, the challenge became clear from the very first trials on novel phosphodiester stationary phases. Instead of neat, sharp peaks, the chromatograms showed tailing and broad peaks, while several compounds co-eluted into a single signal. Adjusting gradients and modifying the mobile phase brought improvement but it was not sufficient: Some analytes continued to remain hidden under overlapping peaks. What to do?

Looking at the problem from another angle

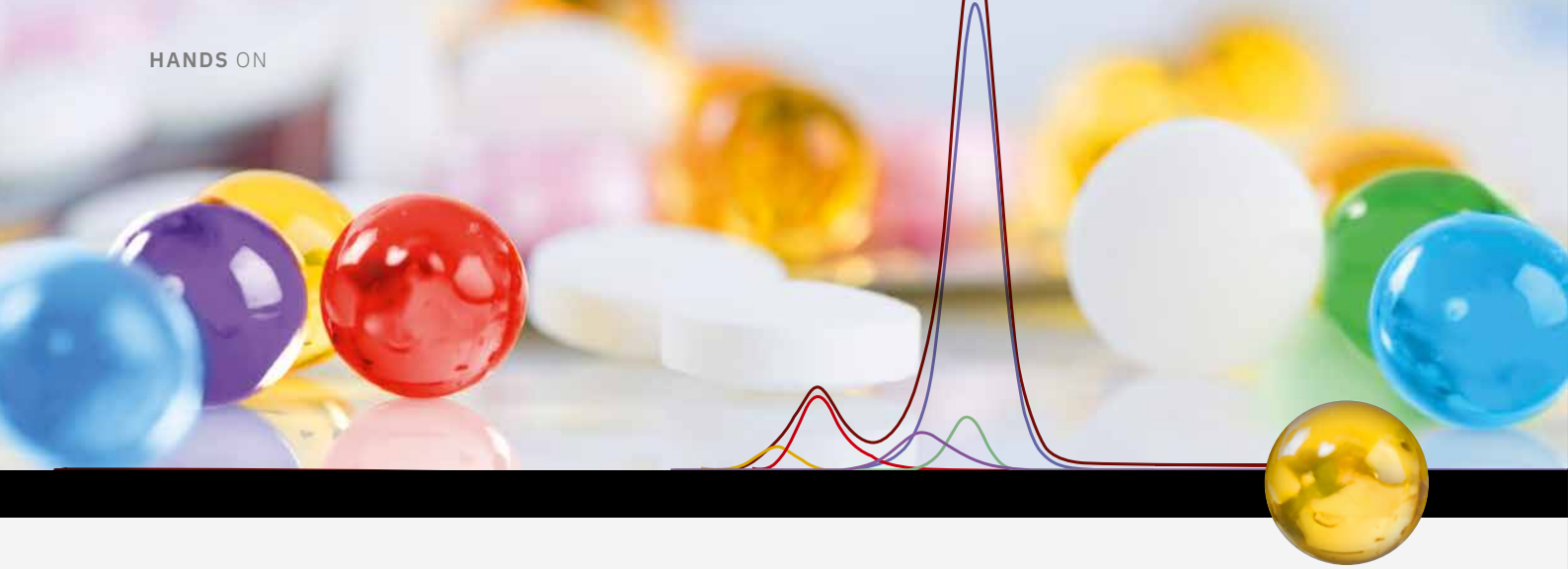
At this point, the PhD student did what anyone might do: She talked with her advisor, who suggested a different approach. *"You don't have to strive for perfect separation if it's not necessary,"* advised Dr. Szymon Bocian (Nicolaus Copernicus University, Toruń, Poland). *"Let's look at the data from a different perspective. Try i-PDeA II."*

i-PDeA II (Intelligent Peak Deconvolution Analysis II) is an advanced data processing technique available in the LabSolutions software. Dr. Bocian was already familiar with i-PDeA II through previous collaboration with Shimadzu. That knowledge led him to suggest a new strategy: Instead of endlessly optimizing the chromatographic conditions, why not use spectral deconvolution to reveal what the chromatogram could not show?

Why not use digital peak separation?

Instead of resorting to extensive method development to achieve baseline separation, the Toruń team now began to use the digital i-PDeA II tool to deconvolute overlapping peaks directly from the acquired chromatographic data. This tool, in combination with photodiode array (PDA) detectors, enabled the use of 3D PDA data to mathematically differentiate overlapping peaks, even if they were not visually separated on the chromatogram.

Instead of relying solely on retention time, i-PDeA II was able to analyze the entire UV-Vis spectral profile on the chromatogram and extract the spectra of the pure compounds that were components of the mixture. This approach not only provided better identification, it also minimized both method development and analysis time. In the case of the PhD student's research, it was a turning point. ⟶



Success no. 1: Resolving oxprenolol and pindolol

One of the most significant challenges the team faced was the co-elution of oxprenolol and pindolol on a Diol-P-C10 stationary phase under HILIC conditions. These compounds had a selectivity factor of only 1.01, meaning their peaks were nearly indistinguishable. Without deconvolution, the detector recorded a single peak, making individual analysis impossible. However, i-PDeA II successfully separated the two components digitally by extracting their individual spectra (see Figure 1).

Success no. 2: Simultaneous deconvolution of five beta-blockers

The application of i-PDeA II also demonstrated that this function is not only effective for resolving two peaks (such as distinguishing an impurity from the main analyte) but also for deconvoluting multiple co-eluted compounds from complex mixtures. In a gradient separation performed on the Diol-P-benzyl stationary phase, five beta blockers – oxprenolol, propranolol, atenolol, acebutolol and cicloprolol – were co-eluted. The i-PDeA II software analyzed the UV spectral differences of each compound, which allowed their identification (see Figure 2, Figure 3).

When traditional separation fails, i-PDeA II succeeds

The PhD student's challenge was that standard HPLC methods could not adequately separate beta-blockers and address the co-elution of structurally similar compounds. This prompted her to look for an innovative solution, which she – with the help of her advisor – found in the integration of i-PDeA II with phosphodiester stationary phases and which were used in this way for the first time in her study.

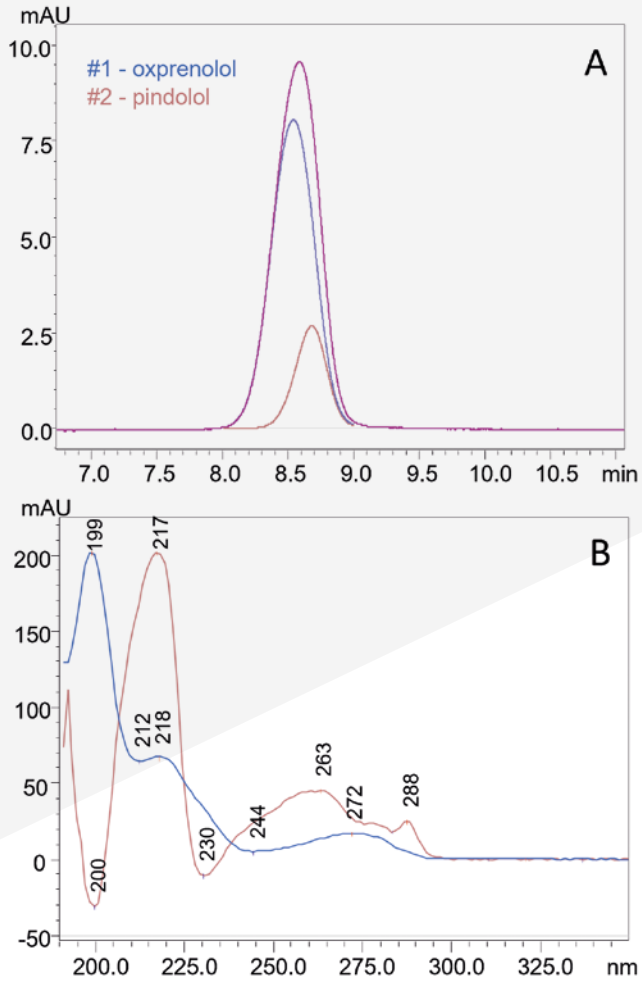
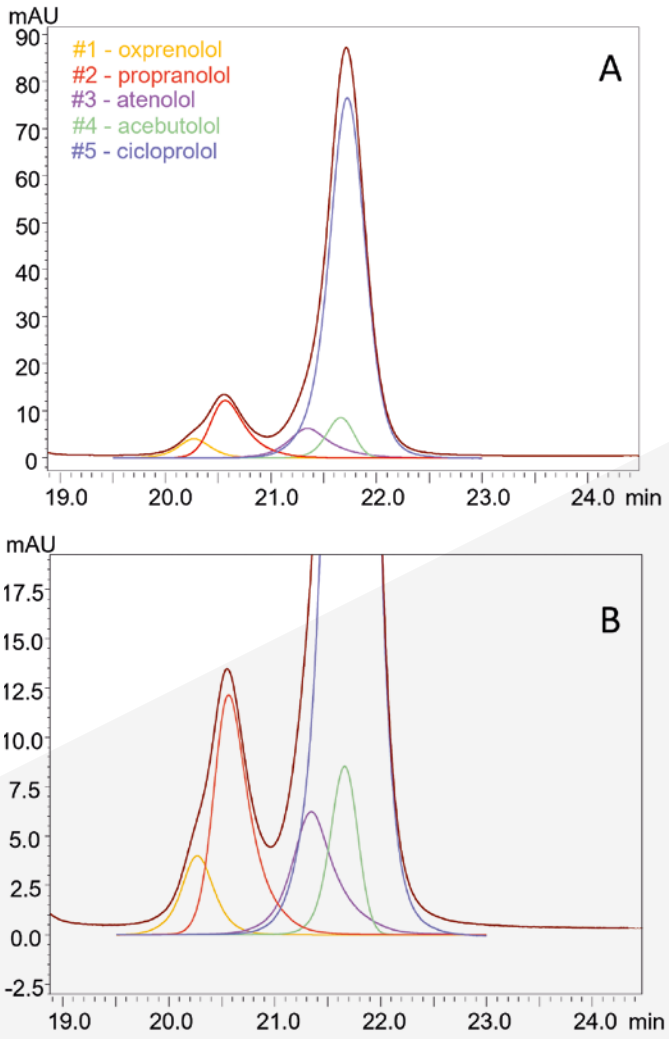
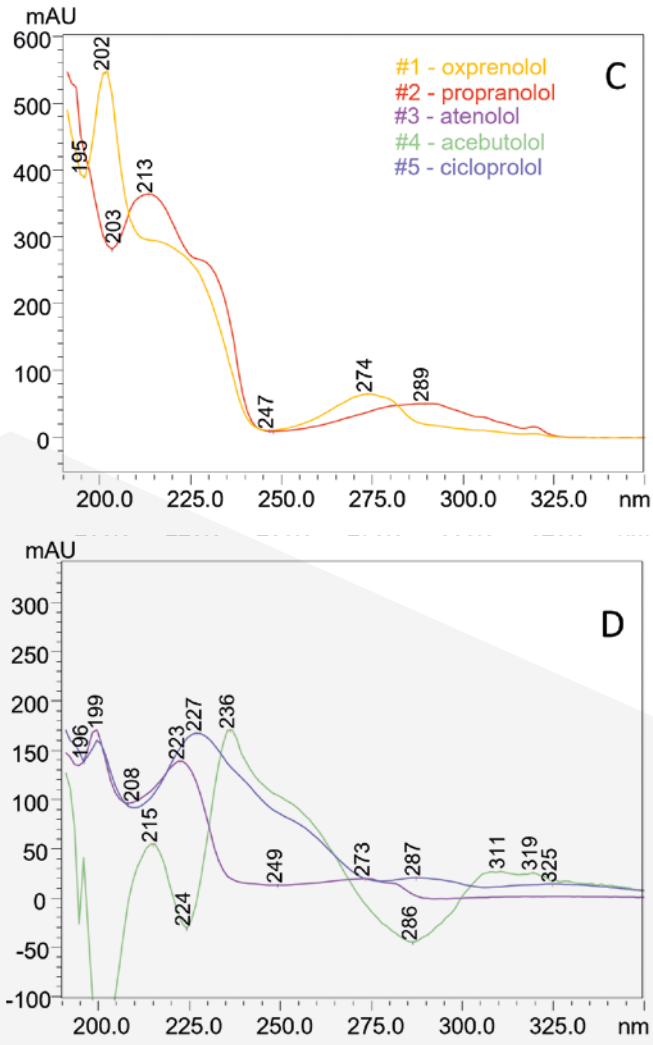


Figure 1: Deconvolution of unseparated oxprenolol and pindolol on the Diol-P-C10 column in 90 % of ACN (A) and corresponding UV spectra (B). The pink line represents the signal obtained from the detector.

▼ Figure 2: Deconvolution of unseparated beta-blockers (A and B, where B is a zoom of chromatogram A) on the Diol-P-benzyl column. The brown line represents the signal obtained from the detector.



▼ Figure 3: UV spectra of compounds identified in the mixture, whose chromatogram is shown in Figure 2



A clear step forward in chromatographic analysis

Among pharmacologically active substances, beta-blockers are a group of drugs used in cardiology. They are also administered to animals during transport to reduce their morbidity, which consequently results in their presence in meat and other animal products. The method developed here provides a beneficial new way to analyze many reference and real samples (blood, urine or wastewater) used in drug metabolism studies, anti-doping tests and trace analysis.

These findings also highlight the broader applicability of i-PDeA II, showcasing its potential as a powerful analytical tool for resolving co-eluting compounds in complex mixtures.

Or, as the PhD student puts it, *"Using i-PDeA II reveals hidden details invisible to the naked eye in traditional LC. It is like putting on spectral glasses that allow you to see individual colors in a beam of light that otherwise appears white."*

Note

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

20 million images per second – built on 20 years of innovation

From the HPV-1 to the HPV-X3: Ultra-fast insights into matter, biology and energy

Dr. Johannes Hesper, Shimadzu Europa GmbH

Figure 1: HyperVision HPV-1

How do atoms move during a chemical reaction? What structures are concealed within viruses? And how does matter behave under extreme conditions, such as those found only in stars or planets? At the European XFEL, the fastest and otherwise invisible processes are brought into the light. The research facility near Hamburg, home to the world's largest X-ray laser, provides ultra-short, high-intensity X-ray flashes – a tool unlocking entirely new opportunities for science and industry. Here, researchers are developing methods to enable and capture these insights – supported by HPV-X2 cameras from Shimadzu, among other things.

Taking ultra-high speed to a new level

Shimadzu's HyperVision HPV-1 transformed the world of high-speed imaging in 2005, marking the beginning of a new era in image capture. With its groundbreaking IS-CCD sensor (in-site storage image sensor), this camera was able to capture an astonishing 1 million frames per second at a resolution of around 81,000 pixels. This remarkable achievement allowed scientists and engineers, for the first time, to see ultra-fast phenomena that were previously invisible, unlocking new opportunities – from materials science to biomedicine. →

Celebrated for its technical innovation, the HPV-1 went on to receive multiple awards. In the year it was launched, it earned the “Best 10 New Products Award” from Nikkan Kogyo Shimbun Ltd., and in 2006 it was honored with an “R&D 100 Award,” which recognizes the world’s most exceptional technological innovations. It was the world’s first ultra-high-speed camera capable of capturing video at an unprecedented speed of 1,000,000 frames per second. These awards highlight the profound impact of the HPV-1 on both industrial development and scientific research.

The HPV series – pushing ultra-high speed one step further

In 2008, Shimadzu introduced the next-generation HPV-2. It offered the same impressive recording speeds as its predecessor, with the added benefits of a lighter, more user-friendly design. The ability to operate in synchronization with up to four cameras facilitated three-dimensional analysis of complex phenomena and advanced research across many scientific fields.

In 2012, the HPV-X series raised the bar: Delivering up to 10 million frames per second – ten times faster than before – the newly developed FTCMOS sensor (Frame Transfer CMOS) provided enhanced image quality and versatile recording modes for all kinds of experiments. For the first time ever, it was possible to capture 256 frames. These cameras played a vital role in the development of new materials and the study of ultra-fast physical processes.

Just a few years later, the next evolution arrived: the HPV-X2. With the upgraded FTCMOS2 chip, it achieved six times greater light sensitivity without sacrificing the recording speed of the HPV-X. This made low-light experiments possible for the first time – even with ultra-short exposure times of only 50 nanoseconds.

HPV-X3 – the latest generation with 20 million frames per second

Introduced in 2025, the HPV-X3 doubles the recording speed to an extraordinary 20 million fps and triples the resolution to 300,000 pixels – no matter the speed. This allows researchers to observe the finest details of ultra-fast processes and gain new insights across numerous fields of study. Utilizing external time synchronization signals also significantly improves data acquisition efficiency.

Figure 2: HyperVision HPV-X3

What sets the HPV-X3 apart from other high-speed cameras is its cutting-edge memory and processing architecture. While other high-speed cameras must store frames on an external drive during recording, often compromising speed or resolution, the HPV-X3 reads the image data from the FTCMOS chip only after the experiment. This enables faster, more efficient processing and is especially advantageous in complex experiments where precise timing is critical.

High speed for science and technology

In addition to research facilities like the European XFEL, ultra-high-speed cameras are used in many other areas of science and technology, particularly in materials research and biomedicine. In materials research, they make it possible to study how materials respond to extreme forces. An impressive example is the study of carbon-fiber reinforced polymers (CFRP): As the samples are stretched, the precise instant of material failure is captured in real time. Studying how cracks develop provides valuable insights into the strength and durability of these materials – crucial for aerospace engineering.

Making the invisible ultra-fast visible

The continuous evolution of the HPV series demonstrates the importance of ultra-high-speed cameras for science and technology. From the pioneering HPV-1 to the latest HPV-X3, Shimadzu has always led the way in technological innovation. By capturing microscopic, ultra-fast phenomena, these cameras deliver priceless insights for research and open the door to practical applications across a wide range of fields.

The HPV-X3, with its unparalleled capability of capturing up to 20 million frames per second, highlights the critical importance of precise observation and analysis of the dynamic world around us today. Be it aerospace, materials research or microscopic cavitation – anything less just can't keep up!

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

Revealing the hidden structural diversity of lipids

Exploring next-generation lipid isomer analysis

Dr. Hidenori Takahashi, Shimadzu Corporation

Conventional MS/MS techniques struggle to determine the positions of carbon–carbon double bonds (C=C) in lipid molecules, making it difficult to distinguish structural isomers. A researcher set out on an ambitious path to develop a more effective way, and one that remained compatible with existing LC-MS workflows and applicable to both ESI and MALDI platforms.

Lipids are vital biomolecules found in every cell of the body and are involved in membrane structure, signal transduction and energy storage. Because of this, they are important in a variety of fields, including nutrition, cosmetics, pharmacology and medicine.

Lipid structural isomers are molecules with the same chemical formula but different arrangements of atoms and arise from variations in carbon–carbon double bond (C=C) positions, sn-positions, and cis/trans configurations.[1] In particular, C=C positional differences, such as those defining omega-3 and omega-6 fatty acids, are associated with diverse biological functions and diseases.[2]

Ways to analyze lipids

Although gas chromatography–mass spectrometry (GC-MS) has been widely used for fatty acid analysis, derivatization to fatty acid methyl esters (FAMEs) often results in the loss of lipid class-specific information.

Liquid chromatography–mass spectrometry (LC-MS), on the other hand, enables intact lipid analysis but lacks structural detail using conventional low-energy collision-induced dissociation (CID), especially for C=C positions.

In the Shimadzu lab of Koichi Tanaka (Nobel Prize in Chemistry, 2002), a new ion dissociation method had previously been developed – Oxygen Attachment Dissociation (OAD) – which utilizes neutral atomic oxygen (O) and hydroxyl radicals (OH•) for radical-induced fragmentation.[5] Radicals are generated by microwave discharge of a water vapor and hydrogen gas mixture under vacuum (Figure 1). Since O/OH• radicals are a charge-neutral species, they are unaffected by electric fields and can be introduced directly into the CID cell (Q2) of a quadrupole mass spectrometer. They do not alter ion charge states, making the method broadly applicable to singly charged or negatively charged ions.

The question though was whether – and how – OAD could be applied to lipid structural isomers.

A new method for an old problem

To demonstrate the applicability of OAD for lipid structural analysis, a researcher at the Tanaka lab analyzed human plasma (NIST SRM 1980, Millipore Sigma) following extraction by the Bligh and Dyer method. Extracts were dried under nitrogen and reconstituted in methanol. LC separation was performed on a Nexera UHPLC system (Shimadzu) using a C18 column (50 × 2.1 mm, 1.7 µm) at 45 °C and 0.3 mL/min. Mobile phase A was ACN:MeOH:H_2O (1:1:3, v/v/v), and B was IPA, both containing 5 mM ammonium acetate and 10 nM EDTA. The total analysis time, including column equilibration, was 25 minutes, and the details followed those described in reference.[6] MS analysis was conducted with an LCMS-9050 Q-TOF (Shimadzu) equipped with the OAD Radical Source I (Shimadzu) in ESI mode. ⟶

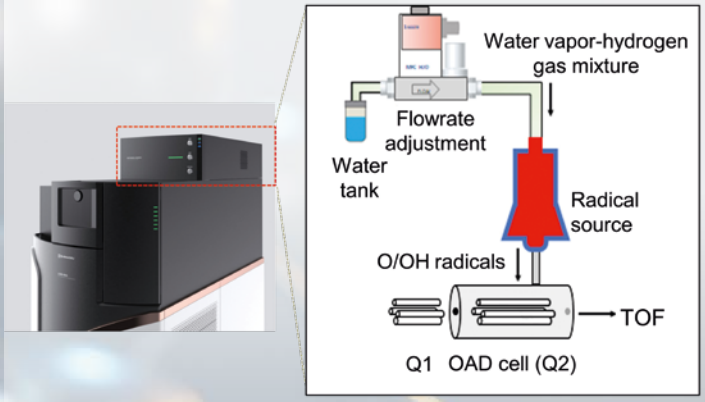


Figure 1: Schematic diagram of OAD system

Achieving differentiation of lipid isomers based on C=C position

Figure 2 shows the OAD-MS/MS spectrum of the target lipid PC 18:1(n-9)_18:1(n-9), where "PC" denotes the phosphatidylcholine lipid class, "18" indicates the number of carbons in each acyl chain, ":1" indicates the number of C=C bonds, n-9 specifies the C=C position relative to the methyl terminus, and the underscore "_" separates the two acyl chains. OAD induces selective fragmentation targeting the C=C bond, resulting in two characteristic fragment peaks (OAD2 and OAD15), as shown in Figure 2. Table 1 summarizes the neutral losses corresponding to OAD fragments for each C=C position.

In targeted analysis, these predicted OAD neutral losses can be preregistered in a compound table, and extracted ion chromatograms (XICs) can be used to differentiate structural isomers. Moreover, when collision energy (CE) is applied simultaneously in OAD, CID-type neutral losses of acyl chains can also be detected.[7] These neutral losses enable the identification of acyl chain length and the number of C=C bonds.[8] Thus, the combination of OAD- and CID-derived fragments allows comprehensive structural elucidation of lipids, including C=C positional information.

Figure 3 shows the XICs of PC 32:2 ([M+H]+ = *m/z* 730.539) obtained using OAD. "32:2" indicates that the two acyl chains contain a total of 32 carbon atoms and two C=C bonds. Figure 3(a) shows the XIC for CID-based acyl chain losses, while Figure 3(b) displays the XICs for OAD-neutral losses corresponding to different C=C positions. Based on Figure 3(a), possible acyl chains such as C18:2, C16:1 and C14:0 were inferred, with structural candidates including PC 18:2_14:0 and PC 16:1_16:1.

From Figure 3(b), C=C positions at n-6, n-7 and n-9′ (where ′ indicates the second C=C from the methyl terminus) were detected. Combining the information from both figures, the acyl chain compositions were determined as PC 18:2(n-6, 9)_14:0 and PC 16:1(n-7)_16:1(n-7).

In a previous study, the sensitivity and quantitative performance of OAD were evaluated using a deuterium-labeled internal standard, PC 15:0/18:1(d7), showing good linearity (R^2 = 0.9921) across a concentration range from 50 fmol to 10 pmol.[7] These results demonstrate that OAD is not only highly specific in terms of structural analysis but also suitable for quantitative applications.

Contributing greater clarity

Discovering the beauty of OAD is like switching from a low-resolution to a high-resolution telescope – what once looked like a single star now reveals itself to be a cluster of many distinct stars. Conventional methods view lipid isomers as one object, but OAD reveals their hidden structural diversity by pinpointing the positions of double

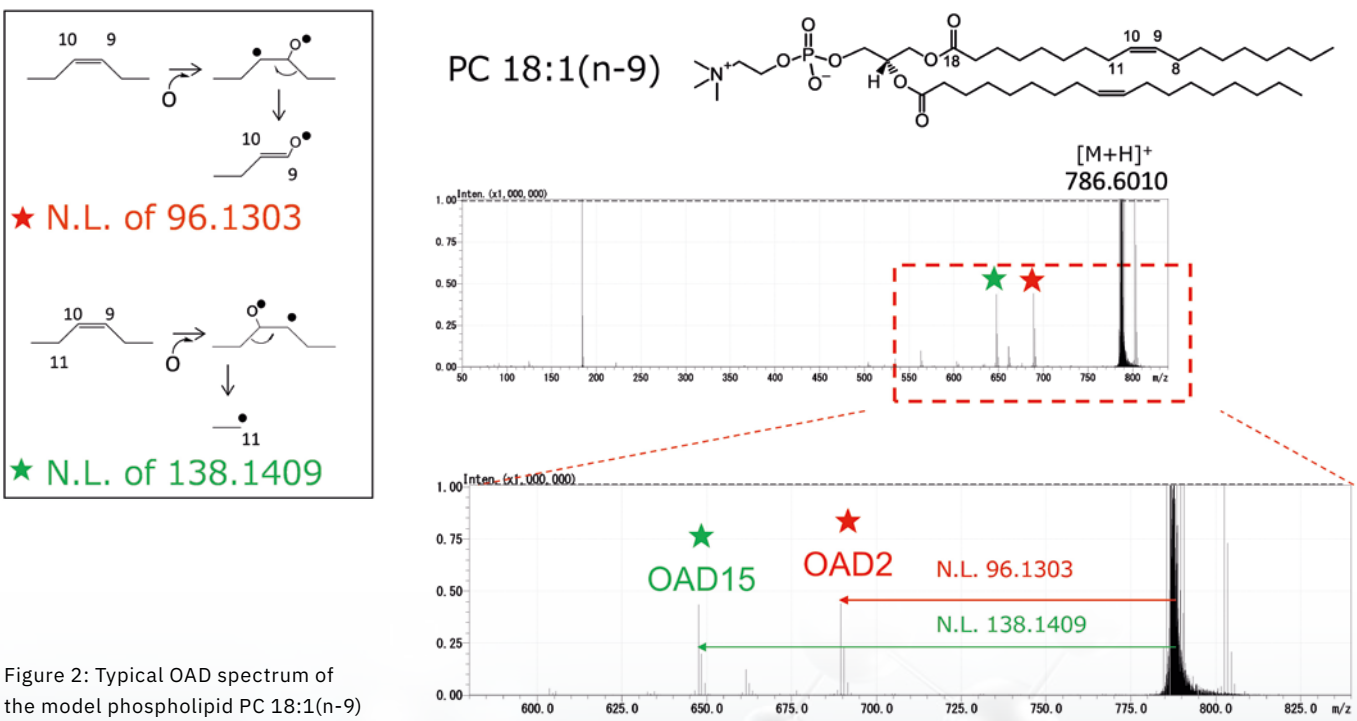
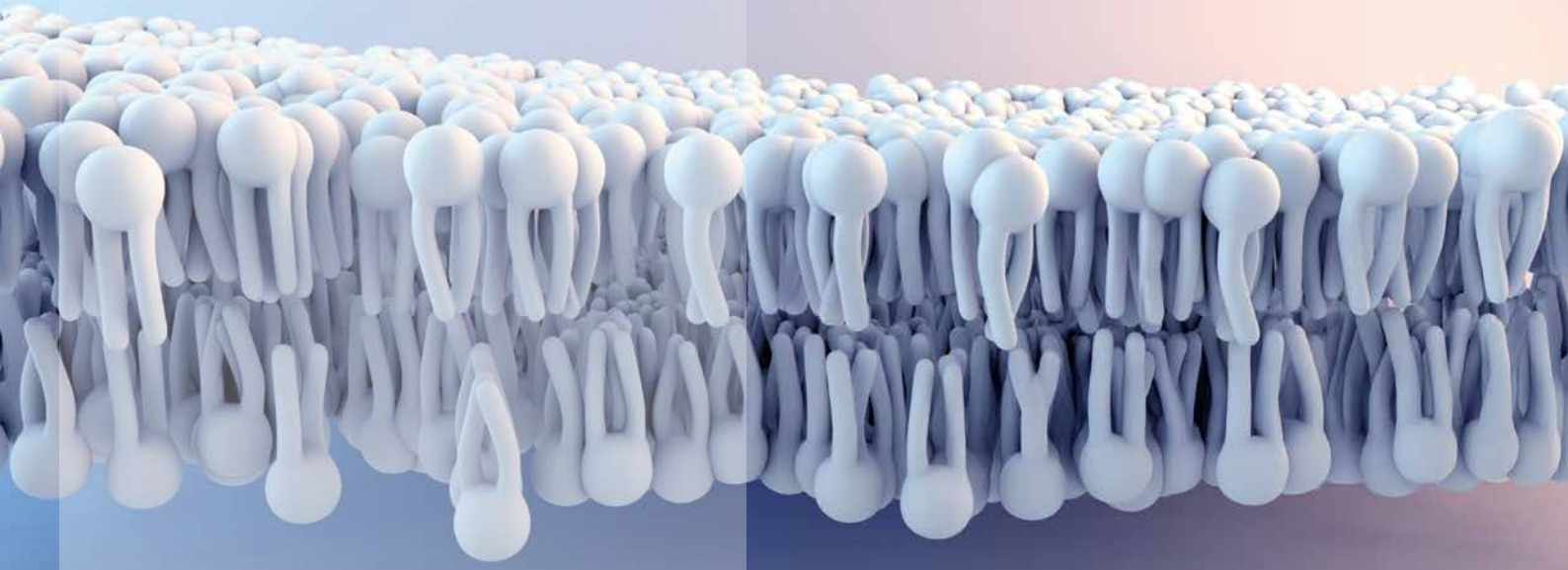


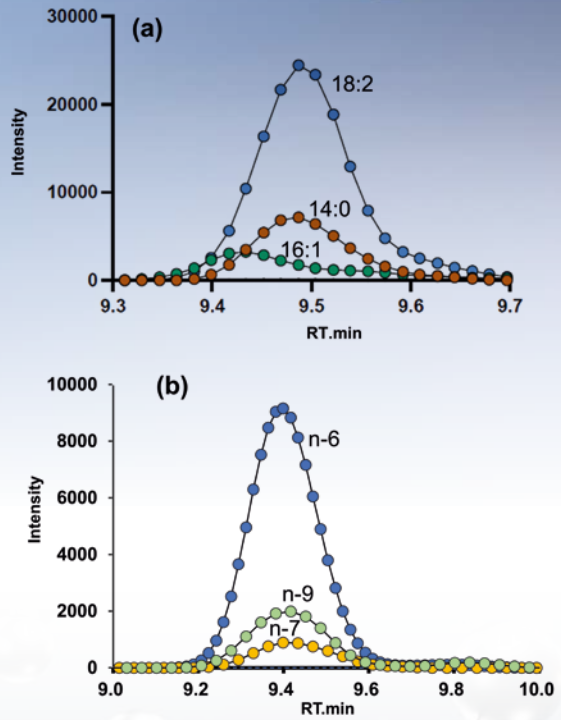
Figure 2: Typical OAD spectrum of the model phospholipid PC 18:1(n-9)

bonds. This improved “molecular resolution” is essential, because even subtle differences in C=C locations can significantly impact biological function.

This development will be valuable for researchers in clinical and pharmaceutical lipidomics, as lipid structure is closely related to disease mechanisms, drug response and metabolic disorders. In addition, it is also relevant to food science, where understanding lipid composition and isomer distribution contributes to nutritional research and food quality assessment.

Importantly, OAD has also been successfully applied to MALDI imaging mass spectrometry using the iMScope system. This advancement enables spatial visualization of lipid isomers in tissue sections, extending the applicability of OAD beyond ESI-based workflows. Overall, OAD holds great promise as a new standard method for lipid structural analysis and is expected to contribute significantly to future advancements in lipidomics, both in research and clinical applications.





	OAD2	OAD15
n-3	–12.0364	–54.0470
n-4	–26.0520	–68.0626
n-5	–40.0677	–82.0783
n-6	–54.0833	–96.0939
n-7	–68.0990	–110.1096
n-8	–82.1146	–124.1252
n-9	–96.1303	–138.1409
n-10	–110.1459	–152.1565

Table 1: Correspondence between C=C positions and neutral loss peaks in OAD fragmentation

Note

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

◄ Figure 3: Extracted ion chromatograms (XICs) of PC 32:2 ([M+H]+ = *m/z* 730.539) obtained by OAD-MS/MS analysis of human plasma. (a) CID-based XICs showing neutral losses corresponding to fatty acyl chains, including C18:2, C16:1 and C14:0. (b) OAD-based XICs displaying characteristic neutral losses specific to C=C positions, indicating n-6, n-7 and n-9′ isomers.

Raising the profile of what we drink – and eat

Improving analysis of amino acids by an automatic pre-column derivatization method

Natsuki Iwata, Shimadzu Corporation

Vadim Kraft, Shimadzu Europe GmbH

Testing food and drink for safety, authenticity, quality and nutritional content is vital to maintaining public health, consumer confidence and business success. A recent proof-of-concept experiment on alcoholic beverages confirmed a streamlined new method for amino-acid assessment that is applicable to other food and drink as well. The method delivers increased accuracy while reducing testing runtimes by up to half.

We've all seen the stories: the 100 % ground beef that contained horsemeat, the fake fruit juice, the expensive branded whisky that was counterfeit, the adulterated honey. Or the 2009 scandal where melamine was added to milk and infant formula to make them appear richer in protein ... and which tragically led to illness and death.

Food and beverage testing helps ensure that regulatory standards are met. Amino-acid testing is one of more sophisticated tools that scientists use to check the quality, safety and authenticity of what we eat and drink, as well as to assess the quantities of various components – protein, for example – for trustworthy nutritional labeling. Amino-acid assessment provides reliable identification – a sophisticated fingerprint – that reveals whether a food product contains what it is supposed to.

Natsuki Iwata works in Kyoto, Japan, and is an expert in the field of amino-acid testing using high-performance liquid chromatography (HPLC), with numerous related patents, awards and publications. She recently conducted an experiment using amino-acid profiling on alcoholic beverages.

Amino acids and their uses

Amino acids exist as two mirror-image forms, known as D- and L-enantiomers. D-amino acids offer distinctive flavor characteristics compared to their L-forms. The specific balance of D- and L-amino acids in foods contributes to the overall complexity of taste.

The ratio of D- to L-amino acids in foods is also useful for evaluating processing techniques and product authenticity, particularly in fermented or aged foods.

Accurately separating and measuring D- and L-amino acids is therefore important – increasingly so – in food science and clinical applications. But traditional analysis methods such as liquid chromatography-mass spectrometry (LC-MS) and multi-dimensional HPLC have limitations. LC-MS may be affected by sample matrix complexities, for instance, and multi-dimensional systems can be time-consuming and complicated to operate. So there is a clear need for analytical techniques that are fast, straightforward and reliable for D-/L-amino acid quantification.

The method investigated in Natsuki Iwata's study was based on a derivatization reaction of o-phthalaldehyde (OPA) and the chiral thiol N-isobutyryl-L-cysteine (NIBC), followed by analysis using liquid chromatography.

Can better technology lead to better methods?

Acutely aware of the great evolution in liquid chromatography in recent years, Natsuki Iwata reasoned that state-of-the-art UHPLC (Ultra HPLC) instruments should enable fully automatic derivatization and highly efficient separation of compounds. So that is what she set out to test.

Two kinds of beer (beer A and B), sake, red wine and white wine were used as samples. Beer A, beer B, red wine and white wine were diluted ten-fold (twenty-fold for sake) with 10 mmol/L hydrochloric acid and then passed through 0.2 µm PTFE membrane filters. →

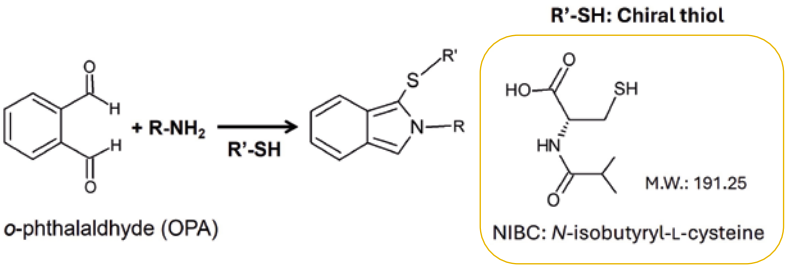


Figure 1: OPA fluorescence derivatization

1	**OPA reagent** Add 0.3 mL of ethanol into 10 mg of *o*-phthalaldehyde and dissolve completely. Then add 0.7 mL of 0.1 mol/L borate buffer (pH 9.1) and 4 mL of ultra-pure water.
2	**NIBC solution** Add 10 mg of *N*-isobutyryl-l-cysteine into 10 mL of 0.1 mol/L borate buffer (pH 9.1).
3	**OPA/NIBC solution** Mix equal volume of OPA reagent and NIBC solution.

Table 1: Preparation of derivatizing reagent

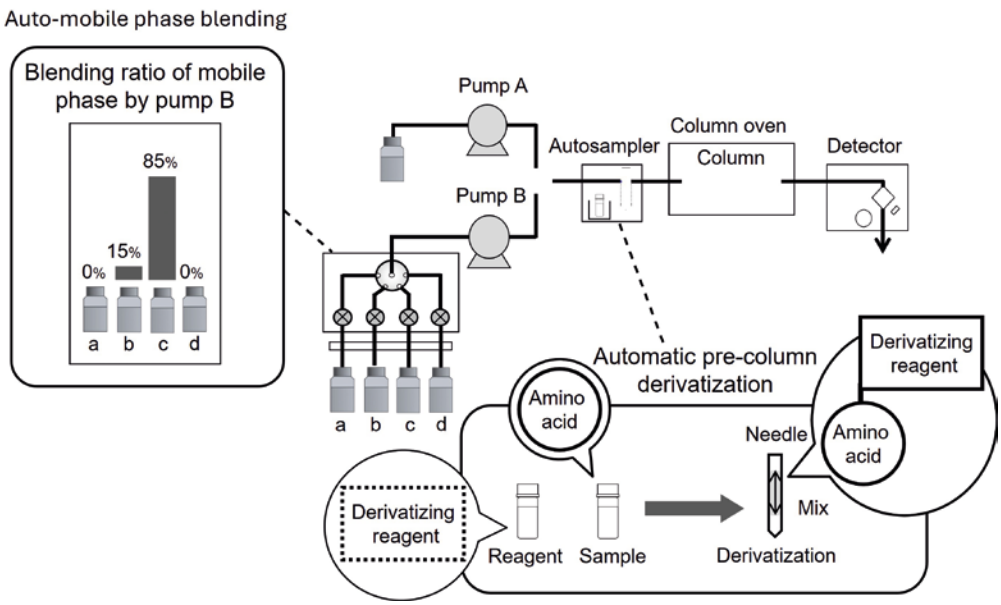


Figure 2: Flow diagram of automated HPLC setup

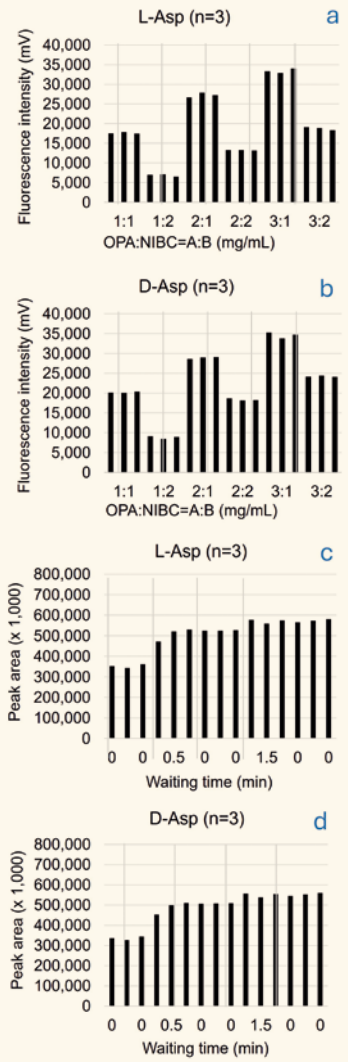


Figure 3: Optimization of OPA: NIBC ratio and reaction time

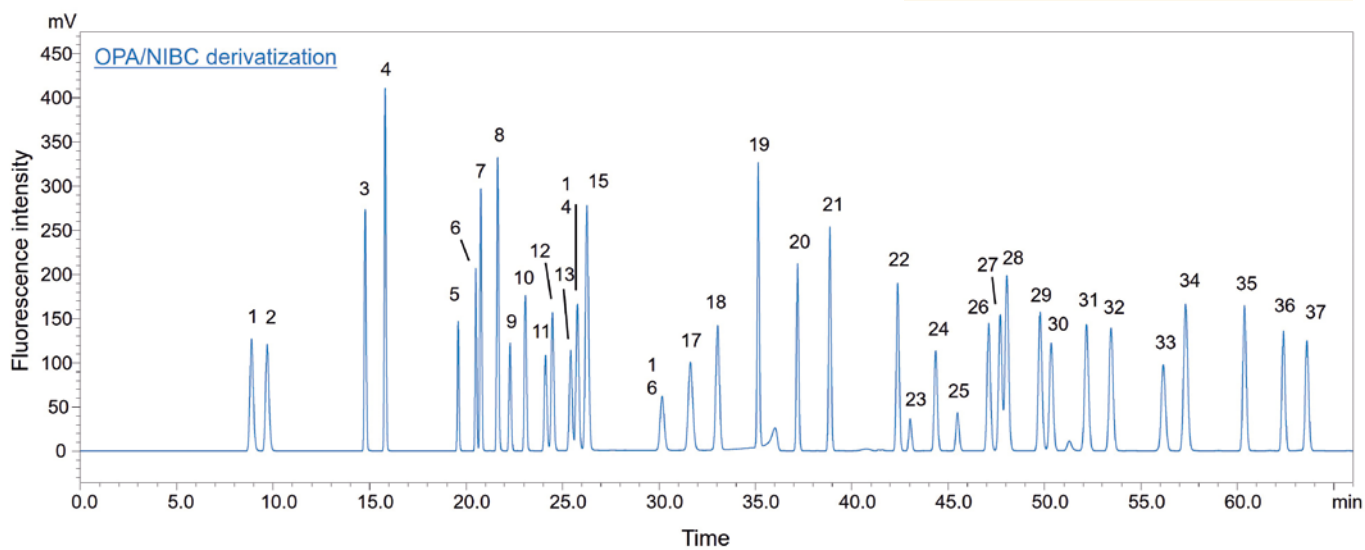


Figure 4: Chromatograms of a standard solution of D-/L-amino acids (5 µmol/L each)

Careful preparation is essential

Fluorescence derivatization of the diastereomers was performed by the reaction with OPA under NIBC conditions. A standard (Shimadzu) UHPLC instrument equipped with a fluorescence detector was used for determination of 37 D-/L-amino acids (Figure 2), and the eMSTAT – Shimadzu's Easy Mass Spectrometric Statistical Solution – was applied for principal component analysis (PCA). The analytical conditions are presented in Table 2. Tables 1 and 3 show the details to the preparation of the reagents and the derivatization procedure of the autosampler.

The optimum combination in concentration of the OPA reagent and NIBC solution was examined using a standard solution of D-/L-amino acids (1 µmol/L each). Therefore, the combination of 2 mg/mL for OPA and 1 mg/mL for NIBC was adopted as the optimum concentration (Figure 3a and 3b).

To promote the derivatization reaction and obtain good repeatability, a constant waiting time after mixing the OPA/NIBC solution and the sample within the injection needle of the autosampler was introduced into the pre-treatment program. Five different waiting times were examined in advance. As the peak area was constant after 1.5 minutes or more, the waiting time was set to 1.5 minutes (Figure 3c and 3d).

Putting the method to the test: Analysis of a standard solution of 37 D-/L-amino acids

A previously reported method with two switching conditions required an analysis time of 120 minutes per sample.[8] In that method, two chiral thiols of N-acetyl-L-cysteine (NAC) and NIBC were independently applied for the reactions. Their products with amino acids were separated and the results from both chromatograms were combined, with a total analysis time of 60 minutes each.

For her work, however, Natsuki Iwata decided to use NIBC alone for the reaction. By increasing the methanol ratio in the mobile phase, setting the column temperature to 20 °C and separation on a unique column, it was possible to separate all 37 diastereomers of D- and L-amino acids in a single analysis within just 66 minutes (Figure 4), cutting overall analysis time almost in half. An overview of the separated amino acids is provided in Table 4. Table 5 shows the description of abbreviations of amino acids.

System	Nexera™ X3
Column	CERI L-column3 C18 (150 mm × 2.1 mm I.D., 2.0 µm) using pre-column filter
Flow rate	0.22 mL/min
Mobile phase	[Pump A] 10 mmol/L (sodium) phosphate buffer (pH 6.9) [Pump B] B) Acetonitrile, C) Methanol B/C = 15:85 using mobile phase blending function
Time program	Gradient elution
Column temp.	20 °C
Injection volume	1 µL
Detection	FL Ex: 338 nm, Em: 455 nm

▲ Table 2: Analytical conditions

1	OPA/NIBC solution 2 µL
2	Sample 1 µL
3	Mix
4	Wait 1.5 minutes
5	Injection

◀ Table 3: Automatic pre-column derivatization

1	L-Asp	20	L-Tyr
2	D-Asp	21	D-Tyr
3	L-Glu	22	L-Val
4	D-Glu	23	L-$(Cys)_2$
5	L-Asn	24	L-Met
6	D-Asn	25	D-$(Cys)_2$
7	L-Ser	26	L-Trp
8	D-Ser	27	D-Met
9	L-Gln	28	D-Val
10	D-Gln	29	L-Ile
11	L-His	30	L-Phe
12	L-Thr	31	D-Trp
13	D-His	32	D-Phe
14	D-Thr	33	L-Leu
15	Gly	34	D-Ile
16	L-Arg	35	D-Leu
17	D-Arg	36	L-Lys
18	L-Ala	37	D-Lys
19	D-Ala		

Table 4: Target compounds

Asp	Aspartic acid
Glu	Glutamic acid
Asn	Asparagine
Ser	Serine
Gln	Glutamine
His	Histidine
Thr	Threonine
Gly	Glycine
Arg	Arginine
Ala	Alanine
Tyr	Tyrosine
Val	Valine
$(Cys)_2$	Cystine
Met	Methionine
Trp	Tryptophan
Ile	Isoleucine
Phe	Phenylalanine
Leu	Leucine
Lys	Lysine

Table 5: Description of abbreviations

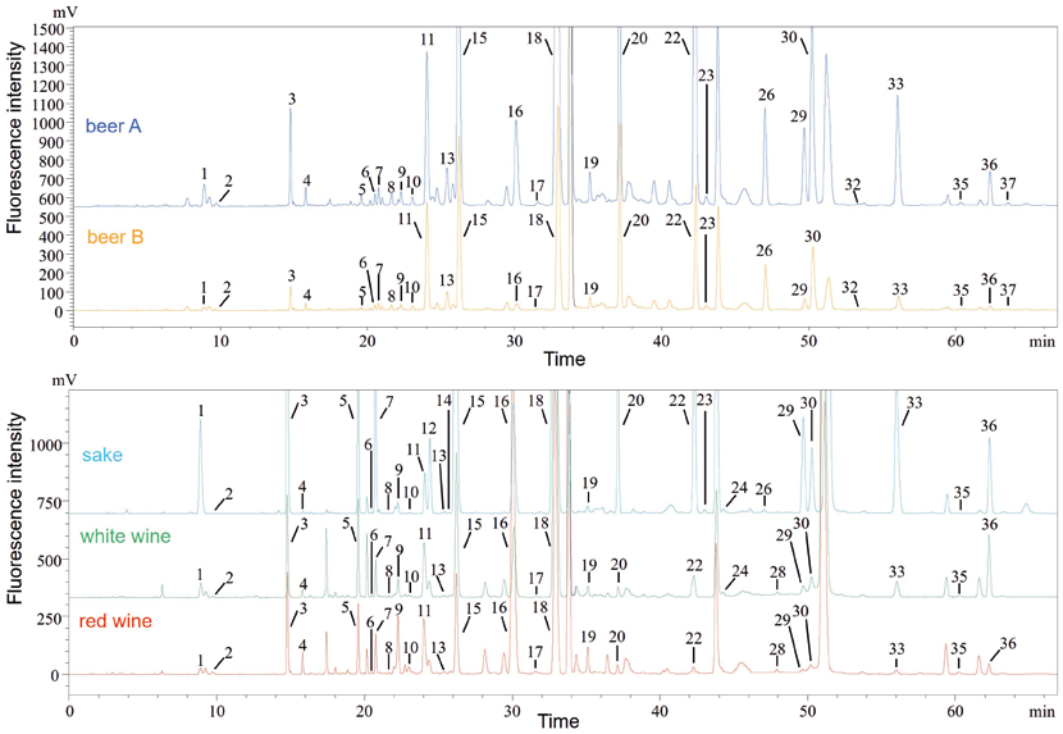


Figure 5: Chromatograms of OPA/NIBC-derivatized diastereomers in alcoholic beverage

Validating the method

The relative standard deviations (%RSD, n = 6) of the peak areas for a standard mixture of D-/L-amino acids (0.1 µmol/L each) were 1.6 % or less, indicating excellent repeatability. This high level of consistency was achieved through the automatic pre-column derivatization, which ensured a constant derivatization time. Calibration curves for all 37 D-/L-amino acids demonstrated good linearity, with coefficients of determination (r^2) of 0.999 or higher. For recovery testing, beer B was selected and spiked with a standard solution containing 31 D-/L-amino acids. The final concentration was set to 1 µmol/L for most amino acids. Six amino acids were instead adjusted to a final concentration of 10 µmol/L each. The six samples were simultaneously pretreated following the established pre-treatment protocol. The recovery rates ranged from 84.9 % to 108.6 %, with %RSD values between 0.8 % and 9.5 %, demonstrating the method's reliability and accuracy.

Applying the method to alcoholic beverages

As shown in Figure 5, 25 to 28 amino acids were separated and detected in five samples. The overall ratio of D-amino acid to D-/L-amino acid (%D) was found to be 6 % or less in all samples. The determination results of D/L abundance ratios of specific amino acids were close to those of the previous studies, using HPLC [9] and GC-MS [10] for various real-sample analyses.

A PCA was performed using the content of each compound (n = 4) in beverages (Figure 6). As a result of the PCA, beer A and beer B were plotted close together on the score plot as well as red and white wines. It was also found that sake showed different characteristics from these beverages. The loading plot revealed that the two kinds of beer as well as the red and white wines contained many D-isomers, while the sake was rich in L-isomers. In particular, L-Trp, D-His and D-Lys contributed significantly to the two kinds of beer, D-Val to the red and white wines and L-Thr to the sake. This suggested that the first principal component (PC1) showed differences in the types of beverages, while the second principal component (PC2) revealed differences in the isomers.

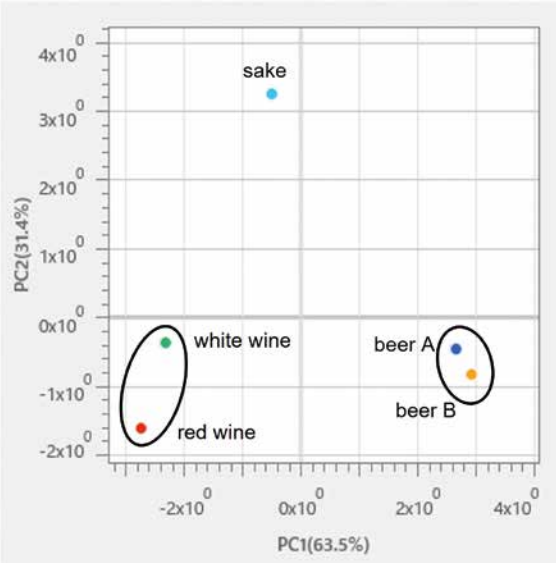
The road to better results

A new method has been developed for the simultaneous separation of 37 OPA/NIBC-derivatized D- and L-amino acids using a straightforward UHPLC system. This method achieves processing results in about half the time required by conventional techniques via a single analysis with automated sample handling and streamlined data.

Besides, it demonstrates strong potential for application in alcoholic beverage profiling and, by extension, for widespread use throughout the entire food and beverage industry.

Rising consumer expectations and greater regulations can now be matched by a highly accurate and reliable, more efficient method for analyzing amino acids, reducing processing time and improving quality control and profiling in the food and beverage industry. It was made possible by progress in technology and by the dedicated curiosity of experts such as Shimadzu's Natsuki Iwata.

(a) Score plot



(b) Loading plot

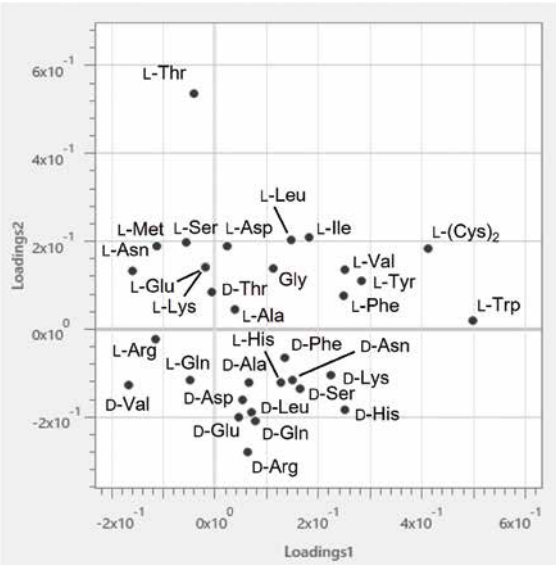


Figure 6: Results of the PCA (n = 4)

Note

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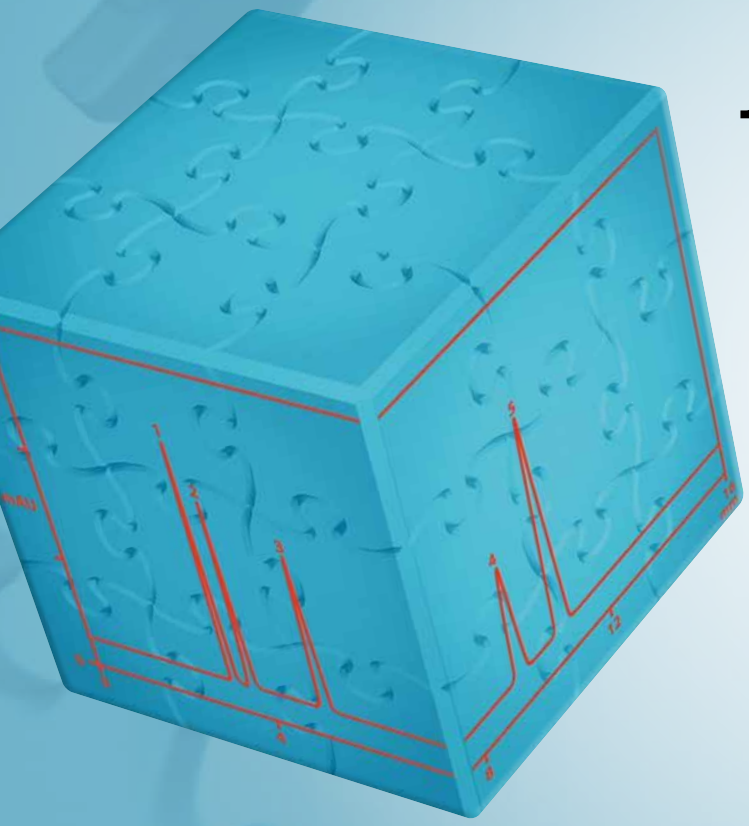


Turning thousands of puzzle pieces into a clear picture

Developing successful methods for complex sample analysis

Dr. Martin Meyer, Shimadzu Europa GmbH

Modern high-performance liquid chromatography (HPLC) is the preferred technique for analyzing complex samples. Its range of applications is remarkably broad – from determining the composition of food products to detecting unwanted impurities in pharmaceuticals, and even identifying trace levels of contaminants in drinking water. For every new question, a tailored separation method must be developed – a process much like piecing together an intricate puzzle. By following a systematic approach, what begins as a maze of endless possibilities gradually takes shape until the full picture emerges.

Structured approach for faster results

If you've ever worked on a tricky puzzle, you know that – to solve one that's truly grand, you always need a careful plan. It's the same when it comes to developing a separation method. Much like starting a puzzle by selecting the pieces with the most obvious differences – corners, edges or brightly colored sections – you start by defining the framework and examining which parameters are even feasible. The foundation is a three-step approach: screening, data evaluation, optimization.

Systematic screening: Column, mobile phase, solvent

Sorting and preselecting the puzzle pieces: It makes sense to start with a rough screening to narrow down the options before getting lost in the details. A key element is the column, which should be chosen based on the properties of the compound you're analyzing.

C18 columns are often the first choice, as they're suitable for nonpolar to moderately polar organic compounds, covering a wide range of substances. Highly polar compounds, on the other hand, are best separated using

polar columns such as amino or silica columns. An interesting alternative is the HILIC method (Hydrophilic Interaction Liquid Chromatography), which is particularly suited for highly polar compounds.

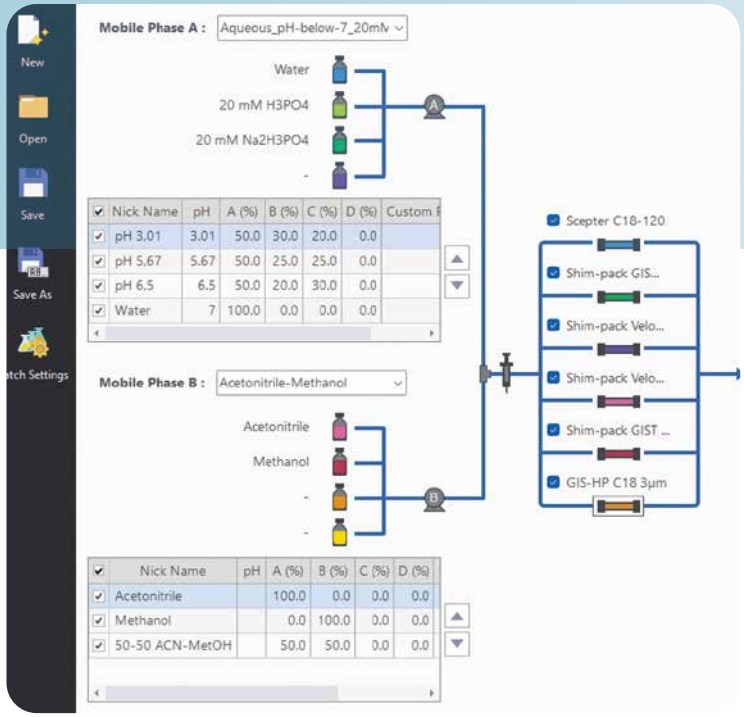
Shimadzu's Shim-pack G series is a great starting point since its range of columns includes 15 different stationary phases designed to address diverse applications. This variety makes it easier to tailor the column selection to the specific requirements of the sample.

Besides the column, the choice of the mobile phase is equally important. The composition of the mobile phase influences not just retention but also peak shape and the reproducibility of the method. Additives such as acids and bases can greatly improve separation performance, while buffered solutions help maintain a stable pH environment. The pH value is especially important for ionizable compounds. Columns with high pH resistance, such as the Shim-pack Scepter series, offer maximum flexibility since they can be used across a pH range of 1 to 12.

Shimadzu also offers various method development column kits that make column selection for method development particularly easy.

Selecting the right solvent is another important step. Acetonitrile often allows for faster methods and sharper peaks, while methanol is more environmentally friendly.

Screening should be performed either isocratically, with constant solvent ratios, or using a simple linear gradient that transitions from mostly aqueous to mostly organic mobile phase. This ratio would be reversed with a HILIC method (Figure 1). →



◀ Figure 1:
Selection of mobile phases and columns in LabSolutions MD

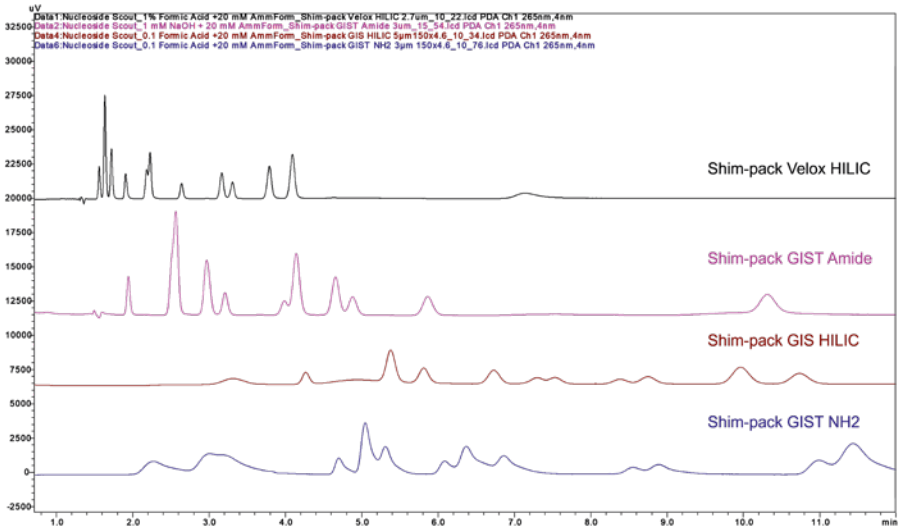


Figure 2: Comparison of the best chromatograms on each column for screening a HILIC method

Response			
Minimum resolution	Peak count	Separated peak count	Evaluation val
0.333	12	5	346.938
0.282	12	5	324.437
0.318	10	5	274.349
0.3	10	5	266.437
0.232	9	5	242.192
0.291	8	5	208.444
0.141	8	5	208.195

Table 1: Criteria for evaluating the measurements

Method development tools: Supported by modern software

Bring helpers on board: Getting the right support can save both time and resources. This is where LabSolutions MD steps in. This software solution, designed specifically for method development, automates the process, making it much simpler. With the software, various columns and mobile phases can be combined and method parameters adjusted in just a few simple steps. The software then automatically generates a batch that can be measured immediately (Figure 2).

When combined with a Method Scouting System, it offers even greater flexibility. Up to eight solvents and twelve columns can be used in one single setup. In this way, a wide range of combinations can be tested without manually changing the hardware. This not only saves time but also creates structure and makes the process more systematic and reproducible.

Data analysis: Systematic evaluation of screening results

Defining the framework: Data gathered during screening are key to identifying the best separation conditions. To efficiently evaluate the many measurements, the Method Development System provides an easy-to-use interface that's perfect for analyzing screening data.

The software evaluates the chromatographic results based on several criteria: The focus is on peak resolution, the number of separated peaks and the total number of detected peaks. The combined metric called the "Evaluation Value" is particularly helpful here. It multiplies the number of separated peaks by the resolution. This way, the best separation conditions can be identified at a glance, without the need to review each chromatogram individually (Table 1).

Optimization: From preselection to the optimal gradient

With the puzzle framework now set in place, related groups are formed: The screening results provide the foundation for the next step – optimizing the method, involving a targeted preselection of columns and mobile phases. This reduces the number of variables, allowing the method to be more effectively fine-tuned.

Now, parameters such as temperature, flow rate, injection volume and gradient conditions are systematically varied. As retention times shift accordingly, the software gathers data to build a precise model of the separation. For the model to be successful, the software needs to correctly identify which signals correspond to each component. LabSolutions MD offers a range of matching criteria to help with building the model. This makes it possible to simulate how method changes will affect outcomes without having to test every variation in the lab (Figure 3).

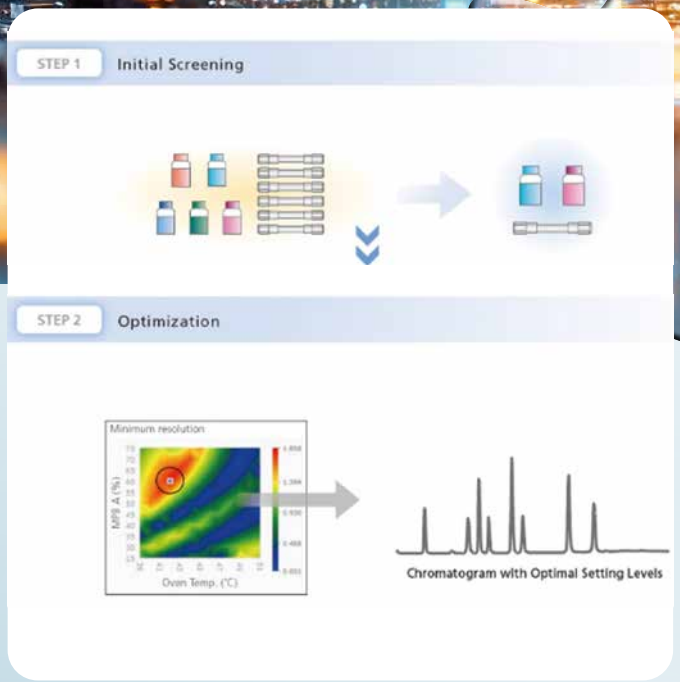


Figure 3: Steps in the method development process

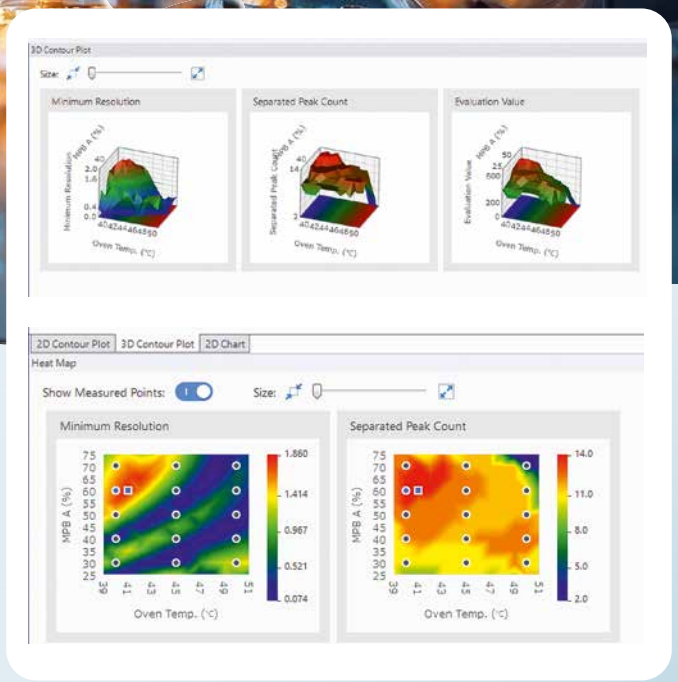
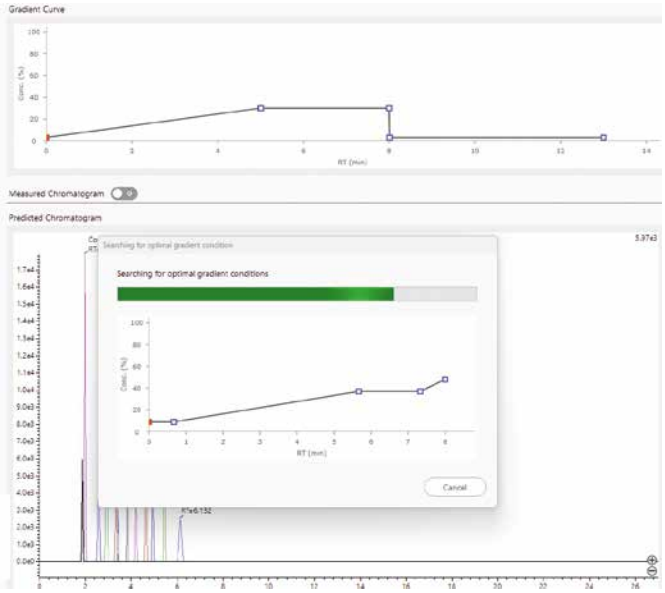
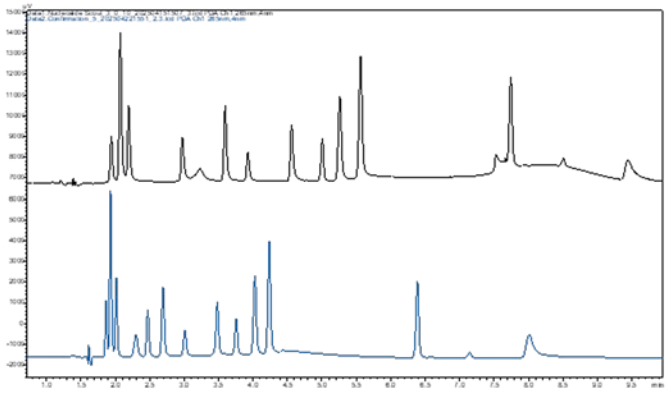


Figure 4: Visualisation of measurement results

Now it's time to fit the final missing pieces exactly where they belong: By comparing parameters, the ideal separation conditions can be identified. The software provides a variety of graphs and interface tools to support this process (Figure 4). ⟶





▲ Figure 6: Comparison between the chromatogram obtained after the optimization phase (top) and the software-enhanced analysis technique (bottom) of a HILIC method

◄ Figure 5: Automated gradient optimization

An optimized gradient can also be calculated, whether prioritizing the fastest possible separation or the best possible resolution, depending on the requirements of the analysis. This step doesn't just save time but also ensures maximum method efficiency (Figure 5).

Once the optimal gradient is calculated, the software performs automatic confirmation measurements to verify the model's predictions. If the results don't meet expectations, the software adjusts the conditions and repeats the measurements (Figure 6).

The combination of model-based optimization and automated monitoring ensures that the developed method is not only precise and reproducible but also perfectly tailored to the specific requirements of the analysis. This approach is especially valuable when it comes to separating complex samples since it offers high flexibility and efficiency.

Choosing the right parameters in method development is much like locking in the first crucial puzzle pieces – once those are figured out, the rest falls into place much more easily. Modern software, systems and columns make this very step easier, streamline the screening process and ensure reproducible results. Building on this, model-based optimization precisely tailors the method to the specific question at hand. In the end, all the pieces fit together to form a clear picture: This is how even the most complex analytical challenges can be reliably mastered. Job done – puzzle solved.

Note

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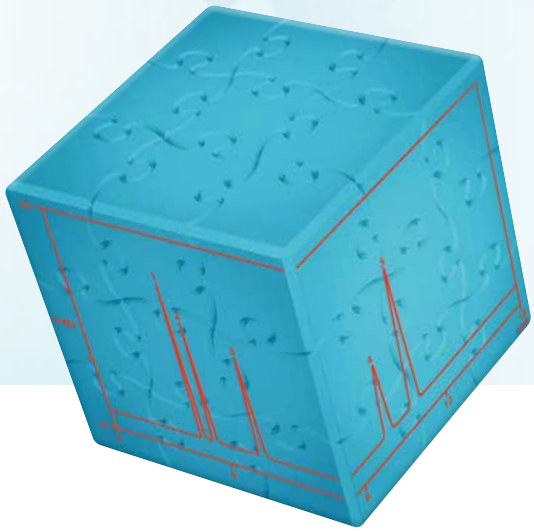
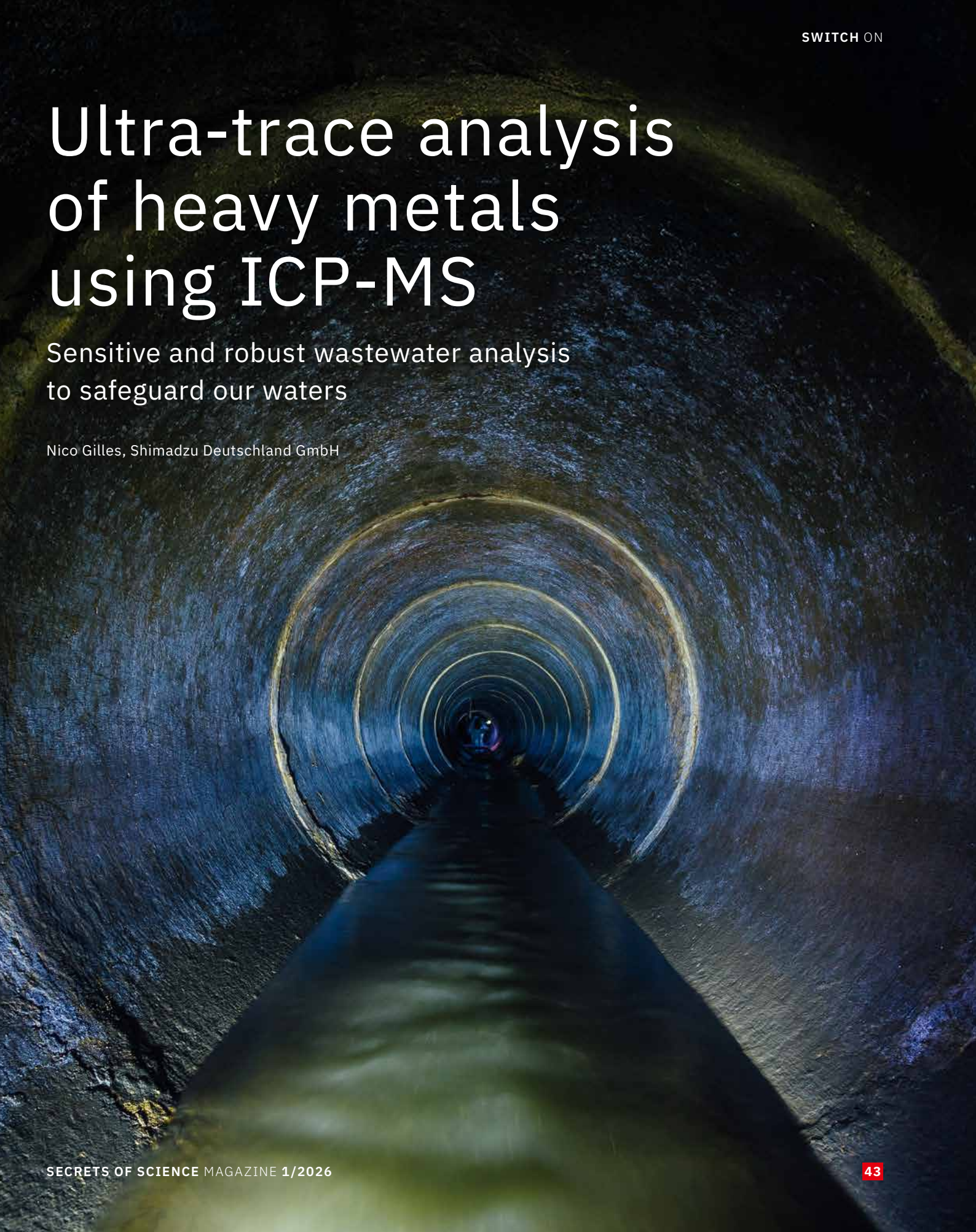


Ultra-trace analysis of heavy metals using ICP-MS

Sensitive and robust wastewater analysis to safeguard our waters

Nico Gilles, Shimadzu Deutschland GmbH



Clean water is our most precious resource – vital for a thriving environment, our food and our health. It only makes sense, then, that wastewater is analyzed with the utmost care, especially when it involves toxic substances like heavy metals. As permissible limits are lowered, the requirements for testing laboratories are increasing. More sensitive measurement methods are the key to success here. ICP-MS technology provides exceptional precision for detecting arsenic, lead, cadmium, mercury and other elements – even in trace amounts.

German Waste Water Ordinance: Setting the bar high – even as permissible limits are lowered

The German Waste Water Ordinance (AbwV) aims to preserve the quality of natural waters and promote sustainable water management. It defines specific discharge limits that depend on both the type of water body (such as surface or coastal waters) and the type of wastewater (whether municipal or industrial). Compliance with these limits is crucial when it comes to safeguarding water bodies from substances that can be harmful, such as heavy metals, organic compounds and nutrients.

Before it is discharged, wastewater must be treated mechanically, biologically and, if necessary, chemically. Municipal wastewater treatment plants play a central role in this, since they handle wastewater from private households. There are also extensive monitoring and documentation obligations: Operators are required to regularly measure pollutant concentrations, document the results and submit them to authorities when necessary. In this way, operators can not only ensure compliance with limits but also identify and address operational problems early on. Industrial wastewater is often subject to stricter requirements because it typically contains higher concentrations of pollutants. This guarantees that the established limits are met there as well.

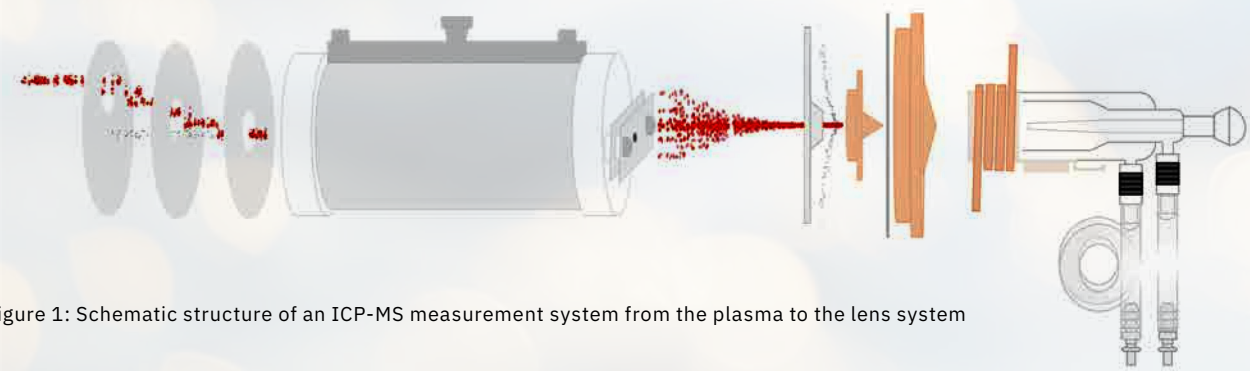


Figure 1: Schematic structure of an ICP-MS measurement system from the plasma to the lens system

Wastewater analysis – correct and critical

Chemical analysis of wastewater is critical when it comes to ensuring compliance with legal regulations and minimizing the impact of pollutants on ecosystems and human health. The German Environment Agency (UBA) reports that around 3.7 billion cubic meters of wastewater are treated in treatment plants in Germany each year. Harmful substances in wastewater can be detected thanks to chemical analysis. These include heavy metals such as lead, mercury and cadmium, as well as organic contaminants like pesticides and pharmaceutical residues, and nutrients such as dissolved nitrogen and phosphorus compounds. Information on pollutant concentrations helps detect potential threats to the environment, our drinking water and human health, and supports taking suitable action. Given the challenges posed by pollution and climate change, the role of chemical analysis in wastewater treatment and monitoring has never been more crucial for safeguarding our essential water resources sustainably.

The challenge of choice in elemental analysis

For decades, atomic absorption spectroscopy (AAS) and inductively coupled plasma optical emission spectroscopy (ICP-OES) have been well established in elemental and heavy metal analysis. Their advantages – high matrix tolerance, sufficient sensitivity and ease of use – remain central to the analytical techniques used. With the inductively coupled plasma mass spectrometry (ICP-MS) techniques, such as the ICPMS-2040/-2050 instruments, wastewater analysis now has a new option that provides an approved method for measuring the majority of heavy metals regulated under the Waste Water Ordinance. The misconception that ICP-MS is only suitable for drinking water and "clean" samples has long been disproven. Thanks to its exceptional sensitivity and ever-improving matrix tolerance, this technique is being used in a wide range of applications. →

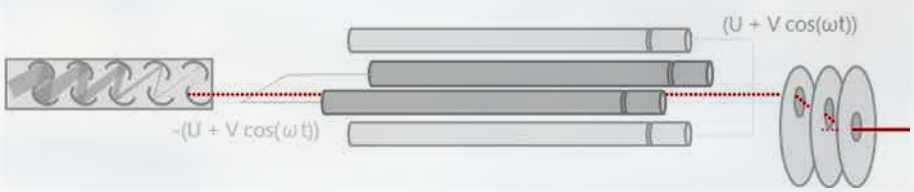


Figure 2: Schematic structure of a quadrupole mass filter and a subsequent multi-electron multiplier as the detector

Each year, around 3.7 billion cubic meters of wastewater are processed in Germany's treatment plants.

Inductively coupled plasma mass spectrometry

ICP-MS is a highly advanced analytical technique for precisely measuring the concentrations of elements across a wide range of samples. Its main advantage: ICP-MS can detect extremely low concentrations, in the range of a few parts per billion (ppb) or even parts per trillion (ppt).

How does ICP-MS outperform other methods?

Compared to traditional measurement techniques, ICP-MS provides huge benefits. It can analyze many elements at the same time, making the process more efficient. Thanks to its ability to detect over 70 different elements in a single sample, even complex wastewater samples can be thoroughly characterized. This is particularly important because wastewater frequently contains a wide range of pollutants that need to be monitored simultaneously.

The method is also impressively fast: The results are ready relatively quickly, which is essential for the timely monitoring of wastewater quality, especially in industrial settings where rapid decisions are needed.

In addition, ICP-MS experiences lower matrix effects compared with other techniques, such as ICP-OES. This results in more accurate and reproducible outcomes, which is particularly important when analyzing wastewater samples that often contain complex chemical mixtures.

Analysis according to DIN EN ISO 17294-2

The Waste Water Ordinance permits ICP-MS in accordance with DIN EN ISO 17294 as an approved method for analyzing heavy metals, with the exception of titanium and mercury. Before ICP-MS analysis, nearly all target elements require the sample to undergo acid digestion in accordance with DIN 15587-2. This ensures that turbid or particulate-containing samples are properly and consistently prepared for analysis. As shown in Figure 3, this method makes it possible to create solutions ready for analysis even from sometimes very turbid samples. If the samples are not prepared in this way, the ICP-MS system's tubing, capillaries and glass components could quickly become blocked, and cross-contamination might occur.

Figure 3:
Example wastewater samples before and after nitric acid digestion according to DIN 15587-2

Highly toxic mercury – an obvious candidate for ICP-MS

Mercury is one of the most critical trace elements due to its extreme toxicity, which can severely affect both humans and animals. It can have acute or chronic effects, primarily damaging the nervous system, kidneys and immune system. Unborn babies and young children are particularly at risk, as mercury can impair brain development and lead to long-term neurological damage.

The environmental persistence of mercury is a critical concern, since it persists in soils, water and the atmosphere and is difficult to break down, resulting in long-term ecosystem contamination. As well as this, mercury also accumulates in the food chain. Predatory fish, at the top of the chain and feeding on contaminated organisms, can contain substantial concentrations, posing a risk to humans who consume them.

The various sources of mercury in the environment, from industrial emissions to mining, waste incineration, and so on, make monitoring and regulation complex tasks. Precise analytical methods are required to reliably quantify mercury in water, soil and biological samples. Due to its toxicity, strict requirements are in place for the monitoring and reduction of mercury. Regulations, including the Substitute Building Materials Ordinance, already employ mercury analysis in accordance with DIN 17294. ICP-MS is particularly well suited for this, offering both the necessary sensitivity and robust performance.

Getting to the point

Two factors work together to safeguard the environment and water quality: The German Waste Water Ordinance sets clear limits for the discharge of wastewater and requires wastewater treatment plant operators to conduct comprehensive monitoring and treatment. Chemical analysis, particularly methods such as ICP-MS, enables the precise identification and quantification of pollutants, including heavy metals and especially critical elements like mercury. These analytical methods are essential for complying with legal requirements and thereby minimizing potential risks to the environment and human health. And that's why the continuous application and optimization of analytical methods will remain of central importance in the future.

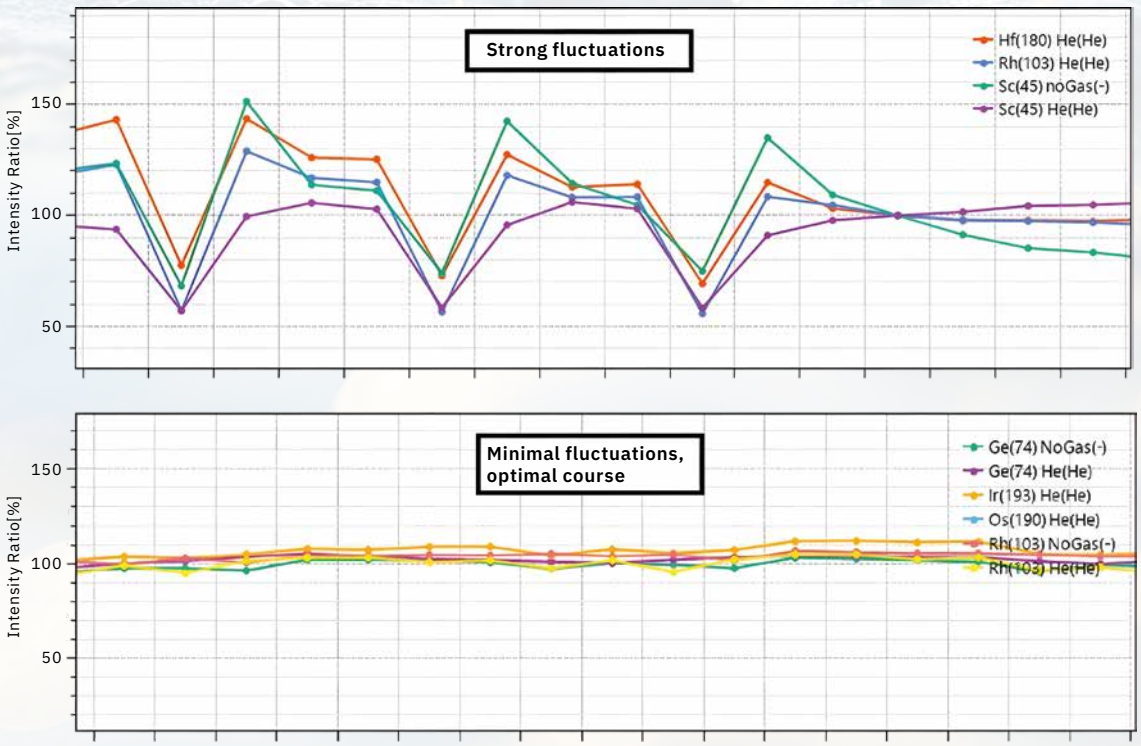


Figure 4: Comparison between the ideal behavior of the internal standard and a highly fluctuating signal caused by excessive calcium levels in a single sample

Note

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