SHIMADZU
Excellence in Science

secrets of science
magazine

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New approach for epitranscriptomics

Quantifying RNA methylations by LC-MS/MS

No need for skepticism

The New Shim-pack Scepter columns for LC/LCMS are robust and extremely pH-stable

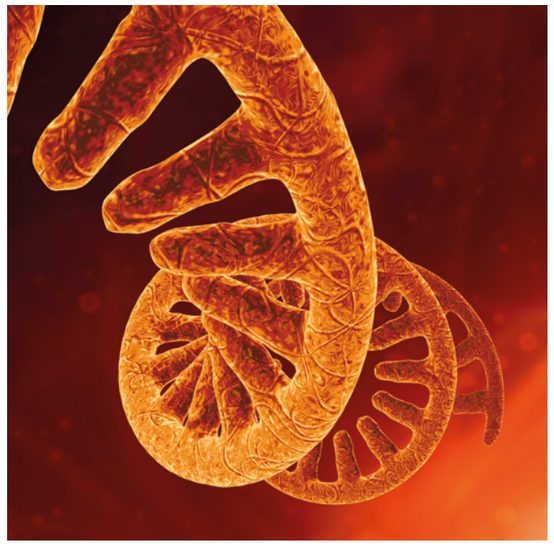
Smallest giant

TOC-1000e online analyzer – specialized for ultra-pure water applications

Secrets of Science
Customer magazine in new splendor **Page 04**

CONTENT

Precision medicine improves safety and efficacy of therapies
Towards simplification of therapeutic drug monitoring with DOSINACO kit for anticoagulants **Page 14**



New approach for epitranscriptomics
Quantifying RNA methylations by LC-MS/MS
Page 46

SECRETS OF SCIENCE magazine

Customer magazine in new splendor

A new name, a new design – but the content as professionally well versed as usual. The Shimadzu “Secrets of Science” magazine replaces the previous Shimadzu-NEWS publication. Whether the secrets of science can actually be solved remains to be seen.

With technical trends such as automation, miniaturization and coupling technologies Shimadzu has given significant momentum to the market in recent years which has initiated many new developments. Digitalization in particular offers technological and creative potential. This applies to medical technology and instrumental analysis as well as to Shimadzu’s customer magazine. Being a voice in the scientific community, further development is also required here. Under the new title, “Secrets of Science” offers even more insights and interaction.

Two in one

Going forward, the magazine will be published in two versions – one as a print edition and one in digital form. The print version is suitable for a quick overview and thanks to the layout of the articles, is also interesting for people unfamiliar with the product and the market.

The digital edition offers more information, such as additional chromatograms and content details – optimized so they can be displayed perfectly on mobile devices. There, experts can also find the insights they need for their subject-specific in-depth studies. It is easy to access via the QR codes in the print version.

Easy allocation, quick access

Categorization of the content has also been adapted. There are sections focusing on different topics – from product- and application-focused articles, to further developments, events, interviews, service topics and tips & tricks.

Staying true to the origin

Just like Shimadzu NEWS, “Secrets of Science” will be published three times a year in German and English. Guest authors from science and industry provide insights into their tasks and applications. The arrangement of information in product and market categories continues and is given a new appearance.

Have fun discovering the new magazine!

Suggestions and criticism are welcome.

SWITCH ON

No need for skepticism

The New Shim-pack Scepter columns for LC/LCMS are robust and extremely pH-stable

Dr Carola Thiering, Shimadzu Europa GmbH

The new Shim-pack Scepter columns with hybrid materials combine the advantages of silica- and polymer-based columns: they are efficient, pressure-stable and tolerate broad pH ranges. They are also available as metal-free versions for sensitive LC/MS measurements of biological samples or chelating substances.

»The chromatographic column is a high-tech product and not ‚a tube filled with sludge'«

Klaus K. Unger, Stefan Lamotte: „Chromatographie: Trennsäulen in der analytischen HPLC" [1]

Rather, it is the core of an analysis, along with the eluent, and specific columns are offered depending on the goal of the analysis. Interdisciplinary knowledge flows into the construction and packing of these columns; their selectivity, for example, depends on numerous material and process parameters.

To support optimal performance interaction of all parts of an HPLC system, seamless quality and transport chains are especially important in high-tech applications. Users benefit from single-source supply and coordinated components and processes. Consequently, accessories and consumables are an integral part of a complete solution from Shimadzu.

Thanks to a new series of LC columns, users get more flexibility. The new columns from the Shim-pack Scepter series are packed with hybrid materials and are available in both metal and metal-free versions. They complement the silica-based, core-shell and polymer column portfolios and cover a wider range of application needs than before. The main advantages of hybrid materials are their pH stability and robustness. They are also available in a broad spectrum of particle sizes and dimensions, making them scalable from UHPLC to HPLC and preparative LC. →

Organo-silica hybrid columns

But what exactly is organo-silica hybrid material? The particles are organic/inorganic silica-based hybrid materials, or in other words, based on hybrid silica gel (figure 1), using tetraethoxysilane and 1,2-bis(triethoxysilyl)ethane as reactants for their production.

The resulting hybrid material is shown schematically. The upper layer represents the surface and the lower layer the internal structure of the particle. Evidently, the silicon atoms are connected not only by an oxygen atom, but also partly by an ethylene bridge. This combination of silica gel with the organic parts makes the material very robust and pH stable.

The silanol groups on the surface are also partially replaced by an ethylene bridge. The resulting decrease in silanol groups on the surface is largely responsible for the increase in stability and inertness of the silica gel, because it is shielded against exposure to chemicals.

As a result, the hybrid materials are suitable for a wide range of HPLC applications. The advantages are

- high durability, even under harsh conditions such as a high pH-value or high temperature
- general high chemical and physical resistance
- good peak shape for all kinds of compounds and
- reproducibility.

Selectivity is also the same across different particle sizes, so method transfer between UHPLC and HPLC is very easy. Due to the preparative columns in the portfolio, the method transfer is even suitable for preparative LC.

Easy method transfer; sensitive measurements

Columns with hybrid materials combine the advantages of silica-based and polymer-based columns: they are efficient and pressure-stable thanks to their mechanical stability. Additionally, they have a high tolerance to basic mobile phases and low silanol acidity. [1]

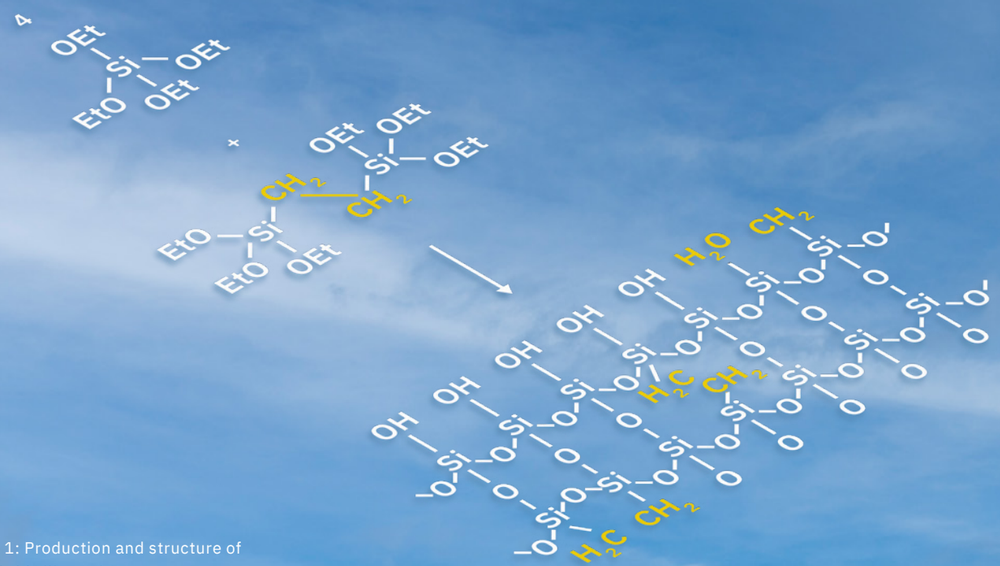


Figure 1: Production and structure of the hybrid silica gel [1]

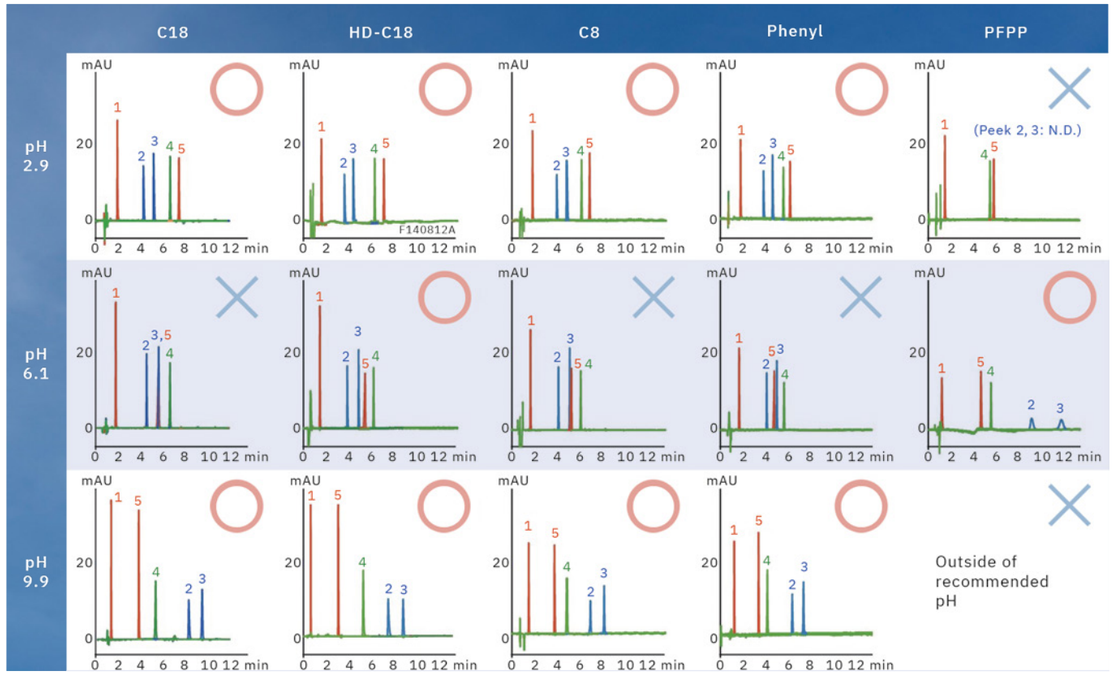


Figure 4: The Shim-pack Scepter columns offer high stability, even under low as well as high pH values. Peaks: 1: Saccharin (pKa = 2.2), 2: Dextrometorphan (pKa = 8.3), 3: Amitriptyline (pKa = 9.4), 4: n-Butylparabene (pKa = 8.3), 5: Ibuprofen (pKa = 4.4).

As a further advantage, the Scepter portfolio is also available as metal-free variants, which are very effective for sensitive LC/MS measurements of chelating substances that would otherwise show strong tailing. So, there is no reason to be skeptical – because overall the Shim-pack Scepter columns comprise a large product range and are extremely robust and durable on account of the hybrid material.

Shim-pack Scepter portfolio: for numerous applications with diverse substances

The Scepter portfolio covers a wide range of stationary phases and dimensions. For each of these stationary phases there is an appropriate precolumn with holder. Table 1 shows all of the phases with their respective specifications.

All columns are robust within a wide pH range. The chromatograms in figure 4 (measurement parameters and methods are explained below) demonstrate the stability. The results of the analyses on the different, stationary phases are shown at different pH values (2.9, 6.1 and 9.1).

Since the same method is used here for all phases at different pH values, not all substances are always separated optimally from each other. In the chromatograms marked with the red circle, all substances are separated out. The chromatograms with the blue X show coeluting or undeterminable peaks. Overall, it is clear that the columns exhibit high stability at different pH ranges and still have very good separation performance. →

The different retention mechanisms also become clear in figure 4: The HD-C18 shows better separation performance than the C18 for critical substances. As expected, the C8 phase shows less retention than the C18 phases, whereas the phenyl and the PFPP phases have completely different separation mechanisms, which partially reverse or strongly shift the retention. In summary, the Shim-pack Scepter portfolio offers the appropriate column for numerous applications with a wide variety of substances.

Measurement parameters and methods

Columns:	Shim-pack Scepter (50 mm x 2.0 mm I.D., 5 µm)
Mobile phase:	0.1 min: 95 % H_2O, 5 % ACN ⟶ 10 min: 10 % H_2O, 90 % ACN 10 - 15 min: 10 % H_2O; 90 % ACN
Oven temp.:	25 °C
Flow rate:	0.2 mL/min
Detection:	230 nm

Metal-free columns in the portfolio

The various columns of the Shim-pack Scepter portfolio are also available as metal-free and complete the product range which includes both, the silica gel-based G-series and the organosilica hybrid-based Scepter series.

The schematic structure of the metal-free columns is pictured in figure 5, showing how the particles as filling material do not come into direct contact with the stainless-steel body, due to a coated layer of polyetheretherketone (PEEK). It also affects the connecting pieces of the column, preventing the stainless steel from contacting the sample being examined.

This is a great advantage especially for biological and chelating samples. Such samples interact with metal and would therefore not show a symmetrical peak shape without a PEEK coating, but rather strong tailing, or even a decrease in signal intensity due to adsorption. The combination of stainless-steel body and PEEK coating is ideal: the stainless-steel body is stable and provides the column with high pressure stability, whereas the PEEK coating provides the necessary inertness. The metal-free columns are also very well suited for LCMS analyses and demonstrate high intensity levels.

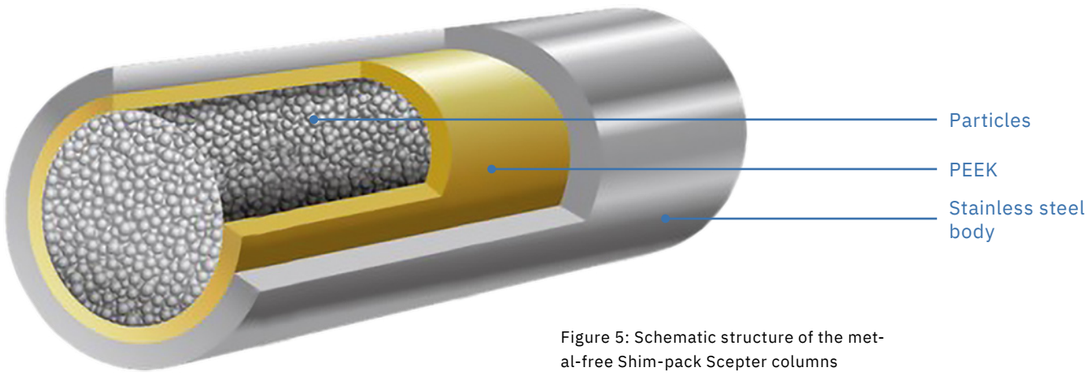


Figure 5: Schematic structure of the metal-free Shim-pack Scepter columns

Shim-pack Scepter	Reversed phase						HILIC
	C18	HD-C18	C8	C4	Phenyl	PFPP	Diol-HILIC
Ligand type	Trifunctional C18 Generic Purpose Type	Trifunctional C18 High Density Type	Trifunctional C8	Trifunctional C4	Trifunctional Phenylbutyl	Trifunctional Pentafluoro-phenylpropyl	Trifunctional Dihydroxypropyl
Particle	Organic Silica Hybrid						
Particle size	1.9 µm, 3 µm, 5 µm						
Pores size	12 nm	8 nm	12 nm	30 nm		12 nm	
End capping			Proprietary			None	
pH range		1 - 12		1 - 10		1 - 8	2 - 10
100% aqueous condition	Yes	No	No	Yes	Yes	Yes	N/A
USP classification	L1	L1	L7	L26	L11	L43	L20

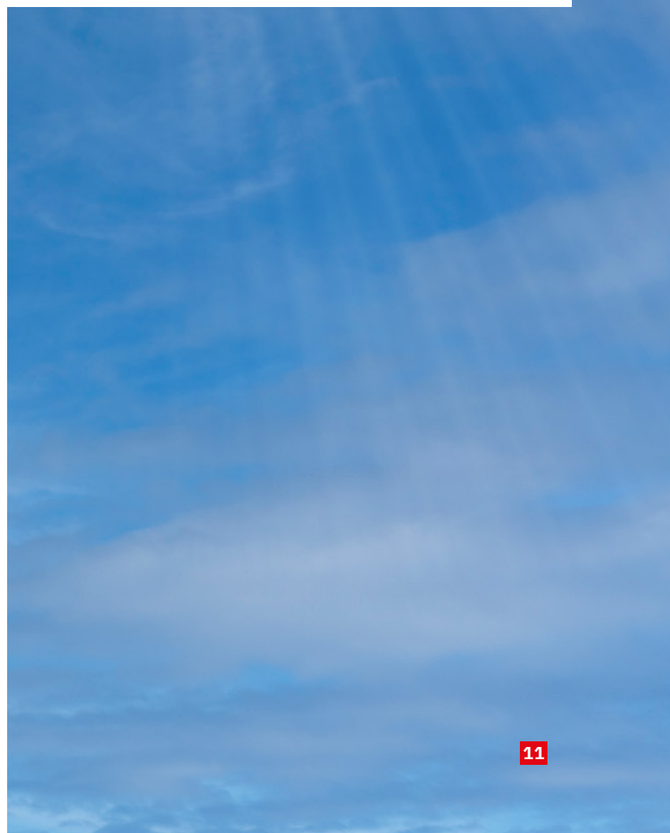
Table 1: The different stationary phases of the Shim-pack Scepter series with the respective specifications

Application

So, when does it make sense to invest in an HPLC column with hybrid material such as the Shim-pack Scepter? It depends on the application, of course. The Scepter series shows its advantages especially when harsh conditions prevail during the HPLC analysis. It offers stability even at a high pH value and high temperatures. The Scepter columns are also ideally suited for method development or method scaling between UHPLC, HPLC and preparative LC, as they are available in many dimensions and particle sizes while delivering the same separation performance.

Note

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



VOICES

Fight against COVID-19

From synthesis, scale up and sequence confirmation of oligonucleotides

Dr Tom Brown Jnr, Director of ATDBio, Southampton / Oxford

ATDBio is an oligonucleotide synthesis company based in UK, specializing in making high quality oligonucleotides for demanding applications including COVID-19 testing kits. To meet the demand for oligonucleotides since the start of pandemic, ATDBio have scaled-up their production and purchased several more Shimadzu LC systems. This has allowed the company to synthesise oligonucleotides on different scales, from minute to very large quantities of primers and probes for COVID-19 tests. ATDBio continues to make a significant contribution to tackling the COVID-19 pandemic.

Synthetic oligonucleotides, small pieces of single-stranded DNA and RNA made by chemical synthesis, have important uses in all aspects of molecular biology, genetics, medicine and beyond. But they have recently made front-page news as the major component in COVID-19 testing kits.

Dr Tom Brown Jnr, Director of ATDBio

Effective solutions to complex problems

ATDBio is an oligonucleotide synthesis company based in Southampton and Oxford, UK, specializing in making high quality oligonucleotides for demanding applications. ATDBio has seen major changes to its business as a result of the coronavirus pandemic. Dr Tom Brown Jnr, Director, explained: »All current genetic COVID-19 testing methods require synthetic oligonucleotide primers and probes. We work with our customers, including UK hospitals and private companies, to help them develop simpler, faster and more accurate diagnostic methods. We've been inundated with enquiries since the advent of the pandemic, and we're scaling up our oligonucleotide synthesis capability significantly in order to meet this demand.«

Shimadzu's reputation for robustness, innovation, product longevity and customer service were just some of the reasons ATDBio selected Shimadzu LC Nexera Prep systems as part of its production workflow.

Dr Brown explained: »Almost every lab I've worked in seems to have had an old Shimadzu UV/Vis spectrometer, still going strong. I also remember reading about Koichi Tanaka at Shimadzu winning the Nobel Prize in 2002. We visited the Shimadzu laboratory in Milton Keynes, met some of the staff, and were particularly impressed with their knowledge, willingness to understand our challenges, and ability to find us a system that was a match for our needs. Shimadzu's appreciation of our needs, along with delivery and support of a trustworthy solution was beyond our previous experience.«

Dr Asha Brown, Director of ATDBio

Dr Brown added: »As part of our recent scale-up, we purchased several more Shimadzu LC systems. We synthesize oligonucleotides on different scales, from very small amounts for research purposes, to very large quantities of qPCR and LAMP primers and probes for COVID-19 tests. Having the ability to work reliably across a wide range of synthesis scales is crucial for us.«

Analyzing nanoconstructs quickly with minimal sample clean-up

When not working on COVID-19, ATDBio engages heavily in research and development, which it views as essential to remain at the forefront of the nucleic acids field. Dr Brown explained: »At any one time we are involved in several research projects. One of our current projects is on DNA nanotechnology, in which oligonucleotides are used as building materials to make large DNA nanoconstructs, which have the potential to be used in drug delivery.«

ATDBio will continue to make a significant contribution to tackling the COVID-19 pandemic, as well as improving thepreparedness for future outbreaks. Shimadzu will surely be there to help.

Note

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

SWITCH ON

Olive oils and their effect on health

Application of GCMS-QP2010 SE to quantify Linoleic (LA) and Linolenic (ALA) acid in olive oil

PhD Joanna Grzelczyk, PhD. Ilona Gałązka-Czarnecka, Prof. PhD. hab. Grażyna Budryn, PhD. Joanna Oracz, Lodz University of Technology, Institute of Food Technology and Analysis, Poland

Omega-6 fatty acids are important for the metabolism of the human body, for bones, healthy skin and hair. As an essential nutrient, they must be supplied from the outside. They are found in many foods, especially vegetable oils and nuts. Their family includes, for example, alpha-linolenic acid (ALA) and the similar sounding linoleic acid (LA). While alpha-linolenic acid plays a role in anit-inflammatory processes and the natural fight, linoleic acid is important for skin health. But how long do they keep their full effect in food, how long are they stable?

This and similar questions are investigated by the Institute of Food Technology and Analysis based at Lodz University of Technology in Poland. It analyzes bioactive compounds in food during growing, rearing, storage, processing and distribution of food. The research interest of members of the group mainly includes bread, meat, legumes, vegetables and fruits, as well as coffee regarding their effects against civilization diseases. Currently, researchers study the stability of omega fatty acids, and they are steadily interested in new techniques for analysis of bioactive compounds in food.

Antioxidant properties of ALA and LA in olive oil

Olive oil is the foremost source of “healthy fats”, used, among others, as a dressing for salads, as a spread for bread or applied for short frying. Olive oil is known for its high antioxidant content, which counteracts the so-called free radicals, i.e. certain oxygen or nitrogen compounds that affect skin aging and the development of certain diseases.

Linoleic acid (LA) and linolenic acid (ALA) are strong antioxidants and must be provided in the daily diet to effectively support the proper functioning of the body. [1] ALA and LA, according to scientific research, protect the body and support against atherosclerosis, inflammation, neurodegenerative and cancer diseases. Interestingly, it was observed that ALA slows down the formation of cancer metastases. [2]

Unfortunately, ALA and LA are not stable compounds, meaning that it is important to properly store products containing these nutraceuticals. Due to their chemical instability, it is important to store olive oil in dark and cool places. In a recent study, the researchers compared the content of ALA and LA in various olive oils stored with and without additives, and the effect of different packaging on the storage of the oil after opening.

Material and sample preparation

Five extra virgin olive oils bought from Poland retailers were tested. Three of them were produced in Italy, and two in Greece. The Italian products contained 98 % cold pressed extra virgin olive oil and 2 % natural additives: extracts of lemon, basil and mushroom from organic agriculture. The olive oils were packaged in slightly tinted green glass. The Greek oils contained 100 % cold pressed extra virgin olive oil. One of them was filled in dark glass packaging, covered with a whole label, while the other one was packed in a can (synthetic material C/LDPE). All samples were stored at room temperature.

The oils were prepared with several solutions for the detection procedure and analyzed immediately afterwards with a GCMS-QP2010 mass spectrometer. (Please see the digital version of the article for more detailed specifications). →

Results

Analysis showed that the highest LA content was found in the 100 % olive oils, and the highest ALA content in olive oil with the basil additive. The results showed that all the oils analyzed in this study met the accepted limits for LA and ALA concentrations according to literature data. [3] (More details and the table with results in the digital version of the article.)

Grażyna Budryn: Member of research team

In the next stage, degradation of LA and ALA after opening during a typical home storage situation was analyzed. The olive oil was kept for a week in a cool, dark place and then tested again. The content of omega 3 and 6 fatty acids decreases with storage of opened containers. Analysis showed that LA was more stable (loss amounted to 5 - 13 %), compared to ALA (with loss of 25 - 45 %).

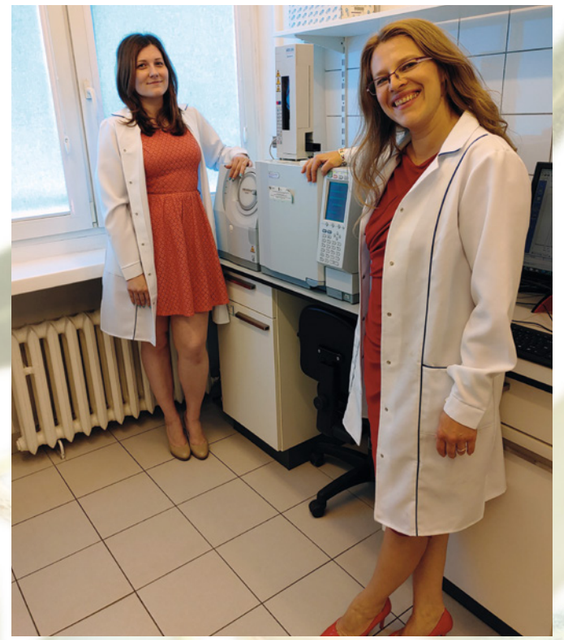
The best packaging for storing olive oil was the can, retaining 95 % of the LA (5.45 g/100 mL). This shows how important it is to store the olive oil properly and consume it as soon as possible, at best within 2 weeks. This allows preservation of the valuable components and maintaining of the properties as a food supplement providing the daily recommended dosage for LA in 15 - 20 mL of olive oil. The analytical method applied allowed easy and fast analysis of the olive oil samples with high sensitivity.

Conclusion

The studies suggest that the best packaging for olive oil is a can, preserving the high content of omega acids in food products in a short time.

Note

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



LA and ALA during storage. Natural flavor additives, such as lemon, basil and mushroom give a taste effect, but did not protect the olive from oxidation causing the degradation of these valuable fatty acids. It can be concluded that to supply the body with the healthy fatty acids, 100 % canned olive oil should be chosen, which will provide high amounts of antioxidant substances having a positive effect on the functioning of the human body.

The application developed enables analysis of different kinds of olive oil using a single method. It allows precise assessment of the content of

Precision medicine improves safety and efficacy of therapies

Towards simplification of therapeutic drug monitoring with DOSINACO kit for anticoagulants

Pablo Zubiaur Precioso, PhD, MPharm, Servicio de Farmacología Clínica, Hospital Universitario de La Princesa

The team led by Francisco Abad-Santos, PhD, MD at the University Hospital of Madrid focuses on pharmacogenetics, therapeutic drug monitoring (TDM) and precision medicine. TDM can be used, for example, to determine whether the dose applied is still within the therapeutic range. For its research, the lab team uses DOSINACO, a reagent kit manufactured by Alsachim, a Shimadzu group company, for quantifying anticoagulants in human plasma.

Dr Francisco Abad-Santos (3rd from left) and his team at the university hospital Madrid

Alsachim team spoke with Dr Pablo Zubiaur (PhD, MPharm) who belongs to the Clinical Pharmacology Department, about the use of the kit and asked what makes DOSINACO so appealing. He gives some insights into his work and the context of applying the kit.

Can you outline the research you are conducting in your laboratory/department? With our research, we aim to advance precision medicine, meaning procedures and drugs that apply individual biomarkers of patients to assess the benefits and risks of therapies. This includes pharmacogenetic studies as well as studies related to therapeutic drug monitoring. Both strategies, when implemented in clinical practice, improve the safety and efficacy of therapies, quality of life of patients and the sustainability of health systems.

I am responsible for coordinating the lab team consisting of clinical pharmacologists, laboratory technicians, predoctoral biotechnologists and several undergraduate students from different disciplines.

How does DOSINACO help you to solve and optimize your daily work? What are your reasons for choosing the Alsachim reagent kit? We have promoted an observational study in which we aim to define the therapeutic range and pharmacogenetic markers of apixaban, dabigatran, rivaroxaban and edoxaban, that are anticoagulants. For this purpose, it is easier for us to carry out the analytical determinations with a commercial kit such as DOSINACO. According to our research, the kit is the only one of its kind on the market.

What are the trends and needs in your field that lead to applying the DOSINACO kit? What are your expectations for LC-MS/MS drug quantification in the future? Precision or personalized medicine is currently under development and will probably continue to expand in the upcoming years and decades. It is therefore expected that the number of labs that need to perform analytical determinations will increase. At least, that is our case. LC-MS commercial kits mean a considerable time saving for laboratories like ours, “small” and without dedicating 100 % of their activities to analytical chemistry.

And the awards go to…

iMScope QT, MALDImini-1 and Packaging Box for GC with Red Dot and iF Design Award

With design making the world a more beautiful place and products more functional, intuitive use of equipment plays a key role in an increasingly technological environment. These aspects relate to user-friendliness, aesthetics and functionality. In Shimadzu´s product development in Japan, an entire team focuses on these design aspects. Their work has repeatedly achieved these most important design awards.

Red Dot Design Award and iF Design Award for Shimadzu products

Shimadzu's design philosophy not only focuses on the usability of a device, but also on how to use a system to achieve results. User experience design (UX) and user interface design (UI) are buzzwords here: they ensure that a product is intuitive to use, and also good looking. The more sophisticated the UX/UI solutions, the happier the users and the better the results with the system.

This perspective plays a major role in the design of new devices. During the development phase, UI designers always put themselves in the role of users and their needs. This results in systems that are both aesthetic and functional, and always focusing on the user experience.

The combination of form and function makes Shimadzu systems convenient and efficient tools for laboratory use. International design organizations have also regularly recognized Shimadzu's work in recent years. In 2021, three Shimadzu systems have been awarded.

Award-winning design

The Red Dot Design Award and the iF Design Award are among the top 3 most important design awards in the world. The international jury members of both awards evaluate the products submitted from all over the world according to criteria such as innovation, functionality, quality, ergonomics, aesthetics and durability. The award-winning products set standards for the future with their unique designs.

Shimadzu was once again able to impress the juries of both awards in 2021. The iMScope QT mass spectrometer imaging system received the "Red Dot: Design Award", while the MALDImini-1 digital ion mass spectrometer was awarded the "Red Dot: Best of the Best" – the highest prize dedicated in the contest. The Packaging Box for GC as well as the iMScope QT and the MALDImini-1 also received the iF Design Award. It is presented by the Hannover-based iF International Forum Design, the world's oldest existing design organization.

The future begins with iMScope QT and MALDImini-1

The iMScope QT combines the LCMS-9030 Q-TOF mass spectrometer with an iMScope series optical microscope. It identifies and visualizes the dispersion of specific molecules and is suitable for medical and pharmaceutical applications as well as for food and agricultural industries. In cancer therapy, for example, the iMScope QT visualizes whether drugs work at their intended position in the body. During operation, it also ensures excellent speed, sensitivity and spatial resolution for data analysis.

The novel MALDImini-1 is a digital ion mass spectrometer which revolutionizes the MALDI market. It combines small floor space, minimal measurement time and microscale sample volume for highly sensitive MS measurements. It allows structural analysis of diverse substances such as peptides and digested proteins with ease. Presently, it is providing great assistance to scientists working on glycan-related substances in the context of COVID-19.

Environmentally-friendly Packaging Box for GC

The packaging design protects the fragile gas chromatography capillary columns from bumps and contamination during international transport as well as storage. Minimization of the material used, and the recyclable cardboard make the Packaging Box more environmentally friendly overall.

Combination of aesthetics and functionality

Design not only reflects pure aesthetics but is always an expression of functionality. In recent years, Shimadzu has received 11 Red Dot and iF Design Awards for its systems. The 2019 Red Dot Design Award-winning spectrophotometer UV-1900, for example, has a control panel designed according to ergonomic principles, positioned at the best possible viewing angle for easy readability. Shimadzu's "Excellence in Science" approach is evident here not only in the technological progress, but also in the functional aesthetics, which ensure outstanding usability for operators.

ORIGINAL SIZE

Smallest **giant**

TOC-1000e online analyzer – specialized for ultra-pure water applications

A world premiere in technology, in the smallest and lightest housing on the market: the new TOC-1000e meets the demand for online analyzers providing high sensitivity and speed to meet the challenges of ultra-pure water applications. It is the first process analyzer in the eTOC series to expand Shimadzu's portfolio of advanced TOC analytics. →

Figure 1: Shimadzu's TOC-1000e is the first process analyzer in the eTOC series which specializes in online analysis for ultra-pure water applications

What do vaccines and microchips have in common? Water is an essential raw material in the production of both. Its molecular structure makes it an ideal solvent for a wide variety of substances. In pharmaceuticals and semiconductor fabrication, however, ultra-pure water, prepared in a complex process, is particularly important. It is necessary to produce medicines and cosmetics as well as memory chips, microprocessors and flat screens.

Water that contains practically no minerals, dissolved organic substances or microbiology, so-called ultra-pure water, does not exist in nature. It must therefore be obtained from pre-purified water in multi-stage treatment plants. But even after its production, constant effort is necessary, because the slightest contact with the atmosphere or even unfavorable dwell times in the distribution plant can lead to purity reduction. For this reason, ultra-pure water plants are monitored very closely and continuously.

Ultra-pure water used in the production of medicines is subject to strict rules and limits as listed in the so-called pharmacopoeias, such as the European Pharmacopoeia (EP) or the US Pharmacopoeia (USP). Two of the parameters are the electrical conductivity to measure the purity of water, and the TOC (Total Organic Carbon), which detects the total carbon from organic compounds. They are the most important and fastest online techniques to detect impurities.

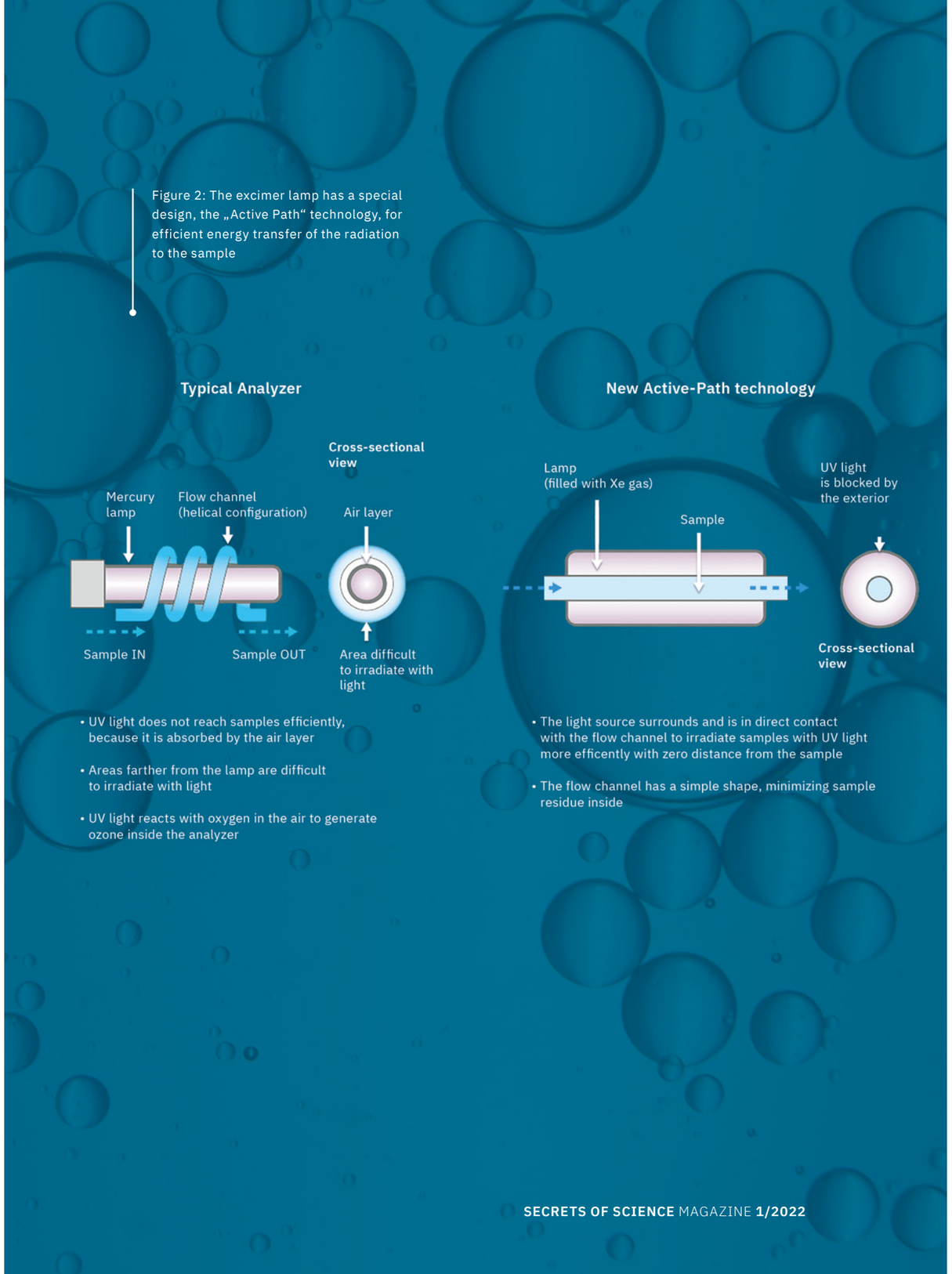
In order to closely monitor the quality of water at all times, process analysis (PAT) is increasingly in demand in addition to laboratory analysis (offline analysis). This requires analytical equipment that sensitively and specifically records the parameters required.

Smallest giant

The new TOC-1000e specializes in online analysis of ultra-pure water applications. It is the first process analyzer in the eTOC series and complements Shimadzu‘s portfolio of advanced TOC analytics, including analyses of a broad range of applications–from ultra-pure water to wastewater, solid samples and swab tests.

The TOC-1000e is small. Very small. It disappears behind a sheet of A4 paper. And weighs less than 3 kg. Nevertheless, its performance is gigantic: it enables highly sensitive analyses and mercury-free oxidation technology.

Figure 2: The excimer lamp has a special design, the „Active Path“ technology, for efficient energy transfer of the radiation to the sample



Environmentally-friendly UV oxidation, optimum sample irradiation

Whereas TOC analyzers have previously used mercury UV lamps as a light source in ultra-pure water applications, the TOC-1000e is the first in the world to use a more environmentally friendly alternative: a mercury-free excimer (short for ‚excited dimer‘) lamp. Compared to conventional lamps, it emits higher-energy light to oxidize organic components.

Featuring „Active Path“ technology, it irradiates the sample optimally with UV light. The sample flow is guided directly through the inside of the excimer lamp, which prevents the formation of ozone in air pockets, favors rinsing of impurities and increases long-term stability.

Easy to operate, low maintenance, on-site service

Despite its small dimensions, the TOC-1000e offers a large clear touchscreen that allows easy, intuitive operation. Flexible output of data and protocols is possible via USB or network transmission, e.g., as text or PDF file (table and trend graph). Maintenance and calibration are scheduled annually. The pump head and excimer lamp can be accessed and replaced without tools via the front cover of the TOC-1000e.

Wide range of data output options

Measured values can be output both in analog (4 to 20 mA) and digital form via fieldbus. In addition, the TOC-1000e systems can be connected to a network and the results can be viewed and downloaded via networked PCs or tablets using a web browser.

The small and ultra-lightweight TOC-1000e is available as a benchtop unit but is also suitable for wall or pole mounting. In any case, an autosampler can be attached to the side to calibrate the analyzer on site. This provides total flexibility in terms of location and eliminates the need to send the instrument in to the manufacturer.

Note

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

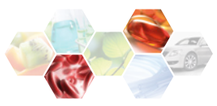
SWITCH ON

The column is crucial

Shim-Pack column enhancement to separate challenging substances

Timofey N. Komarov, Igor E. Shohin, Margarita A. Tokareva, Olga A. Archakova, Dana S. Bogdanova, Alexandra A. Aleshina, Natalia S. Bagaeva, Veronika V. Davydanova, Center of Pharmaceutical Analytics LLC, 117246, Russia, Moscow

Development of new drugs also requires finding new analytical methods to monitor therapies as well as to control the way a drug enters the body, is absorbed and digested, known as pharmacokinetics. The methods must be labor- and cost-efficient. Interaction of the single components of an analytical system such as the chromatograph and the optimal column, is also important.



HPLC with various detection methods is actively used to determine the content of drugs in biological fluids. One of the most difficult practical tasks is the chromatographic separation of poorly retained compounds – drug substances poorly retained on the chromatographic column. Valganciclovir and Ganciclovir are examples of such substances; both are used against herpes viruses – ganciclovir as an infusion and valganciclovir in various dosage forms.

The aim of this study was to develop a method for determination of valganciclovir and ganciclovir in human plasma by LC-MS/MS for pharmacokinetic and TDM studies. This method was developed and validated. Linearity in plasma sample was achieved in the concentration range of 5 - 1,000 ng/mL for valganciclovir and 50 - 10,000 ng/mL for ganciclovir.

HPLC as one of the modern analytical techniques is now widely used to determine the content of medicines in biological liquids. Depending on the chromatographic conditions and detector used, it is possible to separate and identify substances with different properties and, especially, the substances having similar structure.

Challenge: separation of medicines

One of the most challenging practical tasks is the separation of the medicines which are weakly retained on a chromatographic column. The various chromatographic methods could be used for that purpose: HILIC (Hydrophilic interaction liquid chromatography) and ion-exchange columns, ion-pair reagents as eluents etc. However, these approaches may not be effective where a mass spectrometer is used as detector, or when the medicines are measured in complex biological matrices. The case of valganciclovir (VAL, figure 1) and ganciclovir (GAN, figure 2) illustrates this situation.

Ganciclovir was developed initially as a drug for the treatment of cytomegalovirus infection, after which it was discovered that GAN could inhibit in vitro other herpes viruses (human herpes viruses 1 and 2 types, Epstein - Barr viruses, chickenpox virus, human herpes virus type 6 etc). [1] Typically, ganciclovir was used intravenously only during a therapy, and a new prodrug named valganciclovir was developed to increase the bioavailability. Valganciclovir metabolizes easily into ganciclovir which in turn provides a therapeutic effect. [2,3]

Like many other antiviral drugs, valganciclovir and ganciclovir are both rather hydrophilic substances, as clearly shown by the values of their octanol/water partition coefficients (log P, table 1). These properties must be considered in the development of analysis methods including sample preparation and separation techniques.

Previous analytical methods using HPLC with UV detection [7,8,9] and HPLC coupled with mass spectrometer [10] were not able to measure valganciclovir and ganciclovir simultaneously, rather only one substance at a time.

Optimal chromatographic separation with MS detection

The authors of this publication tried to develop a method for simultaneous determination of GAN and VAL using HPLC-UV, but this method required the use of a specific “YMC-Pack Polyamine II” chromatographic column and setting of eluent flow at 2 mL/min, which significantly increased reagent consumption. Moreover, analysis time was quite long (about 26 minutes) to achieve optimal separation of the substances and internal standard. →

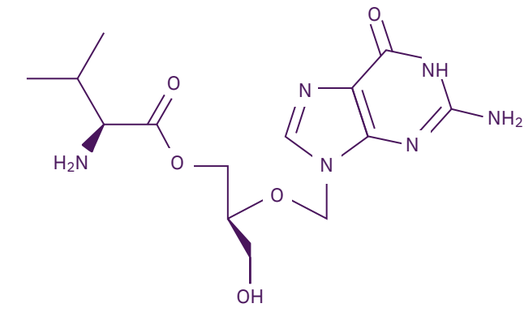


Figure 1: Chemical structure of valganciclovir

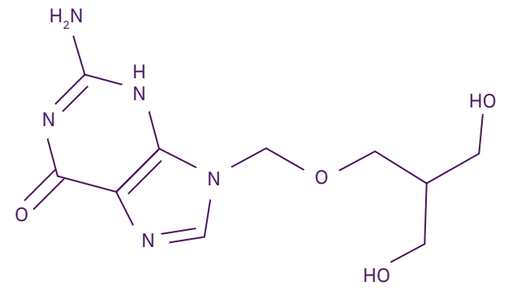


Figure 2: Chemical structure of ganciclovir

	Valganciclovir	Ganciclovir	Acyclovir (internal standard, IS)
Log P	-0.81	-1.66	-0.95
pKa	10.16	8.71	11.98
Reference	[4]	[5]	[6]

Table 1: Log P and pKa for valganciclovir, ganciclovir and acyclovir

Chromatographic column	Total carbon content, %	Surface area, m^2/g
Phenomenex Luna C18(2) 50 × 2 mm. 5 µm	18.2	393
Phenomenex Luna NH_2 50 × 3 mm. 5 µm	10.2	420
Waters XBridge C18 50 × 4.6 mm. 3.5 µm	18.0	178
YMC Hydrosphere C18 100 × 2 mm. 3 µm	12.2	330
Shim-pack GWS 150 × 4.6 mm. 5 µm	9.5	450

Table 4: Some properties of chromatographic columns used in the study

The combination of MS detection and chromatographic separation enables reducing the flow rate and significantly reducing the analysis time. This study provides development and validation of the methods for the determination of valganciclovir and ganciclovir in human blood plasma by LC-MS/MS.

Materials and methods

For the analyses, a Nexera XR UHPLC was coupled to an LCMS-8040 tandem mass spectrometer. Stock solutions were prepared using various reference standards and an internal standard (acyclovir). Mixed GAN and VAL working solutions and an ACI working solution were prepared by dilution to the required plasma concentrations (table 2). Stock and working solutions as well as intact blood plasma samples were stored frozen at -45 °C until use.

Sample preparation

The acyclovir working solution and a calibration sample were added to a solvent, vortexed and then centrifuged. The supernatant was filled into vials and placed into an autosampler.

Level	Analyte concentration, ng/mL		IS concentration, ng/mL
	VAL	GAL	ACI
1	5.00	50.00	1,000.00
2	10.00	100.00	1,000.00
3	25.00	250.00	1,000.00
4	50.00	500.00	1,000.00
5	100.00	1,000.00	1,000.00
6	250.00	2,500.00	1,000.00
7	400.00	4,000.00	1,000.00
8	750.00	7,500.00	1,000.00
9	1,000.00	10,000.00	1,000.00
LLOQ	5.00	50.00	1,000.00
L	15.00	150.00	1,000.00
M	500.00	5,000.00	1,000.00
H	800.00	8,000.00	1,000.00

Table 2: Concentrations of target substances at calibration levels

Results and discussion – Method development

Development of a chromatographic separation method for valganciclovir and ganciclovir is challenging due to their weak retention on “classic” octadecyl columns. Use of the columns with amino groups also did not provide good retention and proper separation of VAL or GAL. An idea was to test C18 columns with different total carbon content (table 4). The best results were achieved using Shim-Pack GWS column with the lowest total carbon content. Despite the insignificant retention of valganciclovir and ganciclovir on this column, the chromatographic peaks were separated from the dead volume, and the method met the requirements of the regulation for the validation, including the “selectivity” parameter.

Method validation

The method was validated based on the guidelines for the examination of medicines [12] as well as FDA [13] and EMA [14] guidelines for the following parameters: selectivity, matrix effect, calibration curve, accuracy, precision, recovery, lower limit of quantification, carry-over and stability.

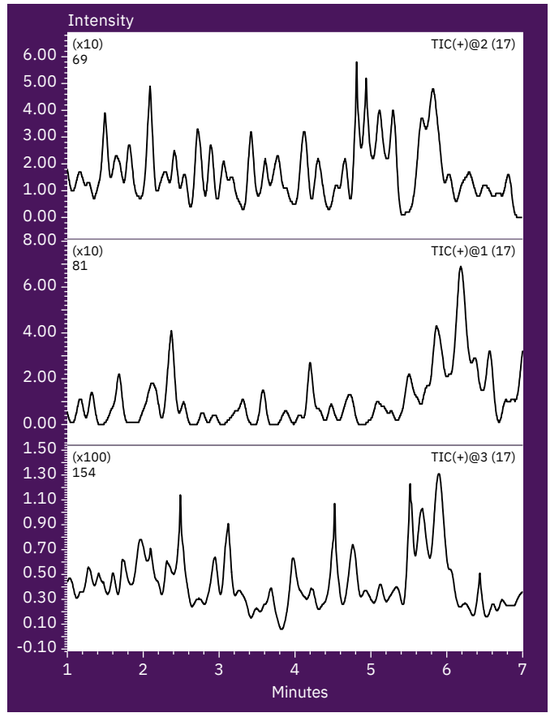


Figure 3: Blank plasma sample chromatogram

Selectivity

Six samples of intact plasma obtained from different sources were tested as well as samples of intact plasma spiked with working solutions of valganciclovir and ganciclovir (5 and 50 ng/mL respectively). Additionally, samples of intact plasma with hemolysis and samples with increased lipid content were analyzed. With blank plasma chromatograms (figure 3) intensity of the peaks with the same retention time as the target substances did not exceed 20 % of the signal at LLOQ (Lower Limit Of Quantification) and 5 % of the internal standard signal.

Matrix effect

The samples including working solutions of valganciclovir, ganciclovir and acyclovir as well as spiked plasma samples were examined to estimate a matrix effect. It was estimated at low (L) and high (H) concentration levels (see table 2) for both valganciclovir and ganciclovir and at concentration of 1,000 ng/mL for acyclovir. The results are shown in tables 5 and 6.

Calibration curves

Based on nine samples of intact plasma spiked with acyclovir, valganciclovir and ganciclovir to the concentration levels 1 - 9 (see table 2) a calibration was created. The calibration curves for valganciclovir (peak area ratio to concentration ratio) and ganciclovir (same coordinates) are shown in figures 5 and 6 respectively. The correlation coefficients obtained meet the regulations (> 0.99). A chromatogram of the sample with concentration level 9 is shown in figure 4.

Accuracy and precision

Spiked plasma samples at LLOQ, high, medium and low concentration levels (see table 2) were analyzed to estimate accuracy and precision of the method developed. Five different samples for each concentration level with triple injections were used. Accuracy and precision have been evaluated within-run, and between-run (two runs and three runs); data is shown in table 7. The obtained values of the relative standard deviation (precision) and the relative error (accuracy) meet the regulations (no more than 20 % at the LLOQ level, no more than 15 % at other levels).

Recovery

Three plasma samples spiked at low, medium and high concentration levels as well as QC samples were tested to estimate recovery value. Additionally, the plasma samples with hemolysis and increased lipid content were tested. The results obtained are presented in table 8. According to the regulations, a recovery should not be 100 %, however, it is necessary to ensure efficient and reproducible extraction of the target substances from the biological matrices. RDS of recovery value should not exceed 15 %.

Lower limit of quantification

LLOQ level was determined based on linearity, accuracy and precision data. The minimum valganciclovir and ganciclovir concentrations in plasma for which it is possible to quantify VAL and GAL with RSD and E values of no more than 20 % were defined as LLOQ for the method. ⟶

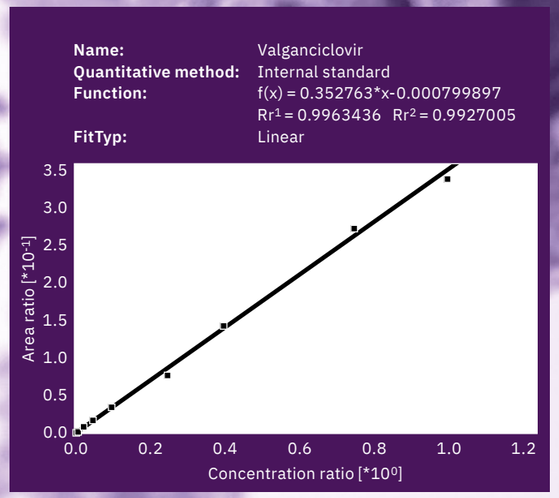


Figure 5: The calibration curve representing dependence of the ratio area peak of valganciclovir to acyclovir on the concentration ratio of valganciclovir to the acyclovir in plasma

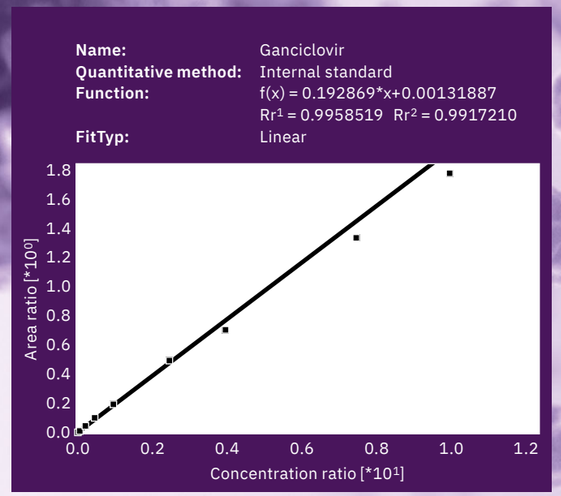


Figure 6: The calibration curve representing dependence of the ratio area peak of ganciclovir to the acyclovir on the concentration ratio of ganciclovir to the acyclovir in plasma

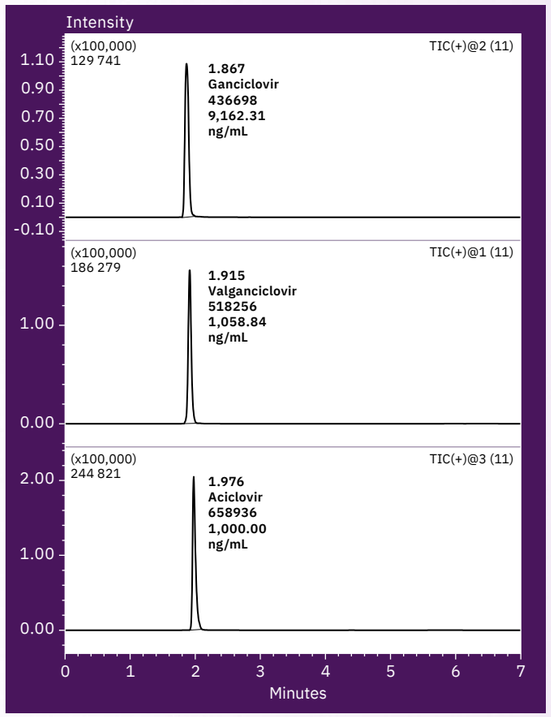


Figure 4: Level 9 plasma sample chromatogram

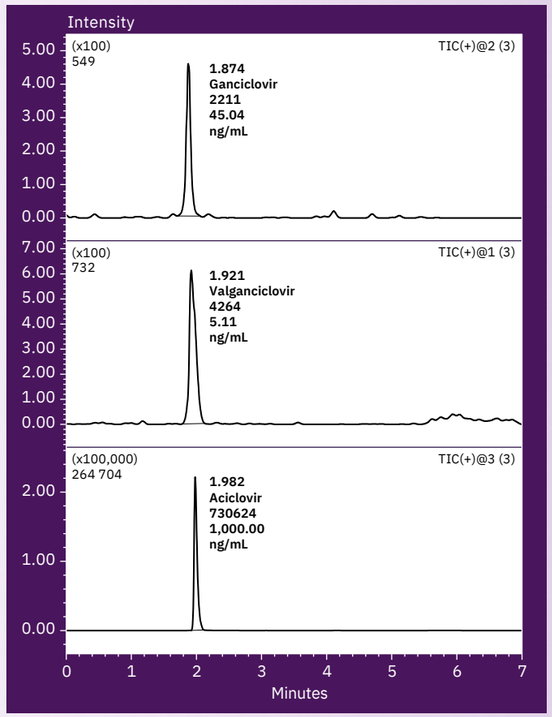


Figure 7: LLOQ plasma sample chromatogram

#	Mf of GAL (level L)	Mf of ACI (level L)	Normalised Mf (level L)	Mf of GAL (level H)	Mf of ACI (level H)	Normalised Mf (level H)
1	0.91	1.00	0.90	1.00	0.99	1.01
2	0.87	1.00	0.87	0.99	1.02	0.97
3	0.91	1.01	0.90	0.98	0.99	0.99
4	0.92	1.02	0.91	1.01	1.01	1.00
5	0.90	0.99	0.91	0.99	1.02	0.97
6	0.94	1.01	0.93	1.01	1.02	0.99
		Average	**0.90**		**Average**	**0.99**
		CV, %	**2.18**		**CV, %**	**1.50**

Table 5: The matrix factor of valganciclovir calculations, normalized by the IS matrix factor

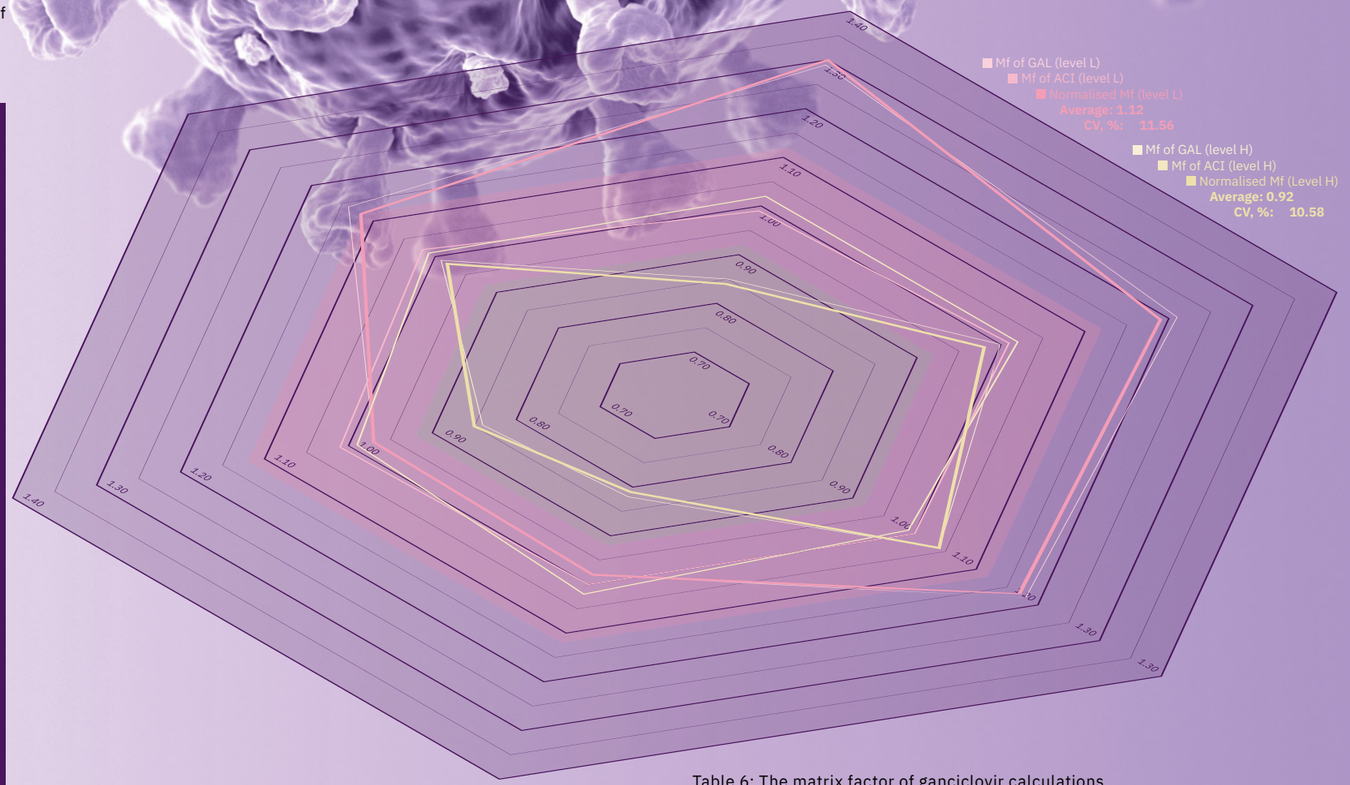


Table 6: The matrix factor of ganciclovir calculations, normalized by the IS matrix factor

Injected (ng/mL)	Average found, ng/mL			SD			RSD, %			E, %		
	(n = 5)	(n = 10)	(n = 15)	(n = 5)	(n = 10)	(n = 15)	(n = 5)	(n = 10)	(n = 15)	(n = 5)	(n = 10)	(n = 15)
						Valganciclovir						
5.00	5.80	5.45	5.29	0.08	0.62	0.56	1.37	11.35	10.53	16.08	8.96	5.89
15.00	14.46	14.08	13.72	0.62	0.75	0.80	4.26	5.31	5.86	-3.63	-6.11	-8.52
500.00	550.73	561.04	563.16	5.21	12.53	10.69	0.95	2.23	1.90	10.15	12.21	12.63
800.00	893.66	901.00	900.88	12.41	13.06	12.59	1.39	1.45	1.40	11.71	12.62	12.61
						Ganciclovir						
50.00	48.32	47.39	47.18	1.13	2.33	2.06	2.33	4.91	4.37	-3.35	-5.21	-5.65
150.00	145.73	143,.35	141.91	6.62	6.30	5.73	4.27	4.39	4.04	-2.85	-4.44	-5.39
5,000.00	4,740.25	4,808.83	4,892.22	77,.26	104.66	153.94	1.63	2.18	3.15	-5.19	-3.82	-2.16
8,000.00	7,198.32	7,372.03	7,482.18	146.29	225.72	252.41	2.03	3.06	3.37	-10.02	-7.85	-6.47

Table 7: Accuracy and precision of the method

LLOQ was 5 ng/mL for valganciclovir and 50 ng/mL for ganciclovir. Chromatograms of plasma containing VAL and GAL at LLOQ level are shown in figure 7. The detection limit of valganciclovir was about 0.93 ng/mL, and the detection limit of ganciclovir was about 0.73 ng/mL (signal/noise ratio about 3 : 1).

Stability

Short-term stability of the samples prepared (autosampler stability and bench-top stability) was confirmed at lower and upper concentration levels. Also, a solution stability of the target substances at three freeze-thaw cycles as well as long-term stability (when stored for 30 and 59 days at a temperature of -45 °C) was confirmed.

Carry-over

There were no peaks with retention time of the target substances on chromatogram when analyzing the blank plasma samples after the calibration samples with the highest concentration level of valganciclovir and ganciclovir.

Conclusion

The LC-MS/MS method for the determination of valganciclovir and ganciclovir in human plasma was developed and validated. Concentration ranges in plasma were 5 - 1,000 ng/mL for valganciclovir and 50 - 10,000 ng/mL for ganciclovir. This enables use of the method for both pharmacokinetics studies and therapeutic drug monitoring.

Note

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

	Recovery (level L), %	Recovery (level M), %	Recovery (level H), %
	Valganciclovir		
Average	88.57	98.94	101.80
SD	6.78	2.76	2.48
RSD	7.66	2.79	2.43

	Recovery (level L), %	Recovery (level M), %	Recovery (level H), %
	Ganciclovir		
Average	93.98	90.95	98.01
SD	8.64	9.83	4.44
RSD	9,.20	10.80	4.53

Table 8: Calculation of valganciclovir and ganciclovir recovery at L, M, H levels from different biological matrix

Joint approach

HPLC and **SFC**: Method development for chiral compounds with the Nexera SFC

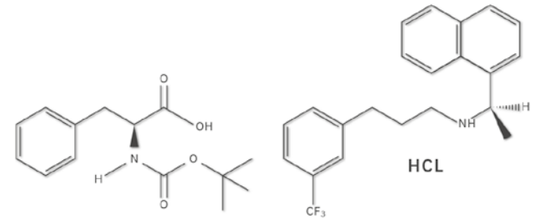


Figure 5: left structure of Boc-DL-Phenylalanine and right structure of Cinacalet HCl (R-Enantiomer)

Dr Brigitte Bollig, Shimadzu Europa GmbH

A general overview of the SFC technique: What are the differences and benefits of SFC compared with conventional UHPLC, and where can advantages be derived when both technologies are combined? A switching system changing between SFC and UHPLC offers users flexibility in method development; this will be shown with some application examples. SFC is applied especially in the separation of chiral compounds. For this, an application for a medicine as well as a compound which can be used as a source for future organic synthesis is developed and shown.

SFC – "green" technology

Supercritical fluid chromatography (SFC) is a separation technique applying a supercritical fluid as the primary mobile phase. Because supercritical fluids have lower viscosity and higher diffusivity than conventional ones, they result in lower column back pressure than separation by UHPLC. That means they can be used for high speed analysis and high separation analysis at high flowrates.

Typically, supercritical carbon dioxide is applied for SFC. The polarity of supercritical carbon dioxide is known to be similar to hexane, a saturated hydrocarbon. However, because it is difficult to elute target components from the column using supercritical carbon dioxide alone, an organic solvent is added as a modifier to the mobile phase. This changes the polarity of the mobile phase, so that the target components can be eluted from the column.

Pharmaceuticals, foods, environmental testing and many other fields require a wide variety of separation methods, such as for separation of chiral compounds and structural isomers. Due to the difference in separation selectivity between UHPLC and SFC using supercritical carbon dioxide and organic solvents, SFC has been highly anticipated in recent years as a new separation method.

Furthermore, an existing UHPLC system can be upgraded to a UHPLC/SFC switching system by adding units required for SFC. By using two separation methods, UHPLC and SFC, for screening during the method development process, the system can configure superior analytical conditions more quickly. [1] ⟶

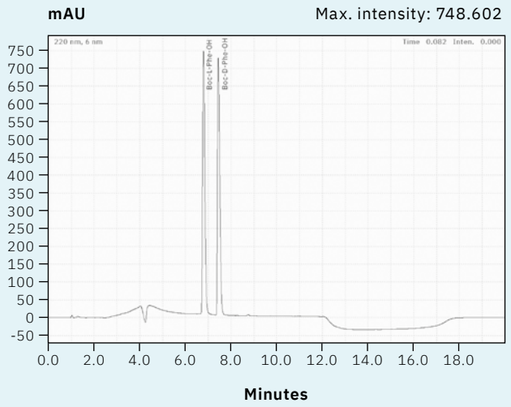


Figure 9: Chromatogram for Boc-DL-Phenylalanine

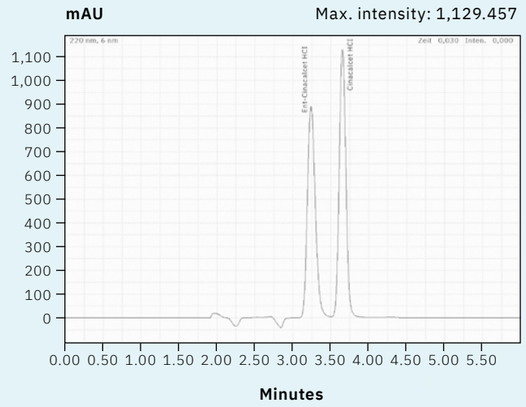


Figure 10: Chromatogram for Cinacalcet HCl [2]

SFC is also known as a “green” technology, due to savings in use and disposal of organic solvents.

Differences between HPLC and SFC separation characteristics

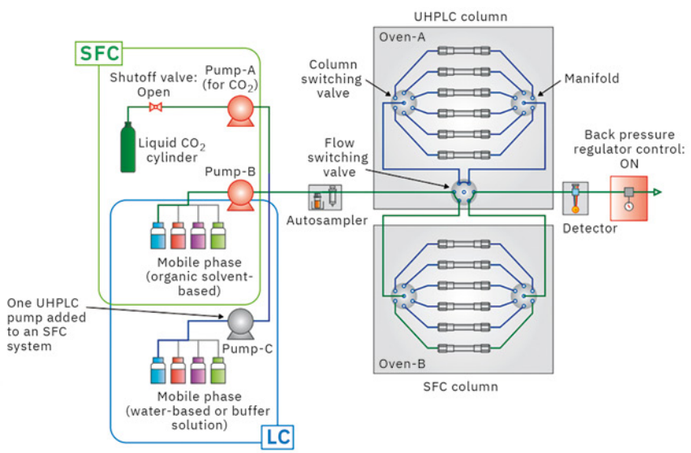
SFC column efficiency does not decrease as much as HPLC, even at high flowrates. SFC therefore enables high speed analysis with shorter analysis times, but can result in a different elution order and different separation selectivity. SFC can improve separation of even the compounds that are difficult to separate by HPLC.

UHPLC/SFC switching system

When considering separation conditions, using both UHPLC and SFC can help to determine even better separation conditions. The Nexera UC/s UHPLC/SFC switching system provides the ability to use both UHPLC and SFC analysis modes in a single system. Figure 4 shows a flow diagram for this system.

The system was configured by adding a supercritical carbon dioxide delivery unit and back pressure regulator unit to a standard UHPLC system. Both UHPLC and SFC analysis modes can be used by switching (control mode ON or OFF) off delivery units and switching the back-pressure regulator pressure. Sharing the solvent delivery unit (for pumping organic solvents), autosampler, column oven, and detector for both SFC and UHPLC analysis minimizes space requirements and equipment cost and improves the equipment utilization rate.

In addition, an existing UHPLC system can be upgraded to this system. Furthermore, by using the mobile phase solvent switching valve in combination with the column switching valve, mobile phase conditions can be changed automatically and continuously for up to twelve columns to enable a wide variety of conditions that improve method development efficiency. [1]



Application development SFC for chiral Boc-DL-Phenylalanine and Cinacalet HCl [2]

Boc-DL-Phenylalanine is a chiral amino acid which can be used typically as structural source for future organic synthesis, resulting later in products in the pharma or chemical industry. Cinacalet is a medicine and is sold in the EU under the brand name MIMPARA to treat parathyroid diseases such as primary and secondary hyperparathyroidism and carcinoma. The chemical structures are shown in figure 5.

As a starting point for the method development, HPLC Normal Phase application is taken, well-known from the literature. See figure 7 for Boc-DL-Phenylalanine [3] and figure 8 for Cinacalet HCl [4] (both shown in the digital version).

Using the SFC part of the Nexera UC/s UHPLC/SFC switching system and with the help of the Method Scouting software, a new method can easily be developed, see figure 6. The software has been designed to prevent human error in writing methods and automatizing this process. For the scouting runs a combination of 4 different modifiers (Methanol, Ethanol, Isopropanol and Acetonitril) with two additives were tested (0.1 % TFA – Trifluoraceticacid, 0.1 % DEA – Diethylamin) with 10 DAICEL columns (Chiralpak IA, IB, IC, ID, IE-3, AD-H, AS-H, AY-H, and Chiralcel OD-H, OJ-H, OZ-3). This combination result in 120 runs in total, and the best separation combinations are used for future improvement like gradient or flowrate adjustments.

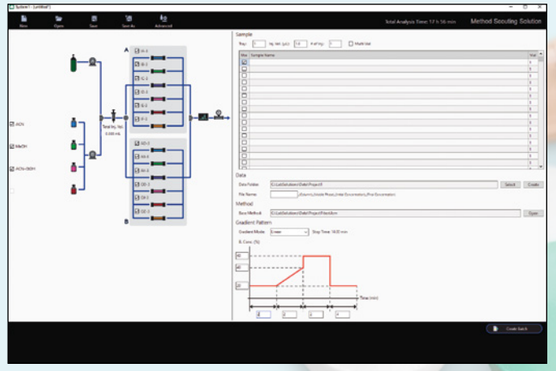


Figure 6: Method Scouting overview

Chromatograms with the ready-to-use SFC method for Boc-DL-Phenylalanine and Cinacalet HCl are shown in figures 9 and 10 respectively.

Conclusion

The Nexera UC/s UHPLC/SFC switching system enables both UHPLC and SFC analysis without reconnecting flow lines. Method Scouting Solution, which is a dedicated software that supports switching between UHPLC and SFC modes, can be used to automatically create and execute a batch table for action required for switching modes, such as purging the mobile phase used for the preceding mode and washing lines with the mobile phase required for the next mode. Consequently, there is no need to perform complicated operations such as creating methods for each combination of column and mobile phase.

Simply by specifying (1) mobile phases, (2) columns, (3) vials, (4) base method used for analysis and (5) gradient conditions (initial as well as final concentration, and gradient), the software can generate a batch table automatically for use in method scouting. This ensures that even first-time users can smoothly apply automatic switching between UHPLC and SFC, and can easily transfer or develop a method with both techniques which has been shown with two simple application examples to separate chiral compounds.

Note

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

360° drinking water analysis – Episode 3

Best things come in small packages

Determination of anions in drinking water using ion chromatography

Uwe Oppermann, Vadim Kraft, Shimadzu Europa GmbH

The determination of inorganic ions in drinking water samples is an important application for drinking water suppliers. For the quality control of drinking water, ion chromatography (IC) with suppressed conductivity is suited perfectly due to its high sensitivity and selectivity. IC is an established method for analysis of anions listed in main standards for analysis of drinking water in accordance with the regulations in US and Europe.

In December 2020, the European Parliament adopted the revised Drinking Water Directive (DWD). The DWD has been in force since January 2021, and Member States have two years to transpose it into their national legislation. The revised Drinking Water Directive replaces the 20-year-old drinking water directive (98/83/EC).

Key features of the revised DWD are reinforced water quality standards which are more stringent than WHO recommendations. The new directive brings many changes: Important from an analytical point of view are the tighter limits for contaminants testing such as the heavy metals lead and chromium. [1]

Introduction

Ion chromatography has been approved for analysis of common inorganic anions as well as some disinfection products in drinking water to complete the regulations of many countries worldwide. Different standards exist to describe procedures of analysis including suppressed conductivity detection as a main detection method. The most relevant methods used in US and European countries are the U.S. EPA 300.1 standard as well as ISO 10304-1 and ISO 10304-4 norms. [2 - 5] Other organizations have also published methods, for example ASTM D4327. [6]

This article describes the determination of seven common inorganic anions and three disinfection by-products in drinking water in accordance with the EPA and ISO methods listed above.

Ion chromatography for anion detection

How does the HIC-ESP ion chromatograph, introduced in 2020, perform in the context of maximum allowable concentration levels of the new European DWD? The system is an ideal tool for sensitive measurement of anions in drinking water samples, and is also suitable for applications in a wide range of fields including environmental science, medicine, chemistry and food science. →

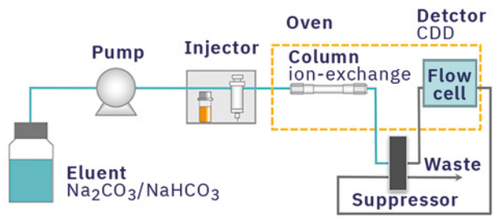


Figure 1: Flow diagram

Compound	Lab tap water [mg/L]	Dispenser water [mg/L]	Bottle water [mg/L]
Fluoride	0.12	0.12	0.42
Chloride	36	36	96
Nitrite	—	—	—
Bromide	0.082	0.080	0.22
Nitrate	12	13	—
Phosphate	0.30	0.27	0.17
Sulfate	70	70	24

Table 1: Concentration of anions in drinking water samples

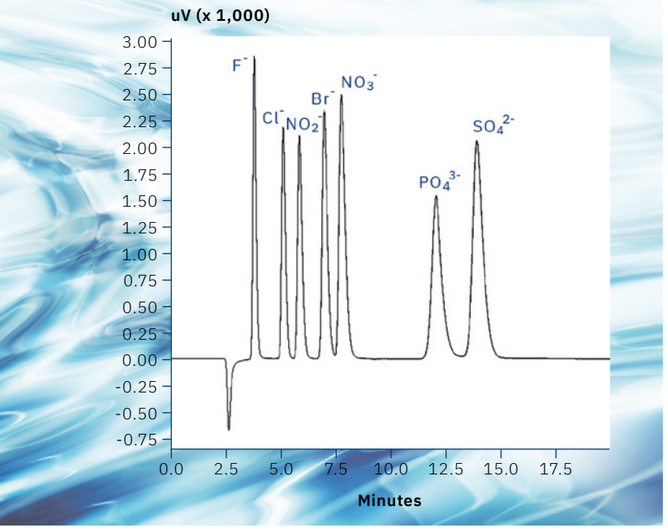


Figure 2: Separation of seven common anions
(Column: Shim-pack IC-SA2)

High sensitivity and reliability

In ion chromatography, an eluent suppressor improves sensitivity by reducing background condcutivity and increasing signal intensity of the analysed ions.

The Shimadzu ICDS-40A suppressor unit is an electrodialysis type, using an electrochemically driven membrane process in which an ion exchange membrane in the suppressor removes sodium ions in the column eluent. The hydrogen ions necessary for regeneration of the membrane are formed by electrolyzing of the aqueous eluent after it has passed through the detector. Figure 1 shows the flow diagram.

Analysis of seven common anions according to ISO 10304-1

Figure 2 shows an overlay of six chromatograms presenting the separation of seven common anions. Very low % RSD for retention time (≤ 0.04 %) and peak area (≤ 0.45 %) for all anions demonstrate the high precision of the analysis system. The low-void-volume ICDS-40A suppressor reduces peak dispersion and improves the separation of fluoride from the water dip peak. The requirement of the ISO standard for resolution R (≥ 1.3) was met, calculated according to US Pharmacopeia (USP). This method is recommended for separation of seven common anions within relative short analysis times.

Linearity

The linear working concentration range used by conductivity detection was from 0.1 to 5 mg/L with the exception of bromide and nitrite, measured from 0.05 to 5 mg/L. The coefficient of determination R^2 was ≥ 0.9993 for all anions.

Sample analysis

The concentration of analyzed anions varied in samples, but all of them showed high contrast in concentration between phosphate, sulfate and partly nitrate to other anions. The samples were therefore measured undiluted and diluted (1/40, v/v) to quantify all target compounds in the working concentration range. The content of highly concentrated phosphate, sulfate and partly nitrate was calculated from diluted samples. The concentration of residual anions was calculated from undiluted samples. Table 1 presents concentration of the anions in the drinking water samples analyzed.

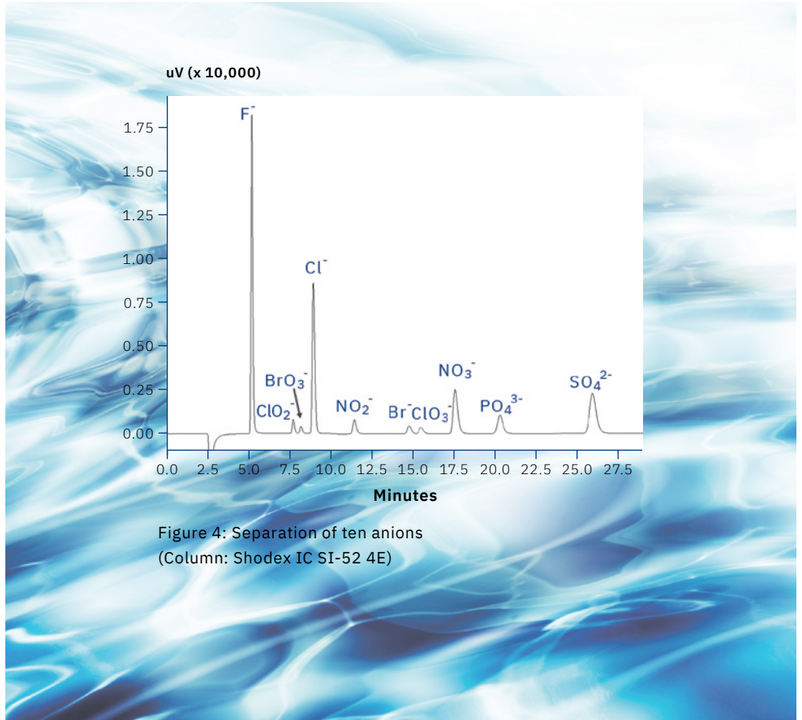


Figure 4: Separation of ten anions
(Column: Shodex IC SI-52 4E)

Compound	Tap water recovery [%]	Dispenser water recovery [%]	Bottle water recovery [%]
Fluoride	99.8	103.6	99.8
Chlorite	107	107	107
Bromate	105	107	105
Chloride	110	83.4	110
Nitrite	97	92	97
Bromide	100	100	100
Chlorate	106	101.5	106
Nitrate	98.5	97.9	98.5
Phosphate	91.7	109.6	91.7
Sulfate	120	99.8	120

Table 2: Recoveries in different water samples

Analysis of ten anions according to EPA 300.1

Figure 4 shows the separation results of 10 anions. This method is recommended for analysis of three disinfection by-products, chlorite, bromate and chlorate, in the presence of seven common anions. All anions were eluted in less than 27.5 minutes. The material of the column used for this method had lower particle size compared to the column material applied for analysis of the seven anions discussed previously.

Although elution time is long, the advantage of this method is the baseline separation of ten analytes. Furthermore, the resolution of bromide and chlorate is higher even with larger injection volume. This method will also be useful for trace analysis of chlorate and bromide.

Linearity and Method Detection Limit (MDL)

To evaluate the method and overall system performance, linearity, method detection limits and reproducibility were also determined. MDL investigation was performed following the procedure described in EPA Method 300.1 by making seven replicate injections of MDL standards.

MDL standards were prepared by fortifying the blank with anion standards to a concentration 3 to 5 times that of the instrument detection limit. MDL was calculated as (t) x (S), where t is student's t value for 99 % confidence level (t = 3.14 for seven replicates) and S is standard deviation from seven injections.

The results of linearity for calibration curves show that the system produces linear response with coefficient of determination R^2 greater than 0.999 for all 10 anions in a broader concentration range. The calculated MDL were at low ppb range from 1.3 µg/L (fluoride) to 12.6 µg/L (chloride).

Reproducibility and Accuracy

This method also showed excellent reproducibility for retention time (< 0.7 % RSD) and peak area (< 0.42 % RSD). Table 2 shows that acceptable recoveries were obtained for all inorganic ions in three water matrices using both methods.

Conclusion

The Shimadzu HIC-ESP ion chromatography system with electrolytic eluent suppression provides sensitive analysis of common inorganic anions and disinfection by-products as listed in ISO 10304-1, ISO 10304-4 or EPA 300.1 standards for different types of water including drinking, surface and wastewater. The results presented demonstrate that a robust and reliable performance of the IC system is possible for the determination of the analyzed anions.

Note

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

Shining green

Tracking down microplastics with fluorescence

Marion Egelkraut-Holtus, Shimadzu Europa GmbH
Albert van Oyen, Erwin Jansen, Carat GmbH

Figure 2: PET bottle bottom (caffeinated lemonade) and the fluorescence under a xenon light source. The bottle was mounted in the sample chamber of the RF-6000.

Figure 3: Screen fractions of a polycarbonate from left to right: 500-, 1,500-, 3,000-µm particle fractions and 5 mm granules.

Where does microplastic come from?

Microplastics are one of the greatest global challenges for humanity, wildlife and the environment. They originate from larger pieces of plastic that have weathered or are already contained in everyday objects, for example in cleaning products, cosmetics or textiles.

Microplastics enter the oceans through wastewater; sewage treatment plants can only filter them out to a limited extent. Once in the sea, these plastics can no longer be removed. Microplastics are found at all depths of the oceans, enter the food chain from there and are ingested by microorganisms that are important food for fish. The fish, in turn, feed marine mammals, birds and humans.

This third part of the series of articles on polymer analysis is about fluorescent microplastics and how they can benefit research using fluorescence spectroscopy.

In the last two issues of Shimadzu Magazine, polymers from beverage bottles in the food sector were detected (1/2020), as well as industrially produced polymer granules (2/2020).

Media, environmental protection organizations and authorities report on microplastics. These particles pose danger to humans and animals and are already found in their excretions. But research on the direct impact of microparticles in the body is in its infancy.

Since many publications keep developing theoretical models and work with “freshly generated” microplastics, the idea here is to recycle used material, shred it and then use it for testing purposes. Thus, instead of putting new and more polymer into the environment, existing polymer is used; it is about micro quantities here, not kilograms or tonnes. And the materials are real-life samples with true properties of the microparticles that the organisms must deal with by necessity.

Microplastics are dangerous to the environment mainly because of their small size, which means that a large number of organisms ingest them, for example because they mistake them for food. ⟶

Figure 1: Three beverage bottles in green from left to right: strongly fluorescent PET bottle for a caffeinated lemonade, bottle made of PP for children‘s juice without fluorescence but with a strong absorption spectrum (red is absorbed in the visible spectrum and the complementary color green remains) and another fluorescent PET lemonade bottle.

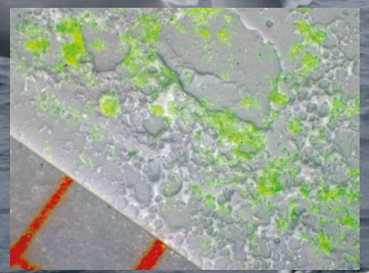


Figure 4a (left) and 4b (right): Microparticles generated from the left PET bottle of figure 1 (4a) and a selection of the smallest particles with graph paper scaling for size comparison, and the particles lying on the $BaSO_4$ bed

A next step in the development of innovations in environmental research could be fluorescent microplastics. They may make it easier to determine whether and where these materials accumulate in organisms. This would then enable a better risk assessment.

According to an application, it should be possible to produce fluorescent microplastics using the lipophilic dye Nile Red. But here the question arises, why produce them when there are enough polymers with fluorophores (part 1 + part 2) (figure 1)? So technical polymers and a very striking greenish-yellow PET beverage bottle (figure 2) were ground to produce microparticles. [1,2]

The Carat company from Bocholt, Germany has developed the idea further. It specializes in the analysis of thermoplastics. For the application at hand, Carat has found a way to break down, specify and certify the polymers. With the RF-6000 spectrofluorometer and a solid sample holder, Shimadzu has supplied a solution that can measure these small particles.

Measurement challenge microplastics

Microplastics lead to special requirements in analytical technology. Fluorescence is known for its high detection limits in liquids. Can this property also be transferred to particle size? In the two previous issues of the Shimadzu customer magazine (see 1 and 2/2020), polymer pieces (2 - 3 cm) and granulates (5 mm) were examined.

Microscopy is useful for samples smaller than 100 µm, for instance in the identification of particles with infrared spectroscopy. This third part of the article series looks at the identification or simply recovery of particles smaller than 500 µm with fluorescence spectroscopy. Common polymer granules in the size of 5 mm show more or less strong fluorescence, which arises from polymers with π-electron molecular structure (double bonds) or from fluorescent additives. Now PC (polycarbonate) and PET (polyethylene terephthalate) are being examined more closely.

In order to simulate microparticles, polymers were reduced to particles smaller than 500 µm with the cryomill, then sieved and divided into three fractions of different particle sizes.

Sample preparation and analyses

Particles from each fraction were scattered into the center of a bed of barium sulphate ($BaSO_4$), acting as a fixative, and pressed down with a quartz plate (figure 4). These measurements consistently showed homogeneity down to below 500 µm and presence of fluorescence (figure 5). Depending on the polymer and/or additive, the fluorescence intensity is attenuated with the microparticles compared to higher concentrations (larger surface areas and layer thickness of the polymers).

In an experiment with PET microparticles, grinding of the bright bottle would be suitable. With a very small amount of microparticles, the fluorescence can still be intense, so a standard fluorescence instrument can detect the presence of the particle (figure 6). Measurement parameters and sample preparation were not changed for comparison reasons.

Conclusion

In this series of articles on the fluorescence of polymers, various fields of application were addressed. Polymers from beverage bottles in the food sector (1/2020), and industrially manufactured polymer granulates (2/2020) were examined. Fluorescence spectroscopy showed the fluorescence of the polymers and their incorporated additives and targeted fluorophores that act as markers. This shows that fluorescence can be used as an excellent instrument for quality control.

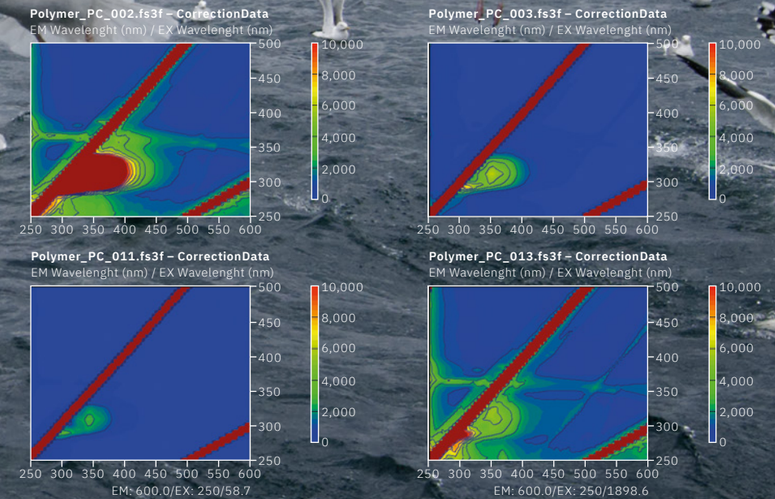


Figure 5: EEM matrices of polycarbonate particles in different sizes, scaling is set to 10,000 intensity units, top left granules, top right 1 particle of 3,000 µm, bottom left 1,500 µm and bottom right smaller 500 µm

For all plastics it could be shown that intrinsic fluorescence occurred or that additives (e.g., Lumicolor admixture yellow, green and others) are responsible for the fluorescence and that their matrix releases fluorescence except for one PP sample.

The detection limits of fluorescence spectroscopy mean that microplastics smaller than 500 µm can also be examined. Here, the concentration of the fluorophores influences the detection limits in relation to the particle size. This is an important argument for polymers that emit weak fluorescence. Residue analysis from filtration, where microplastics are expected, was not considered here. This will be a subject for future analysis.

As a prospect, it is possible to envisage solutions in the near future that can use fluorescent markers invisible to the human eye to better sort recycled PET bottles and increase recycling capabilities to food packaging levels in order to achieve the EU requirement of 95 % purity for food quality in recycled PET. [4]

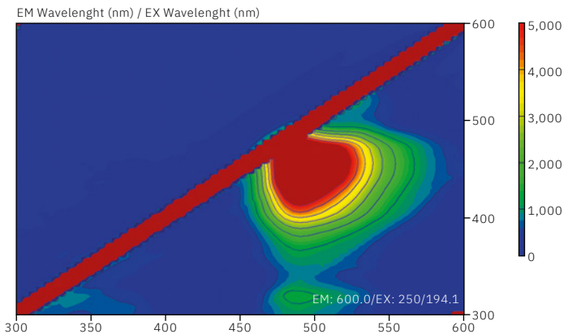


Figure 6: EEM matrix of microparticles from the caffeinated lemonade PET bottle, with scaling to 5,000 intensity units

Note

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

Change is an opportunity

Shimadzu offers virtual trainings as an add-on

Dr Benjamin Thomas, Shimadzu Europa GmbH

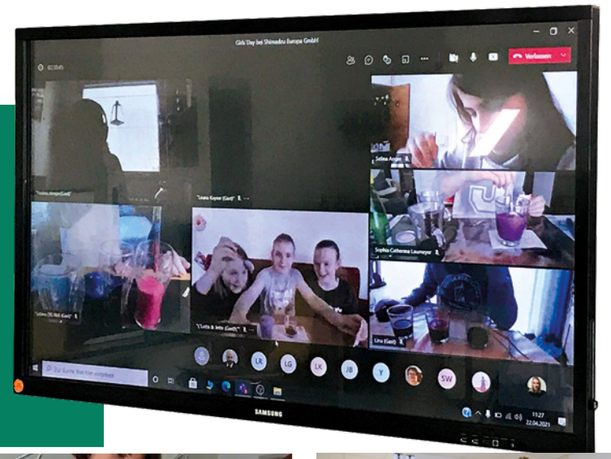
As a result of the Covid-19 pandemic, digital transformation boosts the business world unlike ever before. Target group and service structures are changing, as are communication and collaboration within companies and with customers. Cost-effectiveness and sustainability is reevaluated, business travel, too: the digital world is an opportunity to explore new paths with long-term perspectives.

View into **Laboratory World.** Each area (here UV spectroscopy) is explained by posters for visitors.

How does a sales and service-oriented company like Shimadzu deal with a pandemic situation? A company that is geared towards providing fast customer service wherever needed, delivered by a highly qualified workforce. Which offers training and testing facilities in the European headquarters in Duisburg and the well-equipped “Laboratory World.”

To maintain service excellence, it is essential that an interdisciplinary team of product specialists and service technicians take care of further training of locally operating Shimadzu specialists, e.g. in workshops or targeted trainings on accessories installation. The same applies to the Laboratory World in Duisburg, where customers are invited to test Shimadzu equipment by themselves with real samples they have brought.

shimadzu.eu/
kids-education



Girls Day inspires schoolgirls‘ enthusiasm for technical professions every year and took place virtually for the first time in 2021

Tools such as remote access and video conferencing are well-established in service to provide rapid support on-site. Also, the establishment of the “Shimadzu Academy” internal e-learning platform was already in full swing. However, the measures to contain the Covid-19 pandemic from mid-2020 accelerated the transition to digital working in sales and product support as well.

From physical presence to online

The reduction in contact meant major cuts in the international working environment, which is true for many companies, even medium-sized ones such as the Shimadzu Europe organization. Business trips suddenly became impossible due to travel restrictions, industry trade shows as an important exchange platform were cancelled, and consequently, any form of classroom training was suddenly no longer feasible.

In just a few months, the usual face-to-face training sessions in all Shimadzu departments were converted into online seminars. Camera equipment was quickly purchased, and the know-how to move in a so far unfamiliar, purely digital environment was built up dynamically and pragmatically with mutual support.

The virtual “Girls Day” in April 2021 showed that this form also works beyond the professional community of analytics: Girls from lower and middle school were introduced to chemistry in a playful way with remote experiments – an example with great reach. →

View behind the scenes:
The GC-2030 is staged in front of a green screen

Professional exchange

Internal training courses and product presentations were also transferred successfully to the digital world. In spectroscopy, for example, several “task force meetings” were held for Shimadzu product specialists operating locally in Europe. Selected accessories were presented via video or live sessions, and possible applications or frequent customer inquiries were discussed online.

Screenshot from the **„Task Force Meeting.“** Here, a flow cell for a UV-Vis spectrometer is being prepared for presentation.

Advanced education: hybrid trainings

While the atmosphere at the aforementioned events is informal, certified training courses require a form of performance assessment in order to maintain a high level of support. To this end, the Shimadzu Academy has designed hybrid training courses combining virtual presentation, a video-based installation or measurement, and a final exam. Participants in the live demonstration can learn how to use the software by remote access to the laboratory computer, almost like “hands on” training on-site. A specialist is always present in the lab to change accessories and ensure safe operation.

Making the recorded videos available in the Shimadzu Academy together with the written test offers an additional advantage: Training content can be accessed at any time to refresh knowledge. There is no need for time-consuming scheduling or limiting the number of participants, which greatly simplifies know-how transfer, and training measures can be managed easily.

Virtual equipment instruction for customers

The virtual format of equipment training is also beneficial for the end customer: Usually carried out in person at the customer’s premises en bloc, the online training can be cut into shorter, consecutive sessions.

In this way, partial aspects can be focused per training session (e.g., a certain accessory or a specific measuring mode) and explained in detail. Until the next session starts, users can practice their new skills and address any questions they may have.

Virtual training and on-site presence on demand

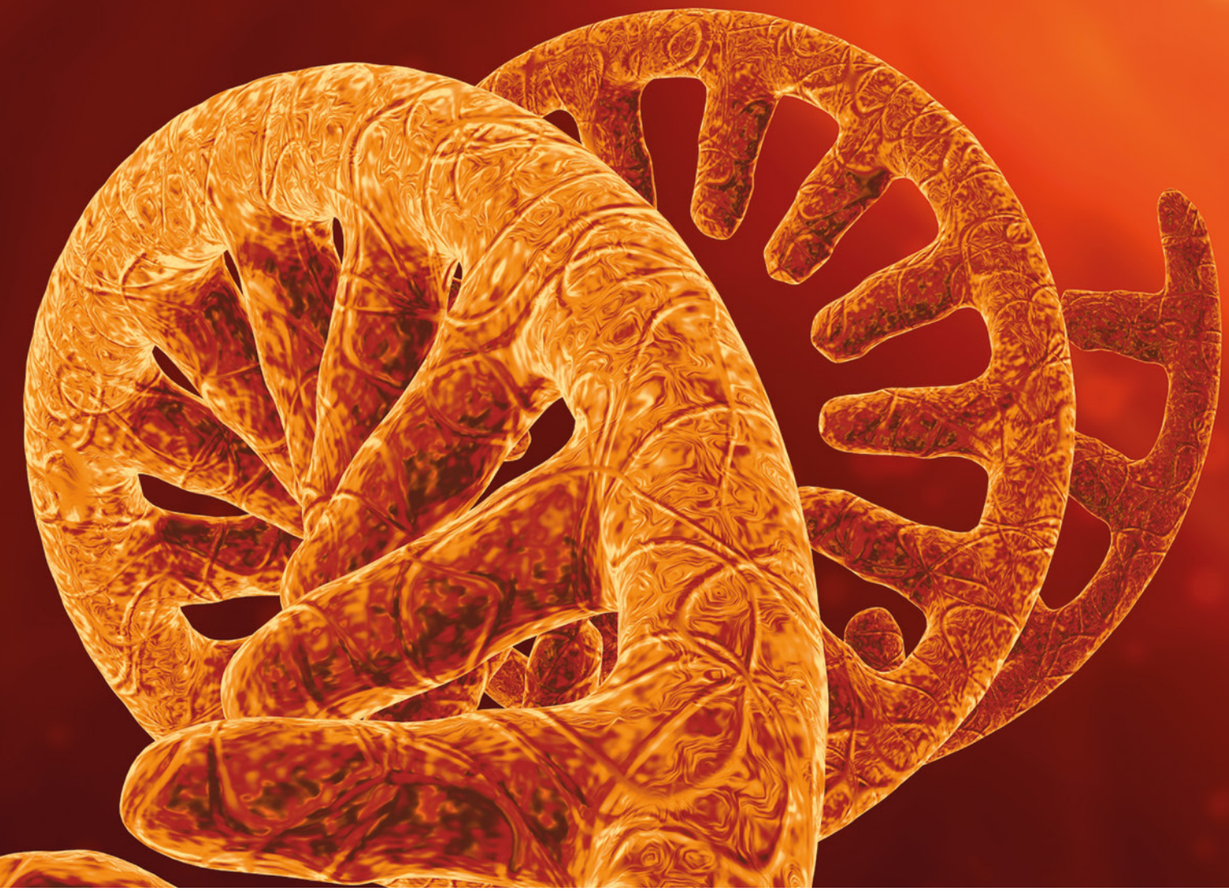
Not everything can be taught digitally. In service for example, the feeling for technical operations (e.g. correct tightening torque of a setscrew or checking of cleaning steps) cannot be learned without practice on the device itself. So, the virtual training sessions can be supplemented with on-site exercises as needed – in compliance with a strict hygiene concept – in response to a demand for a “hands on” component that will be complemented by the mix of videos, chats and e-learning portal after the pandemic.

Some experiences can only be gained by working together, as in this **installation training** for a complex UV-Vis Spectrophotometer

Conclusion

The Covid-19 outbreak accelerated digitization of the workplace and transformation of many industries. Shimadzu was also quick to switch to digital product demonstrations and training. While there are aspects that will continue to require classroom training in the future, the use of digital tools will remain an important pillar in training and rapid customer support.

MOVE ON



New approach for epitranscriptomics

Quantifying RNA methylations by LC-MS/MS

Epitranscriptomics is an emerging and promising research field covering biochemical RNA modifications within a cell. It comprises more than 170 RNA chemical marks, including RNA methylation which can regulate the expression of defined genes, and it is present in all type of RNA, such as messenger RNA (mRNA).

Today, mass spectrometry coupled to a liquid chromatography (LC-MS/MS) has become a method of choice to comprehensively measure modified nucleosides from a biological sample. This application note presents a methodology for quantifying methylated nucleosides from cellular mRNA using LC-MS/MS (Shimadzu LCMS-8060).

Dr Alexandre David, Amandine Amalric, Aurore Attina, Pr Sylvain Lehmann, Dr Jerome Vialaret, Pr Christophe Hirtz, Clinical Proteomics Platform, IRMB-PPC, INM, Univ Montpellier, CHU Montpellier, INSERM CNRS, Montpellier, France

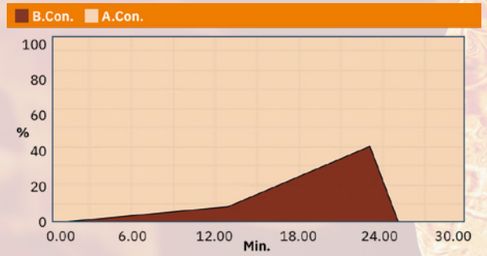
Introduction

Epitranscriptomics investigates modifications to RNA, which is involved in the production of proteins. This branch of research makes it easier to understand the connection between RNA modifications and diseases. For example, the epitranscriptome is modified in many tumors when compared to healthy cells.

The N6-methyladenosine nucleoside (m^6A) - the most common and abundant modifications on RNA in eukaryotes (i.e. organisms whose cells have a nucleus) – is a striking example. This modification occurs not only on mRNA but also on noncoding RNA (rRNA, tRNA, snRNA). As m6A is important for mRNA regulation in normal physiological condition, any dysregulation of its level can lead to disease. [1]

Current technologies to detect m^6A modification [2] use next-generation sequencing techniques and deliver highly valuable information in term of m^6A mapping at the genome-wide level. However, they cannot provide precise quantitative results.

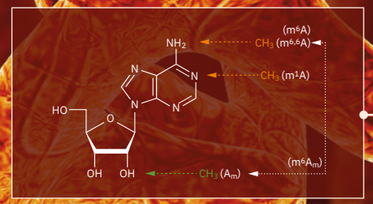
Today, mass spectrometry coupled to a liquid chromatography (LC-MS/MS) has become the method of choice for accurately quantifying modified nucleosides from biological samples. This application note presents a method for precise quantification of methylated nucleosides from cellular mRNA using LC-MS/MS (Shimadzu LCMS-8060). ⟶

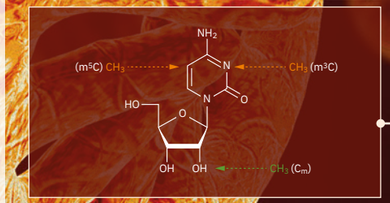
LC analysis conditions	
System	Nexera LC-40
Separation column	Synergi Fusion-RP C18 column (250 mm x 2 mm, 4 µm, 80 Å)
Mobile phase	A: 5 mM ammonium acetate, adjusted to pH 5.3 with acetic acid B: acetonitrile
Gradient elution	

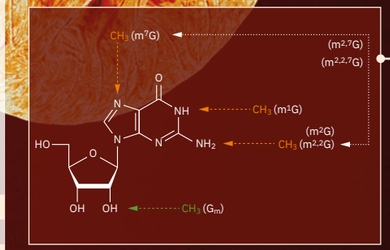
Time (min)	[A] (%)	[B] (%)
0.0	100	0
3.0	100	0
3.1	99	1
13.0	92	8
23.0	60	40
25.0	100	0
30.0	100	0

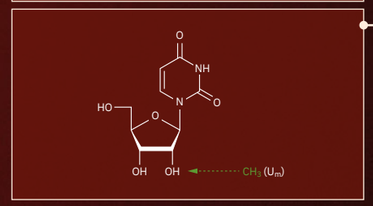
Flow rate	0.4 mL/min
Oven temperature	35 °C
Autosampler temperature	4 °C
Injection volume	5 µL
MS analysis conditions	
System	Triple Quadrupole LCMS-8060
Ionization method	ESI (positive)
Nebulizing gas flow	2.5 L/min
Drying gas flow	3 L/min
Heating gas flow	12.5 L/min
Interface temperature	325 °C
DL temperature	225 °C
Heat block temperature	380 °C

Table 1: Analytical conditions









Molecules	Transitions	Retention time (min)	Target Dwell Time (msec)	Target Collision Energy (E)	Target Q1 Pre Bias (V)	Target Q3 Pre Bias (V)	Interface voltage (kV)
Adenosine (A)	268.0 > 136.0	11.8	197.0	-18.0	-24.0	-24.0	1.0
2'O-methyladenosine (A_m)	282.0 > 136.0	14.6	397.0	-17.0	-14.0	-24.0	1.5
N1-methyladenosine (m^1A)	282.1 > 150.1	5.4	197.0	-21.0	-12.0	-26.0	1.5
N6-methyladenosine (m^6A)	282.1 > 150.1	15.9	397.0	-20.0	-12.0	-16.0	1.5
N6,N6-dimethyladenosine ($m^{6,6}A$)	296.0 > 164.1	18.3	530.0	-25.0	-22.0	-20.0	1.5
N6,2'O-dimethyladenosine (m^6A_m)	296.0 > 150.0	17.6	397.0	-15.0	-18.0	-32.0	1.5
Cytidine (C)	244.1 > 112.0	3.8	197.0	-12.0	-17.0	-29.0	1.0
2'O-methycytidine (C_m)	258.1 > 112.0	7.9	142.0	-15.0	-18.0	-32.0	1.5
N3-methylcytidine (m^3C)	258.1 > 126.0	4.5	197.0	-13.0	-10.0	-14.0	1.5
N5-methylcytidine (m^5C)	258.0 > 126.0	7.3	157.0	-17.0	-14.0	-25.0	1.0
Guanosine (G)	284.1 > 152.0	8.6	142.0	-15.0	-24.0	-23.0	1.5
2'O-methylguanosine (G_m)	298.1 > 152.0	10.9	174.0	-12.0	-12.0	-17.0	1.5
N1-methylguanosine (m^1G)	298.1 > 166.0	10.6	142.0	-15.0	-11.0	-18.0	0.5
N7-methylguanosine (m^7G)	298.3 > 166.0	7.3	157.0	-10.0	-30.0	-18.0	1.5
N2,N7-dimethylguanosine ($m^{2,7}G$)	312.1 > 180.0	10.3	142.0	-12.0	-12.0	-17.0	2.5
N2,N2,N7-trimethylguanosine ($m^{2,2,7}G$)	326.15 > 194	12.4	197.0	-20.0	-15.0	-21.0	1.5
Uridine (U)	245.1 > 113.0	5.2	197.0	-11.0	-28.0	-26.0	1.0
2'O-methyluridine (U_m)	259.1 > 113.0	9.5	142.0	-9.0	-11.0	-19.0	2.5

Table 2: MRM parameters

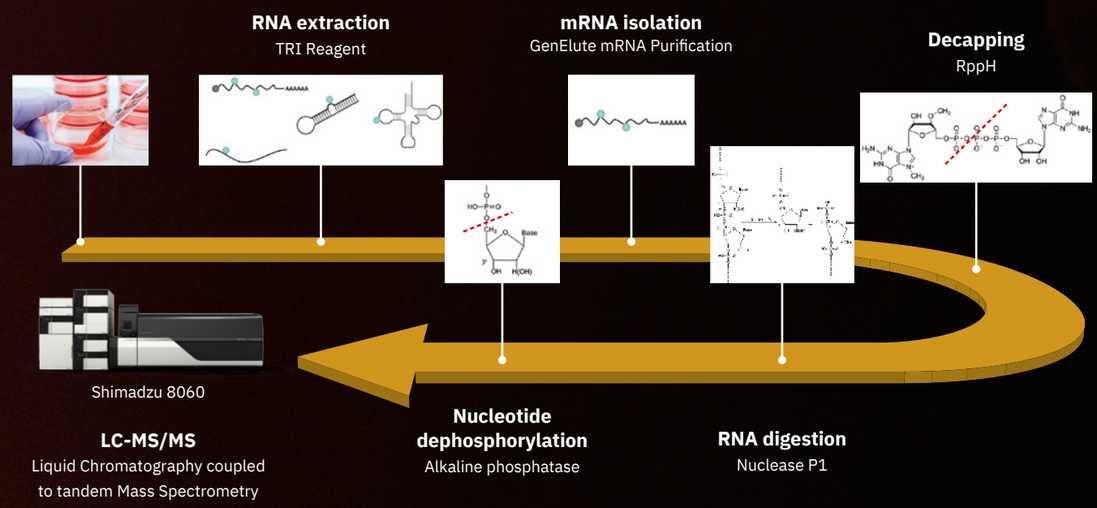


Figure 1: Workflow for label-free quantification of RNA methylations using LCMS-8060

Pre-analytical steps

Experimental workflow for quantification of methylated nucleosides by LC-MS/MS is shown in figure 1. First, the tumor cells are cultured in petri dishes, afterwards the RNA is extracted, isolated and digested into nucleotides, which were then dephosphorylated into nucleosides.

Then, the samples are filtered and injected into an LC-MS/MS.

Analytical conditions

For the measurement of nucleosides using LC-MS/MS, the nucleosides are separated by reverse phase UHPLC (ultra-performance liquid chromatography) and detected by the Shimadzu LCMS-8060 triple-quadrupole mass spectrometer in multiple reactions monitoring (MRM) positive electrospray ionization (ESI) mode. Details of analysis conditions and MRM parameters are given in tables 1 and 2. →

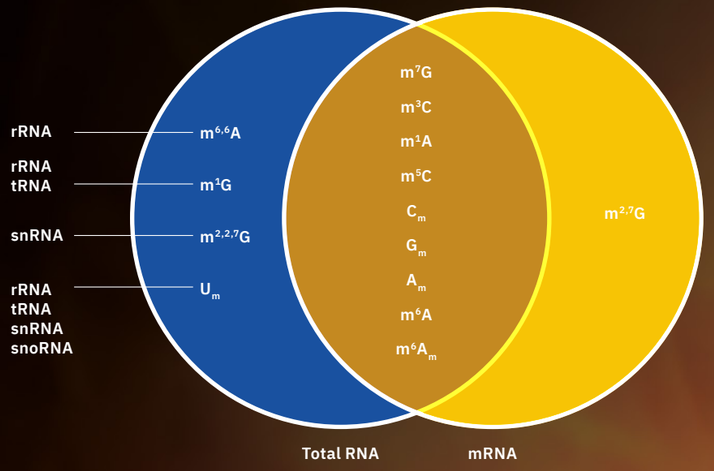
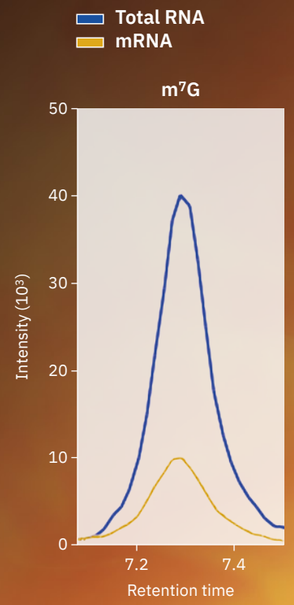


Figure 2: Distribution of the modified nucleoside species in different types of RNA. Ten nucleosides are found in mRNA. Four are only present in other RNA types.



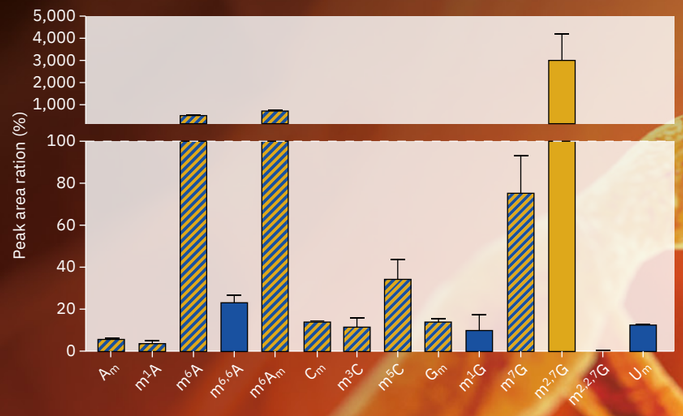


Figure 4: A. Level of RNA methylations in mRNA (histogram) compared to total RNA (dotted line) in CTC44. (n = 3)

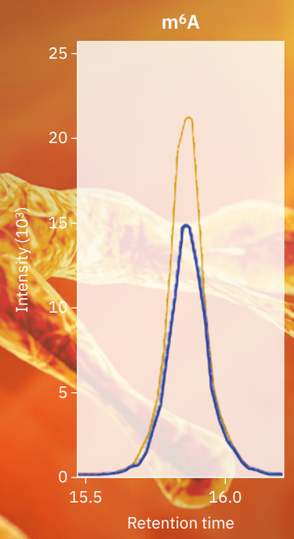


Figure 5: Overlaid extracted ion chromatograms of m^7G and m^6A in total RNA (blue line) and purified mRNA (golden line)

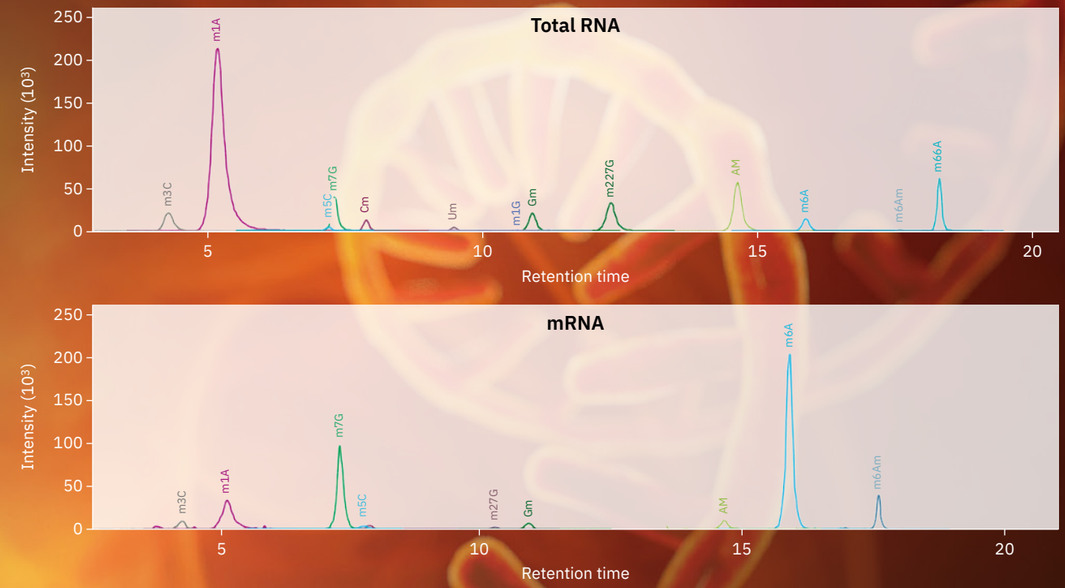


Figure 3: Extracted Ion Chromatograms of methylated nucleosides from total RNA and mRNA analyzed by LCMS

Modomics Database [4] contains information of MRM transitions of numerous modified nucleosides, and enables simultaneous measurement of several methylated nucleosides.

Quantification of methylated nucleosides from messenger RNA (mRNA)

The quantification of methylated nucleosides from mRNA yielded results for 10 nucleoside species from the target panel of 14 RNA methylations (figure 3). The remaining four species have only been observed in rRNA, tRNA, and snRNA, and are – to the author's knowledge – not present in mRNA (figure 2).

Furthermore, for most of the methylations detected, a lower signal is observed for mRNA compared to total RNA (figure 4). For example, m^1A is found in mRNA, which accounts for only 5 % of the total cell RNA, and also in tRNA and rRNA, which respectively represent 15 % and 80 %.

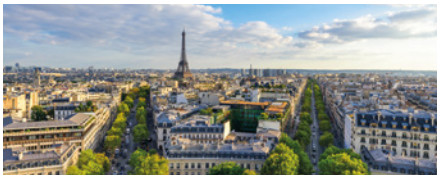
In the case of mRNA purification, the ion signal was enhanced for methylations that are mostly predominantly found in mRNA, such as m^6A and m^6Am (figures 4 - 5).

Conclusion

RNA methylations label free quantification using a triple quadrupole LC-MS/MS system (LCMS-8060 mass spectrometer coupled to Nexera LC-40 in MRM mode) represents a sensitive and reliable approach for epitranscriptomics studies. This application can be implemented for a large panel of biological samples (and RNA species). It is a straightforward and readily applicable experimental setup.

Note

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



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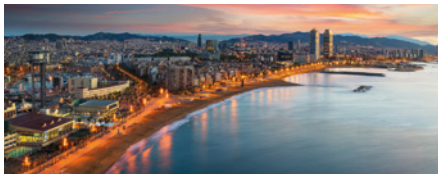

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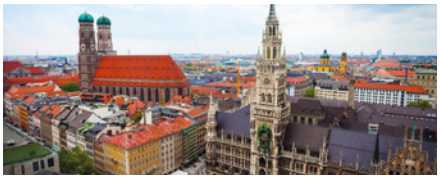

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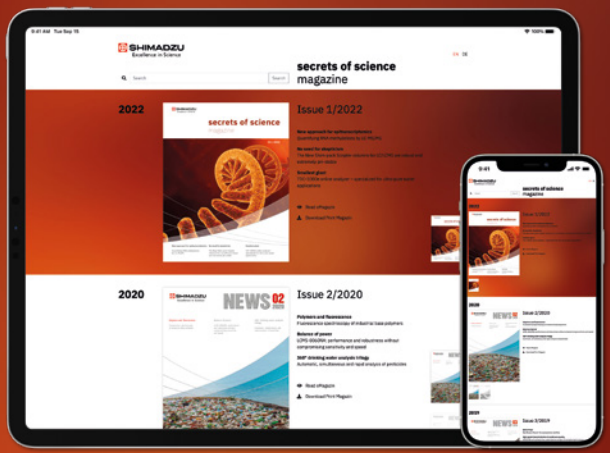

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