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MOVE ON

Shimadzu automates precision testing of ACSR power conductors

Keeping the lights on

Mgr inż. Piotr Szade, Central Mining Institute

An electric power grid is a socially and economically critical installation, and its components must meet exacting performance and safety criteria. The Central Mining Institute (GIG) in Poland tests and verifies the mechanical properties of overhead conductor wires for compliance to current standards. Using Shimadzu equipment, GIG is able to minimize human error in testing via an automated process. The result is a reliable, simplified method.

Transmitting power

Energy is crucial to maintaining life as we know it. Intimately linked to this is the transmission of energy over long distances, from its production sites to its distribution hubs. High-voltage AC (HVAC) transmission lines have been performing this task for more than a century. For the transmission of current at voltages in excess of 100 kV, bare overhead conductors are employed. Aluminium is used as the conducting material.

Two very different materials

To ensure adequate resistance to bending, the conductors are manufactured in the form of a spiral rope containing wires with diameters ranging from about 1.2 to 5 mm, but the most common one is Aluminium Conductor Steel Reinforced (ACSR), in which the conductive aluminium layers wrap around a high-strength steel core made of a single wire or strand. The cross-sectional ratio of aluminium to steel varies and can range from 1.25 to as much as 20 as standard. ⟶

The ACSR cable is therefore an arrangement of conductive, drawn aluminium and steel wires combined to improve its mechanical capabilities. This is extremely important as the cable has to cope with a number of mechanical factors during operation: constant ones such as tension and its own weight as well as variable ones such as icing, temperature effects and wind pressure along with the resulting vibrations.

These materials must work well together, despite having significantly different mechanical parameters: aluminium wire has a nominal strength of 160 to 325 MPa, while steel wires can reach over 1,800 MPa. Add to this the difference in their elastic modulus and elongation at rupture, and the prediction of the mechanical characteristics of a complete conductor becomes rather complicated. The maximum stress exerted on the steel wires in a twisted conductor corresponds to the elongation at which the aluminium wires break. In practice and in normative documents, this elongation is assumed to be 1 %, and it is these values that are used to calculate the nominal RTS (Rated Tensile Strength) breaking strength of ACSR cables.

Conductor testing

Because conductors are such a critical component of power grids, a number of standardized requirements are imposed on them. Within the EU, the leading standard relating to round wire conductors is EN 50182. It specifies the requirements that must be met by any conductor in service and how testing should be carried out. It also includes specific types of tests for the wires that make up the conductor, including a standard tensile test and other tests of mechanical properties. As these results form the basis for calculating the strength of the entire cable, their precise verification is crucial in determining its quality and thus its safe use.

The determination of stress at a 1 % elongation of the steel wire consists of several steps. The first is to mount the wire in a testing machine and subject it to a pre-tension test. Next, an extensometer is mounted on the specimen, and a certain specified initial elongation of the specimen is performed. The final stage is to tension the specimen until the extensometer indicates a total elongation corresponding to 1 % of the gauge base and read the force reading from the testing machine. This procedure is complex and includes potential sources of measurement error. In addition, it applies to straight wires (central core wires or those prior to twisting) that do not have shape waviness.

Figure 1: Example of ACSR cable used for testing

Automated simplicity

This test is regularly performed at Power Grid Systems – an accredited laboratory of the Central Mining Institute (GIG) in Katowice, Poland. The sample is mounted in a Shimadzu AGX-V 300 kN machine equipped with DEMGEN hydraulic grips with automatic pressure adjustment. The subsequent test steps are carried out automatically according to the method entered into the Shimadzu Trapezium software and the specimen geometry measured. The wire is pulled to a force corresponding to the preload, and the Shimadzu SIE-560A extensometer, set to a 250 mm gauge base, is automatically clamped onto the sample.

The specimen is subjected to further uniform stretching until a total elongation value of 1 % is reached. The force and elongation data corresponding to this point – precisely determined by the automated system – are converted by the software into a finished result for the stress at 1 % elongation of the steel wire tested. This serves as the basis for determining compliance. Testing carried out in this way avoids error resulting from manual installation and readout of the extensometer, and its large measurement base results in excellent measurement precision.

As the power needs of the world grow, so too will the physical infrastructure required – including transmission networks. It is therefore vital that efficient and reliable verification of the safety and performance properties of power conductors be as simple to perform as turning on a light. Top-of-the-line measuring equipment and software help make that happen.

Note

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

Figure 2: Professor Jerzy Korol, head of the Laboratory of Material Technologies and Recycling of the GIG Institute

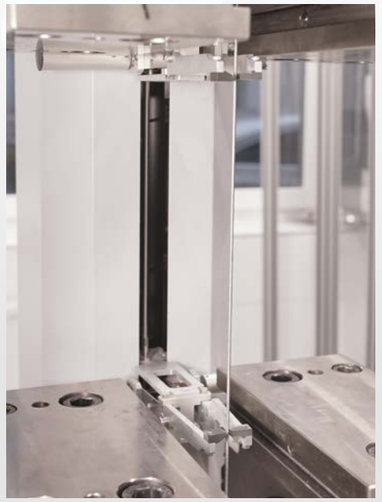


Figure 3: A round steel wire of an ACSR conductor under test

Custom instruments drive major breakthroughs

Custom GC helps UCL researchers explore new frontiers in chemical engineering

Dr. Han Wu, Dr. Nidhi Kapil, UCL Centre for Nature Inspired Engineering, London

The need to improve the environmental credentials of the processes used to make commodity chemicals and fuels and the desire to make use of waste industrial gases such as CO_2 is driving a whole new field of research into novel chemical processes. Foremost amongst the institutions following this path is the Centre for Nature Inspired Engineering (CNIE) at University College London (UCL). We talk to two scientists at CNIE with a unique perspective on the role of analytical instrumentation in this research, and on Shimadzu's custom GC systems in particular.

A passion for instrumentation

It's often said that the progress of science hinges on the performance of the analytical instruments used to acquire measurements – and few people are more keenly aware of this than Dr. Han Wu, based at the Centre for Nature Inspired Engineering (CNIE) within the Department of Chemical Engineering at University College London (UCL).

Since 2018, Dr. Wu has been Research Lab Manager for CNIE as well as for the entire chemical engineering department, overseeing the acquisition and maintenance of a wide range of analytical instrumentation crucial to many research projects. This gives her a unique perspective on how the capabilities of the instrumentation are linked to the success of the research.

Dr. Wu's interest in analytical instrumentation started early. During her Ph.D. studies at Sheffield University into polymorphism in pharmaceutical materials, she used a wide variety of analytical equipment, including synchrotron XRD, HPLC, TGA, DSC, Raman and more. She says: "This helped me realize just how powerful analytical techniques are, and especially how acquiring good data from the instruments in practice is so different from the idealistic scenarios presented in undergraduate textbooks!"

→

During her research, she received a lot of help from the technicians at Sheffield and the synchrotron facility: “I often discussed my work with them and sought advice about how to get the best out of the instrumentation – and as a result, I came to regard their expertise very highly.”

This experience turned out to be a turning point in her career. “Although I had a great passion for research, and indeed continued along that path at UCL, it made me realize that I also loved helping scientists get the best out of their research. So when a job as research technician at CNIE turned up, I didn’t need to think twice!”

The role of chromatographic systems at CNIE

Dr. Wu’s first purchase in her new job was UCL’s first small-angle X-ray scattering instrument, and since then her remit and expertise have expanded greatly. Today, she’s helped by a team of three technicians, who, amongst other responsibilities, look after seven Shimadzu chromatographic systems. Although these are housed in the research labs where they’re used most frequently, Dr. Wu is keen to point out that most of them are shared resources – “making equipment available to whoever needs it is really useful to get the greatest value from it, and of course LC and GC are essential for analyzing all types of chemical reactions.”

These Shimadzu systems include two GC-MS/FID, one liquid-only GC system with a 150-vial autosampler, a recently installed custom GC system with a BID detector and two custom on-line GC-FID/TCD systems. At CNIE, these GC systems and other instruments are used in projects that aim to solve grand challenges in chemical engineering by taking inspiration from nature – such as lung-inspired fuel cells, hierarchically-structured catalysts, self-healing materials and nanoporous catalysts that mimic the confinement effects observed in biological systems.

A more sustainable route to propylene oxide

This latter project is one that’s recently produced a flurry of publications – and it’s all thanks to the work of Dr. Nidhi Kapil, who recently completed her Ph.D. on this topic. With funding from chemicals manufacturer SABIC, she’s been using the custom on-line GC-FID/TCD to study the catalyst-mediated oxidation of propylene to the commodity chemical propylene oxide. The aim is to develop more sustainable routes than the current chlorohydrin and hydroperoxide processes, which generate a lot of waste chemicals.

Dr. Kapil explains her research: “Our reaction involves just propylene, hydrogen and oxygen along with an inert gas and uses a gold catalyst on a fixed bed at 200 °C to generate propylene oxide and water. So it has great potential to be environmentally benign, but the catalyst stability and product yield needed improving, and this has been the focus of my studies.”

“I’ve worked on the project since it started – not only refining the reaction and optimizing the catalyst but designing the reactor too. I started out using nanoparticle catalysts and then proceeded to the meso and macro scales by taking advantage of zeolite supports.”

Fine-tuning the on-line custom GC setup

Naturally, as an on-line system, setting up the analytical equipment needed to go hand-in-hand with the reactor design, and Dr. Kapil explains what the system looks like: “Basically, we have hydrogen, oxygen and propylene oxide gases, mixed in about equal proportions in nitrogen, which as an inert gas is important for safety issues. These pass through the quartz reaction tube in the reactor, and the system runs continuously, with the effluent gases being monitored by a custom-built on-line GC.”

Dr. Kapil explains that, following discussions with Dr. Wu, it was clear that Shimadzu was an obvious choice for the custom GC setup. “We needed to be able to separate not just the reactants and products but several byproducts too – and because it was an on-line system, the columns needed to work efficiently to keep the run time as short as possible. Dr. Wu already had an excellent relationship with Shimadzu, so we got talking straight away.”

Although the challenge seemed formidable, Dr. Wu says that this didn’t faze the Shimadzu team: “Following discussions to fine-tune the requirements and work out the hardware options, we decided on the optimal setup, and they handled it from there. In the end, it took just 6 months from start to finish – they made it all seem so easy!” →

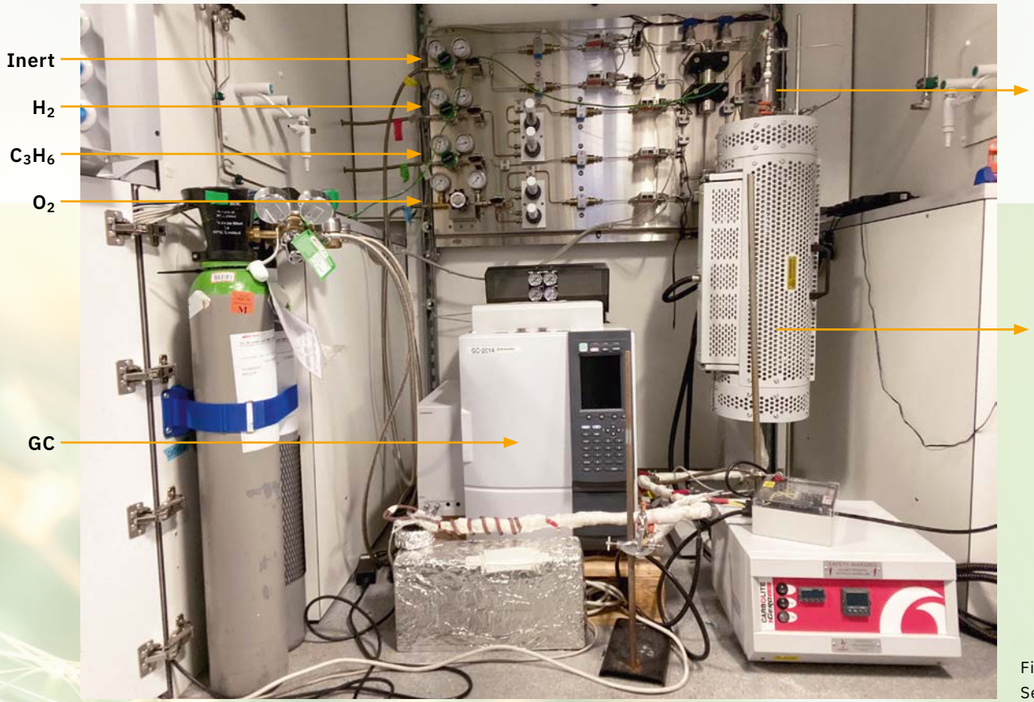


Figure 1: Setup

▶ The custom GC and reactor setup used by Dr Kapil for her propylene oxidation work. The system, which is housed in a walk-in fume hood, is based on a GC-2014 and uses a two-column setup, with a run time of 42 min. A Porapak T column and an FID are used to separate and detect propylene oxide, carbon dioxide, water, propylene and the oxygenated byproducts ethanal, propanal, acetone and acrolein, while the permanent gases hydrogen, oxygen and carbon monoxide used a Molsieve 5A column and a TCD. The photo shows the controls for the reactor gases (and nitrogen) at top left, the reactor itself on the right and the GC in the center.

Efficient analysis, impressive results

Dr. Kapil says that throughout her Ph.D. studies, the analytical setup did exactly what it needed to – "everything worked perfectly!", she says. This enabled her to focus on the core part of her Ph.D., that of finding an efficient route to an optimized catalyst. The results of the reaction itself, published in *Angewandte Chemie*, are impressive, with catalyst lifetime having increased 10-fold to over 20 days and with the selectivity for propylene oxide having raised to nearly 90 %.

One aspect of the system that she was particularly impressed with was its robustness. "To start with, we ran the system for about 4 hours, but ultimately we were able to leave it going for up to 30 days, taking measurements every 40 minutes or so. Its reproducibility meant that we could monitor the performance of the catalyst over longer periods, and the networked GC meant that I could monitor the system – even when I was away for four days at a conference in the USA!"

Reducing detection limits and improving productivity

In addition to the custom GC-FID/TCD used by Dr. Kapil, Dr. Wu helped select and purchase a GC system fitted with Shimadzu's barrier ionization detector (BID) for Dr. Yang Lan's research group within CNIE. This offers detection of virtually any analyte, and at much lower detection limits than conventional detectors. Dr. Wu says this will enable them to use just one detector for their very complex reaction systems, rather than a multi-detector system of one TCD and two FIDs. There are plans to use it for two projects, both led by Dr. Lan: one focused on photocatalytic reduction of CO_2 and the other on the conversion of methane and ethane to their corresponding alcohols.

In initial tests, the sensitivity of the system has impressed them, she says: "As part of setting up the system, the researchers have been injecting the gas manually, rather than running it as an on-line system. This occasionally resulted in the introduction of a tiny amount of air – and with the BID, we were able to see peaks from the CO_2 present in that air, at levels consistent with its atmospheric concentration. That showed us the power of a BID detector!"

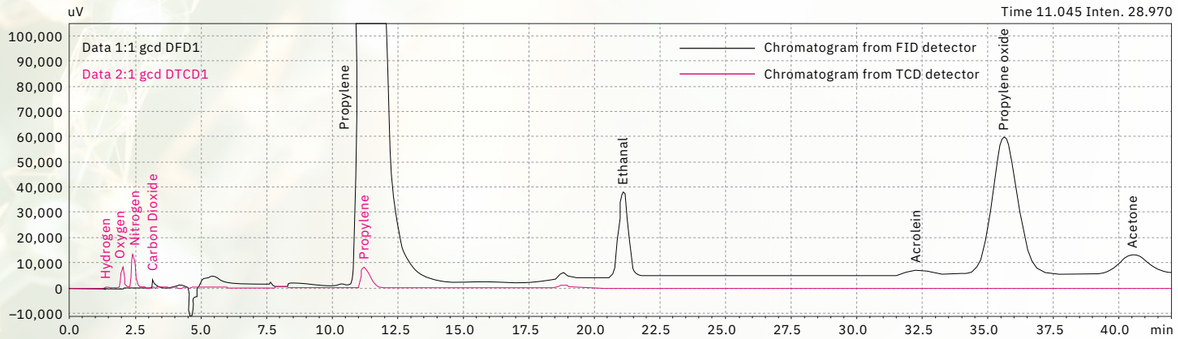


Figure 2: Example chromatogram obtained by Dr Kapil during her work on catalytic epoxidation of propylene using Shimadzu's custom GC-FID/TCD, showing the numerous analytes detected during a single GC run

Personal service … and thriving research

Both Dr. Wu and Dr. Kapil are happy to recommend Shimadzu's dedicated service. Dr. Wu says: "When I first started as a member of the technical staff at UCL in 2013, there was a common viewpoint in academia that there was no major difference between GC suppliers. But once I started to get into discussions with the vendors, I came to realize that there's a huge difference in their way of supporting their customers – and for me that is a big highlight of working with Shimadzu."

She also points out the training provided for students at UCL: "Shimadzu have run special workshops with us, which are super-valuable for those new to GC, because they cover both the theoretical side as well as hands-on practice with different bits of kit. They're highly regarded by our students, who find the topics very relevant to their work. Quite honestly, I'm struggling to think of any other supplier that's kept this capability and dedication in-house and offers it for free to their customers."

It's that personal connection with the subject experts that's important for everyone at CNIE, concludes Dr. Wu: "Unlike many manufacturers, I'm never put through to a customer service center and have to explain everything to a different person each time. I have a couple of key contacts at Shimadzu, and they're always happy to pick up the phone and talk about a technical question or discuss how to adapt an existing system to tackle a new challenge."

"And with the pace of research in UCL being what it is, what's guaranteed is that new ideas will be coming thick and fast. So our collaboration with Shimadzu will undoubtedly be supporting exciting breakthroughs in chemical engineering for many years to come!"

Note

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

Cosmetics – how clean is clean?

TOC-based cleaning validation

Anika Brett, Tilman Stellfeld, Mann & Schröder GmbH

In the cosmetics industry, Total Organic Carbon analysis (TOC) is a meaningful summary parameter for evaluating the cleaning efficiency of manufacturing plants and equipment. What is important to consider?

If cosmetics promise one thing besides beauty, it is cleanliness. The industry stands for hygienic care, well-being and many more pleasant and pristine characteristics. But how can we be certain both that consumers feel good and that the products themselves are good, i.e., of high quality and ultimately produced in compliance with the law? For this, the cosmetics themselves must be one thing in particular: clean. But what exactly does “clean” mean in this context?

All manufacturers of cosmetic products such as soaps, creams or makeup must ensure the cleanliness of the manufacturing facilities and equipment used. That said, how this is to be carried out and validated is only vaguely formulated in the corresponding regulations.[1] It is important that a standardized cleaning procedure exists and that the manufacturer can prove that the cleaning has been effective. The summary parameter Total Organic Carbon analysis (TOC) provides an overview of the cleaning efficiency.

Applicable requirements for cosmetics manufacturers

According to DIN EN ISO 22716:2016 for cosmetic Good Manufacturing Practice (GMP), a suitable cleaning and, if necessary, disinfection program applies to all equipment. This also includes specified effective cleaning and disinfecting agents. The IFS HPC (International Featured Standard – Household/Personal/Care), an international standard for assessing products and processes of manufacturers and suppliers of household and personal care products, further defines the applicable requirements. According to these, the cleaning measures must also be validated accordingly and documented by means of a defined sampling plan using a suitable procedure. The type of validation, on the other hand, is not stipulated. ⟶

Figure 1:
TOC-L with autosampler

The TOC summary parameter

In cosmetic products, a large proportion of the ingredients consist of organic compounds; the same can be said for most cleaning agents. Therefore, TOC is a suitable parameter for checking the cleanliness of equipment following a production run. In order to determine a suitable cleaning and validation procedure, various cleaning steps are carried out and then the “final rinse water” is analyzed. Additionally, rinsing processes can be optimized in this way until they meet the requirements, thus cutting costs.

There are various techniques to determine the TOC. The most common is catalytic combustion oxidation. It oxidizes the carbon components at high temperatures using a platinum catalyst. As a result, the CO_2 produced is detected with a nondispersive infrared (NDIR) detector.

Since inorganic carbon compounds such as carbonates and hydrogen carbonates are detected in addition to the organic compounds, two different TOC determination methods have been established to remove the inorganic compounds from the sample. In the most commonly used method, the so-called NPOC (Non Purgeable Organic Carbon) or direct method, the sample is first acidified using a mineral acid. It converts the inorganic carbon compounds to carbon dioxide, and then a purge gas removes them from the sample. An aliquot of the prepared sample is then oxidized, and the resulting CO_2 is measured.

However, if cleaning agents that tend to foam are used for cleaning equipment, the so-called difference method is ideal. First, the concentration of total carbon compounds (TC) is determined by oxidation. The second step is to measure the inorganic carbon (IC) compounds. Using difference formation (TC – IC = TOC), the TOC concentration can be calculated.

Modern analyzers such as those from the TOC-L series from Shimadzu handle sample preparation (acidification and degassing) fully automatically. The systems operate with a highly effective platinum catalyst at a combustion temperature of 680 °C. The 680 °C combustion catalytic oxidation method was originally developed by Shimadzu and is now used worldwide. In addition to the direct method (NPOC), the Shimadzu systems can also determine TOC using the differential method. The devices have a special syringe unit that allows samples to be automatically diluted when the calibration range is exceeded as well as automatic dilution of standards to generate calibration curves even at equidistant concentration intervals.

0 % alcohol – really?

The required purity of the final rinsing step is determined by means of a risk assessment. Its goal is to ensure legally compliant products. A suitable scenario for risk assessment in the manufacture of cosmetics is the carryover of an ingredient from an ingredient-containing product to an ingredient-free product. Preservatives are a classic example of this. Certain products have explicit claims, such as “free of preservatives”, “0 % alcohol” or “perfume-free”, and this must be ensured during their manufacture. This means that the ingredient must not be detectable in the cosmetic product.

To calculate the TOC acceptance criterion, the first step is to define a risk-oriented indicator substance, in this case the carryover substance. A risk assessment of the production facilities is then used to determine the amount of rinse water from the final rinse that remains in the production vessel in a worst-case scenario and which would then lead to the subsequent production batch becoming contaminated. In the context of this observation, it is assumed that this TOC concentration results exclusively from the leading substance.

Once the acceptance criterion is defined, validation can begin. Samples are taken at various rinse cycles and analyzed for TOC content. As you would expect, the TOC content decreases with each rinsing step (Figure 3). The cleaning process must be considered separately for each manufacturing plant and each product group. Table 1 displays the TOC readings of different products in the same manufacturing plant after running the same cleaning program. If compliance with the specified acceptance criterion is confirmed, even after repeated performance, the cleaning method is considered validated for the respective product.

In the cosmetics industry, TOC is a meaningful parameter for evaluating the cleaning efficiency of manufacturing plants and equipment. It makes analysis simple, accurate and fast. In addition, modern analyzers such as Shimazdu’s TOC-L carry out the necessary sample preparations automatically. Precision measurements can also help to optimize the cleaning steps and, in this way, reduce costs and the use of resources.

Note

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

$$C_{Sub} = \frac{\text{Amount of preparation [kg]} \cdot \text{Detection limit [ppm]}}{\text{Remaining amount of rinsing water [kg]}}$$

$$\text{TOC [ppm]} = \frac{C_{Sub} \cdot 12.0107\ \text{g} \cdot \text{mol}^{-2} \cdot K_{Sub}}{M_{Sub}}$$

C_{Sub} : Maximum permitted concentration of the indicator substance [ppm]

K_{Sub} : Number of carbons of the indicator substance

M_{Sub} : Molar mass substance [g·mol^{-1}]

TOC : Total Organic Carbon [ppm]

Figure 2: Calculation of the TOC acceptance criterion

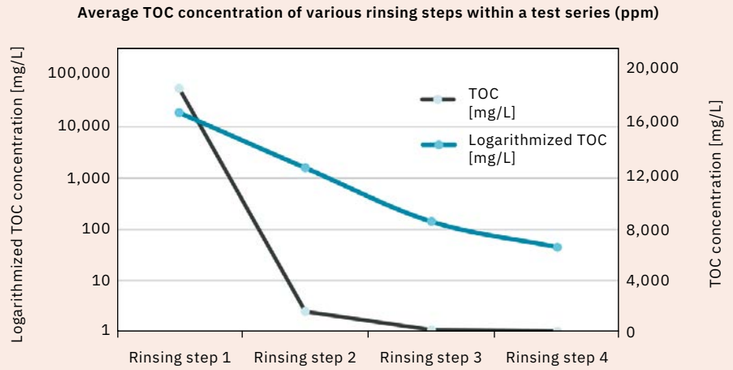


Figure 3: Graphic depiction of TOC concentrations of the rinse water via four rinsing steps

MS parameter	Hair perfume	Sun spray	Hairspray	Facial toner
TOC in rinsing step 1 [ppm]	7,187	14,350	5,521	4,928
TOC in rinsing step 2 [ppm]	189	5,785	1,102	156
TOC in rinsing step 3 [ppm]	19	2,148	50	36
TOC in rinsing step 4 [ppm]		44		

Table 1: Level of TOC in rinse water during cleaning of various products in the same manufacturing plant and under identical cleaning conditions

High quality analysis, even at low concentration

Evaluating MRM analysis by GC-MS/MS of PAH in palm oil

Waldemar Weber, Shimadzu Europa GmbH, Elvi Horiyanto, Cynthia Lahey, Shimadzu Asia Pacific

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds primarily formed from the incomplete combustion of organic materials. PAHs are carcinogenic, teratogenic and mutagenic contaminants that are toxic to human health. A major source of PAH exposure is food, in particular edible oils because of their lipophilic nature and high consumption. This article discusses a new multiple-reaction monitoring (MRM) method capable of PAH detection in palm oil, even at low concentration levels.

Polycyclic aromatic hydrocarbons (PAHs) may be introduced into edible oils naturally or through the production drying process. In 2002, the Scientific Committee on Food identified 15 PAHs that could be regarded as genotoxic and carcinogenic. In 2005, the Joint FAO/WHO Expert Committee on Food Additives added one PAH to that list, creating what is known as the 15+1 EU Priority PAH.

From this list, European Commission Regulation No 835/2011 stipulates the maximum limit of benzo[*a*]pyrene and the sum of the PAHs benzo[*a*]pyrene, benz[*a*]anthracene, benzo[*b*]fluoranthene and chrysene in edible oil to be 2.0 µg/kg and 10.0 µg/kg, respectively. This article describes the establishment and evaluation of a new multiple-reaction monitoring (MRM) method for qualitative and quantitative determination of the 15+1 PAHs in palm oil.

Experimental conditions

A triple-quadrupole GCMS-TQ8050 NX was employed in this work. The details of the system and analytical conditions for the GC-MS/MS method are shown in Table 1. Each of the 15+1 PAHs was monitored via quantitative and qualitative MRM transitions. The details of MRM transitions, collision energy (CE) values and internal standard (IS) grouping are tabulated in Table 2.

Standards and sample preparation

Matrix-matched IS calibration solutions were prepared in the matrix blank. The 15+1 PAH concentrations prepared were 0.2, 0.5, 1, 2, 5 and 10 µg/L (which covers 0.44–22.34 µg/kg). Each of the calibration solutions contained IS concentrations of 5 µg/L chrysene-*d*12 and 5 µg/L perylene-*d*12. To determine the recovery, the matrix blank (spiked with the PAH standard mixture and internal standards before extraction) was analyzed.

Detection and separation

The 15+1 PAHs were separated in the GC-MS/MS using an SH-I-PAH column. The mass chromatograms of all compounds are displayed in Figure 1.

Calibration range, linearity and quantitation

Matrix-matched IS calibration curves were set up for the 15+1 PAHs using prepared calibration solutions. The calibration range of each PAH is shown in Table 3. Calibration curves of all PAHs demonstrated excellent linearity, with R^2 value of at least 0.9979 (Table 3). Quantitation of the palm oil sample was done by analyzing the palm oil matrix blank that was spiked with internal standards. None of the 15+1 PAHs was detected in this palm oil sample. ⟶

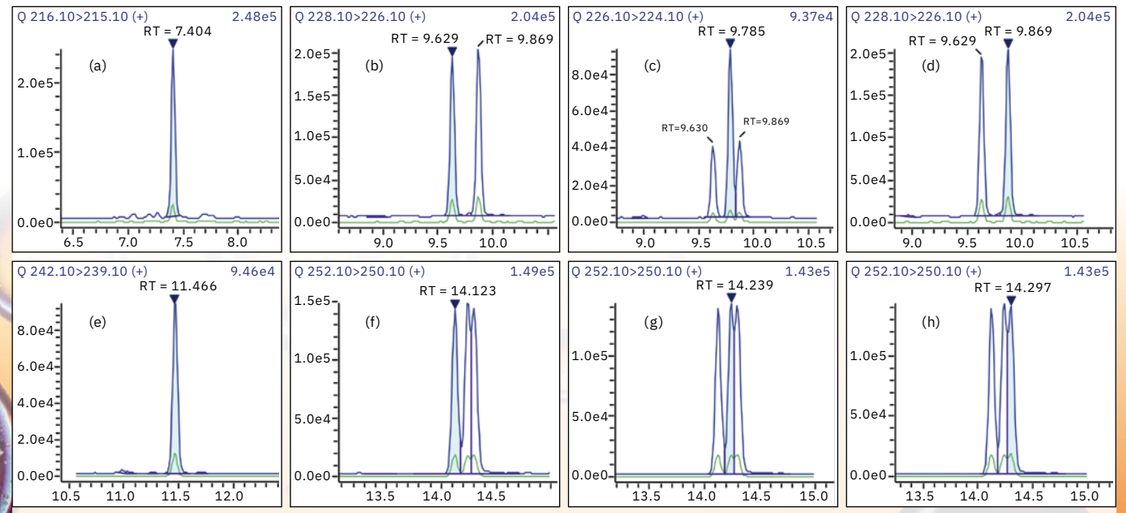


Figure 1: PAH mass chromatograms of: (a) benzo[*c*]fluorene, (b) benzo[*a*]anthracene, (c) cyclopenta[*cd*]pyrene, (d) chrysene, (e) 5-methylchrysene, (f) benzo[*b*]fluoranthene, (g) benzo[*j*]fluoranthene, (h) benzo[*k*]fluoranthene

For recovery calculation, the matrix blank was spiked with 5 µg/L (equivalent to 11.173 µg/kg in palm oil) of both internal standards and PAHs. Concentrations were then quantified using the IS calibration curves.
The recovery of so-called PAH4s (benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene and chrysene) was between 76.15 %–88.11 %, which is within the criteria determined by EU Regulation No 836/2011 (50 %–120 %). The quantitation and recovery results of palm oil spiked with the 15+1 PAHs are presented in Table 3.

LOD, LOQ and repeatability

The limit of detection (LOD) of all 15+1 PAHs in the palm oil sample was determined to be in the range of 0.038–0.327 µg/kg; for the PAH4s in the range of 0.071–0.157 µg/kg. In addition, the calculated limit of quantification (LOQ) for the 15+1 PAHs was in the range of 0.127–1.090 µg/kg; for the PAH4s 0.236–0.522 µg/kg. According to European Commission Regulation No 836/2011, the LOD for PAH4s should be < 0.30 µg/kg, with an LOQ < 0.9 µg/kg. Therefore, the GC-MS/MS method used here demonstrates the capability of MRM detection in quantifying even low concentrations of PAH in palm oil.

Analysis method precision was determined by analyzing eight consecutive runs of the low concentration (0.5 µg/L) and mid concentration (5 µg/L) of calibration levels in the matrix. The %RSD of peak area ratio of all PAHs with respect to their IS were < 8 % and < 5 % for low and mid concentration levels, respectively. For greater insight, the 5 µg/L calibration solution in the matrix was analyzed another 72 times within a 48-hour period. The %RSD of peak area ratio of all PAHs (72) with respect to their IS were < 5 % (except for dibenzo[*a*,*i*]pyrene which was < 8 %).

System configuration	
GC-MS system	GCMS-TQ8050 NX
Liquid sampler	AOC-20i and AOC-20s
Gas chromatography parameters	
Capillary column	SH-I-PAH (30 m X 0.25 mm ID X 0.10 µm df)
Injection mode	Splitless, 330 °C
Flow control mode	Linear velocity 50.0 cm/s
Carrier gas	Helium
Temp. program	110 °C for 1 min, 30 °C/min to 240 °C, 240 °C for 2 min, 3 °C/min to 270 °C, 240 °C for 2 min, 8 °C/min to 340 °C, 340 °C for 8 min
MS parameters	
Ionization mode	EI
Ion source temp.	230 °C
Interface temp.	300 °C
Mode	MRM

Table 1: GC-MS/MS analytical conditions

Analyte	MRM-1	CE (V)	MRM-2	CE (V)
Benzo[*c*]fluorene	216.1 > 215.1	22	216.1 > 189.1	30
Benzo[*a*]anthracene	228.1 > 226.1	28	228.1 > 202.1	26
Chrysene-*d*12 (IS)	240.2 > 236.2	28	240.2 > 238.2	26
Cyclopenta[*c*,*d*]pyrene	226.1 > 224.1	38	226.1 > 200.1	30
Chrysene	228.1 > 226.1	28	228.1 > 202.1	26
5-Methylchrysene	242.1 > 239.1	32	242.1 > 215.1	22
Benzo[*b*]fluoranthene	252.1 > 250.1	28	252.1 > 226.1	30
Benzo[*j*]fluoranthene	252.1 > 250.1	30	252.1 > 226.1	30
Benzo[*k*]fluoranthene	252.1 > 250.1	30	252.1 > 226.1	30
Benzo[*a*]pyrene	252.1 > 250.1	30	252.1 > 226.1	24
Perylene-d12 (IS)	264.2 > 260.2	47	264.2 > 262.2	44
Indeno[*1,2,3-cd*]pyrene	276.1 > 274.1	34	276.1 > 250.1	30
Dibenzo[*a*,*h*]anthracene	278.1 > 276.1	30	278.1 > 252.1	30
Benzo[*g*,*h*,*i*]perylene	276.1 > 274.1	32	276.1 > 275.1	28
Dibenzo[*a*,*l*]pyrene	302.1 > 300.1	36	302.1 > 298.1	60
Dibenzo[*a*,*e*]pyrene	302.1 > 300.1	36	302.1 > 276.1	28
Dibenzo[*a*,*i*]pyrene	302.1 > 300.1	36	302.1 > 276.1	28
Dibenzo[*a*,*h*]pyrene	302.1 > 300.1	36	302.1 > 276.1	28

Table 2: MRM transitions, collision energies of PAHs and internal standards

Analyte	Calibration range µg/L	R^2	Conc. spiked for recovery test µg/kg	Recovery %
Benzo[*c*]fluorene	1.0–10	0.9994	6.53	58
Benzo[*a*]anthracene	0.5–10	0.9995	8.50	76
Cyclopenta[*c*,*d*]pyrene	0.5–10	0.9997	10.32	92
Chrysene	0.5–10	0.9985	9.84	88
5-Methylchrysene	0.2–10	0.9993	9.12	82
Benzo[*b*]fluoranthene	0.2–10	0.9991	8.98	80
Benzo[*j*]fluoranthene	0.2–10	0.9987	8.26	74
Benzo[*k*]fluoranthene	0.2–10	0.9985	9.48	85
Benzo[*a*]pyrene	0.2–10	0.9989	9.35	84
Indeno[*1,2,3-cd*]pyrene	0.2–10	0.9988	9.44	85
Dibenzo[*a*,*h*]anthracene	0.2–10	0.9990	9.49	85
Benzo[*g*,*h*,*i*]perylene	0.2–10	0.9991	9.45	85
Dibenzo[*a*,*l*]pyrene	0.2–10	0.9986	9.01	81
Dibenzo[*a*,*e*]pyrene	0.2–10	0.9981	9.64	86
Dibenzo[*a*,*i*]pyrene	0.2–10	0.9979	9.85	88
Dibenzo[*a*,*h*]pyrene	0.5–10	0.9988	9.25	83

Table 3: Calibration range, R2 value and Recovery % for 15+1 PAHs

These results indicate that the optimized MRM method has high stability and analysis precision. The values discussed above are summarized in Table 4.

An attractive new method

Clearly, this shows the successful development of an MRM method using GCMS-TQ8050 NX for the analysis of polycyclic aromatic hydrocarbons in palm oil, which is suitable for other kinds of edible oils as well. The results show that this method allows the detection and quantification of the 15+1 PAHs even at low concentration: down to 0.071 µg/kg LOD and 0.127 µg/kg LOQ, respectively. Excellent linearity of IS calibration curves with R^2 values was also obtained. Finally, in addition to sensitivity and selectivity, the optimized MRM method also showed good repeatability and robustness – making this new method highly attractive for analytical use by laboratories.

Analyte	Calculated LOD [µg/kg]	Calculated LOQ [µg/kg]	Area ratio %RSD of 0.5 µg/L solution (n = 8)	Area ratio %RSD of 5 µg/L solution (n = 8)	Area ratio %RSD of 5 µg/L solution (n = 72)
Benzo[*c*]fluorene	0.327	1.090	6.2	1.5	2.2
Benzo[*a*]anthracene	0.176	0.588	5.3	1.6	2.4
Cyclopenta[*c*,*d*]pyrene	0.157	0.522	3.1	1.5	4.2
Chrysene	0.175	0.583	5.9	2.0	3.2
5-Methylchrysene	0.112	0.373	2.8	1.6	2.4
Benzo[*b*]fluoranthene	0.088	0.293	3.0	1.8	2.7
Benzo[*j*]fluoranthene	0.077	0.256	5.2	4.1	4.3
Benzo[*k*]fluoranthene	0.077	0.255	5.1	3.9	4.8
Benzo[*a*]pyrene	0.071	0.236	4.0	2.1	2.8
Indeno[*1,2,3-cd*]pyrene	0.049	0.163	1.4	2.2	2.3
Dibenzo[*a*,*h*]anthracene	0.045	0.150	1.9	1.7	2.6
Benzo[*g*,*h*,*i*]perylene	0.038	0.127	2.7	2.2	2.5
Dibenzo[*a*,*l*]pyrene	0.107	0.358	7.3	3.4	4.4
Dibenzo[*a*,*e*]pyrene	0.127	0.423	7.2	2.4	3.7
Dibenzo[*a*,*i*]pyrene	0.114	0.378	5.3	3.0	7.9
Dibenzo[*a*,*h*]pyrene	0.137	0.458	5.3	3.0	4.3

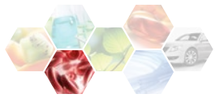
Table 4: LOD/LOQ and repeatability of analyzed PAHs

Note

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

Faster throughput. Together.

New streamlined solution for immunosuppressant analysis by LC-MS/MS



Mikaël Levi, Alsachim / Reagent Kit Business Unit

To face increasing demands on their time and resources, chemical laboratories need easier-to-handle solutions with faster turnaround times, lower costs per sample and full compliance with ever-stricter regulatory standards. Recent work by Alsachim using Shimadzu equipment and software has now led to an optimized high-throughput analytical solution that delivers same-day results for therapeutic drug monitoring of immunosuppressants. The solution combines liquid chromatography and tandem mass spectrometry (LC-MS/MS) with automated sample preparation and a dedicated reagent kit.

Therapeutic drug monitoring (TDM) is a multi-disciplinary science that aims to understand the factors that determine the dose-effect relationship and to use this knowledge to optimize drug treatment.[1–3]

In most laboratories, automated immunoassay platforms dominate in TDM. However, these may produce biased results due to the cross reactivity of the active metabolites. This complication may be compounded by batch-to-batch heterogeneity in antibodies or reagent quality, high-dose-hook effects and a high cost per analysis.[4]

As precision medicine emerges as an approach to individualized patient treatment – i.e., addressing a specific disease and taking into account individual variability in genes, environment and lifestyle – TDM is likely to have an even greater impact on dose adjustment. Already, this new application for TDM is increasing the number of samples to assay, as well as the pressure for rapid turnaround times. Adding to these challenges is the need to comply with European regulations for in vitro diagnostics that came into force in May 2022 and which have dramatically heightened the need for certified analytical solutions.

This article highlights a time-saving, complete and fully compliant high-throughput solution for immunosuppressant analysis – created by a combination of liquid chromatography and tandem mass spectrometry (LC-MS/MS) with automated sample preparation and a dedicated reagent kit.

LC-MS/MS for TDM

Liquid chromatography (LC) techniques have been used in TDM for several decades. However, despite the impact of ultra-high-performance liquid chromatography,[5–7] such detection techniques are limited in terms of specificity – often resulting in extensive sample cycle times and poor sensitivity.[1]

Mass spectrometry (MS) is now regarded as a key technique for laboratories delivering robust platforms with highly selective and sensitive detection.[8] The capability of MS in the development of assays for individual drugs and in multiplexing analyte panels creates new opportunities while expanding the number of drug assays.

Automated sample management and preparation

Biological fluids are highly complex matrices which present challenges in matrix management, as endogenous and exogenous components result in compound and system-specific effects in MS. Negating the effects of the matrix must be carefully considered in all sample preparation and management protocols. Matrix effects can lead to isobaric interference, particulate clogging and ion suppression or enhancement, resulting in a difference between the signal intensity detected in a neat standard solution.

To help reduce the impact of matrix effects on bioanalytical assays, there are several strategies open to the analyst. One of the most important techniques is to use appropriate and validated internal standards (IS), particularly stable-isotope-labelled analogues (SIL-ISTD), to help correct for ion signal changes and handling errors in sampling preparation protocols. However, for many assays the effect of high inter- and intra-patient variability in endogenous molecule concentrations also requires sample clean-up using extraction or purification techniques.

For high-throughput assays, the design of the sample preparation method can benefit greatly from automated systems. Robotic liquid handlers with multiple pipettes, such as the Hamilton Microlab Star, can manage simple or complex sample preparation protocols with a high number of samples.

Immunosuppressant drugs

Immunosuppressant drugs are indicated to reduce the activity of the immune system to prevent transplant rejection. Circulating concentrations of these compounds should remain within a narrow therapeutic window, as overdosing can cause serious toxicity and long-term morbidity, while underdosing can cause graft rejection.[3] As immunosuppressant drugs result in a high pharmacokinetic variability between individual patients, TDM is now an established approach to mitigate the risks associated with organ transplantation.

Several commercial immunoassays are available for the TDM of immunosuppressants; however, all immunoassays show a significant positive bias compared to LC-MS/MS methods.[18] Despite the availability of automated immunoassays, each test is restricted to one analyte per test even though multiple immunosuppressants are used with an individual patient in many clinical settings.[1, 19] The following paragraphs describe a new streamlined and automated LC-MS/MS method for the routine TDM analysis of immunosuppressants. →

Figure 1: Calibrator and control positioning in robot racks

Combining individual solutions

To meet the need for high-throughput analysis, a DOSIMMUNE™ kit (Alsachim) was adapted to robotic liquid handling. This kit, validated and CE-IVD marked with manual sample preparation, provides all the necessary reagents and consumables to monitor immunosuppressants in whole blood by LC-MS/MS. Calibrator and control vials can be directly positioned in robot racks (Figure 1). However, liquid handlers have a larger dead volume that cannot be sucked up, compared to manual pipetting. So, two vials of each calibrator and control level were pooled prior to installation on the racks. With this adjustment, calibrators and controls can be used for an entire week before being replaced.

In order to reduce the number of consumables and protocol steps, an IS solution was mixed directly with the extraction buffer. The mixture can be then dispensed in a reservoir or through a solvent delivery unit.

Workflow optimization of the liquid handler was conducted to reach the shortest possible preparation time, while maintaining the performance obtained with the manual protocol. Most efforts were concentrated on the blood resuspension step. To obtain accurate results, samples should be homogeneous during sampling. Therefore, resuspension of each sample was performed just before sampling. A specific protocol was developed using several sequences of aspiration/delivery using the sample height follow-up function of the robot. With this protocol, samples with volumes ranging from 0.5 to 5 mL and haematocrit values from 30 to 70 % were successfully homogenized with a 60 % time reduction compared to similar protocols observed in laboratories. Vortex and centrifugation time/speed parameters were also optimized and operations parallelized as much as possible. Finally, a complete 96-well plate (blank + 6 calibrators, 4 controls and 85 samples) was provided ready-to-be-assayed in 45 minutes. Compared to traditional robotic solutions for immunosuppressant analysis, this corresponds to a 50 % reduction in preparation time.

Immunosuppressants are complex hydrophobic compounds that are known to produce carry-over in LC. The new Nexera X3 LC system (Shimadzu) has improved rinsing capabilities that dramatically reduce the time needed to clean inside the needle. Consequently, the LC-MS/MS cycle time was reduced from 2.1 to 1.5 min (injection-to-injection). When considering the number of samples to assay, this 30 % reduction greatly improves turnaround time.

Evaluation and validation

Precision and stability of the solution were evaluated by sequentially preparing three plates containing only the same control at low-mid levels (tacrolimus, sirolimus & everolimus at about 8 µg/L, cyclosporin A at about 145 µg/L) as unknown sample. The first plate included calibrators and controls; other plates included controls. Plates were assayed within the same sequence. Finally, 272 data points were obtained across the three plates. Figure 2 shows the measured concentrations for all compounds and the measured IS peak area. The relative standard deviation of concentrations or peak areas were well below the usually accepted criteria of 15 %. This demonstrated that the MS calibration was stable, that the sample preparation precision was excellent and reagents were stable on robot tray. A typical chromatogram of such low-mid level control samples is shown in Figure 3.

The method including sample preparation was validated according to international guidelines for bioanalytical methods (ICH M10[20], CLSI C62-A[21]). In addition, clinical performance evaluation was evaluated by comparing the results obtained on real patient samples using manual preparation. Statistical analysis demonstrated that the methods were in close agreement and commutable. Figure 4 shows the results of Passing-Bablok regression analysis. →

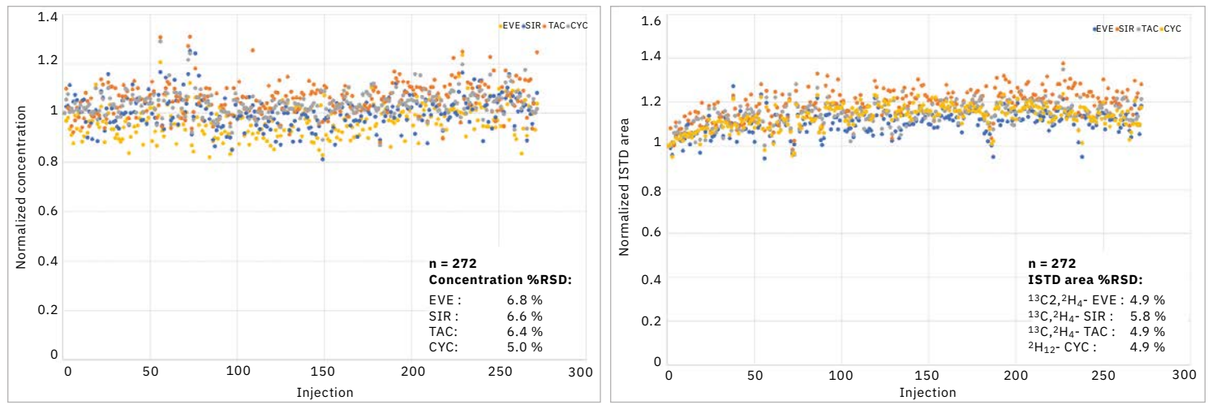


Figure 2: Solution precision and stability for (left) measured concentrations or (right) internal standard area

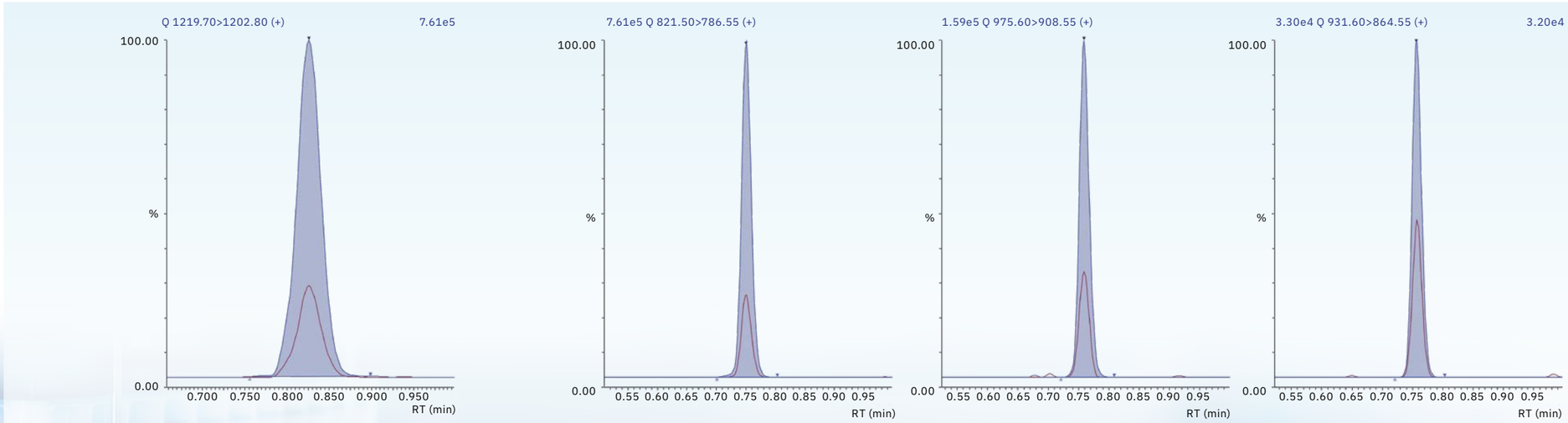
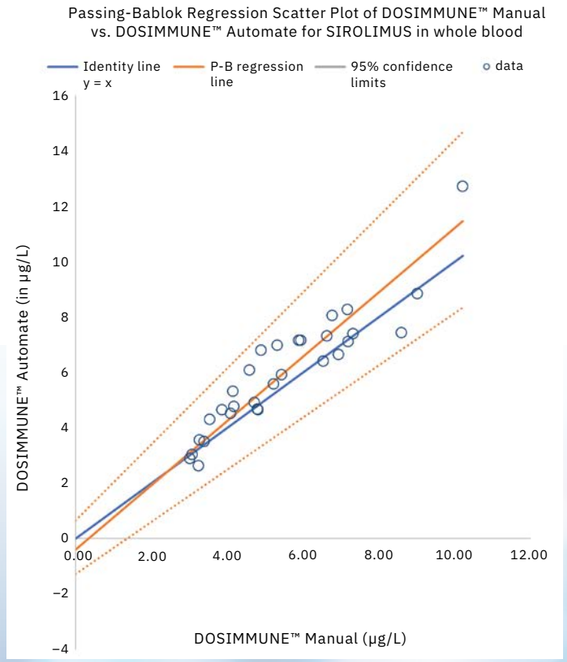
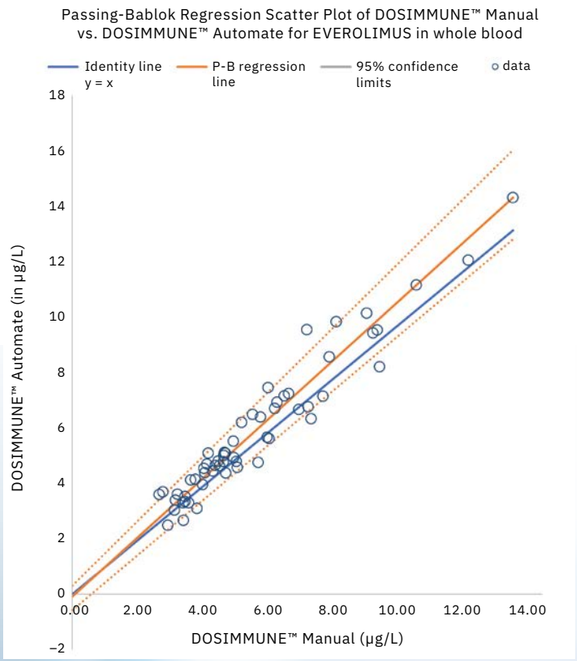
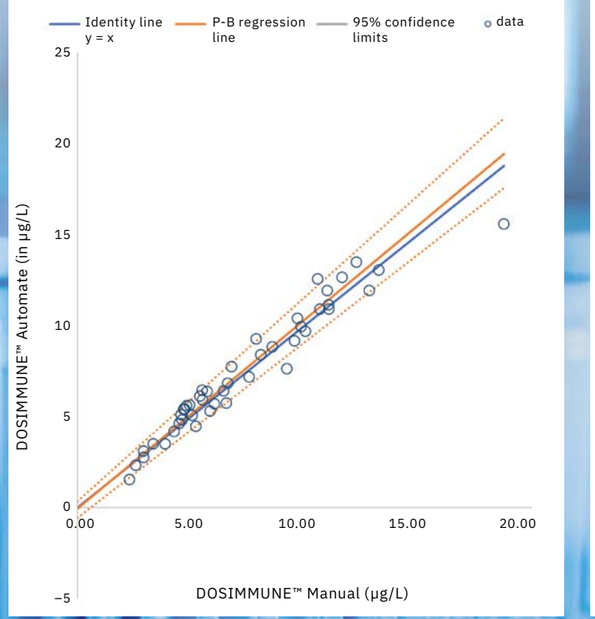
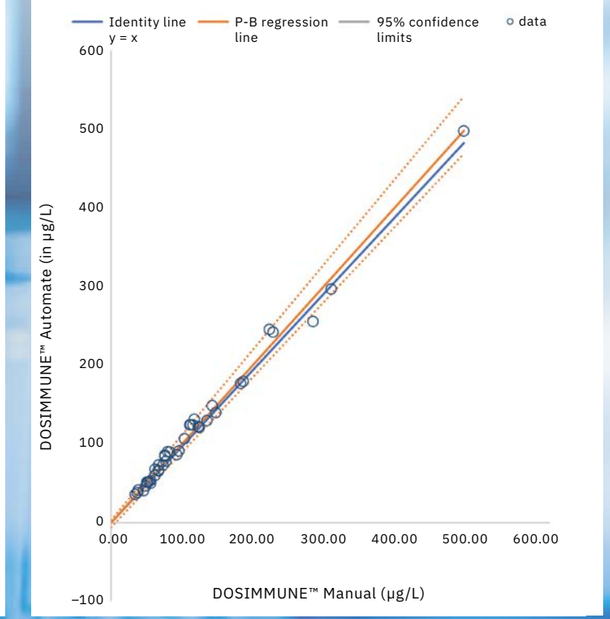


Figure 3: Typical control chromatogram containing (left to right) cyclosporin A, tacrolimus, everolimus and sirolimus









◄ Figure 4: Passing-Bablok regression analysis for comparison of manual preparation and automated method

Throughput and compliance

By adjusting the number of liquid handlers and LC-MS/MS systems, it is possible to assay from 300 to almost 900 patient specimens in less than 10 working hours. Figure 5 shows an example of a suggested configuration to reach very high throughput.

In addition to the analytical optimization, informatic connections were studied to be in line with the system speed. The liquid handler automatically generates the working list by scanning the patient tubes and this list is exported directly in the correct format to the Shimadzu LabSolutions software for LC-MS. With a few clicks, the sample assay sequence is created. Using adaptive flagging rules, the software proposes a review-by-exception that highlights the data that need to be looked at by the analyst. Double data validation is possible before sending the results to the laboratory information system through csv files or HL7 bidirectional connection.

All of the instruments and software involved in this time-saving solution are CE-IVD certified. The DOSIMMUNE™ kit has also been certified as CE-IVD with manual or automated sample preparation.

In summary, an efficient and time-saving new method using an optimized and fully compliant combination of instruments, reagents and software now exists for high-throughput analysis of immunosuppressants.

Note

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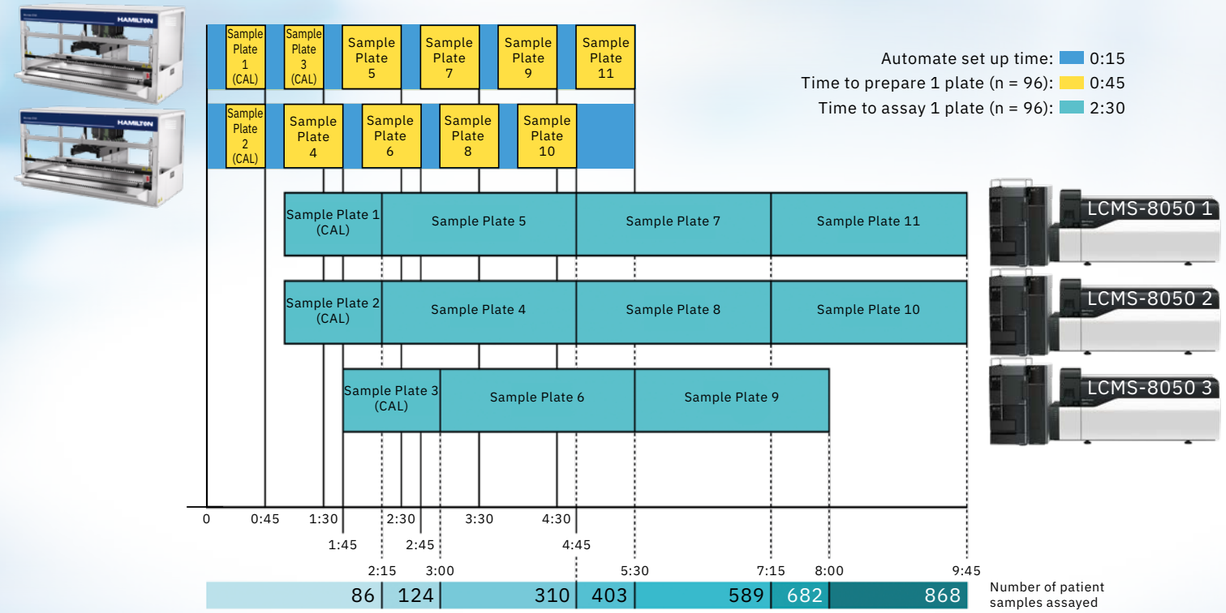

▲ Figure 5: Example of high-throughput configuration

Quicker chloramphenicol detection in honey

New method using DPiMS-8060 source with LCMS-8060NX speeds measurement

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In monitoring honey for antibiotic residues, a novel atmospheric pressure ion source developed by Shimadzu was applied to the determination of chloramphenicol. This innovative method is based on the principle of Probe Electrospray Ionization (PESI-MS) and pioneers LC-MS analysis by eliminating the use of an analytical column. It achieves a limit of quantitation of 1.0 ng/g and offers a dramatically accelerated sample measurement time of two minutes per sample – a 10-fold reduction over established procedures.

During honey production, xenobiotics are commonly used to prevent hive infections. Chloramphenicol, for example, is a broad-spectrum antibiotic used to treat honeybee larvae diseases. However, chloramphenicol has toxic and non-dose-dependent effects in sensitive subjects, and its use is regulated in food-producing animals, including honeybees.[1]

Established methods for monitoring chloramphenicol residues in honey use Electrospray Ionization (ESI). Unfortunately, ESI methods can be time-consuming, so researchers have now developed an ion source that can support a quicker method. This combines the high selectivity of the LCMS-8060NX in the MRM mode with the measurement speed of Shimadzu's new DPiMS-8060 source and eliminates the need for an analytical column.

Detecting chloramphenicol residues in honey

Probe Electrospray Ionization Mass Spectrometry (PESI-MS) applies principles similar to those used in ESI. The mobile phase is replaced by a small volume of solvent that contains the sample, which is transferred onto a plastic plate by the analyst. The ESI probe is replaced by a metal probe which samples the solution, so ionization takes place on the probe. During this PESI-MS method development, three solvents were tested: 100 % methanol; 50 % water – 50 % methanol with 10 mM ammonium formate; and 50 % water – 50 % isopropanol with 10 mM ammonium formate and 0.2 % formic acid. When methanol was used as a solvent, no signal was observed for chloramphenicol and the internal standard (IS). Comparing water-methanol and water-isopropanol, a four-times-higher signal was acquired when the latter was used. This novel method achieves a limit of quantitation of 1.0 ng/g and offers a dramatically accelerated sample measurement time of two minutes per sample. →

Figure 1:
DPiMS-8060 with LCMS-8060 NX

Repeatability, extraction efficiency and matrix effect

Measurement repeatability was investigated by analyzing one spiked sample containing chloramphenicol and chloramphenicol d5 (deuterated internal standard, IS) in triplicate. Initially, relatively high %RSD values were obtained for chloramphenicol (9.5 %) and for the IS (11.1 %). When the analyte/IS (internal standard) ratio was used, %RSD was reduced by a factor of 5, showing that a deuterated IS offers considerable improvement in PESI-MS measurement repeatability.

Since PESI-MS does not use an analytical column, potential sources of ion suppression in the extract can no longer be separated from the analyte and the IS. Therefore, it was important to ascertain the extent of matrix effect of the suggested sample pretreatment protocol. One matrix-matched sample (blank sample, reconstituted with the analyte and IS in solvent) and one spiked sample were prepared at the level of 3 ng/g honey. The results obtained for the two samples were compared to the results obtained from a standard solution to assess matrix effect. Comparing the results for the spiked sample and the matrix-matched sample revealed an extraction efficiency of 74 % for chloramphenicol and 73 % for the IS. Comparison of the results for the matrix-matched sample and the standard solution suggests a total recovery of 54 % for chloramphenicol and 46 % for the IS.

MS parameters and data processing

Negative ionization was used for the detection of chloramphenicol and the IS. For the detection of chloramphenicol, an established MRM transition was used for quantitation, namely 321 > 257.[4] Collision energy (CE) for the quantitation transition was optimized to 11 V. MRM 321 > 194 was used for confirmation, with CE 18 V. The respective parameters for the IS were 326 > 262, CE 12 V for quantitation and 326 > 157, CE 18 V for confirmation. 10 µl of sample extract were used for the analysis, analysis time was 0.3 min per sample and total measurement time was less than two minutes.

Mass spectrometry parameters are described in Table 1. The most notable difference in MS parameters when compared to established LC-MS methods is the significantly lower heat block temperature (30 °C instead of typical values of approximately 250 °C) used in PESI-MS methods. [6, 7] One more difference with established LC-MS methodologies is the presence of one positive and one negative Q3 Scan event (*m/z* 10–2,000) before measuring the analyte and the IS (Figure 2).

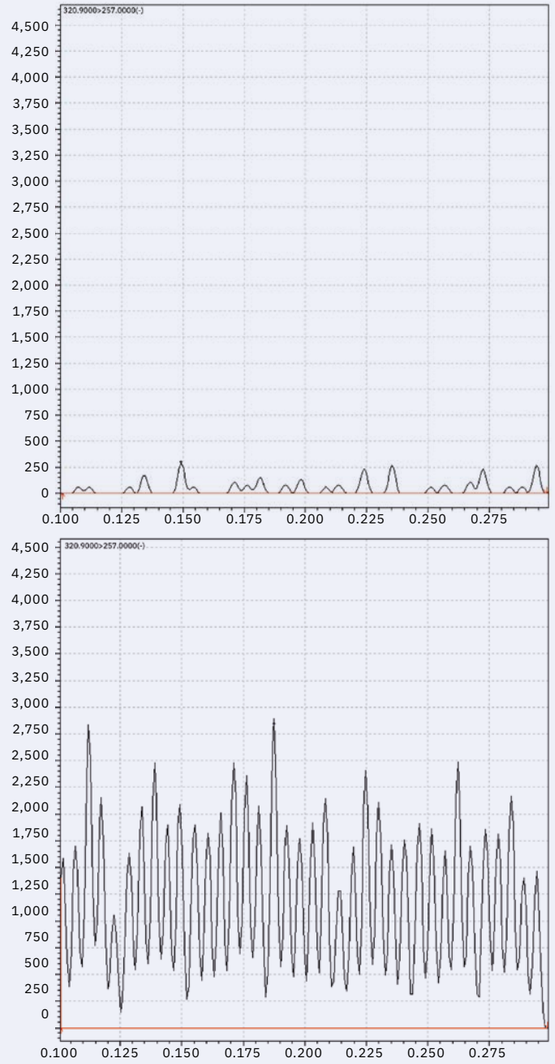
With respect to PESI-MS parameters, two key parameters can be configured in “PESI MS Solution” software: “Ionization outage time” and “Take sample outage time” (Table 2). With respect to the data processing part, using PESI-MS results in a fundamentally different form of raw data. Instead of integrating one chromatographic peak, several tens of peaks must now be integrated. Each peak represents one spontaneous ionization by the PESI needle (Figure 3).

Faster-flowing analysis

In addition to the potentially toxic effects that residual antibiotics in honey can elicit after consumption, antibiotics monitoring is part of a growing global concern concerning the unnecessary use of antimicrobials in agriculture. Faster, simpler and easier sample analysis will help high-throughput laboratories around the world to better perform their essential work in keeping us all safe.

Type	Event#	+/–	Compound name *m/z*	Time (0.000 min – 0.300 min)
Q3 scan	1	+	10.0000:2000.0000	
Q3 scan	2	–	10.0000:2000.0000	
MRM	3	–	CAP 320.9000 > 257.0000, 320.	
MRM	4	–	CAP d5 325.9000 > 262.0000, 3	

Figure 2: Typical MS measurement parameters when PESI source is used



◀ Figure 3: Data obtained for a blank solvent sample (upper) and a 1.0 ng/g spiked honey sample (bottom)

MS parameter	Set value
DL temperature	250 °C
Heat block temperature	30 °C
Dwell time	10 ms
Ionization voltage	2 kV
Q1 resolution	Unit
Q3 resolution	Unit
MRM chloramphenicol (Quantitation)	321 > 257
MRM chloramphenicol (Confirmation)	321 > 152
MRM chloramphenicol d5 (Quantitation)	326 > 262
MRM chloramphenicol (Confirmation)	326 > 157

Table 1: MS parameters for the determination of chloramphenicol in honey

PESI parameter	Set value
Ionization outage time	160 ms
Take sample outage time	50 ms

Table 2: PESI parameters for the determination of chloramphenicol in honey

Note

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

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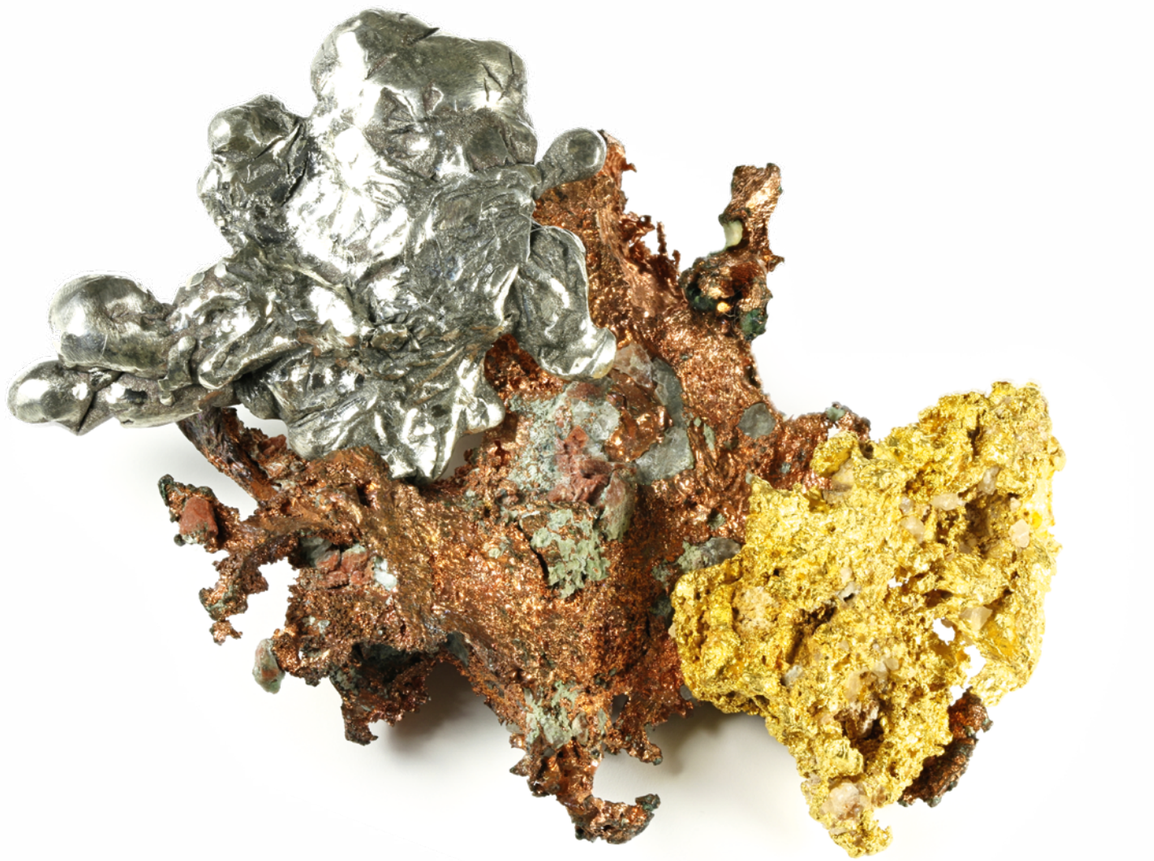


Rust protection with X-rays

The EDX-7200 as a Swiss Army knife in the quality control of electroplating

Dr. Benjamin Thomas, Shimadzu Europa GmbH

Electroplated products have become an essential part of our everyday lives, from rhodium-plated wedding rings to chrome-plated motorbike exhausts. Many of the analytical questions related to electroplating (from the composition of the electroplating bath to the thickness of the applied coating) can be answered in a non-destructive manner using energy-dispersive X-ray fluorescence spectroscopy. Shimadzu's EDX-7200 is a high-speed spectroscopic device that sensitively and precisely performs in a multitude of electroplating applications.

Energy-**d**ispersive **X-r**ay **fl**uorescence (ED-XRF) is a non-destructive analytical technique that can be used to analyze the elemental composition of a sample. During this process, the sample is exposed to X-radiation, better known as X-rays. The energy distribution and flux density of the X-ray photons can be optimized for the particular analysis by adjusting voltage and current at the X-ray tube and by the interposition of absorption filters.

The principle is best described using Bohr's atomic model (Figure 1). The irradiation knocks electrons out of the atomic orbitals ("shells") of the elements contained in the sample (1). This creates a so-called "hole" (2). The energy required for this excitation depends on the atomic number of the element in question. The excited atom now returns to the energetic ground state by filling the resulting "hole" with an electron from a higher shell (3).

According to the principle of the conservation of energy, excess energy must be emitted in the form of light. The energy difference between two shells (and therefore the energy of the emitted photon in kiloelectronvolts (keV)) can be calculated quantum-mechanically and is characteristic for each element. This means the elemental composition of the sample can be calculated from the spectrum and, if the composition of each layer is known, the layer thicknesses can also be calculated. Depending on the composition and density, the X-rays penetrate into the sample up to 100 µm.

Corrosion

A lot of everyday objects as well as building materials are made of metallic substances. The metals are susceptible to reactions with oxygen or acids and, if left in contact with air and moisture unprotected, return to their original state in the long term – i.e., they corrode.

Corrosion is an electrochemical process where water acts as an electrolyte. During the process, the metal releases electrons to oxygen dissolved in the water (Figure 2). →

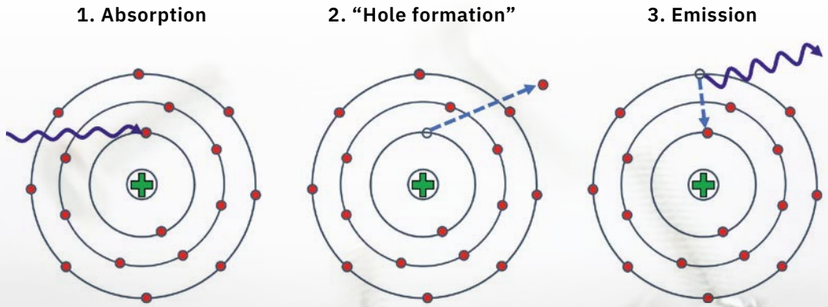


Figure 1: X-ray fluorescence explained on the Bohr atomic model

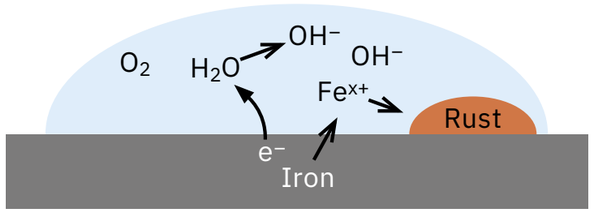


Figure 2: Electrochemical reactions during rust formation

While some metals, such as aluminum, form a continuous, stable oxide layer upon contact with air and, as a result, protect against further corrosion, the oxides of other metals, such as iron, are deposited loosely and are permeable to water. This is how the corrosion literally “eats” its way through the metal body until the structure of the corresponding component collapses. Acids as well as contact with other metals (“bimetallic corrosion”) facilitate this process.

Corrosion protection

There are different approaches to corrosion protection (Table 1). In most cases, corrosion protection is achieved by a coating with other metals, resin or a combination of both (“duplex system”). For example, you can go to the hardware store and find “hot-dip galvanized” screws, i.e. coated in a molten zinc; in the kitchen, you can find galvanized gold-plated cutlery. Even a wedding ring made of white gold is usually electroplated rhodium, i.e. protected from corrosion by a thin rhodium coating.

Electroplating processes

During electroplating processes, an electrically conductive workpiece is coated by an electrochemical deposition of metal from an electrolyte solution. The ions dissolved in the electroplating bath are reduced again by the current applied to the workpiece to form the metal, which is deposited on the workpiece in layers.

The large-scale application of these processes is very complex. The chemical composition of the bath must be closely monitored, including both the current strength and coating time. In practice, several different layers are often necessary to achieve mechanically and chemically stable and durable corrosion protection. The following three examples demonstrate how EDX spectroscopy can be used to quickly and efficiently check original materials, intermediates and end products.

Example 1: Composition of the base alloy

The possible coatings, required parameters and pretreatment depend on the material of the workpiece to be coated. Stainless steel, for example, can be polished to a mirror finish and chrome-plated directly, while other types of steel must first be copper-plated and nickel-plated to create a base for the final chrome layer.

EDX spectroscopy makes it easy to identify the composition of an alloy. A measurement time of 30 seconds is often sufficient, depending on the number of expected elements. A qualitative/quantitative result can also be determined without calibration. Since the position of the element-specific lines is known, all measured lines can be assigned automatically. A distribution is then issued, based on the intensity ratios.

Based on the elements found, the sample is then measured again using a quantitative method to identify the exact composition (Table 2). ⟶

Principle	Mode of action	Advantage	Disadvantage	Example
Coating with precious metal	Protective layer not prone to corrosion	Longevity	Accelerated corrosion in case of damage	Vitrification, chrome plating
Base metal coating	Protective layer acts as sacrificial anode	Protection even in case of damage	Protective layer dissolves slowly	Galvanizing, sacrificial anodes on the hull of the ship
Coating with plastic	Protective layer not prone to corrosion	No bimetal corrosion	Low mechanical stability	Teflon coating, protective varnish
Application of external current	Continuously “resupply” lost electrons	Wear-free	Only feasible on a large scale	Bridges, pipelines, superstructures

Table 1: Comparison of different corrosion protection techniques

Element	Al	Si	Cu	Fe	Zn	Mn	Mg	Ti	Cr
Certificate	85.02	6.90	3.90	1.01	0.99	0.83	0.42	0.29	0.26
EDX	85.31	7.99	2.72	0.94	0.87	0.81	0.00	0.32	0.29

Table 2: Results calculated without calibration in % in comparison to certified concentrations

Example 2: Composition of the bath and wastewater

The correct composition of the plating bath is important for a good plating result. When using an insoluble anode, the base material must be added to the coating in the correct concentration in the form of soluble salts. In the process, the bath is also contaminated, for example by adhering liquid from other production steps. For this reason, samples must be taken regularly and analyzed for their chemical composition.

In addition to concentrated acids, the bath usually contains a large number of toxic and environmentally harmful substances. Therefore, it is also necessary to carefully analyze the wastewater. In contrast to the electroplating bath itself, the smallest concentrations in the ppm range must be reliably determined here.

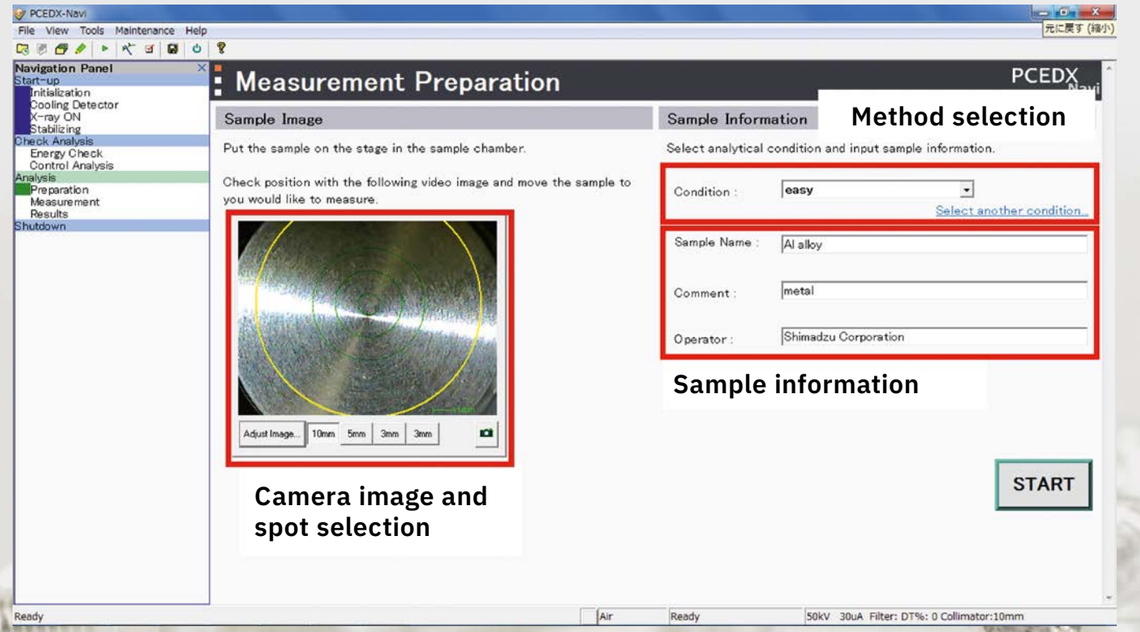
Liquid samples can be easily measured by EDX, without further preparation in a plastic cell. Various cell materials are available, depending on the required chemical resistance. The bottom is made of a thin polymer film to weaken the X-rays as little as possible. Detection limits are typically between 0.1 and 5 ppm, depending on measurement parameters and the quality of calibration.

Example 3: Layer thickness

EDX is also a method of surface analysis. The depth of penetration of the X-rays is below 100 µm, depending on the material. If the sample thickness is below the depth of penetration, the thickness of a line depends not only on the concentration of the corresponding element but also on the layer thickness. This means that if the composition of a coating is known, the layer thickness can be calculated from the spectrum – even without calibration.

It is also possible to analyze multilayer systems, provided that all layers can be irradiated. Using a special method (“Background Fundamental Parameters”), it is even possible to precisely determine layer thicknesses on irregularly shaped components, such as screws.

Figure 3: User interface of the PCEDX Navi software



PCEDX Navi software

At first glance, the possibilities and applications for EDX analysis may seem overly diverse and complex and there is indeed a lot to consider when creating a new EDX analysis method. In practice, however, Shimadzu’s PCEDX Navi software enables quite easy operation (Figure 3). Here, the pre-made methods can be called up and the handles are intuitive. The measuring spot can be controlled and optimized via a camera image.

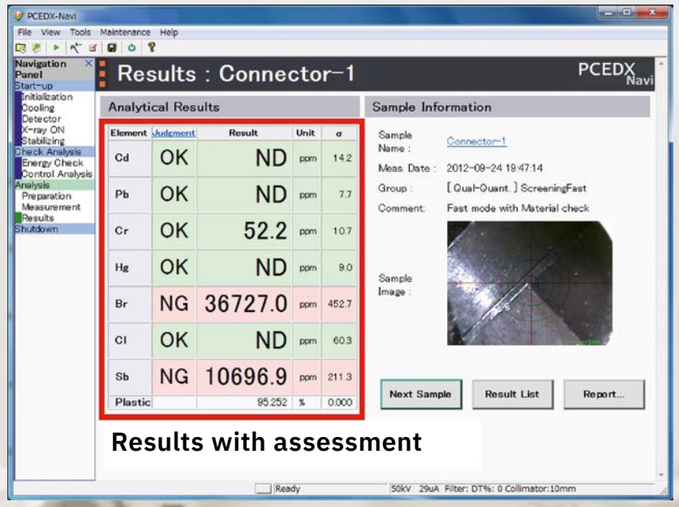
Quality criteria (pass/fail) for the respective sample type can also be stored in each method. This provides the user with immediate feedback as to whether the sample is within the set parameters (Figure 4). The results can then be exported as a report using a template previously created in Microsoft Excel.

Many of the analytical questions facing the electroplating industry can be answered using EDX spectroscopy. With only one device, liquid or solid samples can be measured non-destructively, accurately and quickly. This saves space in the laboratory, is economical and shortens training times. In contrast to methods such as atomic absorption spectroscopy, liquid samples can be measured in an undiluted manner and halogens can also be determined. In the case of solid samples, both the composition and the layer thicknesses can be determined, all without the need for additional equipment. The EDX-7200 – Shimadzu’s Energy Dispersive X-ray Fluorescence Spectrometer – is an easy-to-use, multi-purpose device designed to meet the needs of the electroplating industry.

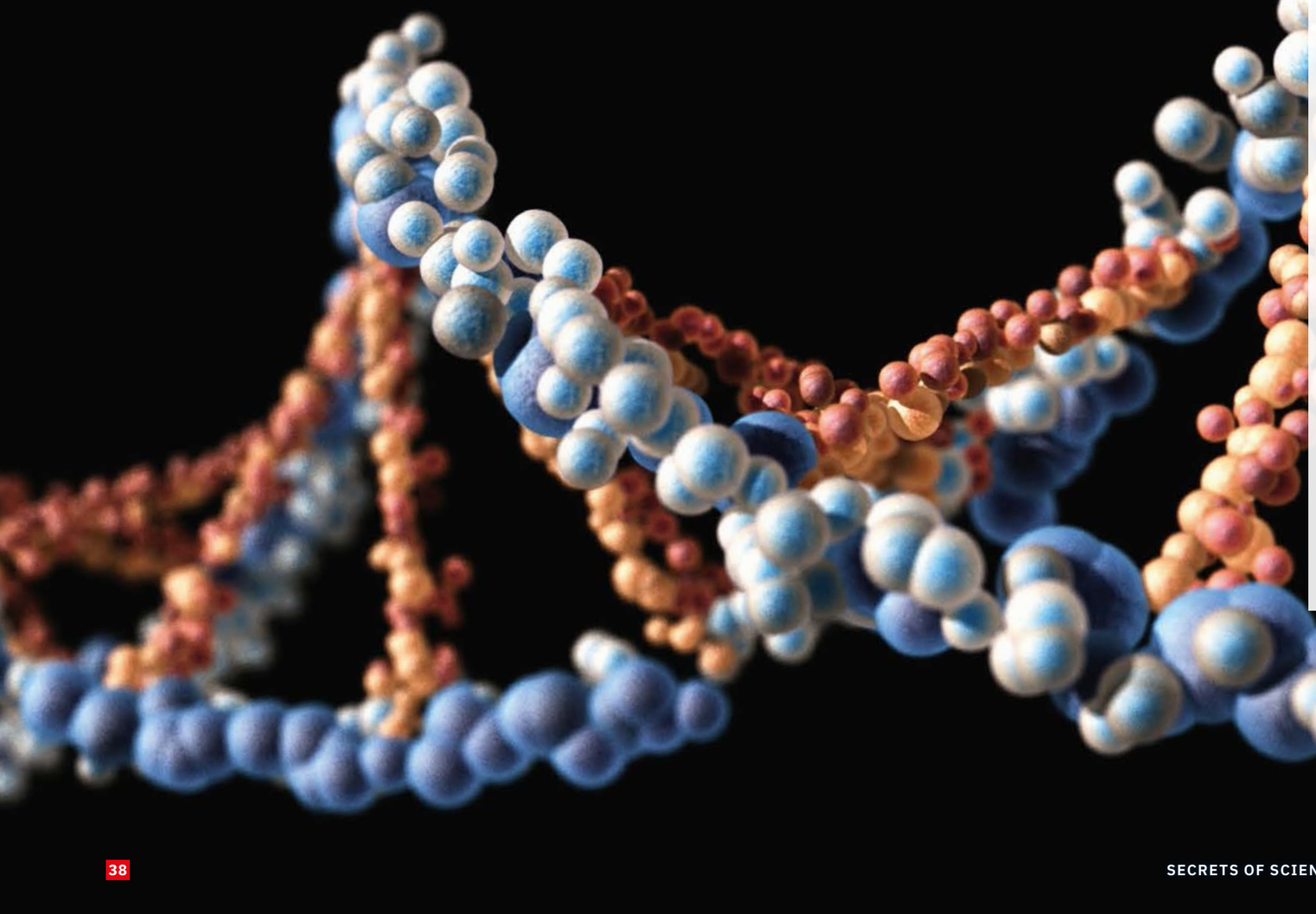
Note

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

Figure 4: Evaluation of a sample with stored quality criteria (here RoHS limit values)



Improving biopharmaceutical drug development



SWITCH ON

Metal-free, high-quality LC-MS analysis of oligonucleotide therapeutics

Dr. Gesa Schad, Dr. Björn T. Erxleben,
Shimadzu Europa GmbH

In recent years, oligonucleotide-based medicines have been recognized for their potential as new treatments for rare and chronic diseases, and several new drugs have been approved by regulatory agencies. Analysis of these nucleic acid polymers to confirm identity and purity by LC-MS is a challenge, as they possess several reactive groups that can lead to adsorption onto metal surfaces in a stainless-steel-based UHPLC instrument. This article introduces the improvement of quantitative performance in the LC-MS analysis of oligonucleotides using the Nexera XS inert, a bioinert UHPLC system.

Introduction

Oligonucleotide therapeutics are characterized by the ability to target specific diseases and are part of the new wave of biopharmaceuticals – most visibly used in the quick and successful targeting of Covid-19. As depicted in Figure 1, the DNA oligomers bind to a target protein or RNA and have successfully been used to inhibit DNA transcription and gene expression. They have also shown efficacy by activating innate immunity. Another distinct advantage is that it takes less time than conventional methods to find new therapeutic candidates as oligo-nucleotides are straightforward to design and synthesize.

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SWITCH ON

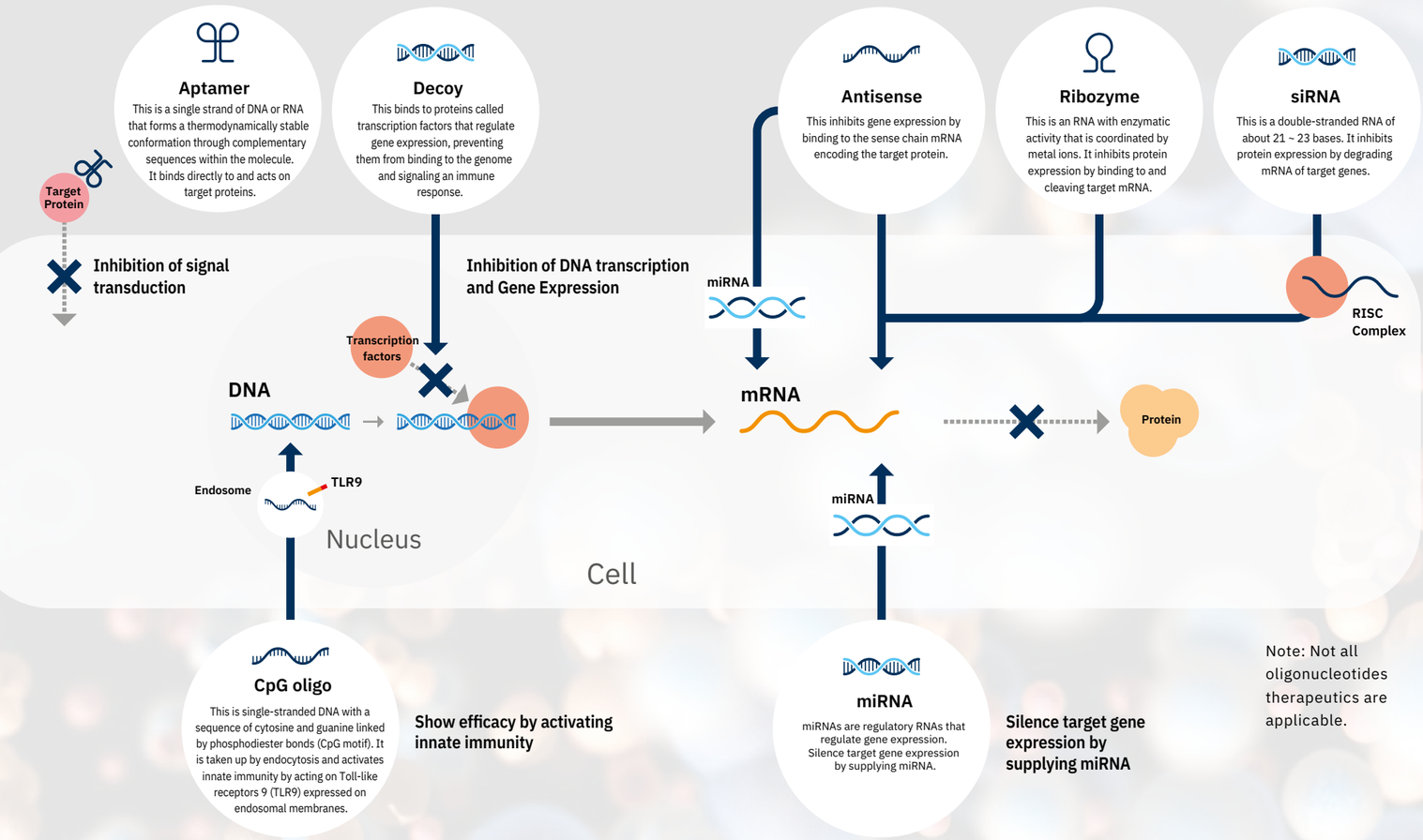


Figure 1: Mechanisms of action of oligonucleotide therapeutics[1]

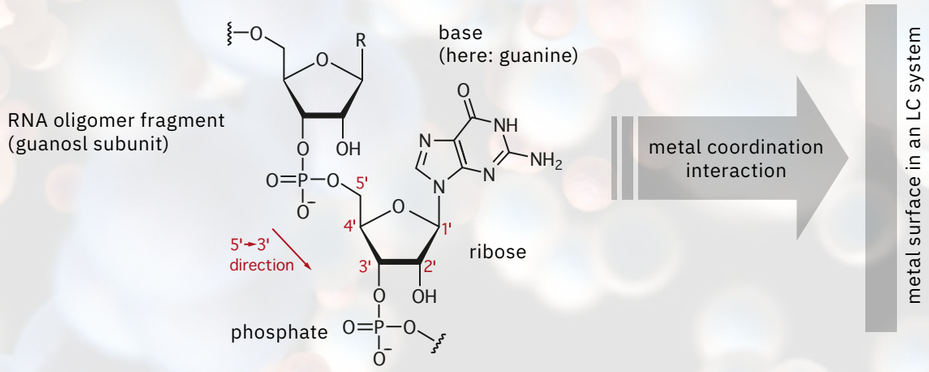


Figure 2: Nucleic acid drugs possess active sites that interact with metal surfaces

Note: Not all oligonucleotides therapeutics are applicable.

For evaluation of a synthesized drug product such as oligonucleotide therapeutics to confirm identity and absence of unwanted impurities, LC-MS precision mass spectrometry is the method of choice. However, analysis of these nucleic acid polymers poses some critical challenges, as they possess several reactive groups that can lead to adsorption onto metal surfaces in a stainless-steel-based UHPLC instrument (Figure 2). This interaction leads to poor peak shape, reduced sensitivity and lack of reproducibility. Repeat injection of a highly concentrated sample can be used to passivate a system before actually starting data acquisition, to “saturate” the adsorption sites on the surface and reduce the effect of sample loss. However, this approach not only wastes time and precious sample, it can also make it extremely difficult to acquire reliable data, due to changes in the state of passivation during continuous analysis. This article introduces the improvement of quantitative performance in the LC-MS analysis of oligonucleotides using the Nexera XS inert, a bioinert UHPLC system.

Experimental

The sample used in this analysis was an oligonucleotide with the sequence 5’-dG-dC*-dC*-dT-dC*-dA-dG-dT-dC*-dT-dG-dC*-dT-dT-dC*-dG-dC*-dC*-3’, where (*) indicates 5-C or 5-U methylation and (d) means 2’-deoxy nucleoside. The molecular weight was 6,431.72.

To evaluate the effect of using a bioinert system with no metal parts in the sample flow path, the analysis was performed using both a stainless-steel (SUS)-based UHPLC system (Nexera XR) with an SUS body separation column and the bioinert Nexera XS inert system using a metal-free column. Detailed analytical conditions are shown in Table 1. HFIP (1, 1, 1, 3, 3, 3-hexafluoro-2 propanol) and DIPEA (N, N-diisopropylethylamine) were used as ion-pair reagents, as is common practice in the reversed-phase analysis of oligonucleotides. →

HPLC conditions (Nexera XR, Nexera XS inert)	
Column:	Shim-pack Scepter C18-120 (100 x 2.1 mm, 3 µm) Shim-pack Scepter C18-120 (100 x 2.1 mm, 3 µm), metal-free
Mobile phase A:	50 mmol/L HFIP + 10 mmol/L DIPEA in water
Mobile phase B:	acetonitrile
Flow rate:	0.3 mL/min
Gradient:	5 % B (0–1 min), 5–30 % B (1–6 min), 95 % B (6.1–7 min), 5 % B (7.1–2 min)
MS conditions (LCMS-8060)	
Ionization:	ESI (negative mode)
Probe voltage:	–4 kV
Mode:	MRM (*m/z* 803.5 > 95.0)
CID gas:	330 kPa
Nebulizing gas flow:	3.0 L/min
Drying gas flow:	8.0 L/min
Heating gas flow:	12.0 L/min
DL temperature:	300 °C
Heat block temp.:	450 °C
Interface temp.:	250 °C

Table 1: Analytical conditions of the LC-MS analysis of an oligonucleotide

Results and discussion

Figure 3 shows chromatograms obtained from the analysis of a 10 ng/mL oligonucleotide standard solution on a standard Nexera XR UHPLC system with an SUS-body column and on Shimadzu's new Nexera XS inert system with a metal-free column. As can be clearly seen, there is a roughly 1.7-fold increase in sensitivity when metal is removed from the sample flow path.

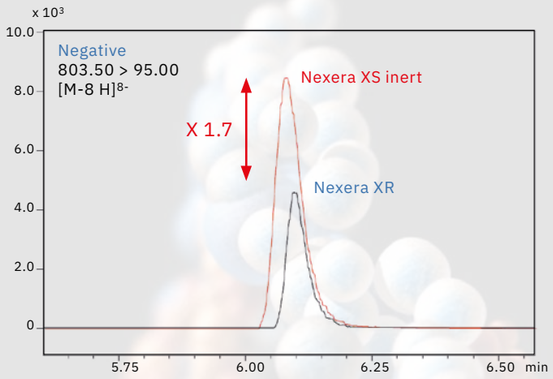


Figure 3: MRM chromatograms of an oligonucleotide standard solution

To compare the quantitative performance of the two systems for the analysis of oligonucleotides, calibration curves in the range of 0.5–1,000 ng/mL were produced. The graph obtained with data from the SUS-based Nexera XR system showed a lower value for linear regression (R^2 = 0.9721) compared to the excellent linearity on the bioinert system (R^2 = 0.9996), emphasizing the effect of metal adsorption on accuracy of the quantification in the highly sensitive LC-MS analysis of oligonucleotides. Results of the evaluation of linearity and accuracy at each calibration point can be found in Table 2 and Figure 4. These values highlight the improvement in reliability achieved when using the Nexera XS inert system with a metal-free separation column, especially when looking at very low sample concentrations.

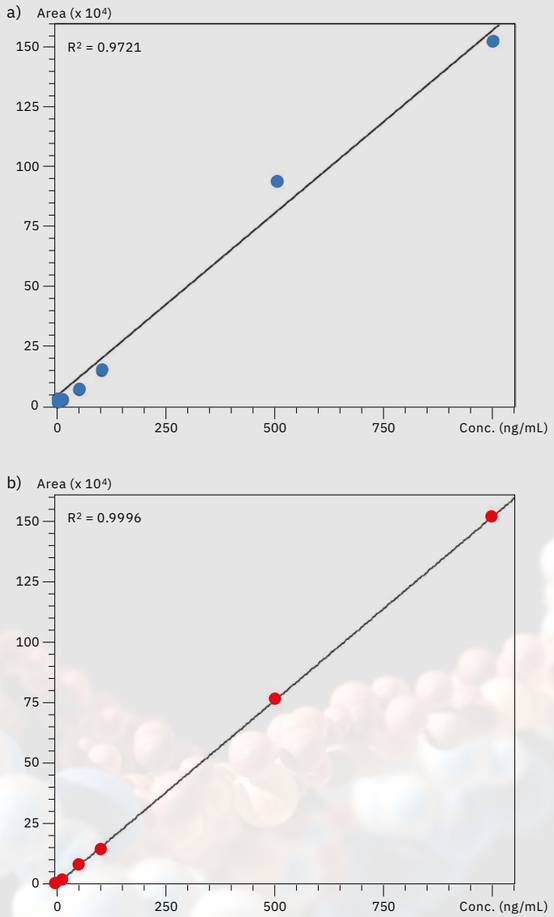


Figure 4: Calibration curve of the LC-MS analysis of oligonucleotide standard using a) an SUS-based Nexera XR UHPLC system and b) a bioinert Nexera XS inert UHPLC system

To determine the system performance with regards to carry-over, a blank (water) was injected right after the 1,000 ng/mL oligonucleotide in water standard solution. Figure 5 shows the comparison of the chromatograms obtained from the blank injection on the SUS-based Nexera XR system (a) and the bioinert Nexera XS inert system (b). The results and the carry-over values of 0.079 % and 0.003 % respectively indicate the absence of metal adsorption in the Nexera XS inert system, which also minimizes carry-over into the next sample run.

Concentration (ng/mL)	Nexera XR		Nexera XS inert	
	ng/mL	Accuracy %	ng/mL	Accuracy %
0.5	2.28	455.7	0.57	113.5
1	–1.04	–104.4	0.93	93.0
5	2.43	48.5	5.42	108.5
10	5.62	56.2	9.13	91.3
50	26.63	53.3	50.24	100.6
100	76.39	76.4	92.77	92.8
500	588.74	117.7	497.04	99.4
1,000	965.46	96.5	1,010.36	101.0

Table 2: Comparison of accuracy of the LC-MS analysis of an oligonucleotide using an SUS-based and a bionert UHPLC system

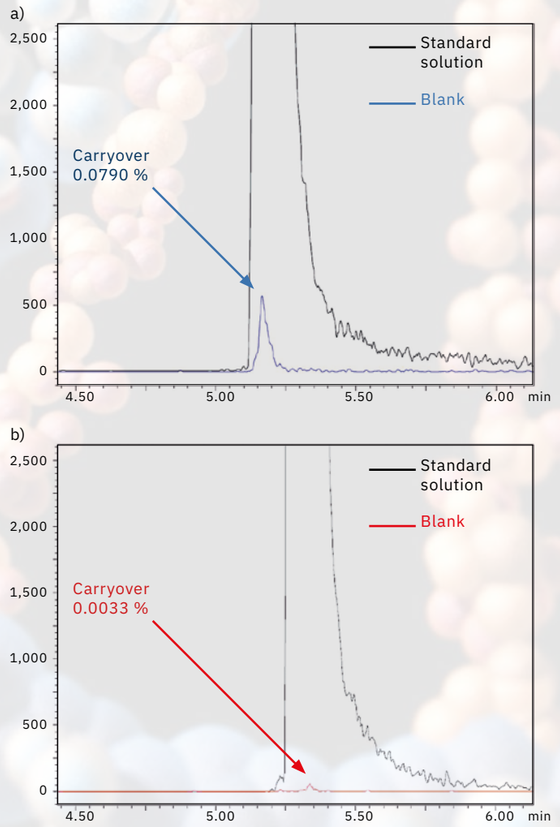
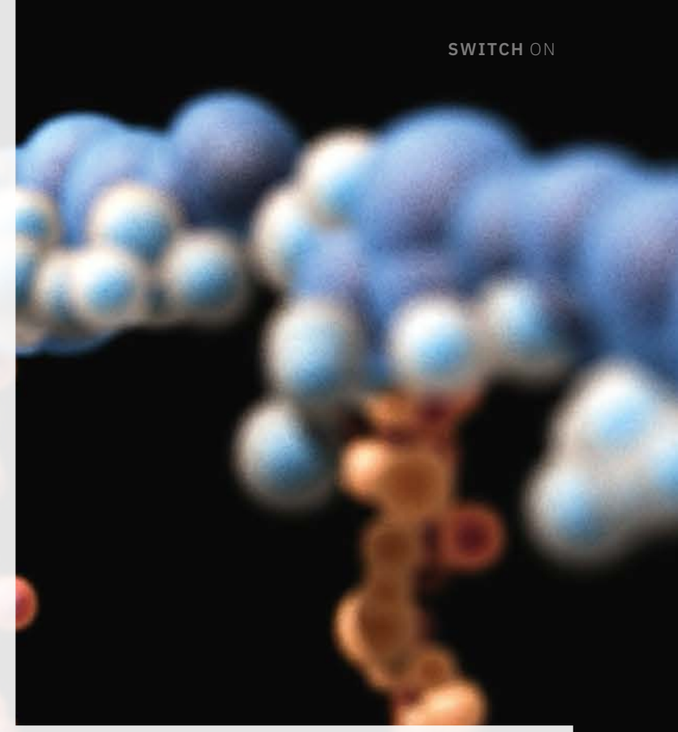


Figure 5: Carryover performance of a) Nexera XR SUS-based UHPLC and b) Nexera XS inert, bioinert UHPLC system



The comparative evaluation of a stainless-steel-based UHPLC system and a bioinert UHPLC system for the highly sensitive LC-MS analysis of oligonucleotides was carried out with regards to sensitivity, quantitative performance and carry-over. With improvements in all aspects when using the bioinert system, the advantages of removing metal from the sample flow path for the analysis of metal coordinating compounds could be clearly shown. This study emphasizes the value of the Nexera XS inert system in combination with a metal-free separation column for most accurate characterization and quality control of oligonucleotide therapeutics.

Note

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

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