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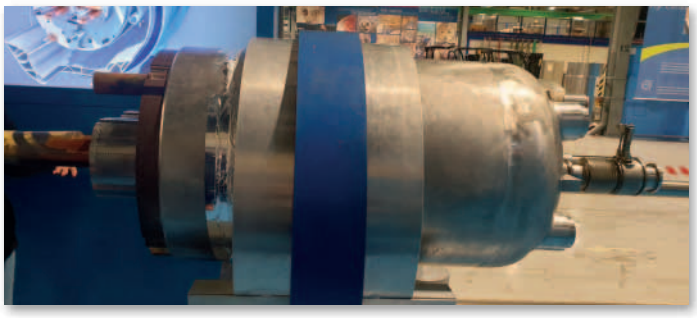
The European Organization for Nuclear Research, Meyrin, Switzerland

Experience high-tech research at first hand

The winners of the »50 Years Shimadzu Europa« competition visit CERN

Visiting the world's largest particle accelerator – Shimadzu Europa made this scientist dream come true. Around its 50th anniversary and a corresponding competition, Shimadzu organized a visit for the winners to CERN in Geneva, the European Organization for Nuclear Research. Antal Erdös traveled with his girlfriend Julianna Szombati from Buda-



Particle accelerator out of service

pest, while Claudio Ghilardi arrived from Milan accompanied by his colleague Giuseppe Scollo. During the three day visit, they had the opportunity to tour CERN and explore the city.

In the footsteps of physics

CERN, located on the Swiss-French border, is the world's largest research center for particle physics. Researchers at the site work with the fundamental laws of the universe. Supported by the world's largest particle accelerator, scientists can examine the smallest matter – elementary particles.

CERN reached international fame through various discoveries. In 2012, a new subatomic particle was researched and later confirmed as the Higgs boson. Peter Higgs, the eponym, received the Nobel Prize in Physics for the theoretical recognition of the particle. For the general public, the research center's best-known achievement is from Tim Berners-Lee. The British scientist invented the World Wide Web at CERN in 1989. He wanted to create a network where information could be exchanged despite large distances.

Claudio Ghilardi and Giuseppe Scollo from Italy

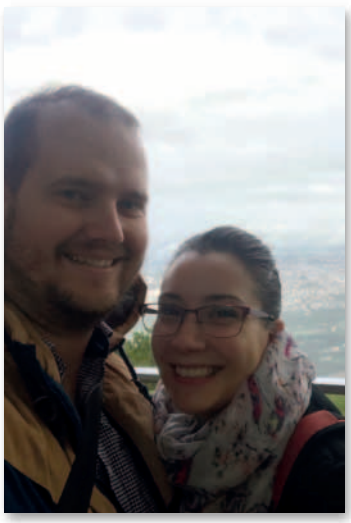
The competition winners and their guests participated in a group tour with 14 employees from the Shimadzu Switzerland subsidiary. The participants received deep insights into nuclear research. Cross-sections and lift simulators were used to demonstrate the famous Large Hadron Collider particle accelerator, which started operation in 2008. Its force is generated by superconducting magnets which are arranged in a circular tube of 27 km below the earth's surface. The CERN tour was completed with a group luncheon and a photograph.

Geneva and surrounding areas

The winners also enjoyed a sightseeing tour of Geneva. The city is a global diplomacy and banking hub and also serves as the headquarters of the Red Cross organization and the United Nations in Europe. Geneva offers panoramic views over the lake and the Alps and Jura mountains behind. Claudio Ghilardi described the visit to CERN as "very cool." Antal Erdös used the short trip to Geneva for a personal matter: he proposed to his girlfriend Julianna Szombati on Mount Salève.

Shimadzu Europa wishes the newly engaged couple all the best in the future.

Group photo in front of the »globe of science and innovation«



Antal Erdös and Julianna Szombati on the top of the mountain »Salève«

Tracking Pesticides

SFC-ESI-MS/MS decreases matrix effects in pesticide residues analysis of orange, leek, and dried spices

Matrix effect is a well-known phenomenon in the pesticide residue analysis field. The ion suppression generated in the mass spectrometer ion source can lead to errors in the quantification of analytes. Other problems regarding detection difficulties and loss of precision and accuracy in the analytical method applied are also associated with these matrix effects.

Supercritical fluid chromatography coupled to mass spectrometry (SFC-MS/MS) is a technology developed in the 1960s and has been continuously updated until it has reached the nowadays robust new generation of systems. The behavior of matrix effects in SFC-MS/MS is different in comparison with reverse-phase liquid chromatography coupled to mass spectrometry (LC-MS/MS).

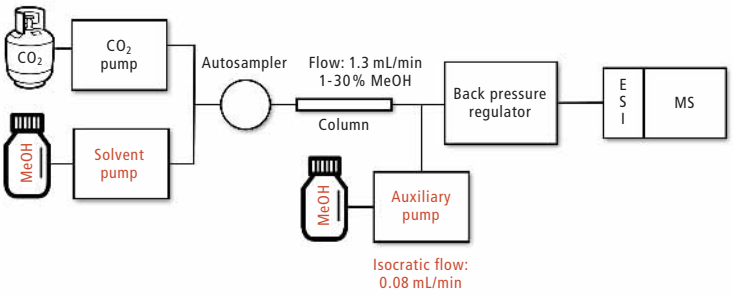


Figure 1: Operation scheme of Shimadzu Nexera UC coupled to LCMS-8060

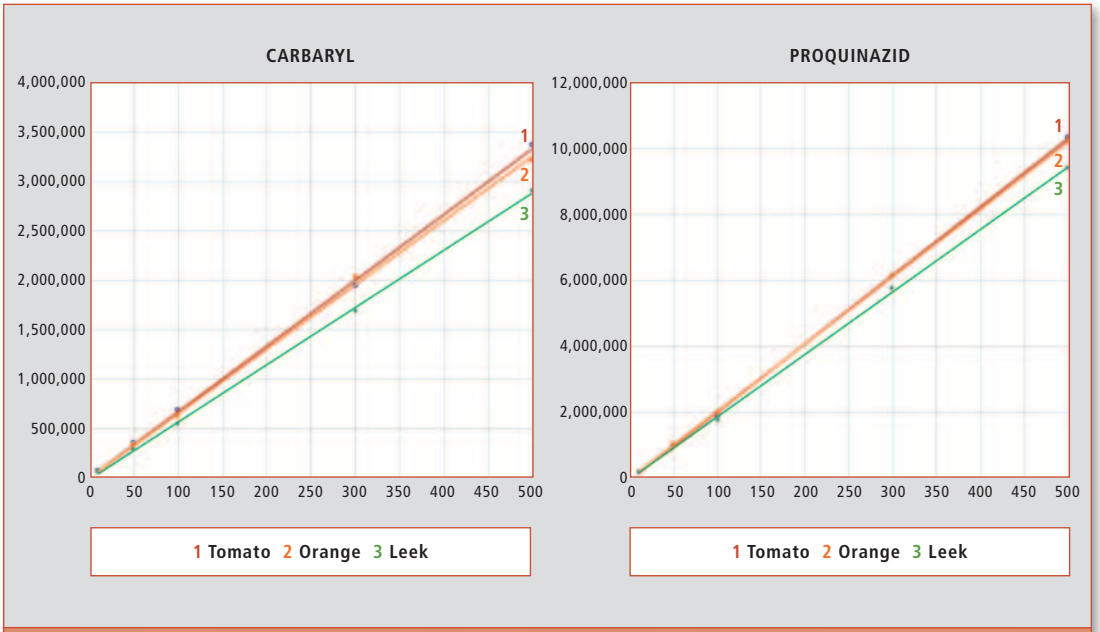


Figure 2: Matrix-matched calibration curves of Carbaryl and Proquinazid in tomato, orange and leek

To understand this, it is necessary to focus on the different chromatographic and ionization conditions of both techniques.

Differences between SFC and reversed-phase LC

The supercritical fluid chromatography system employed during this study is described in Figure 1. The mobile phase presents low viscosity and high diffusivity as supercritical carbon dioxide ($scCO_2$) is used.

Short run times are customary in SFC-MS/MS; the properties of $scCO_2$ allow the application of high flow rates (higher than 1 mL/min) without reaching the pressure limit, and performing of efficient analyte separations.

In reverse-phase LC, the compounds elute in decreasing order of polarity, but in SFC, even though polarity influences their solubility in $scCO_2$, it does not play the primary role in the elution order. Therefore, no sole criterion for the elution order of the analytes in this technique is present, and polarity cannot be used as a reference.

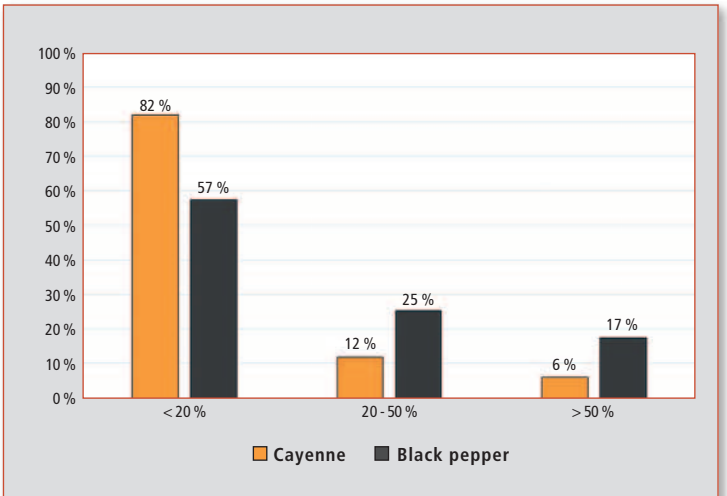


Figure 3: Matrix effect (%) of the 162 pesticides studied in cayenne and black pepper matrix

What is clear is that ionization using electrospray ion source (ESI) in SFC-ESI-MS/MS is more efficient when compared with LC-ESI-MS/MS. Temperature and pressure conditions are controlled by a backpressure regulator (BPR) in supercritical fluid chromatography. However, when the flow with the analytes leaves the BPR, atmospheric pressure is reached. Carbon dioxide then returns to the gas state, and only the small portion of methanol inside the mobile phase reaches the ion source. The advantage of this low flow entering the sources lies in the increment of sampling efficiency due to the production of smaller charged droplets, resulting in increased ionization rates.

Furthermore, the solvent reaching the source plays an essential role in ionization improvement. Methanol together with supercritical carbon dioxide is usually the co-solvent employed. Liquid chromatography customarily uses a mixture of water and an organic solvent. Comparing methanol with water, ionization benefits can be observed as methanol provides a lower density and surface tension that increases solvent evaporation rate. To summarize, the process for the analytes of interest to reach the gas phase is shortened and ionization efficiency is increased accordingly.

The competition in ESI for the available charges decreases the ionization efficiency in the interface. The co-eluting compounds also increase the surface tension and viscosity of the microdroplets. As explained above, this reduces the ability of the analytes to reach the gas phase. Therefore, the ionization improvement of the SFC-MS/MS and the possibility to inject a lower amount of sample due to the high sensitivity results in a reduction of the matrix effect.

Matrix effects in fruit and vegetables

An evaluation of 164 pesticides was performed using SFC-MS/MS in three different matrices of fruit and vegetables. These matrices are representative of different compound compositions: Tomato (high water content matrix), orange (acidic matrix) and leek (high number of co-eluting compounds matrix). The possibility of suppression caused by the matrix depends on the nature of the compounds present; vegetable matrices such as leek contain a large number of compounds in the matrix and are more likely to cause ion suppression.

For evaluation of matrix effect, the slopes of the calibration curves obtained in extracts of the different matrices were compared with those obtained from calibration curves based on solvent and considered as no-suppression reference. An interference substance of the matrix can alter the signal of the analyte and in consequence, the slope of the matrix-matched calibration curve.

QuEChERS extracts from the three matrices studied were spiked with 164 pesticides as follows: 100 µL of each blank extract was evaporated under a gentle stream of nitrogen and reconstituted with 100 µL of acetonitrile containing the mixture of analyzed pesticides at 5, 10, 20, 50, 100 and 500 µg/kg. 400 µL of ultrapure water was then added to dilute the extract before injection. The samples injected therefore contained 0.2 g of matrix per 1 mL of extract, and their pesticide concentrations were 1, 2, 4, 20 and 100 µg/kg. Hence, as 2 µL is the injection volume used, 0.4 mg of the sample was introduced in the system.

	Pear		Zucchini		Orange		Onion		Tea	
	SFC	GC	SFC	GC	SFC	GC	SFC	GC	SFC	GC
Acrinathrin	5	10	5	7	0	94	0	44	-22	52
Bifenthrin	10	4	8	8	-7	33	-24	28	-3	5
Cyfluthrin	10	0	15	-1	-4	40	-2	23	-57	-5
Cypermethrin	-2	1	4	-2	-8	43	-17	26	-32	3
Deltamethrin	1	11	3	-3	-14	23	-63	15	-18	—
Etofenprox	0	1	-1	0	-8	21	-65	15	-10	-12
Fenpropathrin	18	5	20	4	-7	41	-25	30	-1	13
Fenvalerate	0	-1	4	10	-6	24	-40	12	-32	1
Flucythrinate	-5	6	-5	1	-8	52	-37	34	-86	16
Lambda-cyhalothrin	-7	2	-6	2	-9	43	-18	25	-19	18
Permethrin	2	3	8	5	2	48	-11	28	-10	2
Phenothrin	6	9	11	12	-8	53	-17	41	-75	18
Tau-fluvalinate	6	9	10	-4	-6	62	-12	22	-50	35
Tetramethrin	-3	4	3	6	-4	64	-2	35	-11	-38

Table 1: Matrix effect (%) of the 14 pyrethroids in the matrices studied

The zero matrix effect can be considered when calibration curves based on solvent and matrix have the same slope. This situation is more likely to be found in matrices like tomato, where a low number of compounds coelute with the analytes. The complexity of the matrix components in matrices like orange or leek is much higher in comparison with high-water content matrices like tomato. However, using supercritical fluid chromatography, there is a slight difference between the slopes in the different matrices, as can be observed for the examples presented in figure 2.

The percentage of ion suppression/enhancement was taken into account in the statistical study of the matrix effect. Signals within the range 0 - 20 % were considered as low matrix effect, whereas a difference of 20 - 50 % corresponds to medium matrix effect, and > 50 % to strong matrix effect. The percentage of compounds with irrelevant matrix effect (inside the range 0 - 20 % of signal suppression) was 99 % for tomato, 87 % for orange and 62 % for leek.

On the other hand, the percentage of compounds with signal suppression between 20 and 50 % was 1 % for tomato, 12 % for orange and 35 % for leek. Significant suppression was not found in tomato, and only 1 % of the compounds in orange and 3 % in leek had an ion suppression higher than 50 %. These results with SFC-MS/MS show that the ionization benefits bring a reduction of matrix effect even in very complex vegetable matrices such as leek.

Matrix effects in dried spices

This study was also applied to dried spices. These are complex matrices that contain large amounts of essential oils, plant nutrients and secondary metabolites such as flavonoids, terpenes and alkaloids.

Analysis of pesticide residues in spices is complex due to the high ion suppression suffered in the ion source. The spices evaluated (black pepper and cayenne) can be considered as representative of matrix difficulties. The same concentration levels were used for the calibration curves in each matrix, and 0.1 mg was the total amount of sample injected.

As can be observed in Figure 3 (page 5), 132 (corresponding to 81 %) of the 162 pesticides studied showed weak matrix effects in cayenne and 91 (56 %) in black pepper. On the other hand strong matrix effect was only found in 10 (6 %) pesticides in cayenne and 27 (17 %) in black pepper. These results represent an improvement over most of the published literature using LC-MS/MS to analyze pesticides in spices.

The highly efficient ionization in SFC-ESI-MS/MS allows enlargement of the scope to compounds typically analyzed by gas chromatography coupled to mass spectrometry (GC-MS/MS), as is the case of pyrethroids. These synthetic chemical pesticides present difficulties in analysis by reverse-phase LC-MS/MS. In general terms, LC usually provides lower sensitivity, and most of the analytical methods described for the analysis of these compounds use gas chromatography (GC).

After optimization of these compounds with supercritical fluid chromatography, a study of the matrix effect was performed. The extracts of six different matrices (tomato, zucchini, pear, orange, onion and tea) were used to prepare the matrix-matched calibration curves. In all cases, in SFC, the medium and strong matrix effects correspond to signal suppression.

With GC, the situation was the opposite: all pyrethroids with a matrix effect higher than 20 % showed signal enhancement with one exception, tetramethrin, which presented a signal suppression of 38 % in tea. As detailed in Table 1, similar matrix effects were obtained by both techniques. Looking at the results of the orange matrix, no matrix effect was obtained by SFC; however, this matrix produced a remarkable enhancement of the signal for all of the pyrethroids in gas chromatography, with the exception of fenvalerate.

Summary

The low matrix effect in complex matrices like orange, leek or dried spices demonstrates that SFC-ESI-MS/MS can facilitate the analysis of pesticide residues in comparison with LC-MS/MS. The increased ionization efficiency, type of solvent applied and low amount of sample injected provide improved accuracy and precision of the quantitative measurements.

Author

Víctor Cutillas, Amadeo R. Fernández-Alba

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Read for you in eFOOD-Lab 4/19

Pushing the limits

New UV-Vis spectrophotometer combines top performance with network capability

Fast and ergonomic just like its predecessor but now network-compatible with the option of using Wifi: the UV-1900i is the new UV-Vis spectrophotometer designed for the food industry, pharmaceutics, life sciences and chemistry alike. Several measuring modes and numerous accessories support a wide variety of applications, expanding the range of use. Important for pharmaceutical applications is that the optional LabSolutions CS and DB software also meet FDA 21 CFR Part 11 requirements.

UV-1900i spectrophotometer

The UV-1900i is a further development of the acclaimed UV-1900, which won the coveted Red Dot Award in 2019, one of the most treasured design prizes worldwide. The jury emphasized the unique design which supports ergonomic handling.

The UV-1900i is a stand-alone instrument. Its operability can be summarized as “easier and faster than ever before.”

The touch display is ergonomically designed: large and clear symbols show all functions at a glance. The system can be operated in eight languages. With the latest LabSolutions UV-Vis software, spectrum analyses can also be carried out conveniently at a PC.

With the UV-1900i, users can analyze more samples per day than before – also supported by the well-known CETAC ASX autosamplers. The world’s fastest scan function records spectra at up to 29,000 nm/min. The wake-up function allows analyses to start immediately in the morning without the usual half an hour waiting time.

The LO-RAY-LIGH technology patented by Shimadzu ensures high resolution with little scattered light and maximum sensitivity.

Network capability allows the UV-1900i to access a PictBridge printer without an intermediary PC. Several UV-Vis systems can share one printer. For easier operation, Windows-compatible USB keyboards or a barcode scanner can be connected directly to the UV-1900i.

Easy and fast operability

Fully-automated DPD deficiency testing

Each year almost 80,000 new patients in France alone receive fluoropyrimidines, a group of anti-cancer drugs including 5-FU which is normally administered intravenously to treat digestive, breast as well as head and neck cancer

CLAM-2030 + LCMS-8060 in the Innovation Center at CHU Limoges (France)

However, fluoropyrimidines-based chemotherapies can cause severe toxicities (incidence at around 20 %) and sometimes lethal toxicity (incidence between 0.1 and 1 %) with part of these toxicities possibly related to deficiency in the activity of the main enzyme enabling elimination of 5-FU, DPD.

Because of this risk, routine screening for DPD deficiency before treatment with fluoropyrimidine is now necessary.

EH asked Stéphane Moreau, who is responsible for LC/MS in Europe for the Japanese instruments and medical equipment manufacturer Shimadzu to outline what is available for this kind of screening and any new developments.

Two methods are currently available to diagnose DPD deficiency, he said, but underlined that each involves a significant amount of manual work. One is genotyping, though key to that is in knowing the different variants to detect. 'It's not 100 percent accurate,' Moreau observed, 'because you detect only what you know.'

The second technology is LC-MS/MS, which has the advantage that the ratio of uracil and dihydrouracil is measured directly. 'As uracil is metabolised to UH2 by DPD, the ratio UH2/U reflects the DPD activity, thus indicating a risk for the use of 5-FU drug. In this way, you can identify the DPD deficiency, even if you don't know the different genes influencing the DPD deficiency.

'In genotyping,' Moreau added, 'you are looking for the genes that are the cause of the deficiency while, with LC-MS/MS, you estimate the activity of DPD by measuring the ratio of compounds UH2 and uracil. In one case you look at the cause, in the other, you look at the result.

'The advantage of measuring the deficiency in DPD via the ratio U/UH2 is that you will take care of all deficiency cases, thus eliminating the risk of toxicity. In that way, this is safer.'

However, he did acknowledge that the problem with LC-MS/MS is that sample preparation is 'quite tedious, with a lot of manual steps' and has been limiting penetration of LC-MS/MS.

The fully-automated LC-MS/MS method

Potentially leading to toxicity and bone loss by reduction of 5-FU metabolism, dihydropyrimidine dehydrogenase deficiency (DPD) has a big impact on the use of the cancer drug 5-FU (5-fluorouracil). Thus DPD identification is key to identifying those patients who may be susceptible to a negative reaction to 5-FU and help to avoid that serious reaction.

Additionally, with the ever-increasing number of tests needed, automating this process has grown in importance. Fortunately, Shimadzu has now developed a process that promises speed up testing and also deliver greater accuracy, safety and standardization. The answer has been to develop a fully-automated LC-MS/MS method for uracil and dihydrouracil in human plasma, known as indirect phenotyping.

Previous LC-MS/MS methods encountered problems with complex liquid/liquid or solid-phase

extraction procedures. However, Moreau explained that Shimadzu has developed a method in which the extraction is carried out by a programmable liquid handler directly coupled to a liquid chromatograph (LC) MS/MS system.

Specifically, the extraction procedure is performed by a CLAM-2030 coupled with a LCMS-8060 triple quadrupole mass spectrometer. Now, with the increasing number of tests required – and the new French guidelines introduced in December 2018 that state that all patients treated by 5-FU should be checked for DPD enzyme deficiency – coping with demand and throughput is a challenge, due mainly to the long sample prep process.

Due to the complexity of liquid extraction and the manual steps, Shimadzu received a request from a customer using its CLAM robot to develop a method to automate the preparation process prior to LC-MS/MS. 'That completely automated this, Moreau said, 'and reduced the analysis time with a solution for the customer to increase the throughput in their lab.'

First systems installed in France

Recently validated at a hospital in Limoges, the first systems in France are being installed. Moreau believes this will attract widespread interest in other countries. He points to a presentation, last June at the European Association of Pharmacology and Toxicology in Sweden, from scientists in Leiden regarding their systematic genotyping process with a profile for each patient, but also showing that, with this, there are still cases of toxicity because they do not know all the variants that cause this problem.

'So, I'm sure LC-MS/MS is the best solution and that automated sample prep will help a lot of people.' The automated process is timesaving, improves workflow and offers standardization. The Shimadzu LC-MS/MS approach has cut manual sample prep time from 30 to 10 minutes or less, but a major benefit is that the next sample is being prepared while the existing sample is being run on the system, increasing throughput. Sample analysis is also reduced to 15 minutes.

After the sample is placed on the robot there is no need for human manipulation and, as the robot handles every sample in exactly the same way, standardization is high. With so many cancer patients receiving 5-FU-based chemotherapy, Moreau stressed that the importance of finding out quickly about DPD deficiency is now widely recognized.

He believes the Shimadzu solution of automating sample prep and a LC-MS/MS method to measure uracil and dihydrouracil with no human intervention once the sample tube is positioned, is set to play a key role in speeding up this process, and offer better and quicker outcomes for patients.

Read for you in European Hospital (EH) 6/19; interview by Daniela Zimmermann

In 1994, Stephane Moreau obtained his diploma from INSA in fine chemistry and engineering with a specialization in chemical process engineering. He then started his professional career in laboratory equipment distribution before he joined the brand new Shimadzu France subsidiary in 2002. Since then, he has held various positions to develop the MS range business. Since September 2013, he has been product manager for the MS range with Shimadzu Europe.

Luminescent PET bottles

Part 1: Plastic determination using fluorescence spectroscopy

Whether soft drinks, lemonade or sparkling water – PET bottles are used for beverages all over the world. PET stands for "polyethylene terephthalate" and is one of the synthetic polymers commonly known as plastic. Every year, several million tons of polymers are released into the environment.

Together with climate change, pollution by plastic is one of the major challenges worldwide: for humanity, animals, and the environment. Microplastic especially presents a high risk due to its small size, as many creatures and microorganisms may confuse it with food.

PET bottles can be analyzed using fluorescence spectroscopy since these bottles show partial luminescence. As it is a complex topic, the topic of fluorescence effects in polymers in general and the effect of size regarding environmental degradation of plastics into microplastics is divided into three parts. This part 1 of the article series shows the effect of fluorescence on dedicated polymer PET, part 2 widens the focus to different polymers and part 3 investigates particle size and its effect on fluorescence.

Figure 1: Four bottles made from PET. They originally contained beverages. Citrus lemonade, water, lemonade and non-carbonated water

Multicolored polymers

Many polymers are colored with inorganic or organic colorants (pigments and dyes). The group of colorants used depends on the individual plastic and the production form as well as the required consumer preferences. Regulations regarding coloring apply when food comes into contact with plastic, such as candy packaging or beverage bottles [1]. For example, the coloring of PET bottles can range from opaque to transparent.

Figure 1 shows a collection of beverage bottles. All of them contained water-based beverages like non-carbonated water, lemonade, citrus lemonade and sparkling water. Many of these PET bottles have a light or intense blue color. In particular the bottles declared to be based on 100 % recycled PET are more bluish than the others.

Color sells

Marketing reasons also influence the appearance of a bottle. For example, a bottle consisting partly of recycled colorless PET appears unpleasantly yellowish. With the help of colorants, the bottle can be transferred into a bluish color, more attractive to the human eye and enhancing consumer willingness to buy.

Analysis

For a quick screening, rectangular pieces from the bottle walls of approx. 2 x 3 cm were placed in the solid sample holder and measured with Shimadzu's RF-6000 spectrofluorophotometer system. The Excitation Emission Matrix (EEM) of almost all bottle pieces showed a weak fluorescence in the area of 325 to 420 nm.

Quick screening was performed to detect unknown colorants in the PET cuts using the EEM function of the LabSolutions RF software. The advantage of such a screening of unknown colorants is the wide dynamic range of the fluorescence measurements regarding concentration of different fluorophores without changing the parameters.

The variety of PET bottles contributed to identification of PET related fluorescence [1] which was detected in each of the samples, as well as different fluorophores and their concentrations including an overall diverse fluorescence activity.

The quick analysis of the cuts showed higher fluorescence activity for the dark green lemonade bottle. Measurement results are shown in table 1. It contains the

Figure 2: Cuts of plastic sheets from PET bottles

bottle volume, beverage, color, size, highest fluorescence intensity value from the region and the related Excitation (EX) and Emission (EM) wavelength. The three last values only represent one measurement region and are not absolute single values characterizing the polymer or the colorant.

The LabSolutions RF software file version in combination with Shimadzu's RF-6000 was used. Measurement parameters were the same for all samples.

For the double grating system (excitation and emission) the slits for the excitation and emission scan were set to 3 nm. For the excitation energy, a 150 Watt Xenon lamp was used. Step size for the excitation grating movement to receive the EEM was 5 nm.

Scan speed was 6,000 nm/min. The EEM was obtained by moving the excitation grating in 5 nm steps along the defined UV-Vis measurement range and recording the emission spectrum over a fixed range. All spectra collected were then presented with their intensities in a 3D-view of excitation wavelength versus fluorescence wavelength.

Observation range was 300 to 600 nm for both axes (excitation and emission). The intensity scale of the upper and lower limit in the matrices graph is identical. For the table, a more precise value was selected from the highest intensity of a range.

All bottles showed a typical area of weak intensity at about EX 325 nm and EM 320 nm. The intensity of the fluorescence

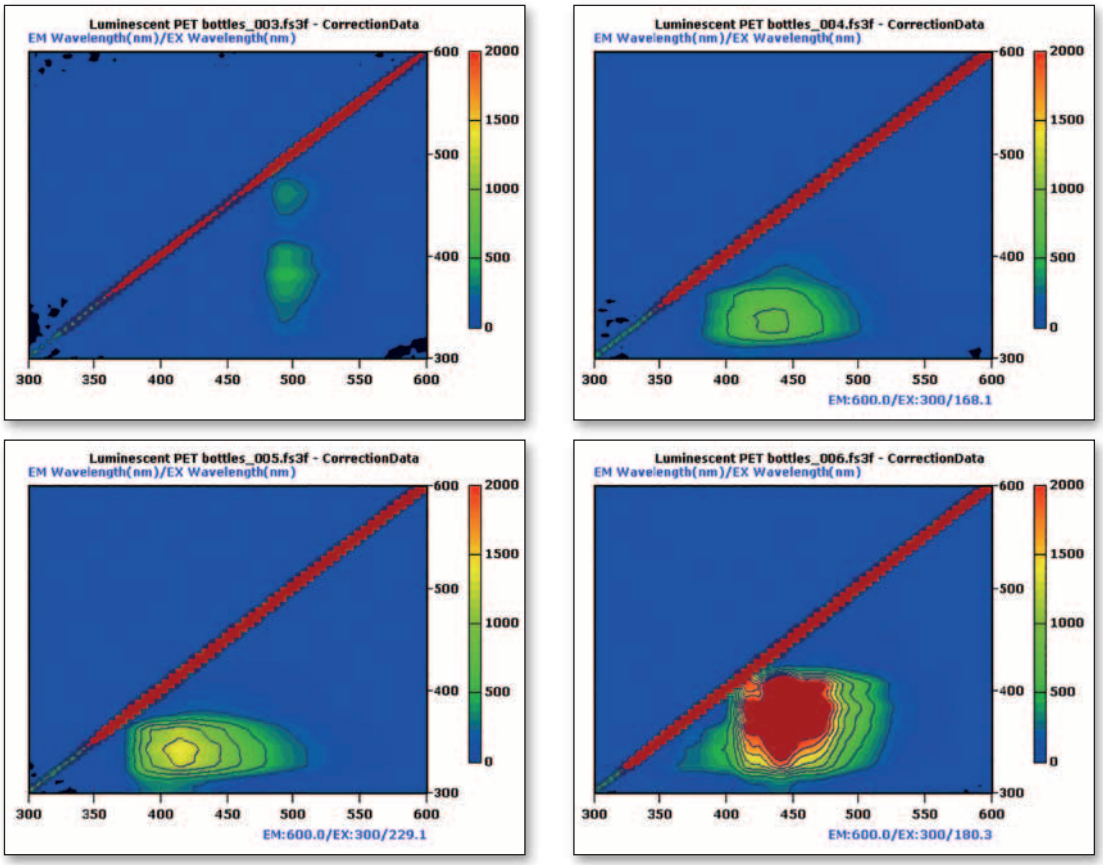


Figure 3: EEM Matrices of the cuts from the four bottles of figure 1, upper left lemonade, upper right water 100 % recycled, bottom left water 25 % recycled, bottom right citrus lemonade

matrix is zoomed to 2,000 intensity units (figure 3).

Generally, PET has its own fluorescence [2]. It may therefore overlap with the colorant. Due to the bottles different structures and thicknesses, fluorescence intensity (table 1) of the hot spots in the matrices is also film thickness dependent.

Conclusion

All bottles made from PET showed fluorescence activity. The dark colored bottles (lemonade-dark green and citrus lemonade-blue) had higher intense signals in the non-PET area. Blue colorants can have their fluorescence in the range of the PET signal, so that an overlapping with a blue fluorescent colorant can be expected. This also explains the shift of the hot spot in the graphics (figure 3) into the region of longer wavelength (table 1).

With this measurement setup, it is possible to analyze fluorescence intensity from colored plastics based on PET. As the measurements were done with low sensitivity and small slits, it is likely that fluorescence with only traces of material can also be expected and detected. The influence of size will be shown in the following parts two and three of this application in the next Shimadzu NEWS issues.

The idea to utilize this measurement came from CARAT GmbH, Bocholt (Albert van Oyen, Erwin Jansen).

Literature

[1] Colorants and Polymers, Food and drug administration HHS 178.3297, 21 CFR Ch. I (4-1-16 Edition)

[2] Absorption and fluorescence spectra of poly(ethylene terephthalate) dimers, M. F. Sonnenschein and C. M. Roland, POLYMER, 1990, Vol 31, November, 2023-2026

Content	Color [transparent]	Fluorescence intensity	Area EX	Area EM
Lemonade	Dark green	500	380	495
		355	460	495
Water (PET 100 % rec., 500 mL)	Light blue	762	335	435
Water (PET 25 % rec., 600 mL)	Light blue	1,385	345	415
Citrus lemonade (1,500 mL)	Blue	3,732	370	445

Table 1: Fluorescence intensity of PET from different sources – in common plastic bottles for beverages (rec. = recycled)

High-performer to support the doctor

Therapeutic Drug Monitoring with LCMS-8040 in Moscow Davydovsky Hospital

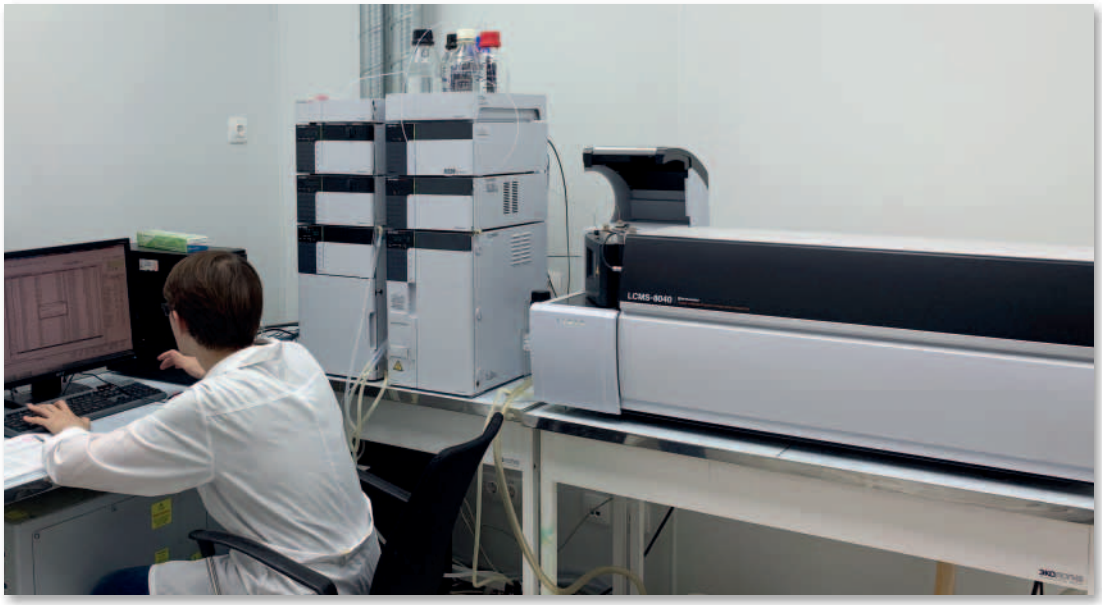


Figure 1: Shimadzu LCMS-8040 installed in the Pharmacokinetics Laboratory at the Moscow Hospital named after I. Davydovsky

The efficient treatment of diseases matters a lot in evidence-based and personalized medicine. One powerful tool is Therapeutic Drug Monitoring (TDM), determining the concentrations of medical substances and/or their metabolites in patients' blood (rarely in urine). Medication dose and frequent adjustment may be done based on the data obtained. Results collected during pharmacokinetic studies indicate that it is impossible to prescribe many medicines according to a "general" template. Individual needs of each patient must be taken into account (figure 2).

TDM mandatory for a wide range of drugs

According to GCP (Good Clinical Practice) regulation, use of a number of medicines has to be accompanied by pharmacokinetic monitoring, especially for drugs with a narrow therapeutic window such as immunosuppressants, anticonvulsants, some antibiotics, antiarrhythmic drugs or cytostatics. Currently, it is prohibited in most European countries and the USA to prescribe some potent drugs (psychotropic, psychostimulants, antidepressants etc.) and long-term drugs (antihypertensive, cardiac glycosides) without monitoring their concentration in the patient's body. According to the Russian Ministry of Health regulation "About improving the clinical pharmacologist's activity" (# 494 on 10/22/2003), it is necessary to perform TDM for a wide range of drugs: anticonvulsants, antiarrhythmic drugs, antibiotics, immunosuppressants, etc.

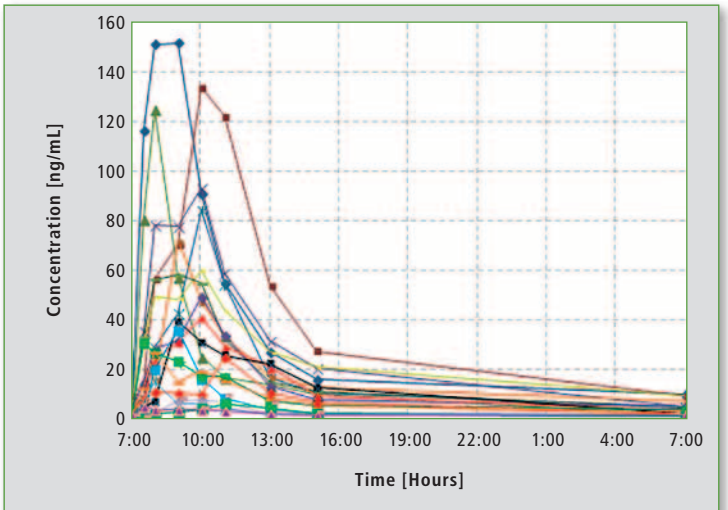


Figure 2: Individual pharmacokinetic curves for daily monitoring of drugs in the blood serum of patients taking the same anti-tumor drug in the same dose at the same time. It shows how significant the differences in pharmacokinetics are among people of the same population.

LC-MS is the method of choice for TDM

The importance of TDM for practical healthcare can be illustrated by routine day-to-day work of the Pharmacokinetics Laboratory located at the Moscow Hospital named after Ippolit Davydovsky where Shimadzu's high-performance liquid mass spectrometer LCMS-8040 was installed in 2013 (figure 1). This triple quadrupole is intended for high-sensitivity and ultra-fast analysis.

Lately, some methods for TDM of several groups of drugs (anticoagulants, anticonvulsants, antihypertensive, hypoglycemic, anti-tumor) were developed in the laboratory (table 1) and many of them have been applied successfully in clinical practice.

TDM also suitable for »simple« drugs?

As mentioned above, TDM is essential for drugs with a narrow therapeutic window, e.g. for carbamazepine. However, the practical work demonstrated that TDM might also be useful for "simple"

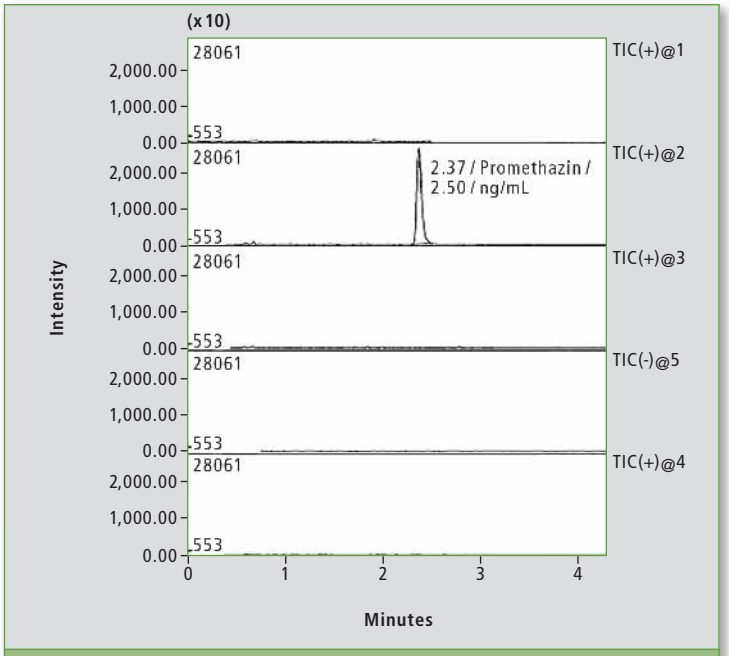


Figure 3: Chromatogram of blood sample of patient with low commitment to treatment by anticoagulants. Only the peak corresponding to the internal standard is present on the chromatogram.

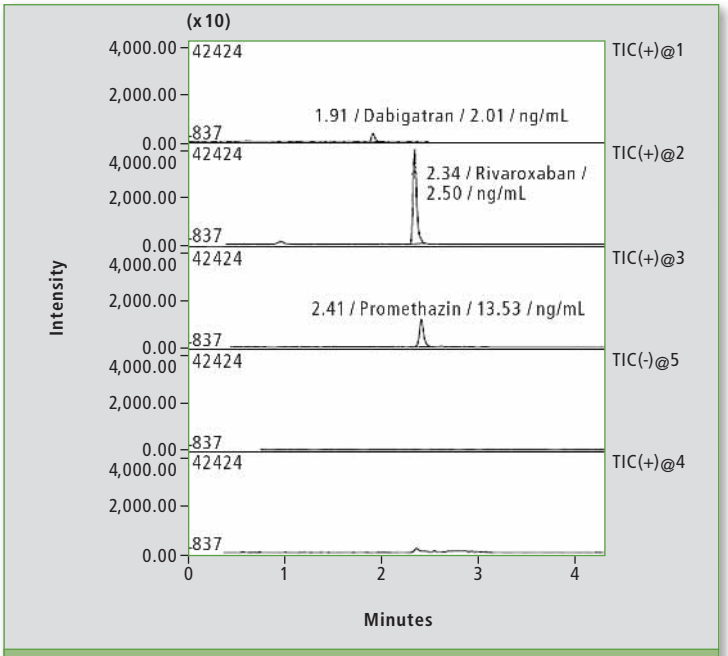


Figure 4: Chromatogram of a blood sample of patient »L« by substituting of dabigatran with rivaroxaban (before taking the drug)

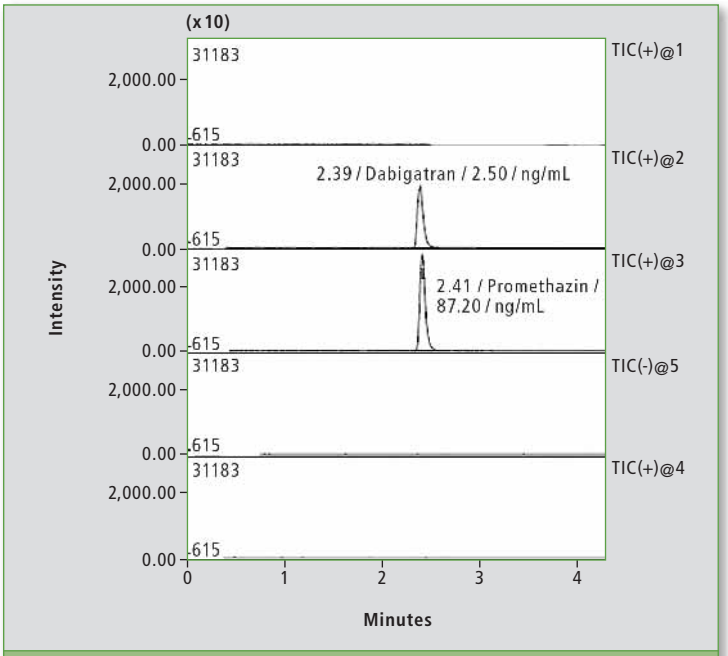


Figure 5: Chromatogram of a blood sample of patient »L« by substituting of dabigatran with rivaroxaban (4 hours after taking the drug)

medicines. For example, enalapril, a well known and widely used drug, is rarely used for monotherapy.

Often, patients take several antihypertensive drugs, and usually need to take additional medicines to relieve side effects. In such cases, it is difficult based on clinical signs only to determine whether side effects/treatment inefficiency are related to the drugs monitored, or induced by other factors.

Enalapril is a prodrug with a weak antihypertensive effect. Its active metabolite, enalaprilat, is formed due to hydrolysis by carboxylesterase. So, the intensity and duration of the hypotensive effect caused by enalapril are determined mostly by the rate of hydrolysis to enalaprilat. This rate may vary significantly from patient to patient. Hypotension and its complications are the highest risks during treatment with enalapril.

TDM helps make the right decisions

With correct dosage, concentration of enalaprilat in blood plasma should remain within the therapeutic window of 10 - 50 ng/mL, and the clinical effect persists up to 24 hours after taking the drug. However, in some cases it is difficult to achieve a stable decrease in blood pressure to the required level as individual patient metabolism (patient phenotype) is not taken into account when prescribing enalapril.

Patient metabolic activity relating to enalapril can be estimated by calculating the enalapril/enalaprilat concentration ratio in blood serum. The absolute value of enalapril concentration in serum is determined in parallel and compared with average (typical) values. The information obtained by TDM helps doctors to make the right decisions concerning dose/frequency adjustment, cancellation or replacement of the drug if necessary.

Patient G: lower risk of hypotension

The algorithm of choosing an individual enalapril therapy is illustrated by the following example. Male patient "G", previously prescribed with 10 mg of enalapril twice daily, was taken to hospital. This prescription achieved the desired therapeutic effect, but TDM showed that the concentration of the active metabolite had increased in the patient's blood.

Medicinal dose was reduced twice in the following days and each time the concentrations of ➧

No.	Analyte	Sample	Sample preparation	Concentration range, ng/mL	Column	Mobile phase	Ionization & MS mode	Reference
1	Enalapril Enalaprilat	Blood serum	Protein precipitation by 50 % of TFA	5 - 250	Phenomenex Synergi Polar-RP 50 x 4.6, 4 µm	A: 1 % FA / water B: 1 % FA / ACN Gradient elution	ESI+, MRM	1
2	Metoprolol Nifedipine		Protein precipitation by ACN	5 - 250				2.3
	Dihydronifedipine Bisoprolol			1 - 250				
3	Carbamazepine			50 - 20,000				4
	Carbamazepine-10, 11-epoxide			5 - 2,000				
4	Dabigatran Rivaroxaban Apixaban		Protein precipitation by MeOH followed by water dilution	1 - 1,000	Waters XBridge C18 50 x 4.6, 3.5 µm	A: 0,1 % FA / water B: 0,1 % FA / ACN		5.6
	Warfarin			10 - 2,000			ESI+, MRM	
5	Losartan Losartan carboxylate Glibenclamide	Blood serum, urine	Protein precipitation by ACN	5 - 1,000			ESI+, MRM	*)
6	Newly developed anti-tumor drug	Blood serum		0.5 - 200	Phenomenex Luna C18(2) 50 x 4.6, 5 µm			**)

Table 1: Electrospray, positive mode, ESI-: Electrospray, negative mode, MRM: Multiple reaction monitoring, ACN: Acetonitrile, MeOH: methanol, FA: formic acid, *): article in the press, **): research in progress

enalapril and its metabolite were determined in blood. Measured concentrations turned to normal values, while the therapeutic effect remained the same as by initial prescription. TDM thus helped significantly to reduce the risk of hypotension.

It is extremely difficult in practice to control how faithfully a patient follows doctor prescriptions. Often, treatment failure is not caused by poor drug quality or individual metabolism, but by weak patient commitment to treatment. This is not only a local Russian problem, but a general global problem.

This phenomenon can even be observed in hospitals. It is impossible to permanently monitor whether each patient took the required drug. TDM with mass spectrometer helps to fight this problem clearly and effectively. While a patient cannot be convicted of neglecting their own health, a chromatogram without peak of the drug even though the patient should have taken it, is strong evidence for doctors and gives the

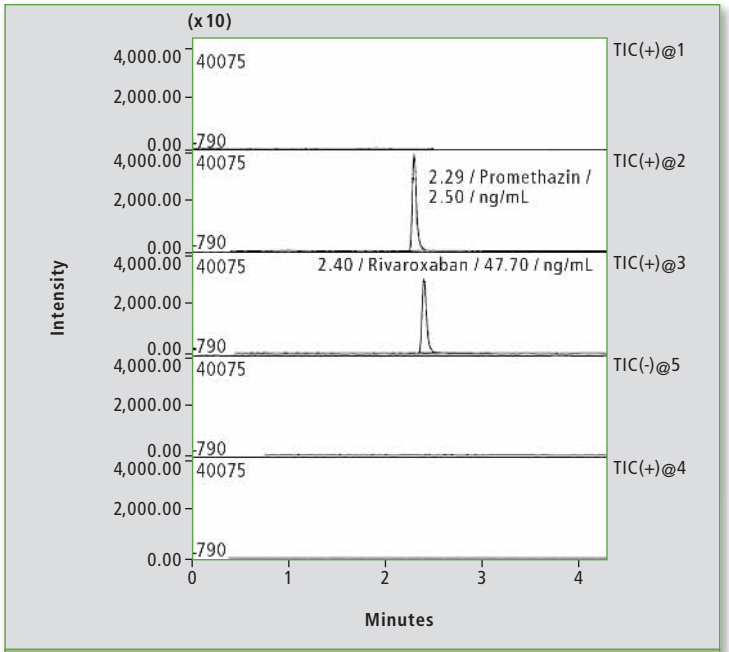


Figure 6: Chromatogram of blood sample of patient »Z« on the first day of the study of background of bleeding

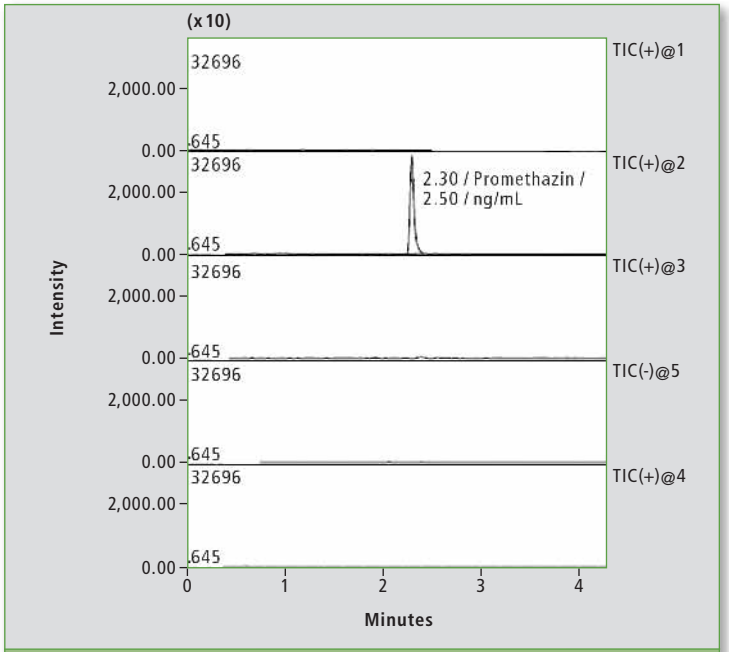


Figure 7: Chromatogram of blood sample of patient »Z« after adjustment of rivaroxaban dosage (before taking the drug)

opportunity to take appropriate action.

Problem: low commitment to treatment

TDM of patients hospitalized for ischemic stroke and prescribed with anticoagulation therapy determined that about 40 % of acute conditions were caused by low commitment to treatment. The new oral anticoagulants and warfarin were either not found in serum samples of the patients or their concentrations barely exceeded LoQ (limit of quantitation). In other cases (about 6 % of examined patients), measured concentrations of oral anticoagulants exceed the average maximum values significantly. The dose for this group of patients was adjusted to eliminate any adverse reactions.

Patient L: avoiding adverse reaction

An important task of TDM is to control forced substitution of one anticoagulant for another, as in the case of male patient "L" diagnosed with chronic kidney disease (figures 4 and 5, page 13). The most common case is a substitute of warfarin for safer anticoagulant. A decrease of warfarin concentration with this replacement is slow (T1/2 of warfarin is about 40 hours), so careful adjustment of drug dosage is required to avoid adverse reaction due to synergism of both medicines. The number of patients who underwent anticoagulant substitution was approximately 10 % of the total number of patients monitored using anticoagulants TDM.

Patient Z: adjusted dose stopped bleeding

An interesting case was observed for the female patient "Z", taking rivaroxaban 15 mg twice daily to treat bilateral thromboembolism of the pulmonary artery. The patient suffered metrorrhagia against the background of simultaneous drug treatment and menstruation.

The first blood analysis was done about 19 hours after the last medication and rivaroxaban concentration was 47.7 ng/mL, a high level considering the time that had elapsed since the last medication (figure 6).

A pharmacokinetic study was repeated two days later (the patient did not take the drug during this period), and rivaroxaban dosage was subsequently adjusted to 20 mg per day. Only traces of rivaroxaban were measured in serum before taking 20 mg of medicine (figure 7), whereas a concentration of 178.9 ng/mL was determined in the sample taken four hours after first medication with 20 mg (figure 8). The last concentration may be considered as a normal level. Adjustment of the rivaroxaban dose led to the patient no longer suffering from bleeding.

Day	Dosage	Sample No. 1 (before taking the drug)		Sample No. 2 (24 hours after taking the drug)	
		Enalapril ng/mL	Enalaprilat ng/mL	Enalapril ng/mL	Enalaprilat ng/mL
1	10 mg 2 times daily	Below LoQ*)	44.32	5.25	131.09
4	7.5 mg 2 times daily	Below LoQ	38.75	Below LoQ	72.68
8	5 mg 2 times daily	Below LoQ	33.55	Below LoQ	34.65

Table 2: Choice of therapeutic enalapril dosage for patient »G«
*) LoQ = Limit of Quantification

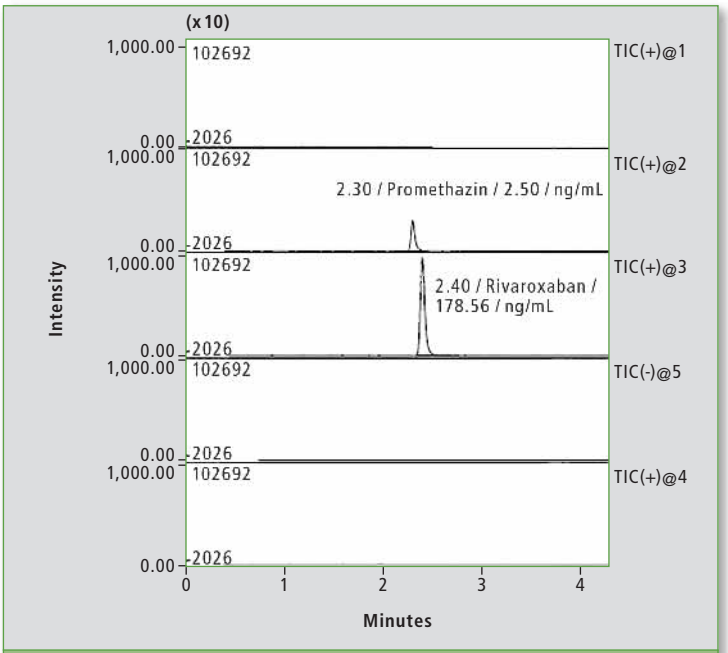
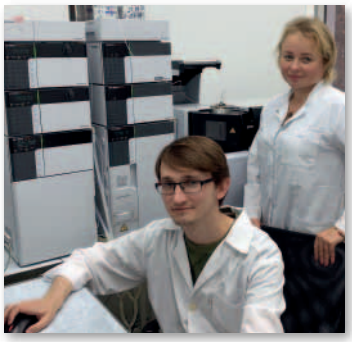


Figure 8: Chromatogram of blood sample of patient »Z« after adjustment of rivaroxaban dosage (4 hours after taking the drug)



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360° drinking water analysis: Episode 1

Qualified water – Contaminants and continuous monitoring

Although two thirds of the Earth's surface are covered by water, only 2,5 % is fresh water with low concentrations of salt. An even smaller portion is suitable for drinking, and only then when purified before consumption. Specific chemical and physical properties make the difference between potable and undrinkable water. Groundwater and surface water from lakes and rivers, so called raw water, is the most readily available resource for drinking water production. But there's a long way to go before the water reaches the glass.

For example, ground water from great depths has a higher content of minerals and carbon dioxide than water from layers closer to the surface, while having a smaller amount of oxygen. To enrich ground water with oxygen, air is blown through the water while at the same time CO_2 escapes from it. Iron and manganese ions are flocculated using pH-sensitive processes. The emerging flakes and other suspended particles affecting color and turbidity are then filtered, often with simple but effective gravel- and sand filter systems with a subsequent active charcoal stage.

Nitrate concentration in the ground water may be elevated through excessive use of fertilizers in agricultural regions. Several methods of denitrification are available, most commonly reverse osmosis or ion exchange processes. Finally, the treated water has to be disinfected to remove waterborne pathogens. Chlorination and treatment with ozone are common means to reduce the microbial risk before drinking water is supplied to the consumer.

Drinking water quality defined

The treatment processes mentioned above are just a few of many examples. And with good reason: The chemical composition of raw water depends on many locational factors such as vegetation, geological properties, surrounding production industries, mining operations and the seasons.

The most suitable treatments are determined by the raw water characteristics, but they need to be applied in a well-balanced way. In chlorination for example, high amounts of chlorine reduce the microbial risk and vice versa, but overdosed water becomes undrinkable. To ensure effective treatment and quality of drinking water, it is essential to monitor each purification step. Together with well-defined quality parameters, this makes water ready for distribution and human consumption.

As one of many quality frameworks world-wide, the European Drinking Water Directive 98/83/EC defines permissible limits for a wide range of parameters such as microbial, chemical and indicators which must be analyzed regularly, for instance aluminium, arsenic, boron, cadmium, chromium, copper, iron, mercury, manganese, nickel, lead, selenium and antimony. For the simultaneous quantitative determination of these elements in raw and drinking water, inductively coupled plasma mass spectrometry (ICP-MS) is the preferred quality control tool. ICP-MS offers a high sensitivity (trace element detection) and wide dynamic range.

Determination of chemical contaminants using ICP-MS

Shimadzu's ICPMS-2030 is one of the smallest ICPMS platforms available today. Many features of this fast and easy-to-use instrument offer peace-of-mind to both operators of the system and laboratory managers with a strict budget to manage.

The ICPMS-2030 is designed for high stability, excellent sensitivity and low interference levels: The optimized internal structure including the newly-developed octupole collision cell enables analysis with excellent sensitivity and minimum spectral interference. The sample injection unit and interface where ionized atoms pass through are easy to remove and maintain.

Shimadzu's proprietary Development Assistant as well as Diagnosis Assistant functions reduce users' burden and improve reliability of data by setting automatically the most suitable analysis conditions and performing checks for spectral interferences. These functions reduce the amount of work significantly in checking data and improving reliability of measurement results.

Low running costs

The ICPMS-2030 can achieve extremely low running costs due to reduced argon gas flow rate (mini torch) which can be minimized even more by applying the "eco mode" during standby, also cutting RF-power output and stopping all aspiration. In addition, the use of 99.95 % pure argon gas is possible. Analysis can therefore be performed with reasonably priced technical argon gas for industrial purposes instead of the expensive 99.999 % high purity argon gas generally used.

To demonstrate the capability for high sample throughput, the long-term stability of the instrument over a typical session was assessed by analyzing a series of known samples repeatedly to ensure minimal deviation of signal intensities over time. The ICPMS-2030 demonstrates stable signals over a 10-hour analytical period, indicating that the instrument will provide robust results in high-throughput laboratories.

Monitoring provides confidence

During water treatment, other problems can be present although not actively searched for. To highlight such problems, the so-called indicator parameters are used. One of these parameters is TOC (Total Organic Carbon), for which no limit value or criterion has been defined, but which can be considered as a cautionary warning for action under unusual circumstances.

Another indicator is the oxidizability parameter, as a measure of the sum of all chemically oxidizable organically bound compounds present in water.

Figure 1: ICPMS-2030

Water is the basis for a healthy life. It plays a crucial role in almost all metabolic processes, as well as in geological and ecological elementary processes. The human body consists of over 70 % water, as does the Earth's surface.

Sophisticated, reliable water purification and supply systems are a prerequisite for human health and the prevention of epidemics worldwide. Every day, a large variety of chemical substances pose a high risk of contaminating our drinking water, potentially causing disease and chronical illness.

This is why rigid controls and continuous monitoring according to strict guidelines, including the European Directive 98/83/EC and WHO Guidelines for Drinking Water Quality, make drinking water the best monitored food in the world.

This and the next issue of Shimadzu NEWS will highlight how drinking water as a basis for life is controlled to guarantee and qualify its quality.

While not a direct cause for concern, it can lead to re-germination or undesirable disinfection by-products. Oxidizability is proportional to the sum of organically bound carbon that are determined as DOC (Dissolved Organic Carbon) and can therefore be replaced by TOC measurement.

When examining carbon compounds in drinking water, it is apparent that the amount of inorganic carbon (IC) such as carbonates and hydrogen carbonates is much higher than the organic fraction which is only 1 % of total carbon (TC).

A TOC determination via the differential method (TOC = TC - IC) will not be appropriate in this case, as the TOC value calculated is prone to large statistical errors. According to EN 1484 (instruction for the determination of total organic carbon and dissolved organic carbon), the differential method can only be applied when the TIC (Total Inorganic Carbon value) is lower than the TOC value.

Identification of organic carbon in water

For drinking water analysis, the NPOC (Non-Purgeable Organic Carbon) method is used. The drinking water sample is first acidified to a pH value of 2. This way, the carbonates and hydrogen carbonates are transformed into carbon dioxide. The CO_2 is then removed via sparging with carrier gas. The amount of volatile and therefore purgeable organic carbon can be disregarded in drinking water. What remains is a solution of non-volatile organic carbon compounds. These can be oxidized to CO_2 and detected via NDIR.

Sample preparation for the NPOC method (acidification and sparging) is done automatically in the TOC-L analyzer, a series of instruments used in laboratory applications. Removing the TIC can be performed either in the syringe of the ISP module or in the autosampler with the external spare kit.

The ISP (Integrated Sample Preparation) module consists of an 8-port valve and a syringe with sparging gas connection. In addition to acidification and sparging in the syringe, the ISP also enables automatic dilution. This feature facilitates an extended measuring range, dilution of highly contaminated samples and the preparation of a series of calibration samples from a stock solution. The robust ISP module therefore considerably reduces time-consuming sample handling steps.

Figure 2: TOC-L analyzer

Conclusion

Shimadzu's ICPMS-2030 and TOC-L analyzers meet all analytical requirements for effective drinking water control with top performance and easy-to-use concepts, while providing an economical solution for the modern laboratory.

Furthermore, Shimadzu provides solutions for other parameters such as pesticides (GC-MS/MS, LC-MS/MS), PAHs (HPLC), trace pharmaceuticals (LC-MS/MS), various chemical parameters by UV-spectrophotometers and much more.

One of those methods will be discussed in Episode 2 of the drinking water trilogy in Shimadzu NEWS 2/2020.

Is it authentic cashmere?

Differentiation and quantification of cashmere with MALDI-MS

Whether sweater, hat or scarf – warm clothing is essential in winter. The shops offer a wide range of garments made of different materials. One luxury fabric is cashmere, coming mainly from China. The fine fiber from the under-fleece of the goat comes from the Kashmir region, situated between India, Pakistan and China.

Unlike sheep's wool, cashmere hair is softer, smoother and one of the most expensive and valuable natural fibers. But in times of counterfeit products, how can vendors and customers be sure that cashmere is actually contained in the textile and not just written on the label?

Conventionally, evaluating the authenticity of cashmere hair

Figure 1: The linear MALDI-8020 shows a great performance with a minimum footprint. Typical applications for the MALDI-8020 are the analysis of proteins/peptides, lipids or hydrocarbons in life science or quality control of polymers.

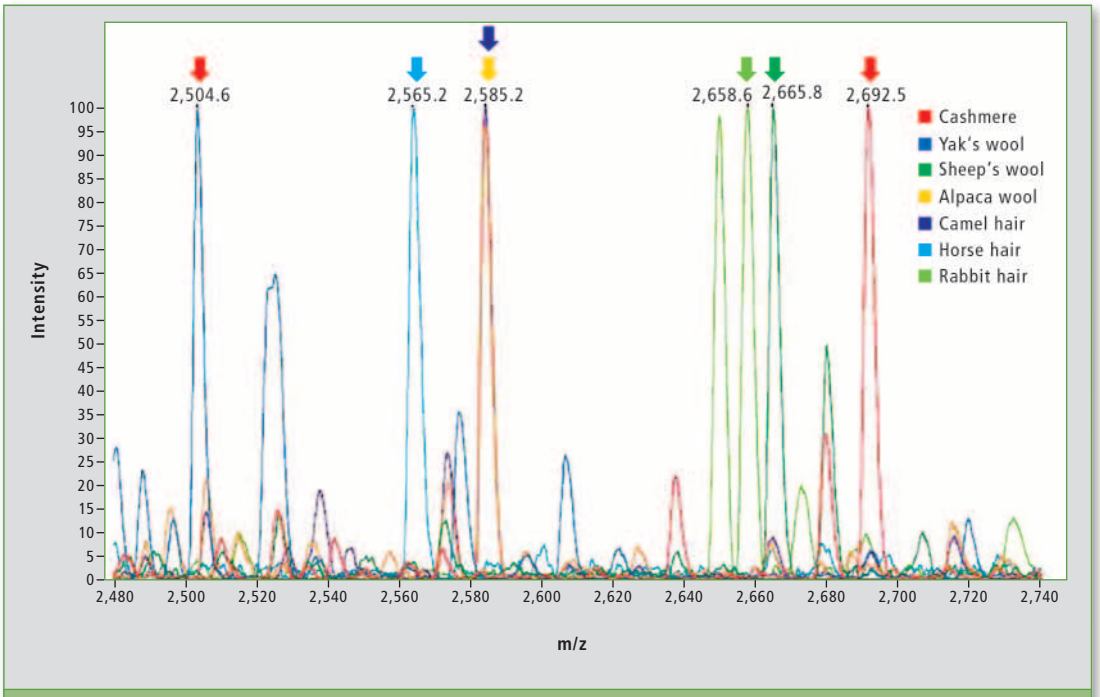


Figure 2: Overlapped MALDI mass spectra of different animal fibers. The mass range around m/z 2,600 shows peaks of tryptic digest products that are specific for different organisms (table 1).

has been investigated by an optical or electron microscope. Recently, the International Organization for Standardization approved a new Matrix-assisted laser desorption/ionization-based method to analyze animal fibers (ISO 20418-2: 2018). This method also allows for quantification to confirm certain mixtures. A single hair is sufficient to determine its origin. In addition, this method also provides potential for the analysis of contaminated food to identify the cause of the contamination.

Shimadzu established this method with its MALDI-8020 Benchtop-mass-spectrometer (figure 1). Due to its compact and affordable design it is the first-choice instrument for such analyses. Fast sample stage and short pump time guarantee a high-speed analysis.

Classical protein sample preparation

Hair fibers were broken up with a scissor or ball mill, denatured with SDS (sodium dodecyl sulfate), disulphide bonds reduced with DTT (dithiothreitol) and alkylated with iodoacetamide. After an optional separation on an SDS-Page, tryptical digest and desalting using a ZipTip, the sample was mixed with matrix and spotted on the target. The following MALDI analysis shows a characteristic peak around m/z of 2,600 (figure 2).

This signal refers to the molecular weight of a digest product of the Keratin Type I protein. Keratin is the predominant protein in hair

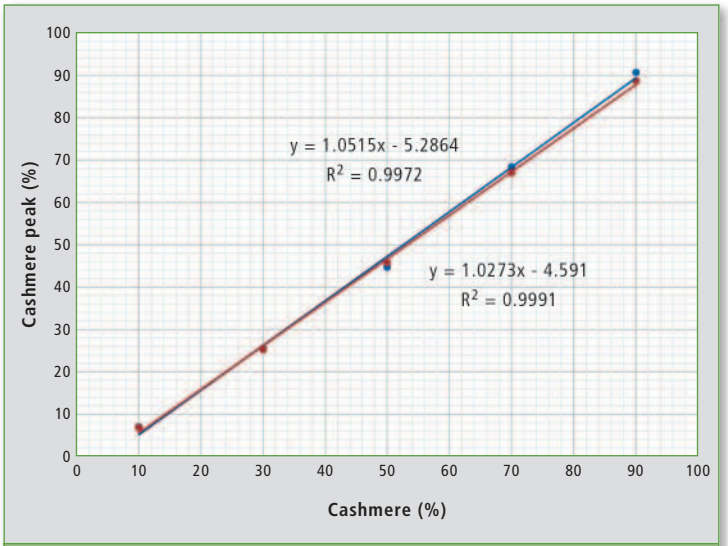


Figure 4: Calibration curve for relative quantitation of cashmere-sheep wool mixtures

fiber and thus shows the highest intensity in the mass spectrum in that mass range. So, it is easy to assign the right peak. As shown in table 1, this signal is specific for different organisms. Only camel and alpaca cannot be differentiated with this method due to their close affinity.

Relative quantitation allows for identification of mixed fabrics

The same method also allows for the identification of mixtures. Figure 3 exemplarily shows mass spectra of cashmere-sheep-wool-mixtures. The different intensities of the specific peaks for cashmere (m/z 2,691) and sheep wool (m/z 2,664) represent the ratios of the different hair fibers in the mixtures.

With these data, it becomes possible to generate a calibration (figure 4). The two specific peaks are evaluated and the portion of the cashmere signal is calculated from the sum of these two signals. The graph shows a good linear correlation.

Conclusion

With the MALDI-MS method, the specification on the label can be checked for its accuracy. It offers qualitative and quantitative analyses. This way, it is possible to verify the presence of cashmere as well as its proportion in the mixed fabric. The technical equipment ensures a high-speed analysis.

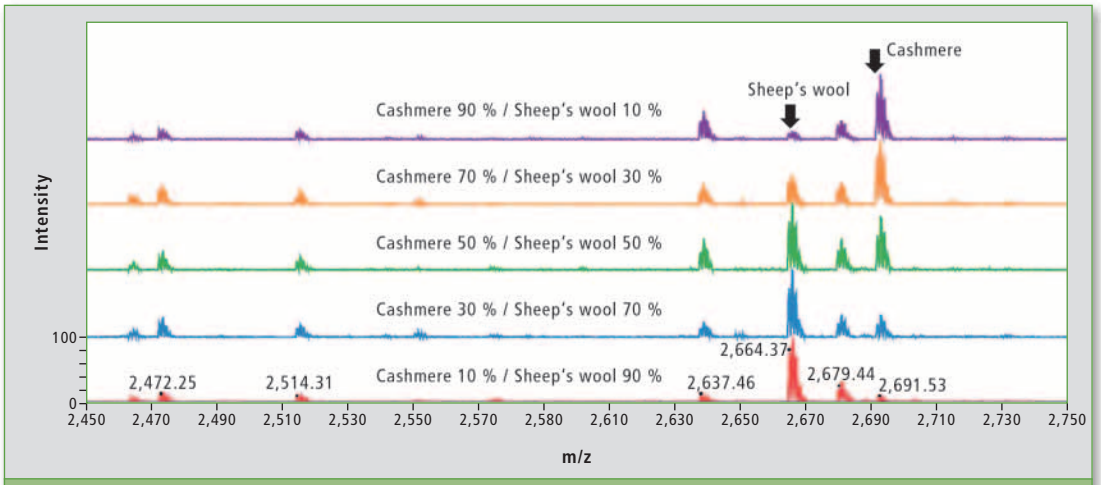


Figure 3: Relative quantitation of cashmere-sheep wool mixtures with different ratios

Species of Organism	Keratin Type I Protein	Amino Acid Sequence	$[M + H]^+$ Average Mass*1
Cashmere	Keratin 33A [Capra hircus]	YSCQLNQVQSLIVNVESQLAEIR	2,692.38
Yak	Keratin type I microfibrillar, 47.6 kDa-like [Bos mutus]	YSSQLAQVQGLIGNVESQLAEIR	2,504.81
Sheep	Keratin 33B [Ovis aries]	YSCQLSQVQSLIVNVESQLAEIR	2,666.03
Camel/Alpaca	Keratin, type I microfibrillar, 47.6 kDa [Camelus dromedaries]	YGSQLSQVQGLITNVEHQLAEIR	2,584.90
Horse	Keratin 33A [Equus caballus]	YSSQLSQVQGLITNVESQLAEIR	2,564.86
Rabbit	Keratin, type I cuticular Ha3-I [Oryctolagus cuniculus]	YSSQLSQVQCMISNVESQLGEIR	2,657.99
Dog	Keratin 33A [Canis lupus familiaris]	YSSQLNQVQCMITNVESQLAEIR	2,713.07
Brown rat	Keratin 31 [Rattus norvegicus]	YSSQLSQVQCLITNVESQLGEIR	2,653.98
Human	KRT34 protein [Homo sapiens]	YSSQLSQVQSLITNVESQLAEIR	2,594.89

Table 1: Specific amino acid sequences of different organisms. *1 The average mass of cysteine residues is calculated after their carbamidomethylation by iodoacetamide.

Further information on this article:
- Application news: LAAN-A-TM-E065 and LAAN-A-TM-E073

Science and Shakespeare

Experimentation outside the lab: near-infrared light to measure cortical activity

Brain imaging during social interactions has classically required participants to remain still in a tightly controlled experimental laboratory. Using new technology by Shimadzu, researchers at the University College London show these experiments can now be done outside the lab, for example, on the stage.

It was only a few days away from the first performance in front of a live audience. During rehearsal, Antonia Hamilton, Professor at the University College London (UCL) Institute of Cognitive Neuroscience, was watching actors rehearse a Shakespeare play. Neuroscientists tend to prefer books with titles like "Neuron" and "Cognition" over "Hamlet" or "A Midsummer Night's Dream", but Hamilton believes studying these actors could give new understanding about human social cognition and how social interactions might be different for individuals with autism.

Autism spectrum condition (ASC) is a neurodevelopmental disorder which impacts social and communication skills. In the United States, one in 68 children is now diagnosed with ASC [1], but there remains a pressing need to improve diagnoses and find effective ways to help these children learn social skills.

Dr. Ilias Tachtsidis and Prof. Antonia Hamilton

The idea to use actors to study ASC came to Hamilton when she was approached five years ago by Kelly Hunter, a director and actor who has been incorporating performance as a therapy for autistics. Hamilton went to see Hunter's work and came away thinking there was a scientific opportunity, especially when considering her access to Shimadzu's functional near infrared spectroscopy (fNIRS) equipment. fNIRS is a non-invasive technique that uses infrared light to measure cortical activity, which may be different in the autistic brain.

»An actor is professionally trained to produce the same interaction repeatedly. This means that we can measure the brain activity patterns of this interaction over many repeats to get a valid brain signal,« says Hamilton.

After watching the work done by Hunter, Hamilton concluded, **»I saw it would make a good experimental stimulus that we could combine with brain imaging. When I had access to fNIRS from Shimadzu and Ilias, I thought, 'Let's put this together.'«**

fNIRS and blood oxygenation

"Ilias" is Dr. Ilias Tachtsidis, Associate Professor at the UCL Department of Medical Physics and Biomedical Engineering and a Wellcome Trust Senior Fellow who is developing fNIRS for clinical purposes. Before applying fNIRS to ASC, Tachtsidis had shown its advantages in neonatology units, where doctors can quickly assess the condition of an unresponsive baby [2]. In these cases, one of the most pressing conclusions that needs to be made is whether the brain is receiving an adequate supply of oxygen.

»The official term is 'hypoxic ischemic episode'; often referred to as "birth asphyxia." Babies that had a severe birth asphyxia episode will have a severe brain injury and probably have serious neurodevelopmental issues later in life,« says Tachtsidis.

»There will be major cues for the medical staff to suspect this. The baby is blue. The baby does not breathe well. The baby is unresponsive. But the doctors do not have any direct brain measurement.«

»Make-up« at UCL (University College London)

Near infrared light can penetrate just deep enough into the brain to measure events about 20 mm from the scalp. Because oxygenated and deoxygenated hemoglobin have different colours, measurements of the light absorption in the cortex can give a quantitative analysis of hemoglobin. Thus, fNIRS is a powerful research tool that can be used to identify brain regions that are unexpectedly inactive, and this information may be helpful towards research and development of new therapeutic strategies, be it for a hypoxic ischemic episode in babies, or, in the new project, for children with ASC.

Several studies have shown the usefulness of neuroimaging to quantitatively measure differences in cognition in ASC, and attempts are underway to use this to improve the sensitivity of behavioural analysis. However, many of the imaging modalities, such as functional magnetic resonance imaging (fMRI), which provides arguably the most detailed images of the brain, require observation of the subject in less than ideal conditions.

»You have to take people down to the basement, put them in a scanner, and they have to stay still. It's a very artificial environment. Not every patient reacts well to fMRI because not every patient can be put in the MRI scanner,« says Tachtsidis.

On the other hand, instruments like Shimadzu's fNIRS are portable, allowing measurements to be taken out of the laboratory while simultaneously monitoring other vitals such as blood pressure, heart rate and body temperature.

In the newest iteration, Shimadzu's LIGHTNIRS*, the portability goes one step further, enabling scientists to observe people in regular social interactions.

Unprecedented portability

Tachtsidis says that LIGHTNIRS is one of the most advanced commercial instruments for research use in the field of rehabilitation and robotics.

"The instrument is quite novel. It uses three wavelengths of light. When you want to resolve two molecules, you need at least two wavelengths. The more wavelengths you have, the better quantification," he says.

Curtain up! »Backpack« and »swimming cap« ready for use

When considering his experience with Shimadzu and the fNIRS industry, these added features do not surprise him.

»In our community, we know Shimadzu. It was one of the first companies to commercialize this technology. That tells us how innovation is applied in this company. I could see that the company took early on high risk,« he says.

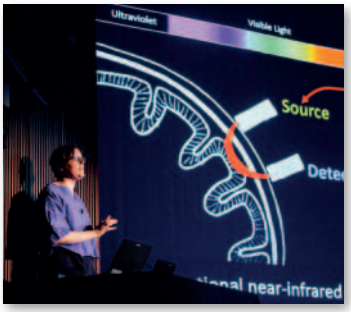
LIGHTNIRS consists of headwear that resembles a black swimming cap and a backpack no bigger than those worn by long distance runners [3]. The headwear is flexible so that it fits all sizes, from children to adults, and the probes can be screwed in at different positions. These features allow fNIRS experiments to be done outside of the lab, including on the stage. To make the data acquired from Hamilton's project worthwhile, it is essential that the experiments are reproducible. Tachtsidis and Shimadzu have adjusted the data collection and analysis software to assure Hamilton that the data are as reliable as if she were doing the experiments in her laboratory.

»We need the interactions to be reproducible. We have our actors play the same scene 8 - 10 times, and that gives us enough signal to see the pattern of brain activity,« she says.

Because actors are extraordinary in their ability to repeat an action, Hamilton hopes to build a database of brain behaviour during specific social interactions that can then be used as a basis to identify brain abnormalities in people with social deficiencies.

Hamilton acknowledges her approach is unique. **»As far as I know, nobody in the world has collected brain imaging data in front of an audience,«** she says. Nevertheless, the main purpose of the project remains clear.

»By observing how the brain behaves in certain social interactions, we want to find behavioural therapies that can make autistic children more comfortable when interacting with people.«



Prof. Hamilton explains fNIRS to the audience

* LIGHTNIRS is Portable functional Near-Infrared Spectroscopy System for Research https://www.shimadzu.com/an/lifescience/imaging/nirs/light_top.html

• The information including affiliates and titles of the persons in this article are current as of the time of interviewing (May 2019).

Literature

[1] Baio, J. et al. Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years – Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2014. MMWR Surveill Summ 67, 1-23, doi:10.15585/mmwr.ss6706a1 (2018).

[2] Bale, G. et al. Oxygen dependency of mitochondrial metabolism indicates outcome of newborn brain injury. J Cereb

Actors in the service of science

Learning in a new sparkle

Social Day 2019: Shimadzu employees renew outdoor area of a special needs school

6th Social Day. In September 2019, 25 volunteers from all over the company met for a day of social commitment in their local neighborhood area in Duisburg, Germany. Supported by the municipality, Shimadzu employees helped to renovate the "Am Rönsbergshof" special needs school.

»It was a great pleasure to support the school with its learning concept on 'support through plants and animals.' We were building a garden house and renovated the outside area for the animals,« explained Jürgen Semmler, Managing Director of Shimadzu Germany.

Learning skills for an independent life

The full-time school, which specializes in the mental development of its pupils, offers various teaching and support options. In small classes, children and young people learn versatile skills to actively shape their future everyday lives independently. Working with the school's sheep and donkeys as well as in the plant garden contributes to the students' individual support. With at least two teachers per class, students receive the attention they need.

Outside area with a new sparkle

To create a pleasant learning atmosphere, the helpers supported the redesign of the exterior area for new students. Building, paving, weeding and painting were on the agenda. Now, a new garden house to store play and exercise materials as well as a newly paved area is available to the school. The pasture fence of the animal area has also been repaired. In sunny weather, participants of the Social Day removed weeds, giving the school grounds a new sparkle. During their time there, the Shimadzu employees also gained insights into the challenges and versatility of a special needs school.

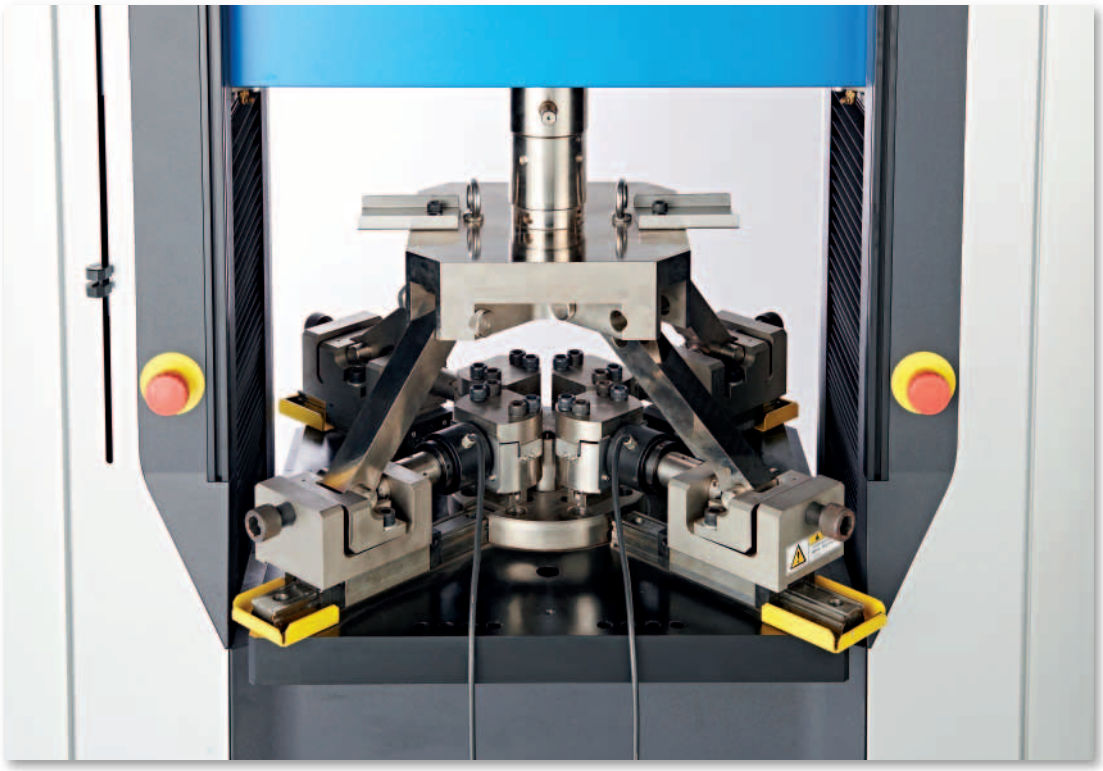
»We were very happy with the commitment of the Shimadzu staff,« said school principal Sirka Justus. **»In the name of all students and teachers, we cordially thank Shimadzu for its support and help. I was also very pleased that the Shimadzu staff are very interested in our work at the school.«**

Joint responsibility

For Shimadzu, it is part of the company's credo to take social responsibility in its neighborhood. Besides the philosophy of "contributing to society through science and technology", it is also important to support the local community and the environment. Shimadzu thanks the 25 employees of the German and European organizations and looks forward to future projects.

Testing machine based on individual needs

New testing machine configurator assembles tailor-made version

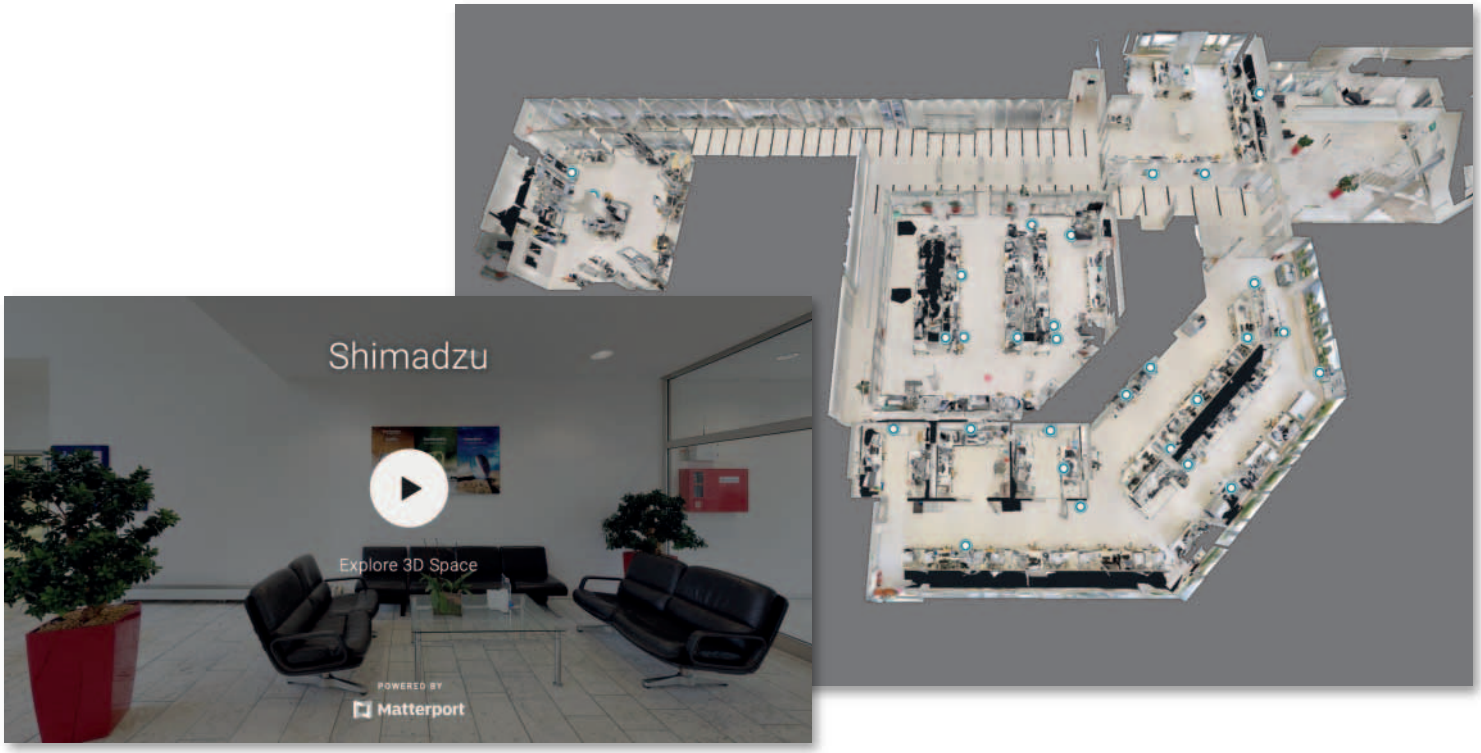


It is known from cars, it is known from muesli, cosmetics, fashion, furniture and more – the individual configuration of products and features serving the buyer's desires and reflecting his or her needs in daily use. Particularly in B2B environments, application-specific products help to improve the efficiency of work stages and processes thereby increasing workplace productivity.

On its website Shimadzu has now released the 'TM configurator', a tool for users to create their own testing machine as required. At https://www.shimadzu.com/an/test/tmc/, users are guided step by step through the configuration menu until completion of an individual version for all required tasks and operations.

At the click of a mouse, users can select from a range of options: the material to be tested, test method, dimensions of the sample, performance of the testing machine, test environment temperature, handles and extensometers. Additionally, a summary of the preferred system with its specifics is saved.

The TM configurator enables getting closer to a tailor-made testing machine as well as trying out of alternatives before discussing details with Shimadzu's product sales & support.



Experience ‘excellence in science’ in three dimensions

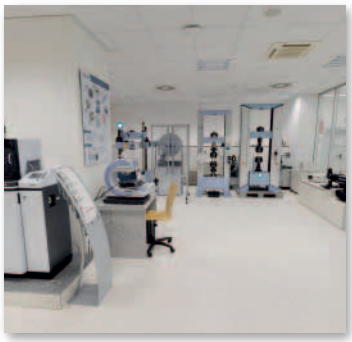
Virtual tour of the Shimadzu Laboratory World

Anyone who hasn’t visited Shimadzu Laboratory World in Duisburg yet can now experience it virtually in the brand-new 3D panorama tour – just enter https://www.shimadzu.eu/virtual-3d-tour. From there, you can head straight to the Laboratory World with over 1,500 m² of real-life testing areas for the entire product range – from chromatography, spectroscopy, sum parameter (TOC) and mass spectrometry to material testing machines.

All laboratories can be accessed conveniently from the lobby with a click of the mouse. Nearly 30 stations invite you to check out instruments, posters and the company. Behind the touchpoints await short videos and detailed views.

The Shimadzu Laboratory World is also used for training, education and demonstration purposes. Furthermore, Shimadzu develops solutions there for the growing challenges of its customers.

Simply scan the QR code and experience the Laboratory World in virtual form.



On a police manhunt with Shimadzu

GCMS assists the investigator duo in Münster at the »Tatort«

Suicide or murder? A GCMS-QP2020 NX helped in solving two mysterious deaths in the most popular television crime series in Germany. In their 36th case, Chief of Police Thiel and forensic scientist Professor Boerne from Münster shifted between the coordinates of the mafia, secret service and jewel smuggling.

Was a Münster globetrotter and diamond smuggler really strangled, as the traces on his neck indicated? And did his sister really commit suicide later? Or were both incidents staged since the victims were previously killed by secret service methods such as injections?

More than 10 million viewers regularly tune in to watch the popular investigator duo from Münster solving cases with wit and humor. They are one of 23 teams that go on police manhunts 35 times per year in various major cities in Germany, Austria and Switzerland – always on Sundays at primetime. The crime scene series has been on air since 1970. Thiel and Boerne have been investigating since 2002.

GCMS – the Scientific Assistant

This time, blood analysis led the investigators on the trail of the perpetrator. The GCMS-QP2020 NX was highlighted for 20 seconds during the program and showed its capabilities. Boerne to Thiel: "Metabolites of a sophisticated pharmacologically modified variant of the active substance ketamine" were present in the bone marrow of the victims. This drug substance for human and veterinary medicine is scientifically a chiral arylcyclohexylamine, used primarily for anesthesia and pain management.

Forensic toxicology and metabolite analysis (metabolomics) are the domains of the latest generation of GCMS-QP2020 NX and GCMS-TQ8040 NX quadrupole and triple quadrupole systems. The youngest member of the product family, the GCMS-TQ8050 NX, was developed specifically for ultra trace analysis and enables femtogram-level analysis (parts per quadrillion). The AOC-6000 automatic sampler as a multifunctional tool supported Prof. Boerne in the analysis, so that the forensic scientist could quickly identify the key findings.

Employees of Shimadzu Germany brought the GCMS-QP2020 NX to the film set in December 2018. At that time, the episode was produced to make it to the screen twelve months later just before Christmas.

During a full day of filming, the employees were able to look behind the scenes of the film production and observe the technical, personnel and organizational efforts required to make just a few minutes of footage. The rule of thumb is that one day of shooting results in 2 - 3 minutes of movie. This also applies to the "Tatort" crime scene series: 25 - 30 days of filming are planned for each episode.

The showdown at the end of the film took place in a mill. Lots of flour and dust, a bluff and then an exchange of gunfire. The perpetrator had no chance and was deservedly arrested.

Shimadzu live

107 Brewing and Engineering Conference
Leipzig, Germany
March 9 - 11, 2020
vlb-berlin.org/frueh2020

ECR
Vienna, Austria
March 11 - 14, 2020
www.myesr.org/congress

Food Integrity
London, Great Britain
March 18 - 19, 2020
foodintegrityevent.com/

Bioprocessing Summit Europe
Barcelona, Spain
March 24 - 26, 2020
bioprocessingeurope.com/

5th Iberian Conference on Structural Integrity
Coimbra, Portugal
March 25 - 27, 2020
ibcsi.pt/

13th Annual Proteins & Antibodies Congress
London, Great Britain
April 27 - 29, 2020
oxfordglobal.co.uk/biologics-series/proteins/

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