

World Premiere: AIM-9000

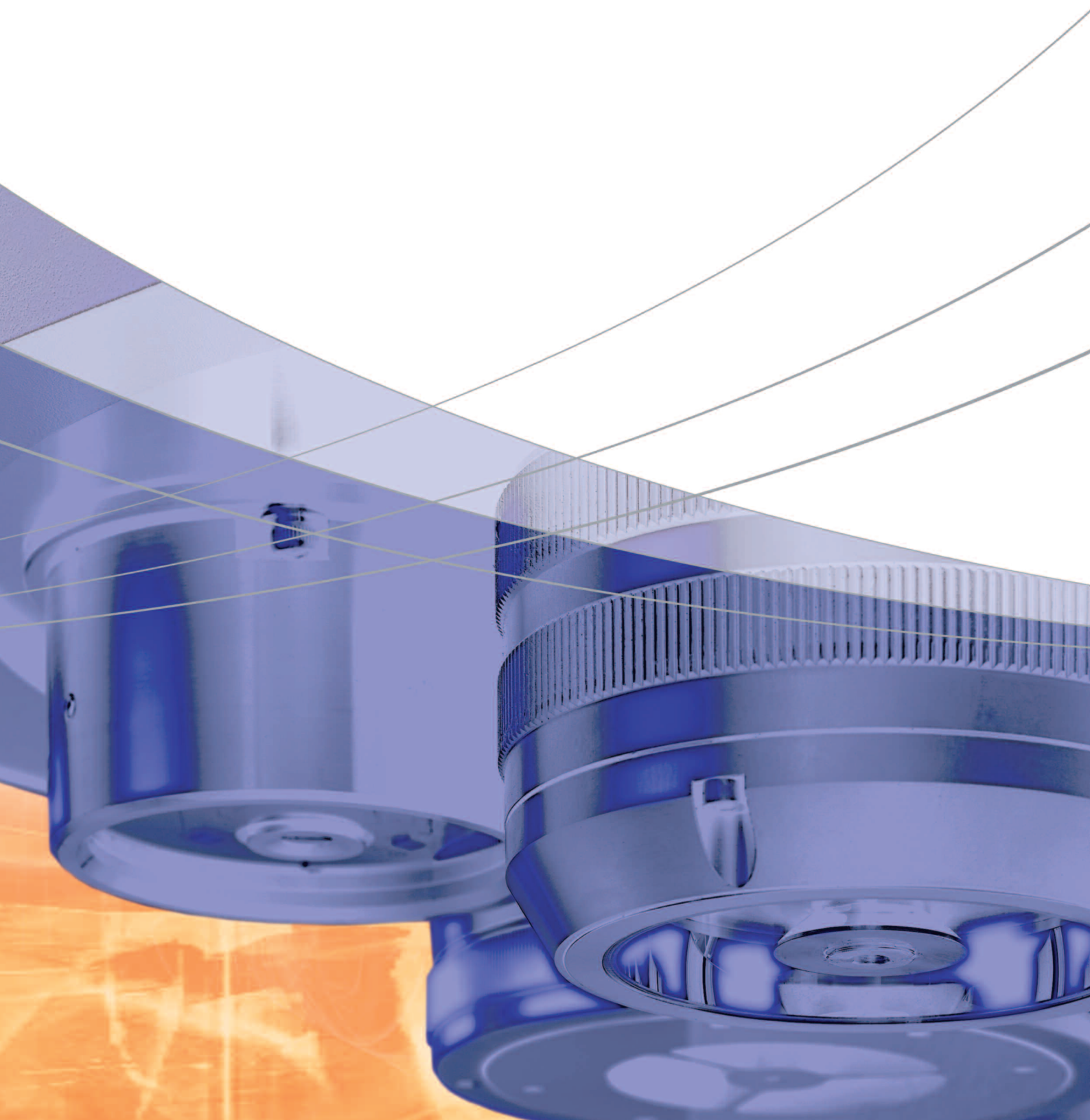
Multipurpose tool for tiny objects

lab4you: Anti-aging for batteries?

LC-MS method development for battery electrolytes

No carry-over: TOC determination

Wastewater treatment plant: high demands on equipment and sampling method





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-  Chemical, Petrochemical, Biofuel and Energy
-  Clinical
-  Environment
-  Food, Beverages, Agriculture
-  Pharmaceutical
-  Plastics and Rubber
-  Automotive

Manipulated steel

Recreating the bi-axial deformation experienced by steel during stamp forming is allowing researchers to engineer the microstructure and yield lighter parts

As the low hanging light-weighting fruits are picked, automotive manufacturers

now have to work that bit harder to shed the pounds. BMW is perhaps one of the more progressive examples of a manufacturer driving composite R&D in a major way. However, it is not simply abandoning metal, and like many others, it is seeking out ways to lightweight vehicles using conventional steel.

This has led to BMW Mini initiating a project between The University of Oxford and Diamond Light Source – the UK's national synchrotron science facility located at the Harwell Science and Innovation Campus in Oxfordshire. The facility harnesses the power of electrons and X-rays to help scientists and engineers gain new insight and understanding into the microscopic and internal structures of materials.

BMW Mini wants answers to a phenomenon that has been witnessed, more broadly, since the 1950s. It is seen when parts are stamp formed, a common automotive process, to make everything from bonnets to doors.

The problem is, for any stamping process that is completed in more than one stage, the deformation becomes highly complex, particularly for a pressed part with strain applied in two-axes. This results in a non-homogeneous arrangement of the crystalline and microscopic defect structures.



Universal testing machine AGS-X

While it may sound arbitrary, the affect can influence grain morphology, crystal orientation and distribution – all of which have significant impact on mechanical properties, including most importantly, how much the material will stretch before it fractures.

Leading the work is Dr David Collins, a researcher at the University of Oxford, who explains the problem. He says: “They can’t stamp form stronger steels at the moment because of this affect. The metals they use on body panels are actually quite weak, say 10 % as strong as the strongest steels on the market. Stronger metals are not ductile and can’t be stamped into the complicated shapes needed.”

It means thicker sections have to be used, so panels end up weighing more. It’s something BMW is keen to understand so it can ultimately reduce the weight of steel chassis.

The problem is complex, meaning testing and analysis is far from straightforward. The deformation is bi-axial, meaning any obvious traditional solution is not suitable. It led to Dr Collins applying to use the synchrotron to carry out tests and shed new light, or X-rays in this case, on the problem. However, he soon faced another problem, the Shimadzu AGS-X 10 kN load frame that was available, was a single axis machine.

Dr Collins decided to do what so many engineers have done before him – innovate. He designed a mechanism to generate the bi-axial deformation needed, which also bolts straight on to the Shimadzu machine.

“I’ve built a mechanism that uses the power of the load frame to generate the stresses,” he says. “There are four diagonal rods at the corners to change the angle and determine the ratio of how much it is deforming in each direction. I can make it more biased towards the horizontal, vertical or even one side.

“I spent time in the workshop machining all the components myself. I started from scratch and it was a very steep learning curve. I broke a lot of tools and upset a couple of people, but I was very determined to make the mechanism and run the tests.”

The request to use the synchrotron was successful and four days of testing commenced. The results show a series of concentric rings, with each ring corresponding to a signal coming from individual lattice planes. The shape of these rings, and their radius, gives an important microscopic insight. “If you deform a bit of material, those rings change in diameter,” he says. “And you can then measure how much strain is being taken up by individual planes. So you capture what is happening on the atomic planar level.”

The cross shaped specimen is 1 mm thick, but in the centre this is reduced to just 300 µm. If it was a uniform thickness, the test would simply pull the arms off. A cross is machined in the middle, which steps down to a thinner circular cross section just 300 µm thick.

“One thing you can see is how strain is being accommodated in the individual grains based on their orientations,” says Dr Collins. “You can monitor the centre on a macroscopic level by putting a camera in front of the rig. But with X-rays, they tell you what the strain is, on all of the individual crystals.”

With the tests complete, the hard work begins. After four intense days of gathering data, it could be a year or more of analysis to find conclusive explanation of the bi-axial deformation phenomenon. What is known is that ‘material texture’, i.e. the orientation of grains in the material, has a big effect on the ductility of the materials and how strain is accumulated. As all sheet materials are rolled during production, it forces most of the crystals to align in one orientation.

“We can tell BMW things like ‘texture is important’, but we don’t yet know how to optimize it,” says Dr Collins. “Eventually, however, we hope to get enough of an idea about the affect to apply it to different materials. We’re sticking with steel for the moment as we don’t want to end up blurring the problem with other complexities. But, there is no reason why this research could not be limited to any alloy.

For a lot of metals, no one has proved if this phenomenon even

exists or not, so there might be massive benefits with lots of different applications outside the automotive world.”

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www.materialsforengineering.co.uk

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The Diamond Light Source

Diamond Light Source is the UK’s synchrotron. It works like a giant microscope, harnessing the power of electrons to produce bright light that scientists can use to study anything from fossils to jet engines to viruses and vaccines.

The machine speeds up electrons to near light speeds so that they give off a light 10 billion times brighter than the sun.

These bright beams are then directed off into laboratories known as ‘beamlines.’

Here, scientists use the light to study a vast range of subject matter, from new medicines and treatments for disease to innovative engineering and cutting-edge technology.



Discover the future of LC-MS/MS

CLAM-2000* – fully automated sample preparation module particularly for biological applications



Figure 1: CLAM-2000 fully automated sample preparation LC-MS/MS system

The trend to use LC-MS/MS technology instead of immunoassays in clinical laboratories can be observed already for a few years. The shift to LC-MS/MS is constantly increasing for several types of analysis, for example immunosuppressants, vitamin D or steroids panels. The progress of LC-MS/MS in that field is mainly due to its ability for high sensitivity detection, high selectivity as well as the possibility to multiplex compounds in one analysis without the risk of inspecific cross reactions inherent to immunoassay tests.

Sample preparation is the bottleneck

LC-MS/MS has proven to be a powerful tool in clinical laboratories and the cost per analysis was decreased significantly – thanks to Shimadzu's UFMS (Ultra-Fast Mass Spectrometry) technologies including ultra-fast polarity switching (5 ms), short pause time (1 ms), multi MRM (555 per second), and fast scanning possibilities (30,000 Da/s), combined with the well-known robustness of Shimadzu systems.

However, the bottleneck still remains sample preparation steps

like deproteinization and dilution which is required to analyze blood samples. These steps are often tedious, introduce the risks of errors, and also increase the potential health risk due to infection for laboratory staff. To overcome this, Shimadzu has developed the fully automated sample preparation module CLAM-2000 (Clinical Laboratory Automated sample preparation Module).

Unique combination of sample preparation and LC-MS/MS analysis

The CLAM-2000 (figure 1) is an automated pretreatment system designed for customers that handle biological samples like blood

serum, blood plasma or urine in pharmaceutical and medical departments, or biological analysis laboratories that are dealing with issues of variability in analytical results or infection risk. It is a fully automated sample preparation module that was developed based on Shimadzu's experience to produce clinical analyzers. The instrument is able to perform the following processes for sample pretreatment:

1. dispensing samples
2. dispensing reagents
3. stirring
4. suction filtration
5. incubation
6. automatic transfer of sample vials to an SIL-30AC autosampler after pretreatment.

The direct detection of diseases related to biological compounds in blood, urine, or other biological samples, the measurement of trace concentration levels of drugs and other applications is possible by mass spectrometry. By additionally using the CLAM-2000 unit in front of the LC-MS/MS system, data quality will not only be improved, but also throughput will be increased. Simply, the blood collection tubes have to be

placed in the system and the CLAM-2000 will perform all other processes from sample pretreatment to LC-MS analysis automatically.

Unlike standard dispensing systems or robots, which are based on batch processing of 96-well plates, the CLAM-2000 is completely automated. No manual steps are required after placing the samples as even the transfer to the LC-MS/MS instrument is done automatically. It processes all individual samples successively. Consequently, it results in uniform pretreatment times for every sample, without slowing down processing speed, and improves data reproducibility and accuracy.

Fully automated analysis of blood serum

Figure 2 shows as an example the workflow for the simultaneous analysis of antiepileptic drugs in blood serum using the CLAM-2000 coupled to a LCMS-8040 mass spectrometer. The blood collection tubes were inserted into the CLAM-2000 and the analysis was started on the touch panel. Additional samples may be inserted while the instrument is already running.

Preparation of the next sample can also be performed in parallel with

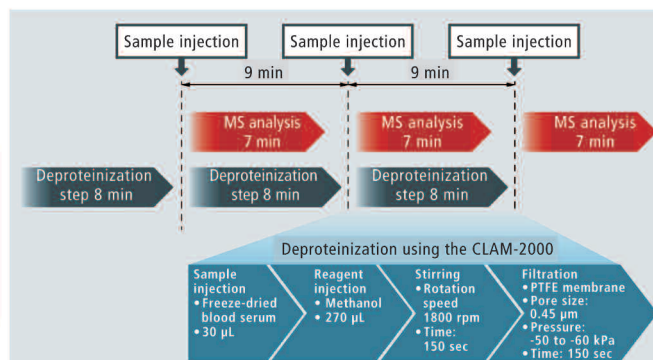
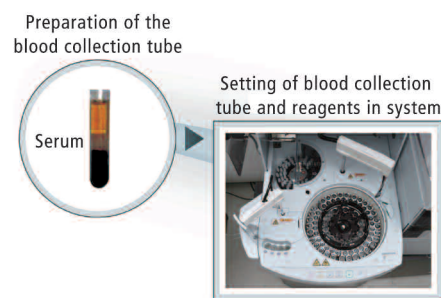


Figure 2: Workflow for simultaneous analysis of antiepileptic drugs in blood serum using the fully automated sample preparation LC-MS/MS system

LC/MS/MS analysis, which can greatly reduce the time required for each sample analysis. In the instant example, a per-sample cycle time including analysis of nine minutes is achieved. The mass chromatogram (figure 3) shows the separation of seven antiepileptic drugs and drug metabolites spiked into human blood serum. Due to the selective detection of the LC-MS based on mass and structure, no interferences from other components in the blood serum are apparent.

Calibration curves were prepared by continuous analysis with fully automated sample preparation and analysis, and used to assess accuracy and precision (repeatability). Good linearity was obtained across the set calibration curve range for each antiepileptic drug (figure 4), with accuracy within

100 % \pm 15 % over the entire measurement range including the minimum limit of quantification. Similarly, precision was measured at a % RSD of within 15 %, showing that good repeatability was achieved (table 1).

Flexible and future-proof

The system is not limited to certain methods and allows the use of homebrew methods as well as commercially available kits as long as the manual preparation steps can be adapted on the system. The required reagents just have to be inserted into the system, and the respective method have to be assigned to the sample.

In addition, to keep a maximum degree of flexibility and to be able to adapt to future needs, the CLAM-2000 is compatible with

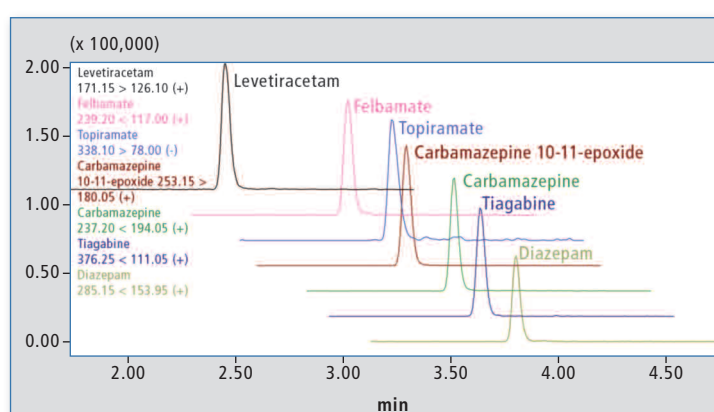


Figure 3: Mass chromatogram of seven antiepileptic drugs and drug metabolites in a control serum sample

the full range of Shimadzu's LC-MS/MS instruments:

- LCMS-8040
- LCMS-8050
- LCMS-8060

The CLAM-2000 can easily be added to an already existing LC-MS/MS system. A simple Graphic User Interface (GUI) via a touch panel simplifies the monitoring of the unit (sample preparation plus LCMS system) as well as tables to follow the work load of the system and tools to organize the maintenance. Samples can be easily entered and tracked by the integrated barcode reader.

Consequently, the new CLAM-2000 is the first system in the world able to perform all steps fully automated from pretreatment of the sample to LC-MS/MS analysis without any further manual interaction. It just requires the simple task of placing the blood, or biological fluids collection tubes, reagents, internal standards and specialized pretreatment vials in the system and start.

It also features excellent management functions that can provide a dramatically improved workflow with better safety for clinical research and higher reproducibility.

**For Research Use Only. Not for use in diagnostic procedures. Not available in the USA, Canada and China.*

Compounds	Range (ng/mL)	QC samples concentration (ng/mL)			Accuracy (%)			% RSD (n = 6)		
		LLOQ	Medium	ULOQ	LLOQ	Medium	ULOQ	LLOQ	Medium	ULOQ
Levetiracetam	10 - 750	10	100	750	94.6	106.1	99.2	3.42	1.23	1.98
Felbamate	25 - 1,000	25	250	1,000	98.6	101.8	99.6	6.28	1.88	1.50
Topiramate	500 - 10,000	500	2,500	10,000	102.3	97.1	100.6	6.71	3.58	2.96
Carbamazepine 10,11-Epoide	5 - 1,000	5	100	1,000	92.9	107.8	99.3	7.48	3.32	1.41
Carbamazepine	10 - 1,000	10	100	1,000	90.6	110.3	99.1	3.79	3.42	1.19
Tiagabine	50 - 1,000	50	250	1,000	98.5	101.9	99.6	1.95	2.00	1.26
Diazepam	5 - 1,000	5	250	1,000	98.1	102.4	99.5	4.61	1.50	1.53

Table 1: Results of validation test for simultaneous analysis of antiepileptic drugs

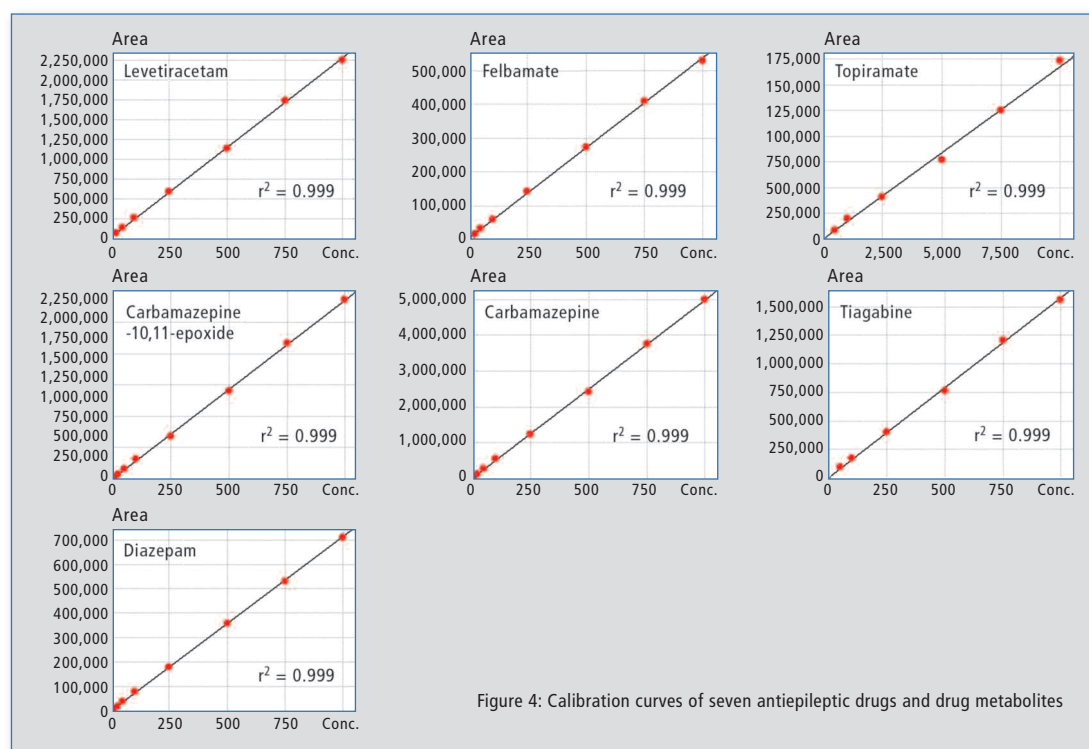


Figure 4: Calibration curves of seven antiepileptic drugs and drug metabolites

Further information on this article

- Application News
- High-Throughput
- Optimization of
- Therapeutic Drug Monitoring





Multipurpose tool for tiny objects

AIM-9000 infrared microscope: automated microanalysis for contaminations and microparticles



Figure 1: AIM-9000 in combination with Shimadzu's FTIR IRTTracer-100

Infrared microscopy is considered to be the ultimate high-light in molecular spectroscopy. With a few simple steps, complex questions about tiny sample particles can be easily answered. 'Tiny' refers to 'micrometer range.' The measurement technology or more specifically, the applied radiation wavelength, limits the sample size. The mid-infrared range of approx. $4,000 - 400 \text{ cm}^{-1}$ can only be applied to measuring spots with a minimum size of at least $5 \mu\text{m}$, because $5 \mu\text{m}$ corresponds to the wavenumber $2,000 \text{ cm}^{-1}$ in infrared spectroscopy.

In the past, key words like contaminations of products, forensics or failure analysis have been used.

Today, discussions focus on the topic of microparticles and their identification. Particles that are smaller than $250 \mu\text{m}$ are of particular interest, for example particles that are found in the stomachs of animals (seals, dolphins, sea birds) or particles in foods (black particles in honey, particles on pizzas or in filled bottles). The relevance of this topic is covered by the application range of Shimadzu's new AIM-9000 fully automatic FTIR infrared microscope.

Good measurement parameters for the analysis of the tiniest spots

The AIM-9000 is an automatic failure analysis system and is optimized for automated observation,

the definition of measurement spots, as well as for the measurement and identification of spectra. To analyze the tiniest spots, the microscope must feature the appropriate measurement parameters.

The AIM-9000 achieves a signal-to-noise ratio of $30,000 : 1$. It can perform and directly display high-speed measurements for 3D or 4D measurements (mapping/screening). An important advantage for the user is simultaneous measurement and visual observation of the sample. Needless to say, the AIM-9000 microscope is available with numerous additional options, such as polarizers for observation and measurement, or wide-field cameras, ATR (attenuated total

reflectance), grazing angle objective and more.

AIMsolutions software and libraries

Automation control in the AIM-9000 is achieved via the AIMsolutions software, which supports the sequence of failure search, measurement and identification. For identification, the AIMsolutions software uses Shimadzu's standard library containing 12,000 spectra, the Tap-Water/Food Contaminants Library, the Thermal-Damaged Plastics Library as well as the Sadtler and the STJ Libraries. The failure analysis mentioned earlier is an automatic search function of distinctive regions in the sample.



Troubleshooting: Highest sensitivity, automatic zoom function and automatic detection of contaminants

Depending on the size of the defect, appropriate aperture sizes can be set.

Compatible with various detectors

The AIM-9000 works in conjunction with Shimadzu's FTIR

IRtracer-100 and IRAffinity-1S spectrophotometers. To detect IR radiation (also called thermal radiation), the microscope can be equipped with various detectors depending on the heat sensitivity required. For high-sensitivity measurements, two MCT detectors with different measuring

ranges are available and for simple and non-sensitive measurement, a TGS detector can be used.

The advantage of the TGS detector is its wide measurement range. Its operation does not require any liquid nitrogen. Its low signal-to-noise ratio, however, is a drawback. While the TGS detector has a signal-to-noise ratio of 100 : 1, the more sensitive MCT detector (detector cooling with liquid nitrogen is required) has a signal-to-noise ratio of 30,000 : 1. Important for these key parameters is the observed area of 100 μm^2 .

Conclusion

The new Shimadzu AIM-9000 infrared microscope can be fully automated to set apertures, to find sample spots and to measure and analyze them. This considerably reduces the microscopy workload. The result is a visible image of the sample area, the infrared spectra and the identification of materials that constitute the defects.

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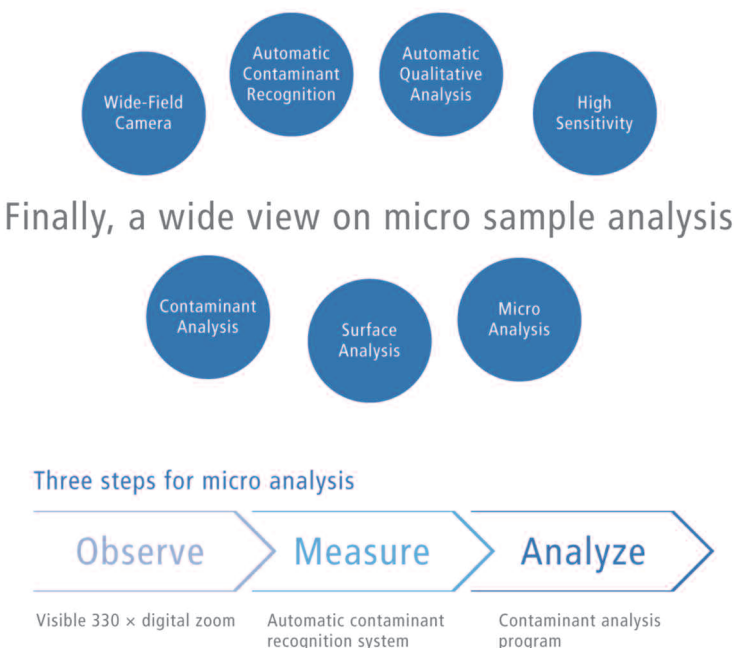


Figure 2: Three steps to success for different tasks



No carry-over: TOC determination in process analysis

Wastewater treatment plant: high demands on equipment and sampling method



The TOC (Total Organic Carbon) parameter for organic pollution of wastewater can serve as an indicator for accurate and efficient control of industrial processes. In addition to carrying out TOC analyses in the laboratory, TOC process analysis is increasingly in demand. Using Shimadzu's TOC-4200 and switching between measuring points, up to six sample streams can be monitored – even in sample

streams of different concentration levels.

Practical example: wastewater treatment plant

In industrial water purification and wastewater treatment plants, a variety of processes are used to purify wastewaters in different sub-processes (for instance, biological or filtration processes). The TOC sum parameter provides

important information on the remaining organic contamination and thus on the efficiency of the

wastewater treatment plant. At the inlet of such plants, TOC concentrations of well over 1,000 mg/L

Sample stream	S # 1	S # 2
Matrix	Ultrapure water	3 % NaCl solution
Target concentration	1 mg/L	1,000 mg/L
Parameter	NPOC	NPOC
Acid added	100 µL	100 µL
Injection volume	50 µL	50 µL
Dilution	None	Factor 10

Table 1: Matrix and measurement conditions of the two sample streams

as well as high salt loads can be expected. Dissolved organic substances are decomposed in biological intermediate stages. Flocculation or precipitation agents added at this stage modify the sample composition (matrix).

At the outlet of such purification or wastewater treatment plants, the TOC content of the effluent water is usually less than 50 mg/L.



TOC-4200

The sample matrices and measuring ranges of these sample streams thus vary widely. Monitoring these sample streams using a single analyzer will place high de-

mands on the instrument and the sampling method used.

ISP module in the TOC-4200

The ISP module (Integrated Sample Pretreatment) is the heart of the TOC-4200 series. It consists of an 8-port valve and a syringe pump (figure 1). The consistent use of inert materials reduces the risk of cross-contamination.

In addition to automated sample pretreatment (acidification and sparging), the module enables dilution of the sample. It is also possible to determine self-cleaning sequences for the module as well as the sample inlets. The automated dilution function serves not only for measuring range extension, but also for matrix reduction. This reduces maintenance and lowers operating costs.

Sampling method

Incorrect measurement values often already occur due to carry-over effects in the sample inlet line or in the sampler. Biological growth and deposits in the sample inlet line constitute a further risk of contamination. To minimize this, the sample should be directed to the sampler at a flow rate of less than 1 m/s. Inspection openings and valves for manual or even automated flushing of the tubing should be provided for. The sampler should be constructed of inert materials.

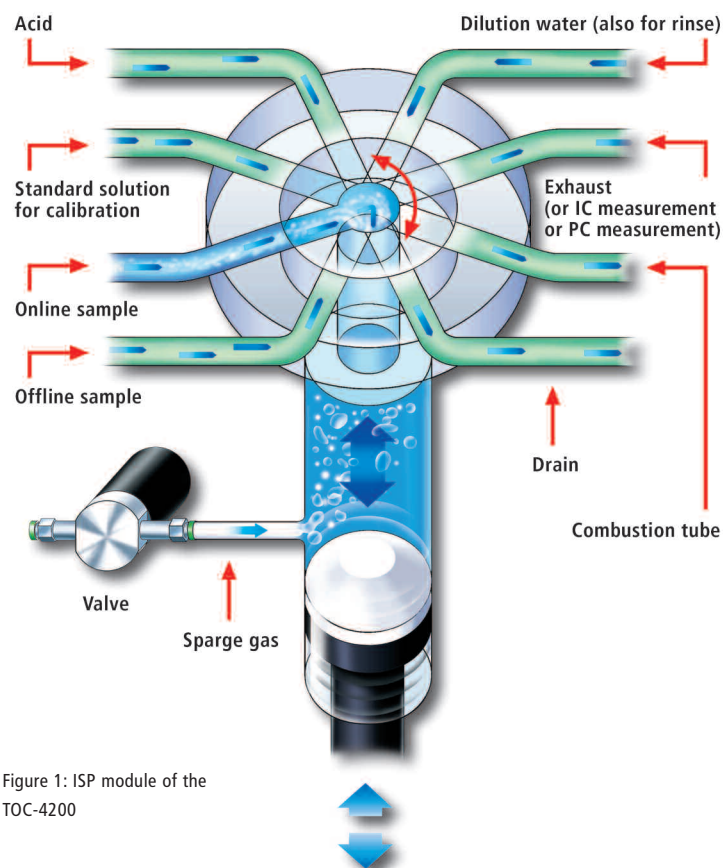


Figure 1: ISP module of the TOC-4200

Automated rinsing steps between individual samplings further reduce time expenditure by the operator. To reduce problems during operation, it is important to pay particular attention to sampling during the project development phase.

Practical test

In a practical test, two sample streams with different matrices

and TOC contents were analyzed (table 1). To demonstrate sample carry-over between these sample streams, they were alternately sampled and analyzed 90 times respectively. The results of these alternating measurements are shown in figure 2. From the measurement results for sample stream 1, a mean value of $1,02 \pm 0,07$ mg/L TOC and for sample stream 2, a mean value of $992,7 \pm 13,32$ mg/L TOC was obtained.

Conclusions

Even with significant concentration and matrix differences between both sample streams, the TOC-4200 is operated free from carry-over thanks to its inert construction and automated rinsing function.

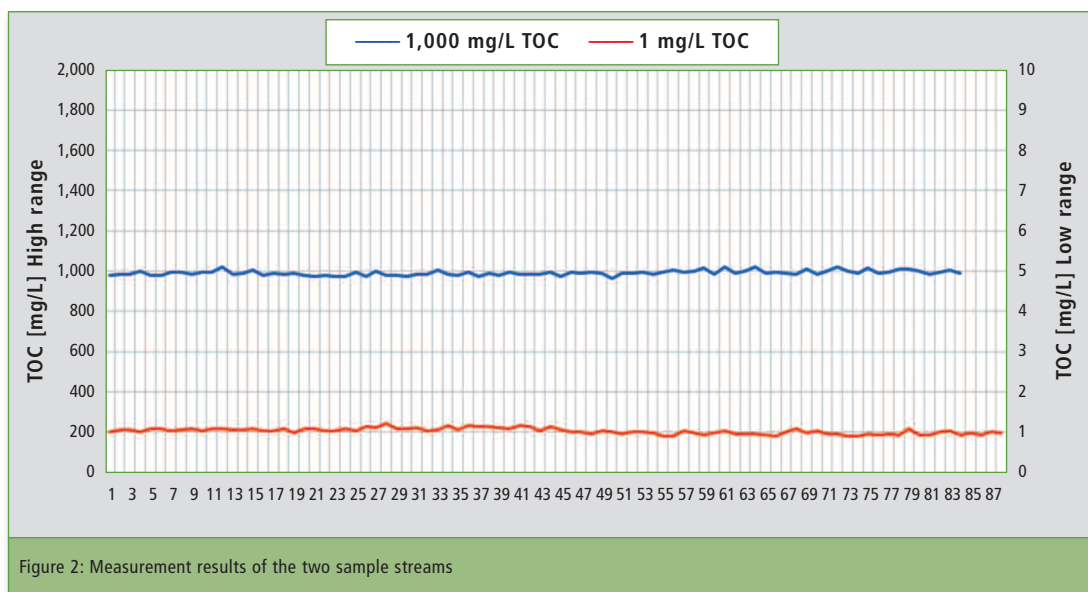


Figure 2: Measurement results of the two sample streams



Salty catalyst?

TOC analysis of salt-containing samples

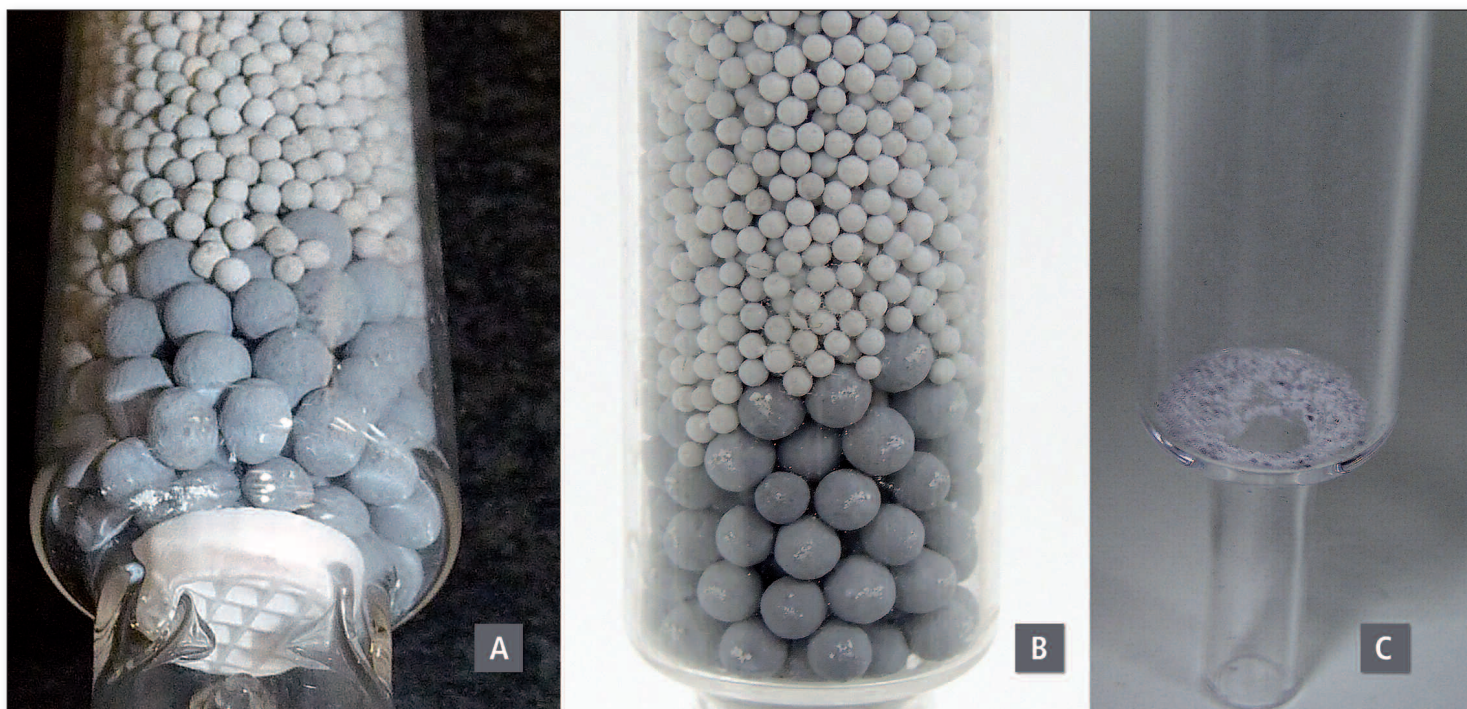


Figure 1: At 680 °C, the salts crystallize and trickle through the combustion tube into the salt trap (figure 1c). The figures show the catalyst before measurement and after measurement of a 9 % salt solution (600 injections).

The determination of the TOC (Total Organic Carbon) parameter has become firmly established in many application areas. Whether in environmental analysis, pharmaceutical or chemical industries – TOC determination is used wherever contamination by organic components plays a role.

In the most frequently used TOC determination method, the sample is first treated with a mineral acid in order to convert the inorganic carbon compounds, such as carbonates or hydrogen carbonates to carbon dioxide, which is then removed via the carrier gas stream.

An aliquot of the sample is subsequently injected onto a hot catalyst where the organic components, in turn, are converted to carbon dioxide and transported to

a NDIR detector via a carrier gas, which detects the resulting CO₂.

Problem: effect of salt accumulation on the catalyst

While water evaporates during combustion oxidation and the organic substances present in water are completely converted to carbon dioxide, salts such as sulfates or chlorides accumulate on the catalyst with each injection.

Salt buildup on the catalyst or in the combustion tube is one of the most frequently occurring technical problem in TOC analysis. Depending on the combustion temperature, the salts can melt and clog the active surface of the catalyst. Residues of the molten salts on glass components, such as the

combustion tube, cause destructive stresses upon cooling or heating. Samples with high-salt content thereby increase the need for maintenance and decrease the lifetime of the analyzer.

Solution: temperature below the salt melting points

To prevent such problems, it is important to use combustion temperatures that are below the melting points of common salts. Already during the 1980's, Shimadzu has developed the 680 °C combustion method. The TOC analyzers oxidize the organic compounds in combination with a highly effective platinum catalyst at a temperature of 680 °C. As shown in table 1, this temperature is below the melting points of common salts.



TOC-L

Analyzers of the TOC-L series also feature an integrated sample preparation module, which makes it possible to perform automatic dilution of the sample. The module cannot only be used to dilute the TOC content of a sample, but also to reduce the concentration of the sample matrix.

Special kits for salt-containing samples

Sometimes, the low TOC concentration in samples such as seawater or pure salt solutions and brines, do not allow for dilution. Special kits for salt-containing samples are available for these sit-

uations. They consist of a combustion tube that has a special geometry. The tube is filled with differently sized catalyst beads that are fixed by a coarsely meshed ceramic lattice. This design allows the salts to crystallize and to trickle down the packing of the catalyst tube as well as the ceramic lattice without clogging the packed tube.

A so-called salt-trap is situated underneath the ceramic lattice (figure 1c). The salt trickling down the catalyst tube accumulates here. Experience shows that the use of this salt kit enables measurement of sample amounts up to twelve times higher compared to the use of a conventional catalyst. Using a salt kit, it is possible to carry out about 2,500 injections of a seawater sample. It is even possible to carry out TOC analyses of highly concentrated sodium chloride brines (28 %) using such a kit.

Also suitable for online process analysis

The use of a kit for salt-containing samples is, however, not only useful for laboratory analysis. Also in process analysis, in which online

Compound	Melting point
NaCl	801 °C
KCl	773 °C
Na ₂ SO ₄	888 °C
MgCl ₂	708 °C
CaCl ₂	782 °C
K ₂ SO ₄	1,069 °C

Table 1: Melting points of common salts

analyzers are working 'around the clock', these kits can be used in order to increase the lifetime of analytical systems.

The kits have been proven particularly suitable for use in wastewater control. To save water in industrial processes, part of the water is recovered from the wastewater. This is why the salt content in wastewaters is often increased.

Conclusion

Salt-containing samples cause salt buildup in catalysts during TOC determination and decrease the lifetime of the analyzer. A combustion temperature below the melting point in common salts reduces the occurrence of salt melts on the catalyst.

Additional kits for the determination of salt-containing samples enable failure-free analysis of samples with high salt loads, such as seawater or brines, and increase the lifetime of the analysis system – in the laboratory as well as in online analysis.

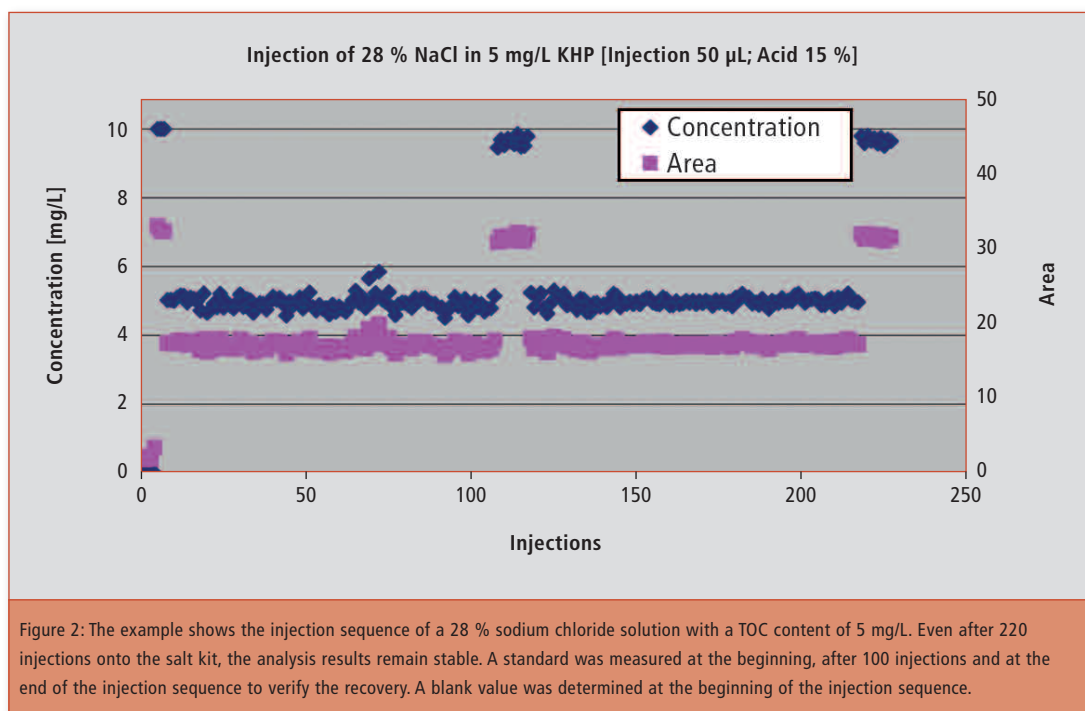


Figure 2: The example shows the injection sequence of a 28 % sodium chloride solution with a TOC content of 5 mg/L. Even after 220 injections onto the salt kit, the analysis results remain stable. A standard was measured at the beginning, after 100 injections and at the end of the injection sequence to verify the recovery. A blank value was determined at the beginning of the injection sequence.



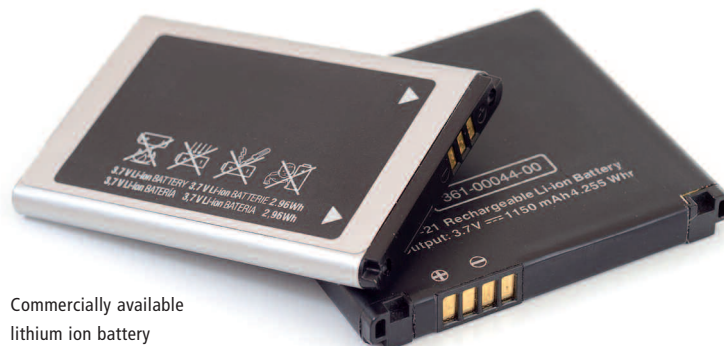
Anti-aging for batteries?

LC-MS method development for battery electrolytes

Carola Schultz from the MEET Battery Research Center at Munster University, Germany, has been the first of young scientists who were awarded with lab time at the Laboratory World in Duisburg, Germany for her research project. Here are some insights in her topic:

Lithium ion batteries are used day-in-day-out in laptops, cell phones or digital cameras. They are also installed in e-bikes and electric cars, mainly due to their low weight compared to other types of batteries such as the lead accumulator. They are characterized by their high specific energy and high energy density. [1]

Figure 1 shows the functional principle of a lithium ion battery. Often, the cathode consists of lithium transition metal oxides. The anode material is usually graphite. During the charge process, lithium ions move through the electrolyte from the cathode to the anode and are incorporated between the graphite layers. The corresponding electrons travel to



Commercially available lithium ion battery

the anode through an external electric circuit. During discharge, the process is reversed and usable electrical power is supplied.

The anode and cathode are separated by an electronically insulating separator. This way, the cell is protected from short-circuiting, the same time it is permeable to lithium ions. The electrolyte enables ion transport between anode and cathode.

The electrolyte contains 1M lithium hexafluorophosphate (LiPF_6) as a conducting salt. The electrolyte consists of cyclic and linear organic carbonates such as ethylene carbonate (EC) and dimethyl carbonate (DMC), ethylmethyl

carbonate (EMC) and/or diethyl carbonate (DEC). The use of cyclic carbonates increases the solubility of the conducting salt. Because EC is solid at room temperature, it is mixed with the linear carbonates to decrease the viscosity. [1] The structures of the individual components are shown in figure 2.

When the electrolyte is aging

The conducting salt, which is chemically and thermally unstable, is subject to so-called aging during the charge and discharge process. This can lead to the formation of a variety of different (for instance phosphate-based [2] or carbonate-based) aging products. An example is the formation of the aging product group of polycarbonates, shown in figure 3 [3, 4]. This is further investigated below.



The fact that batteries age may well be confirmed by anyone who has owned a battery for a while. The battery lifetime is limited because repeated charging and discharging reduces the capacity of the battery. To understand the underlying aging mechanisms and to be able to counteract them requires analytical methods for the identification and quantification of aging products.

Within the lab4you research program for young scientists, it was the aim to develop such a method using LC-MS. The focus of the investigation was on the electrolyte. The following mass spectrometers were used for method development: the LCMS-IT-TOF and the LCMS-8040 (triple quadrupole LC-MS/MS).

Qualitative analysis using the LCMS-IT-TOF

The benefit of the LCMS-IT-TOF is the combination of 3D ion trap and a time-of-flight mass analyzer. This offers a high mass accuracy as well as a high mass resolution in all MS and MS^n stages.

Using the LCMS-IT-TOF, the aging products were qualitatively

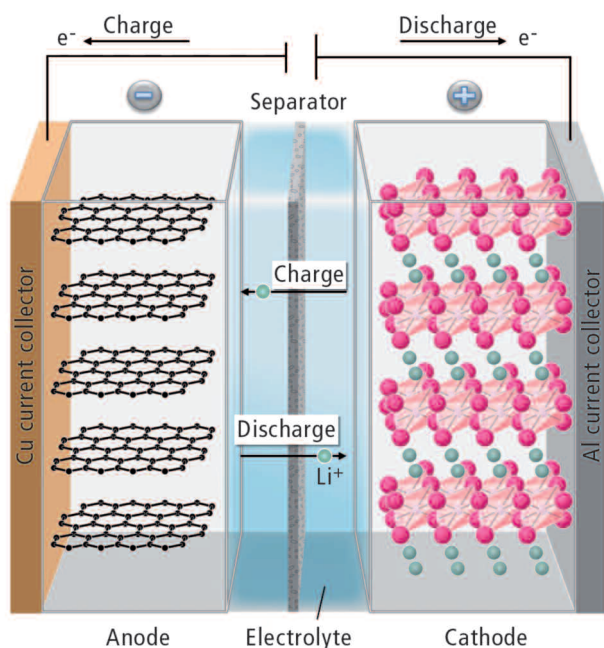


Figure 1: Schematic representation of the functional principle of a lithium ion battery

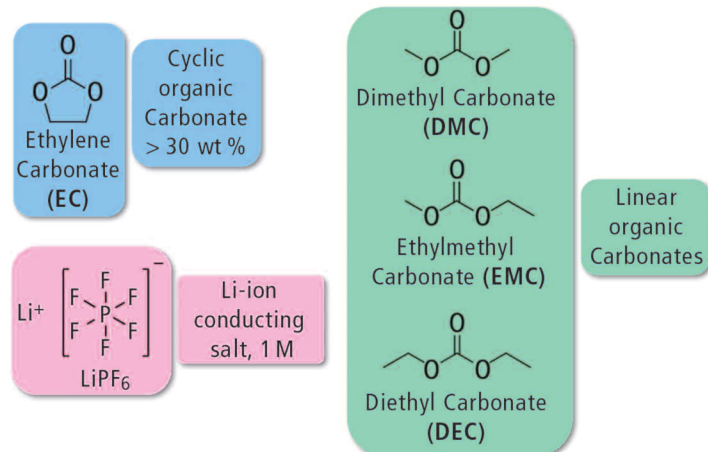


Figure 2: Structures of the main components of an electrolyte

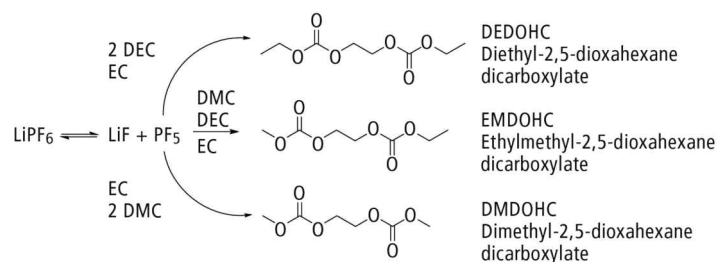


Figure 3: Formation of carbonate-based aging products

analyzed, i.e. the structures were elucidated with the help of the MSⁿ function. An example for the structure elucidation of many aging products via MSⁿ is the fragmentation pattern of diethyl phosphate (DEP) in figure 4 with its corresponding spectra. Despite its molecular mass of 154 amu, the structure of DEP could be elucidated with the help of an MS⁴ analysis. The main molecule with $m/z = 155.046$ was detected via MS¹. The loss of a C₂H₄ group with $m/z = 127.015$ could be measured in the MS² stage. Another C₂H₄ group was cleaved off in the subsequent fragmentation step. The resulting phosphoric acid in MS³ with $m/z = 98.985$ is dehydrated in a final fragmentation step, leading to a molecule with $m/z = 80.972$ in the MS⁴ stage.

More aging products identified in the course of the lab4you program

As there are no commercially available standards for all aging products, the MSⁿ function for structure elucidation is crucially important and useful. Thus, during the lab4you program, many aging products, which are not available as pure substances, could be identified. As an example, some polycarbonates that were detected in thermally aged (60 °C) electrolytes as well as in electrolytes extracted from cycled batteries are listed in table 1.

It becomes clear that this substance class of polymerized aging products alter the function of an aged battery by reducing the conductivity in the electrolyte and increasing its viscosity. Furthermore, organic electrolytes with LiPF₆ are highly water-sensitive. Even brief contact between an electrolyte and air causes the formation of many aging products,

such as DEP whose structure elucidation is shown in figure 4 (page 14).

Charging and discharging of a battery as well as elevated temper-

atures (> 55 °C) lead to the formation of aging products. This could be confirmed, among others, in this study on the various aging processes in the battery and electrolyte.

Quantitative analyses using the LCMS-8040

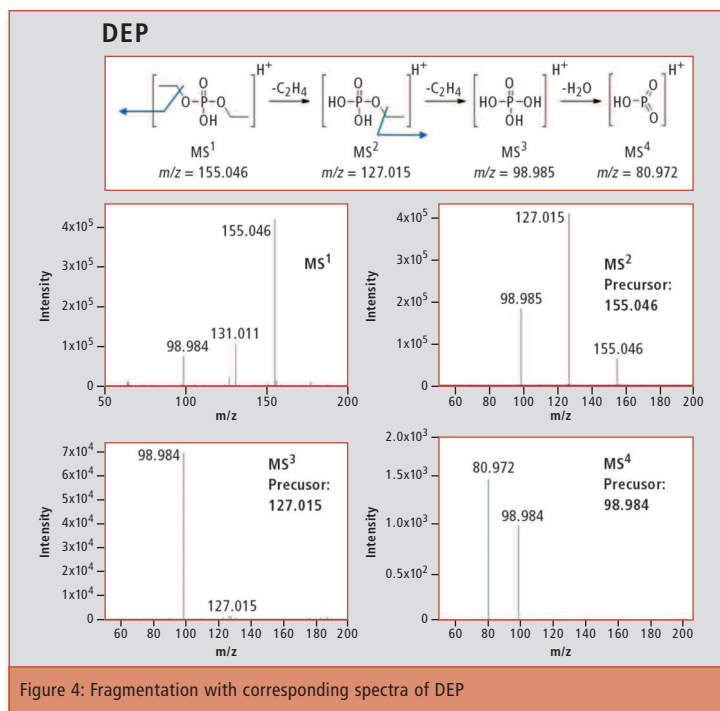
The main components of the electrolyte as well as the commercially available aging products were quantified in the MRM mode using Shimadzu's LCMS-8040 triple-quadrupole mass spectrometer. In addition, structural eluci-

ation via LCMS-IT-TOF was confirmed with LCMS-8040 and further supported by the extended mass range – which in the triple quad is also available for masses < $m/z = 50$.

A total of nine substances, main components as well as aging products were quantified. These included EC, DMC, EMC, DEC, DMDOHC, DEDOHC, triethyl phosphate (TEP), dimethyl phosphate (DMP) and DEP. A C18 pentafluorophenyl phase (2.1 mm x 100 mm; particle size 2 µm) was selected. ♦

Structure	Ion	m/z	Formula
	[M+H] ⁺ [M+NH ₄] ⁺ [M+Na] ⁺	179.05 196.08 201.04	C ₆ H ₁₀ O ₆
	[M+H] ⁺ [M+NH ₄] ⁺ [M+Na] ⁺	193.07 210.09 215.05	C ₇ H ₁₂ O ₆
	[M+H] ⁺ [M+NH ₄] ⁺ [M+Na] ⁺	207.09 224.11 —	C ₈ H ₁₄ O ₆
	[M+H] ⁺ [M+NH ₄] ⁺ [M+Na] ⁺	267.07 284.09 289.05	C ₉ H ₁₄ O ₉
	[M+H] ⁺ [M+NH ₄] ⁺ [M+Na] ⁺	281.09 298.11 303.07	C ₁₀ H ₁₆ O ₉
	[M+H] ⁺ [M+NH ₄] ⁺ [M+Na] ⁺	295.10 312.13 317.08	C ₁₁ H ₁₈ O ₉
	[M+H] ⁺ [M+NH ₄] ⁺ [M+Na] ⁺	— 372.11 377.07	C ₁₂ H ₁₈ O ₁₂
	[M+H] ⁺ [M+NH ₄] ⁺ [M+Na] ⁺	369.10 386.13 391.08	C ₁₃ H ₂₀ O ₁₂
	[M+H] ⁺ [M+NH ₄] ⁺ [M+Na] ⁺	— 400.14 405.10	C ₁₄ H ₂₂ O ₁₂

Table 1: Polycarbonates that were detected via LCMS-IT-TOF in electrochemically and thermally aged battery electrolytes



The mobile phase consisted of water mixed with 0.1 % formic acid and methanol. With this column and mobile phase combination, all nine substances could be separated and quantification of these substances in various electrolytes could be carried out.

Conclusions

Method development for the identification, separation and quantifi-

cation of electrolyte components as well as their aging products using LCMS-IT-TOF and LC-MS/MS could successfully be completed. This method helps to understand battery aging and can thus contribute to 'anti-aging' research for batteries.

For their research project, interested students can apply until October 31st 2016 to be selected for being awarded with free lab

My experiences during the lab4you research program

Because of the interesting work, I can highly recommend the lab4you program to everyone. This program provides a unique possibility to carry out one's own research using the equipment available in Shimadzu's Laboratory World, where participants can specifically use those instruments that will deliver the best possible results for their respective analytes and their desired objectives. For all this as well as with regard to the available measurement time, Shimadzu's Laboratory World offers far more opportunities than most universities could ever offer.

I was able to work independently; there were no preconditions so that I could make all my own decisions.

This had a positive effect on my research, but of course, it also requires very careful project planning in advance.

I have very much appreciated the many possibilities for my research project as well as the excellent technical support regarding the equipment in the Laboratory World. In case of problems or technical questions, I was always helped immediately by the friendly and helpful product specialists or by the service team. All in all, I had a great time at Shimadzu who provided me with valuable results, nice experiences and, of course, interesting insights into the working world.

time at Shimadzu's cutting-edge 'Laboratory World' in Duisburg, Germany. More information: www.shimadzu.eu/lab4you

Author

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... Don't miss this! Limited number of participants ... Don't miss this! Limited number of participants ...

Pyrolysis GC-MS user meeting

October 6th 2016, at Shimadzu's Laboratory World, Duisburg, Germany

Take this opportunity for detailed discussions with experts from research and industry on the numerous pyrolysis GC-MS applications.

The all-day event offers a varied program with lectures and practical examples as well as cutting-edge information on instrument technology and applications. In addition, the tour around Shimadzu's Laboratory World



provides insights into this high-end testing facility for the entire analytical instrumentation product range on over 1,500 m².

However, there will be sufficient time for sharing experiences and for discussions during the breaks.

Please register in time, as the number of participants is limited. The deadline for registration is August 31st, 2016.

We look forward to welcoming you to this special event in Duisburg!

For registration and further information, please use the link below:

bit.ly/pyrolysis-day
www.shimadzu.eu



Metallic contaminations in pharmaceutical packaging

Content and implementation of USP 661.1 with respect to extractable elements



ICPE-9820

Normative directives

While TOC analyses serve as an indicator for extractable organic materials, the determination of extractable metals in materials of construction (plastics) for the production of the packaging or the packaging systems is specifically addressed in chapter 661.1. Chapter 661.2, which addresses finished packaging systems, requires that only materials of construction compliant with USP 661.1 be used and metals are not regarded.

Application

In chapter 661.1, various extraction solutions are used that vary depending on the element or type of plastic to be investigated. These materials of construction are divided into three groups:

1. Polyethylene (PE), polyolefin (PO) and polypropylene (PP)
2. Polyethylene terephthalate (PET) and glycogen-modified PET (PETG)
3. Polyvinylchloride (PVC).

The most complex are the tests for Group 2, PET and PETG, because two different extraction solutions are required: acid extraction and alkaline extraction.

In acid extraction, 20 g of the material to be tested is heated together with 0.1 N hydrochloric acid (HCl) for five hours at 50 °C. After cooling, the solution is analyzed within four hours for the elements aluminum, barium, cobalt, manganese, titanium and zinc.

The plastic materials may not leach more than 1 µg/g (1 ppmw) of the respective element, which corresponds to 0.4 mg/L in the extraction solution.

Furthermore, the PET and PETG are analyzed for antimony and germanium. The limit values required for these metals are the same as for the previously mentioned elements. The extraction process for antimony and germanium, however, is different. Alkaline extraction is used here: 20 g of the material to be tested is heated together with 0.01 N sodium hydroxide solution and is also heated at 50 °C for five hours and analyzed within the same subsequent stability period of the solution of four hours.

Instrumental analysis implementation

In addition to the previously mentioned elements and limit values, lower limit values have been defined for the other substance classes. In the extraction solutions, the limit value for chromium can be at 20 µg/L, while limit values for titanium, vanadium and zirconium can be at 40 µg/L.

To successfully implement the analyses as described in the new USP chapters, the use of Shimadzu's ICPE-9820 is advised, an optical emission spectrometer with inductively coupled plasma. It features highly sensitive axial plasma observation and can, within the same method, also use radial view plasma observation for measurement of higher concentrations. Because in addition to trace elements, calcium for instance, which in the PVC substance class may be present in concentrations

of up to 35 mg/L in the extraction solution, must also be determined.

The ICPE-9820 features, in addition to full flexibility in the measuring range, a particularly low-maintenance Echelle spectrometer. In order to optimally use the entire measuring range (167 - 800 nm) at all times, conventional systems are continuously purged with argon or nitrogen. Because of the sophisticated vacuum technology of the ICPE-9820, this purging process is no longer required, thus completely eliminating any additional costs of consumables.

Conclusion

The ICPE-9820 is ideally suited for the implementation of USP 661.1. Furthermore, Shimadzu offers the ICPMS-2030, a mass spectrometer with inductively coupled plasma, or as an alternative, the AA-7000 atomic absorption spectrophotometer.

All instruments can be operated in a regulated laboratory infrastructure in accordance with FDA CFR 21 Part 11. In addition to TOC, the package can be expanded with the IRAffinity-1S for the identification of polymers or a UV-VIS spectrophotometer, like the UV-1800 for implementation of the listed absorbance tests.

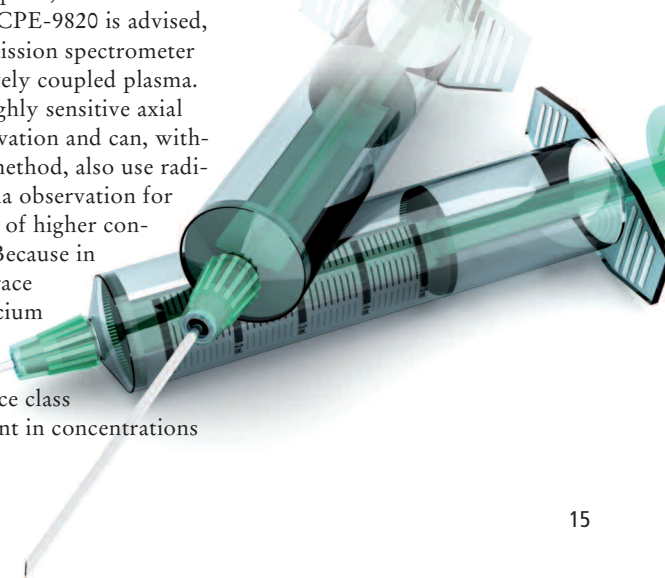
In the pharmaceutical industry, plastic packaging is used in various forms, such as infusion bags, bottles, cartridges or pre-filled syringes. For their particular purposes, these packagings must be tested for suitability.

The United States Pharmacopeia has published two new chapters (661.1 and 661.2) to this effect, which have been valid since May 2016:

- Chapter 661.1 describes the characterization and testing of the individual plastic materials of construction used in the production of plastic packaging.
- Chapter 661.2 deals with the required testing of the final packaging systems, as packaging often consists of more than one plastic material.

Characterization is carried out by the identification and determination of biocompatibility, physicochemical properties and extractable metals.

For the determination of physicochemical properties, Shimadzu offers two suitable TOC systems, the TOC-V and TOC-L, which have already been described in Issue 01/2016 of the Shimadzu News. The present article contains an overview of the analysis of extractable metals.





Healthy fat in chips and sausages?

A new method for extraction, digestion and analysis of fat in food samples

The Regulation (EU) No 1169/2011 of the European Parliament requires the detailed declaration of nutrition values for food as of 2016, especially the differentiation of fat in saturated and unsaturated fatty acids [1].

This differentiation is important in order to protect consumers. For example, the American Dietetic Association (ADA), the European Food Safety Authority (EFSA) and the American "Academy of Nutrition and Dietetics" recommend to cover less than 35 % of the body's daily energy requirements by fat; according to ADA, less than 20 % should be monounsaturated fatty acids (MUFA).

Determination of total fat content as well as unsaturated and saturated fatty acids in food is performed using methods based on ISO standards. Depending on the type of

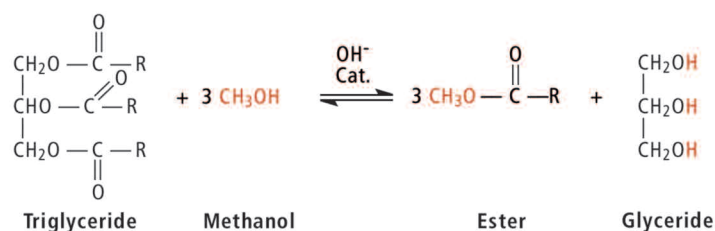


Figure 1: Alkaline Derivatization of a Triglyceride with methanol to ester [5]

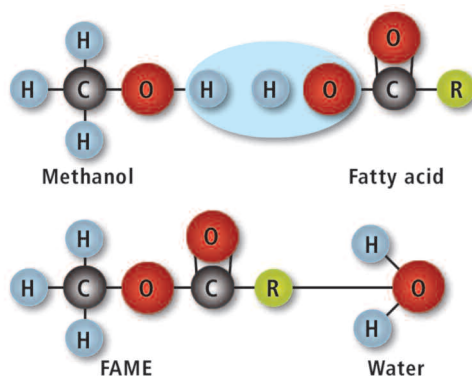


Figure 2: Acidic derivatization of a fatty acid to ester with methanol under formation of water [6]

food, methods like Röse-Gottlieb or Weibull-Stoldt extraction are used for the determination of total fat content [2, 3].

The following analysis of saturated and unsaturated fatty acids is done using gas chromatography

(GC-FID). This analysis requires the derivatization of fatty acids in fatty acid methyl esters (FAMES) based on the ISO norm [4].

Unsaturated fatty acids are sensitive to oxidation processes generated through contact with air and

influence of heat. That's why a gentle microwave process has been developed which is applied in a closed system in two steps for various food compartments. This new microwave procedure is faster, cheaper and more environmentally friendly than the conventional ISO procedure.

It is assumed that the oxidation sensitive unsaturated fatty acids are treated more gently in the microwave process and oxidation is thereby minimized, resulting in a higher analytical result of unsaturated fatty acids.

Methods

Conventional ISO procedure

The conventional standard procedure is based on the ISO extraction method (ISO 8262) for total fat determination in milk and milk products according to Weibull-Berntrop and the derivatization of fatty acids in fatty acid methyl esters (FAMES) based on the ISO method (DIN EN ISO 12966-2).

The ISO 8262 method serves as a base and correlates with the extraction method of Weibull-Stoldt for the determination of total fat content in food samples.

The ISO extraction method according to Weibull-Stoldt is based on a saponified digestion using hydrochloric acid and water, which releases fat bound to proteins in



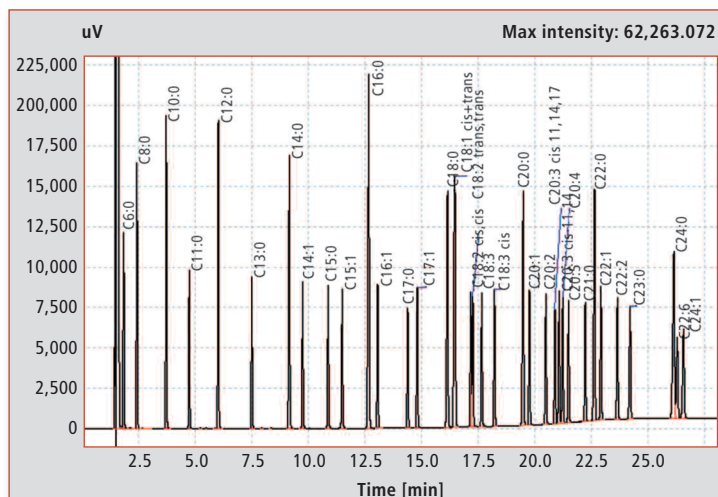


Figure 3: Chromatogram of the 37 Standard-Mix identified FAMES

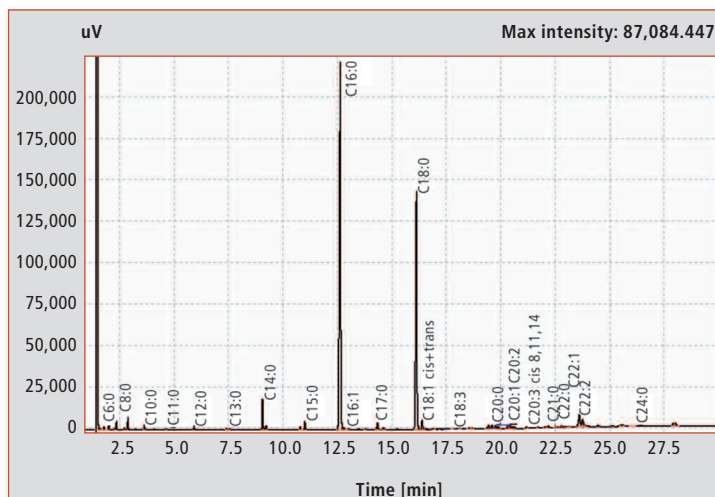


Figure 4: Chromatogram of fatty acids in a sausage sample, derivatization according to ISO procedure and identified FAMES

food samples. In the following hot filtration, the fat released remains in the filter. The filter including the fat is washed to neutral and then dried. Finally, the fat is extracted with a proper solvent in a Soxhlet extractor, taking several hours. As soon as the extraction is finished, the solvent is removed using a rotation evaporator and the remainder is dried. Determination of total fat is performed by weighing the dried fat.

Based on the ISO procedure on sample preparation for GC analysis, the transformation of fatty acids in fatty acids methyl esters (FAMES) requires a sequence of

alkaline and acidic derivatization in water-free methanolic environment.

The alkaline derivatization generates the FAMES out of the fatty acids bound as triglycerides and the saponification of free fatty acids. The following acidic derivatization finally transfers the remaining saponified fatty acids in FAMES.

The reaction equations are displayed in figure 1 and figure 2.

Microwave procedure

The new microwave procedure for determination of fat has been de-

veloped for the microwave system Discover SP-X® (CEM, Kamp Lintfort). It consists of a microwave extraction method (MEM) and a microwave derivatization method (MDM).

The MEM has been developed based on the Weibull-Stoldt method and extracts the total fat in a food sample in a closed system under defined conditions using microwave radiation simultaneously.

In a closed system (including MDM), derivatization of the extracted fatty acids is performed under microwave radiation. Transformation of fatty acids in fatty acids methyl esters (FAMES) also requires a sequence of alkaline and acidic derivatization in water-free methanolic environment for subsequent GC analysis.

GC analysis

Analysis of FAMES of the food samples has been done using a gas chromatograph GC-2010 Plus AF with FID detector (Shimadzu Europa GmbH, Duisburg, Germany).

Influence of the method of sample preparation and derivatization on the content of unsaturated fatty acids in the total fat content of food samples needs to be investigated. In order to compare the contents of unsaturated and saturated fatty acids in both methods,

the total amounts of all fatty acid peak areas are calculated (= 100 %). Peak areas of unsaturated and saturated fatty acids are then summarized, and the ratio of unsaturated vs. saturated fatty acids is calculated. This procedure has been applied for both the ISO procedure and the microwave procedure.

Experimental part

ISO procedure

Extraction method

The food sample is weighed in a 600 mL glass beaker, in the expectation that a total fat weight of 2 to 3 g will be present. The sample is treated with 100 mL water and 150 mL hydrochloric acid (25 weight-%).

The glass beaker is covered with a glass lid, and the sample is then boiled for 30 to 60 minutes. After the digestion process, the hot sample is diluted with 100 mL of water and filtered in a 2 layer round filter which has been wetted before with hot water. Afterwards, the filters and the residue are washed neutral with hot water. Finally, the filter paper and residue are dried in an oven for one hour at 105 °C. The dried filters are then transferred in the extraction sleeve and finally positioned in a 250 mL extractor (Soxhlet-Apparatus). ♦

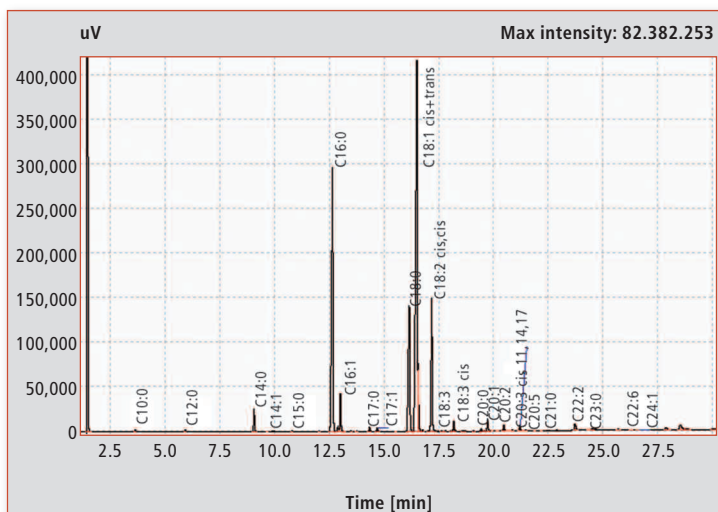


Figure 5: Chromatogram of fatty acids in a sausage sample, derivatization according to microwave procedure and identified FAMES

The round bottom flask of the Soxhlet apparatus (250 mL) is filled with solvent at a volume corresponding to 1.5 times the volume of the Soxhlet apparatus. The temperature of the solvent petrol ether is 40 - 60 °C.

The reflux condenser is then set, the solvent is boiled and the fat is extracted over three to six hours. After extraction, the remaining liquid is fully evaporated in the rotation evaporator.

Afterwards, the open flask is further dried for one hour at 105 °C in order to remove the extraction liquid completely. After cooling, the flask is weighed and the total fat content determined.

ISO based fatty acid derivatization for GC analysis

The extracted fat is transferred in a 10 mL round flask and treated with 2 mL (0.2 mol/L) sodium methanolate in methanol (8 g sodium hydroxide are dissolved in 1,000 mL methanol).

Boiling under reflux is performed until the solution is clear (5 to 20 minutes). The boiling time depends on the chain length of the fatty acids, the longer the fatty acid, the longer the boiling takes. Afterwards the flask is removed from the heating source and two drops of phenolphthaleine solution are added.

Sulfuric acid (1 mol/L) is then added in methanol solution until the solution is clear; another 0.2 mL are then added.

The cooler is reinstalled, and the solution is boiled under reflux for five minutes. The solution is again removed from the heating source and cooled down in water. 4 mL of a saturated sodium chloride solution are now added and shook well. Afterwards, 1 mL n-hexan is added and the flask is shook again for 15 seconds. The solution is left to stand until both phases are separated. Saturated sodium chloride solution is again added until the aqueous phase reaches the lower level of the flask neck, approx. 2 - 3 mL. The upper phase now contains the FAMES generated.

Microwave method

Extraction method

0,5 g of sample is weighed in an 80 mL glass vial and treated with 3,5 mL hydrochloric acid (37 weight-%), 7,5 mL water and 5 mL n-Hexan. A stirrer chip is added. The following parameters are set on the Discover SP-X.

Final temperature: 115 °C
Ramp: 4 minutes
Stirrer: Strong
Hold time: 15 minutes
Power: 200 Watt

After the digestion and extraction, the solvent is transferred in a previously weighed aluminum bowl in order to perform a cold extraction. The digestion in the vial is treated again with 5 mL n-Hexan and then stirred on a magnetic

stirrer for one minute. Afterwards, this solvent will be transferred in the aluminum bowl. This cold extraction is repeated three to four times. The solvent with the extracted fat is dried in the heating oven for twenty minutes at 105 °C and the bowl finally weighed. Calculation of the total fat content is done by following equation 1:

$$F [\%] = \frac{m_2 - m_1}{E} * 100$$

m_1 = Mass in g empty bowl
 m_2 = Mass in g bowl with dried fat
 E = Mass in g weight sample

For the derivatization (MDM), one or two drops of the dried fat

are treated in a glass vial with 10 mL water-free methanolic potassium hydroxide solution (2,5 weight-%), and a stirrer chip is added.

The alkalic derivatization requires the following parameters on the Discover SP-X

Final temperature: 90 °C
Ramp: 5 minutes
Hold time: 10 minutes
Stirrer: Medium
Power: 200 Watt

After finishing the alkaline derivatization, the vial is cooled down in a water bath. 14.25 mL Methanol and 0.75 mL hydrochloric acid (37 weight-%) are added, and the acidic derivatization is generated with following parameters on the Discover SP-X

FAME	Name of acid	Identified findings of the methods	
		ISO	Microwave
C6:0	Caproic	x	
C8:0	Caprylic	x	
C10:0	Capric	x	x
C11:0	Undecanoic	x	
C12:0	Lauric	x	x
C13:0	Tridecanoic	x	
C14:0	Myristic	x	x
C14:1	Myrestoleic		x
C15:0	Pentadecanoic	x	x
C15:1	cis-10-Pentadecanioc		
C16:0	Palmitic	x	x
C16:1	Palmitoleic	x	x
C17:0	Heptadecanoic	x	x
C17:1	cis-10-Heptadecanoic		x
C18:0	Stearic	x	x
C18:1 cis+trans	Oleic + Elaidic	x	x
C18:2 cis,cis	Linoleic		x
C18:2 trans,trans	Linolelaidic		
C18:3	α-Linolenic	x	x
C18:3 cis	γ-Linolenic		x
C20:0	Arachidic	x	x
C20:1	cis-11-Eicosenoic	x	x
C20:2	cis-11,14-Eicosadienoic	x	x
C20:3 cis11,14,17	cis-11,14,17-Eicosatrienoic		x
C20:3 cis 8,11,14	cis-8,11,14-Eicosatrienoic	x	
C20:4	Arachidonic		x
C20:5	cis-5,8,11,14,17-Eicosapentaenoic		x
C21:0	Henicosanoic	x	x
C22:0	Behenic	x	
C22:1	Erucic	x	
C22:2	cis-13,16-Docosadienoic	x	x
C23:0	Tricosanoic		x
C24:0	Lignoceric	x	
C22:6	cis-4,7,10,13,16,19-Docosahexaenoic		x
C24:1	Nervonic		x

Table 1: Identified FAMES of two procedures in direct comparison. Positive findings are marked with X.

Final temperature: 120 °C
 Ramp: 5 minutes
 Hold time: 6 minutes
 Stirrer: Medium
 Power: 200 Watt

After finishing the derivatization, the vial is cooled down in a water bath. 10 mL n-Hexan are added, and the vial is turned twice from bottom to top. The generation of overpressure must be avoided. The glass vial is afterwards filled with saturated sodium chloride solution up to the top. The solvent needs to be transferred with a pipette to a 10 mL GC vial, including sodium sulfate or magnesium sulfate as drying agent (approx. 0.2 g) for possible water residue. This solution is now ready for the analysis of FAMES using GC-FID.

GC analysis:

For identification and quantification of FAMES, a 37 Standard-Mix (Supelco) has been used and analyzed on GC-2010 Plus AF with FID detector.

The following parameters have been set on the GC:

Injector: SPL (Split)
 Injection volume: 1 µL
 Injector temp.: 250 °C
 Column type: FAME WAX
 Inner diameter: 0.25 mm
 Film thickness: 0.25 µm
 Detector temp.: 250 °C
 Mobile Phase: Helium
 Carrier gas speed: 35 cm / sec

The temperature program starts at 130 °C with a hold time of one minute. Afterwards, heating continues with a heating rate of 5 °C/minute until reaching a temperature of 240 °C which then stays constant for ten minutes. The total time is 33 minutes.

Results and Discussion

The gas chromatographic method with flame ionization detection has been optimized for separation of almost all fatty acid derived FAMES.

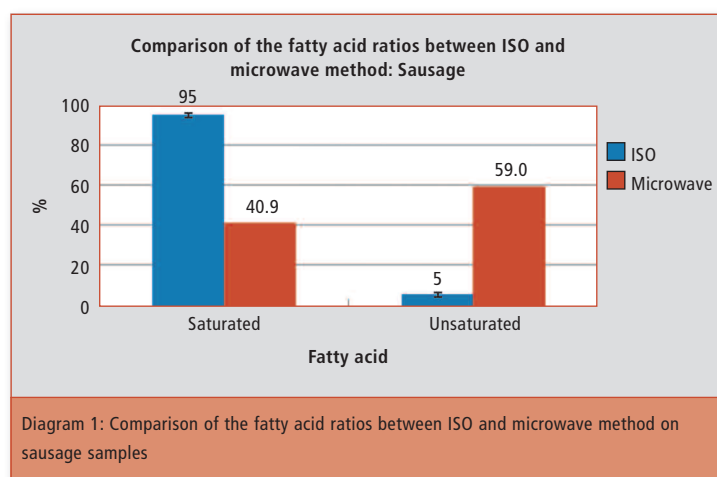
From 37 fatty acids contained in the standard mix, 36 fatty acids have been identified. Two fatty

acids C18:1-cis and C18:1-trans could not be separated, as they co-elute. But this has no influence on the evaluation and differentiation between saturated and unsaturated fatty acids since both co-eluting fatty acids are unsaturated.

Figure 4 (page 17) shows the chromatogram of a sausage sample with the identified FAMES which have been generated according to the ISO procedure. Obviously,

results on unsaturated fatty acids could be observed in the same way during determination of other food samples such as milk powder, chocolate and potato chips, as displayed in diagram 2 (page 20).

In all of the food samples determined, use of the microwave procedure has generated a higher amount of unsaturated fatty acids. This confirms the hypothesis that



fatty acids C16:0 und C18:0 are mainly present. In comparison to the results of the ISO procedure, figure 5 (page 17) shows the chromatogram of the same sample using the microwave procedure.

A comparison of the chromatograms (figure 4 and figure 5) shows a significant difference in the FAMES. The results on C18:1-cis+cis using the microwave procedure are significantly higher in comparison to the ISO procedure.

Table 1 shows that the results of the ISO method have a significant difference in comparison to the microwave method. For a more precise comparison, the peak areas of saturated and unsaturated fatty acids are used and displayed in diagram 1, showing the difference of the fatty acid ratios between the ISO and the microwave method on sausage samples.

Comparison of both methods show that the result of unsaturated fatty acids with the microwave technique is ten times higher than the ISO method. These elevated

the new microwave procedure prevents oxidation of unsaturated fatty acids during the process, and the more gentle treatment results in higher findings when using the new microwave process.

Conclusion

The declaration of food samples is increasingly more complex. For consumers, the declaration is very important and therefore needs a certain transparency. The EU regulation 1169/2011 demands a detailed declaration of food samples. Most important is the differentiation of total fat in saturated and unsaturated fatty acids, which is the top criteria for the consumer to differentiate between healthy and unhealthy food, as has been the subject of two studies. The determination of total fat content as well as unsaturated and saturated fatty acids is regulated in ISO norms.

In this work, two methods have been developed. The first method represents a procedure for digestion and extraction of the total fat

content in a closed microwave system, based on a method of Weibull-Stoldt. Food samples from different areas (dairy products, luxury foods, meat and pastries) have been selected, and the application of the total fat determination using the microwave method in a closed system has been evaluated.

It has been found to be a universal method for the determination of the total fat content in different food areas.

The second method corresponds to a microwave method, also in a closed system for derivatization of fatty acid in FAMES which are then analyzed in a GC-FID system.

The use of the developed microwave extraction method shows that the method is best suited for determination of the total fat content of food samples.

In the second method, food samples have been analyzed for 37 different fatty acid methyl esters. This enables a comparison between the DIN method for digestion and extraction of the total fat content of a food sample with an ISO method for derivatization of extracted fatty acids in FAMES, with the developed microwave procedure consisting of microwave extraction method (MEM) and microwave derivatization method (MDM).

The focus of this comparison is on the ratio between saturated and unsaturated fatty acids (milk powder, chocolate, sausage, chips). Results show that the ratio of fatty acids differs between ISO and microwave procedure. The proportion of unsaturated fatty acids is always higher when the microwave procedure is applied.

The higher rate of findings of unsaturated fatty acids confirms the sensitivity of the unsaturated fatty acids related to the oxidation by air and heat influence. ♦

The DIN procedure is an open system and requires heat over a long period of time which has an influence on the unsaturated fatty acids (oxidation). The microwave system is a closed system with a significant shorter heat application and reduced oxidation of unsaturated fatty acids. The microwave procedure is therefore a more gentle and preferable method as far as unsaturated fatty acids are concerned.

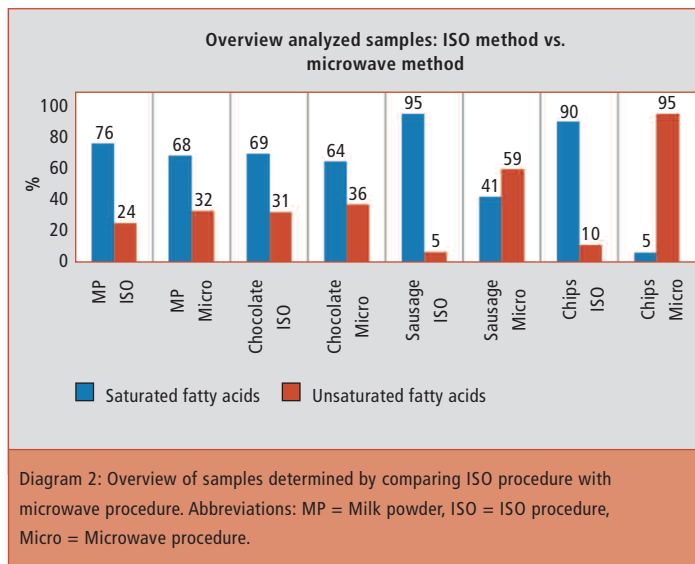
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