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NEWS 01 2016

Mineral oil compounds in paper and cardboard packaging

Investigating the composition of the aromatic mineral oil fraction

New generation of protein sequencer

PPSQ-50 with increased sensitivity and FDA compliance

The game changer redefines productivity

GCMS-QP2020 opens new horizons

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The game changer redefines productivity

GCMS-QP2020 opens new horizons

With the introduction of the QP2010 series in early 2000, Shimadzu made a big step in the GC-MS market and has within a few years reached a main player position in the mass spectrometry segment. Now, after a series of innovative products and technologies (please see separate box), Shimadzu introduces the new GCMS-QP2020 combining all outstanding GC-MS features and opening new horizons for the users.

Three choices of carrier gas

With Fast GC-MS technologies embedded, it is already common to use hydrogen as carrier gas on Shimadzu's GC-MS. The QP2020 now offers a third choice with nitrogen in addition to standard helium and hydrogen.

Figure 1: GCxGC-qMS (ZX2-2010)

SmartSIM reduces development time and improves sensitivity

Users of GCMS triple quadrupoles from Shimadzu are familiar with SmartMRM technology which greatly simplifies the creation of analytical methods. The new GCMS-QP2020 gives access to the same SmartSIM feature, reducing development time and improving the sensitivity of the chosen method by optimizing scheduled windows for SIM analysis and increasing the potential number of compounds that can be analyzed in the same run.

LabSolutions Insight platform accelerates quantitative MS data review

Today, laboratory efficiency is driven by highly automated mass spectrometry platforms, delivering large volumes of high quality data. However, manual data review can limit sample turnaround times and reduce productivity, resulting in a bottleneck. To sustain the increase in throughput which is demanded by most routine labs nowadays, the LabSolutions Insight platform accelerates quantitative mass spectrometry data review while opening a new approach in exception-based reporting. The intuitive design makes it simple to use and easy to adapt to individual workflows in the lab. A collection of configurable flag criteria based on industry-standard quality rules can quickly identify peaks required for data review. A click of a button automatically shows peaks for review.

Figure 2: MDGCMS

The LabSolutions Insight platform supports data review over a network, creating new opportunities in remote data review, and enables multiple pane display for two monitors.

Figure 3: LabSolutions Insight

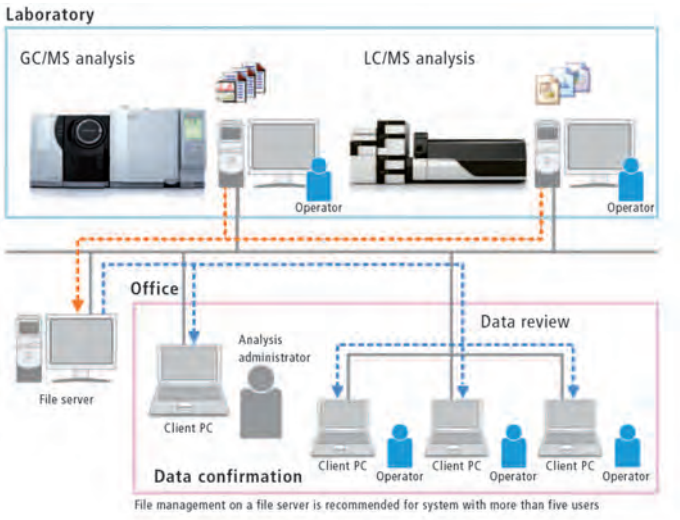


Figure 4: LabSolutions Insight environment

Shimadzu's GC-MS product lines in brief

Fully compatible GC-MS systems

Shimadzu was the first to introduce these instruments for both normal and Fast GC-MS analysis supporting 0.32 / 0.25 / 0.15 / 0.1 mm inner diameter capillary columns without any hardware change. In collaboration with Professor Luigi Mondello's team at University of Messina (Italy), this lead to a compendium on Fast GC showing critical parameters on a system to adjust in order to perform good Fast GC analysis.

GCxGC-qMS known as comprehensive GC-MS

Introducing the fast scanning GC-MS with 10,000 amu/s and later 20,000 amu/s with no skewing in spectra, Shimadzu made a new breakthrough. Together with Chromsquare software dedicated to GCxGC data processing, complex samples such as petroleum extracts, flavor and fragrances samples and environmental or food samples can be screened easily to detect different classes of compounds or identify new compounds in a complex natural extract.

MDGCMS multidimensional technology

This unique tool allows food and flavors companies to check for adulteration of the raw materials they buy.

MDGCMS is an exceptional system for working on flavors, e.g. in wine and coffee, when coupled with sniffers in the first and second dimension. MDGCMS with two ovens and a dean switch tool avoids any retention time shift when selecting a window of time of the chromatogram to be diverted to the second dimension for higher separation.

Twin Line MS

Thanks to the split dual stage turbomolecular design, this configuration allows installation of two different polarity columns on only one GC-MS system, thus reducing pumpdown needed to exchange columns when co-elutions have to be resolved, or when different types of samples requiring either different columns or injection systems are analyzed on the same machine.

Powerful technologies and accessories

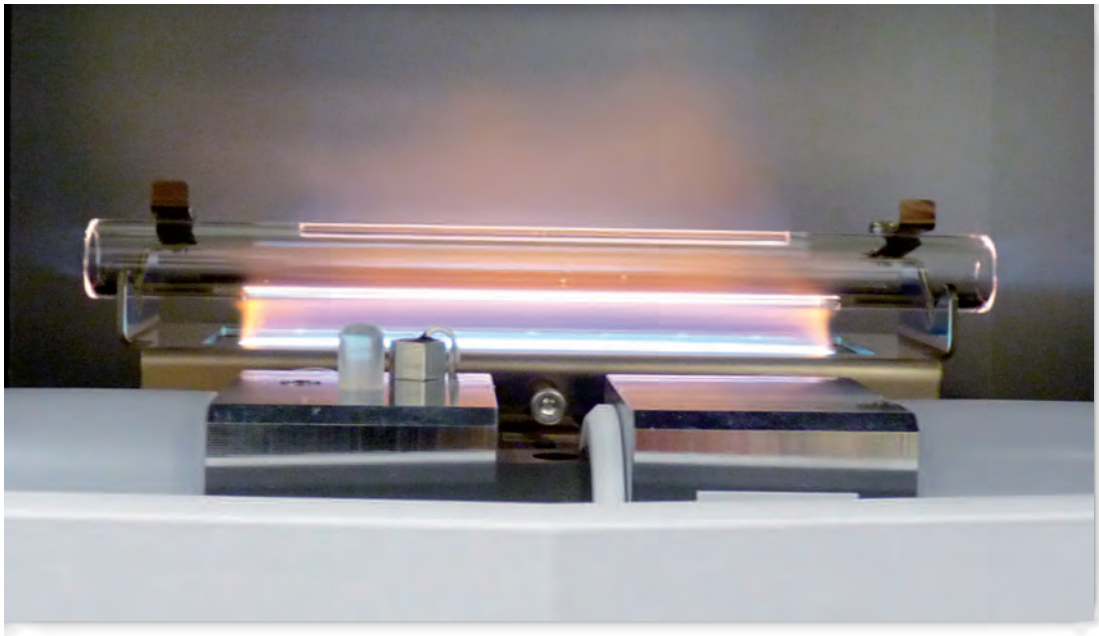
Design of high performance quadrupoles, unique configuration of the ion source with shield plates and dual filament as well as overdrive lens, contribute to the most flexible GC-MS platform available today.

Further information on this article

- Application Handbook Fast GC/GCMS

Higher performance with Atomic Booster

WizAArd software and Atomic Booster optimize AA-7000F sensitivity



Flame AAS is one of the fastest methods in elemental analysis. However, sensitivity of the measuring method often plays a decisive role. How can sensitivity be optimized? This article presents an overview of various optimization approaches using the AA-7000F atomic absorption spectrophotometer.

In order to optimize sensitivity, the flame gas composition as well as flame observation height can be adjusted. These instrument setting parameters are adjusted individually as part of an automated optimization process using the associated WizAArd software.

Fast method creation using the WizAArd software

The WizAArd software provides complete instrument control. Basic parameters for the respective elements are also stored here. In this way, new methods can be created very effectively and require very little time.

Because of the flexibility of the analytical instrument, it is particularly easy to optimize the burner height. It is possible to carry out measurements not only in the flame mode but also in the much more sensitive graphite furnace mode. This option can also be implemented afterwards.

A software-controlled motor enables hands-free switching between flame and furnace mode (AAC: Automatic Atomizer Changer). This motor is also used to adjust the burner height in the flame mode when not using the graphite tube furnace. Flame gas composition can also be adjusted fully automatically via the software.

Optimization of the burner height

In flame AAS, the liquid sample is first nebulized and subsequently desolvated by the thermal energy (temperature) of the flame, and the elements (present in the flame as ionic or molecular species) are further atomized into free atoms. Only in this way the measuring principle does fully apply.

Element-specific light is passed through the flame. The flame acts as a measuring cell, similar to the cuvette used in UV-VIS spectroscopy. The elements present in the flame can now absorb the element-specific light. The more atoms are present, the stronger the attenuation of the light. In addition to qualitative element identification, quantitative measurements can also be carried out.

Depending on the characteristics of the element being investigated, the atoms are present at different heights (temperature zones) in the flame. If the flame observation height (height within the flame where absorbance is measured) is too low, the elements have not yet been atomized and cannot be detected correctly. If the flame observation height is too high, the elements may be present in their excited/ionized state, whereby they are no longer detected.

The sample matrix can have an additional effect on the flame. If, for instance, the sample contains organic compounds such as ethanol or methanol, the flame is generally hotter. This means that the flame must be observed at a lower height, or that the flow rate of the combustion gas must be minimized.

Example cesium

The element cesium (Cs) is used to optimize the burner height.

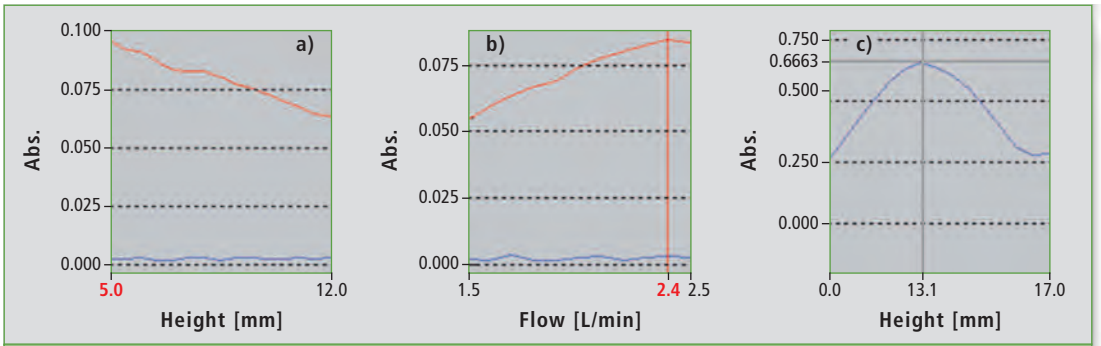


Figure 1: a) Cs – Optimization of burner height; b) Cs – Optimization of flame gas composition; c) Cd Atomic Booster – Optimization of burner height

Cs is quite sensitive to atomic absorption with limits of detection in the double-digit ppb range. In this way, it outperforms ICP-OES (single-digit ppm range). The determination of even lower Cs levels is possible using graphite furnace AAS (double-digit ppt range).

To optimize the burner height, an interval is selected within which various burner heights are tested under simultaneous nebulization of a Cs standard solution. Measuring values are subsequently determined (figure 1a). In this case, the lowest observation height is the optimal choice because Cs is thermally very unstable. Cs atomization already takes place very close to the burner head.

Optimization of the flame gas composition

Two different gas mixtures are mainly used for the flame. In both cases, acetylene (C_2H_2) is the flame gas. Different so-called oxidants are used: air for low flame temperatures and nitrous oxide (N_2O) for higher flame temperatures. For most elements, air will be sufficient to generate free atoms in the flame. For refractory elements with high dissociation energies, higher flame temperatures are required (for instance aluminum or titanium).

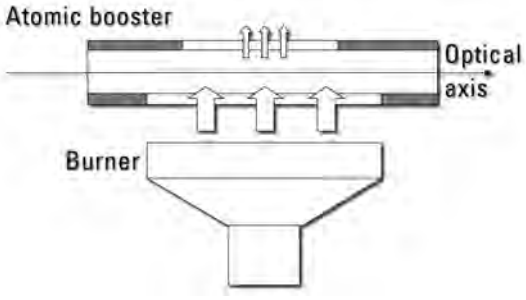


Figure 3: Principle of the Atomic Booster – Dwell time of the atoms in the optical axis is increased

Air-acetylene flames are selected for Cs. This is also stored in the software. Analogous to the optimization of the burner height, an interval is selected for the flame gas flow (acetylene). The resulting plot of absorbance versus flow rate shows a signal maximum at a flow rate of 2.4 L/min (figure 1). This parameter is now stored as the optimum flow rate in the method.

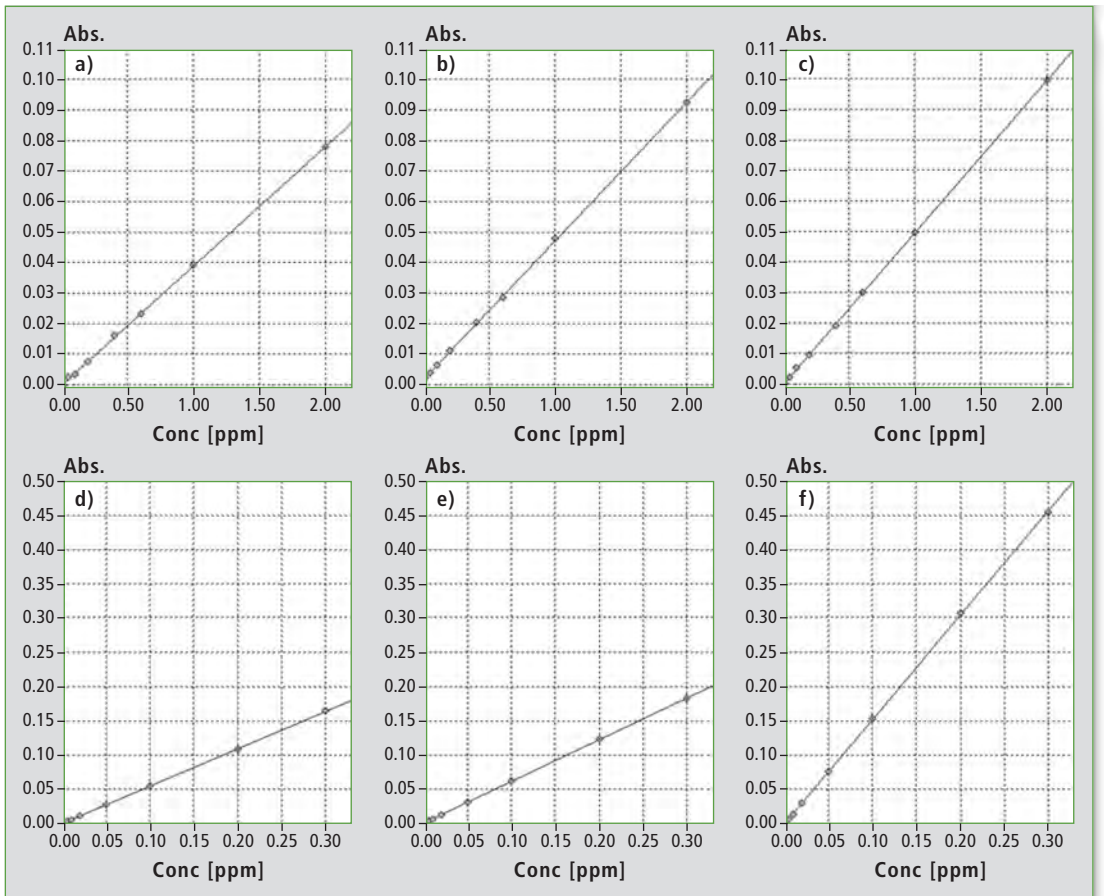


Figure 2: Calibration of cesium and cadmium before and after individual optimizations. Details are provided in table 1 (Page 6)

Effects on the detection limit: cesium

Not only the sensitivity, i.e. the highest measuring signal, but also signal fluctuations have an effect on the detection limit. For this reason, improvement of the detection limit is determined via the linearity of the calibration (DIN 32645). Comparing the values (table 1, page 6), it becomes clear that the detection limit is already significantly improved by adjusting the flame gas composition. Adjustment of the burner height brought about further improvement of the detection limit so that it was possible in standard flame operation to detect concentrations as low as 0.011 mg/L. The effects of the individual steps on the calibration of cesium are shown in figure 1 (a - c).

Atomic Booster – optimization for cadmium

As an alternative to cesium, Cadmium was used for optimization. Cadmium is a heavy metal and is toxicologically very harmful. It is already considered poisonous at low concentrations and has been proven to be carcinogenic as well as mutagenic and teratogenic. For humans it is not required physiologically (non-essential element). This is why trace analysis of this element is of critical importance.

To improve the detection limit of the AA-7000F for this element, the flame gas composition and the burner height were optimized in the same way as for cesium, leading to calibrations with differing sensitivities (see figure 2 d, e). The detection limit for cadmium could also be lowered using these optimization steps. Instead of 1.5 µg/L, a detection limit of 1.0 µg/L could be achieved (table 1). To further increase the detection sensitivity for this toxicologically relevant element, the Atomic Booster is used.

The Atomic Booster is essentially a quartz tube that is positioned in the optical path over the exit slot of the burner. The tube has two slit-shaped openings opposite each other that are different in length. The flame enters the quartz tube through the longer (larger) opening. Parts of the flame can subsequently leave the quartz tube through the shorter opening. However, since the exit opening is smaller, a portion of the flame is held within the optical path or leaves the quartz tube through both open ends (figure 3).

In this way, the dwell time of the atoms in the optical path as well as the 'thickness' of the flame can be increased.

To achieve a maximum energy throughput of the element-specific light (good positioning within the optical axis), the burner height must also be adjusted correctly. The lamp mode is set to emission and the maximum burner height is determined (figure 1c). Measurements using the Atomic Booster take place at a burner height of 13 mm.

By focusing the flame in the optical axis, sensitivity increases significantly, as can be seen in the calibration plot (figure 2f) in comparison to measurements without Atomic Booster (figure 2 d, e). This increase in sensitivity lowers in turn the detection limit by a factor of 2, whereby the detection limit for flame AAS lies within the ppt range (0.52 µg/L).

	Cesium	Cadmium
Standard operation	0.018 mg/L (fig. 2a)	0.00151 mg/L (fig. 2d)
After optimized flame gas composition	0.013 mg/L (fig. 2b)	0.00096 mg/L (fig. 2e)
After optimized burner height	0.011 mg/L (fig. 2c)	
With Atomic Booster	—	0.00052 mg/L (fig. 2f)

Table 1: AA-7000F – Limits of detection of cesium and cadmium before and after individual optimizations (calculated according to DIN 32645). The corresponding calibrations are shown in figure 2.

Conclusion

Using different approaches, the instrument sensitivity of the AA-7000F can be adjusted quickly and easily and optimized to the sample matrix at hand. The supporting functions of the WizAArd software enable automated optimization of burner height and flame gas composition. Detection limits of 1 µg/L (for cadmium) can be achieved in this way. Cesium is also very sensitive with a detection limit of 11 µg/L.

Using the Atomic Booster, further sensitivity increases can be achieved. The principle is quite clear and the results speak for themselves: the detection limit is halved and at 520 ng/L and lies within the ppt range.

The key to success

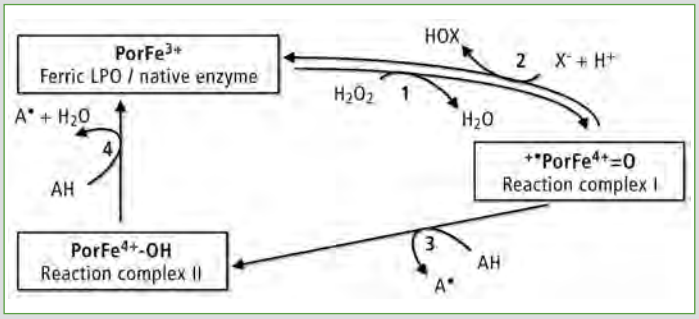
Confirmed by headspace GC-MS: formation of ICN by lactoperoxidase

As part of the innate immune system, the heme protein lactoperoxidase (LPO) is essential for defense against pathogens in mucous membranes and bodily secretions (for instance saliva, tear fluid, milk and respiratory secretions).

This enzyme oxidizes thiocyanate (SCN-) and various small substrates (see box) in the presence of hydrogen peroxide [1]. The resulting hypothiocyanite (HOSCN/-OSCN) is bacteriostatic, passes through cell membranes and specifically oxidizes thiols and selenols. Consequently, these functional groups efficiently activate intracellular proteins [2].

The catalytic and antimicrobial properties of LPO are also used by manufacturers of foods, cosmetics and oral care products, where LPO is used together with a hydrogen peroxide generating system and (pseudo)halogens as preservatives [3, 4]. In some of these systems, iodine is used as the halogen because the resulting hypoiodous acid (HIO) and other reactive iodine species such as I_2, I_3^- and I_2OH^- offer a broader spectrum of activity than HOSCN: thioether groups are also oxidized and iodine is added to tyrosine residues.

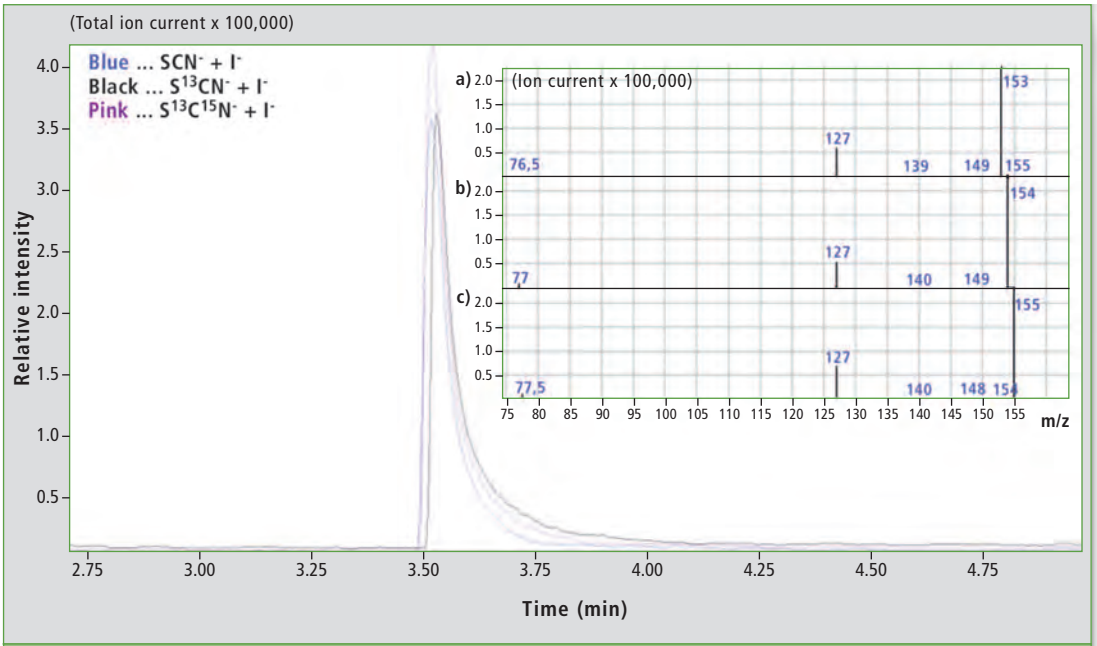
In contrast to the LPO-H_2O_2-SCN^- systems, the I^--containing preparation is also characterized by bactericidal and antiviral properties [5]. This raised the question whether thus far unknown reactive products of LPO were generated. And indeed, the reaction products of LPO-H_2O_2-SCN^-/I systems predicted from the results of ^{13}C nuclear magnetic resonance spectroscopy could be confirmed using headspace gas chromatography-mass spectrometry (headspace GC-MS).



The lactoperoxidase reaction mechanism

LPO belongs to the group of heme peroxidases which oxidize various organic and inorganic substrates in the presence of H_2O_2. Their enzymatic activities are referred to as the halogenation (two-electron oxidation) and peroxidase cycle (two-electron oxidation).

During the halogenation cycle (reactions 1 and 2), the native enzyme is oxidized by H_2O_2 to the reaction complex I, which in turn oxidizes SCN^- and I^-. The myeloperoxidase present in neutrophilic granulocytes was also able to oxidize Cl^- and Br^-. In the case of the peroxidase cycle (reactions 1, 3 and 4), numerous organic substrates, for instance epicathechin present in green tea, are oxidized to their corresponding radicals.



Headspace GC-MS analysis of the reaction product of the LPO-H_2O_2-SCN^-/I^- system. In addition to the chromatograms, mass spectra are shown for the reaction of 4 µM LPO with 10 mM H_2O_2 and 40 mM I^- mixed with (a) 80 mM SCN^-, (b) 80 mM $S^{13}CN^-$ and (c) 80 mM $S^{13}C^{15}N^-$.

Method

In the first step, the compounds of the basic systems consisting of LPO-$S^{13}CN^-$ or I^- were mixed in phosphate buffer pH 7.0, the reaction was initiated by the addition of H_2O_2 and the resulting reaction products were analyzed using ^{13}C-NMR. The second step involved the combined use of SCN^- and I^- in various concentration ratios. It was found that with an excess of iodine, a new unknown reaction product for LPO was formed. Additional analyses revealed that iodide is first oxidized, this oxidized product subsequently reacts with thiocyanate yielding cyanogen iodide (ICN) [6].

Headspace analysis for the detection of ICN

To confirm the ^{13}C-NMR results, a second detection technique was applied. As ICN is volatile, sample analysis using GC-MS was suitable. Due to the high volatility of ICN and the solvent exchange needed from water to an organic solvent, liquid injection was unsuitable for this investigation Headspace analysis was therefore applied for the detection of ICN.

This method is an elegant alternative to conventional GC-MS since, analogous to NMR, no further workup of the reaction mixture was required. Another advantage in the analysis of this reaction with unstable products was the short amount of time needed to introduce the analytes into the system.

The samples were analyzed using Shimadzu's GCMS-QP2010 Ultra and the HS-20 headspace autosampler. By heating the reaction vessel to 60 °C, the analytes could be transferred directly into the headspace and a GC-MS analysis could subsequently be carried out.

Conclusion

It could be shown that a ratio of 1:2 of I^- to SCN^- will lead to the production of ICN (figure 1a), while an equimolar use of both substrates did not result in the formation of a product (not shown). This is probably due to the fact that the value of the rate constant for the oxidation of I^- by reaction complex I of the LPO is only half of that compared to oxidation by SCN^-. Therefore, the required oxidation is not catalyzed.

The formation of ICN at an excess of iodine could be confirmed by comparison with a reference standard regarding retention time and mass spectrum (not shown). Furthermore, $S^{13}CN^-$ (figure 1b) and $S^{13}C^{15}N^-$ (figure 1c) in the system mentioned were measured and supported the identification of ICN. In the underlying spectra for the molecular ion ICN^{++}, shifts of the m/z values of 159.9 to m/z 153.9 and m/z 154.9 corresponding to the 1 u- and 2 u-mass increase of the marked ions were detected.

In addition, the doubly charged molecular ion at m/z 76.5 (figure 1a), m/z 77.0 (figure 1b) and m/z 77.5 (figure 1c) was identified. Likewise, the corresponding fragments were detected. To begin with, I^+ with m/z 127.0 was detected in all spectra. Next, fragmentation within the triple bond led to the formation of IC^+ with m/z 139.0 (SCN^-, figure 1a) and m/z 140.0 ($S^{13}CN^-$ and $S^{13}C^{15}N^-$, figure 1a and c).

The headspace GC-MS measurements confirmed that the combined use of SCN^- and I^- in an H_2O_2 activated LPO system contributes to the formation of a new product. How this product complements the described bacteriostatic or bactericidal properties of HOSCN or HOI/I_2 must be clarified in further investigations.

Authors

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New solution for the UV-VIS-NIR range

ISR-1503 series with large integrating sphere and three detectors in two white standard versions

Inspired by the successful concept of the triple-detector analytics for the UV-VIS-NIR range, the large integrating sphere of the Shimadzu UV-3600 Plus series has been redesigned. It has a diameter of 150 mm, and it is made of either $BaSO_4$ or Spectralon®. It integrates three detectors that can be jointly controlled

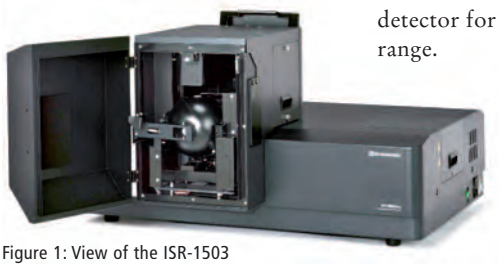


Figure 1: View of the ISR-1503

over the entire measuring range: a PMT (photomultiplier tube), an InGaAs detector and a PbS detector.

In comparison with the predecessor model, its design has a more spherical shape in order to realize an aperture ratio of less than 3 % at 0° reflectance, even in the presence of a third detector. This way, the sphere meets the requirements for applications with a low aperture ratio. This parameter is specified by national and international standard methods such as EN, ASTM and JISZ8722 "Methods of color measurement – Reflecting and transmitting objects".

Two versions of the ISR-1503 are available:

- the ISR-1503 coated with $BaSO_4$
- the ISR-1503F coated with Spectralon (Teflon), which does not take up any moisture. This is an advantage for the NIR range, which sensitively reacts to the presence of surface-bound water. The near-infrared range does not exhibit any absorption of water, as can occur in barium sulfate measurements in this measuring range, when the sample is not embedded in $BaSO_4$.

The ISR-1503/1503F includes a photomultiplier for the ultraviolet and visible range as well as an InGaAs detector and a cooled PbS detector for the near-infrared range.

High measurement stability

The 150 mm integrating sphere is exceptionally well suited for samples with strong light scattering and uneven and rough surfaces. The large sphere collects the strongly reflected light and generates high measurement stability without being too strongly influenced by surface structures.

Due to the design of the sphere, it is possible to place the sample onto the sphere horizontally for transmittance measurements. For reflectance measurements under an angle of incidence of 0° or 8°, the sample can also be put alongside of the sphere (vertically) or under the sphere (horizontally). This way, different physical properties can be measured, such as total transmittance, relative directed reflectance and diffuse reflectance of the sample under investigation.

Properties:

- Wavelength range: 250 to 2300 nm
- Integrating sphere: 150 mm internal diameter
- Detector: integrated PMT, InGaAs and PbS detectors
- Sample positioning: transmittance, 0° reflectance – horizontal; 8° reflectance – vertical
- Aperture ratio: reflectance measurement (0°) 3 %
- Angle of incidence: 0°/8°
- Material of the sphere:
  ISR-1503: $BaSO_4$
  ISR-1503F: Spectralon (Teflon)

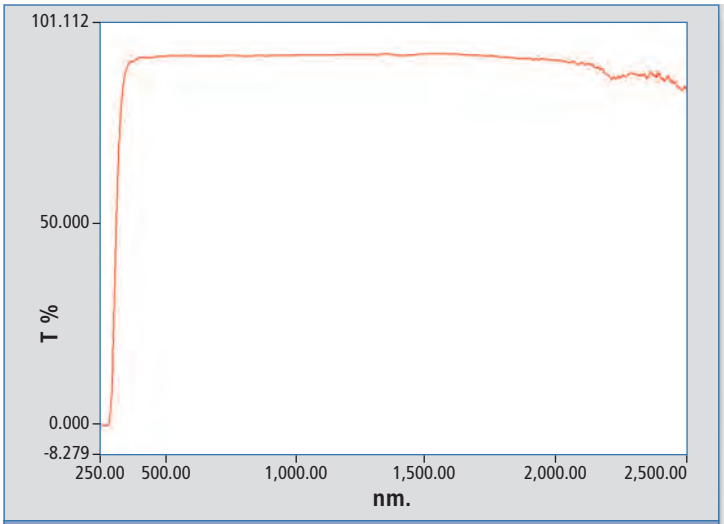


Figure 2: UV-VIS-NIR spectrum of a structured glass measured with the ISR-1503F

Solar glass for photovoltaic thermal solar collectors

Reflectance spectra of solid materials like powders, paper and textiles can be measured using the ISR-1503 series. Transmittance measurements of liquids and transparent solid materials can be carried out.

For solar glass, structures are applied onto the glass surface. In one method, specially designed moulding rollers are used to stamp patterns on both sides of the glass surface. The structures on the surface must ensure a high solar transmittance factor for perpendicular irradiation.

In addition, a minimum loss of reflectance for angles of incidence of sunlight that are different from the perpendicular is required. This is specified as the angular factor. A high angular factor yields a good energy production balance.

Shown here is the measurement of a double-sided structured glass with a layer thickness of approximately 5 mm. The measurement was carried out in the transmittance mode (figure 2). The transmittance baseline is at 92 % transmittance. This corresponds to the luminous efficiency that is measured with flat glass in direct transmittance mode. Flat glass loses approximately 4 % transmittance on each surface under the condition of direct transmittance.

Conclusion

Using the 150 mm-diameter integrating sphere, the determination of the characteristics of a structured glass sample is simple and fast. Combining three detectors within the integrating sphere allows measurements up to the NIR range and also allows for the determination of heat permeability. Various transmittance and reflectance parameters can be determined using the ISR-1503.The aperture ratio allows for stable measurements of the structured surfaces.

New application for the TOC parameter

Testing of plastic packaging in the pharmaceutical industry

In the pharmaceutical industry plastic packaging is used in various forms – for example for intravenous bags, bottles, cartridges or pre-filled syringes. The packaging must be tested for suitability for these particular uses.

The United States Pharmacopeia has published two new chapters to this effect (661.1 and 661.2), which will be valid from May 2016.

- Chapter 661.1 describes the characterization and testing of the individual plastic materials used in the manufacture of the plastic packaging.
- Chapter 661.2 deals with the required testing of the final packaging system since packaging often consists of more than one plastic material.

Characterization takes place by identifying and determining the bio-compatibility, physio-chemical characteristics and extractable metals.

TOC – determination

The TOC parameter as an indicator for extractable organic material is part of the physio-chemical characteristics that must be determined. For this purpose, the plastic material used is weighed, mixed with ultra-pure water and subsequently heated. The amount used and the extraction temperature employed depend on the plastic to be tested. The TOC of the ultra-pure water is subtracted from the measured value of the extraction solution. The resultant TOC value must not exceed 5 mg/L.

For testing the packaging system, it is filled with ultra-pure water, sealed and heated in an autoclave. The temperature and dwell time depend on the plastic used. In order to determine the blank value, ultra-pure water is poured into a glass flask and is heated to the same temperature. The TOC of both solutions is determined. The difference between the two measured TOC values should not exceed 8 mg/L.

TOC determination according to USP <643>

The general TOC determination is described in USP <643>. The TOC systems must be able to differentiate between inorganic and organic plastic; this can be achieved by removing the inorganic carbon (NPOC methods) or by separate determination (difference method). The detection limit is 0.05 mg/L. The suitability of the system must be verified in a system suitability test.

In the controlled ultra-pure water (purified water and water for injection purposes), the TOC must not exceed a value of 0.5 mg/L. In the actual application, the TOC value may be higher. Thus, a linear range of 0.2 to 20 mg/L TOC is required.

TOC determination in pharmaceutical applications

In TOC analytics, two oxidation techniques have gained acceptance: catalytic combustion and wet-chemical oxidation. Catalytic combustion converts the carbon compounds into CO_2 using high temperature and a catalyst; it is then detected with an NDIR detector. Wet-chemical oxidation uses the combination of UV radiation and persulphate to achieve oxidation. Both methods are suitable for TOC determination in pharmaceutical applications.

Two TOC systems for pharmaceutics

Shimadzu offers two systems which are perfectly suited for use in the pharmaceutical industry:

- TOC-$V_{WP/WS}$ applies wet-chemical oxidation
- TOC-L_{CPH} works with the catalytic oxidation method at 680 °C.

With their large measurement ranges from 0.5 µg/L to 30,000 mg/L, they support every application – from ultra-pure water to highly-polluted water (e.g. from cleaning validation to extraction solutions to waste water).

TOC-L series – catalytic oxidation at 680 °C

The ISP module (Integrated Sample Pretreatment) for the TOC-L series significantly reduces workload, since it carries out dilution, acidification and sparging. The measuring range is expanded by automatic dilution from 4 µg/L to 30,000 mg/L.

Additionally, the combustion technology can be coupled with the TNM-L module so that the total combined nitrogen (simultaneous TOC/TN_b determination) is recorded with just one injection. Here it is worth referring to the EN standard determination concerning chemiluminescence detection. Catalytic combustion occurs here at 720 °C. Simultaneous TOC/TN_b determination is particular interesting for cleaning validation since a differentiated consideration between the cleaning substance and the product is potentially possible.

TOC-V series – wet-chemical oxidation

The key technology of the TOC-V series is the powerful oxidation through the combination of sodium persulphate and UV oxidation at 80 °C. Since a persulphate solution is used for the determination, it is important that it does not contain any impurities that could falsify the actual measured value. For this purpose, the TOC-V_{WP} has an automatic reagent preparation which removes potential impurities from the persulphate solution. This ensures that the TOC value recorded actually comes from the measuring sample – and not from the reagents solution used. Together with the large injection volumes (up to 20.4 mL) and the highly-sensitive NDIR detector, this results in an extremely low detection limit and outstanding reproducibility in the lower ppb range. For this reason, the TOC-$V_{WP/WS}$ is particularly suited to TOC determination on an ultra-trace level.

Summary

Both types of device with their various oxidation methods are suitable for TOC determination according to the American Pharmacopoeia, USP <643> and USP 661.1/661.2. Both include the required measuring ranges: the lower range (0.5 mg/L) as well as up to 20 mg/L. The measuring ranges are achieved by adjusting the injection volume and then calibrating accordingly. No further adjustments are necessary.

Literature

Source: www.usp.org

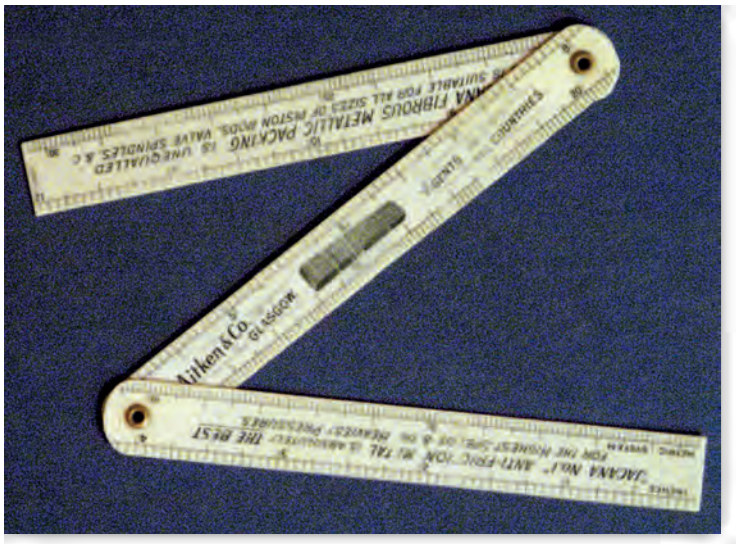


Figure 1: A folding ruler made around 1900

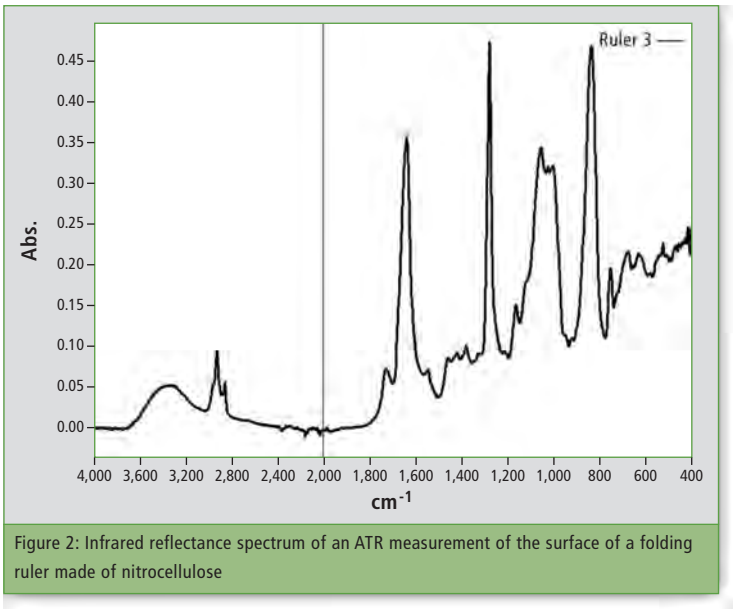


Figure 2: Infrared reflectance spectrum of an ATR measurement of the surface of a folding ruler made of nitrocellulose

Tiny ivory chess piece?

Identification of ivory using infrared spectroscopy

From 1895 until 1910, a sugar mill named 'Oriente' existed on the Caribbean island of Puerto Rico. After the mill closed, many heirloom pieces passed into the hands of the next generation and are considered to be antiques.

Now in the third generation, the question arose as to the value of the objects. Their authenticity has been confirmed. They are made either of natural materials which are now banned (ivory for instance, under the so-called 'Washington Convention', the Convention on International Trade in Endangered Species of Wild Fauna and Flora) or of materials that have been replaced by less flammable, cheaper or possibly more durable substances.

Age and authenticity are criteria used to classify these objects as antiquities. For age the following applies: the object must be at least 100 years old. Occasionally, more than 50 years has been specified.

Natural material or imitation?

The use of historical materials can be a criterion for classifying objects as genuine antiques and determining that they are not an imitation. Table 1 provides a list of 'plastic' materials from the past. Natural materials such as rubber, linseed oil or casein have been used. Cellulose and nitric acid were used to produce the first artificial silk (nitrocellulose). This type of silk was also known as Chardonnet silk.

By the addition of camphor as a solvent, it was possible to produce the first thermoplastics (celluloid) from nitrocellulose. They were not as highly flammable and were used in the production of many consumer goods or as substitute materials (imitations) in the manufacture of objects that would otherwise be made of ivory, amber, horn or mother-of-pearl.

Celluloid or mother-of-pearl or?

The small folding ruler (with metric and inch scale) shown in figure 1 was made by the company Aitken & Co. Aitken, as well as Mirrless Watson from Glasgow, supplied machinery to sugar mills in the United States around 1900. In this way the folding ruler travelled from Scotland via New York to Arecibo, Puerto Rico to the Oriente sugar mill and later on, after the mill was sold, to Bremen, Germany.

To find out if this folding ruler consisted of celluloid, a diamond-

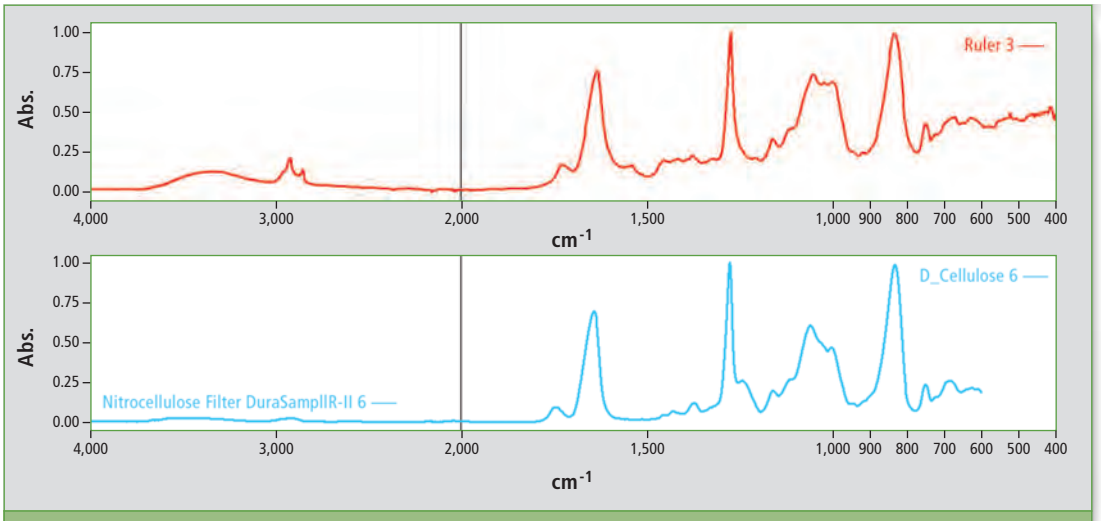


Figure 3: Search result for the surface of the folding ruler – nitrocellulose was identified

Figure 4: Mini chess figure made of walrus tusk, shown with a one Eurocent piece to visualize the size of the figure

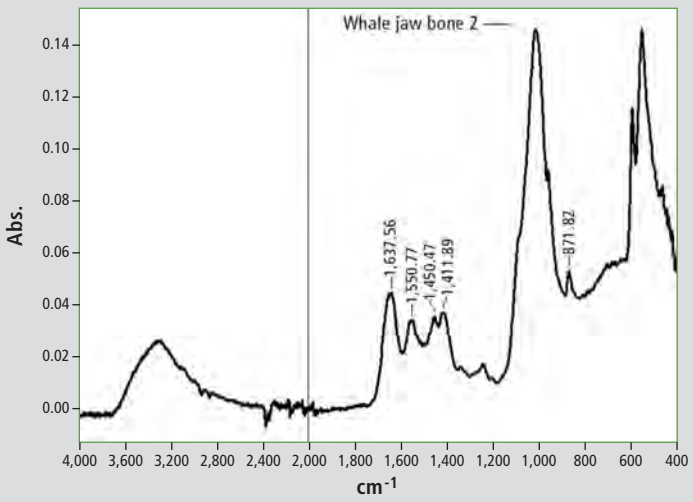


Figure 5: ATR infrared spectrum of walrus tusk ivory, recorded using a diamond single-reflectance unit

ATR unit was used to record an infrared spectrum of the ruler's surface (figure 2). The folding ruler was clamped against the ATR unit and the surface was pressed onto the diamond window. Figure 1 shows the ruler and figure 2 the spectrum of its surface measurement.

Via a library search, the spectrum could unequivocally be assigned to nitrocellulose (figure 3).

Horn, tooth or bone?

Another interesting topic is ivory, which was in the past often used to make jewelry.

Sources of ivory were not limited to elephant tusks and rhinoceros horns, as the long tusks of aquatic animals such as walrus were used as well. The Puerto Rico inheritance also included a tiny chess piece made of walrus tusk (figure 4, photographed next to a one Eurocent coin for size comparison).

A fragment was removed from the bottom of the chess piece and this sample was examined using ATR spectroscopy. The infrared spectrum obtained exhibited inorganic signals. Because ivory is a hard material, the ATR spectrum also exhibited noise in the absorption range of diamond.

This amplifies the CO_2 signal from air at 2,300 cm^{-1} and the noise from water vapor in the air at 3,500 and 1,600 cm^{-1}.

Year	Name	Starting materials
1839	Rubber	Natural rubber vulcanized with sulfur
1860	Linoleum	Linseed oil
1865	Celluloid	Nitrocellulose and Camphor
1885	Chardonnet Silk	Cellulose and Nitric acid
1897	Galalith	Casein and formaldehyde
1910	Bakelite	Phenol and formaldehyde
1913	PVC	Vinyl chloride

Table 1: Chronological assignment of the 'early' plastics

This noise effect is due to the sample not covering the measuring window completely.

The infrared library search led to apatite. This result is correct, as teeth or bone contain apatite. According to the current literature, the apatite here is hydroxyl apatite, a calcium phosphate mineral that, depending on functionality, is found in varying concentrations in bones, hooves, horns or teeth. Figure 7 shows the spectra of different sources (equine hoof, whale jaw bone, apatite mineral and the chess piece).

The spectra shown do not represent pure substances, but as they are natural compounds, their spectra arise from a sum of various substances.

A collagen spectrum may be seen in the apatite spectrum as a further natural substance. Both the apatite region (1,200 – 800 cm^{-1}) as well as the protein region (1,700 – 1,400 cm^{-1}) can be used for more specific determination of the natural material. It helps to distinguish between horn, teeth and bone and to use specific signals for age determination. [1]

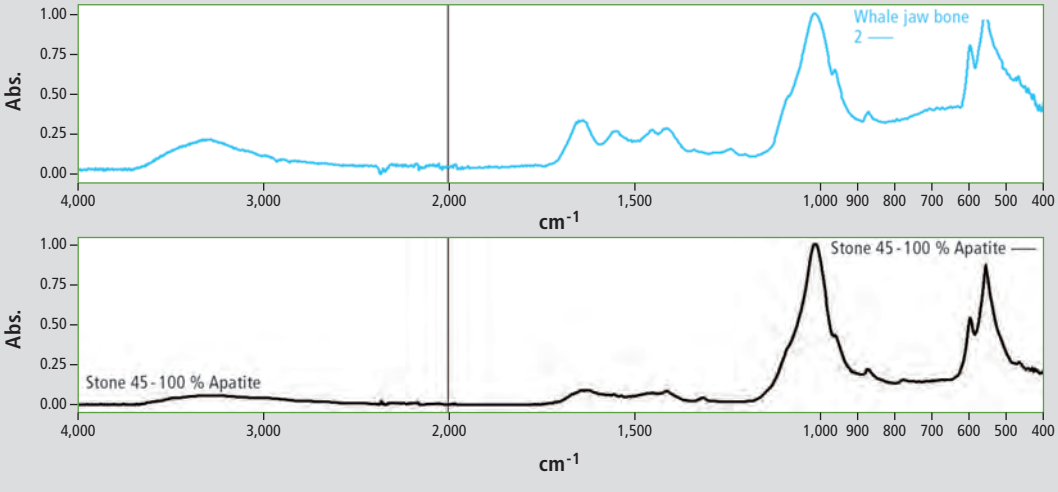


Figure 6: Result of the library search, infrared spectrum of apatite (1430, 860 cm^{-1} carbonate bands, 1050 phosphate bands) with traces of protein deposits (1660, 1550 cm^{-1})

The measurements were carried out using Shimadzu's IRTracer-100 and a Specac Quest diamond unit. The sample stage of the diamond window has a diameter of about 2 mm.

Summary

Infrared spectroscopy can be used for the direct analysis of natural and synthetic materials. Using spectral library searches, identification can be achieved quickly.

In this way, it is possible to determine the material characteristics of antique objects within a very short time. For ivory and wood, it is possible to determine the age of the object.

Literature

[1] „Die Anwendung der spektroskopischen Analyse auf die Datierung von organischen Materialien ist patentiert."
It. Patent Nr. 01266808 – G. Matthaes, 1993

Thanks to Bernhard Westphäling for the interesting topic, his consultation and his participation as co-author.

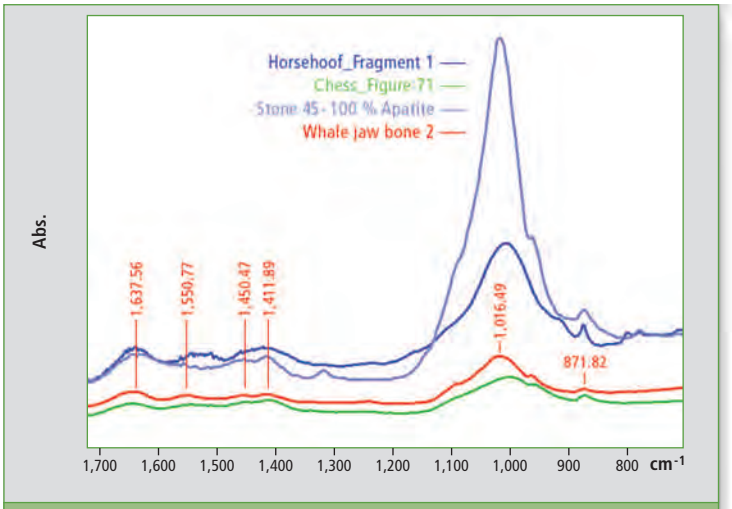


Figure 7: Infrared spectra of bone, teeth and hooves of various origins and an apatite infrared spectrum. In the walrus tusk spectrum, several signals are marked that are relevant for identification.

Simple method development for SFC

Robust, reliable alternative to conventional LC

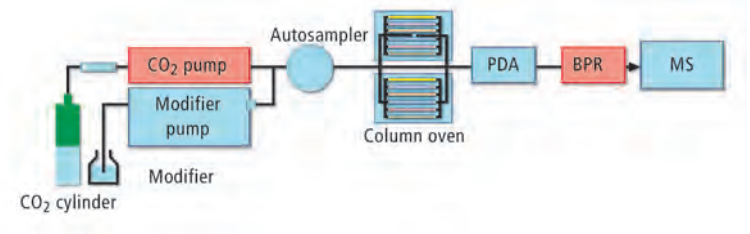


Figure 1: Schematic representation of the Nexera UC method scouting system

Already in the late 18th century, it was discovered that heavy non-volatile organic compounds could dissolve in certain inorganic gases when these gases are present above their critical point, i.e. in their supercritical state. The first ideas to use these gases as mobile phases in chromatography were launched in 1957. In the early 1990's it was shown that the addition of polar solvents could achieve retention time control and this marked the birth of modern supercritical fluid chromatography (SFC).

In supercritical fluid chromatography, so-called 'supercritical' carbon dioxide is used as the mobile phase. Supercritical CO_2 is described as a fluid state of carbon dioxide, whereby it must be held above its critical temperature of 31.1 °C and above its critical pressure of 73.8 bar. In this supercritical state, the density of the medium is vastly influenced by changes in temperature and pressure, and its physical and thermal properties lie between those of the gas and the liquid phase.

The compressibility and the diffusion coefficient of the supercritical solvent are higher, while the viscosity is reduced in comparison to the pure liquid.

Fast, efficient chromatographic separation

By using 'supercritical' CO_2, chromatographic separation becomes faster, more efficient, cheaper and more environmentally friendly than with the use of toxic organic solvents such as hexane, heptane or chloroform, as is customary in normal-phase HPLC. CO_2 in the supercritical state is non-polar and its solvent strength is often increased by addition of a polar modifier. As soon as an additional organic solvent is used, the mobile phase is not truly 'supercritical' but the terminology is used regardless. Many solvents are miscible with CO_2 (for instance MeOH, EtOH [hydrogen bonds], ACN [dipoles]) and offer additional interactions.

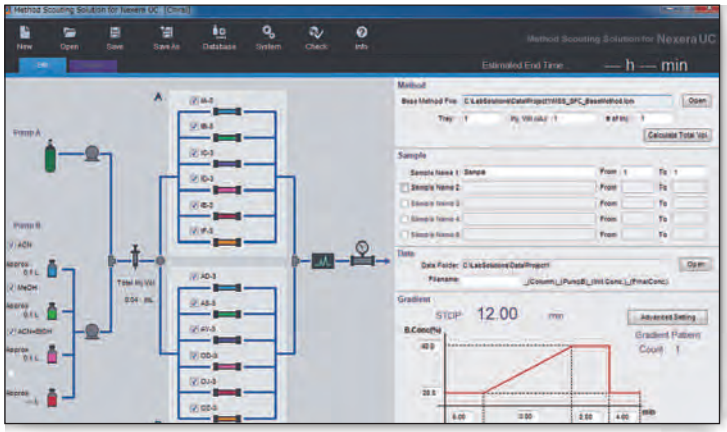


Figure 2: GUI of the method scouting solution of the Nexera UC

In this way, they induce controlled changes in retention and provide a valuable tool to improve the selectivity of a separation.

Method Scouting for automated method development

Method development is greatly simplified using the new Nexera UC system with the method scouting option. This option enables rapid testing of combinations of up to twelve separation columns with up to four modifiers in addition to supercritical CO_2 (schematic representation figure 1).

The associated method scouting solution platform creates all scouting methods automatically in accordance with predefined method parameters (figure 2). The different combinations of modifiers with various columns can also be tested in combination with different gradients.

Separation of chiral compounds

A very good application example is the separation of chiral compounds, which are often best separated using SFC. For two newly developed substances (PS 101 and PS 132) each having a chiral center, a good separation could be obtained quickly and easily using the Nexera UC method development scouting system.

For this purpose, two modifiers methanol and methanol containing 0.1 % formic acid have each been tested at a column temperature of 40 °C, a flow rate of 2.0 mL/min and an applied pressure of 150 bar behind the column and the detector for different columns. Six different chiral separation columns have been tested: Lux Amylose-1 and -2, Lux Cellulose-1 to -4, all with column dimensions 250 x 4.6 mm and a particle size of 5 µm. The individual chromatograms for sample PS 101 are shown in figure 3. The Lux Cellulose-3 column achieves by far the best separation of both enantiomers.

A very similar result in terms of best separation was achieved for sample PS 132 on the Lux Cellulose-3 column as shown in figure 4. For both separations, the simplest and best option is an isocratic solvent composition with 30 % MeOH + 0.1 % formic acid over the entire run.

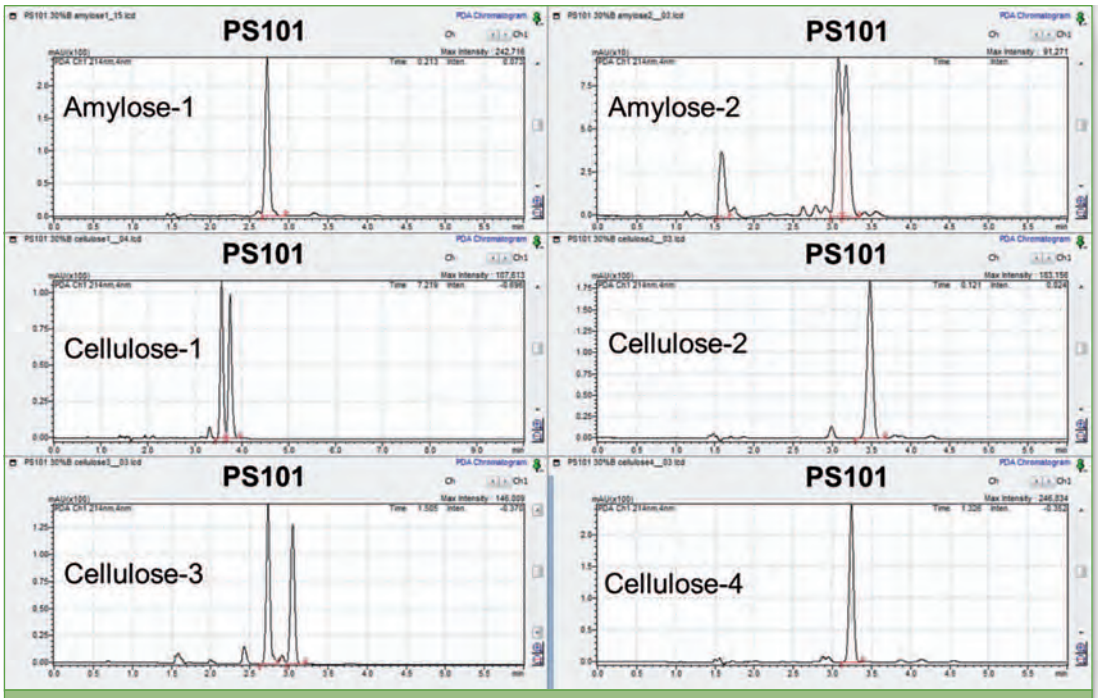


Figure 3: Chromatograms of PS 101 on different columns

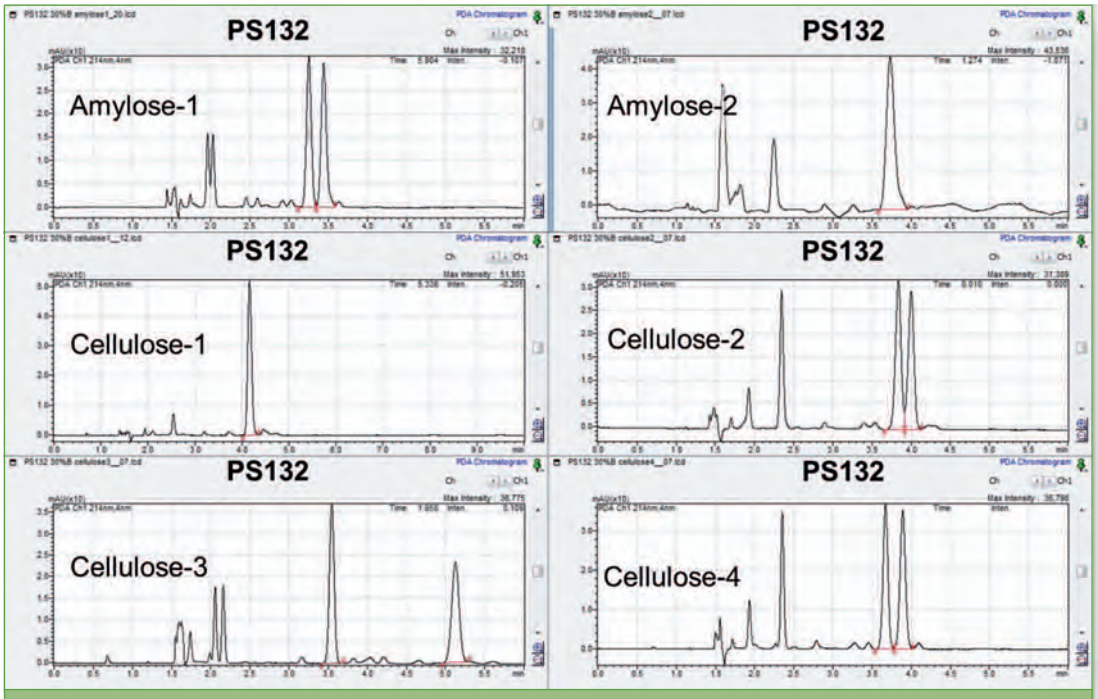


Figure 4: Chromatograms of PS 132 on different columns

Detection can be achieved using a photodiode array detector (PDA/DAD) as well as using a mass spectrometer (LC-MS).

Conclusion

A fast and simple method for screening and separation of chiral compounds on different columns has been developed in a short amount of time. A system was used which allows testing of up to twelve columns in combination with four modifiers as additives to CO_2. The method is optimized in terms of separation and sensitivity. Simultaneous recording of a UV or a PDA signal together with a mass signal via an LC-MS detector is possible within one run. A selectivity of > 1.5 with an RSD < 2 % for the retention time was achieved for all compounds shown.

With this system design, the development of an SFC separation method is very similar to that of HPLC method development. As shown in this example, supercritical fluid chromatography offers a robust, reliable and simple alternative to the conventional LC in use for decades.

Colorful light for good mood...

Emission measurements of glow sticks in party bracelets

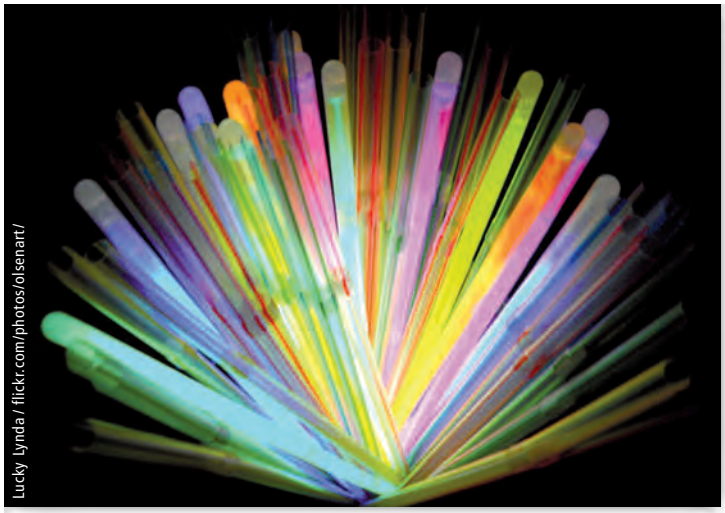


Figure 1: Glow sticks used for party bracelets

Glow sticks are increasingly popular at major events like concerts, carnival sessions or New Year's Eve parties – even birthday parties. But they are also used in high-performance applications, for instance for illumination purposes in professional diving processes. Or in general, as a light source when no other light sources are available. They create colorful lights and bring about good cheer; they light up continuously or flicker, their luminosity can last for a short time or longer (figure 1).

Glow sticks have in common that they light up due to the power of chemiluminescence and that they do not require any external energy sources (power outlet). Chemiluminescence is caused by a chemical reaction between certain substances that results in the emission of light (luminescence). Simple light sticks (glow sticks) typically consist of a polymer tube filled with a dye and a solvent. Inside the tube, there is a glass vial, which contains additional chemicals that are needed to catalyze the chemical reaction.

Which substances and chemicals are present in these glow sticks? What are their chemical properties? Are they hazardous when they get into the hands of children?

Reaction fluids identified using FTIR spectroscopy

FTIR spectroscopy allows, with a minimum of effort, the determination of the polymer tube material. An infrared library search identified polyethylene as the best hit for the polymer tube spectrum (left spectrum). To identify the reaction fluids in a glow stick after completion of the chemiluminescence reaction, the resulting yellowish, oily fluid was placed onto the ATR measurement accessory and measured using a single-reflectance unit. The result is seen in figure 2 (right spectrum), an infrared library search identified dimethyl phthalate as the best hit for this oil.

Phthalic acid esters (for instance dimethyl phthalate) are used as solvents for esters, like butyl benzoate. This is consistent with the FTIR measurement and identification of the oily fluid. According to the packaging specifications, the glow sticks investigated contain the substance butyl benzoate. This substance is an ester of butyl benzoic acid and is one of the chemicals in the chemiluminescence process discussed here. Luminescence is very intense and therefore only a small amount of this substance is needed for luminescence to occur.

What happens inside the glow sticks?

According to the instructions, the polymer stick must be bent. The resulting snapping noise is caused by the glass vial inside the polymer tube breaking. The polymer stick subsequently emits an intense glow. Depending on the fluorescent chemicals used, different colors of light are emitted. A chemical conversion process known as 'peroxy-oxalate chemiluminescence' takes place. An example is the reaction between diphenyl oxalate and hydrogen peroxide, as shown in figure 3.

The oxalate ester reacts with the hydrogen peroxide to produce phenol (2ROH) and the short-lived and high-energy reaction intermediate 1,2-dioxetanedione, which in turn reacts to CO_2 in the presence of the fluorescent dye. At the same time, an electron in the fluorescent dye molecule is promoted to the next unoccupied molecular orbital (excited state). This process is possible because the electron hits the π-electron cloud of the dye molecule (table 1, dyes).

Luminescence occurs when an electron within the electron cloud is promoted into an energetically higher unoccupied orbital level (excited singlet state). From this excited state, the electron falls back to the ground state under the emission of photons (h*√) [1]. This luminescence effect can be analyzed using emission measurements in the visible spectral range. Table 1 lists some of the fluorescent dyes used in this application.

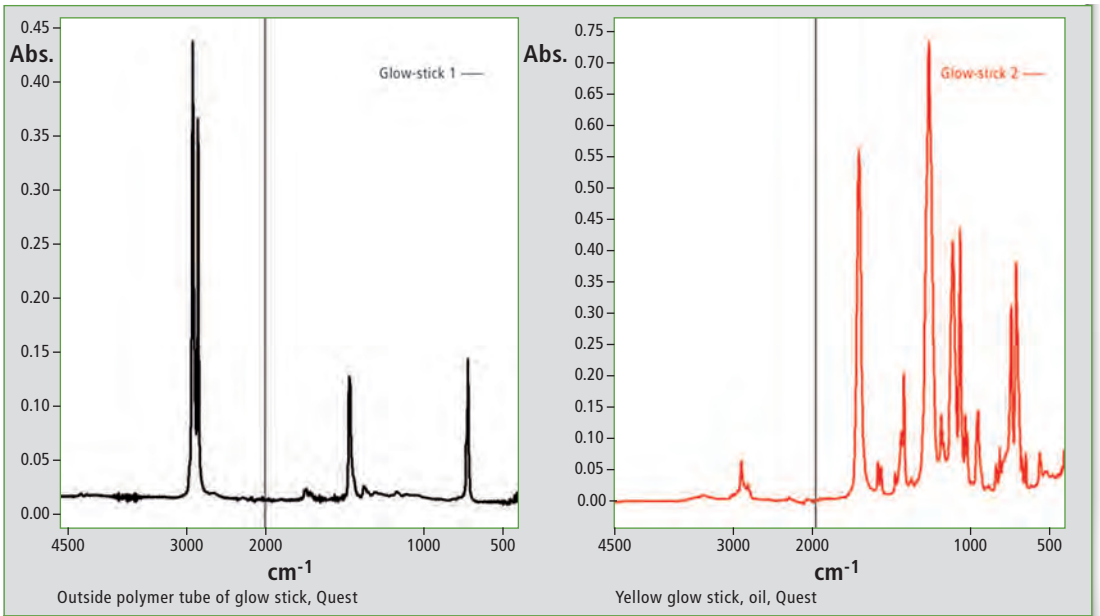


Figure 2: Infrared spectra of the polymer tube and the oily reaction fluid.

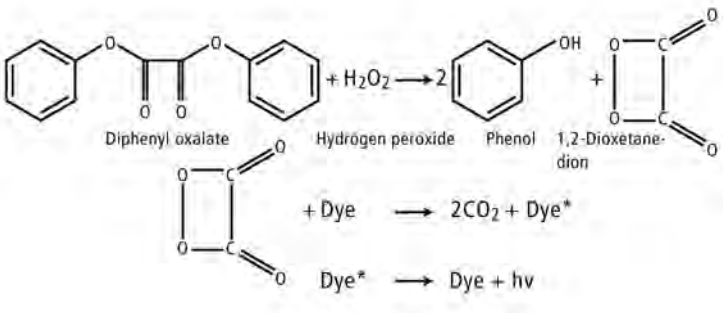


Figure 3: Chemical reaction of the substances in a glow stick

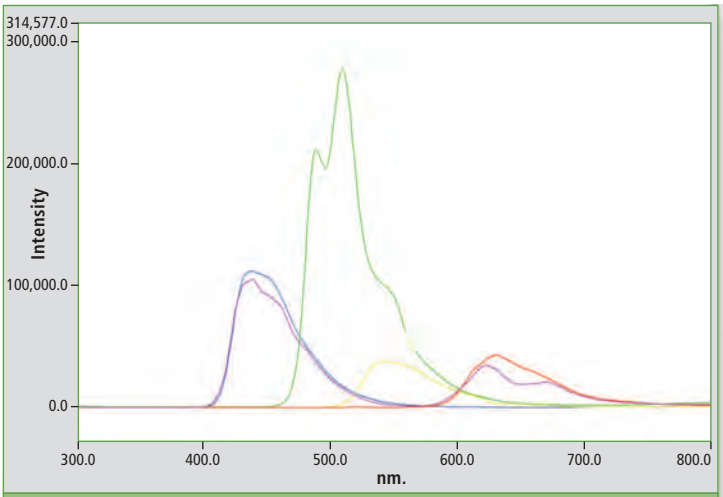


Figure 4: Emission spectra of five party bracelets. The colors of the spectra correspond to the glow stick colors.

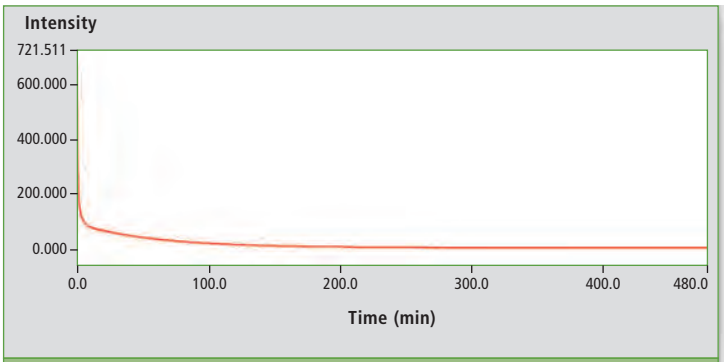


Figure 5: Kinetics of the luminescence of a red glow stick over a time period of eight hours. The curve shows the decrease of intensity versus time.

How is chemiluminescence detected?

Chemiluminescence emissions from chemical reactions can be measured with Shimadzu's RF-6000 spectrofluorophotometer. Luminescence qualification as well as time-dependent observation is possible.

For this purpose, a shutter was placed in front of the excitation light inlet window of the optics of the RF-6000 to prevent excitation light from entering the sample compartment. The second optics for emission measurement can now be implemented for measuring the light emission of the glow stick. As glow sticks are very light intensive, the emission optics are attenuated using optical filters (mesh filters) to reduce the amount of light reaching the detector. Because the glow sticks used in this application are longer than the sample compartment, the small lid inside the sample compartment cover was removed to insert the sticks into the sample compartment. To prevent external light from entering the sample compartment during measurement, the opening was covered by a blackout curtain.

All five bracelets contained in the package in the colors blue, yellow, green, red and pink-purple were measured consecutively. The assignment of color is subjective. It is therefore advisable to process the obtained spectra from the visible spectral region using color determination software. The color pink-purple can then be more unequivocally assigned, as assessed according to standardized color scales.

The emission spectra of the five glow sticks are shown in figure 4. The displayed colors of the spectra correspond to the appearance of the luminescence.

Luminescence decay curve of a glow stick

As the light emission duration was advertised to be approximately eight hours, a corresponding kinetic study was started. For this purpose, a glow stick was placed in the sample compartment for measurement immediately after activation of the luminescence at known luminescence wavelength (629 nm for red). It was found that the highest luminescence intensity was achieved within the first seconds and minutes.

The emission initially decreases strongly and then turns into a faint glow, which continuously decreases over time to a constant value after about 170 minutes (figure 5, total time eight hours).

Conclusion

Glow sticks, which among other things are used as party bracelets, should not fall into the hands of children. The polyethylene tubes contain chemicals that smell strongly and can stain textiles. In addition, after bending the glow stick, the polymer tube contains glass splinters. The polymer tube itself is quite thick but it can be cut easily with scissors. Under normal conditions, everything stays in the tube and can be disposed of after use.

The chemiluminescent colors can be determined using Shimadzu's RF-6000 spectrofluorophotometer and the luminescence time is in agreement with the times indicated on the glow stick packaging: a time period of about eight hours in which the intensity, as shown by the time curve measurement – the kinetics – decreases significantly within the first few minutes.

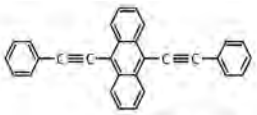
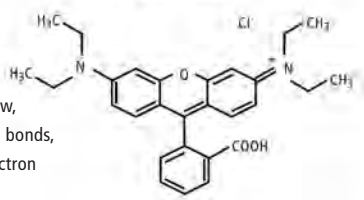
Substance	Structure	Color
9,10-Bis(phenylethynyl) anthracene		Green
Rubren (5,6,11,12-Tetraphenyl-naphthacene)		Yellow
Rhodamine B		Red

Table 1: Fluorescent dyes – examples for red, green and yellow, the ring systems and the multiple bonds, are sources for high-energy π electron clouds

Literature

[1] C.V. Stevani, S.M. Silva, W.J. Baader, Eur. J. Org. Chem., 2000, 4037

[2] Shimadzu LAAN-A-RF-E003, application News No. 493, „Measuring Peroxyoxalate Chemiluminescence Using a Spectrofluorophotometer"

Further information on this article

- Application: Measuring Peroxyoxalate Chemiluminescence Using a Spectrofluorophotometer

Hazardous phthalic acid esters detected quickly with Py-Screener

EU guideline RoHS II will ban the use of four phthalic acid esters in electrical and electronic devices from July 2019

Each year, millions of tonnes of plasticizers are processed worldwide in order to make brittle materials soft, flexible and moldable. The most important field of application by far is plastics, in particular products made of PVC, whereby the largest part is used in the manufacture of films and cables.

The industry uses a broad range of substances as plasticizers, that are chemically very different. In terms of quantity, esters of phthalic acid (identified as phthalates) are currently still predominant.

Figure 1: Pyrolysis GCMS-QP2020

What are phthalates used for?

Apart from use as a plasticizer in plastics such as PVC, nitrocellulose or synthetic rubber, phthalates are applied in many other areas of daily life. They serve as a carrier substance for fragrances in perfumes, deodorants and other personal hygiene products. They are components in nail varnishes and hair sprays. They are used as a formulation agents in pesticides, as industrial solvents and lubricants and as additives in the textile industry. They can be found in various other products such as toys or pharmaceuticals, e.g. as a coating for film tablets or as plasticisers in gelatine capsules (Deutsche Apotheker-Zeitung [German Pharmacist Paper]).

Phthalates are used for external plasticization, meaning that the plasticizer is not bonded covalently into the polymer, but instead interacts with the polymer only via its polar groups, increasing mobility between the polymer chains. Due to the lack of chemical bonding, the phthalates can be extracted relatively easily from the plastic or can migrate out gradually. In this way they then escape into the environment, and, from various products, enter household dust or food.

Health risks

Phthalates have for some time been making negative headlines due to their potential health risk. However, it must be considered that the risks posed by individual compounds varies widely. They are differentiated into low or high molecular compounds according to the length of the esterified alcohol chains.

The low molecular phthalates, including for example di-(2-ethylhexyl)-phthalate (DEHP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP) and diisobutyl phthalate (DIBP) are suspected to have an endocrinologic (hormone-like) effect. These substances, also known as endocrine disruptors, disturb the hormone balance and can cause health problems if they enter a human or animal in a sufficient concentration.

At present, the effect of substances, in particular those which interfere with the sexual hormone system, are being discussed.

It is assumed that they may impair fertility and the development of children in the womb or at certain developmental stages, such as puberty. Their influence in the formation of certain tumours is also being discussed.

Phthalate bans in Europe

In Europe, there are already many bans on phthalates. According to the EU chemicals regulation REACH (2009), plasticizers DEHP, DBP and BBP which are currently classified as toxic to reproduction, must not be present in concentrations greater than 0.1 mass percent in toys and childcare articles for children under three years. This limit also applies to other phthalates (DINP, DIDP, DNOP), the use of which is prohibited as far as possible in toys and childcare articles which children could put in their mouths. Furthermore, some phthalate plasticizers must not be included in mixtures for private consumers or in cosmetic products. The use of phthalates in plastics for food packaging has been restricted throughout the EU.

Finally, due to REACH regulations, companies producing or using the phthalates DEHP, BBP, DBP and DIBP as of 21.02.2015 may only do so with an exceptional permission (approval) that is difficult to obtain. In addition, the European Commission has included these four phthalates in the list of substances whose use is restricted by the RoHS II Directive (Directive on the Restriction of the use of certain Hazardous Substances in electrical and electronic equipment).

Also in this case, a maximum permissible concentration of 0.1 mass percent applies per substance. The envisaged transitional period expires for most device groups on 22.07.2019. For medical devices as

Figure 2: Tool kit for sample preparation

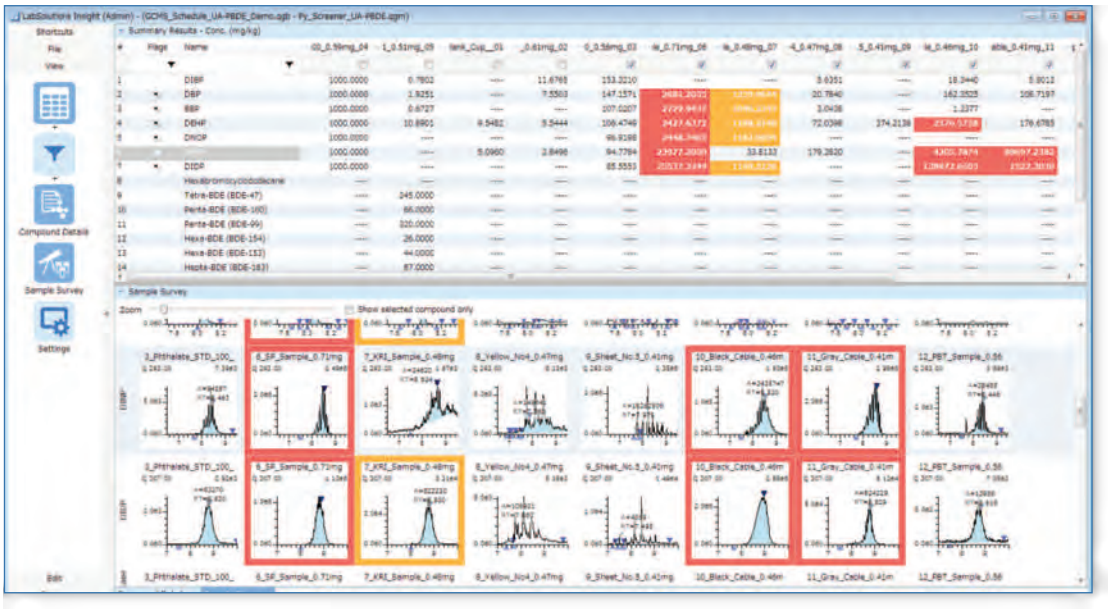


Figure 3: Special evaluation software: For faster optical identification, differing concentrations of indicator substances are color-coded

well as control and measuring instruments, a longer period applies – until 2021.

Fast detection of phthalates in polymer material

Current legislation requires quick and easy identification of phthalates in polymers. Existing standard methods (e.g. EN 14372, ISO 8124-8, ISO 14389 – Analysis of phthalates in toys, childcare articles and textiles) are based on a liquid sample preparation, meaning that the sample is extracted with an organic solvent over several hours and subsequently analysed with a coupled gas chromatograph-mass spectrometer (GC-MS). The 'Py-Screener' presented here is a screening method for phthalates which does not require solvent extraction and offers excellent selectivity and the extension of the method to new target molecules.

The Py-Screener system

Screening is carried out with a coupled pyrolysis GC-MS system. For this purpose, an aliquot of the polymer sample (approx. 500 µg) is placed directly into the pyrolysis furnace. The semi-volatile phthalates are extracted from the polymer with a special heating program. The desorbed phthalates are transported by the inert carrier gas into the gas chromatograph where they are separated on an analytical column and subsequently detected with a mass spectrometer. The screening system includes an in-depth video tutorial in preparation of the analytical standards and polymer samples.

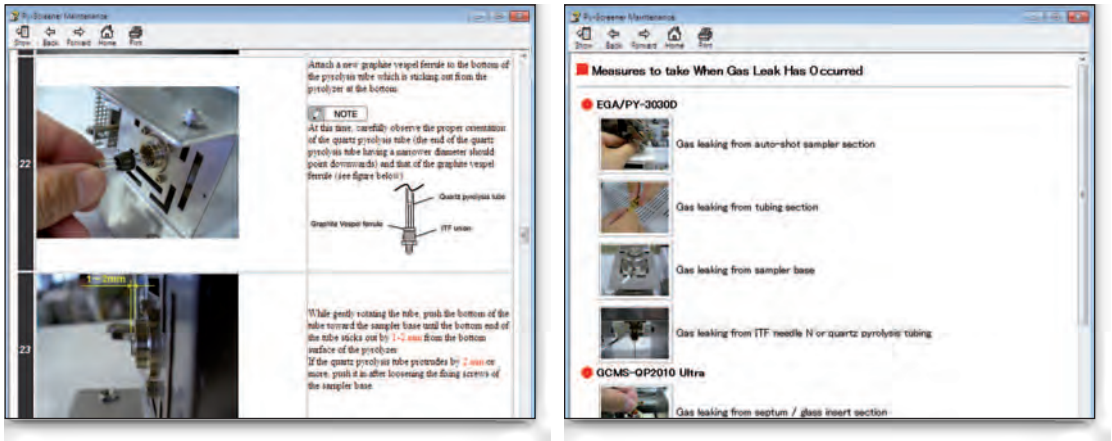


Figure 4a and 4b: Examples of the Maintenance Navigator Window

Since liquid extraction is omitted in the case of pyrolysis GC-MS, a small piece of the polymer sample is cut off, placed directly into a sample cup and weighed. The software specially developed for screening of phthalates provides all necessary, already optimized method and instrument parameters as well as sequences for sample measurement and the complete quantification method including a report template.

Color-coding (Flagging) of noticeable concentrations

When an autosampler is part of the system, samples can be measured easily overnight. The data obtained are displayed in a tabular and graphical overview for quick evaluation, in which the values exceeding defined limits are color-coded. Analytical standards needed to quantify the phthalates and check system performance can be conveniently and easily punched out of the standard material in a defined size using the sample toolkit's micro-puncher.

The so-called 'Maintenance Navigator' supports regular maintenance or troubleshooting procedures with detailed descriptions, comprehensive illustrations and a video.

If required, the 'Py-Screener' system can also be expanded to cover the other compounds already regulated under the RoHS Directive, PBB (polybrominated biphenyls) and PBDE (polybrominated diphenyl ethers), which can be analyzed with GC-MS. The optimized methods are already part of the package.

Summary

The 'Py-Screener' screening system provides a comprehensive package consisting of a toolkit for sample preparation, standards for the analysis of phthalic acid esters and special evaluation software. Videos describing the accurate preparation of standards and test samples as well as regular maintenance of the pyrolyzer and GC-MS round off the system. With the complete Py-Screener method, even inexperienced users can quickly and autonomously learn how to identify phthalates in polymers.

Literature

Official Journal of the European Union 04.06.2015, Commission Delegated Regulation (EU) 2015/836 from 31st March 2015 amending Annex II of the Directive 2011/65/ EU from the European Parliament and of the Council regarding the list of substances subject to restrictions.

Further information on this article

- Brochure: Py-Screener
- Application: Analysis of Phthalate Esters using the Py-Screener
- Technical Report: Comparison of Screening Method (Py-GC/MS) and Quantitative Method (Solvent Extraction-GC-MS) for Phthalate Esters Analysis

Newborn screening – just a few drops of blood for healthy development

Neonatal Solution software for rapid evaluation of screening data

Figure 1: The LCMS-8050 tandem mass spectrometer

Newborn Screening (NBS) is available in many European countries. The blood of newborns is tested for rare metabolic disorders within a few days following birth. When such diseases remain undetected, this can lead to massive health damage or even to death in infancy. Some of these tests are carried out using tandem mass spectrometry (MS/MS). The 'Neonatal Solution' software package supports rapid and routine evaluation of the measurement results.

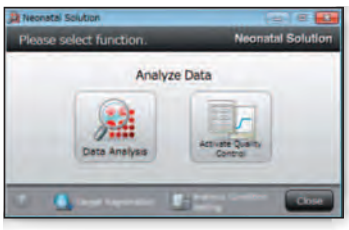


Figure 2: Start window of the Neonatal Solution software

Early screening of metabolic disorders

Newborn screening is a preventive medical measure to detect and effectively treat some important Inborn Errors of Metabolism (IEM) of newborns as early as possible. European countries screen for different types of disorders. In Germany for example, this comprehensive screening test was included since 2005 in the national screening program and since then every newborn child in Germany is currently tested for twelve disorders (see box) after written parental consent.

These disorders include amino acid metabolism disorders such as phenylketonuria (PKU) or maple syrup urine disease, as well as fatty acid decomposition deficiencies in which the transport and oxidation of fatty acids in mitochondria is impaired (medium-chain acyl-CoA dehydrogenase deficiency (MCAD) and very long-chain acyl-CoA dehydrogenase deficiency (VLCAD); carnitine cycle defects). The screening also includes diseases such as the frequently occurring hyperthyroidism, an excessive production of thyroid hormone, or the very rare galactosemia in which too much galactose is present in the blood. Most of these diseases can be treated very well with a special diet, vitamins or hormones.

Extended screening by tandem mass spectrometry

The decisive turning point in this issue (please see box on page 19) was the introduction of tandem mass spectrometry in routine analysis. Using this technology, it is possible to simultaneously identify a large number of disorders of the amino acid metabolism, the metabolism of organic acids and fatty acid degradation within one analytical run. In this way, not only the number of marker substances (metabolites) investigated was increased, but also many more newborns with metabolic disorders could be tested and treated.

List of the target diseases in Germany

- Congenital adrenal hyperplasia (CAH)
- Maple syrup urine disease (MSUD)
- Biotinidase deficiency
- Carnitine metabolism deficiencies
  - Carnitine palmitoyltransferase-I deficiency (CPT-I)
  - Carnitine palmitoyltransferase-II deficiency (CPT-II)
  - Carnitine-acylcarnitine translocase deficiency
- Galactosemia
- Glutaric acidemia type 1 (GA1)
- Hypothyroidism
- Isovaleric acidemia
- Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD)
- Medium-chain acyl-CoA dehydrogenase deficiency (MCAD)
- Very long-chain acyl-CoA dehydrogenase deficiency (VLCAD)
- Phenylketonuria (PKU) and hyperphenylalaninemia (HPA)

All tested metabolic disorders can be attributed to congenital enzyme defects.

Failing or incorrectly formed enzymes lead to degradation disorders in the organism or rather an accumulation of toxic intermediate metabolic products, small organic acids that cause poisoning and in turn lead to irreversible organ damage.

One of the most frequently occurring congenital metabolic disorders is phenylketonuria, an amino acid metabolism disorder that, if undetected, leads to physical and mental development disorders.

With a timely begun low-protein diet, however, the symptoms can be prevented.

At the same time, the number of false-positive test results decreased significantly. Already in 2002, the screening committee of the German Society of Pediatrics and Adolescent Medicine required the use of mass spectrometry as standard method in newborn screening.

The Neonatal Solution software simplifies routine data evaluation

On the third day of its life, blood is drawn from the newborn's heel, applied onto paper filter cards and sent to the respective screening laboratory on the same day.

Due to the tandem mass spectrometer's high specificity, more than 20 metabolites, such as amino acids and acylcarnitine, can be determined within the shortest possible time. Because of continuously improving instrument sensitivity, time-consuming sample preparation, such as derivatization, is now no longer necessary. Chromatographic separation of the marker substances is also no longer required due to the selectivity of the MS/MS technology. With an analysis time of less than two minutes, a high daily sample throughput is no longer a problem.

The amount of data acquired is enormous and a clear and effective data management is urgently required. With the Neonatal Solution data evaluation software, extremely large quantities of data can be rapidly and easily processed. In this way, users can select certain marker substances and create evaluation methods selectively for these substances only.

Concentrations, peak areas as well as concentration ratios for indicator substances can be calculated easily from the LC-MS/MS analysis data. For fast visual identification of conspicuous concentrations it is possible to define limit values for each marker substance. When the limit value is exceeded, the deviating values are flagged using a color-code.

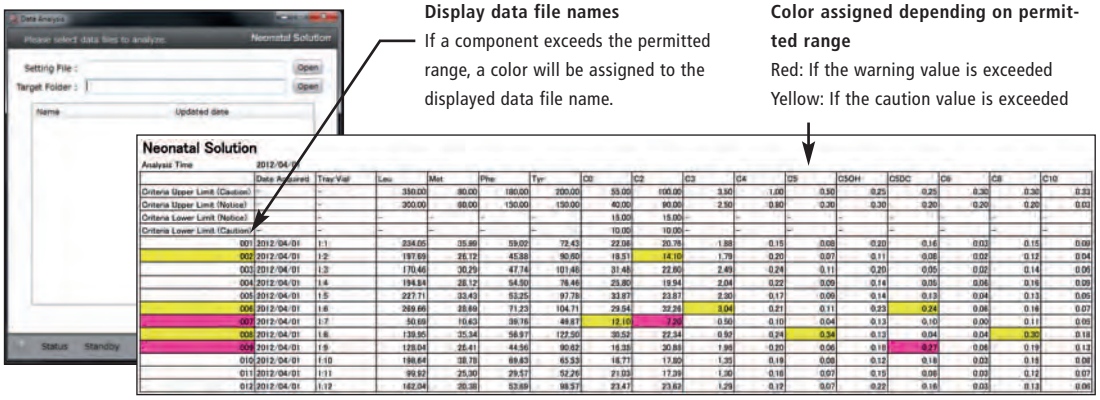


Figure 3: Neonatal Solution – for rapid, visual identification, deviating concentrations of indicator substances are color-coded

Summary

Through newborn screening, disorders that are clinically not yet apparent can be diagnosed and in most cases treated at an early stage. Even when potential disease consequences cannot be prevented fully in all cases, timely treatment will often ensure a largely normal development.

Screening laboratories must meet high demands in terms of quality and speed, as the samples must be analyzed on the day of receipt. Currently, eight of the total of twelve tested disorders can be screened using LC-MS/MS analytics. The Neonatal Solution software with its functions customized to the evaluation of screening data enables rapid and effective processing of large amounts of data.

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

History

Already in 1934, the Norwegian physician Ivar Asbjørn Følling discovered an increased excretion of phenylpyruvic acid in the urine of mentally disabled patients, which could be detected using iron (III) chloride (Følling's test).

It took almost 20 years until the German pediatrician Horst Bickel could prove that the severe developmental disorder, which was later known as the disease phenylketonuria, could be prevented with a diet low in phenylalanine. As was quickly shown, the long-term result of this dietary treatment decisively depends on starting the diet before the onset of clinical symptoms.

In the early 1960's, the American microbiologist Robert Guthrie developed an easy to perform bacteriological test for phenylalanine. Since 1969/70, all newborns in Germany have been comprehensibly tested for elevated phenylalanine blood levels using the test named after Guthrie. Another congenital metabolic disorder, galactosemia, was included in the screening program.

Over the years, additional tests like thyrotropin screening for congenital hypothyroidism and screening for 17-OH-progesterone (AGS) were included in the screening program. The treatment successes in positively tested children speak for themselves, the demand grew and it turned out that more and more diseases could, in principal, be treated presymptomatically, i.e. before onset of the disease, although effective screening methods for early determination were still not available.

Literature

Gudrun Heyn: Ein lebensrettender Test; Pharmazeutische Zeitung 23/2009,
Erik Harms, Bernhard Olgemöller: Neugeborenenscreening auf Stoffwechselerkrankungen und Endokrinopathien; Deutsches Ärzteblatt, 2011, Jg. 108, Heft 1-2, 11 ff

Further information on this article

- Brochures: Neonatal Solution
- Application: Simultaneous Analysis of Amino Acids and Acylcarnitines in DBS (Dried Blood Spot) with LCMS-8040

New generation of protein sequencer

PPSQ-50 with increased sensitivity and FDA compliance

Protein sequencing via Edman degradation is a proven and still very popular method in many laboratories. With the new PPSQ-50 protein sequencer series, Shimadzu has now further developed the successful PPSQ series. Popular features like robustness, reproducibility and low running costs have been retained while sensitivity has been increased significantly by using a far more sensitive detector.

In addition to its customary simplicity of use, the new LabSolutions PPSQ software for instrument operation and data evaluation now also offers optional compliance with the 21 CFR Part 11 guidelines of the US Food and Drug Administration (FDA). Two versions of the sequencer are available:

- the PPSQ-51A equipped with one reactor
- the PPSQ-53A with three reactors enabling simultaneous placement of up to three samples into the instrument with subsequent sequential processing. This ensures the best possible system utilization, for instance during the night or over the weekend.

Sequencing according to Edman degradation

In the main unit of the PPSQ, the N-terminal amino acids of a protein or a peptide which is immobilized on a membrane are derivatized and cleaved off via the Edman degradation reaction.

The remaining protein or peptide is available for the next cycle so that, little by little, all N-terminal amino acids are analyzed.

Meanwhile, the PTH amino acids are injected into the HPLC unit where they are identified chromatographically via the specific retention times of each amino acid and comparison with a standard. Because the separation takes place isocratically on a C18 column, the mobile phase can be continuously

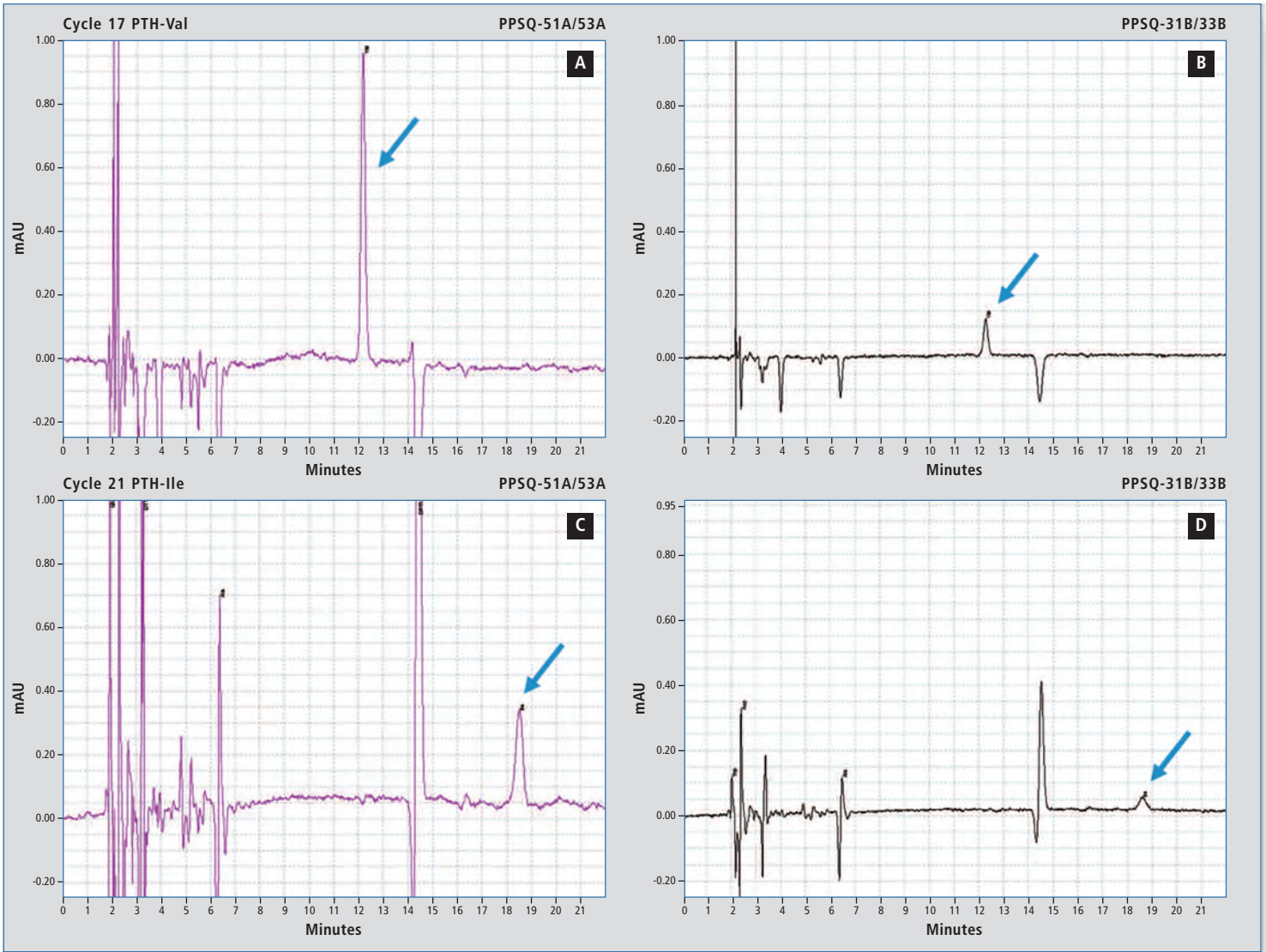


Figure 1: Sample: Horse myoglobin 10 pmol; results show the subtraction chromatograms by PPSQ-50 (A, C) and PPSQ-30 (B, D) connected in tandem after Edman degradation

recycled and reused. This not only reduces purchase and disposal costs of the eluent, but also enables quick startup, even when the system has not been used for longer periods since gradient optimization is not necessary. Another advantage of isocratic analysis is the baseline and retention time stability.

Increased sensitivity – also upgradable

The design of the new PPSQ-50 (figure 2) is more compact than the predecessor series and requires less laboratory bench space. In addition, the new detector achieves a significantly higher detection sensitivity in comparison with the PPSQ-30 series.

Figure 2 shows a tandem analysis in which the sample was analyzed using the new detector as well as that of the predecessor model. It is easy to see that detection via the new unit is far more sensitive. In this way, it is possible to determine long protein sequences, even for samples of which only small amounts are available. As the separation column, the eluent and the analytical conditions are still the same, it is possible to upgrade previously acquired instruments of the PPSQ-30 series and thereby also profit from the increased sensitivity.

Figure 2: More compact design of the PPSQ-50 series with reduced footprint

Software solutions for regulated environments

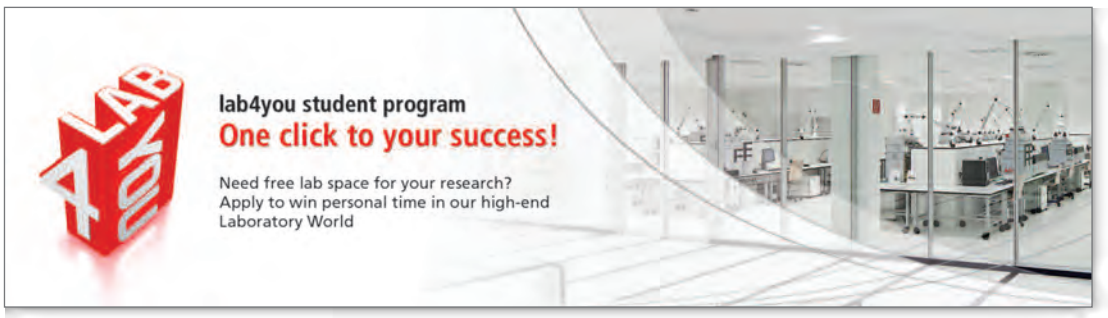
The newly developed LabSolutions PPSQ software incorporates instrument operation as well as data evaluation. In addition to automated sequence determination, the user-friendly software also enables reprocessing and overlaying of chromatograms, as well as a subtraction mode that shows differential chromatograms of two sequential ones for simple and fast sequence determination.

The LabSolutions PPSQ software is available in three versions: The classic version (LabSolutions PPSQ) and a version with integrated database (LabSolutions PPSQ DB), which are both suitable for use in stand-alone instruments. A new feature is the possibility to work in accordance with the 21 CFR Part 11 guidelines of the FDA. This includes security settings, user administration, history documentation (audit trail), as well as data storage in a database.

The third version is a client/server solution (LabSolutions CS) that allows network integration of the instrument so that data evaluation can be carried out from any computer. Clients already using a PPSQ-30 system can easily switch to this new software, which makes working in a regulated environment much easier thanks to the compliance achieved.

Wanted: Clever Minds!

Shimadzu's lab4you student program



In 2015, Shimadzu introduced the lab4you student program for all talents needing free lab space for their research.

Shimadzu's lab4you supports young, enthusiastic scientists with an outstanding topic of research, by offering instrument time on the entire range of the company's state-of-the-art (U)HPLC and (U)HPLC/MS/MS equipment. Many interesting applications were proposed from all over Europe, and decision making was a challenging task.

That is why the lab4you selection committee is happy to announce that not just one, but two promising candidates have the opportunity to advance their research using the equipment in the Shimadzu Laboratory World in Duisburg, Germany.

The researchers are:

Carola Schultz, a PhD student at the MEET Battery Research Center (University of Muenster, Germany) in the competence areas analytic & recycling and aging & safety. Her research aim is the investigation of organic Lithium-ion battery (LiB) electrolytes with HPLC-UV/VIS and HPLC-MS.

Katarzyna Brama, a PhD student at the Warsaw University of Technology, Poland. Her work comprises metal complex and bioligand studies in tomato and garden cress extracts performed by means of hyphenated techniques.

She'll look at the identification of metal complexes with HILIC ESI MS/MS and new determination methods for phytochelatin in natural samples using HPLC with fluorescence detection.

Further information on this article
www.shimadzu.eu/lab4you

Mineral oil compounds in paper and cardboard packaging

Investigating the composition of the aromatic mineral oil fraction

The presence of saturated and aromatic mineral oil hydrocarbons (MOSH – Mineral Oil Saturated Hydrocarbons and MOAH – Mineral Oil Aromatic Hydrocarbons) in paper and cardboard packaging for food and hygiene products is an issue that is being widely discussed at the moment. The MOSH fraction consists of linear and branched alkanes as well as alkyl-substituted cycloalkanes, whereas the MOAH fraction consists of alkylated polyaromatic hydrocarbons with up to four aromatic rings. The main focus is on the aromatic fraction, as it may contain potential carcinogenic and mutagenic compounds [1]. The proportion of the aromatic fraction is approximately 15 - 30 % of the total mineral oil fraction. The main sources of mineral oil compounds in paper and cardboard are printing inks, which are either applied directly onto the packaging or introduced via the recycling processes during which they are not fully removed.

The migration of mineral oil hydrocarbons from packaging into packaged products takes place through direct contact or via the gas phase, by evaporation from the packaging and re-condensation in the packed product. These processes can result in a relevant migration of mineral oil compounds with carbon chain lengths of up to n-C28.

Mineral oil fraction migrates into packed products

The concentration of mineral oil hydrocarbons ≤ n-C28 in paper products is up to 1000 mg/kg [2], of which up to 70 % can migrate into the packed product. Based on the acceptable daily intake of 0.01 mg/kg body weight, as specified by the Joint FAO/WHO Expert Committee on Food Additives (JEFCA) in 2002, a specific migration limit for MOSH of 0.6 mg/kg and for MOAH of 0.15 mg/kg packed product has been defined under assumptions of standard conditions.

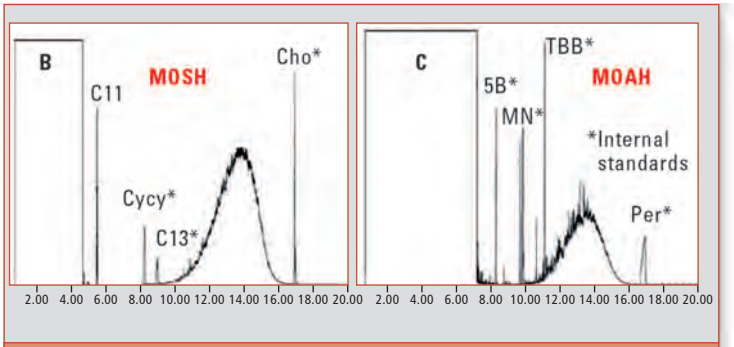


Figure 1: GC-FID chromatogram of a MOSH and MOAH fraction [5]

At present, analytical determination of MOSH and MOAH is mainly carried out via online or offline high-performance liquid chromatography coupled with gas chromatography-flame ionization detection and large-volume injection (HPLC-GC-LVI-FID) [4]. Concentration of MOSH and MOAH is determined in this way.

Large-volume injection (LVI) with flame ionization detection (FID) is used to achieve sufficient sensitivity and to avoid detector discrimination. This, however, causes fractions to elute as non-separated 'mineral oil humps' (broad peaks) and identification of individual compounds is not possible (figure 1). Because of this lack of detailed information, it is difficult to evaluate the mutagenic potential of the MOAH fraction. For this reason, an established evaluation method is at present not available.

Multidimensional GC increases sensitivity and selectivity

Gas chromatography with a mass selective detector offers one possibility to obtain detailed information on the composition of the MOAH fraction. In particular, the so-called comprehensive GCxGC separation is the technology of choice for increased sensitivity and selectivity.

Are newspapers the source of printing ink migration into packaged products?

Newspapers and magazines are part of the raw materials for the production of recycling products for many different applications. The mineral oil containing printing inks are considered to be the main sources of contamination in recycling products.

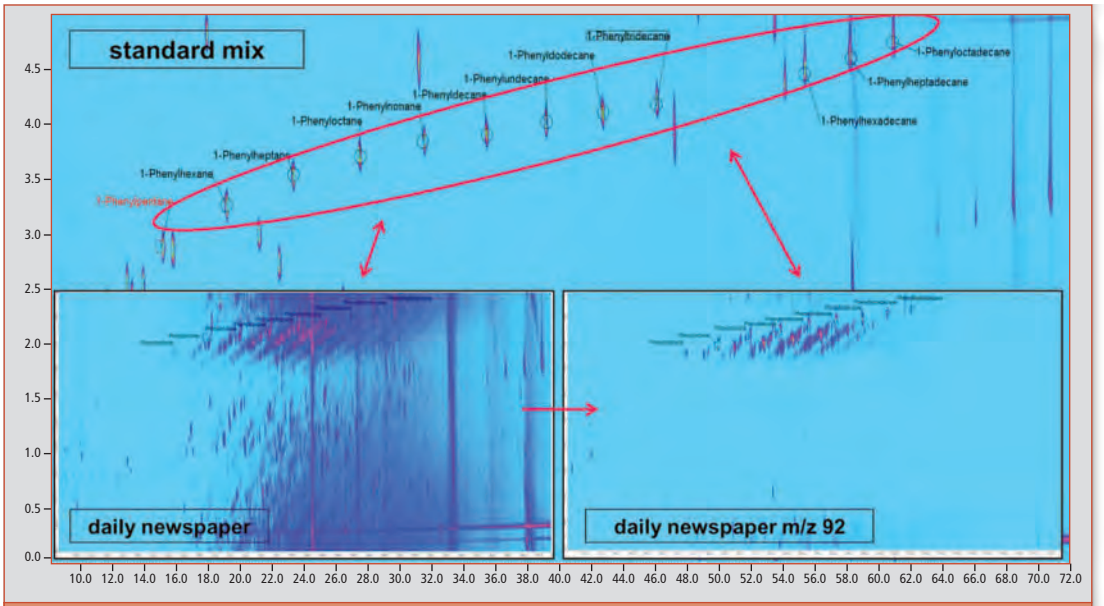


Figure 2: Phenylalkanes (Scan and m/z 92) in standard and sample (newspaper)

As starting material, various daily newspapers were analyzed. In addition, different packaging papers (fresh fiber paper and recycling cardboard) were analyzed.

Sample preparation was carried out by extracting the paper samples and separation of MOSH and MOAH via HPLC. Subsequently, GCxGC-MS analysis of the MOAH fraction was executed using Shimadzu's GCMS-QP2010 Ultra with a Zoex cryogenic modulator. Details of the analytical parameters are shown in table 1.

For identification of individual compounds, a standard mix consisting of the internal standards of the MOSH/MOAH separation [2, 5], alkylated aromates and polyaromatic hydrocarbons (PAHs) as non-alkylated marker substances was used.

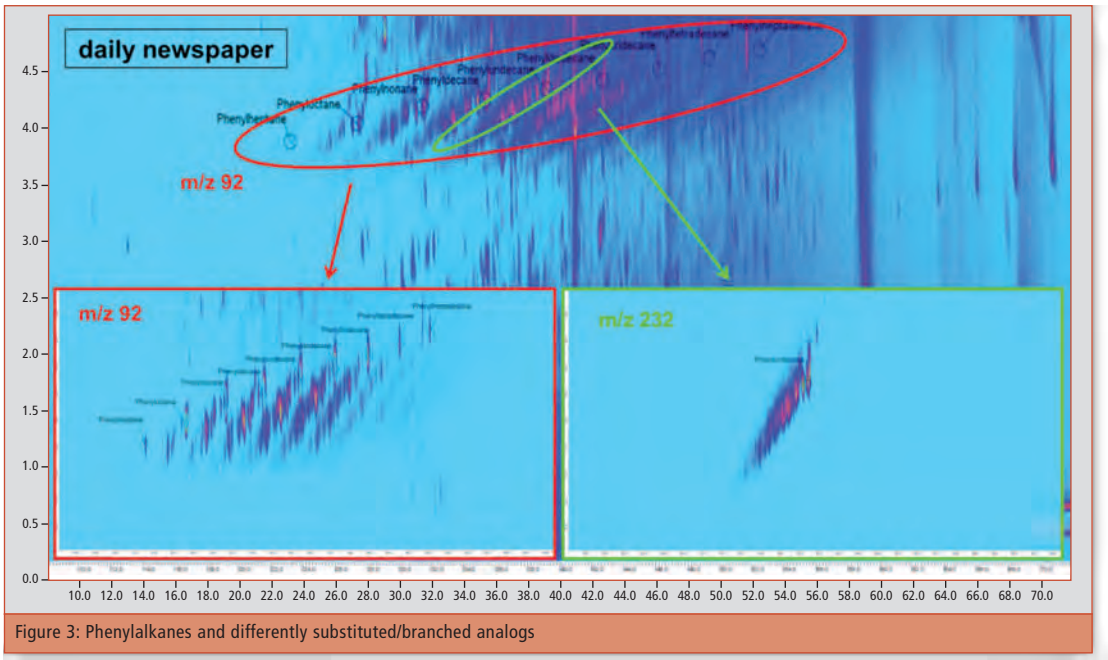


Figure 3: Phenylalkanes and differently substituted/branched analogs

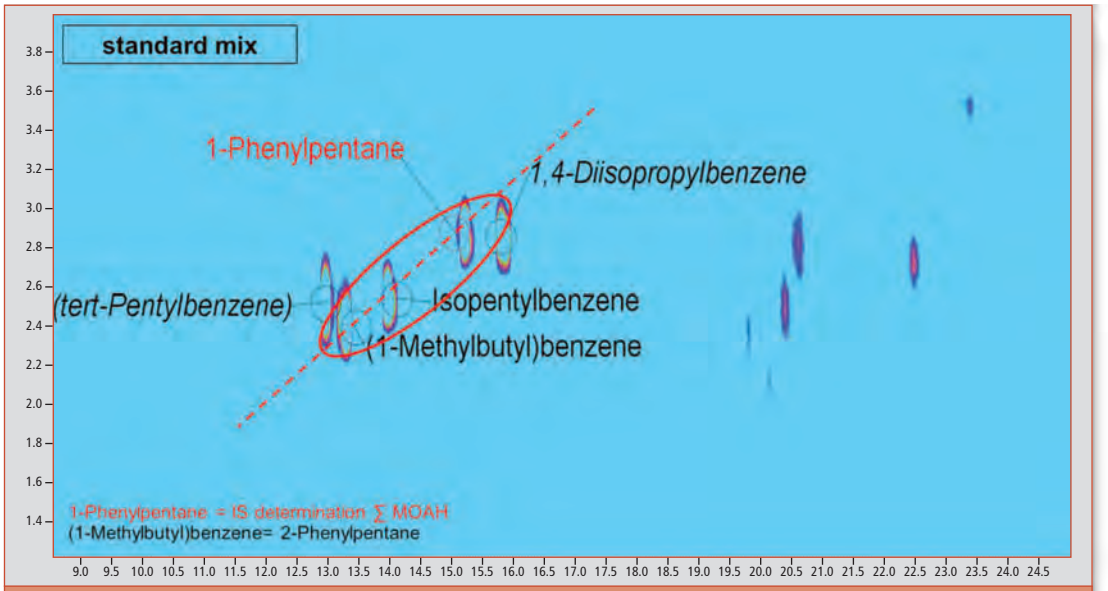


Figure 4: Detection of different branchings and substitutions exemplified by 1-phenylpentane

GCxGC-MS analysis identifies phenylalkanes as dominating aromatic substance group

Using multidimensional GCxGC-MS analysis, it was possible to identify linear and branched phenylalkanes as the dominating aromatic substance group. An unequivocal identification of the phenylalkanes was carried out using a standard mix containing linear phenylalkanes with a carbon chain length of C5 to C18 (figure 2).

Through extraction of the mass fragment m/z 92, characteristic for the phenylalkanes, diagonal lines of unknown compounds become apparent that are also characterized by the mass fragment m/z 92 originating from the linear phenylalkanes. The exact analysis of these unknown compounds revealed that all compounds lying on one diagonal originated from the same mass fragment as the linear marker substance.

This is illustrated for phenylundecane isomers in figure 3. Through extraction of the m/z 92 fraction, it is possible to recognize the diagonals originating from the linear phenylalkanes. By extracting the mass fragment m/z 232 that is typical for phenylundecane, it becomes apparent that all compounds on a diagonal are characterized by the same molecular mass.

An identical molecular mass points towards differently substituted and branched analogs. This could also be shown by verification of the compounds 1-phenylpentane, isopentylbenzene and (1-methylbutyl)benzene contained in the standard mix (figure 4). Extraction of the characteristic molecular mass fragment m/z 148 revealed that these three compounds are on a straight line (dotted line). An unequivocal identification of the unknown differently substituted and branched analogs of linear phenylalkanes with longer carbon chains is, however, currently not possible due to a lack of available standards.

All samples were contaminated with mineral oil components

The results revealed that all samples were contaminated with mineral oil components, whereupon a comparison between fresh fiber and recycled samples showed that in recycled samples there was a considerably higher proportion of mineral oil compounds than in fresh fiber samples (figure 5, page 24).

Phenylalkanes could be detected in all samples analyzed and, therefore, represent a substantial proportion of the MOAH fraction. The highest concentration for this compound class was detected in daily newspapers, followed by recycled papers. The concentration in fresh fiber samples was, as expected, considerably lower although still clearly detectable, which raised the question concerning the source of phenylalkanes in fresh fiber products.

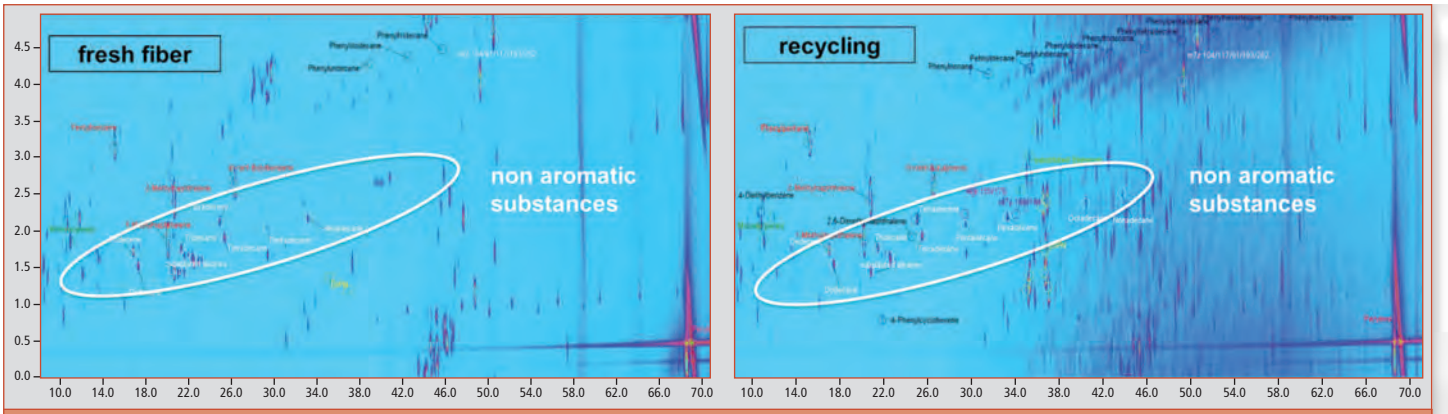


Figure 5: 2D-plot of a fresh fiber and a recycling sample

Non-aromatic compounds also in the aromatic MOAH fraction

In addition to the phenylalkanes and aromatic substance groups such as biphenyls and diisopropylnaphthalenes, various non-aromatic compounds such as saturated hydrocarbons and terpenes were, however, also identified in the aromatic MOAH fraction (figure 5). In particular, saturated hydrocarbons that should have been constituents of the MOSH fraction could lead to false-positive values in the determination of the MOSH and MOAH sum parameters.

In addition to this problem, the complexity of the MOAH fraction becomes clearly apparent even in GCxGC separation, where chromatographically non-separated retention ranges occur. This is especially true for samples with high mineral oil concentrations such as recycling and newspaper samples.

Conclusion

In summary it can be said that multidimensional GCxGC is well able to obtain valuable information on the chemical and structural composition of mineral oil contaminations in paper and cardboard products. Nevertheless, more progress still needs to be made in the elucidation of the composition, which could eventually enable correct assessment of risk.

Authors

Dipl.-Ing. A. Jurek, Ao.Univ.-Prof. Dipl.-Ing. Dr. techn. E. Leitner, Institute of Analytical Chemistry and Food Chemistry, Technical University Graz, Austria

Analytical parameters of the GCxGC analysis	
Column 1st dim.	Zebron HT 1 (30 m x 0.25 mm i.d. x 0.25 µm df)
Column 2nd dim.	SGE BPX 50 (2.5 m x 0.15 mm i.d. x 0.15 µm df)
Injector	OPTIC IV, 1 µl splitless, 270 °C
Modulator frequency	5 s, 280 °C hot jet 350 ms
Temp. program	50 °C (1 min) - 10 °C/min to 120 °C - 2 °C/min to 280 °C (5 min)
Carrier gas	Helium 155 kPa column head pressure, constant flow
Ion source temp.	200 °C
Interface temp.	270 °C
Solvent cut time	7 min
Data acquisition	Scan 50 - 340 amu, 50 scans/s

Table 1: Analytical parameters of the GCxGC analysis

Literature

[1] R. Lorenzini, M. Biedermann, K. Grob, D. Garbini, M. Barbanera, I. Braschi, Food Addit Contam 30 (2013) 760 - 770

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[5] K. Fislier, F. Grundböck, K. Schön, O. Kappenstein, K. Pfaff, C. Hutzler, A. Luch, K. Grob, J Chromatogr A 1271 (2013) 192 - 200