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NEWS 03 2014

The nightmare of doping offenders

Analysis of doping agents using the new LCMS-8050 Triple Quadrupole Mass Spectrometer

i-series – new integrated (U)HPLC systems

Nexera-i / *Prominence-i* – high speed, performance, maintainability and efficiency

New: ICPE-9800 Espresso in Lake Constance

Five application examples of simultaneous elemental analysis sensitivity

Including part 3 of the big breakfast test

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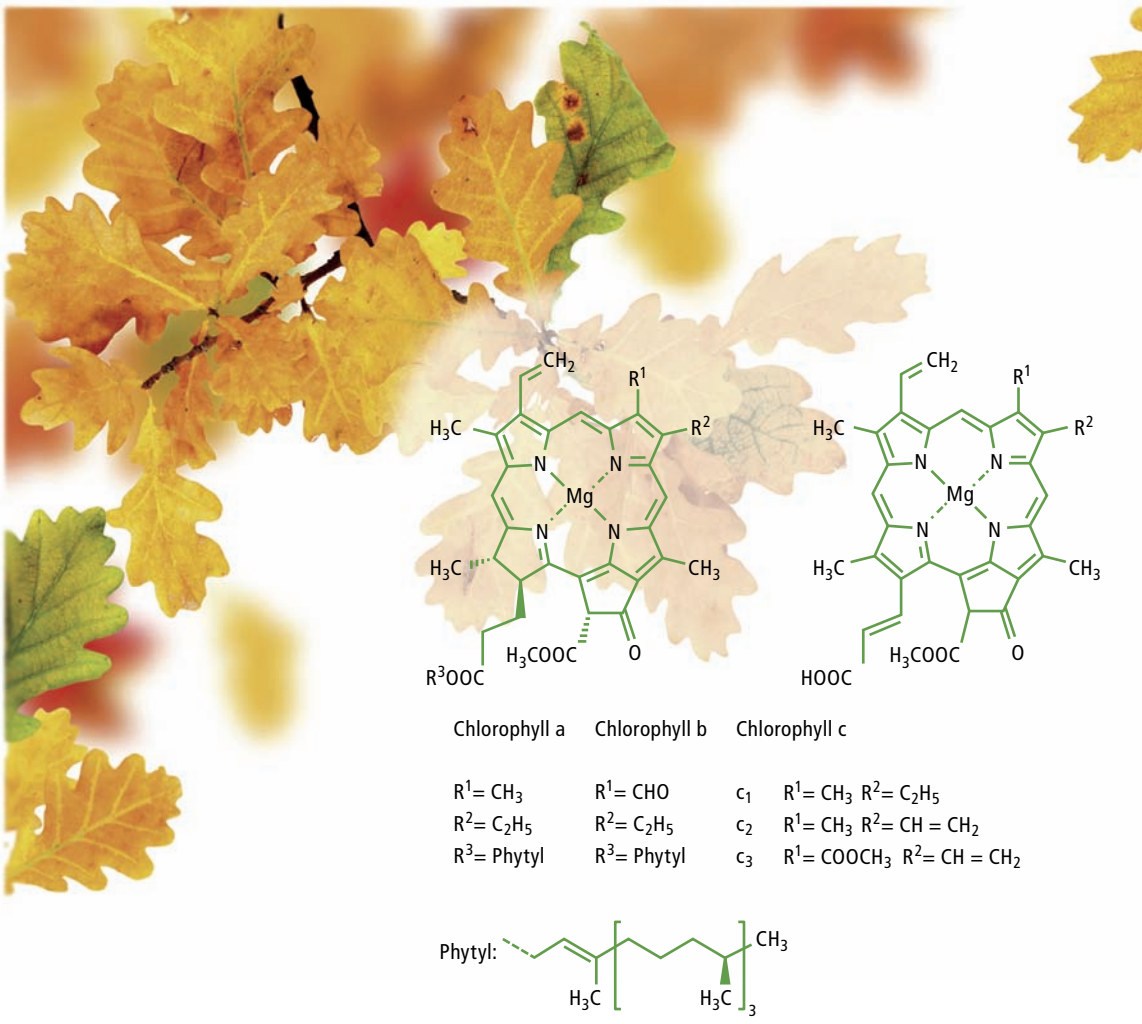

Figure 1: Structure of chlorophyll and its various naturally occurring species [3]

Changing forest – what happens during Autumn?

During Autumn leaves lose their green coloring, a process also known as senescence. This is a generic term for the process of ageing in living organisms. It is assumed that senescence is associated with the chlorophyll contained in plants (see infobox). These interrelationships and processes have been well investigated scientifically and can be described as follows:

During photosynthesis, chlorophylls absorb energy from sunlight to convert carbon dioxide and water into carbohydrates and oxygen. During this process, chlorophylls are bound to apoproteins whereby the absorbed energy can enter metabolism.

With increased ageing, this process becomes less stable and the proteins are gradually removed. Should chlorophylls, at this point, not decompose as well, the energy produced would need to flow into other processes. In this case, oxygen molecules would be available to absorb the energy. Oxygen molecules in the ground state (O_2) and in the triplet state ($^3O_2$) will yield activated (O_2^*) and singlet oxygen ($^1O_2$) [1]. Such an oxygen molecule is much more reactive and can lead to the destruction of vital plant organelles. This is why chlorophyll must be degraded very rapidly after separation from the protein. This process is accompanied by the color change [2]. Each chlorophyll molecule also contains four nitrogen atoms, which can be recycled for new processes in the plant.

Approach

For a more detailed investigation of senescence, the chlorophyll content of the leaves of an oak (Quercus) was determined. The oak leaves were also analyzed with

respect to their other naturally occurring chlorophyll species (see figure 1). Aim of the investigation was direct comparison of green and yellow, already withered (senesced) leaves. In order to assure the direct comparability of samples with different extent of ageing, a tree was sampled in autumn. At that time, the tree bore green as well as yellow, already withered leaves.

Methanol was used to extract the chlorophylls from the leaves. Preliminary experiments showed that an influence on the different chlorophyll species could be largely excluded.

For detection purposes, the UV-VIS activity of chlorophyll is generally taken advantage of. The chlorophyll species are separated using HPLC and subsequently detected using a diode-array detector (DAD). This method would however require the use of reference standards which are only available for chlorophyll a and b, to ensure an accurate analysis. A reliable statement on other chlorophyll species (see figure 1) would not be possible.

A different approach was therefore used, in which the specific structure of chlorophyll was taken advantage of, as each of the molecules contain magnesium as the central atom (see figure 1). As before, the chlorophyll species were separated via HPLC (*Prominence* Modular HPLC). For a more specific detection, flame AAS (AA-7000) was subsequently used. The absorption (output as current in µA) can be tapped off via the analog port of the AA-7000 and can be time-dependently recorded.

As chlorophyll is ashed in the flame and the magnesium is then present in its atomic form, a conventional water-based magnesium standard can be used. This standard is diluted accordingly using methanol, so that the sample matrix and the standard matrix are identical. The water content of the Mg-standard does not cause any problems due to the high dilution factor and can be neglected.

»Chlorophyll and photosynthesis«

The term 'chlorophyll' is derived from ancient Greek and means 'green leaf.' Chlorophyll is responsible for the green coloring of leaves. But it is not only present in plants since other organisms also contain chlorophyll.

It is necessary for photosynthesis, the photochemical process that produces glucose, oxygen and energy from water and carbon dioxide. This is, however not always the case. Oxygen production only occurs in oxygenic phototrophs.

Photosynthesis can also generate compounds other than oxygen and glucose and this type of photosynthesis is carried out by anoxygenic phototrophs. Both the products generated and the chlorophylls involved in these processes differ.

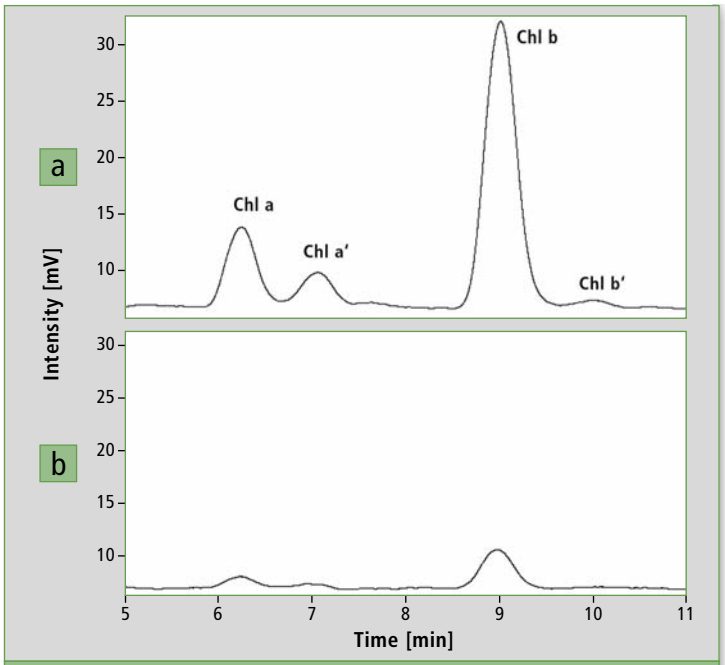

Figure 2: Determination of chlorophyll a and b in oak leaf extracts using HPLC-F-AAS.
a: an oak leaf still green in autumn, b: a withered oak leaf.

Result

Except for one intermediate in the chlorophyll degradation process (chlorophyllide), none of the degradation products contain the bound magnesium. This is why the measurement of senescence was limited to the decrease in intensity of chlorophyll a and b.

The concentration of chlorophyll a decreased by a factor of 8.5 due to senescence. In chlorophyll b, this decrease was even more dramatic. The samples only contained 6.1 % chlorophyll b in comparison to the sample with the non-withered leaves. Before senescence, the ratio between chlorophyll a and b was 4.4 : 1. The natural ratio is 3 : 1. This indicates that the green leaves were also at the initial stage of senescence, which could be confirmed by the time of sampling (autumn). After ageing, the ratio increased to 8.4 : 1. Chlorophyll b was therefore degraded faster than chlorophyll a. Chlorophyll degradation is accompanied by the change of color when other plant substances such as carotenoids (yellow/orange) and tannin (brown) are emerging.

This problem can be addressed using the AA-7000 coupled to the modular *Prominence* HPLC. In particular, the possibility to calibrate using cost-effective water-based magnesium standards instead of expensive chlorophyll standards makes the coupling of HPLC and AAS extremely lucrative. The possibility to study other chlorophyll species and their degradation is also interesting. Chlorophyll c, for instance, is mainly present in algae. Although algae do not age depending on season, they are however limited in their life expectancy by ecological changes.

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Further information on this article:

- Application Note Analysis of Mg by HPLC FAAS (PDF)

www.shimadzu.eu/shimadzu-news-2014

"What can *i* do for you?"

The new *i*-series – *Nexera-i* / *Prominence-i* – new integrated (U)HPLC systems

High speed and outstanding performance, maintainability and efficiency – the new *i*-series with the compact *Prominence-i* (HPLC) and *Nexera-i* (UHPLC) versions meets the needs of large as well as small laboratories. The letter *i* stands for innovation, intelligence and intuitive operation, all of which are combined in the *i*-series concept.

The new *Nexera-i* and the *Prominence-i* systems are perfect for routine analyses in the chemical, pharmaceutical, food and environmental industries. The systems can be operated via workstation for a single user or a large client-server system for multiple-user

Figure 1: Touch panel of the *i*-series

operation. The small surface area reduces space requirements in the laboratory and simplifies possible relocation.

Further development of the LC-2010 predecessor model is characterized by the improvement of all system components (degassing unit, quaternary pumps, autosampler, oven) and also enables, in addition to optional cooling of the autosampler, the configuration of an UV or PDA detector. Furthermore, the *Prominence-i* allows working in a pressure range of up to 440 bar, while the *Nexera-i* allows working at pressures up to 660 bar.

Innovation

The ICM (Interactive Communication Mode), which allows operators to edit and launch methods and sequences directly from a

Figure 2: Placing the RID-20A on top of the *i*-series

touch panel or a PC, is highly innovative (figure 1). Furthermore methods or sequences can be bi-directionally synchronized from the instrument to the software.

The excellent baseline stability, even at fluctuating ambient temperature, is also an innovative feature due to a unique dual-temperature controlled detector unit (detector cell and detector optics). An injection cycle of 14 seconds reduces analysis times and the low

Figure 3: Chromatogram displayed on the touch panel

carryover increases accuracy of the analytical results.

In addition to the absorption detector (UV or PDA) included in the standard configuration, a fluorescence detector or refractive index detector, as well as a light scattering detector, can be added. It is also possible to implement data acquisition via other detectors using an analog input. When combining the *i*-series with the equally innovative RID-20A refractive index detector (figure 2), it is possible to configure a simple GPC system with a minimum footprint.

Besides optional interactive communication modes enable remote monitoring. This allows checking of the current status of the instrument or monitoring of the current data acquisition process. The color touch panel also enables visualization of the recorded chromatograms, in addition to complete instrument control with method and batch creation (figure 3).

Intelligence

Intelligence is embodied in the *i*-series by straightforward method transferability, whereby methods of the previous LC-2010 system or non-Shimadzu systems can be adapted easily. Furthermore, the compact HPLCs of the *i*-series can carry out fully automated analysis workflows.

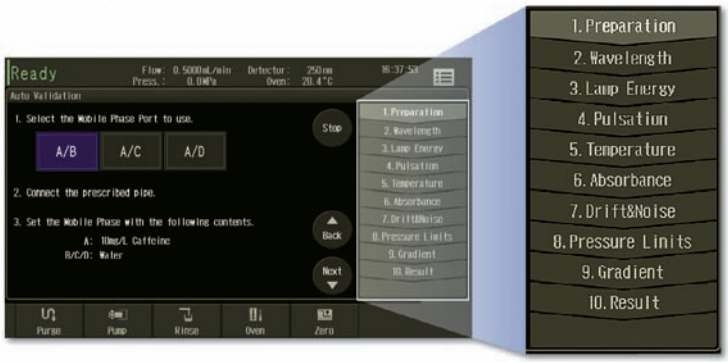

Figure 4: Auto Validation

An overview is presented in table 1.

The 'Auto Validation' function (figure 4) supports the user in obtaining a report on whether the system is working in a stable

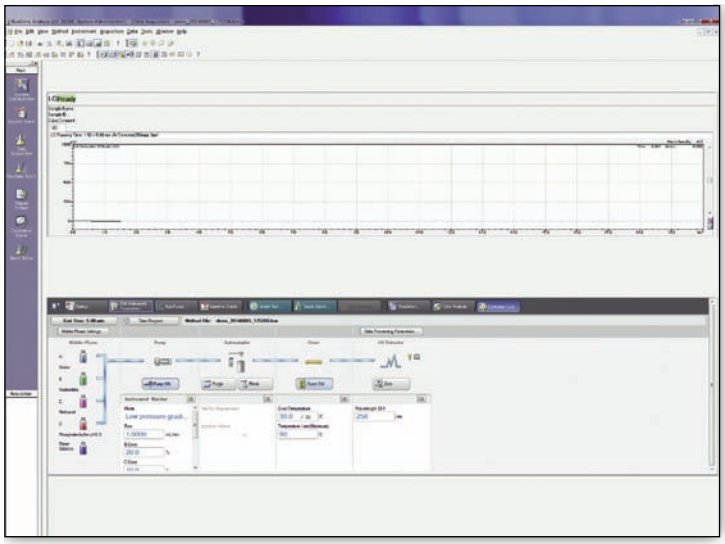

Figure 5: New user interface for LabSolutions software

range. Here, a fixed and thus at any point in time comparable procedure is used that checks solvent delivery stability or the wavelength accuracy of the absorption detector. In addition, it is possible to carry out an automated routine inspection and self-diagnosis of the instrument via the 'System Check' function. Data recorded on consumables used, for instance the number of injections, volume delivered by the pump or usage of the detector lamp, is read. The results of these two status reports can be viewed on the instrument monitor or printed via the software.

Intuition

The new unified user interface of the *i*-series and the new version of the LabSolutions software are highly intuitive. This can be illustrated via a simple comparison between figures 1 and 5, in which the flowline of the *i*-series is visualized, thus enabling intuitive operation of the software via simple selection of the various components.

Another novel software feature is the 'Quick Batch' function where users can easily and quickly create a sequence of analyses by selecting the vial positions on the sample rack which is graphically displayed (figure 6).

Workflow for implementing an HPLC analysis	Automation	Comment
System startup	Auto start-up	Automated system startup by setting a time
Purging of the flow lines	Auto purge	Automated purging of the flow lines with eluents prior to analysis
Column equilibration	Baseline check	Monitors baseline stability and determines when the analysis can be started, based on predefined noise/drift criteria
Starting the analysis	Analysis starts	Analysis is started automatically as soon as the baseline check is completed
Sample measurement	Direct access mechanism	Samples can be added at any time during the ongoing analysis
Ending the analysis	E-Mail notification	After completing the analysis, an email can be sent automatically to laboratory personnel
Column cooling down	Auto shut-down	Automated instrument shutdown with 95 % reduction in energy consumption in the standby mode. Prior rinsing of the instrument or the column can be implemented
Pump delivery and detector lamp switches off		
Column oven temperature control switches off		
System shutdown		

Table 1: Automation of the HPLC analysis workflow using the *i*-series

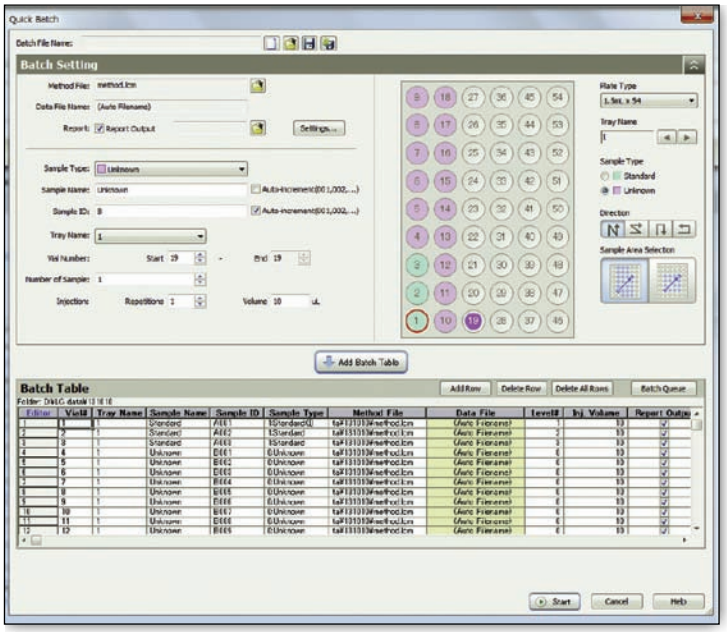

Figure 6: Quick Batch function of the LabSolutions software

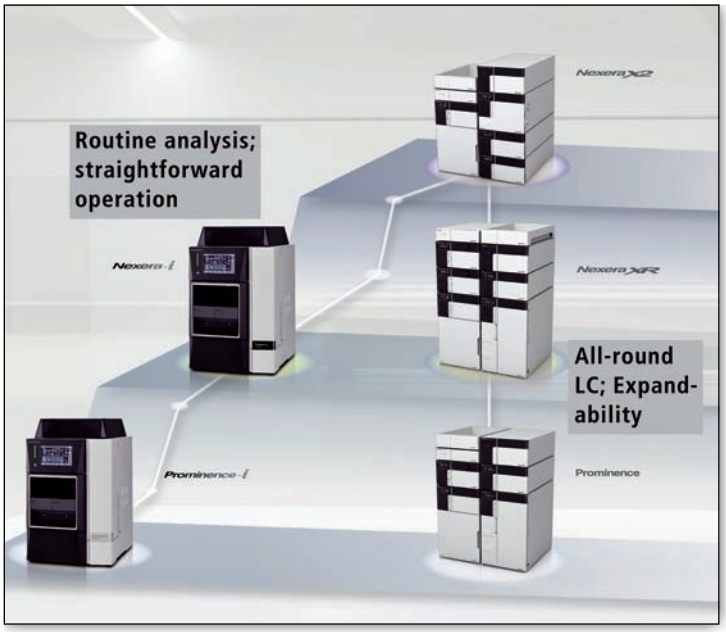

Figure 7: Product lines and categorization of Shimadzu HPLC and UHPLCs

The combination of *i*-series with LabSolutions and the system unified graphical user interface and software allows intuitive operation independently of user experience level.

Shimadzu's comprehensive LC product portfolio meets all analytical requirements, from conventional up to ultra-fast analyses. Next to the modular HPLC/UHPLC systems, figure 7 shows the position of the *i*-series among Shimadzu's LC products.

Further information

Read more about the new *i*-series on www.shimadzu.eu/i-series

Precise temperature control of sample cells

New CPS-100 accessory for UV-VIS spectrophotometers

Figure 1: UV-1800 with CPS-100

Shimadzu offers the most diverse range of accessories for UV-VIS-NIR-spectrophotometers. A wide variety of cell holders, with or without temperature control, allows customized system configurations for all requirements of the environmental, pharmaceutical, food, life science applications and many more.

Whether a single standard cell at room temperature is used or a multi-cell changer for high sample throughput is required – the ideal solution for each analytical problem is always available.

The latest release in the European market is the CPS-100 thermoelectrically temperature-controlled 6-cell changer.

The CPS-100 is a 6-cell changer capable of controlling the temperature of the sample cells in a range from 16 °C to 60 °C with a precision of +/- 0.1 °C. Combined with a spectrophotometer such as UV-1800 working in kinetics mode, the system can be configured to measure the enzyme activity of up to six samples under constant temperature conditions. Figure 1 shows the UV-1800 in combination with CPS-100.

The CPS-100 can also be used in the following spectrophotometers: UVmini-1240 series, UV-2600/UV-2700 and UV-3600.

The UV series is intended for multiple applications. A wide range of accessories enables customized solutions such as emission optics, rotating film holder, sample cups for integrating spheres, diverse selection of sipper units or photomultipliers for the NIR range.

A variety of software solutions for band gap analysis, Bilirubin determination in CSF and sun protection factor for milks, creams and textiles complement the hardware range.

About knackwursts and sausages

The big breakfast test using the EZ-Test-X Texture Analyzer (part 3)

Foods as a basic need of human existence are subject to constant inspections. The Shimadzu News regularly reports on new analytical capabilities.

In addition to taste and the inspection of ingredients, questions are always raised on the physical properties of our food: how quickly does our bread get stale? How crisp are our sausages? What are the differences between eggshells originating from different egg farming methods? ... These are the questions that are addressed in this and in the two previous issues of the Shimadzu News – using the foods that make a continental breakfast.

Those who wish to supplement their breakfast consisting of bread and eggs, might like to try some sausages. Depending on local taste, these may vary in type and can be prepared either fried or boiled. Regardless, however, of their production and preparation: all sausages are expected to be tender but still firm, as in addition to the intense flavor, consumers also want an equivalent bite sensation.

Taste and bite sensation

Additionally to the question which type of sausage best offers this bite sensation, the question on possible differences between industrially produced or home-made type sausages needs to be answered as well. To avoid any differences caused by the method of preparation, the sausages were also tested in the raw state.

All tests were carried out using the EZ-Test-X Texture Analyzer. As cut resistance and firmness are proportional, the sausages were cut using a blade test method, in order to exclude differences in chewing behavior.

As can be seen in the table below, the cutting force for sausages that are mainly eaten warm (for instance Krakauer Polish sausages or Bavarian veal sausages) is significantly higher for the raw state compared to after being cooked. This may be explained by the fact that the raw sausage is considerably softer in its initial raw consistency and, therefore, a much higher force is required to be able to make the first incision. During cooking, the sausage mass expands and the casing tightens.

In contrast, although Vienna sausages show no differences between the raw and cooked state, as they are suitable to be eaten cold as well as warm, there is however a significant difference with respect to cutting force between Vienna sausages obtained from a discount supermarket or from a butcher shop.

The results for Bavarian veal sausages and Nuremberg grilled sausages, however, appear to vary. While the Bavarian veal sausage can be cut into much more easily in the cooked state than when raw, the Nuremberg sausage is much crispier when fried compared to the cold version.

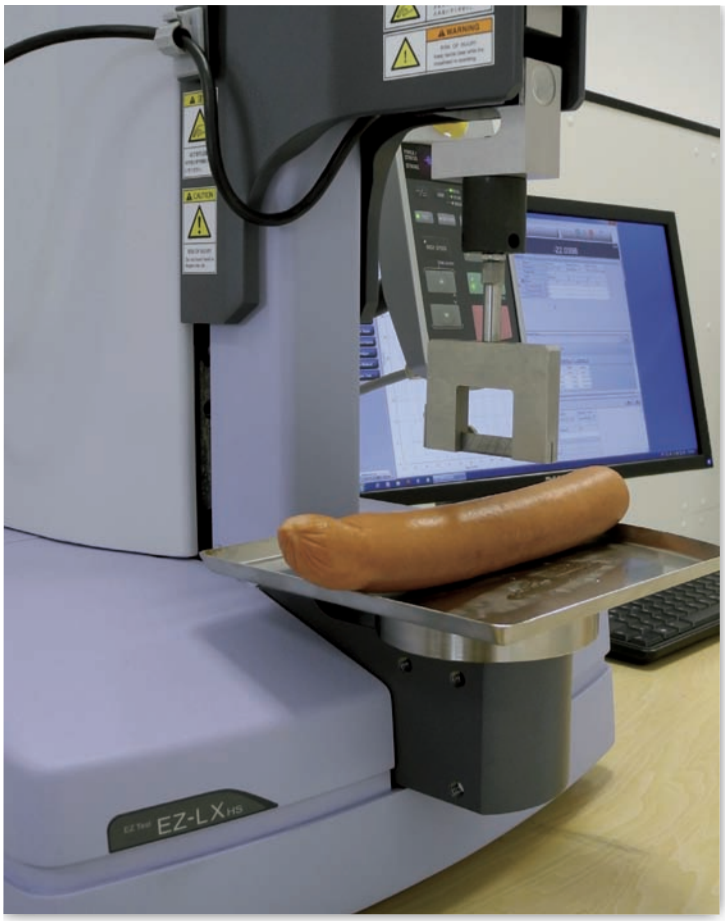

Figure 1: Cutting resistance test with EZ-Test LX

Further information on this article:
- Full big breakfast test (PDF)

www.shimadzu.eu/shimadzu-news-2014

Part 1: How do you like your eggs? – The big breakfast test using the EZ-Test-X Texture Analyzer
Part 2: The daily bread – The big breakfast test using the EZ-Test-X Texture Analyzer

Sausage type	Discount supermarket (A) / butcher (B)	Cutting force [in N] raw	Cutting force [in N] boiled / fried
Krakauer / Polish sausage	A	87	49
	B	94	70
Mettenden / Beerstick sausage	A	111	98
	B	91	84
Vienna sausage (Wiener)	A	17	20
	B	42	44
Bavarian veal sausage	B	43	31
Nuremberg grilled sausage	B	13	27

Tabelle 1: Cutting resistance comparison

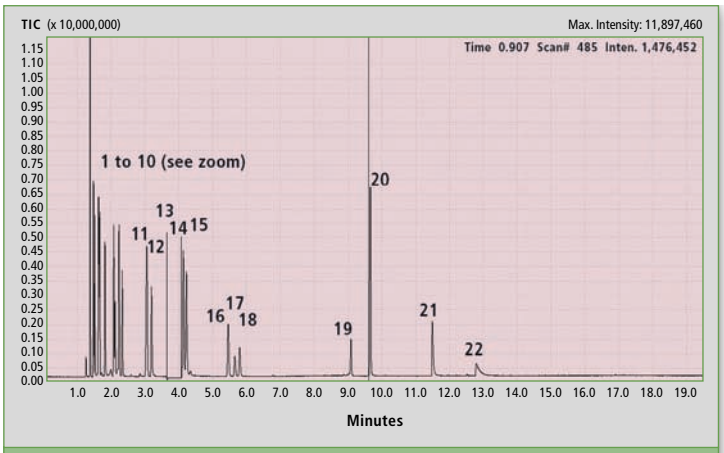

Figure 1: Chromatogram of all separated compounds in the liquid phase (peak assignment in table 2), (TIC = Total Ion Count)

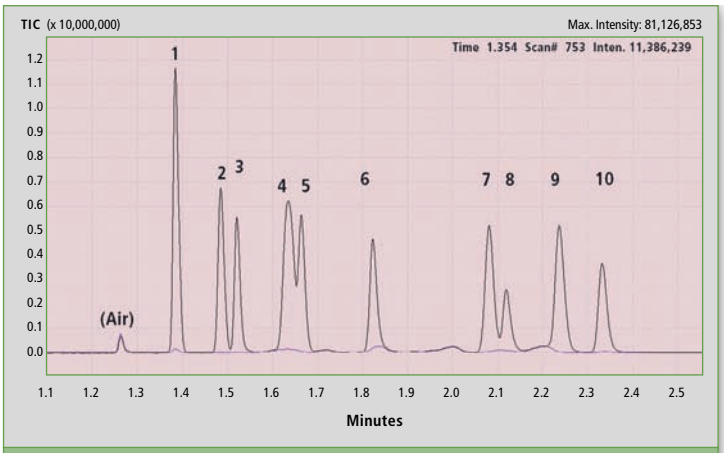

Figure 2: Extension of the range 1 to 3 min in figure 1 with simultaneous representation of the chromatograms of the GC grade toluene

Partial oxidation of isobutane

New analysis method using GC

Peroxides (R1-O-O-R2) and hydroperoxides (R1-O-OH) are increasingly being used in industry and as oxidizing agents in organic chemistry. Hydroperoxides (ROOH) are the initial, generally less stable intermediate products in the oxidation of hydrocarbons (Allara et al. [1968]). For instance, they occur when edible oils turn rancid.

Among the most stable representatives of this class is t-butyl hydroperoxide (TBHP). Current research focuses on improving the efficiency of the industrial production process based on isobutane. Isobutane usually yields several products, as the decomposition of TBHP also plays a role. A prerequisite for increasing the efficiency is, therefore, to carry out a comprehensive study of the processes and a detailed analysis of the complex reaction mixture, which has so far been largely neglected.

Time	Temperature
0 min	80 °C
5 min	80 °C
16 min	190 °C
19 min	250 °C

Table 1: Temperature program

Gas chromatography – the method of choice?

For many years, hydroperoxides have not been analyzed using GC, since it was known that they easily thermally decompose. Later, it became evident that tertiary compounds, in particular (R3C-OOH), were relatively stable. Partial oxidation of isobutane is usually carried out at approximately 130 °C. To increase the rate of the reaction, it would be desirable to carry out the reaction at a higher temperature. However, increasing the temperature also increases the decomposition of TBHP and in addition to gaseous products, alcohols, ketones and aldehydes are formed. This is why a method was needed that could separate the complex mixture occurring during this process. For this purpose, however, only GC is suitable and Shimadzu's GCMS-QP2010 Ultra was selected.

Sample preparation

For method development, potentially liquid and gaseous products reported in the literature were identified. First, a mixture of the identified liquid products was prepared in toluene. It should also be clarified which additional compounds could be considered for the preparation of the analysis samples. This is why, in particular, potential solvents such as hexane, decane, ethanol, toluene, acetonitrile and dioxane, as well as compounds that could be used as internal standards or additives, such as t-butyl methyl ether, methyl ethyl ketone and n-hexane were tested.

The main reaction products are TBHP, t-butanol, acetone and methanol; other byproducts can occur due to strong thermal decomposition. An additional problem is the sensitivity of hydroperoxides to metals (including steel) that can catalyze the decomposition.

Among the gaseous products, particularly isobutane, isobutene, oxygen, nitrogen, carbon dioxide, car-

Nr.	Compound	Retention time /min	Nr.	Compound	Retention time /min
1	Hexane	1.388	12	Acetonitrile	3.201
2	t-butyl methyl ether	1.488	13	Toluene	3.900
3	Acetaldehyde	1.519	14	Dioxane	4.146
4	Di-t-butyl peroxide	1.638	15	Isobutanol	4.231
5	Isobutylene oxide	1.664	16	Ethyl benzene	5.465
6	Acetone	1.825	17	p-Xylene	5.675
7	t-Butanol	2.083	18	o-Xylene	5.779
8	Methanol	2.121	19	t-Butyl hydroperoxide	9.089
9	Methyl ethyl ketone	2.240	20	n-Hexanol	9.648
10	Ethanol	2.335	21	Acetic acid	11.404
11	Decane	3.054	22	Formic acid	12.806

Table 2: Retention times of the separated compounds

bon monoxide and methane – as well as propene at higher temperatures – can be expected. In preparation for the gas analysis, a calibration gas containing all these gases was obtained from Linde Engineering AG. In order to carry out the analysis of both the gas phase as well as the liquid products, a suitable GC configuration is needed.

Application

This problem could be solved via column switching using MDGC-Switching (multidimensional GC). For the separation of polar compounds, an RT Stabilwax column was used (length 30 m, ID 0.25 mm, film thickness 0.25 µm). Separation and detection of the permanent gases is carried out using a molecular sieve column (RT M-Sieve 5A 30 m, ID 0.53 mm, film thickness 0.50 µm), which is connected to the Stabilwax column via the switching valve, and a thermal conductivity detector (TCD). After approximately two minutes, the valve switches over from the TCD to the MS system. The products retained by the Stabilwax column are then detected using a mass spectrometer, after passing through a stainless steel (SilcoTek GmbH, Silcosteel) restrictor (0.45 m, ID 0.15 mm). The restrictors of the switching valve were also manufactured from Silcosteel.

Separation of the mixture

a) Liquid phase

At an isothermal phase of 40 °C for 5 minutes with subsequent heating to 250 °C at 10 °C/min, the components of the liquid sample are only partially separated. The t-butanol and methanol signals overlap each other, although they are separated when using the headspace technique with a retention time difference of approximately 0.2 minutes. In the mass spectrum, the peak is therefore identified as 2-pentanol. Separation is not possible, as the mass of the main peak of methanol (m/z = 31) is the same for both compounds. By increasing the temperature of the isothermal phase from 40 °C to 80 °C (see table 1), all compounds were well separated.

Figure 1 shows the chromatogram of such a separation. Although methanol and t-butanol are not baseline separated, the signals can be clearly distinguished via their mass peaks. This temperature is not yet critical for TBHP and had already been used in the past (Abraham [1959]). The toluene peak was hidden by switching off the filament in the range of 3.8 - 4.0 minutes.

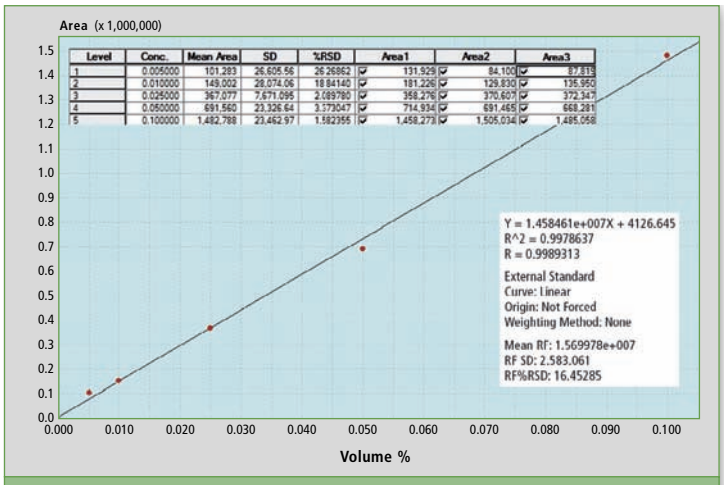

Figure 3: Calibration of formic acid

Figure 2 shows that the GC grade toluene (99.9 %, signal hidden) in addition to the xylene isomers contains other compounds which are responsible for the smaller signals in the lower retention time range. The TBHP exhibits a significantly higher retention time than all the alcohols with a smaller or comparably large alkyl group. In the calibration of formic acid (figure 3), the dependence is linear up to 0.1 vol %.

b) Gas phase

The permanent gases nitrogen, methane, oxygen and carbon monoxide detected via the TCD yield the gas chromatogram presented in figure 4. The remaining gases are retained on the Stabilwax column and – in addition to a nitrogen residue – are detected via the mass spectrometer (figure 5). Calibration of the gases is carried out using one-point calibration based on the mean of three reproducibility measurements, which is common in this case.

Conclusions

All potential liquid reaction products could be separated successfully, whereby the analysis of the strongly polar liquid products as well as the gases could be made possible via column switching. The present configuration, however, does not allow the separation of all gases. Propene was not found. This problem is being addressed further.

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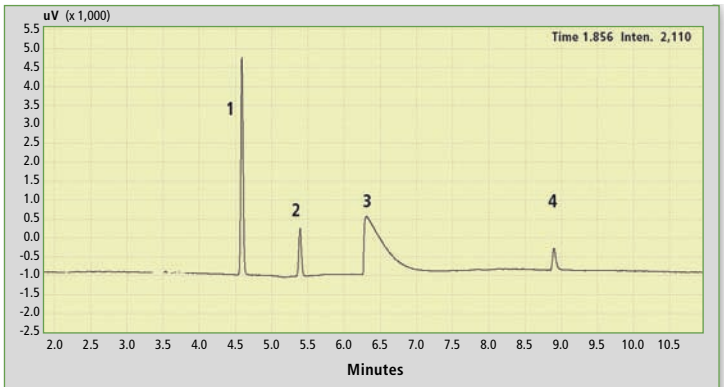

Figure 4: Chromatogram of the permanent gases nitrogen (1), oxygen (2), carbon monoxide (3) and methane (4)

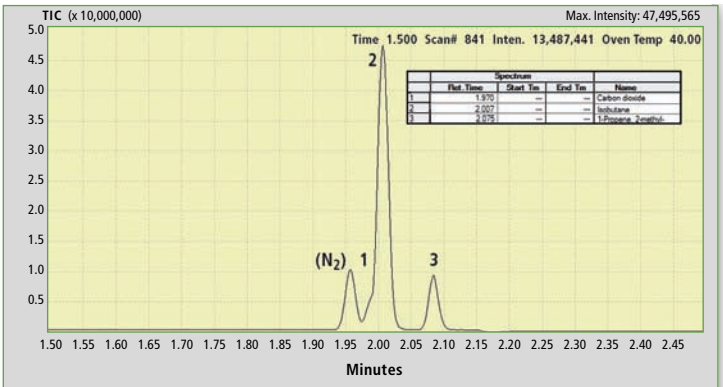

Figure 5: Chromatogram of carbon dioxide (1), isobutane (2) and isobutene (3). (Upon switching it is not completely possible to avoid entry of nitrogen).

Plastics – Water content as indicator for ageing processes

Precise measurements using FTIR microscopy

6 billion tons of plastics have been produced worldwide in the last 50 years. Due to their many versatile properties, plastics can be found in virtually all products. 'Low-density' (LD-PE) and 'high-density' (HD-PE) polyethylenes in particular are almost exclusively used in the packaging industry as they are long-lasting and persistent.

When plastics are used as packaging materials, it is important to test their water content during quality control, as this solvent can potentially transport product components outside of the packaging, as well as components from the environment inward. In addition, the water content can contain information about the condition of the polymer, since water is formed in light-induced ageing processes of polyethylene [1]. Due to capillary forces, water moves between the polymer chains, from the polymer surface into the core and forms water clusters. In the event of damage, a spatially resolved analysis of water within the packaging material could offer indications of potential causes.

In general, ageing processes of plastics can be observed easily using infrared spectroscopy, due to the high detection sensitivity of the resulting decomposition products such as water, aldehydes or carboxyl groups [1]. A great advantage of this type of analysis is its non-destructiveness and its minimal effort required. Complex sample preparation steps are not usually needed. The high spatial resolution required can be achieved using an FTIR microscope. In this way, even the smallest sample components can be examined accurately for possible damage.

In the present analysis, 'low-density' and 'high-density' polymer granulates in various ageing stages have been investigated – from new materials to recycled materials and plastic wastes from the oceans.

In the first step, the samples are split so that the outer surface and the core of the respective granulates become accessible. Small fibers are then cut from the outer and inner surfaces using a scalpel. These can be measured directly with the FTIR microscope.

Reflectance mode with the mirror as supporting surface is particularly suitable for this purpose. For measurements in the transmission mode, the use of a diamond compression cell is recommended. The separated fibers are pressed into a thin film and can be measured without any additional supporting surface.

Evaluation of the spectra

The determination of adsorbed water in polyethylene using FTIR

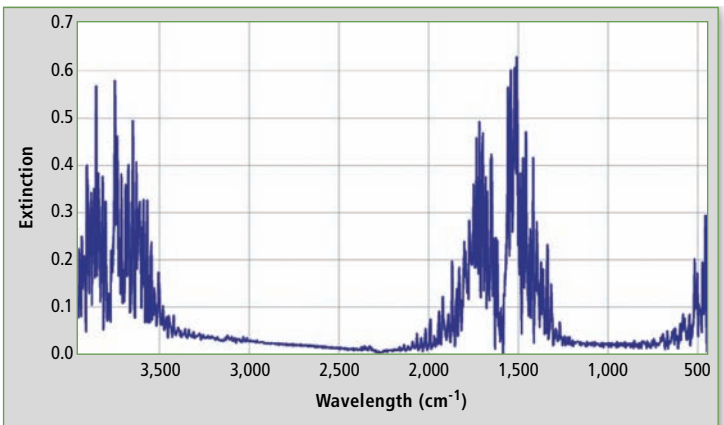

Figure 1: FTIR spectrum (absorption) of water. Typical broadband, finely split absorption patterns in the wavelength range of 4,100 - 3,500 cm^{-1} and 2,000 - 1,200 cm^{-1} [2][5].

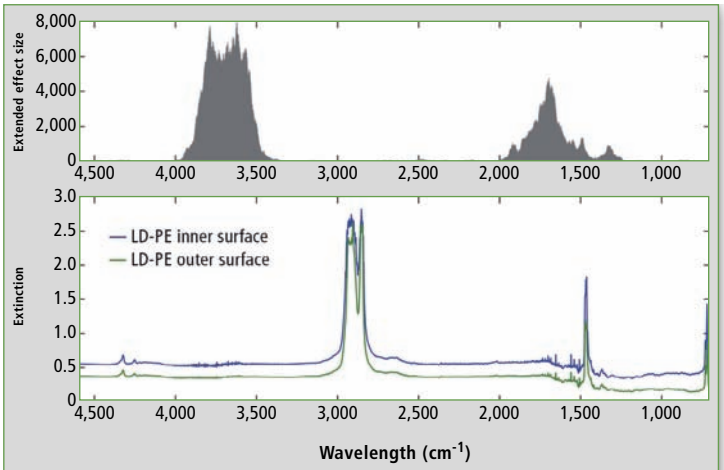

Figure 2: (Bottom) A comparison of mean value FTIR spectra (absorption) of LD-PE granulates (new material) from five measurements each of the outer and inner surfaces. The inner surface exhibits an increased background absorption and a strongly pronounced water absorption pattern. (Top) The extended effect size shows various ranges in the FTIR spectrum, which differ significantly. The water absorption ranges are clearly visible.

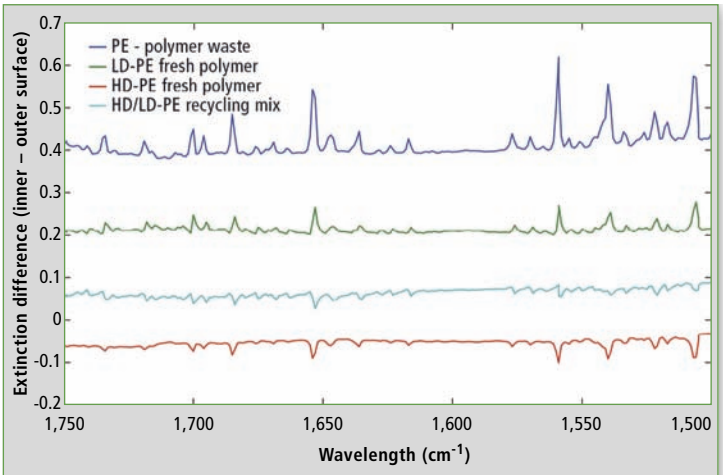

Figure 3: FTIR subtraction spectra (absorption inner surface – outer surface) in wavelength ranges 1,750 - 1,500 cm^{-1} (water absorption) [2][5]. The plastic waste investigated shows the most pronounced water absorption, which can be indicative of the quantitatively highest water content. LD-PE exhibits a slightly increased water content in the interior of the granulate, while HD-PE exhibits even less water in the core. The recycled sample is a mixture from both PE variants and lies between LD-PE and HD-PE.

microscopy requires special chemometric methods. Among other reasons, this is due to water in the form of moisture being contained in the background signal (atmosphere). In addition, water is adsorbed at ambient temperature onto the surfaces of the measurement system.

These effects are counteracted by subtracting the background spectrum as well as using empirical atmospheric corrections. A further challenge lies in the complex vibrational bands of water. At ambient temperatures, there are many different vibrational and rotational states due to van der Waals interactions. This results in very broad and finely split absorption patterns in the wavelength range of 4,100 - 3,500 cm^{-1} and 2,000 - 1,200 cm^{-1} (see figure 1) [2].

As a result of these two effects, a semi-quantitative comparative analysis of the spectra is recommended. In this method, the second derivative of the spectroscopic data, including a Savitzky-Golay smoothing, is first determined. This corrects the baseline and enables a better comparison between the individual samples [3]. Using conventional statistical methods such as the 'two sample t-test' and the 'effect size', the ranges of the IR spectrum exhibiting significant differences between the sample's inner and outer surface can subsequently be isolated (figure 2). The t-test determines the significance of a difference between two mean values, while the effect size describes the extent of that difference [4].

Results and perspectives

Using FTIR microscopy, it can be shown that in terms of water content, there are significant differences between 'low density' and 'high density' polyethylenes, as well as between new materials and plastic wastes. Much smaller water clusters are present in HD-PE, which can be attributed to the increased crystallinity. However, the theory on ageing processes of polyethylene can also be confirmed, as comparatively large quantities of water can be determined in the significantly aged plastic waste samples. A comparison of the spectra of all measured samples as well as a comparative analysis is shown in figure 3. The samples were measured using Shimadzu's AIM-8800 FTIR microscope.

The method presented can be extended through the use of a microtome for improved spatial resolution and reproducibility. In addition, as part of a multivariate data analysis, a calibration of the water content is conceivable.

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IMPRINT

Shimadzu NEWS, Customer Magazine of Shimadzu Europa GmbH, Duisburg

Publisher
Shimadzu Europa GmbH
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Design and Production
m/e brand communication GmbH GWA Düsseldorf

Circulation
German: 5,200 · English: 6,930

The nightmare of doping offenders

Analysis of doping agents using the new LCMS-8050 Triple Quadrupole Mass Spectrometer

Faster, higher, further – doping use has been associated with sports for many centuries. Already during the Olympic Games held in ancient Greece, athletes were using special plant diets to enhance their physical fitness. Beyond the sports, the ancient Incas chewed on coca leaves as a stimulant, and the Chinese swore by the ephedrine-containing Ma-Huang herb.

Figure 1: LCMS-8050

An early form of testosterone doping began with the discovery of the performance-enhancing effects of bull testicles. Even animals were not spared when it came to doping: already in the middle of the 17th century, the performance of horses was affected. This has been shown in an official decree of a small British town. In those days, however, horses were also being poisoned, for example with arsenic, in order to place a bet on a competitor's horse. As it had not been possible to detect illegal substances, it was not until 1812 that the first doping case was discovered – only because the culprit was caught in the act.

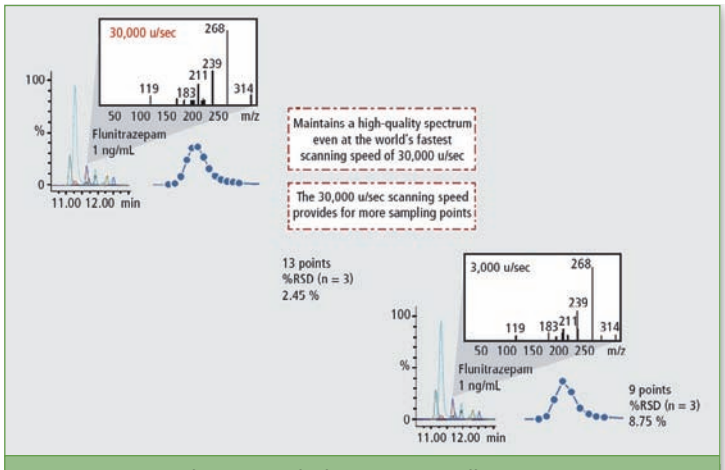

Figure 2: Comparison of data quality for flunitrazepam at different scanning speeds

From nutritional supplement to doping

During World War II, pharmacists discovered how to synthesize derivatives of testosterone: anabolic steroids. Initially, they were used to feed emaciated prisoners of war. Soon athletes discovered these preparations, which were henceforth considered to be the 'breakfast of champions' – but not only for human champions.

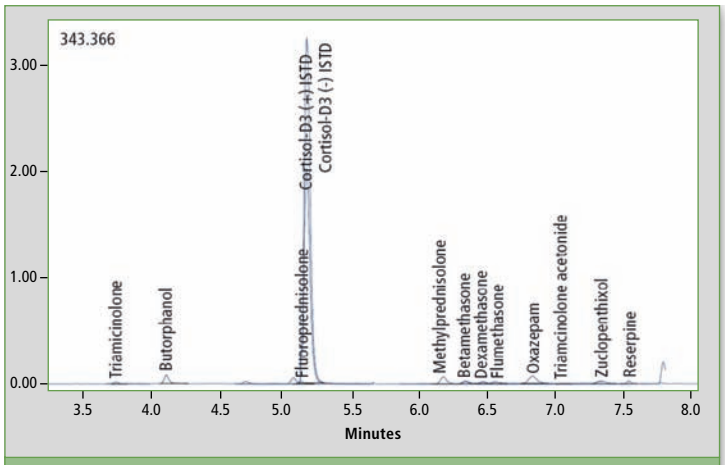

Figure 3: Chromatogram of a horse urine extract, 13 events / 28 MRMs

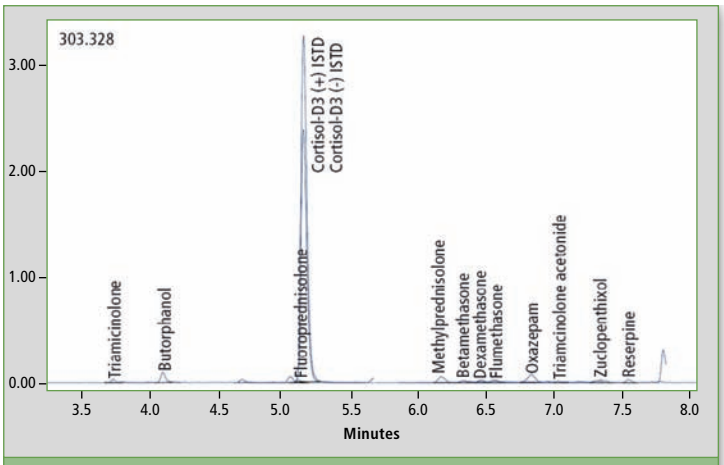

Figure 4: Chromatogram of a horse urine extract, 127 events / 254 MRMs

The American racehorse Holloway won one race after another after receiving testosterone.

Also today, doping incidents are being reported time and again, in spite of all control measures. During the Olympic Games in Beijing, six horses were tested positive. A current example is the most recent doping scandal in endurance racing, a horse racing variant that enjoys great popularity in the Arab countries. The winner of the prestigious UAE President's Cup, which was held in Abu Dhabi in Spring 2013, was banned for two years after a doping-related steroid was detected in his horse. Already in pure mathematical terms, the race across the desert happened at a pace that must have been harmful to the health of the animal.

The seizure of over 120 banned and illegally imported substances including anesthetics, anti-inflammatory drugs and antibiotics, at a British thoroughbred stud farm at the beginning of this year illustrates that the well-being and health of horses are no longer prioritized, but that the animals are being downgraded to 'sports equipment' that must deliver maximum performances during the day of the race.

What is doping?

For years, animal welfare organizations have been demanding consistent actions against the quite lenient anti-doping sanctions in equestrian sports. In Germany, the FN Anti Doping and Drug Control regulations (FN-Anti-Doping- und Medikamentenkontroll-Regeln, ADMR), issued by the German Equestrian Federation (Deutsche Reiterliche Vereinigung, FN) and adapted to international regulations, have been in force since 2010 and are continually updated.

Since April 1, 2011, German top-class riders must be prepared for 'house calls' by the National Anti Doping Agency (NADA), in addition to regular doping controls during tournaments. These controls on horses outside of the tournament are unique worldwide and groundbreaking in the fight against doping.

In general terms, doping refers to the use of substances from prohibited substance classes as well as to the use of banned methods for performance enhancement. Horse racing is also about negative doping, i.e. 'doping to defeat' by decreasing a horse's performance and giving competitors an advantage, or of unauthorized medication. In unauthorized medication, an existing reduction in performance is assumed to be the result of an illness. The horse is, for instance, treated with an analgesic and can thus perform 'normally' again. Just like doping, performance is influenced, which is prohibited in competition.

In unauthorized medication, however, good intentions are assumed, which is reflected in lower sentencing. As broad-ranging as the application possibilities are, so also are the varieties of substance classes used. Stimulants such as amphetamines that make horses run up to the point of complete exhaustion are commonly used. Methyl xanthines which include caffeine also act against signs of fatigue. An opposite effect is attained by sedatives that are used to get extremely nervous horses 'ready for takeoff.' The list of prohibited substances also includes steroid hormones, including testosterone and peptide hormones such as EPO that are used in human doping.

Ludger Beerbaum, one of the most successful show jumpers of the past 20 years, commented on his approach to equestrian sports over the past years in a 2009 'Frankfurter Allgemeine Sonntagszeitung' weekly Sunday paper as follows: "Over the years, I have become accustomed to exhausting all possibilities ... In the past, I had the attitude: anything that is not detected, is allowed" (cited according to Wikipedia as well as Spiegel Online). Today, even the smallest traces of doping agents can be detected in blood and urine. Zero tolerance policies meanwhile apply in equestrian sports, i.e. no prohibited substances may be detected in a horse's blood or urine at the time of the competition.

Advanced analytics extend detection possibilities

The analytical possibilities of a laboratory are crucial for the detection of a substance. Based on optimized detection methods and consistently further developed instruments, substances are now found whose detection had not been possible a few years ago. In addition, these substances can be detected over a much longer time period. Often, the time interval in which these substances can be detected surpasses the effect duration of a substance.

In these cases, a sample can only be considered positive when the amount detected is still effective. This is why doping is sometimes unintentionally a topic of discussion when waiting periods or retention periods are not properly observed, especially when topically applied ointments and gels also lead to positive test results.

Very recently, the idea of a highly sensitive LCMS system, the youngest member of Shimadzu's UFMS (Ultra Fast Mass Spectrometry) family, has become a nightmare for doping offenders. The LCMS-8050 high-performance triple quadrupole mass spectrometer (figure 1) features the world's fastest data acquisition rates and the highest sensitivity, enabling simultaneous quantitative and qualitative analyses. This instrument was developed specifically for increasing quantification requirements at trace level in clinical research, food analysis and environmental analysis.

Excellent sensitivity combined with high-speed analytics is achieved via two improved techniques: the newly designed heated ESI source and the advanced UFsweeper-III collision cell with efficient fragmentation. To optimize desolvation, the newly designed ESI source uses a heating gas in combination with a nebulizer.

New techniques deliver superb qualitative data

The following application example from horse doping illustrates the excellent sensitivity as well as the clear advantage of ultra fast MS technology. Real samples from a horse doping laboratory were tested for various steroid hormones, in the form of free steroids or steroid esters. Small molecules of banned neuroleptics, benzodiazepines and opioids were also investigated.

The samples were analyzed using Shimadzu's LCMS-8050 triple quadrupole mass spectrometer coupled to a *Nexera* X2 UHPLC.

An outstanding feature of the LCMS-8050 is its ultra fast scan speed for MS data acquisition. Figure 2 illustrates the clear advantage of a fast analysis system. Using a scan speed of 3,000 u/sec, only nine data points can be acquired across the marked peak. As a rule of thumb, at least 10 data points should be acquired in order to obtain good reproducibility of qualitative and quantitative results.

A comparison of the standard deviations (RSD) for flunitrazepam, calculated for 3,000 u/sec and 30,000 u/sec, demonstrates unequivocally the advantage of fast data acquisition (figure 2). At these ultra fast scan speeds, quality as well as sensitivity of the simultaneously acquired qualitative data often decreases, assuming that the instrument even allows simultaneous acquisition of MRM and scan data. Both flunitrazepam mass spectra in figure 2 prove that the LCMS-8050 delivers first-class qualitative data, also at the highest possible scan speed of 30,000 u/sec.

High-quality data without loss of sensitivity

In the following example, a horse urine extract spiked with 1 pg/µL of a standard was screened for doping substances using an MS screening method in which 254 MRMs are stored. Due to the high scan speed of the LCMS-8050, it is possible, even with this high number of MRMs, to obtain data of excellent quality and without significant loss in sensitivity. This can be clearly seen when comparing the chromatograms of figure 3 (28 MRMs) and figure 4 (254 MRMs). In addition, detailed assessment of a less intense peak (here: triamcinolone) (figure 5) proves the consistent high data quality despite a considerably higher number of MRMs.

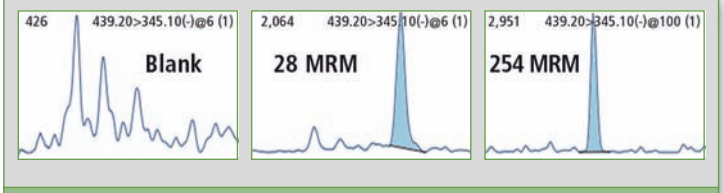

Figure 5: Details of triamcinolone, comparison of chromatograms of 28 versus 254 MRMs

In further measurements, reproducibility (as a measure of good data quality) at simultaneous synchronized survey product ion scan of a doping standard solution was analyzed. Shimadzu's LabSolution software allows simultaneous MRM and product ion scan measurements. The scan does not run permanently in the background but is triggered by an increase of the masses, stored in the MRM method, above a defined threshold.

The synchronized survey scan is not just limited to product ions; it is also possible to define specific masses in the method that will trigger a scan. Table 1 (see further information) shows a comparison of results for reproducibility experiments (n = 6) – working in MRM mode only and then analyzed again in MRM mode with a dwell time of 5 msec for all components plus simultaneous synchronized survey product ion scan (scanning speed 30,000 u/sec). The concentration of the injected standard is equivalent to an extracted sample with a concentration of 2 pg/mL. This comparison also confirms that simultaneous measurements of quantitative (MRM) and qualitative (synchronized survey scan) data are possible without significant losses in sensitivity and reproducibility (see figure 6).

Regular doping controls using modern high-end analytical instruments such as the LCMS-8050 make life difficult for doping offenders in equestrian sports. Statements like "… anything that is not detected, is allowed" are definitely a thing of the past and even recreational riders must be cautious that their animals will not suddenly test positive after careless use of medication.

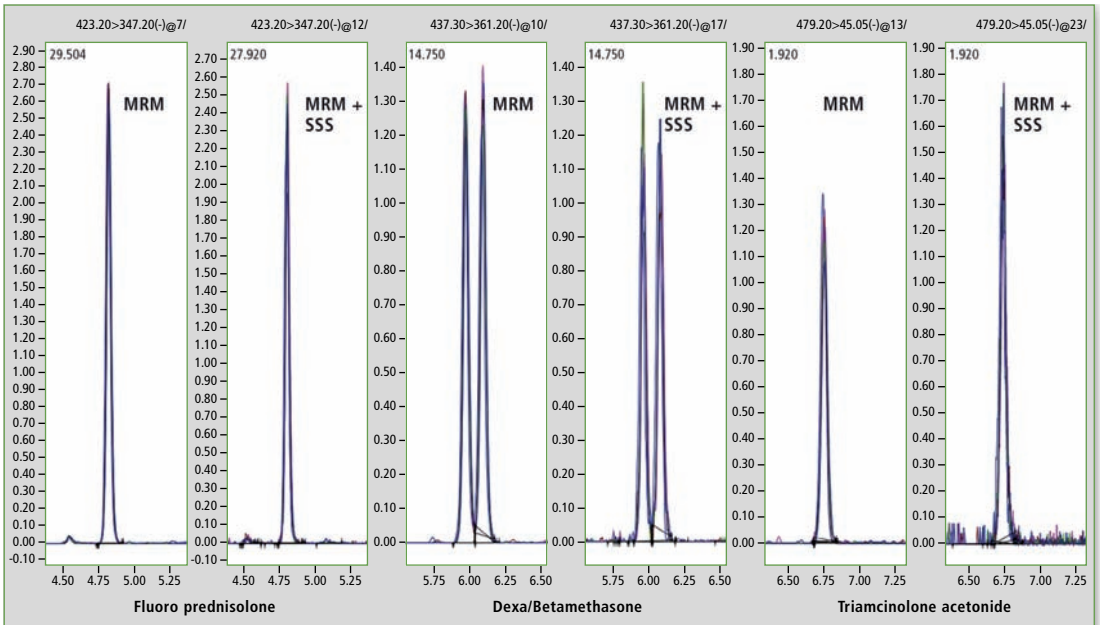

Figure 6: Reproducibilities (6 injections) of an extracted standard acquired using MRM only compared to a combined method MRM and synchronized survey scan with 30,000 u/sec

Further information on this article:
- Poster (PDF)
- Table 1, with comparison of results (Link in text, PDF)

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Espresso in Lake Constance

Five application examples of simultaneous elemental analysis sensitivity

Water is the best-known test medium used in elemental measuring techniques in instrumental analysis. However, modern instrument systems such as Shimadzu's AA-7000 (atomic absorption spectroscopy, AAS) or ICPE-9800 (optical emission spectroscopy with inductively coupled plasma, ICP-OES) offer considerably more application possibilities.

What are these possibilities and what special solutions are available? One answer is provided by the following application overview of the ICPE-9800, which can measure more than 70 elements at the same time for any application within the shortest possible time (simultaneous elemental analysis). Due to the high dynamic measurement range of the ICPE-9800, it is irrelevant whether the elements to be determined are present in the lower ppb range (µg/L) or in the higher ppm range (mg/L).

The ICPE-9800 with its flexible instrument configuration is a true multi-tool. For instance, operation can be switched between hydride system and ultrasonic nebulizer as well as between the various sample types, such as water and organic samples.

Quality assurance of sulfuric acid for special applications

Sulfuric acid (H_2SO_4) plays a major role in synthetically produced chemicals. Its application range is very diverse and the requirements for high purity are not always met, as for instance in the production of phosphate and ammonium sulfate fertilizers. [1]

ICPE-9800 – the multi-tool

However, there are also many special application areas requiring high-purity sulfuric acids such as sample preparation for instrumental analysis. Here, the acid is, for instance, used for sample digestion of polymers, fats or some geological samples such as aluminum oxide. [2]

Using the ICPE-9800, a sulfuric acid sample was measured in a 1:10 and 1:20 dilution. Using the standard addition method as calibration model, matrix effects can be masked. When the results of both dilutions are comparable, it can be assumed that the method is accurate. In order to permit a final validated method, a certified reference material can be measured in addition. The results (summary) are listed in table 1 and are determined for each element at various analytical wavelengths.

Although the diluted sulfuric acid solution still is quite aggressive and its viscosity differs from that of water, measurement can be carried out using the standard configuration (Minitorch). In addition to the consistent results of the different dilutions, the recoveries for samples with added standard are also very good (100 ± 3 %).

Rapeseed oil for biodiesel – determination of phosphorus, sodium and potassium

Vegetable oils in fuels contribute to the sustainable operation of combustion engines. However, they should not influence engine performance and catalyst efficiency in order to guarantee the environmental friendliness of biofuels. [3]

The determination of phosphorus in rapeseed oil is therefore essential, as this element impairs the functioning of catalysts. High levels of calcium and magnesium are also detrimental, since both are associated with increased ash deposits in soot particle filters. [3]

For measurement using ICP-OES, the sample only needs to be diluted. The use of an additional combustion gas (oxygen) is not necessary in this case. Nevertheless, the highly sensitive axial plasma

	Dilution factor	Cadmium, Cd	Chromium, Cr	Copper, Cu	Magnesium, Mg	Nickel, Ni	Zinc, Zn
H_2SO_4 Sample A	20	n.d.	2.62 ppm	0.052 ppm	0.056 ppm	1.68 ppm	0.032 ppm
Spike Recovery		98 %	102 %	99 %	100 %	100 %	99 %
H_2SO_4 Sample A	10	n.d.	2.62 ppm	0.073 ppm	0.038 ppm	1.79 ppm	0.033 ppm
Spike Recovery		97 %	97 %	99 %	98 %	98 %	97 %

Table 1: Analysis results of sulfuric acid. For determination of the recovery, 0.5 mg/L (ppm) of the element was added. n.d. = not detectable.

observation mode can be applied. Measurement results and detection limits are compared in table 2. When selecting a more sensitive working range, additional and more sensitive wavelengths can be

used, whereby the detection limit is further reduced. The stability of measured values for the analysis results is 100 ± 2 % (measuring interval 1 hour).

The results show that the limit value can be determined very satisfactorily using the ICPE-9800 and that the broad measuring range makes it possible to also unequivocally and reliably measure increased concentrations.

Based on the analysis results, the sample can be assessed and is found to be clearly unsuitable for use in biofuels. The considerably increased concentration of the three elements may, for instance, be due to the increased proportion of immature seeds used in production. Other influencing factors are the shelling of the seeds and proportion of broken grains as well as the pressing parameters such as the pressure head temperature.

When using the ICPE-9800 for analysis of other types of organic samples, for instance samples from the petrochemical industry, the use of an Ar/O_2 mixed gas supply kit is recommended.

Along with the standard argon carrier gas, an additional gas is introduced via the quadruple torch for sample transport and maintenance of the plasma. It increases the decomposition of the matrix, thereby reducing background noise.

By using this instrument configuration, the plasma can be observed axially and radially despite the carbon-rich sample material, and detection limits in the lower ppb range can be achieved. For example, for tin in toluene, a detection limit of 2.0 ppb could be attained. Other sample types for this instrument configuration are, for instance, kerosene, xylene, methyl isobutyl ketone (MIBK), isopropyl alcohol (IPA), ethyl alcohol or also the volatile tetrahydrofuran (THF).

Elemental analysis in limited sample volumes – 1 mL and less!

The intelligent instrument design of the ICPE-9800 not only reduces the consumption of argon via its Minitorch, but also ensures efficiency in other aspects: a CCD chip continuously detects the entire spectrum and therefore acquires information on the element content of a sample significantly faster than the earlier sequential ICP-OES technique.

Unlike conventional sample amounts of 5 - 10 mL, the ICPE-9800 only needs 1 mL or less sample volume, depending on the application. In this way, the smallest sample amounts can be investigated without the need for dilution. In addition to the timesaving effect, not having to dilute the samples eliminates the danger of contamination and the loss of sensitivity. Within the shortest possible time, it is possible to obtain meaningful results from a triplicate determination including axial and radial plasma observation.

The measurement of small sample amounts is not only useful for limited volumes. When larger volumes of test media are to be measured for changes in element content over a longer period of time, it is also advisable to only use small samples in order to minimize the effect on the test system.

Rare earths in electronic waste – an alternative source of raw materials?

Which elements are actually contained in waste? This is a key question in a world with limited quantities of raw materials and their continuously increasing consumption. Bottlenecks in the supply of rare earths are already important topics covered in the media.

Rare earths belong to a group of elements that include neodymium, dysprosium or cerium, and add special features to advanced electronic components. They make it possible to design small and compact mobile phones that are, at the same time, quite powerful. But these elements are also used at ton-scale, for instance in the green energy sector as in generators of modern wind turbines.

As raw material sources are becoming limited, recycling possibilities are moving into focus, especially when extraction of rare earth oxides and subsequent purification to pure metal form re-

quires extensive use of chemicals. In 2010, the EU classified rare earths as raw materials with a high supply risk [5]. Todays REE recycling rate is around 1 %.

Is recycling from electronic scrap worthwhile? To answer this question, various electronic components were crushed (homogenized) and digested in a laboratory microwave oven. The digestion solution can be measured using the ICP-OES and yields the results listed in table 3.

This study shows that the analysis of solids using the ICPE-9800 is possible and that modern waste contains many elements which are in high demand on the international raw materials market. Not every sample may contain a variety or a large amount of rare earths, but recycling of used mobile phones (>1 g/kg Nd) could be quite conceivable after selective presorting. For more information on analysis of REE in e-waste please see literature note.

Element	Limit value to DIN 51627-6	Detection limit* [mg/kg]	Analysis result* [mg/kg]
Phosphorus	3.0 mg/kg	0.085 [177.499 nm]	13.6
Calcium	1.0 mg/kg	0.025 [183.801 nm]	17.4
Magnesium	1.0 mg/kg	0.005 [285.213 nm]	1.80

Table 2: Results obtained with the ICPE-9800. Analysis results of rapeseed oil as well as detection limits and maximum admissible content according to DIN 51627-6 (*dilution factor regarded).

Ultra-trace analysis in the ppt range

When elemental analysis in the ppt range (ng/L) rather than in the ppb range (µg/L) is required, questions can often be answered using ICP-MS. But this concentration range can also be covered using advanced ICP-OES such as the ICPE-9800. In this way, detection limits for more than 10 elements (Ba, Be, Ca, Eu, Lu, Mg, Mn, Sc, Sr, Y, Yb) lie within single or double-digit ppt range using the standard configuration including the Minitorch. For more sensitive detection of other elements, various other approaches are available.

Special nebulizers generating sample aerosols more efficiently and reproducibly with a smaller droplet size distribution reduce detection limits very easily without the need to significantly change the instrument configuration. An additional ultrasonic nebulizer can be connected to the ICPE-9800, enabling more sensitive measurements by up to a factor of 20. Besides the elements mentioned above, which can be calibrated in the ppt range using the standard configuration, 27 more elements with detection limits in the double-digit ppt range and lower are added (Ag, Cd, Ce, Co, Cr, Cu, Dy, Er, Fe, Gd, Hf, Ho, La, Li, Mo, Nb, Nd, Pd, Pr, Sm, Ta, Tb, Ti, V, Zn, Zr). Strontium has the lowest detection limit of 1 ppt (parts per trillion – this is the equivalent of a cup of espresso in Lake Constance [Bodensee] surface area = 536 km²).

Elements such as arsenic and mercury that are highly important for the environmental sector are not included in the above list. But it is also possible for these elements to attain detection limits in the ppt range. In this case, the ability of the elements to pass from the liquid into the gas phase after conversion with sodium borohydrate ($NaBH_4$) is taken advantage of. Instead of the aerosol, the gas stream is introduced into the plasma, completely separated from the matrix. As, Sb, Se, Sn and Hg can be detected in the ppt range using this variant.

ICPE-9800 is therefore a good and economical alternative to ICP-MS for detection in the ultra trace range in order to address specific analytical problems.

Sample	Nd	La	Pr	Dy	Y	Er	Ce
Mobile phone	1.040	107	88	48	4,6	4,6	n.d.
LCD (Display)	33	51	n.d.	n.d.	7,8	n.d.	86
Printed circuit board	n.d.	n.d.	n.d.	n.d.	1,3	n.d.	32

Table 3: Analysis results in mg/kg for rare earths in electronic scrap.
n.d. = not detectable.

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Further information on this article:
- Application Note ICP-OES Spectroscopy (PDF)
- Poster Rare Earth Elements in Electronic Waste (PDF)

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Bilirubin determination in cerebrospinal fluid following a subarachnoid hemorrhage

Stroke, aneurysm – UV spectroscopy supports fast response

The UV spectroscopic application presented here is based on the 'National Guidelines for analysis of Cerebrospinal Fluid for Bilirubin in suspected Subarachnoid Hemorrhage' according to Beetham [1].

The causes of cerebral hemorrhage can be diverse, such as a stroke or an aneurysm. In order to take appropriate measures for treatment of a patient, it is important for the physician to determine which type of blood (old, fresh or none) is present in the cerebrospinal fluid. In case of illness, red blood cells will initially enter the cerebrospinal fluid. These cells contain oxyhemoglobin, which is converted to bilirubin by enzymatic processes. In addition to bilirubin, methemoglobin may also be formed.

As the process is time-dependent, the bilirubin concentration and the presence of oxidation products such as oxyhemoglobin provide information on the state of the patient. In addition, it is possible to estimate the duration of the oxidation process.

Subarachnoid hemorrhage is a pathological event in the central nervous system. It is characterized by bleeding into the subarachnoid space, which is filled with cerebrospinal fluid (CSF, liquor cerebrospinalis). Subarachnoid hemorrhage accounts for up to 10 percent of all strokes.

This particular form of stroke is, in most cases, caused by rupture of an arterial vessel due to an abnormality. Subarachnoid hemorrhage is accompanied by sudden onset of a very severe headache and neck stiffness. This can lead to short-term decreased levels of consciousness, but also to severe permanent brain disorders (quoted from Wikipedia).

Bilirubin determination, such as the determination of bilirubin in CSF, is an analytical method used in clinical analysis. In CSF-bilirubin determination, cerebrospinal fluid is analyzed for blood fragments. CSF bilirubin = cerebrospinal fluid bilirubin.

Pigment	Absorption range
Oxyhemoglobin	between 410 and 418 nm
Bilirubin	broad bands of 450 to 460 nm or as a shoulder in the oxyhemoglobin signal
Methemoglobin	more rarely occurring pigment; when present it occurs as a broader signal between 403 to 410 nm

Table 1: Analytical wavelengths for hemoglobin

Base point A = 350 - 400		Base point B = 430 - 530	
365	0.0289	476	0.021
NBA at 475 nm		Limit value	Evaluation
0.00385714		< = 0.007	OK

Table 2: Calculation of the net bilirubin content (NBA) after graphical evaluation

Multi-component measurement required

The color pigments mentioned above are suitable for spectroscopic analysis in the visible range. Quantitative but also qualitative assessment as well as color representation are the domain of UV-VIS spectroscopy. Since cerebrospinal fluid is a mixture not only represented by hemoglobin, it therefore requires a multi-component measurement, as all spectra of the individual components overlap (superposition). In this case, a straight baseline as would be expected in the analysis of liquids is not measured, but rather a rising baseline extending into the UV range. This effect occurs due to substances responding to UV irradiation, such as proteins or other high-energy compounds (pi electrons) in materials whose spectra are expected to be similar to hemoglobin spectra.

This shifting baseline presents a challenge for the automated analysis of very small maxima or shoulders in a signal pattern. Various analytical methods are applied to separate these pigments (HPLC). Fast testing methods can also be performed, such as use of a grating spectrometer for the rapid screening of hemoglobin.

Defined measuring method

Beetham has defined the measuring procedure according to which measurement should be carried out in the visible range from 350 to 600 nm. The analytical wavelengths for the hemoglobin color pigments are listed in table 1. The method requires corrected signal heights, realized via the baseline/tangents under the signals/bands. Net bilirubin absorption is determined using a fixed wavelength of 476 nm.

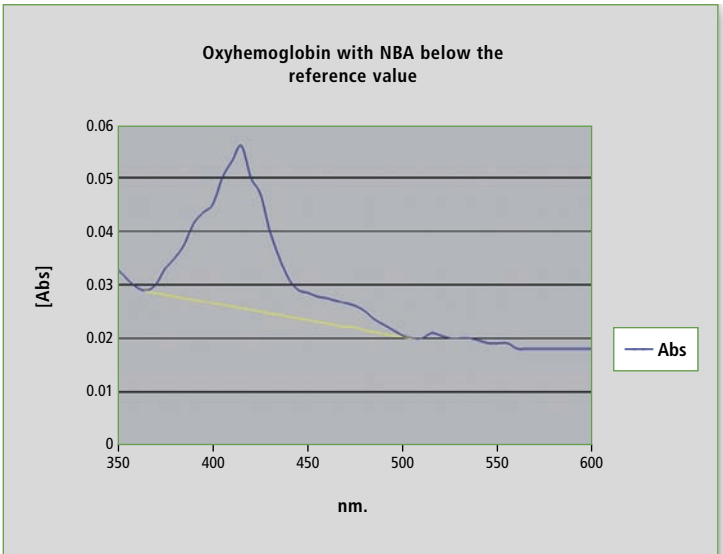


Figure 2: Representation of an absorption spectrum of a bilirubin sample and the representation of the baseline used for the correction of the bands

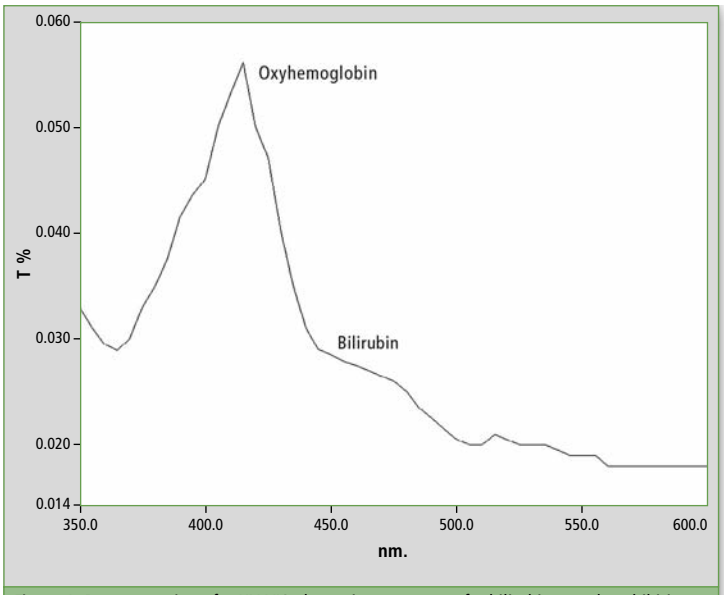


Figure 1: Representation of a UV-VIS absorption spectrum of a bilirubin sample exhibiting an oxyhemoglobin signal at 415 nm and a bilirubin shoulder at 450 to 460 nm recorded using the UVProbe software

Beetham specifies the evaluation of the spectra. One specification is that the baseline under the signal should never intersect the signal itself. This strict instruction infers that automated evaluation cannot lead to the correct result. Manual evaluation is called for, in which the base points under the signal can be specifically adjusted/corrected.

To solve this problem, an Excel spreadsheet was developed in which it is possible to measure, to represent the spectrum, and to manually shift the baseline. Within the evaluation range, all required values of the sample could be represented.

A representation of the spectrum from the Excel sheet is shown in figure 2.

The evaluation is presented in table 2.

Shimadzu's UV-1800 spectrophotometer was used for the analysis. The spectrum was measured in the range of 350 to 600 nm. The sample was measured using a 1 cm cuvette. The limit value in this example was below 0.007 absorption units.

Summary

Using the Excel method, the calculation of the net bilirubin concentration can be easily determined. Using a macro simplifies the adjustment to local conditions, such as different output formats and adaptation to individual forms. On the other hand, it is easy to extend the macro with other applications. The direct instrument control of the UV-1800 allows for direct data evaluation. It is also possible to import the results from the UV-Probe software, which is supplied as standard with the UV-1800.

Literature

[1] „National Guidelines for Analysis of Cerebrospinal fluid for Bilirubin in Suspected Subarachnoid Haemorrhage", Draft 1; R. Beetham, M.N. Fahie-Wilson, I. Holbrook, I.D. Watson, P.R. Wenham, P.A.E. White, P. Thomas, A.M. Ward, A. Cruickshank, G. Keir, W. Egner, K. Allen

New: TOC Application Handbook, Version 2

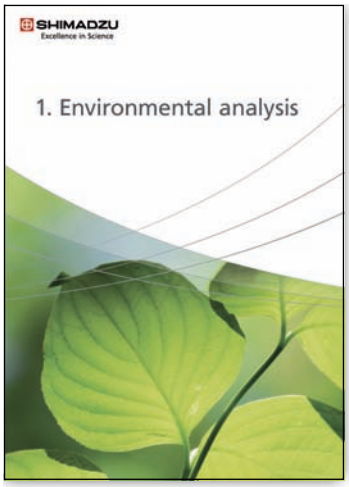
Online and Laboratory TOCs

TOC Application Handbook

As a leader in TOC technology, Shimadzu has been setting standards with its analyzers for decades. The wealth of Shimadzu's experience continuously flows into the development of its TOC systems. In consequence, Shimadzu's online analyzers, as well as laboratory TOC systems offer the highest flexibility, high availability, tremendous robustness and stability, straightforward and intuitive operation accompanied by advanced control and evaluation software. Users benefit from personal support; many additional functions facilitate their work and offer latitude for other important tasks.

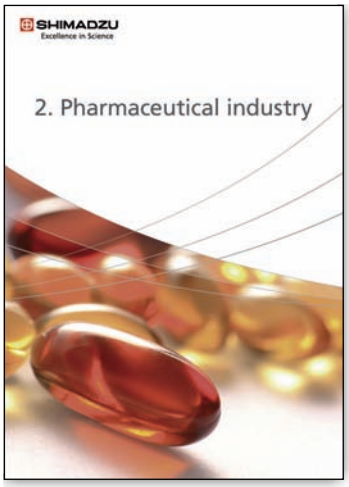
In 2012, Shimadzu has summarized its available experience and information in the first TOC Application Handbook. In the currently published second revision, the existing chapters have been extended and now include 50 application and information notes in more than 100 pages. The TOC Application Handbook contains the following chapters:

1. Environmental analysis
TOC analysis is carried out in a wide variety of environmental matrices – from groundwater to seawater, from drinking water to wastewater, from soils to sewage sludge. This chapter discusses the diversity in environmental applications and their many varying challenges. In addition to the different



concentration ranges, conditions are always diverse, such as salinity or number of particles.

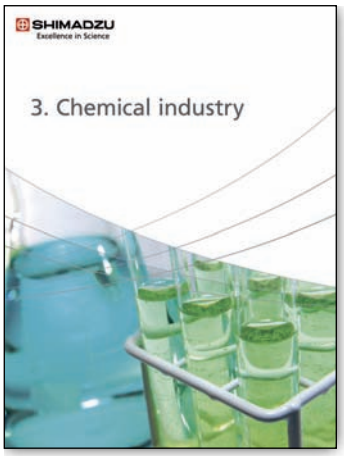
2. Pharmaceutical industry
The TOC determination is described in the European Pharmacopoeia (EP). The sum parameter serves as a measure for contamination by organic components. Not only the method itself is described, but also a test to verify the suitability of a TOC analyzer for the analysis. Recent changes in the American Pharmacopoeia and their effects on validation are presented in a new application note.

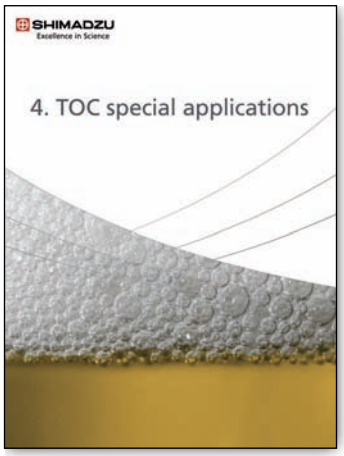


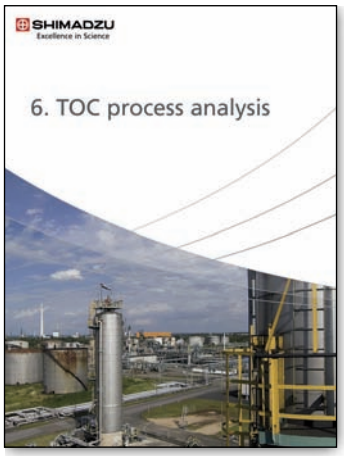
Furthermore, the various samplers for TOC analysis in cleaning validation are highlighted.

3. Chemical industry
Incoming goods control plays an important role in the chemical industry. Impurities present in reagents often also constitute the impurities in products. In addition to the targeted analysis of known compounds, sum parameters can help to assess the raw chemicals with regard to their impurities. TOC is important here: this parameter describes the contamination by organic compounds and specifies the total amount of organic carbon. TOC can, therefore, also be used for the assessment of inorganic chemicals.

Descriptions for TOC determinations in phosphoric acid and in diluted hydrofluoric acid have been added.







4. TOC special applications
Due of its informative significance, the TOC sum parameter is widely applicable. It mirrors the total concentration of organically bound carbon or organic compounds.

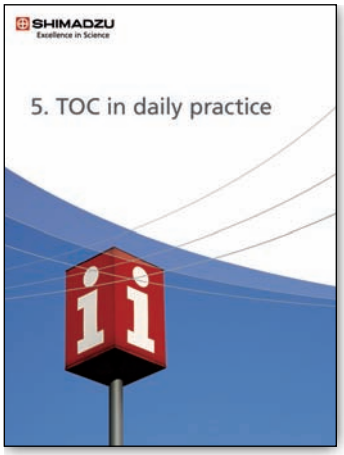
Besides the environmental, pharmaceutical or chemical industry, the TOC parameter is used in numerous other complex applications. Often, it is the scientific curiosity and ingenuity of users who want to solve an analytical problem or simplify analytics that leads to finding the key to the answer in the TOC parameter.

5. TOC in daily practice
This chapter deals with the individual modules, kits, options and supported functions of Shimadzu's TOC analyzers. Concepts and methods are also described. Information on related sum parameters such as BOD (biochemical oxygen demand) and COD (chemical oxygen demand) are included.

Many users want to calculate the COD from the TOC and, therefore, need correlation factors. These correlations depend on various parameters. Examples show how the COD factor can be mathematically determined. They further illustrate why COD factors may be so different, due to the sample components. Hence, the bandwidth of the conversion factors ranges from <1 to >5, depending on how much oxygen is already bound to the organic compounds. In addition, inorganic compounds such as nitrites, bromides, iodides, metal ions and sulfur compounds are also oxidized and co-determined in a COD determination, and therefore may also have an effect the conversion factor.

6. TOC process analysis
Particularly during process control, it is important to obtain fast, continuous and informative data on the organic pollution levels of waters. In TOC process analysis, the sample is continuously fed to the measuring system for subsequent measuring. This way, the control room can react promptly to any possible process changes.

One of the most important attributes of a TOC process analyzer is its versatility. Since a TOC process system is not available 'off the shelf', each measurement must be customized to the particular measuring problem, the matrix and the sampling location. The analyzer can be tailored to the specific measuring task. Many analyzers are used in wastewater control where, depending on the type of industry, the wastewater may have a very different matrix. A further application area is continuous TOC determination of 'clean' water, such as condensates. For this purpose, there is an option for which the specifications were described in a separate application note.



Further information on this article:
- TOC Application Handbook (PDF)

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