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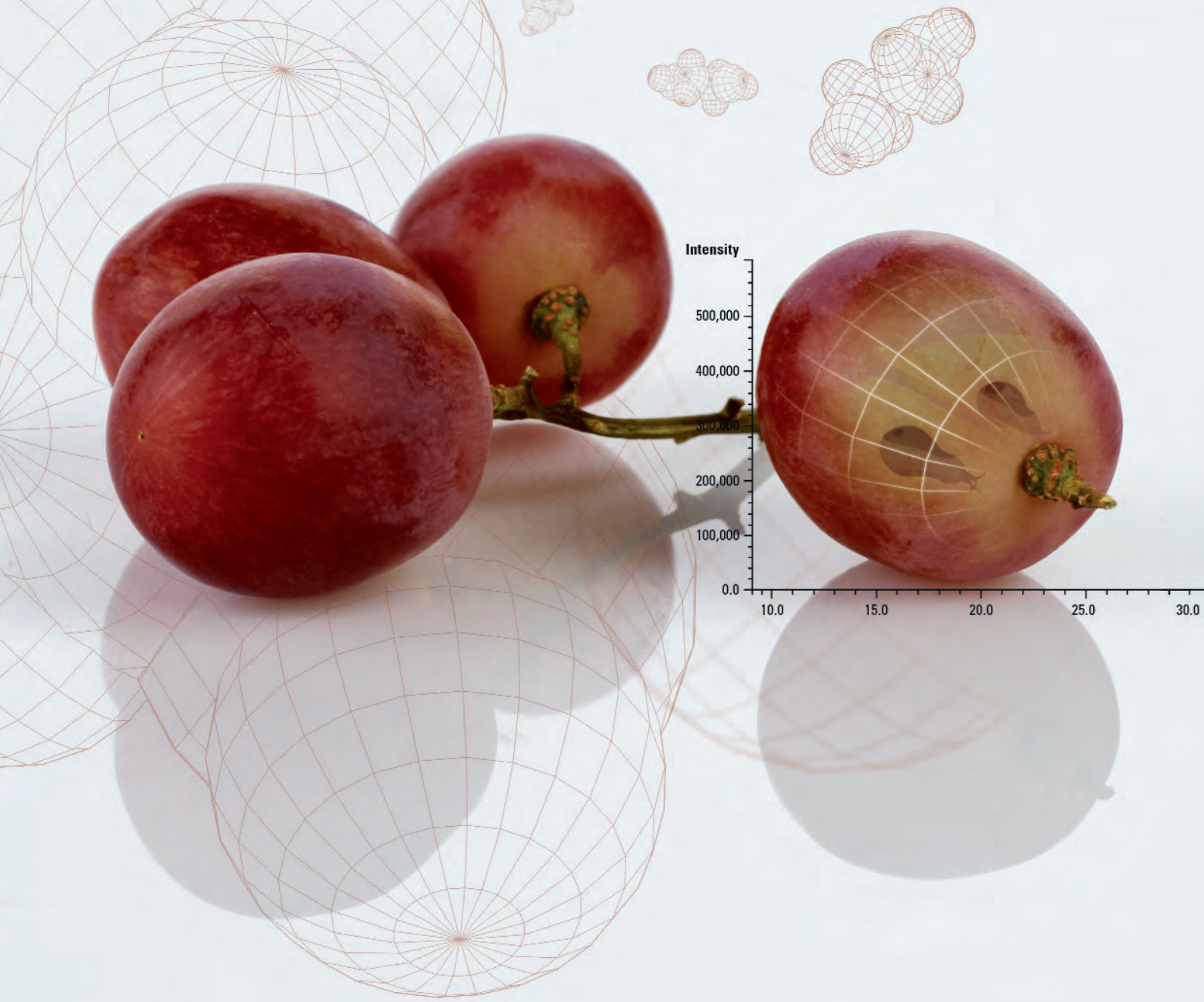

Individually dosed – Therapeutic drug monitoring with LC/MS/MS

Bread packaging – What's hidden in plastic bread packaging?

Hair in your soup? Microchip electrophoresis can help

Acid Test – TOC determination in concentrated hydrochloric acid

2/2012

The change begins …

IMPRINT

Shimadzu NEWS, Customer Magazine of Shimadzu Europa GmbH, Duisburg

Publisher:
Shimadzu Europa GmbH
Albert-Hahn-Str. 6-10 · D-47269 Duisburg
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Design and Production:
m/e brand communication GmbH GWA
Düsseldorf

Circulation:
German: **7,120** · English: **22,070**

Individually dosed

Therapeutic drug monitoring with LC/MS/MS

Therapeutic drug monitoring (TDM) generally refers to determination of specific drug concentrations in blood at specified time intervals. The purpose of TDM is to secure a relatively constant concentration, the so-called steady state plasma concentration, of the corresponding drug in the bloodstream. Many fields of medicine use this method in daily clinical practice to obtain the optimal dosage of drugs with a relatively narrow therapeutic index.

For drugs with a narrow therapeutic index, the effective blood concentration required may be close to concentrations which are already toxic or cause, at least, undesirable side-effects. At the same time, maintaining the therapeutically effective concentration (uptitration) for certain drugs is not as easy as administering a standard dose. Each person absorbs, metabolizes, utilizes and eliminates drugs differently. Factors such as age, gender, general state of health, genetic makeup or interferences with other drugs all play a role.

Life changes – and with it medication

Many drugs that are therapeutically monitored are administered throughout life. And just as life holds many changes with new situations, the drug dosage administered may also need to be adjusted from time to time. The effects of changed life circumstances and, possibly, the altered pharmacokinetics of the drug can be controlled via TDM and the drug dosages can be adjusted accordingly.

Substances determined by TDM include analgesics, antiarrhythmics, antibiotics, antidepressants, antiepileptics, immunosuppressants and cytostatics. In the context of therapeutic drug monitoring, prompt availability of the required drug level, for many critical patients highly important, should be assured. Fast and reliable analytical methods play an important role here.

At the same time, analytical methods for quantitative detection of the various drugs to be

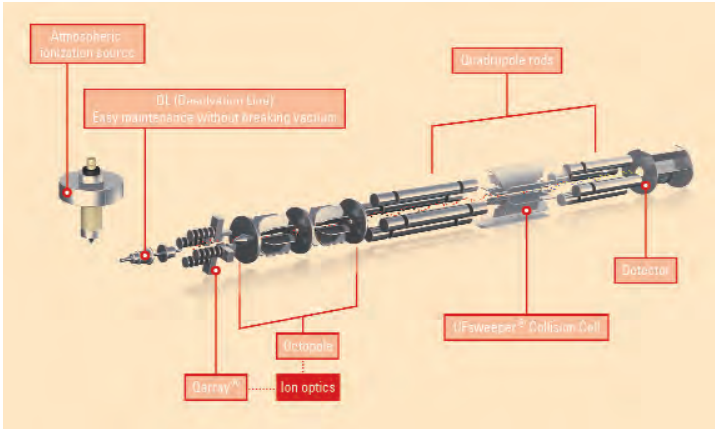

Figure 1: Schematic representation of the triple quadrupole

monitored must be sufficiently sensitive and selective. Immunological methods offer a fast and straightforward solution, as it is often not necessary to separate the drug from the matrix (blood, plasma, serum etc.). A major disadvantage of immunological methods is, however, the very high susceptibility to interfering substances, which under certain circumstances can lead to false results, due for instance to cross-reactivity of metabolites of the active substance. The concentrations of active substances and active metabolites are often expressed as the sum of both.

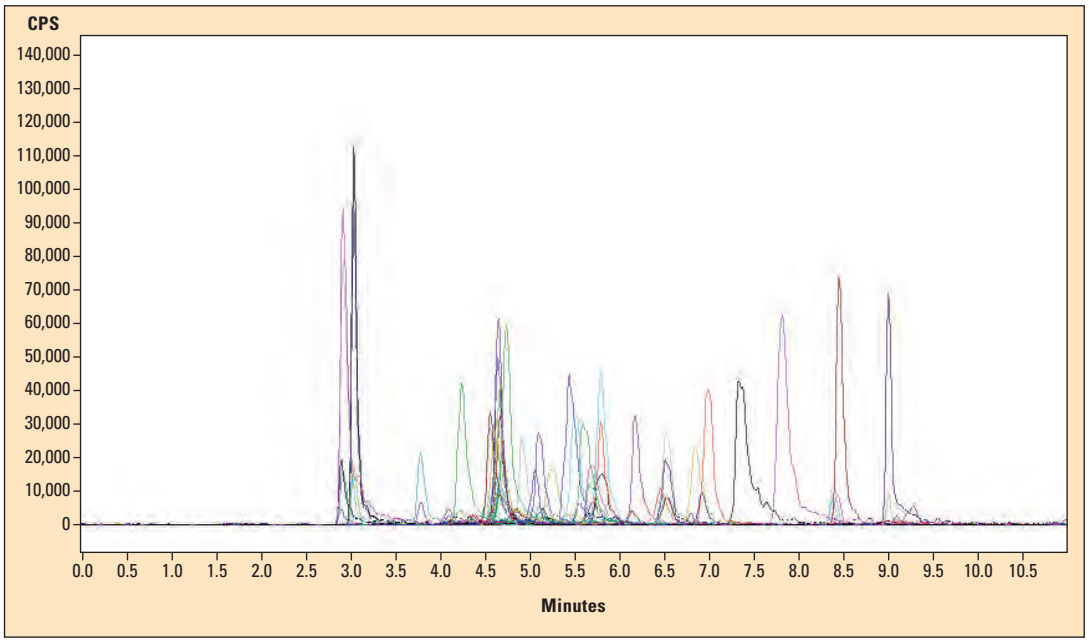

Figure 2: : Example chromatogram of an analysis of 33 benzodiazepines respectively their metabolites using 20 internal standards

Highly selective analysis method: multi-reaction monitoring

The use of liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS) offers an excellent alternative to immunological methods. The underlying methodology of 'multi-reaction monitoring' (selecting the desired parent substance in the first quadrupole [Q1], fragmenting this substance in the collision cell [Q2], detecting one or more specific fragments in the third quadrupole [Q3]) (Fig. 1: Schematic representation of the triple quadrupole) makes this analytical method superior to other methods in terms of selectivity. It is often possible to analyze many compounds using the same method, even without time-consuming sample preparation steps.

LC/MS/MS user kits for routine diagnostics

Shimadzu offers with the LCMS-8030 a triple quadrupole mass spectrometer (Figure 3) meeting all of today's requirements in therapeutic drug monitoring. Since TDM is now part of routine clinical diagnostics, various manufacturers offer complete user kits for the analysis of, for example, immunosuppressants, neuroleptics, antidepressants, benzodiazepines etc. Time-consuming method development and validation is no longer needed when using these types of kits, and even relatively inexperienced users can set up reliable analysis methods for routine diagnostics using LC/MS/MS.

A kit usually includes calibrator sets as well as control samples containing the substances to be analyzed, the required mobile phases and rinsing solutions for HPLC, and an analytical column (depending on the kit, also a trapping column or SPE [solid phase extraction] column) as well as all necessary solutions for sample preparation, which often consists of a less time-consuming protein precipitation. Furthermore, an internal standard is usually added to the samples – ideally these are deuterated standards of the actual analyte or analogues with similar chemical characteristics. They are commonly added to the standards in order to compensate for possible extraction losses or influences that can distort the analysis results.

When speed counts ...

Of course, Shimadzu's LCMS-8030, as well as the manufacturers of analytical kit systems takes fast analysis times into account. It is, for instance, possible to determine 33 benzodiazepines resp. their metabolites together with 20 internal standards (Figure 2, sample chromatogram) in only eleven minutes or to determine the four most common immunosuppressants (sirolimus, tacrolimus, everolimus and cyclosporine A) in 4.5 minutes – or even 1.3 minutes for those who are in a hurry. Fast quantitative analysis can then be carried out using Shimadzu's flexible and user-friendly LabSolution software.

Figure 3: The LCMS-8030 is one of the fastest systems on the market with a polarity switching time of just 15 msec, a scan speed of 15,000 u/sec and dwell times of 1 msec

Bread packaging

FTIR measurement of polymers in the food industry

Figure 1: Edible packaging material: a packaging chip made out of starch

Renewable energy and renewable raw materials are the keywords most often used in relation to fuels. Renewable raw materials are, however, also an important issue for packaging materials. Instead of packaging chips made of polyesteramide or polycaprolactone (which are derived from fossil raw materials), alternative polymer chips from renewable raw materials can be used, for instance of vegetable origin. Examples of target polymers are starch, cellulose and lignin [1].

Packaging chips based on renewable materials have more in common with bread and baked goods than with plastics. In many a delivered package, one can find leaflets pointing out that the packaging chips are edible. These packaging chips consist of starch, or in the case of 'flupis®', of paper foam manufactured from waste paper and starch. Both types of chips can be disposed of easily by composting or in organic waste containers. In the presence of water, the chips immediately disintegrate and form a pulp, similar to that perceived in the mouth when biting into baking wafers or edible paper.

Packaging chip or bread

Can these chips be distinguished from bread or baked goods? For comparison, a piece of crisp bread and a baking wafer have been analyzed using FTIR spectroscopy in combination with single reflectance measurements, allowing fast non-destructive analysis of these types of materials. The infrared radiation applied penetrates approx. 2 µm into the sample surface. The interaction between the radiation and the material provides information on its composition.

The bread mixture is a highly complex composition for infrared spectroscopy, since all materials used such as flour, sugar, yeast, water and other components each exhibit their own distinct infrared spectrum. When these spectra are overlaid, assignment of individual signals is difficult. Starch, cellulose and sugar have similar spectra as they are all polysaccharides. Water is also a difficult material, as it exhibits a highly intense spectrum. For comparison, dry baked goods such as crisp bread and baking wafers (wheat flour and starch) requiring very little water, were used accordingly.

Correlation between spectrum and material hardness

As seen in figure 6, the spectra of the filling materials in the range of 1540 cm-1 show more similarity to the starch spectra than the two baked goods. Furthermore, differences can be observed in the range of the carbonyl bands at 1750 cm-1. In this range, the baked goods can be distinguished

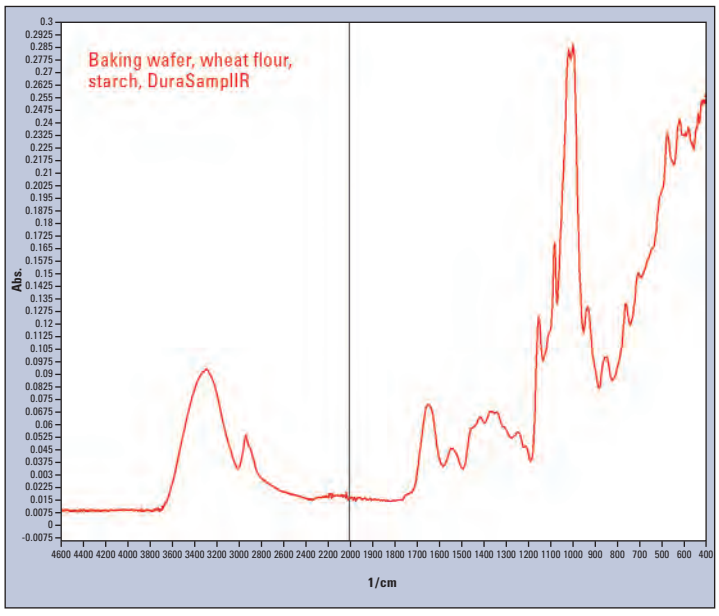

Figure 2: Infrared spectrum of a baking wafer, measured using a single reflectance unit

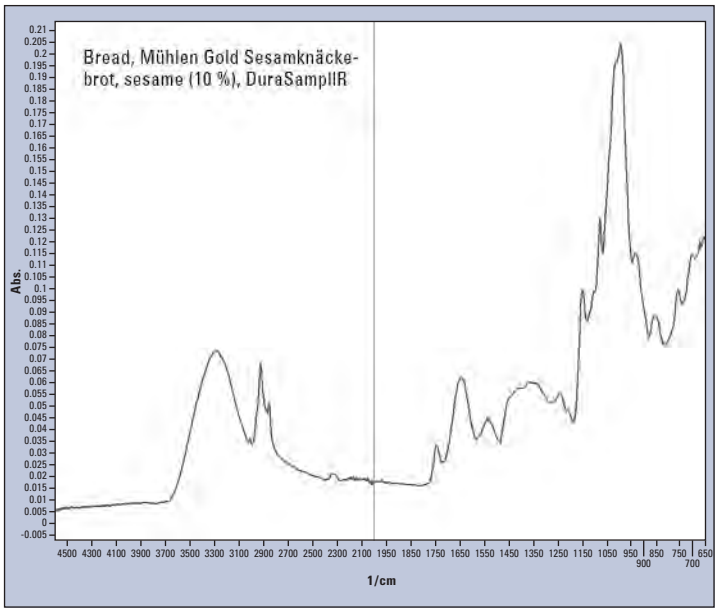

Figure 3: Infrared spectrum of a crisp bread

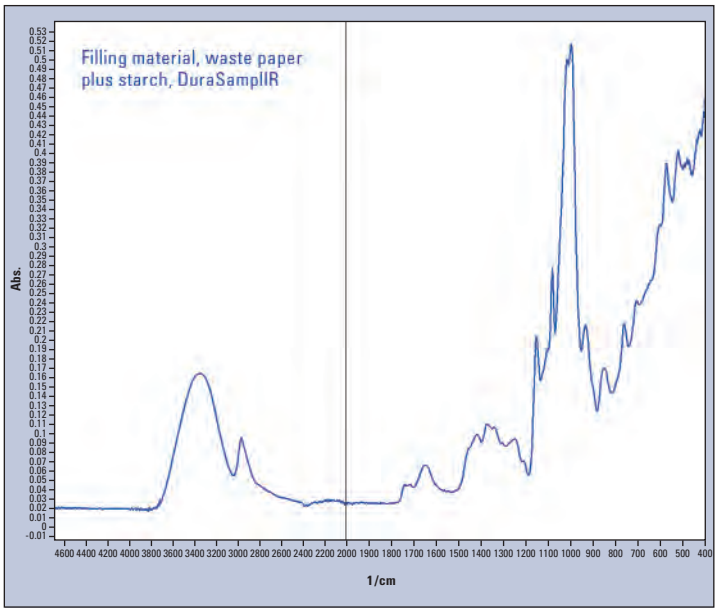

Figure 4: Infrared spectrum of flupis®, filling material made of waste paper and starch

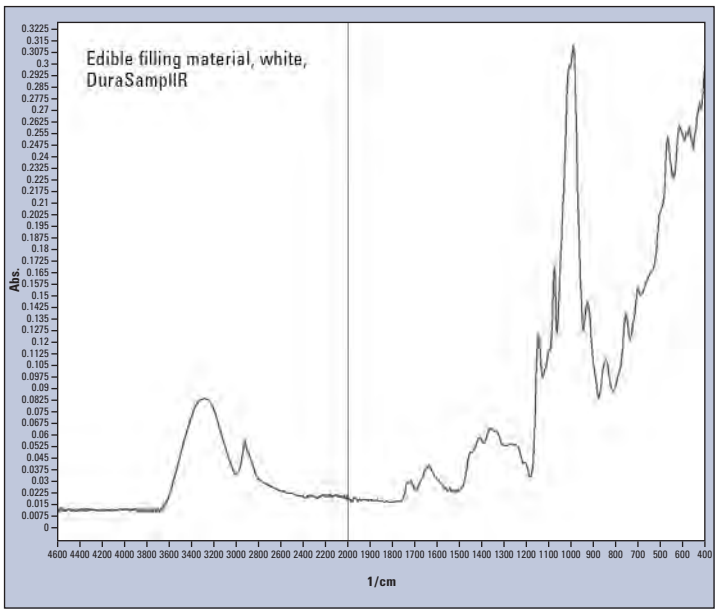

Figure 5: Infrared spectrum of an edible filling material, see Figure 1

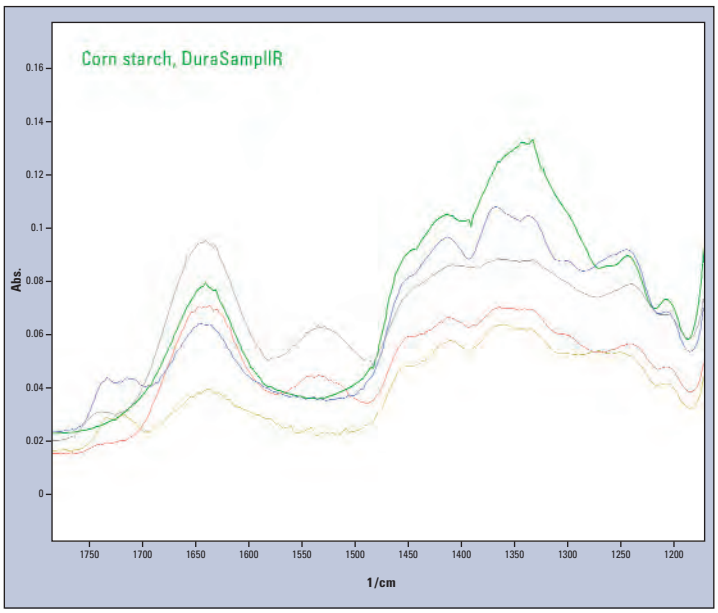

Figure 6: Zoom in the range of 1,900 to 1,150 cm^{-1}. An additional substance (cornstarch spectrum, green line) was used for comparison

from the filling materials as well as from the starch.

The filling materials contain aggregates exhibiting strong signals at 1734 and 1713 cm-1. The various intense spectral signals can be correlated to the hardness of the material. Crisp breads, as well as baking wafers are quite hard compared to the filling materials. Hard materials do not make good contact with the measuring window. As expected, the various compositions of the products are exhibited in the IR spectrum and enable an unequivocal signal assignment.

Conclusion

Using infrared spectroscopy, complex materials can be analyzed directly and non-destructively. Within the shortest possible time, edible filling materials can be distinguished from conventional baked goods or starches (corn starch in this example) using infrared spectroscopy.

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New HPLC-FAAS hyphenated tec of aluminium and aluminium flu

Dr. Marcin Frankowski, Department of Water and Soil Analysis, Faculty of Chemistry, Adam Mickiewicz University

Aluminium as an element commonly found in the earth's crust (8 % by weight) and characterized by strong amphoteric nature [1], can create numerous complex species [2]. The form in which aluminium occurs in the environment affects its mobility, bioavailability and toxic influence on living organisms and vegetation. Toxicity of aluminium is mainly connected with the occurrence of a free Al^{3+} ion, hydroxy forms (including $Al(OH)^{2+}$, $Al(OH)^{+}_{2}$) and inorganic form of complexes. Among inorganic forms, aluminium fluoride complexes dominate [e.g. 3,4]. They are characterized by high values of stability constant (AlF_2^{+}, Log K = 12,600; AlF^{2+}, Log K = 7,000).

The forms of aluminium fluoride complexes (AlF_2^{+}, AlF^{2+}, AlF_3^{0}, AlF_4^{-}, AlF_5^{2-}, AlF_6^{3-}) and their occurrence depend on pH and ligand concentration in solution [e.g. 5]. It should be emphasized that determination of only the total concentration of aluminium does not provide full data concerning the processes the element undergoes in the natural environment and consequently, does not provide information on migration, actual toxicity, bioavailability and cumulation in particular components of the environment.

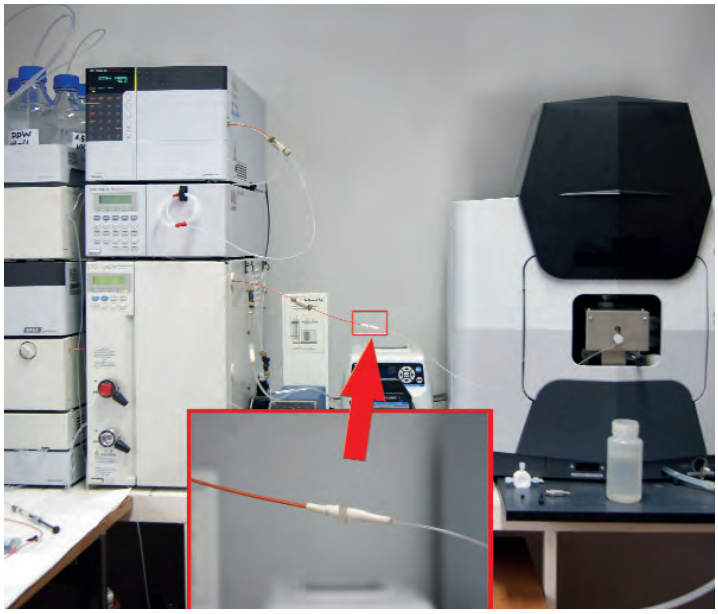

Figure 1: The HPLC-FAAS analytical system used in the laboratory of Department of Water and Soil Analysis, Faculty of Chemistry, Adam Mickiewicz University, Poznań, Poland

Chromatographic separation conditions	
Eluent A	Deionized water
Eluent B	1.5 M NH_4Cl pH ≈ 3.0
Eluents flow	2.0 ml · min^{-1}
Injection volume	200 µL
Reaction loop	1.0 m
Column temperature	20 °C
Sampling	1 Hz (AD2 detector – AAS)
Spectrometer operating conditions	
Wavelength	309.3 nm
Slit width	0.7 nm
Lamp mode	BGC-D2
Lamp current	Low(Peak) – 10 mA
Flame type	C_2H_2 / N_2O
Support gas flow rate	11.0 L min^{-1}
Fuel gas flow rate	6.2 L min^{-1}
Atomiser position	11 mm

Table 1: Basic chromatographic and spectroscopic conditions

One of the best known and most commonly applied procedures of speciation analysis is the Driscoll's method, which enables isolation of a fraction of labile monomeric inorganic aluminium containing an Al^{3+} ion and bonds with inorganic fluoride and sulphate ligands [e.g. 6]. However, this method does not enable direct determination of particular speciation forms of aluminium, including fluoride complexes and Al^{3+}, $Al(OH)^{2+}$, $Al(OH)^{+}_{2}$ species.

HPLC method for aluminium fluoride analysis

The use of liquid chromatography provides many possibilities to separate particular forms of aluminium, both cation and anion. Bertsh and Anderson [7] were among the first researchers who suggested the separation of aluminium fluoride complexes applying ion chromatography. The AlF^{2+} and Al^{3+} forms were separated. The method was not used in environmental samples determination. Also Willet [8] did not obtain a good separation of AlF^{2+} and AlF^{+}_{2} forms, despite the quick separation. Motellier and Pitsch [9] did not achieve proper resolution for the first two peaks AlF^{2+} and AlF^{+}_{2}. So far, research on speciation of AlF_x complexes using ion chro-

chnique for determination oride complexes

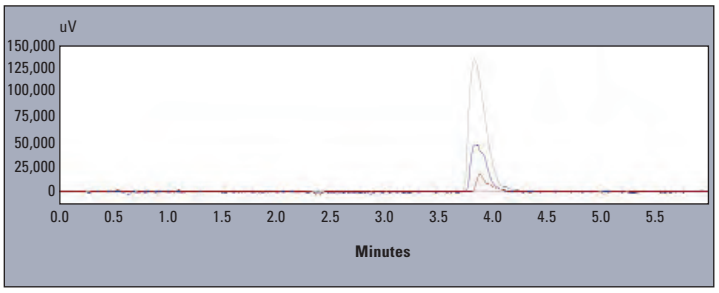

Figure 2: Overlapping chromatograms for standards 10, 50 and 100 mg L^{-1} of aluminium standard solution (Al in HNO_3, Merck)

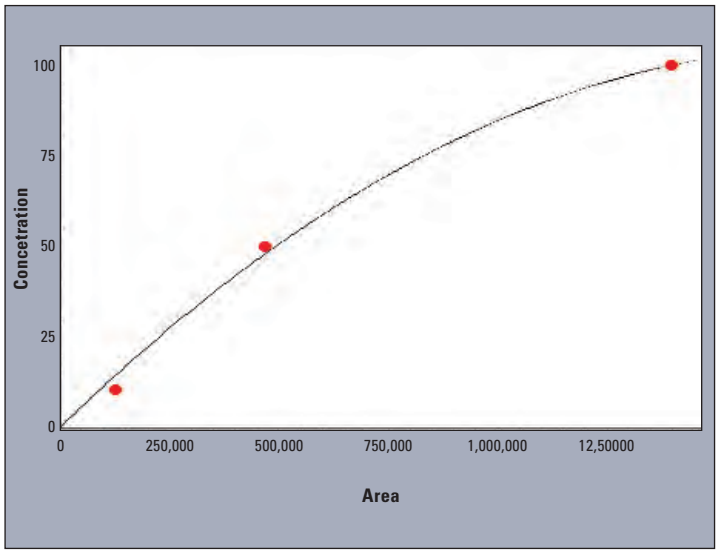

Figure 3: Calibration curve for the sample standard solutions analyzed: 10, 50, 100 mg L^{-1} of aluminum standard solution (Al in HNO_3, Merck)

matography has been based on isocratic elution.

Bormann and Seubert [10] first used UV spectrophotometry and atomic spectrometry ICP-AES for Al-citrate-oxalate complexes. The signals obtained were assigned to the following forms: AlF^{+}_2, AlF^{2+} and Al^{3+}. To date, the combination of HPLC with ICP (mass spectrometry and atomic emission spectrometry (ICP-MS, ICP-AES) in online as well as offline systems has been used. The researchers have also applied atomic absorption spectrometry with electrothermal atomization in the offline system.

The first online systems based on HPLC and FAAS detection were developed by Ziola-Frankowska et al. [11] and Frankowski et al. [12]. These analytical systems encountered some problems with quantitative analysis. Signals from the detector were collected in three replicates for 30 sec. (90 sec. software limited), and absorbance values were then counted manually every 0.5 sec. and saved as a txt file, which was exported to another software for calculation of peak area. However, this method is time-consuming and requires additional tools.

New HPLC-FAAS system

Requirements of high sensitivity and the most universal analytical system for soil and water analysis are known to be very high. A new system has to be capable of separating and determining inorganic as well as organic compounds. On the other hand, there is a demand for the most automated system possible, utilizing different analytical techniques in one run.

Determination of ion complexes in water, use of a chromatographic technique to separate and online atomic absorption technique for detection of concentrations of complexes seem to be the most promising solution. A possible additional advantage of this solution is an increase in analytical throughput.

The experimental system consisted of Shimadzu's LC-10 liquid chromatograph and the new AA-7000 flame atomic spectrometer. The Shimadzu CBM-20A communication module and LabSolution software were the key point in interfacing these two systems. The electronic interfacing necessary was configured in SEG Shimadzu, Duisburg, Germany. The chromatographic separation procedure along with the optimization of the complete HPLC-FAAS system have already been described [11, 12] and are also presented in table 1. The HPLC-FAAS system is presented in figure 1.

Analytical results

The single analysis by means of the solution presented takes four minutes and does not require post-column treatment. The column effluent was connected directly to the AA spectrometer nebulizer using a capillary tube.

Linearity of the tested system is presented in figure 2. The coefficients obtained were; $r^2 = 0.996$ and $r = 0.998$ (Figure 3).

Chromatograms of three different real samples are presented in table 2. The analysis was performed for characteristic solutions with variable concentration of aluminum ion in relation to ion fluorides, so the system enabled observation of the influence of the particular forms of aluminium.

A quantitative analysis carried out using FAAS technique was straightforward with application of the LabSolution chromatographic software for atomic absorption signals. This solution reduced both time of data managing and analysis while increasing accuracy and precision of the determinations. Table 4 shows

speciation analysis of water impurities. The system allows performing of quantitative and qualitative analysis of different aluminium forms. Use of the AA-7000 with LabSolution software enabled overcoming of the usual difficulties encountered due to integration of the atomic absorption signals. The method

- Mobile phases: de-ionized water and 1.5 M NH_4Cl solution (both acidified to pH ≈ 3).

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Chromatogram number	(1)	(2)	(3)
Al [M]	1.85E-03	1.85E-03	1.85E-03
F [M]	2.63E-04	1.32E-03	3.95E-03

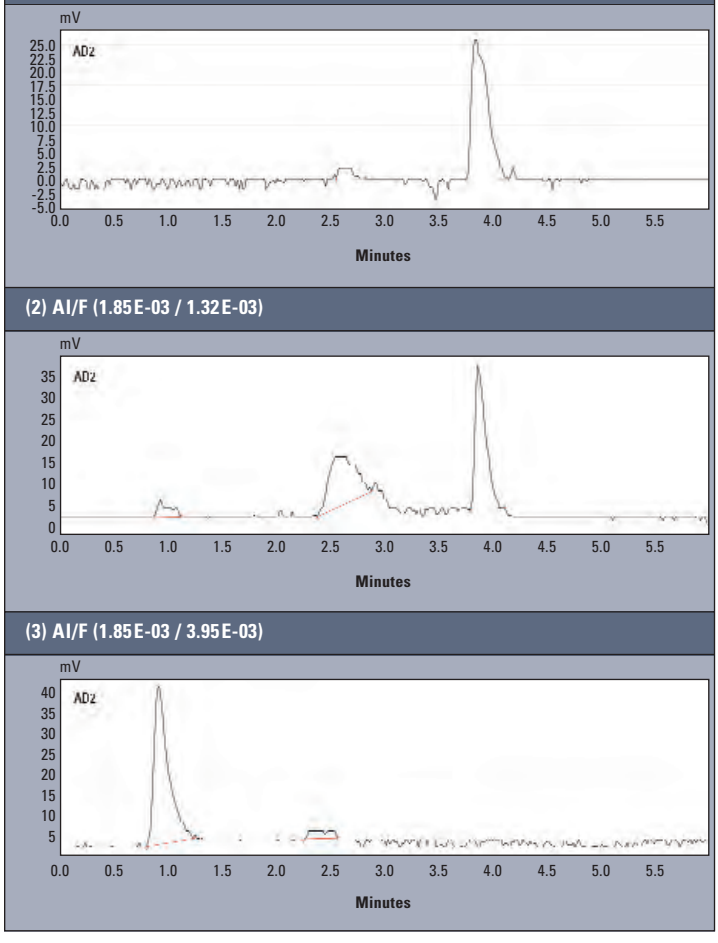

Table 2: Concentration values of Al and F- for AlFx(x-3)

The quantitative results obtained comply with the reference results acquired during analysis of the large number of samples of standard model solutions [11, 12]. The proposed elution sequence is presented in table 3.

results of sample signals obtained for the samples listed in table 2.

Conclusions

The application of HPLC-AAS system allows a fast and accurate

1PA – 1. signal (RT = 0.9)	2PA – 2. signal (RT = 2.5)	3PA – 3. signal (RT = 3.9)
AlF_2^+ and/or AlF_4^-	AlF^{2+} and/or AlF_3^{0*}	Al_3^+

Table 3: Proposed elution sequence of ionic forms: AlF_2^+, AlF^{2+}, AlF_3^0, AlF_4^-, Al^{3+}
*AlF_3^0 – possible elution with +1 and -1 AlF Typen (forms (depending on the ratio Al:F)

Molar ratio Al/F	1PA (RT = 0.9)	2PA (RT = 2.5)	3PA (RT = 3.9)
7.0 (1)	< LOD*	2.39	47.61
1.4 (2)	3.13	22.18	24.70
0.5 (3)	46.26	3.74	< LOD*

Table 4: Determined concentrations of aluminium and aluminium fluoride complexes
*for loop 200 µL

presented can be the basis for analysis of the speciation arrangement of other elements which may be found in the natural environment.

Instrument configuration

- AA-7000 Shimadzu spectrometer with acetylene-N_2O burner
- CBM-20A communication module
- PC 55L electronic interface
- LabSolution software
- Solvent delivery module LC-10 AD_{VP}
- Flow control valve FCV-10 AL_{VP}
- Degasser unit DGU-20A5
- Column oven CTO-10AS_{VPP} with Rheodyne 7725i Injection Valve
- Ion-exchange column – Dionex IonPac CS5A (Analytical column, 250 mm, 4.0 mm i.d., particle size 9.0 µm)
- IonPac CG5A (guard column, 50 mm, 4 mm i.d., particle size 9.0 µm)
- Gradient at 2 ml min^{-1} with an injection volume of 200 µL

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Everything within view

Quality control of camera objectives with UV-2600 and MPC-2600

With the introduction of the new generation of LO-RAY-LIGH® gratings in Shimadzu's new UV-2600/UV-2700 UV-VIS spectrophotometer series, many application areas in the field of optics can now be covered. In addition to single optical elements such as lenses and glasses, composite materials or single-coated systems and even entire assemblies can now be analyzed. Camera objectives are small optical benches, equipped with different lenses and glasses with protective coatings or surface finishes. The quality of an objective is determined by its light intensity and low optical aberrations.

The light intensity can be determined spectroscopically. By combining the UV-2600 or UV-2700 with a MPC-2600 extra large sample compartment, non-destructive analysis of entire camera objectives is possible. This sample compartment is equipped with a V-shaped holder, ensuring stable positioning of the objective. The holder can be positioned in all three dimensions, so that the analytical irradiation hits the center of the objective optics and passes through the optical bench, while the spectrophotometer measures the incident light intensity, which is displayed in the form of transmittance spectra. These spectra show not only the light throughput in percentages but also the transmission range as a function of wavelength for visible and ultraviolet light.

Quality determination of objectives and camera accessories

Using this combination of instruments, it becomes possible to establish a quality determination process that allows production control of a production series.

Figure 1: Two camera objectives from different manufacturers, featuring a standard lens and a macro lens

In addition, it is also possible to check the specifications that characterize the objective. The goal is, after all, to manufacture high-quality objectives. There are several criteria in photography that can be met by implementing such quality control:

- the visible range of an objective for the image quality of the colors, or depth of field in the photographs
- the quality of the coatings on glasses and lenses
- sensitivity in the red or blue ranges.

In addition, a UV-VIS spectrophotometer can be used to qualify camera accessories such as polarizing filters or UV filters.

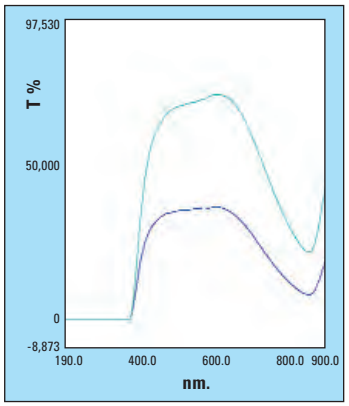

Figure 2: UV-VIS spectra of a zoom objective at a setting of 25 mm (green) and 300 mm (blue)

Testing two objectives

In the experiment shown here, two objectives from different manufacturers were tested. Since the objectives have been designed for different uses, they have different characteristics:

1. Fixed lens system, 50 mm F/1.2
2. Macro lens, 28 - 300 mm F3.5 - 6.3 DG Macro (F = focal length, 1.2 or 3.5 - 6.3 are aperture values.)

The objectives were measured using the UV-2600 and MCP-2600 sample compartment. The objectives were placed on the V-table of the MPC-2600 and were brought to the measuring position. The measuring assembly simulates the incident light for imaging within the camera. The objectives were measured with the incident light from outward to inward.

The detector (photomultiplier) is located in an integrating sphere (Ulbricht sphere) and displays the transmittance, i.e. the light of the transmittance of the object (objective). Furthermore, absorptions by sealings can be expected, as well as effects of reflections and antireflective coatings.

Discussion of the spectra

When the focal length is increased, the light transmittance decreases due to the reduction of the field of view, as can be seen in Figure 2. The spectra displayed are the light transmittance at 28 mm (70.6 %) and 300 mm (37 %). Due to the reduced light transmittance, taking a photograph using the zoom setting needs a longer exposure time, a larger aperture or additional lighting in order to obtain more light intensity.

The quality of the objectives varies according to the function of the objective. An objective with fixed focal length can result in good light transmittance with few components. Figure 3 shows two very different representative examples.

The light transmittance of the fixed focal length results in a value of 86.7 % while the variable focal length results in a transmittance of up to 70.6 %. Assuming the loss of 4 % of the original energy at all surfaces based on the physics of flat glass, it might be concluded that the fixed objective consists of four glass components.

With extrapolation, four glass components should result in a loss of approx. 15 % of transmittance. This corresponds approximately to the measured value of 86.7 %. But this is, of course, a rough estimate for an unknown object in which other aspects, e.g. filtering surface coatings, can have an influence.

Both objectives are distinguished by their wavelength range. The objective with fixed focal length

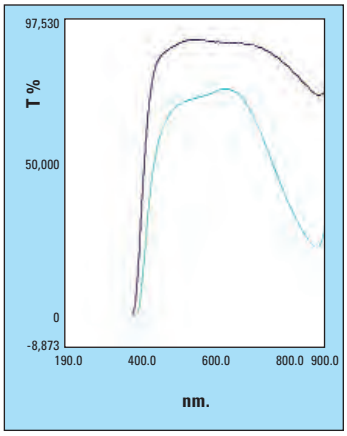

Figure 3: UV-VIS spectra and comparison of the light transmittance of a fixed objective (black) of 50 mm with a zoom objective (green) at a setting of 25 mm

features a high light transmittance. In addition, a profile maximum at approx. 520 nm corresponding to the green wavelength range is apparent. In comparison, the zoom objective is optimized for wavelengths in the red range (approximately 620 nm).

Organic content in liquid manure, fermentation fluids and fermentation residues

TOC suspension method

Biogas is an energy source of the future and can be used in energy generation and supply, or it can be fed into the natural gas networks as biomethane. Energy generation from renewable or regenerative energy sources including water, wind, solar and other biomasses, replaces the use of fossil fuels.

The Institute of Non-Classical Chemistry in Leipzig, Germany, works on the production of biogas from various liquid manures, corn silages and mixtures thereof. Methods for pretreatment of liquid manure and optimization of the fermentation process and biogas yield are investigated. For instance, it has been established that fermentation residues can be further utilized as fertilizers in agriculture. Research is also carried out in this application area, e.g. on methods for ammonia reduction.

Figure 1: Experimental setup for biogas production in the laboratory

Reactors with volumes of 1 to 100 liters are applied for production testing. The prepared liquid manure, or mixtures of other substrates, are used for fermentation. In the laboratory reactors, up to 16 tempered round-bottomed flasks are used in parallel (Figure 1). The biogas generated is diverted via pipelines, the resulting volume is determined pneumatically and the gas composition is analyzed.

How to determine the initial concentration?

To evaluate the efficiency of the reactor and the method, biogas was subjected to different analyses. An important parameter is the gas chromatographic determination of the methane content. In order to compare the biogas

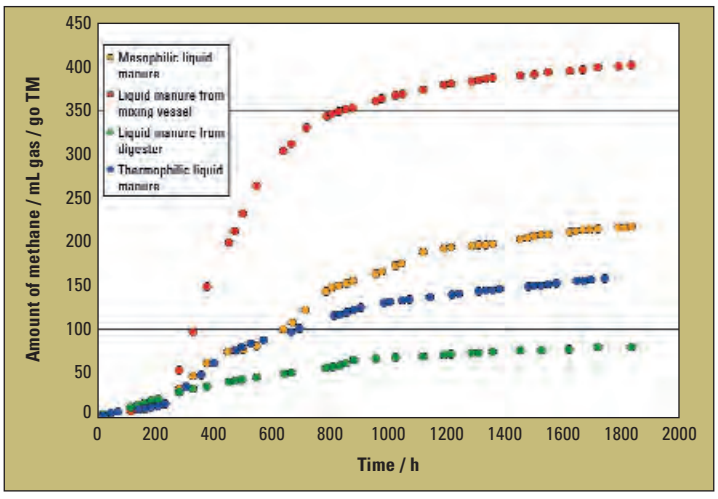

Figure 2: Formation of methane from various pretreated substrate mixtures and during thermophilic (50 °C) or mesophilic (37 °C) operation of the fermenter

yield of the various substrates, biogas volume or methane volume was expressed in terms of the organic dry matter present in the substrate (SL/kg ODM). This requires accurate determination of the initial concentration of the organic substance in the liquid manure.

For this determination, proven methods are available. The dry matter (DM) of the liquid manure is first determined at 105 °C. The dried liquid manure is subsequently annealed at 550 °C in a muffle furnace to a constant mass. The loss of mass during annealing corresponds to the organic content of the liquid manure. The ratio of methane gas concentration and organic content corresponds to the biogas production yield (fermentation). It is a key criterion for the fermentation of various biomasses and for estimating the efficiency of the fermentation process (Figure 2).

Figure 3: The suspension is dispersed

TOC suspension as an alternative method

To avoid long annealing times for ODM determination, an alternative method for the determination of the organic substance was pursued. The TOC suspension method was considered as suitable. The dried sample was weighed into an Erlenmeyer flask and mixed with hydrochloric acid to convert the inorganic carbon compounds, such as carbonates and hydrocarbonates, to carbon dioxide. In the next step, a dispersion device was used to break up and homogenize the suspension (Figure 3). During this process, most of the carbon dioxide generated was also removed.

The final solution is subsequently transferred into the autosampler vials of the TOC-L_{CPN} plus ASI-L system (Figure 4) and analyzed automatically. For this purpose, a small fraction is injected onto the 720 °C hot platinum catalyzer. The organic substances are converted into carbon dioxide and measured using an NDIR detector.

TOC method can be automated

The advantage of the alternative method lies in its suitability for automation. In this way, many samples can be processed automatically in sequence. With the possibility of multiple injections, the method also offers statistical reliability. In the muffle furnace,

Figure 4: TOC-L_{CPH} with ASI-L

a combusted weighed sample yielded an ODM value. The suspensions are generally analyzed at least four times to establish a mean value. Figure 5 shows the peaks resulting from such a multiple determination.

Furthermore, TOC determination using catalytic combustion oxidation allows the simultaneous measurement of total bound nitrogen (TN_b) because, in addition to the carbon dioxide from organic substances, NO is formed from nitrogen-containing compounds. For the conversion of NO to NO_2, the measuring gas ozone was fed to the in-series connected chemiluminescence detector. The photons emitted during this reaction are detected and used in the calculation of the TN_b value. Nitrogen compounds also play an important role in the case of liquid manure.

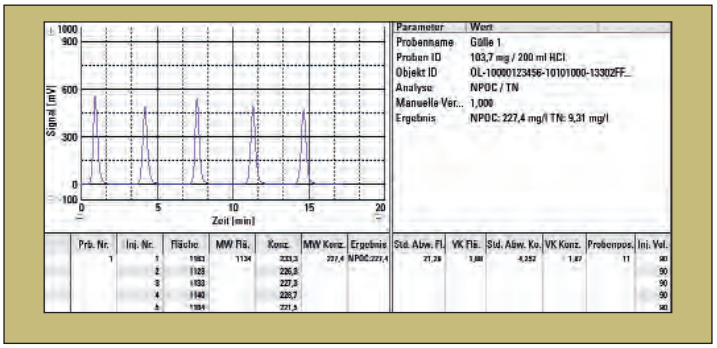

Figure 5: Multiple determination of a liquid manure suspension

Liquid manure (dried and ground)	NPOC [Wt.-%]	RSD [%]
Approach 1	44.1	0.8
Approach 1	44.2	1.9
Approach 2	44.2	1.6
Approach 2	42.5	1.4

Table 1: Determination of organic content in liquid manure (duplicate determination from two different approaches with five separate injections each)

Liquid manure (dried and ground)	TN_b [Wt.-%]	RSD [%]
Approach 1	1.84	1.5
Approach 1	1.80	0.9
Approach 2	1.76	2.2
Approach 2	1.68	1.4

Table 2: TN_b was determined simultaneously with organic content (duplicate determination from two different approaches with 5 separate injections each)

Conclusion

The TOC suspension method offers a good alternative for fast, straightforward and accurate analysis of the organic content of liquid manure samples. The possibility of co-determination of nitrogen content also enables users to acquire additional useful information for the evaluation of liquid manure samples.

Further information on the Institute is available on the following website: **www.uni-leipzig.de/inc/**

Hair in your soup?

Microchip electrophoresis

The production of foods, drugs, cosmetics etc. demands high standards of purity in produced goods. It is essential to rule out all types of contamination. If contamination becomes evident despite all precautionary measures taken, it is necessary to discover and identify the source quickly, reliably and accurately. Only in this way can a standard procedure be developed to prevent the occurrence of future incidents.

Contaminations are usually ascertained by microscopic analysis

which requires extensive experience and specialized knowledge of the staff, particularly when identifying contaminations by animal hair. A reliable and fast alternative is the identification of animal species by polymerase chain reaction (PCR) with subsequent analysis using microchip electrophoresis.

The PCR technique enables fast replication of double-stranded DNA. Using a specific primer, it is possible to synthesize DNA fragments that are specific to different animal species. Analysis and assignment of these fragments is carried out on the basis of their size.

To determine the size of the DNA fragments, agarose gel electrophoresis following PCR is conventionally used. An electric field is applied to the agarose gel so that the negatively charged DNA migrates through the gel. The agarose gel forms a net-like matrix in which the DNA fragments are separated in proportion to their size. They are then stained using a fluorescent dye (usually ethidium bromide [EtBr]) and visualized under UV light.

Modern automated method

A modern alternative to conventional agarose gel electrophoresis is Shimadzu's automated microchip electrophoresis system "MCE-202 MultiNA" (Figure 1). Size determination of DNA fragments as well as quantification is achieved using microchip technology. The reusable quartz microchips (Figure 2) have a 23 mm long separation channel

Figure 1: Shimadzu's fully automated MCE 202 MultiNA microchip electrophoresis system

in which the DNA fragments are separated in a polymer separation buffer.

Filling of the microchips with separation buffer and sample, carrying out of electrophoresis and subsequent rinsing are combined in a fully automated process. A total of four microchips can be installed in the instrument. Using multiple microchips simultaneously, the parallel workflow (rinsing, loading, electrophoresis) enables a reduction in cycle time to 75 seconds per sample.

Up to 120 samples per run can be registered in an analysis plan. In addition to 96-well plates, suitable sample vials are 8-strip or

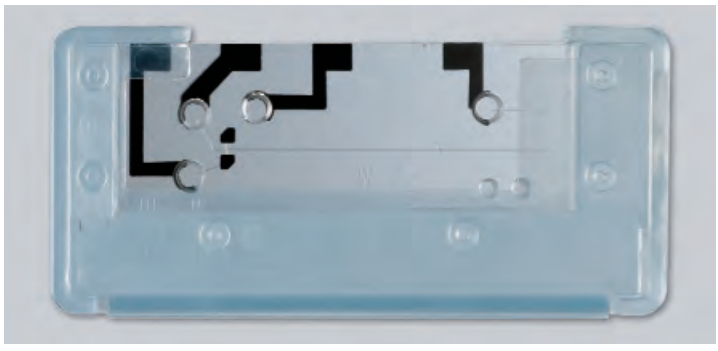

Figure 2: Reusable quartz microchip

Figure 3: Four reagent kits for size determination of DNA and one reagent kit for RNA analyses

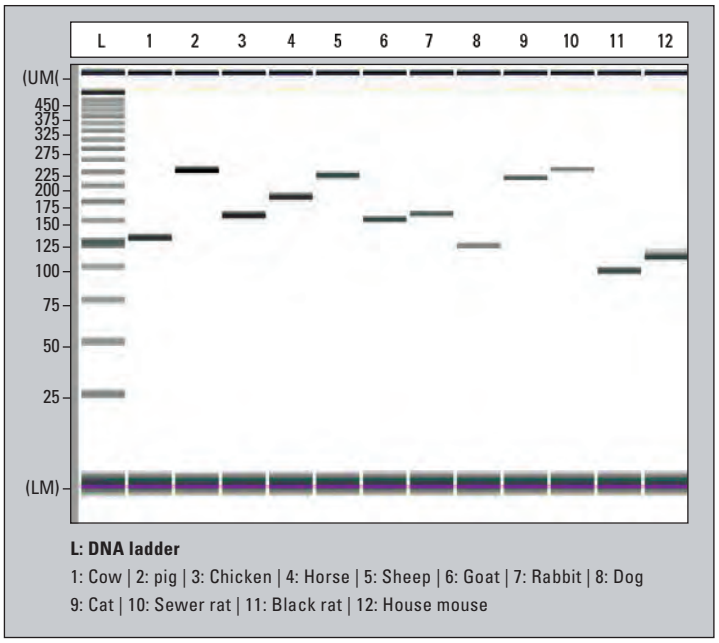

Figure 4: Identification of specific PCR products of different animal species using the MultiNA

12-strip tubes or single tubes. Size calibration curves with individually selectable DNA ladders are recorded for each microchip. These calibration curves enable accurate determination of the fragment sizes in the samples.

To compensate for small production-related variation between microchips, the system automatically adds an internal size marker system (lower marker and upper marker) to the samples prior to each analysis. The lower and upper markers restrict the size range that can be analyzed. In addition to the internal marker system, the size range that can be analyzed is also determined by the separation buffer used. Separation buffer and internal markers are sold together as a reagent kit. Currently, by selecting one of four reagent kits for double-stranded DNA, a size range of 25 base pairs up to 12,000 base pairs is covered. The RNA kit covers a size range up to 5,000 nucleotides (Figure 3).

Advantages of the automated method

Automated microchip electrophoresis offers numerous advantages over conventional gel electrophoresis:

- High sensitivity – the fluorescent stain used and the optical detection system are up to ten times more sensitive than conventional ethidium bromide staining
- Fast fully automated operation – parallel processing by a total of four microchips can reduce the cycle time to 75 seconds per sample
- High reproducibility i. a. due to the low number of manual steps and the associated reduction in sources of error
- Easy operation via straightforward and well designed software
- Low running costs through reusable microchips

When combining a PCR-based verification with subsequent detection using the MultiNA, an automated, fast and reliable method for the detection of contamination by animal hair can be obtained. Hair of six different farm animals (cow, pig, chicken, horse, sheep and goat), three pets (dog, cat and rabbit) as well as three rodents (sewer rat, black rat and house mouse), has been unequivocally identified (Figure 4). DNA of the corresponding animals was extracted from their hair and used for PCR analysis. Specific primer sets generating PCR fragments of specific sizes for each animal were used.

Following the PCR, size determination of PCR fragments was carried out using the MultiNA. In this way, it is possible not only to discover contaminations that have occurred, but also to unequivocally assign them and to identify their source (for instance farm or domestic animals). Only this will ensure that the source of the contamination can be reliably excluded and that, in future, no one finds a hair in the soup, pain reliever or skin cream.

Is all that glitters gold?

Figure 1: Excavation site in Geldern, Germany

Archaeological artifacts are of great historical significance and often emerge in a fragile condition. After being buried for hundreds of years beneath the soil, they have to be dug out and handled very carefully in order to preserve their condition as well as possible. Even where scientific questions need to be answered, the method of investigation should not damage the artifact.

Some 40 km northwest of Shimadzu's European headquarters in Duisburg, Germany, several metal objects were extracted from a 16th century well during the excavation of a medieval town quarter in the city of Geldern (Figure 1). A metal clip was of special interest because of its golden sheen (Figure 2). Could it be made of gold? This needed to be investigated.

Non-destructive analysis

The challenge was to determine the elemental composition of the artifact without damaging it.

EDX in archaeometry

An ideal task for energy-dispersive X-ray fluorescence spectroscopy, for instance using Shimadzu's EDX-720. Its 300 x 150 mm large sample compartment offers sufficient space for even larger artifacts.

What is the metal composition?

The main interest was the composition of the metal. Vacuum conditions or purging of the sample compartment with helium was therefore not required. Both would have positively influenced the measuring results of lighter elements. Measurement of fundamental parameters was carried out without the use of standards.

The instrument determines all elements near the surface of the metal within the concentration range of 100 % down to a few ppm. Small amounts of remaining soil could be recognized in the scratches on the surface of the artifact. The measuring spot was therefore reduced temporarily to 1 mm in order to avoid unintentional measurements. Nevertheless, it should be assumed that elements from the soil were found in the measured results.

The secret of the golden clip

Since a single measurement takes just a few minutes, several measurements on various areas of the artifact were carried out. The following spectrum (Figure 3) shows the results of one of these measurements. Peaks can be recognized at copper (Cu), zinc (Zn), silica (Si), sulphur (S), lead (Pb), calcium (Ca), cobalt (Co), manganese (Mn) and nickel (Ni).

Figure 2: Artifacts retrieved from the excavation site. Below right is the yellow-golden artifact.

Si, S and Ca could be assigned to the soil impurity. However high amounts of copper and zinc are not found in soil at these levels.

Considering only the ratio of copper to zinc, an experimental ratio of 79.88 (Cu)/20.12 (Zn) is obtained. The 'gold' artifact from Geldern was actually made out of brass.

The EDX-720 demonstrated its use in the reliable non-destructive determination of the elemental composition of archaeological artifacts.

We would like to thank Mr. Patrick Jülich at archaeologie.de, the archaeological excavation leader at the Geldern site in Germany.

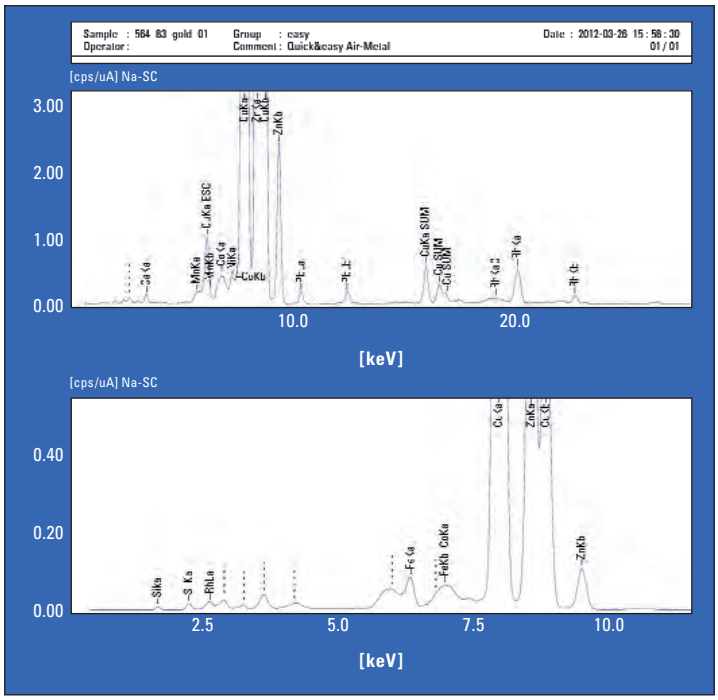

Figure 3: EDX measurement of the archaeological artifact. Measurement was carried out using two different excitation energies. In the lower spectrum, measurement was carried out at lower energy so as to facilitate identification of the lighter elements. The upper spectrum was obtained at higher excitation energy and primarily shows the heavier elements.

Additional information on the EDX-720 is available in the EDX brochure.

Headspace-Cold Trap Samp

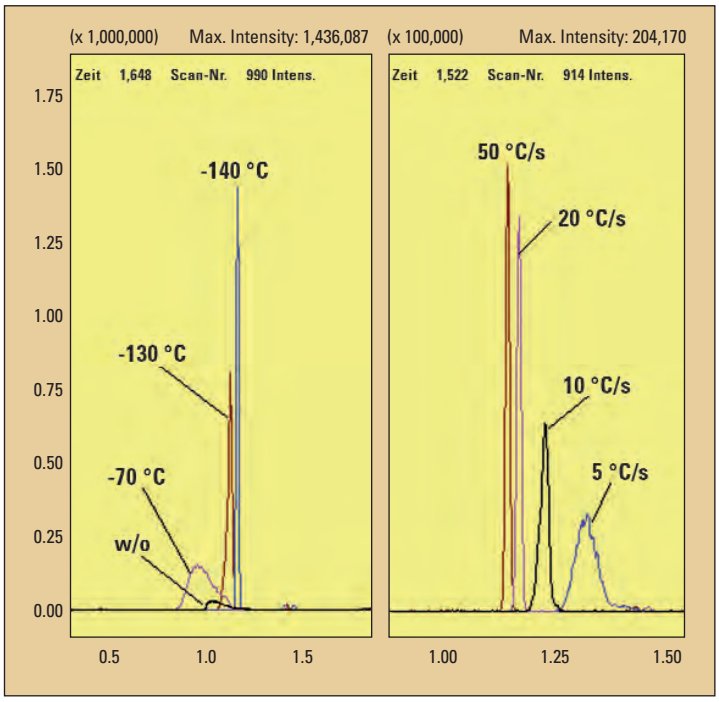

Figure 1: Left: Peak of m/z = 62 (vinyl chloride) for different cryofocus temperatures (without cryofocus, -20 °C, -70 °C, -130 °C and -140 °C). Right: Peak of m/z = 62 for different heating rates of the cryofocus after refocusing.

Hans-Ulrich Baier, Panos Meletis und Stephan Schröder, Shimadzu Deutschland, Duisburg, Germany

Analysis of EPA624 regulated volatile organic compounds in drinking and wastewater is usually performed with headspace or purge and trap technique using a so-called 624 phase with 30 m, 0.25 mm and 1.4 µm. Reducing analysis time (fast GC) but maintaining chromatographic resolution has been applied successfully using narrow bore columns in various fields. However, the reported results were based mainly on liquid injection techniques.

In headspace analysis the transfer of sample from the insert to the column is quite slow as small split ratios are normally used in favor of sensitivity. The spatial distribution of analyte molecules in the glass insert cannot therefore be refocused easily, and fast GC approaches are difficult. A cold trap (cryofocus, ATASGL, The Netherlands) was therefore mounted at the top of the column directly under the injector, cooling the first part of the column in order to refocus volatile compounds showing a broad band during passage through the injector liner. The cooling was established by direct transfer of liquid nitrogen to the trap.

Instrumentation used was a Shimadzu GCMS-QP2010 Ultra with an AOC-5000 Plus headspace sampler. As the column is surrounded by the directly cooled cryofocus, refocusing takes place inside the column. In this study, the inner diameter of the chosen column was 0.18 mm. Length and film thickness were 20 m and 1 µm respectively. Split ratio was 5:1 and the linear velocity was

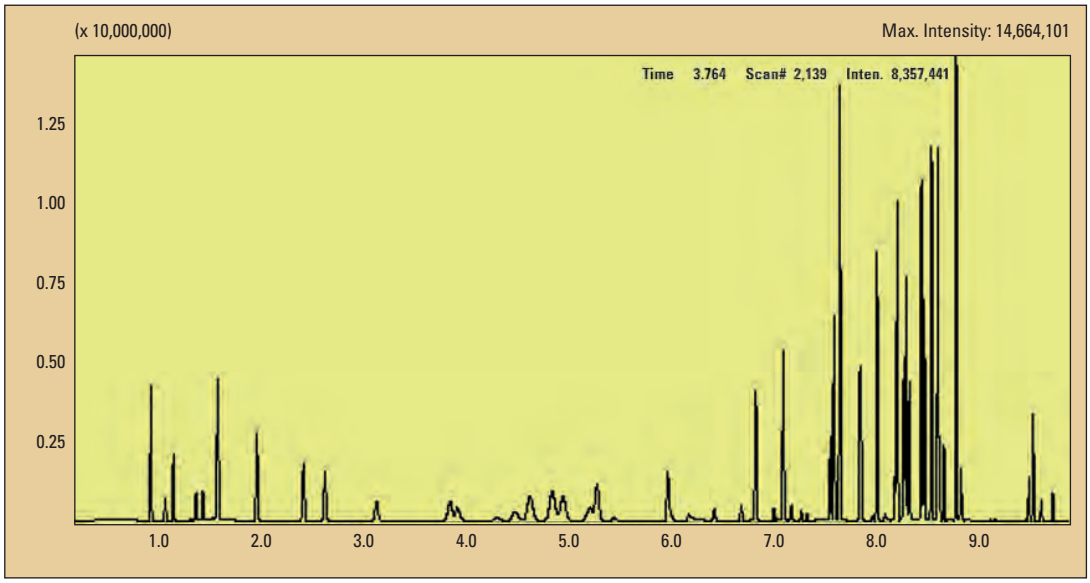

Figure 2: Full scan chromatogram (TIC) of 60 volatile compounds

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Fast GCMS analysis of VOCs in water

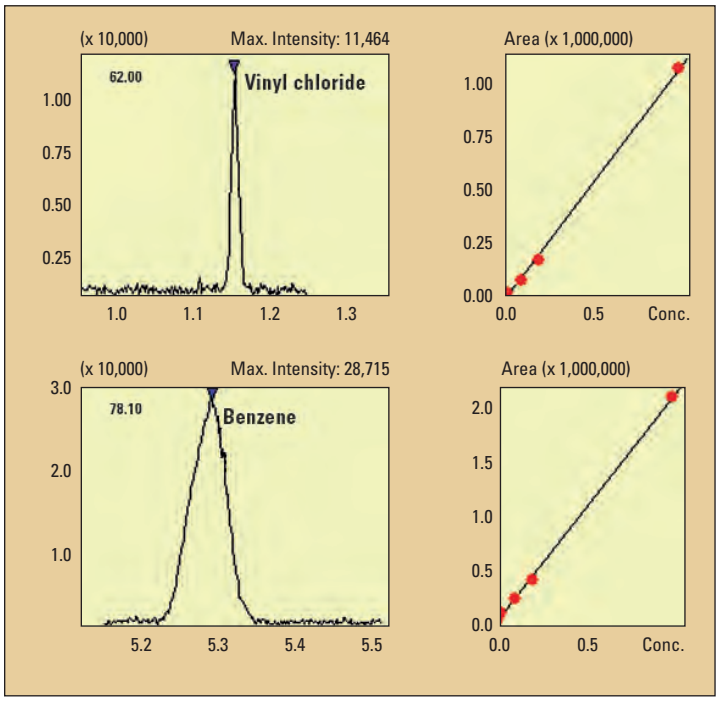

Figure 3: Calibration curves for benzene and vinylchloride

set to 45 cm/s. The GC oven temperature began at 40 °C for 5 min and then ramped with 50 °C/min to 120 °C, 30 °C/min to 170 °C, 60 °C/min to 220 °C. Injection volume was 1 mL headspace from a 20 mL vial filled with 5 mL water matrix. Different cold trap temperatures were set. The mass spectrometer was operated in scan and selected ion monitoring (SIM) mode for highly sensitive analysis.

Good peak shapes at 50 °C/s

Figure 1 (left) shows the m/z 62 relative to vinyl chloride for different cryofocus temperatures. The largest effect of refocusing monitored by measuring the peak profile at the end of the column in the mass spectrometric detector was observed at -140 °C cold trap temperature and subsequent heating to 250 °C at a rate of 50 °C/s. Figure 1 (right) shows the influence of different heating rates for vinyl chloride indicating that 50 °C/s ensures that the releasing process is fast enough to obtain good peak shapes. The peakwidth at half maximum is 8 sec and 0.5 sec for cryofocus temperatures of 0 °C and -140 °C, respectively. The peak height is increased drastically, with considerable improvement to the limit of detection (LOD). The complete chromatogram is shown in figure 2 and the compounds are listed in table 1.

Analysis within ten minutes

Analysis time for 60 volatile compounds was less than ten minutes. Calibration was performed between 0.001 and 1 µg/L. The regression coefficient R showed values of better than 0.998 for all compounds, indicating the high method precision. Two curves are shown in figure 3.

The LOD for benzene and vinyl chloride was determined as 0.005 µg/L and 0.001 µg/L respectively. In figure 4, the selected ion mass traces for tetrachloroethene and 1,1,2-trichloroethane of a real sample (the Rhine River water) is shown. Both concentrations were determined as 0.02 µg/L.

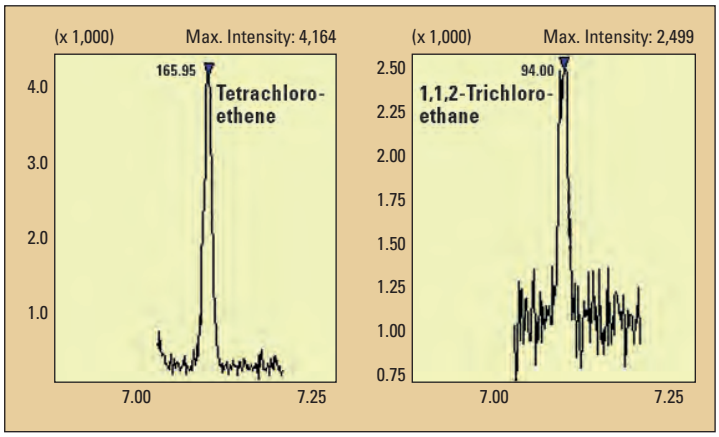

Figure 4: Peaks of tetrachloroethene and 1,1,2-trichloroethane measured for a water sample taken from the Rhine River

ID#	Name	Ret.Time	ID#	Name	Ret.Time
1	Dichlorodifluoromethane	0.945	31	1,2-Dibromoethane	7.319
2	Chloromethane	1.077	32	Chlorobenzene	7.547
3	Vinyl chloride	1.156	33	Ethylbenzene	7.582
4	Bromomethane	1.375	34	1,1,1,2-Tetrachloroethane	7.586
5	Chloroethane	1.443	35	p-Xylene	7.639
6	Trichlorofluoromethane	1.587	36	m-Xylene	7.639
7	1,1-Dichloroethene	1.970	37	o-Xylene	7.835
8	Methylene chloride	2.432	38	Styrene	7.849
9	trans-1,2-Dichloroethene	2.645	39	Tribromomethane	7.957
10	1,1-Dichloroethane	3.163	40	Isopropylbenzene	8.002
11	2,2-Dichloropropane	3.890	41	Bromobenzene	8.177
12	cis-1,2-Dichlorethen	3.967	42	1,1,2,2-Tetrachloroethane	8.186
13	Bromochloromethane	4.346	43	1,2,3-Trichloropropane	8.216
14	Trichloromethane	4.521	44	n-Propylbenzene	8.200
15	1,1,1-Trichloroethane	4.683	45	2-Chlorotoluene	8.262
16	Tetrachloromethane	4.900	46	1,3,5-Trimethylbenzene	8.286
17	1,1-Dichloropropene	5.005	47	4-Chlorotoluene	8.319
18	Benzene	5.313	48	tert-Butylbenzene	8.438
19	1,2-Dichlorethan	5.475	49	1,2,4-Trimethylbenzene	8.466
20	Trichloroethene	6.000	50	sec-Butylbenzen	8.538
21	1,2-Dichloropropane	6.197	51	4-Isopropyltoluene	8.599
22	Dibromomethane	6.284	52	1,3- Dichlorobenzene	8.616
23	Bromodichloromethane	6.414	53	1,4-Dichlorobenzene	8.658
24	cis-1,3-Dichloropropene	6.675	54	n-Butylbenzene	8.780
25	Toluene	6.813	55	1,2-Dichlorobenzene	8.825
26	trans-1,3-Dichloropropene	6.992	56	1,2-Dibromo-3-chloropropane	9.159
27	Tetrachloroethene	7.084	57	1,2,4-Trichlorobenzene	9.489
28	1,1,2-Trichloroethane	7.084	58	1,1,2,3,4,4-Hexachloro-1,3-b	9.532
29	1,3-Dichloropropane	7.165	59	Naphthalene	9.612
30	Dibromochloromethane	7.260	60	1,2,3-trichlorobenzene	9.724

Table 1: List of volatile compounds and retention times in minutes

Acid Test

TOC determination in concentrated hydrochloric acid

Incoming goods control is essential in the chemical industry. The impurities present in reagents often cause impurities in products. In addition to the targeted analysis of known compounds, sum parameters can help to assess the raw chemicals with respect to their impurities. TOC (Total Organic Carbon) plays an important role here: this parameter describes the contamination by organic compounds and specifies the total amount of organic carbon. TOC can therefore be used only for the assessment of inorganic chemicals.

Acids, particularly hydrochloric acids, belong to one of the large

Figure 1: Shimadzu's TOC-L for the determination of hydrochloric acid and OCT-L autosampler for automation of the analysis.

groups of inorganic chemicals frequently used in the chemical industry. Determination of the total carbon content in concentrated hydrochloric acid presents an enormous challenge to the analyzers used. For its new TOC-L series, Shimadzu has developed an application enabling the analysis of low TOC concentrations in concentrated hydrochloric acid.

Acids – a major challenge to materials and methods

The great challenge for TOC measurements in concentrated hydrochloric acid is the development of mechanisms to protect instruments and their components, as well as to prevent damage by acid fumes. For this purpose, the TOC-L series offers several gas washers that bind and eliminate the chlorine gas formed in the flow line of the system in various ways. An additional challenge in this application is to attain a stable and reproducible oxidation so that no fluctuating or tailing peaks are being recorded. Furthermore, the measuring values should remain stable over a larger measurement period.

Low limits of detection are possible

It is usually possible to greatly dilute the substance to be analyzed, in order to eliminate matrix interferences. Sometimes however, it is necessary to attain very low limits of detection (with reference to 37 % hydrochloric acid) of 1 mg/L.

Shimadzu's TOC-L$_{CPH}$ operates with catalytic oxidation combustion at 680 °C. The 37 % hydrochloric acid solution was manually diluted to 1:2 with water, in order to obtain an 18.5 % hydrochloric acid solution. Calibration (Figure 2) was carried out in the range of 0.5 to 10 mg/L. Using the automatic dilution function of the analyzer, this calibration was automatically carried out from a single stock solution. Injection volume in this case was 150 µL.

If TOC contamination of the hydrochloric acid exceeds the measuring range of the calibration, the automatic dilution function of the analyzer readjusts the hydrochloric acid solution to fit the measuring range. After calibration, the TOC content of the concentrated hydrochloric acid was determined. To investigate matrix influences, TOC content of the 18.5 % hydrochloric acid solution was subsequently doped by 5 mg/L using a potassium hydrogen phthalate solution (measuring results in Table 1).

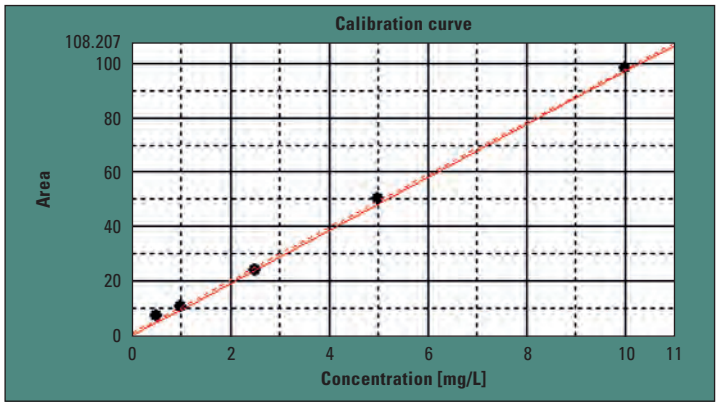

Figure 2: Calibration of the method in the range of 0.5 to 10 ppm

To investigate the long-term stability of the method, the 37 % hydrochloric acid solution was again diluted by 1:2 with water and injected 76 times (150 µL). The relative standard deviation over all measurements was 3.4 %.

Figure 3 shows the progression of the TOC values for the hydrochloric acid injections. Blank values and standards (10 mg/L) were alternately measured between the individual measurements. To automate this hydrochloric acid analysis as much as possible, the TOC-L series offers an autosampler consisting entirely of inert materials, enabling the analysis of up to 16 individual sample vials.

TOC content of the 18.5% hydrochloric acid solution in mg/L		
Injection	Not spiked	Spiked with 5 mg/L TOC
1	4.901	10.46
2	4.858	10.24
3	4.91	10.39
4	4.716	10.64
5	4.728	10.28
6	4.739	10.35
7	4.966	10.34
8	4.71	10.36
9	4.662	10.42
10	4.733	10.33
11	4.659	10.11
12	4.625	10.27
13	4.552	10.06
Mean value	4.75	10.33
Standard deviation	0.12	0.15
Standard deviation %	2.6	1.4

Table 1: TOC measuring values of the 18.5 % hydrochloric acid solution in mg/L

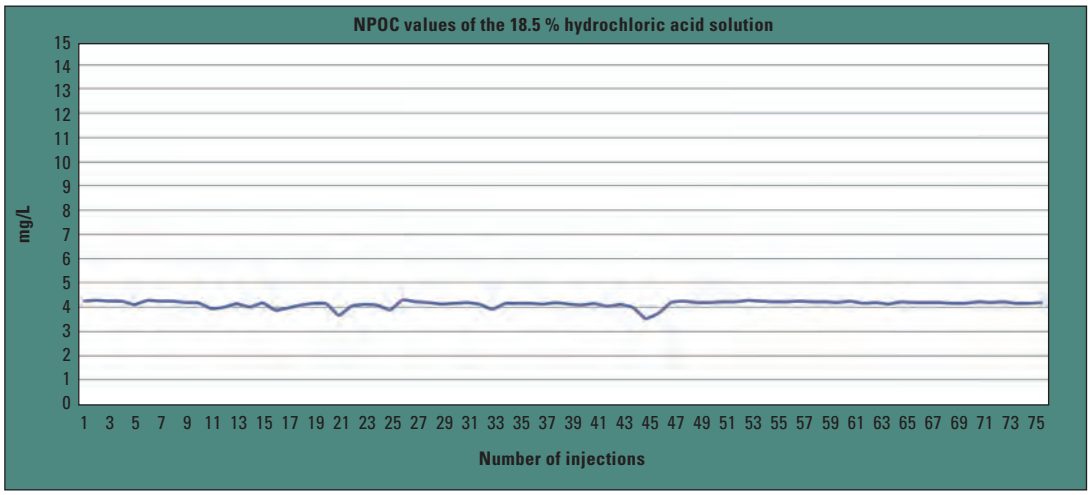

Figure 3: TOC values for 76 hydrochloric acid injections

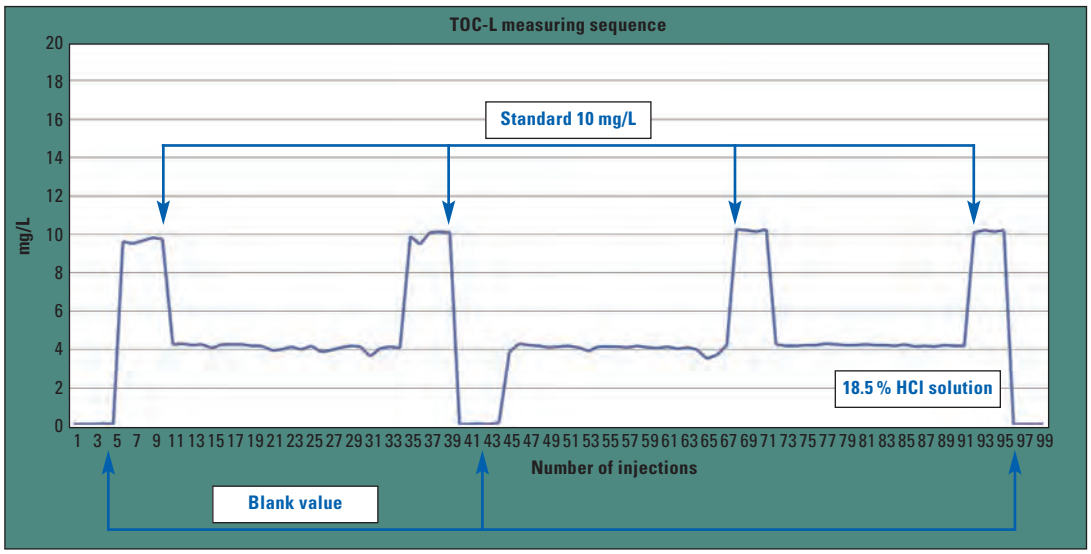

Figure 4: Sequence of a hydrochloric acid measurement. Hydrochloric acid, blank values and standards (10 mg/L) were measured alternately.

TOC analysis of concentrated nitric and sulfuric acid

Further experiments show that the experimental setup was suitable not only for the analysis of hydrochloric acid but also for other high purity chemicals, such as concentrated nitric acid and various salt solutions. A further modification of the system also enables TOC analysis of concentrated sulfuric acid or high-percentage brines. For this purpose, an additional scrubber and a special combustion tube with a special catalyst mixture was used.

TELEGRAM

Red wine and eye diseases

Live presentations at analytica 2012

For the first time, the professional audience could follow daily live demonstrations in the fully equipped laboratory named 'Live Lab' at this year's 'analytica' trade fair. Shimadzu was represented in two discussion sessions: 'Food and water analysis' featuring the '*Nexera* MP' UHPLC system as well as 'Forensics & clinical diagnostics' with the 'MCE-202 MultiNA' microchip electrophoresis instrument.

The live demonstration 'Analysis of Reservatrol in red wine' using HPLC was very well received by visitors to the trade fair. The 'PCR analysis of hereditary eye diseases' demonstration on the microchip electrophoresis system was also met with great interest. The live demonstrations were accompanied by interesting lectures and commentaries of renowned speakers such as forensic and criminal biologist Dr. Mark Benecke.

Dr. Mark Benecke (center) with Shimadzu's product specialists Vanessa Liedschulte and Dr. Klaus Bollig at the MultiNA system in the Live Lab

»Time for solutions«

6th World Water Forum in Marseille

In March 2012, the 6th World Water Forum took place in Marseille, France. Since 1997, this event has been held every three years on a global scale. The World Water Forum conference is the largest event in the world dealing with water topics, and promotes know-how, ideas, and innovation. Its main objective is to add "water" to all political agendas. 2012's forum was headlined "Time for Solutions!"

It is estimated that one billion people still have no access to clean drinking water, although the Right to Water must be guaranteed and implemented according to the responsibility of 193 states in the UN. Hygienic water and reliable water purification and supply are preconditions for health and the prevention of epidemics. It is estimated that more than 100,000 chemical substances contaminate drinking water every day.

During one week of discussions on solutions and best practices in over 250 sessions, 25,000 participants shared information and know-how. In addition to the conference, an exhibition was organized with most modern innovations in water analysis, water monitoring as well as engineering and distribution.

Figure 1: UV-VIS spectrophotometer UV-2600

Shimadzu participated with a booth showing the "state of the art" solutions for water analysis using spectroscopic and chromatographic methods as well as the determination of sum parameters, i.e. TOC (total organic carbon). In particular, spectroscopic methods such as the UV-1800 or the new UV-2600/2700 spectrophotometer series (Figure 1) support the detection and quantification of heavy metals in water. Over 60 years of experience in development of this technology underline Shimadzu's position as the market leader in UV-VIS-NIR spectroscopy.

Furthermore, Shimadzu offers a customized water analysis solution using the ICP-OES spectrometer ICPE-9000 (Figure 2), available for download using the following link:

Figure 2: ICP-OES spectrometer ICPE-9000 – solution for water analysis

www.solutionsforwater.org/solutions/interference-free-drinking-water-analysis-using-icp-oes

We will gladly send you additional information. Please enter the corresponding number on the reply card or order via Shimadzu's News App or News WebApp.
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