Frozen

HPV-1 high-speed camera

Figure 1: Hyper Vision HPV-1 high-speed video camera

One million frames per second – science and technology is impressed. The new HPV-1 high-speed camera "freezes" frames of high-speed phenomena within microseconds (Figure 1). This opens up new ways of studying high-speed phenomena, for example in materials research and development. The new HPV-1 can be applied for studying hydrodynamics, degradation processes, pressure wave propagation and machine processes as well as monitoring fast chemical reactions. Application areas such as the biosciences, medical technology and sports sciences will benefit from the new insights gained by using the HPV-1.

The excellent resolution of 312 x 260 pixels (approximately 81,000 pixels) will not deteriorate even at high speeds. The new chip architecture enables high-speed image acquisition. An IS-CCD chip can store up to 100 individual frames (Figure 2). In addition to each single photodiode on the CCD chip, there are 100 memory registers wherein the pixel information is stored, image by image. At the highest recording speed, only one ten thousandth of a second is needed to record from the first to the last frame.

The special architecture permits so-called post-triggering, whereby the result is already recorded before the start signal has been received. The chip-memory is overwritten continuously, and each newly acquired image replaces the oldest one, so that at any point in time 100 images are stored on the chip. Phenomena that are difficult to trigger will be able to be investigated this way.

User-friendly, multifunctional software

The instrument is ready to record after only a few settings in the control unit software. Recorded films are saved and stored in AVI or BMP format and can be exported to other applications.

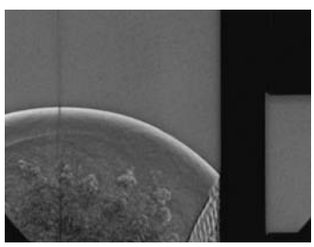

Figure 3: Propagation of a pressure wave with 1,000,000 fps (Schlieren photography)*

**This image was kindly made available by: Shock Wave Research Centre, Institute of Fluid Science, Tohoku University, Japan*

Additional information, as well as demo films are available via our website: www.shimadzu.de

We will gladly send you further information. Please note the appropriate number on your reader reply card or send an e-mail to kamera@shimadzu.de
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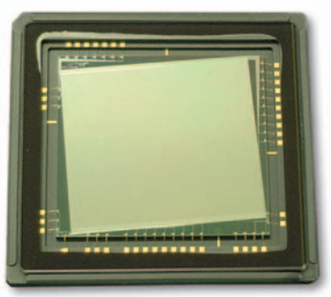

Figure 2: IS-CCD chip, for up to 1,000,000 fps. This CCD technology enables image recording even under relatively low light conditions. No other high-speed CCD camera can record more images at 1,000,000 fps.

SHIMADZU

NEWS

Solutions for Science since 1875

» LCMS-IT-TOF – A new concept for high mass accuracy Tandem Mass Spectrometry

» Straightforward analysis of difficult samples – ICP spectrometer

» Frozen – HPV-1 high-speed camera

1/2006

Analytical Instrumentation – Views into the nano-world

IMPRINT

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Shimadzu Europa GmbH
Albert-Hahn-Str. 6-10 · 47269 Duisburg
Phone: +49-203-7687-0
Telefax: +49-203-766625
Email: shimadzu@shimadzu.de
Internet: www.shimadzu.de

Editorial Team:
Uta Steeger · Phone: +49-203-7687-410
Ralf Weber, Angela Baehren

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CO_2 determinatio n i

Fast help with TOC

Quality assurance is only one of the attributes for brand name products such as beer. And quality is dependent on the quality of the starting materials, strict production processes as well as specialists whose keen eyes oversee the entire production. Finally, millions of consumers are testing their favorite brand of beer every day.

An important player in the production process and a condition for quality is invisible: carbon dioxide, formed during the fermenting process. After filling the beer into barrels and bottles, carbon dioxide ensures that the necessary pressure is maintained. This is an important factor for guaranteeing shelf life and fresh taste of the beer. A constant concentration of carbon dioxide also ensures a steady taste and consistent quality of the beer. The concentration of carbon dioxide also plays an important role in other soft drinks. Beer contains 4 – 6 g/L CO_2; soft drinks contain 4 – 10 g/L CO_2.

Quality control during beverage production therefore requires a routine method for fast and accurate determination of CO_2. Traditional procedures such as titration and manometric methods are usually time-consuming, not very selective and difficult to automate. In collaboration with the König brewery in Duisburg, Germany, a new procedure was developed for the determination of CO_2 content using a TOC analyzing system (Figure 1).

Analytical system and measuring method

For this application, a Shimadzu TOC-V_{CPH} with autosampler (ASI-V) was used. A typical method for TOC determination is the differential method where, initially, the total carbon content (TC) is determined and subsequently the inorganic carbon content (IC). The difference between both parameters represents the organic carbon content.

Stock solution	Dilution factor	Calibration points	Area units
1,000 mg/L C	20	50	205.9
	10	100	407.5
	5	200	803
	2	500	2120

Table 1: IC calibration curve with automatic dilution function

tio n in beer

The IC method was used for CO_2 determination. The sample was injected into a vessel containing a phosphoric acid solution. The phosphoric acid converts all carbonates and hydrogen carbonates to CO_2. Carrier gas is used to transfer the CO_2 from the sample solution to the NDIR detector where it is selectively detected. The peak area of the NDIR analog output signal is then integrated. For evaluation of the correlation between peak area and IC concentration, the TOC system is calibrated using an IC standard solution.

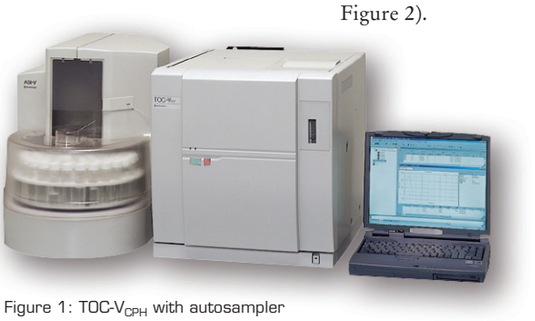


Figure 1: TOC-V_{CPH} with autosampler

Sample preparation for CO_2 determination

In a vessel containing 180 mL beer, 5 mL of a 32 % sodium hydroxide solution was added in order to convert dissolved carbonic acid into carbonates. After mixing, the solution was transferred to the autosampler. As the CO_2 concentration is relatively high, the TOC-V_{CPH} automatically dilutes the sample by a factor of 5 respectively 10. This also minimizes the influence of the aggressive, alkaline matrix.

Calibration and measurement results

For the IC determination, the TOC-V_{CPH} was calibrated with a carbonate/hydrogen carbonate solution. The automatic dilution function again simplifies the calibration procedure. Only one standard solution is prepared manually and the instrument subsequently carries out the entire dilution sequence (see Table 1 and Figure 2).

The calibration curve expresses area units in terms of carbon concentration. The values obtained are multiplied by a factor of 0.00038 in order to obtain the CO_2 content in the original solution (beer).

Figure 3 shows an IC determination of a beer sample.

The CO_2 content of various types of beer was determined using this method. In order to check the plausibility of the data, the results were compared with a reference method (Corning method). Figure 4 shows the results. IC determination resulted in less varying

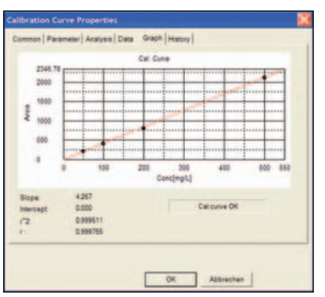


Figure 2: IC calibration curve 500 ppm

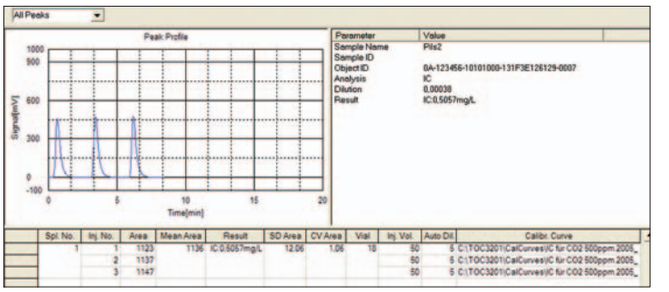


Figure 3: IC determination of a beer sample

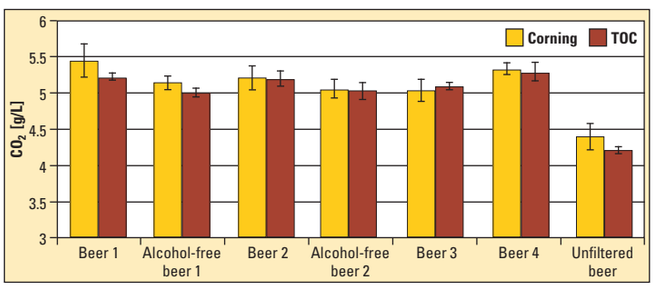


Figure 4: Bar graph

of the data compared to the reference method. In absolute terms, these values were always within the data deviation zone of the reference method.

Summary

IC determination using the TOC analyzer has established itself as a suitable alternative to the classical methods for CO_2 determination. In comparison with the traditional methods, this new method is much more selective, easier to handle and to automate. The excellent reproducibility, the wide measuring range and the fully automatic dilution function of the TOC-V_{CPH} enable its use in routine analyses in an industrial brewery. Furthermore, the TOC system can be used in additional applications in the brewery (for instance for testing of process- and wastewater).

Cheers!

Shimadzu thanks the König brewery in Duisburg for providing their measuring data.

Figure 1: Bee approaching an apple blossom

Figure 2: Phacelia flower with pollen collecting bee

Enjoy without guilt

Honey and GC analysis

Locally produced honey is one of the foods least contaminated with residues of environmental pollutants and pesticides. Although the 1.5 kg per-capita consumption of honey in Germany is negligible in comparison with the amounts of meat, vegetables and fruits that find their way into the shopping basket, honey is nevertheless subject to analytical testing as if it belonged to one of the main foods for human consumption.

Since 1988, pesticide residue analysis has been carried out at the Federal Institute for Apiculture at the University of Hohenheim in Germany on several thousand honey samples every year. Quality control is an important issue but the main focus is the identification of factors which may jeopardize the image of locally produced honeys. The results are primarily important for informing beekeepers. The criteria in honey analysis are very close to those of drinking water analysis, with determination limits in the low ppb range.

Why these procedures?

Where no maximum levels have been assigned, a reliable maximum value, usually 10 to 50 µg/kg, is used for plant food sources. As honey was considered a plant food source (today it is recognized as an animal food source) analytical methods were developed and established that comply with these legal maximum levels. In this way, honey – which as a natural product is regarded as being especially pure – is subject to very stringent quality control using highly sensitive analytical methods.

But even when using very sensitive methods, it is still quite difficult to detect pesticide residues in honey. The problem is that these pesticides are frequently used on cultivated plants in full bloom, while they serve at the same time as an important source of nectar and pollen for honeybees and other insects. Although pesticide contamination of nectar in orchard flowers or rapeseed fields can be detected easily, the search for pesticide residues in harvested honey often produces negative results. Why is this so?

Honey in GC analysis

To investigate this contradiction, laboratory, field and semifield studies as well as tent tests were carried out in recent years where the pesticides were traced from the flower up to the ready-for-harvest honey in the beehive. The availability of reliable miniaturized extraction methods and highly sensitive measuring instruments was indispensable in these studies. The objective was to

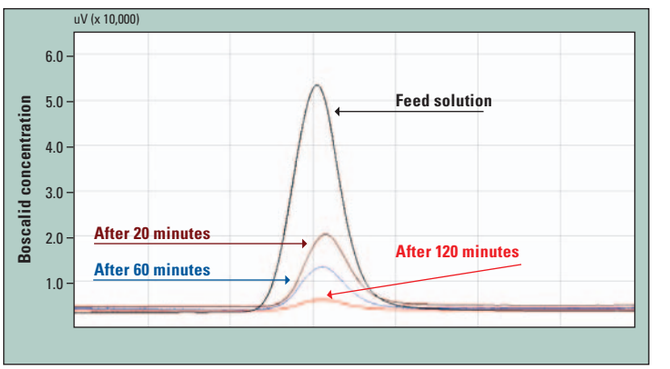


Figure 4: Degradation of the fungicide boscalid in honey sacs, shown via overlaid ECD chromatograms

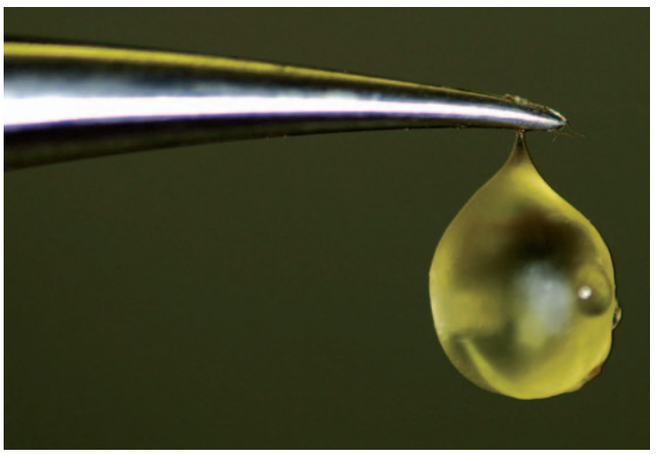


Figure 3: Dissected honey sac filled with nectar

Figure 5: Pollen stored in the honeycomb

detect the active pesticide compounds originally applied to the plants, in the collected nectar of honeybees as transported in their honey sacs.

Analyses were carried out using Shimadzu's GC-17A and GC-2010 gas chromatographs, each equipped with ECD detectors as well as a GCMS-QP5050A.

Depending on the type of plant and weather, honeybees usually visit between 50 and several hundred flowers before they return to the beehive with a full honey sac. During their nectar gathering flights they select one particular type of flower, meaning that they visit only one type of plant and also safeguard the pollination process (Figures 1 and 2). Under suitable experimental conditions, it is possible to guarantee that all collecting bees actually fly into the experimental plot – a rapeseed field that was sprayed with pesticides. Each bee transports on average approximately 40 µL of nectar. This nectar contains the active compound of the pesticide spray subsequently determined in the laboratory.

After returning from the field, the forager bees must be intercepted at the hive entrance. This is done using a converted automobile vacuum cleaner that instantaneously covers the bees with CO_2 snow. In the laboratory the honey sacs, up to 2000 per experiment, are dissected individually (Fig. 3) and the target active compounds then isolated using liquid-liquid extraction methods and subsequently determined using gas chromatography.

The results show that the bees encounter mid ppm range pesticide concentrations in the flowers, which then find their way into the nectar. An interesting observation is that very high fluctuation margins are measured between individual bees in one series, although the bees have all been released into the flowering fields simultaneously. In the honey sacs of some of the nectar collecting bees extremely low pesticide levels – with 0,1 pg/µL being below the quantitation limit – were found. Based on the high number of visited flowers, a more evenly distributed pesticide level was expected in the honey sacs.

Bees under laboratory conditions

In the laboratory, caged groups of bees were fed with sugar solutions containing active pesticides. After a predetermined time frame, the honey sacs of these bee samples were examined in order to determine whether active pesticide levels were being reduced within the honey sacs. Indeed, for some lipophilic compounds a radical decrease in the active pesticide levels could be determined. The active pesticide molecules apparently diffused into the tissue of the honey sac, which in turn suggests that the bees already reduced the pesticide content in the collected nectar during their collecting flights.

As the times that bees spend collecting nectar in the flower fields vary, this may be the cause for the strongly fluctuating measuring values. Bees clearly reduce the pesticide content acquired from the sprayed flowers already during their flight. The nectar delivered when the bees return to their hives already shows clearly reduced pesticide contaminations (Figure 4).

In the beehive, the nectar is processed into honey. The collected nectar is passed on from bee to bee. The worker bees enrich the nectar with endogenous substances and extract water from the honey during honey production. In this way, a self-preserving food substance is produced and stored in the cells of the honeycomb (Figure 5).

Further measuring sequences have shown that additional pesticide reduction processes take place during storage of honey, such as diffusion processes from the collected nectar into the beeswax of the honeycomb cells walls. These processes apply especially to the lipophilic active compounds and take place at the start of honey production when the water content is still relatively high.

The question as to why pesticides rarely present a problem with respect to honey quality, even though beekeepers have always been very critical regarding the use of pesticides in orchards or rapeseed fields, is now close to being answered.

GC/MS

Automated DMI for scree nir cosmetic products

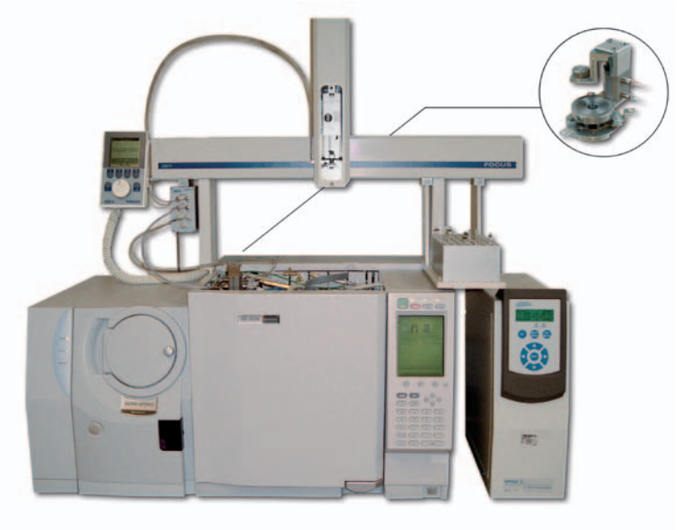


Figure 1: The GCMS-QP2010, AOC-5000, Optic 3 injector and LINEX

Erwin Kaal, University of Amsterdam and ATAS GL International
Hans-Gerd Janssen, University of Amsterdam
Mitsuhiro Kurano, ATAS GL International

Compound	Area RSD %
Diethylene glycol	12.9
Triehtylene glycol	10.9
1.2-Ethanediol, Monoacetate	5.0
Pentaethylene glycol	9.5
Heptaethylene glycol	12.4
Ethylene glycol monododecyl ether	5.7

Table 1: Repeatability of DMI analysis of washing powder

Complex matrices are encountered in many application areas of gas chromatography. Difficult matrices occur for example in environmental analysis, food characterization or in the analysis of home and personal care products. For such complex and difficult matrices the DMI (Difficult Matrix Introduction) technique is a powerful analytical tool.

In the present contribution the DMI technique is applied to the screening of cosmetic products such as lipsticks, lotions, washing powders and shampoos. Pattern recognition, identification of unknown compounds as well as quantification of known ingredients can be done without any sample preparation. If identification of the fragrances in perfumed cosmetic products is necessary, this can be achieved in just one single run during the normal screening since the DMI GC/MS is also coupled to a sniffing port ('PHASER'). At the end of the GC column the carrier gas flow is split into two. One flow is directed to the MS detector whereas the other is sent to the PHASER. Retention times for both detection devices are hence virtually identical.

GC/MS analysis of cosmetic products is generally difficult because the compounds of interest are present at low levels in a complex water and oil-containing emulsion. Transfer of either water or oil to the GC system obviously has a negative influence on the results. A number of labor-intensive sample preparation steps are normally needed for extraction and cleanup if these complex mixtures are to be analyzed using GC/MS.

A different approach to eliminate the matrix is to perform a so-called Difficult Matrix Introduction (DMI). In the DMI technique a small aliquot of the sample is put into a glass vial which is inserted automatically into the injector. The injector is then heated to a temperature just high enough in order to transfer the compounds of interest from the sample onto the chromatographic column. Only the vaporized

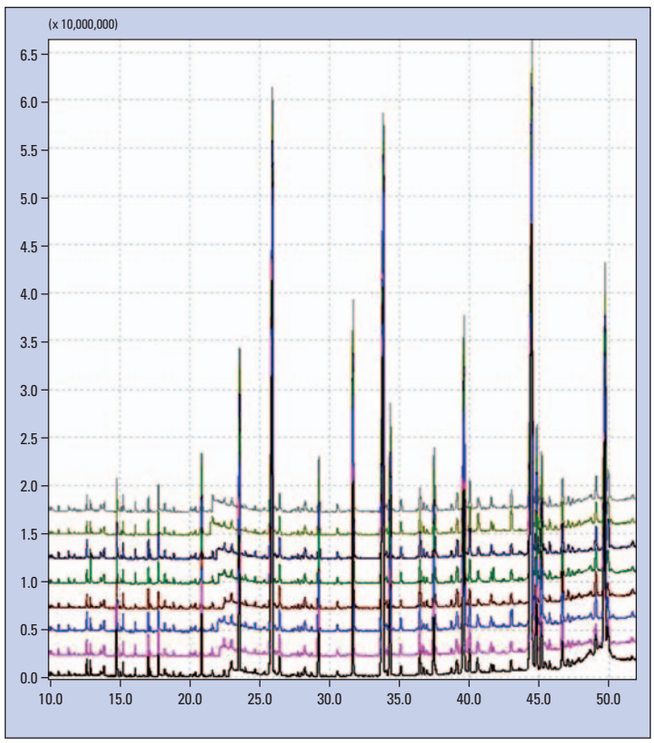


Figure 2: Chromatograms (TIC) of washing powder for the determination of repeatability. GC column: Inertcap wax 0.32 mm x 60 m, film thickness 0.5 µm (GL Sciences); GC temperature program: 40 °C (hold 6.3 min), 15 °C/min to 130 °C, 3 °C/min to 250 °C (hold 25 min); PTV injector: 35 °C to 250 °C rate 5 °C/s.

ee ning of

compounds are transported from the injector onto the GC column, where they are refocussed at the low starting temperature of the GC program. Because the non-volatile matrix species remain in the vial and the injector liner, no column contamination of the GC system occurs.

Instrumentation

The injector used for this study was an OPTIC 3 injector (ATAS GL Int'l, the Netherlands). It was installed into a GCMS-QP2010 (Shimadzu Corp., Japan). The column effluent flow was split into two separate flows, one going to the PHASER sniffing port (ATAS GL Int'l) and the other to the mass spectrometer. Helium was used as the carrier gas in all experiments.

In order to streamline the analysis, an automated liner exchanger unit (LINEX, ATAS GL Int'l) was used (Figure 1). The sample mass weighed into the DMI micro-vials ranged from 8 mg for detergent powders to 12 mg in the case of shampoos.

Reproducibility

One of the advantages of the DMI method is that only minute sample quantities are required. For inhomogeneous samples such as washing powders, however, this could also be a potential drawback. To determine the reproducibility of DMI analysis for such samples a washing powder was analyzed several times. To minimize inhomogeneity problems, the maximum mass of powder was put into the micro vial. Heating was performed in the split mode at a split ratio of 1 to 40. For desorption the injector was heated to 250 °C at a rate of 5 °C/s. The reproducibility (n = 10) of the retention times and the peak areas for most of the compounds was better than 4 % (Rt) and 10 % (areas) as can be seen from Table 1 or Figure 2.

Screening of shampoos

Perfumes in cosmetic products are one of the most common causes of allergic contact dermatitis. For this reason the quantification of allergens is currently receiving a great deal of attention. With DMI it is possible to identify and quantify these perfume allergens at low levels without any sample preparation. This reduces the cost of analysis but it also eliminates potential losses of the volatile target compounds during sample preparation. In addition to the allergens, other ingredients of shampoo can be also be identified and quantified as demonstrated in Figure 3.

Reference

A. Amirav and S. Dagan, Europ. Mass. Spectrom. 3, 105-111 (1997)

ATAS GL International
De Run 4441, P.O. Box 17
5500 AA Veldhoven
The Netherlands

University of Amsterdam
Polymer Analysis Group
van't Hoff Institute for Molecular Sciences
Nieuwe Achtergracht 166
1018 WV Amsterdam
The Netherlands

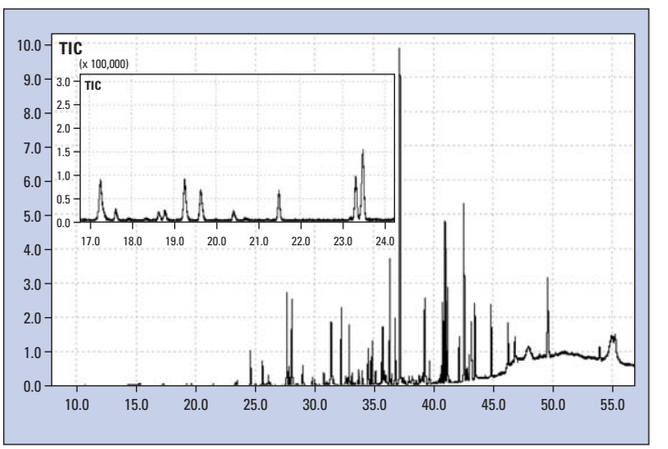
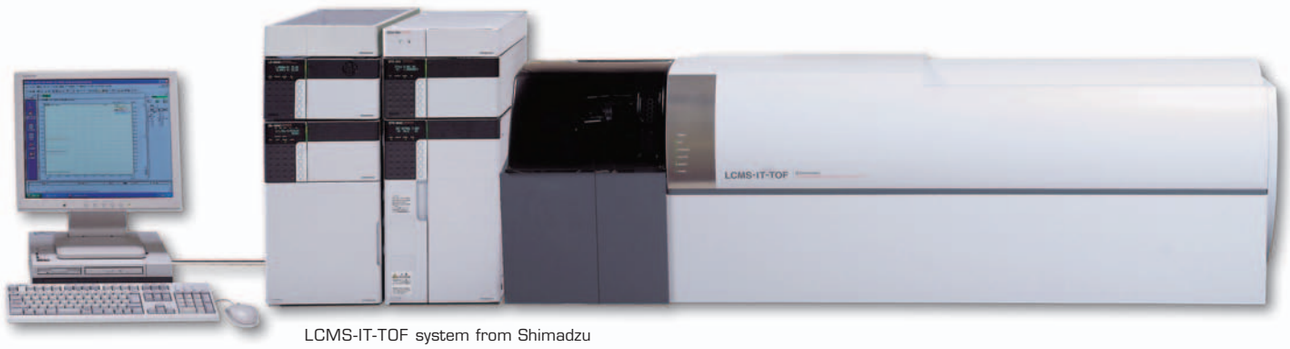


Figure 3: DMI GC/MS chromatogram (TIC) of shampoo for identification of fragrance and allergens. GC column: Inertcap wax 0.32 mm x 60 m, film thickness 0.5 µm (GL Sciences); GC temperature program: 35 °C (hold 8 min), 5 °C/min to 230 °C (hold 10 min); PTV injector: 35 °C to 120 °C, rate 5 °C/s.

01. d-Limonene (Rt 17.1 min)
02. Tetrahydro linalool (Rt 24.6 min)
03. Dihydromyrcenol (Rt 25.6 min)
04. Linalool (Rt 27.7 min)
05. *tert*-Butyl cyclohexyl acetate (Rt 28.1 min)
06. Terpineols (Rt 29.8 min; 31.4 min)
07. Benzyl acetate (Rt 32.2 min)
08. Geraniol (Rt 32.6; 34.7 min)
09. Citronellol (Rt 32.9 min)
10. Nerol (Rt 33.7 min)
11. α-Isomethyl ionone (Rt 34.8 min)
12. β-Ionone (Rt 36.8 min)
13. 2-(4-*tert*-Butylbenzyl) propionaldehyde (38.9 min)
14. n-Hexyl salicylate (Rt 40.0 min)
15. Piperonal (Rt 42.8 min)
16. Cinnamal (Rt 43.0 min)

NEW

A new concept for high mass Tandem Mass Spectrometry



LCMS-IT-TOF system from Shimadzu

Every year mass spectrometry methods are increasingly implemented in the general work flow of laboratories. They offer high selectivity and sensitivity in a broad range of application fields. Nowadays, mass spectrometry is a technique which can be used by most lab workers without requiring extensive training.

The design of user-friendly software (e.g. LCMSsolution) as well as easy-to-use hardware is appreciated by a large user base.

The single quadrupole mass analyzer LCMS-2010EV is well known as a very robust and sensitive HPLC detector for qualitative and quantitative analysis connected to preparative and analytical HPLC systems.

Recently, Shimadzu has introduced a new hybrid type high end mass analyzer to the European market, the LCMS-IT-TOF.

The combination of two mass spectrometric tools (ion trap and TOF) results in capabilities beyond any single mass analyzing technology. Ion trap mass spectrometry allows target molecules to be selected and fragmented easily (MS^n, MS/MS, MS/MS/MS, MS/MS/MS/MS...) while time-of-flight MS provides high mass resolution and accurate mass determination (Figure 1).

More information about a sample

The new instrument delivers more qualitative information about a sample in a single experiment, thus eliminating multiple analyses and avoiding the need to split samples between multiple instruments. Furthermore, both positive-ion and negative-ion information can be obtained from just one sample injection, very useful for analysis of unknown substances.

Thanks to the auto tuning function and the easy-to-use LCMSsolution software, this instrument enables scientists to generate outstanding data for their research projects within a very short time.

Different ionization sources

The range of orthogonal designed atmospheric pressure interfaces for the LCMS-IT-TOF is adapted from the LCMS-2010EV system. These include electrospray (ESI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photo ionization (APPI). Primarily, ionization source choice depends on the chemical properties of the ana-

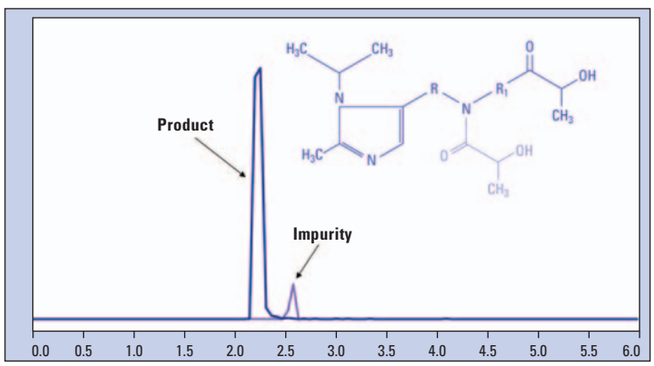


Figure 3a: Auto product and impurity fragmentation experiment: HPLC chromatogram

mass accuracy
etry LCMS-IT-TOF

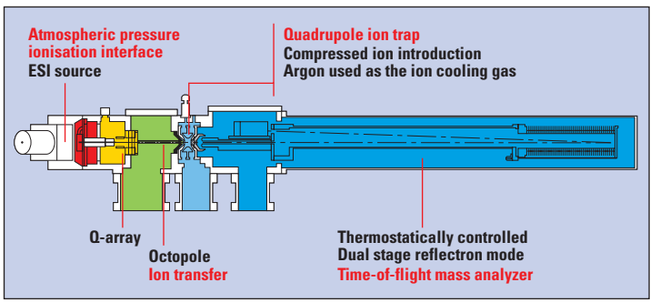


Figure 1: Schematic of the LCMS-IT-TOF instrument

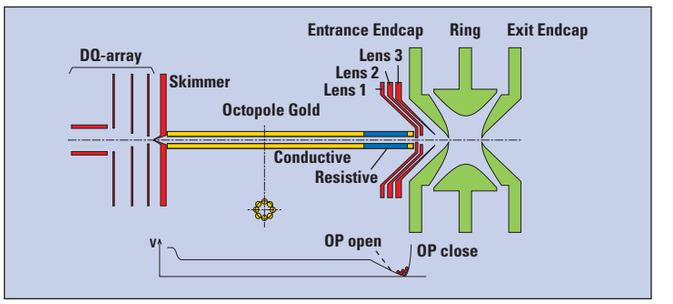


Figure 2: Octopole assembly

lytes – ESI, the most commonly used source, generally works best for polar molecules, APCI for neutral or weakly polar components, and APPI for neutral and difficult to ionize chromophoric compounds.

In addition, a nano-spray source is available which is being used frequently in biochemical applications such as the field of proteomics.

Patented instrument technology

Ions generated in the atmospheric pressure interface region are transferred through a curved desolvation line (CDL) into a focussing area, the Q-array, before they finally reach the so-called octopole region (Figure 2). This patented instrument component is responsible for transfer of a continuous ion beam into discrete ion packages before further introduction into the ion trap. This process, called compressed ion introduction, allows a higher and faster filling efficiency in comparison with traditional ion traps. Furthermore, it is used to control the ion flux into the trap so that an overfill of the trap and resulting difficulties due to space-charge effects do not occur. After enough ions have been collected in the octopole area these get pulsed into the trap as an "ion package". The RF frequency of the ion trap is switched off during ion introduction, to allow the complete ion package to enter the trap, and switched on afterwards to store all ions in the trap. In the trap argon is used as a buffer gas, which results in a faster ion cooling with lower gas pressure.

The ion trap of the LCMS-IT-TOF shows a very accurate precursor ion selection, used in the isolation of an ion of interest for further fragmentation analysis, even with other ion signals in its closest vicinity. MS/MS spectra from instruments lacking such a high precursor ion resolution would suffer from contaminations by fragment signals generated from other precursor ions. This makes an interpretation of those data more difficult.

The trap performs a recursive cycle of cooling an ion package, isolating a precursor ion, exiting the precursor ion and fragmenting it by collision induced dissociation (CID) with pulsed argon.

High mass accuracy

After the chosen cycles in the trap have been completed, the ions are pulsed out of the trap by switching off the RF and applying bipolar voltages to the end caps. This potential gradient forces the ions into the TOF region for further mass analysis. The very sharp starting point of the ion introduction into the TOF analyzer guarantees the high mass accuracy of the LCMS-IT-TOF instrument consistently in all MS and MS^n modes.

In contrast to traditional ion traps that determine the masses of ions by an instability scan of the trap, the LCMS-IT-TOF uses its trap as a very good controllable tool for ion storage, precursor ion selection and CID only. The mass determination is performed subsequently by the TOF analyzer, resulting in superior mass resolution and accuracy.

Figure 3b: 1) Auto MS^5 experiment of the product, 2) Auto MS^6 experiment of the impurity

One of the fastest hybrid systems available

The principle of time-of-flight (TOF) mass spectrometers is straightforward. Ions are accelerated down a flight tube so that those with the same charge have identical kinetic energy; lower mass ions travel with a higher velocity than those of higher mass so they reach the detector more quickly. The highly accurate temperature regulation of the flight tube and related electronics is a special feature of the LCMS-IT-TOF giving the instrument a long time mass stability (approx. +/– 2 ppm over 24 hours) and therefore great mass accuracy – without the need for frequent recalibration or the use of an interleaved external lockmass measurement. The concurrent analysis of an additional reference sample also reduces the number of measurement cycles for the valuable sample during an HPLC experiment. Due to specially developed unique electronic parts, the instrument is able to generate MS spectra every 100 ms, making it one of the fastest hybrid systems available.

Details make the difference

With the new LCMS-IT-TOF, it is possible to fragment various component peaks found during a HPLC separation just as with traditional ion traps. However, because of the high mass accuracy (below 5 ppm with internal calibration), not be obtainable with traditional ion traps, it is possible to determine composition formulas for each compound including the information gathered from related MS/MS and MS^n experiments. This can be very useful when searching for impurities which occur during a synthesis. Very often the core structure of the product and the impurity are similar, but a slight variation in a sidechain can be found. Comparison of the different MS^n spectra of both components often allows identification of the structural difference. The HPLC chromatogram in Figure 3 a shows a compound and its impurity. Beginning with the MS^3 spectra the MS^n sequence of spectra in Figure 3b shows the same fragmentation pattern for both precursor ions, obviously revealing the same core structure.

The integrated, easy-to-use "Composition Formula Predictor" software (Figure 4) assists the user in determination of the correct formula by calculation of a probability score for the formulas suggested. It uses the maximum number of different elements expected in the compound and calculates various formula permutations depending on rules, as for example nitrogen rule or double bond equivalent. If available, the algorithm starts with MS^n information in order to exclude permutations not backed up by fragment spectra.

This is a very powerful approach, because the number of possibili-

ties exponentially decreases to smaller m/z values (Figure 5), and with the option of backward calculation differing formula suggestions can be gradually excluded.

The overlay of the isotopic patterns of the analyzed compound and the predicted formulas can further reduce the number of possible candidates. Among other information, this pattern comparison is included in the scoring scheme which scales the probability of a calculated suggestion.

Numerous software options

Depending on the field of application, MS datasets can be exported and used in various software packages. For example, Shimadzu offers the software package ACD/MS Fragmenter from ACD/Labs, which is able to predict chemical structures related to MS^n fragments depending on the initial structure of a compound. This is very useful for the identification of degradation products from drugs occurring during stability tests or as a result of metabolic reactions in an organism.

In order to find differences on the metabolite level between two or more samples, the software tool Profiler (AM) with accurate mass capabilities from Phenomenome Discoveries is available. Thanks to the high retention time reproducibility of the *prominence* HPLC system in combination with accurate mass information obtained by the LCMS-IT-TOF, a robust and meaningful comparison is feasible. For example, differences in leaf, stem and root cells of plant extracts could be structurally determined and evaluated with the help of advanced statistical tools.

The Shimadzu Proteinlayer software tool embedded in the LCMSsolution package can be used for the identification of proteins and their modifications after digestion. It translates and filters the obtained mass lists for automatic MASCOT searches in protein sequence databases.

This new hybrid mass spectrometer, LCMS-IT-TOF, is expected to find applications in the analysis of small as well as larger molecules in forensics, proteomics, metabolomics, drug development and environmental chemistry laboratories.

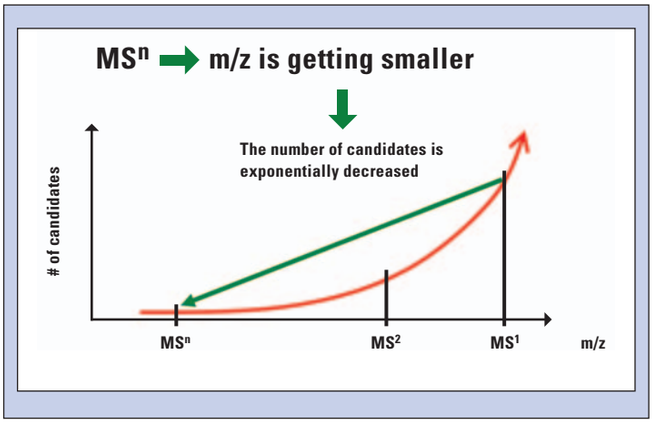


Figure 5: Decrease ratio of possible candidates by smaller mass determination

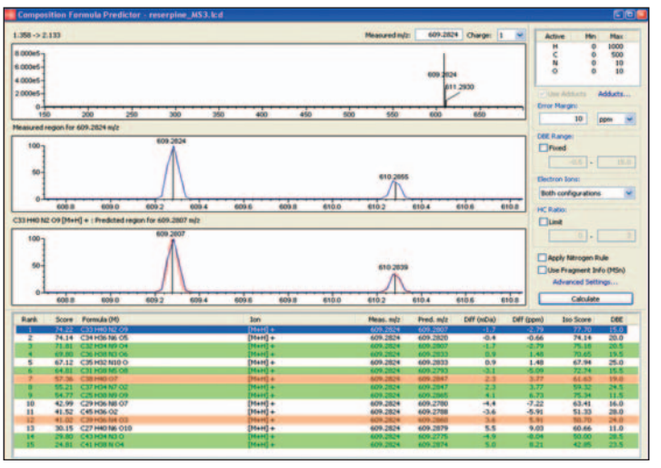


Figure 4: Screen shot "Composition Formula Predictor" software

Blue: highest scored prediction
Green: automatically excluded by nitrogen rule
Orange: automatically excluded by MS/MS information

Acute poisoning with a rat bait

LC-ESI-MS for simultaneous determination of vitamin K antagonists in human

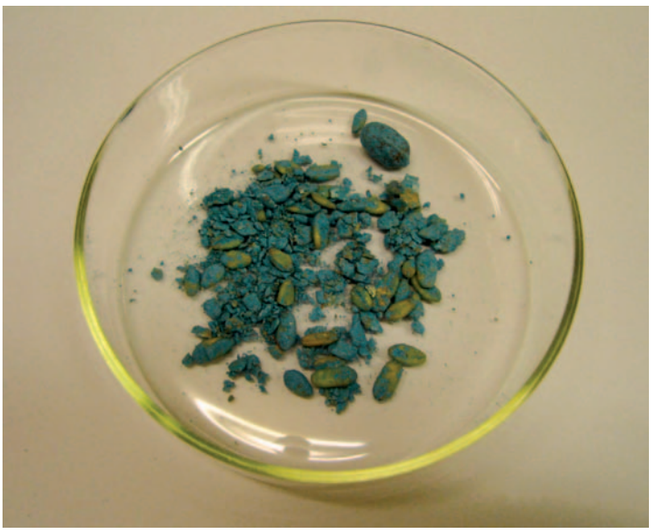


Figure 1: Bait preparation ingested by the patient

Dr. rer. nat. Thomas Grobosch,
Boris Angelow,
PD Dr. med. Dagmar Lampe
Berlin Institution for Central Health Tasks (Berliner Betrieb für Zentrale Gesundheitliche Aufgaben, BBGes), Clinical Toxicology and Pharmacology

Solving acute poisoning cases, as well as therapeutic drug monitoring and drug testing are among the major tasks of the Clinical Toxicology Department of the BBGes. This example describes an LC-MS method for the determination of rat poison intoxications.

A 62-year old man was admitted to the emergency station with symptoms of nausea and vomiting. He admitted to having ingested rat poison bait in a suicide attempt. The patient's medical history indicated an earlier suicide attempt in 2003. No psychiatric treatment had followed. A blood sample of the patient together with a sample of blue rat poison bait (Figure 1) was sent to the BBGes Toxicology Department's laboratory. Analysis using LC-ESI-MS confirmed that poisoning was due to the rat poison's active compound coumatetralyl.

Vitamin K antagonists

4-Hydroxycoumarin, a derivative of the sweet woodruff phytochemical coumarin, and 1.3-indandione belong to a group of indirect anticoagulants (Figure 2). They are also known as vitamin K antagonists as their action is based on a functional disruption of the synthesis of vitamin K-dependent coagulation factors.

Based on the half-life values of the coagulation factors already circulating in the bloodstream, the anti-coagulating effects of the rat poison are evident only after a certain time delay.

Some vitamin K antagonists (acenocoumarol, phenprocoumon, warfarin) are used therapeutically as anticoagulants. The same anticoagulant effects are also being used effectively to combat rodents. Due to increasing resistance of various rat species to these compounds, second-generation anticoagulants – the so-called superwarfarins – have been developed. These are highly effective, even at very low dosages and they have long half-lives associated with long pharmacological action. With increasing commercial distribution of superwarfarins, the number of poisoning cases in humans and animals has also increased. In 2004 the poisoning emergency center in Berlin alone already registered 110 poisoning cases involving anticoagulant ingestion.

Depending on the formulation, the active ingredient concentrations of rat poison baits permitted in Germany are between 0.0025 and 0.79 %. Combination preparations, for instance Racumin® Plus oat flake bait (coumatetralyl and cholecalciferol, Bayer CropScience) or Celaflor® Brumolin® (difethialon and sulfachinoxalin, Scotts Celaflor GmbH & Co.) are also available.

Poisoning cases in humans and animals are indicated by an increased tendency towards bleeding. Death occurs via internal bleeding or haemorrhagic shock. The active compound coumatetralyl has therefore been rated as extremely toxic (T+) in the German Toxic Substance Act. The toxicity data for coumatetralyl and coumatetralyl-containing rat poison baits in various forms of application are summarized in Table 1.

The most important diagnostic parameters are the blood coagulation values (INR [International Normalized Ratio] and the Quick-value). Immediate measures in poisoning cases involving anticoagulants are decontamination and, where necessary, administration of an antidote (vitamin K1). When bleeding is acute and life threatening, the decreased level of coagulation factors must first be replenished.

LC-MS analysis

For the detection and quantification of coumatetralyl, an LC-MS method was used that had been developed specifically for toxicology analyses at the BBGes. This procedure is suitable for simultaneous identification and quantification of 10 indirect anticoagulants in human serum. The method is based on an acidic (pH = 4.2) liquid-liquid extraction using 1-chlorobutane with subsequent detection via LC-ESI-MS. This simple method for the determination of vitamin K antagonists offers broad compound screening and high selectivities with short

Name	LD_{50} Rat (m) oral (mg/kg KG)	LD_{50} Rat (m) transdermal (mg/kg KG)	LD_{50} Rat (m) via inhalation (mg/L)
Coumatetralyl	30.15 (f)	> 100 < 500 (f)	0.05 (dust)
Racumin powder	5000	> 5000	> 3.3
Racumin baits	> 5000	> 5000	– –

Table 1: Toxicity data for coumatetralyl: m = male, f = female

(From "Wirkstoffe in Pflanzenschutz und Schädlingsbekämpfungsmitteln: physikalisch-chemische und toxikologische Daten", 3. Aufl., Industrieverband Agrar e.V. BLV, München, 2000)

s in human serum

analysis times using two masses for identification/quantification. Figure 3 shows the compounds identified using this method.

Based on suspected poisoning with a coumarin derivative, serum samples as well as bait samples were subjected to liquid-liquid extraction and subsequently analyzed according to the LC-MS method described below. In addition, a systematic toxicological analysis (STA) was carried out using HPLC-DAD. The bait samples were first extracted using methanol, the resulting solution was then filtered after centrifugation and 5 µL was injected directly into the LC-MS system (for LC-MS conditions see insert).

For quantification, a 6-point calibration was carried out. The calibration was linear for all calibration points in the range from 10 up to 250 µg/L ($r^2 > 0.995$). The determination limit was 5 µg/L (S/N 10). For the precision the following coefficients of variation were determined based on quality control samples (day-to-day, n = 6, 100 µg/L): acenocoumarol (5.2 %), coumachlor (5.9 %), coumatetralyl (4.8 %), phenprocoumon (6.0 %), warfarin (6.0 %), brodifacoum (14.9 %), bromadiolon (8.2 %), difenacoum (8.4 %), difethialon (11.7 %), flocoumafen (14.2 %).

Results

The selected analytical conditions enable fast elution of the listed compounds within 5 min at a determination limit of 10 µg/L. The experimental conditions (mobile phase, analytical column, ESI interface) conform to the usual LC-MS system configurations at the institute laboratory ⏵

LC-MS conditions

HPLC

Binary pump system with membrane degasser, autosampler, oven, UV detector (Shimadzu)

Mobile phase: A: methanol, B: methanol/0.1 % HCOOH (10/90, v/v) · **Flowrate:** 0.60 mL/min · **Gradient:** 0 – 0.7 min: 95 % B; 0.7 – 1.1 min: 50 % B linear; 1.1 – 3.2 min: 6 % B linear; 3.2 – 3.8 min: 6 % B; 3.8 – 4.2 min: 95 % linear · **Separation column:** Atlantis C18 (2.1 x 20 mm, 3 µm, Waters) · **Oven temperature:** 40 °C · **Gradient for the determination of cholecalciferol (vitamin D3):** 0 – 0.7 min: 20 % B; 0.7 – 3.5 min: 6 % B linear; 3.5 – 3.7 min: 6 % B; 3.7 – 4.1 min: 20 % B linear; 4.1 – 4.2 min: 20 %.

MS

LCMS-2010 system (Shimadzu)

Ion source: ESI positive & negative · **Nebulizer gas:** nitrogen; 4,5 L/min · **Block- and CDL temperature:** 300 °C · **Detector voltage:** 1.9 kV
SIM-masses: acenocoumarol ESI (-): 353 & 352; brodifacoum ESI (-): 523 & 521; bromadiolon ESI (-): 527 & 525; coumachlor ESI (-): 343 & 341; coumatetralyl ESI (-): 292 & 291; difenacoum ESI (-): 445 & 443; difethialon ESI (-) 539 & 537; flocoumafen ESI (-) 543 & 542; phenprocoumon ESI (-): 280 & 279; warfarin ESI (-) 308 & 307, internal standard ESI (+): 432

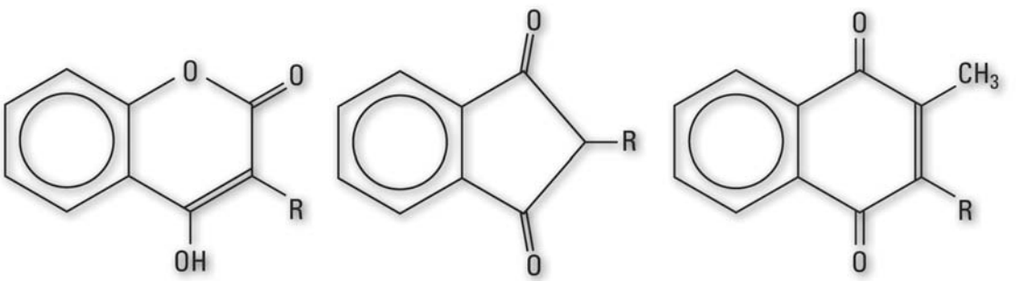


Figure 2: Structural similarity between vitamin K and its antagonists

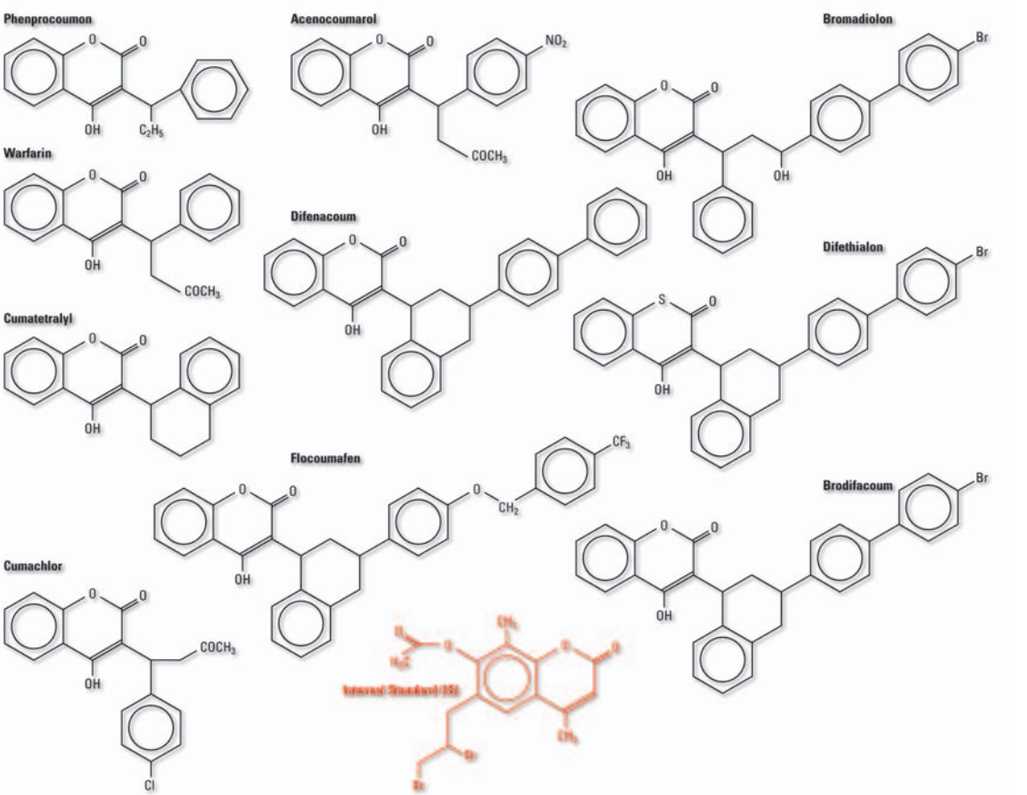


Figure 3: Structures of the vitamin K antagonists and the internal standard

and therefore do not require long reconfiguration times.

Figure 4 shows SIM chromatograms of the 10 established vitamin K antagonists (standard each 100 µg/L). The symmetry of the peaks is acceptable. To safeguard the validity of the analytical result, two masses were used. In marked contrast with the identification after fragmentation in a first run and quantification after a second run, our analytical strategy enabled a simultaneous identification and quantification within one run due to the high sensitivity, whereby a higher sensitivity was obtained for mass [M-1 amu]. Within the scope of method validation, 12 tested blank samples obtained via 6 different sampling methods were free from the above-mentioned vitamin K antagonists.

The upper determination limit of 250 µg/L (3 decades) is not sufficient for the determination of phenprocoumon and warfarin (therapeutic range up to 3000 µg/L). Therefore for the investigation of these compounds, correspondingly less material should be used or the sample should be diluted with coumarin-free blank plasma/serum. The precision of the method is suitable for obtaining acceptable results at low analysis frequency when time is essential.

Based on the suspected coumarin intoxication, the patient sample was investigated using the above-described analytical procedure.

The active compound could be identified unequivocally from the chromatogram (Figure 5) via the retention time (2.0 min) as well as the two masses (m/z 292 and 291). Quantification resulted in a coumarin concentration of 121 µg/L. The control sample (expected value 100 µg/L) showed a coumatetralyl concentration of 94.7 µg/L which was within the required range of 80 – 120 µg/L (± 20 % of the expected value). In addition, the sample underwent screening for basic compounds (HPLC-DAD) whereby no further compounds were identified.

In the bait sample the active compound coumatetralyl was also identified via the retention time and the two characteristic masses. There were no interfering peaks. Both results pointed to a coumatetralyl-containing rodenticide bait. In order to test whether the rodenticide sample was the combination preparation Racumin® Plus oat flake bait (Bayer AG), a methanol extract of the rodenticide sample was analyzed via LC-APCI-MS to detect cholecalciferol. However, no indication on the presence of cholecalciferol was found.

Although a coumatetralyl concentration of 121 µg/L was measured when the patient was admitted, coumatetralyl could no longer be detected in a control sample taken eleven days later (LOQ = 10 µg/L). This is due either to a distinctive distribution behavior or a half-life of less than two to three days.

Summary

This example of a suicide attempt with a coumatetralyl-containing rat poison was presented to demonstrate a sensitive and reliable analytical method for the simultaneous determination of 10 vitamin K antagonists (among them 5 superwarfarins) in human serum/plasma using LC-ESI-MS. The method is based on a simple and fast liquid-liquid extraction with subsequent LC-MS analysis.

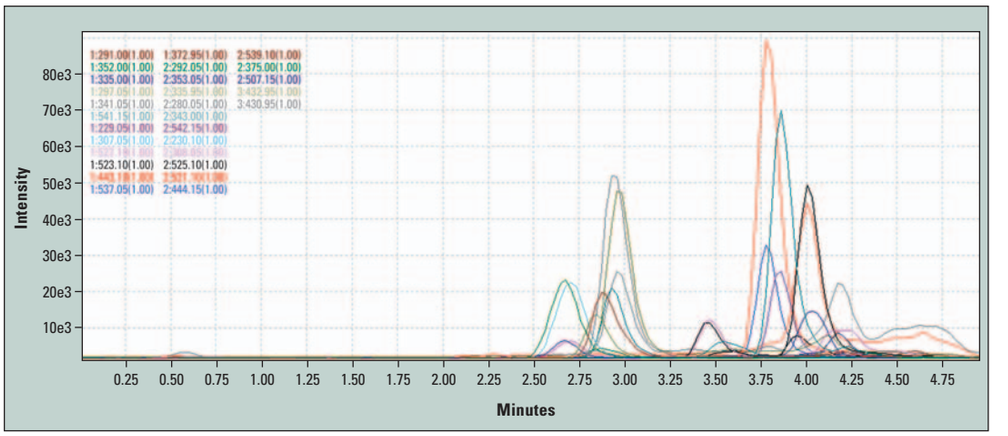


Figure 4: SIM chromatograms of the 10 vitamin K antagonists (each 100 µg/L, ESI negative)

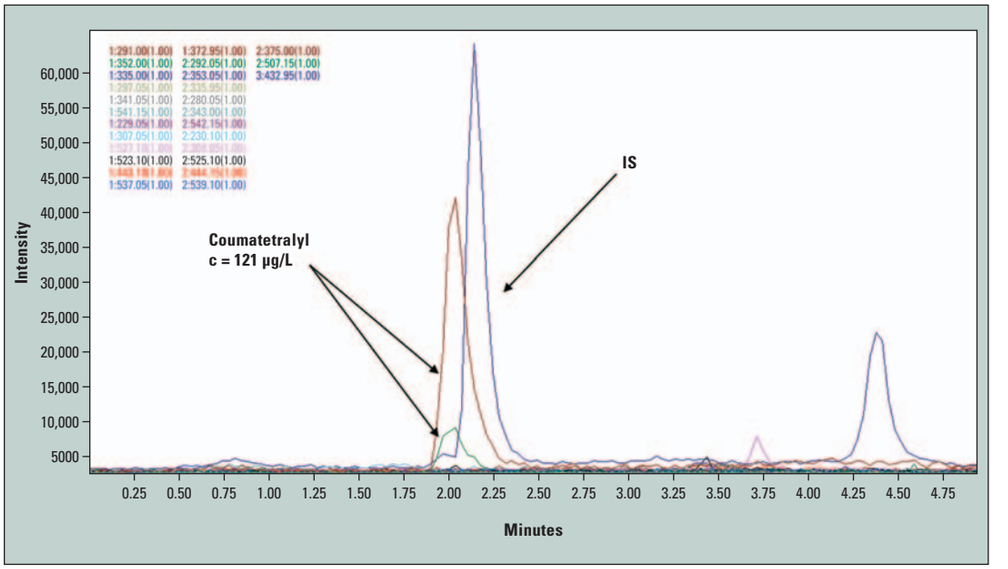


Figure 5: Serum sample of the patient with internal standard (SIM chromatogram)

Straightforward analysis of difficult samples

ICP spectrometer

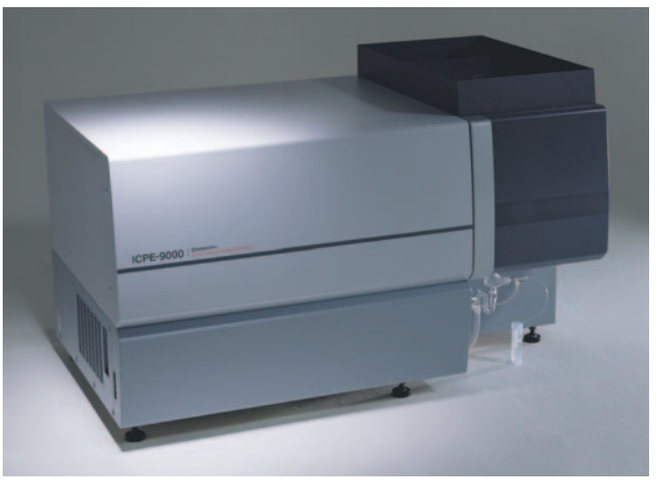


Figure 1: ICPE-9000

Inductively-coupled plasmas (ICP) have been deployed commercially for more than 25 years as excitation sources in optical emission spectrometry. ICP is one of the most important tools for elemental analysis in daily routine testing whenever high sensitivity, a broad dynamic range and high sample throughput is required. With the new simultaneous ICPE-9000 with CCD (charge-coupled device) detector, Shimadzu introduces an ICP spectrometer equipped with a unique optical system which sets new standards with respect to performance and speed (Figure 1).

The new system includes the intelligent ICPEsolution software which easily handles automatic wavelength optimization as well as interference corrections. Even when analyzing difficult samples with complex matrices, users benefit from all new high-performance CCD-ICP technological features. The simple user-interface guarantees reliable and reproducible measurement results independently of user experience.

The new ICPE-9000 operates according to the principle of optical emission spectrometry, where liquid samples vaporize in the plasma torch and the released atoms and ions are excited and subsequently emit radiation. The emitted radiation is then processed by the optical system and measured by the CCD detector (Figure 2), and the emission spectra of all elements present in the sample are displayed. The high sensitivity of the detector enables a highly accurate resolution, even for very closely neighboring wavelengths such as copper (213.60 nm) and phosphorus (213.62 nm). The intensity of the radiation is proportional to the concentration of the elements in the sample.

Quantification of elements such as phosphorus (178 nm), sulfur (180 nm), arsenic (189 nm) and boron (182 nm) occurs in vacuum at excellent stability.

The ICPE-9000 combines the highest quality and measuring accuracy with a very attractive price and minimal operating costs. In this way, for instance, argon consumption is reduced to 12 L/min using the “minitorch” technique.

Broad accessories range

Analysis of many different types of samples is possible with an extensive range of accessories consisting of sample introduction systems such as pneumatic nebulizers, nebulizer chambers and an ultrasonic nebulizer for aqueous and organic solutions. Combination with the ASC-6100 autosampler enables fully automatic multi-element analysis.

Figure 2: Large-scale CCD detector

Fast analysis of hazardous c

RoHS, ELV, ElektroG

Figure 1:
EDX-720, optimal system for RoHS analyses

Since 2003 the WEEE (**W**aste **E**lectrical and **E**lectronic Equipment) and RoHS (**R**estriction **o**f the use of certain **H**azardous **S**ubstances in electrical and electronic equipment) directives have been in force and in August 2004 they were integrated into national German legislature. Based on RoHS and WEEE, the German federal government enacted the ElektroG directive on 23 March 2004. This directive regulates the return of electrical and electronic equipment and its reuse or recycling, as well as enforcing the ban on the use of hazardous substances in these instruments. In this way, threshold values which can be determined using X-ray fluorescence in a fast, non-destructive screening method, have been established for the following substances:

Lead: Solder in printed circuits and their coatings, electro-ceramics, glasses

Cadmium: NiCd batteries, synthetic materials, electric arc contacts, sensors, galvanizings

Chromium (VI): Metal coatings, undercoats for metal coatings, chrome plating, metallic synthetic surfaces

Mercury: Batteries, fluorescent lamps, switches, sensors, relays

PBB and PBDE: Brominated flame-retardants in synthetic materials

In order to identify these banned substances, Shimadzu has developed an improved EDX system. The EDX-720 features improved detection sensitivity for lead (Pb) and cadmium (Cd), the most significant elements in environmental analysis. In addition, the EDX-720 excels with new attractive functionalities such as the "Auto Selection" function for selection of a suitable calibration curve – for instance for PE or PVC – or the "Auto-reduced" measuring function optimizing the measurement time.

EDX-720 highlights

- Sensitivity to the hazardous substances lead and cadmium was improved by a factor of two.
- The "Measurement Time Reduction" function adapts the measurement time to the precision requirements of the user.
- The "Switching Calibration Curves" function automatically selects a suitable calibration curve.
- Large sample compartment, 300 mm diameter x 150 mm height.
- The EDX software features a multitude of fundamental parameter (FP) methods, enabling quantification of practically any type of sample without standards.

Far-reaching application areas

The EDX-720 is a very versatile instrument and is suitable for a wide range of elemental analysis applications. Instrument and software are easy to operate and very little sample preparation ensures fast analysis results.

- **Electronics/automotive materials:** analysis of toxic substances in electrical and electronic components according to RoHS, ELV und ElektroG
- **Chemical industry:** analysis of catalyzer materials, pigments and dyes
- **Petrochemicals:** analysis of nickel, vanadium and sulfur in oils
- **Ceramics industry:** analysis of ceramics, cements and glasses
- **Medicine/agriculture/foods:** analysis of catalyst residues or contaminations
- **Coating technology:** Analysis of coating- and layer thicknesses and their composition

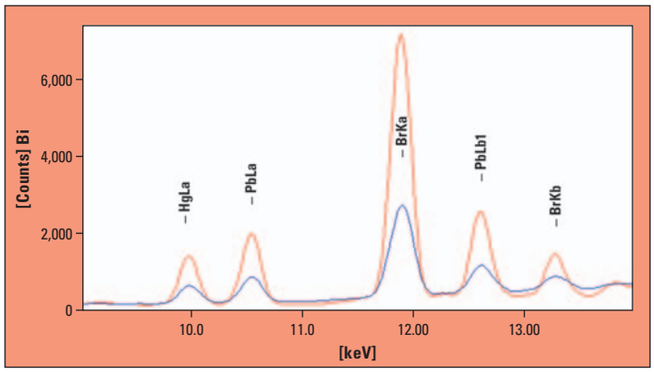


Figure 2: Comparison of two spectra with PVC standards containing 54 ppm Pb. Measurement time is 300 seconds. Blue spectrum: EDX-700HS; Red spectrum: EDX-720.

ou s compounds

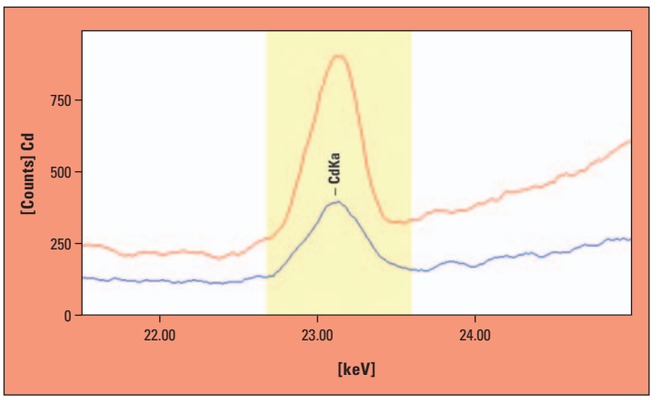


Figure 3: Comparison of two spectra with PVC standards containing 78 ppm Cd. Measurement time is 300 seconds. Blue spectrum: EDX-700HS; Red spectrum: EDX-720.

- **Iron and steel industry:** analysis of alloys, solder and unknown metal samples
- **Environmental analysis:** analysis of soil samples, wastes, wastewater, cinders and ashes
- **Science:** geology and archaeology.

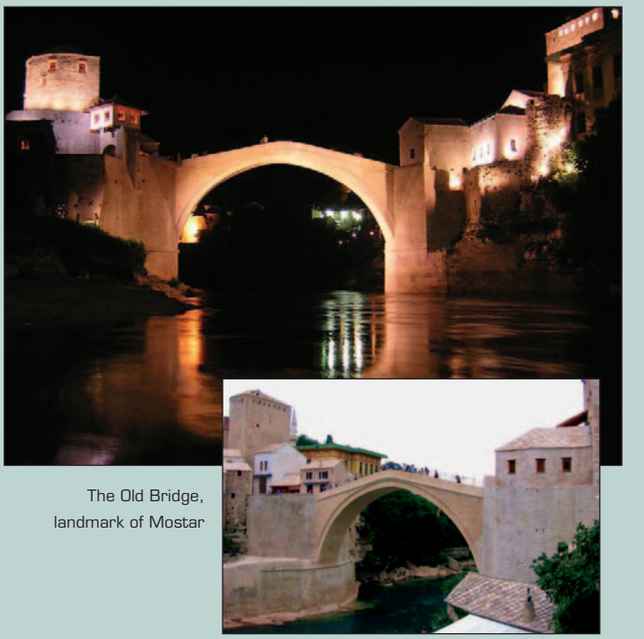
New filters and improved counting rates increase sensitivity by factor of two

- High counting rates increase intensity and improve resolution.
- The new filter types reduce the background signal without appreciably decreasing peak intensities.

In addition to these two new attractive features, the "Measurement Time Reduction" and the "Switching Calibration Curves" functions further contribute to the instrument's success story. The "Measurement Time Reduction" function automatically controls measurement times according to the precision requirements of the user. This is, for instance, very useful for mixed samples as the measurement time can be greatly reduced this way.

The automatic "Switching Calibration Curves" function selects a suitable calibration for unknown samples. When both calibration curves (for example PE and PVC) are stored, a calibration curve suitable for the synthetic polymer is selected based on the chlorine signal. In this way, polymer measurements based on incorrect calibration curves are a thing of the past.

We will gladly send you further information. Please note the appropriate number on your reply card. Info **308**



The Old Bridge, landmark of Mostar

Water quality protection for Bosnia and Herzegovina

AAS systems for European project

Each year during the summer season, many institutions and organizations are busy testing water quality at European beaches. For exemplary water conditions the so-called Blue Flag is awarded, reflecting excellence in environmental protection, water and bathing area quality. Since 1987, the number of participating countries has increased steadily.

This development mirrors a decrease in the presence of hazardous compounds in seawater along European shorelines, due mainly to a general decrease in heavy metal and other hazardous compound pollution levels in European rivers.

Water quality and prevention of water pollution in Europe is subjected to continuous monitoring in order to readily identify any source of water pollution. Shimadzu's analytical systems are often called to the task.

In the course of a European project for water quality monitoring in Bosnia and Herzegovina, Shimadzu equipped four institutes in Mostar, Bijeljina, Trebinje and Sarajevo with atomic absorption spectrometers. The system configurations for drinking water analysis consisted of the fully automatic AA-6300, the GFA-EX7i graphite furnace with digital control, and the ASC-6100 sample preparation station in combination with the ASK-6100 dilution unit.

In order to optimize routine operation of these analytical systems, the Shimadzu branch in Zagreb organized an AAS user training session at the participating institutes in Bosnia and Herzegovina. The three-day seminar took place in September 2005 close to the historical city center with its famous bridge across the Neretva River.

System Linear Performance

prominence HPLC series

Figure 1: HPLC system LC-20A *prominence*

The *prominence* HPLC system, designed for superior performance is setting new limits in terms of performance and reliability (Figure 1). One of these parameters is system linearity resulting from detector and injector linearity. Due to the high quality optical design and a special electronic compensation technique the *prominence* detectors SPD-20A/AV and SPD-M20A provide the best ASTM linearity specification of all current HPLC detectors.

By utilizing a high resolution metering pump for sample aspiration the *prominence* SIL-20A/AC autosamplers provide the highest degree of linearity and precision over the entire working range. Additional parameters such as sample aspiration speed and particular rinse modes support these features even with critical samples and solvents.

The chromatogram overlays in Figure 2 show precision and linearity of the entire *prominence* system. To demonstrate the system linearity a typical Low Pressure Gradient System with the following components has been used:

Vacuum Degassing Unit: DGU-20A5
Solvent Delivery Pump: LC-20AT with LPGE Valve Unit
Autosampler: SIL-20AC
Column Oven: CTO-20A
Detectors: SPD-20A (UV-VIS); SPD-M20A (PDA)
Column: VP-ODS 4.6 mm x 150 mm.

An injection sequence using a range of injection volumes on a 50 ppm caffeine standard was created so that the following three calibration curves (Figures 3 to 5) could be generated.

The calibration curves show that a standard *prominence* system is capable of providing excellent linearity within the entire working range. For all calibration curves a Linear Regression Coefficient (r^2) of better than 0.999 could be achieved. In addition the precision (% RSD) of the Response Factors was below 1 %. The working range of the UV/ PDA detectors with an ASTM linearity up to 2.5 AU is over 30 % higher than conventional HPLC systems. In combination with the excellent precision of the SIL-20A/AC autosampler, which could be achieved even at lowest injection volumes, the system is defining new limits of performance.

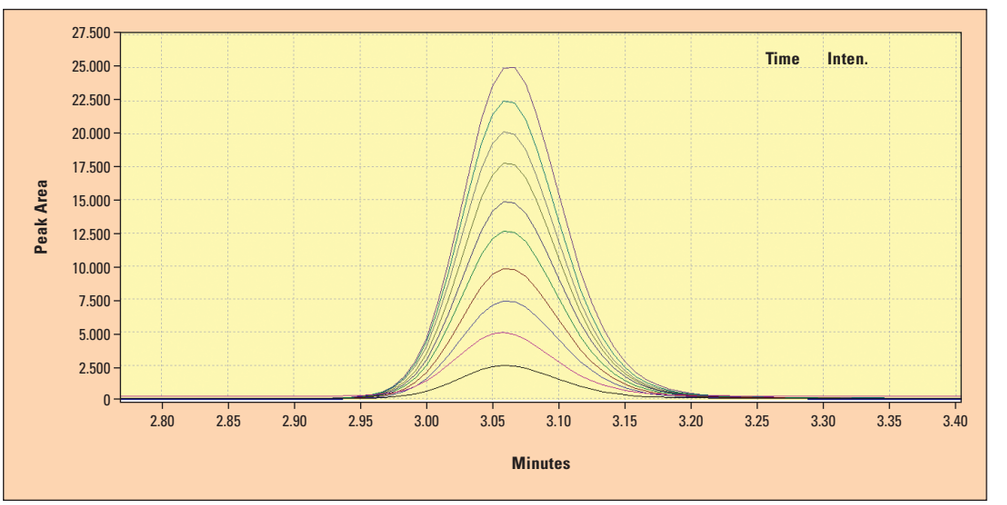


Figure 2: Chromatogram Overlay

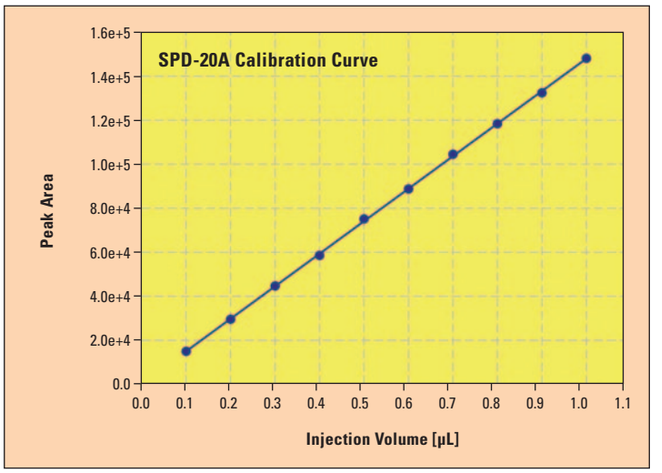


Figure 3a: Results: r^2 = 0.999830 · RF % RSD = 0.893

Injection volume from 0.1 µL – 1 µL (10 calibration points in steps of 0.1 µL)

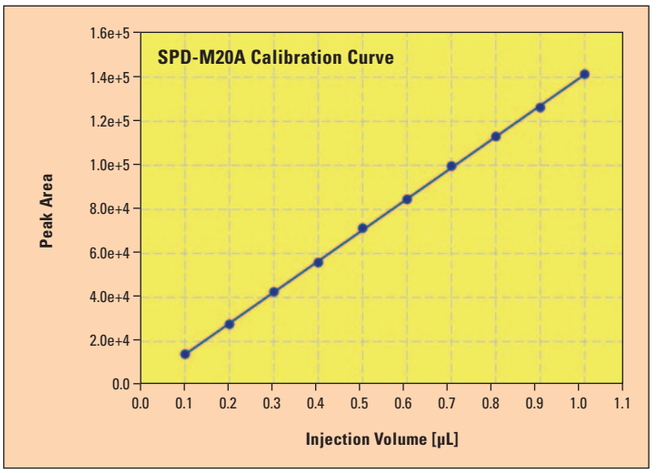


Figure 3b: r^2 = 0.999825 · RF % RSD = 0.795

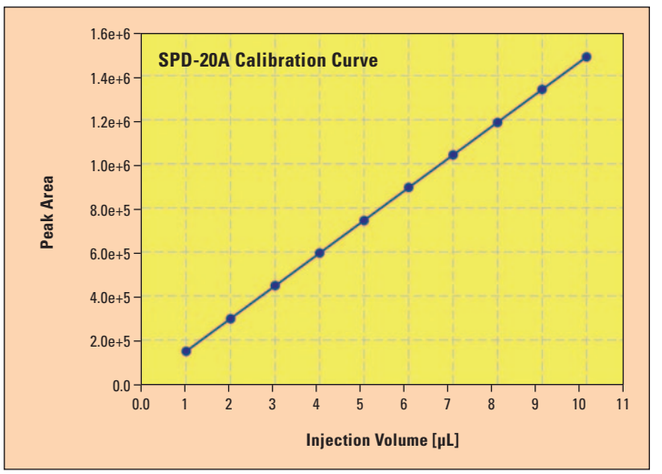


Figure 4a: Results: r^2 = 0.999997 · RF % RSD = 0.143

Injection volume from 1 µL – 10 µL (10 calibration points in steps of 1 µL)

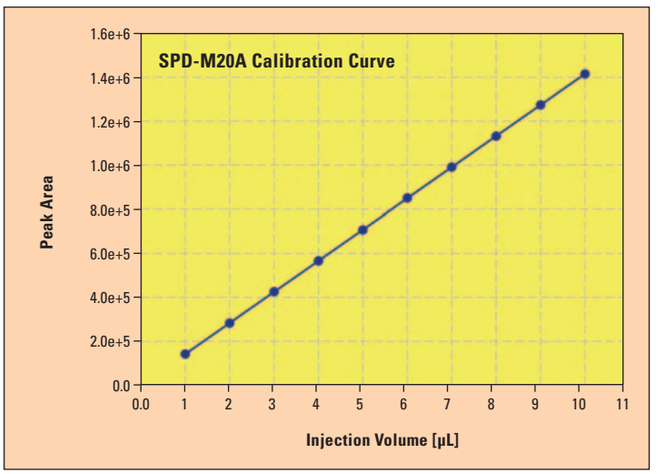


Figure 4b: r^2 = 0.999997 · RF % RSD = 0.178

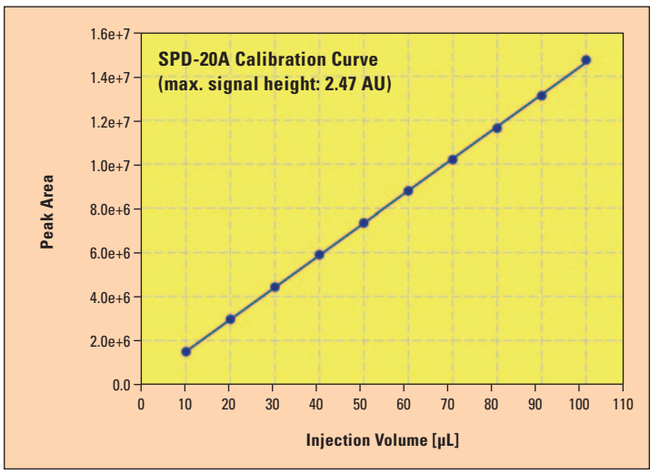


Figure 5a: Results: r^2 = 0.999230 · RF % RSD = 0.951

Injection volume from 10 µL – 100 µL (10 calibration points in steps of 10 µL)

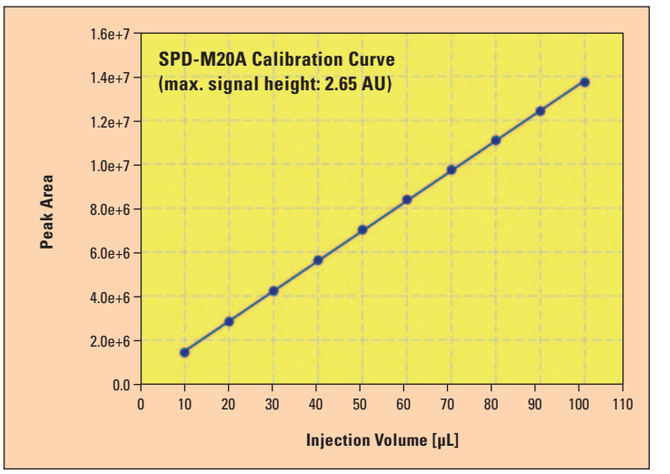


Figure 5b: r^2 = 0.999859 · RF % RSD = 0.770

Perfect symbiosis of hard wa

„panorama Fluorescence“ software

In a joint project between the LabCognition software company in Dortmund, Germany and Shimadzu, the new “panorama Fluorescence” software has been developed. Close cooperation between both companies resulted in the integration of instrument control software for the Shimadzu RF-5301PC Spectrofluorophotometer into LabCognition’s standard “Panorama” software. In addition to general instrument control, the “panorama Fluorescence” software features a number of functions tailor-made to the requirements of fluorescence spectroscopy. Based on the new software, state-of-the-art user control and a well-established fluorescence spectrophotometer seamlessly merge into a reliable and ergonomic laboratory tool.

The “panorama Fluorescence” is a modern software product that meets all requirements of the Windows PC world and is specially adapted to comply with the demands of quality guidelines. GLP/GMP requirements are supported via a multitude of integrated control functions. The software is especially suited for:

- analytical chemistry, biochemistry
- pharmaceutical industry (drug discovery and life sciences)
- foods and agriculture
- research and development.

In addition, the software platform features an object-oriented user-interface. The user workspace always displays the 2D or 3D measurement data requiring further evaluation – either in the form of spectra, time curves or combinations of both. At any time, complete data information and measurement parameters are displayed together with the active spectrum.

Data conversion

The “panorama Fluorescence” software supports many data formats, enabling import of data in older formats, for instance HyperRF- and Shimadzu RFPC formats as well as the ASCII and JCAMP-DX formats into the software. Data formats can be imported and also exported into the corresponding formats.

Data display – an accomplishment by itself

Data display is especially worth emphasizing. Depending on the data set, context-sensitive switching from 2D- to 3D-views is possible, whereby all support and menu functions are shown simultaneously. In this way, it is possible to access the special software features and functions without the need for searching.

Other important display features are the simple intuitive zoom function and the speed control for free rotation of 3D graphics. Using “drag-and-drop” operations, multiple spectra can be overlaid for direct comparison. For support of these operations, an online handbook with context cross-referencing is available. The data can be integrated and printed in professional reports using pre-

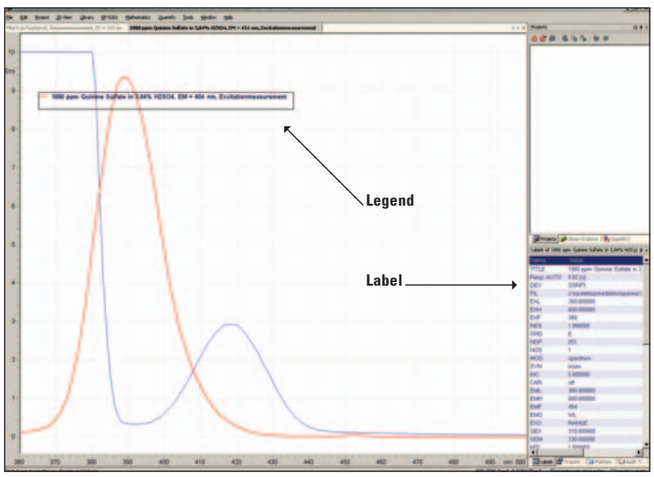


Figure 1: Spectral view in the “panorama Fluorescence” software

rd ware and software

defined templates, which can be adapted individually. When necessary, data or spectra can be copied into other Office applications via the clipboard.

Data handling – project data folders

In general, data can be processed in two different ways. A spectrum can be stored as a standard data set together with its individual information or as project data folders. Here, one or more spectra as well as additional information can be combined. Project data sets allow other formats such as *.pdf, *.doc etc. to be organized in one folder.

Data history – audit trail

Every time data is acquired, entered, processed or deleted in a project, these actions are linked to a CFR 21 Part 11 conform audit trail. This linkage is established for individual users to assure that the spectra will show their own history, which can be visualized in the "Audit Trail" window.

Data manipulation

A large selection of mathematical functions is available to aid spectral processing. All manipulations can be carried out with only a few mouse clicks. Via the UNDO-/REDO function, it is possible to undo or to redo each manipulation.

Below is a small selection of the software functions:
- absorption/transmission
- smoothing functions
- normalization
- arithmetic functions related to individual spectra
- arithmetic functions related to multiple spectra
- peak analysis
- Wizard for univariate calibration.

Spectral library searches and multivariate data analysis are optional.

Figure 1 shows two spectra in one view with the legend for the red spectrum displayed. The information (label) for the active spectrum is displayed to the right, next to the spectrum. The examples shown are: 1000 ppm quinine sulfate in a 3.84 % sulfuric acid solution and an emission spectrum of p-terphenyl in a PMMA polymer matrix.

An enlargement of Figure 1 shows that additional functionality is easily displayed in the task bar. Not only the function "Labels" but also the functions "Properties", "Mathematical Functions" or "Audit Trail" are present (Figure 2).

Data acquisition

The RF-5301PC instrument module has been integrated into the "panorama Fluorescence" software. This high-performance module enables emission- and excitation spectra measurement as well as time-dependent recording in time curves. For both application areas (spectra, kinetics) 2D- and 3D-views can be generated. Time-dependent acquisition of spectra is possible. Using the "Increment" measurement function in the excitation and emission mode, analytical parameters

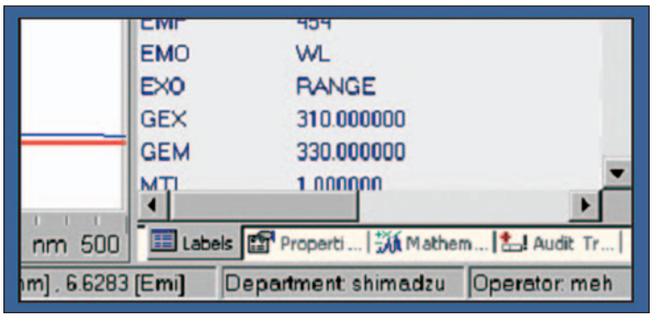


Figure 2: Enlargement of Figure 1, selection of functions for the identification of data, labels, audit trail, etc.

can be obtained for the identification of compounds.

See also Shimadzu NEWS 3/2005, pages 6–8.

We will gladly send you further information. Please note the appropriate number on your reply card. Info **309**

GPC option for data evaluation

LCsolution software

Figure 1: Main menu of the LCsolution software with GPC option

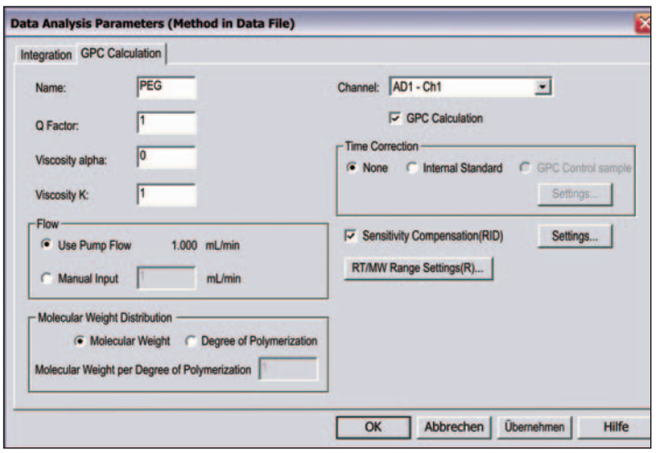


Figure 2: Additional parameters for GPC calculations

Gel permeation chromatography (GPC), more often called size exclusion chromatography (SEC), is a widely applied method for molecular mass determination of natural or synthetic polymers. During the separation process a complete molecular mass distribution of the sample components is obtained. In addition, GPC provides more than just simple numerical values on samples, enabling further characterization of a polymer sample.

However, for these types of calculations such as example molecular mass distribution of polymers, the standard chromatography software is often not sufficient. GPC therefore requires special software which can be quite comprehensive and complex, meaning that users are often faced with different menu navigation options, different labels and sometimes complex and time-consuming data conversions.

Starting with LCsolution software version 1.21, Shimadzu now offers a GPC software package for data evaluation. All program functionalities are already imbedded in the LCsolution standard version. An additional license option will then activate an extra menu button (Figure 1).

Overview of individual features

Data acquired by the HPLC system can be processed without additional conversion steps in the "GPC Postrun" option. GPC specific parameters can be specified for further data evaluation and the data files can be recalculated accordingly. In this way, all detector signals applied in HPLC (2D as well as up to 4 wavelengths [chromatograms] of a 3D-PDA data file) can be used. The GPC method is then generated based on an LC method and/or supplemented with GPC parameters (Figure 2).

For the calibration curve a maximum of 64 points can be used. Several options are available for the calculation. Depending on the characteristics and behavior of the polymer under investigation, point-by-point, linear or higher-order polynomial regression methods are possible.

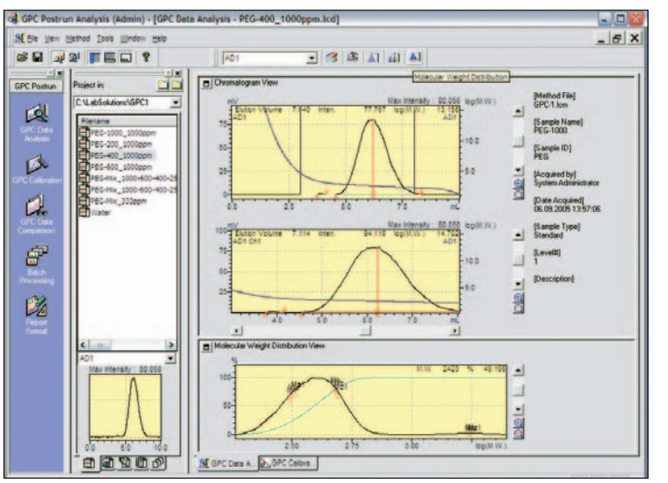


Figure 4: Reanalysis view of a data file in the "GPC Postrun" window, using the parameter in the GPC method

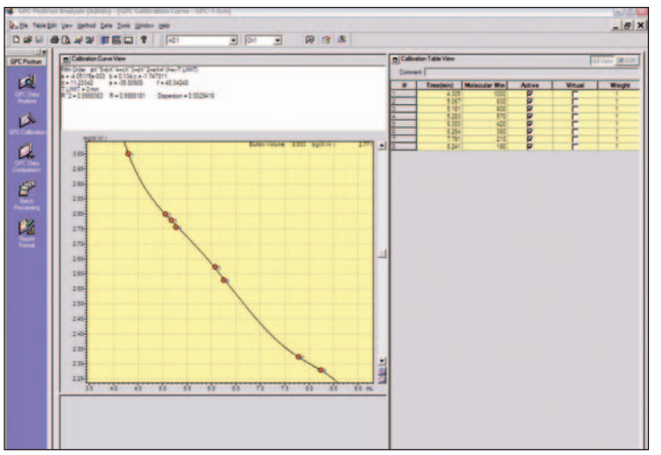


Figure 3: Calibration curve and corresponding line equation, overview of the data files used and table view (molecular mass and assigned retention times)

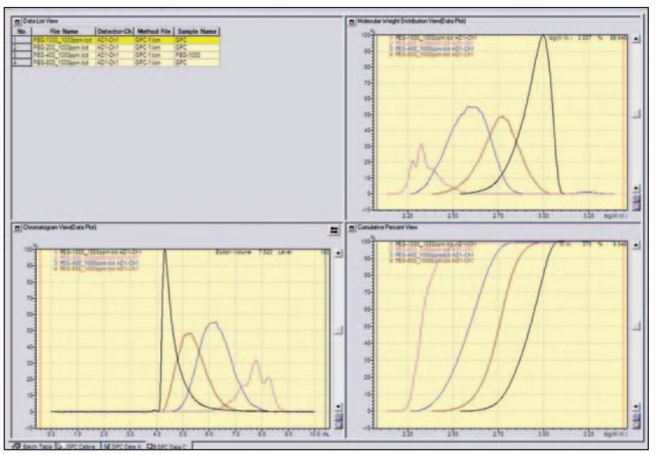


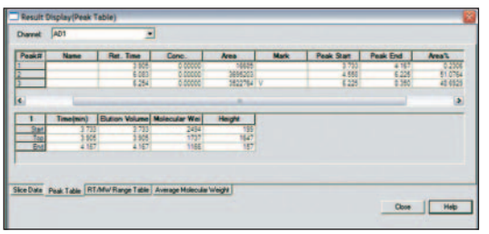
Abbildung 7: Data comparison in the window "Data comparison"

After the GPC method with the corresponding information and calibration curve has been established (Figure 3), the sample table can either be recalculated via the "Batch Reprocessing" function or the data files can be opened individually for recalculation. In addition to the chromatogram, the cumulated molecular mass distribution is displayed instantly (Figure 4). After calculation using the adapted method settings, peak tables (Figure 5) and slice data (Figure 6) are available in report form. These can also be displayed in an additional window in the "Postrun" menu.

For a fast overview of all GPC-relevant information, the "Data Comparison" window is recommended. Up to 10 chromatograms can be opened and the molecular mass distribution as well as the cumulated view can be displayed (Figure 7).

The software supplies standard layouts for the reports. These can be edited within the confines of the LCsolution software. Figure 8 shows a printout of a standard report.

All functions of the LCsolution software as well as database storage and compliance with FDA 21 Part 11 are also available for this module.



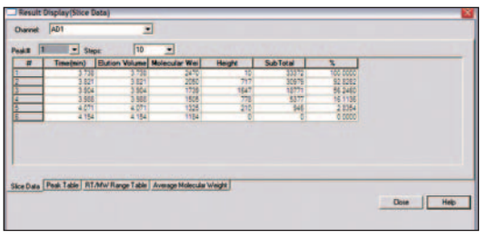


Figure 5 (top): Peak table · Figure 6 (bottom): Slice data

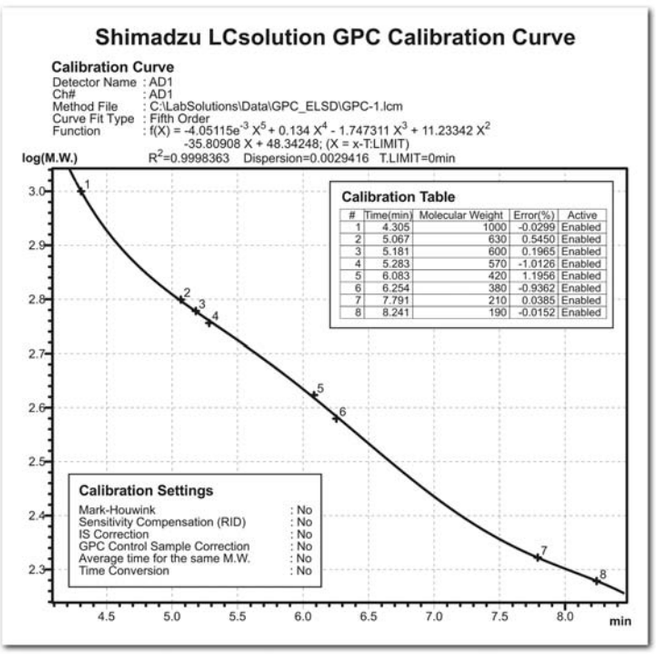


Figure 8: Printed report