The aroma of French fries – fuel from rapeseed oil

ICP spectrometer

Step-by-step within the last decade, biodiesel has established its share of the European fuel market. In Germany, almost 2000 filling stations throughout the country offer biodiesel at their pumps. Biodiesel is considered as an environmentally friendly and attractively priced alternative to conventional diesel fuel – both now and in the future. Biodiesel is one of the most important energy sources originating from renewable raw materials such as rapeseed.

Biodiesel is a mature fuel with a strictly specified qualitative minimum standard as described in the European standard DIN EN 14214. The major producers and distributors of biodiesel have joined forces in the "Arbeitsgemeinschaft Qualitätsmanagement Biodiesel e. V." (AGQM – Working Group for Quality Management of Biodiesel). AGQM has set up a controlled quality management system (QM) ensuring a high and long-term unvarying fuel quality. This describes not only the selection of raw material and production processes but also storage and transfer as well as transport of biodiesel.

Simultaneous ICPE-9000 spectrometer

Quality control according to DIN EN 14214 requires quantitative determination of the elements sodium, potassium, calcium, magnesium, phosphorus and sulfur in the concentration range of 5 up to 10 mg/kg. ICP spectrometers, presently considered as the most important tool in daily routine elemental analysis, are highly suited to carrying out of this task, especially when high sensitivity, a wide dynamic range and high sample throughput are called for.

Using the new simultaneous ICPE-9000 (see Figure) with CCD (Charge-Coupled Device) detector, Shimadzu introduces an ICP spectrometer equipped with a unique optical system setting new standards with respect to performance and speed. The system is highly flexible and is therefore easily adapted to all types of sample material. In the present case the sample is biodiesel diluted with kerosene.

Detailed information is available in the ICP 1 application note, which can be ordered from Shimadzu.

We will gladly send you further information. Please note the appropriate number on your reply card. Info **315**

INTERNAL

Zagreb, Croatia – Presentation for business and academic communities

In November 2005, the Shimadzu Branch Office in Zagreb, Croatia organized a half-day presentation of Shimadzu's product range of analytical instruments. The presentation took place in Pliva Research Institute, with more than 120 people attending. Zagreb is the hub of the business and academic worlds in Croatia. It is also the home of the university and many reknowned scientists work in the city.

The food and chemical industries are among the most dominant players of the Croatian economy. More than half of all the exports are targeted at European Union countries. Since 2004, Croatia has been a candidate for future membership.

The presentation was held in two parts. Speakers from Shimadzu Zagreb as well as Shimadzu Germany gave a detailed overview on technologies and applications in chromatography, spectroscopy, sum parameters and balances. Afterwards lunch was a good opportunity for the business and academic community for discussion and making business contacts.

Participants in Zagreb

SHIMADZU

NEWS

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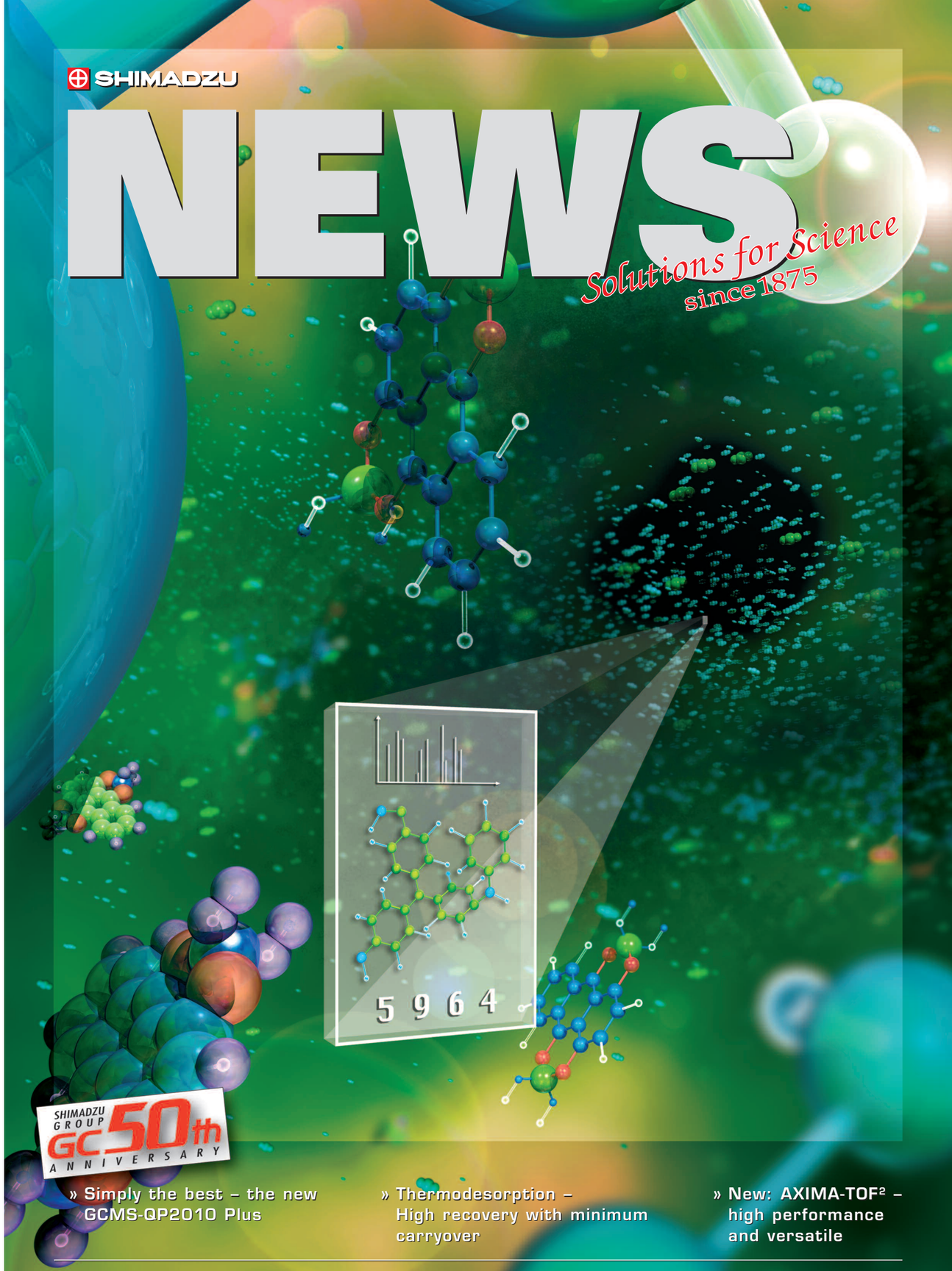

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Sensitive nose provides more Safety in the Workplace

Monitoring Chemical Hazards with GC

Figure 1: GC-2014

The use or generation of hazardous substances is often unavoidable in chemical manufacturing. Nowadays, although it is possible to work in a safe and controlled manner with highly toxic substances, there is always a risk of leaks at critical locations and the possibility that human beings will be exposed to these substances. To provide more safety in the workplace, reliable monitoring of chemical hazards is a must.

Solvias Chemical Hazards Monitors are an integral part of the safety concept used by numerous leading corporations. The monitors record exposures from the ppm to the ppt range and trigger alarms when limits are exceeded.

Solvias Chemical Hazards Monitors are used in refineries, the petrochemical industry, polymer chemicals and basic chemicals as well as fine chemicals, agricultural chemicals or in pharmaceutical production.

Solvias developed SAM GC-600, a Chemical Hazards Monitor on the basis of Shimadzu's GC-14A in 1988. It was developed specifically for continuous chemical hazards monitoring and is used where highly toxic or carcinogenic substances may be present in plant air or in the workplace. Based on its high level of selectivity and sensitivity, as well as its low detection and alarm limits, the SAM GC-600 is a reliable, robust monitor for hazardous substance exposures in the ppm to ppt range. It not only offers the needed reliability, but can also help you to continuously improve production processes.

After Shimadzu's launch of GC-2014 in 2005 this new analyzer technology was chosen as the basis to continue the success story of SAM GC-600 in Chemical Hazards Monitoring.

Operating principle

Figure 2 shows the operating principle:

The heart of the SAM GC-600 Chemical Hazards Monitor is a gas chromatograph equipped with an accumulation module (A). The air to be analyzed is drawn in continuously at up to 15 sampling points by means of two pumps (P) and – depending on the measuring path selected – passes through a multiposition valve (V) (see Figure 3) and the adsorbent-containing accumulation module. Here, all substances not normally found in air are first adsorbed, then thermally desorbed, separated from each other chromatographically and then detected (D).

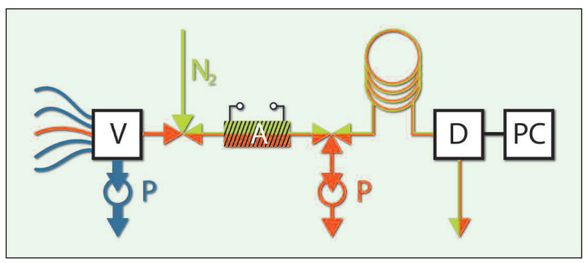

Figure 2: Operating principle

Finally, the software (PC) calculates the substance concentrations from the chromatograms and manages all functions of the SAM GC-600. This controller is based on a PC, running under the Windows® operating system. The software, which was especially developed for this unit, allows all measurement results to be managed, archived, and evaluated statistically, and it also allows the alarm thresholds to be set. It provides high system functionality together with robust operation and can be individually configured. In addition, digital and analog outputs enable communication with process control systems as well as remote maintenance. For enhancing selectivity standard FID may be exchanged with ECD, FPD or PID.

Advantages of Chemical Hazards Monitoring

- Early alarm triggering signalling exposure to hazardous substances – warning and display already well below existing limits thanks to adjustable alarm thresholds
- Highly selective and interference-free measurement – no false alarms and no consequent interruptions in operations
- Individual customized method development in the laboratory plus implementation and optimization on site – development of special solutions for reliable chemical hazards monitoring*
- Wide bandwidth – almost all substances with a boiling point < 250 °C or a vapor pressure > 0.000001 mbar can be analyzed

*To date, analysis methods have been developed for more than 50 substances (see extract of the substance list)

Applications

Dimethyl sulfate and diethyl sulfate

Despite their toxicity, these two substances are widely used as alkylation agents. However, they can be detected reliably at values well below the existing limit values using the SAM GC-600 Chemical Hazards Monitor. The low detection limit (< 0.1 ppb) enables an early response before any serious threat to human health occurs.

Bis(chloromethyl)ether (BCME)

This highly toxic substance is formed spontaneously when formaldehyde is used in the presence of hydrogen chloride (chloromethylation reactions). BCME's workplace limit of just 1 ppb indicates how dangerous it is. Thanks to the ability to reliably detect concentrations as low as 10 ppt, the SAM GC-600 keeps people and companies on the safe side.

Detectable substances		
Substance	**Exposure limit [ppm]**	**Detection limit [ppm]**
Acrylaldehyde	0.1	0.001
Acrylonitrile	3.0	0.03
Benzene	2.5	0.01
Bis(chloromethyl)ether BCME	0.001	0.00001
2-Chloro-1.3-butadiene	5.0	0.05
2-Chloroethanol	1.0	0.01
1-Chloro-4-nitrobenzene	0.075	0.001
1.2-Dibromoethane	0.1	0.001
1.3-Dichlorobenzene	3.0	0.05
1.4-Dichlorobut-2-ene	0.01	0.0002
Diethyl sulfate	0.03	0.0001
Dimethyl sulfate	0.02	0.0001
Epichlorohydrin	2.0	0.01
Ethylenoxide	1.0	0.01
Methyliodide	0.3	0.003
Nitrobenzene	1.0	0.01
Propylenoxide	2.5	0.01
Styrene oxide	1.0	0.02
1.1.2.2-Tetrabromoethane	1.0	0.01
1.1.2.2-Tetrachloroethane	1.0	0.01
Carbontetrachloride	10.0	0.1
Tributyltin chloride	0.002	0.00005
Trichloromethane	10.0	0.1

Table 1: The list shows a selection of chemical hazards and is expandable on request. All values of exposure limits are based on German and Swiss guidelines (MAK, TRGS).

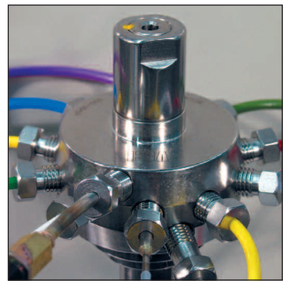

Figure 3: Multiposition valve

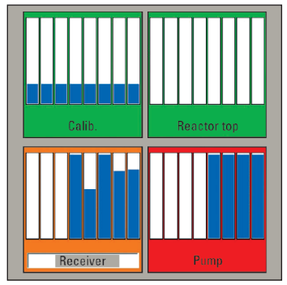

Figure 4: Alarm status

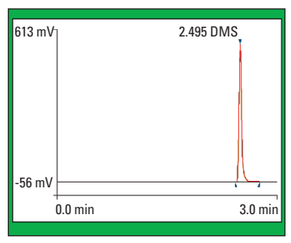

Figure 5: Chromatogram dimethyl sulfate (40 ppb)

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Advanced method develo pment, overnight

LC-20A *prominence*

Figure 1: Shimadzu's LC-20A *prominence* method development system

Figure 2: CTO-20AC column oven with 5-fold column switching

A large part of resources available to research and development in HPLC – hardware as well as working hours – are spent on method development. Many HPLC parameters need to be optimized in order to attain the best possible results: the selection of solvents and concentration gradients, the type and dimensions of the separation column as well as the temperature are among the most important HPLC parameters.

Shimadzu's HPLC series LC-20A *prominence* offers the possibility of setting up a highly automated yet very compact system for in-house method development (Figure 1).

At the core of this method development system is the CTO-20AC column oven that can accommodate two built-in FCV-14 high-pressure valves without the need for an additional control unit. In this way, column switching is possible between a selection of six different columns (or five columns and a bypass [Figure 2]). In addition, the column oven can be thermostatted at temperatures ranging from 10 °C below ambient temperatures up to + 85 °C.

By using one or more LC-20A pumps, low-pressure gradients or accurate high-pressure gradients can be applied with numerous solvents. Depending on the nature of analytes present in the sample, a number of suitable detectors are available – either highly sensitive UV-VIS and diode-array detectors (such as the SPD-20AV and the SPD-M20A) or universal detectors such as the RID-10A refractive index detector, the ELSD-LT light scattering detector and the LCMS-2010EV single quadrupole mass spectrometer.

Using these systems, it is possible to carry out a multitude of measuring parameter variations – including different types of columns – for overnight method development. The chromatograms generated in this way can then be evaluated on the following day.

As a first step during method development it is, for instance, possible to determine which column material is suitable for the separation of the sample components. Figure 3 shows the separation of a mixture of eight sulfonamides under the same separation conditions on five different Pathfinder® phases. Carrying out further optimization steps then leads to the development of a method that is not only fast but also robust. Increasing the temperature, the flow rate or using shorter columns can further reduce the analysis time. This, of course, while maintaining the resolution of the sample components.

Figure 4 shows the result of the optimized separation method for the eight sulfonamides. Compared to the original chromatogram, this chromatogram was obtained after running more than several dozen optimization steps, mainly overnight.

In this way, after finding a suitable column packing material during the test run shown in Figure 3, an optimum combination of column length and particle size was determined for the separation and several gradients were tested. As a result, a method was obtained for the separation of eight sulfonamides using a regular HPLC system in about 100 seconds.

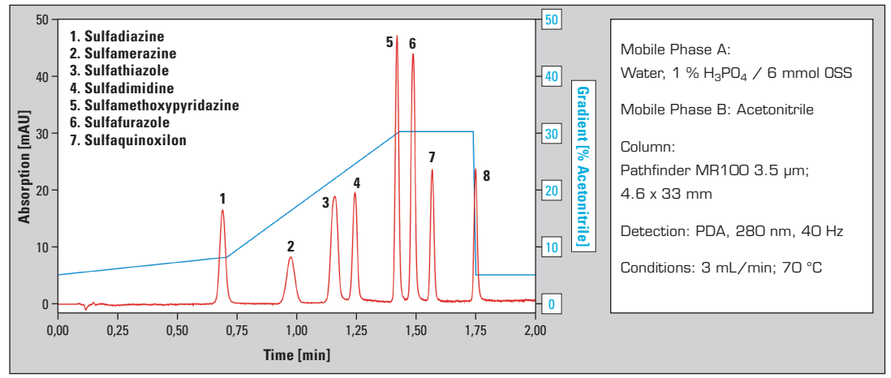

Figure 4: Separation of 8 sulfonamides in 100 seconds

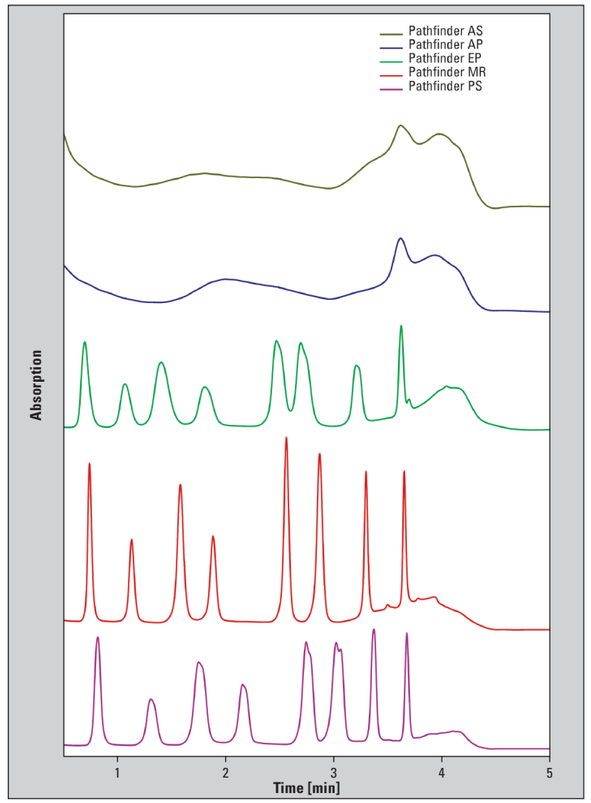

Figure 3: Test run for the determination of the optimum column materials

Lead in lead-free solder – E DX-720

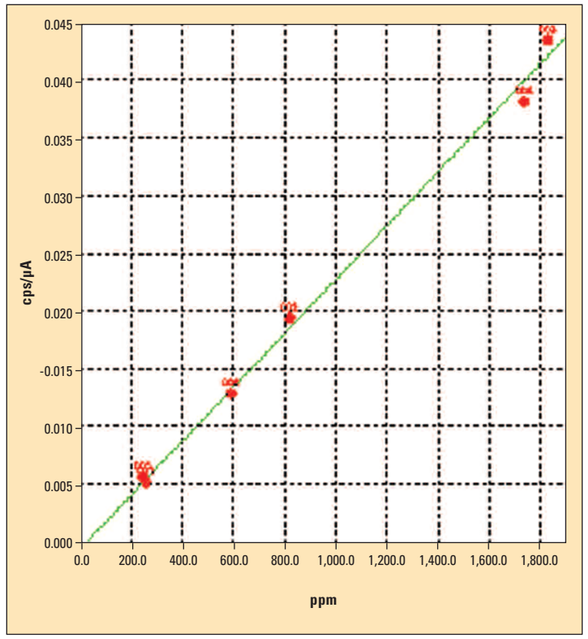

Figure 1: Calibration curve of the six lead-containing lead standards measured via the EDX-720

The EU directives WEEE and RoHS as well as the ElektroG (German Electrical and Electronic Equipment Act) regulation issued by the German Federal Government govern the return of used electrical and electronic equipment, their re-use or their recycling. These regulations also include a ban on hazardous substances including the elements lead, cadmium, chromium (VI) and mercury as well as polybrominated biphenyls and polybrominated diphenylethers (flame retardant in plastics) in the manufacture of electrical and electronic equipment.

Standard	Concentration (ppm) *
	Pb
74X-E	262
74X-HN	820
74X-TC	1830
74X-AM	1740
74X-HA	250
74X-HB	590

Table 1: Pb concentration of the certified standards.
*Obtained using ICP/MS

This article focusses especially on the heavy metal lead, which is still present in many so-called lead-free solders. In its directive of 21st October 2005, the European Commission specified additional exemption clauses for lead in the RoHS directive. The 2005/747/EG directive describes changes in subparagraphs 7 and 8 (cadmium) and includes the new subparagraphs 11 to 15.

Subparagraph 7 contains the following exemptions for lead:

- lead in high-melting solders (solders containing at least 85 % lead by mass)
- lead in solders used in servers, data storage systems and memory arrays as well as network infrastructure hardware for relaying, signal propagation, transmission and network management in the telecommunications sector
- lead in ceramic electronic components (for instance piezoelectronic components).

Subparagraphs 11 to 15 were added to the directive, specifying:

11. lead in press-in connectors with flexible zones
12. lead as coating material for C-rings in heat-conducting devices
13. lead in optical glasses and glass filters
14. lead in solders containing more then two elements with a lead content (mass percentage) of greater than 80 % and less than 85 %, used for connections between connector pins and microprocessor circuits
15. lead in solders which create a stable electrical connection between a semiconductor chip and a circuit board in integrated flip-chip circuits.

EDX-720 – twice the detection sensitivity

For the required monitoring of the use of lead, Shimadzu has developed an improved EDX system. The EDX-720 features sensitivity to lead (Pb) and cadmium (Cd) of more than twice the level of previous models. Based on the measurement of lead-containing solder standards, the sensitivity and reproducibility of acquired data are presented and discussed below.

Standards

The data on lead concentrations of the reference materials listed in Table 1 is supplied by MBH Analytical Ltd., Barnet, England. Tin (Sn) is the main component of the standards followed by Cu, Ag and Sb etc. in order of decreasing concentration.

In order to determine the lower limit of detection (LLD) a calibration curve was obtained via the PbL_{b1} line of lead. Although the PbL_{α} line is more intense, it can lead to inaccurate results due to line overlap phenomena. Therefore, the PbL_{α} line should not be used without prior testing. Based on the calibration curve presented in Figure 1, the detection limit can be calculated as follows:

$$LLD = 3 \times k \times \sqrt{\frac{I_{back}}{T}}$$

k: calibration constant
I_{back}: background intensity
T: measuring time

Detection limit (LLD)

A measuring time of 300 seconds resulted in a detection limit (LLD) of 24.8 ppm for the six standards. In this way, the legal threshold value of 1000 ppm for lead could be adhered to easily (Table 2).

Reproducibility

In addition to the detection limit, reproducibility is especially important as an indicator of the quality of a measurement. The certified reference standard 74X-E containing 262 ppm lead was measured sequentially ten times.

Element	Pb (L_{b1})
Measuring time	300 s
LLD	24.8 ppm

Table 2: Results of the calibration for Pb (L_{b1})

Element	Pb (L_{b1})
Certified concentration (ppm)	262.0
Average value n = 10 (ppm)	259.3
Standard deviation (ppm)	7.4
CV (%) experimental	2.9
CV (%) theoretical	2.5

Table 3: Results of the repeated measurements

The average value of 259.3 ± 7.4 ppm obtained via the EDX-720 reflects an excellent reproducibility (Table 3).

Results

The results show that even without any sample preparation, high accuracy and precision are attained already after a measuring time of 300 seconds. The EDX-720 is therefore, the ideal tool for fast analysis of elements ranging from sodium to uranium in solid and liquid samples. Without adjusting the method, one measurement can cover the entire concentration range from ppm up to 100 %. The possibility of carrying out analyses without using standards (fundamental parameter method) enables the investigation of unknown samples with very high precision. In addition, the large sample compartment (300 mm internal diameter x 150 mm height) offers enough room for non-destructive analysis of most samples without the need for prior sample fractionation.

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Routine determination of hazardous compounds

Atomic absorption spectrometry and WEEE, RoHS, ElektroG

Figure 1: The AA-6300 fully automatic atomic absorption spectrometer

Atomic absorption spectrometry is primarily suitable for quantitative determination of hazardous compounds such as lead, cadmium, mercury and chromium in sample materials according to ElektroG (WEEE/RoHS). AAS is a relative method for quantification and is based on the elemental composition of the sample and absorption according to Lambert-Beer's law. In principle, calibration curves are calculated in the appropriate concentration ranges for each element to be determined. The calibration curves are then used to evaluate all unknown samples. A prerequisite for accurate results is, however, that calibration standards and samples represent the same composition with respect to other elements and matrix. This prerequisite is not always met and can, therefore, lead to problems – for example when, in addition to the elemental absorption, background absorption of the matrix contributes to the signal.

Interferences such as molecular absorption, particulate caused scattering and spectral interferences caused by absorption line overlap can be eliminated via high-performance background compensation techniques. For complete compensation of all known AAS interferences in the flame- as well as in the electrothermal atomization modes, the high-speed self-reversal method is well established. Another widely used method, deuterium background compensation is, however, only usable in the wavelength range up to 420 nm, while self-reversal background compensation can be applied over the entire 185 – 900 nm range.

Cadmium in polymers

Quantitative determination of elemental cadmium in polymers was carried out using an AA-6300 Shimadzu atomic absorption spectrometer (Figure 1), which is equipped as standard with deuterium- and self-reversal background compensation modes. For electrothermal atomization, the highly sensitive GFA-EX7i graphite furnace with digital control was used.

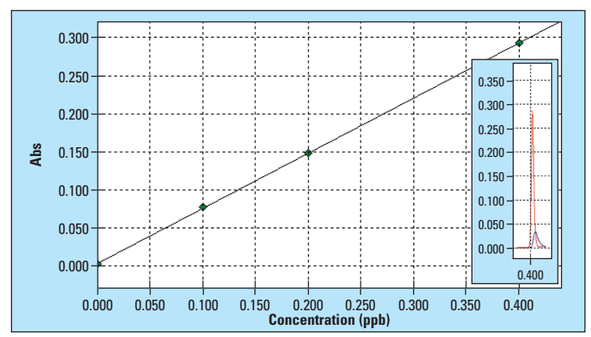


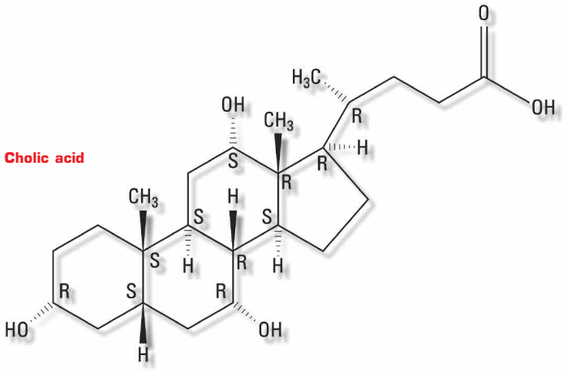
Figure 2: Calibration curve for cadmium

The experimental results were obtained from standard solutions, diluted measuring solutions and dissolved reference materials. For sample preparation of polymers, several dissolution procedures are possible, for example dry ashing or microwave-assisted acidic digestion using nitric acid and, if necessary, hydrogen peroxide under addition of hydrofluoric acid.

Cadmium determination (Fig. 2) was carried out in a concentration range of 0.1 up to 0.4 mg/L using flame atomization and in the concentration range of 0.1 up to 0.4 µg/L using electrothermal atomization. Due to spectral interference of the cadmium line at 228.8 nm by arsenic and iron, the deuterium method can lead to overcompensation. In this case, the self-reversal method was applied for background compensation. In this way, AAS can be applied as a suitable routine analysis method for the reliable determination of cadmium and other hazardous compounds according to the ElektroG directive.

Simultaneous determination of try ptophan, phenol, p-cresol and cholic acid in pretreated human blood

HPLC/DAD/MS method



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In the treatment of patients with kidney and liver problems, extracorporeal blood cleaning processes are very important forms of therapy in modern medicine. The currently applied practice of dialysis with three 4 – 6 hour treatment sessions per week is a pragmatic compromise between the ability to cope with this form of treatment and the well-being of the patient and is based on empirical data that can be transferred to nearly all dialysis patients.

The duration and frequency of a dialysis treatment must be applied in such a way that a maximum of enriched pathogenic metabolic products or toxins are removed from the patient's circulatory system while, at the same time, taking care that important blood constituents are not unnecessarily enriched. Special emphasis, in this case, is on protein-bound metabolic products and toxins, the so-called uremic toxins. The analytes presently under investigation – tryptophan, phenol, p-cresol and cholic acid – are used as representative markers for other important medical indicators.

When important metabolic organs such as the liver and/or kidneys are not functioning properly, a multitude of metabolic waste products will accumulate in the body. As long as these waste products are low molecular mass compounds, soluble in water and do not exhibit high degrees of protein binding, they can be removed via dialysis treatment. Some of these metabolic products are, however, lipophilic or are bound to proteins and therefore cannot be eliminated via the usual dialysis procedures. At the same time, these types of compounds are not usually co-detected in the clinical-chemical routine laboratory, so that clinicians will have no experimental process parameters at their disposal.

Phenol and p-cresol are formed as metabolic end products of the degradation of the amino acids tyrosine and phenylalanine by intestinal bacteria. When liver or kidney failure occurs, phenol and p-cresol accumulate in the blood. Although the patho-physiological significance is still largely unclear, p-cresol seems to negatively impact the intestinal endothelial barrier function, reduce endothelial cell proliferation as well as impair the response of the endothelium to pro-inflammatory stimuli.

Interestingly enough, a prospective clinical study recently showed a significant correlation between the serum level of free p-cresol and mortality probability of patients due to chronic haemodialysis therapy. This has further increased the interest in the possibility of p-cresol monitoring.

Liver failure will, among others, lead to a strong imbalance in the amino acid metabolism. In particular, the essential amino acid tryptophan has already been correlated for over 40 years to the pathogenesis of liver coma, wherein the free tryptophan level is noticeably elevated in the serum of these patients. Changes in the tryptophan concentration in human blood or different endogenous sera have been observed in correlation with metabolic diseases such as Parkinson or Alzheimer.

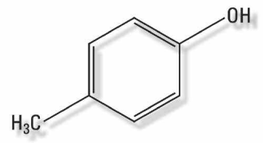
Individual steps in the development of an HPLC/DAD/MS method for the identification, separation and quantification of cholic acid, phenol, p-cresol and tryptophan in human blood

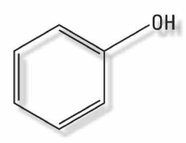
Step I: Development of an isocratic HPLC/DAD/MS method for the identification and separation of the analytes tryptophan, phenol and p-cresol.	**Step II:** Development of an isocratic HPLC/DAD/MS method for the identification of the analyte cholic acid
Step III: Combination and conversion of both isocratic HPLC/DAD/MS methods into a gradient HPLC/DAD/MS method for the identification and separation of the analytes tryptophan, phenol, p-cresol and cholic acid	
Step IV: Optimization of sample pre-treatment. Complete removal of cellular and further solid sample constituents, precipitation and removal of proteins, precipitation and removal of cholesterol from the blood sample and quantitative extraction of the analytes tryptophan, phenol, p-cresol and cholic acid in the diluted blood plasma phase.	
Step V: Calibration of the HPLC/DAD/MS analysis method for the quantification of the analytes tryptophan, phenol, p-cresol and cholic acid	
Standard-calibration based on a statistically sufficient number of PBS buffer solutions containing various amounts of an analyte-sample standard	Standard-addition calibration based on a statistically sufficient number of diluted human blood samples containing various amounts of an analyte-sample standard
Step VI: Statistical comparison and testing of the calibration procedure via an F-test and T-test. Evaluation of both methods for the quantification of the analytes.	

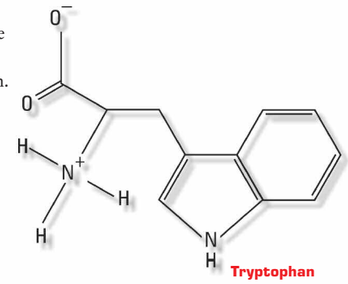
Left: Dipl.-Ing. Jürgen Müller M.Sc., right: Prof. Dr. Hermann Büttner

Method

An HPLC/DAD/MS method has been developed for the simultaneous determination of tryptophan, phenol, p-cresol (4-methylphenol) and cholic acid in human blood. These four analytes represent a selection of hydrophobic albumin-bonded uremic metabolites in human blood. Blood constitutes approximately 8 % of the body weight; for an adult weighing 80 kg, this is approximately 6.4 liters.







Fresh blood appears to be quite fluid although it consists of more than 40 % of solid components such as blood cells. The fluid part is called plasma, which contains numerous proteins necessary for blood clotting and functioning of the immune system. The most abundant plasma protein is albumin, which has a number of different transport functions and can bind to a multitude of compounds, among others the four analytes tryptophan, phenol, p-cresol and cholic acid, under investigation in the present study.

Due to the complexity of the blood matrix, a clear strategy was required in order to develop a robust HPLC method with high analytical reproducibility and high recovery. Method development was carried out in a series of individual steps. Breaking down the method development process was aimed at achieving a clear and thereby straightforward procedure. The method was, in the first place, aimed at the simultaneous determination of tryptophan, phenol, p-cresol and cholic acid in a human blood matrix. The analysis of a variety of blood sample types was, at this point, not yet considered. The available control blood sample (500 mL) originated from a healthy person.

In the first sample pretreatment step, the blood solids were removed. Simultaneously, during this step, all soluble proteins should, if possible, be denatured and precipitated. The most abundant soluble protein is albumin, which in the blood acts as a carrier for a large part of the respective analytes. The procedure must therefore ensure that all analytes that are adsorbed to particulate matter and to all soluble proteins are completely desorbed while quantitatively remaining in the plasma solution. In the subsequent step the cholesterol that has remained in solution is removed. A stock solution containing all four compounds is being used for the preparation of the measuring solutions for the standard-addition calibration series.

In the suggested analytical method, the analytes are separated using a semi-micro gradient HPLC (Shimadzu Liquid Chromatograph LC-10AD; HPLC column: Multispher 120 RP 18 HP-3 µm, mobile phase A: methanol, mobile phase B: 1 N formic acid). Detection is carried out in series, first via UV-VIS spectrometry (Shimadzu DAD SPD-M10A diode array detector) and subsequently via mass spectrometry (Shimadzu LCMS-2010 single-quadrupole mass spectrometer). For ionization APCI (atmospheric pressure chemical ionization) as well as ESI (electrospray ionization) are well suited. The combination of both independent detection modes – diode array and mass spectrometry – results in a high degree of analytical control and reliability especially with respect to the complex sample matrix. The duration of a single measurement takes almost thirty minutes.

Results

For the quantitative determination of the analytes tryptophan, phenol, p-cresol and cholic acid in blood, two methods were used – the standard-calibration and the standard-addition method. Calibration data must, in principle, be free from outliers. The resulting values of the respective corresponding sample series were compared with each other and statistically evaluated using the F- and T-tests. In this way, a distinct agreement between each of the independent sample series for the analytes tryptophan, phenol p-cresol and cholic acid could be observed. According to the F- and T-tests, the four measuring series of the standard calibration could be pooled. When comparing the standard-calibration method in PBS buffer with the standard-addition method in the pretreated blood, a slight matrix-dependent deviation was detected.

The detection limits for tryptophan, using the standard-calibration method were 0.049 mg/L in the matrix-adapted calibration standard PBS-buffer and 0.145 mg/L in human blood.

The detection limits for phenol in the PBS-buffer were 0.097 mg/L and 0.068 mg/L in human blood. The detection limits for p-cresol were 0.027 mg/L in the reference standard and 0.068 mg/L in human blood.

HPLC/DAD/MS method for simultaneous analysis of tryptophan, phenol, p-cresol and cholic acid in pretreated human blood

Analyte	Reference (PBS-buffer) Detection limit analyte (experimental) in mg/L	Pretreated blood sample Detection limit analyte (experimental) in mg/L	Normal concentration Analyte in blood (empirical) in mg/L	Pretreated blood sample Concentration analyte (experimental) in mg/L
Tryptophan	0.049	0.145	13.7 ± 4.5	9.25
Phenol	0.097	0.068	0.6 ± 0.2	< 0.069
p-Cresol	0.027	0.068	0.6 ± 0.2	1.23
Cholic acid	0.063	0.044	< 0.068	0.679

The detection limits for cholic acid were 0.063 mg/L in the reference and 0.044 mg/L in human blood.

The detection limits are approximately one order of magnitude lower than the measured concentrations of the analytes in blood samples of a healthy person, and the literature values for average normal concentrations of these compounds in blood.

Discussion

The developed HPLC/DAD/MS method enables the reliable simultaneous determination of tryptophan, phenol, p-cresol and cholic acid in pretreated human blood. The method is convincing through its simplicity, as derivatization of the analytes is not necessary. All four analytes can be unequivocally determined in their native form, which saves time and money. In combination with an efficient automation of the sample pretreatment, the proposed method is one step towards the development of a "semi-online" HPLC determination of selected toxins and metabolic products in blood during a patient dialysis treatment.

In this application, tryptophan, phenol, p-cresol and cholic acid are examples for other important medical markers whose fast and simultaneous determination in blood can enable optimal patient-customized checking and control of dialysis treatment in order to reduce the physical stress to a minimum. This can restore a certain amount of quality of life to dialysis patients.

In conclusion, it should be noted that the development of a technical implementation of the analysis during patient-dialysis should be coupled to clearly defined and reproducible conditions: an automated, efficient and hygienic sample pretreatment technique combined with a high-performance HPLC/DAD/MS system that can handle the entire analysis sequence – from blood sampling, subsequent removal of cellular and other solid blood constituents, precipitation and removal of most proteins, precipitation and removal of cholesterol, the quantitative extraction of the analytes in the diluted blood plasma phase up to the dilution and injection into the HPLC/DAD/MS system. Such a technical implementation is proposed using Shimadzu's Bio-Sample Analysis System Co-Sense® BA.

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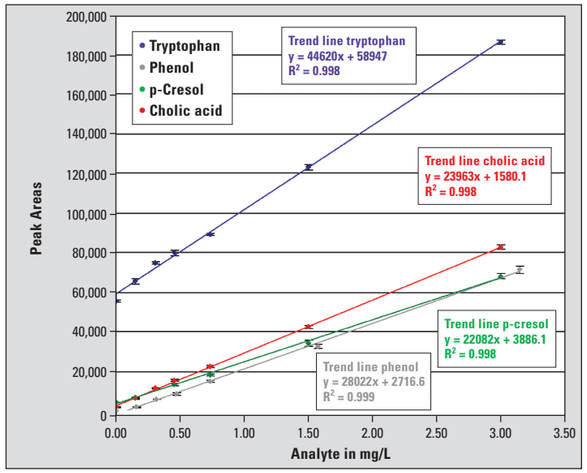
Co-Sense BA



Tryptophan, phenol, p-cresol and cholic acid in pretreated human blood (standard-addition method)

Successful participation in round robin tests

TOC suspension method for sediments and soils

Figure 1: Obtaining of sediment samples from a harbor basin carried out by the German company Schreiber in Duisburg

In accordance with the German AbfAblV Waste Disposal Directive [1], disposal of wastes containing more than 1 mass% (Landfill class I) or 3 mass% (Landfill class II) of organic compounds is prohibited without thermal or mechanical-biological treatment. This also applies to wastes such as soils, sediments or construction waste.

As a direct consequence, the analytical requirements with respect to the TOC parameter (**T**otal **O**rganic **C**arbon) have increased dramatically. Due to its high degree of automation and low operating cost, the determination of TOC is currently the analytical method of choice for the sum parameter determination of organic compounds. Using the new suspension method, solid samples such as sediments and soils can now be analyzed with considerably reduced expenditures in time and costs.

Suspension method

The suspension method was successfully applied to the pre-treatment of several matrices originating from the cement industry [3]. Preparation of suspensions from sediments and soils requires optimization of the sample preparation procedure. The sample material is finely pulverized (< 200 µm) using appropriate grinding methods (for instance a ball mill) and is subsequently suspended in a dilute hydrochloric acid solution.

It is especially important to minimize re-sedimentation of the suspended particles. Particles are effectively homogenized using a suitable dispersion tool such as Ultraturrax®. TOC determination of the suspension is carried out via the NPOC method (**N**on **P**urgeable **O**rganic **C**arbon). The IC (**I**norganic **C**arbon) is quantitatively removed using acidification. Volatile organic compounds can be neglected (drying at 105 °C). For this application the following assumption therefore applies: NPOC = TOC.

Analytical system

TOC measurements on suspensions were carried out using a Shimadzu TOC-V_{CPN} including an ASI-V autosampler (Figure 2). The system works according to the catalytic combustion principle. Due to an optimized sample introduction technique employing the ISP module (**I**ntegrated **S**ample **P**retreatment), suspensions can be ideally quantified using this system.

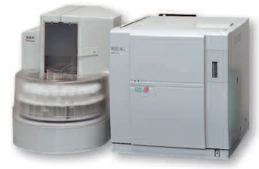


Figure 2: TOC-V_{CPH} with autosampler

Results from round robin tests

In addition to extensive comparative measurements [4], in part carried out using reference materials (see example in Figure 3), the suspension method was also tested by participation in two round robin tests.

The round robin tests involved ISE 2005.4 (**I**nternational **S**oil-analytical **E**xchange Programme) and SETOC 2005.4 (**S**ediment **E**xchange for **T**ests on **O**rganic **C**ontaminants) coordinated by WEPAL (**W**ageningen **E**valuating **P**rogrammes for **A**nalytical **L**aboratories) in Wageningen, the

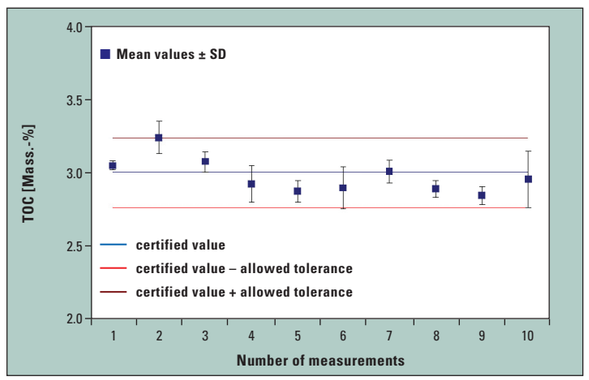


Figure 3: TOC suspension measurements using Reference Standard NIST41b

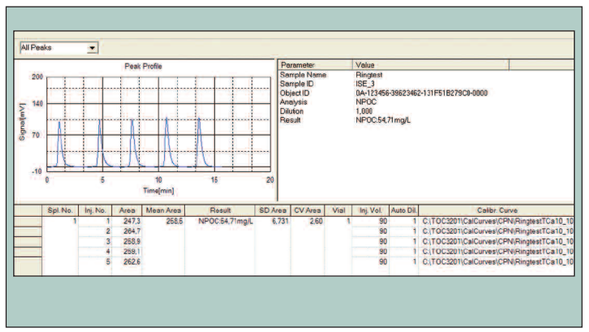


Figure 4: NPOC determination of a suspension sample

Netherlands. Each participating laboratory received 4 soil and sediment samples for testing. Figure 4 shows a NPOC determination of a suspension sample.

Three participants in the round robin tests have determined the TOC values independently of each other using the suspension method:

1. Laboratory for water analysis, Umweltbundesamt (Federal Environmental Agency) in Berlin, Germany
2. ALBO-tec GmbH, Technology Centre for Analysis and Soil Mechanics in Bochum, Germany
3. Shimadzu Europa GmbH in Duisburg, Germany.

Figure 5 shows an overview of the round robin test results of the suspension method compared with the overall result.

The median is based on the results of the round robin tests and the amount of relevant participants (ISE: 24 - 29; SETOC: 12 - 15). The results of the participants in 'Lab 1, 2 and 3' were obtained using the suspension method.

Conclusions

In addition to savings in time resulting from the straightforward sample preparation process and thorough automation via the ASI-V, the suspension method offers high analytical accuracy based on the multiple-injection requirement under AbfAblV. Measuring errors due to contamination are reduced considerably. An additional solid sample module is no longer necessary. This leads to a reduction in acquisition costs.

[1] Verordnung über die umweltverträgliche Ablagerung von Siedlungsabfäl-len (BGBl. S. 305; 24.07.2002, S. 2807) (Ordinance on Environmentally Compatible Storage of Waste from Human Settlements and on Biological Waste-Treatment Facilities)

[2] Verordnung über die Verwertung von Abfällen auf Deponien über Tage (BGBl. Nr. 46 vom 28.07.2005, S. 2252) (Ordinance relating to the recovery of waste at surface landfills and amending the Commercial Wastes Ordinance)

[3] TOC in der Zementherstellung (LABO 12/2004)

[4] Der TOC in Sedimenten und Böden – Fortschritte in der Analytik (WLB 10/2005)

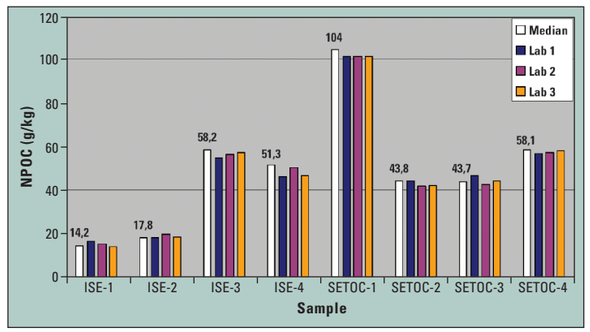


Figure 5: Round robin test ISE 2005/4 and SETOC 2005/4 (WEPAL, Wageningen, NL)

Good day, sunshine

Simultaneous detection of UV filters in sunscreen products

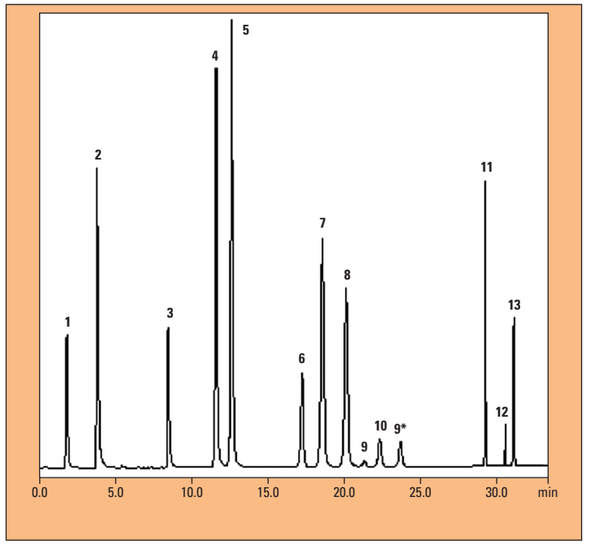


Figure 1: Typical chromatogram of the developed method, with thirteen UV filters separated in one run

When visiting the beach or walking the hills on a sunny summer day, people often apply sunscreen lotions. These are developed to protect our skin by reflection or absorption of solar radiation using UV filters, allowing us to stay longer in the sun.

UV rays are divided into three major groups:

- UVA rays of long wavelengths (380 – 315 nm)
- UVB rays of medium wavelengths (315 – 280 nm) and
- UVC rays of short wavelengths (< 280 nm).

In contrast to UVA and UVC rays, UVB rays cause sunburn of the skin.

Although UV filters have a protective function, their levels in cosmetic products are regulated by the European Union. In order to determine the levels of UV filters within sunscreens, assays have been set up to qualify and quantify these UV filters using liquid chromatography and gas chromatography [1,2].

Several methods for the simultaneous determination of several UV filters using a polymeric bonded silica ODS type reversed phase column are to be found in the literature.

This application describes a method for the simultaneous determination of thirteen internationally authorized organic UV filters commonly found in sunscreen products. For the separation, a Pathfinder MR column (polymeric encapsulated silica based, reversed phase) was used.

The filters determined were:

- 4-amino-benzoic acid (PABA)
- benzophenone-3 (Benz-3)
- 2-phenylbenzimidazole-5-sulfonic acid (PBSA)
- homosalate (HMS)
- 2-ethylhexyl-4-dimethyl-aminobenzoate (ED-PABA)
- 2-ethylhexyl-4-methoxycinnamate (EMC)
- drometrizole trisiloxane (DTS)
- isoamyl-p-methoxycinnamate (IMC)
- ethylhexyltriazone (ET)
- 2-ethylhexylsalicylate (ES)
- diethylhexyl butamido triazone (DBT)
- octocrylene (OC)
- 4-methylbenzylidene camphor.

The measurements have been carried out using a *prominence* HPLC system with PDA detection.

Nr.	Compound	k'	Resolution
1	PABA	0.2	
2	PBSA	1.5	10.2
3	Benz-3	4.6	26.9
4	IMC	6.7	16.2
5	MBC	7.3	4.4
6	OC	10.4	17.3
7	ED-PABA	11.3	4.2
8	EMC	12.3	4.6
9	HMS	13.1	3.3
10	ES	13.8	2.6
9*	HMS	14.7	3.5
11	DBT	18.4	23.8
12	DTS	13.2	14.9
13	ET	19.7	5.0

Table 1: Overview of the UV filters in the chromatogram, with retention factors and resolutions

Figure 1 presents a typical chromatogram of the developed method, where thirteen UV filters are separated in one run. The similar chromatographic properties of the compounds makes it difficult to obtain a complete baseline separation of all thirteen compounds. With the described method, it is possible to resolve all of the UV filters. Resolution of all pairs is larger than 1.5, as can be seen in Table 1. This allows accurate qualification and quantification of UV filters in sunscreens. The two peaks observed for HMS are typical for this compound, since HMS is present in two isomers forms [4].

In contrast to the method developed by Schakel and co., a selectivity difference occurs between the LiChrospher® column used in their method and the Pathfinder column used in this study. The difference can be explained by the fact that Pathfinder can be categorized within the family of polar embedded reversed phase columns [5]. Apart from the hydrophobic interaction model, playing a major role within regular ODS types of columns, Pathfinder alters selectivity due to the polar groups incorporated in the structure. In cases where matrix peaks disturb the analysis, selectivity change with polar embedded columns can be a good alternative.

An additional benefit of Pathfinder columns over regular ODS phases: there is no need to add base deactivating products such as EDTA, as described in a previous study [3], in order to obtain reasonable peak shape. The polymer shield around the silica core prevents analytes interacting with residual silanols.

With the present method it is possible to separate 13 of the most common UV filters found in sunscreen products. The method can be used as an alternative to existing methods when alternative selectivity is desired.

The development of this application was accomplished in cooperation with the research lab for non-food chemicals of the Food and Consumer Product Safety Authority, Groningen, the Netherlands.

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[2] Kazuo Ikeda, Sukeji Suzuki and Yohya Watanabe J. Chromatogr. A, 513, 1990, 321-326

[3] D.J. Schakel, D. Kalsbeek, K. Boer, J. Chromatogr. A, 2004, 1049, 127-130

[4] A. Chisvert, M.C. Pascual-Marti, A. Salvador, J. Chromatogr. A, 921 (2001) 207

[5] J. Layne, J. Chromatogr. A A, 957 (2002) 149 164

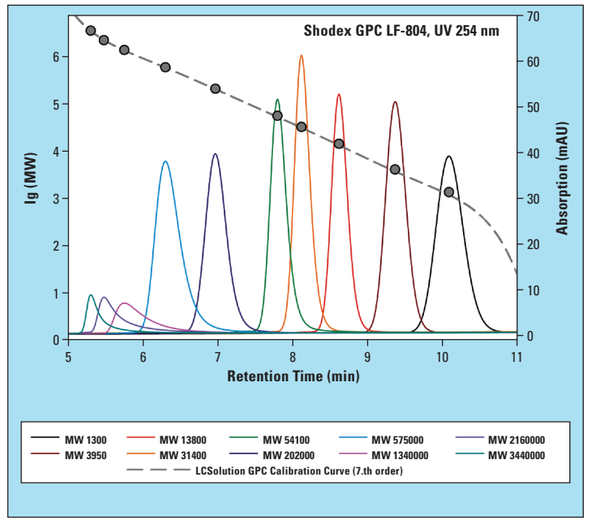


Figure 2: Calibration curve for the measured standards: virtually linear correlation in the range of 1300 – 3.44 million

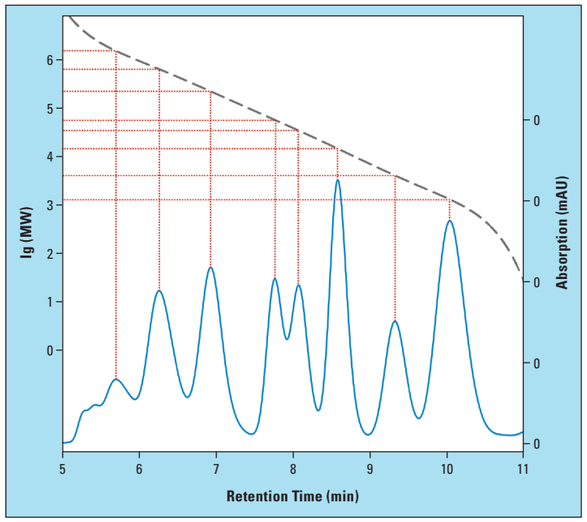


Figure 3a: Elution behavior and molecular mass distribution

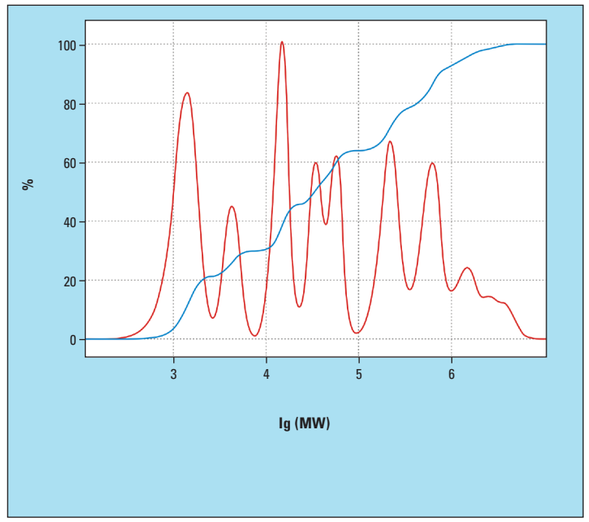


Figure 3b: Molecular weight, distribution view

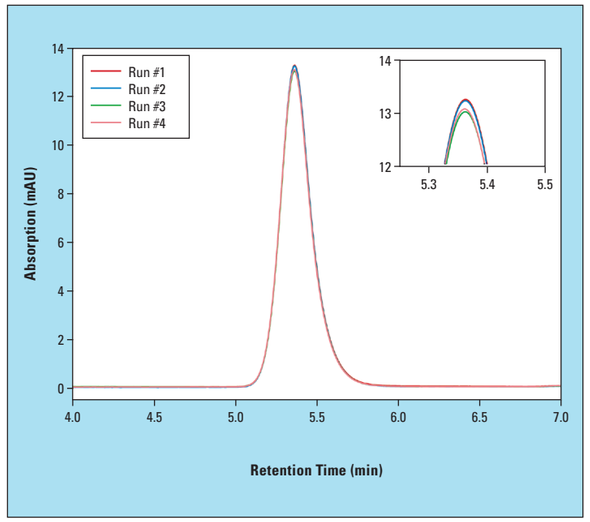


Figure 4: Reproducibility of the system (overlaying chromatograms of different runs)

Tested and approved!

GPC *prominence* system for standard applications

Gel permeation chromatography (GPC) is being applied in many different types of application. A traditional application is the characterization of macromolecules in organic or aqueous media. Other application examples are the pre-separation of sample components prior to GC or GCMS analysis, or general sample preparation.

Specialized GPC systems particularly suitable for these tasks are frequently mentioned without, however, always being able to point out clear differences from conventional HPLC systems. For the LC *prominence* the opposite approach has been taken – in addition to being able to carry out GPC separations using the previously introduced GPC option in the LCsolution software (see Shimadzu News 1/2006), Shimadzu has recently introduced the *prominence* GPC system.

Flexibility through standard components

The system is composed of standard HPLC components (Figure 1). Depending on the application at hand, injection can be carried out via an injection system based on the SIL-20A with high-pressure option or the SIL-10AF loop autosampler. Three types of detectors are suitable for GPC: a refractive index detector, a UV detector and an ELSD-LT light-scattering detector.

Figure 1: GPC system consisting of HPLC standard components

The application range of the *prominence* GPC system covers organic as well as aqueous GPC in the temperature range up to 85 °C.

Every GPC application is challenged when few or no suitable standards are available for the analytes. Polystyrene standards are therefore frequently relied upon. These are available for various molecular masses and in different qualities. The quality of these standards, in turn, is crucial for the creation of a calibration curve for the molecular mass range under investigation and its successful use.

All depends on the column

In addition to the standards, the separation column plays a decisive role: the linear range should correspond to the calibration range and the expected molecular masses. Independently, the GPC hardware system must guarantee consistent high reproducibility and be able to work with all common GPC solvents. For calibration, polystyrene samples (Shodex, SL-10^5, SLM-10^5) covering a molecular weight range of 0.67 x 10^3 up to 3.44 x 10^6 were used. A Shodex KF-801 (8 x 300 mm) GPC column was used for separation.

Figure 2 shows the calibration curve for the measured standards and illustrates the virtually linear correlation with the molecular mass range of 1300 – 3.44 million.

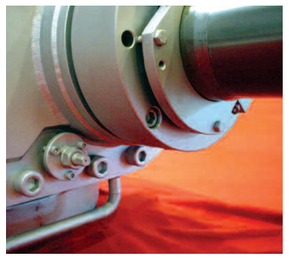
A mixture of the individual standards provides a good overview of the elution behavior and the molecular mass distribution (Figure 3).

Each sample was measured several times. The reproducibility of the system is shown in Figure 4, where multiple runs are overlaid. The stability of the retention times is especially emphasized.

Conclusion

The GPC *prominence* system is optimally suitable for standard GPC applications and more than lives up to its specifications. As far as complex combinations with other techniques are required, the uniform software platform of the LabSolutions software family simplifies switching between LC , GPC or GC application and enables the operation of complex chromatographic systems.

Gear pump for polymerization plant



Figures 1a and 1b: Model SBJV11000LF-803

Today, polymers are part of everyday life. Polymers are used in automotive and electric industry, for packaging and for buildings. The development of the plastics industry is a success story, and production is increasing steadily. 26 percent of all plastics are produced in the European Union. The worldwide demand of plastics materials is predicted to increase by 5 percent by 2010.

Japan is among the top three nations for production of machinery and equipment used in the plastics industry. Pumps are core products in the manufacturing of plastics and polymers. Shimadzu's history of gear pumps dates back to 1925. These systems contributed to the growth of the textile industry and the petrochemical industry behind the scenes (Figure 1a and b).

In order to produce economically, each polymer resin manufacturer needs to select the equipment based on a steady level of performance and maximum reduction of maintenance time and cost.

Developed for pressurized feeding of molten plastics at high temperature and high pressure, the Shimadzu SBJ gear pumps have demonstrated proven performance in many applications such as synthetic fiber, plastics as well as films and sheets. The pumps can be operated under severe conditions (high viscosity, high temperature and high pressure).

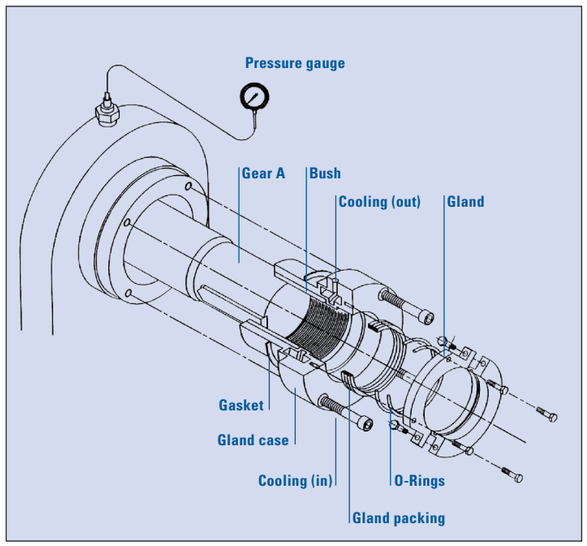


Figure 2: Construction of Labyrinth seal

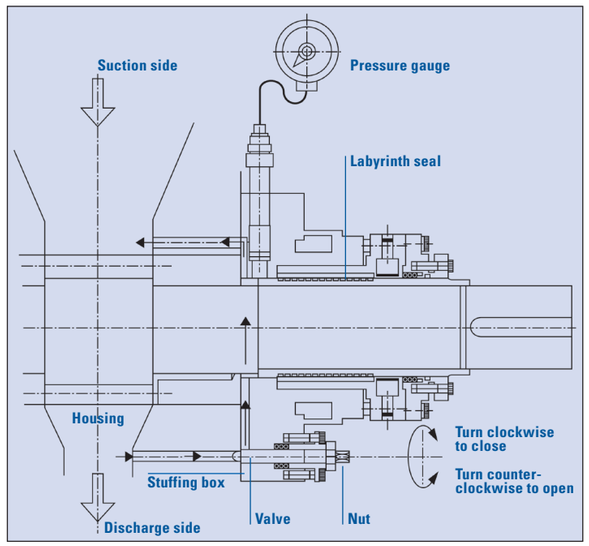


Figure 3: Pressure adjustment mechanism

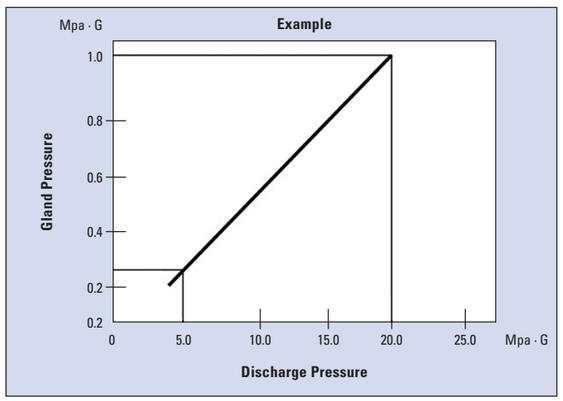


Figure 4: Discharge pressure vs. gland pressure

Their main features are:

1. Operation under vacuum using Labyrinth seal (non contacting seal) with pressure adjustment mechanism (Figures 2 and 3).
2. No maintenance necessary during operation after start up.
3. A flexible capacity range (cm^3/rev) allowing design to fit the customer's application.
4. Flexible suction diameter and distance from suction to discharge flange, according to the customer's standard and specification. This enables simple retrofitting of Shimadzu gear pumps to the existing production line.

	SBJV	SBJLV	SBJ	SBJL	SBJ-LL
Capacity	45 ~ 25,000 cm^3/rev	45 ~ 15,000 cm^3/rev	45 ~ 11,000 cm^3/rev	45 ~ 15,000 cm^3/rev	45 ~ 11,000 cm^3/rev
Viscosity	Up to 20,000 Pas				
Suction Pressure	Full Vacuum ~ 1 MPaG		0 ~ 1 MPaG		0 ~ 5 MPaG
Discharged Pressure	Up to 25 MPaG	Up to 7 MPaG	Up to 25 MPaG	Up to 7 MPaG	Up to 25 MPaG
Design Temperature	Up to 350 °C				
Material:					
Housing	Stainless Steel or Cast Steel				
Gear Shaft	Nitrided Steel			Nitrided or Stainless Steel	Nitrided Steel
Bearing	Tool Steel			Tool or Special Steel	Tool Steel
Shaft Seal	Labyrinth Seal (LF, LM)				
	Gland Packing (GP)				
	Double Mechanical Seal (M)				

Table 1: SBJ series, technical data

Highly reliable

Based on Labyrinth seal structure, reliability is the biggest advantage of Shimadzu gear pumps.

In the degas process for manufacture of Polyester, Polystyrene, ABS etc., a double mechanical seal and a non-contacting seal are generally selected as shaft seal in oil circulating systems to prevent air leakage. However, these seals are expensive and sometimes cause contamination by leaking seal oil into the polymer.

Shimadzu Labyrinth seals can solve these problems using the pressure adjustment mechanism.

The structure of this mechanism is simple. Pressurized polymer flows through the needle valve into the gland from the discharge side. The gland maintains pressure higher than the surrounding atmosphere and prevents air from being sucked in. The polymer in the gland returning to the suction side is provided within the pump. With this circulation of polymer through the shaft seal, the above-mentioned problems do not occur. Gland pressure is adjusted by opening and shutting the needle valve according to Figure 4.

The gland pressure should be set to less than 1 MPaG. As long as the adjustment is in order, no maintenance is required before the next regular one.

The only consumables required with this Labyrinth seal are packing material for shaft seal, and these are only necessary at start-up. No expensive parts need to be exchanged during operation.

This design and configuration for shaft seal has been operating since 1973. Over 1,000 units have been sold up to now. Users appreciate the time-saving operation and the dramatic reduction in maintenance costs.

Simadzu has recently introduced another type of Labyrinth seal together with a lip seal and multiple Labyrinth groove for low viscosity grades. The potential of the Labyrinth seal is expanding.

We will gladly send you further information. Please note the appropriate number on your reply card. Info **313**

NEW Simply the best – the new GCMS-QP2010 Plus

50 years of Shimadzu GC

Shimadzu announces the 50th anniversary of its gas chromatographs in 2006. In 1956, just one year after the first commercial GC was available, Shimadzu produced their first made-to-order gas chromatograph. The GC-1 then went into production in 1957. Ever since, Shimadzu has been developing gas chromatographs continuously, always close to the users and their needs.

Now Shimadzu has released its latest GCMS instrument, the GCMS-QP2010 Plus. GCMS instruments have been produced by Shimadzu since 1970, starting with the GCMS-9000 (magnetic sector). In 1982, the first quadrupole GCMS, the QP-1000, was introduced, followed by the QP-2000. In 1992 the QP-5000 was released as Shimadzu's first bench-top GCMS instrument.

GCMS-QP2010 Plus – Highest sensitivity and flexibility

Shimadzu has been improving the quality and performance of its instruments continuously. In 2001 the GCMS-QP2010 was introduced as the fastest GCMS instrument on the market, featuring high sensitivity. Based on this successful instrument, the new GCMS-QP2010 Plus was designed. The new ion source design as well as the powerful differential pumping system make the GCMS-QP2010 Plus the most sensitive GCMS system market-wide (Figures 1-3).

Highest flexibility in method development is achieved by the increased mass range from 1.5 to 1090 amu and the independently heated ion source in a range of 100 to 300 °C, also for compounds with high boiling points and difficult to analyze samples e.g. brominated flame retardants.

Configurations for EI, positive and negative CI are available. The different ionization modes can be used without hardware changes via the combi ion source. Tuning for all modes, specific to all CI gases, is available.

The GCMS-QP2010 Plus along with its predecessor, the GCMS-QP2010 is the fastest quadrupole GCMS on the market giving the user the benefit of short analysis times and high sample throughput with high chromatographic resolution.

Intelligent software features

The new software GCMSsolution 2.5 was introduced along with the GCMS-QP2010 Plus. It offers the user intelligent software solutions for ease of use.

Unique to Shimadzu: the automatic LRI (Linear Retention Index) calculation gives optimum security for the identification of unknowns from complex samples. Unambiguous results can be achieved using the library search function with integrated LRI.

Apart from the general purpose NIST 05 library, Shimadzu offers a special library for flavors and fragrances (FFNSC Ver. 1.2, **F**lavour and **F**ragrances **N**atural and **S**ynthetic **C**ompounds) with LRIs (Figure 4). All compounds in the FFNSC have been measured with the GCMS-QP2010. Only pure standards were used for the analysis in order to obtain high quality mass spectra.

AART (Automatic Adjustment of Retention Times) identifies and quantifies all compounds automatically after a column change, without compromising analysis parameters (also with constant linear velocity mode for best chromatographic resolution) – easy and fast!

Best quadrupole available worldwide: the new GCMS-QP2010 Plus

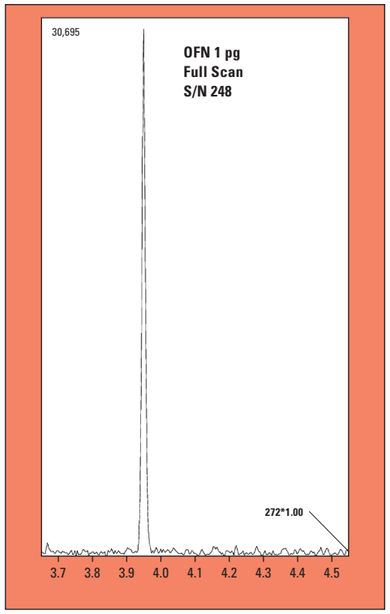


Figure 1: Sensitivity in Full Scan mode

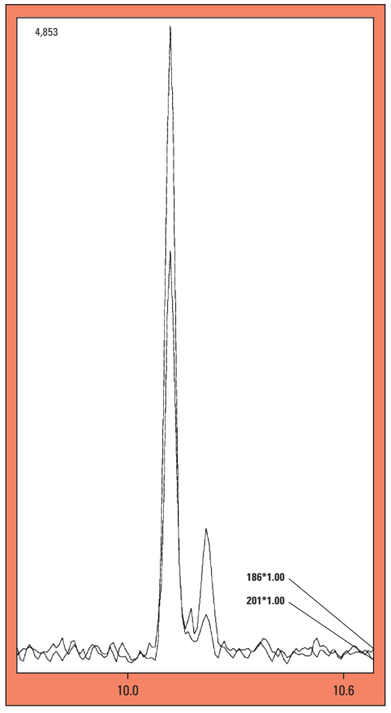


Figure 2: 10 pg Simazine in Full Scan mode

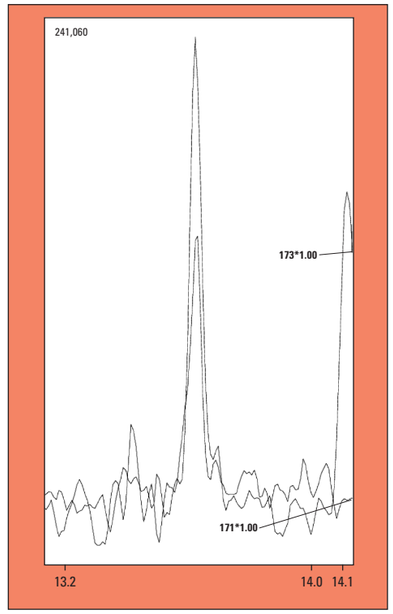


Figure 3: 100 ppt Tribromoform in water (headspace technique) in Full Scan mode

Fast GCMS: FASST (Fast Automated SIM/Scan Type)

With FASST, Shimadzu opens a new chapter of Fast GCMS. The GCMS-QP2010 Plus with its fast quadrupole enables acquisition of Scan as well as SIM data on one peak. Automatic SIM table creation, always a feature of the GCMSsolution software, is extended to automatic creation of the SIM/Scan table (COAST, Creation Of Automatic SIM/Scan Table). Quantitation of both data sets is possible. For quantitative analysis the number of data points on a peak is of utmost importance for the quality of the results. With a data acquisition frequency of up to 50 data points per second in Scan mode and up to 100 in SIM mode, data of excellent quality is obtained. This also plays an important role in qualitative analysis as the quality of the library search is determined by the quality of the mass spectra. Shimadzu with its long experience in Fast GCMS guarantees this highest data quality.

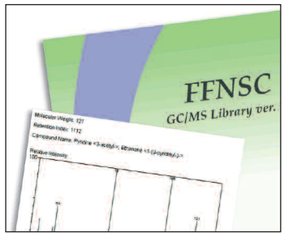


Figure 4: Shimadzu's special library for flavors and fragrances

(FFNSC Ver. 1.2, **F**lavour and **F**ragrances **N**atural and **S**ynthic **C**ompounds)

High recovery with minimum carryover

Thermodesorption system TD-20

New thermodesorption system TD-20

Thermodesorption is an effective method for the analysis of volatiles and semi-volatiles which can be applied in many areas such as organic contaminants in air (Figure 1) or fragrances in food (Figure 2). Shimadzu has now presented its new thermodesorption system TD-20 (left).

High performance

The new TD-20 shows a high recovery rate even for high boiling point compounds (Figure 3). As there are no cold spots in the system, the TD-20 shows minimum carryover even after numerous analyses of high boiling point compounds. All flows in the TD-20 are controlled electronically by AFC-2010 (Advanced Flow Control) for best reproducibility. Settings for flow rate and split ratios can be easily reproduced with the GCMSsolution software. Pressure programs and split ratio programs can also be used.

Effective cooling

Cooling in the TD-20 is performed by a Peltier element, so coolants such as liquid nitrogen are unnecessary. This makes operation easy and gives the user more security as the instrument can run continuously and there is no risk of stopping the analysis because the coolant has run out.

Easy operation

Maintenance on the TD-20 is easy, and it is possible to exchange only the parts that were in contact with the gas.

For full automation the TD-20 comes with an autosampler with 48 sample positions. The TD-20 is controlled by the TD control software which can also work together with the GCMSsolution software.

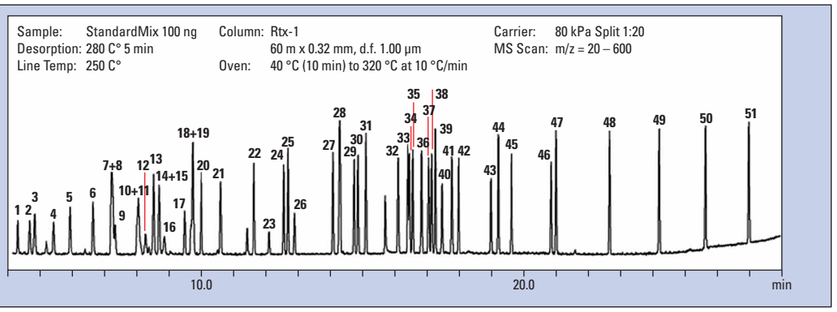


Figure 1: VOC air analysis standard

01: Ethanol
02: Acetone
03: IPA
04: Dichloromethane
05: 1-Propanol
06: MEK
07: Ethylacetate
08: Hexane
09: Chloroform
10: 1.2-Dichloroethane
11: 2.4-Dimethylpentane
12: 1.1.1-Trichloroethane
13: 1-Butanol
14: Benzene
15: Carbontetrachloride
16: Tetrachloromethane
17: 1.2-Dichloropropane
18: Bromodichloromethane
19: Trichloroethylene
20: Heptane
21: MiBK
22: Toluene
23: Dibromochloromethane
24: n-Butyl acetate
25: n-Octhane
26: Tetrachlorethlene
27: Ethylbenzene
28: m,p-Xylene
29: Stylene
30: o-Xylene
31: n-Nonane
32: alpha-Pinene
33: m-Ethylmethylbenzene
34: p-Ethylmethylbenzene
35: 1.3.5-Trimethylbenzene
36: o-Ethylmethylbenzene
37: beta-Pinene
38: 1.2.4-Trimethylbenzene
39: n-Decane
40: 1.4-Dichlorobenzene
41: 1.2.3-Trimethylbenzene
42: D-Limonene
43: Nonanal
44: n-Undecane
45: 1.2.4.5-Teramethylbenzene
46: Decanal
47: n-Dodecane
48: n-Tridecane
49: n-Tetradecane
50: n-Pentadecane
51: n-Hexadecane

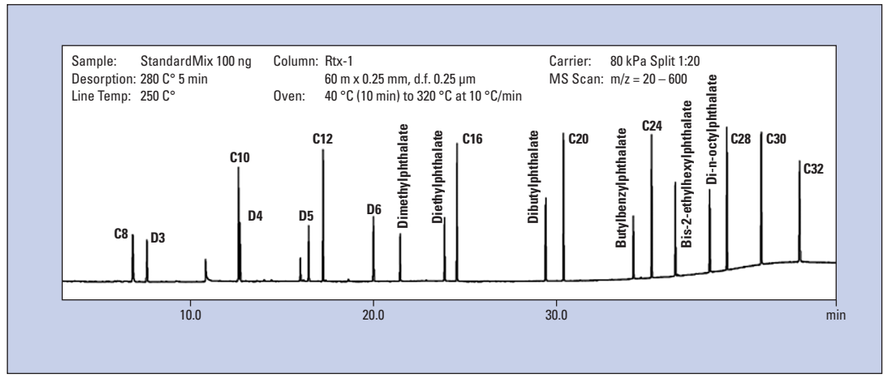


Figure 3: Standard mix containing high boiling point compounds

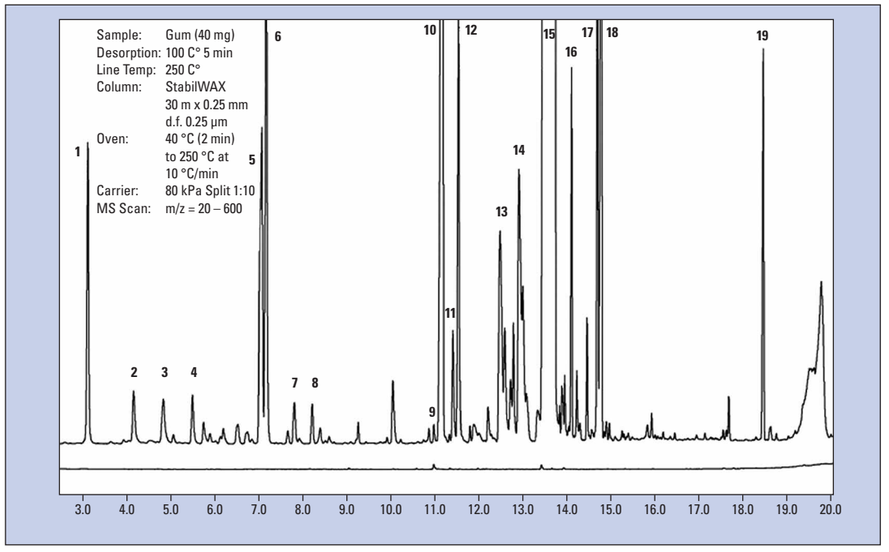


Figure 2: Volatiles in chewing gum

01: Ethanol
02: alpha-Pinene
03: Camphene
04: beta-Pinene
05: Limonene
06: Eucalyptol
07: gamma-Terpinene
08: Cymene
09: Aceticacid
10: Menthone
11: Mentofuran
12: Isomenthone
13: Isomentoacetate
14: Isomenthole
15: Menthole
16: beta-Cital
17: alpha-Cital
18: Carvone
19: Triacetin

The most flexible research grade MA LDI MS/MS mass spectrometer

AXIMA-TOF² – high performance and versatile

Over the past few years, a revolution in MALDI based mass spectrometry has begun. The technique has progressed from a simple laboratory tool providing molecular weight information to a highly specialized research instrument allowing the rigorous investigation of complex mixtures permitting compound identity and composition.

Kratos Analytical Ltd, a Shimadzu owned subsidiary, and Shimadzu Corporation have launched a new high-performance MALDI TOF mass spectrometer for state-of-the-art high energy MS/MS, the AXIMA-TOF².

This system is a new member of the AXIMA family of MALDI mass spectrometers and now incorporates the highest available energy collision MALDI system, effectively providing CID (collision induced dissociation) with a lab energy of 20 keV. This enables efficient fragmentation of all manners of analytes from peptides to sugars to pharmaceutical compounds.

Routine identification of proteins from high energy MS/MS fragmentation of tryptic peptides is easily achieved. In general, the expected a, b and y-type fragment ions are observed, in addition to the valuable immonium ions and a number of diagnostic side chain cleavage ions. A typical MS/MS spectrum is shown in Figure 1, together with the resultant Mascot search result. Resolution and accuracy are consistent across the fragment ion mass range with no stitching of MS/MS fragment and precursor ion spectra.

Sensitivity has also been improved, particularly in MS/MS mode. The unique patented combination of the advanced curved field reflectron design and the high energy collision cell means that all fragment ions formed are observed regardless of where they are generated in the instrument. Both LID and CID ions are accumulated into a seamless spectrum providing maximum sensitivity.

Revolutionary gating technology

Optimal precursor ion selection resolution of 400 (FWHM) is achieved using revolutionary patented gating technology. This is particularly useful when isolating ions which are close in nominal mass for subsequent fragmentation, for example isotopically labelled peptides.

When combined with high resolution MS performance, more information can be extracted from complex mixtures without the complication of contribution from ions of a similar nominal mass. MS/MS spectra are easier to interpret and significant database search hits are more readily achieved. The highest energy collisions (20 keV) of any MALDI system produce information-rich MS/MS data.

The new Low Mass Zoom feature allows rapid enhancement of the region of the spectrum encompassing the immonium ions, used to confirm the putative sequence

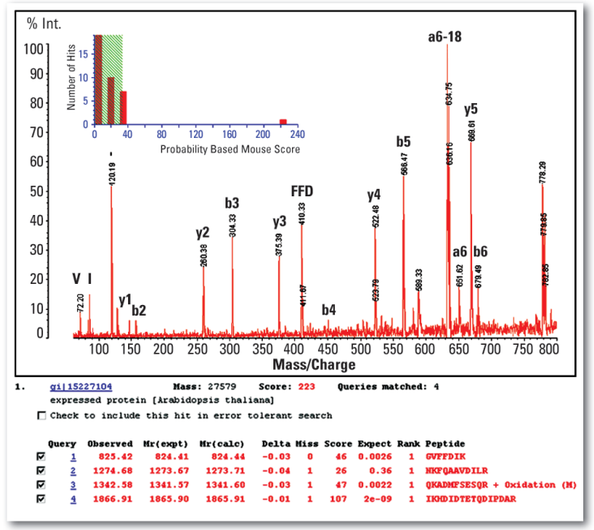


Figure 1: Typical MS/MS spectrum (e.g. first matched peptide m/z 825.42; GVFFDIK)

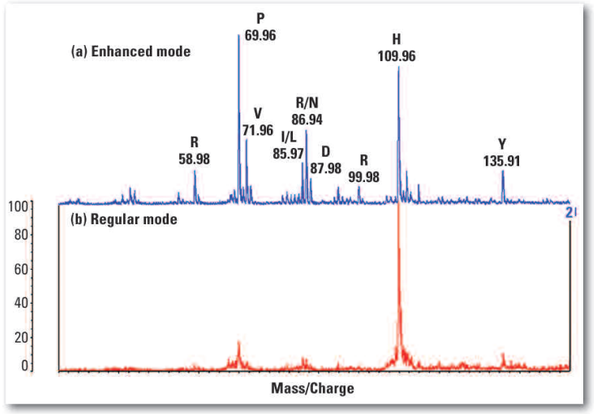
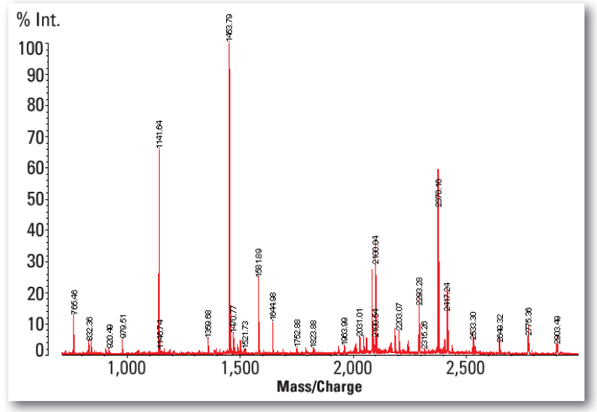


Figure 2: Comparison of regular mode with enhanced mode by Low Mass Zoom feature



of a peptide and isotopically labelled quantitative diagnostic ions (Figure 2).

More accurate and efficient peptide PMF

The high resolution MS data obtained in reflectron mode, shown in Figure 3, may be utilized for more accurate and efficient peptide mass fingerprinting (PMF) and complex mixture analysis. In addition, the linear mass range and sensitivity is uncompromised allowing the analysis of high mass analytes such as in-tact proteins and oligonucleotides. As an example of a high mass protein, an immunoglobulin is shown in Figure 4.

As with all AXIMA systems, on-axis laser irradiation enhances ion transmission and sensitivity in all modes of operation.

This is a mass spectrometer designed to meet a whole range of requirements in many different areas of research. The software is focussed on ease of use and includes integrated packages for a whole host of applications.

Manual or fully automated operation

Continuing the philosophy of producing instruments designed to solve problems, the AXIMA-TOF² has been engineered to allow manual or fully automated operation permitting the analysis of just a handful or hundreds of samples. Fully enabled software for proteomics experiments – the Intellimarque suite – has been integrated for automated data ⏵

292 - 300	920.49	919.48	919.45	0.03	0 SVVANGMAR	Oxidation (M)
339 - 355	2013.99	2012.98	2012.97	0.01	0 ESFDFRPGMMTINLDLK	
339 - 355	2030.01	2029.00	2028.96	0.04	0 ESFDFRPGMMTINLDLK	Oxidation (M)
339 - 355	2045.98	2044.97	2044.96	0.01	0 ESFDFRPGMMTINLDLK	2 Oxidation (M)
339 - 356	2170.09	2169.08	2169.07	0.01	1 ESFDFRPGMMTINLDLKR	
339 - 356	2186.09	2185.08	2185.07	0.02	1 ESFDFRPGMMTINLDLKR	Oxidation (M)
339 - 356	2202.07	2201.06	2201.06	0.00	1 ESFDFRPGMMTINLDLKR	2 Oxidation (M)
365 - 373	979.51	978.50	978.47	0.04	0 TAAYGHFGR	
365 - 387	2649.32	2648.31	2648.30	0.01	1 TAAYGHFGRDDPDFTWEVVPLK	

1. gi|81647 Mass: 43117 Score: 262 Expect: 3.2e-22 Queries matched: 27
methionine adenosyltransferase (EC 2.5.1.6) - Arabidopsis thaliana

Figure 3: High resolution MS data obtained in reflectron mode – for more accurate and efficient peptide mass fingerprinting (PMF) and complex mixture analysis

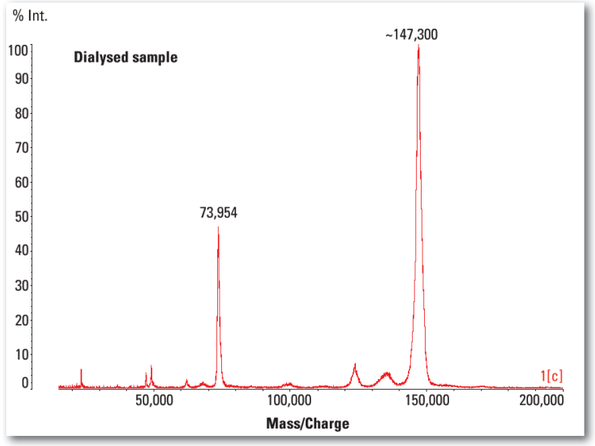


Figure 4: Example of a high mass protein, an immunoglobulin

dependent peptide mass fingerprinting and MS/MS of peptides with incorporated Mascot database searching. Peptide mass fingerprints are acquired and subjected to an online Mascot database search. User definable limits for acceptance of protein identification may be set allowing full control of the quality of data accepted for confident assignment. Using the results of the PMF search, data dependent MS/MS may be performed on those ions that have matched as a significant hit – confirmation MS/MS – in addition to those that have not – investigation MS/MS. Batch searching of these MS/MS spectra is then performed automatically to provide protein assignment.

All data may be reprocessed and re-searched at a later time to provide further information if required.

Software identifies complex mixtures via automated MS/MS

In addition, new LC MALDI software has been included allowing identification of complex mixtures via automated MS/MS. The system provides total support for LC MALDI based experiments. The software suite allows the fully automated acquisition of LC separated samples deposited onto MALDI targets and subsequent identification of proteins via MS/MS of the peptides detected. The workflow automatically provides a provisional intensity map of all sample spots across the target to assess the distribution of peptides and identify the position of the apex of the chromatographic peak.

A candidate list is generated and MS/MS data acquired for all discrete peptide ions. Exclusion lists are used to remove known contaminants or high abundance peptides. All data is then subjected to an integrated Mascot search. This process can be carried out on a single MALDI target or across multiple targets allowing complex 2D HPLC separations to be analyzed.

Low sample consumption allows multiple spectra to be acquired from the same spot increasing the amount of useful data obtained. The combination of the *prominence* HPLC with the AccuSpot automates LC micro-fractionation, spotting and preparation for MS analysis and offers the perfect front end for this application.

Alternative applications

Additionally, the AXIMA-TOF^2 allows the recognition of biomarker patterns and distribution of compounds of interest in clinical proteomics samples. Software generates a "heat map" indicating the presence or absence of a particular mass along with an indication of its intensity. Fully automated acquisition of unusual format samples, for example tissue sections, may be performed followed by visualization of the sample via its total ion current or a specified mass.

Data can also be exported into alternative processing packages to allow comparative experiments. The combination of the ChIP 1000 Chemical Printer for sample preparation with the AXIMA MALDI products is a helpful tool for reproducible biomarker discovery.

We will gladly send you further information. Please note the appropriate number on your reply card. Info **314**

Automatic liner exchange with the new LINEX system

Gas Chromatography

Exchanging of liners in a GC is a routine task for the analyst. Depending on the samples analyzed and especially on their matrix content, the liner may have to be exchanged after a few hundred or even after a few tens of injections. Based on the Optic 3 GC injector, Shimadzu now offers a new automatic system for the liner exchange, called LINEX (ATAS GL).

This new LINEX system works fully automatically with the AOC-5000 autosampler from Shimadzu.

With LINEX, the multi-sample analysis sequence works in a simple way: the sample-equipped liner is fed into the injector and purged with carrier gas after the injector head is closed. The injector is then heated and the sample transferred onto the column. After analysis, the empty liner is returned to the tray and the cycle is repeated. In this way the user is relieved from tedious exchanging of the liner, particularly important for customers handling so-called "dirty" samples with high matrix content, e.g. in clinical analysis where the liner has to be exchanged frequently. Standard liners for the Optic 3 can be used in combination with the LINEX system.

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

DMI (Difficult Matrix Introduction)

The LINEX system does more than just saving valuable time otherwise used for maintenance. With the LINEX system the method of DMI (Difficult Matrix Introduction) can be applied. Liquid or dirty solid samples are loaded into a microvial. The microvial is then inserted into a fritted injector liner and placed in the injector. The analytes are desorbed from the sample directly onto the head of the GC capillary column.

The sample can be used without any sample preparation, so with fewer steps involved there are also less opportunities for analyte losses when compared with conventional sample preparation. Solvents can be removed by venting under controlled conditions.

High boiling point compounds from the matrix are kept in the microvial which is disposed of after use, so the liners can be re-used for future analysis.

Applications for LINEX and DMI

The automatic liner exchange can be used in any application field, particularly where the samples have a high matrix content requiring frequent exchange of the liners, e.g. in environmental or clinical analysis. DMI can be used for the thermodesorption of volatiles from matrices as different as hand cream, tobacco, edible oils, coffee, washing powder etc.

Success factors for high throughput ana lysis – A look behind the HPLC scene

High pressures will not automatically result in higher sample throughput – or – what can be achieved with a "Fast LC"?

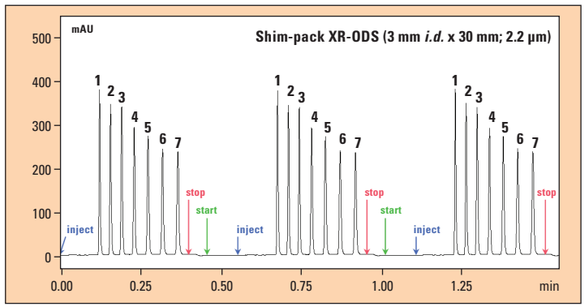


Figure 1: Three separations (injections and gradients) within 1.5 minutes
Chromatographic conditions:
Shim-Pack XR-ODS (3 mm I.D. x 30 mm; 2.2 µm)
Mobile Phase: water/acetonitrile (4/6 up to 2/8 within 0.4 min; convex gradient)
Pressure: max. 21.5 MPa · **Flow rate:** 3 mL/min · **Temperature:** 80 °C
Detection: UV 245 nm · **Injection:** 4 mL (each 800 nmol); 0.1 min time-delayed injection · **Peaks: 1:** Acetophenone · **2:** Propiophenone · **3:** Butyrophenone **4:** Balenophenone · **5:** Hexanophenone · **6:** Heptanophenone · **7:** Octanophenone

"Speed, it seems to me, provides the one genuinely modern pleasure" is a famous quote from British writer Aldous Huxley, author of "Brave New World" and other scholarly works. A view of our world today seems to prove him correct, especially when considering technological developments. Whether automobiles or computers, telecommunication or production processes – everything has been developing at faster speeds. And HPLC is no exception...

When observing these trends at international trade fairs and conferences, conventional column dimensions and particle sizes seem to be a thing of the past, as "Fast LC" is rapidly taking over. With reference to "Fast LC", we are usually talking about smaller particle diameters and higher operating pressures, as these are absolutely essential for these types of separations.

But what exactly is "Fast LC"? Or, differently: what can be accomplished using "Fast LC"? Are high pressures really indispensable when carrying out fast HPLC? Or do we actually mean that we want to analyze more samples within the same time frame, when we speak of "Fast LC"?

One thing is certain: the practical advantages of "Fast LC" separations can only be gained when more samples are analyzed within shorter time frames. In addition, we also want to achieve lower detection limits using less sample material and more selective columns...

But returning to high sample throughput. More effective and faster separations can also be achieved using conventional HPLC and therefore conventional HPLC can be used successfully in high-throughput analysis. What then, is required to analyze more samples in the same amount of time?

1. Short cycle times
2. On-time injection
3. Fast and reproducible gradients
4. Short, selective columns with high stability
5. Temperature control (thermostatting)
6. Fast detectors
7. Fast data processing by the detector and via the associated software
8. A robust HPLC system.

Let's take a closer look at each of these success factors:

Cycle time

The speed of the injection system will have a decisive influence on the cycle time of the HPLC system. To ensure the lowest possible sample carry-over, rinsing of the injection needle and/or the sample loop is usually required. In this case, the time needed to carry out this rinsing step should be taken into account. Ideally, subsequent samples should be immediately ready for injection at the end of each previous run, no matter how long, or in this case how short, the separation is. Figure 1 shows three separations (injections and gradients) within a total analysis time of 1.5 minutes.

Injection

"On-time" injection – an accurately timed injection to ensure that gradient and sample will start on the column simultaneously – can reduce the total analysis time. Sample components will then be eluted faster and more reproducibly from the column, as the system volume will not influence the elution time. An optimized system configuration guarantees a minimum dead volume and therefore minimizes the delay time.

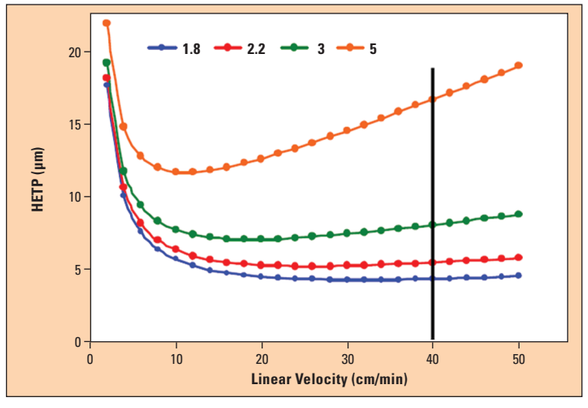


Figure 2: van Deemter curves for several particle sizes (in µm)

Gradients

Fast gradients require accurate pump control and small efficient mixing chambers in order to guarantee reproducible conditions. Suitable modifications are integrated in the newest generation of pumps.

Column selection

A decisive factor in the selection of a suitable column, with respect to particle size, is a practical compromise between maximum plate height and linear flow velocity. In this respect, it is possible that larger particles lead to equal or better separation performances (Figure 2). A small particle diameter does not automatically yield better results. The question is, above all, whether smaller particles will lead to the expected analytical performance in terms of higher sample throughput, i.e. more samples analyzed within less time...

Let's consider that even though we may have the most modern generation of columns at our disposal, the perfect "universal" column for all separations does not exist. Differences in selectivity of individual stationary phases for certain chemical structural classes will still require method development and optimization.

Temperature control

With respect to system optimization, the temperature should also be taken into consideration, as reproducible gradients and separations can only be guaranteed under constant operating conditions. The smallest deviations may jeopardize system stability and reproducibility.

In addition, higher temperatures can speed up the separation and decrease system pressure. In practice, temperatures of over 60 °C are, however, seldom applied, perhaps also because small or rapid changes in the flow line or the exchange of a column can rapidly lead to a "tricky" situation. Speeding up the separation by applying high temperatures should, on the other hand, not be ruled out even if effects with respect to selectivity and retention cannot be predicted in every case. Figure 3 shows separations of a sample mixture at 40, 60 and 80 °C. It is clear that higher temperatures lead to shorter retention times for the individual sample components as well as decreasing peak widths and increasing peak heights, as illustrated for Peak 7.

The potential of "Elevated Temperature LC" is currently only partially foreseeable, especially with respect to the use of aqueous mobile phases instead of organic solvents. Therefore, for temperatures higher than room temperature, it is recommended to consider preconditioning of the mobile phase at the applied temperature. At the same time, preheating the sample can avoid temperature effects on the column caused by a "cold mobile phase and sample", which could otherwise invalidate the entire method. A thermostatted flow cell and sufficient backpressure is recommended in order to prevent bubble formation in the sample in the detector cell. The example in Figure 4 shows the peak capacities at 20, 50 and 85 °C with, and respectively without temperature preconditioning of the mobile phase. In addition to the altered peak shapes, an increase in the peak capacity is, in each case, evident, in addition to the decrease in retention times with increasing temperatures.

Detectors

Fast, narrow peaks require fast data acquisition and optimization of the detector settings. Data acquisition rates similar to those in gas chromatography will be required in order to be able to gather sufficient data points for peak calculation.

Software

The other element of an HPLC system, designed for fast and/or maximum throughput separations, is the software. Stable and fast communication between hardware and software cannot, in spite of modern PC technology, be assured. Likewise, not all software is able to carry out reanalysis and report generation while keeping up with the speed of the separation.

Robustness

Last but not least, let's not forget that a HPLC system is required which will be able to run in a stable and trouble-free manner under routine operating conditions. Especially when sample throughput and speed are essential, instrument failure should be prevented.

Finally, each fast LC separation must still prove its suitability for routine operation over a sufficiently long time period, as is usually the case for the so-called antiquated columns with an internal diameter of 4.6 mm.

So, with respect to questions about fast LC, the answer is that the proven 4.6 mm columns are still being used in many HPLC systems and that their days are certainly not numbered.

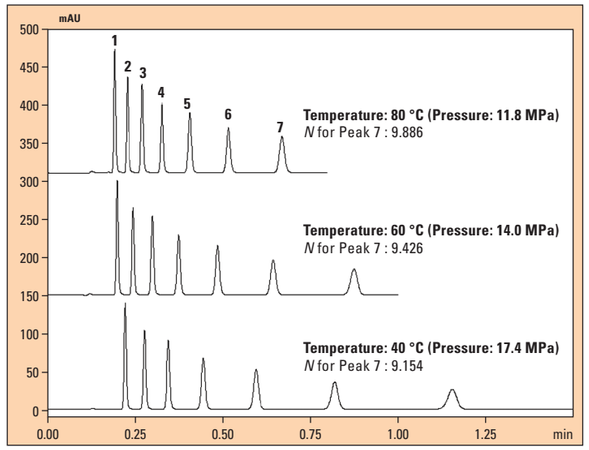


Figure 3: Fast separation at high temperatures
Chromatographic conditions:
Shim-Pack XR-ODS (3 mm I.D. x 30 mm; 2.2 µm)
Flow rate: 1.5 mL/min · **Mobile Phase:** water/acetonitrile (3/7, v/v)
Detection: UV 245 nm · **Peaks: 1:** Acetophenone · **2:** Propiophenone
3: Butyrophenone · **4:** Balenophenone · **5:** Hexanophenone · **6:** Heptanophenone
7: Octanophenone

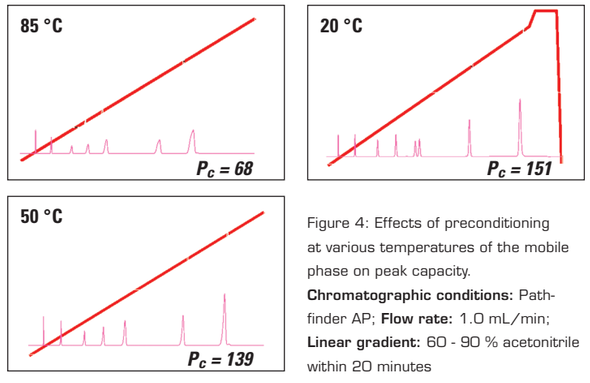


Figure 4: Effects of preconditioning at various temperatures of the mobile phase on peak capacity.
Chromatographic conditions: Pathfinder AP; **Flow rate:** 1.0 mL/min; **Linear gradient:** 60 - 90 % acetonitrile within 20 minutes