

## LabSolutions concept

Cross-system platform for HPLC-, UHPLC and GC systems, as well as for LCMS and LCMS/MS systems

Including part 2 of the big breakfast test

## SALD-7500

New family member for nanoparticle measurement

## Amino acid identification

Fast and easy with the UF-Amino Station for high-throughput analysis

Quality

Sustainability

Innovation



## APPLICATION

Bitumen – it's all in the mix – FTIR spectroscopy determines quality differences 4

Searching for elemental traces in petrochemicals – The high-precision ICPE-9000 inductively coupled plasma optical emission spectrometer 9

Exact measurement of color – New software for color determination 10

Determination of microbial biomass in soils – TOC in soil science 12

Amino acids – fast and simple identification – UF-Amino Station enables high-throughput analysis 14

A small, subtle distinction – Trace analysis in polymers using pyrolysis GCMS 16

The daily bread – The big breakfast test using the EZ Test EZ-X texture analyzer (part 2) 19

Following the traces of climate change – Novel plasma technology offers new detection possibilities 20

Effect of mobile phase pH on reversed-phase HPLC separations of ionizable compounds 22

Customized TOC analysis – The online TOC-4200 with many options and kits 23

## PRODUCTS

How to make invisible things visible? Worldwide unique: The new Hyper Vision HPV-X camera 2

New family member, improved possibilities – SALD-7500 for measuring nanoparticles 5

**New:** LabSolutions DB and CS Software 6

New accessory for UV-VIS spectrophotometers TCC-100 8

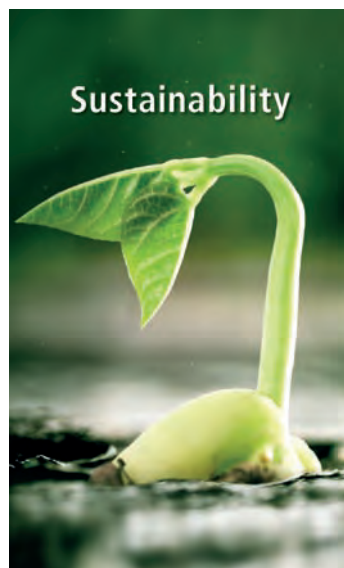


NEWS – Shimadzu's customer magazine is also available as WebApp (for iOS and Android) via: [www.shimadzu-webapp.eu](http://www.shimadzu-webapp.eu) or as app for iPhone in the AppStore.

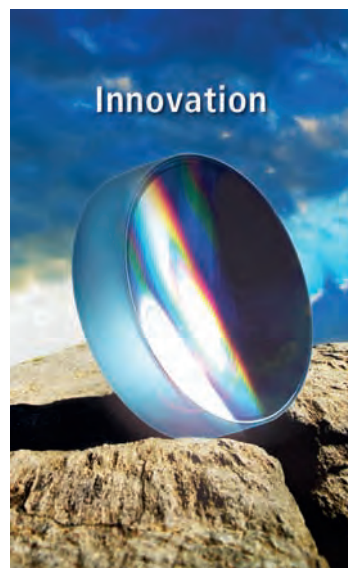
# Added value for you



... means the best. It is apparent in the details. And in our employees. Both contribute to your added value.



... means creating technologies that are compatible with our lives, our values and with nature. This will secure our future.



... means overcoming limitations and preconceptions – technical, psychological, social. This leads to progress.

**P**roduct and service quality of the highest level, easy-to-use instruments, system performance and total costs of ownership (TCO) are important parameters for achieving the best results for maximum cost-effectiveness. With this claim, Shimadzu contin-

uously extends and redefines technological limits. This is confirmed by numerous world premieres and awards. All analyzers are manufactured in accordance with internationally established standards (for instance ISO), so users can work according to GLP, GMP, FDA or

Pharmacopoeia. Shimadzu offers interdisciplinary solutions for many application areas. The motifs shown above represent Shimadzu's claims to trade fairs, conferences and symposia – and offer users the added value in their work for their clients.

## How to make invisible things visible?

Worldwide unique: The new Hyper Vision HPV-X camera with 10 million frames per second

**U**ltra-high-speed recordings with video cameras have become standard tools for characterizing and quantifying

of high speed events in order to make invisible things visible. Shimadzu's HPV-1 and HPV-2 enable recordings at up to one

million frames per second (fps) while maintaining excellent spatial resolution at any recording speed.



The need to capture high-speed phenomena comes from academic institutions and research institutes as well as from various manufacturing industries. Applications include fuel injection in combustion engines, dispersion of ink drops by inkjet printers, studies on hydrodynamics, destruction processes, shock wave development and visualization of machining processes as well as fast chemical reactions.

A simple real-life example helps to illustrate the advantage of the ultra-high-speed recording in comparison to conventional video camera systems:

When a high-speed train passes the station at 180 km/h, the human eye hasn't time to recognize any detail, neither of the train itself nor of anything inside. A conventional video camera with a recording speed of 25 frames per second cannot record individual passengers inside this moving train. A high speed video camera with a recording speed of 4,500 fps (180 times faster) allows the visualization of every detail of the train and every single passenger inside.

aerospace industries in order to understand the generation and distribution of shockwaves on the wings of airplanes. At supersonic flights with velocities of 343 m/s (1,225 km/h) the shockwaves are the subjects of intensive studies.

CFRP (carbon fiber reinforced plastics) have also attracted attention as high-performance composite materials with high strength and rigidity, yet low in weight. They are applied in various fields such as automobiles and civil engineering. With high-speed video cameras it is possible to record specimen fracture, allowing capture of image data of the instant the CFRP material is fractured.

#### Unique: 10 million fps – in high resolution

Nowadays, many phenomena in scientific applications elapse in an instant, leaving no clue to their solution. Ultra-high-speed video cameras record such moments, enabling visualization through slow-motion replay.

The latest development in this application field is the Shimadzu

cessing. The conversion from light to an electric charge can be realized in a very fast and efficient way, but the processing of the analog signal (electric charge) is however the most time-consuming step.

With the high-speed CMOS sensor, the photo diode is connected directly to an in situ-memory. Since there is no sequential read-out operation, there is also no output amplifier limitation, thereby enabling high-resolution recording even at ultra-high speeds.

#### High speed meets high resolution

The new HPV-X can record 128 frames, 20 % more than the HPV-2 at a resolution of 400 x 250 pixels. In HP-mode a double-memory function enables recordings of 256 consecutive frames for recording of even longer periods. This gives the user the choice of prioritizing either resolution or recording time.

While other high-speed camera systems lower the resolution as recording speed increases, the

per second using 100,000 pixels or at a speed of ten million images per second using 50,000 pixels.

In contrast to other high-speed camera systems, the HPV-X is very compact and easy to use. Just like previous models in the Hyper Vision series, the simple system configuration consisting of the HPV-X camera head connected by cable to a laptop computer offers a compact and highly portable design making on-site setup especially easy. The same HPV software that has been so popular with current users all over the world is also retained. It features intuitive and easy-to-understand setting screens making it easy to capture ultra-high-speed videos.



Figure 1: HPV-X system configuration consisting of camera head and power supply unit (Laptop PC optional accessory)

It is even possible to see the titles of books that people in the train are reading.

Many physical phenomena in real life are much faster than the top speed of a high-speed train. During explosions a sudden increase of pressure generates a shock wave. To visualize such effects, recording speeds of 1,000,000 fps or even higher are needed. Shock-wave research is important for the

Hyper Vision HPV-X High-Speed Video Camera equipped with a newly developed proprietary high-speed CMOS image sensor, allowing ultra-high-speed recordings at 10 million frames per second. This CMOS sensor is a unique feature and a key differentiator in comparison to other high-speed cameras systems.

The challenge for all ultra-high-speed cameras is the signal pro-

HPV-X maintains the same high resolution even when recording speed is increased. Analog data is stored within the sensor and processed afterwards. This feature is included in all Shimadzu Hyper Vision high-speed video cameras, allowing recordings of ultra-high-speed phenomena in far more detail.

The HPV-X is able to record at a speed up to five million images

# Bitumen – it's all in the mix

## FTIR spectroscopy determines quality differences



**B**itumen is used in many everyday products, like carpeting or roofing paper. Bitumen is best known from road construction; together with mineral additives, bitumen makes asphalt.

The quality of the bitumen also determines its use. Through the addition of polymers, such as polypropylene and styrene-butadiene-styrene, the material can become very viscous. The blend with polypropylene is called APP plastomeric bitumen and the blend with styrene SBS is called elastomeric bitumen. These blends are used as sealing materials and in

roofing paper. Another polymer is EVA copolymer (ethylene-vinyl-acetate), which is for instance applied in polymer packaging as an odor barrier.

### Quality control using FTIR

Bitumen is the residue from vacuum distillation of mineral oils. The quality of the bitumen is an important analytical criterion in order to be able to better control the amounts of additive used. Non-destructive infrared spectroscopy can be used for the analysis. Combined with single-reflectance accessories from ATR family, this can be easily implemented.

As bitumen can be very viscous by nature, a diamond ATR unit is recommended. Diamond is robust against the cleaning procedures that are required after measurement. One drop of the sample material is sufficient for the 2 mm measuring window, which minimizes complex cleaning procedures. The sample is heated to soften it in order to enable a better contact with the measuring window. This technique makes it possible to control the production process.

### Detection of the additives

In this application, various types of bitumen (base bitumen), graded as 1/10 and 70/100, are explored. The values reflect how the bitumen performs during the specific needle test. The needle penetrates the surface, whereby 70/100 refers to a penetration depth between 70 and 100 tenths of a millimeter. A table with typical values for the needle test and other specifications is presented below (table 1).

The presence of the additives can be easily verified in the infrared spectra. Base bitumens and modified base bitumens are compared with each other.

The spectrum of the blend clearly shows changes in signal structure. The signals at  $630\text{ cm}^{-1}$  that can be

attributed to the EVA polymer, are particularly noticeable. For comparison, the 1/10 base bitumen is shown in figure 2.

An additional group of signals at  $1,693\text{ cm}^{-1}$  clearly illustrates the difference: it is a signal of the carbonyl group ( $\text{C}=\text{O}$  vibration). Using subtraction spectroscopy, it is possible to isolate the additive from the spectrum of the blend. Figure 3 shows the result of this subtraction and the subsequent library search for the identification of the EVA polymer. The subtraction is performed with a factor 1 and can also be further refined.

Quality control of unknown additives can be carried out with the help of infrared spectroscopy, wherein the base bitumen is compared with the unknown bitumen.

The base bitumen in the example is graded as 70/100. Figure 4 shows the base bitumen and a modified base bitumen. Visual comparison shows deviations at  $630\text{ cm}^{-1}$ , which cannot be attributed to the EVA polymer.

In comparison with the associated libraries, the contamination analysis function revealed, cross-linking to polyethylene as main components, which does not present any problem for bitumen, and additives based on phosphate salts.

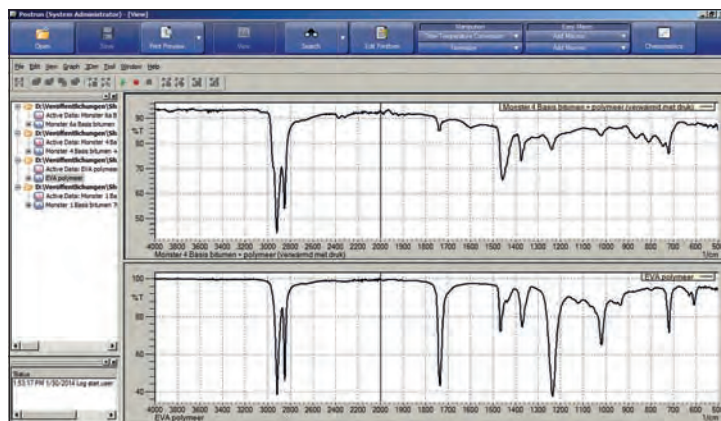


Figure 1: Two infrared spectra of a bitumen with additive (above) and an EVA polymer (below)

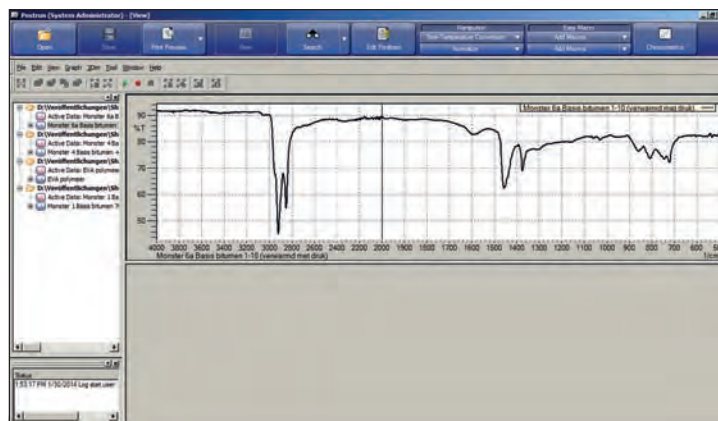


Figure 2: Infrared spectrum of a 1/10 grade base bitumen



# New family member, improved possibilities

## SALD-7500 for measuring nanoparticles



The SALD 7-series has been developed specifically for highly sensitive particle measurement in the submicron and nanometer range. The successful SALD-7101 is now being replaced by the SALD-7500.

The SALD-7500 not only builds upon the excellent qualities of its predecessor – Fourier Optics, one light source, one large detector, and one measuring theory – but also adds new and improved features.

Using additional detector elements, the measuring range is now extended to 7 nm - 800 µm. Samples can be measured in the concentration range of 0.1 ppm - 20 %. Through revised and in part completely new accessories and new software, the SALD-7500 opens up entirely new application areas.

The main application area continues to be the measurement of nanoparticles and nanomaterials that are present in, for instance, sunscreens, clothing, wall paints and even foods. These tiny helpers disinfect or protect the human body from hazardous UV-radiation, for example.

Nanoparticles also impart new properties to materials. Their effects (so-called nanorisks) on the human body are, however, still quite unknown. The actuality of this problem is illustrated by the fact that the EU has, already in mid-2013, introduced labeling require-

ments for nanoparticles in cosmetic products. In 2014, this could be followed by labeling requirements for foods. Labeling, risk assessment and measurement of nanoparticles will become more and more important in the future.

**Accessories increase range of applications**

The 'Aggregation Property Evaluation System for Biopharmaceuticals' (Aggregate Sizer) offers the new possibility not only to quantitatively determine particle size of biopharmaceutical products, but also their concentrations in the range of 100 nm - 10 µm. With a continuous measuring function, changes in size or mass can be monitored in real time. Measurement data can be stored at 1-second intervals. Mechanical stimulation accelerates the agglomeration process.

Nanobubbles are another exciting new application. Here, the tiniest long-term stable air bubbles in water impart amazing new properties to this solvent. Water treated in this way can even exhibit fat-dissolving properties – as if tensides had been added. But as the water is free of tensides, it is now an ideal cleaning solvent for semiconductors.

New and yet fully integrated in the family – the SALD-7500 is ready to take on a wide range of applications.

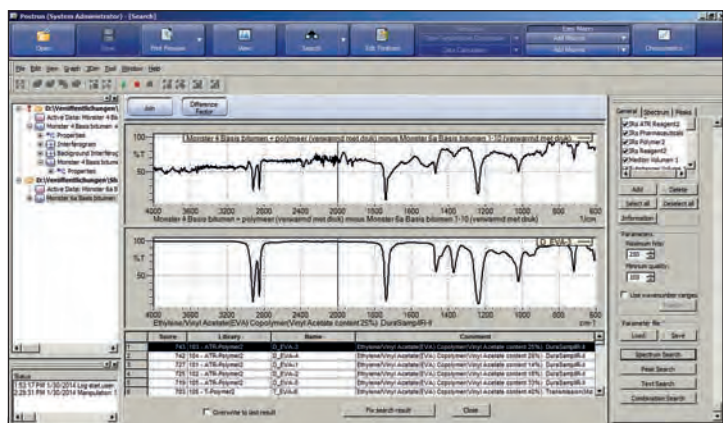


Figure 3: Result of the subtraction of the base bitumen from the modified bitumen and the result of the subsequent library search for identification of the material

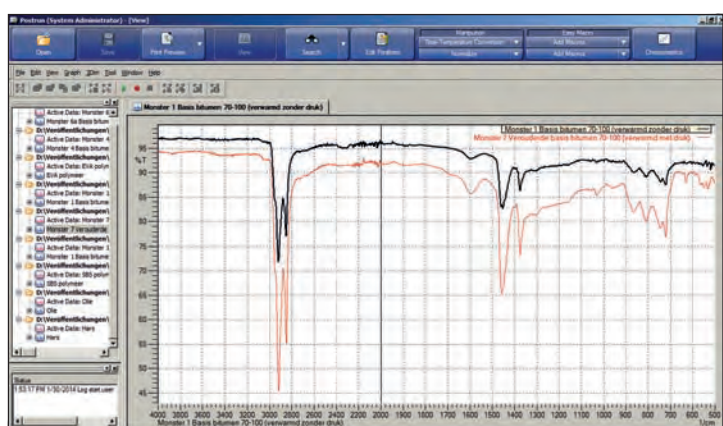


Figure 4: Spectra of a 70/100 grade bitumen with additive (red line) and a base bitumen (black line)

The signal at 630 cm<sup>-1</sup> correlates with the vibrations of these salts.

### Conclusion

The analysis examples presented here clearly show that infrared spectroscopy is suitable for the analysis of various bitumens. Bitumen blends can be analyzed and quality control can be implemented via comparison of the infrared spectra. Shimadzu's IRAffinity-1S and its Golden Gate single-reflection unit, equipped with a diamond window, have been used.

**Further information**  
about the IRAffinity-1S:  
[www.shimadzu.eu/iraffinity-1s](http://www.shimadzu.eu/iraffinity-1s)



	Bitumen 70/100	Bitumen 70/100 + modified clay	Bitumen 20/300
Pen (dmm)	95	88	26
Softening point (°C)	44.6	46.2	56.8
Retained penetration (%)	67	80	69
Increase in Softening point (°C)	4.2	2.2	4.0
Viscosity at 135 °C (Pa s)	0.24	0.56	0.87

Table 1: Typical influence of the additives in bitumen compared to the base bitumen

# LabSolutions concept – one platform for all instruments

## New: LabSolutions DB and CS Software

LabSolutions has been the standard software for new HPLC, UHPLC and GC systems for many years. Shimadzu's LC/MS and LCMS/MS systems operate under the same platform. In this way it is possible to mutually utilize functions and system controls, i.e. there are no restrictions regarding HPLC control or the use of the photodiode-array detector for the MS and MS/MS version. This simplifies, for instance, accessing or switching to a new or unfamiliar analysis technique.

The aim is to gradually enable all Shimadzu's analytical systems to be operated under this software. A unified user interface simplifies laboratory operation.

The new LabSolutions DB and CS software packages are available for use in conventional chromatography and Fourier transform infrared spectroscopy (FTIR) and can be operated from a common user interface in a laboratory network.

### One software for system operation, data acquisition, processing and storage

An integrated database is the heart of the new LabSolutions DB (database version) and CS (client/server) software packages. Depending on varying customer requirements in different countries, Oracle as well as MS-SQL is supported.

For the user, little changes at first glance – method parameters, data descriptions and re-analysis functions remain at the same location and menu structures also remain unchanged. Users will quickly get used to departing from the file-based structure.

This makes it possible to store data of all analytical instruments

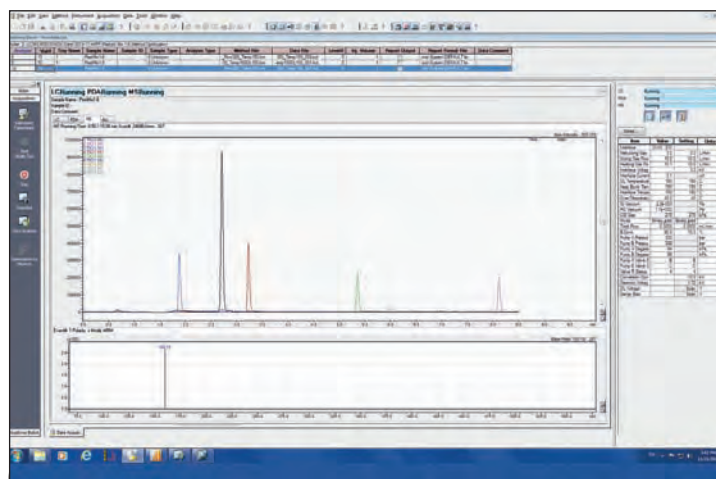


Figure 1: Real time window LCMS/MS and LC

consistently in a common database and to enable faster retrieval in a common report. At the same time, data backup and recovery are becoming more user-friendly. On the positive side for the user, this means one software package only for operating various instruments, their data acquisition, data processing and data storage. Individual functions are clearly still dependent on the analytical system used, but essential workflows and routines can be used without any additional learning effort.

The new LabSolutions DB and CS packages support all functions required by the FDA guideline

21 CFR Part 11: User administration and access rights, audit trails of all actions carried out on the analytical data and in the software, as well as an electronic signature of the data. All of these features which up to now were only available via the CLASS Agent in combination with the respective software.

Existing CLASS Agent systems and their databases can be integrated into a LabSolutions CS database. Just as a reminder: primary responsibility for compliance lies not with the software itself and its functions, but rather with the customer/software user

who must ensure that the entire system is compliant. The software only facilitates compliance.

It is still possible to integrate data from other instruments, e.g. laboratory balances and UV spectrophotometers, in the database via the CLASS Agent. Bearing in mind the compliance issue already mentioned, this is a convenient solution for report generation since it simplifies the compilation of reports, including insertion of additional calculations, graphs and results of various detectors with the help of Microsoft Excel®. In addition to the Agent report which helps to compile reports directly in Excel while allowing for adjustments, a multi-data report creation function is optionally available for the advanced user.

This function can merge all of these data into an integrated report. At first glance, this seems to be a complex task. However, the time invested to explore the various options is worthwhile, as specific calculations can be implemented quickly and in accordance with individual requirements, and can be saved as templates. For entry-level users a Flash animation is available which explains the individual steps from the idea of the report content up to the

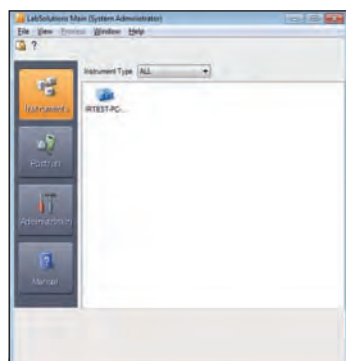


Figure 2: Home screen LabSolutions CS (including IRTSolutions)

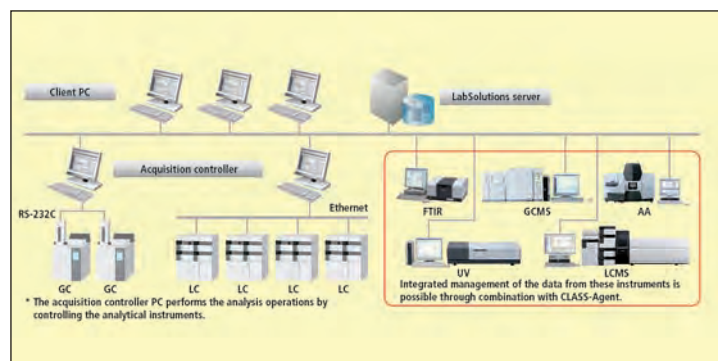


Figure 3: Agent concept



finished template – with time windows for copying and participating. For all users already familiar with Microsoft Excel®, the Agent Report and its template based reporting is still available.

### Controlling instruments of other manufacturers

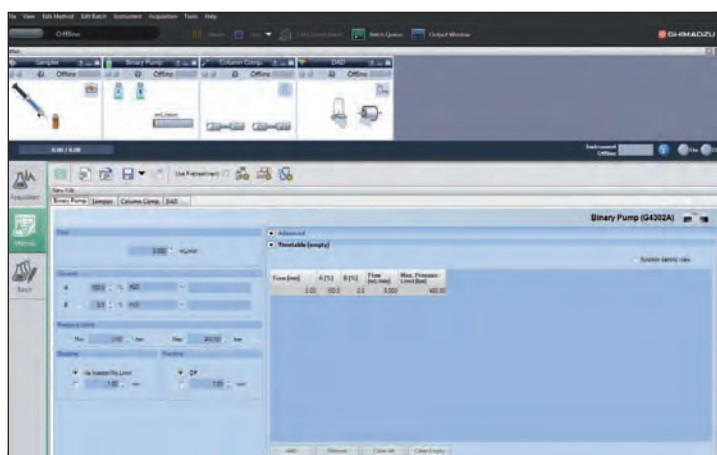
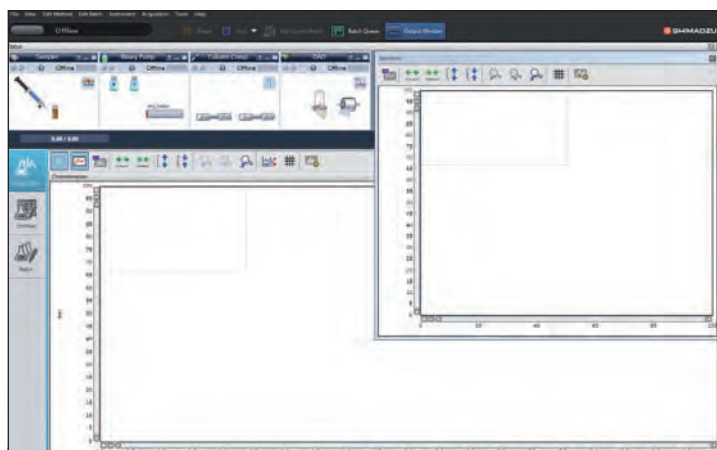
The distinguishing feature of current chromatography data systems is the control of instruments of other manufacturers – each user can control their analytical systems via one single software interface. This requires an exchange of sensitive information between competitors, which does not always proceed as quickly and as easily as all parties would like.

In mid-2013 Shimadzu and Agilent agreed to share the control codes for their LC and GC instruments. Control of Shimadzu's prominence

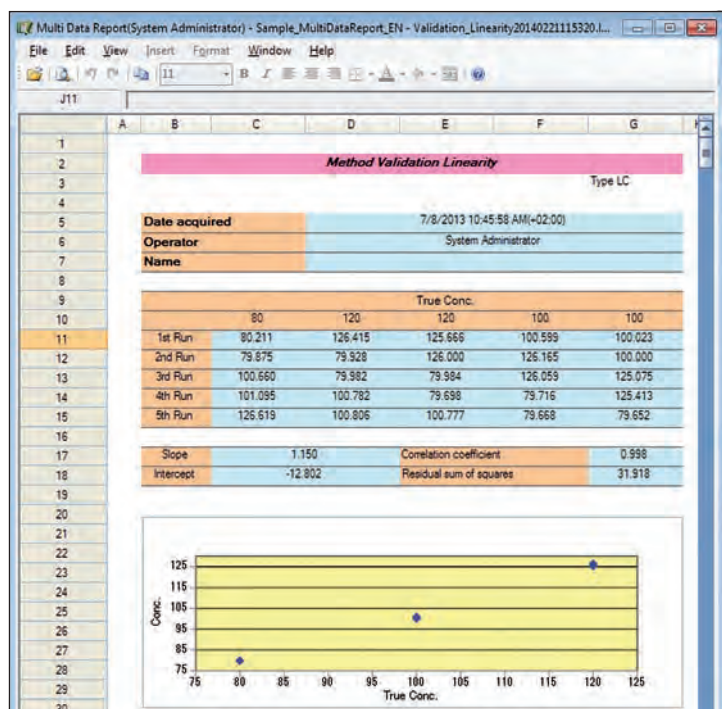
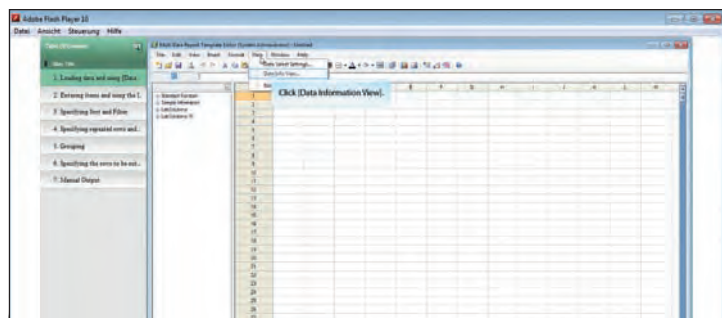
and Nexera is carried out via Agilent's OpenLAB Software; conversely, LabSolutions CS also controls Agilent's LC- and UHPLC systems. This is possible via the consistent implementation of the ICF/RC.Net concept introduced by Agilent several years ago.

### LabSolutions goes iPad

In modern networks, it is common to make all applications available to all users via one terminal server in order to avoid the need for installation of memory- and resource intensive programs on many PC's, while ensuring a high degree of data security. Moreover, this method offers the advantage that in a regulated environment only one PC needs to be validated, as all users log on to this PC (terminal server) and do not work with the data and this program on



Figures 5a and 5b: Screenshot of Agilent's LC control



Figures 4a and 4b: Multi data report and Flash screenshot

their local computers. When installed on a terminal server, the LabSolutions CS software offers the possibility to start data acquisition or re-analysis and data evaluation.

In such a network setup, it is also possible to use the LabSolutions software on an iPad via special apps – an attractive option when working in a confined laboratory space. And since electronic laboratory notebooks and protocols have already been implemented in e-lab notebook applications, connection with a chromatography data system is surely only a matter of time.

The new DB and CS versions of the LabSolutions software meet a wide range of requirements: an attractive entry-level version (LabSolutions LITE), a standard workstation for LC/GC up to a database as well as network design. Since upgrades to higher versions are possible for all versions, a user can switch from a 'simpler' version to a more com-

plex software version depending on available budget and the task at hand.

### LabSolutions DIRECT – the smartphone can carry out LC

Whoever prefers to remotely operate and monitor the HPLC systems can control via the current LabSolutions software version 5.57 (and of course also later versions) the LC instruments, browser-based and wireless via smartphone.

The following instruments and systems are currently supported: iPhone 4S, iPhone 5, iPod touch (4th generation), iPad (1st to 4th generation) as well as Android 2.3 to 4.0; for LC systems a new system firmware may be required.

# New accessory for UV-VIS spectrophotometers

TCC-100 – thermoelectrically temperature-controlled cell holder



Figure 1: UV-1800 with TCC-100

**P**art of Shimadzu's vast range of accessories for UV-VIS spectrophotometers is the wide variety of cell holders. They are available with or without temperature control and allow system configurations tailor-made for all requirements of the environmental, pharma, food and life science applications, and many more. Whether a single standard cell at room temperature is used or a cell holder for high temperature measurements is required – the Shimadzu product portfolio covers each analytical problem.

The latest release in the European market is the TCC-100, a thermoelectrically temperature-controlled single cell holder. The TCC-100

includes a reference cell using a Peltier element for temperature control and does not require an external cooling water device. The temperature control range is from 7 °C to 60 °C with a precision of  $\pm 0.1$  °C.

In combination with a spectrophotometer such as UV-1800 working in kinetics mode, the system can be used to measure enzyme activity. Tests like these are very sensitive to temperature. It is therefore absolutely necessary to maintain a stable temperature during the sample measurement. The ideal solution for regulating the cell temperature is a Peltier-controlled cell holder, without external water circulator.

Figure 1 shows the UV-1800 in combination with CPS-100. Furthermore, the TCC-100 can be used in the following spectrophotometers: UVmini-1240 series, UV-2600/UV-2700 and UV-3600.



# Searching for elemental traces in petrochemicals

## The high-precision ICPE-9000 inductively coupled plasma optical emission spectrometer

To evaluate elemental pollution in petrochemical applications, it is necessary to measure organic samples in a direct way without any dilution, since concentrations may already be very low. For fast multi-element analysis, Shimadzu's ICPE-9000 is most suitable.

The Ar/O<sub>2</sub> Mixed Gas Supply Kit is recommended for obtaining highest sensitivity in the measurement of organic solvents. This kit uses a special 4-way Quadruple torch (figure 1). An additional gas (Ar + O<sub>2</sub>) is mixed with the carrier gas flow, resulting in high oxidizing power. This allows the

measurement of trace levels by reducing the background noise from the organic solvent, which is now separated more easily.

The oxygen kit can be helpful with many different types of organic solvents, such as kerosene, xylene, methyl isobutyl ketone (MIBK), isopropyl alcohol (IPA), and ethyl alcohol.

To show some typical limits of quantification (LOQ), different elements were analyzed in toluene (figure 2). In this case, the limit of detection (LOD) was about a third of the LOQ.

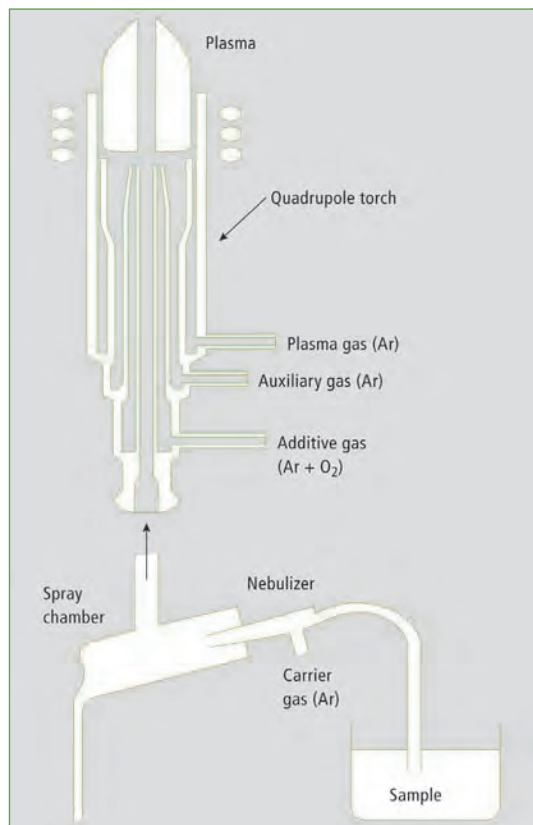


Figure 1: Sample introduction and torch

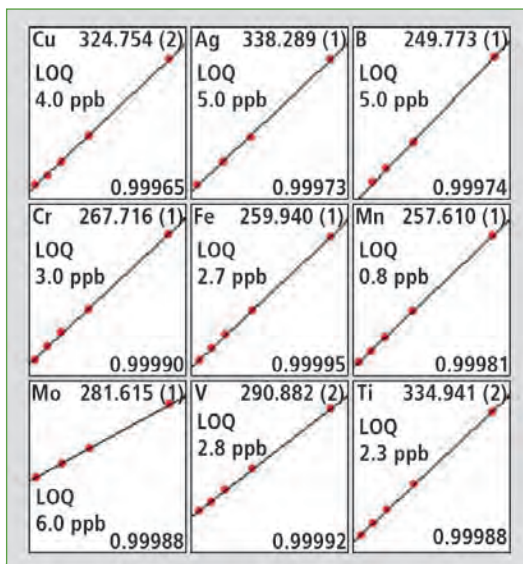


Figure 2: Calibration and LOQ of some elements in toluene

An application note focussing on highest sensitivity of tin (Sn) in toluene is available by scanning the QR code:

[www.shimadzu.eu/sites/default/files/AN\\_SCA\\_115\\_021%20Toluene%20Oxygen%20Kit.pdf](http://www.shimadzu.eu/sites/default/files/AN_SCA_115_021%20Toluene%20Oxygen%20Kit.pdf)

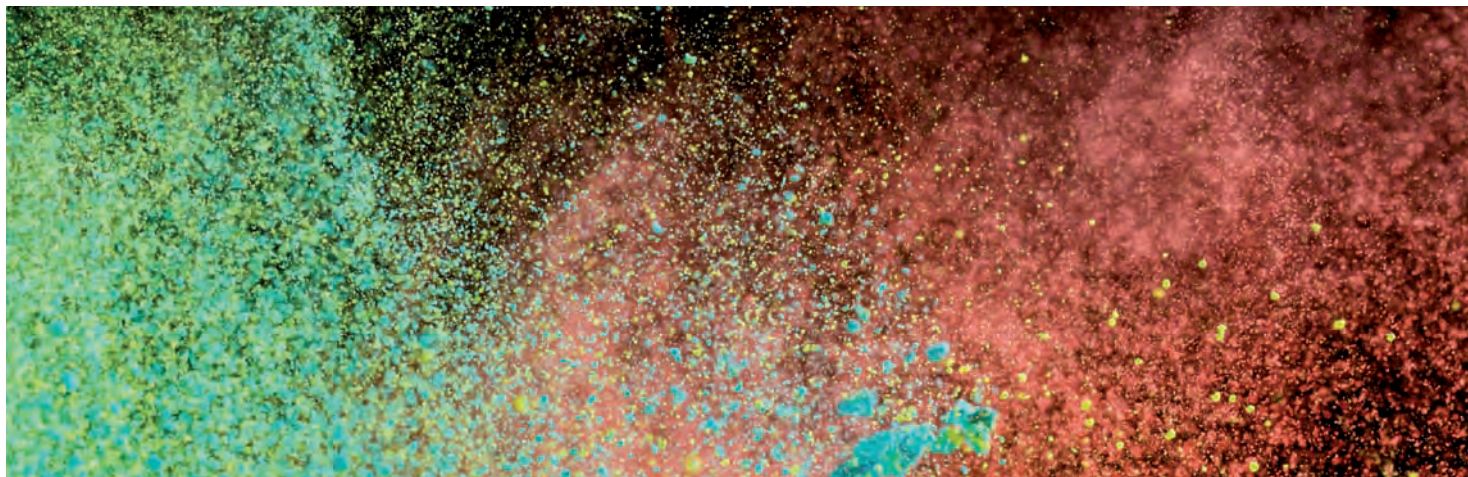
Simply scan the QR code.

Application Note 'Determination of Trace Elements in Toluene using ICPE-9000 and Oxygen Kit'



# Exact measurement of color

New software for color determination in the industrial sector with direct control using Shimadzu's UV-VIS spectrophotometers



Color can be of decisive significance for the marketing success of a product. Especially when using natural or recycled raw materials with fluctuating chemical composition, color can vary strongly – for instance in the production of packaging glasses. Continuous and objective control of the color effect is therefore an essential precondition for an unchanging and reproducible quality.

## Color measurement

Color is not just a physical characteristic of materials but rather a

sensory perception that is strongly dependent on environmental conditions (especially on lighting) and the observer (human). Color is perceived, so to speak, in the eye of the observer. For objective determination of color values, the spectral sensitivity of the human eye for the three primary colors red, green and blue (there is a corresponding number of sensory cell types in the retina) was determined by empirical experiments in the 1930's, and the CIE Color Space was published by the International Commission on Illumination.

To this day, these so-called color-matching functions have been the basis for color determination according to ISO 11664 and other standards.

For the calculation of color values, a suitable spectrophotometer (for instance Shimadzu's UV-1800) was used to record a transmission or a reflection spectrum (depending on the application) in the visible range of the electromagnetic spectrum (380 to 780 nm). This spectrum is subsequently weighted against a standardized light source (D65 for daylight or A for

incandescent light) in order to take into account the influence of the illumination. Subsequently, the spectrum is successively weighted against the three CIE color-matching functions to determine the red component (X), the green component (Y) and the blue component (Z) of the spectrum. Using these three values, any color can be unequivocally characterized.

In practice, however, the tristimulus values X, Y and Z are usually not applied directly. Instead the CIELAB system, in which color perception is specified by the

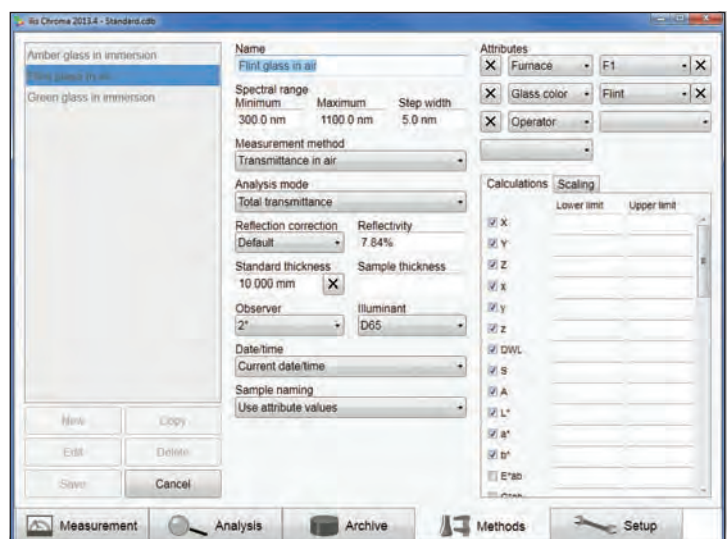


Figure 1: All measurement parameters necessary for measurement are summarized in methods



Figure 2: During measurement, the corrected spectrum is displayed in addition to the raw data



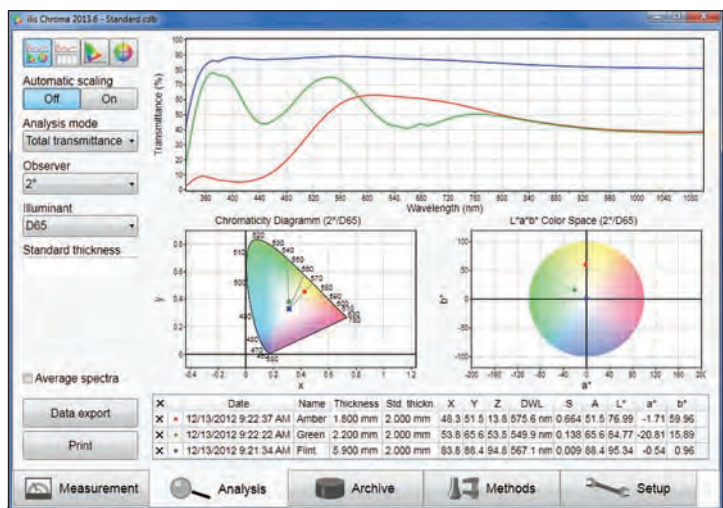


Figure 3: In the analysis mode, all results are numerically and graphically displayed in a well-organized manner

three values  $L^*$  (brightness),  $a^*$  (red-green ratio) and  $b^*$  (blue-yellow ratio) has become established. These values, however, are mathematically derived from the tristimulus values  $X$ ,  $Y$  and  $Z$  so that the information content is the same. Alternatively, the CIE<sub>xy</sub> system in which color perception is expressed by three values  $DWL$  (dominant wavelength),  $S$  (saturation) and  $A$  (brightness) is still applied in many industrial sectors. But these values are also based on the tristimulus values  $X$ ,  $Y$  and  $Z$ .

### Industrial practice

In industrial production processes, color is usually determined at random on the basis of representative samples. Color measurement is often carried out in an on site labo-

ratory, but more frequently color determination is moving closer to the production line, in order to be able to react faster in case of color deviations. Personnel, which has been specially trained for this task, is often not available at the production line, whereby the demands on the software significantly increase. The software must be easy to use, fast to operate and must be able to rule out operating errors as well as possible. In order to recognize trends quickly and to be able to immediately intervene, statistical evaluation in the form of quality control charts is indispensable. The data should, therefore, be automatically transferred to an on site data acquisition system. Ideally, the software itself features an integrated database and corresponding evaluation options.

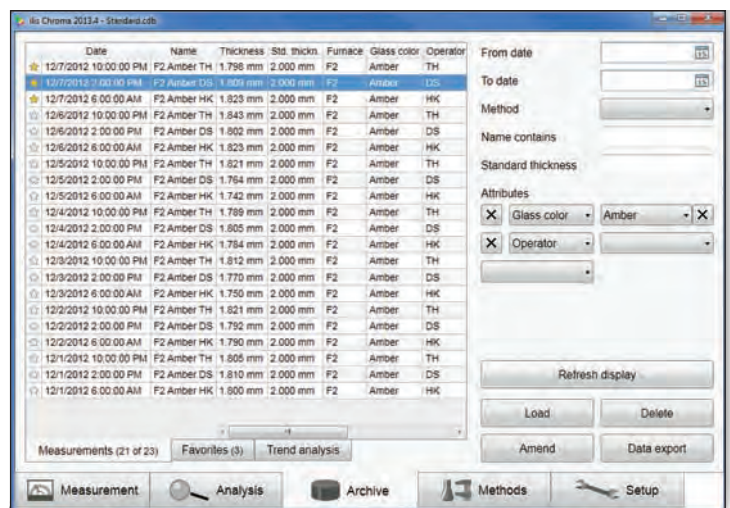


Figure 4: All measurement results are archived in an integrated database

### Color measurement using Chroma

The Chroma software was developed to fulfill the above requirements in routine analysis. The principal feature of the software is the clearly structured user interface that is tailored to the workflow and user roles. All required measuring parameters for the measurement are summarized in so-called methods (figure 1). Prior to measurement, only the missing sample parameters must be added – for example the sample thickness for transmission measurements. The transformations defined in the method, such as conversion to a fixed standard thickness, are already taken into account during the measurement and the corrected spectrum is displayed in addition to the raw spectrum.

After data storage the user is automatically directed to the analysis mode, in which all results are expressed in terms of numbers and graphs (figure 3). Here, the possibility exists to print the results or to export them to other programs for additional processing. Furthermore, certain parameters can be modified later on in the analysis mode, without affecting the results that are already stored in the database.

time. To organize the measurements, it is possible to define any attribute according to which can be filtered in the archive (figure 4). Alternatively to display in tabular format, the data can also be graphically displayed in the form of trend diagrams (figure 5). This way, it is possible to easily recognize trends in the development of certain color values and to take suitable measures.

### Summary

Using the Chroma software package and a Shimadzu UV/VIS spectrophotometer, spectral characteristics of glasses and other materials can be accurately determined and color values can be calculated and graphically displayed. Chroma combines measurement and evaluation with a high-performance database in which all results are stored in a structured manner. Chroma fulfills the requirements in routine analysis and quality control and meets the highest demands in research and development through its flexible evaluation options and statistical functions. Chroma is always intuitive, simple and reliable to use.

### Author:

Henning Katte, ilis GmbH Erlangen

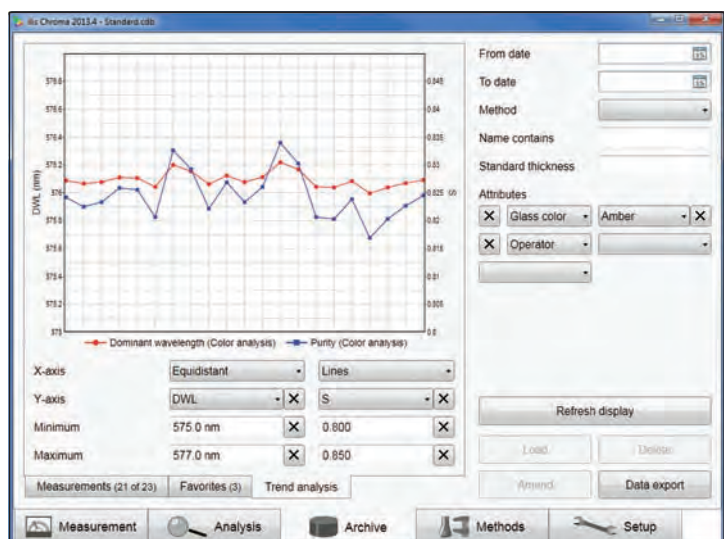


Figure 5: The trend view shows the development over time of a selected color value

# Determination of microbial

## TOC in soil science



Fertile soils contain a multitude of microorganisms. They are responsible for the degradation of organic substances and protection of the nutrient cycle. To evaluate soils in terms of their biodegradability and their fertility, the microbial biomass of the soil, i.e. the organic carbon fraction that is bound to these tiniest organisms, is determined.

The fumigation-extraction method is a commonly used method for the determination of microbial biomass in soils. The microbial biomass is described regarding extractable organic carbon compounds before and after killing off the microorganisms.

treated with chloroform gas in a suitable apparatus (e.g. a desiccator) over an extended period of time of at least 24 hours. This destroys the cell walls and kills off the microorganisms. After fumigation, the chloroform remaining in the soil is removed.

### DOC is determined first

Subsequent to fumigation, each fumigated subsample and one non-fumigated soil sample is mixed with a 0.5 M (mol/L) potassium sulfate solution and then shaken. After filtration of the eluate, the DOC (dissolved organic carbon) of the extracts is determined. Since experience has shown, not all cells are destroyed and extracted, and an empirical correction factor is additionally applied. (An exact description of the fumigation-extraction method is found in the EN ISO 14240-2:2011 standard).

DOC analyses of the potassium sulfate extracts are carried out using a TOC analyzer. In this procedure, the extract is mixed with an acid to remove the inorganic carbon fraction, whereby carbonates and hydrogen carbonates are converted to CO<sub>2</sub>. The carbon dioxide is purged from the sample using a purging gas. An aliquot of the pretreated extract is subsequently injected onto a hot

In the fumigation-extraction method, a subsample of the soil is

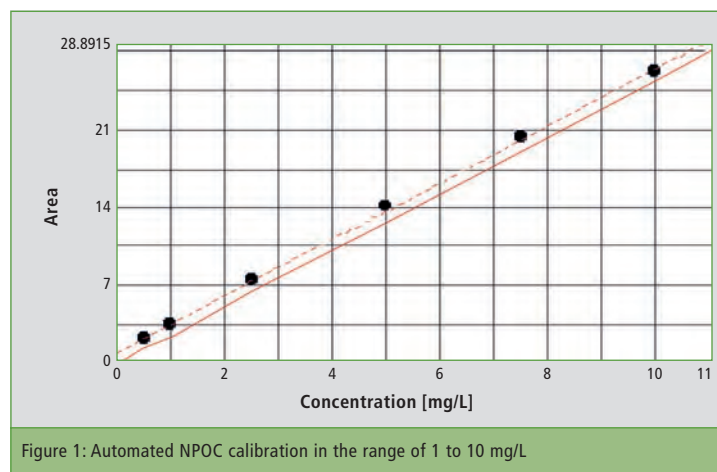


Figure 1: Automated NPOC calibration in the range of 1 to 10 mg/L



# biomass in soils

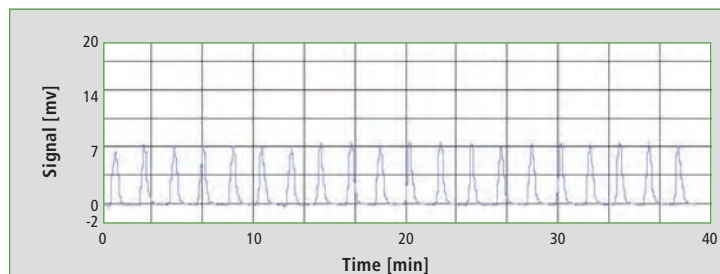


Figure 2: Injection 1 - 20

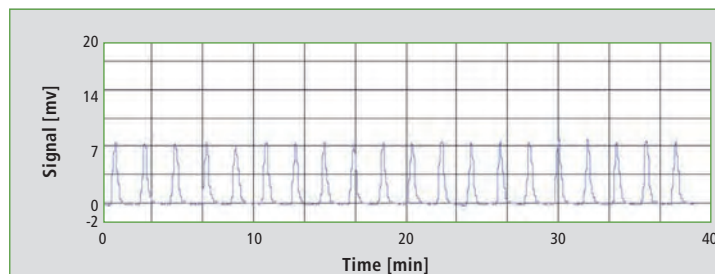


Figure 3: Injection 581 - 600

platinum catalyst, whereby the organic substances are oxidized to CO<sub>2</sub>. A carrier gas transfers the carbon dioxide to an NDIR detector, which detects the amount of CO<sub>2</sub>.

## Kit for high salt loads

For many TOC analyzers, the high salt loads of the extracts can, however, cause problems. The extraction solution alone has a salt load of approximately 87 g/L. High salt loads can lead to clogging of the catalysts and the combustion tubes. When the combustion temperature is too high, the salts form a melt that blocks the active sites of the catalyst.

Therefore, Shimadzu's TOC-L uses a catalytic combustion temperature of 680 °C, which is lower

than the melting point of most salts. In addition, a special kit for high salt load samples is available for such measuring tasks. It consists of a combustion tube featuring a specific geometry and a unique catalyst mixture. This particular catalyst can handle up to 12 times more salt than a conventional catalyst.

## Long-term test with 600 injections

A long-term test should show that an application, such as the analysis of soil extracts from a 0.5 M potassium sulfate solution can be carried out problem-free using a kit for high salt load samples. For this test, a 0.5 M K<sub>2</sub>SO<sub>4</sub> solution (corresponding to 87 g/L) was injected 600 times onto the kit for high-salt samples.

## Method parameters

**NPOC preparation:** automated using the ISP module of the TOC-L analyzer

**Acidification:** 2 %

**Sparging:** 6 minutes

**Injection volume:** 50 µL

**Calibration:** 6-point calibration using the automated dilution function, from a stock solution in the range of 0.5 mg/L to 10 mg/L.

## Results

Even after 600 injections, the peak shapes remain exactly the same as at the start of the injection series. The results remain stable over the entire time period. As one can see, the TOC-L in combination with the kit for high salt load samples is highly suitable for TOC determination of salt-containing samples, such as soil extracts from a 0.5 M potassium sulfate solution.

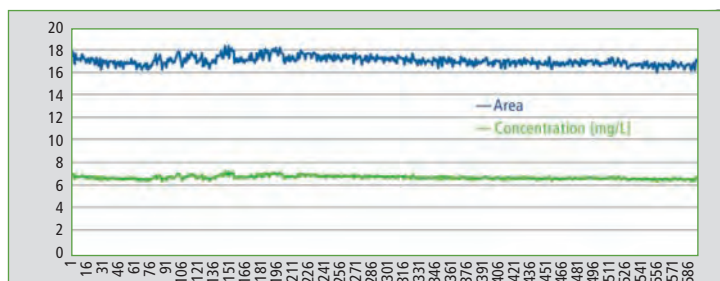


Figure 4: Sequence (area and concentration) of the 600 injections

	Area	NPOC concentration [mg/L]
Mean value	17.01	6.66
Standard deviation	0.392	0.153
Standard deviation in %	2.3	2.3

Table 1: Statistical evaluation of the 600 injections

## IMPRINT

**Shimadzu NEWS, Customer Magazine of Shimadzu Europa GmbH, Duisburg**

### Publisher

Shimadzu Europa GmbH  
Albert-Hahn-Str. 6-10 · D-47269 Duisburg  
Phone: +49-203-76 87-0  
Fax: +49-203-76 66 25  
shimadzu@shimadzu.eu  
www.shimadzu.eu

### Editorial Team

Uta Steeger  
Phone: +49-203-76 87-410  
Ralf Weber, Tobias Ohme

### Design and Production

m/e brand communication GmbH GWA  
Düsseldorf

### Circulation

German: 6,050 · English: 7,280

### Copyright

Shimadzu Europa GmbH, Duisburg,  
Germany – April 2014.

Windows is a trademark of Microsoft Corporation. ©2014

Apple Inc. All rights reserved. Apple, the Apple logo, Mac, Mac OS and Macintosh are trademarks of Apple Inc.

# Amino acids – fast and simple identification

## UF-Amino Station enables high-throughput analysis

Amino acids are of vital importance for human life. They are the basis for each and every metabolic pathway in the body, since they serve as transport or storage medium. Amino acids are components of protein-containing foods and are also used in cosmetics, such as skin care products and shampoos.

Proteinogenic amino acids are the building blocks of proteins in living organisms. The 20 L-amino acids are each encoded by three nucleic acids. Of these, 12 amino acids are synthesized by the human body, for instance by microorganisms that live in the human digestive tract. The remaining 8 amino acids are essential for humans, meaning that they must be absorbed from foods containing animal proteins or a suitable combination of plant proteins.

Valine, methionine, leucine, isoleucine, phenylalanine, tryptophan, threonine and lysine are essential amino acids for humans. Semi-essential amino acids, however, only need to be absorbed from foods in certain situations, for instance during growth or serious injury. The remaining amino acids are either synthesized direct-

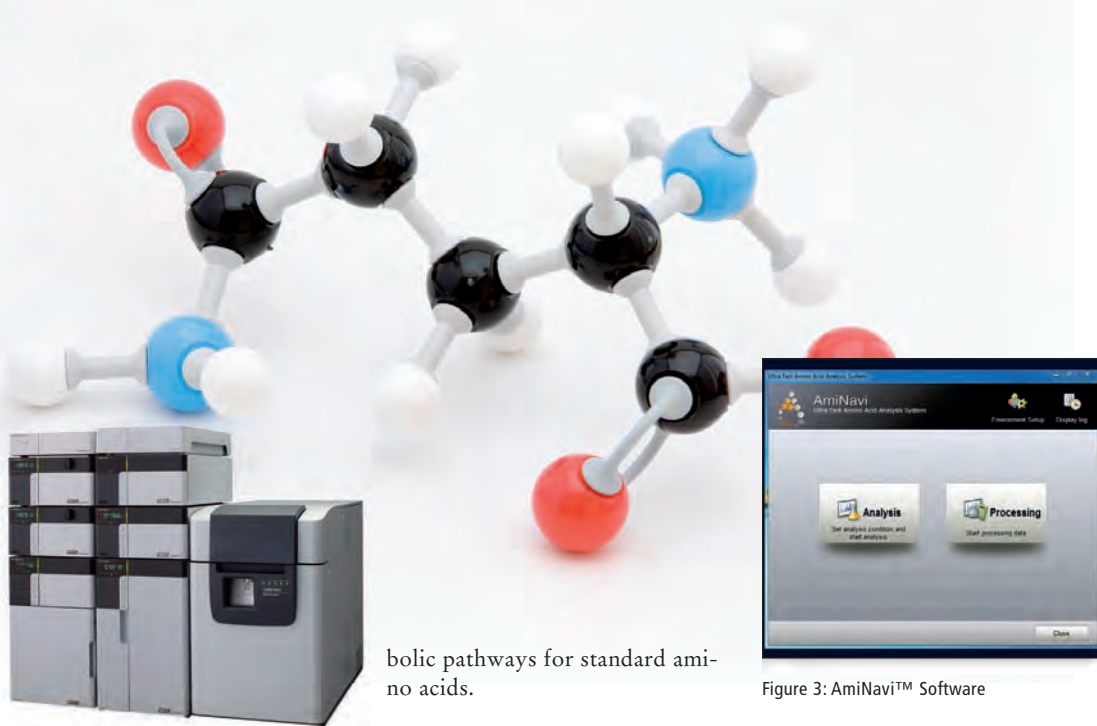


Figure 1: UF-Amino Station

ly or derived from other amino acids through modification and are, therefore, referred to as non-essential.

In addition to 20 standard amino acids, there are many non-standard amino acids which often occur as intermediates in the meta-

bolic pathways for standard amino acids.

### Amino acid analysis

There are many possibilities for analyzing amino acids. The most commonly used methods are carried out via automated pre-column or post-column derivatization. The UF-Amino Station, developed in cooperation with the international food manufacturer Ajinomoto, uses automated pre-column derivatization with a special reaction reagent.

Figure 2 shows the schematic design of the UF-Amino station. All necessary reagents such as amino acid standards, internal standard, reaction reagents and eluents are commercially available from Wako Chemicals, Neuss, Germany. After mixing of all the reagents, the product mixture is left standing in the reaction unit for a short time at 60 °C to complete the derivatization. The derivatized amino acids obtained are injected onto the analytical column (Shimpack UF-Amino 2.0 µm, 2.1 x 100 mm) via an injection loop, installed within a switching valve, and subsequently detected using the

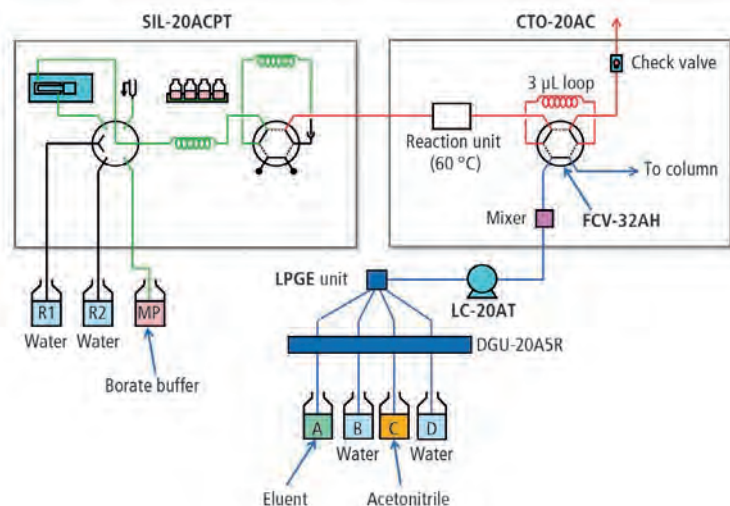


Figure 2: Flow diagram for derivatization and injection

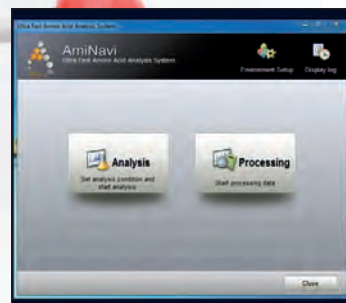


Figure 3: AmiNavi™ Software

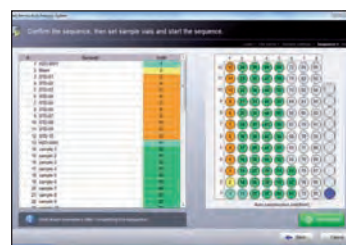


Figure 4: Analysis – Color highlighting of the individual sample positions of the batch



Figure 5: Processing – Calibration curve of the individual substances

Figure 6: Overview table of the concentrations



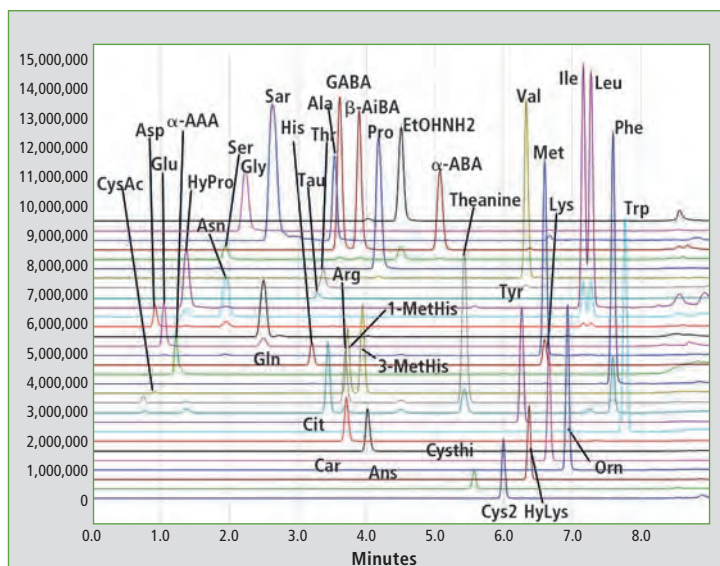


Figure 7: 38 Amino acids

LCMS-2020 single-quadrupole mass spectrometer.

### Specifically developed software

The AmiNavi™ software, specifically developed for the UF-Amino Station, supports users in setting up complete analyses and evaluation of samples. After entering sample names, a batch with the desired number of standards, blank injections and samples is created automatically. This is then illustrated via color highlighting of the positions of the individual substances in a deep-well plate, so that filling of the wells is greatly simplified.

The calibration curve is created automatically according to the preset method and can, whenever required, be very easily modified or adapted via manual integration. An overview table which shows the concentrations of the individual amino acids can be printed as a report

Investigation of a standard mixture containing 38 amino acids with the UF-Amino Station results in good separation between the individual substances and good sensitivities for identification and quantification – which can be evaluated very easily using the AmiNavi™ software.

Amino acid	Three-letter code	Comment	Ø in proteins*
Alanine	Ala	non-essential	9.0 %
Arginine	Arg	semi-essential	4.7 %
Asparagine	Asn	non-essential	4.4 %
Aspartic acid	Asp	non-essential	5.5 %
Cysteine	Cys	non-essential*	2.8 %
Glutamine	Gln	non-essential	3.9 %
Glutamic acid	Glu	non-essential	6.2 %
Glycine	Gly	non-essential	7.5 %
Histidine	His	semi-essential	2.1 %
Isoleucine	Ile	essential	4.6 %
Leucine	Leu	essential	7.5 %
Lysine	Lys	essential	7.0 %
Methionine	Met	essential	1.7 %
Phenylalanine	Phe	essential	3.5 %
Proline	Pro	non-essential	4.6 %
Serine	Ser	non-essential	7.1 %
Threonine	Thr	essential	6.0 %
Tryptophane	Trp	essential	1.1 %
Tyrosine	Tyr	non-essential*	3.5 %
Valine	Val	essential	6.9 %

Table 1: 20 standard amino acids – \*essential for children and pregnant women

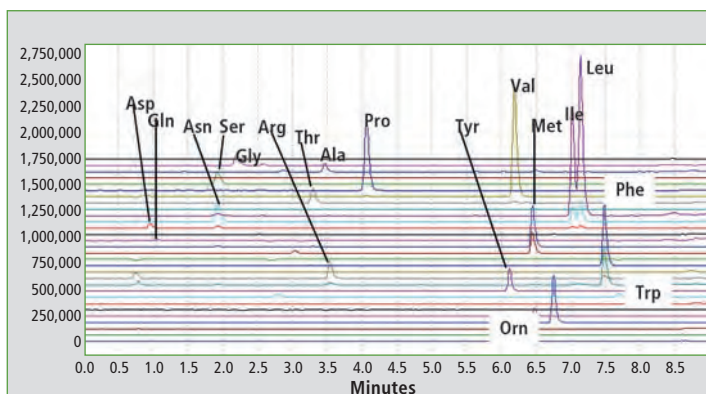


Figure 8: Amino acids in culture media

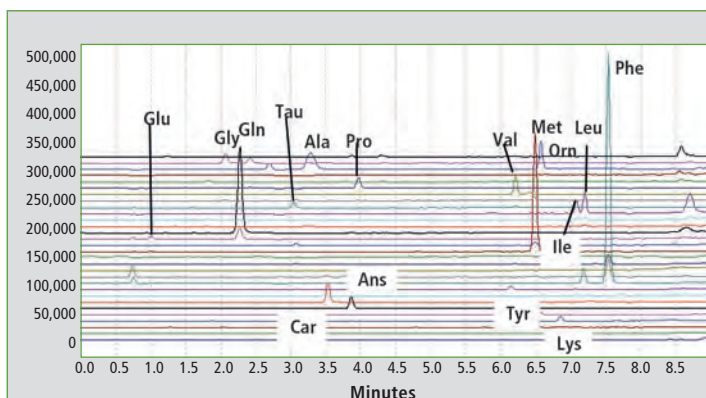


Figure 9: Amino acids in meat

Figure 7 shows the corresponding chromatograms as depicted with the LabSolutions software.

As an example for one of many applications, figure 8 shows the amino acids contained in culture media. Here, it is possible to investigate continuous monitoring of the amino acid. An example for the amino acids contained in meat is shown in figure 9. This method can be used for quality control in the food industry.

### High-throughput analysis

The fully automated derivatization by the UF-Amino Station and the easy-to-use AmiNavi™ software for starting and evaluating sample sequences enables high-throughput analysis of up to 38 amino acids. Compared to other methods, fast chromatography and automatically performed, parallel derivatization can result in considerable savings in time per analysis, as illustrated in figure 10.

[1] Paula Yurkanis Bruice: Organic Chemistry, Pearson Education Inc., 2004, 4<sup>th</sup> Edition, p. 960 - 962

### UF-Amino Station

9 min. → 38 amino acids

### Reversed-phase LC with pre-column derivatization

15 min. → Up to 25 amino acids

### Ion-exchange LC with post-column derivatization

160 min. → 38 amino acids

Figure 10: Time comparison of various amino acid analysis methods

# A small, subtle distinction

## Trace analysis in polymers using pyrolysis GCMS



Figure 1: Pyrolysis GCMS

Synthetic polymers or plastics are omnipresent. They are processed into products such as polyethylene garbage bags, polypropylene bicycle helmets or highly specialized materials for medical technology, sports, construction or research.

Initially, they were only used as substitutes for valuable or rare materials. But they also possess unusual properties that other materials do not offer. For instance, plastics can be tailored to specific application areas in order to attain certain chemical, mechanical or electrical characteristics. This is achieved through selection of starting materials, the addition of additives and the production process. Chemical additives such as antioxidants, stabilizers or flame-retardants can change the characteristics of a product already in very low concentrations.

### Polymer characterization via pyrolysis

Expectations of the material properties of plastics are high – depending on the application area, they should be resistant to weathering, shape-and-temperature sta-

ble, inert to solvents, and fire resistant. This is why verification of the desired properties during material development or post-production control is of great interest.

Physical and chemical test methods help to characterize polymer properties. In order to determine the chemical composition of a plastic or the type of additive

used, specialized progressive analytical methods are applied.

Pyrolysis GCMS (coupled gas chromatography-mass spectrometry) is an excellent technique for the analysis and characterization of polymers. Using this technique, which avoids time-consuming sample preparation, minimal differences between two samples such as impurities or fluctuating

concentrations of the starting materials and additives can be detected.

During pyrolysis GCMS, approximately 200 µg of the sample material is decomposed directly in a preheated furnace at temperatures between 400 and 800 °C typically. The resulting pyrolysis products are separated chromatographically on a GC capillary column and subsequently detected using a mass spectrometer. Due to the way of sample introduction and the chromatographic separation, it is possible not only to analyze very small amounts but also to obtain valuable, detailed additional information – in comparison with other methods in polymer analysis such as FTIR, TGA or HPLC.

### Selecting the detector

Characteristic chromatograms or ‘pyrograms’ are obtained using a flame ionization detector (FID) as well as via mass spectrometry detection. These pyrograms act as ‘fingerprints’ and are used to identify polymers unequivocally via comparison. When using an FID, it is only possible to use retention

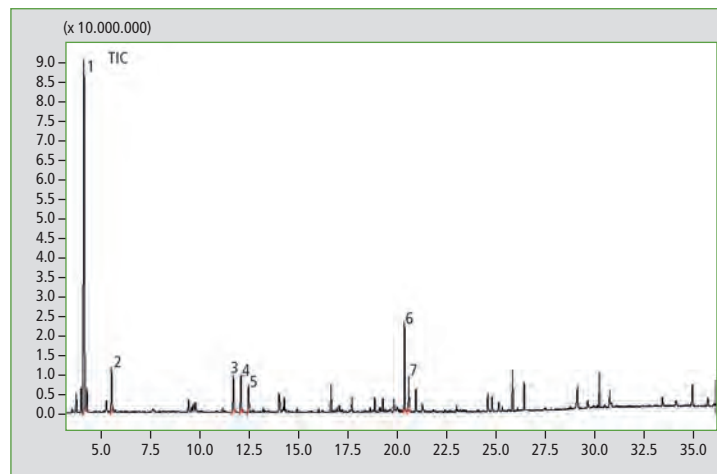


Figure 2: Pyrogram of the elastomer sample A, butyl rubber (result of the library search of several significant peaks: 1. Isobutene; 2. Isoprene; 3. 1,3-Pentadiene, 2,4-dimethyl-; 4. 1-Pentene, 2,4,4-trimethyl-; 5. 2-Pentene, 2,4,4-trimethyl-; 6. Hept-2-ene 2,4,4,6-tetramethyl-; 7. l-Limonene).

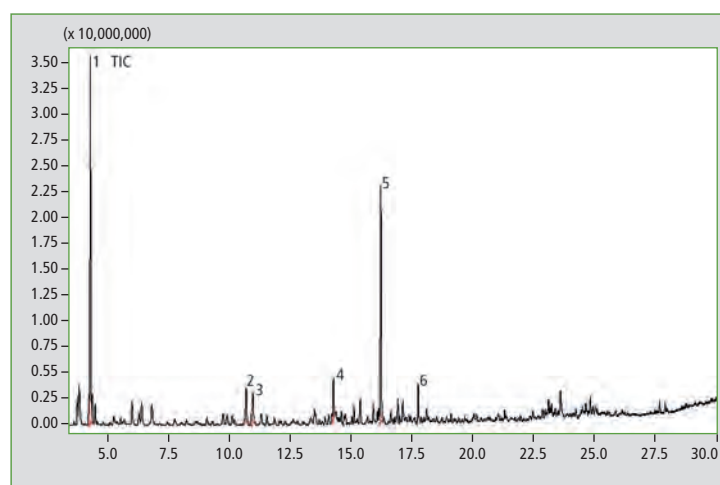


Figure 3: Pyrogram of the elastomer sample D, butadiene rubber (result of the library search of several significant peaks: 1. Cyclobutene; 2. Benzene; 3. 1,3-Cyclohexadiene; 4. Benzene, methyl-; 5. Cyclohexene, 4-ethenyl-; 6. Styrene).



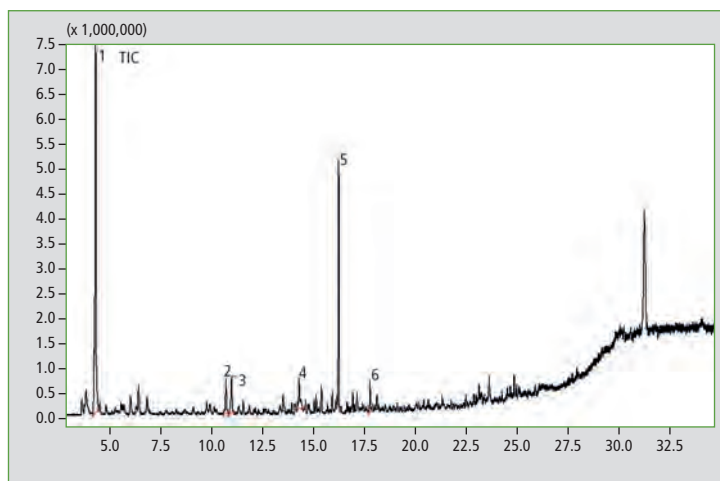


Figure 4: Pyrogram of the elastomer sample C, butadiene rubber with traces of butyl rubber. (Results of the library search of several significant peaks: 1. Cyclobutene; 2. Benzene; 3. 1,3-Cyclohexadiene; 4. Benzene, methyl-; 5. Cyclohexene, 4-ethenyl-; 6. Styrene).

times for the identification of individual peaks. Mass spectrometry detection yields a mass spectrum for every peak, providing information on the structure of a substance. By comparing the mass spectra with those stored in specialized libraries, individual components can be definitely identified. Using characteristic mass traces, it is also possible to detect and quantify very low concentrations of substances in highly complex pyrograms.

### Trace analysis in rubber samples

The following example demonstrates the performance of a pyrolysis GCMS system in the detection of traces of a compound in a polymer sample. Figure 2 shows the pyrogram of an elastomer sample A. The sample is a butyl rubber (copolymer of 95 - 99 % isobutene and 1 - 5 % isoprene). Elastomer sample D is a pure butadiene rubber (see figure 3), which is used widely in the rubber world. Sample C is also a butadiene rubber, as can quickly be identified based on its virtually identical fingerprint (see figure 4). But this elastomer is a blend of butadiene and butyl rubber. The concentration of the butyl rubber is between 0.5 - 3 %, which is in the trace range. Is it possible to detect this minimal amount in the pyrogram of sample C?

The pyrograms of the three samples were directly compared.

When looking closely at individual enlarged sections, peaks can be found in sample C that are unequivocally identified as pyrolysis products of a butyl rubber.

To illustrate this peak assignment more clearly, a certain mass trace (here:  $m/z$  112) is calculated from the TIC (total ion current) and the corresponding section of the pyrogram is enlarged. At retention times 12.17 and 12.56 seconds, there are two peaks that can only be detected in sample A (black mass trace) and in sample C (red mass trace) but not in sample D (blue mass trace) – see figure 5.

Comparison of the mass spectra in figures 5a and 5b shows that it is clearly the same substance. In this way, additional peaks can be found that confirm the presence of traces of butyl rubber in the butadiene rubber (see figures 6 and 6a).

### Data reproducibility

An important requirement for this efficient trace analysis is data reproducibility. In pyrolysis, this depends greatly on the heating-up time of the sample.

Characteristic for a furnace pyrolyzer is the preheated, accurately temperature-controlled furnace in which the sample falls (free fall) and is heated in a fraction of a millisecond. ♦

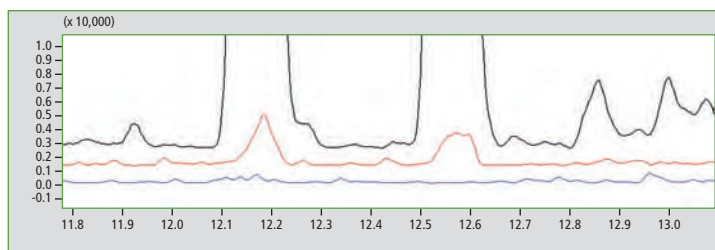


Figure 5: Detail of the comparison of mass traces  $m/z$  112 of 11.8 to 13 minutes of sample A (black), sample C (red) and sample D (blue)

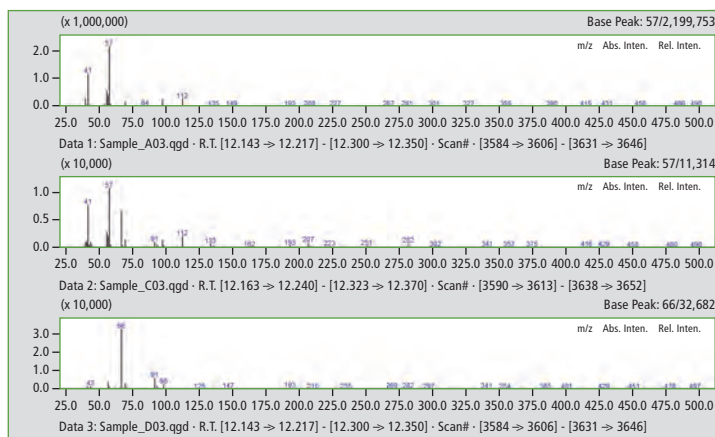


Figure 5a: Comparison of mass spectra of the peaks at 12.17 minutes

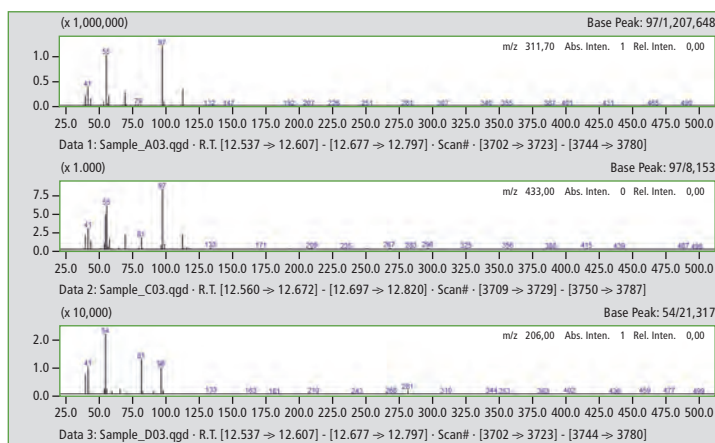


Figure 5b: Comparison of mass spectra of the peaks at 12.56 minutes

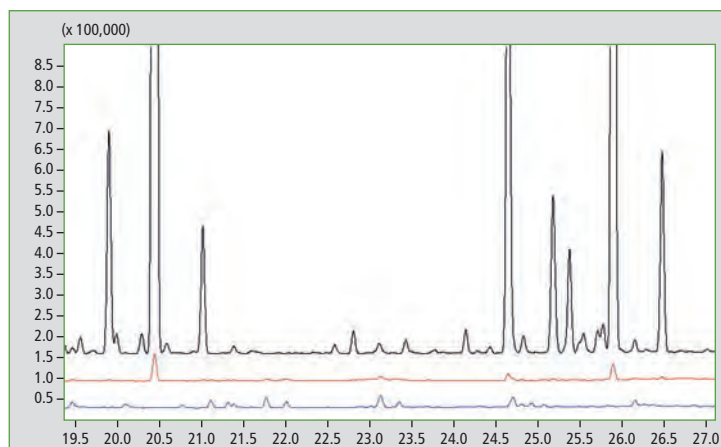


Figure 6: Detail of the comparison of mass traces  $m/z$  97 from 19.5 to 27 minutes of sample A (black), sample C (red) and sample D (blue). Presence of the butyl rubber can be found at 20.45 and 25.92 minutes.

The sample is placed in a deactivated stainless steel cup that is cleaned quickly and easily via red heating with a small torch.

Due to the minimal heating-up time, the loss of volatile components is prevented and high reproducibilities can be achieved. The optimized interface between pyrolyzer and gas chromatograph minimizes dead volumes and cold spots, thereby preventing poorly reproducible peaks.

Figure 7 demonstrates the excellent reproducibility of the pyrolysis GCMS system in eight consecutive measurements of a styrene-butadiene polymer blend. If desired, the system can be equipped with an additional FID (flame ionization detector). Reproducibility measurements of the styrene-butadiene polymer blend were also carried out using this detector.

Table 1 lists some statistical details of the evaluation of one significant peak (peak C: butadiene dimer). Quantification was carried out via the area normalization or area % method, which can yield very good results even when the individual samples could not be weighed. For quantitative analysis of pyrolysis GCMS data, quantification of characteristic SIM mass traces (single ion monitoring) using the internal standard method is recommended.

#### Valuable time savings using an autosampler

For all measurements shown here, an autosampler was used to introduce the samples in the EGA/PY-3030D pyrolyzer. Autosamplers offer a great advantage when many samples need to be analyzed. The sample carousel can be loaded with 48 stainless steel cups, and

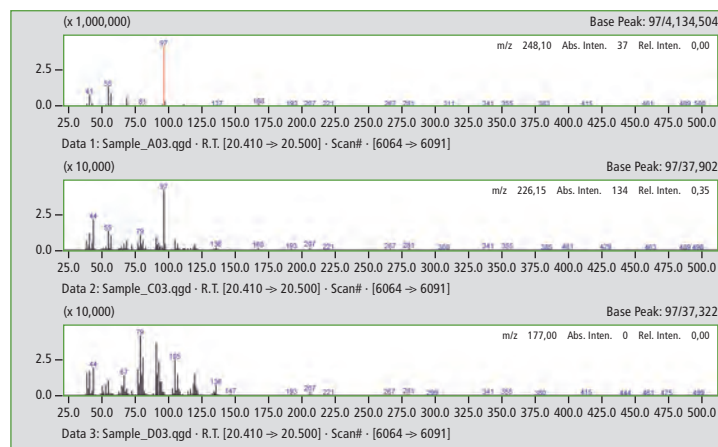


Figure 6a: Comparison of the mass spectra of the peaks at 20.45 minutes

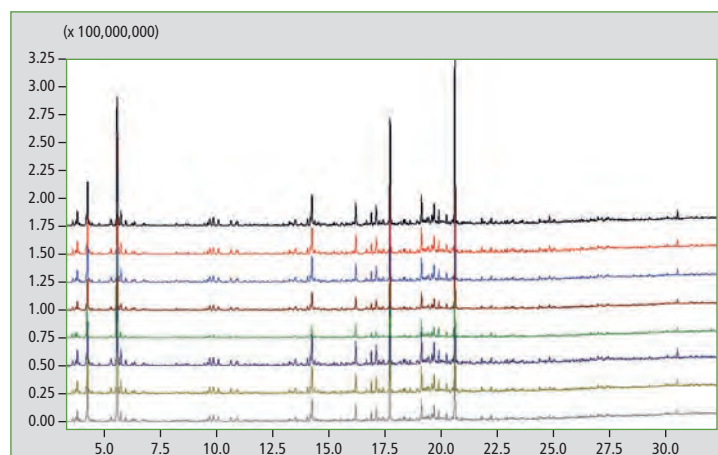


Figure 7: Comparison measurements (8 runs) of a styrene-butadiene polymer blend using pyrolysis GCMS

ID#1 Compound name: C			
Data file	Ret. time	Area	Con. [%]
FID_Sample_E_repro02.gcd	9.314	1160042	22.115
FID_Sample_E_repro03.gcd	9.314	1313027	21.300
FID_Sample_E_repro05.gcd	9.325	1595818	22.281
FID_Sample_E_repro06.gcd	9.316	1308772	21.753
FID_Sample_E_repro07.gcd	9.314	1534222	21.344
FID_Sample_E_repro08.gcd	9.312	1095007	22.265
FID_Sample_E_repro09.gcd	9.321	1516246	22.459
FID_Sample_E_repro10.gcd	9.315	1318044	21.940
FID_Sample_E_repro11.gcd	9.317	1396971	21.947
FID_Sample_E_repro12.gcd	9.319	1512641	22.112
Average	9.317	1375079	21.952
% RSD	0.040	12,110	1.765
Maximum	9.325	1595818	22.459
Minimum	9.312	1095007	21.300
Standard Deviation	0.004	166521	0.387

Table 1: Statistical evaluation of comparison measurements (10 runs) using pyrolysis FID

programming of a sequence table takes place via a simple, user-friendly interface.

#### Summary

Pyrolysis GCMS is a modern, high-performance technique for accurate characterization and quantification of synthetic polymers and biopolymers. The application possibilities are extensive and range from plastics manufacturing and processing, automotive and paper industry, wood and resins analysis to paints, varnishes, adhesives and applications in forensics, art and archaeology.

This application provides just a glimpse of the capabilities of the EGA/PY-3030D pyrolyzer furnace. Using the specialized samplers, it is also possible to select various sampling methods such as the double-shot technique, reactive pyrolysis or sample irradiat-

ing with UV-light in order to simulate aging processes. In addition, standard EGA (evolved gas analysis) measurements using an extremely short capillary column without stationary phase are possible, for instance to determine the optimum pyrolysis temperature of a sample.



# The daily bread

## The big breakfast test using the EZ Test EZ-X texture analyzer (part 2)



Figure 1: Bread firmness test using Texture Analyzer EZ-Test-LX with ø 50 mm compression jig

**F**oods as a basic need of human existence are subject to constant inspection. The Shimadzu News reports regularly on new analytical capabilities.

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

Bread has been a basic food in the Western world for thousands of years. With an average bread consumption per capita of approxi-

mately 66 kg/year, Europe holds a leading position in the worldwide consumption of bread. With nearly 70 percent, fresh baked bread dominates, prior the frozen bakery products, fast food bread and long-life bakery products.

### Bakery or supermarket – which offers the freshest products?

It is not surprising that freshly baked goods are usually found at the European breakfast table. Depending on local tastes, these could be breads, baguettes or ciabattas; the main issue is the freshness of the products. In addition to traditional bakeries, freshly baked goods from on site baking ovens are increasingly being offered in supermarkets.

Yet, how fresh are these baked goods and are there any differences in texture and shelf life? Shimadzu has tested several baked goods from a bakery and supermarket chosen at random.

### To get upon the freshness' track

Various baked goods were subjected to a compression test using the EZ Test-X Texture Analyzer, where pressure plates simulate a pressure test applied with the palm of a hand. In this way, the stiffness/chewiness of the crust as well as the crumb is tested. The baked goods were removed from their commercial packaging, and each set was tested one hour after purchase, thereafter 24 hours and 72 hours after purchase:

As expected, a very strong increase in the texture of all breads has been measured. This is mainly

due to drying out of baked goods that were packaged in paper bags. Only in the case of the crusty rye bread bought at the supermarket could a lower value be measured after 24 hours of storage.

As a slight increase could also be measured for the crusty bread obtained from the bakery, it can be concluded that the crumbs in these baked goods have a much higher moisture content compared to the small baked goods. This ensures that the bread dries out much more slowly. For baked goods from the discount supermarket, the crust apparently softened over 24 hours. This is why a decrease in force was registered. This could, however, also indicate that the baking time was too short so that the bread was not completely baked.

Overall, it can be concluded that the measured values for products purchased at the supermarket and the bakery were similar and were still edible after 24 hours. Significant changes could only be observed after 72 hours. Many products had by then become so hard that they were no longer fit for consumption. Another proof of why Europeans like to enjoy their baked goods freshly baked each day ...

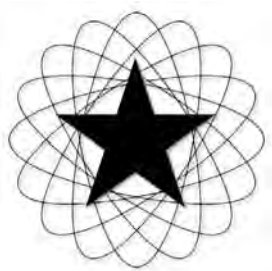
To be continued ...\*

\*For those of you who cannot wait for the next breakfast test, the entire text can be downloaded in advance.

[www.shimadzu.eu/breakfast-test](http://www.shimadzu.eu/breakfast-test)

Bakery products	Discount supermarket (A) – bakery (B)	Max. force (N) after 1 hour	Max. force (N) after 24 hours	Max. force (N) after 72 hours
Croissant	A	5	16	85
	B	4.5	24	82
Ciabatta	A	47	255	700
	B	36	291	305
Wheat bread rolls	A	35	57	160
	B	30	44	80
Multigrain roll	A	65	142	1,700
	B	100	160	1,900
Baguette	A	50	125	670
	B	47	100	520
Crusty rye bread	A	36	32	125
	B	20	26	90

Table 1: Compression test of bread



The Analytical Scientist – Innovations  
Award 2013

# Following the traces of climate change

## Novel plasma technology offers new detection possibilities

Climate change due to global warming is taking place much faster than expected [1] – according to the clear warning in the most recent report of the ‘Intergovernmental Panel on Climate Change’ (IPCC). This is a reason for developing more efficient methods for monitoring

contributed to a gas chromatographic system with only one column and one detector (Tracera System, figure 1).

### Innovative plasma technology

The barrier ionization discharge detector (BID) is a helium ionization detector based on a novel plasma technology. The helium plasma is generated inside a quartz tube, which serves as a dielectric barrier (figure 2). In this way, the electrodes used for plasma generation are protected against possible contamination due to column bleeding, the sample and the plasma itself, which considerably improves the long-term stability of the detector. The light emitted from the plasma has an ionization energy of 17.7 eV, which enables the BID to detect all substances except helium and neon. The ionized substances are detected via electrodes made of a specially developed Kovar alloy.

Through innovative technology, a highly sensitive universal detection system with a hitherto unsurpassed long-term stability has been developed. In this way, the BID bridges an application gap to the flame ionization detector (FID), the most commonly used detector in gas chromatography.

The FID is popular because of its exceptional combination of sensitivity, reproducibility and long-term stability. Unfortunately, the FID detection spectrum lacks permanent gases such as oxygen, nitrogen and carbon dioxide. Sensitive measurement of permanent gases in the presence of volatile hydrocarbons, therefore requires complex GC configurations using multiple detectors.

As the BID is up to 100 times more sensitive than a thermal conductivity detector (TCD) and twice as sensitive as a FID, it offers an ideal solution for such applications without any significant



Figure 1: GC-2010 Plus with barrier ionization discharge detector (BID, Tracera System)

contributors to the greenhouse effect, such as methane ( $\text{CH}_4$ ), carbon dioxide ( $\text{CO}_2$ ) and nitrous oxide ( $\text{N}_2\text{O}$ ).

Up to now, this seemingly straightforward task required a considerable analytical effort. This is due to the strongly varying concentrations of these components in the atmosphere, which range from the high ppm range for carbon dioxide, to ppm and ppb levels for methane, and even lower to the trace level below 100 ppb for nitrous oxide. To meet these analytical requirements, complex GC systems were needed using two or three detectors. With the new barrier discharge ionization detector (BID), this approach can be sim-

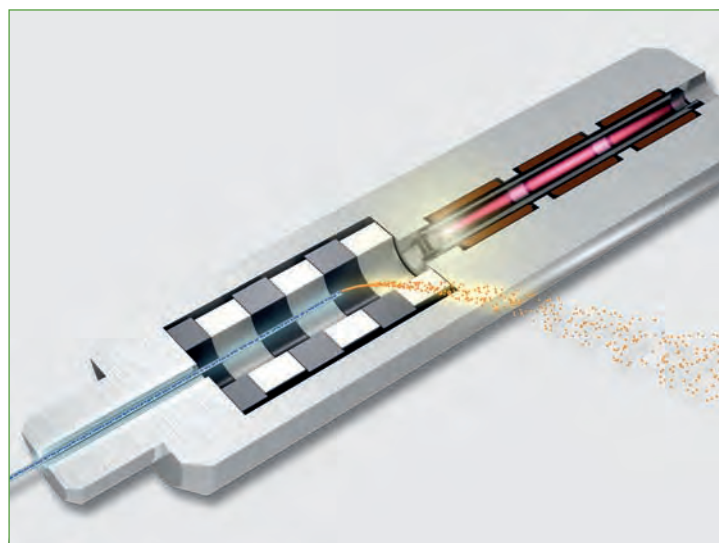


Figure 2: Cross sectional drawing through a barrier ionization discharge detector. The lower half of the detector contains the capillary column and Kovar electrodes for detection. Plasma generation takes place inside the quartz tube in the upper half of the detector.



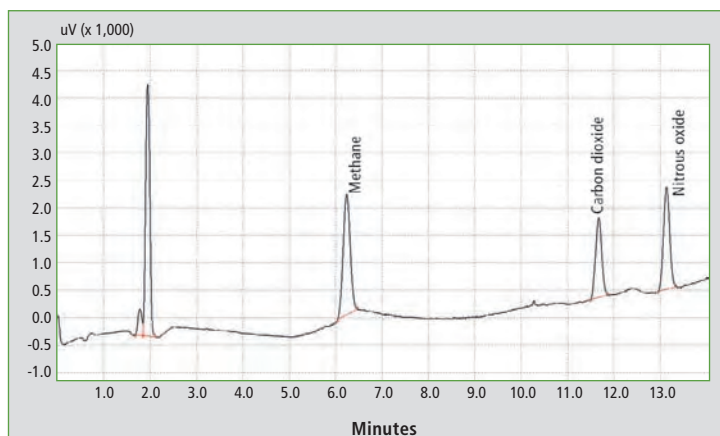


Figure 3: Chromatogram of the standard, 500 ppb methane, carbon dioxide and nitrous oxide in helium

loss in precision or long-term stability. For this reason, leading European scientists voted the BID at rank four of the most innovative products in the year 2013 [2].

and thus detection limits well below 100 ppb. Sampling and injection was carried out using a VALCO six-port valve. As leakage of the valve in the trace range

Name	Ret. Time	Area	Height	Noise	S/N	DL	Unit
Methane	6.236	23,251	2,192	57	38	43	ppb
Carbon dioxide	11.67	13,356	1,448	57	25	65	ppb
Nitrous oxide	13.132	16,997	1,857	57	32	51	ppb

Table 1: Evaluation of the standard sample in figure 3. Detection limits (DL) are calculated at a signal-to-noise ratio of 3.3.

Name	Ret. Time	Area	Height	Conc.	Unit
Methane	6.203	77,362	6,144	1,664	ppb
Carbon dioxide	11.664	932,381	1,060,978	371,820	ppb
Nitrous oxide	13.138	8,972	1,010	264	ppb

Table 2: Results of the real sample in figure 4

### Trace analysis of greenhouse gases

The chromatographic separation of methane from oxygen and nitrogen in ambient air represents a challenge to the capacity of any capillary column. To achieve detection limits below 100 ppb, split-analysis is not a common alternative. For direct analysis of volumes greater than 500  $\mu$ L, PLOT (porous layer open tubular) columns with an internal diameter of 0.53 mm are often overloaded by nitrogen and oxygen, and separation from methane can no longer be satisfactorily achieved.

The best results in terms of detection limit and chromatographic separation were obtained using a 2 m 1/16" micro-packed ShinCarbon ST column. This allows injection volumes of 1,000  $\mu$ L

can lead to sample variation by ambient air, the valve was equipped with a helium-purged rotor housing.

1,000  $\mu$ L of sample was injected directly from the sample loop onto the column. Helium with a purity of 6.0 was used as carrier gas and for gas discharge of the BID. The carrier gas flow was kept constant at 15 mL/min and a helium flow rate of 80 mL/min was set for the BID discharge gas.

The best chromatographic separation was achieved using a temperature program starting at 30 °C. Depending on the characteristics of the ShinCarbon ST column, a starting temperature of 40 °C is also possible (Temperature program: 30 °C for 5 minutes isothermally, followed by a rate of 10 °C/min to 120 °C). The long isotherm

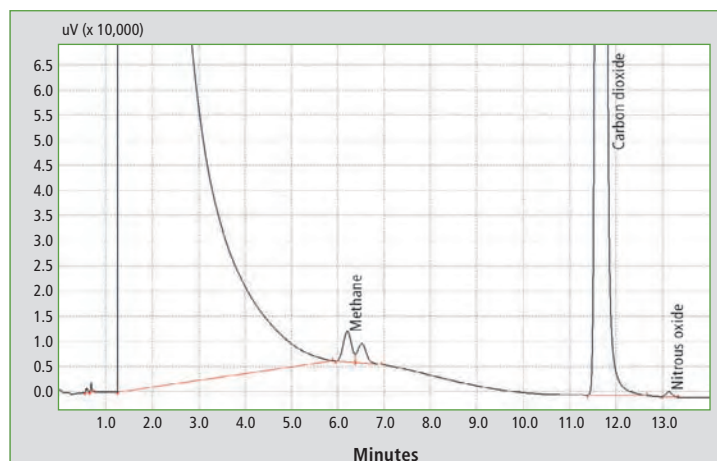


Figure 4: Real ambient air sample from the vicinity of the city of Duisburg, Germany

at 30 °C is necessary to sufficiently separate methane from the nitrogen/oxygen signal.

### Results

A standard of 500 ppb methane, carbon dioxide and nitrous oxide in helium was used for calibration (see chromatogram in figure 3). Regardless of the low standard concentration, a reproducibility of the measurement results with a standard deviation of 1.1 % for CH<sub>4</sub>, 1.4 % for CO<sub>2</sub> and 1.1 % for N<sub>2</sub>O could be determined. Based on a signal-to-noise ratio of 3.3, a detection limit below 100 ppb could be calculated for all components (table 1).

Figure 4 shows the chromatogram of a real ambient air sample from the vicinity of the city of Duisburg, Germany. Methane is sufficiently separated, not only from the matrix but also from an unknown substance that elutes immediately following methane (resolution R = 0.92). Low N<sub>2</sub>O concentrations are also well separated from a nearly 400-ppm broad CO<sub>2</sub> signal (resolution R > 400). Additional results of the real sample are listed in table 2.

To date, sensitive measurement of greenhouse gases often required complex gas chromatographic systems equipped with a thermal conductivity detector (TCD), flame ionization detector (FID) and electron capture detector (ECD). Using the barrier ionization discharge detector (BID), the GC system could be simplified to

one column and one detector, without having to accept any loss in precision and detection limit.

### Reference literature

- [1] 5<sup>th</sup> IPCC Report Final Draft (accepted): <http://www.climatechange2013.org/report/review-drafts/>
- [2] The Analytical Scientist, Volume 11, December 2013, The Analytical Scientist Innovation Awards 2013

**Additional information** to this article can be obtained via the QR code or by sending an email to [shimadzu-news@shimadzu.eu](mailto:shimadzu-news@shimadzu.eu).



# Effect of mobile phase pH on reversed-phase HPLC separations of ionizable compounds

N-acetylprocainamide, pKa  $\approx$  8.3

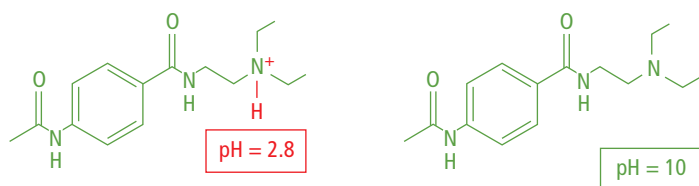


Figure 1: Ionization of N-acetylprocainamide in acidic or basic pH

Changing the pH of a mobile phase is a powerful tool to obtain significant changes in the selectivity of a reversed-phase HPLC separation. Ionizable compounds, meaning acidic, basic or zwitterionic analytes will show extreme changes in retention depending on their pKa and pH of the mobile phase. Therefore, a wide pH range of the HPLC equipment offers additional possibilities for HPLC method optimization by exploiting selectivity changes at low, intermediate and high pH.

The mobile phase pH reflects the hydrogen ion [H<sup>+</sup>] concentration in solution. An acidic pH < 7 represents an increased [H<sup>+</sup>] concentration, while adding a base to pH > 7 lowers the [H<sup>+</sup>] concentration and suppresses ionization of basic analytes, as shown in the example of N-acetylprocainamide in figure 1 and methylparabene in figure 2.

As the extent of analyte ionization changes, so does the retention behavior in reversed-phase HPLC separations. Since the ionized form is more polar, it is retained less on alkylated, non-polar stationary phases. Ion suppression by decreasing the pH for acids, or increasing it for basic analytes will significantly lengthen retention as demonstrated in the application example shown in figure 3.

Methylparabene, pKa  $\approx$  8.4

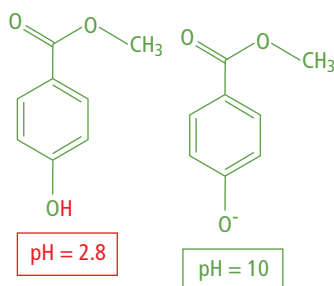


Figure 2: Ionization of methylparabene in acidic or basic pH

A mixture of seven pharmaceuticals was separated in acidic or basic mobile phase conditions. While the neutral analytes showed no marked change in retention time, the basic analytes, namely nizatidine, N-acetylprocainamide and reserpine were retained much longer in basic conditions. Methylparabene moved forward once the phenol group was ionized (figures 2 and 3).

Ion suppression can also be used to considerably improve the peak shape of ionizable compounds, as charged analytes undergo secondary interactions with the stationary phase, which can lead to strong peak tailing. Neutral or uncharged compounds will normally elute as sharp, symmetrical peaks. In any case, care should be taken to adjust the mobile phase pH in a range that is about 2 pH units

away from the analyte pKa, where a compound is 50 % ionized. This can give rise to split peaks, due to different retention behavior of the ionized and non-ionized form.

Instrumentation: Nexera X2 series UHPLC system, wide pH version

Column: ACE Excel 3 Super C18, 100 x 2.1 mm

Mobile phase: a) acidic: A: 10 mM HCOONH<sub>4</sub>, pH 2.8 in H<sub>2</sub>O and B: in MeCN/H<sub>2</sub>O (90 : 10 v/v)  
b) basic: A: 0.1 % NH<sub>3</sub>, pH  $\sim$  10 in H<sub>2</sub>O and B: in MeCN/H<sub>2</sub>O (90 : 10 v/v)

Gradient: 3 - 100 % B in 7 min

Cycle time: 11 min

Flow rate: 0.42 mL/min

Temperature: 40 °C

Injection volume: 2  $\mu$ L

Sample:  $\sim$  0.3 mg/mL of each compound in MeCN/H<sub>2</sub>O (5 : 95)

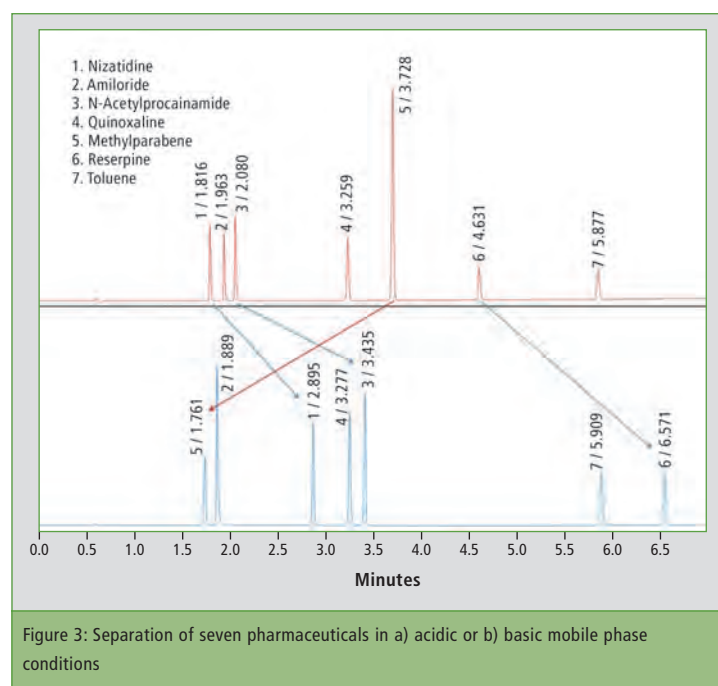


Figure 3: Separation of seven pharmaceuticals in a) acidic or b) basic mobile phase conditions



# Customized TOC analysis

## The online TOC-4200 with many options and kits

In monitoring of wastewaters or quality control of ultrapure water – wherever the organic load of water plays a major role, TOC analyses are used. The TOC (Total Organic Carbon) sum parameter defines the carbon concentrations in organic compounds in a matrix and is therefore considered to be a measure of contamination by organic compounds.

However, different applications and measurement tasks also place different demands on the analyzer. Some waters contain high concentrations of organic substances while other waters contain hardly any, or only very small amounts of TOC. Some waters are clear, others contain particulate matter, still others contain large amounts of salt or inorganic carbon compounds such as carbonates or hydrogen carbonates.

### Process analysis enables fast response

It is often important to monitor water quality very closely and in real-time. Conventional laboratory analyses will not be adequate here. It often takes too much time from collecting a sample and analyzing it in the laboratory to having the results available. Process analysis has been developed for such cases. Here, a special analyzer operates at close distance to a wastewater discharge line or a process, to withdraw a sample periodically (for instance every ten minutes), process it, and analyze and evaluate it. The analyzer then sends the analysis value automatically to a process control system. The wastewater plant can now respond promptly to any changes or abnormalities.

The TOC parameter can be determined easily and securely in on-line process analysis. The analyzer withdraws an aliquot of the sample and mixes it with an acid. The acid decomposes inorganic carbon

such as carbonates and hydrogen carbonates compounds. The resulting  $\text{CO}_2$  is sparged from the sample using a sparge gas.

Subsequently, an aliquot of the sample is injected onto a hot platinum catalyst ( $680^\circ\text{C}$ ). The organic carbon compounds are oxidized to carbon dioxide and transferred by the carrier gas to an NDIR detector, which detects the amount of  $\text{CO}_2$  produced. A single analysis takes about 3 to 4 minutes, so the TOC can also be measured in the shortest time intervals.

### Numerous applications for process analysis

- Wastewater treatment plant inflow is monitored to prevent damage to the biology of the plant by high organic loads with associated high costs. To check the operation of the wastewater treatment plant, the plant's outflow is also examined.
- In industrial applications, the organic load in a wastewater is often used to calculate the sewage charge. Therefore, the wastewater streams are often monitored online.
- In the steam-water cycle of a power plant, the water is allowed to evaporate and condense again and again. The organic substances can accumulate and damage the components of the turbines. Online monitoring of the condensate protects the plant components from damage.
- During cold spells, aircrafts are treated with de-icing agents shortly before takeoff. This results in wastewater with very high concentrations of organic substances. Airport operators therefore examine their wastewater streams during the winter months using online TOC systems.
- In some applications, the TOC serves as a quality parameter. Starting materials or products are examined for possible organic impurities. In critical applications, this also needs to be monitored around the clock.
- To cool industrial processes or plant components, water from flowing watercourses is often used. This water is subsequently led back. It is hard to imagine what environmental damage could occur when plant components start to leak and let environmentally hazardous substances enter our watercourses –

not to mention the damage to the plant and the company's reputation. Here also, online TOC analyzers are of great help in protecting the environment as well as the company.

These are just some examples of the many application areas of TOC process analyzers. ♦



Figure 1: The TOC-4200

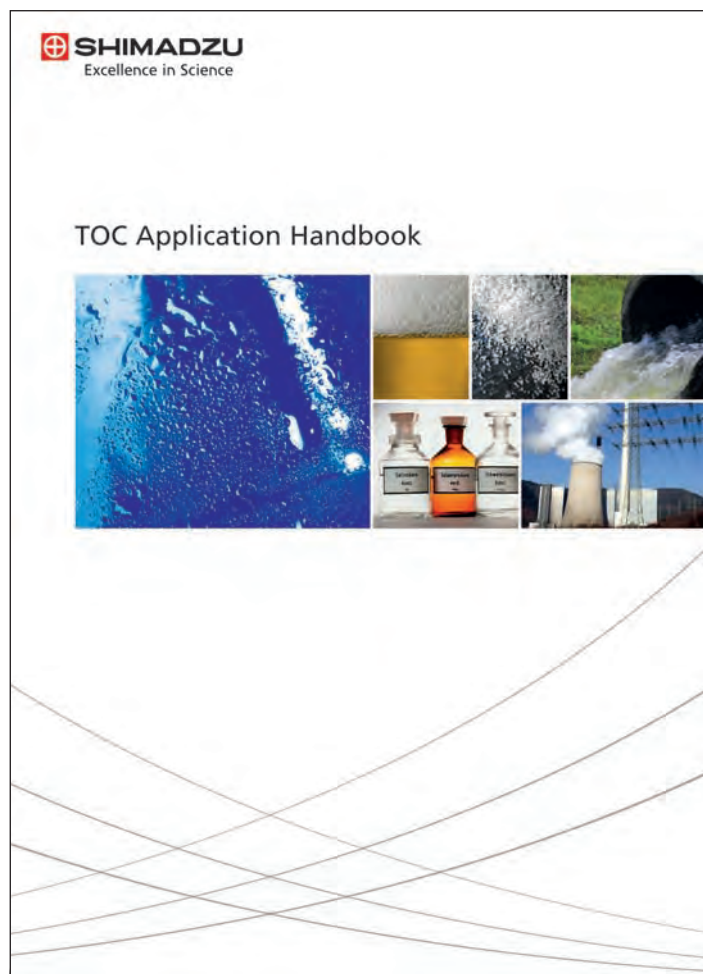


Figure 2: TOC Application Handbook

To meet the requirements of each application, one needs systems that can be customized to the respective measurement task. To do so requires many special options and kits.

#### Universally applicable and adaptable

Shimadzu's TOC-4200 is a TOC process analyzer featuring all of these characteristics. The univer-

sal system can be customized to any measuring task using many accessories. Various types of sampling systems are available. There are samplers with homogenizers that homogenize particle-containing wastewaters as well as special sampling stations for ultra-pure water. Various rinsing systems such as an acid or base rinse can be used to clean the sampling system. Sample stream exchangers enable monitoring of up to six different sample streams using a TOC-4200.

The analyzer also features an automatic dilution function. This not only increases the already wide measuring range, but also enables the creation of multi-point calibrations from a standard solution.

Process analyzers must be able to largely operate autonomously. Maintenance requirements should be as low as possible and service life as long as possible. High salt loads in the sample reduce the maintenance interval. A high-salt kit has been developed specifically for this purpose. It consists of a catalyst tube with a specific geometry and a special catalyst mixture that can handle up to twelve times the amount of salt-containing sample, compared to a conventional catalyst. In addition, the dilution factor can also help to reduce sample matrix effects. A TN module enables the simul-

taneous determination of the total amount of nitrogen compounds.

#### Communication supports versatility

The communication options of the analyzer are also versatile. In addition to the conventional 4 - 20 mA line, the TOC-4200 also features Modbus communication. This enables wired communication between multiple analyzers and the control room. An additional web-based option enables access to measurement results and the status of the analyzer from any networked computer.

It is clear that the many options and kits make the TOC-4200 a universally applicable analyzer suited to any measuring task.

## Shimadzu live

### Wire

07.04. - 11.04.2014  
Duesseldorf, Germany  
[www.wire.de](http://www.wire.de)

### Control

06.05. - 09.05.2014  
Stuttgart, Germany  
[www.control-messe.de](http://www.control-messe.de)

### Setac

11.05. - 15.05.2014  
Basel, Switzerland  
[www.basel.setac.eu](http://www.basel.setac.eu)

### ISCC

18.05. - 23.05.2014  
Riva del Garda, Italy  
[www.chromaleont.it/iscc](http://www.chromaleont.it/iscc)

### Pyrolysis

19.05. - 23.05.2014  
Birmingham, Great Britain  
[www.pyro2014.co.uk](http://www.pyro2014.co.uk)

### EXTECH

25.05. - 28.05.2014  
Crete, Greece  
[www.extech2014.tuc.gr](http://www.extech2014.tuc.gr)

### Nordic Plasma

01.06. - 04.06.2014  
Loen, Norway  
[www.nordicplasma.com](http://www.nordicplasma.com)

### EPRW Pesticide

30.06. - 04.07.2014  
Dublin, Ireland  
[www.eprw2014.com](http://www.eprw2014.com)

### IMSC

24.08. - 29.08.2014  
Geneva, Switzerland  
[www.imsc2014.ch](http://www.imsc2014.ch)

### WOTS 2014 (former HET)

30.09. - 03.10.2014  
Utrecht, The Netherlands  
<http://wots.nl/>

If you would like to receive the Shimadzu News on a regular basis, please email us your postal address via: [shimadzu-news@shimadzu.eu](mailto:shimadzu-news@shimadzu.eu)

You can also subscribe to our newsletter via: [www.shimadzu.eu/newsletter](http://www.shimadzu.eu/newsletter)



Shimadzu News & Events



@ShimadzuEurope