SHIMADZU
Excellence in Science

NEWS 01 2014

Greek wine

Determination of organic acids in wine to ensure proper fermentation

New series: The big breakfast test part 1/3

How do you like your eggs? Hardness test using the Texture Analyzer EZ-Test-X

Masterpiece in speed and sensitivity

The new LCMS-8050 triple quadrupole

CONTENT

Farmland or flame?

Analysis of heavy metals in sewage sludges

In Germany, 3.6 million tons of sewage sludge (dryed matter) arises each year. Due to high nutrient levels of nitrogen and phosphate, this waste product of sewage treatment plants is of particular interest to agriculture. But before it can be used as a fertilizer, certain conditions must be met. The heavy metal content, for instance, must not exceed specified limit values. Should this be the case, other applications are still possible, such as the use as fuel and subsequent landfilling. All disposal routes for sewage sludges as well as their potential percentages are summarized in figure 1.

	Cd	Cr	Cu	Ni	Pb	Zn
Sample 1A	2.0	32	490	31	57	1,100
Sample 1B	2.1	32	490	30	55	1,100
Sample 2A	1.5	13	130	28	< NWG	730
Sample 2B	1.5	13	140	29	< NWG	770
Limit value	10	900	800	200	900	2,500

Table 1: Analysis results of unknown samples including the corresponding limit values according to the German Sewage Sludge Ordinance (AbfKlärV, § 4, section 12). All data refer to the dryed matter of the corresponding sewage sludge sample (mg/kg dryed matter).

SHIMADZU
Excellence in Science

Fast determination of heavy metals in sewage sludges

How can the determination of heavy metal content in sewage sludges be carried out within a short time? For this purpose a simultaneous measurement method is the best suitable one, like it can be performed with Shimadzu's ICPE-9000, an inductively coupled plasma with optical emission spectroscopy (ICP-OES). After a single measurement the information of all calibrated elements is available, whereat more than 70 elements can be chosen, depending on the problem at hand, like in this case the heavy metals. This multi-element measurement is possible due to a large 1 inch^2 CCD chip whereby the entire wavelength range of 167 nm up to 800 nm is detected simultaneously.

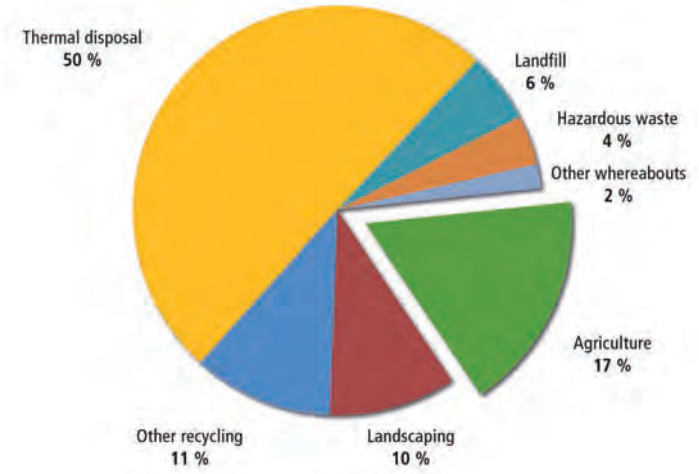


Figure 1: Disposal routes for sewage sludges from communal wastewater treatment plants in Germany in 2010 (source: German Federal Statistical Office, 08/2013)

Within a very short time, the concentration of many elements present in sewage sludge is determined, including the phosphorus content – a criterion for selecting the ICPE-9000 over atomic absorption spectrometry. Other aspects for selecting appropriate elemental analysis methods can be found on page 16 of this issue.

In the framework of this investigation the elements lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni) and zinc (Zn) are determined. For these elements, limit values are defined in the German Sewage Sludge Ordinance (AbfKlärV, § 4, section 12). When these limit values are exceeded, the sludge may not be used as a fertilizer and is to be used in an alternative way (see figure 1).

Sample preparation and measurement

Sample preparation was carried out in accordance to DIN ISO 11446. Essentially, this means that 2 g of the dried sample is boiled under reflux in 28 mL of aqua regia for two hours at 150 °C. The resulting extract is subsequently filtered (0.45 µm membrane filter) and diluted to a volume of 100 mL. After another 1:5 dilution the samples are placed with the calibration standards to the ASC-6100 autosampler and the fully automated measurement can start.

A special feature of the ICPE-9000 is that measurements can be carried out under both radial and axial plasma observation, without the need for any instrument conversion. This fully automated switching between the observation directions becomes important especially with respect to additional elements, as radial plasma observation is less sensitive, and this way it is possible to determine high-concentrated elements without further sample dilution. In addition to these savings in time, the use of the Mini Torch contributes significantly in minimizing the consumption of argon gas resulting in low level running costs.

Results

For method development, a certified European Reference Material ERM®-CC136a (sewage sludge) was investigated prior to the measurement of real samples. When the certified values can be determined, the accuracy of the method and suitability of the ICPE-9000 is ensured.

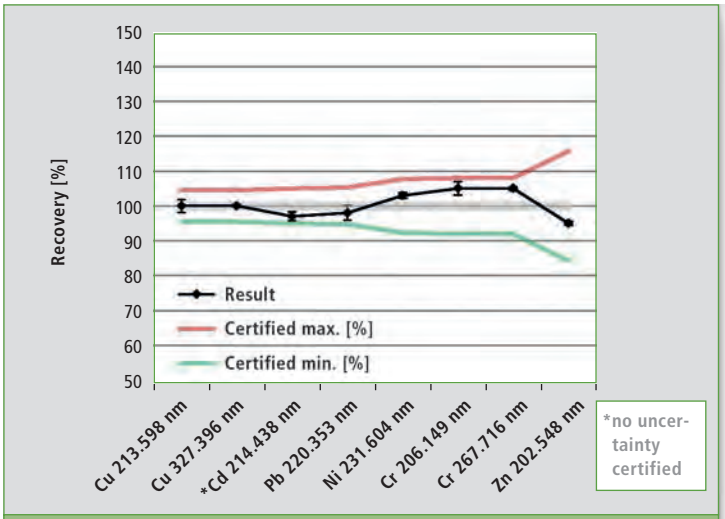


Figure 2: Results of the sewage sludge analysis for the ERM®-CC136a reference material. The inaccuracies of the analytical results of the certified material (red, green) and those determined using the ICPE-9000 (black) including error bars (relative standard deviation of a trip-licate determination) are indicated.

All elements could be determined with good recoveries (see figure 2) and the accuracy of the results (relative standard deviation of the analysis results) even clearly exceeds the permitted tolerances of the reference material. The tolerances of the reference material are specified in the certificate of analysis and based on a previously conducted multiple determination of the material by several laboratories (ring trial). That is why there always is a scattering of the certified results.

Additionally more unknown samples are now also investigated using the developed method (see table 1).

Summary

The good recoveries for the certified reference material in accordance with the certificate of analysis, as well as the good recoveries of the measurement results show that the ICPE-9000 is excellently suited for the analysis of sewage sludges. Other elements (Ca, Co, Fe, K, Mn, Na, P) were also included in this investigation, and the determined values are in accordance with the certificate of analysis. The simultaneous measuring technique ensures that results can be obtained within a very short time.

All results in table 1 are in compliance with the corresponding limit values. Provided that, in addition to the investigated heavy metals, all other parameters also comply with certain specifications. For instance this further specifications are the determination of the organic fraction by loss on ignition (LOI) test. If all points are checked and in the ranges, the sewage sludge can be safely used as a valuable source of nutrients in agriculture.

The background data on the topic of sewage sludge are obtained from:

- The report 'Abwasserbehandlung – Klärschlamm – Ergebnisbericht 2010' of the German Federal Statistical Office, 02-08-2013
- The website of the German Federal Ministry for the Environment, Nature Conservation and Nuclear Safety: www.bmu.de/P608/-1 and www.bmu.de/N39804/

Masterpiece in speed and sensitivit

The new LCMS-8050 targets clinical research, food analysis and other markets

Figure 1: The new triple quadrupole mass spectrometer LCMS-8050

The latest member of Shimadzu's UFMS family (Ultra-Fast Mass Spectrometry) continues the evolution of the company's UF technology. The high-end LCMS-8050 triple quadrupole mass spectrometer features the world's fastest data acquisition rates and best-in-class sensitivity allowing quantitative and qualitative analysis simultaneously.

UF-sensitivity

The LCMS-8050 has been developed based on the groundbreaking LCMS-8030/8040 to meet the growing demand for trace-level quantitation in clinical research, food analysis and other markets. Outstanding sensitivity combined with high-speed analysis are achieved through two improved technologies, the newly designed heated ESI source and the enhanced UFsweeper™ III collision cell featuring a better CID efficiency. In order to increase desolvation efficiency, the newly developed ESI probe uses a high-temperature gas (heating gas) in combination with the nebulizer spray (figures 2 and 3). This facilitates ionization of a wide range of compounds and expands the LC-MS/MS application range.

Durable high-sensitivity performance

Shimadzu continuously enhances the proprietary UFsweeper™ technology by optimizing gas pressure within the collision cell, resulting in a 6-fold improvement in sensitivity compared to the UFsweeper™ II of LCMS-8040. The UFsweeper™ III cell accelerates ions out of the collision cell without loss of momentum. Fast collision cell sweeping on successive events is achieved while maintaining signal intensity and suppressing cross-talk even for high-speed multi-component analysis at a dwell time of 0.8 sec. High-speed MRM transitions up to 555 channels per second accelerate laboratory throughput for simultaneous multi-component analyses.

These technical improvements combined with Shimadzu's patented ion optics system deliver durable high-sensitivity performance, enabling excellent reproducibility even at attogram levels. Table 1 shows the high-precision quantitative results obtained with the LCMS-8050 in the analysis of

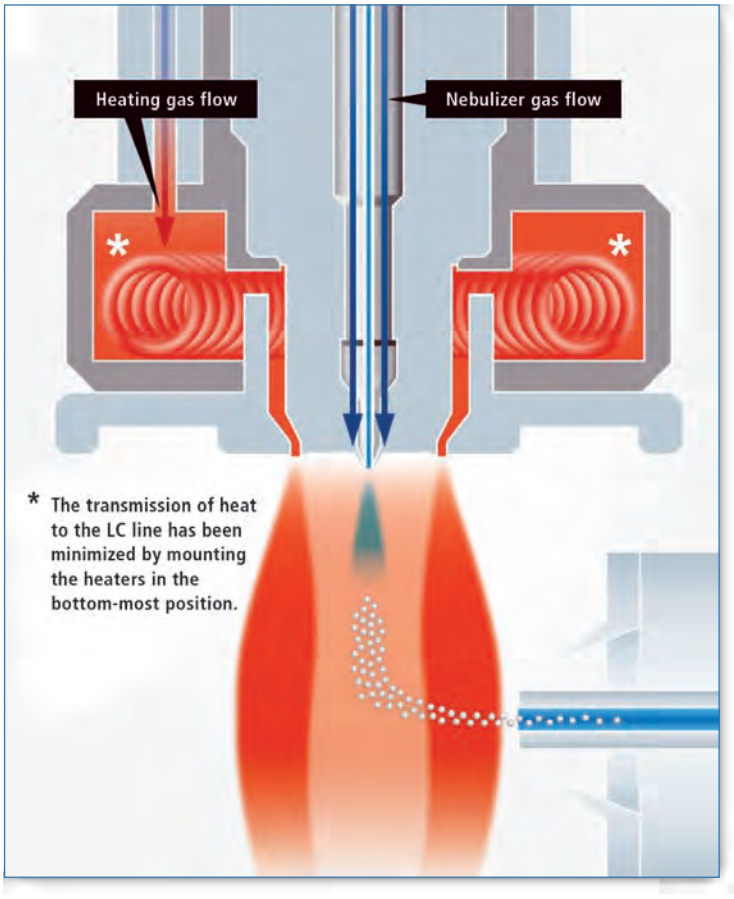


Figure 2: Illustration of the newly developed heated ESI source

y

Verapamil in blood plasma at levels between 500 ag and 50 pg.

UF-switching and UF-scanning

Fast polarity switching is essential when measuring positive and negative ions simultaneously. With the world's shortest switching time of only 5 msec, the LCMS-8050 is able to collect sufficient data points even for the narrow peaks obtained by UHPLC. In addition, the world's fastest scanning rate of 30,000 u/sec allows true high-speed analysis by obtaining quantitative and qualitative information in a single run without compromise in sensitivity and

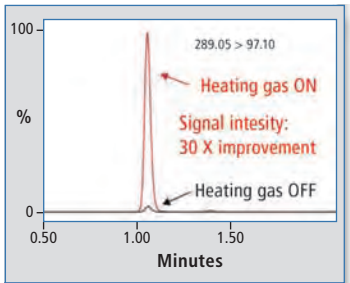


Figure 3: Effect of heating gas – MRM chromatogram of testosterone

mass accuracy. A scan step of 0.1 u even at full speed ensures quantitative accuracy even when performing MS/MS scans and MRM measurements concurrently.

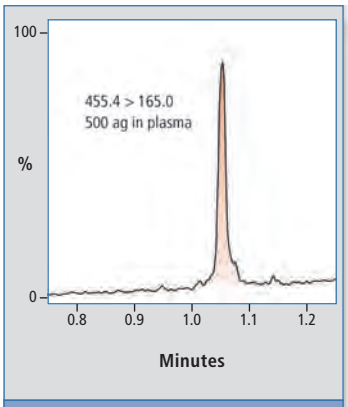


Figure 4: MRM chromatogram of Verapamil

Easy maintenance

The new "plugin and play" design (figure 5) of the ionization unit has another big advantage: the source housing is cable-free and tubeless, allowing quick removal and easy maintenance. Changing the ionization probe is simple: only one lever has to be released and the probe can be lifted out. No tools are necessary to remove the corona needle in APCI or DUIS probes.

As known from Shimadzu's other triple quad systems, replacing the desolvation line (DL) and ESI capillary is quick and easy. It is possible to replace the DL without breaking the vacuum, resulting in longer uptime and usability.

User friendly operation

LabSolutions LCMS Version 5.60 software provides intuitive, easy-to-use operation. It seamlessly integrates the operation of Shimadzu's LC product lines (*Nexera* and *prominence*) as well as the LCMS-8030, LCMS-8040 and LCMS-8050 triple quad systems. MRM transitions already optimized with LCMS-8030/8040 can also be used for LCMS-8050 since parameters for MRM such as Q1 and Q3 pre-rod bias or collision energy are identical in all triple quad systems. Accordingly, the existing method packages can be used, e.g. for Rapid Toxicology Screening System, Primary Metabolites or Lipid Mediators.

Concentration (ng/mL)	Calculated Conc. (ng/mL)	% RSD (n = 6)	Accuracy % (n = 6)
0.0005	0.000501	2.77	100.2
0.005	0.00496	3.98	99.2
0.05	0.0506	1.21	101.2
0.5	0.493	1.31	98.6
5.0	4.89	1.81	97.8
50.0	51.6	0.65	103.2

Table 1: Quantification results of Verapamil

Interface setting support software

The new optional interface setting support software is a tool for automizing optimization of source parameters. It automatically creates a batch file to perform analysis and acquire data while changing the heater temperature and gas flow rates in the interface. In this way, optimal conditions for the target compound can be determined, enabling higher sensitivity of analysis. This software can also be used with LCMS-8030 and LCMS-8040.

Summary

The new LCMS-8050 is a milestone in triple quad technology, enabling quantitative and qualitative analysis simultaneously. It combines the world's best speed parameters with high sensitivity, enabling higher data quality. The system meets the growing demand for trace-level quantitation in clinical research, food analysis and other markets.

Figures 5: Cable- and tubeless: newly designed sources for easy mounting and dismounting

An acidic challenge

Online TOC determination in concentrated hydrochloric acid

Cleaning and disinfection agents, pesticides, pharmaceuticals or plastics such as PVC – they all consist of chlorine compounds and have meanwhile become indispensible. Chlorine is one of the most important basic chemicals in the chemical industry.

Various processes for the production of chlorine, such as chlor-alkali electrolysis, have become established. The membrane electrolysis process is used about in 2/3 of commercially run plants, since the end-products chlorine, hydrogen and sodium hydroxide (NaOH) occur with almost the same purity as when using the amalgam electrolysis process – yet a clearly lower energy input is required. In addition to the membrane process, hydrochloric acid electrolysis according to the ODC (Oxygen Depolarized Cathode) process has become established.

Hydrochloric acid is generated as a by-product in some processes and is, therefore, present in high amounts. The membranes used are sensitive to certain contaminations in the hydrochloric acid, such as organic compounds. This is why it is important to determine if hydrochloric acid contains organic contaminations prior to its use. Quality assurance also plays an increasingly important role in the sale of hydrochloric acid.

TOC – the sum parameter

The TOC (Total Organic Carbon) sum parameter is a measure of contamination by organic compounds. It provides an indication of how much of the carbon present in the sample originates from organic compounds.

Specialized TOC analyzers are used for this purpose. The sample is acidified in the analyzer in order to destroy inorganic carbon compounds present, such as carbonates and hydrogen carbonates. The resulting carbon dioxide is subsequently removed using a sparging gas.

An aliquot of the pretreated sample is injected onto a heated catalyst (680 °C). The organic com-

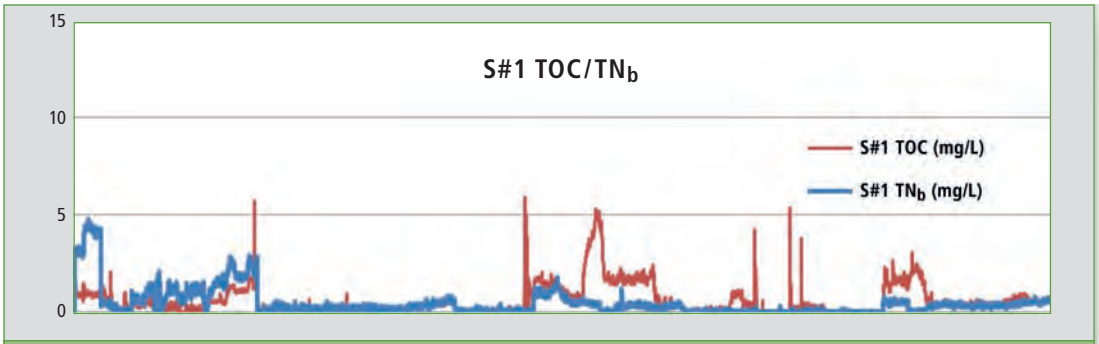


Figure 3: Trend graph: TOC and TN_b -results measured over a period of four months

Figure 1: Concentrated hydrochloric acid is diluted 1:3

Figure 2: TOC-4200 in a protective cabinet

pounds are converted to CO_2 and detected via an NDIR detector.

In addition to conventional laboratory analysis, TOC determination can be simply and securely implemented online. Such process analyzers operate autonomously in close proximity to the sample stream to be analyzed. Depending on the parameter setting, they take a sample automatically every few minutes, prepare and analyze it and send the results to a control station or issue a warning when a limit value is exceeded.

TOC in concentrated hydrochloric acid

These types of process analyzers are used routinely in many application areas, such as wastewater monitoring. Online TOC monitoring of concentrated hydrochloric acid, however, remains a major challenge.

As the extremely acidic sample does not contain any hydrogen carbonates, the sample preparation step (acidification and sparging) can be omitted. Reliable TOC determination in concentrated hydrochloric acid is, nevertheless, anything but easy.

In close cooperation with the engineering department of a Shimadzu costumer, a project was realized determining TOC in hydrochloric acid.

Simultaneous TN determination

A major advantage of catalytic combustion oxidation for the determination of TOC is the possibility to simultaneously detect the amount of nitrogen compounds. During combustion, the nitrogen compounds are converted to nitrogen monoxide (NO). A chemoluminescence detector (CLM) is connected in series following the CO_2 selective NDIR detector. The incoming NO is oxidized to nitrogen dioxide (NO_2) using ozone, and the photons emitted during this reaction are detected by the CLM detector. This combination of detectors enables simultaneous detection of TOC and nitrogen compounds.

Protection and safety

To protect the analyzer from aggressive hydrochloric acid fumes, various gas washers are needed. Shimadzu's TOC-4200 is equipped with several such gas washers that chemically bind the aggressive chlorine gas.

Also needing to be considered in this analysis, is that hydrochloric acid is a gas being released from a concentrated solution. The concentrated hydrochloric acid must therefore be diluted prior to reaching the analyzer. This requires a dilution apparatus located outside of the analyzer (figure 1), which dilutes concentrated hydrochloric acid 1:3 with ultrapure water.

Another safety issue is occupational safety. Employees should not come into contact with aggressive vapors. This is why these types of analyzers must be surrounded by a protective cabinet equipped with hydrochloric acid sensors. An alarm signal is issued when HCl (hydrogen chloride) gas is detected. This protects employees as well as materials.

Taking these issues into account, a reliable system for the determination of organic load in hydrochloric acid is designed. And what has long been a routine laboratory application can now also be analyzed online.

In a test phase, concentrated hydrochloric acid was monitored over a period of four months. The TOC as well as the TN_b was calibrated over a range of 1 - 10 mg/L. The TOC-4200 features an automatic dilution function enabling creation of a multi-point calibration from a standard solution. As the acids need no further acidification, sample preparation (acidification and sparging) is not necessary. The injection volume is 50 µL.

Changes in the US Pharmacopoeia <643>

What are the impacts on TOC determination in the pharmaceutical industry?

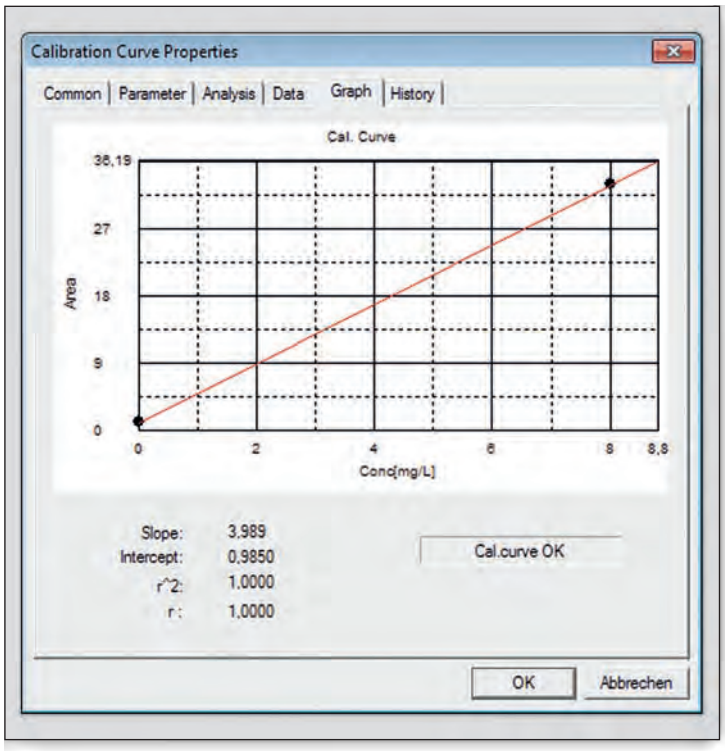


Figure 1: Calibration curve of sucrose, 8 mg/L

In 1996, the US Pharmacopoeia has introduced the TOC parameter for the determination of impurities in purified water and water for injections. For other waters used in the pharmaceutical industry, the wet-chemical potassium permanganate test continued to be used. Meanwhile however, TOC determination has proven to be so effective that it now replaces the wet-chemical test.

In the current version of the UPS <643> (USP 36-NF 31) a distinction is made between 'bulk water' and 'sterile water.' The chapter 'Bulk Water' includes purified waters that are to be used right away as purified water, water for injection, water for hemodialysis and as condensate of pure steam. The following known conditions apply to TOC determinations:

- Limit of detection of the TOC system used: < 0.05 mg/L C
- Blank water for the preparation of standards, r_w: max. 0.1 mg/L C
- Standard solution (sucrose), r_s: 0.5 mg/L C
- System suitability solution (benzoquinone), r_{ss}: 0.5 mg/L C
- Permitted response: 85 - 115 %
- Limit response for the waters, r_u: < (r_s-r_w)

The chapter 'Sterile Water' is new. It includes sterile purified water, sterile water for injections, sterile water for irrigation and sterile water for inhalation. Sterile water can be stored in various packaging configurations.

In comparison to bulk water, however, other conditions for TOC determination apply:

- Limit of detection of the TOC system used: < 0.1 mg/L C
- Blank water for the preparation of standards, r_w: max. 0.1 mg/L C
- Standard solution (sucrose), r_s: 8 mg/L C
- System suitability solution (benzoquinone), r_{ss}: 8 mg/L C
- Permitted response: 85 - 115 %
- Limit response for the waters, r_u: < (r_s-r_w)

Impact of the new determination

The present requirements of the UPS <643> (bulk water) are consistent with the requirements of the European Pharmacopoeia (limit of detection, concentration of the standard solution (sucrose) and system suitability solution (benzoquinone and response). Validation of the TOC system for both determinations is therefore sufficient. In accordance with the new USP <643>, the implementation of a system suitability test using higher concentrations is required. For users of Shimadzu's TOC systems, this just means the creation of an additional calibration curve (sucrose, 8 mg/mL, see figure 1) and control sample (benzoquinone, 8 mg/mL, see figure 2) as well as extension of the current validation process with these data. Additional modifications of the TOC system are not necessary.

TOC determination in ultrapure water

Two oxidation techniques are now commonly used in TOC analysis: catalytic combustion and wet-chemical oxidation. In catalytic combustion, carbon compounds are converted to CO_2 using high temperatures and a catalyst, with subsequent detection of the resulting CO_2 using an NDIR detector. Wet-chemical oxidation uses a combination of UV irradiation and persulfate oxidation. Both methods are suitable for TOC determination in ultrapure water.

Shimadzu TOC system

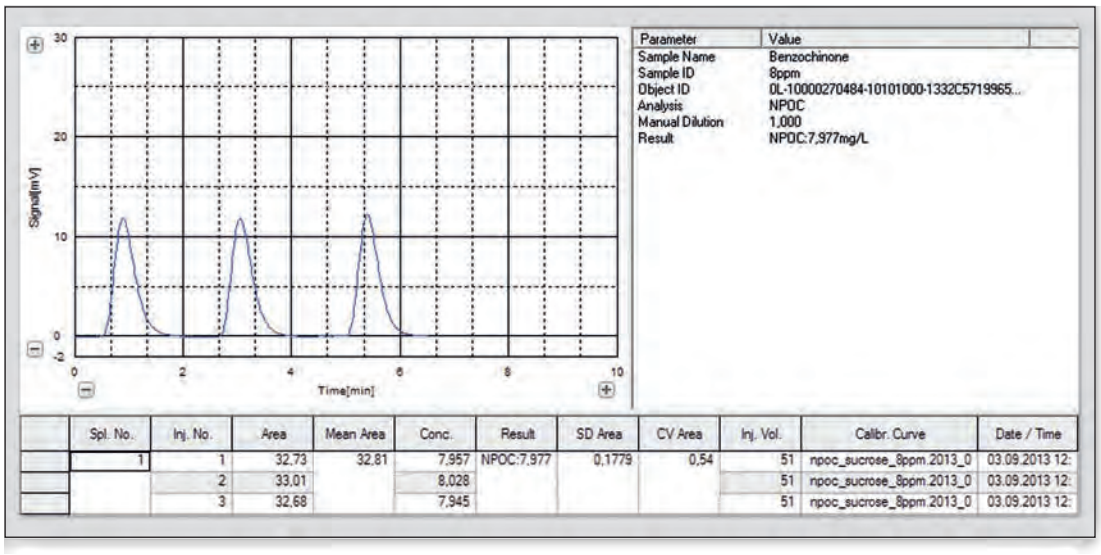
Shimadzu offers two systems that are ideally suitable for TOC determination in ultrapure water. The TOC-$V_{WP/WS}$ uses wet-chemical oxidation, whereas the TOC-L_{CPH} uses catalytic combustion at 680 °C. With their wide measuring range of 0.5 mg/L up to 30,000 mg/L, the instruments support any application – from

ultrapure water (for instance in cleaning validation) to highly polluted waters (such as wastewaters).

TOC-L series – catalytic oxidation at 680 °C

The ISP (Integrated Sample Pretreatment) module for the TOC-L series significantly reduces the user's workload as the instrument carries out dilution, acidification and sparging. The fully automated dilution from 4 µg/L up to 30,000 mg/L extends the measuring range.

In addition, the combustion method can be used in combination with the TNM-L module, whereby a single injection is sufficient for simultaneous determination of the total bound nitrogen (simultaneous TOC/TN_b determination). The EN-compliant determination via chemiluminescence detection is applied. Catalytic combustion is carried out at 720 °C. Simultaneous TOC/TN_b determination is highly suitable for cleaning validation, as it enables a differential determination between the cleaning agent and the product.



Spl. No.	Inj. No.	Area	Mean Area	Conc.	Result	SD Area	CV Area	Inj. Vol.	Calibr. Curve	Date / Time
1	1	32,73	32,81	7,957	NPOC:7,977	0,1779	0,54	51	npoc_sucrose_8ppm.2013_0	03.09.2013 12:
	2	33,01		8,028				51	npoc_sucrose_8ppm.2013_0	03.09.2013 12:
	3	32,68		7,945				51	npoc_sucrose_8ppm.2013_0	03.09.2013 12:

Figure 2: Graphical representation of benzoquinone peaks, 8 mg/L

TOC-V series – wet-chemical oxidation

The key technique of the TOC-V_{WP} series is the powerful oxidation via the combination of sodium persulfate and UV oxidation at 80 °C. As a persulfate solution is used for the determination, it is important that it does not contain any contaminations that could distort the actual measuring value.

The TOC-V_{WP} features an automatic reagent preparation function that eliminates possible contaminations of the persulfate solution. This ensures that the average TOC value truly originates from the sample – and not from the reagent solution used.

The large injection volume (up to 20.4 mL) in combination with the highly sensitive NDIR detector, leads to an extremely low limit of detection and excellent reproducibility in the lower ppb range. The TOC-$V_{WP/WS}$ is therefore highly suitable for TOC determination in the ultra-trace range.

Conclusions

Both types of instrument with their different oxidation methods can be used for TOC determination in accordance with the new United States Pharmacopoeia (USP <643>) and the European Pharmacopoeia (EP 2.2.44). The advantage of the combustion method is its high oxidation potential, especially for samples containing particulate matter. Moreover, simultaneous TOC/TN_b measurements can be carried out, leading to a higher information content of the analysis. The advantage of wet-chemical oxidation is its very high injection volume, which leads to higher sensitivity and therefore enables high precision measurements in the lower ppb range.

TOC-L_{CSH}

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: 6,270 · English: 15,920

N-terminal protein sequencing in drug development

PPSQ-30 protein sequencer for peptide-containing beads isolated from peptide libraries

Figure 1: The main user of the PPSQ Robert Cordfunke (left) and Huybert van de Stadt (right) both from LUMC Leiden, who developed the reaction chamber design for bead analysis

Protein sequencing is a simple and robust method based on Edman degradation. As medical drugs are increasingly protein-based, it is necessary for quality control to determine the N-terminus. N-terminal amino acids from immobilized proteins or peptides are cleaved, derivatized and chromatographically identified according to their retention times.

The Edman reaction starts in the reaction chamber under basic conditions with the coupling of phenylisothiocyanate (PITC) to the N-terminal amino acid of the peptide/protein. This amino acid is cleaved from the peptide/protein via a conversion reaction under acidic conditions and is subsequently transferred to the conversion flask. It is then converted to a phenylhydantoin amino acid (PTH-AA) and injected onto a reversed phase chromatographic column. The isocratic separation enables PTH-AA identification based on retention time related to a PTH-AA standard.

While the conversion is still in progress, the next cycle starts in the reactor by coupling of PITC to the next amino acid of the peptide/protein. The exact order of amino acids, i.e. the protein or peptide sequence, can then be determined in this way. Even isobaric amino acids like isoleucin and leucin which have the same mass, can be separated and identified.

The samples are usually already immobilized, e.g. by western blotting or are in solution and immobilized onto a membrane before analysis.

The research group of Jan Wouter Drijfhout from the Leiden University Medical Center (LUMC) in Holland uses PPSQ for analysis of rather unusual samples. They synthesize peptide libraries using a mix-and-split protocol. This generates millions of different peptides still attached to small polymeric beads. Each bead has a size of about 100 µm and is loaded with 50 - 100 pmol of peptide. Every bead in the library contains a peptide with a unique sequence leading to a peptide library with large diversity/complexity.

This library can be used to screen for new active substances. Thousands of peptide-containing beads are incubated simultaneously with a molecule of interest, such as an antibody, in solution. Antibody-binding beads are visualized to stain for the presence of antibody on particular beads. Such beads contain possibly interesting peptides of which the sequence is not known at this stage. The sequence of the peptide is determined by sequencing the peptide-containing bead.

Testing peptides for antibiotic properties

Another application is the testing of peptides for antibiotic properties. The library beads are synthesized in such a way that a part of the peptide can be liberated from the bead by irradiation with UV light. Beads are spread in a bacterial culture, and beads containing bactericidal peptides are identified by the presence of an area of killed bacteria around the bead (a halo). Again, the sequence of such an antibacterial peptide is unknown in this stage and needs to be determined by Edman sequencing of the peptide-containing bead.

As the peptides are randomly synthesized using mix-and-split technology, the exact sequence of the peptides of interest is unknown and needs to be disclosed. In order to sequence the peptides while still coupled to the bead, the reactor of the PPSQ had to be rebuilt. This now enables sequencing of peptide-containing beads.

Data evaluation is supported by the analysis software. Easy-to-use handling, such as reprocessing of chromatograms, individual reports and automatic estimation of amino acid sequence simplify analysis. An own software window allows control of hardware, combining the sequencing unit with the HPLC.

Figure 2: The protein sequencer PPSQ-30 consists of the Edman reaction unit and a HPLC unit. Both units are controlled by one unified software.

The PPSQ-30 protein sequencer has the following features:

- stable base line and reproducible retention times due to isocratic separation
- high sensitivity
- easy-to-use software and hardware
- suitability for low throughput due to easy start-up
- reduced costs due to low solvent consumption based on recycling of mobile phase.

How do you like your eggs?

The big breakfast test using the EZ-Test-X Texture Analyzer

Figure 1: Texture Analyzer EZ-Test-LX with customized compression jig

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

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

Eggs are part of a rich, healthy breakfast – whether boiled, fried or as an omelet. On average, the egg consumption per capita per year is 218 in the European Union. Whereas the Spanish eat 285 eggs per person per year, each Briton consumes only 176. Germany nearly meets the average of 217 eggs per person per year.

This high level of consumption requires a high production rate, which can only be attained by keeping high populations of laying hens in egg farming systems. Ever since the ban on battery cages, a change in mindset has been triggered with respect to egg production, and the number of production facilities for organic eggs or free-range eggs is increasing. What is the influence of egg farming methods on the product itself?

The eggshell could be used as an index for natural egg farming methods, since it can be assumed that due to better animal welfare conditions, eggs may grow at a slower rate, and in turn the eggshell can absorb more calcium and thus may be stronger.

Hard center, hard shell?

The rigidity of eggs from various egg farming methods have been compared. Eggs were obtained from a random supermarket and were subjected to a pressure test using the EZ-Test-X Texture Analyzer. The eggs were from barn-raised, free-range and organically raised laying hens as well as from free-range laying hens that received special grain feeds. All eggs were clamped in the testing machine and compressed at a constant crosshead movement until the break. To level possible natural unevenness of the shell, the bottom support surface as well as the bottom of the pressure plate was covered with a 40 mm thickness foam board.

All test measurements were carried out on raw eggs as well as eggs that had been hard-boiled for ten minutes.

Egg farming methods and eggshell strength

Table 1 shows inconsistent values. While there were hardly any differences between the values for boiled and raw barn-raised eggs and free-range eggs, the values between raw and boiled organic eggs and for grain-fed eggs increased significantly.

Also the strength profiles between the individual egg farming methods were not as expected. While eggs produced according to increasingly more ecological farming methods, have harder shells when boiled, this was not the case for raw eggs. Surprisingly, organic eggs exhibited significantly lower values than free-range eggs.

Consequently, it cannot be concluded that the egg farming method has a significant influence on the eggshell. However, it must be noted here that organic eggs exhibited the lowest measured value fluctuations and the most uniform break patterns.

To be continued ... *

*Those of you who cannot wait for the next breakfast test, the entire text can be downloaded in advance.
www.shimadzu.eu/breakfast-test

Egg farming method	Raw (in N)	Boiled for 10 min (in N)
Barn-raised, Grade A	142.74	135.39
Free-range, Grade A	181.27	172.26
Grain-fed free-range, Grade A	162.79	193.30
Organic eggs, Grade A	109.41	161.39

Table 1: Test measurements of raw and boiled eggs

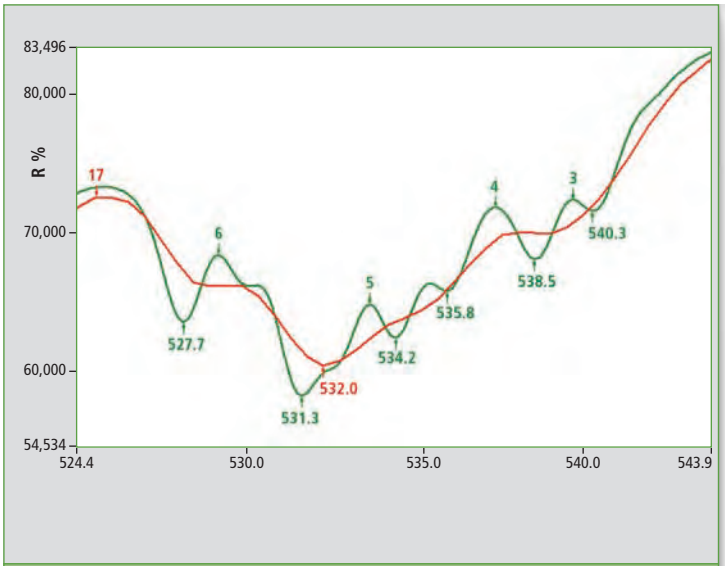


Figure 2: UV-VIS spectra from $NdVO_4$ powder under conditions of 1 nm (green) and 5 nm (red) color. The identification and uniqueness of such substances is of interest. A target application is to prevent copying of the printed graphics on currency.

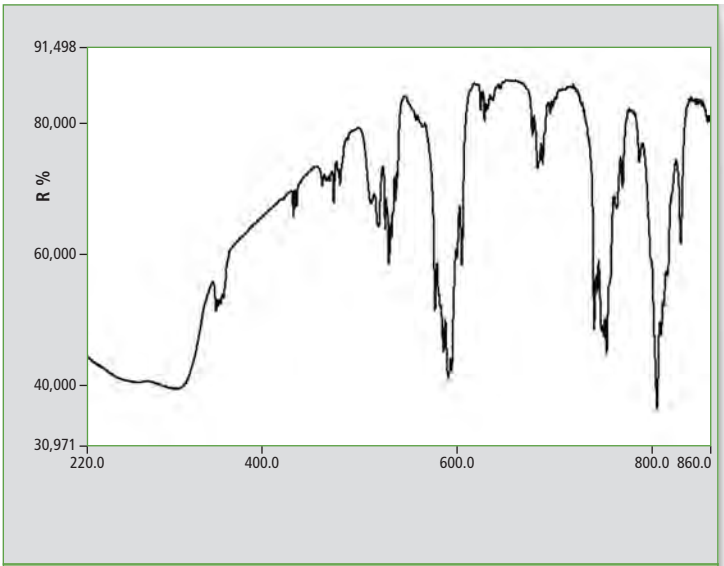


Figure 3: UV-VIS Reflectance spectrum of Neodymium vanadate, 1 nm slit width, ISR-3100 with three detectors, range 220 to 860 nm

Rare earth salts prevent counterfeit money

High resolution spectra in diffuse reflectance for powder samples measured with integrating sphere in the UV-VIS-NIR range

Rare earth elements are located everywhere, but are seldom concentrated in significant amounts at one point (rare earth); their extraction is time-consuming and expensive. Rare earths include 17 elements belonging mainly to the group of Lanthanides. They are used in various industries such as illuminants, magnets, glasses, catalytic converters, polishes and in metallurgy. A short approximation is given in an article from BGR in 2009 [1]. The pie chart shows the distribution of rare earth elements over various industries. In many industrial applications the earth element used to color a target object specifically or even make it unique.

Figure 1 does not include niche markets or applications. One such use can be the coloring of paper, e.g. banknotes. Paper money is copied very often. One attempt to solve this problem applies the use of colors which are unique and difficult to prepare. This can be achieved using rare earth salts. Their structures bear a unique color. Small changes in the crystal configuration will change the appearance. This application shows the effect of different Neodymium and Samarium salts in UV-VIS spectroscopy.

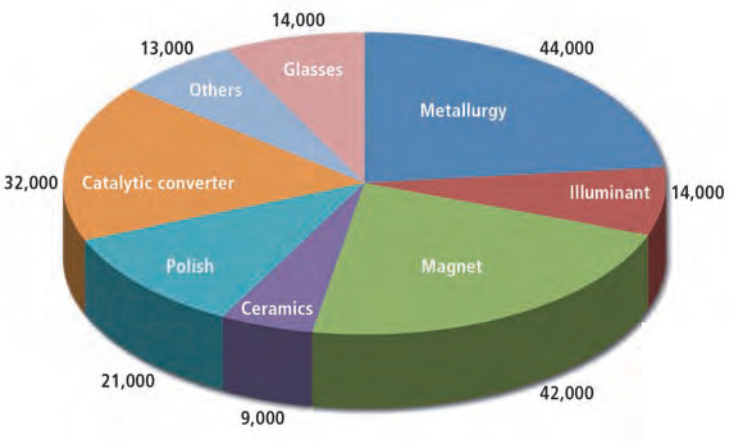


Figure 1: Usage of rare earth metals in t (REO) and by commercial use 2012 (Total consumption 189,000 t approximately) [1], source Kingsnorth (2007)

Rare earth salts are a product's finger prints

The salts from the rare earth elements form UV-VIS spectra which have similarities to the so called "finger print" range of the mid infrared spectra. Normally, the UV spectrum of liquids forms broad signals with low resolution. High resolution in a UV-VIS spectrum is expected with gas phase spectra such as the benzene gas phase or for the well-known mercury, deuterium or halogen iodide lamps. In this case, the sample was a solid in powder form pressed behind a quartz window and diluted with $BaSO_4$. Even under this condition, the salts form a finger-

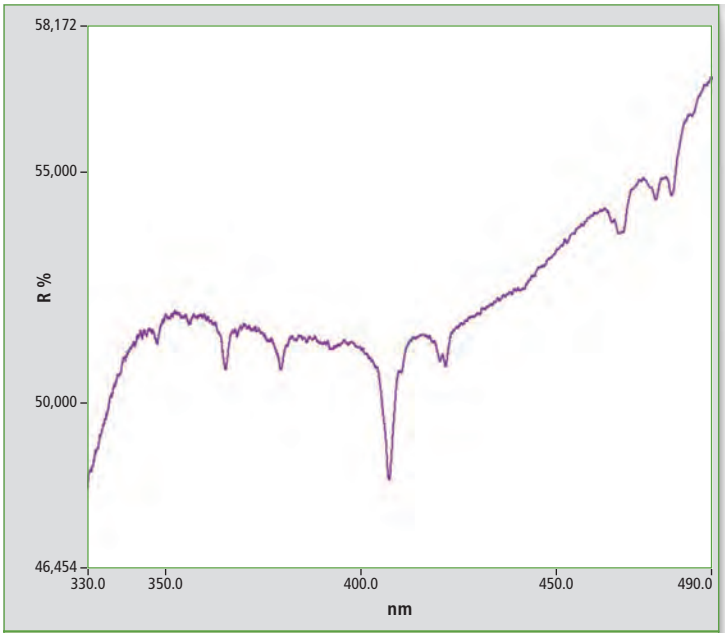


Figure 4: UV-VIS spectrum from Samarium vanadate measured under conditions of diffuse reflectance ($SmVO_4$)

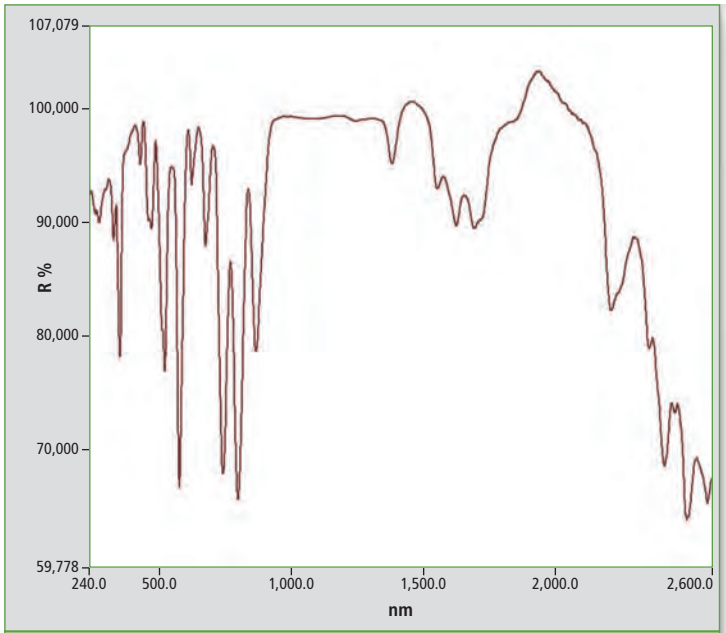


Figure 5: UV-VIS NIR spectrum from Neodymium phosphate $NdPO_4$, 5 nm measurement in the range from 240 to 2,600 nm

print spectrum in the UV-VIS range. The signals reflect not only colors but also energy transfer transitions from the salt pattern. The energy modes shown are characteristic for the lattice or crystal form. They are probably so unique that a specific identification is possible.

Due to the rough surface of a powder, the ISR-3100 integrating sphere was used for the analysis. The integrating sphere collects diffuse reflected light from the powder surface. The sphere has a size of 60 mm, and the diffuse reflectance can be measured.

The sample is placed for this purpose in the 0 degree position at the sphere. Under this condition specular reflected light is excluded from the measurement, and only diffuse reflectance is measured.

In this case, the possibility of obtaining a 1 nm resolved spectrum depends on a good response from the material. Looking at the range of 520 to 550 nm shows substructures in high resolution.

Figure 2 includes two spectra in different resolutions. The red and green colored lines are the 5 nm and 1 nm spectra respectively. In the case of a signal group, the resolution can be shown.

The Neodymium salt yielded successful spectra with many signal groups. For reference, $NdPO_4$ and (for the Lanthanide Group) a Samarium vanadate spectra are presented.

Measurement Properties	
Wavelength Range (nm.)	220 to 2,600
Scan Speed	Medium
Sampling Interval	0.01
Instrument Properties	
Instrument Type	UV-3600 Series
Measuring Mode	Reflectance
Slit Width	1.0 nm
Light Source Change Wavelength	310.00 nm
Detector Unit	External (3 Detectors)
Detector Change Wavelength	870 nm 1,650 nm
Grating Change Wavelength	720 nm

Table 1: Parameter settings for the UV-3600

Homogeneous sample in short time

Sample preparation was completed in short time. The salts were in a ratio of 1:10 volume percent diluted with BaSO4. The powder mixture was transferred into a cup with quartz window. The cup was closed using manual pressure onto the sample mixture to generate a homogeneous layer, pressed with even distribution of the sample over the quartz window.

Objective was to create a homogeneous surface of the sample. The cup was placed into the sample position at the integrating sphere. As reference a cup filled with $BaSO_4$ only was used. The ISR-3100 integrating sphere (with three detectors) was equipped with PM, InGaAs, and PbS detectors for the complete range of UV-VIS-NIR.

The benefits of such rare earth salts are the uniqueness of the crystal lattice which forms a spectrum in the visible range dependent on the lattice. Charge transfer transitions are mainly measured. The color of the material depends on all of these characteristics, thereby being unique. Identification can be done using UV-VIS-NIR spectroscopy.

Literature

[1] Seltene Erden; Maren Liedtke, Harald Elsner; http://www.bgr.bund.de/DE/Gemeinsames/Produkte/Downloads/Commodity_Top_News/Rohstoffwirtschaft/31_erden.pdf?__blob=publicationFile&v=2

Acknowledgment

Many thanks to Prof. Dr. Robert Glaum (Inorganic Chemistry Department at the University of Bonn) for the neodymium and samarium salts and the discussions regarding this topic.

Identify different sources of contamination

Applications with IRTracer-100 and »Contaminant Analysis« software

IRTracer-100

Infrared spectroscopy as classical analysis has been a major technique for the identification of solid materials with KBr pellet and cell techniques for liquid samples. Nowadays, other reflection style measurement methods are increasingly being used.

Users need a new view on spectra, because the measurement results not only include air from the atmosphere, but also extra signals from the accessory, e.g. a diamond profile. Finally, the spectrum is contaminated by accessory absorbance or reflection.

A different class of contaminants can be particles in materials which as impurities restrict the quality of use (uneven, broken etc.). Contamination due to organic and inorganic compounds can be present in different media, such as tap water. In this application, methods are shown which are designed to assist in the identification of contaminants.

Contaminant analysis software for complex challenges

The new IRTracer-100 system with its new LabSolutions IR software platform enables complete application analysis, e.g. a contaminant analysis. In such cases, it often happens that the contaminant consists of a mixture of several substances. A conventional search result covers just one of the main components and needs further expertise to identify the remaining unknown substances.

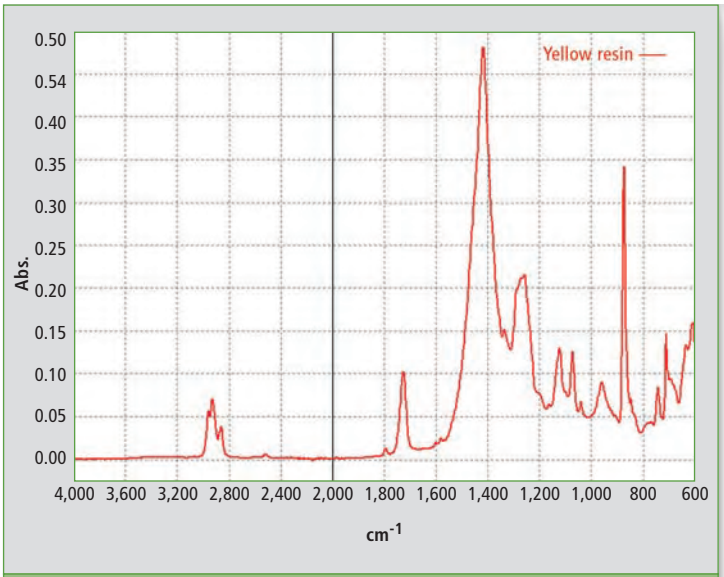


Figure 1: ATR spectrum from PVC resin measured with a diamond reflection unit

The Contaminant analysis software sorts out such complex identification.

The LabSolutions IR program offers two main application fields, i.e. Transmission and ATR (Attenuated Total Reflection) mode. 533 spectra (typical contaminants) are contained in specific libraries which are used for sophisticated searching. The search algorithm has been improved to also enable precise re-identification of inorganic material. As an example, a resin was selected.

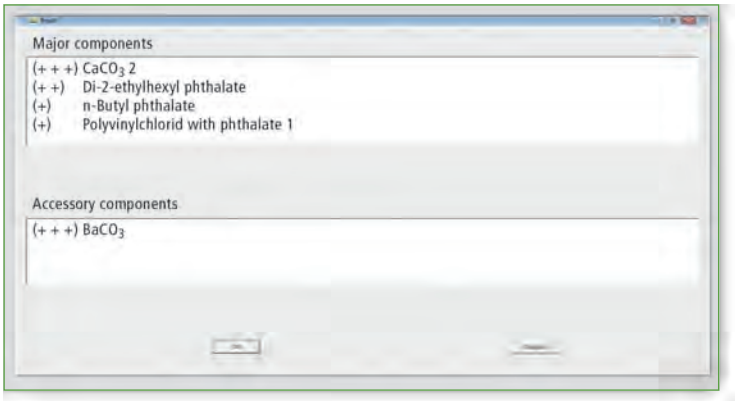


Figure 2: Analysis results using the contaminant analysis software, listing with judgment levels

Several infrared spectra in vinyl chloride resin

Several types of additives are added to resin materials to enhance their material properties. Each one has its own infrared spectrum overlaying the main material spectrum.

In the following article, results of a vinyl chloride resin are presented. The sample was prepared with a diamond-based single reflection unit, the DuraSamplIR, and analyzed by Contaminant analysis program using ATR measurement technique. Result of the analysis indicates phthalic ester present in PVC and calcium carbonate (see figures 2 and 3). These additives are commonly used as plasticizer and non-reinforcing filler respectively in PVC products.

Contaminants in tap water

A different approach in identifying unknown materials is the analysis of inorganic and organic contaminants in tap water. They can originate from different sources, such as tap water system materials, minerals and microorganisms. Major contaminant sources include rubber and metallic components resulting from degradation of the water supply system.

The tap water analysis program includes two databases for FTIR and EDX analysis. As soon as a run is started, the system identifies the IR-Spectrum and gives additional information based on the EDX analysis of various materials.

An example of analysis is shown on the right (figure 4).

Conclusion

With dedicated knowledge bases such as libraries or databases, it is easy to analyze contaminants. Mixtures, e.g. polymers, are easy to identify. The software can be adapted for contaminant analysis in environmental, petrochemical and food fields.

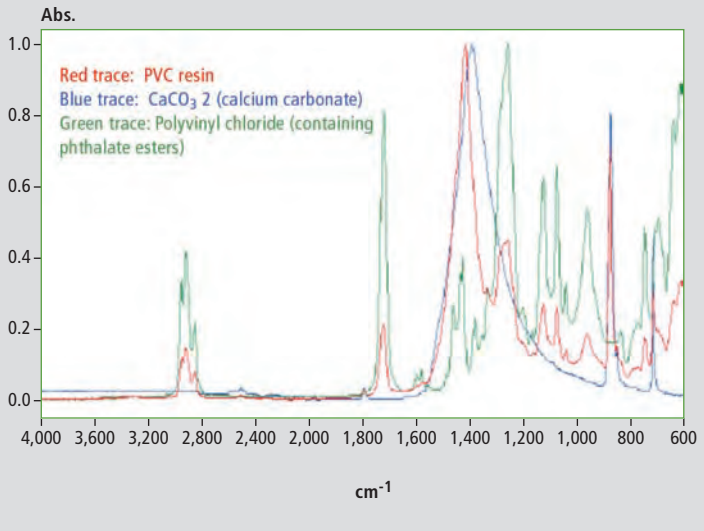


Figure 3: Spectra output window for direct comparison of search results from the library

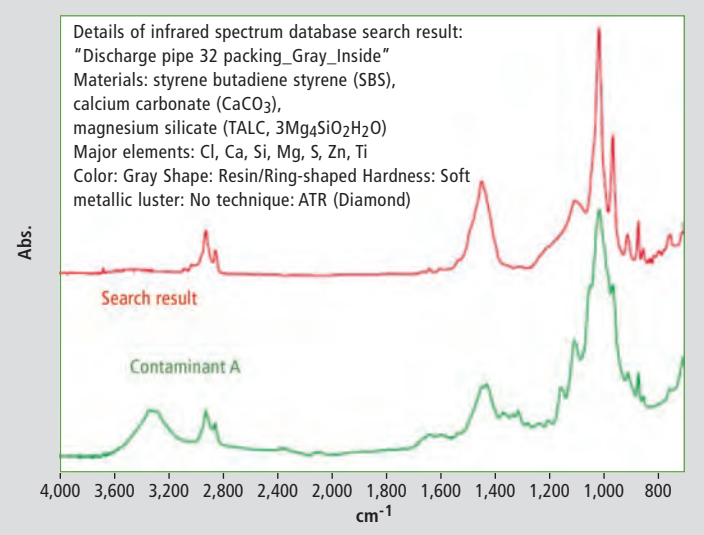


Figure 4: Combination with the EDX database allows judgment of the contaminant's color or other characteristics

The best technique for elemental analysis

Criteria for selection

Figure 1: Fully automatic atomic absorption spectrometer AA-7000

Atomic spectroscopy includes a variety of technologies that are designed primarily for the determination of the elemental composition of samples from many industries, e.g. environmental, geochemical, metallurgical, pharmaceutical, food, agriculture and more. Among these techniques, the most popular ones in routine applications are atomic absorption- (AAS), inductively coupled plasma- (ICP-OES), and X-ray fluorescence- (XRF) spectroscopy.

Which of these techniques for qualitative and quantitative determination of element concentrations is best suited for solving a particular analytical problem? Due to the techniques' specific strengths and advantages, but also limitations and disadvantages the optimum solution for a particular problem may not always be clear. Selecting the right technique requires consideration of a variety of important criteria such as detection limits, analytical working range, sample throughput, interferences, ease-of-use and the investment budget available. This article gives an overview of the most important techniques and provides the information required to help in selecting the best solution to a specific analytical problem.

Atomic Absorption Spectroscopy

AAS quantitates concentrations of elements in a vapor, when a ground state atom absorbs light energy of a specific wavelength and is elevated to an excited state. The amount of light energy absorbed at this wavelength is increased when the number of atoms of the selected element in the light path increases. The relationship between the amount of light absorbed and the concentration of the element present in known standard solutions can be used to determine unknown sample concentrations by measuring the amount of light they are absorbing.

Quantitative analysis of elements is typically carried out using atomic absorption spectrometers such as the Shimadzu AA-7000, consisting of a primary light source for the target element, an atomization unit, a monochromator to set the specific wavelength of light to be measured, a photomultiplier detector to measure the light, and electronics for the data processing as well as reporting of the results. The light source is a hollow cathode lamp containing the element to be determined in the cathode. The atomization unit is either a burner head for flame atomization or a graphite furnace for electrothermal atomization.

The typical flame system operates a titanium burner head with an air/acetylene or nitrous-oxide/acetylene flame. The sample is introduced as an aerosol into the flame by the sample-introduction system consisting of a nebulizer with Pt/Ir capillary and a spray chamber which is inert to aqueous and organic solvents. The burner head is aligned so that the light beam passes through the flame, where the light is absorbed. Unfortunately, sample introduction using a nebulizer and spray chamber is not very efficient, so that only a small amount of sample is delivered to the flame, thereby limiting the sensitivity of such system.

In order to improve the sensitivity of atomic absorption spectrometers, another atomization unit is needed consisting of a high sensitivity graphite furnace such as the GFA-7000, where the sample is introduced directly into a graphite tube before it is heated in a programmed sequence to remove the solvent and matrix components and to atomize the element of interest. All of the analyte is atomized, and the residence time of the atoms within the tube is extended resulting in a high sensitivity and significantly improved detection limits in comparison to flame atomization.

The key differentiator between electrothermal atomization and flame sampling is the analysis time, which is much longer when using the graphite furnace system. On the other hand, the enhanced sensitivity of the graphite furnace which is typically 100 to 1,000 times better than flame makes this an ideal solution for the analysis of ultra low concentrations. Figure 1 shows the AA-7000 which is a fully automatic double beam dual atomizer system in combination with the graphite furnace GFA-7000 with digital control and the ASC-7000 sample preparation station.

The AA-7000 in the dual atomizer version includes both flame and

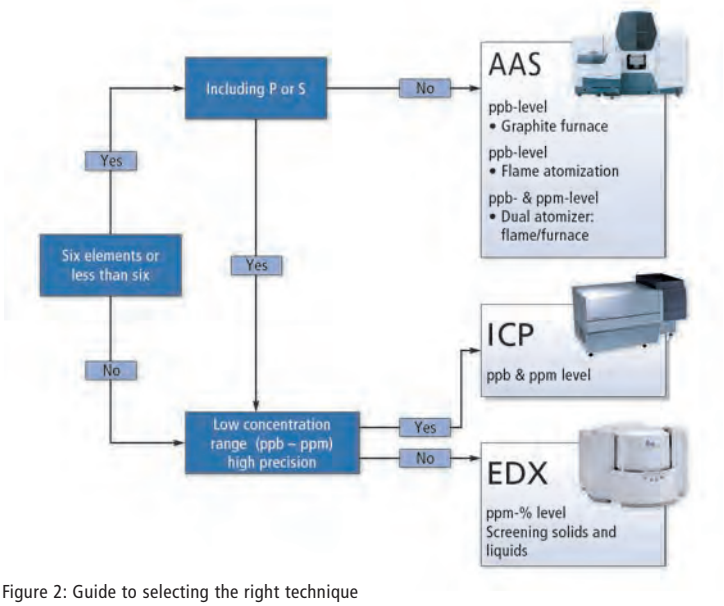


Figure 2: Guide to selecting the right technique

Technique	Strengths	Limitations	Applications	System
Flame AAS – Flame Atomic Absorption Spectroscopy	Very easy-to-use Widely accepted Reference method in many fields Wide application range	Low sensitivity Sequential analysis Use of flammable gas	Ideal for laboratories analyzing many samples for up to six elements and for the determination of concentrations in ppm level	**AA-7000F**
GFAAS – Graphite Furnace Atomic Absorption Spectroscopy	Inexpensive Low detection limits Wide application range Unatteneded operation	Limited analytical working range Sample throughput limited in comparison to flame AAS or ICP-OES	Ideal for laboratories analyzing many samples for up to six elements at low detection limits with typical concentrations in ppb level	**AA-7000G + GFA-7000**
ICP-OES – Inductively Coupled Plasma Optical Emission Spectroscopy	Fast analytical speed Simultaneous multi-element analysis Highest sample throughput Widest analytical range Good documentation Wide application range Unatteneded operation Qualitative and quantitative analysis	Higher initial investment	Ideal for laboratories doing multi-element analysis on a large number of samples in ppm to ppb level	**ICPE-9000**
ED-XRF – Energy Dispersive X-ray Fluorescence Spectroscopy	Easy-to-use Non destructive analysis Fast screening No need for sample pretreatment	Lower precision at low concentration levels	Ideal for laboratories doing fast analysis in the upper ppm to % level with no sample preparation	**EDX-720P/800P**

Table 1: Overview of strengths and limitations

graphite furnace atomization unit, allowing fully automatic changeover from flame to graphite furnace mode and element-specific optimization of the atomizer position. The system includes two methods of background correction for the determination of element concentrations in samples with complex matrices. The Deuterium background correction is useful for compensation of spectral interferences generated by molecular absorption and particulate caused scattering. In addition, the high speed self-reversal technique (high current pulse technique) is useful for compensation of interferences caused by overlapping absorption lines and structured background.

ICP-OES: Inductively Coupled Plasma Optical Emission Spectroscopy

Inductively coupled plasma optical emission spectroscopy (ICP-OES) is the measurement of light emitted by all elements present in a sample introduced into an ICP source. The emission intensities measured are then compared with the intensities of standard samples of known concentration to obtain the elemental concentrations in the unknown samples. The argon plasma is generated by an RF field and ionized argon gas. The advantage of the plasma in comparison to other energy sources is the high temperature of 10,000 K, enabling complete atomization of the elements in a sample while minimizing interferences.

The ICPE-9000 applies a mini-torch with reduced argon consumption which is mounted in a vertical position. This setup allows two ways of viewing the light emitted from the torch. In the typical ICP-OES configuration, the light across the plasma is viewed axially from the top, resulting in the highest sensitivity. In radial view from the side, the sensitivity is reduced by a factor of 5 to 10. The most effective way of operation allows the plasma to be viewed in either orientation in a single analysis, providing the best detection capabilities and the widest working range, the so called dual view mode.

The vacuum optical system of ICPE-9000 consists of a spectrometer including a diffraction grating and a prism for separation of the individual wavelengths of light and focusing onto the surface of the large CCD detector (Charge-Coupled Device). This setup allows true simultaneous multi element analysis with highest sample throughput. In addition to quantitative measurements against calibration curves of standard samples, the system also enables qualitative analysis.

X-ray Fluorescence Spectroscopy (XRF)

XRF allows analysis of element composition of samples in a wide variety of applications. This technique provides non-destructive and fast measurements of liquid and solid samples and is best suited for analyzing the elemental range from sodium/carbon to uranium, which covers the majority of the metallic elements.

In a typical energy dispersive XRF-spectrometer such as EDX-720P/800P, the tube directs X-rays onto the sample, ejecting electrons from the inner electron shells of the atoms. When outer electrons fill in the hole left by the ejected electron, energy is emitted as X-rays and measured on the detector of the XRF spectrometer. The strength of the signal from each element reveals the relative concentration in the sample. Energy dispersive XRF spectrometers such as EDX-720P/800P are multi-purpose instruments for quantitative analysis using standard samples for calibration with high accuracy, as well as the fundamental parameter method when standard samples are not available. Simultaneous screening in the elemental range from sodium/carbon to uranium is also possible.

Shimadzu is one of the worldwide leading manufacturers of analytical instrumentation and has been setting milestones in the field of atomic spectroscopy for more than 40 years. With a state of the art product line ranging from flame atomic absorption and high performance graphite furnace atomic absorption to simultaneous ICP-OES- and X-ray fluorescence systems, Shimadzu offers a total hardware and software solution for the determination of element concentrations in every type of sample.

Greek wine

Determination of organic acids in wine

Organic acids contribute greatly to the composition, stability and organoleptic qualities of wine, especially white wine. Their preserving properties enhance microbiological stability. So dry white wines not subjected to malo-lactic fermentation are more stable in terms of potassium hydrogen tartrate and calcium tartrate precipitation. Red wines are stable at lower acidity levels due to the presence of phenols.

Since the composition of the organic acids directly influences the flavor and color of wine, it must be controlled carefully to ensure proper fermentation and to prevent spoilage (table 1).

Organic acid	Reasons of interest for wine
Acetic	Monitored to prevent spoilage
Citric	Monitor for export limits
Fumaric	Added to prevent malo-lactic fermentation and adjust acidity
Malic	Determined to measure the progress of malo-lactic fermentation
Tartaric	Concentration is useful for deacidification and might be applied to cold stability testing

Table 1: Reasons of interest of organic acids in wine

The Union of Agricultural Cooperatives of Peza in Greece is located in the prefecture of Heraklion, Crete. Its main products are wine and olive oil. By collecting high quality raw materials from local producers, the Union of Agricultural Cooperatives of Peza Crete ensures excellent quality of these traditional products for its customers. The red wines have P.D.O. Peza (Protected Designation of Origin Peza) status since 1971, and the white wines have had this status since 1982.

In collaboration with N. Asteriadis S.A., the Peza Union Crete has developed in its quality control laboratory a green, fast HPLC method for the determination of organic acids in wine based on research of the University of Calabria [1]. This method requires no hazardous organic solvents. In addition no buffers are used, thereby eliminating the need for additional time to clean the HPLC system.

Experimental

The analyses were performed on a Shimadzu *prominence* HPLC system equipped with a PDA detector (SPD-M20A). Chromatographic separation was achieved on a 250 mm x 4.6 mm, 4 µm Phenomenex Synergi Hydro-RP column (table 2). Detailed analytical conditions are presented in the following paragraph:

Analytical conditions

Analysis mode: Isocratic
Mobile phase: Formic acid 0.1 % in water
Mobile phase flow rate: 0.5 mL/min
Column: Phenomenex Synergi Hydro-RP, 250 mm x 4.6 mm, 4 µm
Column temperature: 35 °C
Detector: PDA SPD-M20A
Detection wavelength: 210 nm
Total run time: 20 min
Injection volume: 20 µL

Column selection

The goal was to select a column that could operate at 100 % aqueous conditions without the need for phosphoric buffer addition. Using water as a mobile phase makes initial equilibration faster and total time of everyday analysis shorter, as it eliminates the additional time needed to clean the system from unwanted salts. In addition, a mobile phase compatible with a LCMS detector and an ESI probe was tested since this instrument is included in the Union's future plans. Formic acid was selected because of its appropriate properties. Two different concentrations (0.1 and 0.5 % formic acid) were tested and the best separation was obtained at 0.1 %. This is in agreement with a previous study conducted by Gamoh et al [2].

Polar analytes are not always retained and often do not separate with a satisfactory capacity factor on conventional C18 columns. Synergi Hydro-RP is a C18 bonded phase endcapped with a polar group to provide extreme retention of both hydrophobic and polar compounds under 100 % aqueous conditions. The high 4 µm silica surface area (475 m^2/g) combined with a dense bonded phase coverage allows substantial interaction between the sample analyte and the bonded phase. The result is a very retentive C18 phase, well suited to separate organic acids.

Running a 100 % aqueous mobile phase on a C18 column can provide improved retention of polar compounds. However, conven-

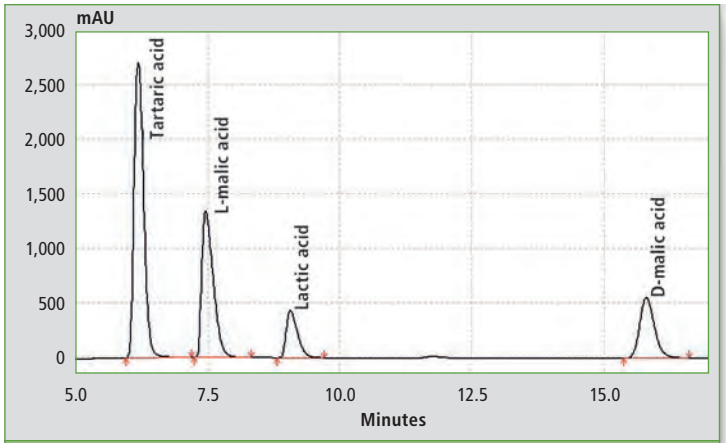


Figure 1: Chromatogram of a mixed standard solution of D-malic acid 5 g/L, L-malic acid 5 g/L, lactic acid 5 g/L and tartaric acid 10 g/L

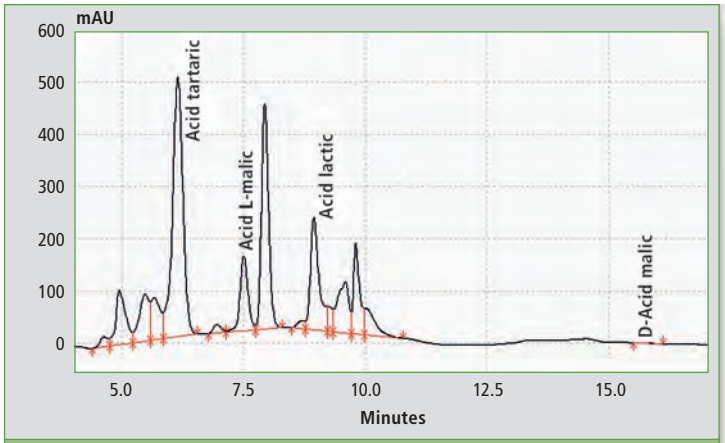


Figure 3: Typical chromatogram of a wine sample

tional C18 phases are poorly wetted by highly aqueous mobile phases, causing the C18 ligands to mat down on the surface of the silica with the consequence that over time, retention is lost completely. Organic acids are often difficult to separate as their polarity hinders interaction with conventional C18 ligands, but it was observed that this is accomplished using Synergi Hydro-RP under 100 % aqueous conditions.

Procedure

Working standard solutions of D-malic, L-malic, lactic and tartaric acid were prepared in ultrapure water. Standard solution concentrations for D-malic, L-malic and lactic acid were 0.5, 1, 2 and 5 g/L. Standard solution concentrations for tartaric acid were 1, 2, 4 and 10 g/L. In figure 1, a chromatogram of a mixed standard solution of D-malic acid 5 g/L, L-malic acid 5 g/L, lactic acid 5 g/L and tartaric acid 10 g/L is shown.

Calibration curve creation and quantitation were performed automatically using LC solution software.

Correlation coefficients and QC parameters for all organic acids are shown in table 3. Correlation coefficients for all calibration curves were excellent as can be seen in figure 2.

Wine samples were diluted 1 : 10 with ultrapure water and filtered using 0.45 µm pore size prior to analysis. Because of the dilution, a dilution factor of 10 was used for quantitation. In figure 3, a typical chromatogram of a wine sample is shown.

Quantitative results and QC parameters for the most typical wine products are shown in table 4.

Conclusions

The method described above has been developed and successfully implemented in the Union's Quality Control laboratory. It has proven to be fast and reliable while requiring no organic solvents and minimum time for equilibration and cleaning. Linearity of the calibration curves for all organic acids was excellent in the range of the measurements, providing a solid base for the determination of organic acids in wine. Using a Phenomenex Synergi Hydro-RP column with particle size of 4 µm proved to be an excellent choice for this type of analysis. This method, although developed using UV detection, is also suitable for LC/MS systems. Using such a system in the future, the Union expects to achieve even better LODs* and LOQs.**

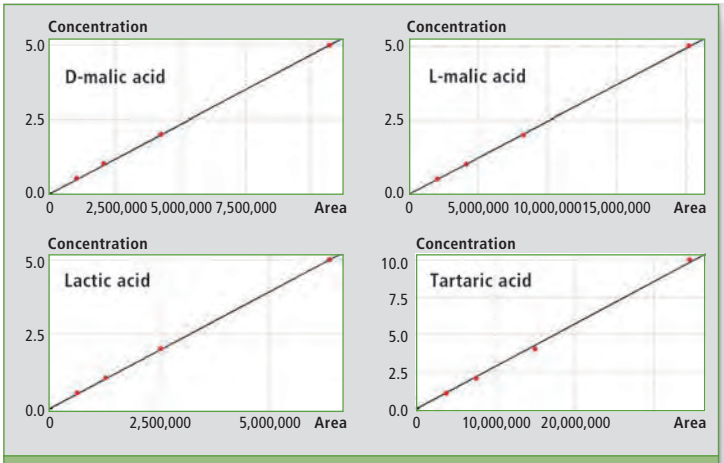


Figure 2: Calibration curves for D-malic, L-malic, lactic and tartaric acid

* LODs: Limits of detection
** LOQs: Limits of quantification

References

[1] RT-OR5-ALTRO-CHIM/2, Rapporto Technico: Verifica Delle Metodiche Analitiche E Loro Applicazione A Vini Calabresi DOC, Progetto: LOGICA, Laboratorio Tecnologico Della Logistica In Calabria, Laboratorio Di Cosenza, R&D.LOG, Italy

[2] K. Gamoh, H. Saitoh, H.Wada, Rapid Commun. Mass Spectrom. 17 (2003) 685-689

[3] A. Rodríguez-Bernaldo de Quirós, M.A. Lage-Yusty, J. López-Hernández, Talanta 78 (2009) 643-646

Packing material	Particle size (µm)	Pore size (Å)	Pore volume (mL/g)	Surface area (m^2/g)	Carbon load (%)	Calculated bonded phase coverage (µmol/m^2)	End capping
Synergy Hydro-RP	4	80	1.05	475	19	2.45	Hydrophilic

Table 2: Column technical specifications

Organic acid	Slope (a)	R^2	Resolution	k'	Theoretical plates	LOD (g/L)	LOQ (g/L)
D-malic	4.6697×10^{-7}	0.99997	15.8	2.410	16500	0.05	0.14
L-malic	2.4624×10^{-7}	0.99991	4.0	0.621	7000	0.08	0.23
Lactic	7.8443×10^{-7}	0.99999	4.5	0.964	10000	0.03	0.09
Tartaric	2.8536×10^{-7}	0.999	—	0.333	6300	0.55	1.68

Table 3: Calibration curve slopes ([Organic acid] = a *Area), correlation coefficients and QC parameters for organic acids. Limits of detection and quantitation are calculated by multiplying the residual standard deviations of the calibration curves (Sy/x) by 3.3 and 10 respectively.

Wine	[D-malic acid] (g/L)	[L-malic acid] (g/L)	[Lactic acid] (g/L)	[Tartaric acid] (g/L)
Retsina	0.53	4.85	13.35	21.52
TTV	0.08	4.03	20.88	21.15
3-150	2.85	7.67	8.34	56.64
QC parameters				
Average resolution	7	1.6	1.0	0.9
Average k'	2.5	0.7	1.0	0.35

Table 4: Quantitative results and QC parameters from some wine samples

Emmanouil G. Barbounis*, Konstantinos Tampouris*, Kleanthi Garefalaki, Georgios Koumantakis****

* Application Department N. Asteriadis S.A

**Union of Agricultural Cooperatives of Peza, Crete

New approach for analysis of pesticide residues

Scientific and economical chromatographic analysis of pesticide residues in organic products with difficult matrices

Organic products differ from other conventional products in the way that they are produced and processed while observing the rules of organic agriculture. These rules, among others, forbid the use of pesticides, and as Regulation EC No 889/2008 states: the quantifiable presence of a pesticide residue in an organic product (positive analysis result) leads to "substantiated suspicion."

Differing controlling authorities don't share the same opinion regarding maximum allowable quantity in mg/kg of pesticide residues in organic products, while the „quantifiable presence" (probably corresponding to the limit of quantification) also differs for pesticides. Since the LOQ for most pesticides is currently around 0.010 mg/kg, it is common for the control authorities to take this value as determinant when declaring a product to be BIO or not. One of the largest associations of organic processors – Bundesverband Naturkost Naturwaren

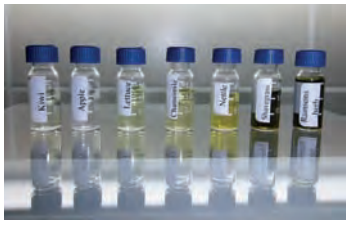


Figure 1: Vials with different vegetable and fruit matrices

(BNN) e.V. from Germany has introduced the following guidelines for decision-making regarding pesticide residue findings in agricultural products: „...The orientation value for each substance (active ingredient) is 0.010 mg/kg, and applies to the original unprocessed product ... No more than a total of two pesticides may be present ..."

Plant species with very complex chemical composition

It is well-known that some plant species have very complex chemical compositions, which result in very difficult sample matrices in QuEChERS sample preparation during pesticide residue chromatographic analysis. As a result, a serious over/underestimation of the quantity of pesticide residues found in such plant samples can occur in gas and/or liquid chromatographic analysis if no proper matrix-matched calibration curves are used.

This is shown in the report issued by European Union Reference Laboratory for Pesticide Residues: Pesticide analysis in teas and chamomile by liquid chromatography and gas chromatography tandem mass spectrometry. Validation data of 86 pesticides using a multiresidue method by LC-MS/MS and GC-MS/MS in green tea, red tea, black tea and chamomile, have shown that the GC response for these pesticides was up to nine times higher in matrix than it was in the pure solvent, whereas the LC response was up to four times lower in matrix than in the pure solvent.

Furthermore, it is well-known that the multiresidue methods currently used for pesticide residue analysis in foods were developed based on the need to search for a large number of pesticides at a reasonable price. In addition, the use of proper matrix-matched calibration is not often seen in common laboratory practice as this could lead to higher expenses, if proper blank commodities were to be purchased for each measurement. Nevertheless, Document No. SANCO/12495/2011 strictly prescribes the use of exact matrix-matched calibration for difficult matrices.

Inaccurate quantification possible

Everything mentioned so far can lead to inaccurate quantification of pesticide residues if multiresidue methods and non matrix-matched, or even solvent calibration curves are used for analysis of plants with difficult matrices, while on the other hand organic certification authorities can in the case of a minimal violation of maximum residue limit (MRL,

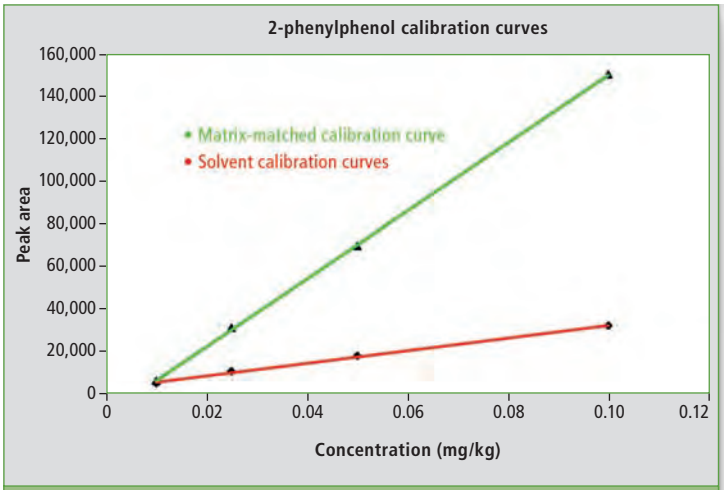


Figure 2: The relationship between matrix-matched and solvent calibration for 2-phenylphenol

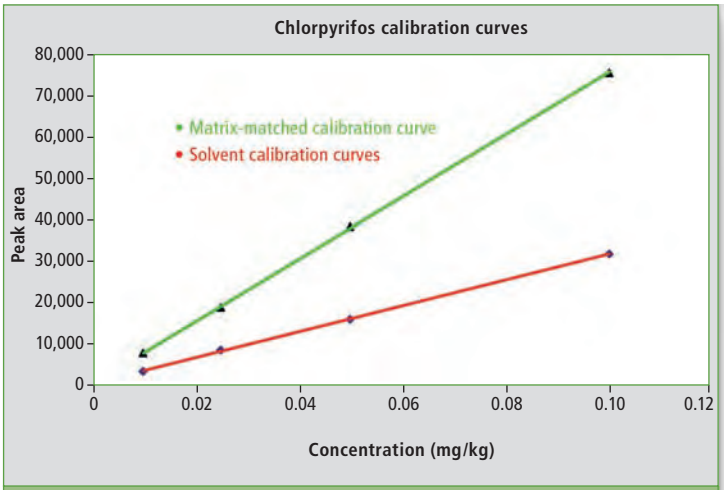


Figure 3: The relationship between matrix-matched and solvent calibration for chlorpyrifos

0.011 mg/kg instead of the allowed 0.010 mg/kg) stop the sale of the commodity in which excessive residue has been found, and organize challenging inspections.

When considering that medicinal herbs and teas (commodities that often have very complex matrices) are mostly acquired by "wild collecting" from areas where no pesticides are used, but which can be present in high quantities in the case of an unpredictable pollution from the surroundings by air or water or in case of unfavorable atmospheric conditions, it is clear that there is a greater risk of finding high pesticide residues reaching MRL-s in these commodities. Methods more accurate than the multiresidue methods for determination of quantity of pesticide residues should be used in these cases.

New approach in four steps

For analysis of pesticide residues in organic products with difficult matrices, the authors suggest the following scientific and economical approach – analysis in several steps with respect to the results obtained:

1. Use of multiresidue methods for the analysis of organic commodities and possible identification of pesticide residues.

2. Rough estimation of whether the sample analyzed is „too bad", or records quantities of pesticide residues which are clearly under the MRL-s (in which case further analysis is not necessary).

3. Recalculating the quantities of residues found using multiresidue method, by using the factors showing the difference in response of the respective pesticides in matrix compared to those in pure solvent (these factors can be obtained by in-house experiments or found in the literature).

4. If the values recalculated show quantities for one or two pesticide residues around 0.010 mg/kg (BNN guidelines), perform the method of standard addition for the most accurate determination of the quantity of the pesticides found.

In order to perform the third step of the proposed approach, the author's laboratory obtained matrix-matched calibration curves for difficult matrices, particulary for herbal teas. As a blank matrix, a mixture of 13 blank herbs, fruits, flowers and roots was used for matrix-matched calibration. These commodities were confirmed to be blank by own and external laboratories. Ratios of calculated concentrations with matrix-matched calibration curve, and concentration calculated with solvent calibration curve were obtained for different concentrations of standard solutions of pesticides, at 0.010, 0.025, 0.050 and 0.100 mg/kg, and named „matrix effect" (ME).

New method in practice: analysis of daisy

To demonstrate the proposal, the authors conducted analysis of one Bellis Perennis (daisy) sample. This analysis was conducted on Shimadzu's GCMS-TQ8030 with Optic 4 injector, using a multiresidue method for determination of 124 pesticides (including isomers). Sample was prepared using the European style QuEChERS sample preparation method.

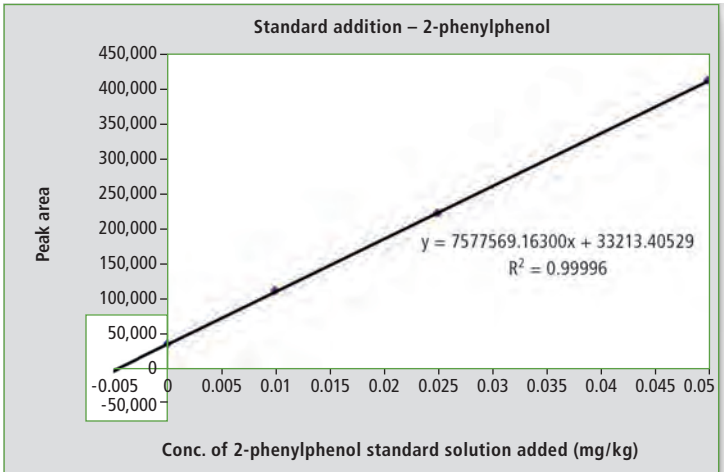


Figure 4: The results of standard addition method for 2-phenylphenol

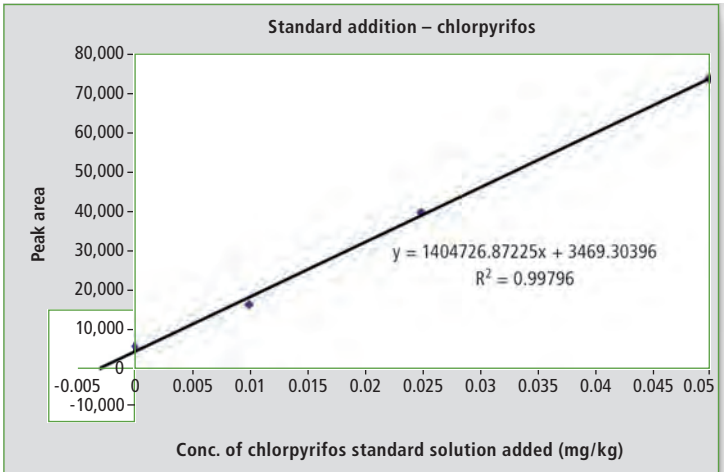


Figure 5: The results of standard addition method for chlorpyrifos

Step 1

Multiresidue analysis of the sample investigated showed positive identification of two pesticides in concentrations above 0.010 mg/kg as calculated by solvent calibration, specifically 2-phenylphenol at 0.011 mg/kg, and chlorpyrifos at 0.012 mg/kg.

Step 2

Elimination of further analysis does not apply.

Step 3

The authors obtained the following ME factors for the identified pesticides at 0.010 mg/kg: 2-phenylphenol 1.22, and chlorpyrifos 2.39. Both concentrations would therefore be lower than 0.010 mg/kg after dividing the results by the ME factors.

Step 4

To confirm these findings, the method of standard addition for these two pesticides was performed later. The daisy sample extract was divided into three equal parts, after which increasing volumes of pesticide standard solutions were added into the three portions of an unknown sample, resulting in three different concentrations of added pesticides, 0.010, 0.025 and 0.050 mg/kg respectively.

After analysis of the prepared solutions was completed and calculations were made, the results confirmed (as can be seen from the graphs 2 and 3) that the true quantities of the pesticide residues found in the daisy sample were smaller than the maximum allowed quantity of 0.010 mg/kg. Corrected values were 0.0044 mg/kg for 2-phenylphenol and 0.0025 mg/kg for chlorpyrifos.

The proposed approach showed that the product which would not have been marketable as organic when analyzed using multiresidue method and external calibration (based on positive identification of two pesticides with quantities > 0.010 mg/kg), satisfies the organic regulation criterion after all. This knowledge was gained by investing in moderately higher analytical costs while acquiring much more precise results.

Time savings in protein analysis

Online digestion of proteins with Perfinity iDP

Figure 1: Perfinity iDP system

Protein analysis is complex and time-consuming. Most of the proteins needing to be characterized are relatively large molecules that are not easily analyzed without pretreatment. Enzymatic cleavage into smaller fragments is a common procedure. In addition to chemical and thus relatively drastic methods, enzymatic reactions are widely used in biochemical laboratories, and have proven to generate reproducible results. The problem is that enzymatic cleavage processes are often time consuming, as they require incubation of the protein with a specific enzyme. It can take 24 hours or longer to selectively cleave a protein without denaturing it.

Tryptic digestion is frequently used. The name is derived from trypsin, an endopeptidase that cleaves proteins at specific locations in the molecule.

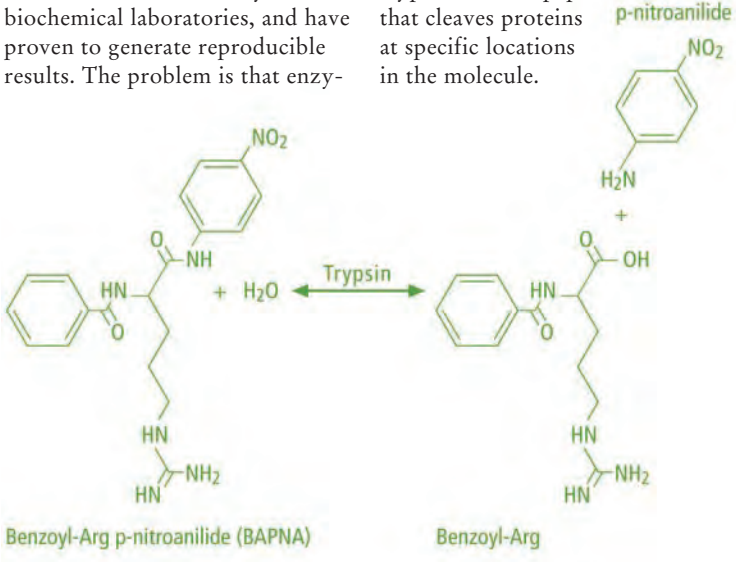


Figure 2: Reaction equation of the trypsin activity test by BAPNA

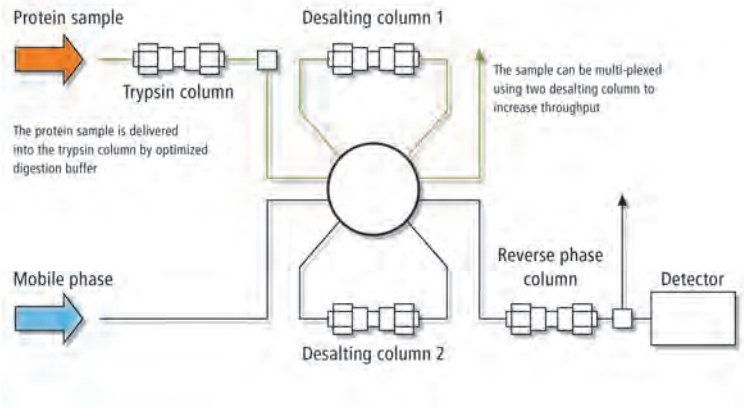


Figure 3: Flow schematics of the Perfinity iDP system

The function of endopeptidases

Endopeptidases are important substances in chemical analytical protein sequencing. The cleaved (denatured) proteins are easily hydrolyzed, and bind water molecules. In the intestinal tract, trypsin selectively cleaves peptide bonds into the basic amino acids lysine, arginine as well as modified cysteine.

Similar reactions occur in the human intestinal tract during digestion of proteins. These are well known in terms of mechanism and functioning. By experience it is, however, much more difficult to predict exactly or to describe such processes for unknown proteins and then to carry them out in a test tube. Nor is it possible to predict reproducibilities or even statistical data for such reactions.

Special column for tryptic online protein digestion

Experiments to speed up enzymatic digestion or to carry this out on a stationary phase are not new. But thanks to advanced developments in HPLC stationary phases, it is now possible to produce a specialized column for tryptic online protein digestion.

From the initial idea of an immobilized trypsin column to the

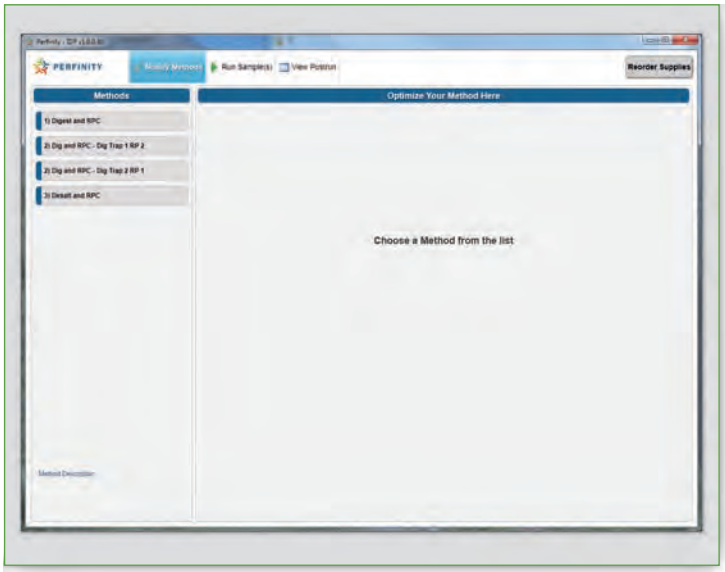


Figure 4: Start screen of the Perfinity iDP software

Perfinity iDP™ system (Perfinity iDP – integrated Digestion Platform) described here, it was and still is a long way: on-column digestion times must be optimized for individual proteins. Questions in this context include: How long should the protein remain on the column in order to be fully digested? What is the optimal digestion time, and what concentrations can be handled? How many digestion cycles can be performed using one column? How can the method be tested and what is involved in quality control?

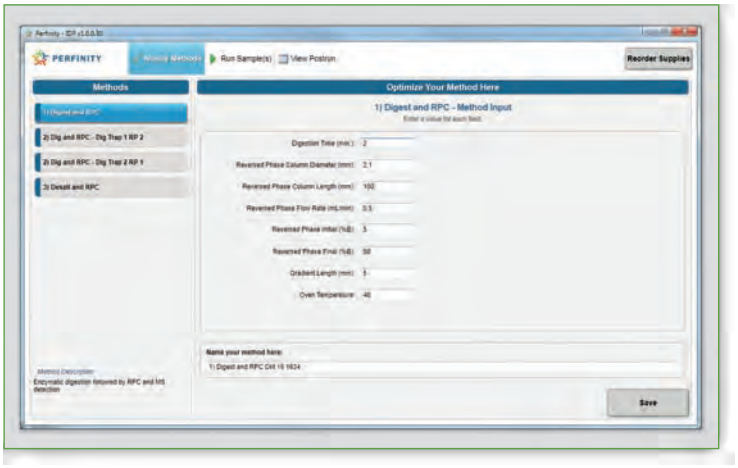


Figure 5: Method details of the standard pre-built method

Hardware, software, application kit

We should not try to answer all these questions ad hoc here, but the Perfinity iDP system offers a combination of HPLC hardware, columns, buffer mixtures and a user-friendly software interface that simplifies an important part of protein analysis: sample preparation for MS/MS analysis, or quality control for biotechnological production of proteins.

In addition to the hardware mentioned here, the system also features an application kit. The kit includes the trypsin column and the appropriate buffer mixtures as a set for every 1-liter ready-made buffer solution, as well as software and a series of prebuilt methods that facilitate system operation. All that is required is to place the samples in the autosampler and start the analysis.

Detecting the enzyme activity of trypsin

The BAPNA test is used as a functionality test. The enzyme activity of trypsin is detected via N-benzoyl-D,L,-alanine-p-nitroalanine (BAPNA). BAPNA is cleaved by trypsin at arginine to form p-nitroaniline. The p-nitroalanine concentration can be detected spectroscopically at a wavelength of 405 nm.

Briefly returning to the system flow line: the previously cleaned protein is loaded onto the trypsin column via a switching valve and is 'parked' on the column using a low flow rate suitable for digestion. In a subsequent step, a higher flow and a different mobile phase are used to transfer the digested protein fragments via an additional valve and a downstream desalting column onto the actual separation column. Separation takes place in an acetonitrile/buffer mixture suitable for LCMS/MS. In addition to the UV detector that is part of the system, a mass spectrometer can easily be connected. This is certainly the detector of choice when as many of the peptides as possible are to be detected and identified.

Simple user interface for LabSolutions

Since most protein analysts find the control and the complexities of a HPLC system only of limited interest, straightforward user interfaces and omitting of all non-essential parameters are an absolute requirement for routine use of the system.

The start screen of the Perfinity iDP software meets all these requirements (figure 4):

The software features a specially adapted user interface for the LabSolutions LC Workstation software.

For everyone interested in HPLC, it goes without saying that all settings can also be completed in the LabSolutions software itself. Being able to start the analysis using pre-built methods is an enormous help to novices in this analytical application area, and should not be underestimated. In addition to the main menu, it is possible to adapt individual method parameters and to save the user-created methods. Just like the standard pre-built methods, these user-created methods are subsequently available in the combo box. Being able to adapt the digestion times in particular can provide interesting results in order to be able to assess the true value of the system for protein analysis.

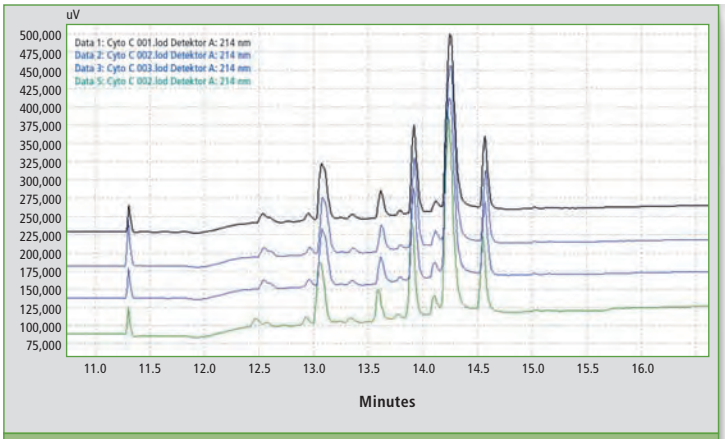


Figure 6: Chromatograms obtained using Perfinity: Tryptic digestion of cytochrome C and examples of their reproducibilities

Fast results

Using the standard pre-built method, it is possible to quickly obtain results for various proteins. The following chromatogram shows the tryptic digestion of Cytochrome C. In addition to the considerable savings in time (8 minutes digestion time 'on-column' compared with 20 hours at 37 °C using the conventional approach), the reproducibility of the method is remarkable. One can only wonder whether experienced protein analysts can obtain such results using a manual approach and how often this actually succeeds. The reproducibility refers equally to results obtained on various non-consecutive days for the same sample. This illustrates the merit of the method.

What the UV detector does not show, however, are the many smaller digestion products which can subsequently be detected using a highly sensitive MS/MS. On the other hand, the limited complexity was also advantageous during the initial experiments using the system (and is certainly also the case elsewhere).

The use of trypsin and tryptic digestion is just one possible approach in protein analysis to somewhat simplify this complex application area. Of course in practice, a variety of enzymes are used for protein digestion.

Acknowledgment

Shimadzu Europa sincerely thanks its colleagues at Shimadzu Scientific Instrument (Maryland, USA) as well as Perfinity Biosystems (Purdue University, Indiana, USA) for the development of the system.

Rapid Scan Software and IRTracer-100

The new IRTracer-100 Fourier Transform Infrared (FTIR) spectrophotometer is a highly versatile instrument in combination with the new LabSolutions IR software. Combining high speed, sensitivity, and resolution with enhanced expandability and easy-to-use

IRTracer-100

software, the IRTracer-100 quickly and easily obtains high-quality data for samples in such fields as pharmaceuticals, foods, chemicals and electronics.

Using the additional 'Rapid Scan' software, the IRTracer-100 demonstrates its advantages in fast kinetics analyses. Up to 20 spectra per second are recorded and are evaluated in terms of signal intensity, area, ratio of two signals according to intensity and area, as well as the TAC (Total Absorbance Curve). These values are basic requirements for kinetics analyses.

The resolution, the number of measurements and the mirror velocity are parameters that affect the measurement speed. A resolution of 16 cm^{-1} and a mirror velocity of 40 mm/s results in an acquisition time of 0.05 seconds per spectrum.

'Rapid scan' is suitable for far-infrared (FIR), middle-infrared (MIR) and near-infrared (NIR) measurements. In the NIR, measuring range data points affect the speed of measurement.

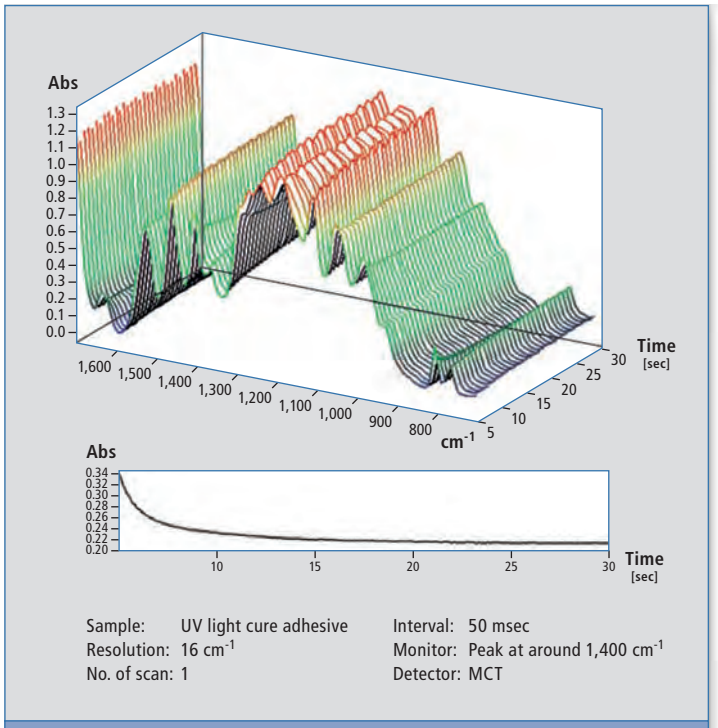


Figure 1: Analysis of the curing reaction of a resin under the influence of UV light

Figure 1 shows the fast kinetics of the changes in a resin under the influence of UV light. The spectra are recorded at 50 ms intervals. Below is the corresponding signal analysis at 1,400 cm^{-1} and the absorption profile over time – 30 seconds in the present case. The 'Rapid Scan' software is therefore recommended for fast kinetics analysis within a time frame of less than one second.

Shimadzu live

HTC-13 & HTSP-3	JEC Composites	Nordic Proteomics Conference (NPC)	ESAS & CSSC	analytica 2014
29.01. - 31.01.2014 Bruges, Belgium htc-conference.org	11.03. - 13.03.2014 Paris, France jeccomposites.com	11.03. - 13.03.2014 Turku, Finland nordicproteomics2014.org	16.03. - 21.03.2014 Prague, Czech Republic esas-cssc2014.spektroskopie.cz	01.04. - 04.04.2014 Munich, Germany analytica.de

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

You can also subscribe to our newsletter via: www.shimadzu.eu/newsletter

YouTube
@ShimadzuEurope