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# NEWS 03 2017

**Joy and fear of Christmas baking ...**

Determination of mycotoxins in bakery products

**The new IRSpirit**

makes infrared spectroscopy easier than ever before

**Behind the scenes**

Users' insights on working with CLAM-2000

# Ultra-small footprint meets exceptional ease-of-use and functionality

## IRSpirit makes infrared spectroscopy easier than ever before


Fourier Transform Infrared Spectrophotometer IRSpirit

The new IRSpirit series complements Shimadzu's well-known FTIR instruments line. Designed for high specification on a small footprint, IRSpirit series adds new possibilities combined with the robustness of its higher-class family members. With the new dedicated software IRPilot, IRSpirit sets a new standard in terms of ease of use. This provides outstanding efficiency in infrared spectroscopy and helps users to save time during analysis development procedures.

### Space-efficient and flexible

In modern laboratories, effective space planning is a permanent challenge. Aware of this problem, IRSpirit has been developed to be as small as possible. Thus, the footprint of the IRSpirit series is smaller than an A3 sheet of paper.

The design of the IRSpirit series also allows to conduct measurements with the unit positioned horizontally or vertically in order to adjust to the smallest spaces, such as glove boxes. In fact, the start button and the humidity indicator are accessible and visible from both setups. Moreover, IRSpirit is especially designed to offer an user easy access of sample compartment from two sides.

Despite this small size, the sample compartment width is the same as on higher-end models: 200 x 140 x 100 mm (WxDxH). This makes the IRSpirit series compatible with many Shimadzu and other commercial accessories and usable for a wide variety of applications.

### High reliability through technology inherited from high-end models

Based on Shimadzu's long and in-depth experience with the FTIR interferometer, the IRSpirit series optical system is constituted by a Michelson interferometer which is well-known for its high performance associated with a ceramic light source. Efficient

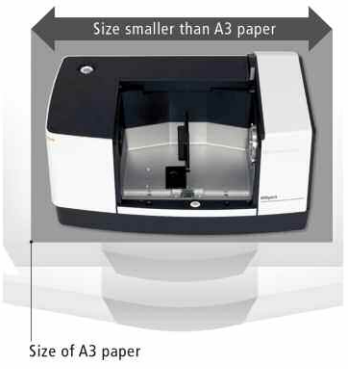



Figure 1: IRSpirit size versus A3 paper size. The A3 paper size is represented by the gray rectangle.

Figure 2: Examples of hardware options for the IRSpirit series: QATR-S (left) single-reflection ATR measurement attachment and DRS-8000A (right) for Diffuse Reflectance Spectroscopy (DRS).

features like the dynamic alignment and the high brightness of the light source support the optical system. With its DLATGS detector with temperature control functions [1], the IRSpirit series is declared the best of its class with a sensitivity up to 30,000 : 1 (with a KBr-window). This system shows high stability and throughput in order to generate reliable results.

High flexibility is ensured by the different window materials available as well as different detector variations. [2] Even though the instrument is small and compact, it is fully controlled the same way as higher-end versions. Status monitoring is also available as well as the diagnostic function. IRSpirit has an automatic accessory recognition which is able to assist users during the parameter selection needed for a specific accessory.

## LabSolutions IR: more than a software

In addition to the accessory automatic recognition, LabSolutions IR software is able to control all the different parameter settings for measurement and analysis.

In order to minimize the users workload, LabSolutions IR provides IRPilot, an assistant software for standard analysis. Guiding through parameter settings, supporting the measurement and ending in the final result – nearly one-stop analysis.

With its 23 integrated applications, IRPilot makes sample analysis easy, even for operators with minimal FTIR experience.

LabSolutions IR will help to solve the specific demands of an infrared

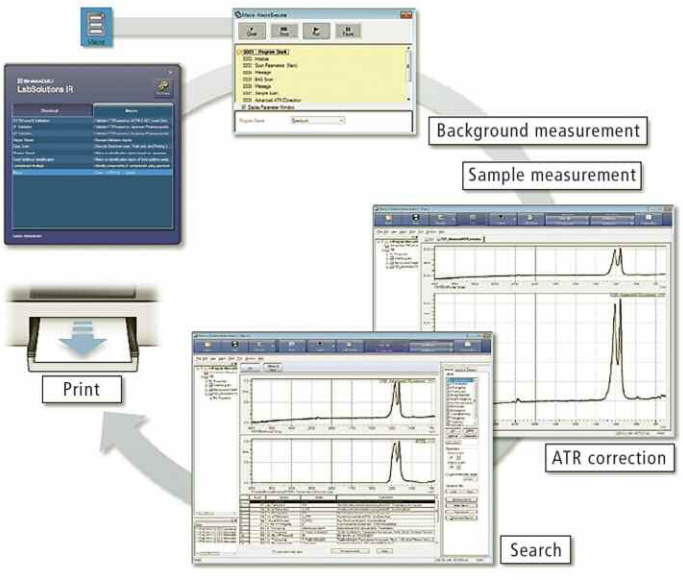



Figure 4: Overview of LabSolutions IR Macro programming

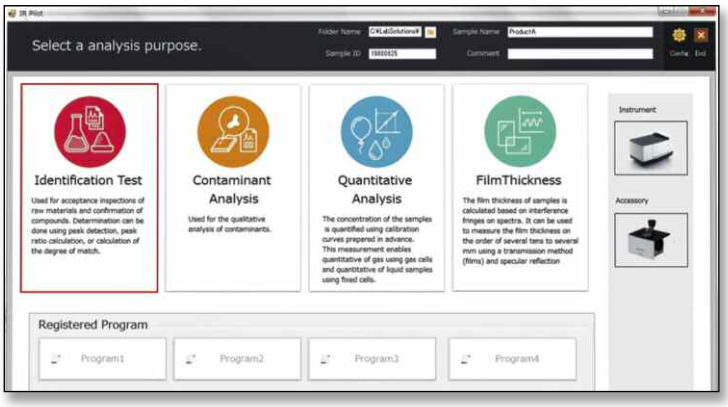



Figure 3: IRPilot diagram and workflow

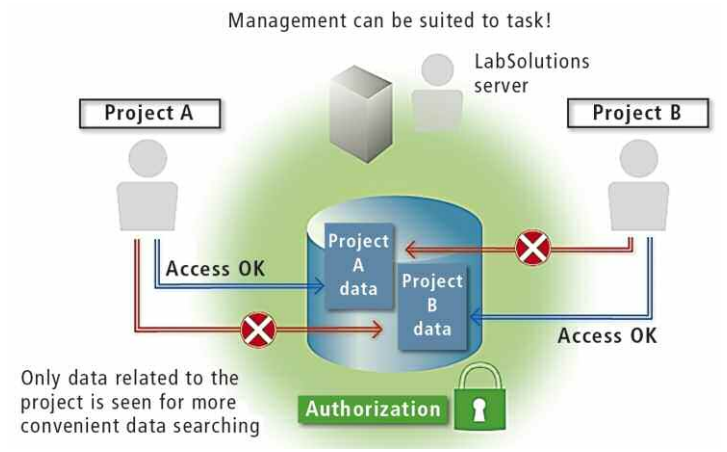



Figure 5: LabSolutions DB/CS Database Management System

spectrum. A part of this software is, for example, dedicated to the "Identification Test Program" and the "Contaminant Analysis Program". Both tools testify the ability of the IRSpirit series to identify pure or contaminated samples. Of course, LabSolutions IR is also able to utilize quantitative analysis.

LabSolutions IR has an additional macro-function helping operators to create more personalized automation for the analysis. Some software options, like specific libraries or kinetic studies, are integrated to provide for all user wishes. Finally, the exclusive optional software EDXIR is available, which overlaps EDX and IR measurements to identify contaminants.

The compliance with ER/ES regulations is granted with the Shimadzu LabSolutions DB or CS platform. These tools provide a project management function suited to tasks and system operations. This function enables equipment and user management, security policy, and data processing to be set on a project-by-project basis, thereby improving the efficiency of data searches and management tasks.

## Conclusion

The IRSpirit series defines a new standard in the world of compact IR spectrometers. The combination of its small size and high performance makes it an ideal solution for many different applications. IRspirit series is then a valuable system for quality control even in highly regulated fields, e.g. the pharmaceutical industry. These spectrometers also help to solve sophisticated problems, such as the identification of solid, liquid and gaseous materials. The simplicity of software and instrument handling also make IRSpirit a perfect support for education purposes.

## Literature

[1] Available on IRSpirit-T.
[2] IRSpirit-T: DLATGS-detector with temperature control function ; IRSpirit-L: $LiTaO_3$ detector.


Figure 1: LabSolutions splash screen

# Compliance made easy

## TOC determination in a regulated laboratory environment

Since the American and European Pharmacopeia requirement of TOC determination for testing ultrapure water (water for injection, highly purified water), TOC measuring systems have been used extensively in the pharmaceutical industry. In addition to the ultrapure water analysis application, TOC determination is used for cleaning validation and in the testing of plastic packaging and its materials. This is why TOC instruments are used in a regulated laboratory environment and why these systems are subject to various regulations that apply particularly to instrument software, which is focused in this article.

### GLP/GMP refers to the organizational process

One of these regulations is "Good Laboratory Practice" (GLP), which refers to the organizational process and the conditions involved in the planning, implementation and monitoring of laboratory studies and testing as well as the recording and reporting of analytical results. This means that the instrument software authorizes access to the system and fully documents data and parameter changes as well as events and results.

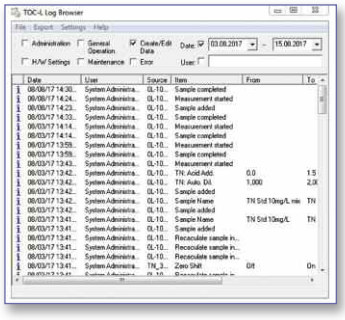



Figure 2: Audit trail

### FDA 21 CFR Part 11 covers the use of electronic records

In 1997, the American Food and Drug Administration initially developed guidelines on the use of electronic records and electronic signatures, in order to drastically

reduce the necessity for paperwork. As electronic information is easier to falsify, 21 CFR Part 11 defined criteria under which applications and documentation must be digitally filed and electronic signatures can be recognized. This is to guarantee that electronic documents are as trustworthy and reliable as paper records.

Control mechanisms and directions on procedures must safeguard data authenticity – and if necessary also data integrity. Most Part 11 regulations involve safety measures against illegal system access, user management, data security, data archiving and the electronic signatures themselves. The TOC-Control L software running the TOC-L series provides full support for complying with CFR 21 Part 11, while still remaining extremely user-friendly.

### TOC-Control L software supports analysis work and data reliability assurance

Already during software installation, the operating criteria of the software are defined. The parameters selected cannot be deactivated afterwards. The TOC-Control L software provides a wealth of functions to fully support analysis work and to support data reliability assurance.

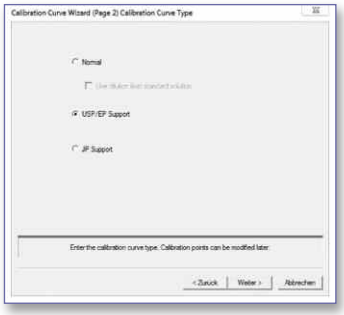
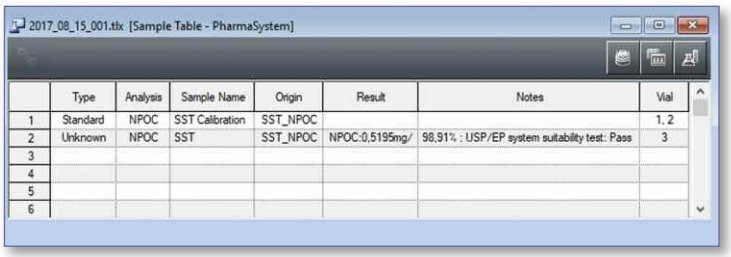



Figure 3: Template for EP/USP calibration curve

### User administration

Software utilization is enabled via user access rights. It offers individual accounts on four different levels, each protected through own passwords. The administrator can change access rights for each user. TOC-Control L allows changing of login during ongoing operations. This is especially important for laboratories working in multiple shifts. User administration is carried out in an external management software, where parameters for passwords like minimum length, complexity and validity period can be specified. To prevent illegal access, it is possible to set lockout functions and automatic email transmissions in case of specified events.




| | Type | Analysis | Sample Name | Origin | Result | Notes | Vial |
|---|---|---|---|---|---|---|---|
| 1 | Standard | NPOC | SST Calibration | SST_NPOC | | | 1, 2 |
| 2 | Unknown | NPOC | SST | SST_NPOC | NPOC:0,5195mg/ | 98,91% ; USP/EP system suitability test: Pass | 3 |
| 3 | | | | | | | |
| 4 | | | | | | | |
| 5 | | | | | | | |
| 6 | | | | | | | |

Figure 4: Executed system suitability test with automatic calculation

### Audit Trail for documentation purposes

All software operations are stored automatically in the audit trail (figure 2) which operates in the background. Entering of a comment can be set as mandatory when parameters are modified. Data storage takes place in a relational database. Through targeted queries specific operations can easily be referred to. It is possible to sort data according to time periods as well as user names or topics such as maintenance, administration or sample measurement. It is possible to manually enter maintenance operations that are not recognized by the software (e.g. replacement of the catalyst).

### Raw data management provides data acquisition security

In a regulated laboratory environment, data acquisition security is particularly important. Data acquired may not be altered by users not recognized by the software. TOC determination starts with peak integration, which cannot be influenced by the user. The peak area obtained is subsequently converted into a concentration value via a calibration curve. Recalculation applying a different calibration curve is possible, if permitted by the administrator.

All data is stored automatically, and the respective data files contain all important information: the system utilized, the user, the methods and calibration curves applied, the measured results (areas and concentrations) and the peak profile. For data protection and backup, these data files can be exported into a database directly after creation. Output is possible in human-readable form in .txt or .pdf file format.


Figure 5: Template for the system suitability test

### LabSolutions: cross-functional data management

For solid data security and improved management efficiency, Shimadzu offers the LabSolutions software platform. LabSolutions is a cross-functional data management concept for FDA 21 CFR Part 11 compliance and is applicable across the entire laboratory. Using LabSolutions, all data generated by various Shimadzu systems can be administered, signed and archived centrally in a relational database. This enormous amount of data can be structured project-wise to enable easy search and management.

The possibility to create customizable multi-data reports from different types of analysis simplifies reporting to decision-makers and contractors. Support for computerized system validation (CSV) and predefined validation templates within TOC-Control L, facilitate easy system validation while minimizing possibilities of error. The LabSolutions platform can be set up on a single-PC base (LabSolutions DB) for smaller laboratories or in a company-wide client server network system (LabSolutions CS).

### Support for TOC determination according to EP 2.2.44

The TOC-Control L software simplifies the implementation of tests with integrated templates for the creation of calibration curves (figure 3) and measurement of control samples.

The system suitability test is predefined in a method template (figure 5). Following measurement of the control sample (benzoquinone), recovery is calculated automatically, compared with the predetermined limits (85 - 115 %) and documented (figure 4).

### Conclusion

The TOC-Control L software combines all necessary functions for secure data handling while supporting user-friendly compliance with existing regulations in the pharmaceutical industry.

**Further information on this article:**

- Whitepaper: "Compliance of Shimadzu Total Organic Carbon (TOC) analyzer with FDA 21 CFR Part 11 regulations on electronic records and electronic signatures – TOC-Control L Ver. 1 / LabSolutions DB/CS Ver. 6"

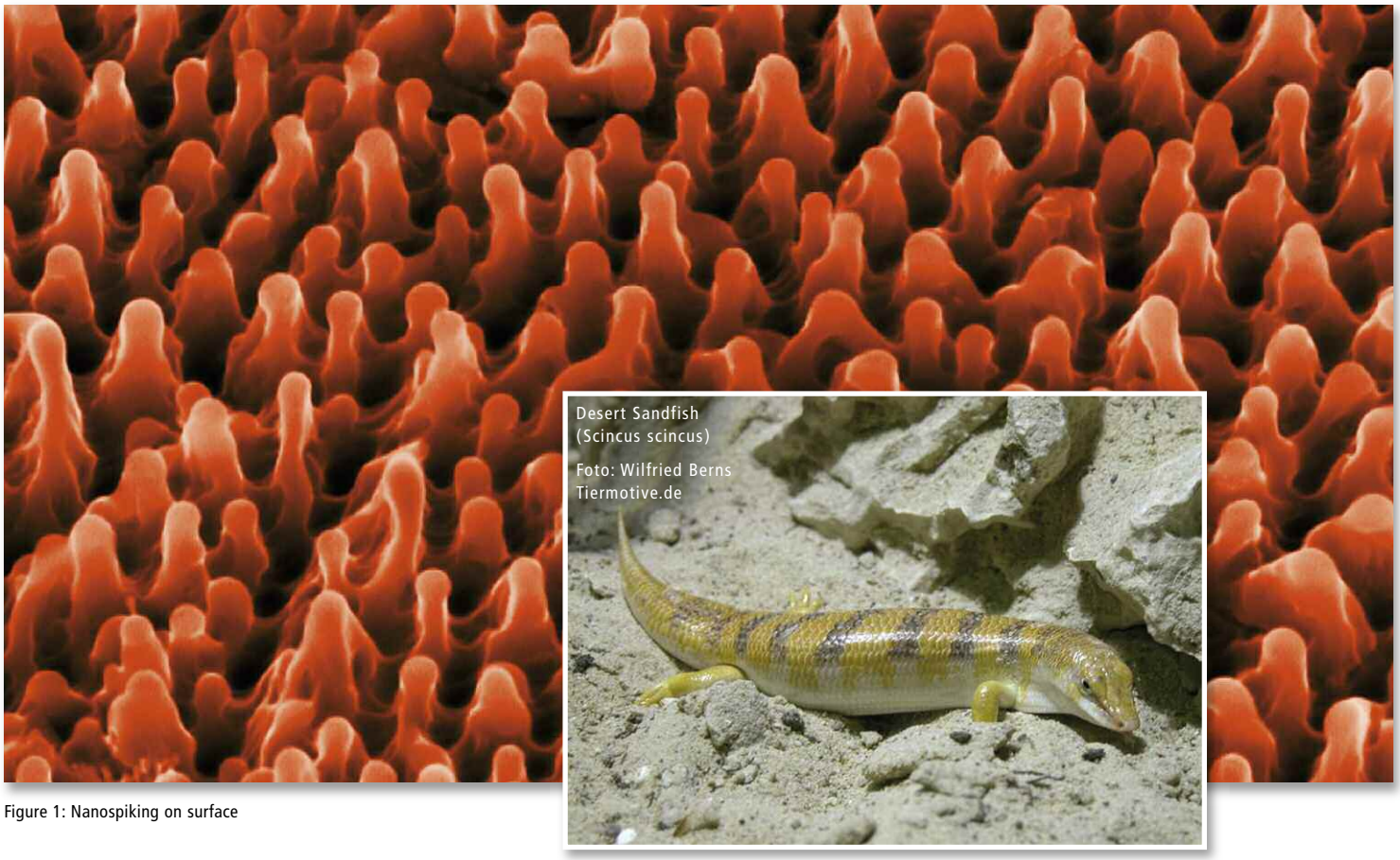



Figure 1: Nanospiking on surface

# Sandfish and teeth: the power of bionics

## UV-2600 and ISR-2600Plus: analysis of nanospikes from $TiO_2$ and sodium on surfaces from dental materials

What has a reptile that swims through sand got in common with paint? Both are linked through bionics, an interdisciplinary science which applies biological methods and systems found in nature to design modern technologies. A well-known application is the so-called lotus effect imitating the nanoscopic properties of plants for coatings, paints, and roof tiles to support self-cleaning features.

Paint whiteners and dental implants based on Titanium might seem a world away from the world of the desert sandfish (Scincus scincus), a little reptile that swims through sand like through water. But advances in the world of bionics makes the reptile's skin and the surface of the dental implants share the same secret: nanospikes.

The skin of the desert sandfish has evolved spiky nanostructures which produce a virtually frictionless surface, offering only a tiny fraction of the normal surface area of the creature in contact with the hard grains of dry sand. [1] In the dental implant world, the nanospikes exploit almost the exact opposite of this property, provid-

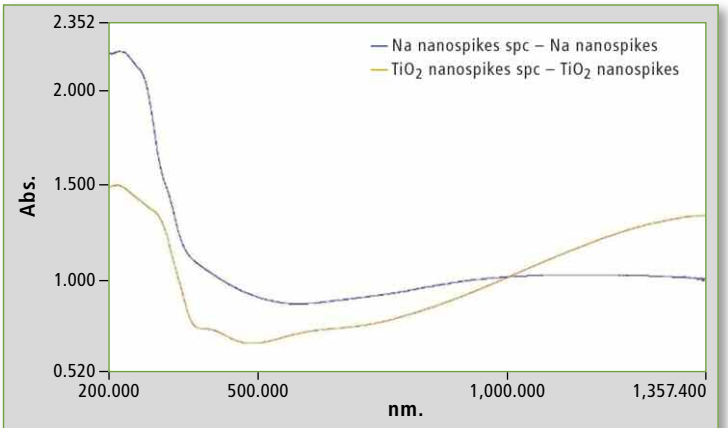



Figure 2: UV-VIS-NIR spectra from nanospikes (Na dark blue line, Titanium Dioxide orange line)

ing a very large contact area for the implant to establish contact with the soft surface of the tissues.

This application discusses the analysis of $TiO_2$-based nanospike material for dental purposes.

## New approach: nanostructures on surfaces related to or with $TiO_2$

For decades, $TiO_2$ has been a well known substance for different areas of application. Very typical is its use as a whitener, based on excellent reflectance properties. Nanospiking opens up additional applications for $TiO_2$. Nanospiking is an approach generating thin active surfaces with $TiO_2$ to get the antibacterial properties of the $TiO_2$ into very small areas. Nanospiking also produces a high surface reflectance composed of many tens of thousands of tiny spikes. The illustration on page 6 depicts titanium nanospikes at high magnification using Scanning Electron Microscopy (SEM) (figure 1). This spiking gives the surface a very high intense and bright white appearance.

However, the unusual surface structure when presented in the nanospiked form can alter the reflectivity. Titanium Dioxide also exhibits fluorescence. For medical use, it is usually advantageous to use as little material as possible. A new approach is to use nanostructures on surfaces related to or with $TiO_2$. The nature of the nanospikes represents that of a field of needles which, in principal, is an ideal diffuse reflector. Hence, the expectation is that such surface has a higher diffuse reflectance level than a normal unspiked surface.

## Application

Several samples were measured with UV-VIS-NIR spectroscopy in reflectance mode. [2, 3] There were Na and $TiO_2$ nanospiked samples, one blue-grey and one brown-beige shiny material. Due to the nature of the material, an integrating sphere was used to collect as much diffuse reflectance as possible. These samples were small in size, so in order to mount the samples on the reflectance port of the ISR-2600Plus integrating sphere, a background mount was made with $BaSO_4$ white standard. The sample was large enough for the beam image of the instrument to fall only onto the sample.

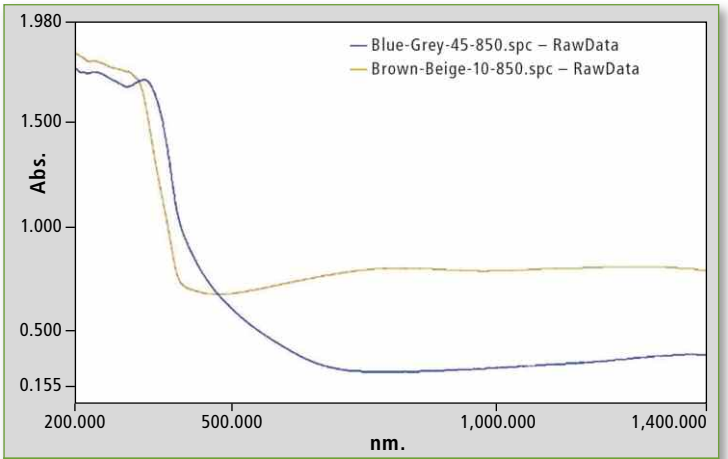



Figure 3: Brown-beige material (paler trace) and blue-grey material (darker trace)

The sample was mounted on a white reflector, so that the reflection losses from the sphere were minimized. Scans were made from 220 to 1,400 nm using the unique dual detector integrating sphere with the sample mounted at the diffuse reflectance port, which is at a zero degree incident angle to reject any specular reflectance components of the measurement.

## Summary

The nanospike traces show high absorbance (low reflection) at short wavelengths; it is likely that the trace at 200 nm is limited by the reflectivity of the inside coating of the sphere: (Barium sulfate) which mainly falls in the region between 220 and 200 nm. Even in this region however, the results show that the instrument was still operating within range as the data remains smooth. If the limit of detection had been reached, the trace would have become 'noisy' in the region below 220 nm.

The remainders of the traces are well within the photometric range and the stray light range of the system. The reflectance traces are very clear with minimal noise.

These traces are recorded in 'normal' specular excluded mode, so the specular reflectance of the sample is rejected. (The sample is illuminated at near-normal angle and mounted in a way that specular reflectance is lost through the sphere transmission port.)

With materials which do not reflect an image at anything but very shallow viewing angles, this measurement is generally the most accurate as the specular component is minimal. In the event that the sample is somewhat specular, an additional measurement can be made using the 'specular included' reference port which illuminates the sample at eight degrees from normal, allowing specular reflectance to be reflected back to the sphere wall and thus included in the measurement.

Reviewing these results, the instrument clearly delivers accurate results for the entire 200 - 1,400 nm range. The results are all obtained using barium sulfate as a reference material. This is a very good neutral reflector. All Integrating Sphere measurements have to be made in Percent of Reference mode as there is no way of measuring diffuse reflectance as an absolute value. [2, 3] The instrument normalizes all the reflectance values for the barium sulfate to 100 % (0 Abs) during background acquisition, but consideration should be given to the reflectivity of the reference material.

## Literature

[1] The Sandfish of the Sahara – A Model for Friction and Wear Reduction, Ingo Rechenberg, Abdullah Regabi El Khyari, Technische Universität Berlin, Bionik und Evolutionstechnik, 2/2004.
[2] Standards in Absorption Spectrometry, Burgess and Knowles (ISBN 0-412-22470-4).
[3] Fundamentals of Molecular Spectroscopy, Banwell and McCash (ISBN 0-07-707976-0).

# Climate research in the ocean

## An alternative method for the determination of carbon dioxide in seawater

The world's oceans have been carbon dioxide sinks since eons. $CO_2$ from the air is permanently bound in the sea by creatures that use calcium carbonate to build their skeleton. An American scientist team conducted a long-term experiment with corals at the Leibniz Center for Marine Tropical Research (ZMT) in Bremen, Germany. The TOC analyzer based DIC (Dissolved Inorganic Carbon) method demonstrated its suitability as a safe, exact and fast alternative method for the investigation of seawater.

One of the world's greatest challenges today is the containment of anthropogenic global warming. The agreement ratified at the Paris Climate Summit stipulates, inter alia, to limit global warming to below 2 °C of the pre-industrial level. Hopes are that the effects of climate change such as weather extremes, glacier melting or rising sea level can be reduced significantly.

One of the main causes of climate change is presumed to be the emission of so-called greenhouse gases. The gas most emitted by industrialized countries is carbon dioxide, and it is generated by burning fossil fuels. By way of comparison: In the 1960s, the average volumetric carbon dioxide content of the air was approx. 280 ppm, today it is over 400 ppm.

### Coral in the climatic cycle

Two thirds of the Earth's surface are covered by water. The world's oceans have functioned as important $CO_2$ sinks for millions of years. A part of the carbon dioxide contained in the atmosphere is first dissolved in seawater until it finally dissociates and reaches the chemical carbonic acid equilibrium. Within the pH-environment of the oceans (pH approx. 8.2), it is present as almost 100 % hydrogen carbonate. Much of these carbon deposits are permanently bound by organisms that use calcium carbonate to build their skeleton, such as stony corals.

Climate changes and the increasing concentration of carbon dioxide in the atmosphere have several effects on the oceans and the seas. On one hand, increases in $CO_2$ concentration lead to the so-called acidification of the oceans. On the

Figure 1: Prof. Dr. Justin Ries from the Northeastern University (USA) uses stony corals for experiments in the test facility of the ZMT Bremen, Germany.

other hand, the solubility of $CO_2$ decreases as sea temperature rises.

### Corals in various climatic environments

Particularly in tropical latitudes, corals which are important for the global carbon cycle grow and thrive. In the marine water research facility (MAREE) of the Leibniz Center for Marine Tropical Research (ZMT) in Bremen, Germany, a tropical marine environment can be simulated and ecophysiological tests can be performed.

In order to study the possible effects of warming and increased $CO_2$ concentration of seawater on corals, a series of experiments were undertaken where corals were observed in different environments. Varying temperatures were set in the test tanks, and preindustrial, present, and future $CO_2$ concentrations were investigated using a gas mixing system. This way, growth rates of the corals were determined over many weeks.

### Various corals

The experiments were carried out on the tropical stony corals *Stylophora pistillata, Pocillopora damicornis* and *Seriatopora hystrix*, as well as on the cold-water coral *Lophelia pertusa*. They are part of the scientific studies of Prof. Justin Ries of the "Northeastern University" (Boston, Massachusetts, USA). For this purpose, Prof. Ries and his team visited the ZMT for several months to conduct and evaluate the experiments.

In order to characterize the water conditions in the test tanks, temperature, pH, alkalinity (acidity buffering), salinity (salt content) and DIC (dissolved inorganic carbon) were used. During these experiments, especially the alkalinity and the DIC were analyzed continuously.

### DIC determination

A coulometric reference method has been established as an international standard to determine the DIC. In this process, an aliquot of the seawater sample is acidified with phosphoric acid. The resulting $CO_2$ is introduced into the measuring cell of a coulometer by means of a stripper gas and is absorbed in a reaction solution (ethanolamine in dimethyl sulfoxide). The $CO_2$ reacts with the ethanolamine to form hydroxyethylcarbamic acid and leads to a decoloration of the indicator contained in the reaction solution. The resulting hydroxyethylcarbamic acid is neutralized by the electrochemical formation of OH ions. The current consumed is used to calculate the concentration of the DIC.

Figure 2: TOC-L

Analysis of a seawater sample (single determination) typically takes about 15 minutes by means of the reference method. Additional time is consumed for tempering the samples and the long equilibration of the analysis system. About ten samples can be analyzed during an 8-hour working day. In Prof. Ries' experiments, 36 seawater samples per day had to be analyzed for DIC.

An alternative analytical method was therefore proposed which would provide comparable results, have high reproducibility, be automatable and offer shorter analysis times. In addition, the use of smaller sample quantities was requested (25 mL instead of 100 mL in the reference method).

### Alternative measuring method using TOC/TIC-analyzers

One procedure established in the environmental sector to detect the organic load of water is TOC-Determination (Total Organic Carbon). An aliquot of the water sample is injected onto a 680 °C heated platinum catalyst. The carbon dioxide arising during the combustion is guided by a carrier gas to an NDIR detector and thereby detected. With this method, it is necessary to remove the inorganic carbon fraction completely before combustion or to determine it separately and to take it into account (subtraction).

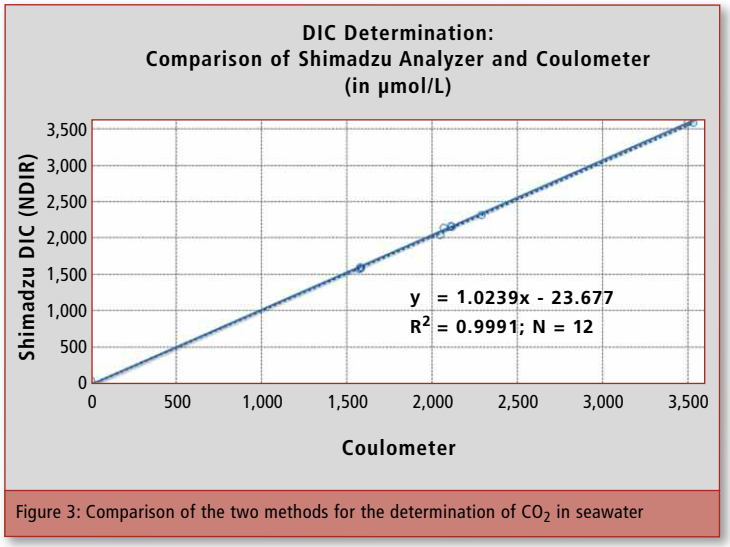



Figure 3: Comparison of the two methods for the determination of $CO_2$ in seawater

The ZMT uses a highly sensitive Shimadzu TOC analyzer capable of determining DIC separately. An aliquot of the sample is injected into a phosphoric acid receiver. The resulting $CO_2$ is conducted to an NDIR detector by means of carrier gas and quantified. The single determination of a DIC concentration takes about 3 minutes. The time advantage of this method was obvious. However, before it could be used, equivalence to the reference method had to be demonstrated.

For this purpose, both methods should have consistent accuracy and correctness. In addition, reproducibility should be < 1 %. ZMT Development engineer, graduate engineer Matthias Birkicht and his colleague Dieter Peterke carried out a large measurement range to ensure equivalence. Synthetic standard solutions of the "Dickson Sea Water Reference Standards" as well as seawater and tap water were measured with both methods at different concentration levels. Values of both methods were plotted against each other in a diagram (figure 3; concentration in µmol/L).

### Evaluation

Evaluation of the equivalence assessment showed a clear conformity between the two methods. They showed good reproducibility (± 6 µmol/L) and excellent accuracy (± 4 µmol/L) for this type of experiment. Moreover, the alternative DIC method did not consume expensive and toxic reagents compared to the reference method. Consumption costs for an analysis could be reduced to 20 % while saving valuable analysis time. It can be fully automated when used with an autosampler.

### Conclusion

In order to be able to assess the risks and effects of climate change, many scientific studies are needed requiring fast, accurate, reproducible and cost-effective analysis methods. The OSPAR international treaty dealing with the protection of the North Sea and the North Atlantic also describes the determination of $CO_2$ in seawater in its guidelines for the Study of Sea Water (JAMP Guidelines for Monitoring Chemical Aspects of Ocean Acidification [2014]). DIC measurement using a TOC analyzer represents a safe, accurate and fast alternative method.

### Acknowledgements

The author thanks Matthias Birkicht and Dieter Peterke (ZMT) for valuable data.

**Read for you** in GIT Labor-Fachzeitschrift 6/17

# Fast GC without a handbrake

## Filter time constant and sampling frequency improve the signal-to-noise ratio

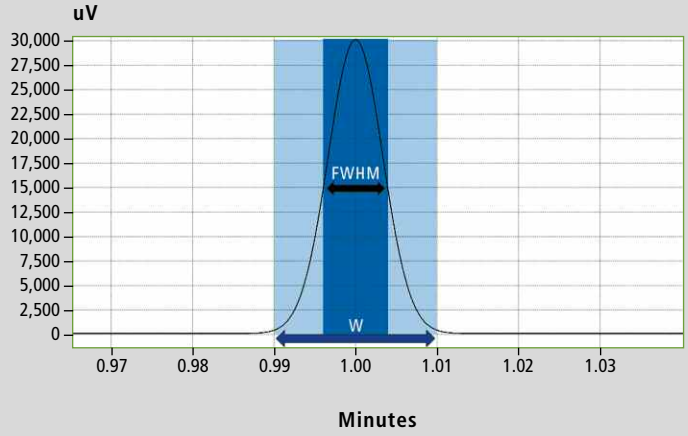



Figure 1: Definition of full width at half maximum (FWHM) and base width (w) of a signal

The fast response time of detectors used in gas chromatography is often characterized solely by the maximum sampling frequency. However, the latest techniques using short-duration signals require noise filtering (filter time constant) to be adjusted as well. If this parameter remains unchanged, the response time of the detector is too slow and the mapped signal is falsified.

### The ratio between the signal and the noise is decisive

In analytical applications, the ratio between the signal height and the baseline noise generally determines the achievable limit of detection. Developments in gas chromatography thus lead to ever sharper and thus higher signals. The sharpness of a signal is expressed by the full width at half maximum (FWHM) or the signal base width (W; see figure 1). The sharper the expected signals are, the faster the response time of the detector must be set up in order to correctly map the signals in the chromatogram.

In this case, the first step is to adjust the sampling frequency. Shimadzu recommends calculating with around 25 measuring points per signal so that even asymmetrical signals (e.g. due to tailing) are shown correctly. A sampling frequency of 10 Hz is sufficient for signal base widths > 2.5 s. The baseline measured by the detector is thus an extrapolation over measuring points that were all sampled at 0.1 s. Since unnecessarily high sampling frequencies increase baseline noise, the frequency should be suitable for the signal widths but not unduly high.

Fast GC techniques can achieve signal base widths of < 1 s. If the signal width approaches one second, the sampling frequency must be increased to 25 Hz. However, a second parameter also becomes essential: the noise reduction.

Electronic noise filters in a gas chromatograph reduce detector noise. In principle, high-frequency components in the baseline are attenuated by the filter in such a way that the measured signals should remain unaffected as far as possible. If the signals become sharper, noise filtering has to be adjusted via the filter time constant. However, this has the disadvantage that reducing the filter time constant also lowers noise reduction and thus baseline noise increases. Nevertheless, all the effort is worthwhile if sharp signals are mapped correctly, thus yielding a better signal-to-noise ratio.

### When signals disappear due to noise reduction

The Nexis GC-2030 allows a maximum sampling frequency of 500 Hz for each detector in combination with a minimum filter time constant of 2 ms. Theoretically,

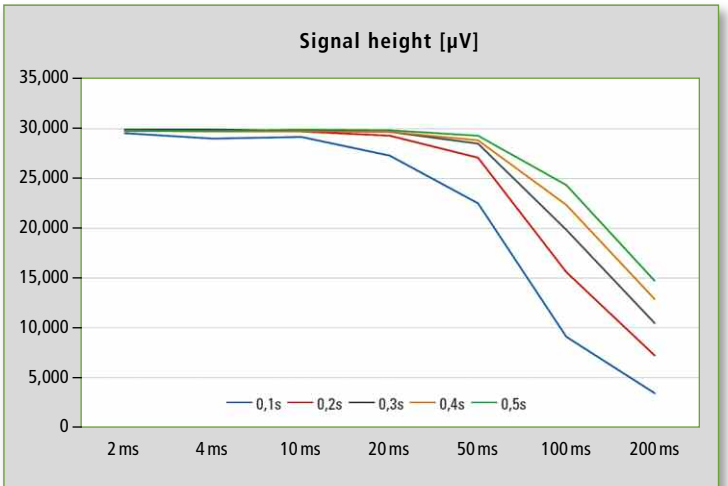



Figure 2: Decrease in the signal height for incremental increases of the filter time constant from 2 to 200 ms. The measured signal heights for the predefined signals with FWHMs of 0.1 - 0.5 s are depicted. The sampling frequency was kept constant at 500 Hz.

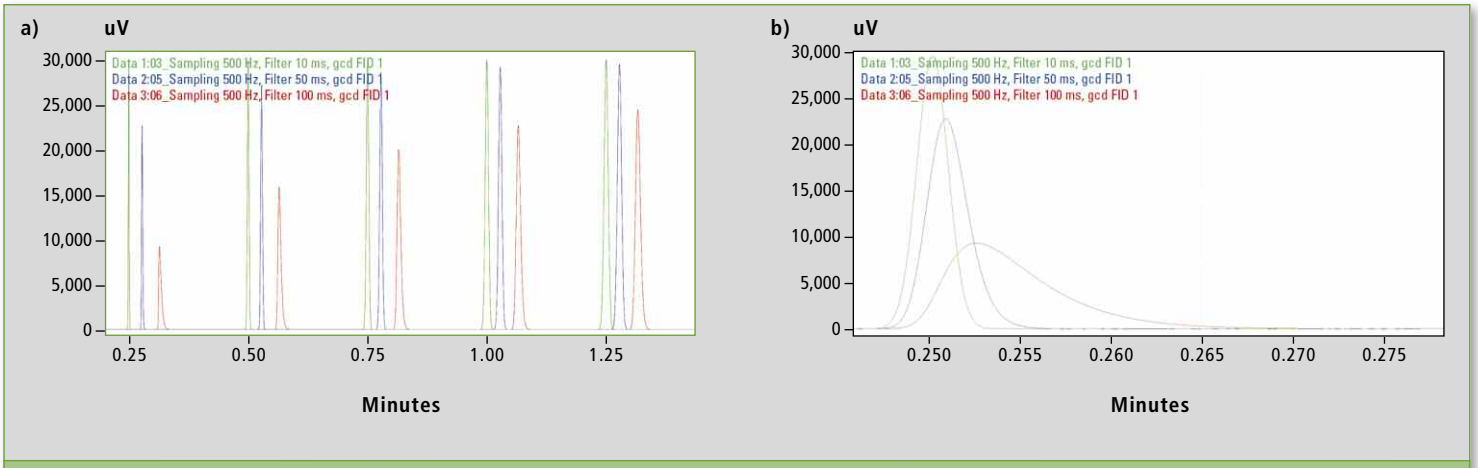



Figure 3: In (a), triplets of the five signals with FWHMs of 0.1 to 0.5 s are shown. The colors within a triplet represent the different filter time constants (green 10 ms, blue 50 ms, red 100 ms). In general, increasing the filter time constant leads to broadening and loss of signal height, which is exhibited particularly clearly by the sharpest signal with a FWHM of 0.1 s (see zoom in b).

this allows correct mapping of signals with a base width of 0.05 s, which is more than enough for all current techniques.

To determine how important adjustment of the filter time constant is, signals were generated with five different FWHMs using the integrated peak generator of the Nexis GC-2030. These were subjected to noise filtering just like any normally measured signal. With respect to effects due to the sampling frequency, it must be pointed out that the artificial signals are highly symmetrical and can thus be mapped reasonably well even at low sampling frequencies.

The influence of the filter time constant and sampling frequency on five signals generated with FWHMs of 0.1, 0.2, 0.3, 0.4, and 0.5 s was determined. The resulting base widths are 0.39, 0.76, 1.09, 1.59, and 2.04 s. All five signals have a fixed height of 30,000 µV. Any deviations from this value are mapping errors arising from the response behavior of processing the detector signals.

In the first series of measurements, these five signals were measured at a constant sampling frequency of 500 Hz using different filter time constants. Figure 2 shows the effect on the signal height. All signals remain virtually unaffected up to a filter time constant of 10 ms. As the filter time constant increases, sharper signals become increasingly smaller. From 100 ms upwards, signals with a FWHM of 0.5 s are then also affected by the noise filter and decrease in size. For a filter time constant of 200 ms, only 10 % of the actual signal height of 30,000 µV remain for the sharpest signal (FWHM of 0.1 s).

Figure 3 shows how the increasing filter time constants (10, 50, 100 ms) affect the shape of the signals. For a filter time constant greater than 10 ms, the signal width is affected if noise filtering is increased even further. For a filter time constant of 200 ms, the FWHM of the signal increases by a factor of ten from the original 0.1 s to 0.96 s; the base width of the signal increases even more strongly from the original 0.39 s to 5.43 s.

## Smaller effects on changing the sampling frequency

So what effect does changing the sampling frequency have under the same conditions? Once again, five signals are generated with FWHMs of 0.1, 0.2, 0.3, 0.4, and 0.5 s. In this case, the filter time constant is kept constant at 2 ms, whereas the sampling frequency is reduced incrementally from 500 Hz to 10 Hz. The result (figure 4) shows a less drastic effect than that of changing the filter time constant (figure 2). Up to 50 Hz, only a slight decrease in the signal height is observed for the signal with a FWHM of 0.1 s.

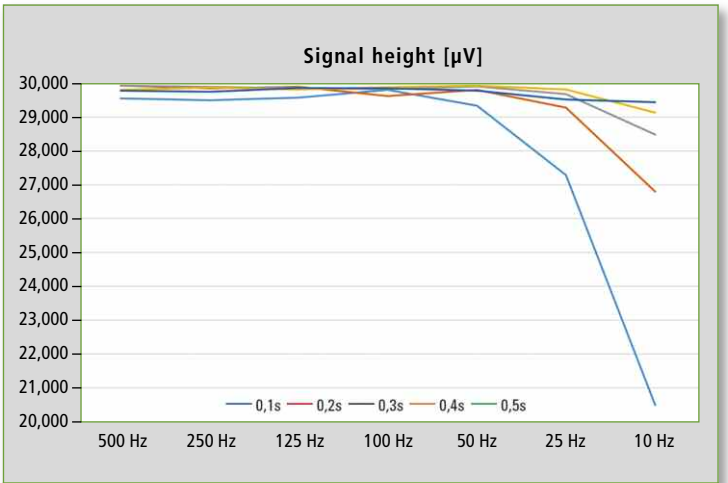



Figure 4: Decrease in the signal height with decreasing sampling frequency from 500 Hz to 10 Hz for the predefined signals with FWHMs of 0.1-0.5 s. The filter time constant was kept constant at 2 ms.

Decreasing the sampling frequency even further decreases the height of the signal from the original 30,000 µV to about 20,000 µV.

## Summary

An experiment that can be carried out on any Nexis GC-2030 shows that when using the latest chromatography techniques it is not enough to adjust only the sampling frequency to the resulting signal sharpnesses. On the contrary, time constants as well as the sampling frequency leads to the best signal-to-noise ratio, which ultimately defines the achievable limit of detection and thus allows fast GC – without a handbrake.

## Literature

J. V. Hinshaw, LCGC (2002), volume 15, page 152.

# Optimal handling of analytical data in regulated laboratories

## How to deal with stricter requirements of cGMP

Shimadzu Laboratory World in Duisburg

The increase in digital evaluation and approval of measured data in recent years has led to data integrity – i.e. the unalterability of data - becoming more and more important. Electronic data processing enables data to be modified intentionally or unintentionally, e.g. changes to evaluations or substitution of datasets. For example, if these data are used for the release of medicinal products or active substances, such manipulation could lead to serious consequences for the health of the patient. Regulatory auditors therefore pay particular attention to data integrity measures during audits.

Controls by the US FDA (Food and Drug Administration) have revealed a number of instances in which companies could not demonstrate compliance regarding data integrity, prompting the FDA to publish a guideline in April 2016. "Data Integrity and Compliance with cGMP" emphasizes that the FDA regards data integrity as a key quality topic.

As a result, numerous measures have been published in recent years, e.g. "warning letters". They discuss the most common problems regarding Guideline 21 CFR 211 & 212 as well as the principles of electronic record keeping according to Guideline 21 CFR Part 11. In other cases, import bans were imposed as a consequence of contraventions.

The Guideline also includes precise explanations of commonly used terms such as data integrity, audit trails and metadata. Even where meeting of requirements is not specified in detail, the topics the FDA regards as most important are discussed in 18 questions and answers.

Data integrity is not a new concept; it is an integral part of cGMP requirements such as:

- § 211.68 requiring that backup data is exact and complete, and secure from alteration, inadvertent erasures or loss
- § 212.110(b) requiring that data be stored to prevent deterioration or loss

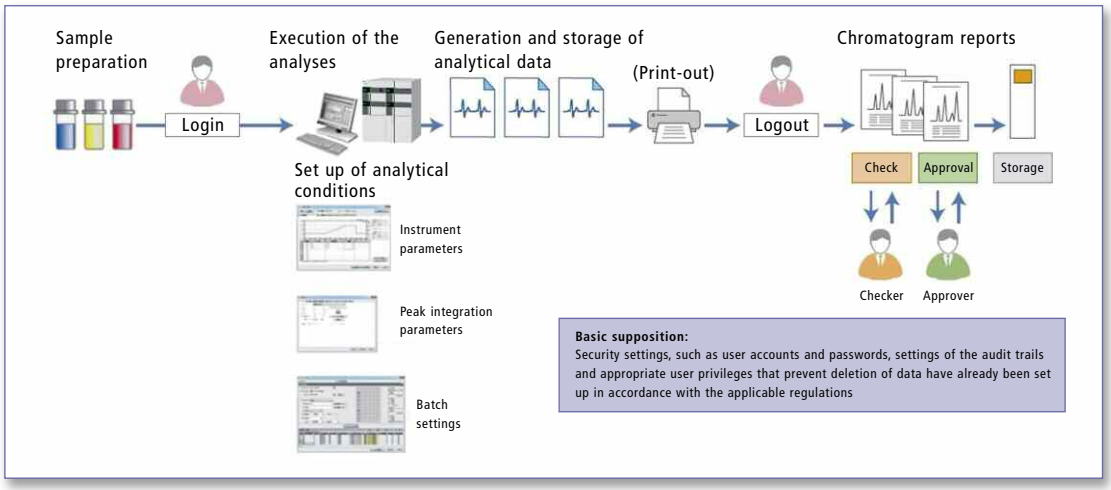



Figure 1: Schematic workflow for acquisition and processing of data using the example of an HPLC analysis

- § 211.100 and § 211.160 requiring that certain activities be documented at the time of performance and that laboratory controls be scientifically sound

- § 211.180 requiring true copies or other accurate reproductions of the original records; and

- § 211.188, § 211.194, and § 212.60(g) requiring complete information, complete data derived from all tests, complete record of all data and complete records of all tests performed. [1]

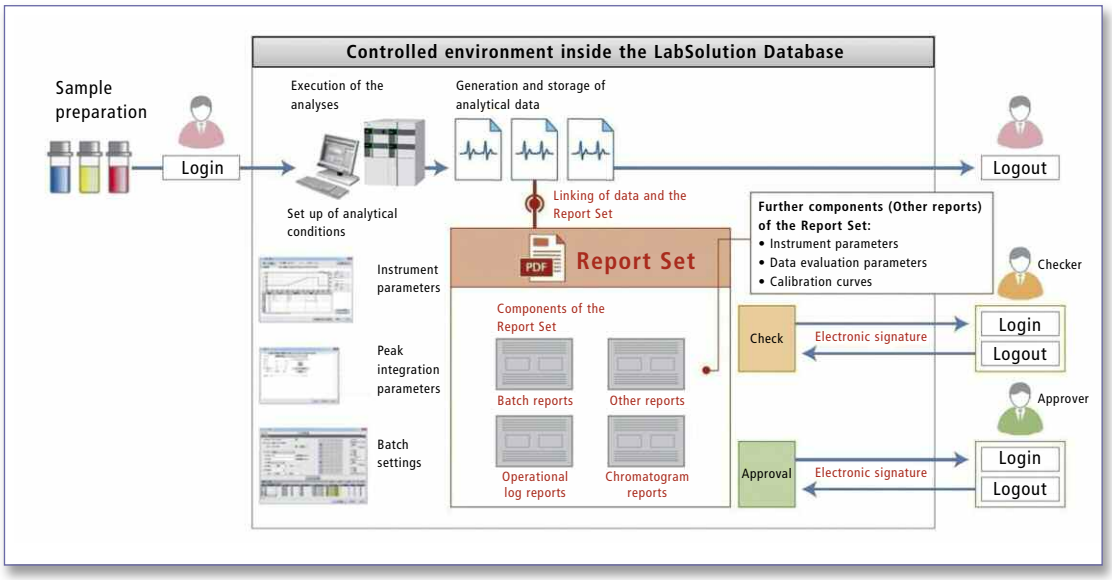



Figure 2: Compliant data integrity using the LabSolutions DB/CS Report Set

## ALCOA – good data handling practice

The "ALCOA" principle applies to good data handling practice. The acronym stands for **A**ccurate, **L**egible, **C**ontemporaneous, **O**riginal and **A**ttributable (to the respective person). This principle can be applied to the entire life cycle of the data.

- "Accurate" refers, for example, to the validation of instruments to prove that the measured data are recorded correctly, the data are approved by a laboratory manager using the four-eyes principle and that the software and electronic systems are validated to verify that data are also stored correctly.

- "Legible and permanent" primarily concerns the security measures to protect data against change. Handwritten data must be recorded legibly in indelible ink, and corrections to the data must be carried out in an appropriate manner. Furthermore, all data must have a change history by means of an audit trail.

- "Contemporaneous" refers to acquisition of the data. This must be carried out immediately after the respective process, for example, a measurement in the laboratory, and must not be predated or backdated. In electronic systems, it must not be possible to change the time or the date, and every change to the dataset must be saved immediately after it has been entered.

- Since the "original" data must also be available, the respective set of raw data from every measurement must be kept and remain archived, and any changes must be traceable.

- The last item, "attributable to the respective person", means that all the previously mentioned processes must be attributable to the person who carried it out. Anyone who records or processes data must identify themselves with a dated personal signature. If electronic systems are used, this means that a personalized access as well as electronic signature control are absolutely essential.

For further information, please refer to "Data Integrity and Compliance with cGMP". [1]

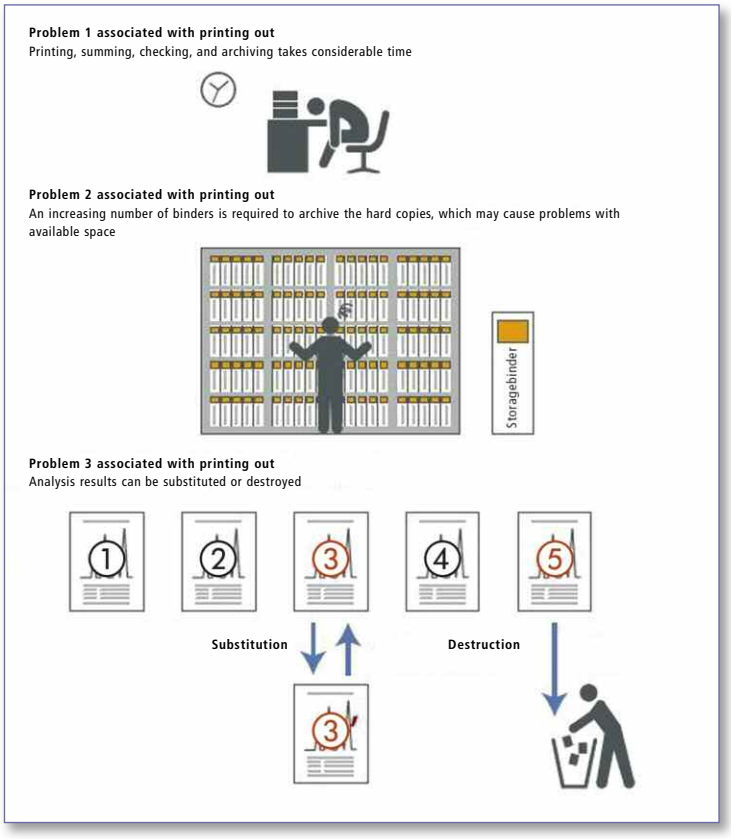



Figure 3: Problems associated with printed analysis reports

## Instrument manufacturers support the integrity of measured data

Due to the many contraventions in recent years, FDA auditors meanwhile apply a "guilty until proven innocent" approach during their inspections, meaning that they assume non-compliance with the regulations. As a consequence of this change compared to previous audits, end-to-end proof of the integrity of measured data has become essential in a controlled environment. [2]

Suppliers of chromatography data systems, i.e. software for acquisition, processing, administration and storage of data arising from analytical measurements have also adapted to these changed circumstances by implementing dedicated functions for data integrity to support companies operating in a controlled environment with regard to data integrity and FDA compliance.

### Pitfalls associated with data integrity exemplified by an HPLC analysis

To illustrate the necessary functions and precautions during day-to-day analytical work, the possible pitfalls associated with data integrity are highlighted using an HPLC analysis as an example. In a purely paper-based laboratory, the workflow follows the scheme shown in figure 1.

A lab technician logs in on the PC, records data, processes and prints them. The printed chromatograms are checked, approved and then archived in a filing system. At first glance, it appears as though this procedure would be acceptable, provided that the corresponding security settings such as access data, settings for the audit trail and user privileges for deleting data have been chosen appropriately.

In the normal case of paper-based documentation, only the printed chromatograms are evaluated. The settings for the instruments, data processing, sample table or other parameters are usually not taken into account. However, all these factors are necessary to obtain a reliable evaluation. [3]

The printout does not show the exact structure of the total dataset at the time it was printed. Regardless of how secure an electronic data processing system or software is, it always depends on the entries made by the user. Thus, even if the strictest security measures are implemented on the computer, it is very difficult to prevent inadmissible actions, e.g. specification of analytical parameters or evaluation of the analytical data.

### Clear visualization of every manual manipulation

How can it be proven that no inadmissible actions whatsoever occurred during acquisition or evaluation of the data that would falsify the analytical results? Such proof can only be furnished if all calculations and operations during data processing are easy to see. This can be achieved by gathering all human-mediated processes, such as setting of the chromatography parameters or evaluation of analytical data, and making them easily recognizable as manual steps. By making every manual change visible, any inadmissible processes, such as modification or substitution of data, can be easily identified.

Shimadzu's LabSolutions CS or DB uses the so-called "Report Set" function. It creates a set of PDF reports automatically, such as batch analysis, operational log, and chromatograms that are subsequently converted into a single document. This Report Set also includes information on manual and automated actions so that any data manipulation leads to documented deviation from defined procedure that is immediately recognizable.

Figure 2 shows a schematic workflow for computer operations using the "Report Set" function in the LabSolutions DB/CS software, which links and displays data.

The three main features of the "Report Sets" are described below.

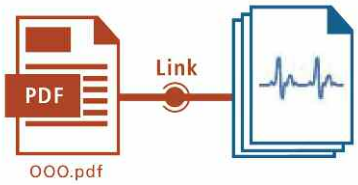
**Feature 1:**

Visualization of the individual analysis steps makes it easier to check results while providing reliability.


The "Report Set" function includes not only the results of a sequence of analyses (batch), but also the results and steps during processing in the postrun. The Operational Log Report tracks data capture and evaluation from start to finish of the analytical process. Similarly available as an e-book, the individual steps can be followed from page to page without the user having to switch back and forth between windows to check user steps and settings. This makes the work not only easier, but also more reliable.

**Feature 2:**

Batch results are protected automatically against modification.




Once a digital link has been generated between a sequence run, analytical results (digital data) and the report set, further data manipulation is no longer possible. This ensures that modifications such as substitution or deletion of data are prevented.

This digital linking of the individual data set offers not only a unique relationship between the report and the analytical results (digital data), but also allows the data to be searched and checked easily.

**Feature 3:**

Increased productivity thanks to digitization of the verification process of analysis reports.


Verification of the reports (Report Confirmation function) serves as proof that the analysis reports have been reviewed in the PDF report. If this has not taken place, an integrated monitoring function generates an error message.

### Advantages of the paperless system:

Electronic signatures are used for reviewing and releasing reports, for which the original digital analytical data are consulted at the same time. The use of an electronic signature obviates the need to print out the reports for a manual signature. Accordingly, this avoids problems with printed analysis reports, as shown in figure 3, and also saves time.

### Conclusion

For users operating in a controlled environment, data management and data integrity offer the necessary security to comply fully with official regulations. The "Report Set" function of LabSolutions DB/CS supports data integrity while drastically simplifying routine laboratory work.

### Literature

[1] https://www.fda.gov/downloads/drugs/guidances/ucm495891.pdf
[2] http://www.pharmtech.com/data-integrity-analytical-laboratory?pageID=1, (accessed March 7, 2016).
[3] Keiko Bansho. Chapter 5 Operations and Management for Paper-Based and Electronic Data for Compliance with Regulations by the Three Authorities. Recording and Managing Data and Migration to Digitization at Laboratories Based on the Issues Raised by the Three Authorities. Science & Technology, May 15, 2015, pp. 127-146.

# Behind the scenes

## Users' insights on working with CLAM-2000 after one year in duty

Video shoot for CLAM-2000

Since its launch at the 2016 Analytica trade exhibition in Munich, CLAM-2000 has been a whirlwind during trade events such as MSACL shows and EuroMedLab, and revolutionized the world of clinical analysis allowing easier access to the use of LC-MS/MS.

The CLAM-2000 (Clinical Laboratory Automated sample preparation Module) automates the pretreatment of blood or other biological samples before LC-MS analysis. It has been designed for pharmaceutical departments, medical departments or biological analysis laboratories dealing with risk of human error in sample preparation or infection risk.

In September 2016, CLAM-2000 made its official debut in a private sector laboratory in Cologne, Germany. After one year of service in different places across Europe, it is time to share the experience how CLAM-2000 is breaking the wall between the world of clinical analyzers and the associated technology of LC-MS/MS liquid chromatography coupled to tandem mass spectrometry.

Shimadzu's Clinical Bio Division has a long-standing history in producing clinical analyzers marketed as OEM equipment by other well-known suppliers, such as Werfen Group, Sekisui and Abbott. At the same time, the Mass Spectrometry Division from Shimadzu made an important breakthrough in the LC-MS/MS market by introducing Ultra-Fast Technologies, sensitivity and robustness for highly demanding applications like therapeutic drug monitoring in whole blood or plasma. The benefit of coupling both divisions' technologies to simplify access to LC-MS/MS in the clinical field became obvious in order to fill the gap, because the main restriction for the penetration of mass spectrometry is the sample preparation prior to LC-MS analysis.

### Cutting-edge scientists' and researchers' insights

In August 2017, a video team toured through several laboratories in Germany, France, and Italy to collect users' experiences on working with CLAM-2000. The footage covers applications in university hospitals, testing and validation in clinical laboratory environments, toxicology analysis, and private sector laboratories. Cutting-edge scientists and researchers provide insights into their daily routine applications with CLAM-2000.

Prof. Franck Saint-Marcoux, Full Professor of Toxicology at University of Limoges, France, describes CLAM-2000's automated sample pretreatment as the new revolution. Its main advantage is the increased speed of the workflow: it can be fully programmed, which reduces the need of explanation towards the staff or the technicians. "The global capability of LC-MS/MS is no longer a problem," he says.

"CLAM-2000 is a kind of fire & forget system: it does all the work for you," explains Dr. Lars Kröner, department of toxicology of the Cologne, Germany based Dr. Wisplinghoff laboratory. The company is specialized on full service of clinical toxicology and clinical analysis for different diseases. The lab works for over 40 hospitals, and applies CLAM-2000 particularly for the 50 - 100 emergency samples per day. This way, the up to 1,000 high-throughput sequences running each day do not have to be interrupted.
Dr. Kröner describes the use of their own in-house method as the system's main benefit. "We can put all methods of other systems on the CLAM system. Changing parameters of a method is no problem as we can do all programming on our own."

For the clinical research department at University Hospital in Göttingen, Germany, CLAM-2000 is the ideal tool for measuring samples even if the staff lacks any specific chromatographic and mass-spec experience.

CLAM-2000 is controlled via VPN (Virtual Private Network) which enables method development also from other places. Dr. Frank Streit, Department Head, says that "accuracy and precision for the determination of the analytes is better than using an LC-MS system and offline sample preparation."

Prof. Paolo Brambilla, Head of Clinical Chemistry Laboratory, DESIO hospital, Milan, Italy, also appreciates the higher accuracy especially at lower concentrations, and the increased sensitivity. According to him, CLAM-2000 avoids a lot of interferences. He says: "The system is exactly what we need in clinical analysis."

The brief experts' reviews on CLAM-2000's benefits are available in full length through the QR code.

**Further information on this article:**

- www.shimadzu.eu/clam-2000-references-customers
- www.shimadzu.eu/clam-2k

# Joy and fear of Christmas baking ...

## Determination of mycotoxins in bakery products using LC-MS

Outside it is dark, wet, and cold, indoors warm and cozy, with coffee or tea and homemade biscuits – quite traditional. As much as cookies warm the soul, within their ingredients may lurk invisible dangers: mold fungus toxins, also called mycotoxins. In addition to grain products, these ingredients include nuts and almonds, dairy products, spices, fruit, and cocoa. For example, aflatoxins are frequently found on fat-rich vegetable products such as nuts, almonds, and corn, while ochratoxin A, fumonisins, deoxynivalenol, and zearalenone can often be detected in cereals and also in corn.

### But what exactly are mycotoxins?

These secondary metabolic products of mold have different chemical structures; they can neither be seen nor smelled. They are largely heat-stable and are therefore not destroyed during frying, cooking, or baking. They can lead to different diseases in humans, the so-called mycotoxicoses – in contrast to mycoses, i.e. diseases triggered by fungi themselves. In addition, mycotoxins promote the development of cancer, damage of the kidneys and liver, affect the immune system, or cause diarrhea and vomiting.

Mycotoxins represent, besides antibiotics, the second largest group of active ingredients synthesized by microorganisms. As well as antibiotic-forming microorganisms, mycotoxin-forming mold fungi are distributed worldwide. The toxins, which are contained in certain higher fungi (e.g. amanita), are not included among

Figure 1: Shimadzu Nexera X2 with LCMS-8060

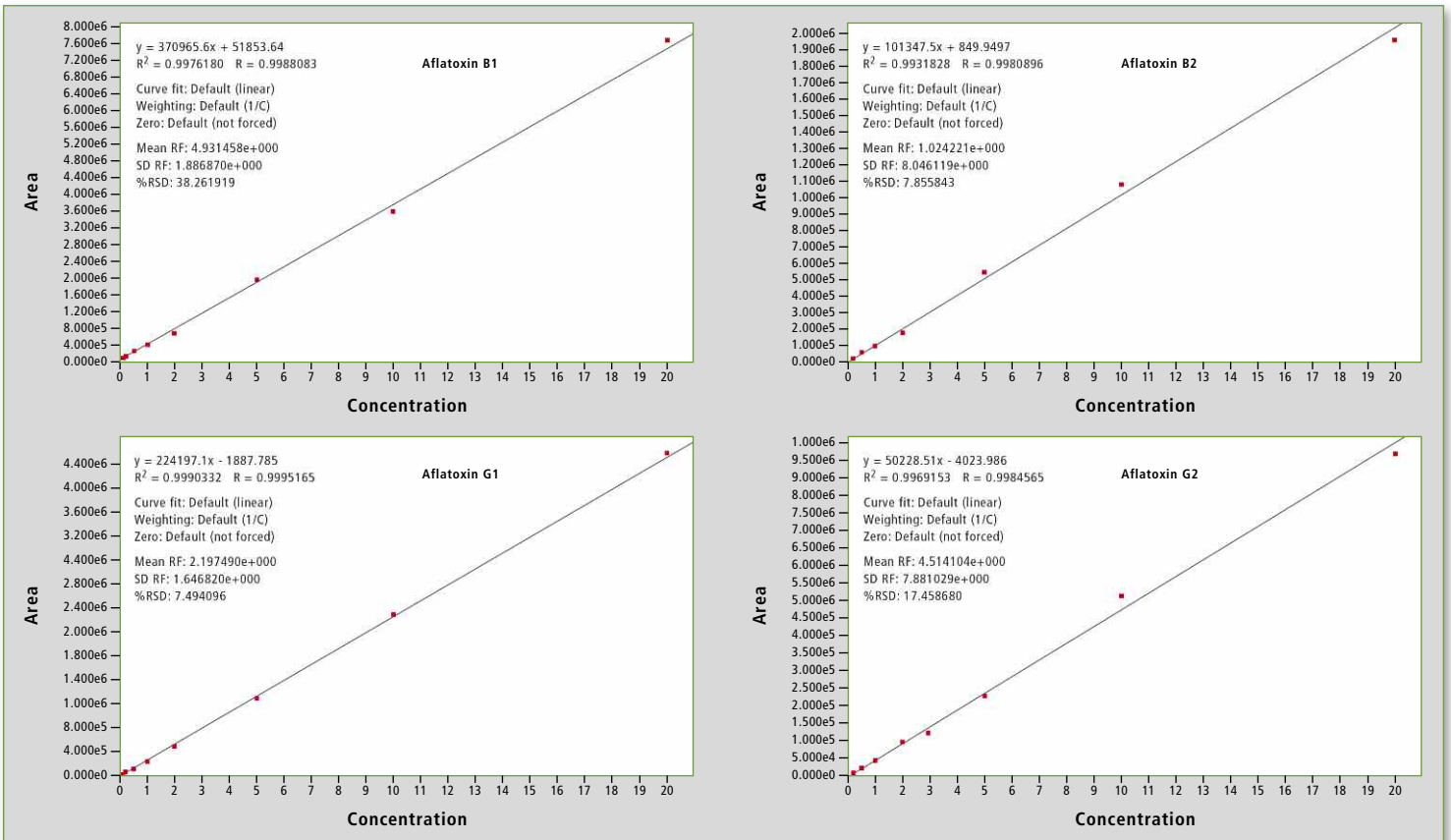



Figure 2: Calibration curves of aflatoxins B1 and B2 as well as G1 and G2, concentration range 0.1 µg/kg for B1 and G1 resp. 0.2 µg/kg for B2 and G2 – 20 µg/kg

the mycotoxins in the narrower sense. [1]

## Contamination and transfer of mycotoxins

Mycotoxins are usually produced when a fungus finds particularly favorable growth conditions, such as moist, warm weather conditions. A distinction is made between primary and secondary contamination. Primary contamination is the direct infestation of the plant on the field with so-called field fungi (Fusarium). This leads to plant diseases and to a lower harvest yield. Unfavorable storage can also lead to mold fungi, the so called storage fungi (*Aspergillus* and *Penicillium* species).

Secondary contamination, on the other hand, is referred to when the already finished food becomes moldy due to being stored too long or incorrectly. This is easily recognizable by the well-known mold growth.

Mycotoxins can also enter our food products by transfer (carry-over). Via their feed, productive livestock are contaminated with mycotoxins, and from there these can reach (partly in metabolized form) food products obtained from animals, such as meat, eggs and dairy products.

## Puzzling turkey and trout death

The year 1960 was of central importance for mycotoxin research. That year, 100,000 young turkeys in England and one million young trouts in the United States perished from the mysterious "Turkey X Disease". The feeding of the residues of peanut pressing, originating from the processing of moldy peanuts, could be identified as a trigger for this mass mortality. The aspergillus flavus, which had grown, had formed so far unknown mycotoxins in high concentrations, the so-called Aflatoxins. [2]

| | Sample 1 almond | Sample 2 almond | Sample 3 walnut | Sample 4 walnut | Sample 5 peanut | Sample 6 hazelnut | Sample 7 cashew | Sample 8 wheat flour | Sample 9 wheat flour | Sample 10 wheat flour | Sample 11 wheat flour | Sample 12 spelt flour | Sample 13 spelt flour | Sample 14 oat flakes | Sample 15 oat flakes | Sample 16 oat flakes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aflatoxin B1 | < LOQ | 0.31 | -- | -- | -- | 1.65 | < LOQ | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| Aflatoxin B2 | -- | -- | -- | -- | -- | 0.46 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| Aflatoxin G1 | -- | -- | < LOQ | < LOQ | -- | 2.74 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| Aflatoxin G2 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| Total Aflatoxins | -- | 0.31 | -- | -- | -- | 4.85 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| Ochratoxin A | -- | -- | -- | -- | -- | -- | -- | -- | -- | 6.77 | -- | 0.64 | -- | < LOQ | 0.14 | < LOQ |
| Fumosinin B1 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| Fumosinin B2 | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| HT-2 Toxin | -- | -- | -- | -- | -- | -- | -- | -- | < LOQ | < LOQ | -- | -- | -- | 9.80 | 24.59 | 6.01 |
| T-2 Toxin | -- | -- | -- | -- | -- | 2.39 | -- | -- | -- | -- | -- | -- | 5.70 | 3.32 | 23.05 | 2.80 |
| Zearalenone | -- | -- | -- | -- | -- | < LOQ | -- | < LOQ | < LOQ | 6.35 | -- | < LOQ | -- | < LOQ | < LOQ | < LOQ |

Table 1: Measurement results (in µg/kg) of all samples tested – results highlighted in red exceed the maximum permissible quantities.

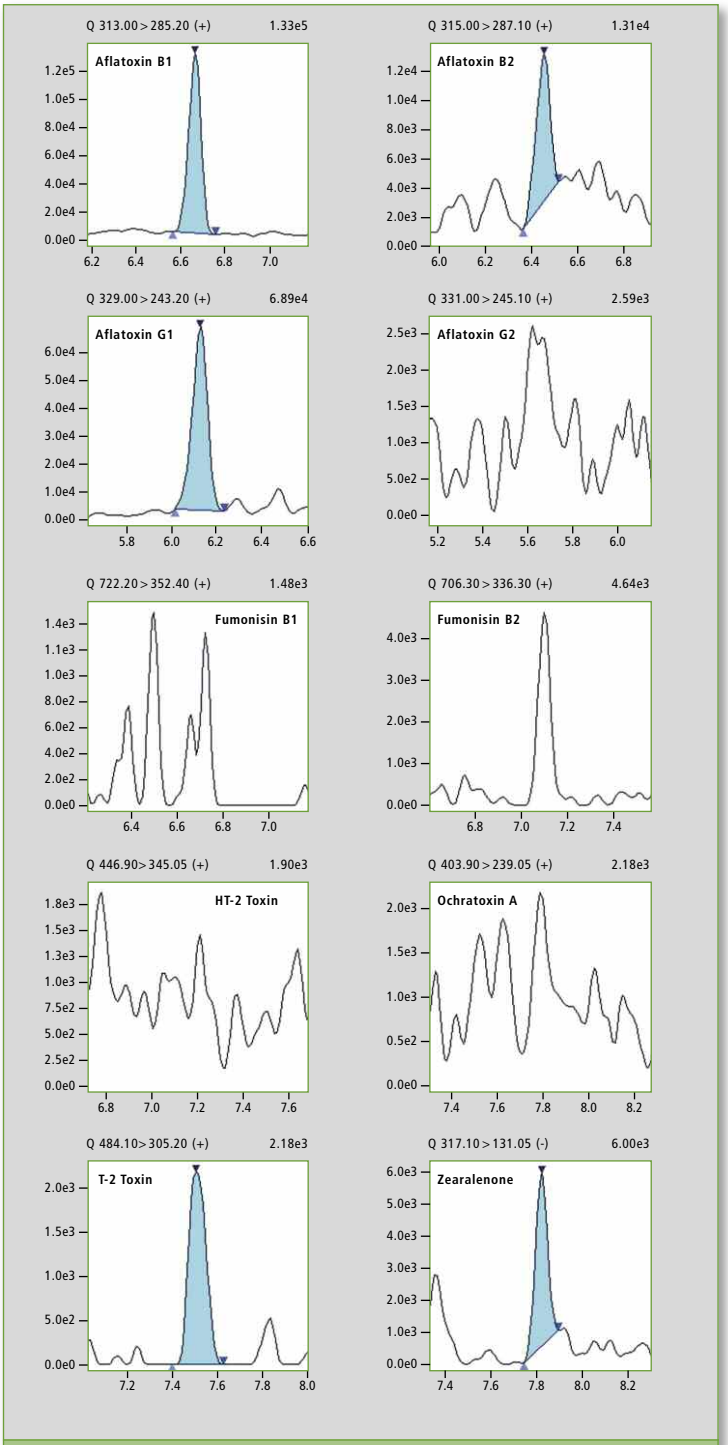



Figure 3: Mycotoxins in a hazelnut sample

## Good agricultural practice, good manufacturing practice, and limits

Since food and animal feed contaminated with mycotoxins can no longer be purified and therefore should not be used, it is important to prevent fungal attack and thus mold in the first place by adhering to good agricultural and manufacturing practices.

The final consumer should make sure that the food is stored in a dry and cool location and that discolored nuts with an unpleasant smell are not consumed. Cutting off the moldy areas of the affected food often does not help because the fungus has already spread out invisibly.

In case of doubt, moldy goods should no longer be consumed.

Because of the risk of mycotoxins, the European Union and many other countries have set limits. In the European Union, this is done by regulations of the EU Commission setting the maximum levels for certain contaminants in food products (Regulation [EC] no. 1881/2006 and supplements). [3] Aflatoxins in nuts, for example, are limited to a maximum content of 2 µg for aflatoxin B1 per kg and 4 µg for the sum of aflatoxins B and G per kg or 50 µg of zearalenone per kg in bread, pastry, and biscuits.

It is therefore desirable to be able to determine as many of the mycotoxins to be controlled as possible, using only one analytical method, independent of concentration range and sample matrix. The analysis of such multi-analytical samples is facilitated by the increasing use of liquid chromatography, coupled to a triple quadrupole mass spectrometer (LC-MS/MS).

## Sensitive detection of mycotoxins by means of LC-MS

The following application example shows a process which allows almost all relevant mycotoxins for baking ingredients to be determined with only one method. (In case of deoxynivalenol, a modified extraction method must be performed due to the polarity of the molecule.)

The sample preparation was carried out using a simple "catch-and-release" solid phase extraction (SPE) method.

5 g of the ground sample (nuts, almonds, flour, etc.) were mixed with 20 mL of a water/acetonitrile mixture (50/50 v/v) and treated in an ultrasonic bath for 5 minutes. The sample was then shaken for 30 minutes at room temperature before a part of the supernatant was further processed after centrifugation.

The supernatant was diluted with water and, after appropriate conditioning of the solid phase cartridge (ISOLUTE® Myco, Biotage, Cardiff, UK), transferred, washed and eluted with solvent. The eluate was completely evaporated and reconstituted with 300 µL of a 20 % acetonitrile/methanol + 0.1 % formic acid solution. Before the sample is

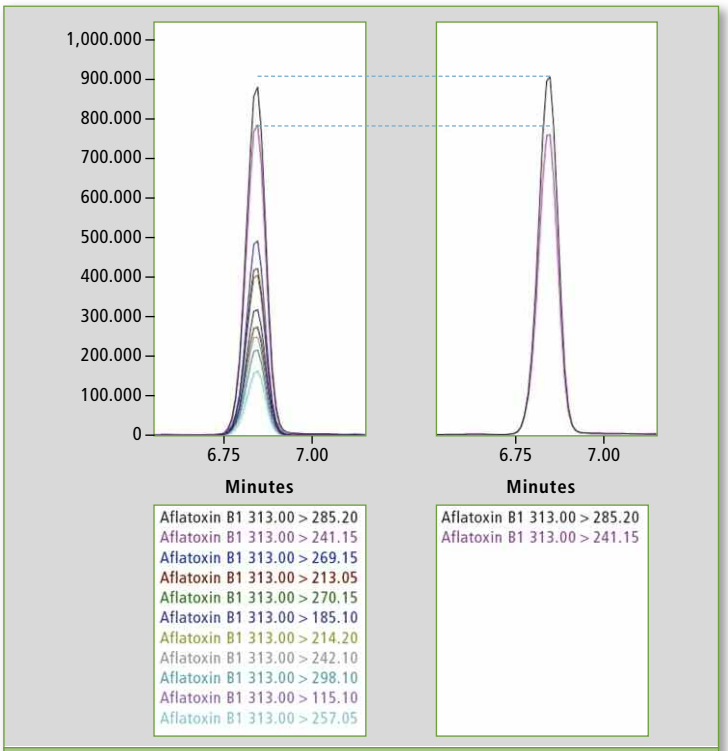



Figure 4: The figure shows MRM chromatograms for aflatoxin B1, one recorded with the usual 2 fragment ions, and compared with a method with higher number (11) of fragment ions which, despite this fact, have the same sensitivity.

injected into the LC-MS/MS system, it is recommended to filter it through a filter vial (0.45 µm PTFE).

For the analysis of the extracted samples, a Nexera X2 UHPLC system was used, coupled to an LCMS-8060 Triple Quadrupole Mass Spectrometer (both Shimadzu; figure 1, page 16).

The LC conditions used originated from an already described method. [4] A detailed description of the method can be downloaded using the QR code at the end of this article. In the case of the described measurements, a Mastro C18 chromatography column (150 x 2.1 mm, 3 µm, Shimadzu GLC Ltd.) was selected, which is able to produce excellent peak-shape due to its internal structure (figure 6), even in chelate-forming substances such as fumonisins.

Thanks to the described method, the limits required by the EU regulation were easily met for all mycotoxins analyzed. Figure 2 (page 17) shows the calibration curves of aflatoxins B1 and B2 as well as G1 and G2 extracted from ground almonds.

## Analysis of Real Samples

16 commercial samples of various baking ingredients (wheat flour, spelt flour, oat flakes, almond, hazelnut, peanut, walnut, and cashew nut) from various local shops were examined for common mycotoxins. In some samples, mycotoxins could be detected (table 1, page 17; figure 3, page 18). In two samples the maximum levels recommended by the European Union for individual mycotoxins were even exceeded.

In addition, the so-called "MRM spectrum mode" was used for analysis. Here, not only the fragments of the quantifier and the qualifiers are determined, but also a higher number (typically 6 - 10) of MRM fragment ions. Using this MRM spectrum mode, conventional MRM quantification is combined with a high-quality MRM product ion spectrum, which can be used in a library search routine, thus increasing the specificity and verification of results (figures 4 and 5).

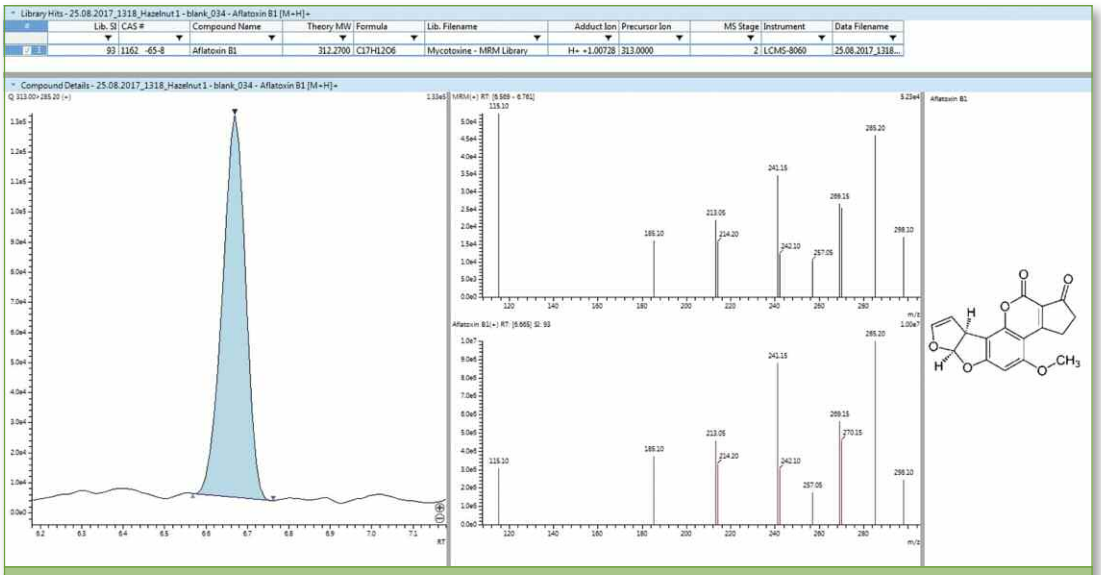



Figure 5: Result of the library search, presented with LabSolutions Insight Screening software

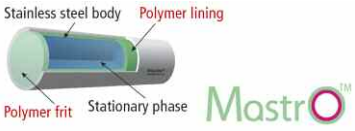



Figure 6: Structure of Mastro™ column

## Conclusion

The manufacturer is responsible for the safety of food products. They must ensure by internal checks that the food products produced do not have any negative impact on the health of the consumer. [3] In order to ensure this and to ensure the maximum levels for certain contaminants, rapid and sensitive multi-analytical methods are essential for food analysis. This begins with monitoring the raw materials and should help to make sure that contaminated food does not enter the market in the first place.

Nevertheless, some, almost unavoidable, contamination with mycotoxins could be demonstrated in the examined samples. Two samples even exceeded the applicable EU limits for individual mycotoxins.

In spite of these exceptions, everybody should enjoy baking and eating their own cookies. If excessive consumption threatens health, then it is most likely due to weight gain!

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[4] Baker et al, Shimadzu Application News No. C138, LAAN-A-LM-E109.

**Further information on this article:**

- Application note: Multi-Residue Analysis of 18 Regulated Mycotoxins by LC/MS/MS

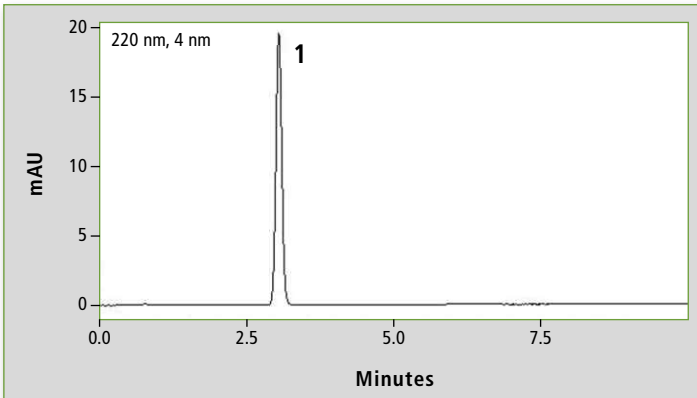


Figure 1a: Chromatogram of acetaminophen (0.01 mg/mL) obtained through the original USP method. Column: (a) Shim-pack GIS C18 (250 x 4.0 mm, 10 µm).

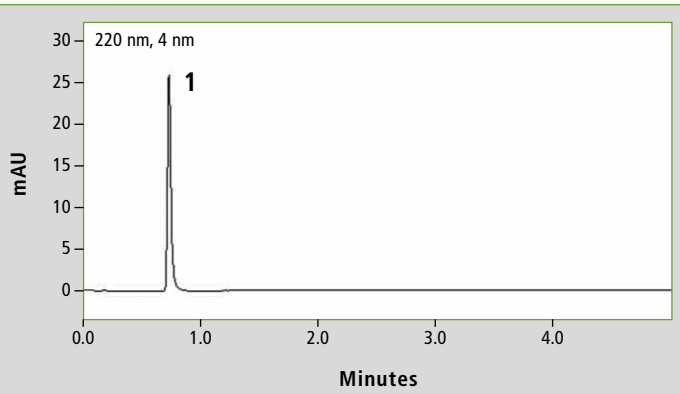


Figure 1b: Chromatogram of acetaminophen (0.01 mg/mL) obtained through the accelerated USP method. Column: (b) Shim-pack GIS C18 (100 x 3.0 mm, 3 µm).

# Small but powerful!

## Small columns accelerate conventional USP methods for medical use and save solvents, time, and money

In order to increase productivity and efficiency in laboratories, high-throughput analyses have become very important in recent years. Especially in the pharmaceutical industry, fast methods are an advantage as this industry faces many challenges: cost-cutting measures in the European and American healthcare sector limit growth and profit potential. The development of new drugs has become increasingly difficult and expensive. [1, 2]

Therefore, it is important to reduce costs in other ways. For example, by performing analyses as quickly as possible and more economically. This can be achieved in liquid chromatography (LC) by using a smaller column. Using examples from the official pharmacopoeia of the United States Pharmacopeia (USP), the possibilities were investigated. The pharmacopoeia contains general chapters on tests as well as individual monographs. A monograph consists of tests on active ingredients and their specifications.

In particular, the chapter "Chromatography" [3] (USP 621) is paramount, since the pharmacopoeia has been adapted for the use of smaller column dimensions and now allows, to a certain extent, the modification of HPLC and GC parameters in order to obtain faster methods.

### Chapter USP <621> is focused on chromatography

This chapter of the USP is of a general scope and contains information on all types of chromatography, such as gas, thin-layer, paper, and liquid chromatography. The principles of chromatography, including the basics as well as the equipment, and procedures used for the analyses are listed here. In addition, all important parameters which are necessary when analyzing a chromatogram are explained.

These USP application parameters, such as retention time, resolution, or plates, have certain target values, which must be met by applications modified from the original method. Table 1 shows the parameters, which may be changed in reference to USP 621. Additionally, the permitted ranges within these LC parameters are listed.

In the following, isocratic, conventional analyses of different medicines were modified while observing the permitted adaptation criteria of the USP. As shown in the example, the investigations of acetaminophen, ibuprofen, and

| | |
|---|---|
| Particle size (L/dp) | Constant L/dp ratio or number of theoretical plates: |
| Column length (L) | - 25 to + 50 % |
| Columns ID (dc) | Any, as long as the linear speed remains constant |
| Flow rate | Combination* of dp and dc: ± 50 % |
| Injection volume | Can be varied, but must match precision and detection limits |
| Column temperature | ± 10 °C |

Table 1: Permitted range of changes of the LC parameters in accordance with USP <621>
*$F_2 = F_1 \times [(dc_2^2 \times dp_1)/(dc_1^2 \times dp_2)] \cdot F_1$ and $F_2$ are the flow rates of the original and the modified method; dc1 and dc2 are the column diameters according to these methods; dc1 and dc2 are the particle size.

| Method | Acetaminophen, Glibenclamide | Ibuprofen |
|---|---|---|
| Instrument | LC-2040C 3D (Shimadzu) | Nexera X2 (Shimadzu) |
| Detection | PDA: D2 at 190 - 350 nm | SPD-M30A at 254 nm |
| Oven temperature | 40 °C | 40 °C |

Table 2: Instruments and parameters used


Shimadzu Nexera X2 SPD-M30A

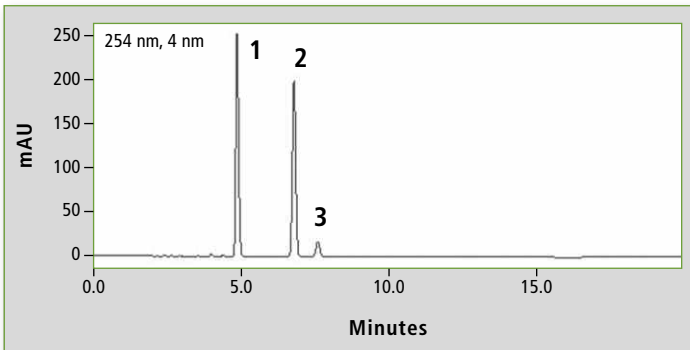


Figure 2a: Chromatogram of ibuprofen (peak 1) (12 mg/mL) obtained through the original USP method. Column: (a) Shim-pack GIST C18 (250 x 4.6 mm, 5 µm). Peak 2: vale-rophenone (0.35 mg/mL), peak 3: 4 isobutylacetophenone (0.012 mg/mL).

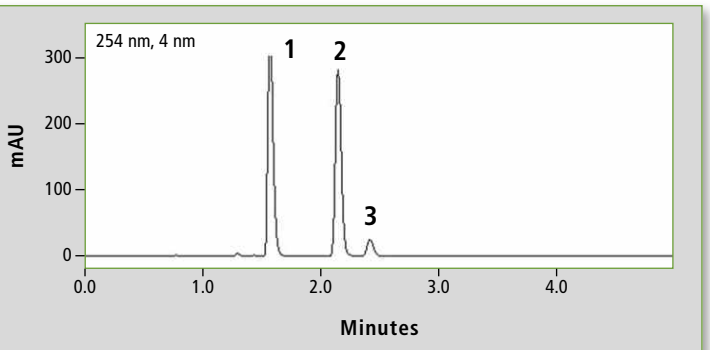


Figure 2b: Chromatogram of ibuprofen (peak 1) (12 mg/mL) obtained through the accelerated USP method. Column: (b) Shim-pack GIST C18 (100 x 2.1 mm, 2 µm). Peak 2: Valerophenone (0.35 mg/mL), peak 3: 4 isobutylacetophenone (0.012 mg/mL).

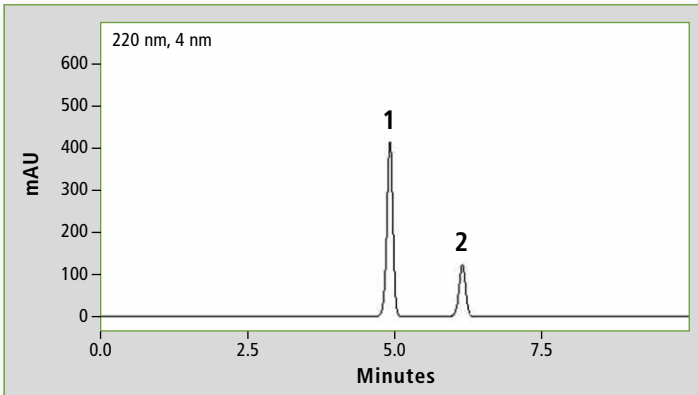


Figure 3a: Chromatogram of glibenclamide (peak 1) (0.44 mg/mL) obtained through the original USP method. Column: (a) Shim-pack GIST C8 (250 x 4.6 mm, 5 µm). Peak 2: progesterone (0.2 mg/mL).

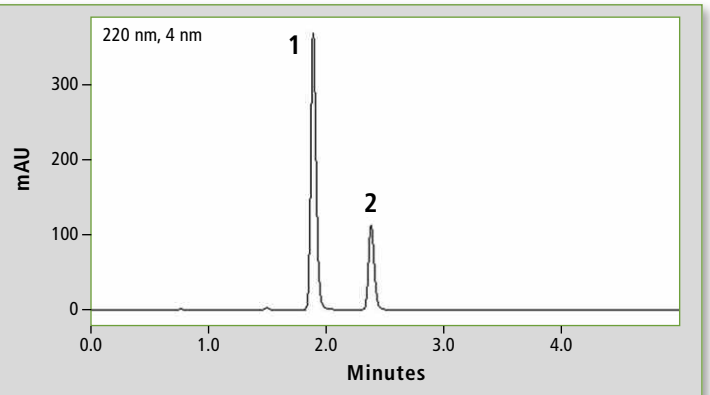


Figure 3b: Chromatogram of glibenclamide (peak 1) (0.44 mg/mL) obtained through the accelerated USP method. Column: (b) Shim-pack GIST C8 (100 x 2.1 mm, 2 µm). Peak 2: progesterone (0.2 mg/mL).

glibenclamide could be carried out faster than described in the original monograph.

### Advantages of shorter analysis times – achieved with smaller column dimensions

When replacing standard columns (250 or 150 x 4.6 mm) with smaller equivalents (e.g. 100 x 3 mm) packed with 2 - 3 µm instead of 5 µm particle sizes, different effects occur: the efficiency of the separation performance increases, resulting in narrower signals, and the analytes elute faster from the column. At the same time, the flow rate must be reduced, otherwise the pressure increase in the system would be too high. This provides two major improvements: a much shorter analysis time, which results in a higher sample throughput and a higher workload of the device, as well as a lower consumption of solvents.

With the saving of time and solvents, the down-scaling offers two advantages, which in turn greatly reduce expenses.

### Adaptation of USP methods in accordance with the guidelines

The permitted ranges within which the analytical conditions may be modified can be found in USP chapter <621> Chromatography. By changing the analytical conditions in accordance with the guidelines, the analysis time can be significantly shortened. Here, a shorter column with a smaller inner diameter was selected and the flow rate was correspondingly reduced in order to maintain the linear velocity. In order to maintain the resolution of the separation, the length and the particle size of the column may be modified as long as the ratio of the column length (L) to the particle size (dp) remains within the specified range (permissible range: - 25 % to + 50 %).

### Reduction of analysis time

Various medicines, which can be identified by LC methods described in the USP, have been tested. The original methods were modified by using smaller columns and lower flow rates according to USP guidelines. All analyses were measured under isocratic conditions.

### Used medicines

To demonstrate how much faster this new approach is, compared to the conventional methods of the pharmacopoeia, three different medicine analyses were chosen:

- Acetaminophen, better known as paracetamol, is a medication to treat fever and pain. [4]
- Ibuprofen is a non-steroidal anti-inflammatory drug. The drug is used to relieve fever, inflammation, and pain. [5]
- Glibenclamide acts as an antidiabetic agent. The active substance belongs chemically to the group of the sulphonylureas. The drug increases insulin release from the pancreas in order to lower blood sugar. [6]

| | | Acetaminophen | Ibuprofen | Glibenclamide |
|---|---|---|---|---|
| Column | conventional | Shim-pack GIS C18 (250 x 4 mm, 10 µm) | Shim-pack GIST C18 (250 x 4.6 mm, 5 µm) | Shim-pack GIST C8 (250 x 4.6 mm, 5 µm) |
| | accelerated | Shim-pack GIS C18 (100 x 3 mm, 3 µm) | Shim-pack GIST C18 (100 x 2.1 mm, 2 µm) | Shim-pack GIST C8 (100 x 2.1 mm, 2 µm) |
| Mobile phase | | A: Methanol; B: Water; A/B = 1/3 v/v | A: 1 % chloroacetic acid solution; pH 3.0; adjusted with ammonium hydroxide<br>B: acetonitrile; A/B = 40/60 | A: 2.6 g Ammonium dihydrogen phosphate in water/acetonitrile = 450/550 mL |
| Flow rate | conventional | 1.5 mL/min | 2.0 mL/min | 2.0 mL/min |
| | accelerated | 0.5 mL/min | 0.6 mL/min | 0.5 mL/min |
| Injection volumes | conventional | 10 µL | 5 µL | 10 µL |
| | accelerated | 5 µL | 1 µL | 1 µL |
| Concentrations | | Acetaminophen (0.01 mg/mL) | Ibuprofen (12 mg/mL), Valerophenone (0.35 mg/mL), 4-Isobutylacetophenone (0.012 mg/mL) | Glibenclamide (0.44 mg/mL), Progesterone (0.2 mg/mL) |

Table 3: Columns and method parameters used

## Measurement parameters and methods

The measurement parameters as well as the methods are shown in Tables 2 and 3.

## Results

The results are shown in figures 1 - 6 and in table 4. These indicate shorter retention times. The original USP methods have been greatly accelerated by changing the length, the internal diameter, and the particle size of the columns. The flow rate was adjusted to keep the linear velocity always constant. The retention times were thus shortened, resulting in time savings of 77 % for acetaminophen, 67 % in the analysis of ibuprofen and 61 % for glibenclamide. The solvent consumption of the three analyses was also significantly reduced: 67 % for acetaminophen, 70 % for ibuprofen, and 75 % for glibenclamide.

## Conclusion

The USP methods for acetaminophen, ibuprofen, and glibenclamide have been significantly improved by using smaller columns compared to the original USP methods. All analysis times are shorter, and less solvents are consumed. As a result, the costs per analysis are significantly reduced. The method transfer from larger columns to small columns offers a lot of advantages altogether, hence the headline: small but powerful!

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[3] United States Pharmacopeia (USP) 39, <621> Chromatography, August 2016.

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[5] Melzer, M.: Ibuprofen: Wirkung, Anwendung, Nebenwirkungen. Apotheken Umschau 2017.

[6] Davidson J.A., Scheen A.J., Howlett H.C. Tolerability profile of metformin/glibenclamide combination tablets (Glucovance): a new treatment for the management of type 2 diabetes mellitus. Drug Saf, 2004, 27(15), 1205-16.



Shimadzu LC-2040C 3D

| | Acetaminophen | Ibuprofen | Glibenclamide |
|---|---|---|---|
| $t_R$ fast method | 0.7 min | 1.6 min | 1.9 min |
| $t_R$ original method | 3.1 min | 4.8 min | 4.9 min |
| **Time saving** | **77 %** | **67 %** | **61 %** |
| Flow rate fast method | 0.5 mL/min | 0.6 mL/min | 0.5 mL/min |
| Flow rate original method | 1.5 mL/min | 2.0 mL/min | 2.0 mL/min |
| **Eluent saving** | **67 %** | **70 %** | **75 %** |

Table 4: Retention times and flow rates of the accelerated, as well as the original USP methods including calculated savings

# Just paper, isn't it?

## Analysis of paper-based food packaging materials using infrared ATR spectroscopy

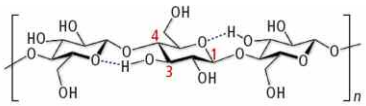

Figure 1: Cellobiose unit of a cellulose polymer

Anyone working with food packagings cannot do without one basic constituent: cellulose. This is a polymer composed of cellobiose units, which are glucose molecules linked together with β1,4-glycosidic bonds (figure 1). Cellulose is obtained from wood and is commonly known as (card)board or paper. Paper makes up the largest proportion of packaging materials. Its advantages are clear: not only is it a cheap, renewable raw material, but waste paper can be recycled and used in the production of new packaging materials.


IRTracer-100

Food-safe paper is available, for example, as wrapping paper for direct contact with foods such as fresh cold cuts or as laminated paper as drinking cups for beverages. The range of applications can be illustrated with the following keywords: bio-, to-go, and fast-food packagings. The use of paper-based packagings in the form of cardboard is limited to secondary packaging or for dry foods such as flour, sugar, pasta, and muesli.

On its own, paper does not provide a barrier against moisture and oxygen. Cardboards used for packaging foods are constructed of several layers. The outermost layer contains fillers such as kaolin ($SiO_2/Al_2O_3$) and talcum (a magnesium-containing sheet silicate) as well as titanium dioxide as a white pigment. These provide smooth, white surfaces that are more suitable for printing than the rough untreated board layer. [1, 2]

### ATR determines the main components of packaging surfaces

In the following, ATR-FTIR spectroscopy is used to quickly determine the main components in the surfaces of packagings. The acronym ATR stands for "**A**ttenuated **T**otal **R**eflection", which describes the applied measuring principle: IR radiation is passed through an optical element, e.g. a diamond crystal, before it reaches the sample. Most of the incident radiation is reflected by the interface between the sample and the ATR crystal, whereas a small fraction penetrates the sample and interacts with it. The resulting attenuation of the total reflection is measured.

The penetration depth of the IR radiation depends on the angle of incidence and, in the present experimental setup, it is approx. 2 µm. However, multilayer systems cannot be analyzed adequately in this way, because the layers are often only a few microns thick and cannot be separated from each other. IR microscopy, however, can be used to make ATR measurements of the cut edge of a multilayer system.

The measurements shown here were carried out with an IRTracer-100 with a diamond ATR (Quest). Several components can be identified in the infrared spectrum (figure 2a) of the outer surface of a cardboard box for chocolate bars.

The first database search identifies a copolymer composed of styrene and allyl alcohol monomers. These are constituents of the colored print. By generating a difference spectrum between that of the sample and that of the identified components, it was also possible to identify kaolin and calcium carbonate (figure 2b - 2d).

Paper packagings are stabilized against moisture by laminating them with PE (polyethylene) layers, which extends the application range for paper packagings. Examples of this include milk cartons and coffee cups.

Furthermore, the PE film makes the packaging more tear-resistant. However, since PE is permeable to $CO_2$ and $O_2$, its use is limited to fresh foods that are consumed within a short time. [3]

### Packaging carries advertising and creates reputation

The use of paper in packagings can contribute to shaping its image and also boost the reputation of the food itself. For example, the FSC logo can be awarded for using wood from "sustainable sources". This logo characterizes the product as being particularly environmentally friendly. [4]

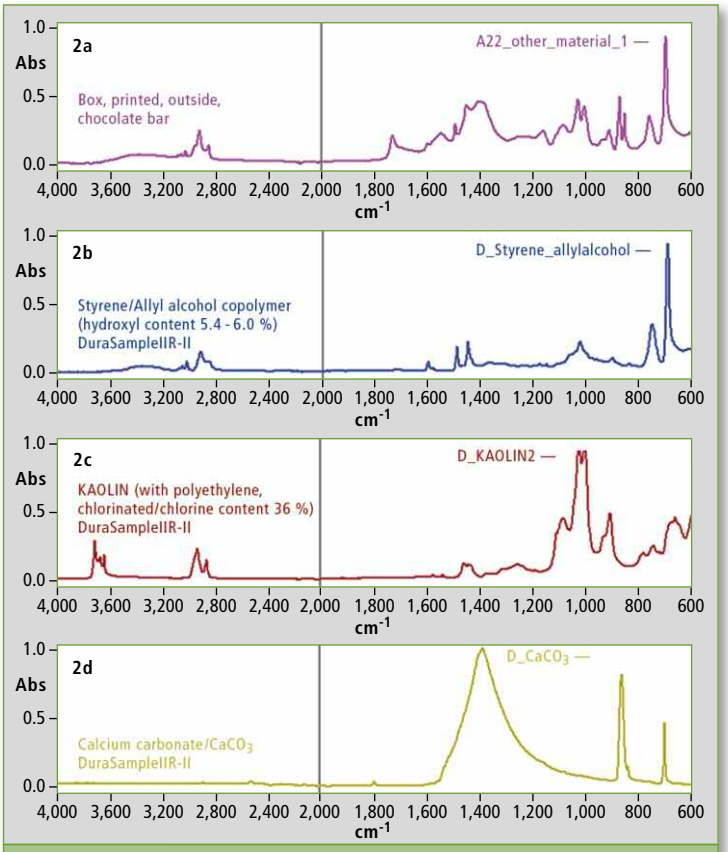


Figure 2a to 2d: Spectrum of a cardboard box (2a) and the constituents identified in it: styrene/allyl alcohol copolymer (2b), kaolin (2c), and calcium carbonate (2d)

Paper is also often used as a component of packagings for high-quality products. Only a very few of our purchasing decisions are based on logic; neurological research has even shown that all decisions are driven by emotions. [5] In addition to the copy text of ads, visual and haptic effects also play a major role in influencing purchasing behavior, and this leads to the use of packaging systems with a complex composition.


Figure 3: Paper bag for throat lozenges whose components were investigated with FTIR. [6]

## FTIR analyzes several layers of the packaging

Examination of a bag used for Em-eukal throat lozenges (see figure 3) revealed that both the yellow lozenge as well as the "Em-eukal" lettering on the packaging had a smooth shiny surface. An FTIR analysis showed that this was due to a PP (polypropylene) film. Furthermore, the inside of the bag is coated with an ethylene/acrylic acid copolymer, which is used as an adhesion promoter or for sealing packaging films. In addition, nitrocellulose, which is a typical constituent of printing inks, was found on the printed outer surface of the bag.

Thus, this simple 'paper bag' was composed not only of cellulose, but also three other materials, which gives the consumer the impression of a high-quality product. [3]

## IR microscopy analyzes components

IR microscopy can also be used to analyze the components of packagings, for example, using Shimadzu's infrared microscope AIM-9000 with a germanium-based ATR objective. The germanium crystal is used as the optical element. A cut is made through the layers of the Em-eukal bag. This reveals fibers close to the purple surface and a plastic film. The ATR crystal can be used to record spectra of 100 µm² areas of the corresponding layers. The investigated object and two of the measured spectra are shown in figure 4.

## Summary

Paper is a popular material for packaging foods. Many different materials are used in combination with paper, and each of these can be identified quickly and nondestructively with FTIR spectroscopy. As demonstrated here, some paper packagings consist of several layers as a combination of polymer coatings around the paper. The infrastructure of such a multilayer system can be investigated by means of infrared microscopy, which can be used to analyze layer thicknesses in the µm range.

## Literature

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AIM-9000

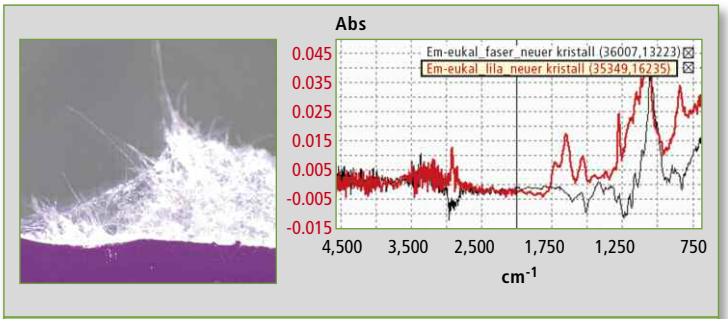


Figure 4: Red: micro-ATR spectrum of the purple surface (nitrocellulose); black: micro-ATR spectrum of the white fibers (cellulose).

# Time-efficient method for beer analysis

## Fully automated derivatization and quantification of Glyphosate and AMPA using a standard LC-MS/MS system

By using a standard LC-MS/MS system set-up, the analysis of Glyphosate and AMPA in beer could be simplified, without requiring an additional instrument, e.g. a liquid handling system for sample pretreatment. By staggering the pretreatment and the LC-MS/MS analysis, the method is very time-efficient. Calibration curves showed excellent precision and accuracy, and even in a complex matrix such as beer, Glyphosate and AMPA can be quantified at or below 5 ng/mL, which is below the EU maximum residue levels (MRL). 60 % of all tested beer samples contained traces of Glyphosate, but all of them were far below MRL.

### Introduction

Glyphosate is currently one of the most common pesticides used worldwide. In spite of its approval by regulatory bodies all over the world, the concern about its harm to humans and the environment persists. [1, 2] Therefore, the strict control of Glyphosate and its metabolite aminomethylphosphonic acid (AMPA) in food and environment is mandatory.

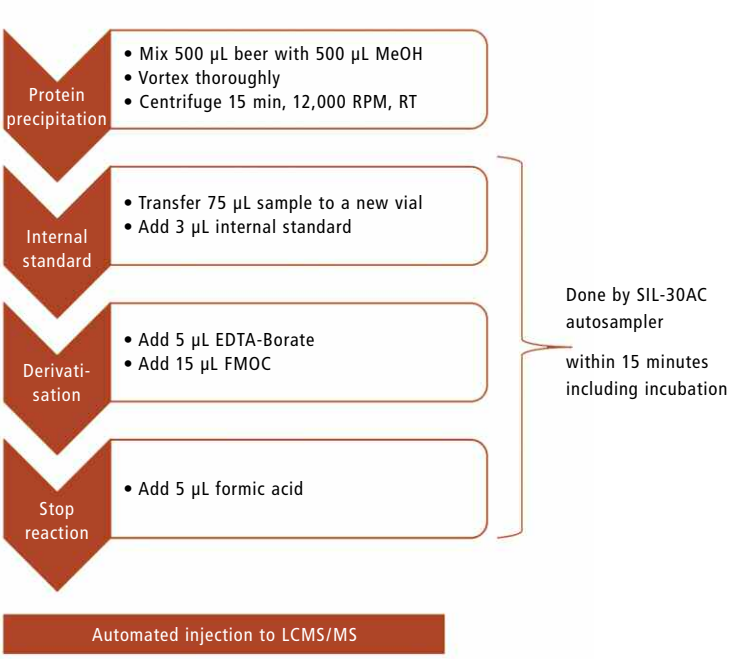


Figure 1: Workflow of sample pretreatment. Addition of internal standard as well as all remaining derivatization steps are done by the autosampler.

In 2016, the year of the 500th anniversary of the German Beer Purity law, Glyphosate has gained dubious fame after being found in many German beers. [3] While there are defined maximum residue levels (MRL) for drinking water and some food products, there is no dedicated MRL for beer. [4] In this case, the MRL of the individual ingredients apply, e.g. the MRL for barley which is used for malt production (20 mg/kg) or for hops (0.1 mg/kg). These values are far above the MRL for drinking water (0.1 µg/L).

## Fully automated derivatization followed by LC-MS/MS analysis

The quantification of glyphosate and AMPA is very challenging. On the one hand, both molecules are highly polar which does not allow enough retention on reversed-phase columns. On the other hand, there are not many transitions for LC-MS analysis available for these molecules due to their small molecular weight. Additionally, beer is a complex matrix that requires good chromatography.

In order to overcome the low retention of Glyphosate on reversed-phase columns, there is a well-established method that includes a derivatization step with 9-fluorenylmethyl chloroformate (FMOC) [4] followed by LC-MS analysis. FMOC derivatization leads to a decreased polarity allowing chromatography on a standard reversed-phase column. The derivatization requires several pipetting steps. They can be executed manually, which is tedious and prone to errors, or automatically, which requires dedicated additional hardware.

This article reports a fully automated derivatization followed by LC-MS/MS analysis of beer samples. The instrumental set-up does not require any additional hardware for sample pretreatment, but uses the built-in pretreatment function of the autosampler.

| Temps (min) | % B | Flow rate | Valve position |
|---|---|---|---|
| 0.00 | 5 | 0.4 | to waste |
| 2.50 | | | to MS |
| 5.00 | | | to waste |
| 7.00 | 50 | 0.4 | |
| 7.01 | 95 | 0.6 | |
| 12.00 | 95 | 0.6 | |
| 12.01 | 5 | 0.6 | |
| 14.00 | 5 | 0.6 | |
| 14.01 | 5 | 0.4 | |
| 15.00 | 5 | 0.4 | |

Table 1: UHPLC method

## Experimental

### Sample preparation

A beer sample of 500 µL was mixed with 500 µL methanol in order to precipitate proteins. After vortexing, the samples were centrifuged (15 minutes, 12,000 g) and set into the autosampler, which handled all further sample pretreatment steps fully automatically.

### Pretreatment steps:

### UHPLC method

Instrument: Nexera UHPLC, Shimadzu
Column: Gemini 5 µm C18, 150 x 2 mm
Mobile phase A: 2 mm $NH_4HCO_3$, pH 9.5
Mobile phase B: acetonitrile
Injection vol.: 50 uL
Column temperature: 35 °C

### MS conditions

Instrument: LCMS-8060, Shimadzu
Ionization: pos/neg ESI
Nebulizing gas: 3 L/min
Heating gas: 15 L/min
Drying gas: 5 L/min
Interface temperature: 325 °C
DL temperature: 150 °C
Heat block temperature: 400 °C
CID gas: 270 kPa
Interface voltage: 4 kV/-3 kV

## Results and discussion

### Method development for automated derivatization

The highly polar Glyphosate as well as its degradation product AMPA are very well soluble in water, but not soluble in other solvents like methanol, isopropanol or acetonitrile. In contrast to that, the non-polar FMOC is not soluble in water but can be solved easily in organic solvents. Bringing together the analyte and the derivatization agent in an environment that allows solubility for both of them is crucial for the whole analysis.

One way to achieve this is incubation at a higher temperature. This increases the solubility of FMOC in water and is therefore applied very often for the analysis. [5] For an automated approach, this requires a dedicated instrumental setup enabling heated incubation.

In this report, a good solubility could be achieved for FMOC,

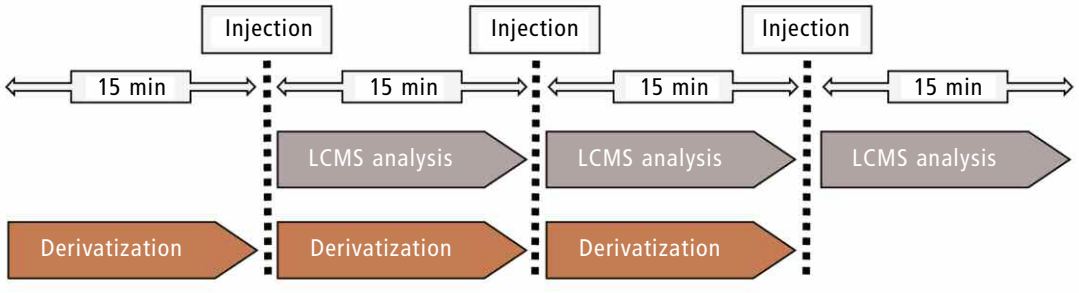


Figure 2: Overlapping sample pretreatment and analysis done by SIL-30AC. Total time per sample is reduced to 15 minutes.

Glyphosate and AMPA in a solution with 50 % methanol (data not shown) without heating the sample during incubation. This allowed a fully automated pretreatment of the samples by using the pretreatment function of the autosampler. The only manual pretreatment required was adding 500 µL methanol to 500 µL beer, vortexing the sample and centrifugation.

The supernatant was set into the autosampler, which executed all following steps (adding FMOC and internal standard, stopping the reaction) automatically (figure 1). After derivatization the sample was injected directly to the LC-MS/MS and analyzed accordingly. The time required for all derivatization steps is only 15 minutes.

The following chromatography also requires 15 minutes. Due to the overlapping sample pretreatment functionality, the next sample was already pretreated during the on-going analysis in order to maximize sample throughput (figure 2). Except for the first and the last sample, the total time per sample for automated pretreatment and analysis can be reduced to 15 minutes.

Figure 3 shows typical chromatograms for LOQs of Glyphosate-FMOC (2.5 ng/mL) and AMPA-FMOC (5 ng/mL) and their calibration curves (Glyphosate 2.5 - 100 ng/mL; AMPA 5 - 100 ng/mL). The method shows excellent linearity for Glyphosate-FMOC ($R^2$ = 0.9986) and AMPA-FMOC ($R^2$ = 0.9995). By analyzing QC samples in three different concentrations (3 ng/mL, 15 ng/mL and 75 ng/mL), the accuracy of the method was proven to be very high.

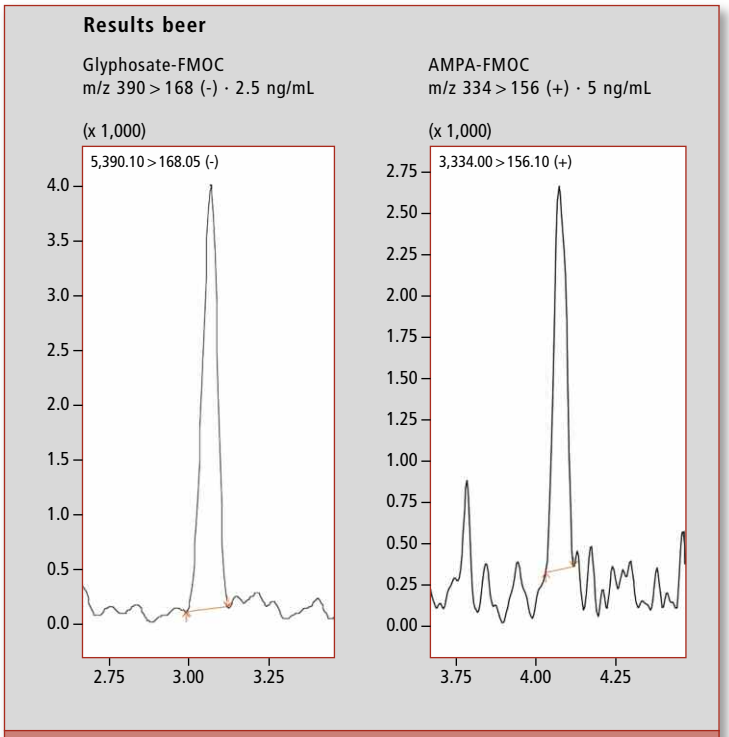


Figure 3: Chromatograms of Glyphosate-FMOC (2.5 ng/mL) and AMPA-FMOC (5 ng/mL)

The relative standard deviation was below 10 % for all QC except the smallest QC for AMPA-FMOC, which was extrapolated as it was below LOQ (table 1).

## Quantitative analysis of 40 beer samples

After the successful development of a fully automated method, a total of 40 commercially available beer samples were analyzed. Among these samples, there were 21 samples of beer brewed according to Pilsener style, three samples of organic beer, ten samples of other types of beer, and six samples of alcohol-free beers or non-alcoholic beer mix drinks.

All samples were analyzed in duplicate in two consecutive runs. While Glyphosate was detected in 60 % of all samples, its metabolite AMPA was below LOQ in all samples (table 2).

There is no correlation between the kind of beers (Pilsener style, alcohol-free and others like wheat beer) and the detection of Glyphosate, as there were samples containing Glyphosate among all kinds of beers. Only the three organic beers tested were completely free of Glyphosate. But even for the beers tested positively, the amount was far below the MRL so that none of the beers is a health hazard – at least with regard to Glyphosate levels.

| | Glyphosate-FMOC | | | | | | AMPA-FMOC | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | QC 3 ng/mL | | QC 15 ng/mL | | QC 75 ng/mL | | QC 3 ng/mL | | QC 15 ng/mL | | QC 75 ng/mL | |
| Batch | Conc. | Acc.% | Conc. | Acc.% | Conc. | Acc.% | Conc. | Acc.% | Conc. | Acc.% | Conc. | Acc.% |
| A | 2.60 | 86.5 | 14.89 | 99.3 | 74.14 | 98.9 | 4.76 | 158.5 | 15.66 | 104.4 | 80.80 | 107.7 |
| A | 2.87 | 95.7 | 14.96 | 99.7 | 81.22 | 108.3 | 2.71 | 90.3 | 16.16 | 107.7 | 85.65 | 114.2 |
| A | 3.41 | 113.5 | 15.14 | 100.9 | 77.94 | 103.9 | 3.15 | 105.0 | 15.99 | 106.6 | 81.38 | 108.5 |
| B | 2.81 | 93.7 | 16.00 | 106.7 | 79.18 | 105.6 | 4.11 | 137.0 | 15.33 | 102.2 | 78.40 | 104.5 |
| B | 3.20 | 106.7 | 16.08 | 107.2 | 76.19 | 101.6 | 3.49 | 116.2 | 15.20 | 101.3 | 82.23 | 109.6 |
| B | 3.46 | 115.3 | 15.42 | 102.8 | 83.74 | 111.6 | 3.02 | 100.8 | 15.66 | 104.4 | 84.15 | 112.2 |
| C | 2.82 | 93.9 | 14.94 | 99.6 | 67.88 | 90.5 | 3.48 | 115.9 | 15.48 | 103.2 | 83.97 | 112.0 |
| C | 2.73 | 91.1 | 15.67 | 104.5 | 76.89 | 102.5 | 3.25 | 108.3 | 16.55 | 110.3 | 79.72 | 106.3 |
| C | 3.27 | 109.0 | 15.87 | 105.8 | 84.87 | 113.2 | 3.38 | 112.6 | 16.87 | 112.5 | 82.65 | 110.2 |
| D | 3.19 | 106.2 | 16.42 | 109.5 | 82.82 | 110.4 | 2.73 | 90.9 | 16.85 | 112.3 | 75.46 | 100.6 |
| D | 3.33 | 110.9 | 16.00 | 106.7 | 85.29 | 113.7 | 3.31 | 110.4 | 14.35 | 95.7 | 72.06 | 96.1 |
| D | 3.23 | 107.6 | 17.14 | 114.3 | 84.74 | 113.0 | 3.55 | 118.3 | 15.50 | 103.3 | 75.97 | 101.3 |
| Mean | 3.08 | | 15.71 | | 79.57 | | 3.41 | | 15.80 | | 80.20 | |
| SD | 0.2915 | | 0.6816 | | 5.2735 | | 0.5676 | | 0.7306 | | 4.0615 | |
| **RSD (%)** | **9.5** | | **4.3** | | **6.6** | | **16.6** | | **4.6** | | **5.1** | |
| | | | | | | | Extrapolated | | | | | |

Table 2: QC sample results


## IMPRINT

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## Conclusion

The reported method is able to derivatize Glyphosate and AMPA with FMOC fully automatically within 15 minutes. The only manual pretreatment required is protein precipitation. No additional hardware is required as all pretreatment steps are performed by the autosampler of a standard LC configuration. The method is robust and reliable for samples

even in a complex matrix like beer, which makes it suitable for high-throughput analysis. Additionally, the throughput was doubled by using the overlapping sample pretreatment functionality. This allows sample pretreatment while the previous sample is analyzed.

| | Glyphosat-FMOC | | | | | AMPA-FMOC | |
|---|---|---|---|---|---|---|---|
| | Conc. ng/mL | Conc. ng/mL | Mean | SD | % RSD | Conc. ng/mL | Conc. ng/mL |
| **Pils** | | | | | | | |
| Sample 1 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 2 | 8.37 | 8.95 | 8.7 | 0.4087 | 4.7 | <LOQ | <LOQ |
| Sample 3 | 20.85 | 20.28 | 20.6 | 0.4038 | 2.0 | <LOQ | <LOQ |
| Sample 4 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 5 | 6.78 | 6.57 | 6.7 | 0.1549 | 2.3 | <LOQ | <LOQ |
| Sample 6 | 11.34 | 12.08 | 11.7 | 0.5240 | 4.5 | <LOQ | <LOQ |
| Sample 7 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 8 | 8.61 | 9.41 | 9.0 | 0.5706 | 6.3 | <LOQ | <LOQ |
| Sample 9 | 4.74 | 4.63 | 4.7 | 0.0834 | 1.8 | <LOQ | <LOQ |
| Sample 10 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 11 | 10.81 | 12.03 | 11.4 | 0.8627 | 7.6 | <LOQ | <LOQ |
| Sample 12 | 13.95 | 14.65 | 14.3 | 0.4943 | 3.5 | <LOQ | <LOQ |
| Sample 13 | 33.06 | 27.61 | 30.3 | 3.8509 | 12.7 | <LOQ | <LOQ |
| Sample 14 | 20.29 | 18.68 | 19.5 | 1.1377 | 5.8 | <LOQ | <LOQ |
| Sample 15 | 25.28 | 22.09 | 23.7 | 2.2578 | 9.5 | <LOQ | <LOQ |
| Sample 16 | 3.23 | 2.93 | 3.1 | 0.2171 | 7.1 | <LOQ | <LOQ |
| Sample 17 | 3.66 | 3.48 | 3.6 | 0.1308 | 3.7 | <LOQ | <LOQ |
| Sample 18 | 5.25 | 5.65 | 5.4 | 0.2807 | 5.2 | <LOQ | <LOQ |
| Sample 19 | 2.67 | 2.93 | 2.8 | 0.1881 | 6.7 | <LOQ | <LOQ |
| Sample 20 | 3.87 | 4.39 | 4.1 | 0.3698 | 9.0 | <LOQ | <LOQ |
| Sample 21 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| **Organic Beer** | | | | | | | |
| Sample 22 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 23 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 24 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| **Others** | | | | | | | |
| Sample 25 | 2.79 | 3.26 | 3.0 | 0.3323 | 11.0 | <LOQ | <LOQ |
| Sample 26 | 4.61 | 4.15 | 4.4 | 0.3260 | 7.4 | <LOQ | <LOQ |
| Sample 27 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 28 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 29 | 2.52 | <LOQ | | | | <LOQ | <LOQ |
| Sample 30 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 31 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 32 | 8.06 | 7.27 | 7.7 | 0.5621 | 7.3 | <LOQ | <LOQ |
| Sample 33 | 11.19 | 11.57 | 11.4 | 0.2737 | 2.4 | <LOQ | <LOQ |
| Sample 34 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| **Non alcoholic** | | | | | | | |
| Sample 35 | 4.75 | 4.47 | 4.6 | 0.1952 | 4.2 | <LOQ | <LOQ |
| Sample 36 | 16.05 | 15.71 | 15.9 | 0.2454 | 1.5 | <LOQ | <LOQ |
| Sample 37 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 38 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 39 | <LOQ | <LOQ | | | | <LOQ | <LOQ |
| Sample 40 | 2.50 | 2.85 | 2.7 | 0.2482 | 9.3 | <LOQ | <LOQ |

Table 3: Analysis of beer sample

## Literature


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# New solutions to complex problems

## Global Innovation Summit – World-leading research meets cutting-edge technology


Innovation Center in the United States

Mass Spectrometry Center in China

Working with scientists and academia to explore new grounds for providing advanced technological systems has been a long and good tradition within Shimadzu to become a worldwide-leading manufacturer of analytical instrumentation and medical technology.

Many industry-firsts have today turned into standards and norms of a well-equipped laboratory and underline Shimadzu's approach of "Excellence in Science".

To maintain itself as a global leader and expand its technology, Shimadzu has placed an increasing emphasis on strengthening its current academic partnerships and establishing new ones.

The establishment of Shimadzu Innovation Centers combining scientific and technological know-how in order to use Shimadzu's expertise to provide even more customer-focused service is the next step towards these partnerships. Meanwhile, there are Innovation Centers in the United States, China, and Singapore. In 2017, the European Innovation Center was opened in Duisburg, Germany. It applies a decentralized structure to be close to

Innovation Center in Laboratory World Europe, Duisburg, Germany

scientists and related markets, and is a strong statement by Shimadzu about its commitment to working with academic institutes. The result is faster response times for the development of technologies that meet its partners' needs.

"We have many good scientists and engineers. However, it is not easy to develop solutions, so we must collaborate with more researchers. Only then can we continue to offer outstanding technologies, products, and services," said Shuzo Maruyama, general manager of the Analytical and Measuring Instruments Division at Shimadzu Corporation.

Shimadzu has invested tens of millions of dollars into projects with universities around the world. Examples include the Shimadzu Center for Advanced Analytical Chemistry (SCAAC) at the University of Texas at Arlington (United States), the Shimadzu Analytical Innovation Research Laboratory at Osaka University (Japan), and the HMSTrust Analytical Laboratory at Monash University (Australia). Through Shimadzu instruments, these open-access, non-profit laboratories provide not only advanced research, but also high-level training to students and future scientists at the beginning of their careers.

Initiatives like these are the reason why nearly 40 % of Shimadzu's 11,000 employees and nearly 50 % of its customers are located overseas.

### Global Innovation Summit on human health, food safety, and environmental conservation

Apart from this new generation of collaboration with scientists, the Shimadzu Global Innovation Summit was born, a conference-style multilateral engagement between key opinion leaders (KOL), Shimadzu scientists, engineers, and executives, and many emerging thought leaders (ETL). In July 2017, the Summit brought 11 KOLs to the Shimadzu global headquarters in Kyoto to speak about their research in medical innovation, environmental issues, and food evaluation, for example.


Dr. McIntosh in her lab at the Monash Institute of Pharmaceutical Sciences

The Summit's aim was to facilitate collaborations among scientists using similar techniques but rarely having the opportunity to interact with one another. The research themes presented focused on global issues of human health, food safety, and environmental conservation, and highlighted the importance of analytical technologies for solving these problems.

Most representatives attending the Summit came from universities or national research institutes. The Summit was deliberately kept small, with just over 100 partici-

pants, but was internationally diverse, as scientists from 16 different countries participated. The reason for the small size was to encourage as much interaction as possible between the attendees. Following the academic model, along with KOL presentations, ETL were welcomed to present their data at a poster session, and several open-panel discussions were held to facilitate interaction between ETL and KOL.

"The conferences I attend are mostly on pharmaceuticals and rarely about food science. The talk I heard at the Summit about extractables and leachables in food packaging described the same issues we encounter with pharmaceutical products and packaging. I exchanged business cards after the talk to discuss potential collaborations," said Monash University Associate Professor Michelle McIntosh from Melbourne, Australia.

### Hope for new mothers: new treatment of postpartum hemorrhage

Dr. McIntosh's talk is a perfect example of how Shimadzu hopes to see its technology bring solutions to some of the world's crises.

Each year, 100,000 women will die from postpartum hemorrhage, most of them in developing countries. The gold standard for preventing and treating postpartum hemorrhage is oxytocin, a drug that costs only a few cents. Despite this cost, access to oxytocin is not extensive. The reason is the drug's storage and delivery. Because it is a peptide, oxytocin must be stored under controlled temperatures and must be injected, which requires qualified medical staff. However, in poorer nations, it has been estimated that nearly 90 % of mothers in the lowest income brackets give birth at home and do not have access to pristine oxytocin or adequately trained professionals.

As an alternative, Dr. McIntosh has been researching aerosolized versions of oxytocin. Although systemic drug delivery via the lungs has remained a challenge, drug absorption and onset of action can be equivalent to injections. She therefore investigates a dry powder version of oxytocin, which removes the need for refrigerated storage and simplifies the administration of the drug. A major piece of this work is understanding the degradation of oxytocin, for which Dr. McIntosh uses a number of Shimadzu instruments. These studies include understanding how the liquid and solid versions of oxytocin respond to the high temperatures common in the regions most in need of the aerosolized form. Through this research, she and her team now conduct Phase 2 clinical trials.

Dr. Schug (right) at the University of Texas at Arlington

### New anti-cancer therapy known as near-infrared photoimmunotherapy (NIR-PIT)

Another KOL who spoke was Dr. Hisataka Kobayashi, Senior Investigator at the Center for Cancer Research, National Institute of Health in the United States, within easy reach of where the first Shimadzu Innovation Center was established. There, Dr. Kobayashi has been developing a new anti-cancer therapy known as near-infrared photoimmunotherapy (NIR-PIT). NIR-PIT uses conjugates of fluorescent dyes and antibodies to attack the cancer cells. These conjugates are injected into the patient and will bind to the surface of the cancer cell. A NIR stimulus causes a reaction in the dye to activate the antibody. This activation permeabilizes the cell membrane and triggers necrosis. NIR-PIT achieves extraordinarily high specificity for cancer cells. It is scheduled for Phase 2 clinical trials and soon will proceed to the registration trial.

Shimadzu analytical equipment supports Dr. Kobayashi in this research by providing data on the photosynthetic reaction that releases the antibody. The antibody must transition from a hydrophilic to hydrophobic environment to cause the necrosis. Shimadzu mass spectrometry equipment is being used to understand the chemical mechanism of the reaction in order to optimize the light intensity that maximizes the cytotoxic effect.

The latest Shimadzu NIR technology was also presented at the Summit. In this system, indocyanine green (ICG) is injected into the patient. NIR light is shone onto the patient to fluoresce ICG, illuminating blood perfusion and lymph vessels in real time for the doctor conducting the surgical procedure under ambient light. This technology is currently used at Japanese hospitals in an experimental setting on patients.

### New diagnostics for prostate cancer

KOL Dr. Alex J. Rai, associate professor at the Columbia University College of Physicians and Surgeons (United States), sees these and other talks as a big boost to his research on new diagnostics for prostate cancer.

One major challenge in cancer relates to the heterogeneity of the disease process, which demands analytical tools that can reveal biomarkers for molecular stratification. These biomarkers can be used to assist in diagnosis, prediction of therapeutic response, and for monitoring purposes. From what doctors currently know, there is no single gene that is responsible for the majority of prostate cancers. Thus, it is of great interest to identify biomarker signatures that can provide insight into the disease process, to understand how genetic mutations are translated into dysfunctional proteins that result in aberrant signaling in tumor cells.

Prostate cancer is the second leading cause of cancer death. Dr. Rai is searching the constituents of exosomes for biomarkers that can stratify patients for better diagnosis and treatment plans. This research has not only benefited from Shimadzu technology but also from Shimadzu education programs like 'lab4you', which invites graduate students and other scientists to Shimadzu facilities and provides quick access to equipment for experiments.

### Environmental conservation: potential impact of hydraulic fracking

Also, key opinion leaders studying environmental issues and having long relationships with

Dr. Santos during her presentation at the Global Innovation Summit

Shimadzu University Laboratories presented their research. One was Shimadzu Distinguished Professor of Analytical Chemistry Kevin A. Schug of the University of Texas at Arlington.

His lab researches the potential impact of hydraulic fracturing, or fracking, on the environment in the State of Texas. The SCAAC has allowed the Schug lab to conduct some of the largest studies on groundwater contamination, providing invaluable information for policies that will establish best practices for this increasingly common energy extraction method.

While his own talk was technical and focused on advances in the analysis of intact proteins, Dr. Inês C. Santos, an ETL in the Schug lab, presented an award-winning poster about changes in the microbiome in groundwater wells near fracking sites.

### Evaluation of complicated food function

Professor Eiichiro Fukusaki of Osaka University also gave a technical talk, which was about metabolic fingerprinting and its application to food evaluation.

"It is very difficult to evaluate food function using conventional reductionism. The metabolomics technique is very useful to evaluate complicated food function, particularly flavor," he explained.

By creating a metabolic matrix using mass spectroscopy data, Dr. Fukusaki showed that metabolic fingerprinting can predict the scoring of various attributes, such as aroma, flavor, and appearance of different foods by expert taste testers. These fingerprints can be used by companies to modify flavors for different markets.

Prof. Erich Leitner (right), University of Technology, Graz, Austria

### The future

The Global Innovation Summit is the latest example of Shimadzu's recognition that to continue to innovate groundbreaking technologies, it must work closely with scientific leaders in analytical research of different fields. The Summit was a natural growth from the relationships the company had forged through its many laboratories and centers around the world. These facilities have allowed Shimadzu scientists and engineers to work closely with KOL, establishing a level of trust that could not be attained otherwise.

"Shimadzu is starting to communicate much more, especially in my field," said Prof. Erich Leitner, a professor at Graz University of Technology (Austria) and a KOL at the Summit, who spoke about his research on food aroma and food contamination. "It makes a difference. They give us great opportunity for measurements. It's really nice to go there. Nice to work there. They really try to speed up things."

**Further information on this article:**
- www.shimadzu.com/an/gis/

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