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No. 16

Metabolomics

Automated Sample Preparation for Lipidomics

Forensic Science and Toxicology

Automated determination of THC, CBN and CBD in human hair

Flavor, Fragrance, and Odor Analysis

A new Twist: Free acids and phenols

Dear Reader,

The task may be to monitor food safety by determining individual identities and amounts of various chemical compounds in food or beverages; or to analyze blood, urine or hair for residues of drugs and pharmaceutical compounds; or even to determine the type and amount of microplastic particles in environmental samples. In chemical analysis as in competitive sports, the credo is maximum efficiency, highest productivity and sustainable use of available resources.

Eberhard G. Gerstel

Holger Gerstel

Ralf Bremer

Analytical laboratories that want to meet and exceed current and future requirements should turn to simple-to-use, reliable automation, ideally requiring only minimal amounts of sample and solvent to reach low limits of detection.

GERSTEL is among the leading providers of automation for GC/MS and LC/MS, specializing in sample preparation and -introduction among other things. GERSTEL solutions are used in a broad range of application areas. If you are considering automating parts of your laboratory work, or if you are looking for an autosampler, the article „Looking for Automation?“ on page 11 offers valuable tips and insights on what to look for.

We hope that this issue of GERSTEL Solutions worldwide magazine will give you food for thought and stir your interest in starting a conversation with our experts. GERSTEL partners and representatives in your area can be found under www.gerstel.com or by sending a mail to gerstel@gerstel.com. Contact details are also listed on the back cover of this magazine.

GERSTEL Solutions worldwide No. 16 consists of a series of informative and easy-to-read articles from a broad range of analytical laboratories, most of the work has been done by GERSTEL users. The content is listed on this page. Additional articles and material can be found under www.gerstel.com.

We hope you will enjoy the magazine.

Sincerely,
GERSTEL Management

Images (Frontpage): Dr. Malte Reimold, Jan Garbe-Immel / GERSTEL

CONTENT

NRW State Governor visits GERSTEL

Hannelore Kraft, Governor of the State of North Rhine-Westphalia (NRW), paid GERSTEL a visit to get first-hand information about the conditions under which technology companies in the German "Mittelstand" operate and to ask the company to add even more young people to its vocational training program. With 18 million inhabitants, NRW is the most populous of the German states (Länder) and the one with the largest economy.

By Guido Deussing

If you measure the German Small and Medium Enterprises (SMEs) by their number, their revenues, added value, and by the money they invest each year, these companies make up the major group of players in the thriving German economy. In Germany, SMEs are referred to as belonging to the "Mittelstand" or group of medium sized companies.

The Governor means business: NRW Governor Hannelore Kraft visited GERSTEL to listen, learn, and garner support for her political programs helping school graduates join the ranks of trained professionals. Mrs. Kraft is shown here with Holger R. Gerstel (front), Eberhard G. Gerstel (center) and General Manager Ralf Bremer to the right.

In 2014, the German Federal Bureau of Statistics published a report: „Mittelstand, the engine of the economy, numbers and facts about German SMEs". These SMEs generate 36 percent of the total revenues of all German companies and 55 percent of the added value. In addition, the SMEs are responsible for 16.8 Million, or 62.3 percent, of the employees who contribute to the German equivalent of Social Security taxes. These numbers are out of a total population of 81 Million (2014).

This means that politicians should pay attention to and support SMEs, which form a key pillar of the German economy and the social fabric. This is exactly what Governor Hannelore Kraft of North Rhine Westphalia was doing when she visited GERSTEL Headquarters in September 2015. Incidentally, Kraft means Force in German. The NRW Head of State wanted to get first-hand information about the operations of GERSTEL, a global player in the field of Laboratory Instrumentation for Chemical Analysis.

GERSTEL core competence fine mechanics: For some products, a magnifying glass helps to appreciate the fine details shown to the Governor by Dennis Arkeveld (center) from the GERSTEL production team.

In addition, Governor Kraft wanted to drum up support for a State Government project called "No graduation without continuation, transition from school to professional life in NRW". The idea behind the slogan is to pave the way for school- or high school graduates to enroll in professional training and education programs in companies. The German two-pronged approach ("Duales System") combines training and work in the company with classes at professional or vocational schools. At the end of the process, the trainee goes through an official exam to become a certified professional. The political class is well aware that the "Mittelstand" is the educator of the nation where four out of five professionals receive their training. Governor Kraft's appeal to GERSTEL was to provide even stronger support for her program. GERSTEL currently has five such trainees and has a history of taking them on as full employees when they pass their exams.

GERSTEL has 200 employees world-wide, 160 of these are in Germany mainly at Headquarters in Mülheim an der Ruhr, NRW. About one third have a B.Sc. or higher education including 14 Ph.D. scientists. Two thirds have professional training including fine mechanics and office/commercial staff, many of whom received their training in the company. "We train and qualify employees in order to support and extend the GERSTEL know-how", says Holger Gerstel, President and co-owner of the company. Governor Kraft and GERSTEL upper management stressed the importance of the dual educational system to ensure professional training on a high level. The system is recognized as unique and many nations are looking to replicate it. "GERSTEL has received several delegations from China who wanted to study the German system of professional training and to see how it is implemented in practice", says Eberhard G. Gerstel, President and Co-Owner. Management ensured Governor Kraft that they will maintain and expand GERSTEL's role as a leading company in its field while supporting and training young people for their professional future. However politics should refrain from adding restrictions to SMEs but rather let them focus their energies on core competences, allowing them to retain and gain competitiveness in the global marketplace.

Ralf Bremer (2nd from left) shows Governor Kraft the Thermal Desorption Unit (TDU), the centerpiece of many GERSTEL systems. Application Chemist Jochen Vandenberg (left) demonstrated the use of the TDU in combination with the GERSTEL Twister® for flavor and fragrance analysis.

Images: GERSTEL

A Sea of Polymers

Microplastic particles in the marine environment could have a bigger impact than previously thought: To get an idea of the extent of the plastic waste pollution in the marine environment, and of how quickly, if at all, it breaks down, science must rise to the challenge and find ways to determine qualitatively and quantitatively what is floating amongst the aquatic wildlife that makes up a good part of our food supply. Pyrolysis GC/MS has been found to be a useful tool for the characterization of microplastics in environmental samples.

By Guido Deussing

Image: istock / enviromatic

During the International Coastal Cleanup in 2013, 648,015 volunteers worked along a stretch of around thirteen thousand miles of ocean coastline to remove more than 5,500 tons of waste. The top ten waste items were: Cigarette butts (913 tons), paper candy wrapping (794 tons), plastic bottles (426 tons), plastic caps and lids (385 tons), plastic soda straws (252 tons), standard plastic shopping bags (200 tons), glass bottles (179 tons), other plastic bags large and small (176 tons), paper bags (167 tons), and beverage cans (154 tons) [1].

There is no way to correlate the recorded amount of these different types of waste to the total amount of waste released annually into the oceans, but it does paint a grim picture if one considers that we are only looking at the tip of the iceberg. Another large source of pollution is plastic micro beads (less than 5 millimeters in diameter) used in personal care products mostly as exfoliating agents. During use they enter the waste water stream; however, they pass though waste water treatment plants and end up in our water ways and oceans where fish ingest them at an alarming rate. It seems safe to assume that plastics are one of the major sources of environmental pollution. Not just environmental action groups, but also polymer material producers see cause for concern and the need for action as an increasing number of scientific journal papers and reports are referenced in the press documenting the detrimental effects of microplastics on the environment [2]. What then needs to be done – and what can the individual consumer do to avoid contributing to microplastics pollution or even to help improve the situation?

A responsible and sustainable use of polymer materials is a good start for individual consumers, companies and organizations alike. On a grander scale, well organized collection and recycling systems must be available. Various projects have been initiated with the aim of reducing the problem of waste in the oceans [2-6]. In the meantime, inland waterways and water bodies are also becoming the focus of attention since they seem to be impacted significantly as well [7-8].

Obviously, for any long term strategy to have a chance of success, it needs to be built on solid and reliable data. At this time, too little is known about transport routes, transformation processes, as well as effects and whereabouts of polymer residues in the environment. The scientific community is working to close this knowledge gap with a special focus on microplastics [7, 9-12].

The big problem posed by the tiniest particles

Polymer materials are very rugged. Even when exposed to energy-rich solar radiation, chemicals, mechanical force, or microbial attack, they never really seem to degrade or disappear from the environment. Sooner or later, polymer materials become brittle and are ground into ever finer particles until they end up in the microplastics range.

Microplastic particles, due to their small size, ranging from a few micrometers to a few millimeters, and their irregular shape and color, are often mistaken for food and are ingested by aquatic organisms and sea birds.

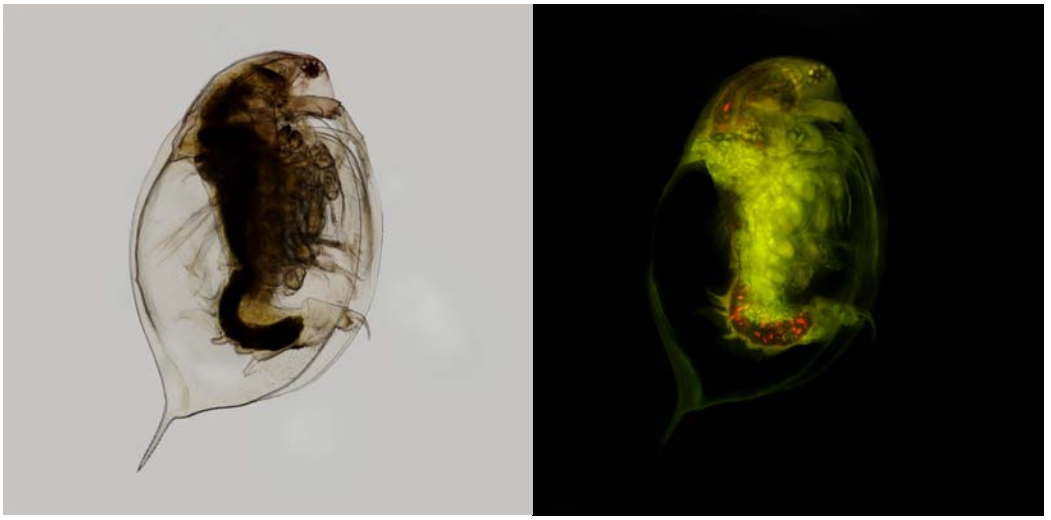
Increasingly, substantial amounts of polymer fragments are being found in the digestive tracts of dead seabirds. Whether the microplastic particles caused the death of the animals is uncertain. Nevertheless, it is not unreasonable to assume that animals which often ingest plastic material could suffer from malnutrition. Scientists predict that in 2050 we will find plastic residues in around 80 percent of all seabirds. Further, microplastic particles can contain unhealthy or toxic additives as well as pesticides, heavy metals or other toxins that are concentrated in the polymer from the surrounding environment. Since humans are at the top of the food chain this could eventually become a threat to us as well.

International efforts to learn more

The European Commission's Marine Strategy Framework Directive (MSFD) [13] focuses on protecting the seas and on managing natural marine resources efficiently. According to the MSFD, the type and composition of micro particles, and microplastic particles in particular, need to be characterized. This is exactly what researchers at the Universities of Osnabruck and Darmstadt, both located in Germany, have set out to do. As part of their research [10, 11], Professor Elke Fries and her colleagues investigated marine microplastic particles, which they had extracted from sand samples collected on the North Sea Island of Norderney, Germany. In order to determine the material composition of the microplastic particles collected as well as any additives used or pollutants that had been absorbed by the particles, Prof. Fries and her colleagues turned to pyrolysis-GC/MS as the first scientists ever to do so for this type of sample [10-12]. Previously, mainly spectroscopic methods had been used to establish

Plastic waste floating in surface water is mistaken for food or ingested with food by aquatic organisms and seabirds. Scientists speculate and project that in 2050, plastic residues will be found in almost all seabirds.

Image: istock / Micha Klootwijk



Comparing a picture taken with visible light and a fluorescence picture brings the truth to the fore: The red dots in the latter (right hand side) are microplastic particles in the digestive tract of the animal.

Images: Prof. Christian Laforsch, Universität Bayreuth

the structure and composition of polymers, Fries et al. wrote, and organic additives were extracted using supercritical fluid extraction or Soxhlet extraction. For polymer particles that are not easily dissolved, extracted or hydrolyzed, pyrolysis GC/MS can be a highly useful complementary technique.

Pyrolysis GC/MS serves to provide structural information about macromolecules based on the fragments formed in a controlled thermal decomposition process [14].

Serial pyrolysis is performed in two or more steps. Initially, volatile compounds are thermally extracted from the sample at a relatively low temperature. The technique is referred to as thermal desorption. Following the first step, the same sample is pyrolyzed at a higher temperature; ideally both steps are performed without the need to reconfigure the instrument. By collecting GC/MS data for both steps, organic additives and pyrolysis breakdown products can be determined during a single sample run. As Prof. Fries et al. note, this is a very efficient means of gathering information on both polymer type and the additives it contains.

Image: GERSTEL / Wolfram Schroll

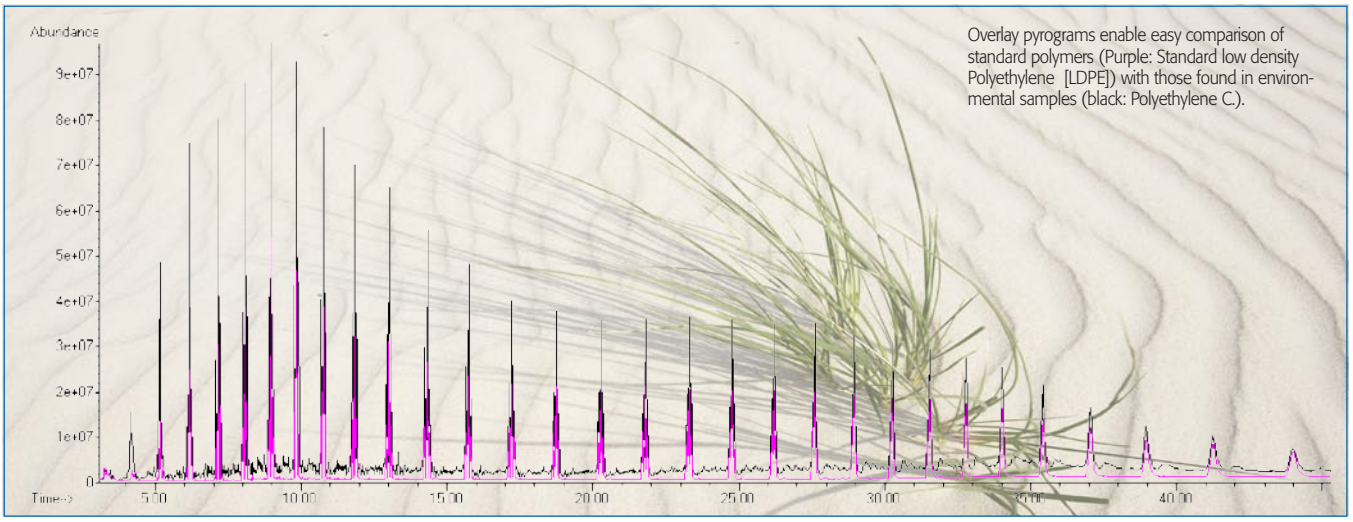
The TDS is well suited for performing serial pyrolysis GC/MS on a small number of samples. When the TDS is fitted with a special module, the PM 1, it can be used for both thermal desorption and pyrolysis on the same sample. When a larger number of samples need to be analyzed by serial pyrolysis GC/MS, the Thermal Desorption Unit (TDU) in combination with the MultiPurpose Sampler (MPS) shown here is the best option.

For their work, the researchers used a 7890 GC (Agilent Technologies) equipped with a Cooled Injection System, PTV-type inlet (GERSTEL CIS) and a Thermal Desorption System (GERSTEL TDS) fitted with a Pyrolysis Module (GERSTEL PM 1). This instrument combination is well suited for performing pyrolysis and serial pyrolysis manually. If a larger number of samples need to be analyzed, the process can readily be automated using the GERSTEL MultiPurpose Sampler (MPS) in combination with a GERSTEL Thermal Desorption Unit (TDU) and the GERSTEL PYRO module.

Technical and application details

Fries et al. performed the analysis as follows: A microplastic particle was placed in a pyrolysis sample tube and transferred to the PM 1. The PM 1 was then inserted into the TDS and sealed using the cone locking mechanism. The TDS temperature was set to 40 °C and heated at a rate of 10 °C/min to 350 °C (10 min); during this period, volatile compounds were thermally extracted from the matrix and cryofocused in the CIS at -50 °C; subsequently, the CIS was heated from -50 °C at a rate of 12 °C/min to 280 °C (3 min) and the analytes transferred to the GC column (30 m HP 5MS with 250 µm ID and 0.25 µm film thickness). The GC oven temperature program used was 40 °C initial temperature; 15 °C/min to 180 °C; 5 °C/min to 300 °C (12 min). Helium carrier gas was used. After the GC/MS run for the volatile compounds had been completed, the sample was pyrolyzed as follows: The TDS was set to a starting temperature of 60 °C (1 min) and heated at a rate of 180 °C/min to 350 °C. Pyrolysis was then performed in the PM 1 at 700 °C (1 min).

The pyrolysis fragments formed were transferred to the CIS and cryofocused using liquid nitrogen. Following the pyrolysis step, the fragments were then transferred from the CIS to the GC column using a temperature program as described above. Mass selective detection followed using a MS 5975C from Agilent Technologies. In addition to the chromatogram of the



Overlay pyrograms enable easy comparison of standard polymers (Purple: Standard low density Polyethylene [LDPE]) with those found in environmental samples (black: Polyethylene C.).

Image: istock / Anschiwil; Fries et al.

volatile compounds, the pyrogram containing the pyrolysis products was collected.

Obtaining valuable information efficiently

Based on mass spectral libraries as well as comparison of retention times and mass spectra of analyzed standards, Fries *et al*. were able to identify a variety of plastic additives in the microplastic particles found on the North Sea island of Norderney. Among these were plasticizers, including the phthalates DEHP, DBP, DEP, DIBP and DMP, the antioxidant 2,4-di-tert-butylphenol, and aromatic compounds such as benzaldehyde, which is added as fragrance to cosmetics and to polymers directly.

The polymer type was determined by the researchers by comparing pyrograms from the collected microplastic particles with those from standard polymers. They identified polyethylene (PE), polypropylene (PP), polystyrol (PS), polyamide (PA) as well as chlorinated and chlorosulfonated PE.

Concerning their method for the determination of organic polymer additives, the researchers came to the following verdict: Compared with traditional solvent extraction based methods, the serial pyrolysis method offers the advantage of determining both the volatile compounds in the material, including additives, and the type of polymer in a single process step – without having to use solvents and without background contamination from the material. The pyrogram is free of interfering compounds that offer no structural information since these have been removed in the thermal desorption step.

Final words from the researchers

Serial pyrolysis GC/MS has sufficient sensitivity to determine plasticizers, antioxidants, and fragrances in microplastic particles with a mass of less than 350 µg. This makes it possible to determine the chemical, toxicological or endocrine disrupting risk potential of such particles. According to Fries et al., Serial pyrolysis could be used for the implementation of the Marine Strategy Framework Directive (MSFD) by enabling the determination of the chemical composition of microplastic particles. Just for good measure, in addition to the organic compounds, the researchers also determined the following inorganic polymer additives: Titanium oxide, as well as barium-, sulfur- and tin compounds [10].

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Automated Sample Preparation Using the GERSTEL MPS WorkStation

Automated Lipid Fractionation Using Solid Phase Extraction

Metabolomics studies focus on the analysis of small molecules (MW<2000) in biological matrices, processing relatively large sets of samples to allow differentiation between sample types. In order to obtain statistically meaningful results, analytical variability should be much lower than biological variability and automation of sample preparation can significantly contribute to improved repeatability of the total analytical procedure.

By Koen Sandra, Ruben t'Kindt, Christophe Devos, Bart Tienpont, Pat Sandra, Frank David
Research Institute for Chromatography, President Kennedypark 26, 8500 Kortrijk, Belgium

In a typical metabolomics workflow, extraction of the sample is followed by fractionation or clean-up, and if needed, derivatization, concentration, and finally GC or LC separation and MS detection. In a series of articles, we describe a number of automated methods that are currently applied in our laboratories. In the first article, automated ultrasonic assisted liquid extraction and filtration using the GERSTEL MPS Workstation were discussed [1]. In this second article, an automatic fractionation procedure based on solid phase extraction (SPE) is described. This method was used in a lipidomics study, focusing on the characterization of plant material based on the relative composition of different classes of lipids, including neutral lipids (triglycerides, sterols), free fatty acids and polar lipids. Due to the fact that these classes are present in the plant material at substantially different concentration levels, it was observed that fractionation and selective enrichment of lipid classes prior to LC-MS analysis resulted in a much better coverage of lipids [2]. After liquid-liquid extraction, based on the Folch method [3], a concentrated lipid fraction was obtained. Next, fractionation was performed in a "normal phase LC" mode on an aminopropyl SPE cartridge. Three fractions of increasing polarity were obtained and the extracts were concentrated using a Multi-Position Evaporation Station (*m*VAP) installed on the MPS Workstation. Finally, the concentrated extracts were analyzed by LC-QTOF.

Lipidomics

Lipidomics is the large-scale study of pathways and networks of cellular lipids in biological systems. The word „lipidome" is used to describe the complete lipid profile within a cell, tissue or organism and is a subset of the „metabolome" which also includes the three other major classes of biological molecules: proteins/amino-acids, sugars and nucleic acids. Lipidomics is a relatively recent research field that has been driven by rapid advances in mass spectrometry (MS) and other analytical technologies, as well as computational methods, coupled with the recognition of the role of lipids in many metabolic diseases such as obesity, atherosclerosis, stroke, hypertension and diabetes. This rapidly expanding field complements the huge progress made in genomics and proteomics, all of which constitute the family of systems biology.
Source: Wikipedia

Experimental

Automated Extraction.
A one gram sample of plant material was extracted with 6 mL chloroform:methanol (2:1). Next, 4 mL water was added and 1.5 mL from the bottom chloroform layer was filtered into a high recovery vial. The solvent was evaporated in an *m*VAP station. Extraction, filtration and concentration was performed on a separate MPS WorkStation unit.

Fractionation by Solid Phase extraction.
Automated SPE and concentration were performed using a MPS Dual Head WorkStation configured as illustrated in Figure 1 and listed in Table 1.

The extracts obtained from the extraction and filtration steps were reconstituted in 300 µL chloroform. These extracts were fractionated using the SPE protocol shown in Figure 2. Basically three fractions of increasing polarity were obtained, containing neutral lipids (NLs), free fatty acids (FAs) and polar lipids (PLs),

Table 1: MPS Dual Head WorkStation configured for automated SPE and analyte concentration

MPS Module	Description
Left Arm	500 µL syringe
Right Arm	2.5 mL syringe
Tray and Holder	10 mL headspace vials for SPE fractions
Tray and Holder	SPE cartridges
Wash Station	Needle wash
Stacked Tray	1.5 mL high recovery vials for filtered extracts (samples)
SPE Module	Performs SPE using replaceable, standard dimension packed bed cartridges
*m*VAP	Vacuum assisted evaporation of extracts and SPE fractions
Solvent Filling Station	SPE Solvents – Hexane, 2:1 Chloroform/IPA, Diethyl ether (2% acetic acid), and MeOH

Image: RIC

Figure 1. MPS Dual Head WorkStation configured for automated SPE and analyte concentration.

respectively. These three fractions (collected in 10 mL vials) were concentrated to dryness in the ^{m}VAP station and reconstituted in chloroform:isopropanol for LC-MS analysis. Solvent amounts were optimized according to the concentration of the lipids in the extracts [2].

LC-MS

An Agilent Technologies 1290 Series UHPLC System coupled to a 6540 Q-TOF LC/MS was used for the analysis of the extracts (Agilent Technologies, Waldbronn, Germany). A reversed-phase separation was performed on a C18 column using 20 mM ammonium formate in water and methanol as the mobile phase constituents [4]. In total, 4 LC-MS methods were used, applying slightly different gradients and different MS conditions. Fraction 1 was analyzed using positive electrospray ionization (ESI POS), fraction 2 was analyzed in negative ESI mode (ESI NEG), and fraction 3 was analyzed both in ESI POS and ESI NEG modes.

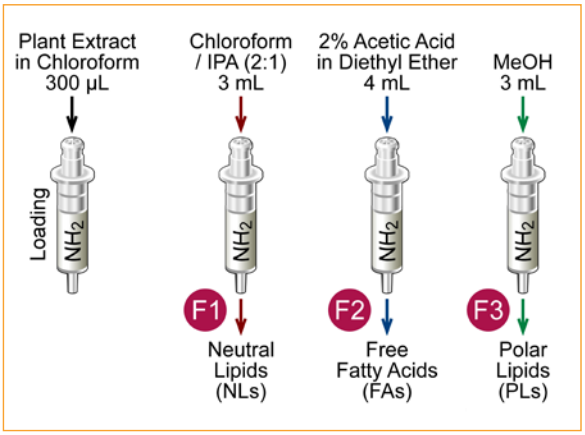

Figure 2. Automated SPE procedure

Results and Discussion

For a plant lipid study, 84 samples were prepared using the automated SPE method described above. Samples from 22 individual plants, belonging to 3 main types, were each prepared in triplicate. In addition, 18 quality control (QC) samples were analyzed to assess the reproducibility of the sample preparation and LC-MS protocol. Photos of reconstituted SPE fractions of three plant samples (each belonging to a different main class) are shown in Figure 3.

Typical LC-MS chromatograms are shown in Figure 4. The upper trace shows the analysis of fraction 1 (neutral lipids) in ESI POS mode. Monoglycerides (MGs), diglycerides (DGs), triglycerides (TGs) and plant sterols are detected. Trace B shows the analysis

Suggested reading

Development and validation of a robust automated analysis of plasma phospholipid fatty acids for metabolic phenotyping of large epidemiological studies.
Laura Yun Wang, Keith Summerhill, Carmen Rodriguez-Canas, Ian Mather, Pinal Patel, Michael Eiden, Stephen Young, Nita G Forouhi and Albert Koulman, Genome Medicine (2013) 5:39, DOI: 10.1186/gm443, http://genomemedicine.com/content/5/4/39

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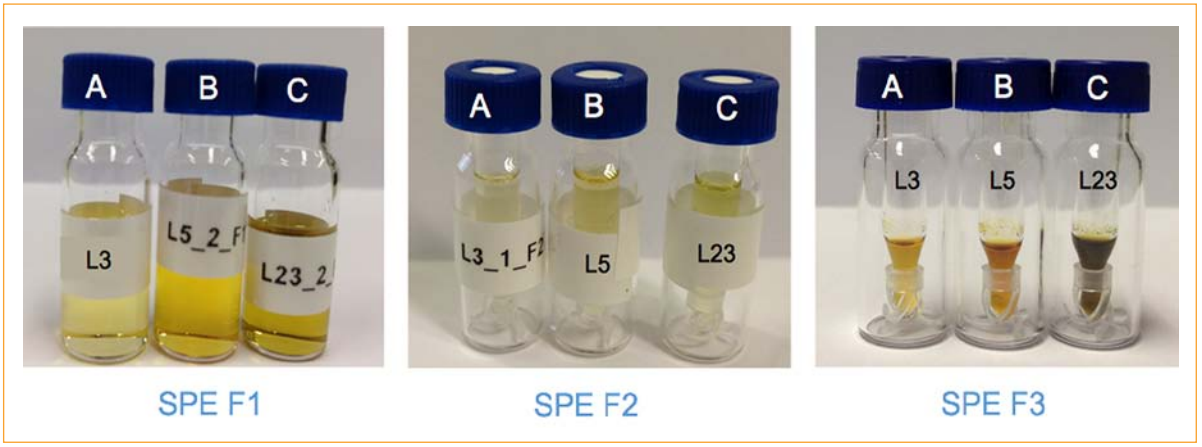

Image: RIC

Figure 3. Reconstituted plant extracts after SPE fractionation and ^{m}VAP concentration. A, B and C correspond to three different types of plants. F1: Neutral Lipids (NL); F2: Free Fatty Acids (FF); F3: Polar Lipids (PLs).

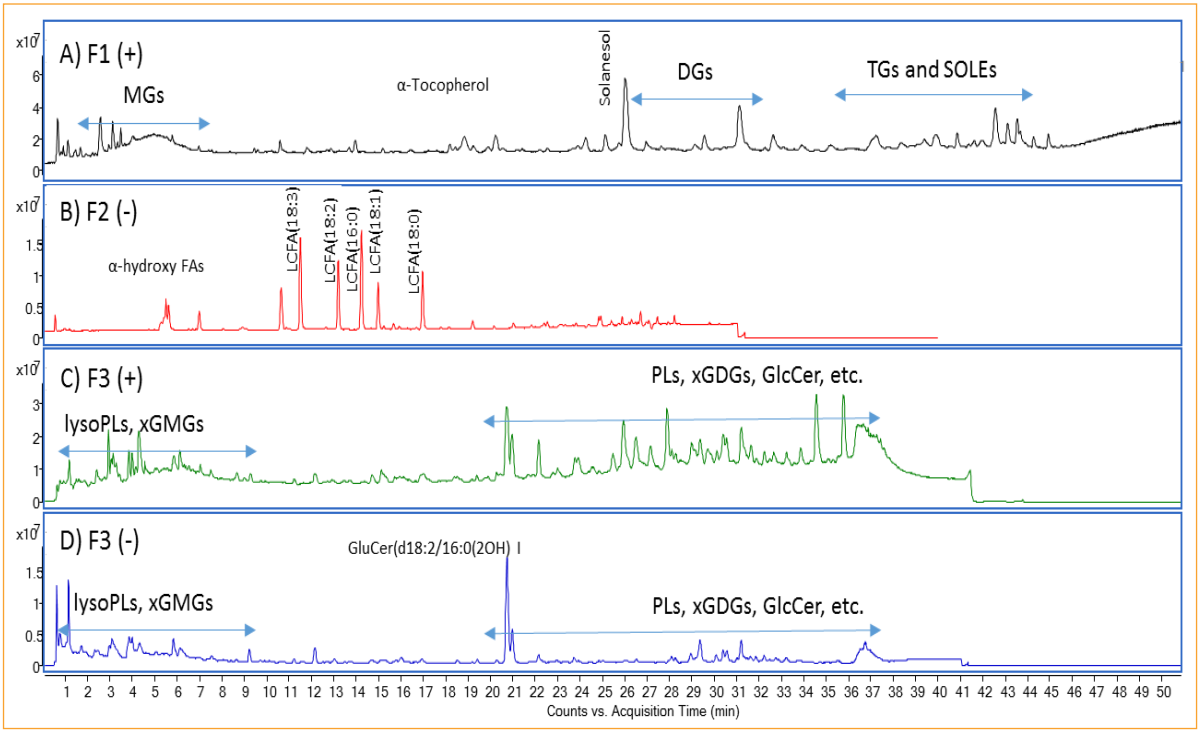

Image: RIC

Figure 4. Total ion chromatograms of LC-QTOF analyses of the SPE fractions. A: fraction 1 in ESI POS mode, B: fraction 2 in ESI NEG mode, C: fraction 3 in ESI POS mode, D: fraction 3 in ESI NEG mode

of the long chain free fatty acids (LCFAs) present in fraction 2 using ESI NEG mode. Traces C and D show the detection of phospholipids (PLs), sphingolipids and other polar lipids in ESI POS and ESI NEG modes respectively.

To evaluate the precision, a number of identified compounds were selected, and the area RSD% of each calculated. The results are presented in Table 3. It should be noted that for large-scale lipidomics studies, the cutoff for area RSD values is typically 30 % [5]. As can be seen from Table 3, the results obtained for the 18 QC samples gave an area RSD of less than 20 % in most cases.

Conclusions

The GERSTEL MPS dual head WorkStation is particularly useful for the automation of sample preparation in metabolomics studies. A lipid class fractionation method based on solid phase extraction was fully automated on a dedicated platform, including concentration of the SPE fractions by solvent evaporation. The LC-QTOF analysis results for the fractions showed excellent repeatability. In an upcoming article, the automation of a derivatization protocol combined with GC/MS analysis applied in metabolomics will be described.

Table 3. Precision of lipidomics methods including automated sample preparation.

Fraction	Lipid	Mass	t_R [min]	% RSD Area
F1 (+)	MG (18:3)	369.2879	6.389	9.6
	Solanesol	647.6005	29.097	8.6
	LANE (18:3)	703.6267	38.749	5.5
	SOLE (18:3)	907.8145	44.918	7.7
F2 (-)	LCFA-OH (18:3)	294.2210	7,540	11.7
	LCFA (18:3)	278.2259	14.497	5.4
	LCFA (16:0)	256.2414	17.245	6.2
F3 (+)	MGMG (18:3)	531.3407	8.137	22.1
	LysoPC (18:1)	521.3481	9.783	21.6
	GlcCer (d18:2/16:0)	697.5493	25.470	23.4
	PC (36:2)	785.5935	30.794	18.7
	MGDG (36:0)	803.6486	34.540	8.2
F3 (-)	MGMG(18:3)	560.3197	8.186	10.1
	LysoPC(18:1)	567.3550	9.868	19.4
	GlcCer(d18:2/16:0)	713.5471	24.494	15.2
	PC(36:2)	831.5980	30.745	10.3
	MGDG(36:0)	832.6212	32.410	5.8

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Automation technology

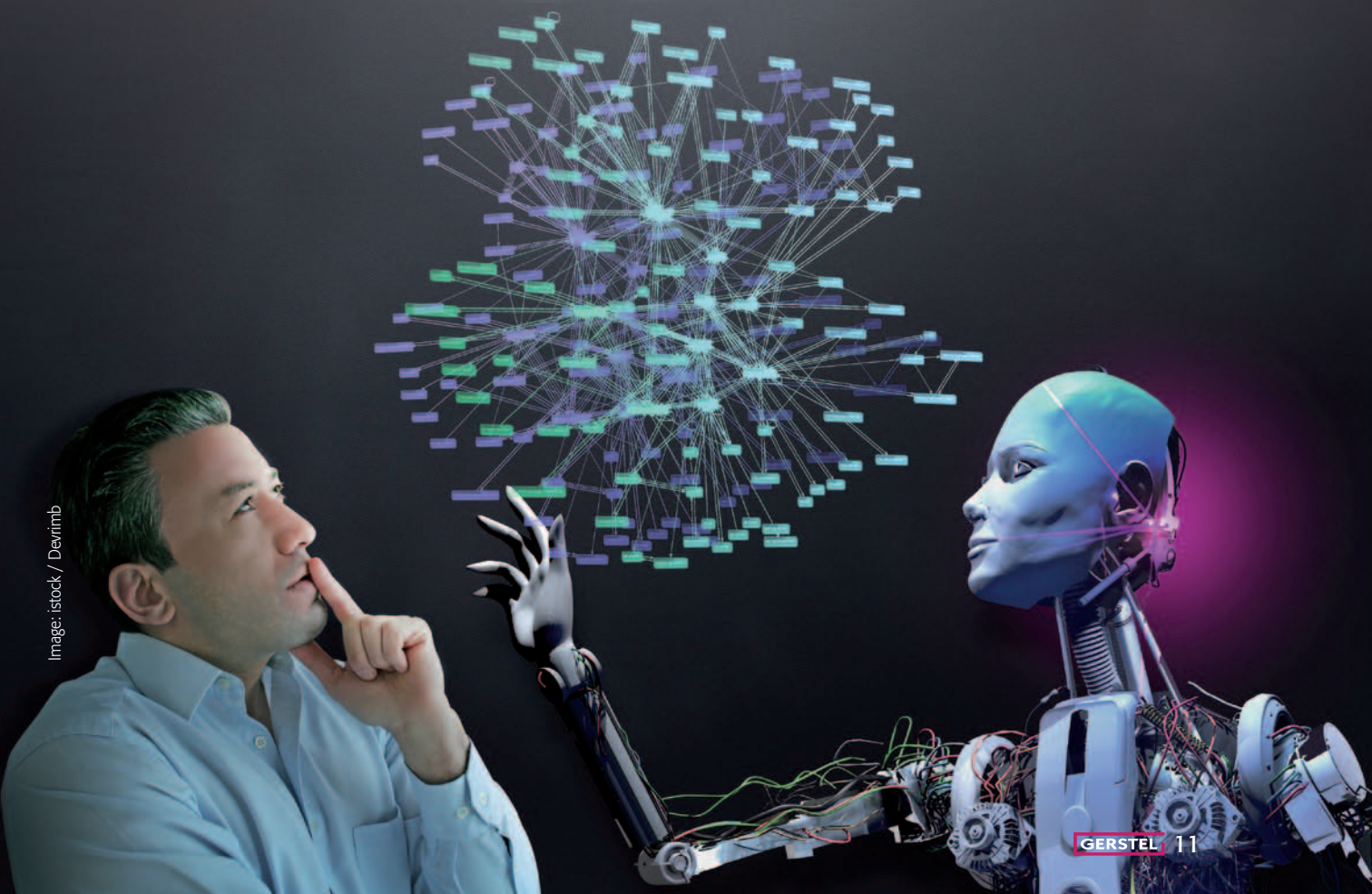
Looking for Automation?

What should you look for when buying a new autosampler for GC/MS and LC/MS

Laboratories carefully analyze their current requirements for productivity and efficiency before investing in autosamplers for GC/MS and LC/MS. It also makes sense to look at automation potential, specifically automated sample preparation.

By Ralf Bremer

Every GC/MS and LC/MS laboratory today faces ever-increasing productivity and efficiency demands. Typically the number of samples keeps increasing, but staff is not added or is even reduced. To make matters even more challenging, there is constant pressure to reduce cost, save energy and to eliminate or reduce the amount of potentially toxic solvents used. Also, it is necessary to have maximum flexibility as new sample types require new methods for sample preparation and analysis, but with the same turnaround as standard analyses. To meet all demands and keep the customer satisfied, a good start is to automate manually performed routine sample preparation tasks. Typical examples are generating standards or dilution series - or adding an internal standard or derivatization reagent. But the possibilities are, if not endless, more extensive than most people would think, provided you have the right automation platform. So when you are trying to decide which autosampler is best for your needs, it is a good idea to carefully determine which manual steps in your



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workflow can be automated and make sure you find an autosampler that fits these needs. There are significant differences between automation platforms – especially in the sophistication of the software used to automate more advanced sample preparation steps, so it is critical that these features are thoroughly evaluated.

Basic autosampler functionality is a good indicator of success

A modern autosampler based on an X-Y-Z robot, such as the GERSTEL MultiPurpose Sampler (MPS), can perform almost unlimited tasks since it moves unrestricted in three dimensions and can access a large number of sample positions. Standard tasks include sample introduction to GC/MS or LC/MS or simple sample preparation steps like adding an internal standard. This is a good starting point, but one needs to look deeper. Maybe you need a sampler to perform dilution series or generate a series of calibration standards. All this must be accompanied by enough syringe- or valve rinsing possibilities to eliminate sample-to-sample carryover and cross contamination.

GC/MS system from Agilent Technologies Inc. equipped with a GERSTEL MPS roboticpro for automated sample preparation. The MPS is configured with Dynamic Headspace (DHS) option. The standard DHS system uses 20 mL vials – a version that uses 1 Liter sample containers (DHS Large) is also available.

Image: GERSTEL / Wolfram Schroll

Advanced functionality on demand

If more advanced sample preparation tasks need to be performed at a later time, can these be added to the system in a modular fashion? Which options and accessories are typically added? Examples include filtration or solid phase extraction (SPE), liquid-liquid extraction (LLE), centrifugation, solvent evaporation, solid phase micro-extraction (SPME), stir bar sorptive extraction (SBSE) as well as static and dynamic headspace (HS/DHS). A fully loaded autosampler is rarely needed, but it is very useful to be able to add these capabilities on demand with little effort or additional expense.

On the sample storage side, a wide range of sample vessels should be accommodated. These should include deep well plates, and standard vials and even customized trays for any container you might need in your method. The possibility of storing samples cold in order to eliminate analyte degradation and preserve sample or reagent integrity - or of heating and agitating after a reagent has been added - should be available as options.

The Dual Head version of the MPS can perform different tasks simultaneously for improved productivity and flexibility; this includes handling vastly different liquid volumes using two different syringe sizes. Having both of these mounted in the Dual Head MPS offers a higher degree of flexibility and productivity since you do not have to manually change the syringe or wait for the syringe to be changed (available with the MPS robotic pro) during the sample preparation method.

The little differences that mean a lot

Can the autosampler be fitted with a separation technique such as a centrifuge? Can it do vortexing and solvent evaporation? Can liquids be added under highly controlled conditions and the amount determined using a balance? The availability of any of these capabilities can be the deciding factor in finding the right solution for your needs when automating manual sample preparation processes and making them fully traceable on a 24/7 basis.

Application and integration requirements

Is the autosampler needed for sample introduction directly into the GC/MS or LC/MS system? If so, wouldn't it be helpful to perform sample preparation and analysis in parallel? This would ensure that samples are always prepared immediately before being analyzed and could maximize system utilization. Or will the system mainly be used as a separate workstation to generate standards and dilution series for the analysis instrument(s)? The clearer the definition, the easier it is to select the right autosampler, both for current and for future requirements. The main sources for information and comparisons are Laboratory Magazines and their websites. Laboratory Instrumentation Exhibitions such as PittCon, ASMS

Image: GERSTEL / Wolfram Schroll

A modern GC/MS Laboratory. The analysis systems are equipped with high performance autosamplers that can automate all steps in sample preparation and introduction. The high degree of automation helps ensure productivity and throughput while offering enough flexibility to easily adapt to new sample types, analysis methods and performance requirements.

and Analytica in Europe can also serve to enable comparisons in a short time span. Obviously vendor websites offer detailed information, typically with a lot of application examples. GERSTEL is among the leading providers of intelligently automated solutions for GC/MS and LC/MS. If you are considering automating your sample preparation and -introduction, please contact your local GERSTEL representative. We have many years of experience applying our automation solutions to real world samples and applications. If you let us know what you want to achieve, we will listen carefully before proposing a solution that truly fits your needs.

What you should consider when you choose your next autosampler

1. Keep it simple. It may sound nice to have an autosampler that can do everything, but it can add unnecessary cost and operational complexity.
2. The software package is your everyday interaction with the instrument. Make sure it is as easy as possible to operate and that it adapts directly to the instrument configuration to eliminate unnecessary complexity, it should be "plug and play". Also, you should be able to change and adapt methods and sample preparation processes without the need for programming steps and external support.
3. Plan your purchase and your laboratory automation with your mid-to-long term strategies and goals in mind. Limit your investment to fit your current needs, but make sure that technologies can be added at a later time as your needs change without having to purchase completely new systems and rendering the initial investment obsolete. Even if the plan is to automate liquid-liquid extractions, it can be beneficial to be able to update the system to perform evaporation, filtration, SPE or even headspace.
4. Make sure you get good support. Laboratory autosamplers are high-tech products and they often need individual implementation depending on the sample types and performance requirements. Make sure the supplier has the experience and resources to provide efficient technical and application support without delay.
5. Consider existing systems in the laboratory. New autosamplers can upgrade existing GC/MS or LC/MS systems to a higher performance level by removing interfering matrix compounds or concentrating analytes. Relying on existing systems in the laboratory could provide significant savings.

Images: GERSTEL / Wolfram Schroll

Automated Liquid-Liquid Extraction (LLE)

Speeding up the workflow …

In addition to SPE workflows, the GERSTEL MultiPurpose Sampler (MPS) can perform fully automated liquid-liquid extraction. Several functionalities can be added as modules.

By Oliver Lerch

An important stepping stone on the path to improved productivity is the automation of manually performed sample preparation steps. Whether these can be transferred to an automated sampler system of course depends on whether the sampler has the individual capabilities needed to perform each step in the process. Multiple application examples have been reported over the years using the GERSTEL SPE system based on the MultiPurpose Sampler (MPS) [1, 2].

OPEN ACCESS ARTICLE!

Suggested reading

Determination of THC and its metabolites 11-hydroxy-THC (THCOH) and 11-nor-9-carboxy-THC (THC-COOH) in blood serum
K. Purschke, S. Heinl, O.Lerch, F. Erdmann, F. Veit, Anal Bioanal Chem, DOI 10.1007/s00216-016-9537-5
Link: http://link.springer.com/article/10.1007/s00216-016-9537-5

Recently, a similar effort has been under way to automate several aspects of and complete workflows for liquid-liquid extractions (LLE).

Application examples include determination of Tetrahydrocannabinol (THC), the active compound in cannabis, and the cannabinoids cannabinol (CBN) and Cannabidiol (CBD) all in human hair (A scientific publication has been accepted by the Journal of analytical Toxicology). In addition, an article on the determination of THC and its metabolites 11-hydroxy-THC (THCOH) and 11-nor-9-carboxy-THC (THC-COOH) in blood serum has been published in the Journal of Analytical and Bioanalytical Chemistry [3]. The GERSTEL GC/MS-solutions configured for those applications are being used successfully by Forensic Toxicology Institutes. In addition to these more special analyses, a much wider array of applications require liquid-liquid extraction. All steps required in such a process can be automated using the GERSTEL MPS. Key elements in such systems are the CF 200 centrifuge, the MultiPosition Evaporation Station (*m*VAP) and *quick*Mix. The only step that must be performed manually is the addition of the liquid or solid sample into a vial and placing the vial in the MPS sample tray. All other steps are performed automatically. The analytical method and sample sequence is simply set up with a few mouse-clicks using GERSTEL MAESTRO software.

GERSTEL MultiPurpose Sampler (MPS) equipped with centrifuge, *m*VAP and *Quick*Mix.

The MPS adds internal standards to the sample followed by extraction buffer and extraction solvent. The extraction is performed in a few minutes while the MPS agitates the sample vigorously in the quickMix ensuring thorough mixing. The phases can then be separated efficiently using the CF 200 centrifuge, or alternatively, a more powerful Sigma® centrifuge. The MPS aspirates the extract and transfers it to a clean vial. As needed an additional extraction step is performed using a new volume of clean solvent. Depending on the analytical workflow requirements, the MPS can inject an aliquot of the resulting extract into the analysis instrument or evaporate it to dryness in the *m*VAP. If evaporated to dryness, the residue can then be taken up in an HPLC compatible solvent with the option of adding a derivatization reagent. The steps to include in the final workflow are totally up to the user. These examples show how the MPS can be used as a highly flexible and rugged tool for automated liquid-liquid extraction processes. An additional application example based on veterinary samples can be found online [4].

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Automated determination of THC, CBN and CBD in human hair

In a joint research project with the Institute of Forensic Medicine at the University of Giessen, Germany, GERSTEL has successfully automated a validated manual analysis method for determining Δ^9-tetrahydrocannabinol (THC) and the cannabinoids cannabinol (CBN) and cannabidiol (CBD) in human hair.

By Oliver Lerch

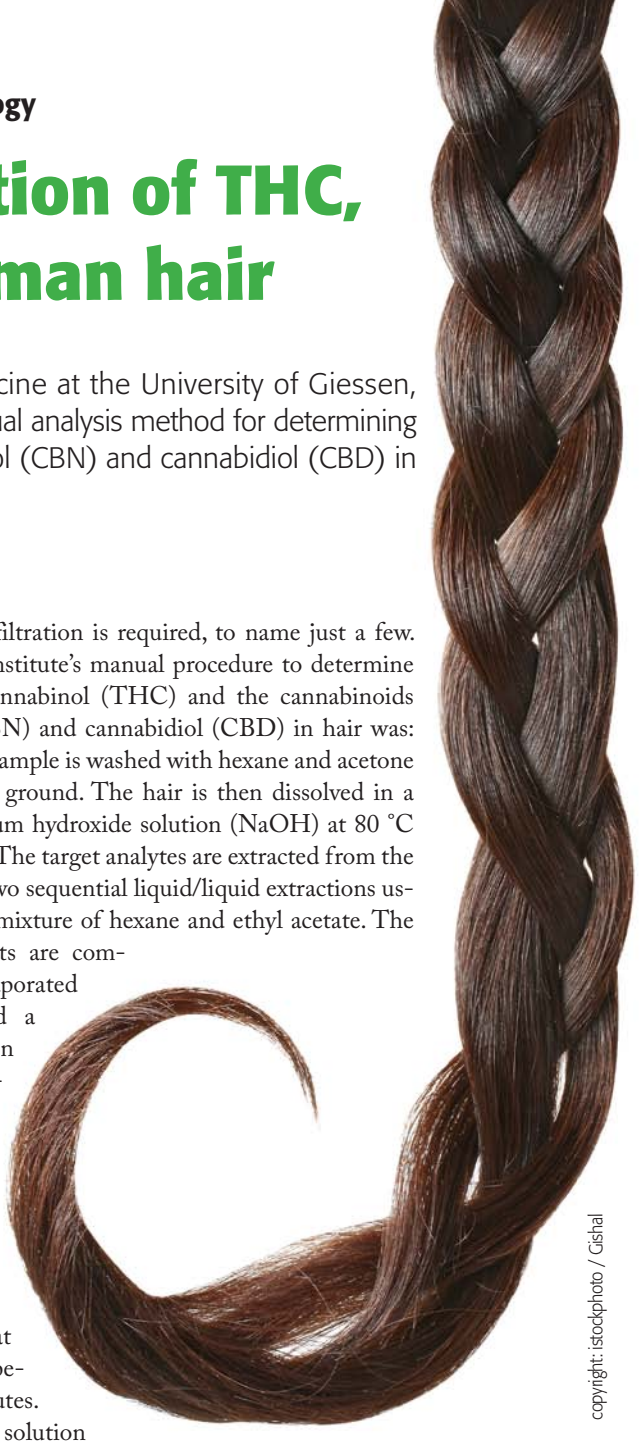
From a forensic toxicology point of view, human hair samples are extremely well suited for determining a person's consumption pattern of medication or drugs of abuse over a period of time. A human hair grows between 0.25 and 0.4 millimeters per day, in other words between 7.5 and 12 millimeters per month. Once consumed, drugs, active ingredients from medications as well as the metabolites formed, are distributed throughout the body and also incorporated into human hair as it grows. In cases of one-time or short-term drug use, the position of these substances within each hair stays fixed as the hair grows. In other words, prior drug use is no longer detectable after the hair has fallen out or you've gone for a haircut and the hairdresser has thrown away the evidence. Hair analysis can be used to detect drug abuse and to determine, which drugs have been consumed over which time period. By the same token, hair analysis can be used to confirm drug abstinence.

Hair today, gone tomorrow: How to determine cannabis consumption over time

Detecting prior cannabis consumption through hair analysis is a complex and labor-intensive process. The residual substances stored in hair must first be released from the matrix and converted into a form that is suitable for GC/MS analysis. At the Institute of Forensic Medicine at the University of Giessen, the steps required to prepare human hair samples are performed manually. Δ^9-tetrahydrocannabinol (THC), the active compound in cannabis, and the cannabinoids cannabinol (CBN) and cannabidiol (CBD), are then determined by GC/MS. GERSTEL's goal in working with the Institute of Forensic Medicine was to assess the potential for automation of the analysis processes used and to develop a solution based on a GC/MS system with integrated automated sample preparation and sample introduction.

The manual procedure is the starting point

Determining whether an existing manual method of analysis is suitable for automation requires thorough evaluation of the entire process. All aspects of the method need to be examined such as sample throughput required, reagent type, volumes of liquids used, and whether heating, mixing or filtration is required, to name just a few. The Forensic Institute's manual procedure to determine Δ^9-tetrahydrocannabinol (THC) and the cannabinoids cannabinol (CBN) and cannabidiol (CBD) in hair was: A 100 mg hair sample is washed with hexane and acetone and then finely ground. The hair is then dissolved in a one molar sodium hydroxide solution (NaOH) at 80 °C for 25 minutes. The target analytes are extracted from the hair matrix in two sequential liquid/liquid extractions using a 9/1 (v/v) mixture of hexane and ethyl acetate. The resulting extracts are combined and evaporated to dryness and a derivatization reagent consisting of BSTFA and TMCS is added to the residue at a ratio of 99/1 (v/v); derivatization is performed at 110 °C over a period of 20 minutes. The resulting solution is again evaporated, the residue taken up in ethyl acetate and an aliquot injected into a GC/MS system for analysis in single ion monitoring (SIM) mode.



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Automated hair analysis

The efforts to fully automate the manual method used for determining THC, CBN and CBD in hair were successful. The primary focus of the project was automating the sample preparation. For this task, the dual-head version of the GERSTEL MultiPurpose Sampler (MPS) was selected. The Dual Head version allows two different syringes to be used simultaneously, allowing the MPS to handle different

Suggested reading OPEN ACCESS ARTICLE!

Determination of THC and its metabolites 11-hydroxy-THC (THCOH) and 11-nor-9-carboxy-THC (THC-COOH) in blood serum
K. Purschke, S. Heinl, O.Lerch, F. Erdmann, F. Veit, Anal Bioanal Chem, DOI 10.1007/s00216-016-9537-5, http://link.springer.com/article/10.1007/s00216-016-9537-5

GC/MS system configured for liquid-liquid extraction. Shaker (left), evaporation station (center) and centrifuge (right).

Using the GERSTEL method, the extraction of THC, CBN and CBD from hair is fully automated and is accelerated using the GERSTEL *quick*MIX module mounted on the MPS.

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Sample vial after hair digestion, extraction and centrifugation. A clear phase separation between the upper organic extract phase and lower aqueous phase (dissolved hair) is achieved.

solvents, different volumes and make injections into an analytical system quickly and efficiently by eliminating the need to stop the sample preparation process in order to change syringes. The MPS can be configured with a wide range of optional modules to meet almost any need, including a solvent evaporation module (*m*VAP) for sample concentration, a centrifuge, and a vortex shaker to name a few. The automated procedure involves placing a vial containing a ground hair sample and internal standard in the MPS sample tray, after which all subsequent steps in the method are performed by the MultiPurpose Sampler under MAESTRO software control.

The following steps were performed:

- A 100 mg sample of ground hair (plus ISTD) was placed in 1 molar NaOH and incubated at 85 °C for 13 minutes until dissolved
- Two extractions with hexane/ethyl acetate (9/1, v/v) were performed for 4 minutes each at 200 rpm in the GERSTEL *quick*MIX
- Phase separation by centrifugation at 4500 rpm for 3 minutes
- Extracts evaporated to dryness at 65 °C (*m*VAP)
- Introduction of MSTFA / ethyl acetate (GERSTEL *quick*MIX)
- Injection of 2 µL (inlet derivatization)
- GC separation was performed using a DB-5MS 30 m x 0.25 mm x 0.25 µm capillary column (Agilent Technologies) with mass spectrometric detection (MSD) in single ion mode (SIM).

Results

Any method must prove its worth in practical use. The automated method for determination of THC, CBN and CBD in hair using the MPS to prepare and inject the samples was validated in accordance with GTFCh guidelines. The limit of determination for THC was 0.01 ng/mg; the extraction efficiency (at 0.02 ng/mg) was 102 % and the precision (at 0.02 ng/mg) was 4.2 %.

The author wishes to thank Sonja Heinl, Freidoon Erdmann and Florian Veit from the University Medical Center, Giessen, Germany for the excellent cooperation.

Conclusion and outlook

In close cooperation with the Institute of Forensic Medicine at the University Medical Center, Giessen, GERSTEL successfully automated the manual method previously used at the institute for determination of THC, CBN and CBD in human hair. The required 0.02 ng/mg limit of detection for THC was achieved. As planned, the manual sample preparation steps were automated using a GERSTEL MultiPurpose Sampler (MPS) under MAESTRO software control. The PrepAhead feature optimizes throughput by preparing the next sample in parallel during the ongoing GC/MS analysis. This ensures that each sample is treated in the same manner and injected immediately after it has been prepared. GERSTEL's *quick*MIX, centrifuge and *m*VAP modules were able to reproduce the manual steps previously used in the method, from mixing and extraction to evaporative concentration of the extracts. It was found that derivatization of the analytes was best performed in the hot GC inlet. In addition to the determination of THC, CBN and CBD in human hair, the steps automated in this method can be applied to a variety of other manual liquid/liquid extractions and sample preparation methods.

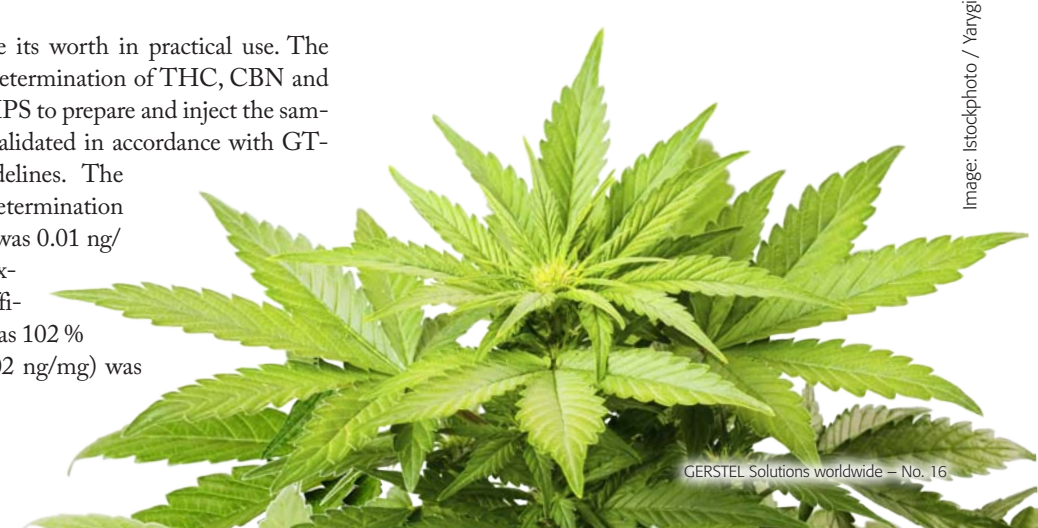


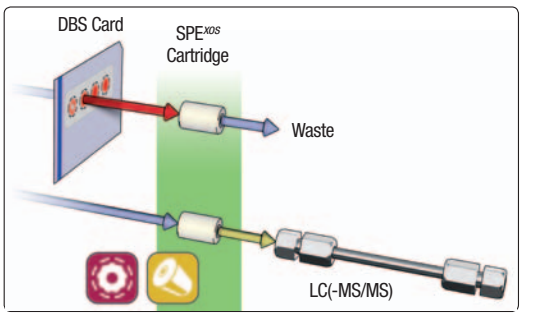
Image: istockphoto / Yarygin

What a drop of blood reveals

In blood analysis, precision, sensitivity and the required sample amount are important factors – along with the analysis time. In all these areas, Dried Blood Spot (DBS) sampling and analysis is a convincing alternative – especially when automated with the GERSTEL MultiPurpose Sampler (MPS).

By Oliver Lerch

Since the 1960s, Dried Blood Spot (DBS) sampling has been used. Initially, especially neonatal screening for metabolic disorders was performed based on the DBS technique. In recent years, especially since more powerful GC/MS and LC/MS system became generally available, DBS has become useful in pharmaceutical research [1], forensic toxicology and doping analysis [2-4] as well as veterinary analysis [5].



Schematic diagram of DBS flow-through desorption (FTD™) and SPE clean-up. FTD is patented by, and is a registered Trade Mark of, Spark Holland B.V.

Just a few drops of blood taken from a fingertip are sufficient to perform DBS analysis. The drops are placed onto small circular fields on special cards used for DBS work. As soon as the drops have dried, the cards are used to store and transport the samples, which are extracted directly from the card using dedicated sampling equipment.

A DBS sample typically contains between 15 and 30 µLs of blood evenly distributed across the spot. This means that a representative sample – and a defined amount of blood – can be taken by simply punching out a small area of the blood spot. Traditionally, a small, well defined disc of a few millimeters across has been punched out from the blood spot and transferred to a vial or micro-titer plate in which it was extracted using a suitable solvent. The resulting extract was centrifuged and the supernatant cleaned or analyzed directly following solvent exchange. Most often, LC-MS/MS or GC-MS/MS are the analysis techniques used. The described procedure is used for manual sample preparation based on DBS cards.

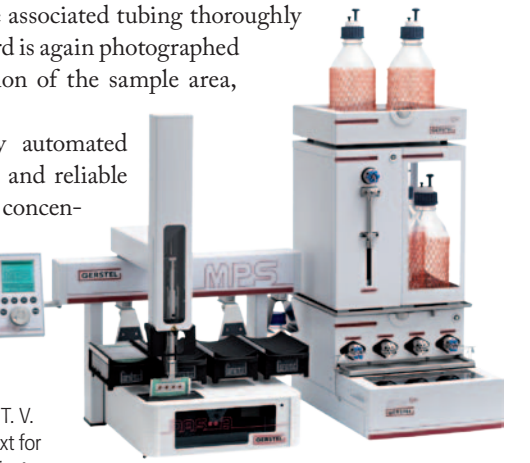
Automation improves productivity

In order to improve throughput and simplify the routine analysis workflow, the DBS technique must be properly automated. In cooperation with Spark Holland B.V., GERSTEL has developed an integrated system based on the Spark DBS system, with fully automated sampling from up to 240 DBS cards. Automation is performed using the GERSTEL MultiPurpose Sampler (MPS) under MAESTRO software control. The system can operate as stand-alone workstation preparing samples for LC/MS analysis - or integrated with the LC-MS/MS system. Slightly simplified, the DBS-MPS-LC-MS/MS System* works as follows: The MPS transports a DBS card to a camera. An image recognition software evaluates the dried blood spot. The card is loaded into the desorption interface and clamped into position. A desorption eluent then flows through a defined area of the blood spot desorbing the analytes based on the patented Flow Through Desorption technique (FTD™); an internal standard can be added to the desorption eluent for quality purposes if required.

An eluate clean-up step can further be performed based on an on-line SPE module which can be integrated into the overall analysis system. The online SPE system (GERSTEL-SPE^{xos}) is based on replaceable SPE cartridges, ensuring minimal sample to sample carry over while efficiently transferring the analytes to the LC-MS/MS system with best possible recovery for best possible LODs. Finally, the card is released and the associated tubing thoroughly rinsed with solvent. The card is again photographed to enable full documentation of the sample area, which has been desorbed.

Conclusion: The fully automated DBSA system enables fast and reliable determination of analyte concentrations with excellent LODs.

Images: GERSTEL



MPS-based GERSTEL DBSA Autosampler (to the left) with SPE^{xos}-Module (right hand side) for direct connection to the LC-MS/MS system (not shown).

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Image: istock / Tuned_In

Flavor, Fragrance, and Odor Analysis

A new Twist: Free acids and phenols

Sensitive SBSE determination of free acids and phenols - along with the usual suspects

By Ray Marsili and Charles Laskonis, Rockford University, Rockford, Illinois, USA.

Stir Bar Sorptive Extraction (SBSE) and Headspace Sorptive Extraction (HSSE) have regularly been applied successfully to the analysis of odor-active chemicals in a wide variety of sample matrices at concentrations as low as ultra-trace levels. The addition of the Ethylene Glycol-Silicone (EG-Silicone) Twister® has significantly enhanced the usefulness of these techniques by enabling the efficient extraction of polar hydrogen bond donors like phenols and carboxylic acids – important odor and flavor compounds. Various SBSE and HSSE extraction techniques using different combinations of PDMS and EG-Silicone Twisters were used to analyze samples discussed in this article. Versatile application of Twister technology combined with Thermal desorption – GC/MS and peak deconvolution are adding up to a significant analytical tool-kit to assist the flavor chemist in off-flavor and malodor elucidation, detecting both non-polar and polar analytes spanning a wide range of volatilities and concentrations.

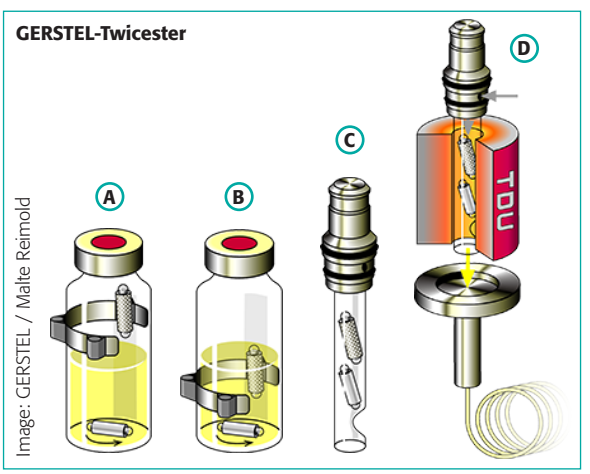

Figure 1: Schematic diagram of the multi stir bar extraction process using Twicester® (A) Magnetic positioning of up to three Twisters using Twicester Showing 1 Twister in HS & 1 stirring (B)^{m}SBSE using two Twisters submerged in liquid (C)Transfer of Twisters to TDU Tube (D) Simultaneous thermal desorption of the Twisters, cryofocusing in the CIS and GC/MS analysis.

Examples presented in this article include the determination of off odors in Beer, Pretzels, and Casein, a widely used food ingredient. Last but not least, axillary off odors were determined in well-worn T-shirts to determine the odor-reducing impact of antibacterial fabrics.

A simple, solvent free, quantitative, and sensitive analytical method for studying off-flavor development in beer based on SBSE using the GERSTEL PDMS Twister has previously been published [1]. Beer contains dozens of odor active chemicals in concentrations in the parts per billion range. Developing an analytical technique to cover such a wide range of volatiles and concentration levels is challenging. In the work presented here, recently developed SBSE techniques were combined with gas chromatography-time of flight mass spectrometry (GC-TOFMS) incorporating peak deconvolution software.

Beer off-flavor chemicals

The aim was to determine which approach would be most appropriate for detecting low levels of potential off-flavor chemicals in aged beer. Four Twister methods were used to analyze Blue Moon beer, a Belgian-style wheat ale. Ten milliliters of beer were extracted in all cases:

(a) One PDMS Twister (1 cm x 0.5 mm) submerged in the beer inside a sealed 20 mL vial, stirring at 900 rpm for 2 hours;
(b) Same as (a) with a second PDMS Twister submerged in the beer, attached to the inside of the vial using a GERSTEL Twicester® magnet clip as seen in Figure 1;
(c) Sequential SBSE with two PDMS Twisters (1 cm x 0.5 mm) stirred in sample for 1hr [2], followed by salt addition (20%) and stirring for an additional hour; and finally
(d) Extracting the beer sample with one immersed PDMS Twister used for stirring and one EG-Silicone Twister attached to the inside of the vial submerged in the beer using Twicester®.

Our results show that using the combination of the PDMS and EG-Silicone Twisters enabled significantly more sensitive determination of polar compounds including carboxylic acids compared with the other methods. In figure 2, peak areas for fourteen odor-active compounds are shown (normalized to PDMS + EG-Silicone results) for the four SBSE methods used on the same Blue Moon beer. For all but one of the compounds, (2-methyl-2-pentenoic acid), the PDMS + EG-Silicone method provided the highest recovery. Sequential SBSE was the second most favorable. The higher recoveries for carboxylic acids with the PDMS + EG-Silicone method make the Twister combination method an excellent choice for the determination of this important difficult-to-detect class of compounds. The compounds in question are: 4-vinyl guaiacol (clove flavor); linalool (floral); phenylethyl alcohol (rose); *alpha*-terpineol (pine, woody); 2-phenylethyl acetate (floral, honey); *o*-thymol (woody, camphor); and 2-phenylethyl esters of hexanoic, octanoic, and decanoic acids.

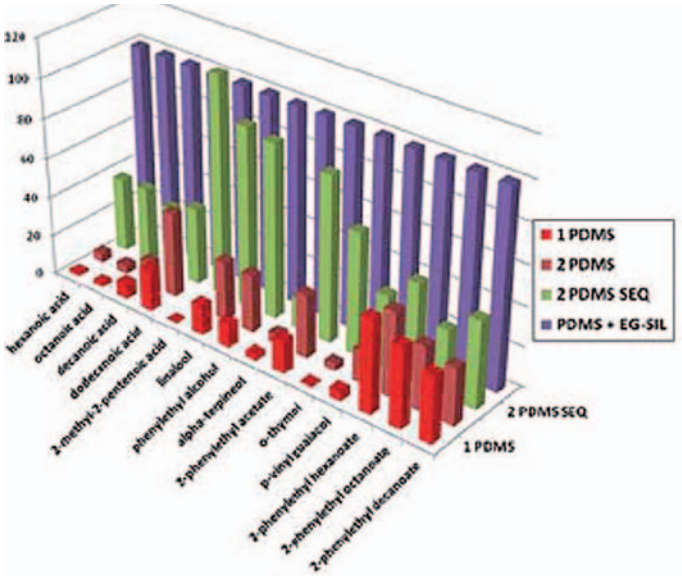

Figure 2: Comparison of four SBSE extraction techniques for odor-active chemicals in beer (normalized to PDMS + EG-Silicone peak areas).

Image: Dr. Ray Marsili

Axillary malodors in clothing fabrics

An understanding of human body odor chemicals may be desirable for many reasons. For example, knowledge of the composition of VOC metabolite excretions may be a useful health-related diagnostic tool. Our studies were undertaken to determine the ability of new fabrics to minimize axillary malodors.

Instrumentation

The thermal desorption-gas chromatography/mass spectrometry (TD-GC/MS) analysis was performed using a GERSTEL Thermal Desorption Unit (TDU) combined with a MultiPurpose Sampler (MPS) and a GERSTEL Cooled Injection System (CIS 4) programmed temperature vaporization (PTV) type inlet. Two GC-MS systems were used: an Agilent 7890A GC with an Agilent 5973B MSD and a GC-TOF/MS system.

GC parameters
Column: DB-5MS (30 m x 0.25 mm x 0.25 µm).
Temperature program: 40 °C (3 min), 10 °C/min to 270 °C (10 min).
Carrier gas flow: 1.0 mL/min, splitless.
The same GC conditions were used for DHS and SPME.
The same column type and GC parameters were used on both the Agilent and Leco systems.

Thermal desorption parameters
GERSTEL CIS 4: PTV Solvent Vent mode at a flow of 50 mL/min. Initial temperature -100 °C (0.50 min) ramped to 280 °C (3.0 min). GERSTEL TDU: Initial temperature 30 °C (0.40 min) 60 °C/min to 280 °C (4.00 min hold time) when only PDMS Twisters were used. A final temperature of 220 °C (4.0 min hold time) was set when EG-Silicone Twisters were used. TDU transfer line temperature: 300 °C.

Materials
GERSTEL Twisters used were PDMS 1cm x 0.5 mm; PDMS 2 cm x 0.5 mm; and EG-Silicone Twister. A GERSTEL Twicester® magnetic clip was used to hold multiple Twisters inside the sample vial.

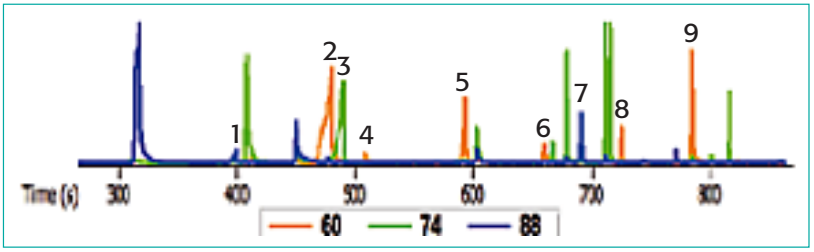

Image: Dr. Ray Marsili

Figure 3: Chromatogram of cotton shirt worn during exercise and analyzed by an HSSE dual Twister method
(1 PDMS + 1 EG-Silicone). Masses plotted for 60, 74 and 88 amu. Peak identities are: (1) isobutanoic acid; (2) 3-methyl butanoic acid; (3) 2-methyl butanoic acid; (4) pentanoic acid; (5) hexanoic acid; (6) heptanoic acid;
(7) 2-ethyl hexanoic acid; (8) octanoic acid; (9) nonanoic acid. None of these axillary malodor chemicals were present in the same shirt prior to exposure to an exercising person.

Malodors form various sites on the body when natural secretions are converted to volatile odorous chemicals through microbial activity. Short chain volatile fatty acids VFAs (C_2-C_5) are the primary axillary malodors formed; 16-androstene steroids and thioalcohols have also been identified as contributors along with 3-Methyl-2-hexenoic acid (3M2H) [3]. Following the successful combined PDMS and EG-Silicone Twister extraction of volatile organic acids from beer, the same Twister combination was used for analyzing functional T-shirts treated with antimicrobial chemicals and contaminated with perspiration to monitor production of various axillary malodor chemicals.

Experimental

The combination Twicester® approach was investigated using HSSE. One gram of soiled fabric was suspended from a paper clip protruding through the septum of a 40 mL glass vial. A Teflon coated micro-stir bar was placed at the bottom of the vial (to agitate the air in the vial) and one 1 cm x 0.5 mm PDMS Twister and one EG-Silicone Twister were attached to the sides of the vial with magnetic clips (GERSTEL Twicester®). The Teflon stir bar was stirred at 800 rpm, and the vial was thermostated at 50°C. Extraction was conducted for 2 h. The two Twisters were removed from the vial and desorbed in the GERSTEL TDU using the method parameters previously described for the beer analysis except with a maximum desorption temperature of 220 °C (instead of 280 °C) to protect the EG-Silicone Twister from thermal decomposition.

Results and discussion

Several volatile acids were detected at low ppb (µg/L) levels from the sweat residue on worn T-shirts. These included acetic acid, propanoic acid, isobutanoic acid, 3-methyl butanoic acid, 2-methyl butanoic acid, pentanoic acid, hexanoic acid, 3-methyl-2-hexenoic acid, octanoic acid, and nonanoic acid. In addition to these acids, several pyrazines were detected along with 2-nonenal, an unsaturated aldehyde with an unpleasant greasy and grassy odor, which was primarily found in subjects 40 years old or older. Results from one study indicate that 2-nonenal is generated by the oxidative degradation of omega-7 unsaturated fatty acids and suggest that 2-nonenal may be involved in the age-related change of body odor [4]. According to the study, the change of the monounsaturated fatty acid composition of skin surface lipids and the increase of lipid peroxides associated with aging may be involved in the formation of this characteristic odor component.

Table I shows results for unsoiled control cotton shirt samples spiked with five levels of various volatile acids and 2-nonenal ranging from 10 to 500 µg/L. Excellent least square correlation coefficient and detection limits are demonstrated for all acids and 2-nonenal. Figure 3 shows a chromatogram of axillary malodor volatile acids extracted from a soiled shirt by HSSE with PDMS and EG-Silicone Twisters.

A separate SBSE method was developed to determine androstenone, in which the PDMS Twister was desorbed at 270 °C for 5 min (instead of 220 °C for 5 min used for the EG-Silicone Twister-based work). It is worth remarking that Soini et al. [5] developed a novel tool for using a PDMS Twister to extract volatile organic malodorants directly from the skin. The stir bar, which is attached to a miniaturized holder that looks like a miniature paint roller, is rolled over a determined area of a human skin while collecting a representative sample of VOCs from the skin surface.

Table I: Calibration curve results for five concentrations of axillary malodorant standards in the 10 to 500 ppb (µg/L) range analyzed by HSSE method with 1 PDMS + 1 EG-Sil Twister. XIC = extracted ion mode; SIM = selected ion mode.

Standard	Mass Ion	Detection Limit (ppb)	Linear Least Sqauares Correlation Coefficient	Analytical Method
butyric acid	60 (XIC)	5	0.9921	HSSE
isovaleric acid	60 (XIC)	5	0.9731	HSSE
pentanoic acid	60 (XIC)	5	0.9737	HSSE
hexanoic acid	60 (XIC)	5	0.9972	HSSE
octanoic acid	60 (XIC)	10	0.9911	HSSE
2-methybutanoic acid	74 (XIC)	5	0.9655	HSSE
3-methyl-2-hexenoic acid	113 (XIC)	10	0.9901	HSSE
2-nonenal	70 (XIC)	5	0.9900	HSSE
Androstenone	272 (SIM)	0.4	0.9914	SBSE

Complaint Casein Powders with Musty Off-Flavor

Due to its flavor stability and functional properties, casein powder is commonly used in cheese analogues, bakery products, meat products, confectionery products, desserts, nondairy coffee creamers, and beverages. Several complaint casein powders from various international food and beverage companies were received in our laboratory. These had extreme musty taints and were implicated as causes of expensive product recalls involving cereal bars, nutritional beverages, a chocolate-flavored beverage, and nondairy coffee creamers in the U.S.

Musty off-flavors are commonly associated with microbial metabolites such as trihaloanisoles, geosmin (trans-1,10-dimethyl-trans-9-decalol), 2-methylisoborneol, and 2-isopropyl-3-methoxy pyrazine. These compounds have such low odor detection thresholds that they must be detected in the ng/L range making determination challenging (2,4,6-trichloroanisole odor threshold: 0.15-2.0 ng/L; geosmin: 1-10 ng/L). These compounds have relatively large log Ko/w values, however, allowing PDMS Twisters to extract them very efficiently from an aqueous sample or aqueous suspension of a solid sample. Indeed, SBSE GC-TOFMS was found to be a sensitive and simple technique for the determination of these compounds in casein powders.

Experimental

1 g of casein + 25 mL water was stirred 30 min at room temperature with a Teflon coated stir bar, which was then replaced with a 2 cm x 0.5 mm PDMS Twister and stirred for three hours. The Twisters were thermally desorbed in a GERSTEL TDU using the parameters previously indicated.

Results and discussion

The primary musty off-flavor chemicals found in the casein were trichloroanisole isomers formed by microbial methylation of 2,4,6-trichlorophenol, a common ingredient in fungicides, pesticides, and wood preservatives. In extracted ion chromatograms at 212, 195, 196 and 161 amu, haloanisole peaks were readily apparent. The major haloanisoles identified were 2,4-dichloroanisole, 2,4,6-trichloroanisole (at 7 µg/L), and 2,3,6-trichloroanisole contaminated with coeluters.

In resolving the product recall lawsuits, it was important to provide supporting evidence that the substrate 2,4,6-trichlorophenol was also present in the complaint caseins. The source of the trichlorophenol was found to be the wooden shipping pallets that the bags of casein were stacked on during overseas shipment.

Interestingly, testing of additional complaint casein samples revealed the presence of 2,4,6-tribromoanisole (2,4,6-TBA), the source of which was identified as the slip sheets used when stacking the bags of casein during transport. These sheets were manufactured from recycled plastic, mainly high-density polyethylene, contaminated with tribromophenol (TBP), likely stemming from the use of organobromine flame retardants, such as polybrominated diphenyl ethers (PBDEs). Standard calibration curves for 2,4,6-TCA demonstrated correlation coefficients (R2 values) greater than 0.99. For TBA testing, samples were spiked with deuterated TBA at 133 µg/L. Stable Isotope Dilution Analysis (SIDA) is arguably one of the most accurate quantitation techniques for the determination of organic compounds by GC-MS.

Conclusion

The application examples presented in this article demonstrate that SBSE and HSSE have been successfully extended to a wide variety of sample matrices. The new polar phase EG-Silicone Twister has significantly extended the application potential of SBSE and HSSE to include analytes that were difficult to extract at high recoveries with PDMS. Twister technology combined with thermal desorption-GCMS can be applied to a broad spectrum of sample types using SBSE and HSSE, making the Twister a significant analytical tool to assist the flavor chemist in off-flavor and malodor elucidation of a wide profile of non-polar and polar analytes covering a broad range of analyte volatilities and concentrations.

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Suggested reading

Written from a practical perspective, the second edition of Flavor, Fragrance, and Odor Analysis highlights the powerful SBSE technique and emphasizes the range of applications available.

Ray Marsili (Edi.),
Flavor, Fragrance, and Odor Analysis,
Second Edition, 280 Pages,
ISBN-10: 1439846731 and
ISBN-13: 978-1439846735.

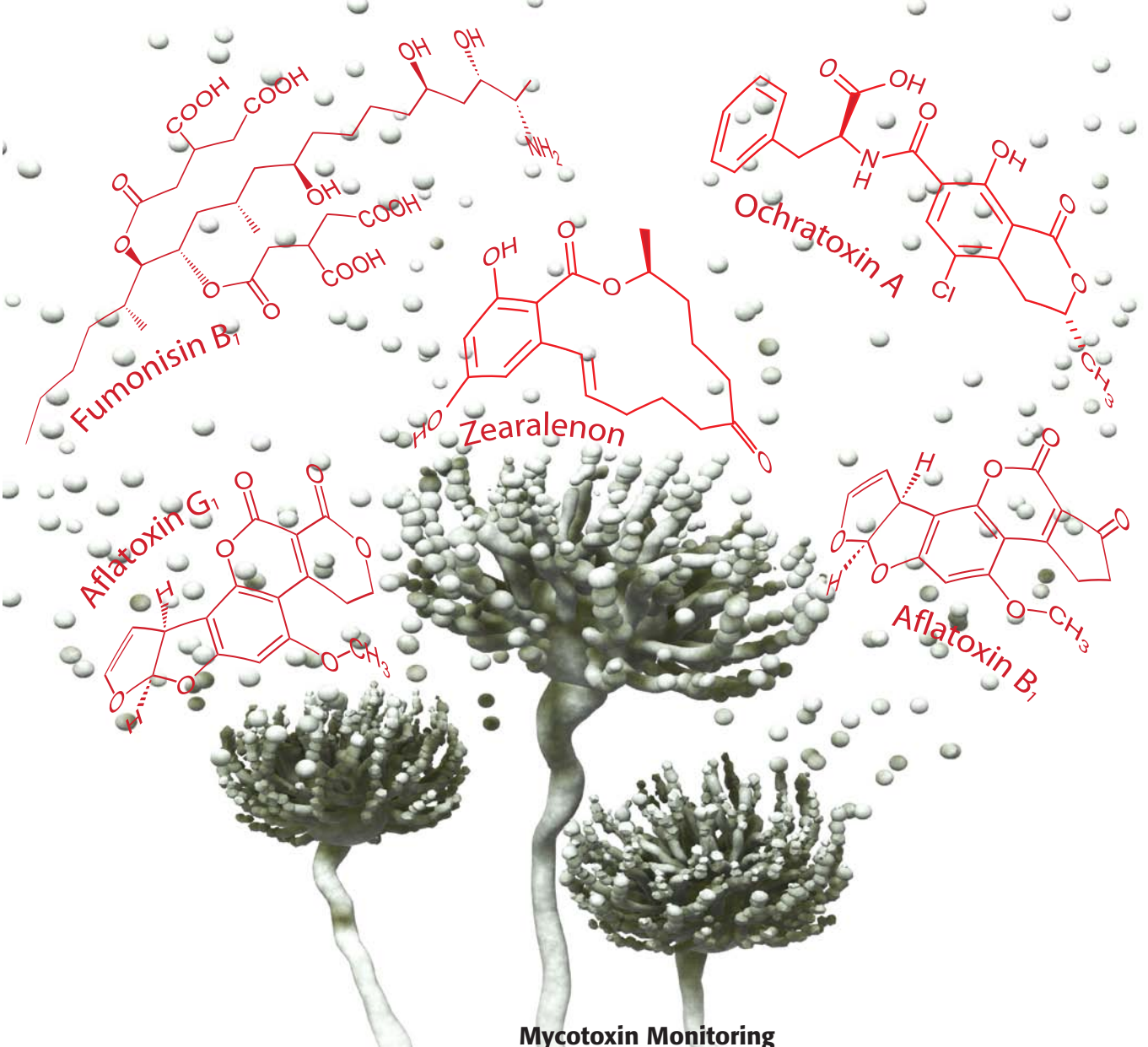

Image: istock / selvanegra, PauraDesign

Mycotoxin Monitoring

Nine steps forward

Laboratories are constantly under pressure to improve productivity – many times without the addition of human resources. To support this demand, more and more scientists are turning to automation of their time consuming and costly manual sample preparation procedures. This article describes how scientists at a contract laboratory specializing in food and environmental analysis developed and implemented an automated SPE-HPLC-MS/MS multi-method to determine nine mycotoxins in one run without relying on immunoaffinity cartridges or human intervention.

By Franziska Chmelka, Mariia Matkovskaia and Norbert Helle,
TeLA GmbH, Geestland, Germany

Their Latin names seem almost regal, and those who know their powers are aware that the molds Aspergillus parasiticus and Aspergillus flavus should be treated with the utmost respect. They should be admired only from a safe distance, with proper protection, and never ingested. Luckily, the visual and olfactory appearance of molds is not appealing to humans and we are genetically programmed to avoid them, but they are not always visible to the naked eye, nor do they necessarily have an odor. Among the metabolites of these molds are potent toxins known as mycotoxins. Apart from being acutely toxic they have carcinogenic, genotoxic and endocrine disrupting traits [1].

Only the dose makes the poison

While Paracelsus obviously was right for most poisons, much more detailed toxicological knowledge at a mo-

lecular level is available to today's scientist. The dose considered toxic for some mycotoxins would have surprised the old master, since for these it is safe to assume that any measurable amount could be unsafe. Whoever processes grain, fruit, spices and other agricultural products should know that is not a question of whether these contain molds, but rather how much they contain. Molds are ubiquitous and cannot be eliminated, only controlled. Accordingly, depending on their individual toxicity, European Union (EU) lawmakers have established maximum levels of mycotoxins in food and feed with a special view to protecting the youngest humans [2]:

Aflatoxin B_1: 8,0 µg/kg (Peanuts), 0,1 µg/kg (Baby food); Sum total of aflatoxins B_1, B_2, G_1 and G_2: 15 µg/kg (Peanuts), 4,0 µg/kg (Grain)

Ochratoxin A: 10 µg/kg (Coffee, raisins), 0,5 µg/kg (Baby food)

Zearalenon: 200 µg/kg (Corn/Maize), 20 µg/kg (Baby food)

T-2-/HT-2-Toxin: not yet established

Fumonisin B_1: 2000 µg/kg (Corn/Maize), 200 µg/kg (Baby food)

Challenges in standard mycotoxin analysis

When maximum levels for toxins are established, this implies that adequate analysis methods and technologies are available with which these levels can be accurately determined and our food quality monitored. Standard methods prescribe the determination of mycotoxins in food and feed in separate groups of analytes using HPLC and fluorescence detection with a clean-up process based on Solid Phase Extraction (SPE) using immunoaffinity cartridges. For some, namely aflatoxins and fumonisin, a derivatization must be performed [3]. An HPLC-MS/MS method is specifically required only for T-2 and HT-2. Standard methods used for the determination of mycotoxins are of course effective in that they produce correct results, but they generally offer significant potential for optimization in terms of productivity. In addition, the use of immunoaffinity cartridges is quite expensive and several different cartridges and clean-up steps are required for each compound group making the total analysis very time consuming.

Realizing the optimization potential

Upon closer inspection, it seemed feasible to dramatically increase the efficiency and productivity of our mycotoxin analysis. Several steps of the process were analyzed and the optimization potential determined. Among other things, it became clear that a major bottle neck was the need for multiple SPE process steps for different groups of mycotoxins. If these could be combined into one SPE step based on a standard sorbent cartridge, the need for expensive immunoaffinity cartridges could be eliminated

Image: TeLA GmbH

The mycotoxin multi-method for the determination of nine mycotoxins was successfully automated on an LC-MS/MS system from Agilent Technologies in combination with a GERSTEL MultiPurpose Sampler (MPS Dual Head version). The solvent gradient used was (A: 5 mM formic acid, B: Acetonitrile; Flow: 0.2 mL/min, 50 °C). The stationary phase used was a C18 reversed phase (RP) material. Mycotoxins were detected in positive ESI Mode.

and the time spent per sample could be reduced significantly, of course provided the quality of results were comparable or could even be improved. A further positive point was that all steps could be automated using a standard sampler

Most Unwanted

Aflatoxins

Aflatoxins are formed as secondary metabolites by different Aspergillus species, including Aspergillus flavus and Aspergillus parasiticus. These are mainly found in nuts and spices. Aflatoxins are highly toxic and carcinogenic; the most critical Aflatoxins are the types B_1, B_2, G_1 and G_2. Aflatoxin B1 is the one most frequently found in food. Aflatoxins are thermally stable and are therefore not destroyed by cooking.

Ochratoxins

Next to the Aflatoxins, Ochratoxin A is counted among the most dangerous mycotoxins. Ochratoxin A is known to cause kidney damage and animal experiments have shown it to be carcinogenic. This mycotoxin is often found in coffee, cereals, beer and dried fruits. As is the case for Aflatoxins, Ochratoxin A is thermally stable and is not even destroyed during coffee roasting.

Fusarium toxins

Fusarium toxins are produced by various Fusarium species among others and are frequently found in cereals. Among these are Zearalenon, T-2 Toxin and HT-2 Toxin. Their acute toxicity is considered low.

Fumonisins

Fumonisins are strongly polar mycotoxins formed by the molds Fusarium verticillioides and Fusarium proliferatum, the most potent of these toxins is Fumonisin B1. Fumonisins are found everywhere in the world. They are formed especially on corn/maize, the amount formed on grains or in foods depends mainly on environmental factors and on storage conditions. Fumonisins are water soluble and are not deactivated by most food preparation processes.

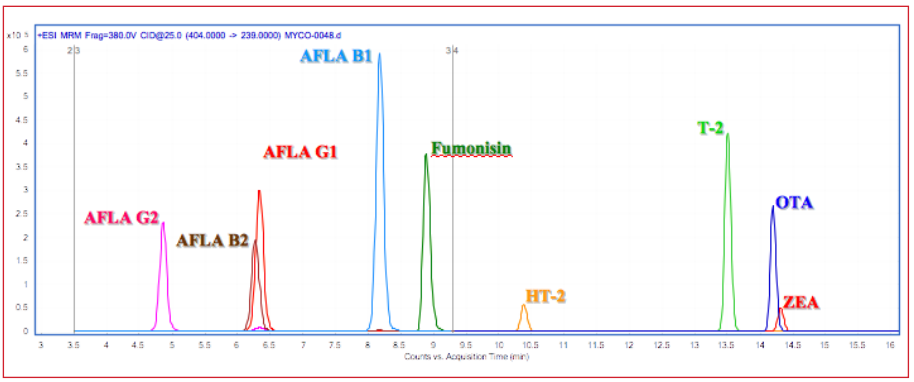

Image: TeLA GmbH

Chromatogram of a standard mixture of the mycotoxins Aflatoxin B_1, B_2, G_1 and G_2, Ochratoxin A, Zearalenon, T-2- and HT-2 Toxin as well as Fumonisin B_1.

Automated Sample Preparation makes the difference

for sample preparation and introduction to the LC-MS/MS system, reducing the laboratory workload. Finally, using MS/MS technology instead of fluorescence detection would improve both the specificity and the sensitivity of the analysis. Following a great number of carefully calibrated experiments, the mycotoxin multi-method was established for the determination of the mycotoxins Aflatoxin B_1, B_2, G_1 and G_2, Ochratoxin A (OTA), Zearalenon (ZEA), T-2- and HT-2 Toxin and Fumonisin B_1. The system used for the analysis consisted of an Agilent 1290 HPLC with 6495 Triple Quadrupol Mass Spectrometer from Agilent Technologies in combination with a GERSTEL MultiPurpose Sampler (MPS) in Dual Head configuration equipped with two towers. This version enables different types of process steps to be processed efficiently without the need to change tools.

Following the method development work, the automated sample preparation process was implemented as follows: The SPE cartridge was conditioned using methanol and water and a 7 mL sample was added to the cartridge. The packed bed is washed with 4 mL of water and dried with nitrogen gas. The analyte elution is performed using 1.5 mL acetic acid ester. The vial with the eluate is transferred to the MultiPosition Evaporation Station (*m*VAP) and the eluate evaporated to dryness. The residue is taken up in 500 µL of the mobile phase and injected to the separation column. In summary, the goal of developing a fully automated multi-method for the determination of mycotoxins was reached and the method implemented for the following compounds: Aflatoxin B_1, B_2, G_1 and G_2, Ochratoxin A, Zearalenone, T-2 and HT 2 Toxin as well as Fumonisin B1. The simplified analysis process with unified sample preparation steps for all mycotoxins lead to significant time savings compared

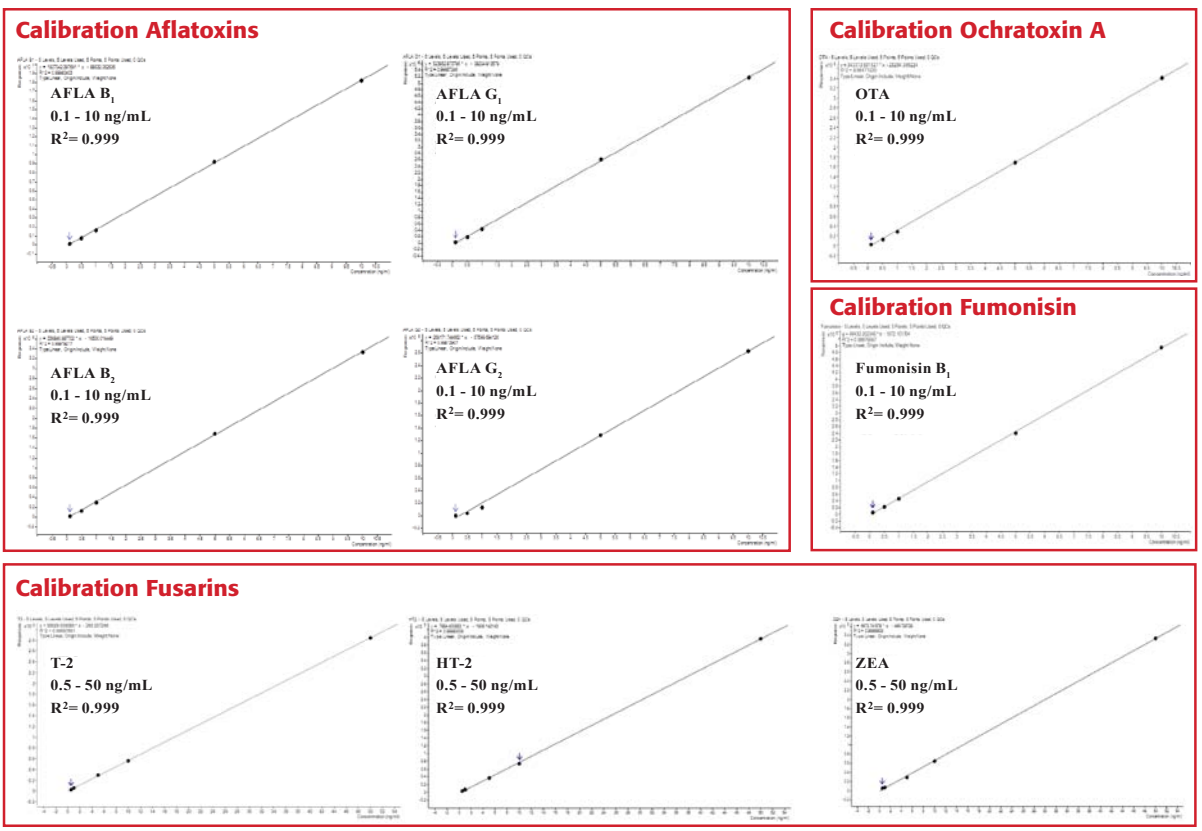

Image: TeLA GmbH

Excellent linearity over a wide range of concentrations for all compounds.

Mycotoxin	Level [µg/kg]	Recovery [%]	RSD [%]
Fumonisin	2	79	5.4
T-2	2	105	6.1
HT-2	2	103	6.5
OTA	2	88	4.3
Aflatoxin G_2	2	94	5.7
Aflatoxin G_1	2	91	4.8
Aflatoxin B_2	2	95	4.5
Aflatoxin B_1	2	89	3.9
ZEA	2	108	5.9

Validation data for the mycotoxin Multi-method for the determination of nine mycotoxins.

with the traditional manual sample preparation process. By using parallel processing of sample preparation steps and chromatography analysis (PrepAhead function) the analysis time was reduced to 52 minutes for the first sample and 30 minutes for all subsequent samples.

Results more than satisfactory

Using a solid phase extraction for sample clean-up instead of performing costly clean-up steps with immunoaffinity cartridges enabled us to reduce the cost per analysis significantly. Furthermore, fluorescence detection was replaced by much more sensitive and selective MS/MS detection, leading to lower limits of determination (0.1-0.3 µg/kg). Statistical values were good, average recoveries ranged from 94.6 percent and upwards and Relative Standard Deviations averaged 5.2 percent. Calibration curves for all mycotoxins showed excellent linearity from 0.1 to 10 ng/µL (Aflatoxins, Ochratoxin A and Fumonisin) and 0.5 to 50 ng/µL respectively (T-2, HT-2 and Zearalenone). Our routine analysis implementation showed that the Mycotoxin Multi-Method was rugged and reliable.

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GERSTEL NEWS

Thermal Desorption Unit – TDU 2

The new GERSTEL Thermal Desorption Unit (TDU 2) is the heart of the most flexible and powerful sample introduction platform available for your GC/MS.

The TDU 2 is a fourth generation Thermal Desorption instrument from GERSTEL, building on more than 20 years of experience. The TDU 2 offers the highest performance, new low split pneumatics for improved LODs, built-in alignment support for easy liner change, and automation performed by the MPS robotic offering high precision delivery of samples, Barcode reading capability and up to 240 TDU tube sample capacity in standard configurations.

The TDU 2 enables ultra-trace analysis of a wide range of samples. Mounted directly on top of the CIS inlet without the need for a transfer line, the TDU provides a completely inert sample path for best possible analyte recovery.

The GERSTEL MultiPurpose Sampler (MPS/MPS robotic) is extremely versatile. The system performs automated spiking of adsorbent tubes with liquid standards as well as multiple sample introduction techniques such as Liquid, HS, SPME plus the following seven:

- Thermal Desorption of sorbent packed tubes
- Direct Thermal desorption/extraction of solids
- Thermal Extraction of liquids in µ-vials
- Dynamic Headspace – DHS and DHS Large (1 L)
- Hot injection and trapping (HIT) for HS
- Stir Bar Sorptive Extraction (SBSE)
- Pyrolysis using the GERSTEL PYRO

It is extremely easy to add – or switch between – sample introduction techniques. All techniques are fully controlled by GERSTEL's powerful, yet easy to use MAESTRO software. The complete integration of MAESTRO into Agilent's GC/MS software allows set up through dropdown menus and combined storage and reporting of all method parameters.

Image: GERSTEL / Wolfram Schroll

Image: Istockphoto / joyt

Company News

GERSTEL expands in Southeast Asia

Ever increasing demand for intelligently automated solutions from GERSTEL has made it necessary to expand into larger offices in Singapore to more efficiently support customers and partners/distributors in South East Asia. GERSTEL LLP held an opening celebration at the new premises that was attended by prominent local officials as well as by key GERSTEL partners and customers. Everybody agreed that this was an important step towards a promising future for GERSTEL in the region.

By Guido Deussing

Singapore is not just a great place to live, boasting one of the highest standards of living in Asia; it is also an excellent place to run a company. The "Lion State" has for decades been an important and reliable partner for German businesses according to Professor Dr. Thomas A. Lange, Chairman of the Board of Directors of the Nationalbank AG in Essen, Germany, GERSTEL's partner bank since 1967, when the company was founded by Eberhard Gerstel Sr. Professor Lange was speaking at the inauguration of the new and larger GERSTEL LLP offices in Singapore, congratulating GERSTEL management, Eberhard G. Gerstel, Holger Gerstel and Ralf Bremer, on their choice of location and praising the company's decision to increase the presence in the region as a sign of sound judgment and a basis of further growth. Prof. Lange stated that the Southeast Asia business hub offers a first class business environment with significant competitive advantages including a second to none infrastructure and a highly qualified workforce.

Image: GERSTEL

Left of the pillar, the guests of honor (from left) Dr. Tim Philippi, Executive Director / Board Member of the German/Singaporean Chamber of Commerce, Dr. Sascha Kienzle, Head of the Science and Technology Department of the German Embassy in Singapore, Professor Dr. Thomas A. Lange, Chairman of the board of NATIONAL-BANK AG in Essen, Germany, Professor Hian Kee Lee, Faculty of Sciences of the National University of Singapore, Dr. Khim Hui Ng, Scientist of Firmenich Asia Private Ltd., Hooi Yan Moy, envoy for the Health Sciences Authority in Singapore. To the right of the pillar (from left): Ralf Bremer, General Manager GERSTEL GmbH & Co. KG, Ms. Tan Surakanpinit Regional Sales Manager South East Asia, Eberhard G. Gerstel and Holger Gerstel, Managing Directors and Co-Owners.

For the inauguration of the new GERSTEL LLP offices, GERSTEL management and the regional team led by Ms. Tan Surakanpinit had invited customers, part-

GERSTEL management (from left): Ralf Bremer, Holger Gerstel and Eberhard G. Gerstel go through a Singapore style dedication ritual.

Image: GERSTEL

ners and guests from business and government. GERSTEL LLP was founded in 2010 as the fifth GERSTEL company outside Germany. The four others are based in the USA, Switzerland, Japan and Brazil. GERSTEL is also represented in over 70 other countries by carefully selected and trained distributors.

Image: GERSTEL

Impressions from the inauguration of the new GERSTEL LLP offices in Singapore. Guests got a behind the scenes look at the application laboratory.

According to the GERSTEL management team, the expansion in Singapore was overdue: "The international business revenue has steadily grown over the past years and in 2014, it made up more than 60 % of total company revenues", says Eberhard G. Gerstel. "Our GERSTEL K.K. subsidiary in Tokyo has supported the Japanese market extremely successfully over the past ten years. The demand for GERSTEL systems and solutions in Singapore, China, South Korea and large parts of South East Asia have made it mandatory to have a solid support structure in the region outside Japan, offering technical and application support for GERSTEL customers and partners from the same time zone".

Global growth of a German company

Since the company was founded in 1967, GERSTEL Headquarters have been in Mülheim an der Ruhr, Germany, where products are developed and produced. "The label "Made in Germany" is well recognized and respected world-wide and it certainly applies to GERSTEL products", says Holger Gerstel. "But the label is not a guarantee for a successful global presence that requires first class products, which are well supported". Ralf Bremer adds: "If you want success internationally, you have to go where the markets and the customers are and this is exactly what we are doing, while making absolutely sure not to neglect our strong home base in the German market".

"GERSTEL is experiencing good growth as shown by the fact that we have had five consecutive years of increased revenues, with international sales growth of 20 percent from 2013 to 2014", says Eberhard Gerstel.

Ms. Tan Surakanpinit, Regional Sales Manager South East Asia is responsible for GERSTEL LLP activities. Ms. Surakanpinit has worked in the analytical instruments business in Asia, the US and Europe and is intimately familiar with the South East Asia region. The GERSTEL LLP offices have separate service and application support departments with highly qualified regional staff to assist customers in the time zone. At the same time, the support staff is well connected with GERSTEL colleagues world-wide such that they have access to company-wide product knowledge. Other application labs are located in Europe, the US and Japan, and all cooperate very closely since analytical chemists are facing more or less the same challenges all over the world.

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Well stocked: When they compose new flavor and taste compounds, Lars Grohmann (left) and Uwe Schafer from Symrise AG in Germany have access to thousands of ingredients.

Image: Guido Deussing

Automated Sample Preparation

The Perfect Blend

In order to always be one step ahead of the competition, Symrise AG relies on innovative product concepts combined with strategies that take a long term view. By automating and miniaturizing a key process, the Germany based global player has succeeded in increasing the productivity of its flavors and fragrances development in a sustainable manner, reducing the use of costly ingredients.

By Guido Deussing

When management spurs an organization on to question standard procedures and to try new approaches in order to improve products and processes, an innovation drive can be set in motion that releases a lot of creative energy within a short period of time. This is what happened in the German company Symrise AG. The Symrise company group was formed in 2003 by a merger between the German companies Haarmann & Reimer and Dragoco. Today, Symrise is among the top three companies in the global flavors and fragrances market. It is the stated intention of the company to continue growing faster than the market.

The key to success

Creating conditions for growth is easier said than done, but a company that can afford to take a long-term view on investment while continually working on process improvement is off to a promising start. Key parameters in working towards continual improvements and success are productivity, flexibility and sustainability, the latter meaning reducing the amount of energy and other resources spent at all levels. This is the approach taken by the Analytical Department of Symrise, which has a key role in the development of flavors and fragrances for different applications and markets such as the food and consumer goods industries. When they create new flavors and fragrances, the flavorists rely on a large number of very different raw materials and ingredients, each of which can consist of a multitude of compound mixtures. The ingredient concentrations, depending on their flavor intensity, can range from a few ppm to as much as 90 percent in the final mixture. Accurately producing a new flavor or fragrance with such a wide concentration range of components requires technical know-how and the right equipment in addition to the flavorist's creativity.

Miniaturization improves flexibility and reduces cost

Blending new flavors for sensory evaluation is typically a time consuming and labor intensive task that requires a significant amount of expensive ingredients. Speeding up flavor development requires automation. If automation can be combined with miniaturization of the blending process, significant savings can be achieved. If the automated system is capable of running 24/7, good efficiency and productivity is also ensured.

Automated blending devices used in flavor and fragrance development are obviously capable of reducing the

amount of labor required, but they typically require a lot of bench space as well as large amounts of the often very expensive ingredients. Since one key goal of Symrise for the entire organization is to operate with minimal use of resources, miniaturization of the blending process was a key objective from the very beginning of the project.

The MultiPurpose Sampler – a powerful blending device

For many years, the analytical laboratories at Symrise have been relying on the GERSTEL MultiPurpose Sampler (MPS) to automate sample preparation and sample introduction in combination with GC/MS analysis. The MPS-based systems in the lab are, among other things, configured for liquid injection, various static and dynamic Headspace techniques as well as extraction techniques including Solid Phase Micro-Extraction (SPME). Since 2005, the company has also used an MPS WorkStation equipped with an integrated balance for simple extraction procedures. The MPS is extremely well suited for handling and transporting the tiniest amounts of sample and standard solutions and dispensing them with high accuracy. This gave the flavor experts Uwe Schaefer and Lars Grohmann the idea that they could miniaturize the flavor blending process using the MPS, and greatly reduce the quantities of ingredients used in the process. "The results of the initial experiments we performed clearly indicated that we were heading in the right direction", the flavor experts reported.

Key capabilities for clean and accurate work

From a technical point of view, the MPS Dual Head WorkStation offers a wide range of sample handling options. The system can be configured with many different tools as well as with a range of heated or cooled trays of different dimensions. If liquid addition steps have to be verified and documented with the highest accuracy, a laboratory balance can be integrated into the workflow. To prevent contamination and sample to sample carry over, Symrise chose to configure the MPS WorkStation with Dynamic Load and Wash (DLW) technology: "Between the needle and the syringe barrel, there is an inert sample loop through which a well-defined amount of flavor ingredient is sampled", Lars Grohmann explains. At no point does the sample or ingredient get into contact with the liquid syringe. Finally, before the next liquid volume is aspirated, the sample loop and needle are thoroughly rinsed from above using solvents that are approved for use within the flavor industry. The solvents are dispensed from a dedicated DLW solvent station.

Even innovative hardware requires thoughtful implementation

When developing flavors and fragrances, Symrise relies on approximately 2500 different ingredients. Among these are pure raw products, essential oils, and extracts. Because many of these ingredients can degrade when exposed to heat, it was decided that Peltier cooled tray stacks were the best option for ingredient storage. Three stacks with the capacity to hold 6 trays per stack were used in the final configuration. These were vented in order to eliminate the possibility of flavor and fragrance emissions and possible cross contamination. This provided the MPS Dual Head WorkStation with a capacity of 204 to 918 ingredients depending on the vials and trays used. For example, VT12 trays hold up to twelve 10 mL vials; VT54 trays offer 54 positions for 2 mL vials. If only VT12 trays are used, a maximum of 204 ingredients can be stored in the system that was configured in cooperation with Symrise; if only VT54 trays are used up to 918 positions are available. The remaining 18th tray is used for diluents and solvents. Through careful optimization, the flavor experts at Symrise and their GERSTEL project partners succeeded in accommodating all essential raw products normally needed for flavor development work using a single MPS WorkStation.

To assist in the technical implementation of the blending process on the MPS Dual Head WorkStation, Symrise developed a database tool, which stores and takes into account specific product data including relevant ingredient specifications such as specific density and viscosity. In addition, the Symrise database keeps track of ingredient usage and stock levels in the MPS WorkStation. An integrated export function directly transfers the "recipe" of ingredients and their associated quantities for a particular fragrance blend directly to a MAESTRO Software Prep Sequence for automated blending.

In the development project, a strict and indispensable condition laid out was the ability to identify raw products by unique product numbers as well as to pinpoint their

Image: Guido Deussing

The MPS WorkStation accelerates the development of new fragrances and flavors, increasing both productivity and throughput. The system can operate around the clock – 24/7.

Symrise AG

Symrise develops, produces and sells fragrances, flavors, cosmetic active ingredients, raw materials and functional ingredients as well as sensorial and nutritional solutions. These are typically key functional ingredients in the final products produced by the customers of Symrise. The bulk of the approximately 30,000 products available are based on natural raw products such as vanilla, citrus fruits, flower petals and other plant material. Symrise' customers include manufacturers of perfumes, cosmetics, food and beverages, the pharmaceutical industry and producers of nutritional supplements as well as pet food and baby food.

Image: Guido Deussing

A strong team: During the development of the MPS blending device, GERSTEL R&D manager Dirk Bremer (Middle.) and GERSTEL Sales Manager Michael Groeger (right) worked closely with Lars Grohmann (left) and his colleague Uwe Schaefer.

exact vial and tray position. The overall implementation and integration of the database with the MAESTRO control software required multiple project steps in which the impact of the parameters chosen on the wider software project always had to be considered carefully. "The system alerts the user when ingredient stock is low and should be replenished or if an ingredient is missing altogether and should be added", Uwe Schaefer and Lars Grohmann report.

Successful cooperation

The flavor experts from Symrise and the application and software experts from GERSTEL were able to draw on each other's expertise, pooling their knowledge to produce an impressive solution. The task was among other things to expand the possibilities of the MAESTRO software and to enable the desired features such as stock management, data transfer, weighing, data tracking – and, last but not least, miniaturization. "Instead of dispensing milliliters or larger volumes by hand, the MPS performs the job dispensing only a few µL of an ingredient in order to create a new fragrance", Uwe Schaefer and Lars Grohmann report: "The automation and miniaturization of the process using the MPS WorkStation resulted in savings in raw product usage of up to 80-90 %".

According to the flavor experts, the MPS WorkStation works day and night, eight days a week when needed. That means the MPS is working overtime and the flavor experts have more time to challenge conventional wisdom in the operation and to try out new things. In short: More time for creative work – the most important thing in the flavor and fragrance business.

GERSTEL NEWS

GERSTEL MPS with SID 1D/2D Barcode Reader

The new Sample ID (SID) 1D/2D Barcode reader for the GERSTEL MultiPurpose Sampler (MPS) uses a dual camera setup and image analysis for positive identification of samples. SID can be connected to a PC or via LAN using USB connectivity. Fully implemented into the MAESTRO software, SID enables independent sample logging or fully integrated sample ID transfer to the data file with multiple user-defined options for sample verification and the handling of deviations.

Images: GERSTEL / Wolfram Schroll

GERSTEL *quick*Mix

The *quick*Mix is an option for the GERSTEL MPS family of samplers. It enables extremely fast and efficient mixing and extraction of a sample as part of the automated sample preparation process. The mixing power is comparable to that of vortex mixing. The sample is agitated in a special tray on the module, depending on the vial size holding up to 6 samples at a time. The tray can be exchanged to operate with 2 mL, 4 mL, 10 mL and 20 mL vials. If needed, *quick*Mix can be configured with a heated tray. All sample preparation steps are set up by mouseclick in the MAESTRO software in stand-alone operation, fully integrated with Agilent MassHunter or ChemStation, or coupled with software from SCIEX™ or Thermo Scientific®.

Conducting E&L Studies more efficiently

To safeguard the health and well-being of patients, it must be ensured that no harmful chemical compounds can leach from packaging into a pharmaceutical product before it is administered or taken. This is done by conducting Extractables & Leachables (E&L) studies on pharmaceutical products in their packaging and by screening the packaging for potential extractable and leachable compounds, for example, using direct thermal desorption of the packaging material. Thermal Desorption (TD) coupled with GC/MS analysis has proven a highly efficient and sensitive screening method. Chemical compounds that are extractable and leachable are likely to be suitable for thermal extraction. TD Screening gives a first rate overview of potential contaminants covering a wide polarity range [1].

Application experts from Agilent Technologies and GERSTEL have now expanded the range of TD-related methods used for E&L studies to polymer based blood bags (IV bag systems) [2]. In this work, direct thermal extraction of the IV bag system material was used to provide an overview of the volatile compounds present. Hoffmann et al. then went one step further to extract and concentrate leached compounds from the IV bag content (simulants) using Stir Bar Sorptive Extraction (SBSE) based on the patented GERSTEL Twister. The added value of this approach: Labor and time-intensive solvent-based extraction and concentration procedures are eliminated. The GERSTEL Twister has a large sorbent phase volume enabling efficient extraction with good recovery resulting in high overall sensitivity of the analysis and low detection limits. In addition, the absence of an extraction solvent reduces the number of interfering compounds and eliminates solvent peak masking. During thermal desorption, analytes are concentrated in the cold trap and subsequently transferred quantitatively to the GC/MS column whereas only an aliquot of liquid extracts are analyzed resulting in less favorable limits of detection. Results obtained on a quadropole GC/MS system were controlled using high resolution GC-QTOF-MS leading to a highly interesting correction of the results reported. In summary, TD screening enables a wide range of analytes to be determined with much lower limits of detection and GC-QTOF can help remove any doubt as to the identity of a leachable compound.

Image:: GERSTEL

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GERSTEL NEWS

Image: GERSTEL / Wolfram Schroll

GERSTEL MPS liquid

The new MPS liquid is a highly efficient liquid autosampler for GC/MS and LC/MS analysis work. Focusing on the core tasks of an autosampler, the MPS liquid offers large sample capacity, modern intuitive software control, as well as the capability to perform key sample preparation steps such as the addition of internal standards or a derivatization reagent. The MPS liquid helps you ensure that your laboratory offers fast, responsive and productive analysis work while delivering reliable and accurate results.

Preview – coming up in the next issue

Chlorinated water and the consequences

Water is chlorinated to eliminate or at least reduce the presence of potentially harmful bacteria. In the process unwanted disinfection byproducts (DBPs) are formed such as halogenated acetic acids (HAAs), which could themselves be harmful. Read more about efficient monitoring of HAAs and how polymer materials react with disinfection chemicals in the next issue of GERSTEL Solutions Worldwide Magazine.

Image: Jan Garbe-Immel

Image: GERSTEL / Wolfram Schroll

Polymer break-down products

Using a clever combination of a widely used extraction technique for GC/MS analysis, breakdown products from thermogravimetric analysis (TGA) is efficiently concentrated and accurately determined by GC/MS. The role of Stir Bar Sorptive Extraction (SBSE) based on the GERSTEL Twister in this work is revealed in the next issue of GERSTEL Solutions Worldwide Magazine.

Efficient multi-vitamin analysis

Determining fat soluble vitamins can be cumbersome, but it doesn't have to be: Using an intelligent sample preparation strategy and the right technology, fat soluble vitamins can be efficiently determined using a multi-vitamin analysis method. Franziska Chmelka, Mariia Matkovskaia and Norbert Helle, Ph.D. from TeLA GmbH in Germany report how it is done.

Image: Creativ Collection

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