GLOBAL ANALYTICAL SOLUTIONS

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No. 15

Automated sample preparation

Don't Work Day and Night!

The MPS handles the night shift p. 16

Material Characterization
Medical Implants – A Product Quality Check p. 4

Metabolomics (part 1)
Automated Extraction and Filtration on the MPS Dual Head WorkStation p. 22

Forensic Toxicology
Automated Determination of Opioids, Cocaine and Metabolites in Blood and other Matrices p. 13

Water Analysis
Clean and Sensitive: On-line SPE-LC/MS Determination of Pesticides p. 19

Dear Reader,

Eberhard G. Gerstel

Holger Gerstel

Ralf Bremer

Did you ever consider adding a second or third shift in your laboratory to keep up with the workload? In many organizations around the world, the laboratory workload is increasing and at the same time results must be delivered ever faster to keep up with production and on-time shipping requirements. The analytical laboratories of the pharmaceutical company Schülke & Mayr near Hamburg, Germany were faced with these same increasing demands and management considered all possibilities to solve the problem, including adding a night shift. In the end, a more attractive solution for laboratory staff was found:

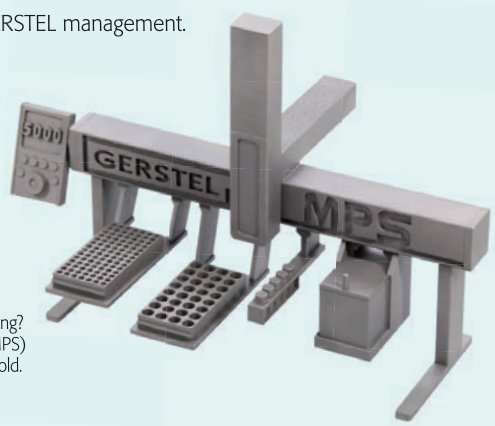
The degree of automation in the laboratory was increased based on the GERSTEL MultiPurpose Sampler (MPS), of which to date, over 5000 have been installed in laboratories worldwide. Whoever invests in automating GC/MS or LC/MS analysis based on the MPS gets sample preparation work done reliably and efficiently – any time, day or night. Individual requirements are easily incorporated into the workflow, often significantly reducing solvent usage, and no one has to watch over the instrument. This is what we would refer to as an intelligent productivity boost. And why did Schülke & Mayr decide to go for the MPS? Hint: Our automated weighing option played a key role. You can find more information in the article on page 16.

Although it seems like yesterday, the GERSTEL Twister was introduced in 2000 and we are celebrating our 15th anniversary with stir bar sorptive extraction (SBSE). In recognition of this important milestone, this issue will feature a number of articles on the technique. The inventor, Professor Pat Sandra from the Research Institute for Chromatography (RIC) in Belgium has provided a guest editorial with insights to the recent SBSE Technical Meeting in Paris, which hosted experts from all over the world. Other Twister articles include the determination of Leachables from whole medical implants and using the multi-SBSE technique to achieve good recovery of a wide polarity range of flavor volatiles in beverages.

Further information on applications, news, workshops and events can be found on our website under www.gerstel.com where you can also sign up for the GERSTEL Newsletter. More content is listed on the right hand side of this page and a preview of articles in the next GERSTEL Solutions Worldwide Magazine (No. 16) is offered on the back cover. We hope that the selected topics are of interest to you and that you will enjoy reading the magazine.

Yours sincerely,

GERSTEL management.



A peek into the future of manufacturing? 3D printed MultiPurpose Sampler (MPS) model in celebration of 5000 units sold.

GERSTEL Solutions worldwide 15

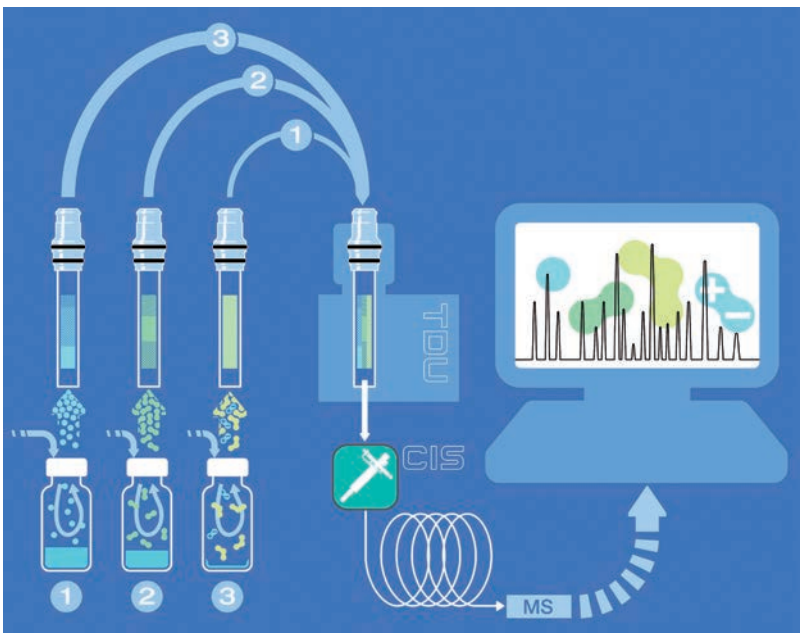
Contents

Flavor Analysis

Tracking the Whole Range of Flavor Compounds

Sequential dynamic headspace sampling of brewed coffee and other beverages provides more information in a single GC/MS run.

Aroma profiles of brewed coffee and other beverages are composed of a wide range of compounds, representing a multitude of compound classes. These span not only a wide volatility range, but also a wide solubility range in aqueous solution. Until now, it has been extremely difficult to combine and determine all these compounds in a single GC/MS run with adequate recoveries and useful results for all compound classes. In the following, an overview is given of the Multi-Volatile Method (MVM), developed by GERSTEL application experts, which relies on a new analyte concentration technique and automated sample preparation to overcome the problems of determining all compounds from beverages in a single GC/MS run.



Flavor analytes are extracted from the sample in three separate DHS steps and the analytes concentrated on three separate TDU liners packed with different adsorbents. The three TDU liners are desorbed separately and the analytes are concentrated in the CIS inlet. When the CIS is heated, the analytes are transferred simultaneously to the GC/MS and determined in one run.

Headspace gas chromatography (HS GC) is frequently used for flavor analysis due to the volatility of flavor compounds. Several established HS techniques are available, for example, static headspace (SHS), dynamic headspace (DHS), and headspace using solid phase microextraction (HS-SPME). However, in terms of analyte recovery, these techniques tend to discriminate in favor of more volatile and/or hydrophobic compounds. Recently, a full evaporation DHS (FEDHS) method, based on a classical full evaporation technique (FET) was demonstrated for uniform enrichment of aroma compounds from several sample types [1]. FEDHS of 10-100 µL of sample at 80 °C using a valve-less short-path DHS system, enables near complete vaporization and uniform recovery of aroma compounds, while largely eliminating non-volatile matrix. However, the FEDHS method often requires a large purge volume in order to remove water from the adsorbent trap. This can lead to loss of volatile compounds due to breakthrough in the adsorbent trap during the purge step. The loss of volatile compounds can be overcome by using replaceable adsorbent traps for analyte concentration, enabling sequential sampling from the same HS vial under different trapping conditions. This method is made practical through the use of a completely automated GERSTEL DHS system mounted on a GC/MS system using a highly flexible MultiPurpose Sampler (MPS). The flexibility of the system allows different adsorbent traps to be used. This enables the extraction and trapping of more volatile compounds in a traditional DHS step prior to the FEDHS extraction step. A novel multi-volatile method (MVM) was developed based on sequential DHS sampling (and desorption) using a variety of trapping conditions for the determination of a wide range of aroma compounds in aqueous samples. The MVM method consists of three different DHS sampling steps including a final FEDHS step. The DHS parameters were examined with the model aroma compounds spiked in 100 µL of water. Feasibility and benefits of using the MVM method is demonstrated through the determination of key odor compounds in brewed coffee [2].

For more information

[1] Flavor and Fragrance Analysis of Consumer Products – Dynamic Headspace Compared to Some Traditional Analysis Approaches, GERSTEL AppNote 06/2012: www.gerstel.com/pdf/p-gc-an-2012-06.pdf

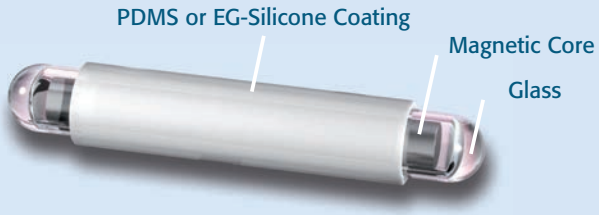
[2] Multi-volatile method (MVM) for aroma analysis using sequential dynamic headspace sampling with an application to brewed coffee, Jun Tsunokawa, Nobuo Ochiai, Kikuo Sasamoto, Andreas Hoffmann. Journal of Chromatography A, 1371 (2014) 65–73. Free Download: www.sciencedirect.com/science/article/pii/S0021967314016859

Extractables & Leachables

Medical Implants – A closer look

In order to assess the quality and compatibility of a polymer based implant, it is vital to perform simulated in-vivo studies to provide qualitive and quantitive information concerning the compounds that are leached out of the device. These compounds are referred to as Leachables. Since they are present at very low concentrations, analysts often turn to Stir Bar Sorptive Extraction (SBSE) followed by Thermal Desorption GC-MS/MS for an accurate answer. In the following, we report on a joint effort to test polymer based tibial knee inserts for Leachables.

A look inside: Implants at work



The patented GERSTEL Twister® serves as extraction medium based on the Stir Bar Sorptive Extraction (SBSE) technique. The Twister is coated with a relatively large volume of sorbent, either polydimethylsiloxane (PDMS), mainly for non-polar compounds, or Ethyleneglycol silicone (EG Silicone Twister), which provides enhanced extraction of various polar compounds in addition to non-polar compounds. Analyte extraction is performed while the Twister stirs the sample or, in the case of the Twicester, with one Twister immersed in the sample, but held in place on the vial wall, while another Twister stirs the sample.

Pharmaceutical products in general should serve to maintain the health of the patient. Under no circumstances should they endanger or adversely impact the patient's health. Manufacturers are required to perform extensive testing on pharmaceutical products, including the packaging used, over an extended period during the development phase and regularly once it has been approved and is in production. If unallowed Leachables from the packaging are found, or if they are found in too high concentrations in the product, it will not be approved, for example, by the US FDA, which means it cannot be sold in the US.

In simulated "worst case" scenarios, Extractables studies are performed on complete, undamaged packaging and the extracted chemical compounds determined. The packaging is exposed to solvents of different polarities at elevated temperature to extract a wide range of compounds. Those compounds identified as potentially hazardous that could leach into the product are then studied further in formalized Leachables studies based on the actual product using validated methods [1].

Implants must be safe as well

Implants are generally made of both metal and polymer materials and they must be analyzed for the presence of contaminants. Further, it should be studied whether additional contaminants could be formed while the product is inside the patient (*in vivo*), endangering the health of the patient. Up until now, manufacturers of medical implants have not had an easy way to perform such analyses on medical device implants. However, since implants remain inside the body for an extended period of time in direct contact with body fluids, these analyses are even more important. Leached contaminants from implants can gain direct access to the patient's blood, unlike most pharmaceuticals, which are ingested orally and have to go through the digestive tract.

Unlike the situation for pharmaceutical products, until now there are no legally binding regulations for implants and prosthetics, or indeed any standardized methods for performing Extractables or Leachables studies according to Gyorgy Vas. For this reason, the researcher who works for the US based company Intertek, followed regulations for performing E&L studies on pharmaceutical packaging when describing the leaching properties of implants. They further adhered to guidelines of the „Product Quality Research Institute" (PQRI), a US non-profit organization, which recommends a Maximum dose for genotoxic or carcinogenic leachables of 150 ng/day [2].

Not identical but comparable

In its guidelines, the PQRI refers to results obtained from testing of inhaler products. As Gyorgy Vas and his colleagues report in their article in *Journal of Pharmaceutical and Biomedical Analysis* [3], they based their studies on the PQRI guidelines, which represent a more "conservative" approach in the sense of erring on the side of caution in order to ensure the greatest possible safety for the patient. The researchers report how they determined leachable compounds in medical implants, in this case tibial knee inserts, based on Stir Bar Sorptive Extraction (SBSE) followed by GC/MS analysis. The goal of their study was to develop a systematic procedure, by which antioxidant-related leachables can be determined accurately. These are formed in knee implants during sterilization with gamma radiation.

Of Additives and Guidelines

The tibial knee inserts that were investigated by Vas et al. are produced from ultra-high molecular weight polyethylene (UHMWPE). After the molding process, these are cross-linked and sterilized using a very high dose of gamma radiation. During this process, the mechanical properties of the polymer are improved, but free radicals are formed, which can attack the material and negatively impact its chemical and mechanical long term stability. To prevent oxidation processes in the material and improve product stability, an antioxidant is added, such as pentaerythritol tetra-

Twisters are desorbed in the Thermal Desorption Unit (TDU) shown in this picture or in the Thermal Desorption System (TDS). The process is automated.

Image/s: GERSTEL, Istockphoto
Author: Guido Deussing

Stir Bar Sorptive Extraction using the GERSTEL Twister is followed by thermal desorption in the Thermal Desorption Unit (TDU), cryofocusing, and GC/MS analysis – fully automated using the GERSTEL MultiPurpose Sampler (MPS) under MAESTRO control.

kis (3-[3,5-di-tert-butyl-4-hydroxyphenyl] propionate). The purpose of adding this compound known as PBHP is to remove free radicals, prevent oxidation processes and improve the long-term stability of the product. However, PBHP can degrade and the degradation products formed leach from the implant into the surrounding tissue. A total of 16 such PBHP degradation and by-products have been identified in simulated in-vivo experiments with knee implants that were between ten and thirty years old. Because of these findings, the researchers stress that implants need to be tested for leachables. Studying the leaching behavior of larger implants, however, requires large volumes of aqueous simulants. This results in the extracted analytes being present at very low levels. As reported by Vas *et al.*, a concentration step is required prior to GC/MS or LC/MS determination in order to enable quantification as per the PQRI guidelines. Typically, such a concentration step would be time consuming and would require highly pure organic solvent. These factors make the whole process expensive, challenging, and environmentally unfriendly.

Turning to Stir Bar Sorptive Extraction (SBSE)

Evaporative concentration of organic solvent extracts inevitably leads to loss of volatile analytes. Additionally, a solvent peak typically masks peaks of interest in the chromatogram and a solvent could easily introduce interfering compounds leading to errors and uncertainty. Stir Bar Sorptive Extraction (SBSE), on the other hand, relies on the GERSTEL Twister to extract organic compounds from an aqueous solution with high recovery without any use of solvent.

Following an extensive evaluation of various widely used methods, Dr. Vas and his team decided to use SBSE and the PDMS Twister as extraction medium combined with GC/MS analysis. The Twister extracts and concentrates analytes in a single step, no further sample preparation is required before GC/MS analysis. Thanks to the large PDMS sorbent volume and the efficient stirring of the sample by the Twister stir bar, analyte recovery is far higher than what is achieved with Solid Phase Micro-Extraction (SPME). In the words of Vas et al.: "Case-by-case, we have found SBSE to be significantly more sensitive than SPME for the determination of organic compounds in aqueous matrices".

Technical details

This is how the scientists approached the project: Whole implants were immersed in 500 mL of an aqueous extraction solution (90:10 water/acetone). Extraction times of 24 hours and 30 days were used. The resulting extract was stirred with a Twister for two hours at 1,000 rpm, during which time the organic compounds in the extract solution were absorbed into the PDMS coating of the Twister. The Twisters were collected from the aqueous extract, briefly rinsed with deionized (DI) water, dried using a lint-free paper cloth, placed in a TDU liner, and transferred to a TDU liner tray. The next step was thermal desorption in the Thermal Desorption Unit (TDU), fully automated with the GERSTEL MultiPurpose Sampler (MPS). The desorbed analytes were cryogenically trapped in the GERSTEL Cooled Injection System (CIS), a PTV-type inlet, mounted in an Agilent 7890 GC (Agilent Technologies). Analytes were determined using an Agilent Triple Quad MS/MS detector. As an aside, three different sources of DI water were tested. All had detectable background concentrations of antioxidants. Monitoring and controlling the blank level, and choosing the right DI water source, is clearly very important in order to obtain accurate results at these low concentration levels.

End of a successful study

The SBSE-GC-MS/MS method developed and validated by Vas *et al.* enabled them to determine Leachables in medical implants at trace level concentrations equivalent to leaching rates below 150 ng/day. The scientists reported that no genotoxic or carcinogenic compounds were determined to leach from the tested implants at rates higher than 150 ng/day in the 24 hour extraction tests. For the PHBP degradation products, the linearity of the developed method was tested over the 1–150 ng range (equivalent to 40–6000 pg/mL) for all relevant components. Calibration curves with internal standard were prepared using the extraction media as the matrix. The linear range for each component was 1.0–150 ng with good RSDs and the method was subsequently used to analyze eleven tibial knee implants. Unlike other methods, which can only analyze fragments, the developed method can analyze the entire implant, which is the approach preferred by regulatory authorities to eliminate sampling errors, according to Dr. Vas. Most of the sample preparation was automated; only minor manual steps were needed. The method had the added benefit of eliminating the use of expensive, toxic solvents that are also expensive to dispose of and which can negatively impact the laboratory air quality.

References

[1] GERSTEL Solutions No. 10 (2010) 20-22
[2] Summary of the PQRI Leachables and Extractables Reconnedations (www.pqri.org/workshops/leach_ext/imagespdfs/posters/PQRI_Recommendations_Poster.pdf)
[3] J. Pharm. Biomed. Anal. 74 (2013) 62-70

Extractables and Leachables

In the case of pharmaceutical packaging, Leachables and Extractables studies are required in order to determine if packaging will leach contaminants into the product under normal storage conditions or if contaminants can be extracted by the product under well-defined more aggressive conditions.

Stir Bar Sorptive Extraction (SBSE)

Established, Useful and Quite Often Simply the Extraction Technique of Choice

Stir Bar Sorptive Extraction (SBSE), based on the patented GERSTEL Twister, is a fully established technique in the world of chromatography. Quite often, SBSE is simply the method of choice. Professor Pat Sandra, SBSE inventor and founder of the Research Institute for Chromatography in Kortrijk, Belgium, casts some light on the beginnings of SBSE, the current status and what the future may hold.

By Professor Pat Sandra,
founder of the Research Institute for Chromatography (RIC), Kortrijk, Belgium

Professor Pat Sandra The Inventor of SBSE, Professor Emeritus Pat J. Sandra received his Master's degree in Organic Chemistry in 1969 followed by a Ph.D. degree in Analytical Chemistry in 1975 from the Ghent University, Belgium. He joined the Faculty of Sciences of the Ghent University in 1976 as Assistant Professor and was promoted to Full Professor of Separation Sciences in 1988. In 1986 he founded the Research Institute for Chromatography in Belgium, a center of excellence for research and education in chromatography, mass spectrometry and capillary electrophoresis. He was Co-founder of the Pfizer Analytical Research Center (PARC) that he directed during the period 2003-2011. During this time he has authored or co-authored over 500 scientific publications. Among numerous awards are the ACS Chromatography Award (2005) and Doctor Honoris Causa degrees in Pharmaceutical Sciences, in Food Safety and in Chemical Engineering. In 2013, he was appointed member of the Research Council of President Barroso of the EU commission.

Stir bar sorptive extraction (SBSE) was developed as a reaction to a negative comment on one of our publications related to the partitioning mechanism occurring between polydimethylsiloxane (PDMS) and water [1]. Theoretically, absorption (sorption) and not adsorption is taking place but this was obscured by adsorption of polychlorinated biphenyls (PCBs) on a PTFE stir bar. Coating the stir bar with PDMS resulted in 100 % absorption of the PCBs proving the partitioning mechanism and the relation with the octanol/water distribution coefficient. SBSE was introduced in 1999 as a solvent-less sample preparation technique combined with thermal desorption coupled online to capillary GC. It immediately became clear that this new method offered unprecedented sensitivity for compounds with a log P value larger than 3. For compounds with log P < 3, several in-situ derivatization methods were developed to cope with more polar compounds in different matrices e.g. food, beverages, biological fluids, etc. SBSE combined with liquid desorption was the next step in its development opening the combination of SBSE with liquid chromatography and the electrically-driven separation methods. Initially SBSE was mostly used for the extraction of compounds from aqueous matrices. The technique has also been applied in headspace mode for liquid and solid samples and in passive air sampling mode. Initially it was not our intention to commercialize SBSE – our hope was simply to have a good scientific outcome of this work. With approximately 1000 citations in the literature that hope has certainly been realized. However, GERSTEL showed strong interest in the technique and commercialized SBSE under the name "Twister®". Thanks to this move, the great potential of the technique has been shown over and over again.

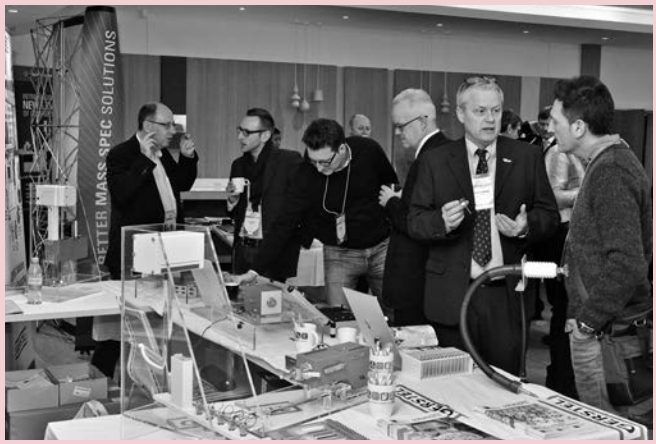
The benefits of the Twister technology

The main features and advantages of the SBSE technique are that it is a green sample preparation technique; analyte recovery is predictable and can be calculated from the log P value; very low detection limits (ppq's have been mentioned e.g. for organotin compounds) can be reached as well as excellent reproducibility; different *in-situ* derivatization methods are available, broadening the technique to compounds with low log P values and at the same time, improving chromatographic performance; a wide variety of matrices can be handled; and further clean-up steps are not needed (as opposed to other recently introduced sample preparation techniques). Moreover, the method is cost efficient since the Twisters can be reused many times. More recently, GERSTEL has developed an additional coating: Ethylene glycol – silicone (EG-Silicone) Twisters have become available, leading to high recovery extraction of specific solutes in a wide variety of matrices (beverages, biological flu-

ids, etc.). Any new analytical method needs high visibility to become successful. GERSTEL considered the Twister an important development and marketed the product accordingly. Soon after its commercialization, David Benanou from Veolia Water in Paris demonstrated the enormous potential of the Twister for detection of off-odors in drinking water and became one of the first proponents of the technique. Moreover, through his organization, he introduced the technique in several laboratories worldwide. He organized the first SBSE symposium in Paris in 2011 and decided to make this a biennial event. This year we had the third SBSE Technical Meeting with a strong international presence including speakers from laboratories in France, Belgium, the US, Japan, the Slovak Republic, the Czech Republic and Italy. This symposium series has definitely contributid to the spread of the Twister technology. In the past, one of the comments often heard about the Twister was: "It is too sensitive; we do not like to see all those compounds at ultra-trace levels." However, in recent years environmental concerns have increased tremendously, reducing the maximum allowable levels of several contaminants to extremely low values (ppq's for organotin compounds in water samples, zero tolerance of pesticides in baby food, etc.). The application of techniques like SBSE with its extreme power of concentration, will become mandatory to ensure the required accuracy and precision at these levels. Also, new analytical challenges are emerging for which Twister technology can be extremely useful. To mention a few examples: domestic passive sampling to elucidate endocrine disrupting chemicals, detection of allergens in cosmetic products, detection of toxic substances in shipping containers, release of chemical products from packaging materials, etc. Also, new Twister coatings can be developed for untargeted or targeted analysis. A polymer based on ionic liquids can be the next candidate for coating that small stir bar. The Twister technology is definitely on the move!

Literature

[1] E. Baltussen *et al.*, Study into the Equilibrium Mechanism between Water and Poly(dimethylsiloxane) for Very Apolar Solutes: Adsorption or Sorption? Analytical Chemistry 10 (1999) 71(22). DOI: 10.1021/ac990313g



Impressions from the SBSE technical meeting: Stimulating discussions about applications, trends, and possibilities of SBSE.

▲ SBSE Technical Meeting organizer David Benanou poses a question.

SBSE methodology was actively discussed in smaller groups during session breaks.

It is always worth listening to experienced users. GERSTEL Marketing Manager Kaj Petersen in conversation with Naïke Noyon, Research Engineer from CIRSEE, SUEZ environnement's main Research and Expertise Centre near Paris. Mr. Noyon presented his work on interaction of disinfectants with polymer pipes at the SBSE conference.

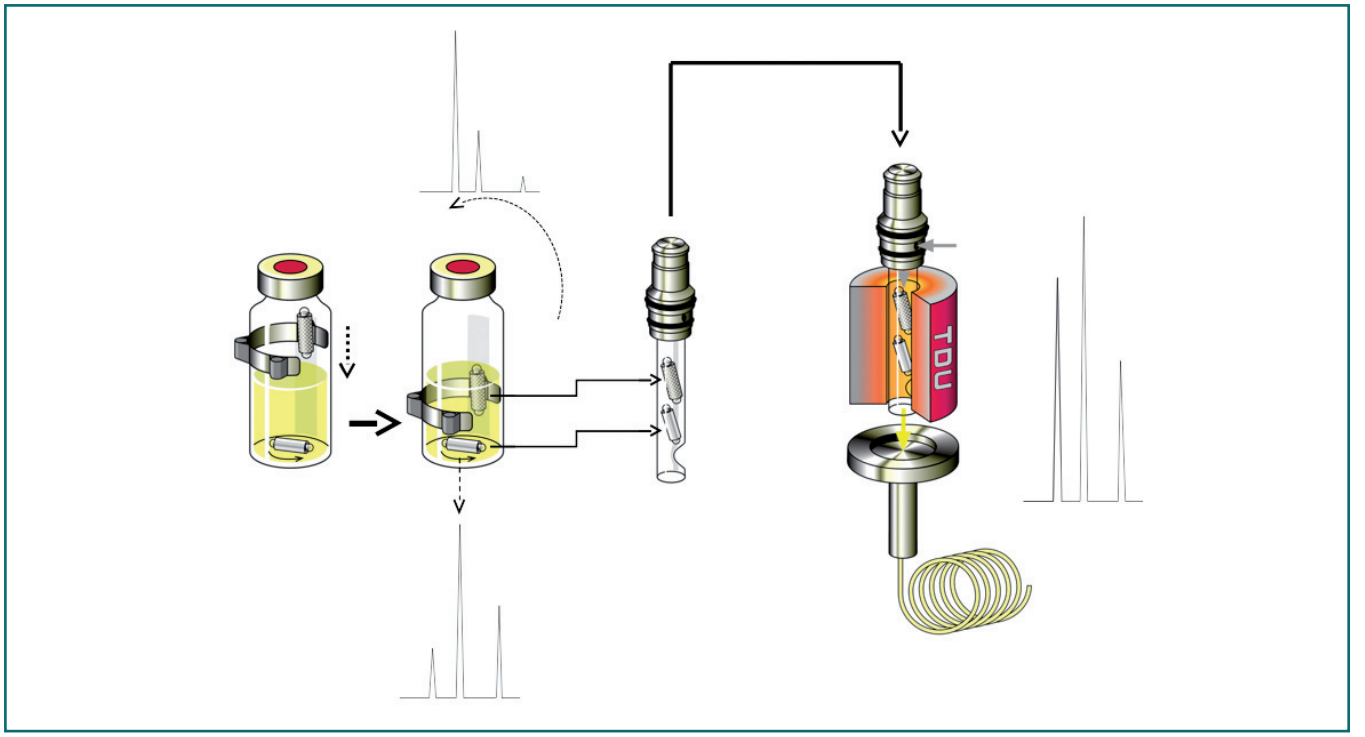


Figure 1: Multi-SBSE (^{m}SBSE) procedure.

Beverage Analysis

The Twister Sisters pick up the Flavors

By utilizing Multi-SBSE and SBSE with in-situ derivatization, you can determine non-target analytes across a wide polarity range as well as target off-flavors and key flavor compounds. If a high-end GC/MS system is used, the amount of information gained is significantly increased

By Nobuo Ochiai, GERSTEL K.K., Tokyo, Japan

Stir bar sorptive extraction (SBSE) has been successfully applied to food analysis including aroma analysis. For aroma analysis, SBSE has been applied to various sample matrices, such as water, beverages, fruits, herbs, plant material, essential oils, and vinegar [1–3]. These applications have been mostly performed using polydimethylsiloxane (PDMS) coated stir bars, because this was the only available phase for commercial stir bars (Twister®) before 2011. SBSE recovery can be estimated if the octanol–water distribution coefficient (K_{ow}) of the analyte is known. Hydrophobic solutes with a high K_{ow} can be extracted with high recovery, while hydrophilic solutes with a low K_{ow} show lower recovery [1]. Therefore, SBSE using PDMS phase is generally more selective for hydrophobic solutes, often resulting in a partial chromatogram biased towards less hydrophilic solutes. In 2011, a new Twister phase coated with polyethyleneglycol-modified silicone (EG Silicone) on a metal mesh support was introduced and applied to various sample types including whisky, wine, essential oils, and brewed coffee [4, 5]. This new polar coating showed good performance for the extraction of polar/hydrophilic solutes. A novel SBSE procedure termed multi-SBSE (^{m}SBSE) was developed in 2013 [6]. ^{m}SBSE consists of the PDMS Twister stirring at the bottom of the vial and the EG Silicone Twister attached to the inner side wall of the vial (a magnetic clip is used for the set-up). Compared to conventional SBSE, ^{m}SBSE provides more uniform enrichment of a wide range of aroma compounds in aqueous medium since the two Twister phases complement each other.

For the extraction (and analysis) of specific hydrophilic/polar solutes, SBSE in combination with *in-situ* derivatization (e.g. acylation, esterification, and oximation) can also be used (derivat-SBSE). For polar/hydrophilic solutes with low K_{ow} values, the corresponding derivatives generally have higher K_{ow} values, resulting in higher recovery and

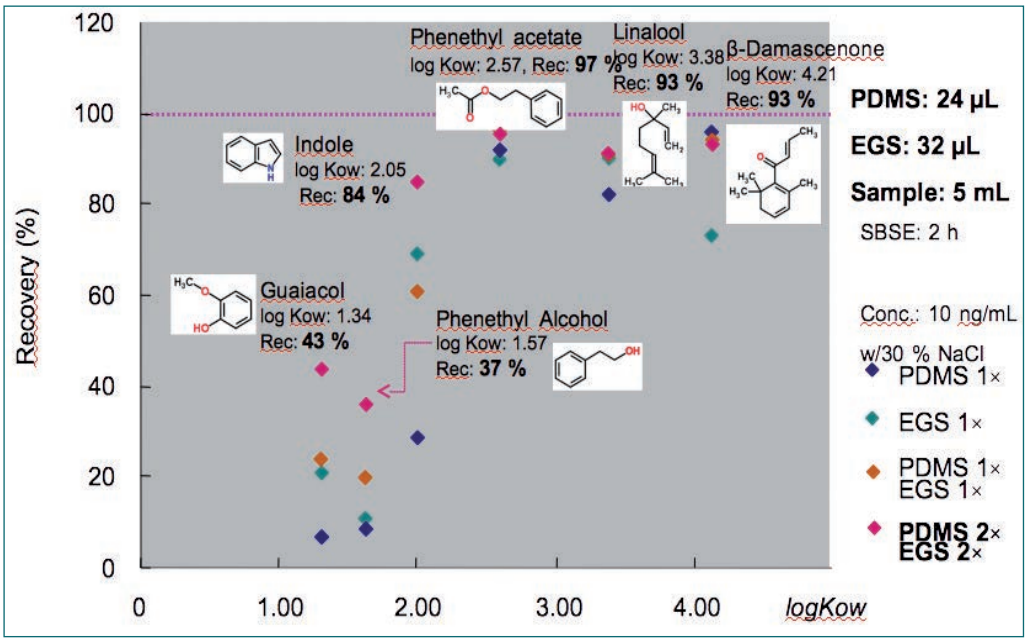


Figure 2: Comparison of recovery for test aroma compounds between single SBSE and ^{m}SBSE.

sensitivity [1]. Also, the higher molecular weights of derivatives provides higher selectivity in GC-MS analysis.

In this paper two SBSE approaches, ^{m}SBSE and derivat-SBSE will be described for aroma/off-flavor analysis of beverages. ^{m}SBSE shows more uniform enrichment of aroma compounds covering a wide polarity range in roasted green tea. Also, derivat-SBSE will be used to demonstrate trace analysis of key aroma compounds and off-flavors in beer.

Multi-SBSE (^{m}SBSE) for non-targeted analysis of aroma compounds

Comparison of recovery between single SBSE and ^{m}SBSE

Fig. 1 describes the multi-SBSE (^{m}SBSE) procedure. The extraction was performed by using a 24 µL PDMS Twister and a 32 µL EG Silicone Twister on a 5 mL sample after addition of 30 % NaCl. After extraction, the Twisters were thermally desorbed in split mode with a split ratio of 1:1 using the low split option controlled by the pneumatic box of the TDU system, and analyzed on a 30 m length × 0.25 mm i.d. × 0.25 µm df DB-Wax column using MS detection in scan mode.

Recoveries obtained by ^{m}SBSE for test aroma compounds in water, including various types of chemical classes (e.g. alcohol, ester, hetero-cyclic, ketone, and phenol), were compared with those obtained by conventional single SBSE. The log K_{ow} values of the test compounds were in the range of 1.34 (guaiacol) to 4.21 (β-damascenone). The concentration of the test compounds was 10 ng/mL each. Fig. 2 demonstrates a recovery comparison between 4 different SBSE conditions; (a) single SBSE using the PDMS Twister (1×), (b) single SBSE using the EG Silicone Twister (1×), (c) ^{m}SBSE using the PDMS Twister (1×) and the EG Silicone Twister (1×), (d) ^{m}SBSE using two PDMS Twisters (2×) and two EG Silicone Twisters (2×) (one PDMS Twister is stirring, while another PDMS Twister and two EG Silicone Twisters are attached on the inner side wall of the vial). For the condition (a) single SBSE using the PDMS Twister (1×), the solutes with log K_{ow} > 2.5 showed more than 80 % recoveries, while the solutes with log K_{ow} < 2.5 showed low recoveries, especially for guaiacol (log K_{ow}: 1.34, recovery: 8.3 %) and phenethyl alcohol (log K_{ow}: 1.61, recovery: 10 %). For the condition (b) single SBSE using the EG Silicone Twister (×1), the solutes with log K_{ow} > 2 showed higher than 70 % recovery, while the solutes with log K_{ow} < 2 also showed low recovery. However, compared to the condition (a), the recoveries for guaiacol (log K_{ow} 1.34) and indole (log K_{ow} 2.05) increased from 8.3 % to 21 %, and 29 % to 71 %, respectively, while the recovery for β-damascenone (log K_{ow} 4.21) decreased from 96 % to 74 %. Meanwhile, the condition (c) ^{m}SBSE using the PDMS Twister (1×) and the EG Silicone Twister (1×) showed higher recoveries for all test solutes except for indole (log K_{ow} 2.05, recovery 60 %) compared to single SBSE approaches. Moreover, the condition (d) ^{m}SBSE using two PDMS Twisters (2×) and two EG Silicone Twisters (2×), which has the highest phase ratio, showed the highest recoveries for the solutes with log K_{ow} of less than 2.5. Consequently, the ^{m}SBSE approach not only combines the extraction power of the PDMS Twister with the EG Silicone Twister, but also results in higher recovery due to increased phase volume (smaller phase ratio).

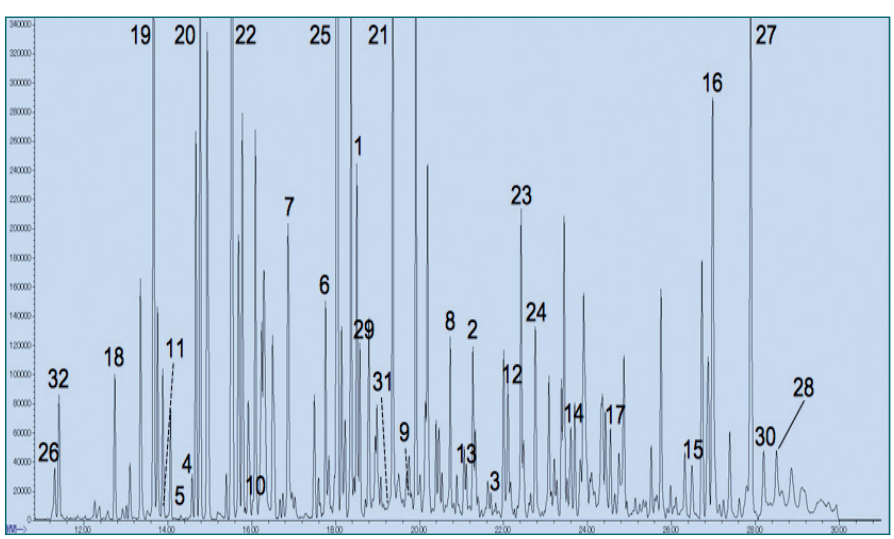


Figure 3: Total ion chromatogram of roasted green tea obtained from ^{m}SBSE-TD-GC-MS.

1. Furfuryl alcohol (log K_{ow} 0.45), 2. Benzyl alcohol (log K_{ow} 1.08), 3. Phenethyl alcohol (log K_{ow} 1.57), 4. *cis*-3-Hexenol (log K_{ow} 1.61), 5. 1-Hexanol (log K_{ow} 1.82), 6. 2,6-Dimethyl-1,3,7-octatrien-6-ol (log K_{ow} 3.24), 7. Linalool (log K_{ow} 3.38), 8. Geraniol (log K_{ow} 3.47), 9. Citronellol (log K_{ow} 3.56), 10. Furfural (log K_{ow} 0.83), 11. 6-Methyl-5-hepten-2-one (log K_{ow} 2.06), 12. *cis*-Jasmone (log K_{ow} 3.55), 13. Guaiacol (log K_{ow} 1.34), 14. *p*-Cresol (log K_{ow} 2.06), 15. Vinyl Guaiacol (log K_{ow} 2.24), 16. *p*-Vinyl phenol (log K_{ow} 2.41), 17. *p*-Ethyl phenol (log K_{ow} 2.55), 18. 2-Methyl pyrazine (log K_{ow} 0.49), 19. 2,5-Dimethyl pyrazine (log K_{ow} 1.03), 20. 2-Ethyl-5-methyl pyrazine (log K_{ow} 1.53), 21. 5,6,7,8-Tetrahydroquinoxaline (log K_{ow} 1.90), 22. 2-Ethyl-3,5-dimethyl pyrazine (log K_{ow} 2.07), 23. 2-Acetyl pyrrole (log K_{ow} 0.56), 24. 2-Formyl pyrrole (log K_{ow} 0.60), 25. 1-Ethyl-2-formyl pyrrole (log K_{ow} 1.14), 26. 1-Ethyl pyrrole (log K_{ow} 1.92), 27. Indole (log K_{ow} 2.05), 28. 2-Methyl indole (log K_{ow} 2.60), 29. 2-Acetyl thiazole (log K_{ow} 0.67), 30. Coumarin (log K_{ow} 1.51), 31. 2-Formyl thiophene (log K_{ow} 1.53), 32. 2,4,5-Trimethyl oxazole (log K_{ow} 1.86).

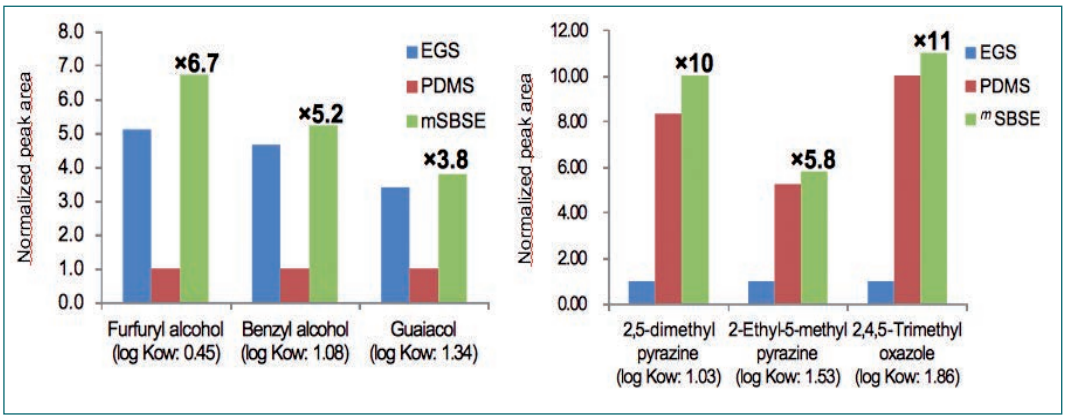


Figure 4: Comparison of the normalized areas of some aroma compounds between single SBSE and ^{m}SBSE.

Analysis of roasted green tea

Aroma compounds in green tea are present at trace level (from pg/mL to ng/mL), and therefore analytical methods should include powerful extraction and enrichment steps before GC analysis. Several sample preparation techniques, e.g. liquid phase extraction, gas phase extraction/distillation, and solid phase extraction, have been proposed for isolation and extraction of aroma compounds in green tea. The major drawbacks are, however, large sample volumes, e.g. 3-30 L [7], and the fact that the enrichment factor (original sample amount versus final extract volume) obtained with these techniques are rather limited and require additional evaporative concentration to a very small volume (<1 mL).

Roasted green tea (Houji-cha) was analyzed as an example of trace analysis of a wide variety of aroma compounds. The Maillard reaction roasting process of Houji-cha replaces the fine green and vegetative tones of standard green tea with more complex aroma (e.g. toasty, nutty, and caramel-like) [8], but those additional aroma compounds are still only present at trace level. Fig. 3 demonstrated a total ion chromatogram (TIC) of roasted green tea obtained from ^{m}SBSE using the PDMS Twister (1×) and the EG Silicone Twister (1×). A variety of solutes which contribute to the aroma of roasted green tea were detected in the chromatogram from only 5 mL of sample, including coumarin (log K_{ow} 1.05), guaiacol (log Kow 1.34), *p*-cresol (log K_{ow} 2.06), indole (log K_{ow} 2.05), 2-ethyl-3,5-dimethyl pyrazine (log K_{ow} 2.07), linalool (log K_{ow} 3.38), geraniol (log K_{ow} 3.47), and *cis*-jasmone (log K_{ow} 3.55). Most of these solutes were determined in the range of 7.0 to 43 ng/mL with the standard addition calibration method [6].

Fig. 4 shows a comparison of the normalized areas of some aroma compounds between single SBSE using the PDMS Twister, single SBSE using the EG Silicone Twister, and ^{m}SBSE using both the PDMS Twister and the EG Silicone Twister. These data suggest that solutes which form a hydrogen bond were mainly recovered by EG Silicone Twister, while some heterocyclic solutes (2,5-dimethyl pyrazine, 2-ethyl-5-methyl pyrazine, and 2,4,5-trimethyl oxazole) were mainly recovered by PDMS Twister.

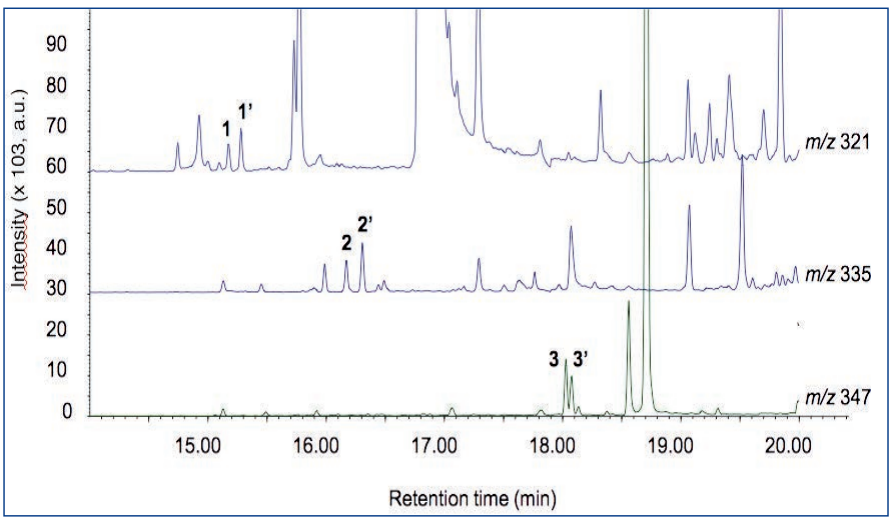


Figure 5: Selected ion monitoring (SIM) chromatograms of spiked beer at 500 pg/mL obtained from derivat-SBSE-TD-GC-MS. 1,1': *E*-2-Octenal, 2,2': *E*-2-Nonenal, 3,3': *E,E*-2,4-Decadienal

SBSE with in-situ derivatization (derivat-SBSE) for targeted analysis of off-flavors and key aroma compounds

Analysis of stale flavor aldehydes in beer

Oxidatively produced unsaturated aldehydes play a major role in the development of stale-flavor in beer. *E*-2-Nonenal has been considered as the major source of the papery/cardboard stale-flavor in beer because of its very low odor threshold level at 0.1 ng/mL [9]. Analysis of *E*-2-nonenal and similar congeners in beer is generally rather challenging taking into account the relatively high levels of matrices (e.g. fusel alcohols, fatty acids, and esters). A simple and effective method to decrease the interference caused by beer matrices during both sample preparation and GC analysis is to use derivatization. *E*-2-nonenal and similar congeners can be enriched and selectively detected by GC-MS using SBSE with in-situ derivatisation (derivat-SBSE). For *in-situ* derivatization, pentafluorobenzylhydroxylamine (PFBHA) was used to derivatize the targeted aldehydes (log K_{ow} 2.57-3.33) into the corresponding oximes (log K_{ow} 5.36-6.13), resulting in a highly selective and sensitive method (LOD 21-32 pg/mL) [10]. This is illustrated in Fig. 5 showing the analysis of a beer sample spiked at 500 pg/mL with a mixture of 3 aldehydes (*E*-2-octenal, E-2-nonenal, and *E*, *E*-2,4-decadienal). The extraction was performed by derivat-SBSE using a 47 µL PDMS Twister on a 30 mL sample (10-fold diluted with water) after addition of 0.45 mL of PFBHA solution (10 mg/mL). After extraction, the oximes were thermally desorbed in splitless mode and analyzed on a 30 m length × 0.25 mm i.d. × 0.25 µm df HP-5MS column using MS detection in selected ion monitoring (SIM) mode. The excellent sensitivity is clearly illustrated.

Analysis of tropical aroma thiols in beer

Polyfunctional thiols in food and beverages have received special attention due to their extremely low odor threshold levels and high sensory impact. Several thiols, e.g. 4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH), and 3-mercaptohexyl acetate (3MHA), are well known for their contributions to the

fruity/citrus/tropical aroma. For strongly hopped beer, the odor threshold levels are in the low ng/L range [11], and consequently very sensitive and selective methods are needed. A derivat-SBSE method was developed for these thiols by Ochiai et al. [12] using a 24 µL PDMS Twister and the simple ester of propiolic acid as derivatization reagent. After extraction, thermal desorption (TD)-GC-QQQ-MS in selected reaction monitoring mode (SRM) was performed. In Fig. 6, SRM chromatograms of a beer sample spiked in the range from 1-10 ng/L are superimposed on the SRM chromatogram obtained for the non-spiked beer sample. At 17.18 min, thioacrylates of 3MHA (*cis*-derivative) appears in the SRM chromatogram of the beer samples. This compound was detected below its odor threshold level of 5.0 ng/L.

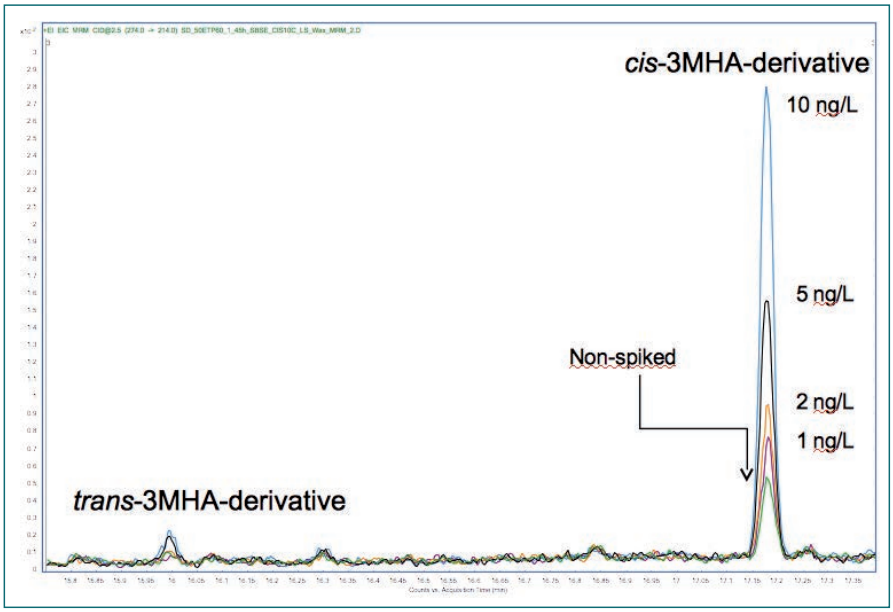


Figure 6: Selected reaction monitoring (SRM) chromatograms of spiked (1-10 ng/L) and non-spiked beer obtained from derivat-SBSE-TD-GC-MS/MS.

Conclusion

Multi-SBSE (^{m}BSE) and SBSE with *in-situ* derivatization (derivat-SBSE) can be successfully applied to the non-targeted analysis of a wide range of aroma compounds, and target analysis of off-flavors and key aroma compounds, respectively. These two SBSE modes can be considered as very complementary for aroma/off-flavor analysis of beverages, and can offer even more information content and/or improved sensitivity/selectivity when combined with high-end GC-MS (e.g. ^{m}SBSE with GC-high-resolution TOF-MS, and derivat-SBSE with GC-QQQ-MS).

Acknowledgements

All co-authors of the references [6, 10, 12] and Dr. Kevin MacNamara (Dublin, Ireland) are thanked for their contributions.

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[12] N. Ochiai, K. Sasamoto, T. Kishimoto, in preparation.

Dynamic Headspace (DHS) and DHS Large

Improved Limits of Detection

The GERSTEL DHS is an accessory module for the MultiPurpose Sampler (MPS) in combination with the Thermal Desorption Unit (TDU). DHS offers significantly improved limits of detection combined with the ruggedness and ease of use of static headspace analysis. The headspace above a solid, viscous or liquid sample is purged with inert gas and analytes are transferred to, and concentrated on, a replaceable adsorbent trap. The process is fully automated, including trap desorption in the TDU and GC/MS analysis.

The GERSTEL DHS Large (DHS L) is an extension of the DHS option for sample containers with a volume of up to 1 L. A single sample DHS L extension or an autosampler for up to 11 samples can be chosen.

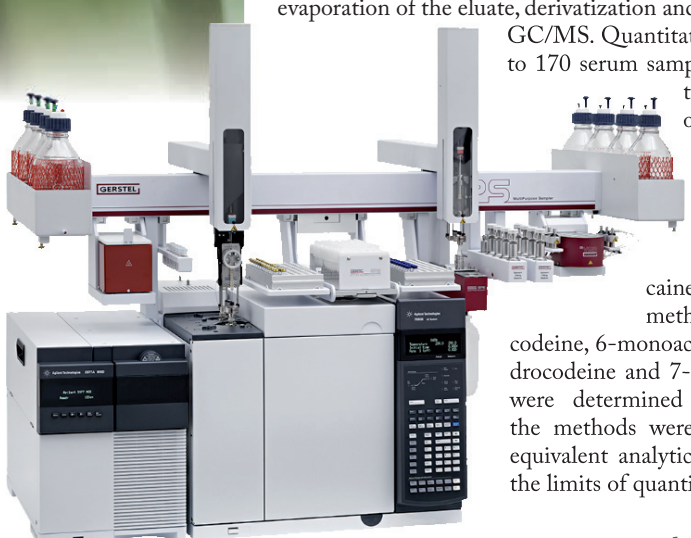
DHS Large can be used for material emissions screening and for volatiles in consumer products mong other application areas.

Efficiently Automated Drug Analysis

Comprehensive automation of SPE-GC/MS based analysis of serum and other matrices for opioids, cocaine and metabolites.

By Oliver Lerch, GERSTEL GmbH & Co. KG, Eberhard-Gerstel-Platz, 45473 Mülheim an der Ruhr, Germany, and Oliver Temme, Thomas Daldrup, University Hospital Düsseldorf, Institute of Legal Medicine, Department of Forensic Toxicology, Moorenstrasse 5, 40225 Düsseldorf, Germany

Analyzing blood serum for opioids, cocaine and metabolites is a routine task in forensic laboratories. The most commonly used methods involve several manual or partly-automated sample preparation steps such as protein precipitation, solid phase extraction, evaporation and derivatization followed by GC/MS or LC/MS determination. In the work reported here, a comprehensively automated method is compared with a validated, partly-automated routine method. Following manual protein precipitation, the automated method relies on a GERSTEL MultiPurpose Sampler (MPS) to perform all remaining sample preparation steps. These include solid phase extraction (SPE), evaporation of the eluate, derivatization and introduction to the GC/MS. Quantitative analysis of close to 170 serum samples, as well as more than 50 samples of other matrices like urine, different tissues and heart blood, was performed using both methods. Cocaine, benzoylecgonine, methadone, morphine, codeine, 6-monoacetylmorphine, dihydrocodeine and 7-aminoflunitrazepam were determined quantitatively and the methods were found to produce equivalent analytical results even near the limits of quantification.



GC/MS System used for the determination of opioids, cocaine, and metabolites in serum, as well as for THC and metabolites in serum [3]. The system performs automated SPE, evaporative concentration, derivatization as well as introduction to the GC/MS.

Instrumentation

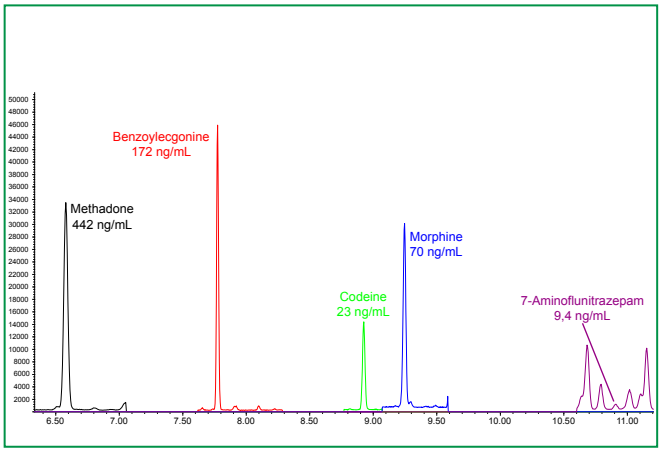
A GERSTEL MultiPurpose Sampler (MPS) was used, configured with a 2.5 mL syringe with gas supply for the sample preparation steps and a 10

Source

[1] O. Lerch, O. Temme, T. Daldrup: „Comprehensive automation of the solid phase extraction gaschromatographic mass spectrometric analysis (SPE-GC/MS) of opioids, cocaine, and metabolites from serum and other matrices“, Anal. Bioanal. Chem. 406 (2014) 4443; Free download of the article under: http://link.springer.com/article/10.1007/s00216-014-7815-7

[2] O. Lerch, O. Temme, T. Daldrup: „Comprehensive Automation of the SPE-GC/MS Analysis of Opioids, Cocaine and Metabolites from Serum and Other Matrices“, GERSTEL AppNote 07/2013 (www.gerstel.com/pdf/p-gc-an-2013-07.pdf)

Standard solid phase extraction cartridge (bottom) adapted for automated SPE.



Extracted ion chromatogram resulting from a real serum sample. Quantified compounds are named.

µL syringe used for sample injection into a GERSTEL Cooled Injection System (CIS 4) coupled to a 7890 GC/5975 MSD (Agilent Technologies). The MPS was equipped with modules for solid phase extraction (GERSTEL SPE), for evaporation of solvents under controlled vacuum and temperature (GERSTEL ™VAP), for shaking under controlled temperature conditions (Agitator) and for supplying large volumes of solvents (GERSTEL SFS).

Materials

All analytes and deuterated analogues were certified standards purchased from Lipomed AG or LGC Promochem GmbH. All solvents and salts were of analytical grade and purchased from VWR. N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) for silylation was purchased from Macherey-Nagel or Sigma-Aldrich. Bond Elut Certify 130 mg, 3 mL format SPE cartridges from Agilent Technologies were used. For automated SPE these cartridges were cut at the top, equipped with a transport adapter and a disposable syringe needle (canula). Blood, urine and tissue samples were taken from authentic forensic cases at the Institute of Legal Medicine in Düsseldorf.

Preparation of standards and solutions

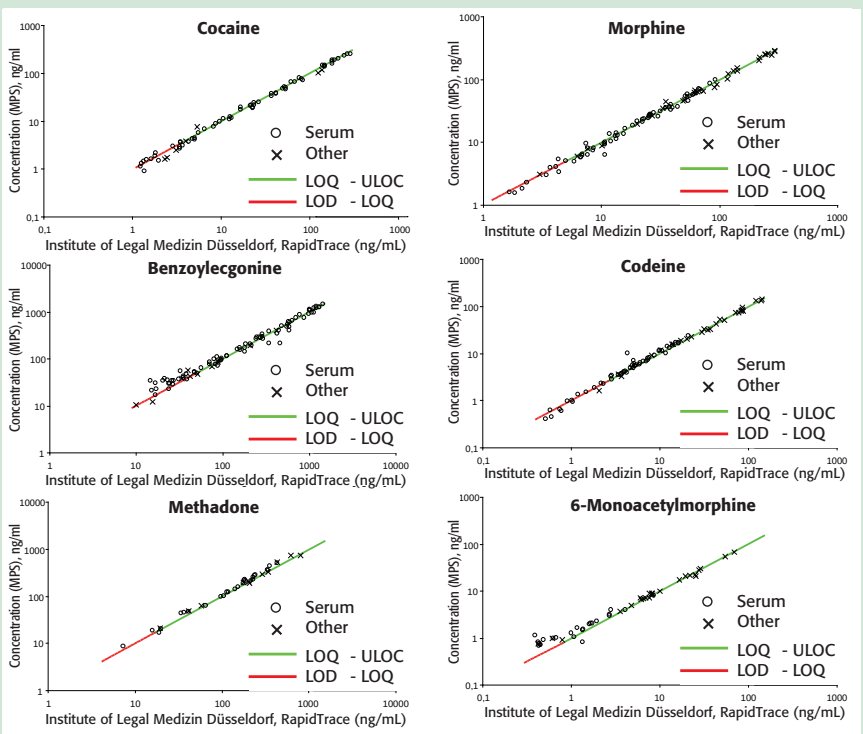
For calibration, multi-compound calibration solutions and one multi-compound internal standard solution containing deuterated analogues of every analyte were prepared in methanol. The calibrations ranged from 25 to 1500 ng/mL (methadone), from 50 to 1500 ng/mL (benzoylecgonine), from 5 to 150 ng/mL (codeine), from 5 to 300 ng/mL (cocaine, dihydrocodeine, morphine), and from 2.5 to 150 ng/mL (7-aminoflunitrazepam, 6-monoacetylmorphine) respectively and were calculated for 0.6 mL serum sample (nine levels). A 20 µL aliquot of the internal standard solution was added to each individual sample, calibration sample and quality control sample.

In agreement with the guidelines of the Society of Toxicological and Forensic Chemistry (GTFCh, Germany) a blank injection of pure derivatization solution was performed after every sample, quality control or calibration sample.

Results and Discussion

The validated, partly-automated routine analysis method was successfully automated using the MPS – from dilution of the sample after protein precipitation to injection into the GC/MS. Some modifications were necessary:

Dilution of the supernatant after protein precipitation was partly performed in the autosampler syringe. A 0.75 mL aliquot of the supernatant was diluted with 0.75 mL phosphate buffer and 0.75 mL of this mixture was aspirated. After that, another 1.75



Correlation of determined analyte concentrations in double logarithmic scale. Line with a slope of one – representing complete equivalence of results – is shown.

ng/mL: Nanogram per milliliter or nanogram per gram for tissue respectively; Other: Other matrices than serum - urine, blood, lyophilized kidney tissue, heart blood, lyophilized and native brain tissue; LOD: Limit of detection; LOQ: Limit of quantification; ULOC: Upper limit of calibration.

Analysis Conditions

MPS

Syringe:	10 µL
Injection Volume:	2 µL

CIS inlet

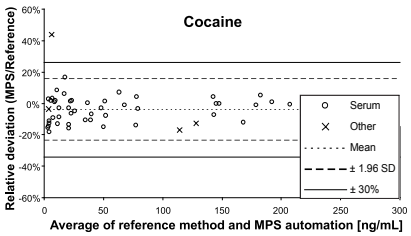
Temperature:	50 °C -12 °C/s - 280 °C (5 min)
Pneumatics:	Splitless 3 min
Liner:	Quartz wool deactivated

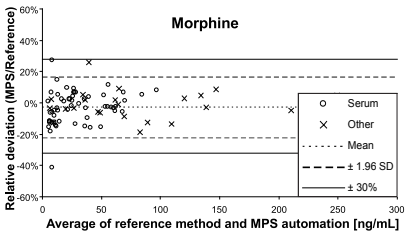
GC

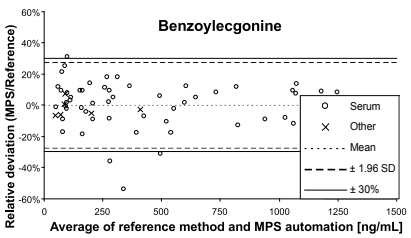
Oven Temperature:	140 °C (1 min) - 120 °C/min - 225 °C (5.29 min) - 120 °C/min - 275 °C (5.2 min)
Post Run:	300 °C (2.5 min)
Column:	Rxi-5Sil MS (Restek) 30 m, di = 0.25 mm, df = 0.25 µm
Pneumatics:	Helium, constant flow, 1 mL/min

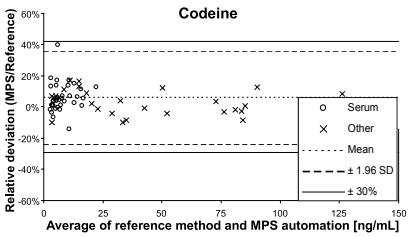
MSD

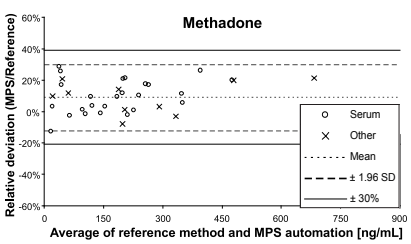
Detection mode:	SIM mode

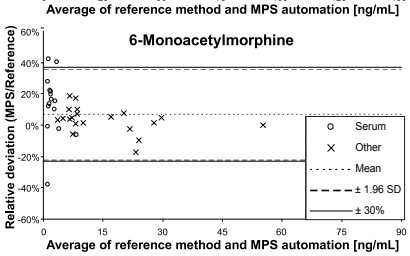












Relative deviations of measured concentrations displayed in Bland-Altman-plots.
ng/mL: Nanogram per milliliter or nanogram per gram for tissue respectively; Other: Other matrices than serum - urine, blood, lyophilized kidney tissue, heart blood, lyophilized and native brain tissue; LOD: Limit of detection; LOQ: Limit of quantification; ULOC: Upper limit of calibration; SD: Standard deviation of relative deviations.

Limit of detection (LOD), limit of quantification (LOQ) and upper limit of calibration (ULOC) for each compound using the validated, partly-automated reference method.

Analyte	LOD [ng/mL]	LOQ [ng/mL]	ULOC [ng/mL]
Cocaine	1.1	3.5	300
Benzoylecgonine	9	47	1500
Methadone	4.2	16.7	1500
Morphine	1.2	4.9	300
Codeine	0.4	2.6	150
6-Monoacetylmorphine	0.3	0.8	150
Dihydrocodeine	0.8	4.2	300
7-Aminflunitranzepam	0.6	2.5	150

Quantifier and qualifier ions for analytes and internal standards.

Compound	Quantifier [m/z]	Qualifier [m/z]
Cocaine	182	303, 198
Cocaine-d_3	185	306, 201
Benzoylecgonine	361	256, 346
Benzoylecgonine-d_3	364	259, 349
Methadone	223	294, 236
Methadone-d_3	226[a], 303[b]	303[a], 318[b], 242
Morphine	429	220, 401
Morphine-d_3	432	223, 404
Codeine	371	234, 343
Codeine-d_3	374	237, 346
6-Monoacetylmorphine	399	340, 400
6-Monoacetylmorphine-d_3	402	343, 403
Dihydrocodeine	373	315, 358
Dihydrocodeine-d_8	379	318, 364
7-Aminflunitranzepam	326[b], 355[a]	326[a], 356[a], 327[b], 354[b]
7-Aminflunitranzepam-d_7	362	333, 363

[a] Qualifier ion used in partly-automated analysis method.
[b] Qualifier ion used in fully automated analysis method.

mL phosphate buffer was aspirated resulting in the final dilution (same as in the reference method). This solution was added to the SPE cartridge and the process was repeated once to transfer the entire sample. The elution volume was reduced from 2 mL to 1.9 mL. The first 0.6 mL was discarded and the last 1.3 mL was collected based on an established elution profile. The derivatization time was shortened from 30 min to 5 min with shaking at 90 °C by employing a mixture of isooctane/pyridine/MSTFA 14/5/1 (v/v/v) instead of the isooctane/MSTFA 19/1 (v/v) mixture originally used.

Close to 170 serum samples and more than 50 samples of other matrices like urine, different tissues and heart blood were analyzed by both methods. Results are equivalent as can be seen in the double logarithmic line- and Bland-Altman-plots. This is true for serum samples and also for alternative matrix samples. Although results between the limit of quantification and the limit of detection may not be reported routinely, they are included in the line plots. Even in this concentration range the method equivalence is obvious. Since only a couple of samples were positive for dihydrocodeine and 7-aminoflunitrazepam these results are not plotted. Samples and quality control samples were also in good concordance for these compounds.

No carryover for any of the compounds could be detected when extracting blank serum after real samples. By overlapping sample preparation steps with the GC/MS run a throughput of around 29 samples per day could be achieved, which is comparable with the partly automated reference method.

The analyses were performed in different laboratories by different personnel at different times proving the ruggedness of the instrumentation and methods and the suitability for routine forensic analysis tasks.

Automated sample preparation

Condition SPE cartridge with 2 mL methanol and 2 mL phosphate buffer (pH 7.9)

Dilute the supernatant of the protein precipitation in the SPE syringe and add the diluted sample to the SPE cartridge

Wash the cartridge with 2 mL water, 2 mL acetic acid, and 2 mL methanol.

Dry cartridge briefly using a flow of nitrogen

Elute with 1.9 mL of dichloromethane/isopropanol/ammonia. The first 0.6 mL are discarded and the following 1.3 mL are collected in a vial

Evaporate the eluate to dryness at 70 °C, 8 kPa and 300 rpm in the *m*VAP station

Reconstitute in 200 µL isooctane/pyridine/MSTFA 14/5/1 (v/v/v)

Shake for 5 min at 90 °C for derivatization

Inject 2 µL into the GC/MS

Calibration solutions were treated analogous to the eluates.

Manual sample pretreatment

All liquids (urine, blood, serum) were treated identically:

1. Protein precipitation by drop-wise addition of a mixture of 0.6 mL sample, 0.1 mL water and 20 µL internal standard solution to a mixture of 1 mL acetonitrile and 0.1 mL isopropanol.
2. Mixing and centrifugation.
3. Transfer of an aliquot (0.75 mL) of the supernatant to individual vials for both analysis methods.

Tissues (brain and kidney, native and lyophilized) were homogenized. An aliquot of approximately 0.6 g was weighed and handled like the liquids above though the acetonitrile/isopropanol solution was added to the sample/standard mixture. The protein precipitation steps could be automated using a centrifuge option with the MPS, but this was not within the scope of this study.

Conclusions

The following achievements were made:

- Comprehensive automation of a validated, partly automated analysis method for opioids, cocaine and metabolites from blood serum and other matrices.
- Analysis results of the methods were found to be equivalent based on GTFCh recommendations.
- The automated method proved to be rugged and suitable for routine analysis in forensic laboratories.
- The automated method saves manual work and reduces the risk of human error. It generates a throughput of 29 samples per day, which is similar to the reference method and is well sychronized with the GC/MS analysis time.
- The analysis system is highly flexible and can reproduce manual sample preparation workflows. Therefore it can be used to automate other validated GC or LC analysis methods or for stand-alone automation of sample preparation.

Inside the laboratories of Schülke & Mayr GmbH

The MPS handles the Night Shift

Analytical Quality Control (QC) of pharmaceutical products follows a strict set of guidelines. The quantity of ingredients, standards and solvents used must be accurately determined; stability tests must be performed; analytical methods must be validated, the performance of analytical instrumentation must be verified and certified; and replacement of consumable parts and preventive maintenance must be scheduled at regular intervals. A key element in the analytical Quality Assurance program at the Pharmaceutical producer Schülke & Mayr GmbH in Germany is the GERSTEL MultiPurpose Sampler (MPS) with integrated Weighing Option.

Schülke & Mayr GmbH was founded in 1889 in Norderstedt near Hamburg, Germany and is today a wholly owned subsidiary of Air Liquide. The company is a leading international provider of products for industrial hygiene, infection prevention, Microbiological Quality Management (MQM), as well as chemical preservatives or stabilizers for technical products such as fuels and oil drilling liquids. The company has around 800 employees worldwide, of which 550 are located in Germany. Around ten percent of staff work in R&D at company Headquarters in Norderstedt.

Schülke & Mayr produces and sells disinfectants, antiseptic agents, preservation agents, biocides, medical skin care products, active agents for deodorants, and system cleaning liquids. Among their customers are hospitals and other medical facilities, as well as pharmaceutical companies, cosmetics producers, and food companies. Fuel producers add preservatives from Schülke & Mayr to their diesel fuels, and emulsions from the company are used for oil drilling and production.

Since the bulk of the products from Schülke & Mayr are used for medical, clinical or pharmaceutical purposes, the company is officially classified as a pharmaceutical company. A requirement for getting and maintaining this classification is that guidelines for product monitoring and quality control must be strictly adhered to:

- Good Manufacturing Practice (GMP)
- German Medicinal Products Act (AMG)
- German Pharmaceutical and Active Ingredient Production Guidelines (AMWHV)
- European Union GMP Guidelines
- EU Regulation 1223/2009 (Cosmetics Regulation)
- EU Regulation 528/2012 (Biocides Regulation).

Schülke & Mayr Headquarters in Norderstedt near Hamburg, Germany.

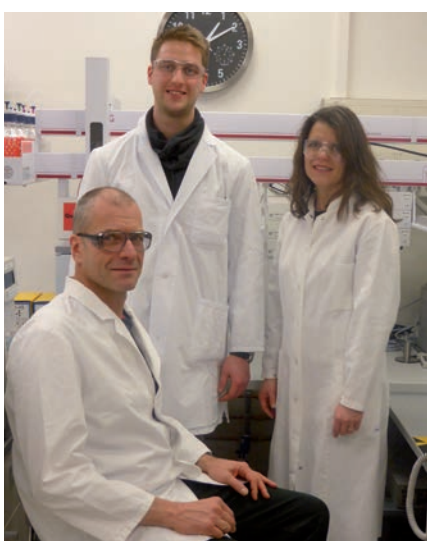
These regulations require strict control of incoming raw materials, continuous monitoring of product intermediates and control of active ingredient concentrations in the final products. Finished products regularly undergo stability testing to ensure that there is no significant change in product safety and performance as a result of aging processes. The regulations also require regular scheduled inspection and validation of measuring and testing equipment.

Text and images: Guido Deussing

A view of the Schülke & Mayr laboratories.

No Quality Control without instrumental analysis

Schülke & Mayr perform all chemical analysis in-house. The analytical techniques used for most of this work are Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC). Analytical methods are developed in the company's Quality Control (QC) laboratory and validated according to regulatory guidelines. Long-time employee Andreas Teevs from the Quality Control and Process Control Department is the key person charged with overseeing analytical method development, method validation, instrument qualification, as well as method implementation and routine analysis. Schülke & Mayr has around 40,000 samples to analyze each year, 90 % of which are performed using GC and HPLC. The GERSTEL MultiPurpose Sampler (MPS) performs automated sample preparation of ten thousand mainly liquid samples every year. "The number of samples has grown dramatically over the past years," reports Mr. Teevs, "and this has been a sizeable challenge for the company and the QC laboratory team. Just in the past five years, the laboratory staff has doubled in size." However, in spite of the growth in the number of samples that must be analyzed, the number of employees in the laboratory will not change in the foreseeable future. In order to increase lab productivity without adding staff, while continuing to meet the required high standards, introducing a night shift was seriously considered. "An extra shift would of course have improved the utilization of the laboratory instrumentation and laboratory productivity, but having to work nights was considered by most to be an unattractive option and an extra burden. Every one of us would have had to make big adjustments," said Andreas Teevs. After considering the alternatives, a more attractive solution was found: It was decided to increase the level of automation in the laboratory. Today, the GERSTEL MPS handles the night shift.



As part of their daily tasks, Andreas Teevs (left) and his colleagues Michael Bosnak and Beate Teevs-Aschinger are responsible for developing and implementing analytical methods and qualifying the analysis instruments.

No reliable analysis without precise weighing

"Analyzing a large number of samples overnight without the need for a staff presence to prepare samples, generate standards, and to monitor the process is not just a great idea in theory, it actually works!" says Andreas Teevs. But there was one condition that had to be met in order to ensure the success of implementing such a significant amount of automation: The automated sample and standard preparation system had to include an integrated balance. This is required, not only to weigh samples, but also to generate standard and reagent solutions as well as extensive dilution series for calibration and quality assurance purposes. Today, in many cases, Schülke & Mayr have replaced using standard pipettes with precise liquid dispensing based on the GERSTEL MPS and its automated Weighing Option. "It is easy to see why," says Mr. Teevs: "The accuracy of a 1000 µL syringe is greater than 99.5 % with a relative standard deviation of less than 0.2 %. This means that the MPS provides more accurate results with higher precision than a person using a manual pipette."

When they prepared a laboratory restructuring plan that focused specifically on improving laboratory automation, Mr. Teevs and his team started searching for an autosampler with an integrated weighing option. "We found what we were looking for in the GERSTEL MPS, specifically the Dual Head version, which enables the use of two different syringe sizes that can dispense a wide range of volumes accurately and efficiently without time-consuming syringe changes." The MPS weighing option offers the possibility to monitor, control and document the volumes dispensed. But the MPS also performs all standard sample preparation steps for GC and HPLC and injects the sample into the chromatography system.

A well balanced selection of laboratory equipment

Andreas Teevs and his colleagues currently work with several MPS systems: A standalone MPS WorkStation is used to add internal standards and generate dilution series. An XL version of the MPS, called "the bridge", handles sample preparation for two GCs. This is combined with sample introduction to the GC on the left and delivering a vial with a prepared sample into the standard GC autosampler on the right hand side. A third MPS is mounted on another GC system. Each MPS is equipped with

the Weighing Option, i.e. with a laboratory balance, in this case a Sartorius ME 235S OCS. Thermostatically controlled trays are used to store standard and reagent solutions at a constant temperature of 20 °C. Finally, each sampler has two towers (Dual Head version), enabling the simultaneous use of two different syringe sizes. The weighing step is freely selectable in the PrepSequence method settings in the GERSTEL MAESTRO Software (please see workflow diagram). "The resulting weighing protocol can be exported as a Microsoft Excel file or as semicolon separated (CSV) text file for further processing, for example, in a LIMS or Chromatography Data Handling System," says Andreas Teevs.

"Does the complete automation save time? Probably not a lot in the sense that analyses are finished faster", says the laboratory manager. "But the MPS with weighing option has enabled us to work both more efficiently and more productively". The MPS performs analyses overnight without staff watching over it. When the staff comes to work in the morning, analysis results are quickly processed and evaluated and the production batches released. This has led to reduced waiting periods in production, which is of course important. "All in all," says Mr. Teevs, "the MPS handles the complete analysis, which is something we could only dream of before." The staff is still trained and able to perform all sample preparation steps manually in case any problems should arise with the laboratory automation system.

Monitoring instrument performance – required and necessary

Where pharmaceuticals or products for medical use are manufactured, a high level of precision and accuracy is required. "All analysis instruments must undergo regular detailed checks and controls in order to find potential sources of errors and inaccuracies before they develop into something bigger," says Andreas Teevs. This is another important aspect in which the MPS with automated weighing option plays a key role. Analysis instruments can significantly influence the accuracy of the analysis results, as Mr. Teevs has seen in his 35 years at Schülke & Mayr.

Since the GERSTEL MPS was introduced to the Schülke & Mayr laboratories, it has enabled a more comprehensive control of variations in analytical data resulting from system deviations and instrument wear. As an example, a gas bubble could form inside a syringe when sample is drawn leading to a deviation in sample volume. "This could happen," says Andreas Teevs, "If the syringe barrel has been worn from extensive use and as a result, air is being drawn in between the barrel and the plunger." If the sampler is equipped with a balance, the resulting variations in sample volume can be seen directly, trends can be followed and countermeasures such as replacement of consumable parts can be scheduled. This helps to ensure that the laboratory always delivers reliable and accurate data. "Without a weighing option," says the expert, "A regular or ongoing system monitoring and control would never be this easy – or even possible."

Stability Test

Pharmaceuticals are subjected to stability tests on a regular basis: During product development, during the approval phase, and after the product has been approved for sale into the market (ongoing stability tests). It is established, whether contamination is formed or introduced into the product and, if so, which compounds are found and in which concentrations. Even after years on the market, product stability can potentially change leading to changes in estimated shelf-life or even product recalls. Samples of different batches of a product are stored under various storage and temperature conditions. During this process, samples are regularly taken and extensive analysis performed.

Every GERSTEL MPS in the Schülke & Mayr laboratories is equipped not only with a Weighing Option, but also with two towers (Dual Head version) in order to accurately dispense a wide range of volumes without having to go through the process of changing syringes. "The Bridge", an XL version of the MPS spans two GC systems. Prepared samples can be introduced to the GC on the left and vials with prepared samples can be placed into the autosampler of the GC on the right hand side.

Steps that are performed automatically in the MPS weighing option

Vial is placed in the vial weighing position in the laboratory balance

Weighing is performed, the value is exported and entered into the weighing protocol

The vial is returned to the MPS tray and sample is dispensed into the vial

The vial is returned to the weighing position

Weighing is performed, the value is exported and entered into the weighing protocol

The MPS returns the vial to its position in the sample tray

When they prepared their laboratory restructuring, Schülke & Mayr focused on automated sample preparation. Mr. Teevs and his team specifically wanted an autosampler with integrated weighing option that can dispense a wide range of volumes accurately and efficiently without time-consuming syringe changes while offering the possibility to monitor, control and document the dispensed volumes. They found all this and more in the GERSTEL MultiPurpose Sampler (MPS).

Clean and Sensitive Determination of Pesticides

Online SPE sample clean-up based on replaceable cartridges provides higher sensitivity and lower limits of detection – without the risk of carry-over.

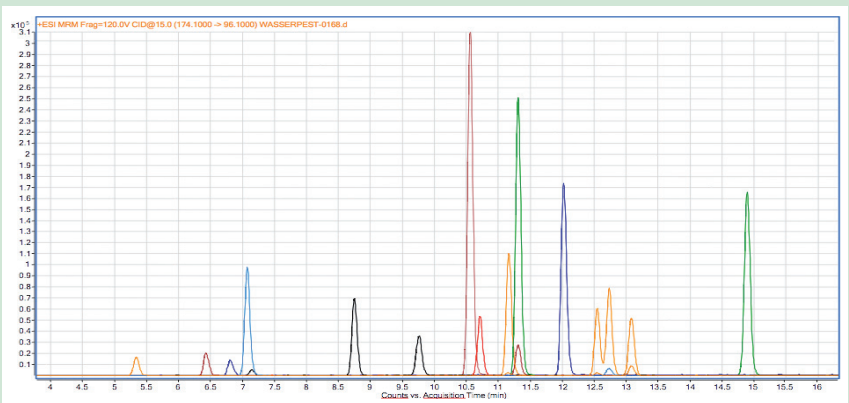
By Norbert Helle and Franziska Chmelka, TeLA GmbH, Geestland, Germany, norbert.helle@tela-bremerhaven.de

Analyzing food, water and soil for pesticide residues constitutes a significant part of the workload in laboratories that specialize in food safety and environmental analysis. Given the vast and increasing number of samples, efficiency is key and the strategy has to be automation – sensible and efficient automation.

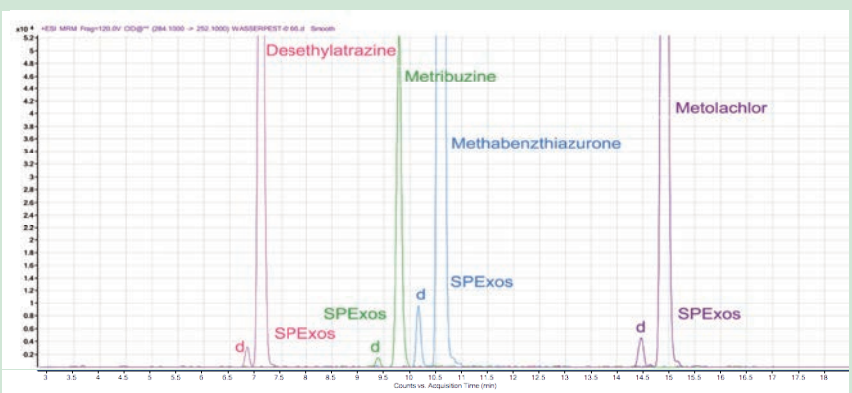
The weed killers (herbicides) most frequently used for crop protection in fruit production are based on phenyl urea or triazine compounds. Both these compound classes enter the plant through the roots and are transported to the chloroplast where they interfere with the process of photosynthesis, ultimately leading to the death of the plant. It is in the nature of weed eradication through chemical agents that residues applied to the upper soil layers will reach deeper layers where the crop roots are located and will be transported into both the wider environment and the food chain. Pesticides and herbicides accumulate in ground and surface waters which are also our drinking water reservoirs. To avoid any danger to human health, governments have limited the maximum allowable concentrations for such residues in water to 0.1 µg/L with a required limit of determination ten times lower at 0.01 µg/L. Reaching this limit of determination normally requires direct introduction to a highly sensitive HPLC-MS/MS system, but not all compounds can be determined

Pesticides are found in surface and ground water.

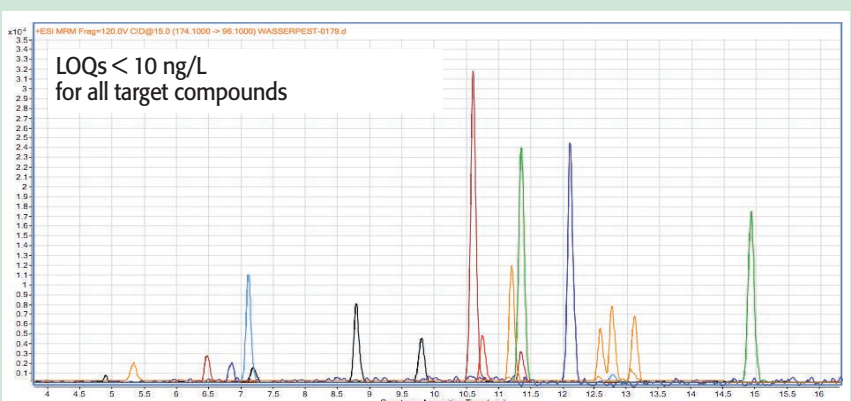
Sample preparation and LC-MS/MS analysis are performed simultaneously in parallel.



Standard mixture of the target analytes (100 ng/L) following extraction and cleanup in the SPE^{XOS} unit.



LC-MS/MS peak overlays resulting from SPE^{XOS} clean-up and analysis of 1 mL (SPE^{XOS}) and direct injection of 50 µL (d) of a pesticide standard-mix (100 ng/L). Compared to direct injection of 50 µL, a factor of 50 increase in sensitivity was achieved without peak broadening. Please note, the SPE^{XOS} process leads to retention time delays of up to 30 s.



Standard mixture of the target analytes (10 ng/L) following extraction and cleanup in the SPE^{XOS} unit. A Limit of Quantitation (LOQ) below 10 ng/L was achieved for each analyte.

this way, especially not early eluting compounds. To circumvent these obstacles, larger volumes (up to 100 µL) are injected directly into the LC-MS/MS system – or the compounds in question are concentrated on fixed on-line SPE cartridges, a widely used procedure. However, both these alternatives are associated with certain drawbacks: The introduction of a large sample volume frequently leads to peak broadening. In addition, highly sensitive and expensive analysis instrumentation would typically be needed to reach the required limits of determination. Analyzing a series of samples using only a single fixed cartridge to concentrate analytes, on the other hand, will regularly lead to sample-to-sample carry-over and incorrect results with the need to re-analyze especially high concentration samples along with several of the following samples. Since the cartridge is typically loaded with sample from one side and eluted from the other side, the clean-up effect is also limited because the analytes don't have to traverse the entire column.

Online Solid Phase Extraction with clean-up

The goal of this project was to reach the required limits of determination based on injecting only 1 mL of water sample. In order to combine the advantages of on-line SPE concentration with the required

Automated sample prep workflow

[LOAD]
Load the SPE^{XOS} cartridge

[SPE PREP]
Condition with 4 mL of methanol

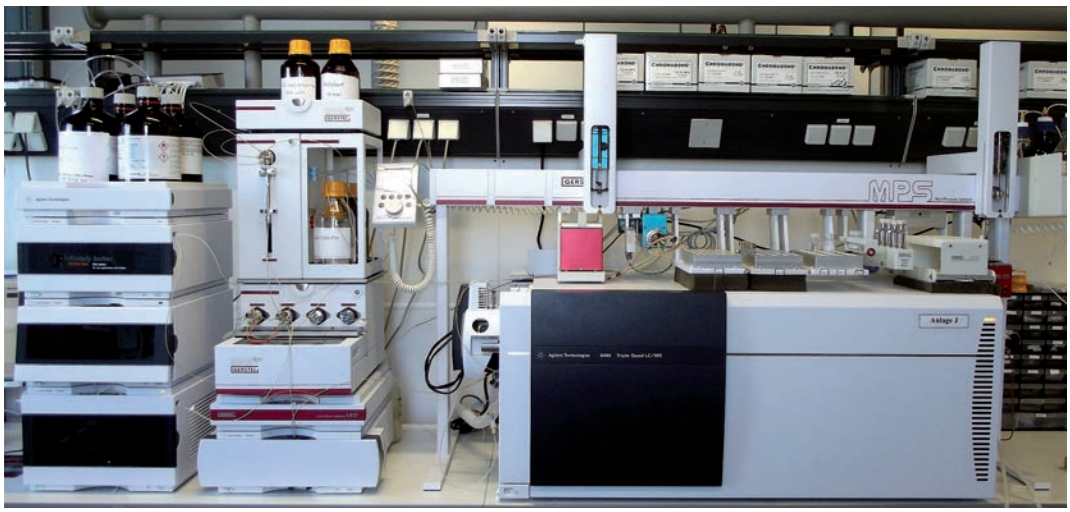
[SPE PREP]
Condition with 4 mL of water

[ADD]
Load 1 mL of sample into the MPS injection valve loop.

[SPE PREP]
Transfer the sample from the loop to the SPE^{XOS} cartridge using 1.5 mL of water

[SPE PREP]
Valve switch: The flow from the binary pump is switched to the SPE^{XOS} cartridge

[INJECT]
Start signal for the Agilent MassHunter Software and the LC-MS/MS system.



The SPE^{XOS}-HPLC-QqQ-MS system used for the determination of phenylurea and triazine herbicides.

Action	MPS	Method / Value	Source	Vial	Destination	Vial
PREP Vials 1-5		No Overlap				
CARTRIDGE	Left MPS	LOAD	Left Rack		Left Clamp	
SWITCH INJ	Left MPS	Active			LC Vlv1	
SPE PREP	Left MPS	Cond MeOH 4000µl				
SPE PREP	Left MPS	Cond_AquaUltraPure 4000µl				
ADD	Left MPS	Sample Introduction 1000µl-10ml/Vial	Tray2,VT32-10		LC Vlv1	
SWITCH INJ	Left MPS	Standby			LC Vlv1	
SPE PREP	Left MPS	Rinse with 1500µl AquaUltraPure				
SWITCH INJ	Left MPS	Active			LC Vlv1	
SPE PREP	Left MPS	Valve Switch and Elution with LC Pump				
INJECT	Left MPS	WATER_PEST-SPExos-0µl.mth	Tray2,VT32-10		LC Vlv1	
ADD	Left MPS	Wash valve MeOH	SFS2Wsh1		LC Vlv1	
ADD	Left MPS	Wash valve H2O	SFS2Wsh2		LC Vlv1	
END						

Screenshot of the sample prep work flow as seen in MAESTRO software. Method and sequence set-up is easy and uncomplicated based simply on selecting the necessary steps from a pull-down menu or using copy-paste from existing methods and sequences.

clean-up, we configured our LC-MS/MS system with a separate online SPE Module (GERSTEL SPE^{XOS}), which is based on replaceable cartridges. Some technical detail: SPE^{XOS} cartridges contain only 50 mg of sorbent compared with 100 to 1000 mg of sorbent used in regular SPE cartridges. This means that the SPE process can be completely integrated into the HPLC process since significantly less solvent is required for analyte elution. SPE^{XOS} is integrated into the system between the autosampler (GERSTEL MultiPurpose Sampler, MPS) and the LC-MS/MS system (Agilent 1260 HPLC/6460 Triple Quad MS). Sample introduction to the HPLC follows online, i.e. the SPE eluate, and thus 100 % of the analytes, is transferred directly and quantitatively into the HPLC mobile phase. In practice, the analysis requires only a very small amount of sample, in the order of 1-5 mL, and the complete process is fast enabling high throughput. System control for the complete process from sample preparation through introduction to the LC-MS/MS is conveniently controlled by mouse-click using the GERSTEL MAESTRO software. Sample preparation and analysis can be performed in parallel using the PrepAhead function to ensure that the next sample is always prepared and ready for introduction when the LC-MS/MS system is ready for the next run.

We had anticipated that the addition of the SPE^{XOS} module would provide several interesting and useful benefits. For example, since the cartridges are exchangeable we expected carry-over to be eliminated. Further, we expected the clean-up effect to be superior since analytes had to travel the entire length of the sorbent bed. Further, different – even specific – clean-up steps were conceivable since we could freely select sorbent materials. Finally, focusing the analytes on the analytical column after they had been transferred quantitatively from the SPE column would lead to sharp peaks and improved separation and sensitivity – thus the theory and the high expectations.

LC/MS method parameters

Mobile phase:

Flow: 0.35 mL/min; A - Formic acid 5 mmol/L; B - Acetonitrile; 0 min: 5 % B – 10 min: 50 % B – 22 min: 100 % B – 22.1 min: 5 % B - End: 28 min.

Column Oven Temperature:	60 °C
Column material:	C18
MSD Source:	Agilent Jetstream, ESI positive
Gas Temperature:	300 °C
Gas Flow:	9 L/min
Nebulizer:	45 psi
Sheath Gas Temperature:	270 °C
Sheath Gas Flow:	12 L/min
Capillary:	5500 V
Nozzle:	300 V

A glimpse at the technical details of the analysis

The practical analysis was performed as follows: The one and only manual sample preparation step was to load water samples into vials and place them in the proper positions on the MPS autosampler. All further steps were performed automatically, as specified in the instrument method.

Results and discussion

A method is only useful if it can prove itself in practiceal use. The idea of using online SPE for analyte concentration and as a clean-up step proved highly useful and SPE^{XOS} reliably replaced SPE cartridges between samples. In samples of only 1 mL volume, the following analytes were determined: metolachlor, metazachlor, diurone, terbuthylazine, metoxurone, methabenz-thiazurone, chloridazone, atrazine, metribuzine, chlorotolurone, isoproturone, metamitron, desethylatrazine and desisopropylatrazine.

Compared to a direct injection of 50 µL, we achieved a factor 50 increase in sensitivity – without peak broadening. Only the retention time was shifted with a delay of 30 seconds. Carry over effects were not observed when comparing injections of a standard [c = 100 ng/L] through the SPE^{XOS} system with subsequent blank injections. Apart from the increase in sensitivity, the additional clean-up step resulted in a significantly cleaner solution, which should have a positive long-term effect on system stability. Not least, a limit of determination of < 10 ng/L was reached for all compounds. The calibration resulted in good linearity throughout.

Metabolomics

Automated Sample Preparation Using the GERSTEL MPS Dual Head WorkStation

In metabolomics studies, relatively large sets of samples are processed to allow differentiation between sample types and the analytical variability must be lower than the biological variability. In order to achieve this, automating the sample preparation is a good first step, which can contribute significantly towards improving the repeatability of the total analytical procedure. Part 1of a series of 3 takes a closer look at Automated Ultrasonic Assisted Liquid Extraction and Filtration

By Koen Sandra, Frank David, Christophe Devos, Bart Tienpont, and Pat Sandra,
Research Institute for Chromatography, President Kennedypark 26, 8500 Kortrijk, Belgium

Metabolomics studies focus on the analysis of small molecules (MW<2000) in biological matrices from micro-organisms, plants, animals, and of human origin. Relatively large sets of samples are processed to allow differentiation between sample types and it is of course critically important to ensure that the analytical variability is lower than the biological variability. In order to achieve this, automating the sample preparation is a good first step, which can contribute significantly towards improving the repeatability of the total analytical procedure.

A typical metabolomics workflow includes extraction, fractionation or clean-up, derivatization, and a concentration step, followed by 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 this first article, we focus on extraction and filtration. In a second article, an automatic fractionation procedure based on solid phase extraction will be described and in a final article, we will describe the use of an automated derivatization procedure prior to GC analysis.

For the extraction of plant material, ultrasonic assisted liquid extraction is a well-established method. However, ultrasonic extraction is mostly performed manually. This is in part due to the fact that solid particulates can create a suspension in the extraction solvent, which can easily block syringes, making automated collection of the extract and subsequent injection into the GC or LC unreliable. Applying recently introduced tools for the GERSTEL MultiPurpose Sampler (MPS), extraction, filtration and further processing of samples can be automated.

This is illustrated by an automated sample preparation protocol developed for the ultrasonic extraction of glycosides and phenolic compounds from plant material for a metabolomics study. The implementation of screen filters to prevent blockage of the MPS syringe along with 0.45 µm replaceable filter cartridges to filter the extract have enabled direct injection of the sonicated and filtered samples into an LC/MS system without the risk of system contamination with sample matrix.

Experimental

Automated Extraction

A 60 mg sample of ground plant material is weighed into a 10 mL headspace vial. Before capping the vial, a 17 µm stainless steel screen filter (GERSTEL p/n 020006-050-00) is placed inside the vial. Next, automated extraction and filtration is performed using an MPS Dual Head WorkStation (Figure 1). Extraction solvent (5.8 mL of 75/25 methanol/water) is added using a 2.5 mL syringe, followed by 0.2 mL internal standard solution using a 1.0 mL syringe. The vial is then transported by the MPS to the ultrasonic bath (Figure 2) and sonicated for 30 min. An aliquot (400 µL) of the extract is transferred from the sample vial (from inside the stainless steel screen filter) and filtered by the MPS using a disposable 0.45 µm filter cartridge (Figure 3). Figure 4 shows the sample vials before and after sample preparation. The MPS configuration is detailed in Table 1, and the MAESTRO Prep Sequence is described in Table 2.

Figure 1: MPS Dual Head WorkStation configured for automated ultrasonic extraction and filtration.

LC/MS

An Agilent Technologies 1290 Series UPLC 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 water, acetonitrile and formic acid as the mobile phase constituents.

Results and Discussion

For a metabolomics study of glycosides and phenolic compounds in plant material, 86 samples were prepared using the automated Prep Sequence described above. Of the 86 samples, 18 were quality control (QC) samples that were used to assess the reproducibility of the

Table 1: MPS Dual Head WorkStation Configuration.

MPS Module	Description
Left Arm	2.5 mL syringe with magnet for 10 mL vials
Right Arm	1.0 mL syringe with gripper
Tray and Holder	Ultrasonic bath with holder for 10 mL vials (6 positions)
Tray and Holder	10 mL headspace vials (VT-32)
Solvent Filling Station	Extraction solvent (75/25 Methanol/Water) + Wash (Methanol)
Tray and Holder	Filtration Tray (0.45 µm filters)
Tray and Holder	1.5 mL high recovery vials (VT-98) with filtration cover
Waste	Waste unit for used filters

Table 2: MAESTRO Prep Sequence used for Automated Sample Preparation.

Action	Arm	Description
Add	Left MPS	5,800 µL 75/25 Methanol/Water (Pre-rinse)
Add	Right MPS	200 µL IS (Pre-rinse Methanol)
Move	Left MPS	Tray 10 mL → Ultrasonic
Ultrasonic		30 min
Move	Left MPS	Ultrasonic → Dry*
Move	Left MPS	Dry* → Tray 10 mL
Get	Right MPS	Get filter (Gerstel # 017450-103-00)
Filtrate	Right MPS	400 µL + 600 µL air, Filter from above
Put	Right MPS	Transfer filter to the waste receptacle

*Dry is the name of the position in the ultrasonic tray, in which excess water is removed from the outside of the vials.

Table 3: Targeted analysis of the 18 QC samples.

Compound	Accurate Mass	tR (min)	%RSD Area
Rutin	610.1450	4.444	5.32
Chlorogenic acid	354.0950	3.319	5.95
Salicylic acid D5 (IS)*	142.0570	3.349	13.67
Kaempherol*	286.0480	7.197	13.60

*Low intensity.

Figure 3: Picture of the MPS picking up a 0.45 µm filter from the filtration tray.

Figure 4: Sample vials at the start (left) and end (right) of the automated sample preparation protocol.

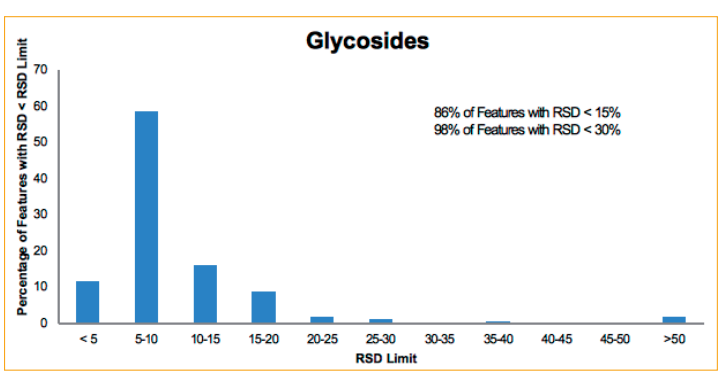


Figure 5: Bar plot showing the percentage of features with an area RSD value lower than the RSD limit (untargeted analysis).

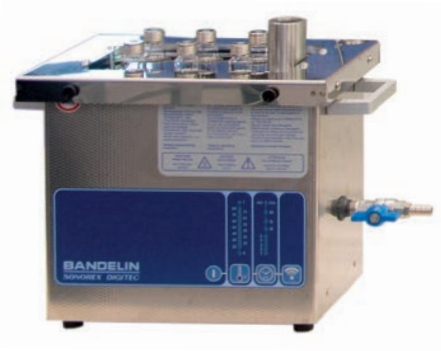


Figure 2: Ultrasonic bath option for the MPS WorkStation used for automated extraction of the plant material.

sample preparation and LC-MS protocol.

The combination of the consecutive filtration steps allowed unattended error-free sample preparation and injection sequences of 86 samples to be executed. The reusable stainless steel screen filter inside each sample vial prevents clogging of the MPS syringe needle by sample particulates dispersed in the extract. The liquid extract (methanol/water solution) was turbid after the ultrasonic agitation (see Figure 4) and additional filtration was needed. This was efficiently performed using the 0.45 µm disposable cartridge filters. Finally a clear extract was obtained that could be injected into the LC-QTOF system. All 86 samples were analyzed without any pressure increase on the 1290 UHPLC system.

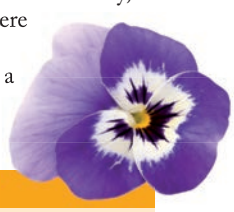
Both targeted and untargeted data analysis was performed on QC samples. For targeted analysis, the internal standard and a number of known compounds were selected and the area repeatability calculated; the results were excellent (Table 3). It should be noted that for metabolomics studies, the cutoff for area RSD values is typically 30 %. As can be seen from Table 3, the targeted analysis results obtained from the QC samples gave an area RSD of less than 14 % for the low intensity peaks, well within the limit for metabolomics data, and less than 6 % for Rutin and Chlorogenic acid.

For untargeted analysis, 590 features were considered. Plotting the area RSD values against RSD limits (Figure 5), it is clear that the results from the untargeted analysis were also excellent. As can be seen, 98 % of all features had area RSD values lower than 30 %, making them useful for further statistical evaluation.

Conclusions

The GERSTEL MPS Dual Head WorkStation is particularly useful for the automation of sample preparation in metabolomics studies. The combination of automated ultrasonic assisted liquid extraction and a dual filtration process results in extracts that can be analyzed directly by LC/MS. For the extraction of glycosides from plant material, in-vial stainless steel screen filters were successfully utilized to prevent blockage of the MPS syringe. Extracts were aspirated from inside the screen filter inserts, and taken through a further automated filtration step based on 4 mm 0.45 µm syringe filters before being injected into an LC/MS system. Following analysis of the quality control samples used in a metabolomics study, it was determined that the results obtained were highly repeatable.

In a following article, the automation of a SPE fractionation protocol applied in lipidomics will be described.



Glycosides

Glycosides are a class of compounds that contain a sugar and a non-carbohydrate moiety. The non-carbohydrate moiety is typically a small organic molecule. When the non-carbohydrate moiety is a phenol, the glycoside is for instance a flavonoid. In general, glycosides are secondary plant metabolites that are not involved in plant growth, development, or reproduction, but rather, in the interaction of a plant with the environment, such as UV protectants, pigment sources, and interactions with insects. In many plants, glycosides are important precursors of flavor-related compounds.

Preview - coming up in the next issue

Determining Microplastics in the Environment

Recent research has documented that the presence of microplastics is a growing problem for aquatic life and anyone relying on aquatic life forms for nutrition. Polymer products have been – and continue to be – released into our oceans on a huge scale in various ways. They are then slowly ground up into small particles. Until now, it has been difficult to gauge the consequences for the environment let alone for animals and humans. Increasingly proof is surfacing that plastic micro-particles find their way into organisms and into the food chain. The political class, industry and environmental organizations increasingly see the need for action to stop and reverse this development. When it comes to finding and characterizing microplastics, Pyrolysis-GC/MS can help provide answers.

Determining THC Residues in Hair

In cooperation with renowned forensic toxicologists, GERSTEL has produced a series of Applications and solutions over the past five years. A further project is underway and will be reported in the next GERSTEL Solutions Worldwide Magazine. The topic is the determination of THC, CBN and CBD in hair samples. This type of drug screening and analysis is used, for example, to determine whether or not a person has been consuming drugs regularly over a period of time.

Composing Flavors Efficiently

GERSTEL Solutions has again been on the prowl, looking for novel and interesting applications of our technologies in the wider laboratory world. In the next issue, we report from Symrise AG in Holzminden, Germany, a leading worldwide producer of flavors and Fragrances for food and cosmetics, where the MPS is used to compose flavors. Something to look forward to….

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