GERSTEL solutions worldwide

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No. 18

Green Analysis

Stir Bar Sorptive Extraction (SBSE)

Stirred, not shaken

Powerful water analysis based on the EU-WFD

Metabolomics
Watered down taste

Forensic Dating Services
Gunshot Residue Dating

Food Safety Analysis
Refined Risk 3-MCPD & glycidol

Presenting the Lab
The DOW Ecology Lab in Stade, Germany

GERSTEL 18 worldwide solutions

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Cooling options

Chilled out

The GERSTEL Cooled Injection System (CIS) is a PTV-type GC inlet that is also used as cold trap in GERSTEL GC sample introduction solutions such as thermal desorption. To maximize performance, a cooling system that matches the application is needed. The available cooling options and where they best fit are described below.

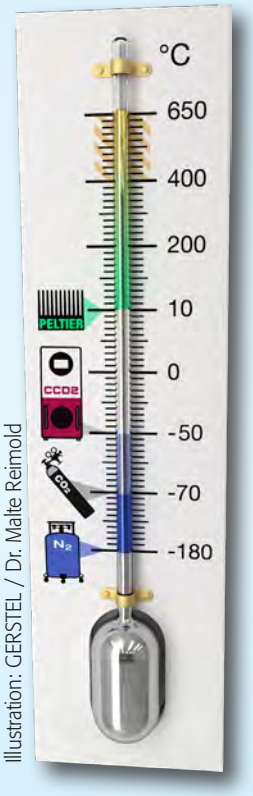


Illustration: GERSTEL / Dr. Malte Reimold

The GERSTEL product range includes cooling options to meet every application need. To find out which cooling option is best for you, contact GERSTEL to discuss with our experts.

Liquid Nitrogen (LN_2)

LN_2 based cooling provides the widest range of trapping temperatures, and greatly simplifies method development. Thanks to being able to cool the CIS to as low as -180 °C, you can always be sure you are trapping even the most volatile compounds. Physics-based cryogenic trapping with LN_2 ensures that all unknown analytes are trapped without chemical transformation and are consequently transferred to the GC column discrimination free. The use of LN_2 eliminates the need to use chemical sorbents that have specific trapping characteristics and are designed to trap a range of compounds or a specific compound type (i.e. sulfur compounds). Solving critical problems requires that you see all compounds in your sample, but with chemical trapping you can never be sure that you have selected the right adsorbent/adsorbents. They should never be used unless performing target analysis where you validate trap performance through the use of standards

Carbon Dioxide (CO_2)

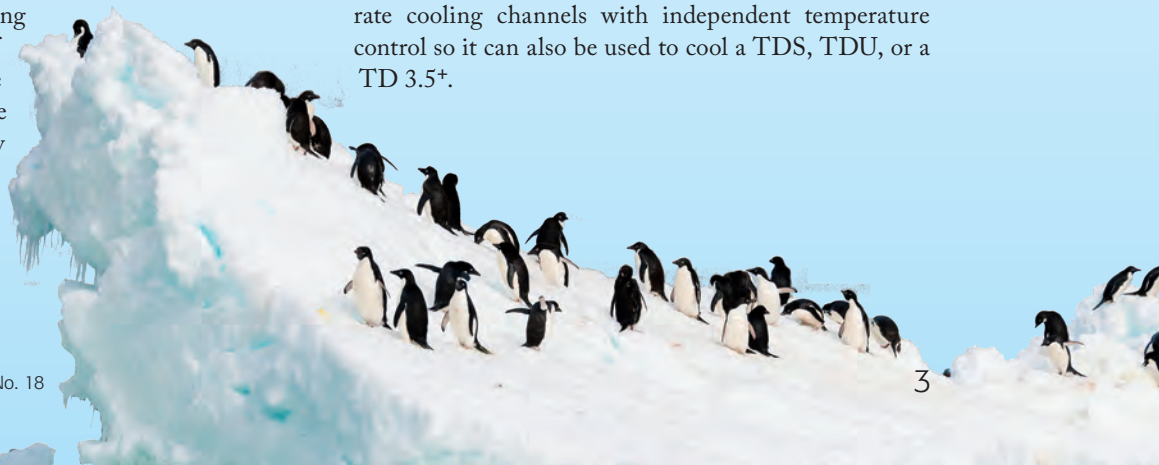
When you know that your compounds of interest are in a higher volatility range, but you still do not want to use adsorbents for trapping, liquid CO_2 can be used to reach temperatures as low as -70 °C. Liquid CO_2 has the advantage of being stored conveniently in a gas cylinder at ambient temperature. However, compared to LN_2, LCO_2 has less cooling capacity; the number of analysis cycles that can be performed using a single cylinder is significantly smaller when compared to LN_2.

Universal Peltier Cooling (UPC Plus)

When using the CIS strictly as a PTV-type GC inlet for liquid injections the electrically powered Universal Peltier cooling module (UPC Plus) can be used. The UPC plus is based on Peltier Cooling and it continuously circulates coolant to remove heat. UPC Plus requires very little maintenance and operates quietly in the background. Temperatures as low as 10 °C can be reached reliably and the UPC Plus doesn't require additional bench space, it is mounted on the GC. The UPC Plus is also used to cool all GERSTEL modules where cooling aids in decreasing analysis cycle time such as the TDS, TDU, TD 3.5+, and DHS. It can also be used when cooling is needed for sample trays and agitators.

Cryostatic Cooling Device (CCD 2)

When performing targeted analysis where the use of adsorbent traps is acceptable or non-target analysis of, for example, SVOCs, the GERSTEL Cryostatic Cooling Device (CCD 2) is a perfect replacement for cryogenic cooling. It provides continuous, reliable cooling of the CIS to as low as -40 °C. The CCD 2 provides two separate cooling channels with independent temperature control so it can also be used to cool a TDS, TDU, or a TD 3.5+.



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Watered down taste

Extreme weather with alternating periods of drought and heavy rainfall can negatively affect yield and quality of agricultural crops. Recent research into the impact of climate variations on the quality of tea have shown how dramatic the influence can be.

By Guido Deussing

Second only to water, tea is by far the most widely consumed beverage in the world. Apart from actual or imagined physiological effects, the main and most important aspects for a tea drinker are thought to be flavor and enjoyment. There may be regularly recurring trends towards consuming only the healthiest beverages, but generally, consumers' main concern is that their drinks taste good. However, sensory characteristics such as taste and aroma are exactly the qualities affected by weather, as Professor Albert (Al) Robbat, Director of the Tufts University Sensory and Science Center in Medford, Massachusetts explains to GERSTEL Solutions worldwide. As it turns out, this is especially the case when the harvest literally falls into the water.

Tea – an ideal model plant for climate effect studies

In cooperation with an interdisciplinary team of colleagues, Al Robbat investigated tea samples from Yúnnán province in Southwestern China, an area renowned for excellent tea. The aim was to determine why tea leaves picked during the rainy monsoon season are less aromatic and frequently have off flavor issues compared with tea leaves picked during the dryer spring season. This was found to be the case even when comparing leaves picked from the same plant. The chromatography expert explains that not only do tea drinkers suffer when exposed to inferior quality brews; tea farmers incur significant losses since they are forced to sell crops grown during the monsoon season at much lower prices compared with the spring crop.

There is an urgent need for more insight into causes and effects since the Yúnnán region is increasingly faced with extreme weather conditions that can affect quality and yield and in extreme cases lead to total loss of harvest. As Al Robbat explains, there has already been a noticeable shift in the start of the summer monsoon and its duration, which means that periods with ideal harvest conditions are becoming shorter. The scientists set out to determine the root causes for changes in quality and flavor of Yúnnán tea as a function of rainfall, temperature, and elevation. Their findings are reported in Journal of Chromatography A [1].

Preliminary investigations showed the way

Initial investigations of tea harvested in the mountains of Yúnnán from spring until the onset of the monsoon period brought the following results: With increasing

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amounts of rainfall, the catechin compound concentrations dropped by more than 50 percent. Among these are: catechin, catechingallate, epicatechin-3-gallate, epigallocatechin, epigallocatechin-3-gallate, gallic acid, gallocatechin and gallocatechin-3-gallate. The same effect was found for methylxanthines (caffeine, theobromine and theophylline) Al Robbat reports [2]. Although catechins and methylxanthines, astringent bitter compounds characteristic of poor quality teas decreased in concentration, other polyphenolic compounds (also astringent and bitter) increased in concentration. "Initially, we assumed that the loss of quality was related to a kind of dilution effect, in other words that the plant growth would outpace the production of secondary metabolites to which the flavor compounds belong", the scientist explains. However, when they determined that the total concentration and activity of antioxidants in teas harvested in spring was lower than in comparable teas harvested during the monsoon period, Al Robbat and his colleagues concluded that the plant chemistry, i.e. metabolism and physiology had changed completely and had adapted to the change in precipitation. This had resulted in changes in metabolism and, therefore, in the associated flavor determining metabolites. The idea was obvious, Prof. Robbat explains, to not just focus on significant flavor compounds in tea, but rather look at the bigger picture, including determining the entire group of flavor relevant compounds and to generate a profile of as many metabolites as possible in order to understand how environmental conditions influenced tea plant chemistry.

A closer look at plant metabolism

Taste and aroma of tea are a result of complex interactions between hundreds of chemical compounds. Al Robbat: "Extending our knowledge of potentially sensory relevant metabolites and monitoring them over a period of time is key if we want to develop an understanding of how environmental and climate factors influence tea quality." We know that seemingly unimportant compounds can significantly influence the organoleptic quality. Most studies listed in literature have focused on seasonal changes of non-volatile compounds, but we now know that volatile organic compounds (VOCs) with low odor thresholds contribute significantly to the total sensory impression, Al Robbat points out. This realization had led the group towards using GC/MS as the technique of choice for monitoring metabolites.

Tea harvester in a Chinese highland region.

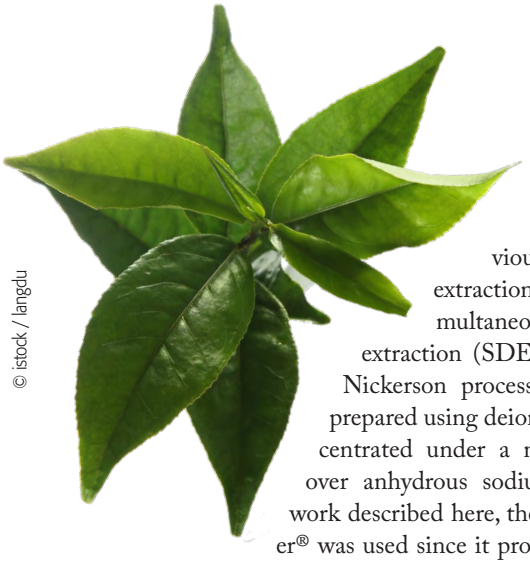
Flavor research needs GC/MS analysis, and GC/MS methods are widely used in metabolomics research (see page 9). In the case of tea plants, the sample matrix is highly complex and a huge number of potentially relevant

flavor compounds are involved. Literature sources count around 600, according to Prof. Robbat. This is the reason that a sequential, multidimensional GC-GC/MS method was used in order to comprehensively determine all detectable volatile metabolites in spring- and monsoon teas. The acquired data were used to build a metabolite database that relies on a deconvolution software to solve highly complex analysis tasks very quickly using a standard GC-MSD system. This is even possible when multiple compounds co-elute and also have overlapping signals.

The deconvolution technology developed by Professor Robbat and his colleagues offers added value in comparison with conventional approaches. This includes the possibility to obtain and store retention times and spectra of neat metabolites that aren't skewed by matrix influence. It is also possible to identify perfectly co-eluting compounds. Professor Robbat and his group routinely measure the relative concentration of more than 400 compounds from infused tea leaves.

Technology that enables successful analysis

Initially the aim had been to use the spring harvested tea samples to find as many metabolites as possible and build a solid reference database. Al Robbat: "In order to build a comparative database, we instead analyzed tea samples consisting of buds and leaves that had been collected over three day periods in both spring and summer". In other words, the periods spanned both the dry season (spring) and the rainy season (monsoon). Collected tea samples were briefly microwaved in the field to stop enzymatic activity and subsequently sent to the laboratory in sealed plastic bags. In the laboratory, they were vacuum sealed, wrapped in aluminum and stored in a deep freezer awaiting analysis. GC-GC/MS analysis was performed on



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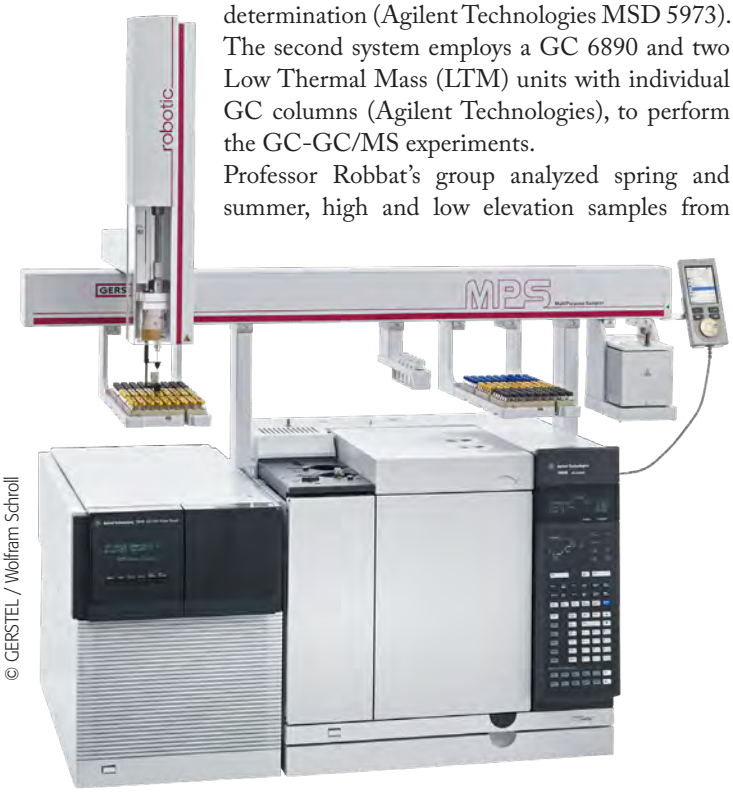
extracts of the tea samples. The previously used classical extraction procedure was simultaneous distillation and extraction (SDE) using the Likens-Nickerson process on a tea infusion prepared using deionized water and concentrated under a nitrogen atmosphere over anhydrous sodium sulfate. For the work described here, the GERSTEL Twister® was used since it provides a simple, clean method to extract organic chemical compounds from the tea infusion requiring no or almost no use of organic solvent. The Twister is a glass coated magnetic stir bar fitted with a PDMS sorbent phase. While stirring the sample, the Twister efficiently extracts organic compounds. The Twister was also used to collect organics in the field by placing it directly under the leaf and holding it in place with a magnet [3]. For the tandem GC-GC/MS analysis Al Robbat and his colleagues used two 6890 GCs from Agilent Technologies. The first system was fitted with an FID; analyte separation was performed using a column with polar stationary phase. Sample introduction to the Cooled Injection System (CIS) PTV-type inlet was automated using the GERSTEL MultiPurpose Sampler (MPS). The second GC system was configured with a non-polar capillary column and a Cryo-Trapping System (CTS) in order to enable heart-cutting and cryo-focusing of fractions separated on the first column and to deliver these to the second column as a narrow band for best possible quality of separation as well as MS determination (Agilent Technologies MSD 5973). The second system employs a GC 6890 and two Low Thermal Mass (LTM) units with individual GC columns (Agilent Technologies), to perform the GC-GC/MS experiments.

Professor Robbat's group analyzed spring and summer, high and low elevation samples from both Yunnan and Fujian Provinces, temperatures during sampling varied by up to 10 °C. The samples were taken to study locational differences over a 3-year period (2014-2016)[4]. The data analysis software developed by Dr. Robbat and sold through GERSTEL provided the means to analyze these samples by GC/MS and track compositional changes in tea under stress conditions [5].



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Analysis results confirm sensory impression

Al Robbat: "Our analysis results confirm what tea farmers tell you: The spring tea is of much higher quality and has a sweet floral flavor compared with the monsoon tea, which is described as green or earthy. Using tandem GC-GC/MS analysis we succeeded in identifying hundreds of metabolites in the spring and monsoon teas". Professor Robbat's group found 169 metabolites that were common to both tea types and more than 100 compounds that were unique seasonal compounds. A further 163 compounds were detected, but could not be identified. In future, we will increasingly have to contend with extreme weather conditions. This means that plant research must develop tools that can monitor the influence not only on plant growth and yield, but also on taste, flavor and nutritional value of crops. "Our work has delivered a set of tools to monitor seasonal variations in the metabolism of tea plants", says Al Robbat, "and I'm certain these tools can be used for similar metabolomics studies of crops and human systems".

Using a comparable MPS-GC/MS system, Professor Al Robbat performed the analysis of tea plant extracts and determined secondary metabolites as described in this article.

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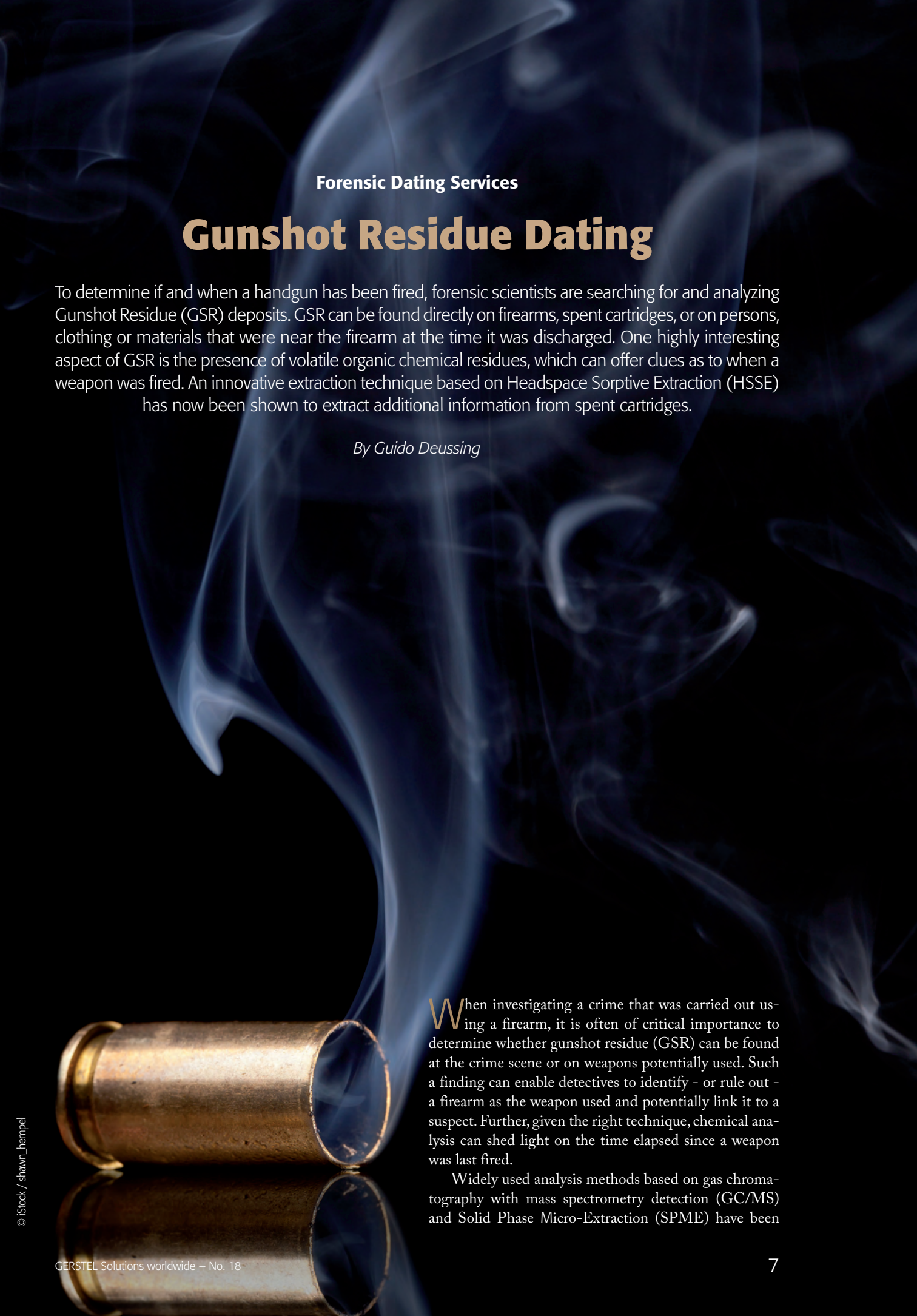
Forensic Dating Services

Gunshot Residue Dating

To determine if and when a handgun has been fired, forensic scientists are searching for and analyzing Gunshot Residue (GSR) deposits. GSR can be found directly on firearms, spent cartridges, or on persons, clothing or materials that were near the firearm at the time it was discharged. One highly interesting aspect of GSR is the presence of volatile organic chemical residues, which can offer clues as to when a weapon was fired. An innovative extraction technique based on Headspace Sorptive Extraction (HSSE) has now been shown to extract additional information from spent cartridges.

By Guido Deussing



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When investigating a crime that was carried out using a firearm, it is often of critical importance to determine whether gunshot residue (GSR) can be found at the crime scene or on weapons potentially used. Such a finding can enable detectives to identify - or rule out - a firearm as the weapon used and potentially link it to a suspect. Further, given the right technique, chemical analysis can shed light on the time elapsed since a weapon was last fired.

Widely used analysis methods based on gas chromatography with mass spectrometry detection (GC/MS) and Solid Phase Micro-Extraction (SPME) have been

evaluated by collaborating scientists at the University of Lausanne, Switzerland; the Sapienza University in Rome, Italy; and King's College in London, England. In addition, a novel method using Headspace Sorptive Extraction (HSSE) followed by thermal desorption-GC/MS (TD-GC/MS) was compared with the SPME based method. The scientists were pleased with the results. [1].

The Content of Gunshot Residue

Every firearm emits gunshot residue when discharged. The compounds involved are released at explosive speed and deposited or adsorbed on the hand, body and clothing of the shooter. Depending on the distance to the firearm, residue can also be found on the victim and, obviously, on the weapon used and on spent cartridges that were ejected.

Sources of GSR are the ignition- and propellant charge powders in the cartridge. In addition, metallic powder is formed by abrasion from the bullet and cartridge and added to the mix. GSR consists of various inorganic and organic chemicals. Due to aging processes and volatilization, changes in the concentrations and amounts of organic chemicals as well as certain compound to compound ratios of amounts can be helpful in determining the time of discharge of a weapon according to Gallidabino et al. as reported in their paper in Analytical Chemistry [1].

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To perform Headspace Sorptive Extraction (HSSE), a GERSTEL Twister is suspended inside a sealed vial placed in the headspace above the sample. In the case shown here, a spent handgun cartridge (left) is being analyzed. During extraction, volatile and semi-volatile organic compounds (VOCs and SVOCs) are concentrated in the sorption phase of the Twister. The Twister shown here is the Polydimethylsiloxane (PDMS) version. Following the extraction period, the Twister is removed, placed in a glass liner, and analyzed by thermal desorption-GC/MS. The GERSTEL Thermal Desorption Unit combined with a GC/MS-System (Agilent Technologies) was used in the work reported here.

The compounds identified as GSR from literature references are, among others, nitroglycerine, diphenylamine, ethylcentralite, dibutylphthalate and 3-Ethyl-1-Hexanol as well as organic reaction byproducts, especially derivatives of benzene, and polycyclic aromatic hydrocarbons (PAHs). To determine these, the SPME technique has been widely used in combination with GC/MS. To perform GSR-based dating, Gallidabino et al. performed repeat extractions of analytes from the interior of a firearm and/or spent ammunition with the aim of establishing an aging profile, which can be compared with reference profiles. Naphthalene and decomposition products of nitrocellulose were proposed as target reference compounds.

The problem with handguns – and the solution

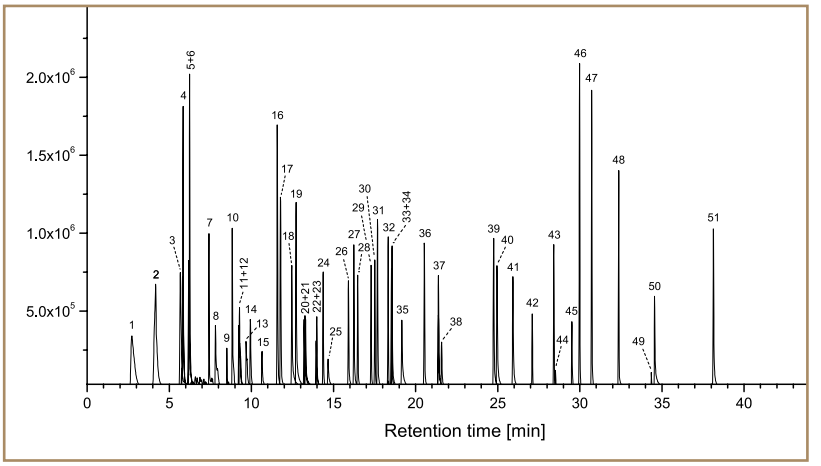
SPME is an established technique for time estimates concerning discharge of ammunition from rifles and other larger firearms. However, its usefulness for forensic dating of firing of smaller handguns is more limited. The repeatability is insufficient, as reported by the scientists, and degradation curves for the target analytes quickly fall below their limits of detection with SPME, as Gallidabino et al. [1] report. For these reasons, SPME is not the ideal technique for the investigation of GSR in combination with handguns.

In their search for an alternative extraction and analysis method with significantly higher sensitivity for the target analytes, the scientists' attention was caught by Headspace Sorptive Extraction (HSSE). HSSE is based on the GERSTEL Twister, a glass coated magnetic stir bar with a PDMS sorption phase. The Twister is most often used for Stir Bar Sorptive Extraction (SBSE) of aqueous samples. In HSSE, the Twister is suspended in the Headspace above the sample inside a sealed 20 mL headspace vial. HSSE is thus very similar in principle to Headspace Solid Phase Micro-Extraction (HS-SPME). The main difference is that the Twister offers a much larger sorption phase volume than SPME fibers which consist of a thin sorption phase coating on a metal or glass fiber contained within a syringe needle. Consequently, SBSE provides better recovery, better repeatability, and lower limits of detection. After the extraction step, analytes are released from the Twister by thermal desorption in the GERSTEL Thermal Desorption Unit (TDU 2) immediately followed by GC/MS determination of the analytes. These steps are fully automated using the GERSTEL MultiPurpose Sampler (MPS) under MAESTRO software control. "The Twister can provide up to 1000 times higher sensitivity than SPME, depending on the application", says Oliver Lerch, Ph.D., Application Scientist from GERSTEL.

SBSE can be performed with different Twisters to cover a wide range of analytes from non-polar to polar. Desorption can be performed by thermal desorption – as is most often the case - or by liquid desorption combined with either GC/MS or LC/MS.

Preparing the GSR analysis

To determine whether HSSE is a suitable extraction technique for the analysis of GSR, different types of cartridges were inserted into a semi-automatic pistol, discharged, and the spent cartridges placed in sealed HS vials with GERSTEL Twisters suspended in the headspace above the sample. The cartridges contained propellant charges based on either nitrocellulose or a combination of nitrocellulose and nitroglycerine. The results from the HSSE-TD-GC/MS analysis were compared with results from the analysis of a reference mixture based on 55 selected substances, identified as GSR compounds. This is how Gallidabino et al. approached the analysis: In the headspace above the spent cartridge to be analyzed, a perforated glass insert containing a conditioned Twister was positioned and the headspace vial was sealed. Different Twister techniques enable different sampling approaches along with the use of different phases to collect a wider range of analytes. In this case, the analytes were extracted at 80 °C for 72 hours. The Twisters were removed from the vial and placed in individual sealed glass tubes ready for Thermal Desorption-GC/MS analysis. The glass tubes were fitted with individual transport adaptors, which seal the tubes and enable the MultiPurpose Sampler (MPS) gripper to transfer them to the Thermal Desorption Unit (TDU) for temperature programmed desorption. The released analytes are focused in the GERSTEL Cooled Injection System (CIS) PTV type GC inlet at -80 °C. The CIS is then heated using a temperature program and the analytes transferred to the GC column in splitless mode using a helium carrier gas flow of 1.3 mL/min for highly sensitive GC/MS determination. The CIS was installed in a gas chromatograph (Agilent GC 7890A) connected to an Agilent 5975 Mass Selective Detector (MSD). The column used was an Agilent HP-5MS (30 m x 0.25 mm x 0.25 µm). The GC oven initial temperature was 40 °C, the program progressing to a final temperature of 280 °C. The GC run including cool-down and equilibration lasted 46 minutes. Mass Selective Detection (Agilent 5975C MSD) was performed in EI mode in full scan mode across a range of m/z 40 to 500.



Extracted Ion Chromatogramm (EIC) of the standard solution containing 55 substances injected by Gallidabino et al. Using HSSE-GC/MS, the researchers were able to determine 51 of the 55 compounds in GSR. The "missing compounds" were thermally labile substances including nitroglycerine and N-nitrosophenylamine.

HSSE delivers

The results of their study clearly showed that by using HSSE, the scientists were able to determine and track the concentration degradation curves of 51 of the selected 55 GSR target analytes from discharged cartridges. HSSE proved to be significantly more sensitive while delivering better reproducibility than SPME. Among the 4 compounds not found were thermally labile compounds such as nitroglycerine that are decomposed during thermal desorption. Further studies are to be performed to optimize the analysis method parameters with the aim of increasing the number of target analytes that can be determined.

As part of their project, Gallidabino et al. generated aging curves in order to develop a method for determining the approximate time of discharge including determining whether the firearm was discharged at all. These aging curves are based on GSR compound concentrations on spent cartridges from handguns and their degradation curves. The scientists report that based on the use of HSSE on aged samples, several GSR compounds have shown significant aging profiles. In addition, compound-to-compound ratios can be used to extend the time periods that can be covered. This approach also contributes to making the determination more rugged and to reducing variability, making the method more useful for the forensic scientist entrusted with the case. Gallidabino and his colleagues concluded that their results were very encouraging for the development of a new and complete forensic dating technique based on GSR.

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Reference

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Food Safety

Herbal warfare

Plant based food and feed can contain toxic pyrrolizidine alkaloids (PAs) due to co-harvesting of weeds that contain PAs. Experts therefore recommend testing crops known to co-exist with PA containing weeds before use in food or feed. The method of choice for direct determination is solid phase extraction (SPE) cleanup followed by LC-MS/MS analysis.

By Guido Deussing

Green and lush pastures and fields present a wonderful sight. A closer look, however, can reveal more than just healthy greens and harmless edible plants. Among the crops we grow for use as food and feed, wild herbs crop up that are a thorn in the flesh of farmers and horse breeders. In temperate zones in Europe, ragwort is a ubiquitous, particularly toxic wildflower, which looks similar to plants of the arugula family. Unlike arugula, however, it is anything but wholesome. Ragwort and related species contain toxic pyrrolizidine alkaloids (PAs), presumably synthesized by the plants as a defense against herbivorous enemies. PAs are natural toxins whose toxicity is not limited to insects: Many are toxic both to humans and to the herbivores we rely on for meat and dairy products. Some PAs cause liver damage and are both genotoxic and carcinogenic. Regular intake of even small amounts can lead to a creeping process of progressive damage to internal organs, as can be observed in horses and cattle. PA poisoning can lead to serious illness and even death.

How big is the threat?

The challenge facing producers and consumers is significant: A total of more than 6,000 plant species produce PAs, equal to about 3 % of the total number of flowering plants world-wide as calculated by BLL, the German Federation for Food Law and Food Science. BLL is the main industry association of the German food sector.

More than 600 PAs and their N-oxides have been found and characterized in more than 350 different plant species Worldwide, according to the Federal German Institute for Risk Assessment and Consumer Safety (BfR) in Berlin. PA toxicity has long been established; a recent scientific publication by US FDA scientists also established the toxicity of a number of PA N-oxides, recorded to induce Hepatic Sinusoidal Obsctruction Syndrome (HSOS) in humans, and further confirming PA N-oxide-induced hepatotoxicity on mice with hepatotoxicity similar to, but potency much lower than, the corresponding PAs.

The FDA scientists conclude that the levels of both PAs and PA N-oxides in herbs and foods should be regulated and controlled [2]. As an example, PAs have been found in herbs used for herbal infusions - also referred to as herbal teas. Examples are fennel-, chamomile-, herbal-, peppermint-, nettle-, and garden balm (Melissa) tea. In 2013, the BfR assessed the situation and concluded that PA levels in some foods (herbal tea, rooibos tea, black and green tea as well as honey) can pose a threat to children and adults alike when these foods are consumed on a regular (chronic) basis and that effort should be made "to minimize the PA contents in herbal teas and teas in order to minimize the putative higher cancer risk of frequent consumers and in particular of children" [1].

Consumer protection

The widespread occurrence of PA-synthesizing plants worldwide means that we as consumers should accept the fact that the risk of having PAs in food and feed cannot be totally eliminated. This may be part of the reason that until now, no country has introduced legally binding maximum concentration levels, relying instead on recommendations. In 2001, the United States Food and Drug Administration (FDA) issued a ban of comfrey based products marketed for internal use, and a warning label for those intended for external use. In an opinion published by the BfR, food producing companies have an obligation to take steps to ensure that PA levels in food are reduced. In preparation for such action, and based on a request from the European Commission, the European Food Safety Authorities (EFSA) in 2016 published a scientific report entitled: Dietary exposure assessment to pyrrolizidine alkaloids in the European population [3]. A further extensive publication in the form of a "Statement" was published by the EFSA in 2017, entitled: Risks for human health related to the presence of pyrrolizidine alkaloids in honey, tea, herbal infusions and food supplements [4]. In summary, the EFSA recommends gathering more toxicological data, expanding the proposed list of 17 PAs to be monitored, and developing more sensitive analysis methods. EFSA currently sees no possibility of establishing a tolerable daily intake (TDI) value. In Germany, the BfR therefore recommends a zero-tolerance approach. While this is commendable, it is highly unlikely to be implemented in practice. According to the BfR, the aim of following this approach is simply to keep PA levels in food and feed as low as possible and to support this effort by monitoring, which requires efficient and highly sensitive chemical analysis including sample preparation.

Analysis methods

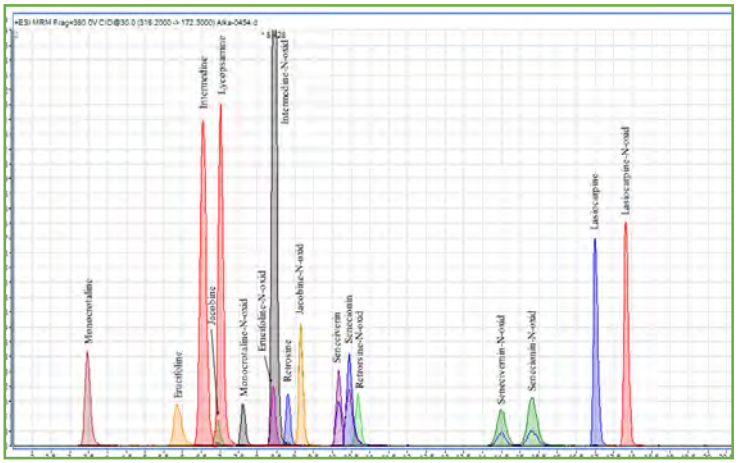
Monitoring PAs in agricultural products, such as food and feed, is not exactly easy: PAs are structurally diverse, they are present in a wide range of products and the analysis poses a real challenge. Over the past years, scientists at the BfR have developed accurate and reliable methods for the determination of PAs. These methods have been validated in round-robin tests. The methods could be implemented in food and feed monitoring performed by individual German states, but currently only a limited number of PA standards are commercially available. This has led the BfR to develop additional analysis methods that can be used to estimate the total amount of PAs in a sample. To determine PAs in plant material, the BfR recommends analyte concentration using solid phase extraction (SPE) followed by LC-MS/MS determination: The PAs are extracted from plant material in an ultrasonic bath using diluted sulfuric acid. The material is extracted twice and the extracts combined and centrifuged. An aliquot of the supernatant is taken for solid phase extraction (SPE) using a C18-phase. Following elution with methanol, the eluate is evaporated to dryness and the residue taken up in a methanol-water mixture. Finally, the sample is injected for chromatographic separation and MS/MS detection [1].

Automation provides added value

A contract laboratory that specializes in the analysis of food and feed requires efficient sample processing in order to be competitive. That also applies to PA, of course. "This is where I see a deficit of the BfR methods", says Franziska Chmelka, Food Technologist at TeLA GmbH, "the sample preparation is quite labor intensive". TeLA is an accredited contract laboratory for food and environmental analysis in Northern Germany. In order to improve the productivity of the BfR analysis method, TeLA automated it, including sample preparation. Franziska Chmelka and her colleagues used the GERSTEL MultiPurpose Sampler (MPS) to automate the required sample preparation steps for the PA determination. A key component of the system is automated SPE in combination with LC-MS/MS (Agilent Technologies 1290 HPLC and 6495 Triple Quadropol LC/MS).

Instrument setup used for the automated determination of pyrrolizidine alkaloids: GERSTEL MultiPurpose Sampler (MPS) configured for automated SPE in combination with an LC-MS/MS system from Agilent Technologies (1290 HPLC and 6495 Triple Quadrupole LC/MS).

Separation of the analytes was performed using a Standard-RP-Phase column (Nucleodur C18 HTec 250 x 2 mm x 5 µm, Macherey-Nagel) and gradient elution with 5 mM formic acid (Eluent A) and methanol (Eluent B): 0 min (5 % B) – 3 min (5 % B) – 7 min (20 % B) – 13 min



Analysis of a standard mixture of 17 pyrrolizidine alkaloids resulted in a clean chromatogram with well resolved peaks. Good separation of the individual PA compounds is extremely important. Many exhibit very similar mass transitions and can only be distinguished and identified unequivocally when peaks are fully resolved. (Source: TeLA GmbH)

(20 % B) – 16 min (65 % B) – 17 min (95 % B) – 20,1 min (5 % B). The flow rate was 0.25 mL/min and the column temperature 28 °C. A 5 µL aliquot of the eluate was injected. Target analytes were detected in Multiple Reaction Monitoring (MRM) mode, ESI positive.

For method development, an aqueous standard solution was used, containing 17 different PAs: Monocrotaline, erucifoline, intermedine, jacobine, lycopsamin, monocrotaline N-oxide, erucifoline N-oxide, intermedine N-oxide, retrorsine, jacobine N-oxide, senecivernine, senecionine, retrorsine N-oxide, senecivernine N-oxide, senecionin N-oxide, lasiocarpine and lasiocarpine N-oxide.

Automated SPE to the rescue

"Our focus when developing the method was on automating the most labor intensive step in the process, which is without a doubt the solid phase extraction (SPE)", said Franziska Chmelka. After completing their initial experiments, the application experts from TeLA decided to use a C18-RP sorbent (Macherey-Nagel C18 ec 3 mL/500 mg).

© TeLA

Chopped Chamomile (left), spiked with 0.1 % ragwort (right).

All SPE steps were successfully automated: Conditioning the sorbent with 5 mL of methanol and 5 mL of water; injecting 5 mL sample onto the sorbent; as well as eluting the analytes with 5 mL methanol. "We also succeeded", says Norbert Helle, Ph.D., President and Owner of TeLA GmbH and established LC/MS expert, "in automating the remainder of the process: Evaporating the eluate to dryness, taking up the residue in 1 mL of a 10 % methanol solution, and injecting 5 µL of the resulting extract into the LC-MS/MS-System".

Analysis of the standard mixture resulted in a clean chromatogram with well resolved peaks. Franziska Chmelka explains the focus: "Good separation of individual peaks is essential. Many of the compounds have similar mass transitions and we must be able to differentiate between the different analytes. That is only possible if we have retention time differences." The application experts from TeLA then put both the method and the instrument setup to the test by running real samples, in this case, ragwort leaves. "All statistically relevant parameters confirmed that our automated SPE-LC-MS/MS method is delivering accurate results", says Franziska Chmelka. Good linearity was achieved across a wide concentration range, going as low as 1 ng/mL, and recoveries ranged from 85 to 98 percent for all analytes. The overall method reproducibility, including sample preparation, was in the range from 1.3 to 4.8 percent for all analytes. Franziska Chmelka: "It was especially important to us to achieve good retention time stability as this is a key indicator of method ruggedness. Using our method, retention times for the analytes only varied between 0.063 and 0.35 percent over a period of several days. Finally, the limits of determination we can reach are between 0.05 and 0.5 µg/kg".

In a further step, the TeLA team tested how many PAs are actually transferred to the herbal infusions we brew and drink and how much PA is transferred in total: Dry Chamomile material was spiked with different levels of Ragwort; the amounts added were 10.1 and 0.1 percent w/w relative to the chamomile. As Franziska Chmelka reports: "Just adding 0.1 percent ragwort resulted in our finding a significant amount of PAs in the brewed herbal infusion".

Conclusion

The automated SPE-LC-MS/MS analysis method was successfully used to determine PAs in various food and feed samples. In all cases, the method worked well and provided good results. Looking forward, the method needs to be extended. A larger number of PAs will need to be monitored and the sensitivity of the method must be improved. In its current form, however, the method provides highly satisfactory results, as Franziska Chmelka reports.

Suggested bedside reading (292 pages):
US Food and Drug Administration, FDA. Bad Bug Book, Foodborne Pathogenic Microorganisms and Natural Toxins. Second Edition.
https://www.fda.gov/downloads/Food/FoodborneIllnessContaminants/UCM297627.pdf

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Metabolomics

Analytical Mass Movement

When epidemiological studies require analysis of large sets of blood, plasma, or urine samples under uniform conditions, with good accuracy, and within a limited time frame, manual sample preparation may not be the best approach. Metabolomics studies generally require efficient, fully automated sample preparation.

By Guido Deussing

According to experts, cardio vascular disease, obesity, Type 2 diabetes, and various types of cancer are often linked to a lack of exercise and to an unhealthy diet. Especially certain fatty foods can have a lasting influence on your health and well-being, but individual humans respond differently and are affected to different degrees. That, at least, is one way to explain why a fast food dominated diet causes some people to become overweight and increases their risk of developing diseases while others seem unaffected. The reason could be differences in genetic make-up with respect to metabolic processing of, and energy recovery from, nutrition in the organism.

In order to determine the effects of nutrition on health, as well as determining the role and influence of the gene pool, large epidemiological studies are undertaken that examine the resulting metabolites. The entirety of metabolites is referred to as the metabolome, the study and quantification of metabolites is referred to as metabolomics.

Profiling thousands of individual compounds

When searching for knowledge, in the form of correlations between health, genetics and nutrition, researchers use metabolite profiling hoping to find biomarkers that can provide information on metabolic processes in the organism. The tools they typically use are high level analytical instruments such as mass spectrometers, combined with gas chromatography (GC) or Ultra-High performance Liquid Chromatography (UHPLC), the combined instruments are typically referred to as GC/MS or LC/MS systems. In the case reported here, fatty acid methyl

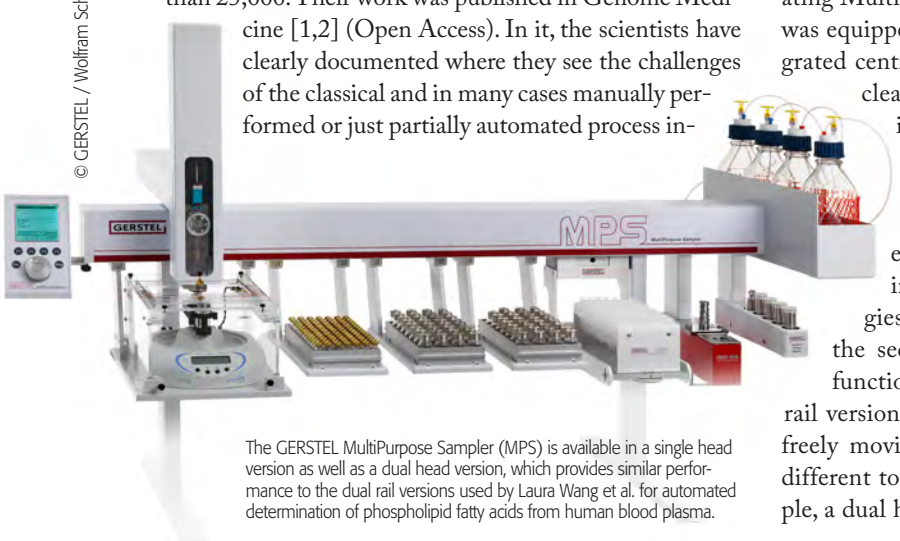
For quality control purposes, QC samples with a defined concentration of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were included and analyzed in every sequence. The table shows the median values in %. N is the number of sequences processed, SD the standard deviation, and % CV is the coefficient of variation in percent. The numbers demonstrate the excellent long term stability and reproducibility of the systems. [1]

Month	DHA			EPA		
	Mean	SD	% CV	Mean	SD	% CV
1 (n = 36)	2.14	0.06	2.60	0.46	0.02	4.22
2 (n = 36)	2.14	0.06	2.66	0.46	0.01	2.22
3 (n = 38)	2.13	0.06	3.02	0.47	0.03	7.28
4 (n = 38)	2.15	0.08	3.58	0.47	0.04	9.16
5 (n = 37)	2.16	0.06	2.66	0.47	0.03	7.13
6 (n = 38)	2.15	0.05	2.47	0.48	0.04	8.46
7 (n = 38)	2.16	0.04	1.78	0.47	0.03	6.54
8 (n = 38)	2.15	0.07	3.23	0.47	0.02	5.30
9 (n = 38)	2.15	0.06	2.71	0.46	0.03	6.90
10 (n = 38)	2.17	0.05	2.55	0.46	0.03	6.31
Comparison of mean values	$P = 0.062$			$P = 0.064$		

esters were quantitatively determined using a GC with flame ionization detector (GC/FID), due to the larger linear and dynamic range of the FID.

Metabolomics calls for automation

In order to obtain scientific evidence of possible health effects, you need large, comprehensive, and reliable data sets. It is not unusual for metabolomics studies to involve the determination of hundreds or even thousands of individual compounds. Just processing the large number of samples required within a reasonable time frame represents a challenge. In addition, it must be ensured that the results generated are extremely accurate in order to be able to draw any meaningful conclusions. To achieve all this, metabolomics experts agree, you cannot rely on manual sample preparation, you need as much automation as possible. As an example, Laura Yun Wang and her colleagues from the Elsie Widdowson Laboratories and the Institute of Metabolic Science in Cambridge, England, have reported on their work developing and validating a fully automated method for the determination of phospholipid-bound fatty acids in human blood plasma. The goal was to help perform metabolic phenotyping as part of a large epidemiologic study involving a sample set of more than 25,000. Their work was published in Genome Medicine [1,2] (Open Access). In it, the scientists have clearly documented where they see the challenges of the classical and in many cases manually performed or just partially automated process involved. They also report on how they have fully automated and validated the process steps needed.



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The GERSTEL MultiPurpose Sampler (MPS) is available in a single head version as well as a dual head version, which provides similar performance to the dual rail versions used by Laura Wang et al. for automated determination of phospholipid fatty acids from human blood plasma.

The Sample Preparation Challenge

The use of nutrition related biomarkers in large-scale epidemiological studies is only possible, "if the analysis is sufficiently fast, relatively cheap, robust and precise", according to Wang et al. It was decided to develop an automated method for fatty acid profiling of the phospholipid fraction in human plasma. A number of steps would have to be automated: The lipids would have to be extracted in total from the plasma using solid phase extraction (SPE) and converted into free fatty acids and then volatile fatty acid methyl esters (FAMEs), which can be determined using a GC with a flame ionization detector (FID). Using this method, stereo-isomers (cis- and trans fatty acid isomers) can be determined by GC in an acceptable time frame, according to the scientists.

When performed by hand, the large number of complex steps requires an inordinate amount of time and the process is prone to human error, as pointed out by Wang et al. As they found in their literature searches, multiple research groups had previously reported automated GC/FID analysis methods for fatty acid profiling in human plasma running a large number of samples. However, none had previously managed to automate the entire analysis, including extraction of the phospholipid fraction and hydrolysis and derivatization of the free fatty acids for a large set of samples as needed for epidemiological studies.

All Steps Completely Automated

Using a combination of three automated systems, the scientists succeeded in achieving the required high throughput combined with sufficient long-term stability and accuracy for the determination of phospholipid fatty acid fractions of human plasma samples. This was done as part of epidemiologic studies designed to correlate genetic and nutrition related factors with the development of type 2 diabetes. Each of the three automated systems consisted of two independently operating MultiPurpose Sampler (MPS) systems. One MPS was equipped with automated SPE option and an integrated centrifuge to perform automated extraction and cleanup of the lipid fraction. The second MPS in each system was integrated with the GC/FID and was used to perform sample preparation, i.e. the steps required for hydrolysis and derivatization to form fatty acid methyl esters (FAMEs) as well as introduction to the integrated GC 7890 N (Agilent Technologies, Little Falls, DE, U.S.A.). When choosing the second autosampler, Wang et al. focused on functionality. The MPS in the dual head or dual rail versions features two independently operating and freely moving towers that can simultaneously operate different tools, or syringes of different sizes. For example, a dual head system can simultaneously handle large

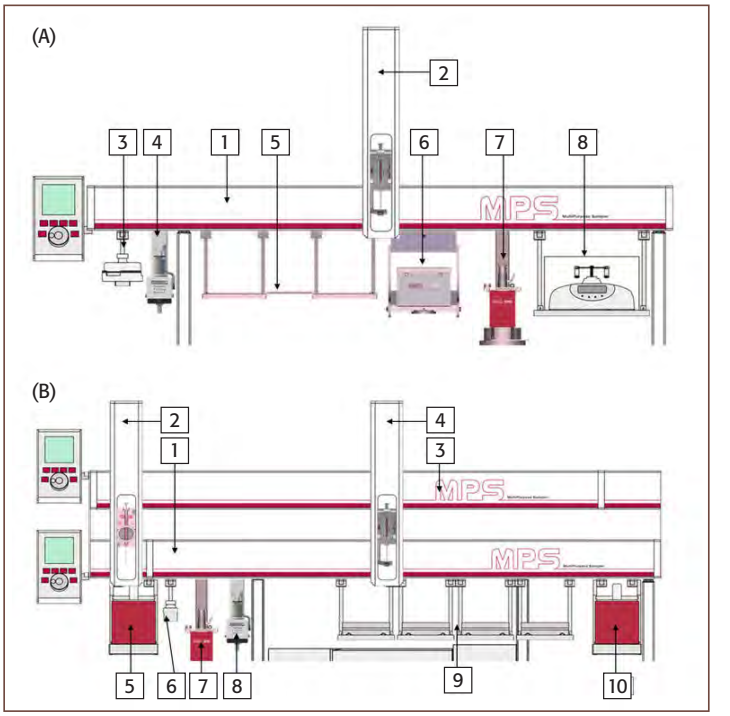
volume sample preparation and µL scale introduction to the GC analysis system. In order to achieve the required accuracy and reproducibility, reliable and accurate timing is necessary, especially when analyte derivatization is performed and the reaction product may not be stable. In such a case, the time period that elapses between analyte derivatization and introduction to the GC/FID system must be exactly the same for each sample in order to ensure accurate and reproducible results. In practice this means that samples are best prepared immediately prior to sample introduction. The MAESTRO software, which is used to operate the MPS systems by mouse-click automatically optimizes the process timing and ensures that every step is performed on time without having to be watched over by laboratory staff.

Final Words

Laura Yun Wang and her colleagues were confronted with a huge set of samples as part of the EPIC InterAct Project (See box below) for metabolic phenotyping of human plasma samples. Even with efficient automation, the analysis work took several months to complete. The three combined sample preparation and analysis setups used by the team performed extremely well over this extended period, delivering highly reproducible, reliable and stable results, including good instrument to instrument performance.

In direct comparison with the manual method, automation based on the MultiPurpose Sampler (MPS) came out ahead in many respects including improved standard deviation, as reported by Wang et al.; another aspect is the processing time per sample and the achieved throughput: Using the manual process, a team of two people could analyze 350 samples per month, or 4200 samples per year. Automating the process with three MPS systems operating in parallel enabled a team of four to process up to 90 samples plus standards and QC samples per day. On a monthly basis, 1,200 samples were analyzed, doubling the number of samples processed per person. Over a two-year period, 860 sequences were processed analyzing more than 25,000 samples in total [1]. The systems were described by the authors as both rugged and user-friendly, suitable for the determination of fatty acids in plasma phospholipids for both epidemiological research and routine analysis purposes. In addition, the method can easily be adapted to the analysis of other matrices such as cell extracts, tissue homogenates and food samples.

The work reported on in this article was part of a large case-cohort study of diabetes incidence nested within an even larger investigation into cancer and nutrition that includes 350,000 participants from 10 European countries. EPIC (European Prospective Investigation into Cancer & Nutrition) was designed to investigate the relationships between diet, nutritional status, lifestyle and environmental factors and the incidence of cancer and other chronic diseases.



MultiPurpose Sampler (MPS) Systems for automated sample preparation, derivatization of fatty acids from human plasma phospholipid fractions as well as sample introduction to GC/FID.
(A) MPS Single Rail for extraction of the phospholipid fraction, configured with: 1.) Solid phase extraction (SPE) unit; 2.) Syringe holder; 3.) Salt solution reservoir; 4.) Solvent reservoirs; 5.) Three tray holders; 6.) SPE cartridge tray; 7.) SPE / Evaporation; 8.) Vortexer / centrifuge.
(B) MPS DualRail/DualHead for hydrolysis, derivatization and injection of phospholipids 1.) Derivatization unit; 2.) Derivatization syringe holder; 3.) Injection unit; 4.) Injection syringe holder; 5.) Heated Zone; 6.) Wash bottles; 7.) SPE / Evaporation; 8.) Solvent reservoirs; 9.) Four tray holders; 10.) Agitator. [1]

InterAct

Investigating how our genes and lifestyle interact to lead to diabetes. The InterAct project started in 2006 and is an EU funded large scale collaboration between nine European Countries and India, designed to:

- discover how genetic and lifestyle behavioral factors, particularly diet and physical activity, interact in their influence on the risk of developing type 2 diabetes
- investigate how these discoveries may help to prevent the development of diabetes

More information: www.inter-act.eu

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green analytic

Water analysis

Stirred, not shaken

A highly sensitive method for quantitative determination of around 100 contaminants in surface waters with and without sediment has been developed. The key element of this GC-MS/MS-based method is Stir Bar Sorptive Extraction (SBSE) using the GERSTEL Twister. The SBSE technique is the method of choice for analyte concentration, providing simple, yet powerful, water analysis based on the EU Water Framework Directive (EU-WFD) (2013/39/EU).

By Oliver Lerch, Ph.D. and Jasmin Zboron, B.Sc.

In 2000, the EU Parliament and the Council released Directive (2000/60/EC) [1] that focuses on the protection and improvement of the quality of surface waters and ground water. The directive in its own words "lays down a strategy against the pollution of water. That strategy involves the identification of priority substances that pose a significant risk to, or via, the aquatic environment at (European, Ed.) Union level". The directive was extended and amended in 2008 (2008/105/EC) [2] and in 2013 (2013/39/EU) [3]. In essence, the Water Framework Directive (WFD) of the European Union (EU) makes it mandatory for all member states to take measures to continually monitor and improve the condition and quality of water bodies in their territory: Various parameters must be monitored, including a range of identified key chemical pollutants. In the EU WFD, priority substances are listed.

EU Environmental quality standards (EQS) specify maximum allowable concentration (MAC) levels for pollutants in surface waters that must be adhered to, at least within a foreseeable future. As the directive is worded: "with the aim of achieving good surface water chemical status in relation to those substances by 22 December 2021 by means of programs of measures included in the 2015 river basin management plans…". In the Environmental Quality Standards that apply to surface waters, there is

differentiation between annual average concentration (AA-EQS) and Maximum Allowable Concentration (MAC-EQS) levels specified for "inland surface waters", such as rivers and lakes, or on the other hand for "other surface waters", listed as transitional, coastal and territorial waters.

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GERSTEL MPS-SBSE-TDU-GC-MS/MS system used for the determination of around 100 contaminants including priority pollutants as specified in the EU Water Framework Directive (EU WFD).

A closer look at the requirements

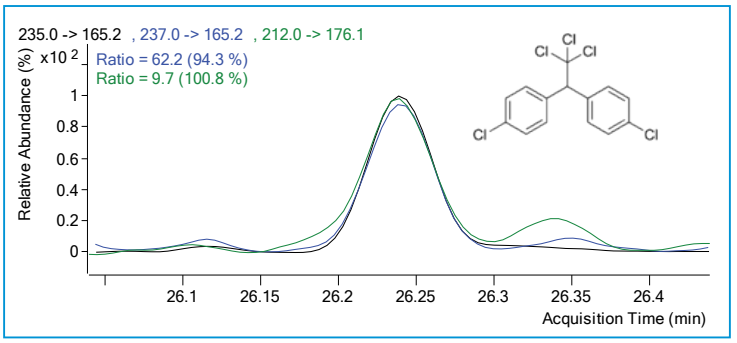
Commission Directive 2009/90/EC provides technical details for the chemical analysis and monitoring specified in the EU WFD. Minimum performance criteria for analytical methods are specified, such as limits of quantification (LOQs). Compound LOQs lower than 30 % of the respective Annual Average EQS (AA-EQS) values are required, for some compounds in the low- to sub-nanogram range per liter. For example, the required LOQ for benzo[a]pyrene is 0.051 ng/L. Reaching such levels requires a highly efficient analyte concentration technique, such as Stir Bar Sorptive Extraction (SBSE), combined with a highly sensitive analysis technique. In addition, the standard deviation (SD) must be lower than 50 % (k=2) of the specified EQS concentration value.

If these criteria cannot be met, due to a lack of suitable analysis methods, the EU-WFD offers some freedom: Monitoring should then be performed using the best available method that doesn't lead to excessive cost.

Proven water analysis technique

Stir Bar Sorptive Extraction (SBSE) with the patented GERSTEL Twister® has long been used for environmental water analysis and has performed well in round robin tests. In 2009, the Environmental protection agency of the State of Bavaria, Germany demonstrated SBSE performance in full compliance with the requirements of the German Federal drinking water regulation (TrinkwV 2001). Furthermore, SBSE was previously successfully used for the determination of priority pollutants in surface waters following the EU WFD 2000/60/EU.

As a result of a significant investment of time and resources, the SBSE method has recently been optimized for highly sensitive determination of the GC compatible priority pollutants listed in EU Directive 2013/39/EU – including particle adsorbed pollutants - using one comprehensive method.



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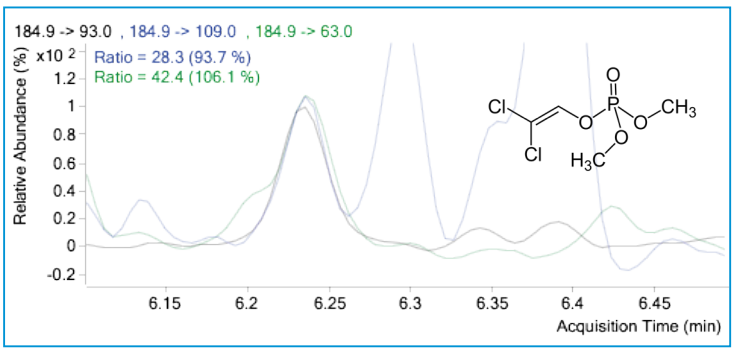
Chromatogram of p,p'-DDT at a concentration of 0.068 ng/L spiked in river water.



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Chromatogram of dichlorvos at a concentration of 0.12 ng/L, spiked in river water.

A detailed look at SBSE

The extraction medium used in SBSE is the patented GERSTEL Twister®. It is a glass coated magnetic stir bar mounted with a substantial sorbent layer. The sorbents used are either polydimethylsiloxane (PDMS) or an ethyleneglycol-silicone polymer (EG-Silicone Twister).

The extraction principle of SBSE, like that of liquid–liquid extraction (LLE), relies on partitioning, reaching

Table 1: Limits of quantitation, determined according to DIN 32645, reflecting the results for precision and accuracy. In case the water samples were free from - or had only very little - contamination with individual analytes, the limits of quantitation were determined in the matrix surface water.

Analyte	Limit of Quantitation [ng/L]
Acenaphthene	1.00
Acenaphthylene	0.10
Aclonifen	0.56
Alachlor	0.42
Aldrin	0.066
Ametryn	0.069
Anthracene	0.061
Atrazine	0.18
Benz[a]anthracene	0.076
Benzo[a]pyrene	0.033
Benzo[b]fluoranthene	0.078
Benzo[g,h,i]perylene	0.049
Benzo[k]fluoranthene	0.081
Bifenox	0.47
Biphenyl	9.00
Bis(2-ethylhexyl)phthalate (DEHP)	134
Chlordane, cis	0.052
Chlordane, trans	0.026
Chlorfenvinphos	0.084
Chlorpyrifos-Ethyl	0.024
Chrysene	0.027
Cybutryn (Irgarol 1051)	0.030
Cypermethrin (Isomerenmix)	0.12
p,p´-DDD	0.020
p,p´-DDE	0.017
o,p´-DDT	0.052
p,p´-DDT	0.067
Dibenz[a,h]anthracene	0.073
Dichlobenil	2.10
Dichlorvos	0.073
Dicofol	0.15
Dieldrin	0.034
Diflufenican	0.16
2,6-Di-tert.-butyl-4-methylphenol	5.90

Analyte	Limit of Quantitation [ng/L]
alpha-Endosulfan	0.070
beta-Endosulfan	0.059
Endosulfan sulfate	0.052
Endrin	0.043
Endrin ketone	0.052
Ethofumesat	0.073
Fenitrothion	0.024
Fenpropimorph	0.13
Fluoranthene	1.00
Fluorene	0.45
Heptachlor	0.052
Heptachlorepoxide	0.052
Hexachlorobenzene	0.10
Hexachlorobutadiene	0.043
alpha-Hexachlorocyclohexane	0.052
beta-Hexachlorocyclohexane	0.13
gamma-Hexachlorocyclohexane	0.052
delta-Hexachlorocyclohexane	0.052
Indeno[1,2,3-cd]pyrene	0.044
Isodrin	0.16
Methoxychlor	0.083
Metolachlor	0.064
Naphthalene	5.00
Nonylphenol	8.80
Octylphenol	0.46
Oxadiazon	0.082
PBDE 28	0.018
PBDE 47	0.015
PBDE 99	0.050
PBDE 100	0.011
PBDE 153	0.032
PBDE 154	0.020
PBDE 183	0.13
PCB 77	0.041

Analyte	Limit of Quantitation [ng/L]
PCB 81	0.039
PCB 105	0.043
PCB 114	0.036
PCB 118	0.012
PCB 123	0.037
PCB 126	0.050
PCB 156	0.046
PCB 157	0.047
PCB 167	0.044
PCB 169	0.054
PCB 189	0.054
Pendimethalin	0.094
Pentachlorobenzene	0.075
Pentachlorophenol	3.00
Phenanthrene	2.50
Picolinafen	0.26
Prometon	0.18
Prometryn	0.13
Propazine	0.057
Propiconazol	0.14
Propyzamide	0.35
Pyrene	0.45
Quinoxyfen	0.087
Simazine	1.90
Terbutryn	0.10
Triallate	0.084
Tributyl phosphate	9.70
1,2,3-Trichlorobenzene	0.95
1,2,4-Trichlorobenzene	1.20
1,3,5-Trichlorobenzene	0.18
Triclosan	1.40
Trifluralin	0.19
Tris(2-chloroisopropyl)-phosphate (TCPP)	29.00

equilibrium between the aqueous sample and the sorbent phase. In the work described here, PDMS Twisters were used. Analyte extraction and concentration takes place while the Twister stirs the sample. Subsequently, the Twister is removed from the sample, quickly dried, placed in a glass liner for thermal desorption, and the liner placed in a sealed sample tray position on the MultiPurpose Sampler (MPS). The MPS performs fully automated processing of large sample batches in combination with a thermal desorber. Following thermal desorption, analytes are focused in a GC inlet (GERSTEL CIS 4) and subsequently transferred to the GC column in split or splitless mode using programmable heating. This approach ensures that extracted analytes can be transferred up to 100 % to the column for extreme sensitivity. The PDMS Twister is well suited for non-polar to medium polarity compounds while the EG-Silicone Twister is mainly used for polar compounds that form hydrogen bonds as proton donor, for example phenols, alcohols and organic acids.

Simple and efficient water analysis

In this work, SBSE, using the GERSTEL Twister, was our method of choice for the GC compatible compounds listed in the EU Water Framework Directive (WFD). In addition to reaching the prescribed LOQs, we also wanted to perform quantitative determination of compounds adsorbed on particulate matter, as demanded by the EU WFD, including polyaromatic hydrocarbons (PAHs). The overall analysis requires a special approach: At first, we performed classical SBSE on individual 100 mL samples, which is a large enough volume to generate meaningful results. After adding an internal standard, we let a Twister stir the sample for five hours to extract free organic compounds. The Twister was then removed, an organic modifier was added to the sample along with a second Twister, and the sample extracted at elevated temperature overnight. Using this approach, compounds adsorbed on particulate matter were successfully released and extracted. The two Twisters were transferred to a TDU glass liner and simultaneously desorbed in the Thermal Desorption Unit (TDU) using a temperature program (90 – 300 °C). The analytes were focused in the Cooled Injection System (CIS 4) at – 40 °C. The CIS is a PTV type Universal GC inlet, which can be heated very rapidly in order to release analytes to the GC column, in our case a HP 5ms Ultra Inert (30 m x 0.25 mm x 0.25 µm) from Agilent Technologies. Following separation, the analytes were deter-

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Analytes are extracted and concentrated in the sorbent phase while the Twister stirs the sample. The stir bar is then removed, dabbed dry, and placed in a TDU liner, which is placed in an individually sealed tray position in the GERSTEL MPS. Thermal Desorption of the concentrated analytes is performed in the Thermal Desorption Unit (TDU), the process is fully automated using the MPS. After being focused in the GERSTEL Cooled Injection System (CIS), analytes are transferred to the GC column where they are separated and finally determined using a mass spectrometer.

mined using a Triple Quadrupol MS (Agilent Technologies 7010) in Multi-Reaction Monitoring (MRM) Mode.

Successful determination of water contaminants

The newly developed SBSE-GC-MS/MS method presented here is both efficient and highly sensitive. We were able to determine the GC compatible compounds listed in the EU WFD at the levels specified – or even well below - with three exceptions: Cypermethrin, heptachlor, and heptachlorepoxide. Those three are also notoriously hard to determine at the required levels down to sub-pg/L using conventional techniques.

In the following, we list a few examples of required limits of quantitation for surface water following the EU guidelines. For dichlorvos: 0.18 ng/L; using SBSE, we reached 0.073 ng/L. For benzo(a)pyrene: 0.051 ng/L; using SBSE, we reached 0.033 ng/L. For pentachlorobenzene: LOQ of 2.1 ng/L; with SBSE, we lowered it to 0.075 ng/L (see table 1). Comparable results were achieved for around 100 other contaminants relevant for surface water analysis.

Relative standard deviations (RSDs) near the respective limits of quantitation were between two and ten percent for the vast majority of compounds with a median of 6.9 percent. Trueness was between 90 and 110 percent in most cases. The extractability and quantifiability of particle adsorbed analytes was tested and confirmed using a certified reference sediment (WEPAL SETOC 745). Certified analysis results were available for PAHs and a few chlorinated compounds.

Assessing SBSE

SBSE using the GERSTEL Twister is simple to perform and highly efficient, resulting in high sensitivity, not only in the case described here, in which we successfully determined priority pollutants listed in the EU WFD. The high sensitivity is mainly due to the large volume of Twister sorbent phase, in our case 63 µL, which guarantees a high concentration factor when doing trace analysis. When the Twister is thermally desorbed, the analytes extracted from the sample are transferred quantitatively to the GC, whereas when performing liquid extraction and injection, only a small aliquot is injected. SBSE performed on a 100 mL water sample coupled with thermal desorption and a highly sensitive triple quadrupole mass spectrometer provides extremely low limits of detection, reaching the sub-ng/L range. A further bonus when using Twister technology: The entire method requires only a very small amount of solvent. And since many solvents are toxic, hazardous and harmful to the environment, a reduction in the amount used would seem particularly meaningful and fitting in order to reach environmentally sustainable solutions for chemical analysis.

References

[1] DIRECTIVE 2000/60/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 23 October 2000 establishing a framework for Community action in the field of water policy

[2] Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council

[3] DIRECTIVE 2013/39/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCILof 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy.

Presenting the Lab: The Ecology Lab at DOW in Stade, Germany

Always one step ahead

Chemical production and reaction processes, as well as storage and transportation of the resulting products, represent a risk potential. Leaks into the environment, or unwanted exposure of workers to contaminants, must immediately be rectified. The law provides minimum requirements on how to proceed in such a case. A key role is played by instrumental chemical analysis, which is used by Public Health authorities and industry alike for monitoring air, soil, and water for potential contamination. GERSTEL Solutions Worldwide magazine visited the Ecology Services Laboratories of DowDuPont at the Chemistry Park Stade in Germany. The lab is responsible for water and environmental analysis at the site and we had a look around.

By Guido Deussing

A little less than one hour by car west of Hamburg, Germany, on the Southern banks of the lower Elbe river, lies the old Hanseatic town Stade, population 46,000. Stade is a county seat in the state of Lower Saxony. Right next door is the "old country" area, which is known for its many orchards and abundant supply of fruit and also serves as recreational area for the people of the region – as well as a flood area when the Elbe rises much above normal levels. The Schwinge, an Elbe tributary, runs through Stade. The meadows along the upper Schwinge and those in Stade along the rivulet are protected nature reserves due to both their biodiversity and their serene beauty. Here you find plenty of agricultural land and picturesque villages with quaint names that reinforce the impression of visiting unspoiled countryside. The impression is accurate, but there is more to the story. Stade may be in the countryside, but it is an industrial hub, home to global players. Airbus has produced airplanes here since 1959. The 2,500 Airbus employees in Stade are specialized in the production and development of carbon fiber reinforced materials for aircraft and space craft. Among other things, they produce the vertical stabilizers (fins) for all Airbus aircraft. Around Airbus, a reliable supply chain of industries has developed.

The Dow Chemical Company also established a presence here. Since 1972, the largest chemical producer by revenue in the world has been producing in Stade at the address Bützflether Sand, which to Germans sounds like the name of the local beach on the river. Over time, Dow transformed its huge premises to an industry park and opened it to outside companies. The conversion was part of a quest to achieve higher efficiency: Share resources, develop and use synergies, and reduce cost by spreading the burden of expenses on multiple shoulders. The concept seems to work. Multiple companies are acting in synergy and coexisting at Bützflether Sand: Trinseo is operating a Polycarbonate facility; Olin is producing epoxides and chlorinated organic compounds; Air Liquide produces and supplies process-relevant technical gases; Air Products supplies hydrogen; Evides handles water treatment; Talke Carriers handles the central stock facilities; and the nearby Elbe Port serves as their gate to the world. Well connected to the freight depot train station and the container terminal, an almost perfect scheduling can be achieved for just in time transport of important goods, raw products and final products. These are shipped in all directions by rail, which unlike the express freeways in the area are generally unclogged. The industrial infrastructure here has been developed and perfected over decades, including freight forwarders that are specialized in the handling of hazardous goods. These and many other aspects make the Chemical Park Stade stand out as extremely well connected, even in global comparisons, though it is embedded in the countryside. The Ecology Lab in Stade is also in a good place: This environmental laboratory receives a lot of attention globally within the Dow organization because it, among other things, has developed outstanding methodology for monitoring volatile organic compounds (VOCs) using Thermal Desorption (TD).

Dow combines the power of science and technology to passionately innovate what is essential to human progress. The Company is driving innovations that extract value from material, polymer, chemical and biological science to help address many of the world's most challenging problems, such as the need for fresh food, safer and more sustainable transportation, clean water, energy efficiency, more durable infrastructure, and increasing agricultural productivity. Dow's integrated, market-driven portfolio delivers a broad range of technology-based products and solutions in high-growth sectors such as packaging, infrastructure, transportation, consumer care, electronics, and agriculture. The Dow Chemical Company („Dow") is a subsidiary of DowDuPont (NYSE:DWDP). DowDuPont was formed by Dow and DuPont to create three strong, independent, publicly listed companies in the areas of Agriculture, Material Science and Specialty Products.

The Ecology Lab at Dow in Stade rely on the Thermal Desorption System (TDS) from GERSTEL for their TD analysis work. And for many other applications that are performed in the lab, GERSTEL instruments and systems play a key role. Reason enough for me to visit and to look behind the scenes. I made the appointment with Michael Gröger, GERSTEL Sales Manager for Germany, Austria and Switzerland and account manager for Dow. Michael has known and looked after the Dow colleagues since… forever and he is here for a meeting with my hosts Sandra Hirsch and Andreas Köhler, who are established experts in matters environmental and water analysis at the Ecology Lab. They are considering investing in a GERSTEL MultiPurpose Sampler (MPS) WorkStation to be used for automated generation of analysis standards. At this point, some questions remain open and my scheduled visit to the Ecology Lab offers Michael Gröger a good chance to join and provide some answers. We are meeting shortly after ten on a Wednesday morning in January in light snowfall. Sandra Hirsch will pick us up in her car at the visitor parking area. We cannot access the Chemistry Part Stade premises in our own vehicle. As outside visitors, a pre-arranged permit is needed even though Michael Gröger has paid regular visits for more than 16 years. Everything follows set rules. We need the permit and that requires time and patience. First time visitors – and those who haven't been back within the past year – must acquaint themselves with the safety rules by watching a video while the person at the reception fills in the paperwork and secures personal information from the visitor's ID card. After being registered in the Dow system, it is time for the test: Three questions about security procedures in the Chemistry Park must be answered correctly. The computer delivers the result on a small piece of paper that I hand to the receptionist who checks it and hands me a permit. So far, so good. We meet Sandra Hirsch in the visitor parking lot, she greets us with a big smile and a cheerful „Moin, Moin", a sure sign that we are now in Northern Germany and the gut feeling says we're in good hands. Ms. Hirsch drives an American car, Dodge Charger, with ample room for all. Do you have to drive a US-made car even in Germany when you work for a US company? "I also like Jim Beam", she responds, she clearly likes the car, it's that simple. The gatekeeper checks all permits and then opens. The Dodge surrounds us with a deep growl and begins moving. We are met with a surprisingly green landscape, the Elbe flows nearby at the Northern edge of the Chemistry Park and I can see the endless meadows at the river bank powdered with fresh snow. "We even have deer and wild boar here", says Sandra Hirsch. I don't see any, but I'll take her word for it. But where do the 1,200 Dow employees work? And

Andreas Kohler (left) and his colleague Sandra Hirsch assess a water sample extract. Before entering the Ecology Lab, clear and clearly visible signs alert you to workplace safety rules that must be followed at all times. Work safety is the highest priority at Dow, and not only in the laboratory, but throughout all production areas and the entire Chemistry Park Stade.

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The GC Lab, part of the Ecology Laboratory within the Chemistry Park Stade, relies on GERSTEL solutions for much of its automation

where do they produce the three million tons of Dowanol, Methocel or MDI?

Where is the huge plant process equipment so typical for larger industrial chemistry sites where they produce and process allylchloride, chloroform and methylene chloride, sodium hydroxide and hydrochloric acid, propylene and… ? The stacks and huge cylinders that require massive amounts of power, steam and compressed air? Where hydrogen, oxygen and nitrogen are produced or added to the chemical processes? Where are the pipes through which countless cubic meters of process and cooling water are pumped in and waste water discharged? No sign of Trinseo, Air Liquide or Evides, companies that generate added value here. "The area covers 550 hectares or almost 1400 acres", says Sandra Hirsch, "things are less visible in these wide-open spaces."

The Ecology Lab, where Sandra Hirsch, Andreas Köhler and Ute Schomacker work is located in the ground floor of a two story building at the edge of the Chemistry Park Stade, a few minutes by car from our meeting point. Since its inauguration in 1975, the lab has regularly been expanded and updated. The laboratory deals with environmental and water analysis in the Chemistry Park Stade, including monitoring workplace air, and ensuring compliance both with maximum allowable concentrations, as per German Occupational Safety & Health rules for workplace air, and with regulations for hazardous materials.

The lab monitors water to ensure compliance with legal requirements for water quality including adherence to maximum allowable concentrations laid down in the regulations issued by the Lower Saxony State Office for Water Economy, Coastal and Environmental Protection. Every year, the Ecology Lab team analyzes around 6,000 samples, making sure that maximum allowable concentrations of various chemicals are not exceeded. 60 % of the samples are water, mainly waste water. Air samples that are required to be drawn regularly as part of routine monitoring protocols, or whenever a leak or too high workplace concentrations are suspected in the production area, make up 30 % of the samples. Soil samples are taken and analyzed, for example, whenever a new building is being planned or if doubts about earlier contamination need to be alleviated. Soil samples make up approximately 10 % of the annual sample load. "Added to all this, we get about 10 samples per month that are sent to us by the environmental protection agencies for comparative analysis. This is done in order to check the accuracy of our analysis results. The agencies can call anytime and draw samples anywhere in the entire Chemistry Park in order to perform their legally required controls", Sandra Hirsch explains. Laboratory routines are well established here, but it never gets boring for the three persons who run the lab.

Occasionally there are even tasks outside the plant area, for example, when the Company Fire Brigade are cleaning up after a spill and need to know its chemical make-up. Or maybe the Fire Brigade from the town of Stade is handling an emergency or potential emergency, involving a road truck or a freight ship on the river transporting hazardous materials. "If they need chemical analysis, we are here to support them", says Andreas Köhler.

Occasionally, we have even had the Police Crime unit request an assessment from us as to the nature of substances found at the scene of a suspected suicide. The Ecology Laboratory takes on such tasks as technical support. Good to know: Technical analytical expertise can be found when needed. It is time for a tour of the laboratories. Sandra Hirsch and Andreas Köhler lead the way. The first stop for our small procession is the Thermal Desorption Laboratory.

The lab is remarkably orderly, it almost seems as if it had been cleaned up and made extra neat for our visit, but apparently that is the way they always work. Sandra Hirsch explains: "Searching takes a lot of time, right?"

I answer: "Right!". "And we don't have time", says Andreas Köhler. "And we don't need to either", adds Sandra Hirsch, "Because we know where everything is located". The method by which everything is organized originally came from Japan. It is termed the 5S or 5A method. The method specifies that everything must be in its place, must be clean, and must be ready for use. A clear sign that the 5A method is in use is the clear markings on the lab benches around important tools or instruments: Yellow lines marking areas on the lab benches, on the floor, on drawers, and on cabinet doors make it easy to locate the right tool or instrument and to handle it correctly. It reminds me of the areas designated for smokers in Railway Stations. An optically clearly distinguishable element can discipline people operating in the area, even though there are of course lots of yellow lines and signs in the lab.

On the lab bench across the aisle, two Tube Conditioners (TC 2), used to condition sorbent tubes, are standing ready within their yellow line frame. Right next to them are two Tube Standard Preparation Systems (GERSTEL TSPS), used to load sorbent tubes with standard mixtures. "For quite a while, we experimented with different sorbent materials, and today, using just one method, we are able to determine more than 50 analytes in the range from C10 to C40 with limits of determination in the single ppb range", Andreas Köhler says. "And we are far below the required limits of determination specified by the authorities", Sandra Köhler adds. The two colleagues clearly take great pride in their work and in reaching ambitious goals. To be ahead of regulations, and always stay ahead in the analytical race, drives them to great performance. This is also wise as a precaution: "We know from experience", says Sandra Hirsch, "that rules and regulations change at short notice". Instead of waiting and reacting, the two experts are proactively meeting future challenges with steadily lower required limits of determination in order to be ready if these are enforced. "That approach has always served us well", says Andreas Köhler, "whenever stricter guidelines were enforced, we were prepared and ready". As to the method, he adds: "From volatile organic compounds (VOCs) with boiling points around -20 °C to semi-volatile compounds (SVOCs) with boiling points of 180 °C, we can generally determine them all using just one method". And the word is out within the worldwide Dow organization about the results the team has

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The Thermal Desorption laboratory of Dow in Stade. Sandra Hirsch: „Colleagues from other sites are sending us their samples for analysis".

Another color that dominates is GERSTEL magenta. Even a lay person would recognize that the lab has a high affinity towards GERSTEL products. On the lab benches along the windows, there are three complete GC/MS systems, each equipped with a GERSTEL Thermal Desorption System (TDS) with Autosampler (TDS A) mounted above a Cooled Injection System (CIS). "Apart from ensuring that the lab is well organized and neat, automation is the other way to improve efficiency", Sandra Hirsch remarks, "and this is especially important when you are not free to hire more people", as is the case in most labs. Compounds are detected using MSD and FID, connected to the GC column via a column effluent splitter.

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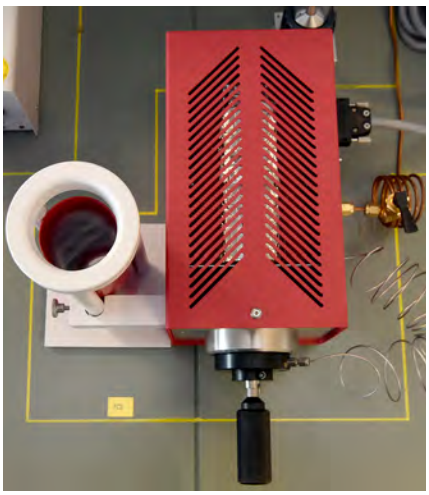
Standing side by side as they have for many years, from right to left: Andreas Köhler and Sandra Hirsch from the Ecology Laboratory with GERSTEL Sales Manager Michael Gröger

achieved. The Ecology Lab in Stade is the center of excellence for thermal desorption analysis world-wide. Sandra Hirsch: "Colleagues from other sites are sending us their samples for analysis, that is quite a recognition". But not just Occupational Safety and Hygiene samples are received from other Dow sites. Highly labor intensive AOX analyses, the results of which must be reported to authorities, are performed by the lab, which receives samples from sites in various countries. This is the work area of Ute Schomacker who has extensive knowledge in the field. By the way, Andreas Köhler discovered Thermal Desorption for his work before the year 2000. Sandra Hirsch, who today shares his enthusiasm, later joined the team. When their "homemade" TD had reached its capacity limits, they decided to purchase a commercially made system: "We needed a system that enables accurate determination of the many compounds we must to report with low limits of detection and without sample to sample carry-over" says Sandra Hirsch. Apart from that, Dow needed the potential to reach even much lower limits of determination as regulations grow stricter, coupled with efficient automation.



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Structure promotes efficiency – also when it comes to operating the GERSTEL TC 2. The team in the Dow Ecology Lab keeps the lab orderly following the Japanese 5S method. The areas framed by clearly visible yellow lines are designated exclusively for one instrument. No other instrument or unrelated tool is allowed to be stored within this area.

Early on, Andreas Köhler was interested in the GERSTEL TDS and in 2001 he contacted Michael Gröger, beginning what has today developed into a friendly professional partnership. The cooperation is not limited to the determination of VOCs in air. GERSTEL is also supplier of solutions for the analysis of water samples that make up the bulk of the samples in the Ecology Lab. In the GC lab, I count at least six GC systems with individual GERSTEL MultiPurpose Samplers (MPSs) mounted on top. Just like in the TD lab we just toured, the 5S yellow markings and GERSTEL Magenta stripes here accentuate the beige colored GC lab, which somehow exudes quality work. And as to the underlying quality of the analysis systems, Andreas Köhler reports that in the field water analysis, the Ecology Lab has equally been faced with steadily tightened requirements, which they have been able to meet. Waste water with its high salt content and the various polar compounds that need to be determined had posed quite a challenge to the analysis experts. Many compounds can be determined with standard headspace GC analysis resulting in perfectly adequate results in terms of sensitivity and accuracy. Other compounds are less simple to determine, especially higher boiling compounds or those that are more water soluble. "The solution came in the form of GERSTEL's HIT technology", says Andreas Köhler. The acronym HIT signifies Hot Injection and Trapping. "The method ensures that higher boiling compounds are kept in the gas phase during sample introduction", Andreas Köhler explains, "they don't condense on the syringe needle and, more importantly, are not removed and lost from the inlet when the needle is retracted following the injection".

In addition, analytes from multiple injections can be trapped and transferred together to the GC column for a single GC run. HIT has provided the Ecology Lab with a way to achieve better sensitivity, allowing them to stay ahead of the game for a long period of time. In the never-ending race for lower limits of determination, clearly, the team in the Ecology Lab takes great pleasure in being a step, or more exactly several steps, ahead of the authorities in meeting regulatory requirements. Sandra Hirsch: "one of our goals was to lower the limit of determination to 1 µg/L even though the requirements are 2 µg/L. We actually reached 0.2 µg/L". And while others are struggling with standard headspace or liquid/liquid extraction techniques, the Ecology Lab staff has automated their analysis quite efficiently. This means more time is available for data handling, data interpretation and reporting – or for method development. "Or to ponder how much work an MPS WorkStation needs to perform in the lab", says Sandra Hirsch, a friendly reminder that the tour through the lab is coming to an end and urgent work is waiting – I hear you.

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Food Safety Analysis

Refined Risk

In the refining process for edible oils conditions must be carefully controlled to avoid the formation of toxic process contaminants, such as 2-MCPD, 3-MCPD, glycidol and their fatty acid esters. An automated GC/MS-method now enables highly efficient determination of these compounds based on standard methods such as ISO 18363-1, AOCS Cd 29c-13, and DGF C-VI 18 (10).

By Guido Deussing

The European Food Safety Authority (EFSA) has recently updated their risk assessment for 3-monochloropropanediol (3-MCPD), 2-MCPD, fatty acid esters of these compounds, as well as glycidyl fatty acid esters (GE) in food [1]. The result is based on data submitted by 23 member states: "Glycerol-based process contaminants found in palm oil, but also in other vegetable oils, margarines and some processed foods, raise potential health concerns for average consumers of these foods in all young age groups, and for high consumers in all age groups." [2]. According to the press release: "The highest levels of glycidyl esters, as well as 3-MCPD and 2-MCPD (including esters) were found in palm oils and palm fats, followed by other oils and fats. For consumers aged three

and above, margarines and 'pastries and cakes' were the main sources of exposure to all substances." Incidentally, the FDA refers to GE as glycidol fatty acid esters.

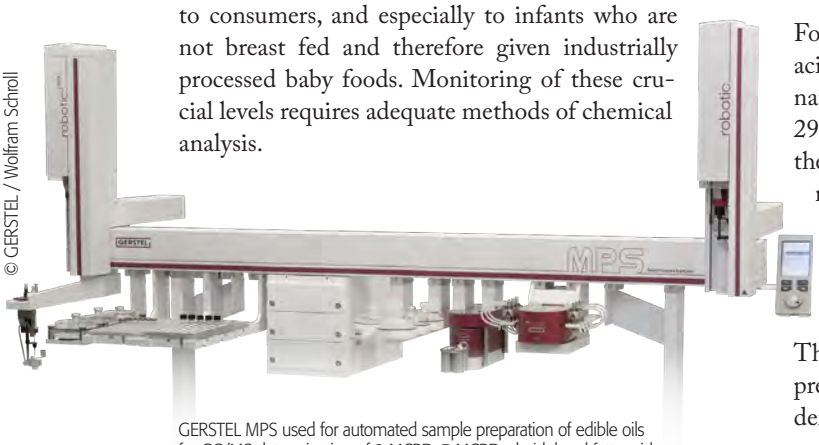
Glycerol as building block

All fats and edible oils contain glycerol in the form of fatty acid esters (triglycerides). Not all oils are ready for consumption in their native form; processing is generally required to remove off-odors and to ensure sufficient shelf life. For deodorization, as part of the refining process, steam is used to gradually heat the oil under vacuum to around 200-230 °C to ensure that unwanted flavor and taste-intensive compounds are removed along with other unwanted VOCs and even pesticides. When chloride is present, heat treatment accelerates the substitution of fatty acids in triglycerides with chlorine atoms, leading to the formation of 2-MCPD and 3-MCPD fatty acid esters. Under these conditions, diglycerides also react to form the glycidyl fatty acid esters.

Assessing the risk

The EFSA risk assessment of the above-mentioned glycerol derivatives is based on findings from animal experiments: In rats that had been fed 3-MCPD, cell changes were found, especially in the kidney area. Higher dosages led to benign tumors, as reported by the BfR in Berlin. According to the EFSA, the tolerable daily intake (TDI) value is 0.8 µg per kg body weight for 3-MCPD; due to a lack of adequate toxicological information, no assured TDI value for 2-MCPD can be given.

The risk assessment of glycidyl fatty acid esters is based on the assumption that these are fully transformed to free glycidol in the body. Since the free compound is known to be both genotoxic and carcinogenic, the experts from the panel on contaminants in the food chain (CONTAM)[3] organized by EFSA could not provide any guidance on a safe level of glycidyl fatty acid esters. When a safe level doesn't appear to exist, there is all the more need for action toward minimizing the level of these contaminants in order to minimize any health risk to consumers, and especially to infants who are not breast fed and therefore given industrially processed baby foods. Monitoring of these crucial levels requires adequate methods of chemical analysis.



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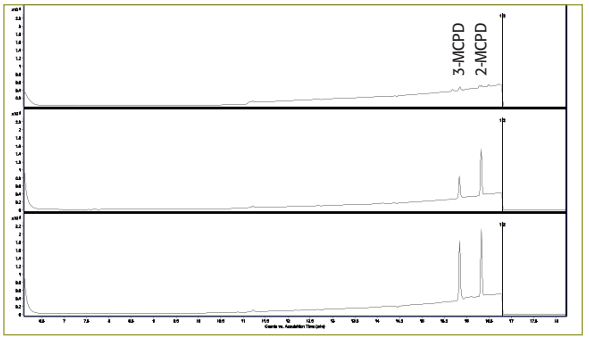
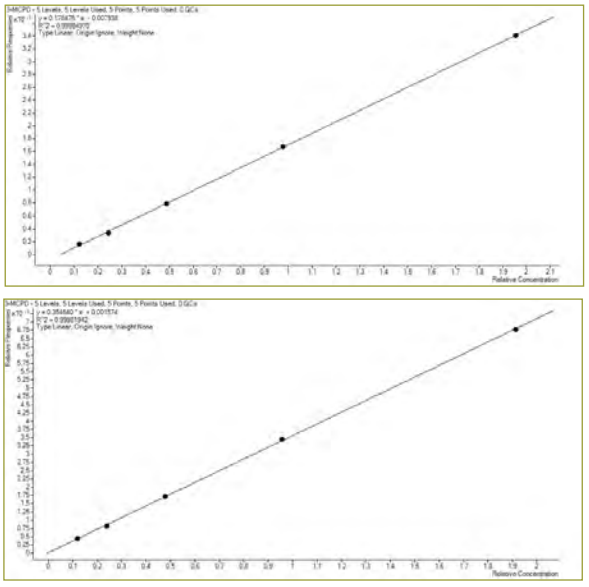
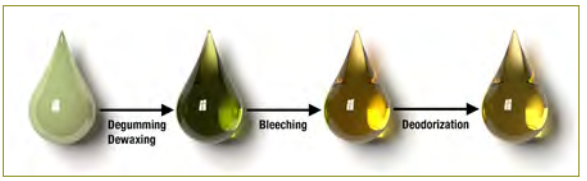
GERSTEL MPS used for automated sample preparation of edible oils for GC/MS determination of 2-MCPD, 3-MCPD, glycidol and fatty acid esters of these.



SIM chromatogram m/z 198 : Top: Virgin olive oil used as blank oil. Middle: Edible oil sample assay B (3-MCPD). Bottom: Edible oil sample assay A (3-MCPD + glycidol).



Linearity study for 3-MCPD assay B (top) and glycidyl assay A (bottom), 0.12-1.9 mg/kg each.



Refining process for production of edible oils.

A challenge for the analytical chemist

For the determination of 2-MCPD and 3-MCPD fatty acid esters, as well as glycidyl fatty acid esters, internationally, the ISO 18363-1 [4] and the AOCS Cd 29c-13 [5] methods are widely accepted. In Germany, the German Society for Fat Sciences (DGF) recommends a unified method, DGF C-VI 18 (10) [6]. The mentioned methods are all based on the use of gas chromatography with mass spectrometric detection (GC/MS). The DGF unified method C-VI 18 (10) is almost identical to the AOCS Cd 29c-13 method: The 3-MCPD content of the 3-MCPD fatty acid esters present is determined following alkaline hydrolysis and derivatization with phenylboronic acid (assay B). These methods also enable determination of glycidyl esters (as

bound glycidol) by indirect calculation after hydrolysis and conversion to 3-MCPD. Glycidol is determined as the difference between the total amount of 3-MCPD present including converted glycidol (assay A) and the amount of 3-MCPD determined in assay B. This in short highlights the added value of the unified DGF C-VI 18 (10) and the AOCS Cd 29c-13 methods. These methods enable the determination of the amount of glycidol in the sample and reading through them provides a fair impression of the effort required to transform the compounds such that they can be determined by gas chromatography, in this case relying on a manual sample preparation process that includes hydrolysis, extraction and derivatization. In addition, duplicate analysis of each sample prepared in two different ways is needed in order to determine both 3-MCPD and glycidol.

Higher efficiency and better sensitivity through automation

GERSTEL experts have developed a fully automated sample preparation solution, integrated with the GC/MS system, that replicates the AOCS Cd 29c-13/ DGF C-VI 18 (10) methods step by step on a robotic sampler. "The automated sample preparation is performed on a Multi-Purpose Sampler (MPS robotic*pro*) Dual Head version with two independently moving towers fitted with different syringe sizes. This means that larger amounts of liquid can be handled for sample preparation with one syringe while another smaller syringe handles smaller volumes, for example, used for injection into the GC/MS", says Dominik Lucas, formerly Application Specialist, now member of the German sales organization within GERSTEL.

In addition, method steps such as liquid-liquid extraction, solvent evaporation, solvent exchange to a GC-compatible solvent and analyte derivatization, are fully automated and very efficiently integrated in the overall method (see listing on the right hand side). Finally, the MPS introduces the prepared sample to the GC/MS system. Dominik Lucas adds: "The analysis results we generated using the described automated method showed good correlation with reference analysis results obtained from independent laboratories. Relative standard deviations for repeat analyses were at 5 percent for 3-MCPD and 6.4 percent for glycidol for the complete process". As to using the described sample preparation and system solution for determination of 2-MCPD, 3-MCPD, glycidol and their fatty acid esters: "The evaporation step in the method means that users can reach the required sensitivity and stability even when using a single quadropole mass spectrometer", Dominik Lucas states. The evaporation step ensures better sensitivity by concentrating analytes. In addition, it provides improved overall system stability by removing excess derivatization reagent before it can enter and destabilize the MS ion source.

MANUAL

- Weigh a 100 mg sample into a vial
- Fill a second vial with sodium sulfate as drying agent (drying vial) – optional

GERSTEL MPS

- Add MTBE to the sample
- Add ISTD solution and mix, or melt and mix (solids)
- Add MeOH/NaOH mixture
- Agitate and incubate
- Add acidic NaCl solution (Assay A)
- Add acidic NaBr solution (Assay B)
- Add n-hexane for matrix extraction
- Agitate and incubate
- Discard hexane phase
- Repeat extraction with n-hexane twice
- Perform multiple analyte extractions using MTBE/Ethylacetate 3:2 (v/v), transfer the organic phases to the drying vial
- Add phenylboronic acid solution
- Evaporate to dryness and derivatize in the ^{m}VAP at 50 °C and subambient pressure
- Take up the derivatives in isooctane
- Introduction to GC/MS(/MS) if integrated with sampler

Listing of the required manual sample preparation steps, which were transferred to the GERSTEL MPS and automated. The MPS automates the AOCS Cd 29c-13 and DGF C-VI 18 (10) methods for the determination of 2-MCPD, 3-MCPD, glycidol and fatty acid esters of these. Depending on the instrument configuration, the prepared extracts can be introduced directly to the GC/MS system for analysis.

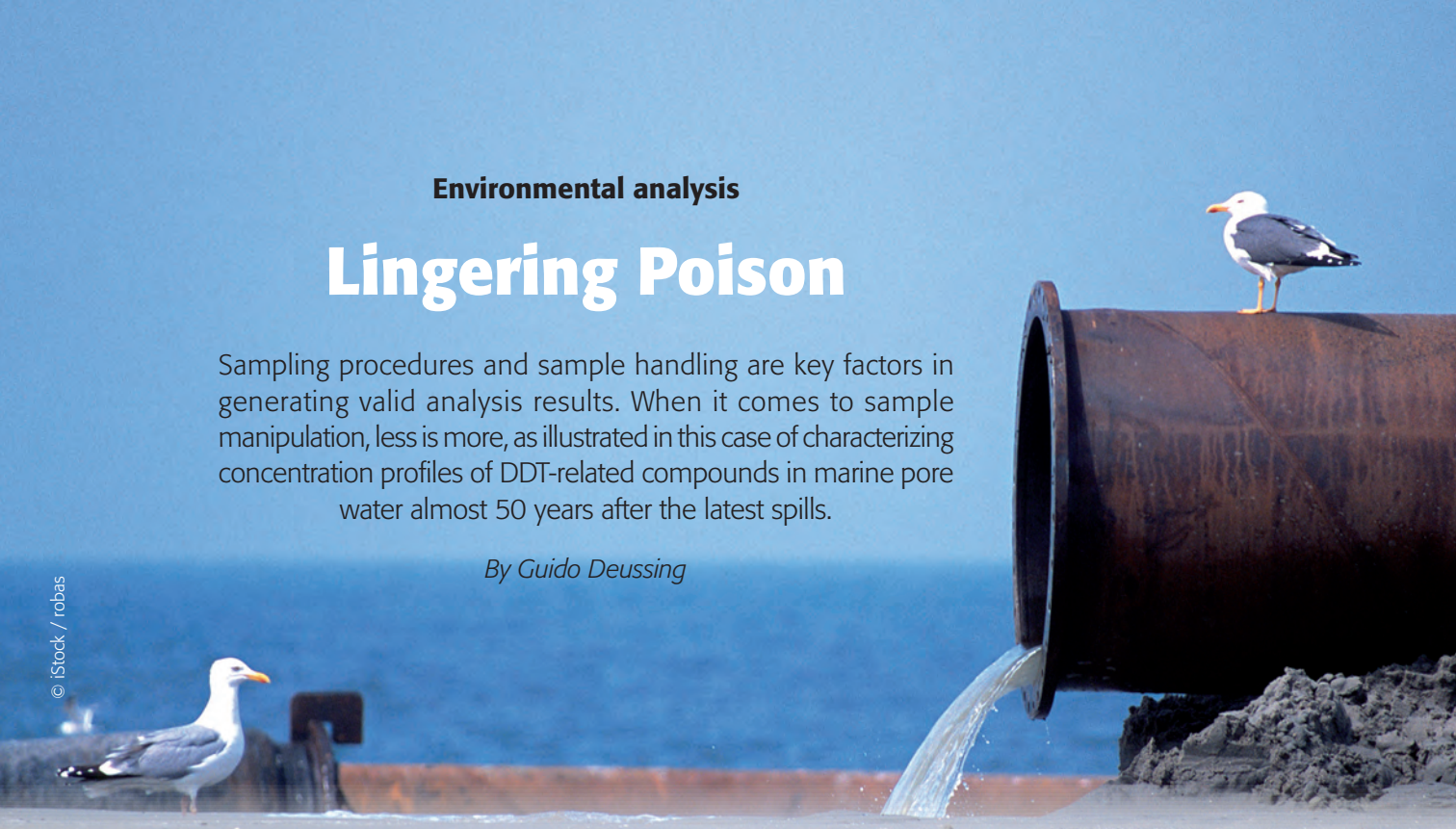
References

[1] www.efsa.europa.eu/en/efsajournal/pub/4426
[2] www.efsa.europa.eu/en/press/news/160503a
[3] www.efsa.europa.eu/en/panels/contam
[4] ISO 18363-1:2015 Animal and vegetable fats and oils -- Determination of fatty-acid-bound chloropropanediols (MCPDs) and glycidol by GC/MS -- Part 1: Method using fast alkaline transesterification and measurement for 3-MCPD and differential measurement for glycidol
[5] AOCS Official Method Cd 29b-13, Revised 2017: 2- and 3-MCPD Fatty Acid Esters and Glycidol Fatty Acid Esters in Edible Oils and Fats by GC/MS (Difference Method)
[6] DGF Unified Method C-VI 18 (10) (Available only in German language).

Lingering Poison

Sampling procedures and sample handling are key factors in generating valid analysis results. When it comes to sample manipulation, less is more, as illustrated in this case of characterizing concentration profiles of DDT-related compounds in marine pore water almost 50 years after the latest spills.

By Guido Deussing



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When bodies of water are found to contain pollutants, it may not necessarily be a result of ongoing or recent contamination. It could be related to past spills that occurred during a time when "dilution" was considered an acceptable means of disposing chemical waste. Unfortunately, these persistent organic pollutants (POPs) can be adsorbed on sediment particles and then can slowly and steadily return to the marine ecosystem over a long period of time. If you want to assess the degree of contamination in any part of the ocean floor in order to appraise the risk of contamination of aquatic ecosystems, the bioavailability of contaminants in the sediment must be determined. U.S. scientists Robert P. Eganhouse and Erica L. DiFilippo successfully developed a new method for determination of ten DDT-related compounds in marine pore water based on extraction with disposable PDMS-coated fused silica fibers. After extraction, the analytes on the fibers were determined using automated Thermal Desorption GC/MS (TD-GC/MS). As they report in their journal paper, the method is simple, and "improves the ability to meet quality objectives at a significantly lower cost" [1].

Characteristics of sampling procedures

As part of a multi-disciplinary investigation of the factors controlling the fate of DDT in sediments of the Palos Verdes Shelf in the Pacific Ocean near Los Angeles, it was the task of Eganhouse and DiFilippo to assess the whereabouts and fate of the insecticide dichlorodiphenyltrichloroethane (DDT), which was prohibited in the US in 1972. The project was called into life following public discussion of the need for highly cost intensive clean-up [2]. Between 1947 and 1971, the Montrose Chemical Corporation released waste water containing hundreds of tons of DDT from their plant into the county sewer system from where it was released into the ocean. Eganhouse and DiFilippo set about determining the concentrations of ten DDT-related compounds in marine sediment pore water. As a first step, they identified a suitable sampling procedure and analysis method, which would give accurate results while not being too labor intensive or time consuming and preferably keeping cost and environmental impact to a minimum. Samples were taken slightly above the ocean floor and from ocean floor sediment. Sampling was conducted at three different locations on the Palos Verdes Shelf off the coast of Los Angeles, the scientists took sediment cores at a water depth of 60 meters using specially designed coring boxes. Subcores were subsequently taken using core tubes with sealed ports along their lengths for later insertion of analyte sampling devices. The cores were finally sealed at both ends and overlying seawater was expelled through a vent hole. The whole process was performed without compacting the core or altering its composition or structure, as Eganhouse and DiFilippo report. The cores were kept cool at the original sediment temperature (11 °C) and transported to the Laboratories of the United States Geological Survey (USGS) Water Science Center in San Diego, where they were stored in vertical position at a temperature of 11 °C until they could be sampled.

Extraction with disposable fibers

Eganhouse and DiFilippo succeeded in preserving the original sample structure and in avoiding changes to the natural distribution of contaminants between pore water and sediment in the sample. The scientists then focused their attention on determining the DDT related compound concentrations in the pore water. The next step was to extract and concentrate the analytes on a suitable carri-

er material for later analysis by TD-GC/MS. The extraction technique of choice was solid phase microextraction (SPME) and after several test runs, they decided to use 10 cm long pieces of fused silica fiber coated with a thin layer of polydimethylsiloxane (PDMS) (Fiberguide, Dr. M. T. O. Jonker). An important criterion was that the extractive capacity of the fibers should not disturb the system by significantly depleting the sample of contaminants. The fibers were inserted into the core through holes on the side, which were resealed, and were left for up to 79 days in order to allow equilibrium to be established between the fiber, pore water and sediment. Fibers were recovered after different extraction periods and prepared for TD-GC/MS analysis: They were rinsed, dried and cut in 2 cm long pieces that were placed in micro-vials and stored contamination free at -20 °C until they could be analyzed.

Intuitive Software Control

Open micro-vials, each containing three pieces of a single SPME fiber, were transferred to individually sealed glass thermal desorption tubes. The TDU tubes were fitted with transport adapters for automated processing using the GERSTEL MultiPurpose Sampler (MPS). Thermal desorption of the SPME fibers was performed using a GERSTEL Thermal Desorption Unit (TDU) connected to a Cooled Injection System (CIS), which was used for cryofocusing and subsequent transfer to the GC column for highly sensitive GC/MS determination. The intuitive MAESTRO software control enabled Eganhouse and DiFilippo to quickly set up and automate processing of the samples as well as automating spiking of the TDU tubes with liquid calibration standards or adding internal standard. The CIS was used to transfer analytes to the GC column inside the 6890 GC plus (Agilent Technologies) using programmed temperature vaporization (PTV). Separation was performed on a 30 m DB 5 capillary column, 0.25 mm ID and 0.25 µm film thickness, which was connected to the mass selective detector (5973 MSD from Agilent Technologies) through a heated interface kept at 275 °C. The quadropole was kept at 150 °C and the ion source at 230 °C.

Data acquisition was performed in full scan mode (FS) ranging from 50 to 500 amu at 1.68 scans per second. Setup and control of the MPS-TDU-GC/MS system as well as data acquisition and processing was performed using the MAESTRO software integrated with Agilent® Technologies ChemStation.

Verification of individual compound identities was based on MS and retention time data as well as comparisons with mass spectra in the NIST MS library database, Eganhouse and DiFilippo report.

Reaching the set goals

Following extended method development, during which Eganhouse and DiFilippo worked out the best extraction medium for the analysis and optimized desorption and analysis conditions, the scientists succeeded in determining ten DDT-related compounds in marine pore water using their TDU-GC/MS system. Among the analytes were: 4,4‘-DDNS, 4,4‘-DDNU, 4,4‘-DDMU, 2,4‘-DDE, 4,4‘-DDMS, 4,4‘-DDE, 2,4‘-DDD, 4,4‘-DDD, 2,4‘-DDT, and 4,4‘-DDT.

Method Detection Limits (MDLs) for all DDT-related compounds in the fiber coating were in the range between 0.177 ng/µL (4,4‘-DDNS) and 1.66 ng/µL (4,4‘-DDT) in FS and between 1.90 pg/µL (4,4‘-DDNS) and 4.98 pg/µL (4,4‘-DDT) in single ion monitoring mode (SIM). These units are per µL fiber coating, which translates to MDLs in seawater of 0.05–2.4 ng/L and 0.67–16 pg/L for FS and SIM respectively, calculated based on compound-specific PDMS-water partition coefficients at 11 °C. It should be noted that the GC/MS system used was an older model; current models will provide at least an order of magnitude better sensitivity and thus should generate significantly better MDLs.

In addition to the DDT-related compounds, other hydrophobic organic contaminants (HOCs) can be determined at sub-parts per trillion levels and monitored over extended time periods using the presented method. In their work analyzing marine pore water, Eganhouse and DiFilippo found the dominant contaminants to be 4,4‘-DDNU, 4,4‘-DDMU, 4,4‘-DDE and 2,4‘-DDE. Furthermore, a range of other compounds, both of natural and anthropogenic origins, were identified, among which were fatty acids, steroids, tensides, gasoline additives, antioxidants, plasticizers, and polychlorinated biphenyls (PCBs).

Performance data on the final method confirmed the high quality of the overall SPME-TD-GC/MS method used. The authors concluded that the method is relatively simple, cost effective, efficient, accurate and precise. Method recovery can be adjusted quite easily by adjusting the length of fiber inserted for analyte extraction and, as a precaution, multiple fibers can be inserted in each section in order to have a back-up sample in case of loss or breakage. Finally, the following statement from the authors should speak for itself: “Moreover, the TDU-CIS system appears to be effectively inert; we detected little or no evidence of degradation of these thermally sensitive compounds”.

References

[1] Robert P. Eganhouse, Erica L. DiFilippo, Determination of 1-chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl)ethyl] benzene and related compounds in marine pore water by automated thermal desorption gas chromatography/mass spectrometry using disposable optical fiber, Journal of Chromatography A, 1415 (2015) 38–47

[2] www.scientificamerican.com/article/the-mystery-of-the-vanishing-ddt-in-the-ocean-near-los-angeles

TD 3.5+: Steely and 20 percent more capacity

The Thermal Desorber TD 3.5+ is a key component of automated thermal desorption and extraction solutions. The TD 3.5+ fits on top of any modern GC without the need for additional bench space and is perfectly suited for the analysis of material emissions and air. The TD 3.5+ handles 3.5" tubes, as prescribed in standard methods, and GERSTEL plus tubes with up to 20 % more sorbent for enhanced break-through volume and improved recovery and limits of detection.

The TD 3.5+ incorporates the latest advances in thermal desorption technology. Intelligently designed and based on a "Liner-in-Liner" concept it has no valves or transfer lines. The TD 3.5+ is connected directly to the GERSTEL Cooled Injection System (CIS), which serves both as a cryo-focusing trap and as a temperature programmable GC inlet. Active sites are eliminated, reducing the risk of analyte loss, discrimination and memory effects to an absolute minimum.

The TD 3.5+ can be operated in single split, dual split or true splitless mode enabling it to cover the widest range of concentrations, to protect the column from water and contamination, and to reach the lowest possible limits of detection. For extreme sensitivity, multi-desorption mode is simply selected in the method.

The MAESTRO software combined with Agilent Technologies MassHunter or ChemStation controls the complete process from DHS to thermal desorption to GC/MS analysis with one integrated method and one sequence table ensuring efficient and error-free operation. Samples can be analyzed using one or more methods and priority samples inserted as needed. Techniques supported by TD 3.5+:

- Thermal desorption of adsorbent tubes used for air sampling
- Dynamic Headspace (DHS 3.5+) based on standard headspace vials
- DHS 3.5+ Large based on sample containers up to 1 L in volume
- Thermal extraction of solid samples placed in fritted TD tubes
- Twister desorption following stir bar sorptive extraction (SBSE)

The TD 3.5+ can be removed in seconds to enable direct liquid sample introduction into the GERSTEL Cooled Injection System (CIS), a PTV-type universal GC inlet.

When configured with the GERSTEL MultiPurpose Sampler (MPS robotic), up to 40 samples are stored per TD 3.5+ tray in individually sealed sample positions. The system can easily be scaled up by using up to three TD 3.5+ trays per tray holder. The number of tray holders depends on size and configuration of the MPS robotic.

Hot in the vial: Pyrolysis for HPLC and more

The GERSTEL PyroVial enables pyrolysis procedures in a dedicated sample vial up to 800 °C. Volatile pyrolysis products can be sampled directly from the headspace for GC/MS determination. Less volatile pyrolysis products can be taken up in a suitable solvent for subsequent GC/MS- or LC/MS determination – or for analysis using other techniques. The pyrolysis process is fully automated based on the GERSTEL MultiPurpose Sampler (MPS). Placing the sample into the reaction chamber is very simple and the PyroVial can be used as a micro-scale reaction chamber.

The gas phase in the vial can be replaced by an inert gas or a reactant as needed. Food preparation processes, such as the Maillard reaction can be simulated in small scale. The addition of reagents or catalysts before pyrolysis even enables the simulation of complex industrial processes.

DHS large 3,5+: Automated micro-scale chamber

The DHS large 3.5 allows samples to be placed in individual inert chambers up to 1 liter in volume with precise temperature and purge gas flow control. Analytes are automatically collected at user-defined intervals followed by thermal desorption in the TD 3.5+ and GC/MS determination. Emission profiles can be established automatically and automated spiking of standards onto sorbent tubes can be performed for calibration and qualification purposes using the TSS option for the MPS robotic .

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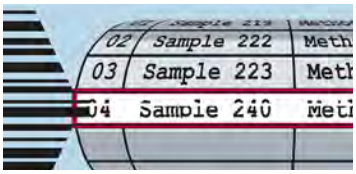
Sequence by barcode: Type it all in? No, just load up the samples



In addition to sample logging, new automation efficiency and QC options have been integrated into the MAESTRO software based on the GERSTEL Sample ID (SID) Barcode reader:

- Automated analysis by predefined methods
- Automated generation of sequence tables
- Single sample mode or Batch Analysis Mode (BAM) selected by mouse-click
- Trigger vials introduced at user defined intervals, activating solvent injection(s) followed by one or more check standard runs as part of the QC routine operation.
- Trigger function requires only one vial position enabling high overall analysis throughput
- Multiple options for sample verification and handling of deviations
- Password restricted access, no unauthorized changes
- Sample ID transfer to the data file
- Sample ID incorporation into the data file name
- Data import and export functions enable LIMS or Data Base synchronization.

SPE 2: Rugged automation

The new GERSTEL SPE 2 is available for the MultiPurpose Sampler (MPS robotic Pro). All SPE steps are performed automatically using the MAESTRO PrepAhead function for maximum efficiency and throughput. SPE can be followed by sample introduction to a dedicated GC/MS or LC/MS system resulting in the exact same treatment of all samples. Or SPE can be performed off-line for independent analysis using one or more techniques. Liquid volumes are dispensed highly accurately; eluates are transferred using individual syringe needles and collection vials, minimizing sample-to-sample carry-over. Samples are processed using individual standard dimension 1, 3, and 6 mL SPE cartridges for easy transfer of established SPE methods to the automated system. Set up by mouse-click includes added sample prep steps such as evaporation, dilution, and mixing, as well as adding standards and reagents or introducing the clean eluate to the analysis system.

The Solvent Filling Station (SFS 3) holds up to four 1 L solvent bottles; up to two SFS 3 modules can be mounted on the MPS robotic pro. Solvent waste can be collected in separate receptacles for optimized waste disposal.

*Quick*Mix: Shake it up

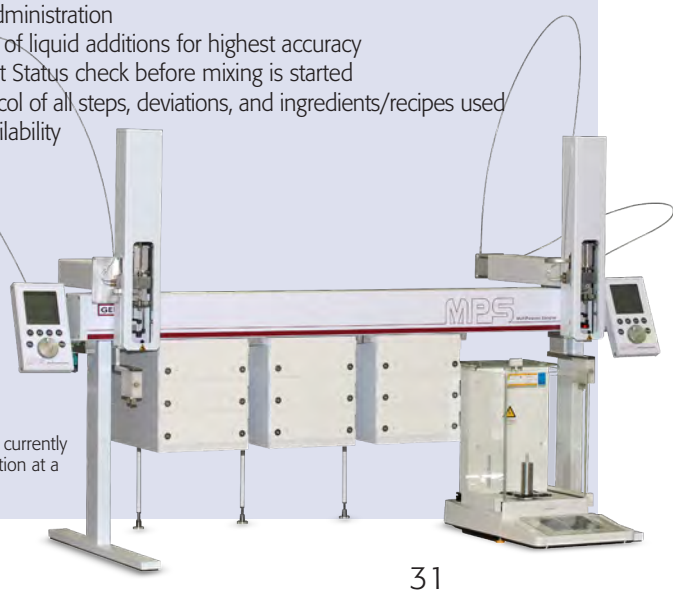
*Quick*Mix is now available for the GERSTEL MPS robotic series. *Quick*Mix enables extremely fast and efficient mixing and extraction of samples as part of the automated sample preparation process. The mixing power is comparable to that of vortex mixing. *Quick*Mix can be used with 2 mL, 4 mL, 10 mL, and 20 mL vials. If needed, *Quick*Mix can be configured with a heated tray. All sample preparation steps are set up by mouse-click in the MAESTRO software in stand-alone operation, fully integrated with Agilent Technologies MassHunter or ChemStation, or coupled with software from SCIEX or Thermo Fisher Scientific.

Benchtop Mixture Maker for Flavorists

The Mixture Maker from GERSTEL is a bench-top blending station with dedicated software, designed for highly accurate automated generation of flavor and fragrance blends from large numbers of ingredients.
More than 900 raw materials (essential oils, extracts...) can be stored protected from light in 3 cooled stacks with 3 drawers each.
The Mixture Maker generates blends in the µL range, resulting in significant savings on the costly ingredients often required in flavor and fragrance product development.

Weighing is performed automatically and is used for individual ingredient delivery calibration or for general liquid additions when highest possible accuracy is needed. The Mixture Maker software includes detailed ingredient information, with individual correction factors, recipe checks and ingredient stock monitoring. All steps in the process and deviations in weight are logged, allowing the flavorist to take corrective action. The Mixture Maker is designed for uninterrupted 24/7 operation and highest productivity.

- Miniaturization, blending in µL range
- Raw material savings up to 90 %, excellent ROI
- Easy automated generation of series of flavors
- Automation level for common flavors > 75%
- Automation level up to 100% for test or calibration mixtures
- Recipes can be generated in LIMS and imported
- Recipe administration
- Weighing of liquid additions for highest accuracy
- Ingredient Status check before mixing is started
- Full protocol of all steps, deviations, and ingredients/recipes used
- 24/7 availability



Mixture Maker currently in 24/7 operation at a customer site

Preview – coming up in the next issue

Caution: E-cigarettes

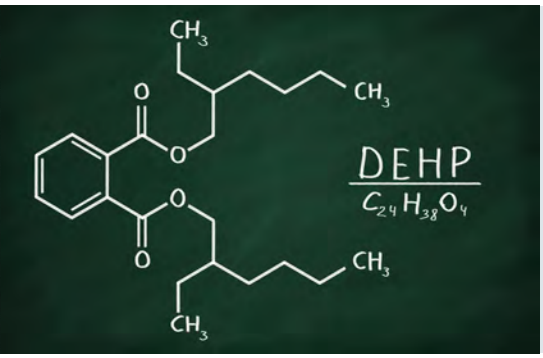
E-cigarettes and e-liquids have one thing in common with classical tobacco products: They must be closely monitored to ensure that they do not expose consumers to health risks. The analysis of e-liquids is not at all trivial, however. It requires both the right strategy and the right technology.

Efficient Anti-Doping analysis

The Center for Preventive Doping Research at the German Sport University in Cologne, Germany is racing to stay ahead of doping offenders by upgrading the laboratories with fully automated Dried Blood Spot-LC-MS/MS Systems.

Wellness in the woods

Even a short stay in the woods supposedly brings about healing - or is it all in your head? Apparently not: Japanese researchers have found that Natural Volatile Organic Compounds (NVOCs) emitted from trees have healing properties and they are looking at setting up customized natural wellness areas.



Additives on the fly

They are volatile, and they pose health risks: Phthalates are used in many industrial processes and must be monitored in the environment. Based on extensive literature search, scientists have been working to determine, which air monitoring methods deliver the best results.

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Imprint

Published by
GERSTEL GmbH & Co. KG
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ISSN 1619-0076

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