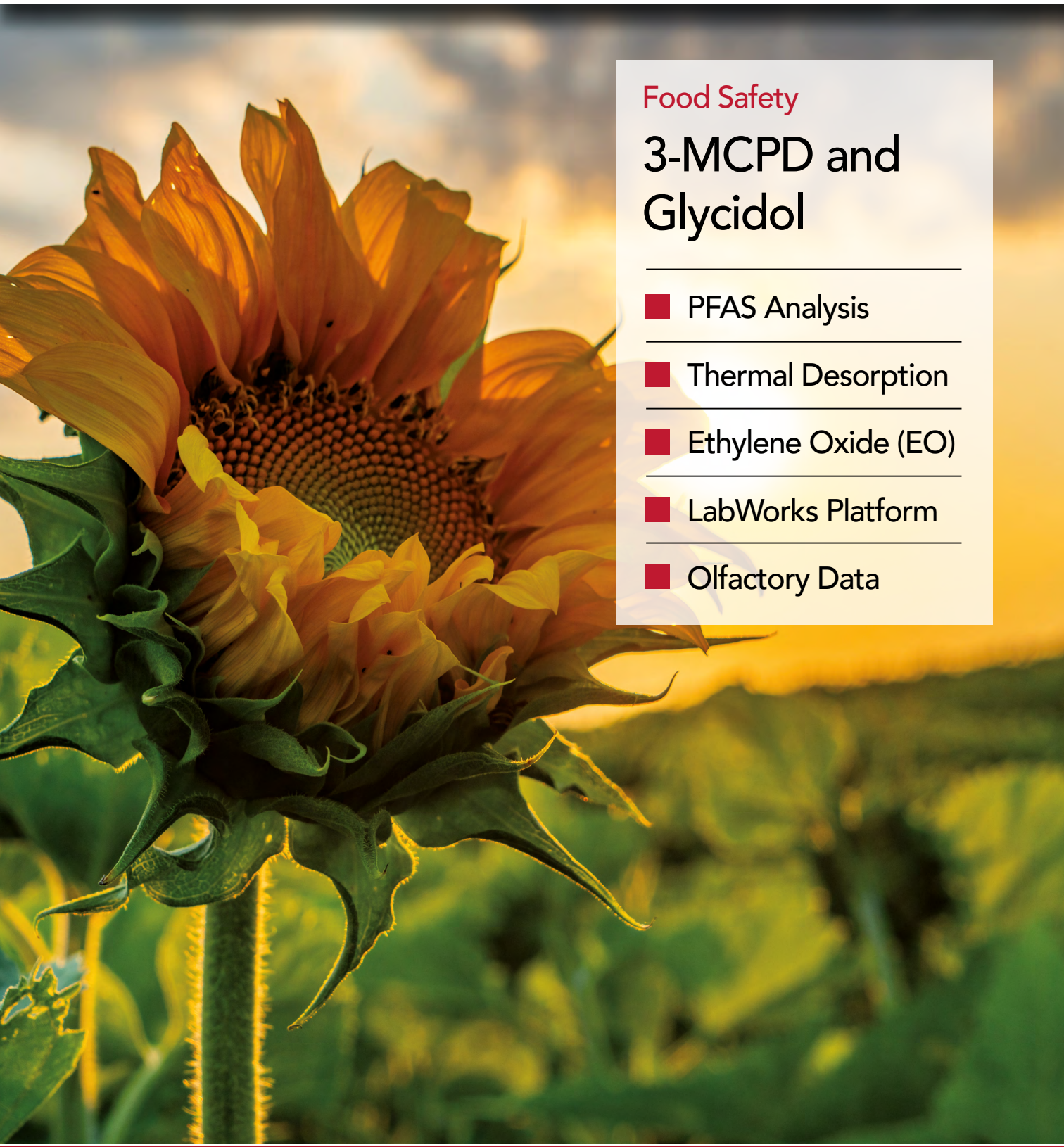
GERSTEL

MAKING LABS WORK

No. 19

solutions worldwide



Food Safety

3-MCPD and Glycidol

- PFAS Analysis
- Thermal Desorption
- Ethylene Oxide (EO)
- LabWorks Platform
- Olfactory Data

Prepared for further growth

These are memorable times. Change around us is leading to change inside every organization. Over the past two years, we have prepared GERSTEL, restructuring and readying the company and our processes for new opportunities and challenges.
Steps have also been taken that are important for a family operated business. A lot is at stake, nothing less than retaining and expanding know-how and passing it on to the next generation. To continue to be a reliable, competent, and responsive partner for you, our customers, we continually strive to fully understand and support your needs and your applications. Further we constantly follow developments in how lab operations can be optimized to meet the relentless demands for productivity growth and higher performance. Our company wide processes have been scrutinized, and structures and communication tools optimized to make the entire organization more responsive and more flexible. This has been done with a view to making new product developments available faster, and to be able to respond more quickly to requests and to changes in laboratory requirements. Our key areas of technology continue to be thermal desorption and automated sample preparation for GC-MS and LC-MS. You can count on GERSTEL to deliver unique solutions supported by technical and application know-how, which are second to none based on 50 years of accrued experience in the market. This is your guarantee that the requirements you are faced with can be met and expectations exceeded. To give you an impression, we cordially invite you to take a closer look at solutions for various applications that are presented in the following pages of this 19th issue of the GERSTEL Solutions Worldwide magazine. Will you also profit directly using a product or a solution to make your operation even more efficient? Let's have the conversation. Maybe you can visit us at one of the many conferences we attend – or just contact us to find out how GERSTEL technology can benefit you.

GERSTEL Management, from left to right: Peter Wiersdörfer, Holger Gerstel and Ralf Bremer

We look forward to meeting you.

GERSTEL Management

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Introducing the GERSTEL Management Board

For 55 years, GERSTEL has been developing and producing technology that is highly regarded in laboratories worldwide. During that time, our products and solutions have provided added value to analysts working in research and development, quality control, food safety, environmental and forensic analysis, and product development.
Our actions, characterized by mutual respect, are aimed at building, and maintaining long-term relationships with our customers and our partners. Our goal is to make it an inspirational experience to work with our company and with our products and services. It is a great honor for us to be entrusted with the implementation of technical and application innovation. Our committed, skilled employees ensure that we live up to the trust placed in us.
To address the welcome challenges of a steadily growing company, we have expanded the company's management team. The aim is to improve cooperation between all areas of the company to further increase productivity, efficiency, product quality and delivery reliability.
The key to mutual success is people - our customers, our business partners, and our employees. As a responsible employer, we attach great importance to actively developing and supporting our team.

Holger Gerstel

Holger Gerstel (Owner and Managing Director) joined the company, which was founded by his father Eberhard Gerstel Sr. (1927-2004), 35 years ago. He has held key responsibilities as Owner and General Manager since 1998, establishing GERSTEL GmbH & Co. KG and developing it into a worldwide leading technology provider, specializing in thermal desorption GC and automated GC/LC sample preparation and sample introduction. During this time, the company has expanded both nationally and internationally and has established subsidiaries in the USA, Japan, China, Singapore, and Switzerland. GERSTEL is present in more than 70 countries. Now, as the sole owner, Holger Gerstel is working to shape the future of the entire GERSTEL Group. He is guided by a quote by the German poet and thinker Johann Wolfgang von Goethe: "It isn't enough to know – you must apply your knowledge. It isn't enough to want to do something – you must act".

"It isn't enough to know – you must apply your knowledge.
It isn't enough to want to do something
– you must act."

Holger Gerstel
Owner and Managing Director

Ralf Bremer

Once Eberhard Gerstel Senior's right-hand man, Ralf Bremer took over responsibility for the further development and expansion of the GERSTEL product portfolio as Managing Director in 1998 after the company founder's retirement. Since then, he has been driven by the idea of developing analytical solutions that make the daily work of laboratory personnel easier and that contribute to greater sustainability with low environmental impact. Ralf Bremer's activities span decades with worldwide reach. His countless mutually beneficial cooperation projects with customers and business partners have expanded and sharpened his sense of the achievable analytical solutions, innovations, and markets. Ralf Bremer's competence and experience have greatly contributed to our business success. Transferring his knowledge to the next generation of employees is a matter of particular importance to him.

"Make the daily work of users in the laboratory easier for greater sustainability and environmental protection."

Ralf Bremer
Managing Director

GERSTEL Management Board

Peter Wiersdörfer

"Those who say it can't be done should get out of the way of those doing it."

Peter Wiersdörfer
Chief Financial Officer

For 35 years, Peter Wiersdörfer worked in various banks, most recently as a director and divisional manager at a leading independent regional bank. The appeal was great to use the experience he had gained to help grow a medium-sized company. For more than two years now, Peter Wiersdörfer has been part of GERSTEL's upper management. True to the motto „Who dares wins", he understands and conveys change as an opportunity. His responsibilities include business finance, but also the establishment and development of internal structures and processes that generate added value for the company as well as for GERSTEL's customers and business partners. One of his overriding goals is to expand the company's leading market position and to guide its transition to the family's third generation.

Dr. Sascha Giegold

"My job is to brighten the stars and to make us all shine."

Sascha Giegold, Ph.D.
Director Global Sales
and Marketing

As a former application and sales specialist, product manager, and branch manager of a leading global player in the analytical industry, the Ph.D. chemist Dr. Sascha Giegold knows what matters in laboratories - and he has a proven performance record. As Director of Global Sales and Marketing, his stated goal is to optimize and focus the organization using the latest communication and process tools for best-in-class efficiency and responsiveness. Leading through motivation and curiosity is his mantra, making sure that the team effort is supported by all to reach our market leadership goals. "My job is to brighten the stars and to make us all shine", says Dr. Giegold.

Dr. Eike Kleine-Benne

„Key to GERSTEL's success has been listening to our customers and working closely with them to develop solutions that meet their critical challenges."

Eike Kleine-Benne, Ph.D.
Director Innovation
and Technology

Dr. Eike Kleine-Benne, involved in research and development from the start of his career at GERSTEL in 2000, initially focused on cooperation with external partners. In recent years, his focus has increasingly been on technical product management. In joint leadership with Dirk Bremer, Dr. Kleine-Benne has taken on the task of further developing the company's products and services, leveraging the existing knowledge base, and pursuing new opportunities. "Key to GERSTEL's success has been listening to our customers and working closely with them to develop solutions that meet their critical challenges", says the Ph.D. chemist, who plans to continue this tradition and to further expand GERSTEL's expertise in providing innovative products.

Walter Mertzen

GERSTEL is a leading technology supplier and a responsible business partner. This includes comprehensive, end-to-end service and support. Walter Mertzen knows how important service and support is, based on his experience over 27 years at GERSTEL, building both the local and worldwide support organizations. The chemical engineer has deep knowledge of GERSTEL technology and experience in designing and conducting customer training courses, as well as in overcoming technical and application challenges. In his view, two ingredients are essential for success: Enthusiasm and balance. Those attributes are Walter Mertzen's guiding principles in his dealings with customers and in the management of his team. He draws on a wealth of experience gained through work for GERSTEL both nationally and worldwide.

"Two ingredients are essential for success: Enthusiasm and balance."

Walter Mertzen
Director Service and Support

Dirk Bremer

Based on his many successful years at GERSTEL, Dirk Bremer is one of the most experienced employees in the company. During his 30-year career, he has worked in all technical areas of the company. For the past 20 years, as R&D Manager he has also been responsible for custom-made products and solutions that have successfully been implemented in laboratories throughout the world. There is hardly a device or system in GERSTEL's product portfolio that Dirk Bremer has not been involved in developing. His extensive knowledge of GERSTEL technology, especially in the field of thermal desorption and automated sample preparation and sample introduction, makes Dirk Bremer an excellent counterpart to Dr. Eike Kleine-Benne, with whom he is jointly responsible for the Innovation and Technology Division.

„Those who want to achieve something find a way; those who don't find a reason."

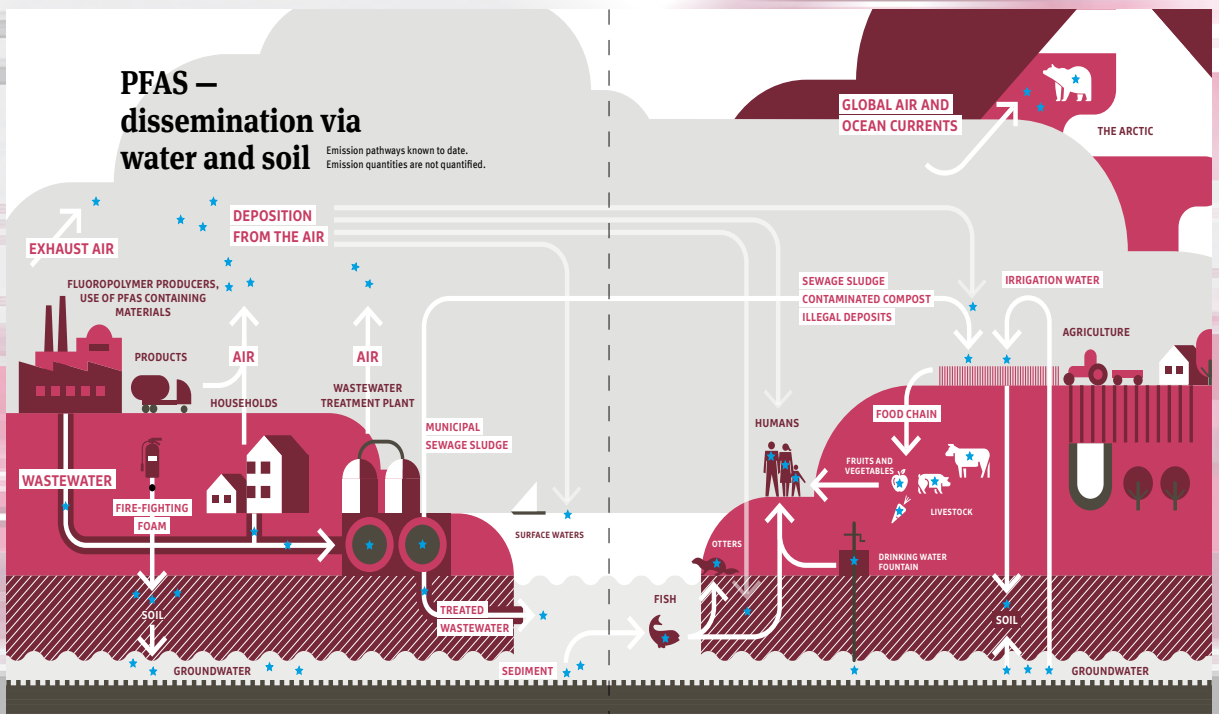
Dirk Bremer
Director Innovation and Technology

Marcel Müchen

A company whose core business is the development, production, and supply of sought-after high technology solutions is constantly faced with challenges that can be described in just a few words: Productivity, efficiency, product quality and on time delivery. What is easy to put down on paper requires skill, experience, depth of knowledge, and assertiveness in business practice. Marcel Müchen took the reins two years ago as Head of Production and Quality Assurance and has made it his task to harmonize existing processes company wide, making them more efficient through process oriented management and control. Marcel Müchen is also responsible for purchasing, logistics, order planning, quality management and production, which enables an even more targeted and sustainable approach throughout the entire internal process chain. In this respect, Marcel Müchen builds on experience gained as a production manager and project manager in previous jobs. The results of his efforts are added value for both GERSTEL and the company's customers.

"Productivity, efficiency, product quality and on time delivery require skill, experience, depth of knowledge and assertiveness."

Marcel Müchen
Director Business Operation



PFAS analysis miniaturized and automated

The EU Drinking Water Directive (EU 2020/2184) includes limits for total PFAS of 0.5 µg/L and sets a limit of 0.1 µg/L for the sum of 20 per- and polyfluoroalkyl substances (PFAS) of most concern. The Directive entered into force in January 2021. EU Member States have a 2-year transitional period to develop national laws. The listed perfluorinated carbonic and sulfonic acids can be determined by an automated method based on miniaturized solid phase extraction with weak anion exchange sorbent combined with LC-MS/MS. Surface adsorbed analytes are included using rinse cycles and effluent recovery, at the same time minimizing sample-to-sample carry-over. Based on just 1 mL sample volume, as opposed to the normally used 250 mL, the required quantification limit of 1.5 ng/L is reached for all listed analytes with good method accuracy.

By Thomas Brandsch, Ph.D.

It occasionally takes a while, before we as consumers realize that synthetic chemicals, which make our lives easier and more convenient, are harmful in addition to being useful. The category of synthetic chemical troublemakers includes Persistent Organic Pollutants (POPs). Once released, the members of the so-called dirty dozen can hardly be recovered and neutralized since mother nature has no effective mechanism to do so. Per- and polyfluoroalkyl substances (PFAS) should now be added to that list. Their resistance to photolytic, hydrolytic, oxidative, and reductive breakdown mechanisms are the exact properties designed into these compounds to make them fulfill their purpose. PFAS belongs to a family of highly fluorinated anthropogenic organic chemicals with special physical chemical properties. Adding PFAS to the surface makes a material oil and water repellant in addition to heat resistant. These are useful properties in many household and industry applications. They are used in commercial products like cooking utensils, food packaging, clothing, carpets, cleaning products, and in firefighting foams. And there is more to PFAS than just thermal and chemical stability.

Peeking into the molecular cosmos

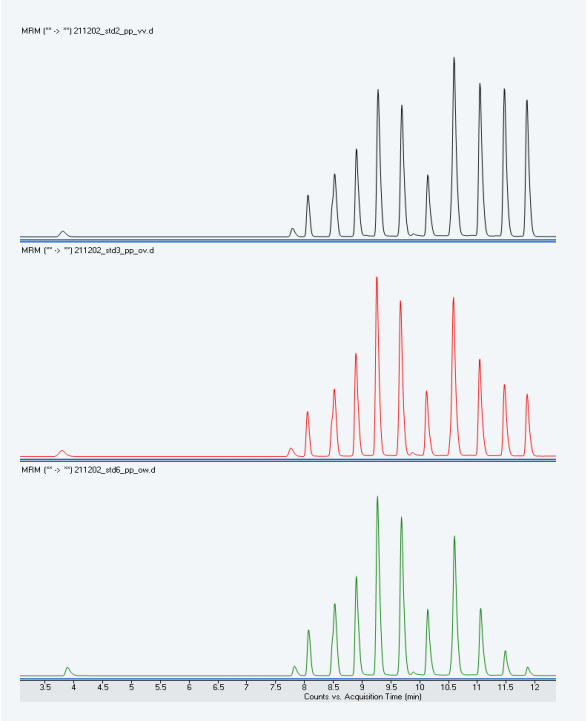
The PFAS carbon atom chain is hydrophobic, whereas the head of many PFAS molecules is hydrophilic. The resulting amphiphilic character is what makes a compound useful as surfactant. As opposed to classical surfactants, the hydrocarbon chain of the PFAS is also oil repellent, which is why PFAS is used as water, oil, fat, and dirt repellant. At the same time, PFAS are sufficiently water soluble to access and accumulate in the food chain, ground water, rivers, and surface waters, which means in our main drinking water reservoirs.

Clear and present danger

An estimated 4700 chemical compounds are classified as PFAS. 20 of these have been targeted in the EU Drinking Water Directive 2020/2184 due to their toxicity [1]. They are strongly suspected of causing liver damage, thyroid disease, adiposity, fertility disorders, and cancer [2] leading to their inclusion in the EU Directive:

- To minimize the risk of adverse effects through potentially contaminated drinking water, the EU Drinking Water Directive 2020/2184 establishes a limit of 0.5 µg/L for the sum of all PFAS.
- The Directive also sets a limit of 0.1 µg/L for the sum of the 20 per- and polyfluoroalkyl substances (PFAS) of most concern.
- The lower limits of quantitation (LOQ) subsequently required are: 30 ng/L for the sum of the 20 PFAS and 1.5 ng/L for the compounds individually.

To minimize the overall environmental impact and to limit adverse effects on humans, livestock, and wildlife, not only drinking water will need to be monitored for contamination with the relevant PFAS compounds, but also ground-, surface-, and wastewater. Unlike drinking water, the latter types can be expected to contain up to significant amounts of particulate matter and solid matrix, which will impact the analysis. Extracting adsorbed PFAS from solid matrix material and subsequently eliminating the solids from the extract will become key objectives of the sample preparation.



The importance of rinsing and recovering the rinse effluent can be seen in the above comparison of chromatograms obtained through direct injection of 1 mL of PFAS standard solutions into the online-SPE-LC-MS/MS system. The uppermost chromatogram resulted from subsequently rinsing the vial, syringe and injection loop and adding the rinse effluent to the SPE cartridge before the analysis. For the middle chromatogram, only the syringe and injection loop were rinsed, and the effluent recovered. Finally for the bottom chromatogram, no rinsing was performed resulting in significantly decreased recovery of relevant analytes.

Solid Phase Extraction

The separation technique of choice specified in German standard procedures for the analysis of waste water and sludge (DIN 38407-42) [2] is solid phase extraction (SPE). Due to the anionic properties of the analytes the extraction is performed using weak anion exchange (WAX) resins. The matrix is purged from the SPE cartridge, and retained analytes are subsequently eluted using a methanolic ammonia solution. How efficiently the SPE and thereby the analysis is performed depends to a large extent on the SPE technique used.

Chemistry of PFAS

Per- and Polyfluoroalkyl compounds are purely synthetical man-made chemicals. They are formed by chemical reactions substituting hydrogen atoms with fluorine atoms in carbonic and sulfonic acids with a chain length from C4 to C18. Two categories of PFAS are especially relevant for environmental and food analysis: Perfluorinated alkyl sulfonates (PFAS) with perfluorooctanoic sulfonate (PFOS) as the most widely known representative, and perfluorinated carbonic acids (PFCA), for which perfluorooctanoic acid (PFOA) is the most widely known. Among the estimated 4700 PFAS compounds, are alkaline, neutral, and poly- rather than perfluorinated compounds.

As opposed to standard dimension SPE cartridges, as described in the DIN 38407-42 method, the Online-SPE (GERSTEL SPE^{XOS}) relies on smaller cartridges from which the eluate is transferred directly and quantitatively to the HPLC mobile phase for 100 % analyte recovery and significantly lower limits of detection and of quantification. In other words, the required limits can be reached even with substantially smaller sample amounts.

Online SPE the method of choice

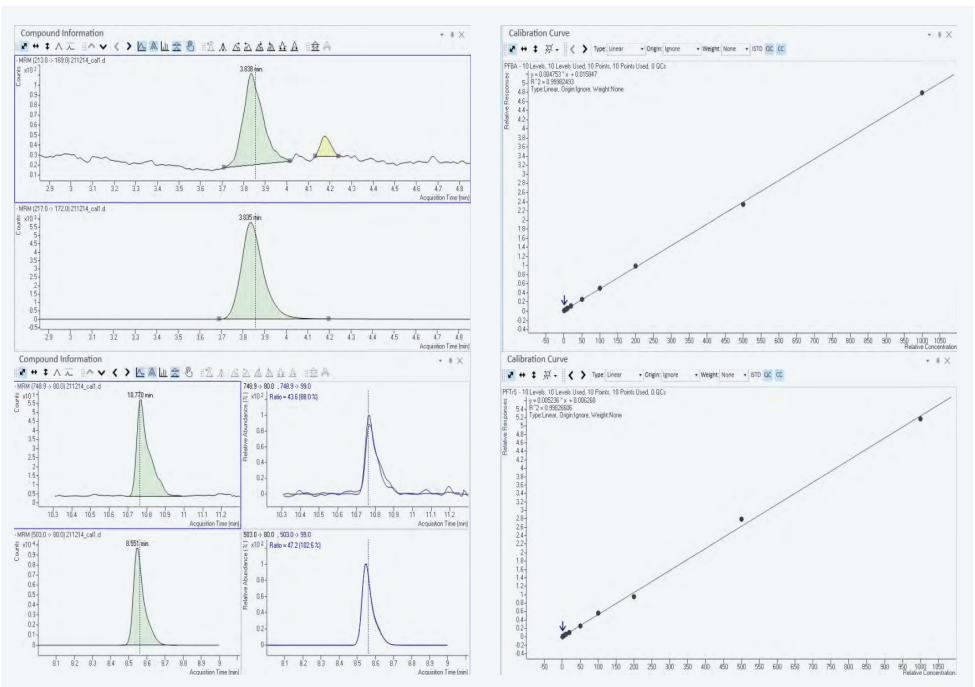
The combination of the GERSTEL SPE^{XOS} and the MultiPurpose Sampler (MPS) robotic makes PFAS analysis both efficient and simple to perform. SPE^{XOS} automates all steps normally associated with standard SPE. These include conditioning, loading, rinsing, eluting into the HPLC mobile phase, and finally exchanging the cartridge. The MPS, on the other hand, injects the sample into the SPE system and then rinses the vial, syringe, and injection volume to recover surface adsorbed PFAS. The rinse effluent is transferred to the SPE cartridge for inclusion in the analysis leading to improved analyte recovery and reduced sample to sample carry over. Following analyte elution, SPE^{XOS} removes the cartridge from the HPLC mobile phase flow path and prepares the system for the next analysis in parallel to the ongoing HPLC-MS/MS analysis.

Parallel sample preparation and analysis (PrepAhead) ensures maximum efficiency and throughput.

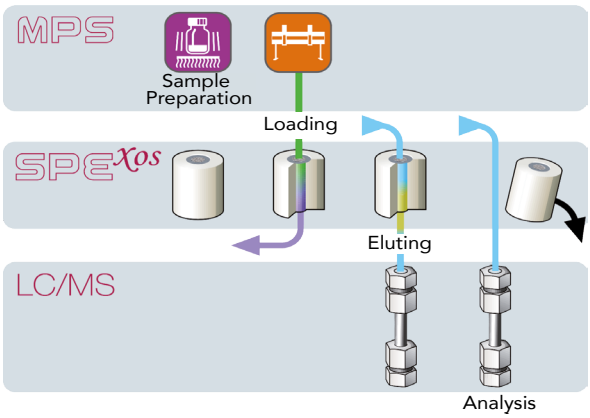
SPE^{XOS} method details

During the development project, the method was extensively tested and validated, resulting in a rugged and robust miniaturized method for determination of the PFAS listed in the EU Drinking Water Directive 2020/2184:

- An Agilent Technologies HPLC-MS/MS system was used (1260 Infinity II LC and ULTIVO LC/TQ MS) combined with a GERSTEL MPS robotic and SPE^{XOS}, which performed all sample preparation and introduction, including SPE cartridge exchange (SPE^{XOS} Polymer WAX, 25-35 µm) in parallel to the ongoing analysis.
- Analyte elution was performed using a 0.25 % solution of ammonia in Methanol. HPLC separation (duration 15 min) was performed using a Poroshell 120 EC-C18 column, 3.0 x 100 mm, 2.7 µm (Agilent Technologies) with a gradient of 0.1 % formic acid in water and 0.05 % formic acid in a 0.25 % solution of ammonia in methanol at a flow rate of 0.6 mL/min.
- Analyte detection was performed in dynamic Multiple Reaction Monitoring Mode (dMRM). For every target compound and every isotopically labelled internal standard (ISTD), two MRM transitions were selected, one Quantifier and one Qualifier, with the exception of PFBA and PFPeA, for which only one transition was available.



Calibration was performed using water spiked with a standard solution containing the 20 relevant PFAS (carbonic- and sulfonic acids ranging from C4 to C13) in the range from 1–1000 ng/L. To each reference solution and each sample, a standard mixture of isotopically labelled compounds was added. All calibration curves were linear in this range with correlation coefficients R2 > 0.998. Calibration curves for the first and last eluting compounds (PFBA and PFTrDS) are shown in the figure.



The MPS-Online-SPE system automates liquid sample preparation as well as SPE with automated cartridge exchange

Successful implementation

The combined MPS-SPE^{XOS}-HPLC-MS/MS system was successfully validated for the target compounds listed in EU Drinking Water Directive 2020/2184. To prove its usefulness for standard laboratory analysis work and the accuracy and trueness of the results, drinking water (tap water) samples as well as surface water samples from the river Ruhr were spiked at two concentration levels (5 and 100 ng/L) and analyzed. The fivefold analyses showed only minimal concentrations of some short chain PFAS (below 10 ng/L). These results were confirmed by determining low concentrations spiked into the samples. Trueness was determined at between 90 and 110 %, except PFPeA for which trueness was at 70 %. The higher-level spiked samples were analyzed resulting in trueness for all compounds between 70 and 130 %, as well as relative standard deviations with a median of 2.6 % and an upper range of 8.6 %, demonstrating the good performance of the method.

Summary

The presented online SPE-LC-MS/MS-System enables the fully automated determination of the 20 target PFAS compounds listed in EU Drinking Water Directive 2020/2184 at low ng/L concentrations while meeting the requirements of DIN 38407-42. The added value of the dedicated analysis system includes vastly simplified handling, dramatically reduced solvent consumption for improved laboratory sustainability, as well as excellent accuracy and reproducibility. In addition, water samples need not be filtered. By rinsing the sample vials with methanol, PFAS compounds adsorbed on the glass surface as well as fine particulate matter are recovered and transferred to the SPE cartridge where particle adsorbed PFAS compounds can equally be desorbed and included in the analysis for a true picture of the PFAS load in the entire water sample.

For more details on the analysis, please consult GERSTEL AppNote 237: Determination of PFAS in Water according to EU 2020/2184 and DIN 38407-42 using online-SPE-LC-MS/MS. Download AppNote 237: https://gerstel.com/en/Determination-of-PFAS-in-Water

Limits of Determination (LODs) and Limits of Quantitation (LOQs) determined by sixfold injection of 1 mL zero blind water sample into the Online-SPE^{XOS}-LC-MS/MS-System according to DIN 32645.

Compound	Acronym	Formula	LOD [ng/L]	LOQ [ng/L]
Perfluorobutyric acid	PFBA	$C_4HO_2F_7$	0.4	1.2
Perfluoropentanoic acid	PFPeA	$C_5HO_2F_9$	0.1	0.3
Perfluorohexanoic acid	PFHxA	$C_6HO_2F_{11}$	0.3	0.8
Perfluoroheptanoic acid	PFHpA	$C_7HO_2F_{13}$	0.2	0.5
Perfluoroctanoic acid	PFOA	$C_8HO_2F_{15}$	0.4	1.2
Perfluorononanoic acid	PFNA	$C_9HO_2F_{17}$	0.2	0.5
Perfluorodecanoic acid	PFDA	$C_{10}HO_2F_{19}$	0.2	0.5
Perfluoroundecanoic acid	PFUnDA	$C_{11}HO_2F_{21}$	0.3	0.8
Perfluorododecanoic acid	PFDoDA	$C_{12}HO_2F_{23}$	0.3	0.9
Perfluorotridecanoic acid	PFTrDA	$C_{13}HO_2F_{25}$	0.4	1.1
Perfluorobutanesulfonic acid	PFBS	$C_4HO_3F_9S$	0.2	0.5
Perfluoropentanesulfonic acid	PFPeS	$C_5HO_3F_{11}S$	0.1	0.4
Perfluorohexanesulfonic acid	PFHxS	$C_6HO_3F_{13}S$	0.2	0.5
Perfluoroheptanesulfonic acid	PFHpS	$C_7HO_3F_{15}S$	0.1	0.3
Perfluorooctanesulfonic acid	PFOS	$C_8HO_3F_{17}S$	0.2	0.5
Perfluorononanesulfonic acid	PFNS	$C_9HO_3F_{19}S$	0.2	0.5
Perfluorodecanesulfonic acid	PFDS	$C_{10}HO_3F_{21}S$	0.4	1.3
Perfluoroundecanesulfonic acid	PFUnS	$C_{11}HO_3F_{23}S$	0.3	1.0
Perfluorododecanesulfonic acid	PFDoS	$C_{12}HO_3F_{25}S$	0.5	1.4
Perfluorotridecanesulfonic acid	PFTrS	$C_{13}HO_3F_{27}S$	0.3	0.9

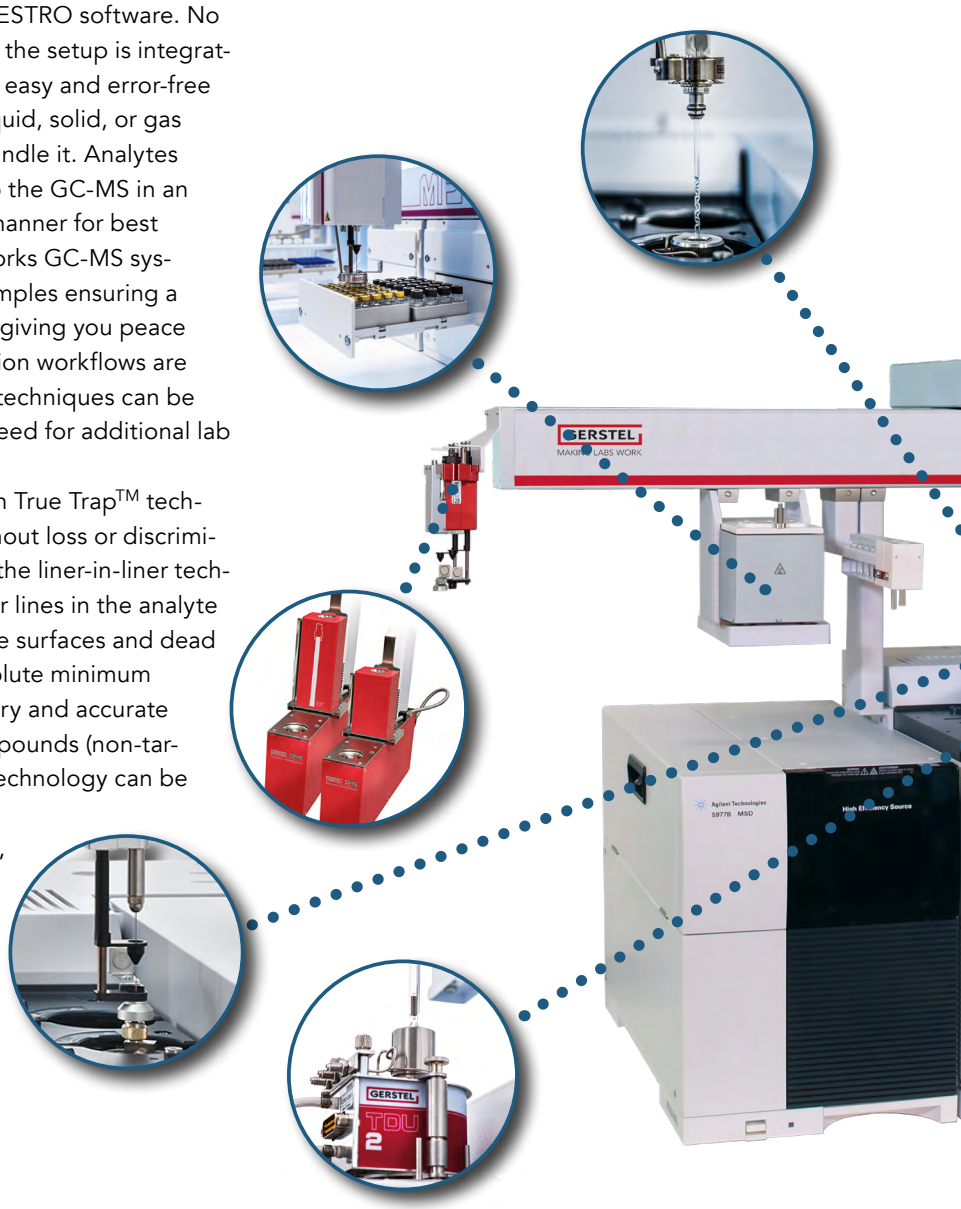
GERSTEL LabWorks Platform

The basis of your analytical success

Sascha Giegold, Ph.D.
Director, Global Sales
and Marketing

With this system in your lab, you are prepared for whatever comes your way: The GERSTEL LabWorks Platform is the only truly universal system for GC-MS sample introduction. LabWorks incorporates highly efficient automation with modular flexibility that enables the GC-MS lab to meet critical challenges at short notice. LabWorks Basic enhances the potential of GC labs by incorporating 10 fully automated sample introduction techniques that are intuitively selected, set up and controlled using MAESTRO software. No programming skills are needed, the setup is integrated with the Agilent software for easy and error-free system operation. Whether a liquid, solid, or gas phase sample, LabWorks can handle it. Analytes are extracted and introduced to the GC-MS in an accurate, reliable, and precise manner for best possible results. A single LabWorks GC-MS system handles a wide range of samples ensuring a good return on investment and giving you peace of mind. Even complex application workflows are easily automated, while further techniques can be added as needed without the need for additional lab bench space.

The LabWorks platform relies on True Trap™ technology for analyte trapping without loss or discrimination. The system is based on the liner-in-liner technology without valves or transfer lines in the analyte flow path. This means that active surfaces and dead volumes are reduced to an absolute minimum for best possible analyte recovery and accurate results – even for unknown compounds (non-target analysis). The True Trap™ technology can be used with Headspace, Thermal Desorption, SPME, SPME Arrow, SBSE, and TF-SPME for highly efficient analyte enrichment and unsurpassed limits of detection and quantification. The LabWorks platform further includes a range of sample preparation capabilities such as adding internal standard to your sample, diluting, derivatizing, as well as generating calibration curves and updating the method with the new calibration. And LabWorks is easily expanded to include more than 20 sample preparation and introduction techniques that can be accessed as needed. For laboratories that need to respond quickly and flexibly to meet critical challenges, the LabWorks platform offers the most comprehensive and flexible solution available today.



Techniques included in the GERSTEL LabWorks platform

- Liquid injection
- Large volume injection (LVI)
- Headspace
- Thermal desorption (True TD) with TDU and True Trap™ Technology
- True headspace enrichment
- Twister
- TF-SPME (Thin Film SPME)
- Direct thermal extraction
- Thermal desorption of tubes packed with sorbent
- Thermal extraction of liquids in µ-vials
- Derivatization
- Adding internal Standards
- Generating calibration standards
- Generating dilution series

Important features of the LabWorks platform

- 10 sample introduction techniques included in LabWorks Basic
- True Trap™ technology requires only one cryogenic trap for all applications
- Cryogen free focusing of target analytes
- True enrichment for HS, SPME, TD, Twister, TF-SPME and DHS techniques
- No valves or transfer lines – best possible system for nontarget analysis (unknowns)
- Simple modular expansion path for more than 20 additional sample introduction techniques
- Very simple addition of advanced analysis techniques (ODP, 1D/2D etc.)
- No need to reconfigure the GC inlet when changing between techniques
- Space saving system, no further lab bench space required in addition to the GC-MS.
- MAESTRO integration into the Agilent Technologies Software platform

LabWorks platform hardware components

- MPS robotic: Automates the sample preparation and all sample preparation techniques
- Thermal Desorption Unit (TDU 2): Enables the analysis of all sample matrices
- Cooled Injection System (CIS): PTV type GC inlet, highly suitable as universal cold trap for thermal desorption.

Source: istock / Kuzihar

Focusing on 3-MCPD and Glycidol

When fat containing foods are processed and heated, fatty acid esters of 3- and 2-monochloropropanediol (3-MCPD and 2-MCPD), and of glycidol can be formed, all of which are unwanted process contaminants. To ensure a safe food supply, the presence of these compounds in food must be monitored. Given the abundance of food and food products, monitoring should be efficient and therefore automated. GERSTEL offers a wide spectrum of validated, proven automated analysis solutions.

By Oliver Lerch, Ph.D. and Jasmin Zboron

3-Monochloropropanediol (3-MCPD), 2-Monochloropropanediol (2-MCPD) and glycidol - as well as their fatty acid esters - are unwanted substances in food due to their health effects. These compounds can all be generated during food processing – especially when heating is involved. The compounds have been found in fatty bakery products, baby food, soy sauce, as well as edible fats and oils [1]. Free 3-MCPD and 2-MCPD can be formed when fat and salt containing food is exposed to high temperatures in the production process. The ester bound form of 2-MCPD, 3-MCPD as well as Glycidol are formed during refining of oil types that are not edible in the native form. Refining, and especially deodorization involves heating the oil or fat to temperatures between 200 and 300 °C to remove off-flavors and contaminant residues.

Dangerous process contaminants

The International Agency for Cancer Research (IACR) has designated 3-MCPD a potential carcinogen [2]. During food production and processing, it is nearly impossible to completely avoid the formation of 3-MCPD, and therefore the European Food Safety Agency (EFSA) aims to limit the exposure, having defined a tolerable daily intake (TDI) of 0.8 µg of 3-MCPD per kg body weight [3]. For 2-MCPD and its fatty acid esters, an assessment of the health risks is currently not possible due to a lack of toxicological data [1]. The IACR categorizes glycidol as a probable carcinogen and as genotoxic.
The contaminant levels in food must be reduced to minimize consumer health impact – especially for infants who are not breastfed and are instead given

food produced in an industrial process. Reaching such ambitious goals requires use of highly sensitive laboratory instruments for chemical analysis.

Maximum Levels in certain foods:

The European Union (EU) Commission has defined maximum levels in Directive 2020/1322 [4]:

3-MCPD

- Plant based oils and fats (palm-, sunflower-, rape-, and olive oil etc.): 1250 µg/kg
- Other plant- and animal-based oils / fats: 2000 µg/kg
- Baby food/infant formula and ingredients used: 15 to 750 µg/kg

Glycidol

- Plant based oils and fats, fish oil, oil from marine organisms etc.: 1000 µg/kg
- Baby food/infant formula and ingredients used: 6 to 500 µg/kg

Choosing the right method

In collaboration with food producers and contract laboratories, GERSTEL has developed fully automated analysis solutions that meet the requirements of key international methods for determination of 3-MCPD, 2-MCPD and Glycidol present in their fatty acid ester form. The solutions are being used successfully in the laboratories of leading international companies and of reputable contract laboratories and public institutes.

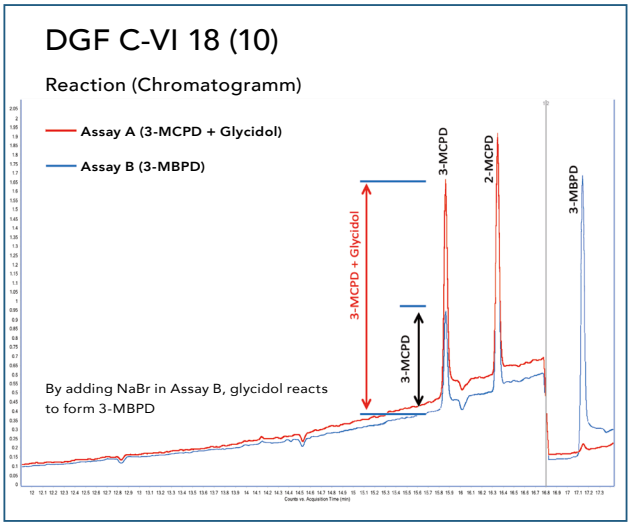
All methods listed and presented in this article are based on the same chemistry through hydrolysis. Further, glycidol is transformed to 3-MCPD or 3-MBPD (brominated form) respectively. After derivatization with phenylboronic acid (PBA), the analytes are finally determined directly or indirectly by GC-MS or GC-MS/MS. Differences between the listed methods are mainly found in the reaction control, the internal standards added, and the data handling and calculations performed. Incidentally, if the sample is a complex food type, such as a bakery product, a nougat cream that contains nuts, a chocolate bar, or similar, a further sample preparation step is required: Extraction of the fat contained in the product. The 3-MCPD, 2-MCPD, and glycidol levels are then determined in the extract.

Differential method

The "original" 3-MCPD solution, described in GERSTEL Application Note No. 191 (2017) [5] performs fully automated determination of 3-MCPD and glycidol fatty acid esters in plant-based oil following the official AOCS* method Cd 29c-13 [6], the DIN EN ISO 18363-1 method [7], and the DGF** Unified method C-VI 18 (10). The system is available from GERSTEL as customer specific solution based on the GERSTEL MultiPurpose Sampler (MPS). Two sample or extract aliquots are saponified and the reaction stopped in the presence of an acidic chloride- or bromide solution respectively. This step influences the conversion of the ester-bound analytes in their free form and enables the determination of the original levels of 3-MCPD and glycidol in the sample. This method, referred to as the differential method, enables the fast and efficient determination of the analytes. It is the most frequently used method and is implemented worldwide in incoming raw material quality control (QC) as well as quality QC of the final product in oil mills, in the food industry, and in contract laboratories.

AOCS Cd 29c-13
DIN EN ISO 18363-1:2015
DGF C-VI 18 (10)

*The American Oil Chemists' Society
**Deutsche Gesellschaft für Fettwissenschaften



Unilever method

AOCS Method Cd 29a-13, respectively the DIN EN ISO 18363-3:2017 method also known as the Unilever Method. In collaboration with a customer laboratory, GERSTEL application scientists developed an automated application solution for the Unilever method, described in AppNote 217 [8]. The main difference from the differential method lies in the sample preparation. Just one analysis run per sample is required and the analytes in question are released gradually in steps: Glycidyl esters react with acidified sodium bromide solution to their corresponding 3-Monobromopropanediol-fatty acid esters (3-MBPD). The fatty acid esters are extracted with hexane the extract is then subjected to evaporative concentration, and the residue is taken up in tetrahydrofuran (THF). Subsequently, 3-MBPD-, 2-MCPD- and 3-MCPD fatty acid esters are hydrolyzed to their free form in acidic media at 40 °C for 16 h. Following derivatization with PBA in an ultrasonic bath, the analytes are extracted with hexane. The extract is again evaporated, and the residue taken up in another solvent and finally separated and determined using GC-MS. The automated sample preparation meets the requirements of the standard method and greatly simplifies the analysis thanks to the significant degree of automation.

AOCS Cd 29a-13
DIN EN ISO 18363-3:2017

Kuhlmann or SGS 3-in-1 method

Known as the "Volvo" among the 3-MCPD and glycidol fatty acid ester methods, the DIN EN ISO 18363-2:2018, respectively AOCS Cd 29b-13 method, are collectively referred to as the Kuhlmann- or SGS 3-in-1 method [11], a rugged method that delivers good results reliably. It is a relatively slow, but highly precise method that requires sample cooling to between -22 °C and -25 °C. The method including sample preparation and analysis can be fully automated using an integrated GERSTEL system complete with GC-MS.

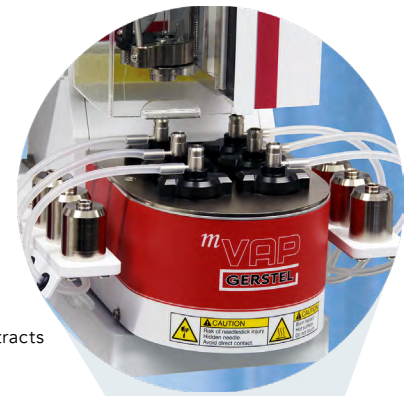
AOCS Cd 29b-13
DIN EN ISO 18363-2:2018

Zwagerman / Overman method

ISO 18363-4:2021 [9] is the most recent standard method for the determination of 3-MCPD, 2-MCPD and glycidol fatty acid esters in edible oils. The method was devised by two Dutch scientists, Zwagerman and Overman [10]. Several internal standards are used to compensate for even minor deviations in chemical reactions and in sample preparation. This concerns, among other things, an overestimation of glycidol that has been observed in the presence of large amounts of 3-MCPD. The use of a triple quadrupole GC-MS/MS is required, analytes are determined in MRM Mode. The standard method including all sample preparation steps specified therein have successfully been transferred to a GC-MS/MS system directly coupled to a GERSTEL MPS leading to a fully automated workflow described in GERSTEL AppNote 239 [12]. Important: A high power mixing module is required for efficient vortex-like mixing during extraction, a cooled vial tray for accurate temperature control during transesterification and a fast wash station, all of which are integrated in the automated system.

DIN EN ISO 18363-4:2021

The only manual step that remains is to weigh the sample in an autosampler vial and transfer it to the MPS tray. To keep the system clean and reduce memory effects to a minimum, a backflush system prevents high-boiling matrix residue and reagent from reaching the analytical column and the MS/MS system.
The method was successfully validated and implemented for the analysis of plant- and animal-based oils and fats. Successful participation in round robins has demonstrated the high quality of the automated sample preparation process and the analysis systems. Relative Standard Deviations were between 0.1 and 10 % for all analytes in different matrices, with only a few values above 5 %. The Limit of Determination of 0.1 mg/kg required in the ISO method was reached. Lower LOQs are possible if a Multi-Position Evaporation module (^{m}VAP) is installed on the MPS. The chromatograms are simple, "clean ", and easy to interpret due to the high selectivity of the triple quadrupole GC-MS/MS that largely eliminates nontarget signal. Automation enables 24/7 operation and priority samples can easily be inserted into the running sequence as needed, adapting to urgent changes.



The GERSTEL mVAP enables automated evaporative concentration of extracts as specified in standard methods. Up to 6 extracts can be concentrated in one batch.

AOCS Cd 29c-13 · ISO 18363-1
Differential method

Selecting a method

Compared with DIN EN ISO 18363-1, the 18363-4 (Zwagerman/Overman) method is somewhat faster (one assay per sample and calibration is required) and it is much faster than the DIN EN ISO 18363-2 and -3 methods. The DIN EN ISO 18363-4 method relies on multiple expensive internal standards but it delivers highly accurate and precise results with good trueness, for example, compared with DIN EN ISO 18363-1. The methods DIN EN ISO 18363-1 and -4 are most often used where results are needed quickly in a production laboratory or for incoming or outgoing quality control, accepting or releasing a shipment; the ISO 18363- 1 (differential method) is used more widely than the ISO 18363-4 (Zwagerman/Overman method), mainly because DIN EN ISO 18363-1 was accepted as a standard method much earlier. Many operations are reluctant to change from a well-known method that delivers good results in a reliable manner – even when a new method offers advantages. The transition to the Zwagerman/Overman method is therefore typically a drawn-out process – unless the analytical requirements involved are changed and updated. This means that laboratories are well advised to prepare and broaden their offering to meet ever changing market demands. If you are interested in learning more about updating your lab to perform automated 3-MCPD analysis, please contact GERSTEL to discuss which solution will best meet the requirements of your organization and how to best approach the analysis of your particular samples.

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DYNAMIC HEADSPACE DHS 3.5

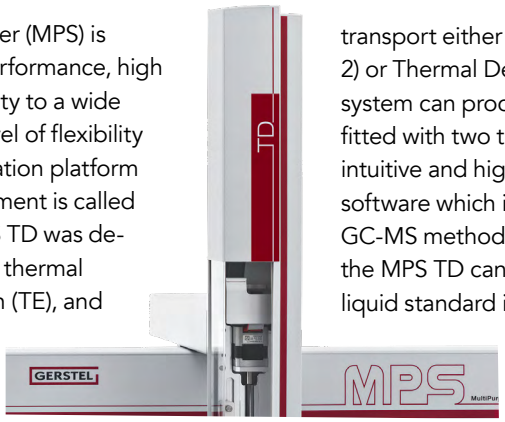
The DHS 3.5 is based on ¼" x 3.5" tubes with up to four times more sorbent than standard DHS tubes leading to improved recovery of volatile organic compounds (VOCs). GERSTEL Plus 3.5" tubes, for example, hold up to 240 mg Tenax TA. Improved recovery leads to higher precision and lower limits of determination, including for very volatile organic compounds (VVOCs). During the dynamic headspace extraction and concentration step, the sorbent tube is held at a user defined temperature between 10 and 70 °C to optimize analyte trapping. Following the DHS step, a user activated dry purge can be included as needed. The GERSTEL MultiPurpose Sampler (MPS) roboticPRO automatically transfers the sorbent tube to the Thermal Desorber (TD 3.5+) for analyte desorption and GC-MS determination. The standard size MPS processes up to 120 samples in one efficient analytical sequence. For larger batches, larger MPS versions can be chosen. The DHS 3.5 system processes samples in 10 mL or 20 mL vials. The DHS large module can be added for processing of samples in 250, 500 and 1000 mL vessels.

MPS PYRO – Automated Pyrolysis System

The GERSTEL MPS PYRO performs automated pyrolysis of up to 40, 80, or 120 samples at temperatures as high as 1000 °C in standard pulsed (instant) mode, or in multiple steps (fractionated) for separate determination of VOCs, SVOCs, and pyrolysates. Smart Ramp Pyrolysis (SRP) enables analysis of unknown samples and polymer mixtures without method development and without interference from compounds that are formed in secondary reactions when a single "compromise" pyrolysis temperature is used. In Evolved Gas Analysis (EGA), gases formed as the sample is heated are determined. The valve-free liner-in-liner flow path provides excellent recovery, as well as minimized contamination and sample-to-sample carry-over. Temperature verification and calibration in the sample position is performed by the user, ensuring accurate and reproducible results from instrument to instrument and from laboratory to laboratory, and liquid injections can be performed for GC-MS performance tests or for trouble shooting.

MPS TD – DEDICATED TO THERMAL DESORPTION

The GERSTEL MultiPurpose Sampler (MPS) is characterized by its automation performance, high degree of flexibility, and adaptability to a wide application range. When a high level of flexibility is not needed, a dedicated automation platform that requires a more limited investment is called for. With this in mind, the new MPS TD was developed specifically for automated thermal desorption (TD), thermal extraction (TE), and dynamic headspace (DHS).
The MPS TD is equipped with a GERSTEL gripper that can transport either Thermal Desorption Unit (TDU 2) or Thermal Desorber 3.5+ (TD 3.5+) tubes. The system can process up to 240 TD samples when fitted with two tray holders. System control is intuitive and highly efficient using MAESTRO software which is integrated with the Agilent GC-MS method and sequence table. If required, the MPS TD can also be reconfigured to perform liquid standard injections.



Kurt Thaxton
International Product Manager
Thermal Desorption and Pyrolysis

Solutions for Thermal Desorption

Comprehensive and flexible analysis

For many types of analyses, a key objective is to determine both known (target) and unknown (nontarget) analytes, for example, to help safeguard product quality and acceptance or to monitor material emissions. For volatile and semivolatile organic compounds (VOCs and SVOCs) the analysis is ideally performed without using potentially toxic solvents and without analyte loss or degradation. In many such cases, thermal desorption is the technique of choice.

Whether a nontarget analysis is successful largely depends on the sample handling and analysis technique used, but it also requires choosing a suitable analytical instrument for the task. Ideally, it should be flexible enough to allow fast adaptation to new and unexpected challenges, for example, whenever a critical product quality issue emerges that must be resolved quickly. Flexibility is best achieved in systems that are modular and offer a wide range of techniques. Whenever nontarget (unknown) compounds must be identified, the ability to cover a wide range of chemical compounds without discrimination or loss is critically important. As an example, off-odors are frequently caused by compounds with extremely low odor thresh-

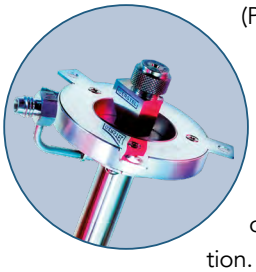
olds. These compounds are easily detected by the human nose but require extreme analyte concentration either by preextraction or by the analytical instrument/technique used for the analysis to be definitively identified by a mass spectrometer. To top off the wish list, the analytical system must be automated, reliable and rugged, and its operation user friendly. When it comes to the determination of VOCs, thermal desorption (TD) in combination with GC-MS has emerged as the technique of choice – and for obvious reasons: No need for potentially toxic solvents that dilute the sample and are expensive to both purchase and to dispose of. GERSTEL has provided instruments and systems for solvent free, highly sensitive and environmentally friendly thermal desorption analysis for more than 30 years and is a recognized global leader in this field.

A deeper look at TD technology and instrumentation

Thermal desorption instruments generally consist of four core elements: An autosampler, a thermal desorber, a flow path, and a focusing trap - not necessarily in that order.

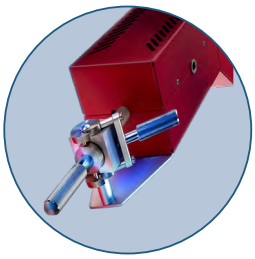
The trap

The heart of every GERSTEL thermal desorber has always been the Cooled Injection System (CIS), a PTV type GC inlet used to concentrate and transfer analytes efficiently and without discrimination or loss. The CIS was originally developed for GC analysis to perform discrimination-free liquid injection at low temperature followed by programmed temperature vaporization (PTV). This approach was also widely used for large volume injection (LVI) for improved detection limits. In thermal desorption systems, the CIS is used as a cold trap, concentrating analytes before transferring them to the GC column in a sharp band for optimal separation and compound detection. Ideally, analyte focusing is performed at low temperatures without having to rely on sorbents. Sorbent traps can cause chemical reactions and analyte degradation of more labile compounds and should be avoided when an application requires the identification of nontargeted (unknown) compounds.



True Trap™

GERSTEL's True Trap™ technology is applied when the CIS is cooled with liquid nitrogen to -180 °C and only inert glass beads are used to enhance compound trapping. This is the ideal way to trap even the most volatile compounds and obtain a "true" profile of the analytes in a sample. This single trap configuration can be used for all applications and does not require the CIS to be reconfigured as the sample type changes. Since the technology does not rely on sorbent material, it eliminates the challenge of selecting the "right" sorbent which requires the use of standards and a cumbersome trial and error approach to ensure the compounds of interest are being trapped without significant loss or degradation. There are other features that contribute to making the CIS the ideal cold trap. The slender design and short analyte flow path free of valves and active surfaces minimizes the risk of analyte loss and sample-to-sample carry-over. The user defined temperature and programmed temperature vaporization during analyte release enable the simplest possible operation. In some cases, the use of True Trap™ technology may not be necessary. For example, if the compounds to be determined are known (target analysis) or the compound type and boiling point range is well defined, other cooling options can be used. Cooling with carbon dioxide provides trap temperatures to -70 °C, the cryogen-free Cryostatic Cooling Device (CCD) provides trap temperatures as low as -40 °C and Peltier Cooling goes to +10 °C. These options provide trapping temperatures that allow the TD system to be optimally configured for any application or laboratory requirement.



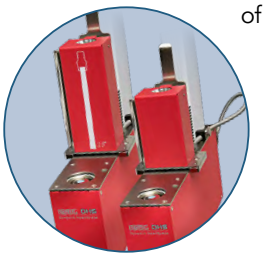
Flow path

When selecting your TD instrument, always consider the analyte flow path between the thermal desorber, the trap and the GC column. It should have low dead volume while being inert, rugged, and leak free. As always "the best transfer line is no transfer line", since void volumes and active surfaces are eliminated for best possible analyte recovery and quality of GC separation. These requirements were developed to perfection in the TDU and TD 3.5+ by connecting the

modules directly using the "liner in liner" approach and having the GC column inserted directly into the CIS trap liner. Connecting the elements of a thermal desorption system in such a compact manner eliminates multiple elements from the analyte flow path: No o-rings; no valves; no polymer stator or rotor/slider; no connecting tubing; no protective PTFE filters used to protect the valves. Protect the valves? Yes, valves must be protected from sorbent particles using PTFE filters in the analyte flow path to avoid surface scratching and leaks. Such instruments may be specified to a maximum desorption temperature of 400 °C, but the PTFE filters will melt even at temperatures well below that level, so best not to try it. The compact liner-in-liner flow path eliminates complexity and provides the inertness required for good recovery across the volatility range even for more labile compounds.
Liquid samples or standards can be injected directly into the TDU and TD 3.5$^+$ or introduced using µ-vials placed in the TD tube. The CIS can also be used for liquid injection, for example during GC-MS method development, when validating GC-MS performance using a liquid standard, or when performing trouble shooting. Further, the Tube Spiking System (TSS) module enables the MultiPurpose Sampler (MPS) used for the TDU 2 and TD 3.5$^+$ to spike sorbent tubes with liquid standards as required in various air monitoring methods.

Choice of thermal desorber

The CIS can be combined with any of the three different GERSTEL TD systems: Thermal Desorption Unit (TDU 2), Thermal Desorber 3.5$^+$ (TD 3.5$^+$), and Thermal Desorption System (TDS 3). Sample introduction is performed by inserting an TD tube best suited for the sample type (gas, liquid or solid) or preliminary extraction technique (GERSTEL Twister® or Thin Film SPME). During primary (tube) desorption, a high carrier gas flow and programmed temperature desorption should be used to reduce the risk of analyte overheating and decomposition. The GERSTEL TD systems, uniquely, use this approach. To meet all requirements,



GERSTEL TD systems support use of three of the most widely used tube dimensions worldwide: 2.4" (60 mm), 3.5" (89 mm) and 7" (179 mm) long. The most suitable instrument is simply selected based on the analysis requirements. As to choosing the tube size, we offer the following recommendations: 2.4" TDU tubes are especially well suited for thermal desorption of the GERSTEL Twister® and for Thin Film SPME devices. The 3.5" (1/4"OD) tubes used in the TD 3.5$^+$ enable the use of significantly more sorbent providing larger sample capacity resulting in improved recovery during whole air sampling or, for example, when performing Dynamic Headspace sampling (DHS) of very volatile species.

Autosampler

All GERSTEL TD systems can be operated with highly efficient autosamplers. For the TDU 2 and TD 3.5$^+$, the dedicated MultiPurpose Sampler MPS TD version can be used, or the MPS robotic Smart series is available if a fully flexible configuration is required. Further, the LabWorks basic system offers 10 techniques including TD, and the LabWorks advanced system is scalable to more than 30 techniques, in one system. These range from liquid injection through headspace and dynamic headspace, SPME and TF-SPME as well as Stir Bar Sorptive Extraction using the GERSTEL Twister®.

Conclusion

Users that need to perform nontarget analysis as well as determination of labile or adsorptive compounds as part of their daily tasks are well equipped to meet their challenges with a GERSTEL TD solution. Our powerful TD platforms further help to maximize the use and performance of powerful mass spectrometers such as TOF-MS. The simplicity and ruggedness of the TD systems enable routine quantitative determination of a wide range of VOCs and SVOCs. Whether you are looking for a few compounds in a packaging sample, a wide range of analytes in air, a full aroma profile of a beverage, or a critical off-odor in your product, the GERSTEL TD technology will quickly provide the results you need.

Ethylene oxide in the sights of food safety inspectors

In large numbers of sesame seed shipments, especially from India, EU food inspectors have found residues of the highly toxic compound ethylene oxide, leading to food product recalls with associated public information campaigns. Many years ago, the fumigant was widely used as a disinfectant for foodstuffs, but its use for food and feed has been strictly prohibited in the EU since 1991. To safeguard the food supply, fast, safe, and reliable analysis methods are clearly needed.

By Tatiana Cucu, RIC Technologies

Ethylene oxide (EO) is a sweetish smelling, colorless, highly flammable gas, which in many countries outside the European Union EU is still in use as a biocide to eliminate insects, bacteria, and fungi from the food supply. The fumigant is especially used for dry foodstuffs, such as herbs, spices, nuts, or oily seeds. In the EU, the use of EO to treat food has been prohibited since 1991 due to its carcinogenic and genotoxic properties. Nevertheless, the EU commission's Rapid Alert System for Food and Feed (RASFF) has received a large and increasing number of notifications relating to EO contaminated foodstuffs found at EU entry points since 2020, initially mainly regarding sesame seed shipments from India. The determined EO amounts exceeded maximum residue level (MRL) of 0.05 mg/kg, specified in EU Commission Regulation 2015/868 [1], by a wide margin leading to further increased monitoring and, in turn, a significant number of product recalls in various EU member states. Incidentally, recalls were issued both for conventional and organic food products and the list of afflicted food products and of countries of origin has grown with the number of samples taken. The US Food and Drug Administration and Canadian authorities specify tolerance limits of 7 ppm for Ethylene oxide and 940 ppm for 2-CE for a range of spices, dried herbs, and vegetables.

2-CE, also known as ethylene chlorohydrin, counts among the most toxic halogenated organic compounds. Incidentally, 2-CE seems to be the dominating compound in foods treated with EO. This is widely attributed to the extreme reactivity and volatility of EO.

The active ingredient and its metabolite

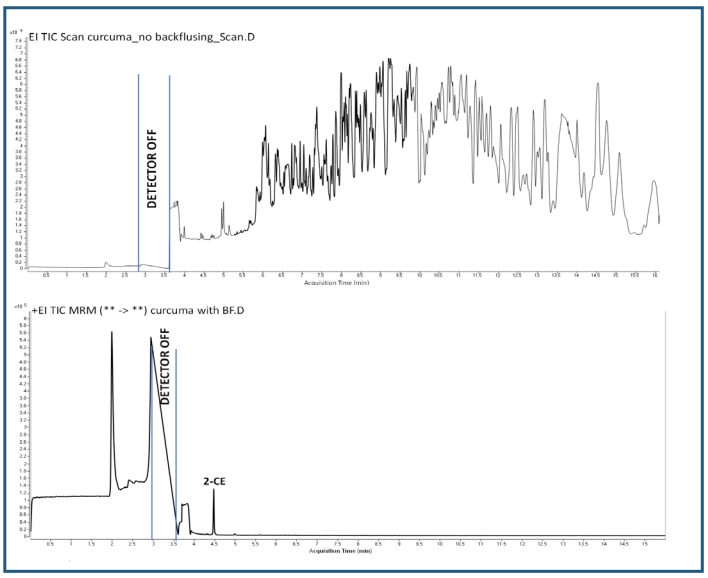
To safeguard a healthy food supply, industry and food safety inspectors need a proper toolkit of highly sensitive analysis methods. They must be able to reliably determine ethylene oxide (EO) and its main metabolite 2-chloroethanol (2-CE), at concentration levels well below the specified MRLs (see above). Literature searches reveal different methods used to determine EO or, in some cases, the sum of EO and 2-CE. Some methods, such as the official German method (§ 64 LFGB, L53.00-1) rely on converting 2-CE to EO under alkaline conditions and then converting the formed EO to 2-Iodoethanol (2-IE), which is in turn determined by GC-ECD or, more frequently nowadays, by GC-MS. Other methods rely on converting EO to 2-CE under acidic conditions followed by extraction of the resulting 2-CE

with ethyl acetate and GC-MS determination. In December 2020, the EU Community Reference Laboratory for Single Residue Methods in Stuttgart, Germany (https://pesticides.cvuas.de/) proposed a combination of a QuEChERS sample preparation method and GC-MS/MS determination [2].

Meeting the challenge

When analyzing food products for EO and 2-CE, there are challenges to overcome. The very volatile EO, for example, requires a special GC column to avoid co-elution with acetaldehyde, frequently found in fatty foods. To sharpen the challenge, acetaldehyde and EO not only have similar retention indices, but also similar mass spectra. At the other end of the molecular size spectrum, even after QuEChERS cleanup, food extracts contain large amounts of nonvolatile matrix material. Such matrix residue accumulates in the GC inlet liner impacting analyte recovery and thereby method accuracy and ruggedness. When nonvolatile material is then transferred to the GC column, it accumulates and impacts separation performance before eventually contaminating the MS ion source.

At the RIC Technologies laboratories, we took up the challenge of developing and validating an automated method for the determination of EO and 2-CE in matrix laden QuEChERS extracts while reducing or eliminating instrument downtime for cleaning and maintenance. The analysis method upon which our solution is based on was developed by the EU Community Reference Laboratory for Single Residue Methods at CVUA Stuttgart, Germany (https://pesticides.cvuas.de/). The method performs quantitative determination of EO and its main metabolite 2-CE in foodstuffs, specifically in sesame seeds and curcuma. It was found that to obtain sufficient method ruggedness, residue from the sample matrix must not be allowed to accumulate in the GC inlet liner, GC column or reach the MS ion source. The automated system we used consisted of an Agilent 8890 GC, Agilent 7010 Triple Quadrupol MS and a GERSTEL MultiPurpose Sampler (MPS) fitted with the Automated Liner Exchange (ALEX) option. ALEX removes and replaces dirty liners at user defined intervals. In addition, the analytical column was protected by integrating a pre-column backflush option, which improved ruggedness and reduced instrument downtime for maintenance.



GC-MS traces of curcuma extracts analyzed in scan mode without backflush (upper trace) and in MS/MS mode with backflush (lower trace). Source: Tatiana Cucu / RIC Technologies

A look at the analysis details

Method development was performed using EO and 2-CE standard solutions, the method validation was performed based on real samples that had been mechanically homogenized and spiked with deuterated standards. Quantification was performed using deuterated EO and 2-CE as internal standards. The QuEChERS extraction was performed manually according to DIN EN 15662.

To demonstrate chromatographic stability, extracts of sesame seeds and curcuma were spiked with EO and 2-CE resulting in concentrations of 10, 40, and 100 ng/mL. These were repeatedly injected from the same vial to the same GC inlet liner. A moderate reduction was observed in the EO peak areas for the sesame extract injections, slightly more for the curcuma extract injections. This was explained by evaporative loss of EO from the sample, which was kept at ambient

temperature over a period during which multiple septum punctures were performed to aspirate the samples. Nevertheless, repeatability was a respectable 5-6 %, indicating that the loss was adequately compensated for using internal standards in the calculation. Unexpectedly, the less volatile 2-CE also exhibited some loss over the period. The 40 % reduction in absolute 2-CE peak area seen between the first and fifth injection was clearly caused by increasing activity in the GC inlet liner. Further, peak distortion was observed after injection of samples with particularly high matrix loads, for example in the case of curcuma extracts. The injection of relatively "dirty" QuEChERS extracts influenced the recovery of 2-CE prompting the decision to use Automated Liner Exchange (ALEX) in the EO and 2-CE analysis system. Using the ALEX option, clean liners were inserted after 20 injections (user defined) to ensure that extracted nonvolatile matrix material couldn't accumulate to a degree that would influence 2-CE recovery and compromise the quality of the analysis results. Further, installing a pre-column backflush option clearly prevented a large amount of high boiling material from reaching the GC column and by extension the MS ionization source. When the backflush option was activated, MS source contamination was negligible. If pre-column backflush was activated shortly after the target analytes had reached the analytical column, the sample would have no or almost no impact on the analytical column, extending column life and protecting the MS ion source from contamination that would otherwise impact system stability and require down time for cleaning.

Succesful ethylene oxide determination

The described method for determination of ethylene oxide and 2-chloroethanol was validated by performing recovery experiments in triplicate at three different concentrations (0.05, 0.2, and 0.5 mg/kg) in actual samples. Recoveries ranged from 84.5 to 100.6 % for EO and from 88.8 to 106.2 % for 2-CE in sesame seed and curcuma samples. To demonstrate the practical usefulness of the method, representative food samples that had been purchased in a local food store were analyzed.

Result: None of the samples tested positive for EO, but all tested positive for 2-CE, some even exceeding the MRL levels. The precision of the developed method was tested using sesame seed reference samples kindly provided by a partner laboratory and the established value was close to the reported average of 4660 µg/kg (27.3 % CV). Recovery was between 85 and 106 %, with good repeatability for both EO and 2-CE. Concerning the sensitivity, the developed method achieves LOQs for sesame and curcuma (representative for food category spices) below the currently valid MRLs. Results were stable both for sesame and for curcuma samples, RSDs for triplicate combined extractions and analyses for the recovery experiments were well below 20 %. The sample preparation and the optimized GC-MS/MS method reliably delivered results and performance that would indicate the usefulness of the method for the quantitative determination of EO and 2-CE down to well below the regulated concentrations for sesame and spice samples. The process delivers high sensitivity, selectivity, precision, and especially high sample throughput with minimal downtime for cleaning and maintenance of the inlet system, replacement of GC columns, and MS ion source cleaning.

REFERENCES:

[1] Regulation (EU) 2015/868 of 26 May 2015 amending Annexes II, III and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for 2,4,5-T, barban, binapacryl, bromophos-ethyl, camphechlor (toxaphene), chlorbufam, chloroxuron, chlozolinate, DNOC, di-allate, dinoseb, dinoterb, dioxathion, ethylene oxide, fentin acetate, fentin hydroxide, flucycloxuron, flucythrinate, formothion, mecarbam, methacrifos, monolinuron, phenothrin, propham, pyrazophos, quinalphos, resmethrin, tecnazene and vinclozolin in or on certain products. Off. J. Eur. Union L 145/1 – 71 (2015).

[2] EURL-SRM-Analytical Observation Report: Analysis of Ethylene Oxide and its metabolite 2-Chloroethanol by the QuOil or the QuEChERS method and GC-MS/MS. Dezember 2020. Link: https://www.eurl-pesticides.eu/library/docs/srm/EurlSrm_Observation_EO_V1.pdf

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Olfactory Data Interpreter (ODI)

The novel Olfactory Data Interpreter (ODI) software brings a new level of ease to data handling and interpretation of Olfactory data obtained in combination with GC/MS. ODI is part of GERSTEL Enterprise Edition powered by OpenChrom.

The GERSTEL Olfactory Data Interpreter (ODI) enables fast and reliable evaluation of olfactory information including identification of unknown odor-active compounds. Chromatography data is typically acquired using a mass spectrometer / mass selective detector (MSD), a Flame Ionization Detector (FID) or a Sulfur Chemiluminescence Dectector (SCD) or a combination thereof. The chromatogram is loaded into the ODI software, which automatically recognizes the chromatogram data format before importing the data. In parallel, an olfactogram is imported with information provided by the user as he or she assessed odors eluting from the GC column in parallel with the detection process during the GC run. The olfactogram combines spoken comments from the sensory analyst and the registered intensities along a time axis. The „Olfactory Intensity Device" (OID) conveniently enables the user to indicate the intensity of the olfactory impression as it transpires. Text comments are added using voice recognition software to translate spoken descriptors. The combined information is presented in the ODI Software in a clear and concise overview including a chromatogram, an olfactogram and a table. In addition, the detailed report of the signals registered during olfactory detection at the sniff port includes important parameters such as retention time (RT) and retention index (RI) as well as NIST library search results for compound identification. The audio file with descriptors is stored for later evaluation, as needed. If changes to the initial impression have been realized, the text comments can be edited. Regarding the MS data, results from database searched can be aligned with Retention indices (RIs) calculated by the ODI Software. To evaluate olfactory information and determine the identity of a critical or key odor active compound or compounds that result in an olfactory impression, the Olfactory Data Interpreter (ODI) provides the analyst with several helpful software features and functions.

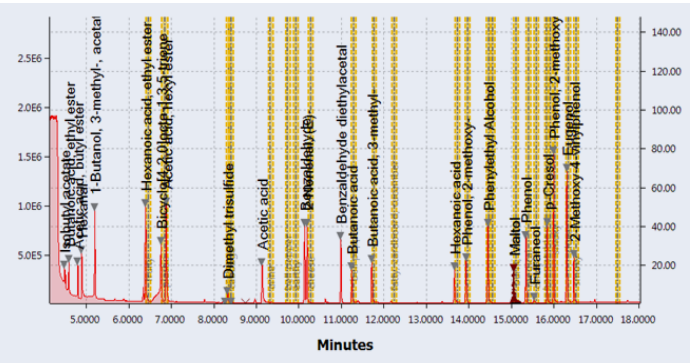

Figure: Chromatogram with peak annotations and overlay olfactogram

Cumulative Olfactogram presentation

When a sample is evaluated in multiple dilutions using Olfactory GC (GC-O), the resulting olfactograms are conveniently combined and presented in cumulated form: The ODI software adds the respective odor intensities and displays the cumulative value. Substances that are still above the odor threshold in the highest dilution will be represented with the highest values in the cumulative olfactogram. The cumulative olfactogram function is a simple yet efficient tool that instantly provides the odor analyst with reliable information as to which compounds belong to the group of more potent odor active compounds that influence the flavor or odor impression even at very low concentrations.

Aroma Extract Dilution Analysis (AEDA)

The ODI Software enables efficient evaluation of classical Aroma Extract Dilution Analysis (AEDA) with defined dilution factor (Flavor Dilution, FD). The ODI software performs all calculations and determines the FD value, which is a measure of the intensity of an odor active compound within a mixture. The software transfers the established value to a ready to print report. The AEDA report provides the sensory analyst a tool for simpler and more efficient evaluation of panel data.

Sensory Panel Analysis

If a sample undergoes olfactory evaluation by a sensory panel, the resulting data will show how many panel members perceive a given impression or compound. This information can be very helpful as a supplement to GC-O data or to correct GC-O data, as well as for further analysis and aroma identification. Manually generated reporting can be both cumbersome and labor intensive, requiring a lot of time. The Olfactory Data Interpreter helps to perform the panel analysis evaluation by mouse-click and delivers the detection frequency of each compound without delay.

MS Library Search

The ODI Software integrates numerous functions relating to extraction, cleanup, and interpretation of mass spectra. Spectral recognition is performed using existing libraries; various data formats can be used. The GERSTEL Application Laboratories recommend the NIST AMDIS software, which is coupled directly to the ODI Software enabling efficient and convenient compound identification and spectral deconvolution of co-eluting compounds.
In summary, the GERSTEL ODI software simplifies and supports fast determination and elucidation of unknown flavor active compounds and mixtures, determined by GC/MS-Olfactory (GC/MS-O) analysis or panel analysis. When it comes to handling and processing Olfactory Data, the ODI software offers the necessary combination of incisiveness and flexibility, required to reach the right answer when confronted with inherently fuzzy olfactory data. The ODI is a novel highly useful tool for the flavor chemist.

ODP 4 and Olfactory Data Interpreter (ODI) Software

The GERSTEL Olfactory Detection Port (ODP 4) enables simultaneous detection of compounds with the human nose and a mass spectrometer. The ODP 4 has a uniformly heated highly inert transfer line connected to a heated mixing chamber, resulting in good recovery and sensitive olfactory detection even for high-boiling and polar compounds. Makeup gas and humidification can be added to the GC column effluent as required. The ODP is mounted on a multi-jointed arm with a centrally placed fixation knob for optimized ergonomics and easy positioning. Nasal positioning guideposts are available for each user to ensure consistent results through reproducible nasal positioning. For improved safety and hygiene, each analyst can use his or her own PTFE outer cover, positioning guideposts and glass funnel; these can be exchanged in a few seconds. The ODP can be operated with or without a glass cone, and sorbent tubes can be inserted to trap and concentrate interesting fractions for further analysis. The ODP 4 is supplied with Olfactory Data Interpreter (ODI) software enabling time-aligned sensory evaluation of compounds determined by the GC-MS, complete with intensity levels and qualifiers displayed in a combined olfactogram. For more information on the ODI software, please see the previous article.

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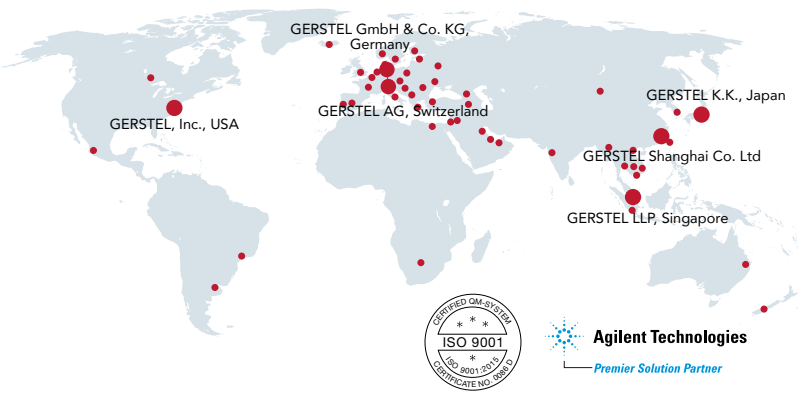

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