Twister® and Metabolomics

Of flavors and off-flavors

Doing Drugs II
Automated Sample Prep and LC/MS/MS with Dynamic MRM
Olfactory Detection Port (ODP)

New GC Olfactory Detection Port with Voice Recognition and Voice-to-Text Capability

The new GERSTEL ODP 3 Olfactory Detection Port with Voice Recognition enables sensory detection of odors by the human nose simultaneously with analytical detection by any GC detector, including MSD, FID, and FPD. Voice recognition software allows the sensory analyst to describe odors and fragrances in real time – these voice descriptors are recorded and converted to editable text files. For each GC run a complete report is generated, including a chromatogram superimposed with an annotated olfactogram. Text of the descriptors spoken by the analyst is placed above each olfactogram peak. The analyst can assign any of four levels of intensity to each eluting component, and the olfactogram is recorded with this intensity information. The ODP 3 is an effective tool for obtaining simultaneous sensory and analytical information in determining odors in foods, beverages, fragrances, and other complex samples.
The most widely sold PTV inlet in the world

Since Martin and Synge invented gas chromatography (GC) in 1940, a steady stream of innovations has improved performance and reliability of GC instrumentation. The GC technique is one of the most widely used for the determination of volatile and semi-volatile organic compounds (VOCs and SVOCs). Among the significant developments along the way were the temperature programmed GC oven, capillary columns, selective detectors and modern sample inlets, such as the Programmed Temperature Vaporizer (PTV). The PTV improved performance by ensuring discrimination free analyte transfer to the GC column and enabling analyte concentration. The GERSTEL Cooled Injection System (CIS) with the patented septum-less head (SLH) was first introduced 25 years ago. Today, the 4th generation CIS is the most widely sold and installed PTV-type inlet in the world.

With the introduction of the 5890 GC in the 1980s, Hewlett Packard set itself firmly on the path to becoming the leader in GC instrumentation. The 5890 GC was designed for capillary columns, it had a powerful autosampler and it was equipped with Electronic Pressure Control (EPC) for the carrier gas, enabling optimized GC separation for analytes over a wide boiling point range. These advances clearly exposed the limitations of the sample inlets available at the time. In hot inlets, solvents and volatile analytes would evaporate extremely quickly in an uncontrolled manner giving rise to discrimination and reduced recovery, especially for high-boiling compounds that would condense in unheated areas around the GC inlet. In addition, thermally labile analytes were also prone to decomposition when exposed to temperatures needed to quickly vaporize a sample.

In 1984, GERSTEL introduced the Cooled Injection System (CIS). The patented CIS is a Programmed Temperature Vaporizer (PTV), often referred to as a “Universal GC Inlet”. In contrast to permanently heated GC inlets, the CIS enables introduction of liquid sample a low temperature followed by programmed heating with controlled evaporation and transfer of analytes to the GC column. The result is better separation, improved recovery, lower detection limits, and more accurate quantitative results.

The description “Universal GC Inlet” refers to the unique flexibility of the CIS. It can be used for either hot or cold injection combined with split, splitless, or direct on-column analyte transfer. The CIS can be cooled to temperatures as low as – 180 °C making it uniquely suitable as cryo-trap for VOC analyte concentration in combination, for example, with Thermal Desorption. Additionally, the CIS can be used to concentrate analytes through Large Volume Injection (LVI) with solvent venting. This provides to substantially lower limits of detection. PTV and LVI injections are typically performed using GERSTEL exclusive cryogen-free peltier or cryostatic cooling. Depending on the required starting temperature, cryogenic cooling may also be used. At the other end of the temperature scale, the CIS 4 operates up to 450 °C for determination of SVOC, PAHs and some waxes. The high temperature version (CIS 6) scales the heights up to 650 °C, enabling pyrolysis of polymers in suspension following initial evaporation of the solvent. Whichever version the analyst is using, all CIS inlets are based on patented heating and cooling systems and optimized inlet liner dimensions that provide uniform temperature profiles throughout the entire – 180 °C to 650 °C temperature range for controlled evaporation and best possible results.

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CIS benefits

- Improved quantitation due to controlled evaporation and discrimination free analyte transfer.
- Improved limits of detection, improved identification and improved quantitation through sharper peaks.
- Lower background signal, no septum bleed, improved detection limits thanks to patented Septum-Less Head (SLH).
- Best possible transfer and recovery of thermally labile analytes due to patented heating system and freely programmable heating rates.
- Reliable analysis of samples with high matrix load using Automated Liner EXchange (GERSTEL ALEX).
Advanced Flavor and Fragrance Analysis

An extra GC dimension at your finger tips

Gas chromatography (GC) experts rely on sharp peaks and baseline resolution to provide accurate answers. To perform chromatographic analysis of real-world samples, analysts often must deal with either complex sample types such as essential oils and petroleum fractions, or complex matrices like biological fluids, foods, sludge, or polymers. Once the sample has been prepared for analysis, separation of all the individual compounds present by means of a single chromatographic separation can be challenging due to the compounds having different ranges of polarity, boiling point, solubility, MW, and concentration. It is therefore necessary to use innovative yet robust techniques that go beyond using a single chromatographic dimension to achieve compound separation. This sounds simple enough, but until now it hasn’t been, since techniques that require a second dimension (column) require a lot of additional hardware, including an extra GC. Not anymore: The patented GERSTEL Selectable 1D/2D-GC/MS System enables the best of both worlds. The system can be used for routine single dimensional GC/MS analysis, and with the click of a mouse, can be switched to perform two-dimensional separation when needed for more complex matrices. This allows interesting sections of the chromatogram to be collected and concentrated from multiple runs to better separate and isolate trace compounds. This can, for example, be used for trouble shooting when off-odors are detected in a product. All this is performed using just one GC/MS system.

Food analysis is certainly not a trivial matter. The typical matrix is complex, often requiring several sample preparation steps and extensive sample clean-up. However, even well prepared samples can produce forests of overlapping peaks making it a case of not being able to see the trees for the forest. If a case of unresolved peaks is clearly at hand, or if an odor detected by using an Olfactory Detection Port (ODP) doesn’t match the peaks in the chromatogram, the user needs to have a good tool kit at her or his disposal. In this case, selectable multidimensional GC can be the technique that cuts through the thicket and provides clear, reliable answers whenever one-dimensional GC does not.

An attractive alternative to standard systems

Until now, multi-dimensional GC required the use of a dedicated system with two GCs coupled to each other. Due to the extra cost, and to the often limited utilization in the laboratory, such solutions didn’t always provide the best return on investment (ROI).

SBSE – 2D GC-O/MS analysis of odor-active compound in beer

1D GC-O/MS analysis
GC-O/MS can help locate the region of odor-active compounds within a complex Chromatogram, but insufficient resolution may still prevent reliable compound identification. A heart-cut of the region of interest followed by separation on a second column (second dimensional separation) provides the resolution needed to accurately identify individual compounds.

Heart-cutting and Backflushing
The system can be configured to perform 2D GC-O/MS analysis without any hardware or column connection changes. After heart-cutting, the 1st dimensional column can be backflushed. An additional cryo-trap device is available if necessary.

Simultaneous MS and olfactory detection is possible in both 1D and 2D GC analysis modes.
GERSTEL now offers a solution that can be used for routine analysis as well as for special challenges. The patented GERSTEL Selectable 1D/2D-GC/MS is a flexible system, based on a single standard GC/MS instrument. It is both a routine analysis system and a complex problem solving system that offers heart-cutting and two-dimensional separation on demand. Because of this dual functionality, when questions arise regarding a section of the standard one-dimensional chromatogram, this section in question can be transferred to a 2nd dimension, i.e. a GC column with different polarity to further increase separation. Both columns are installed in the same GC and are heated independently using Low Thermal Mass (LTM) technology. The process of cutting a section of a chromatogram and introducing it to another column is called heart-cutting. The 1D/2D system can be used to determine analytes in either the 1st or the 2nd dimension in a flexible manner. Neither the GC run, nor analyte detection is interrupted during the run. Detection of the analytes that were transferred to the 2nd column follows using the same detector(s) used for the 1st dimension: MSD, Olfactory Detection Port (ODP), PFPD etc. etc. Should lower detection limits be required for the analyte in question, the system enables heart-cutting from multiple repeat injection, with cryofocusing of the sections that were cut, on a GERSTEL Cryo Trap System (CTS). The accumulated sections are then transferred to the 2nd dimension once there is sufficient mass on column to perform the determination.

Wide Range of Applicability

Until now, Selectable 1D/2D-GC/MS systems have mainly been sold to, and used for food, flavor, and fragrance analysis, but any application that occasionally requires a 2nd dimension separation to solve a puzzle would benefit from having such a system available at a reasonable cost. Further, detection of analytes in both dimensions is performed using one or more detectors simultaneously including MSD, ODP, FID or PFPD. The complete system is efficiently and conveniently controlled by mouse-click through the GERSTEL MAESTRO software, integrated with the Agilent ChemStation. It couldn’t be simpler.

For more information
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**Selectable 1D/2D GC-Olfactrometry (O)/MS System**

**1D GC-O/MS analysis**
1D GC-O/MS is operated using LTM-GC Column 1. At the same time, carrier gas is supplied to the LTM-GC Column 2 by the PCM.

**Heart-cutting**
User selected peaks eluted from LTM-GC Column 1 are transferred onto LTM-GC Column 2. The transferred peaks are focused at the head of the LTM-GC Column 2 with or without cryo-trapping depending on the compound volatility.

**2D GC-O/MS analysis**
After heart-cutting, the bulk of the sample can be effectively back flushed. A few minutes after the heart-cut, the temperature program for LTM-GC Column 2 is started.

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**Library search**
A Wiley library search tentatively identified the peak as a \( \beta \)-Damascenone.
Off-flavor compounds collected on tap

Based on stir bar sorptive extraction (SBSE), a solvent-free extraction technique, French scientists have developed and patented an innovative new passive sampler for drinking water. Off-flavor compounds are extracted and concentrated directly at the tap under standard usage conditions and are subsequently determined at ultra-trace levels using thermal desorption GC/MS.

**Off-flavors and bad taste in drinking water – whence they come?**

In today’s modern world, tap water is generally completely safe to drink and mostly odorless. At the time of Shakespeare, people were used to a heavy background of unpleasant flavors and odors. In those days, water would also often be unsafe to drink. Those who could afford it would drink beer or wine to avoid the threat of being infected with bacteria or parasites. With improved general hygiene and sanitary conditions, however, consumers are no longer used to off-flavors and are unwilling to accept these. In terms of product quality, taste and odor have come to play an important role for drinking water companies. “Consumers assess the organoleptic (sensory) quality of their tap water while drinking it and an unpleasant smell or taste is often mistakenly associated with health hazards”, explains David Benanou. Drinking water companies therefore work hard to identify sources of unpleasant odor or taste compounds in order to take corrective action and/or make recommendations about how to eliminate them.

Off-flavor compounds in drinking water include geosmin, 2-methyl isoborneol and 2,4,6-trichloroanisole, which normally cannot be attributed to a single source. These may well have been introduced by the feed water that is processed into drinking water. Other conceivable sources are algae, waste water, and leaks. Off-flavors are often the product of microbial activity in today’s extensive water distribution networks or directly in small household systems.

“In most cases, a concentration level of the solutes in the sub-nanogram per liter range (ng/L = ppt) is sufficient to upset consumers’ satisfaction. Their invention „Advanced Relevant Investigation Sampler for Taste & Odor at Tap“ (ARISTOT) has enabled true passive sampling of off-flavors directly from the consumers water faucet. From left, Thomas Thouvenot, David Benanou and Christophe Tondelier from Veolia Environment in Paris, France.

Snooping out the causes of off-flavors: Analyte concentration is a must. ARISTOT on the test-bench. Water usage patterns had to be established and subsequently simulated in the laboratory.

The satisfaction you get from a job well done clearly shows.
The contents of two, three, and four Twisters were analyzed in single GC/MS runs using the multi-shot method; the results were compared with standard single Twister desorptions. The Twisters were spiked with 2 ng/L 2,4,6-Trichloroanisole (TCA). The 2,4,6-TCA peak areas were plotted as function of the number of Twisters desorbed. Linear regression of the results showed a correlation very close to 1. “This shows”, says David Benanou, “that the Multi-Shot Method will enable sensitivity enhancements proportional to the number of Twisters used – and with just one GC/MS run”.

**Analyte concentration is necessary in order to determine off-flavor compounds in water**

The key to successful determination of off-flavors in water is using the correct sample preparation technique. Analytes must be concentrated prior to GC/MS analysis in order to provide adequate detection limits and rugged results. Methods that have been widely used are closed loop stripping analysis (CLSA) and Headspace SPME. CLSA involves concentrating analytes on activated charcoal or similar adsorbents after stripping them from the sample, a relatively lengthy process that also requires extensive cleaning of glassware. Headspace SPME is performed by placing a fiber coated with adsorbent or sorbent material in the headspace of the sample inside a vial. Volatile Organic Compounds (VOCs) are extracted and trapped in the SPME fibre followed by thermal desorption in a GC inlet and GC/MS determination.

Stir bar sorptive extraction (SBSE) has in recent years increasingly been used for extracting and concentrating trace level VOCs and SVOCs from aqueous matrices. SBSE relies on the GERSTEL Twister, a magnetic stir bar coated with polydimethylsiloxane (PDMS). Organic compounds are extracted into the PDMS phase while the Twister stirs the aqueous sample. The stir bar is then removed, dabbed dry and desorbed in a thermal desorption system (GERSTEL TDU or GERSTEL TDS) prior to GC/MS determination.

“Our experience has shown that SBSE is an attractive alternative to conventional stripping methods and SPME”, says David Benanou. “SBSE is based on the same PDMS phase used in SPME fibres, but the sorbent phase volume is significantly bigger leading to much better enrichment and lower limits of detection – even for polar compounds – SBSE helps us determine organic compounds in aqueous matrices dependably and reliably”, the scientist points out.

**Developing a passive sampler for tap water monitoring**

There was agreement within the Veolia R&D team about which extraction and analysis technologies to use, but developing a passive sampler for tap water proved to be a complicated assignment: “Uncharted waters had to be navigated...”
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Innovation really is. Small scale tests were practical use reveals just how good an practice makes perfect. avoiding solute losses. Thouvenot adds that seven positions were chosen to ensure best possible flow conditions, while optimizing the interaction surface between water and PDMS and avoiding solute losses.

ARISTOT on the test bench: practice makes perfect

Practical use reveals just how good an innovation really is. Small scale tests were first performed to determine whether the new passive sampler was suitable for routine operation. The scientists developed a pilot unit, which was used to simulate consumers' habits, which were determined with the help of a questionnaire. To test the sampling efficiency, computer models were used to develop a mixing chamber, that could mix tap water with a defined amount of an off flavor compound standard solution.

Extensive consumer feed-back from questionnaires had revealed that cold water would flow from most kitchen taps about 15 minutes per day at an average rate of 2 liters/min. "Hydrodynamic computer modeling was used to verify that the ARISTOT sampler produced the optimum flow pattern with efficient exchange between water and the PDMS phase", reports Thomas Thouvenot. The team also made sure that the cavities holding the Twister stir bars remained filled with about 18 mL of water after the water tap had been closed, in order to block contamination from the air reaching the PDMS phase.

The scientists reproduced standard water usage patterns on a pilot unit in the laboratory. Optimal conditions were determined to be a tap water flow rate of 2L/min, cold water, large stir bars – 2 cm long x 1 mm PDMS thickness – and with an enrichment time of up to 120 mins. While the water was dispensed, it was spiked with a standard ethanol solution of off-flavor compounds (200 pg/L). The solution included 2,4,6-trichloroanisole (2,4,6-TCA-d5) as the internal standard, 2,4-dichloro-6-bromoanisole (2,4-DC-6-BA), 2,6-dichloro-4-bromoanisole (2,6-DC-4-BA), 2-chloro-4,6-dibromoanisole (2-C-4,6-DBA) and 4-chloro-2,6-dibromoanisole (4-C-2,6-DBA). A GC 6890 with MSD 5975 (Agilent Technologies) was used for the analysis in combination with a GERSTEL thermal desorption system (TDS) fitted with an autosampler (GERSTEL TDS A) and a Cooled Injection System (GERSTEL CIS). 20 mm Twisters with a 1 mm PDMS coating were used for the extractions. The stir bars were subsequently desorbed in splitless mode: 30°C (0.8 min), 60°C/min, 250°C (8 min). The desorbed analytes were cryofocused at –50°C in the CIS. The CIS was then heated up to 300°C at 12°C/min (2 min) and the analytes transferred to the column in splitless mode. The carrier gas was helium with a constant flow through the system of 1.5 mL/min.

To demonstrate how simple it is to use the ARISTOT passive sampler, Veolia generated an animated film dip in English language. In it, “Professor Benanou” and his assistant explain how the sampler works and how it is used. (www.gerstel.com/en/aristot.htm)

while meeting fundamental conditions”, comments Christophe Tondelier. A passive sampler relies on analyte diffusion from the sample into the trap, active pumping of the sample is not used. In addition, the sampler had to be visually appealing and easy to integrate in bathroom and kitchen workflows. The main question was of course how one could insert a passive sampler into the path of the water without influencing or changing the normal flow and water handling conditions at the tap.

In the end, Mother Nature provided the model for the patented passive sampler ARISTOT (Advanced Relevant Investigation Sampler for Taste & Odor at Tap). Inspired by the petals of daisy flowers, seven cavities, each 3 mm in diameter and 30 mm long, designed to hold the Twisters were made in a 40 mm long piece of stainless steel. The material was chosen for its inertness to avoid contamination or reactions with the substances in the water. “ARISTOT is like the cylinder of a revolver, except that instead of bullets it contains seven GERSTEL Twisters held in place by a nozzle in the cavity”, says Thomas Thouvenot, flow expert and a member of the ARISTOT team. Mr. Thouvenot adds that seven positions were chosen to ensure best possible flow conditions, while optimizing the interaction surface between water and PDMS and avoiding solute losses.

To further improve detection limits, we decided to investigate the effects of desorbing multiple Twisters for each GC/MS run”, explains David Benanou. Initially, two Twisters were in the TDS desorption tube instead of one - and the results were encouraging. Sensitivity was doubled with excellent recovery and reproducibility. “When we then moved to desorb all seven twisters and introduce the combined analytes into the GC/MS in just one run, we reached detection limits close to the low ppq range (pg/L). You just can’t get better sensitivity than that.”

For more information

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Asparagus on the menu

Efficient detection of Sulfur-Containing Metabolites of Asparagus in Urine by SBSE-GCxGC-TOFMS.

Consumption of asparagus has been known to cause a distinct odor in the urine of humans. This odor can be present in urine in as little as 10 to 15 minutes after consumption. The compound that is unique to asparagus and is responsible for the odor is asparagusic acid. Subjects who consumed asparagusic acid, without consuming asparagus, produced the characteristic odor associated with asparagus consumption. The asparagusic acid is metabolized to S-methyl thioesters and ultimately to methanethiol, dimethyl sulfide, dimethyl disulfide, dimethyl sulfone, and dimethyl sulfoxide. Traditionally, samples are prepared using heated solvent extraction over periods as long as 48 hours. Our goal was to eliminate the use of potentially hazardous solvents and reduce overall extraction time through the use of GERSTEL’s Twister Stir Bar Sorption Extraction (SBSE).

Instruments and Methods

In this study, measurements were made with a LECO Pegasus 4D GCxGC-TOFMS system. This system consists of an Agilent 6890 gas chromatograph equipped with a LECO dual-jet thermal modulator between the primary and secondary columns and a LECO Pegasus IV Time-of-Flight Mass Spectrometer (TOFMS) as a detector. The primary analytical column was a GERSTEL-MACH LTM 10.0m x 0.18mmID x 0.20 µm df Rtx-5. The secondary column was a 1.00 m x 0.10 mm ID x 0.10 µm df DB-17ms and was housed in the GC oven. The modulator temperature offset for this study was +30 °C. Helium was used as the carrier gas at a constant flow of 1.5 mL/minute. The transfer line to the TOFMS consisted of the last 20 cm of the analytical column and was kept at 280 °C. For the one-dimensional study, the modulator was turned off and the GC oven was maintained isothermally at 280 °C.

Urine samples of approximately 250 mL were collected prior to consumption of asparagus and approximately 90 to 120 minutes after consumption of asparagus. The samples were immediately placed in 250 mL amber glass bottles, each containing a 10 mm x 0.5 mm GERSTEL Twister and extracted on a stir plate at 800 rpm. Upon completion of the extraction, the stir bar was placed in the GC oven.
removed, rinsed with DI water, and placed in a GERSTEL Thermal Desorption Unit (TDU) tube for analysis.

The tube containing the stir bar was loaded into the TDU. The TDU was then heated, and the analytes were desorbed from the stir bar. The analytes were then cryo-trapped in the GERSTEL Cooled Injection System (CIS). The CIS 4 was then heated to desorb the analytes onto the GC column.

The TDU was operated in splitless mode. Its initial temperature was 30 °C and was held at this temperature for an equilibration time of 120 seconds. It was then heated to 280 °C at a rate of 700 °C/minute and held at this temperature for 120 seconds. The CIS 4 was cooled to a temperature of -120 °C. It was heated to 280 °C at a rate of 12 °C/second and held for 120 seconds.

**Results**

In this study, the urine samples were not derivatized. Analytes of interest in the post-asparagus samples are sulfur-containing compounds not present in the pre-asparagus urine samples. In Figure 1, the 1D traces of pre-asparagus and post-asparagus samples are overlaid to highlight their differences.

Some of the most prominent peaks, visible in red, include A) acetone, B) acetic anhydride, C) 4-heptanone, D) 5-methyl-2-(1-methylethyl)cyclohexanone and E) 1-(1,5-dimethyl-4-hexenyl)-4-methyl benzene. Trace peaks not present in the pre-asparagus sample, but present in the post-asparagus sample include S-methyl propene thioate and 1,4-bis(methylthio)-butane.

In the GCxGC-TOFMS analysis of pre-asparagus and post-asparagus samples, contour plots are shown in Figure 2. The target compounds in this analysis are sulfur-containing metabolites of asparagusic acid. S-methyl thioesters are listed as being one of the primary metabolites. S-methyl 2-propenethioate had retention times of 205 seconds in the first dimension and 1.7 seconds in the second dimension. Its peak true and library spectra are shown in Figure 3.

**Conclusions**

This study has shown an improvement in not only overall analysis time, but in decreased complexity of sample preparation, as well as elimination of extraction solvents. The automated nature of the GERSTEL MPS 2–TDU–CIS4 system decreases the amount of manual work necessary for each analysis by allowing for automation of the process. The use of GCxGC-TOFMS also allows for better resolution of analyte peaks and decreased coeluions. Any remaining coelutions can be resolved through True Signal Deconvolution, made possible by the high-speed, fullrange spectra available from the LECO Pegasus TOFMS.

For more information

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Figure 2: Contour plots of pre-asparagus urine (A) and post-asparagus urine (B). Relevant compounds not present in the pre-asparagus sample are highlighted in the post-asparagus sample.
CN (potassium cyanide) and NaCN (sodium cyanide) are salts of hydrocyanic acid better known as hydrogen cyanide (HCN). HCN is among the strongest and fastest working poisons. The lethal dose is around one to two milligram per kilogram body weight and can be incurred by either oral ingestion or inhalation.

Seconds after ingesting just 120 to 250 mg of KCN or NaCN, the victim goes into shock. Breathing is initially difficult, then impossible and after approximately one minute the victim will lose consciousness. Even immediate medical assistance will typically not help. The patient will die of acute respiratory paralysis within minutes.

**Complexing agent of the first order**

Pure hydrogen cyanide (HCN) is a colorless, highly volatile liquid with a boiling point of 26 °C and a characteristic bitter almond odor. Not all humans are genetically predisposed to being able to smell HCN, though. Even if a person is able to detect the smell, their olfactory sensory system will quickly cease to function when exposed to high HCN concentrations. The toxic effect of cyanide is mainly based on its ability form stable complexes with metals such as iron (III), which is charged with the vital task of making oxygen available to cells inside the human body. As a consequence, cyanide blocks transport of oxygen into the cells and leads to what is sometimes referred to as inner asphyxiation.

To a certain degree, this process can be reversed through appropriate and prompt therapeutic counter-measures. In addition, humans can metabolize small amounts of cyanide enzymatically in the liver and dispose of the metabolites through the urinary system, which is why we can eat apple pits without worrying even though...
they contain minute amounts of cyanide. Other products such as bitter almonds, peas and beans contain cyanogenic glycosides, from which HCN can be formed in the body through enzymatic or chemical hydrolysis.

As an aside: Not all cyanide compounds are toxic, potassium ferrocyanide, for example, is not. The chemical forms relatively stable complexes and is used as a stabilizer. In the European Union (EU), potassium ferrocyanide is known as food additive E536, but can only be used as an additive in table salt products and only at very low concentration.

The most dangerous cyanide salts are those formed with alkaline metals or alkaline earth metals. These salts are highly soluble and are often used to extract raw silver or gold from ground ore. This process is known as cyanide leaching. In the gold-extraction process, ore is ground, mixed with water. Cyanide is then added to “leach” the gold out, precious metals form complexes with cyanide and are extracted into solution. Even though the extraction is performed in a closed loop process, residues of the highly toxic cyanide leaching solution can occasionally spill into the environment where they contaminate soil, surface and ground water. An example of worst case damage by a cyanide spill was seen in January 2000, when heavy rainfall combined with melting water run-off damaged a retaining wall at a gold extraction processing plant in Baia Mare, Roumanian. According to estimates, more than 100 000 m³ of poisonous waste water flooded into the Luples, Somes and Tisza rivers, tributaries to the Danube, killing all life.

The need to monitor Cyanide in drinking water

Poisoned water bodies sooner or later regain their equilibrium thanks to the work of Mother Nature: Cyanide is oxidized to nitrogen and carbon dioxide. In chemical processes, the reaction is accelerated by adding sodium hypochlorite (NaOCl) or hydrogen peroxide (H₂O₂). Without help from human hands, the process takes longer and time is always of the essence.

Even limited amounts of cyanide ingested through our food or drinking water over years can accumulate in our bodies and can cause significant damage to our health. The United States Environmental Protection Agency (U.S. EPA) has therefore established a Maximum Contaminant Level, (MCL) which must not be exceeded. The MCL for cyanide is 0.2 milligram per liter or 200 parts per billion (ppb). The German drinking water regulation (TrinkW2001) specifies a maximum concentration of 0.5 mg/L. If this concentration level is exceeded, the water supplier must take measures to ensure adequate consumer protection.

**HS-GC/MS as the method of choice**

To determine the concentration of dissolved cyanide in drinking water, the most suitable technique is headspace gas chromatography using mass selective detection (HS-GC/MS). The technique captures all cyanide compounds that form HCN under acidic conditions. Jim Eaton, Ph.D., from the H&E Testing Laboratory in Augusta, Maine has recently established an official test method for the State of Maine. Method ME355.01 is entitled Determination of cyanide in drinking water by gc/ms headspace analysis. The method was originally made available by the Centers of Disease Control (CDC) for the analysis of cyanide in whole blood, a method that is part of the technology transfer group at CDC involved with chemical terrorism. Method ME 355.01 has now been promulgated by the US EPA. The method involves the following steps:

- Samples are taken in 40 mL brown glass vials. 1 mL of a 1 M NaOH solution is added to preserve the sample, which is then stored at 4 °C in darkness until it is analyzed. The sample must be analyzed within seven days. Prior to the analysis, a 2-methyl aniline solution is added to the preserved sample. If the sample turns yellow it is discarded. To ensure efficient and accurate analysis, a suitable autosampler capable of automated sample preparation should be used; the Dual Rail PrepStation version of the GERSTEL MultiPurpose Sampler (MPS) is listed in the official method. The MPS PrepStation is a two-in-one autosampler, capable of performing both headspace sampling and liquid handling. The liquid handling steps can include, for example, the addition of an internal standard, a reagent, or a diluent. A standard GC/MS system was used for separation and determination of the analytes.

**Sample preparation:** From each sample collection vial, a 1 mL aliquot is taken and transferred to a 10 mL standard headspace vial, which is capped. The prepared headspace vials are then transferred to the MPS PrepStation for analysis. All further steps are performed automatically, set up by mouse-click through the GERSTEL MAESTRO software, fully integrated with the GC/MS software. Just one method and one sequence table conveniently control the entire process, from liquid handling sample preparation to the HS-GC/MS analysis. In the MAESTRO PrepBuilder, the user can easily set up all necessary steps by selecting them from a drop down menu and adding them to the method. To ensure maximum efficiency and sample throughput, the PrepAhead feature enables overlapping sample preparation of up to 6 samples while the current analysis is in progress. All samples are introduced to the GC/MS system just as it becomes ready after analyzing the preceding sample, which means that the GC/MS system is never idly waiting for the next sample to be prepared.

Back to the method: The MPS automatically adds 50 µL internal standard, an aqueous solution of K₁³C₁₅N, to the sample, followed by 200 µL ascorbic acid and 200 µL phosphoric acid, which are added to release HCN. After thermostating the headspace vial for four minutes at 60 °C, the MPS draws an aliquot of the headspace in the vial. The aliquot is introduced to the GERSTEL Cooled Injection System (CIS) inlet, focusing the analytes at -10 °C. After approximately 1.5 minutes, the CIS is quickly heated to 220 °C and the analytes are transferred to the GC/MS system for determination.

**Round robin**

The method was tested by three independent laboratories. The following sample types were analyzed by each laboratory, each type was laced with 50 and 200 ppb cyanide respectively:

- Reagent grade water
- High salt concentration water
- Drinking water with high Total Organic Carbon (TOC) level.

All three laboratories reported results that were well within the requirements, confirming the usefulness of the method.

Method ME355.01 was specifically developed to require only small amounts of reagents and standards thus minimizing the potential hazard to both the environment and the analyst.

**For more information**

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In order to analyze biological specimens for drugs such as benzodiazepines, it is necessary to perform sample preparation to eliminate matrix interference and ion suppression. Solid-phase extraction is generally the preferred sample preparation technique, in this study Disposable Pipette Extraction (DPX) was utilized. DPX is a fast dispersive solid-phase extraction technique that uses loosely contained sorbent in a disposable pipette tip. The sample is aspirated into the tip where it is actively mixed with the sorbent and forms a suspension. The main advantages of the DPX technology are that the extraction is rapid, minimal solvent waste is generated, and the entire process can be fully automated including introduction of the extract to the chromatographic system. The GERSTEL MPS autosampler performs DPX extractions in approximately 5 minutes using reversed phase (DPX-RP), cation exchange (DPX-CX) or immunoaffinity sorbent material. For determination of target drugs, GC/MS or HPLC/MS/MS are generally the preferred techniques. The advantage of LC/MS/MS is that chemical derivatization of the analytes is not required, making sample preparation simpler and less time consuming. In addition, highly efficient ionization, in combination with tandem mass spectrometry results in high sensitivity and selectivity. This study focused on performing automated extraction of reduced sample volumes coupled with LC/MS/MS to provide high throughput analysis “one sample at a time”. The sample preparation time was decreased sufficiently to allow the extraction of a sample during the chromatographic analysis of the previous sample in the sequence.

###Experimental

**Instrumentation:** All Analyses were performed using an Agilent 1200 HPLC with an Agilent 6410 Triple Quadrupole Mass Spectrometer with ESI source. Sample Preparation and Sample Intro-

###Urine Sample Pretreatment

- Pipette 260μL of hydrolyzed urine sample into a clean 12 x 75mm culture tube.
- Pipette 250μL of 1.0M HCl into the tube and vortex mix for a few seconds.
- Pipette 250μL of acetonitrile into the tube and vortex mix for a few seconds.
- Filter the sample using an Agilent 2 in 1 syringe filter and collect the filtrate into a clean 12 x 75mm culture tube.
- Place the filtered urine sample onto the GERSTEL MPS 2XL multi-purpose sampler with DPX Option.

###Whole Blood Sample Pretreatment

- Pipette 200μL of whole blood sample into a clean 12 x 75mm culture tube.
- Pipette 800μL of acetonitrile into the tube and vortex mix for a few seconds.
- After centrifugation for 10 minutes to pellet the precipitated proteins, transfer the supernatant into a clean 12 x 75mm culture tube.
- Place the sample onto the GERSTEL MPS 2XL multi-purpose sampler with DPX Option.
Automated DPX process

All steps are performed automatically by the MPS. If needed, the sorbent is conditioned with solvent prior to the extraction process.

Sample is drawn into the pipette tip for direct contact with the solid phase sorbent. There is no contact between the sample and the syringe used to aspirate the sample and therefore no risk of cross contamination.

Air is drawn into the pipette tip from below through the frit. Turbulent air bubble mixing creates a suspension of sorbent in the sample, ensuring optimal contact, highly efficient extraction, and high recovery.

The extracted sample is discharged, typically after 30 seconds. If needed, the sorbent can be washed to remove unwanted residue.

4 Extracted analytes are eluted using a suitable solvent, which is added from above for most efficient elution. The eluate is collected in a vial for subsequent sample utilization.

The total time required for extraction in the examples shown in this article was always less than 6.5 minutes. Sample preparation and GC/MS or LC/MS determination can be performed in parallel for best possible throughput and system utilization.

Materials

Benzodiazepine Multi-Component Mixture 8, containing Clonazepam, Temazepam, Nitrazepam, Alprazolam, Diazepam, Flunitrazepam, Lorazepam, and Oxazepam at 250 µg/mL each in acetonitrile, Nordiazepam, Clobazam, Bromazepam, Estazolam, Flurazepam, Midazolam, and Triazolam at 1.0 mg/mL each in methanol, and α-hydroxyalprazolam, at 100 µg/mL in methanol, were purchased from Cerilliant. These stocks were combined and diluted with water to be used as internal standards during analysis.

Deuterated analogues d5-nordiazepam, d5-α-hydroxyalprazolam, d5-oxazepam, d4-clonazepam, and d5-estazolam, at 100 µg/mL each in methanol, and α-H-alprazolam, were purchased from Cerilliant. Intermediate stock solutions of the sixteen benzodiazepines were prepared in water from appropriate dilutions of these stocks.

Extraction

A GERSTEL MultiPurpose Sampler (MPS) was set up with 1 mL DPX-CX tips (DPX Labs, LLC, Columbia, SC) for extraction of hydrolyzed urine samples. The following automation method was used: 250 µL of 30% acetonitrile/water was slowly added through the top of the DPX tip at a rate of 50 µL/s to wet the sorbent. The sample was then aspirated into the DPX tip and mixed with the sorbent by drawing in an additional 1.3 mL of air. After a 20 s equilibration time to allow analyte binding, the resulting solution was dispensed to waste. To wash off excess matrix, 500 µL of a 10% acetonitrile/water wash solution was added to the sorbent material through the top of the DPX tip and dispersed to waste followed by an additional wash using 500 µL of 100% acetonitrile. For elution of

Mass Spectrometer Acquisition Parameters

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<tr>
<th>Analyte</th>
<th>Precursor Ion [m/z]</th>
<th>Product Ions [m/z]</th>
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<th>CE (V)</th>
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<td>288 (315)</td>
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Extracted Ion Chromatogram of DPX extracts of urine samples spiked at 7.5 ng/mL.

Representative Calibration Curve of Triazolam and Clonazepam.

Calibration Curve Results
Calculated using
- $d_5$-$\alpha$-Hydroxyalprazolam (A),
- $d_5$-Nordiazepam (B),
- $d_5$-Oxazepam (C),
- $d_5$-Estazolam (D) and
- $d_4$-Clonazepam (E).

<table>
<thead>
<tr>
<th>Compound</th>
<th>$R^2$</th>
<th>LOQ [ng/mL]</th>
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Results and Discussion

The DPX-CX extractions were readily performed using the GERSTEL MultiPurpose Sampler. The entire extraction process took approximately 6.5 minutes per sample. Because a basic eluent is used with the cation exchange sorbent, the eluents had to be solvent exchanged into the HPLC mobile phase. In light of the world-wide acetonitrile shortage, it was found that the use of 10% isopropyl alcohol in methanol as the organic modifier of the mobile phase provided an alternative to using acetonitrile. All HPLC/MS spectra were collected using dynamic multiple reaction monitoring (MRM) providing good sensitivity for the analysis of these drugs at low concentrations. A rapid resolution HPLC column was chosen in order to perform the chromatographic data in approximately 10 minutes.

Conclusion

Automated DPX extraction of benzodiazepines from urine can be performed successfully using the GERSTEL MPS. In the work presented here, the total ext-

the analytes, 700 µL of 78/20/2 (v/v) of methylene chloride/isopropanol/ammonium hydroxide was added to the sorbent material through the top of the DPX tip and the eluate dispensed directly into a clean 2 mL autosampler vials. All eluents were dried and reconstituted with 50 µL of water before injection.
Matrix effects on the ionization of benzodiazepines in hydrolyzed urine and whole blood compared to the neat standard solutions in pure water (100%).
Amino acids are vital to humans since they are involved in many important metabolic processes. In the middle of the last century, experts came up with the logical idea of diagnosing metabolic disorders by determining amino acid concentration levels. The ideal tool for the determination of amino acids in biological matrices is gas chromatography combined with mass spectrometry (GC/MS), but in order to meet the efficiency requirements of modern diagnostic laboratories, the analytical procedure needs to be highly automated.

Amino acids are equally important to human beings and animals, but they can only be produced by the organism to a limited extent. We humans have to obtain several amino acids with our food. Amino acids are building blocks for proteins and they perform a large number of other functions in our organism. The amino acid tyrosine, for example, is converted into catecholamine, i.e. into a hormone, that has a stimulating and stabilizing effect on the cardiovascular system; glutamate, another key player, is a neurotransmitter which handles vital communication in our body.

Due to their involvement in many different metabolic processes, amino acids are generally useful as markers for metabolic disorders, including, for example, congenital metabolic disorders, among which Phenylketonuria (PKU) is one of the most well-known. A person suffering from PKU is unable to metabolize phenylalanine; this means that the amino acid is accumulated in the body, adversely effecting mental development. A PKU test can be performed by simply determining whether the patient has a significantly elevated phenylalanine concentration level. Amino acid concentrations are determined in body fluids such as blood, plasma or urine.

Routine monitoring of amino acid concentrations in biological samples is frequently performed using dedicated amino acid analyzers; the analyzers are laboratory instruments that are based on cation exchange chromatography combined with post-column derivatization and UV detection. The main disadvantage of this type of analyzer is that the overall analysis is quite time-consuming. Gas chromatography with mass-selective detection (GC/MS) represents an attractive and efficient alternative; Amino acids are derivatized using propyl-chlorofor-mate; the resulting compounds are volatile and can be determined by GC/MS.

“We have completely automated the sample preparation, the method is suitable for high sample throughput”, says Katja Dettmer, Ph.D., Metabolomics Project Manager at Professor Peter Öfner’s Institute of Functional Genomics at the University of Regensburg, Germany. All sample preparation steps from addition of the internal standard (IS) to derivatization and finally introduction of the prepared sample into the GC/MS system, are performed automatically using the dual-rail PrepStation version of the GERSTEL MultiPurpose Sampler (MPS PrepStation). Dr. Dettmer: “Automation enabled us to minimize the amount of manual work, while at the same time improving reproducibility.”

A more detailed explanation: the MPS PrepStation has two robotic arms that operate independently of each other. One of these is, in this case, fitted with a 1 mL liquid syringe used to add derivatization reagent. The other robotic arm holds a 10 µL liquid syringe, used for adding internal standard (IS) and introducing the prepared sample to the GC/MS analysis system. Apart from these hardware details the key component of the system is the GERSTEL MAESTRO software. In the MAESTRO PrepBuilder, the user simply selects sample preparation steps by mouse-click from a drop down menu and adds them to the sample preparation method. Sample preparation and GC/MS analysis is then synchronized in a combined sequence table and performed in parallel for optimum productivity thanks to the PrepAhead function; and finally the Scheduler overview enables the user to check the progress of the analysis at any time. Clear information is provided at a glance on how much time
is required to finish the batch in order to help the laboratory workflow and facilitate planning.

Sample preparation and GC/MS analysis

This is the procedure Dr. Dettmer and her colleagues adopted in their amino acid determination project: blood or urine samples were placed in individual autosampler vials, which were sealed with crimp caps and placed in the cooled sample tray of the MPS PrepStation. All further steps were performed automatically, including transport of the vials to and from the vial agitator, which can stir, shake, heat or cool samples as per individual method requirements. Depending on the sample matrix, a sample volume of between 20 and 50 µL was used to perform the analysis. The following steps are performed automatically by the MPS PrepStation: 1. Add a sample volume of between 20 and 50 µL was used to perform the analysis. The following steps are performed automatically by the MPS PrepStation: 1. Add internal standard (a mixture of 20 amino acids labelled with $^{13}$C and $^{15}$N and two deuterated standard compounds). 2. Add diluent. 3. Add a sodium hydroxide solution. 4. Add a picoline solution (catalyst). 5. Add propyl chloroformate (derivatization reagent). 6. Mix the vial contents in the agitator and 7. Add isocyanate to extract the derivatized compounds into the organic phase after centrifugation. 8. Inject 2.5 µL of the supernatant to the GC/MS system using the 10 µL syringe. Separation was performed on a ZB-AAA column (Phenomenex, Inc.). An Agilent GC 6890 equipped with the GERSTEL Cooled Injection System (CIS) inlet, a PTV-type inlet was used. Detection was performed in simultaneous SIM/SCAN mode on an Agilent MSD 5975, with two characteristic mass fragments being recorded for each amino acid.

Method description and validation

Dettmer and her colleagues determined concentration levels of 33 amino acids and dipeptides using the MPS-CIS GC/MS method: Alanine, sarcosine, glycine, $\alpha$-aminobutyric acid, valine, $\beta$-aminoisobutyric acid, leucine, alloisoleucine, isoleucine, threonine, serine, proline, asparagine, thiaproline, aspartate, methionine, hippuric acid, hydroxyproline, glutamate, phenylalanine, $\alpha$-aminoadipinic acid, $\alpha$-aminopimelic acid, glutamine, ornithine, glycyl-proline, lysine, histidine, hydroxylysine, tyrosine, proline-hydroxyproline, tryptophane, cystathionine and cystine.

Quantification was performed from calibration curves using a series of $^{13}$C and $^{15}$N labelled internal standards: Alanine, glycine, valine, leucine, isoleucine, threonine, serine, proline, asparagine, aspartate, methionine, glutamate, phenylalanine, glutamine, lysine, histidine, tyrosine, cystine, tryptophane and cystine. In addition, $[2H_5]$ hippuric acid and $[2,5,5-2H_3]$ $\alpha$-aminoadipic acid were used. Concentrations of amino acids for which isotopically labelled internal standards were not directly available were calculated based on the response from the isotopically labelled amino acid eluting closest to them”, explains Dr. Dettmer. The use of stable, isotopically labelled amino acids as internal standards resulted in a considerable improvement in both reproducibility and correlation coefficient of the calibrations. “The GC/MS analysis run for the 33 amino acids lasted less than ten minutes, a significant improvement over the conventional method”, the scientist is pleased to report.

Calibration curves for most of the amino acids were generated over the range from 0.3–2 µM based on 50 µL samples of biological fluid. The limit of detection (LoD) ranged from 0.03 µM for the amino acids alanine, glycine and tryptophane to 12 µM for glutamine and proline-hydroxyproline; the limit of quantification (LoQ) was in the range from 0.3 to 30 µM. Dr. Dettmer used the method successfully to analyze various body fluids, while also determining reproducibility. Human and mouse urine and human plasma were investigated, performing determinations in decaplicate. Relative standard deviation (RSD) ranged from 2.0 to 8.8% for human urine; from 0.9 to 8.3% for human plasma; and from 1.3 to 9.1% for mouse urine.

Practical Applications

Dr. Dettmer concludes: “Biological samples like urine, cell cultures, cell extracts, and plasma can be analyzed easily and reliably with our method”. The determination of amino acid concentrations in body fluids in order to diagnose congenital metabolic disorders, such as the ones listed earlier, is just one of the possible applications. The determination of amino acids plays a particularly important role not only in clinical diagnostics but also in food analysis. It is a well-established fact that the organism has to obtain essential amino acids through food under normal circumstances, these cannot be synthesized by the body. Dr. Dettmer notes that her MPS-CIS-GC/MS method could also be used to determine the concentration levels of amino acids in milk, beer and fruit juice. To demonstrate the applicability of their method to food analysis, Dettmer and her colleagues analyzed apple juice, beer and soy sauce. Incidentally: soy sauce is an Asian condiment consisting of water, soybeans, cereal and salt. The analysis revealed, that soy sauce contains numerous amino acids – in very large amounts in some cases – with glutamate dominating at 48 mM. The main amino acids in apple juice are alanine, proline, asparagine, aspartate and glutamate, with asparagine standing out at a concentration of 3.16 µM. The amino acids alanine (1.13 mM) and proline (3.56 mM) are the main components in beer. Dr. Dettmer summarizes: “These results make it clear that our automated MPS-CIS GC/MS method is evidently suitable for detecting amino acids in biological matrices, whether in blood, urine or food stuffs.”

For more information

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Consumer protection requires that harmful chemicals be prevented from gaining access to food products or pharmaceuticals designated for human consumption. While packaging generally keeps products fresh and protects them from direct contamination, materials used to manufacture the packaging can themselves be an unintended source of migrating chemicals. Apart from performing routine migration studies, food producers or packaging suppliers need to determine barrier properties of primary food packaging. In the case of pharmaceutical packaging, leachables and extractables studies are required to determine if packaging will leach contaminants into the product under normal storage conditions or if contaminants can be extracted by the product under well-defined more aggressive conditions. Thermal desorption combined with GC/MS has proven to be an efficient and sensitive screening method for contaminants in packaging.

Modern packaging and packaging materials offer tremendous advantages with respect to hygiene, product freshness, easy handling, efficient transport, extended storage time, low price and a clear product declaration. But as with every advantage in life, there is a trade-off. Use of these materials can involve risk: Solvents, additives and pigments are used in the production of and printing onto packaging materials and these may contaminate the packaged product. A careful assessment of potential risks needs to be made in order to protect consumers and/or patients against harmful migration of unwanted by-products of packaging production.

**Food packaging:** the aim of migration studies is to demonstrate whether chemicals contained in or on the packaging, such as plastic additives, pigments, printing solvents or bonding systems, remain where they are or migrate into the food. The studies are generally carried out with three food simulants under standard conditions (e.g. 10 days at 40°C). The substances used are: aqueous acetic acid, an aqueous alcoholic solution, and edible oil. These studies determine the concentration of the target compound in the food simulant during and/or after the incubation period. The results are used to define specific migration levels (SML) for the target substance. The target compounds are approved in general for the plastic tested and/or for the application in question based on the SMLs. Approved and/or acceptable limits for SMLs are defined in regulations issued by the appropriate agency (see box).
Pharmaceutical packaging: the aim of what is known as “extractables & leachables” (E&L) studies is to demonstrate whether the packaging of a pharmaceutical product is safe and/or whether the drug contained is being contaminated by migrating chemicals. The necessary tests are performed in two stages.

1. “Extractables” study: a worst-case scenario is simulated, in which the pack is extracted with solvents of varying polarity and at a high temperature without destroying it. Extensive analytical characterization is performed of the obtained extracts, in order to gain as complete a picture as possible into all the compounds that might potentially contaminate the pharmaceutical.

2. “Leachables” study: following a toxicological evaluation of the “extractables” study, substances classified as critical are analyzed in the pharmaceutical itself using validated methods. This is generally performed during stability testing.

Incidentally: there are no official upper concentration limits for “extractable” substances; each E&L study has individual characteristics and has the aim of identifying potentially risky compounds in each specific case and of investigating them in detail.

Migration tests and E&L studies are to some extent performed based on official standard methods and regulations. Numerous very different techniques and methods are used in order to get a complete picture. Once the complete puzzle has been meticulously put together, the information can be used to optimize any packaging system with regard to migration properties.

Thermal desorption GC/MS (TDS-GC/MS) plays a key role in seeing the whole picture. The technique is more efficient than practically any other in determining the emission potential of volatile and semi-volatile organic compounds from packaging materials. In addition, thermal extraction is a solvent-free extraction technique, which means that peaks of interest are not masked by solvent. Potential analytes are oligomers from polyolefins, anti-oxidants and their degradation products, plastic additives, solvents from printing inks, plasticisers, monomers from bonding systems, contaminants from pigments, photoinitiators, countless compounds and compound classes from recycled board like diisopropynaphthalene, phthalates or hydrocarbons. If required, the initial screening with TDS-GC/MS can be followed by further analyses using liquid extraction combined with GC/MS, LC/UV, LC/MS, elemental analysis, TOC etc.

Migration from food packaging
A food product is stored in nine different plastic primary packs, including polyole-
Migration from laminated pharmaceutical packaging

An “extractables” study was performed on a laminated multicomponent pack consisting of the following individual components: flexible bag, sealing system, closure, catheter system etc. The pack contained a liquid, lipophilic pharmaceutical product. The study strategy involved initially determining individual compounds in the lipophilic product by TDS-GC/MS. The multicomponent pack was then emptied, refilled with a non-polar solvent, and stored at high temperature (worst-case scenario). The resulting extract was subsequently analyzed and several compounds were identified and quantified.

One component, present at a concentration of >100 ppm, could not be identified, however, comparison of the extract chromatogram with the chromatogram of the individual components finally revealed the source: the laminated polymer material. The mass spectrum of the unknown component indicated that it was a phthalate, since the main fragment was observed at m/z = 149. However, no commercially available phthalate was identified that corresponded to the unknown compound in terms of its chromatographic characteristics and mass spectrum.

The molecular weight of the unknown compound was determined by Chemical Ionization (CI)-MS and the empirical formula was subsequently determined using high-resolution Electron Impact Ionization (EI) -MS and the main fragment (m/z = 149) was clearly associated with the empirical formula C8H5O3, which is a typical phthalate fragment. The TDS-GC/MS analysis of the individual polymer materials and adhesives finally showed that the compound came from an adhesive component. According to the manufacturer of the adhesive, it was based on a polyester diol, consisting of phthalic acid and diol units, which allowed conclusions to be drawn about the proposal structure (low-molecular degradation product of the polyester diol). The compound was subsequently synthesized, characterized, and structurally characterized (1H NMR and 13C NMR), and analyzed as a reference by GC/MS at Ciba Expert Services.

Result: It was determined that the DINP concentration in all packaged foods increased over time. In other words: no polymer-based primary packaging material represented an absolute migration barrier even though some were very good. Simple polyolefin systems tended to be much poorer barriers to migration than, for example, complex laminates or metallized polymer films.

Migration from printed pharmaceutical packaging

A further extractables study focused on polymer-based pharmaceutical packaging, onto which information had been printed. The content was a liquid, aqueous pharmaceutical product. The strategy followed in this study was the same that was used in case study II: At first, TDS-GC/MS screening of the printed and unprinted polymer was performed. Subsequently, the packaging was filled with a polar solvent and stored at an elevated temperature (worst-case scenario). The extract was then analyzed using a variety of different methods. Apart from typical extractables from polymer systems (oligomers, plastic additives), components of the printing formulation such as solvent residues and degradation products of the photoinitiator – a triaryl sulfonium salt – were found in the printed polymer. Benzene (a carcinogenic compound) was one of the substances involved, a targeted analysis was performed to determine the benzene concentration in the polar extract of the printed polymer using HS-GC/MS, with quantification in the ppm range.

The HS-GC/MS method was validated in accordance with ICH rules in a subsequent leachables study. Benzene in the 1 ppb range was quantified in stability samples of the pharmaceutical product (stored in the printed pack). The presence of this carcinogenic substance requires a risk assessment to be performed: whereas the administration of about 1 mL of a pharmaceutical product (typical for pre-filled syringes) per day (corresponds to 1 ng of benzene) represents a comparatively negligible risk for patients, the administration of one litre of a pharmaceutical product – which happens in infusions, for example, and corresponds to the intake of about 1 µg of benzene – proves to be a risk the patient should not be expected to take.

Conclusion: benzene was easily identified in a polymer pack with the help of TDS-GC/MS screening. The printed packaging system was then extracted and the extract analyzed to determine the benzene concentration specifically. Finally, the benzene concentration in the pharmaceutical product was determined as well.

Thermal desorption GC-MS can provide detailed information on properties of packaging material in terms of barrier functions as well as migration, leaching or extraction of chemicals from the material into the packaged product. TDS-GC/MS enables efficient screening for troublesome shooting as well as support for development of suitable packaging of food and pharmaceutical products as demonstrated in the case stories described in this article.

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