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No. 14

Focus on intelligently automated sample preparation

Material analysis
Amber, glue and polymers

Toxicology
Pain management drugs in urine

Flavor analysis
Rotundone in wine

Dear reader,

Eberhard G. Gerstel

Holger Gerstel

Ralf Bremer

In close cooperation with our users in industry, research institutes and government agencies, GERSTEL has developed numerous innovations in the fields of GC/MS- or LC/MS related instrumentation and their application to real world analytical work, in a word: Practical solutions.

Many of these innovations were the result of a request for a solution to a specific problem, including requests to automate sample preparation. Solutions for specific tasks have frequently proven useful in other application areas, sometimes unexpectedly, leading to entirely new standard products and solutions.

In recent decades, countless projects and cooperations between GERSTEL experts and laboratory analysts have been successfully completed. These cooperations generally proved fruitful for both sides by combining our technical and application know-how with the in-depth knowledge of the customer as to the products that need to be analyzed. At GERSTEL we strive to regularly surprise the world with innovations that offer significant added value and benefits to the GC/MS and LC/MS analyst.

When we develop new solutions today, we draw on a variety of resources that have grown and developed side by side under one roof leading to efficient communication and exchange. The R&D Department includes Technical Construction, Prototyping, as well as Electronics, Mechanical, and Software Development. Analytical Services, Customer Support, and Customer Training are equally important departments. The close cooperation within our organization is evident in the many articles on customer applications published in the GERSTEL Solutions Worldwide magazine and other magazines and journals over the past several years.

The GERSTEL product portfolio is mainly based on three pillars. One of them is Thermal Desorption (TD) used for solvent-free extraction and concentration of VOC and SVOC analytes from a range of matrices. If you can combine TD with an automated pyrolysis option, a whole new world of possibilities opens up, such as analysis of polymer material structures. You will find more on this key topic on the pages 6 to 14.

A further pillar and regular main topic in almost all our publications is automated sample preparation. This area offers the greatest optimization potential in almost all laboratories. With the MultiPurpose Sampler (MPS), we offer our customers a highly productive, efficient and flexible autosampler. The MPS is easily expanded and adapted to many different tasks using the available modules and options. In combination with GC-MS and LC-MS/MS systems we arrive at the third pillar: Customized solutions that match the customer requirements perfectly – we call them Sample Prep Solutions. If you are considering automating your sample preparation or have already made plans to do so, contact the experts at GERSTEL – the conversation will most probably prove useful for you.

With this, we would like to wish you much pleasure and enjoyment reading the latest GERSTEL Solutions worldwide Magazine No. 14, which will again provide glimpses of what GERSTEL technology and GERSTEL solutions offer the modern laboratory. Enjoy the magazine!

Yours sincerely, **GERSTEL management.**

GERSTEL Solutions worldwide 14

Content:

Vineyard in One Tree Hill near Adelaide, Southern Australia.

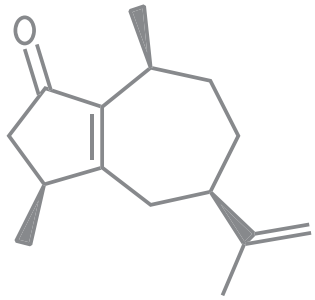
Searching for the pepper in Shiraz

Rotundone is a key flavor compound with a distinct black pepper note. Rotundone occurs naturally in pepper, but also in wines such as Australian Shiraz and others from around the world, for example in the Austrian Green Veltliner. Australian wine scientists are searching for clues as to how the peppery compound is formed in the hope that this knowledge would enable them to optimize both growth conditions for the Shiraz grape and the ensuing wine-making process. Finding answers requires an efficient analysis method. Researchers turned to Membrane Assisted Solvent Extraction (MASE) combined with heart-cut GC/MS for more efficient determination of rotundone in wine and grapes.

When it comes to wine, tasting beats reading reviews. But a little reading could offer a good starting point when you set out to differentiate noble drops from decent everyday wines or even inferior ones. When mingling with real or would-be connoisseurs, the following vocabulary samples could help you fit in: A wine scientist is called an oenologist; wine expert in a classy restaurant: Sommelier; and while merry wine drinkers enjoy wine tasting, highbrow tasting is referred to as a degustation. But even experts can find themselves at wit's end when sensory impressions do not solve the puzzles they face and hard facts are required to ensure best possible product quality and product acceptance.

When faced with a wine with its hundreds of volatile compounds that influence the flavor, the technique of choice for the analyst is gas chromatography with mass selective detection (GC/MS). This is the tool used by Tracey E. Siebert and Sheridan R. Barter from the Australian Wine Research Institute in Glen Osmond (Adelaide), Australia. The scientists conduct basic research; they are not trying to outperform sommeliers.

Siebert's and Barter's goal is to find out how wine flavor compounds are formed in order to find ways to optimize growth conditions for best taste and bouquet. The Australian scientists had set their sights on rotundone, a bicyclic sesquiterpene containing a conjugated ketone group. With its characteristic black pepper note, rotundone is considered a key flavor compound in Australian Shiraz wine. Shiraz is the number one grape grown in Australia. It is highly valued due to its high yield and resistance to cold weather, and of course due to the



Structural formula of (-)-Rotundone

GC/MS-System comparable to the one used by Siebert and Barter for automated MASE and 2D-GC/MS Rotundone determination

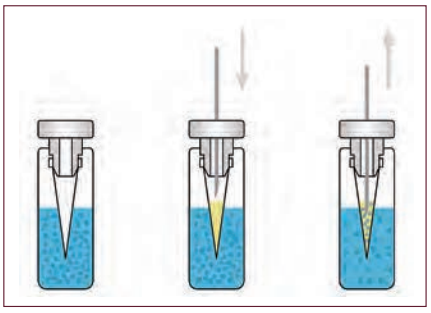
excellent tasting wine it produces. Shiraz vines were originally cultivated in France (Syrah). Today the wine is not only popular in Australia, but also widely grown in South Africa, Latin America, the United States, and Canada.

Wine under the influence

Many factors influence quality and character of the final wine; it is generally accepted that controlling the grape maturation process is key. Knowledge of specific factors that influence, for example, how much rotundone is formed in the vine and how growth conditions could be optimized could help improve quality, taste, flavor, and ultimately product value. Such knowledge might enable wine producers to provide more uniform year-to-year wine quality and taste. But Sieber and Barter are convinced that such insights can only be gained based on access to efficient and sensitive analysis methods.

The scientists presented their work in May 2013 in Palm Springs, California, USA during the 37th International Symposium for Capillary Chromatography (ISCC) [1]. Previously used analysis methods were labor intensive and time consuming. Large sample volumes had to be handled and extensive sample preparation was required for matrix elimination and analyte concentration since Rotundone is present only at very low concentration levels.

According to literature: "The flavor threshold of Rotundone is at 16 ng/L in red wine and 8 ng/L in water. A limit of quantitation (LoQ) of < 8 ng/L would be necessary for juices, grapes, mash and wine. These are complex aqueous samples that contain sugars, ethanol, anthocyanins or polyphenols." [2].



Schematic diagram of Membrane Assisted Solvent Extraction (MASE): The semi-permeable membrane insert is immersed into the sample (left). Extraction solvent is added into the MASE insert and analytes diffuse through the membrane into the extraction solvent while particulate matter is kept in the sample (center).
The extract is aspirated by the syringe for introduction to the GC/MS (right).

Critical factor for success: The extraction

The critical factor that helps ensure analytical success or failure is the extraction step, according to Siebert and Barter.

Searching for a suitable technique, the scientists came across automated Membrane Assisted Extraction (MASE). MASE was developed in cooperation between the Environmental Research Institute Leipzig-Halle and GERSTEL.

This type of liquid – liquid extraction is based on using a semi-permeable membrane as a phase boundary, which keeps particulate matter and other matrix components out of the extraction solvent. Further sample clean-up steps such as filtration and centrifugation are no longer needed. MASE extracts – even of heavily matrix laden samples are clean and can be injected directly to the GC/MS or LC/MS system for analysis. Additionally, MASE enables extraction using sample-solvent mixtures that do not normally result in phase separation. As an example, HPLC compatible polar solvents can be used to extract aqueous samples in which the solvents are normally miscible. MASE extends the analytical possibilities considerably [3,4]

Experimental

The grape juice/extract or wine sample was added to a 20 mL autosampler vial, the MASE membrane bag placed inside the vial, and the vial capped. Sample preparation and introduction were performed automatically by the GERSTEL MultiPurpose Sampler (MPS). MASE was performed by adding 750 µL of hexane into the MASE membrane bag. The sample was extracted for 60 min at 35 °C. A 100 µL aliquot of the resulting extract was injected (Large Volume Injection - LVI) into a GERSTEL Cooled Injection System (CIS 4) inlet mounted in an Agilent GC 7890.

Sample preparation:

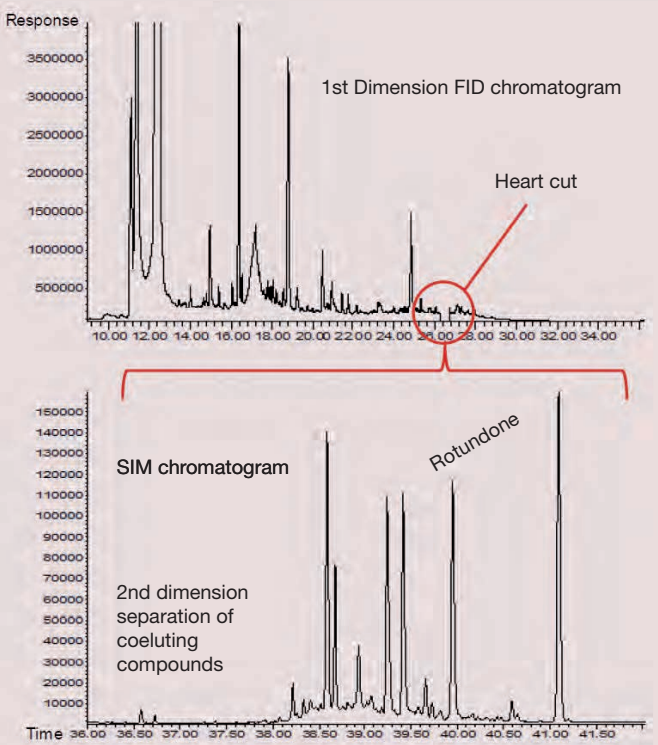
Grapes: A 100 g sample of berries was gently blended and centrifuged and the juice supernatant collected. The solid residue was further extracted by adding 60 mL of an ethanol/water mixture (50:50) and internal standard (IS) to the solids and shaking for 24 hours before centrifuging and collecting the supernatant. The supernatant and the juice initially collected were combined and topped up with water to a volume of 200 mL. Of the final volume, a 15 mL aliquot was taken and used for analysis.

Wine: A 15 mL sample of wine was taken and used directly for analysis.

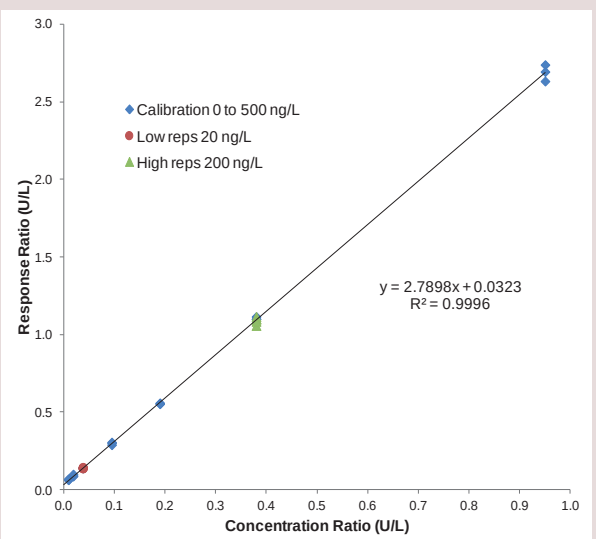
The separation took place on a multidimensional heart-cut GC system, based on an Agilent Capillary Flow Technology (CFT) Deans' Switch configured with two separation columns. A CryoTrap System (CTS, GERSTEL) was connected between the first and second dimension columns for analyte focusing prior to the analytical separation. An FID monitor detector was used on Column 1 and an Agilent 5975C MSD (Agilent Technologies) was used in Selected Ion Monitoring (SIM) mode for the analytical separation on Column 2. Stable isotope dilution was performed using d5-Rotundon as the internal standard.

According to Siebert and Barter, the described method combining MASE and LVI minimizes sample preparation workload significantly. Problems with interferences were compensated using internal standards. By heart-cutting the key part of the chromatogram for separation onto a second column, a cleanly separated and clearly defined rotundone peak is obtained. The scientists reported the analysis method to be accurate, precise, rugged, and sensitive leading to limits of quantitation in the low ppt range and high sample throughput. The statistical data speak volumes: "In the Shiraz grapes (0 to 2000 ng/L) as well as in wine (0 to 500 ng/L), the limits of quantitation (LOQs) were 5 ng/L. The linear correlation (R^2) was >0,999 and the reproducibility expressed as standard deviation was below 3 % (n=6) both for low and high concentration values – a very good result", the wine scientists reported. They went on to say that "the described method will allow more detailed research on the formation of rotundone in the grape. This includes the maturation processes involved as well as whether yeast plays a role by influencing the extraction process. Such knowledge will make it more likely that we will be able to describe how rotundone is synthesized in the grape and ultimately control the rotundone concentration in the wine." Further investigations are planned and a scientific publication of the research results is scheduled to appear in the near future.

Main FID-Chromatogramm and heart cut MSD chromatogram showing a clearly defined rotundone peak. The method developed by Siebert and Barter was used to determine rotundone in Shiraz grapes and in wine following MASE analyte extraction.



Rotundone in red wine: calibration and repeatability



Instrument method parameters

GERSTEL MPS XL, CIS and CTS	
LVI:	100 µL at 0.58 µL/sec
He carrier gas flow:	100 mL/min
CIS:	Glass wool packed liner
CIS:	20 °C, solvent vent, 20 psi (0.12 min); splitless, 47.6 psi, 12 °C/sec to 240 °C (2.5 min); split, 12 °C/sec to 275 °C (2 min)
Column 1:	VF-35ms (30 m x 0.25 mm x 0.25 µm), medium polarity phase; He 47.6 psi
Column 2:	VF-200ms (30 m x 0.25 mm x 0.25 µm), unique selectivity with dipole-dipole interactions He 37.7 psi
Column 3:	Deactivated fused silica (0.70 m x 0.1 mm)
GC Oven:	80°C (1 min), 5°C/min to 210°C, cool 15°C/min to 130°C (2 min), 10°C/min to 280°C (10 min)
Heart-cut:	26.25 to 26.75 min
CTS:	-20°C (34 min), 20°C/sec to 300°C (1 min)
MSD SIM:	m/z 147, 161, 163, 203, 208, 218, 223

Literature

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[4] www.gerstel.com/en/automated-membrane-extraction-mase.htm

Amber fascinates by its color, refraction of light, and golden glow, but how can you tell if it's the real thing?

Material analysis

Amber authentication, a closer look

Amber is cherished for its beauty and is used in jewelry and works of art. Amber has been thought to have healing powers and has been widely used for charms to keep bad luck at bay. However, it is not easy to know if the gem in your hand is real amber or a well-produced fake. Looks can be deceiving and so can the texture of the surface. Age is what matters and older is better: A million years is the bare minimum - orders of magnitude more the norm. While amber doesn't easily reveal its age or origin, instrumental analysis can shed light on the matter: Pyrolysis coupled with gas chromatography (GC) and mass spectrometry (MS) is known as a strong complement to spectroscopic techniques. If you automate the pyrolysis and add a suitable reagent to perform thermochemolysis, detail on the amber's origin is revealed.

The town of Ribnitz-Damgarten lies beautifully situated where the river Recknitz flows into Lake Ribnitz in the German State of Mecklenburg-Vorpommern. The town of 15,000 inhabitants is a very special place as can be seen by visiting its homepage. Since 2009, Ribnitz-Damgarten has been officially designated as the "Amber City." The reason is the Amber Manufacture in the Damgarten section of town and the German Amber Museum in the Ribnitz section. The town boasts an amber shopping mile where amber of every shape and hue can be acquired. In addition there is the annual Amber Queen Contest; the International Amber Art Competition; and the world's longest amber necklace at 120 meters. At the Baltic Seashore not far from town, visitors can spend the day hunting for amber, the "Gold of the sea". Whether or not amber found on the beach is the real thing can be determined by a simple density test as recommended by the German Amber Museum. The following experiments will provide good indications as to the type of amber found and whether it is real:

- Produce a saturated salt solution by adding two soup spoons of salt to a glass of water.
- Amber will float in this solution, stone, glass and polymer materials will sink.
- Compare: Among the different amber

types, clear amber is the heaviest, whereas "amber bone" is the lightest.

- Rub larger amber chunks against wool. They will build up an electrostatic charge and attract paper snippets.
- Carefully tap the find against your front teeth. If it is amber, it is not unpleasant and will produce a dull sound. Amber produces the same sensation as if you tap your finger nail on the teeth. A yellow pebble or a glass shard will sound light and their contact with the teeth will produce a distinctly unpleasant sensation.

The Johannes Gutenberg University in Mainz, Southwestern Germany is far away from the Baltic coast, but even here amber is high on the agenda. Dr. Oluwadayo O. Sonibare and Prof. Dr. Thorsten Hoffmann from the Department of Inorganic and Analytical Chemistry along with Prof. Dr. Stephen F. Foley from the Dept. of Geosciences and Earth System Research Centre are searching for a more accurate method to determine origin and authenticity of amber. The recommendations from the German Amber Museum are useful for tourists and for those who casually collect amber, but of course not for university level scientific research on the matter. The stated goal of the scientists is to determine "The molecular composition and chemotaxonomy aspects of Eocene amber from the Ameki Formation in Nigeria [1], the project is sponsored with a research grant from the Alexander von Humboldt Foundation. For the geochemical characterization and biochemical classification, Sonibare and his colleagues have previously used Infrared (IR) Spectroscopy and Gas Chromatography (GC) with Mass Spectrometry (MS) detection to determine the molecular composition of different fossilized plant resins. They are now looking to automated pyrolysis as well as thermochemolysis to help move the project forward.

What is known about amber?

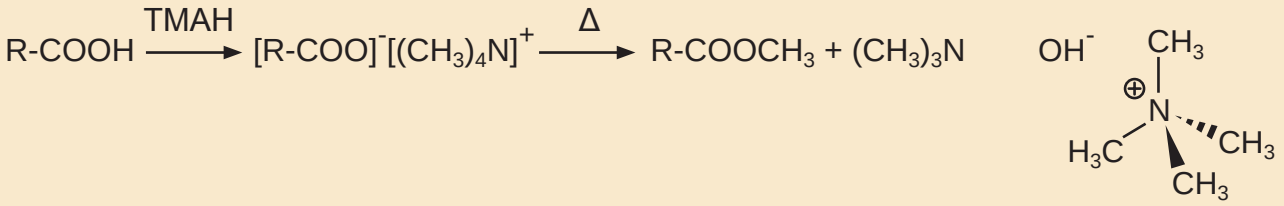
Amber, in mineralogical terms also known as succinite, was formed when tree resin from a fir or other coniferous species was hardened through contact with air millions of years ago. Large quantities of dried resin wound up in the oceans and sank into deep sediment layers, which were in turn covered by more layers of sand and rock. Over time and under huge pressure in an anaerobic environment, amber was formed. Most amber finds have been dated to the tertiary period, around 55 million years ago. However, "younger" species are known as well. To be classified as amber, at least a one million year maturation period is required. Baltic amber is among the most ancient of known ambers. Amber is found in many countries, for example, in England, Portugal, Spain and Italy, but also in Canada, Mexico, Japan, the Dominican Republic, in Madagascar, and in Borneo. Amber fascinates through the varying coloration, light refraction, and the golden yellow glow after polishing. Amber feels sensuous and warm, and is surprisingly light. It makes you want to touch it, have it against your skin, and use it as a hand charm, letting your fingers run over it. Even today, people on the Baltic shore actively collect amber, which is also said to have healing, pain-killing and calming properties. Sometimes, million-year-old insects or plants are clearly visible, encased and fossilized in the amber.

Amber, in mineralogic terms called succinite, is fossilized tree resin, which seeped from firs or other conifers millions of years ago

The remains of insects and plants that lived millions of years ago are sometimes found encased and fossilized in amber.

Delving into the details

At GERSTEL Headquarters, R&D Project Manager, Dr. Eike Kleine-Benne and his colleague Yunyun Nie met with Dr. Sonibare from the Johannes Gutenberg University to exchange information on the properties of amber with a view to determining both origin and authenticity. In short: What are the possibilities for determining the composition and specific properties of amber and thus finding markers for different types of amber? Deciding on which technique to use is easier if you have detailed knowledge of the composition of the material. According to Dr. Sonibare, the fossilization of plant resin is a complex curing and maturation process involving



Methylation of a carboxylic acid using tetramethylammonium hydroxide (TMAH). Shown to the right: TMAH structural formula.

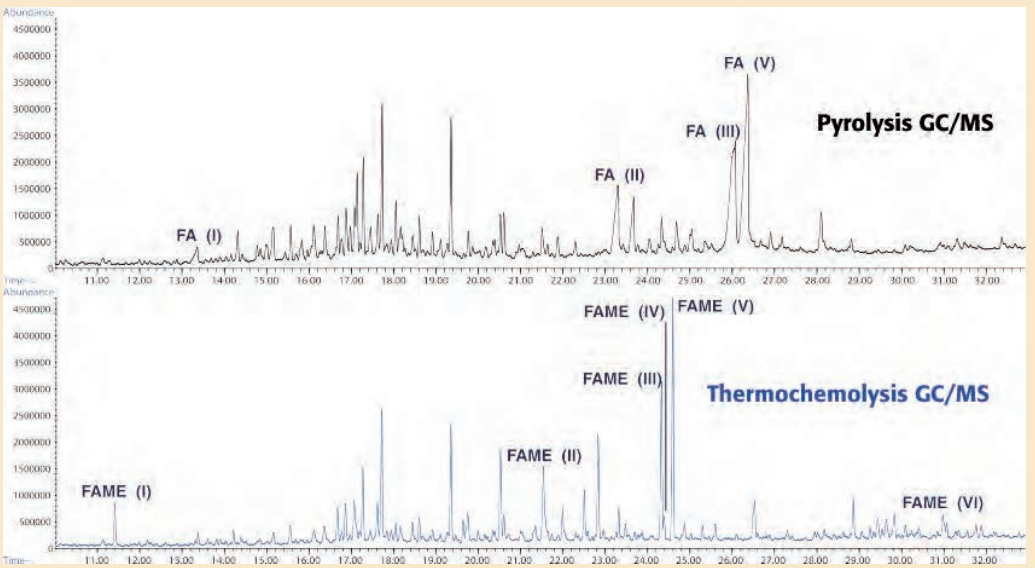
loss of volatile content, polymerization, and crosslinking of terpenoids over a period of up to 100 million years. Amber is sometimes considered a natural polymer, but chemically speaking ambers are heterogeneous mixtures of polyesters classified as terpenoids (mono-, sesqui-, di- and triterpenoids), all of which are derived from isoprene, a compound that occurs naturally in plants. In contrast to terpenes, terpenoids contain functional groups. The analysis techniques mainly used for the characterization of amber are infrared (IR) spectroscopy, Raman spectroscopy, GC/MS analysis of solvent-based extracts, and pyrolysis-GC/MS. Based on the chemical and physical properties of amber, the scientists decided to use automated pyrolysis-GC/MS, but also to take it to the next level: Thermochemolysis, combining pyrolysis and a derivatization reagent, in this case tetra methyl ammonium hydroxide (TMAH), a technique previously described in literature. The hope and theory was that the derivatized pyrolysis fragments could be more extensively characterized, providing deeper knowledge of the composition and enabling a more accurate classification of amber types and their geographical/geological origin.

IR spectroscopy is used to differentiate Baltic amber from other ambers, says Eike Kleine-Benne. The main useful IR differentiator is the so-called "Baltic shoulder" at 1250 to 1175 cm-1. This absorption band is associated with succinic acid, which is found in Baltic amber. However, with regard to amber classification and differentiation in general, the IR method is of limited use, due to the fact that different amber types often yield similar patterns.
Raman spectroscopy is useful for the determination of differences in amber maturation processes, not for geographical origin.
GC/MS enables a molecular – structural elucidation of the soluble part of amber, which unfortunately only constitutes 20 % of the total.
Pyrolysis-GC/MS gives the analyst the possibility of generating fragments of the polymer parts, i.e. to split insoluble and non-volatile macromolecules into individual fragments, separate these and determine their identity based on a detailed mass spectrum. The fragment pattern and certain markers are then used to determine the type and origin of amber samples.
Thermochemolysis combines pyrolysis and a derivatization reagent. This means that as soon as certain pyrolysis fragments are formed, they are derivatized in order to enable or improve their determination by GC/MS.

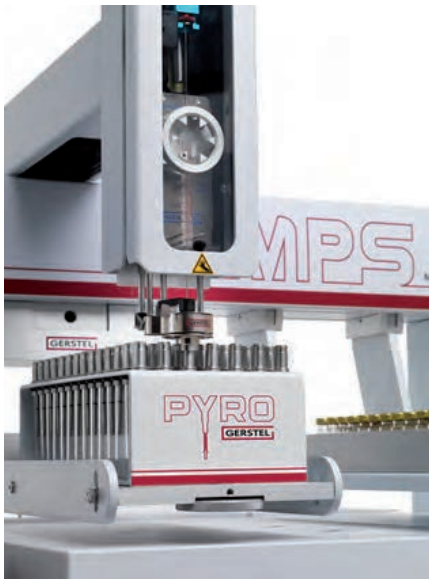
Amber is flammable, it burns generating a distinct aromatic smell and it was long used as substitute for incense. Amber shows limited solubility in alcohol, ether, chloroform, and turpentine oil. Ground amber is negatively charged and has a melting point of 375 °C.

Hunting for data and gathering information with pyrolysis GC/MS

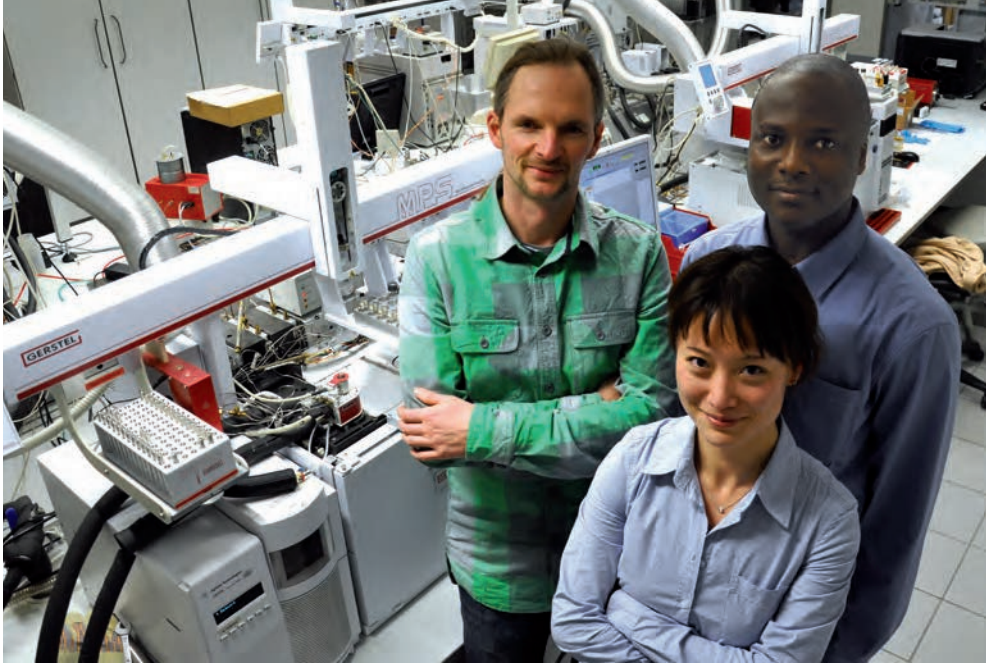
Eocene amber samples from the Ameki formation in Nigeria were kindly made available for the project by the University of Glasgow Hunterian Museum. The sample material was ground and 200 µg portions were individually pyrolyzed at 480 °C for 20 seconds. The following pyrolysis-GC/MS system was used: Agilent GC 6890N with GERSTEL Cooled Injection System (CIS), Agilent 5795B Inert



Comparison of chromatograms resulting from pyrolysis GC/MS and thermochemolysis GC/MS, respectively, of Eocene amber from Nigeria (FAME: Fatty acid methyl ester / FA: Fatty acid).



GERSTEL PYRO provides more in-depth information on the analyzed amber



The amber team from right to left: Oluwadayo O. Sonibare, Ph.D., Yunyun Nie, and Eike Kleine-Benne, Ph.D..

XL (Triple Axis) MSD, GERSTEL Multi-Purpose Sampler (MPS), GERSTEL Thermal Desorption Unit (TDU), and GERSTEL PYRO pyrolysis module mounted on the TDU. PYRO is a plug-in unit, which can be mounted on the TDU in just a few minutes and allows automated sample introduction when used with the MPS. Technical detail: The TDU was operated in split mode in order to vent excess solvent and volatile organic compounds (VOCs). The CIS was set to an initial temperature of 300 °C and used as heated split interface, with a split ratio of 1:20.

The column used was a ZB-5MS fused silica capillary column (30 m x 0.25 µm x 0.25 mm) from Phenomenex. The GC oven temperature program was set as follows: 60 °C (2 min) – 6 °C/min – 300 °C (10 min) using Helium carrier gas at 1 mL/min. The MSD was operated in Electron Impact (EI) ionization mode with an ionization energy of 70 eV, and source and quadropole temperatures set to 230 °C and 150°C respectively. Full scan spectra were acquired from 50 to 650 Da.

Data acquisition and data handling were performed using ChemStation Software (Agilent Tecnologys) and compound identification performed using both the integrated mass spectral library and information from literature.

Gaining deeper knowledge step by step

Initially, a ground amber sample was pyrolyzed. In the chromatogram, a long line of the "usual suspects" were identified, which were previously described in literature. In this case, a series of fatty acids (FAs), among them:

- Norchrysanthemic acid (FA I)
- Naphthalene-1-carboxylic acid-1,2,3,4,4a,7,8,8a-octahydro-1,4a,6-trimethyl (FA II)
- Naphthalene-1-Carboxylic acid-1,2,3,4,4a,7,8,8a-octahydro-1,4a,5,6-tetramethyl (FA III)
- Naphthalene-1-carboxylic acid-1,2,3,4,4a,5,8,8a-octahydro-1,4a,6-trimethyl-5-methylene (FA V).

To gain better chromatographic performance and more information, amber samples were pyrolyzed in the presence of tetramethyl ammoniumhydroxid (TMAH) for in situ derivatization (methylation) during the 20 s pyrolysis period. This process is called thermochemolysis; fatty acids are converted into their corresponding methyl esters (FAMEs):

- Norchrysanthemic acid methyl ester (FAME I)
- Naphthalene-1-carboxylic acid-1,2,3,4,4a,7,8,8a-octahydro-1,4a,6-trimethyl methyl ester (FAME II)
- Naphthalene-1-Carboxylic acid-1,2,3,4,4a,7,8,8a-octahydro-1,4a,5,6-tetramethyl methyl ester (FAME III)
- Methyl-1,2,3,4-Tetrahydro-1,5,6-Trimethyl-1-Naphthalenecarboxylate (FAME IV)
- Naphthalene-1-carboxylic acid-1,2,3,4,4a,5,8,8a-octahydro-1,4a,6-trimethyl-5-methylene methyl ester (FAME V).
- Methyl-16,17-Dinorcallitrisate (FAME VI)

The difference in performance between pyrolysis and thermochemolysis is explained by Eike Kleine-Benne as follows:

„In the pyrolysis chromatogram, the acids peaks (FA (I), FA (II), FA (III) and FA (V) are broad, and partly saw tooth shaped, a typical phenomenon for highly polar or active compounds such as free fatty acids (FFAs) separated on a non-polar column. FA (IV) and FA (VI) are not found at all. In contrast, the corresponding methylated acids elute as well separated sharp peaks in the thermochemolysis-based chromatogram, including FAME (IV) and FAME (VI). It is clear that pyrolysis with TMAH brings out more detailed information about the analyzed material." As Dr. Sonibare reports, an earlier study yielded the first available information on the molecular composition of fossilized resins from the Eocene Ameki formation in Nigeria with pyrolysis GC-MS analysis clearly indicating that the amber belongs to class I b, which is derived from regular labdatriene structures. Another characteristic of class I b ambers is that they do not contain succinic acid. The pyrolysis products of the amber were dominated by labdan-like diterpenoids and a small number of sesquiterpenoids. The exclusive presence of labdane diterpenoids and the absence of plant triterpenoids in the Eocene Ameki amber pointed to a conifer (Gymnosperm) origin of the resin. With the described method, Dr. Sonibare, Prof. Hoffmann and Prof. Foley have a tool which helps them categorize amber by origin while delivering further information for their amber research: New research results were recently published in the Journal of Analytical and Applied Pyrolysis [2].

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[2] O. O. Sonibare, R.-J. Huang, D. E. Jacob, Y. Nie, E. Kleine-Benne, T. Hoffmann, S. F. Foley: Terpenoid composition and chemotaxonomic aspects of Miocene amber from the Koroglu Mountains, Turkey, Journal of Analytical and Applied Pyrolysis 105 (2014) 100-107

Making it stick

Thermal desorption combined with gas chromatography is a simple yet powerful tool that can quickly extract low levels of volatile organic compounds (VOCs) from a wide range of solid matrices, including polymers and adhesives, providing comprehensive information about VOC content. The addition of a pyrolysis module to the tool kit enables the analyst to see more, looking deeper into the structure of the polymer or adhesive material while using a standard GC/MS system.

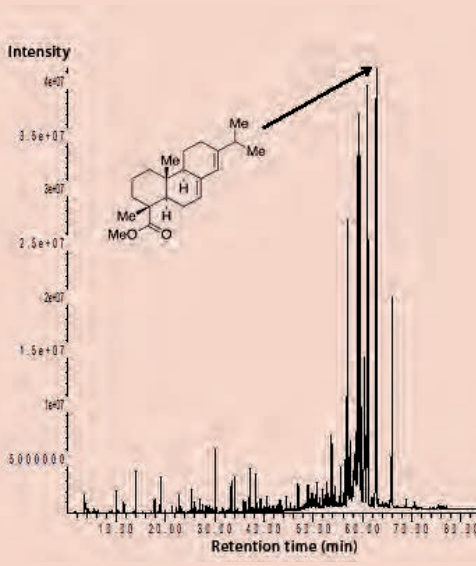
Fastening, bonding, joining, and mounting of materials is done in a number of ways. For example, materials used in industrial applications and building materials used in homes and offices are increasingly bonded using adhesive solutions. These replace more traditional fastening methods, such as screws, bolts, rivets and even welding. The reasons that adhesives are making such inroads into these applications are manifold: Adhesive tapes, for example, are flexible, light, and easy to position or even reposition and are largely immune to mechanical impact. They are widely used in the automobile-, electronic-, and paper industries, among professional builders and by do-it-yourself handymen or -women. Adhesive solutions are as varied as the applications they are used for. In many industries, it is critically important to know the composition of the adhesive material used since it can greatly influence the quality of the final product. This information can also be used to identify cheap imitations that can negatively impact revenues and ruin the reputation of a quality brand.

A typical adhesive tape consists of a carrier film, an anchor coating and the adhesive material. If it is a double-sided tape, one side is typically protected with a separating film, called a liner. Depending on the application, different types of liners can be used, such as polyethylene (PE), polypropylene (PP) or polyethylene terephthalate (PET). Carrier films can be made of PE, PP, PET, or of soft or hard poly vinyl chloride (PVC). In addition, various additives are used to tailor material properties to the specific application. These include plasticizers added to produce, for example, soft PVC, anti-aging agents, or anti-blocking agents. The adhesive material is typically produced from polyacrylate, synthetic caoutchouc, or natural caoutchouc, all of which may contain one or more of the following: Adhesive resin, fillers, crosslinking agents, and/or plasticizers. As an example, a double sided tape may be based on a soft PVC carrier with a resinous polyacrylate adhesive layer on both sides coated with a PE film as shown in the diagram on the following page.

If the soft PVC carrier and the PE film are purchased materials, analytical laboratory resources will be needed for quality control to identify the raw materials used. Often, all additives contained in all materials used must be identified in order to ascertain their compatibility with the adhesive, which of course must also undergo compositional analysis. The focus of the product quality control efforts would typically be the determination of the polymer composition and the adhesive resin content. For these and similar tasks, Pyrolysis-GC/MS has proven particularly useful. Thermal fragmentation followed by GC separation and MS determination offers a range of possibilities for qualitative and quantitative analysis as the three following examples show.

Using pyrolysis-GC/MS for polymer analysis

Initially, the PE film used for the double sided tape described above was analyzed. Material testing had revealed that the mechanical properties did not meet requirements – even though the infrared (IR) spectrum for both the front and back side of the tape showed only the absorption bands typical for PE. To clarify the matter, pyrolysis GC/MS was used with the following instrument configuration: A GERSTEL Multi-Purpose Sampler (MPS) for automated sample preparation and introduction mounted on top of an Agilent 7890/5975B GC/MSD system. The setup was extended with a GERSTEL Thermal Desorption Unit (TDU) mounted on a GERSTEL Cooled Injection System (CIS 4), PTV-type inlet. The PE film was pyrolyzed at 700 °C and the formed fragments separated using a Restek Stabilwax DA column. Both linear and branched aliphatic hydrocarbons were found. A comparison with a user-generated pyrolysis library of various polymers revealed: Linear aliphatic hydrocarbons typically occur in PE pyrograms; branched aliphatic hydrocarbons, on the other hand, typically occur in polypropylene (PP). Finally, the analysis of a PE/PP reference mixture revealed that the unknown

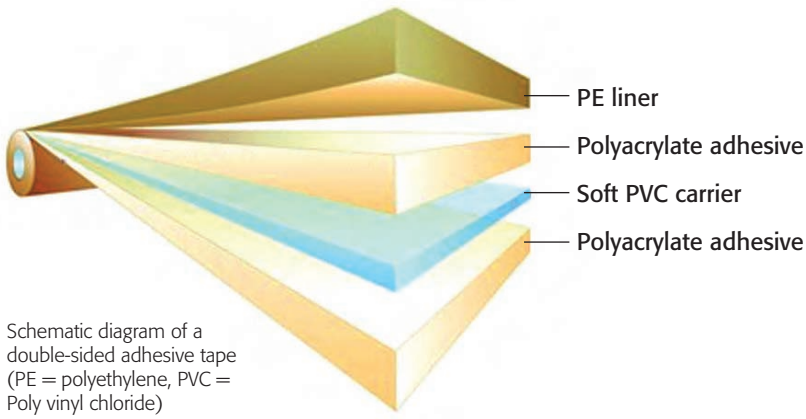


Pyrogram of a rosin methyl ester resin after pyrolysis at 800 °C. The peak with the highest intensity was determined to be methyl abietate (m/z = 316).

"PE" material was in fact a mixture of PE and PP. Since IR spectra of the front and back of the tape only produced PE adsorption bands, it was concluded that the unknown material was a three layer film with a PP layer between two PE layers.

Using Pyrolysis GC/MS to determine additives in polymers

To help assess, whether an adhesive would be compatible with a PVC carrier film, the plasticizer used in the PVC was determined.



Schematic diagram of a double-sided adhesive tape (PE = polyethylene, PVC = Poly vinyl chloride)

A combined analysis procedure of IR spectroscopy and pyrolysis GC/MS was used; in both cases, methods were selected, which didn't rely on solvent-based extraction. Based on the IR analysis, it was determined that there was polyurethane in the PVC, but no phthalate. The PVC film was then pyrolyzed at 800 °C and the fragments identified. In addition to the hydrochloric acid fragment, which is to be expected when pyrolyzing PVC, a high intensity peak for the hexamethylene diisocyanate (HDI or HMDI) fragment was found. This information made it clear that a polyurethane-based plasticizer, which contained HMDI as isocyanate component, was used for the PVC. Based on experience, the plasticizer was deemed compatible for use with the adhesive.

Quantifying additives with pyrolysis GC/MS

In order to quantify adhesive resin content, in this case rosin aka colophony resin, a pyrolysis GC/MS method based on matrix-matched calibration was developed. The main challenges during method development were to identify and select suitable pyrolysis fragments as markers for individual adhesive resins and to optimize sample preparation in order to achieve adequate reproducibility. Methyl abietate (m/z = 316) was selected as marker for rosin methyl ester (see figure). For hydrated rosin resins, norabietan (m/z = 262) was used as quantification marker. Both methods were developed as Single Ion Monitoring (SIM) methods in order to achieve better signal to noise ratios and higher sensitivity. The sample preparation was optimized as follows: The adhesive material sample was weighed on silicone-coated release paper, i.e. anti-adhesive paper (sample weight: 0.5-1 mg). Then the sample was sprinkled with a fine glass powder, which prevents the sample from adhering to the pyrolysis tube without interfering with the pyrolysis process and without impacting the analysis result. For all samples and calibration standards, duplicate analyses were performed and the mean values used. Calibration standards were generated by adding 5 to 40 % (w/w) rosin resin to resin-free polyacrylate adhesive material made up of n-butyl acrylate, 2-ethylhexyl acrylate and acrylic acid. The calibration standards were pyrolyzed at 800 °C resulting in a calibration curve with a correlation coefficient of 0.9916. The calibrated methods were subsequently used to determine both the rosin resin and the hydrated rosin resin contents in polyacrylate adhesives. Additional hydrated resin types were analyzed to determine whether hydrated rosin glycerol ester resin or other ester-based resins could be analyzed quantitatively. Multiple rosin methyl ester resins from different manufacturers were finally analyzed. For both methods it was found that the rosin resin content could be determined with a standard deviation below 4 %. Given the highly heterogeneous nature of such adhesive resins, the developed pyrolysis GC/MS methods for quantitative determination of resin content are considered sufficiently accurate. Using this method, the rosin resin content of polyacrylate adhesives can be determined for quality control purposes. Further investigations will determine whether the methods can be applied to other matrices such as synthetic caoutchouc materials. It should also be possible to expand the use of the method to determine other resin classes such as polyterpene resins and hydrocarbon resins.

Conclusions

Pyrolysis GC/MS is a versatile technique, which can be used for qualitative as well as quantitative analysis of polymers in general and, as shown in this work, also of adhesive materials. Of particular benefit to the analyst is the very limited effort required for sample preparation as well as the considerable depth of information obtained – even from just a single analysis.

Authors

Aylin Meß, Vjaceslav Frank and Andreas Westphal, tesa SE, Quickbornstraße 24, 20253 Hamburg, Germany

Phthalates

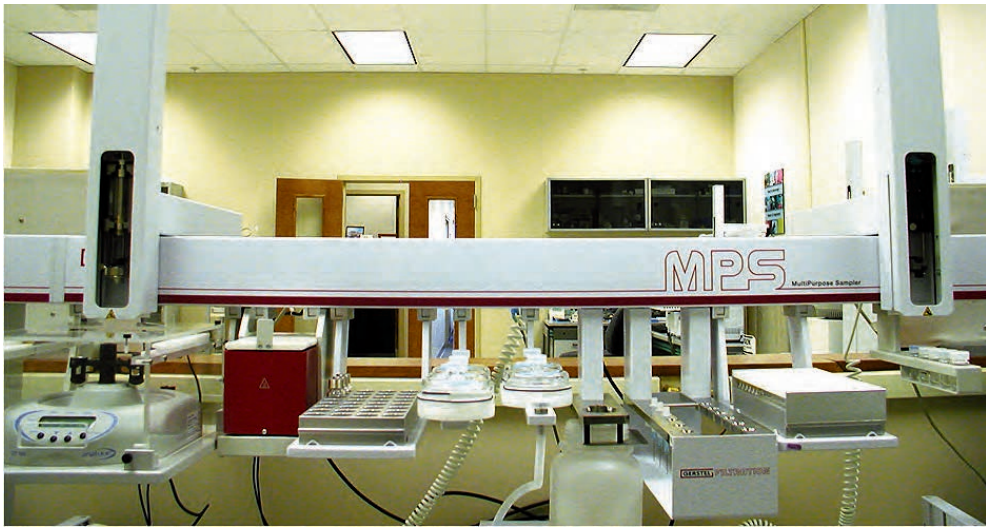
Easy extraction of plasticizers from toys

The US Consumer Product Safety Commission's (CPSC) Test Method CPSC-CH-C1001-09.3 [1] is used by testing laboratories for the determination of phthalate content in children's toys and child care articles covered by the standard set forth in the Consumer Product Safety Improvement Act Section 108. The general manual approach is to dissolve the sample completely in tetrahydrofuran, precipitate any PVC polymer with hexane, filter and dilute with cyclohexane, and then analyze by Gas Chromatography/Mass Spectrometry (GC/MS). A combined autosampler and sample preparation robot can be used to automate these sample preparation steps.

Chemical additives are widely used to impart desirable properties to many products. For some compounds, however, due to toxicity issues, the amounts used need to be closely monitored.. Phthalic acid esters, also known as phthalates, are among these. They are widely used as plasticizers in polymers, making the material more pliable. Phthalates influence the physical properties of the polymer material significantly making it softer and more flexible. PVC, for example, is a highly durable material. Plasticizers give PVC the flexibility and elasticity that have made it so widely used and appreciated. Applications where PVC is used range from flooring, artificial leather, shower curtains, baby care articles, toys, packaging, shoes, sport and leisure materials, and medical products such as IV bags used for medical infusions and blood storage. Some further uses are: Cable coating, roofing materials, truck tarpaulins and undercoating of vehicles. However, there is a flipside to these benefits: Some phthalates, such as Di(2-ethylhexyl)phthalate [DEHP] as well as dibutylphthalate [DBP] and Benzylbutylphthalate [BBP] are considered harmful to human health and are banned by the European Union (EU) for a wide range of uses [3]. According to EU guidelines, DEHP is harmful to reproduction. Within the EU, restrictions apply to items used by children who are less than 3 years of age. Toys for this age group as well as baby care articles that contain the aforementioned phthalates at individual concentrations higher than 0.1 % w/w are not allowed into the market place.

The US Congress has permanently restricted the use of three phthalates (DEHP, DBP and BBP) as well as (on an interim basis) three additional phthalates (DINP, DIDP and DnOP) in any amount greater than 0.1 percent (computed for each phthalate, individually) in children's toys and certain child care articles. The risk of a negative health impact due to the transfer of the additives from PVC based products is deemed particularly great in small children.

In order to ensure compliance with governmental health and safety regulations, products that fall under the regulation must be monitored for these compounds.



Sample preparation is key

Sample preparation of polymers being monitored for additives is the most time consuming part of the overall analysis. When phthalates are determined in polymer matrices following manual extraction procedures described in CPSC-CHC1001-09.3, these procedures are often the rate-limiting step in terms of overall productivity and throughput, and they involve the use of hazardous chemicals and solvents. Typically, liquid dissolution of the polymer is first performed in order to quantitatively release additives and other compounds of interest from the material. A solvent, in which the polymer is not soluble, is then added to precipitate the polymer while leaving the analytes of interest in solution. Filtration of the final extract is performed prior to analysis by either GC or HPLC. This general procedure can be applied to a wide variety of polymers and plastics. In this work, we show that the manual procedure established for the extraction of phthalates from consumer products produced from PVC is easily automated using the GERSTEL Multi-Purpose Sampler (MPS). The entire process is conveniently controlled using MAESTRO software.

Weigh a 50 mg sample of polyvinylchloride (PVC) in a sealable glass vial.

Add 5 mL of THF to the sample

Mix the sample for at least 30 minutes, to ensure complete dissolution. Ultrasound and/or mild heating can be used to accelerate the dissolution process. If the sample hasn't been completely dissolved, the mixing period should be extended by another 2 hours before continuing.

Precipitate the PVC polymer using 10 mL of hexane for each 5 mL of THF used during the dissolution step.

Shake and wait at least 5 minutes for the polymer material to precipitate and settle.

Filter the THF/Hexane solution through a 0.45 mm PTFE filter and collect a few mL of the filtrate in a separate glass vial.

Combine a 0.3 mL aliquot taken from the THF-hexane mixture with 0.2 mL of the internal standard, in case this is used, and top up to 1.5 mL with cyclohexane.

Inject 1 µL of the solution into the GC/MS.

As explained by Ed Pfannkoch, Technical Director, GERSTEL, Inc., Baltimore, MD: "It was our aim to transfer the manual steps one by one and automate the entire process while using the PrepAhead function in MAESTRO to perform sample preparation simultaneously with the GC/MS run and increase productivity". To this end, Ed Pfannkoch and his team used the Dual Head version of the GERSTEL MPS. One head was fitted with a 2.5 mL syringe for liquid transfer used for sample preparation, the other with a 10 µL syringe used for liquid injection into the GC/MS system (Agilent 7890GC/5975MSD); the PTV inlet used was a GERSTEL Cooled Injection System (CIS 4). The MPS was further equipped with a heated agitator, GERSTEL Automated Liner Exchange (ALEX) as well as a Filtration Option. Method and sequence table setup including all sample preparation steps were performed using the GERSTEL MAESTRO Software, completely integrated with the Agilent ChemStation.

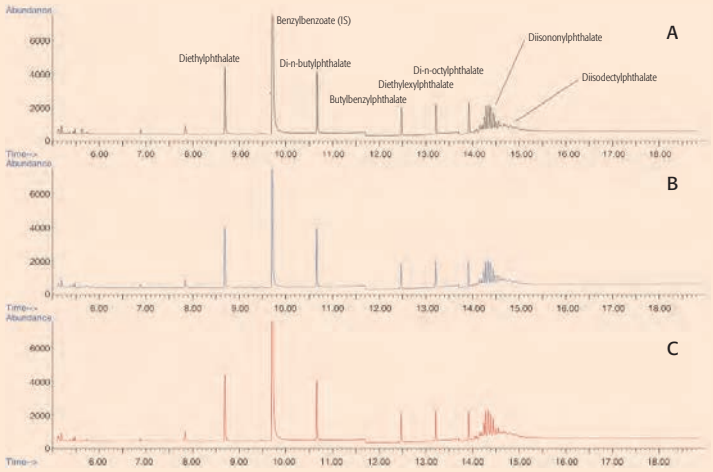
For this method, the PVC sample is dissolved in tetrahydrofuran (THF) and the PVC precipitated using hexane followed by filtration, dilution with cyclohexane and finally analysis using GC/MS. The test procedure is listed in the box to the left on this page.

Greetings from the rubber duck

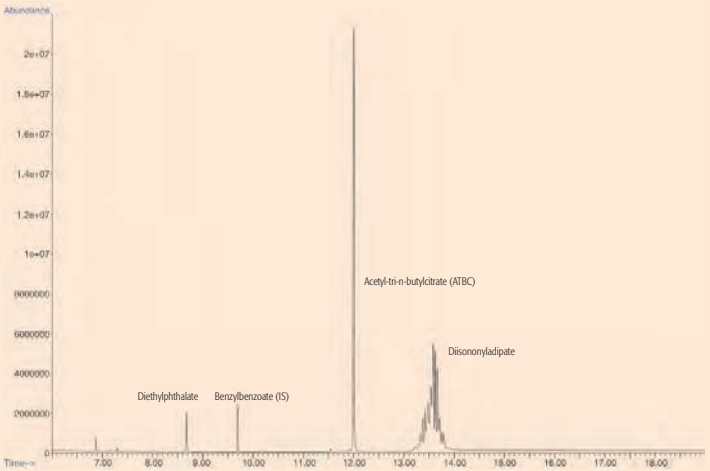
As research objects, Ed Pfannkoch and his team chose ordinary rubber ducks as well as other toys made of PVC. Three other samples were obtained from a local CPSC testing station (CPSC-1, CPSC-2, and CPSC-3). All PVC samples to be extracted were initially cut into small pieces of no more than 2 mm particle size. These were weighed in 10 mL glass vials fitted with screw caps and placed in the MPS sample tray. Analysis method parameters were established as shown in the box on the right. The method was calibrated using standard solutions covering two concentration ranges: Lower range (50-1000 ng/mL) and upper range (5-100 µg/mL). "Initially, we tested whether the solvents used for the extraction were free of phthalates", Edward Pfannkoch explained: "We went through an entire blank extraction procedure, without any sample present, and no phthalates were detected in the final extract." Following the initial experiment, the certified reference samples were extracted – first using the manual sample preparation method and then using the automated extraction procedure. The results obtained using the two methods were found to be in good agreement. The automated sample preparation method combined with GC/MS analysis of the sample extracts worked reliably and pro-

Analysis conditions

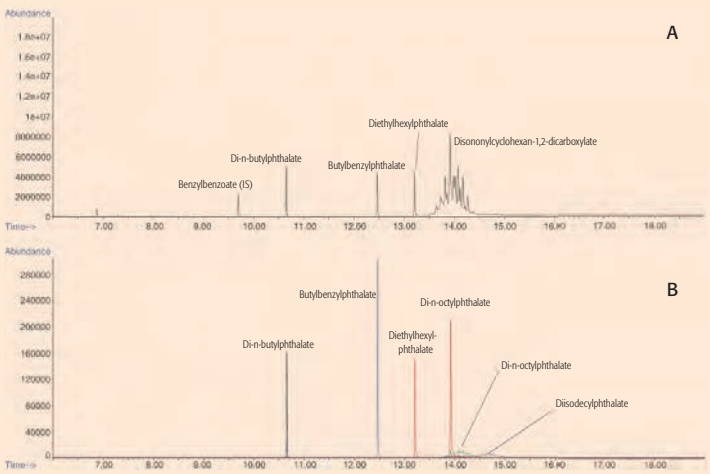
PTV (CIS 4):	Baffled liner, split (20:1) or splitless, 50 °C; 12 °C/s; 280 °C (3 min)
Column:	30 m HP-5MS (Agilent) di = 0.25 mm df = 0.25 µm
Pneumatics:	He, constant flow = 1.0 mL
Oven:	50 °C (1 min); 20 °C/min; 310 °C (5 min)
MSD:	Full scan, 40-350 amu
SIM Parameters (Mass [m/z], Dwell time [ms])	
Group 1:	5 min (91, 10), (105, 10), (149, 10), (167, 10), (194, 10), (194, 10), (205, 10), (212, 10), (223, 10)
Group 2:	11.7 min (91, 10), (149, 10), (167, 10), (206, 10), (279, 10)
Group 3:	13.7 min (149, 10), (167, 10), (261, 10), (279, 10), (293, 10), (307, 10)



Chromatogram resulting from automated preparation of a 50 mg sample (A); automated preparation of a 25 mg sample (B); and manual preparation of a 25 mg sample (C). Conclusion: The chromatograms are equivalent.



Full Scan chromatogram of a rubber duck sample. The chromatogram illustrates the benefits of using SIM/Scan mode: Even plasticizers, which are not selected as target analytes appear in the chromatogram. These include: Diethylphthalate, acetyl-tri-n-butylcitrate and diisononyladipate.



Full scan chromatogram for sample CPSC-2. Figure B shows extracted ion chromatograms of SIM data. Target analytes are identified in the SIM chromatogram. The full scan chromatogram shows the presence of another compound: Diisononylcyclohexane-1,2-dicarboxylate.

duced accurate results. To increase the overall throughput, and to reduce the use of toxic solvents, Pfannkoch and his team reduced the amount of sample and solvent used by 50 % without any measurable impact on the analytical results. Finally, the rubber duck and the CPSC samples were taken through the complete automated procedure.

Extracts were analyzed by GC/MS in split and splitless mode. The MSD was operated in SIM/Scan mode. For the lower concentration range, the instrument was operated in splitless mode. For the higher concentration range, the split was set to 20:1. Analytes were identified based on their mass spectrum and retention times. Quantification was performed based on the calibration curve.

No need to "duck" hard questions

At the end of every method development, the user has to face up to the critical question: Was I successful? Edward Pfannkoch is happy with the results. "Our main aim, to transfer the extraction to the MPS and to automate it completely was achieved", he states. The automated analysis of the certified reference materials gave accurate results. The automated GC/MS method for the determination of DEHP, DBP, BBP, DINP, DIDP and DnOP delivered accurate results with a relative standard deviation (RSD) of 1.9-5.5 percent for all determined phthalates, which is highly precise. In short, the automated GC/MS analysis following test method CPSC-CH-C1001-09.3 performed flawlessly. The results we achieved were highly satisfactory – also for real samples. In the samples CPSC-1 and CPSC-2, we found all six target analytes. Sample CPSC-3 on the other hand contained DEHP at a high concentrations level.[4]

Literature

[1] United States Consumer Product Safety Commission, Test Method: CPSC-CH-C1001-09.3 Standard Operating Procedure for Determination of Phthalates, April 1st, 2010.

[2] German Federal Environmental Protection Agency (Umweltbundesamt): Phthalates: Useful plasticizers with undesired properties, 02/2007; www.umweltbundesamt.de/sites/default/files/medien/publikation/long/3541.pdf

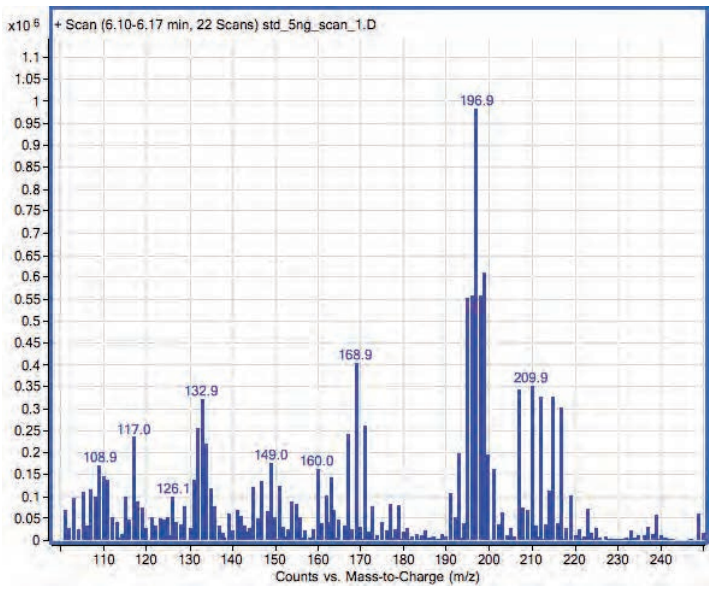
[3] Directive 2005/90/EC of the European Parliament

[4] Fredrick D. Foster, John R. Stuff, Jacqueline A. Whitecavage, Edward A. Pfannkoch: Automated Extraction and GC/MS Determination of Phthalates in Consumer Products, GERSTEL AppNote 4/2013, www.umweltbundesamt.de/sites/default/files/medien/publikation/long/3541.pdf

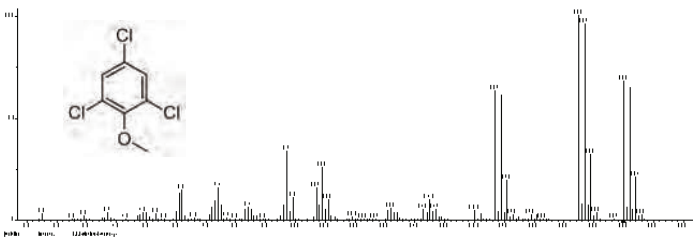
"Corked" pills – no thanks!

Pharmaceutical products with an unpleasant smell may be perfectly safe and effective. However, patients instinctively associate off-odors with inferior quality or even the possibility that the product may harm them. When this happens there is a good chance that the medicine will not be taken, but simply thrown away. This hurts the image of the manufacturer and can even prompt extremely costly product recalls. To track down off-odors and their sources in pharmaceutical products and their packaging, scientist have developed and validated a highly sensitive GC-MS/MS method based on Stir Bar Sorptive Extraction (SBSE).

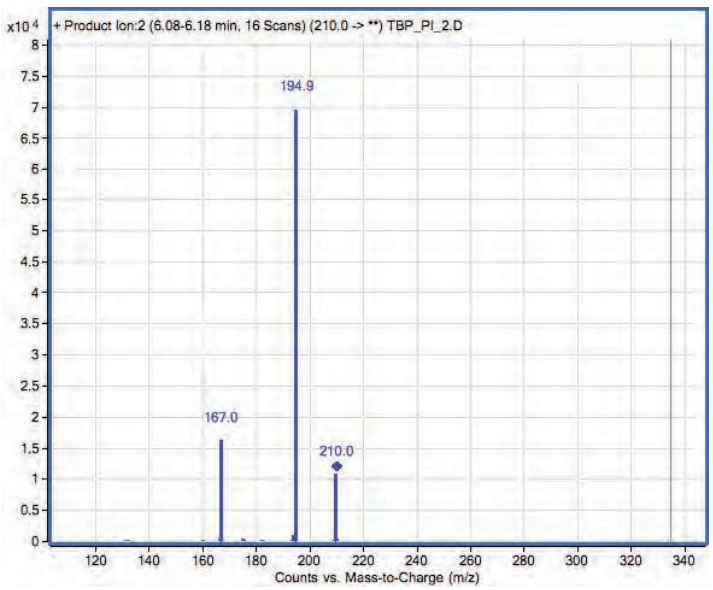
When it comes to quality control, the devices given to us by Mother Nature are hard to beat. Anything we ingest passes through several sophisticated anatomically based inspection steps. First a visual inspection, then a sensory evaluation of "mouth feel" combined with the taste sensed on the tongue and flavor or odor sensed by our olfactory bulbs in the nose. The combinations of all these senses produce a number of responses: Things that provide an overall pleasant experience are favored and eagerly consumed - sometimes in



Electron Impact (EI) spectrum following a 5 ng direct injection of 2,4,6-trichloroanisole.The molecular ion cluster appears as m/z = 210(M), 212 (M+2) and 214 (M+4). The main cluster at m/z=195 (M) is the methyl loss peak cluster. Due to siloxane interferences, the isotope ratio for the three chlorine atoms in the molecule is not properly detected.



Electron impact NIST library spectrum of 2,4,6-trichloroanisole (# 333450). The molecular ion cluster appears at m/z=210 (M), 212 (M+2) and 214 (M+4) with the isotope ratio to be expected from an organic molecule containing three chlorine atoms.



Collision induced dissociation (CID) spectrum of 2,4,6-trichloroanisole with precursor ion m/z=210 (M).

larger amounts than advisable. However, it only takes the presence of a pungent or unpleasant smell to set off our neural alarm network, making us sniff at food and instinctively turn it away. If you look at the greater picture, olfactory evaluation has played a central role in our survival through the ages and continues to be a key guardian of our safety. Evolutionary biologists contend that especially the expansion of the olfactory center lead to the decisive increase in mammal brain size [1].

The nose decides

In order for a product to reach the stomach and the heart of the consumer, it needs to get by the nose. Products taken orally should have a pleasant or at least a neutral flavor. This applies to food and food ingredients as well as to condiments, sweets, beverages, and of course, pharmaceutical products. Some off-odors can set off the olfactory alarm even at ultra-trace levels due to our highly sensitive olfactory early warning system. Threshold levels can be extremely low and vary widely from person to person. In order to accurately and reliably determine the identity and concentration of off odor compounds at relevant levels, gas chromatography in combination with tandem mass spectrometry (GC-MS/MS) is a widely used technique. This is due to its ability to provide low detection limits and accurate mass spectral data, especially for known (target) compounds. For best results, GC-MS/MS is coupled with an extraction/concentration technique that allows for the unadulterated compounds to be introduced into the system. Ideally, off-odor compounds should be sniffed out and the product held back before it is under the nose of the consumer and before a situation develops that includes negative headlines and product recalls that can cause extensive and expensive damage to a brand. Case in point: A couple of years ago, a global pharmaceutical company was forced to recall tens of thousands of packages of different brand name products following customer complaints concerning a musty, moldy off-odor. The malodorous culprits in question turned out to be halogenated anisoles and phenols, well-known repeat offenders in the wine industry leading to corked wines. The line-up included the following identified suspects: 2,4,6-trichloroanisole (TCA), 2,4,6-tribromoanisole (TBA) and 2,3,4,6-tetrachloroanisole (TeCA), metabolic offspring of their equivalent phenols, some of which were also caught in the dragnet: 2,4,6-trichlorophenol (TCP), 2,4,6-tribromophenol (TBP) and pentachlorophenol (PCP). The odor thresholds, the lowest concentrations at which compounds are detected by the human olfactory system, in this case in wine or water, are as follows: 1.4-4 ng/L for TCA; 3-8 ng/L for TBA; and 4-24 ng/L for TeCA [2]. For TCP and PCA the values are approximately 4000 ng/L [3,4].

On the origins of corky off-odor

The usual suspect as a source of 2,4,6-TCA in wine is the cork stopper made from the bark of the cork oak tree (Quercus

suber). TCA is a microbial metabolite, formed by methylation of trichlorophenol (TCP) that may have been applied to the bark as a pesticide. To suspect the cork stopper of introducing TCA to the wine is therefore only logical, but when wine drinkers started experiencing corkiness in wines with modern polymer-based stoppers, experts knew that they had been barking up the wrong tree.

Over the course of the ensuing research projects, it was found that various compounds, mainly halogenated anisoles, would give a musty, moldy note to the wine. These compounds could be formed from other chlorinated chemicals that are used for cleaning of wine production equipment or for treating wooden transport pallets or packaging material.

Until the end of the 1980's, pentachlorophenol (PCP) was used as a fungicide to protect wood, including wooden pallets, from microbial decay. Among other by-products, PCP contained 2,3,4,6-tetrachlorophenol (TCP), a compound that is metabolized microbially to 2,3,4,6-tetrachloroanisole (2,3,4,6-TeCA, TeCA), which causes corkiness in wine.

In animal tests, PCP was found to be carcinogenic. In Germany, the use of PCP has been prohibited since 1989. PCP was substituted by 2,4,6-tribromophenol (TBP), a combined fungicide and flame retardant, which is often used to protect cardboard packaging, polymer materials, paints and coatings even for building modules used in pre-fabricated homes. As it turns out, microorganisms metabolize TBP to 2,4,6-TBA, a compound given the sensory attributes musty, earthy, and chemical with a smell of solvent. TBA is well known to impart a corky odor and taste to wine.

TBA was indeed the main culprit when the above mentioned pharmaceutical products fell from grace with discerning consumers. The contamination with 2,4,6-TBA, it was speculated, could have been introduced through wood protection agents used in the production of transport pallets. The off-odor compound would then have been transferred to packaging materials transported or stored on those pallets and subsequently into the pharmaceutical products. History is said to repeat itself, and in this case, the scenario was well known from previous incidences in the brewing industry, in which beer bottle caps had been contaminated leading to the recall of truckloads of product.

Whenever a cause is identified beyond reasonable doubt, effective remedies can be put into place to prevent a reoccurrence.

With this in mind, scientists charged with finding the root cause of the packaging debacle decided to develop and validate a highly sensitive GC-MS/MS method for the determination of 2,4,6-TCA, 2,4,6-TBA, 2,4,6-TBP and 2,4,6-TCP in tablets as well as 2,4,6-TBA in packaging material [3].

Searching for the best possible extraction technique

To meet the stringent sensitivity requirements in the method they developed, Gyorgy Vas and his team of colleagues from Johnson & Johnson and from McNeil Consumer Healthcare were looking for a powerful extraction and analyte concentration technique, which could span a wide volatility range and result in reliable quantification at ultra-trace concentration levels. During their literature search, the scientists determined that results based on headspace solid phase micro-extraction (HS-SPME) were mainly reported for the relatively volatile halogenated anisoles. In their paper in Journal of Chromatography (A) [3], the scientists summarize the possibilities with this technique as follows: "Compared with liquid-liquid, solvent based extraction methods, HS-SPME has the advantage that it is easy to automate, simple to perform and can be used for a wide range of compounds". The limitation, however, was determined as lack of extraction efficiency from solid and liquid samples for many less volatile compounds. To enable the sensitive and reliable determination of both volatile and less volatile compounds, the scientists turned to Stir Bar Sorptive Extraction (SBSE) using the GERSTEL Twister®, which relies on a significantly larger volume of sorbent phase and had already been shown to be effective in determining the target compounds in the wine industry [2]. "We have found SBSE to be highly efficient for the determination of trace level analytes due to the fact that the extraction phase volume (of the Twister) is relatively large compared with that of SPME (about 5 µL for the 10 mm length Twister) relative to that of SPME (about 65 nL for the 100 µm fiber)," Dr. Vas and his colleagues write in explaining their choice.

Predicting the extraction efficiency

How efficiently analytes can be extracted using SBSE and the PDMS-based Twister, is best predicted based on their octanol/water distribution coefficient ($K_{o/w}$), a dimensionless value. The $K_{o/w}$ value represents the ratio of the concentrations of that analyte in the respective phases in a 1-octanol- water two-phase system at equilibrium. $K_{o/w}$ serves to describe the hydrophobic or hydrophilic properties of a chemical. The logarithmic value log Kow of a compound allows predictions of its distribution in a PDMS-Water system, and is a reliable indicator of how well a compound will be extracted into the Twister stir bar. A high log $K_{o/w}$ value represents a highly hydrophobic compound which would be extracted very efficiently from an aqueous matrix using the GERSTEL Twister.

The straight and narrow path: Focused development of validated methods

Dr. Vas and his colleagues developed their methods based on standard addition quantification. To this end, standard solutions containing halogenated anisoles at 20, 40 and 200 pg/µL were generated as well as solutions containing halogenated phenols at 500, 1000 and 2000 pg/µL and finally an internal standard solution containing d5-TBA at 100 pg/µL. These solutions were generated on a daily basis.

Standards were added to pharmaceutical tablets of different weight as well as to different packaging materials: Cardboard, polyethylene, polycarbonate and pallet wood. Quantification of the target analytes was performed with isotope dilution using 2,4,6-d5-tribromoanisole as internal standard. Compounds were identified and quantified using tandem mass spectrometry detection in Multiple Reaction Monitoring (MRM) mode. The following mass transitions were monitored:
TBA 346 -> 331 (quantifier) and 346-> 303 (qualifier)
TCA 212 -> 197 (quantifier) and 212 -> 169 (qualifier)
TCP 196 -> 132 (quantifier) and 196 -> 160 (qualifier)
TBP 330 -> 222 (quantifier) and 330 -> 250 (qualifier)
d5-TBA 349 -> 331 (quantifier)

Validation of the method was performed in accordance with the requirements of the

Agilent 7890A / 7000B Triple Quadrupole GC/MS. The system is mounted with a GERSTEL MPS Dual Rail sampler with agitator, MultiFiber Exchange (MFX), Thermal Desorption Unit (TDU), Twister Option, SPME fiber bake-out station, and Dynamic Headspace System (DHS).

ICH Q2 (R1) guidance (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) as well as in accordance with the with US Food and Drug Administration (FDA). Given the fact that the ICH Q2 (R1) guidance does not fully apply to analytical validation of methods for the determination of trace concentration and since TCP is an herbicide, the European Council Directive 96/23/EG (Commission Decision 2002/657/EC of 12 August 2002) was followed concerning the analysis method and data analysis. The GC-MS/MS system used was an Agilent 7890/ 7000B Triple Quadrupole GC/MS system. The GC inlet used was a GERSTEL Cooled Injection System (CIS) used for cryofocusing and temperature programmed transfer of analytes to the GC column (DB-5 MS, UI, 20 m, 0.18 mm, 0.36 µm film thickness). A GERSTEL Thermal Desorption Unit (TDU) mounted on top of the CIS was used to desorb the GERSTEL Twisters (10 mm length, PDMS: 1.0 mm film thickness). Introduction of the TDU Liners with the Twisters was performed automatically using the GERSTEL Multi-Purpose Sampler (MPS), which holds up to 196 TDU liners in sealed sample tray positions.

Four tablets were placed in separate sample vials each containing formic acid (0.1 %) and 5 µL of standard solution. The vials were placed in an ultrasound bath for 30 min and subsequently extracted for 90 min with Twister stir bars at a stirring speed of 1000 rpm. The Twisters were removed from the sample vials, gently dried with lint-free paper cloth and placed in individual TDU liners, which were placed in the MPS sample tray for subsequent GC-MS/MS analysis.

The packaging material to be analyzed was cut in pieces of one square centimeter each, the pieces were then added to a sample vial along with 100 pg/g 2,4,6-TBA. The vials were sealed and left for 48 hours to enable TBA to penetrate the packaging material and to be absorbed by it. An internal standard was added and the sample left to equilibrate for one hour before the extraction procedure. The sample was then transferred to a 125 mL vial and 100 mL of a water-acetone mixture was added. The vial was placed in an ultrasound bath for 30 min and subsequently the water-acetone mixture was extracted for 90 minutes using a Twister stir bar with a stirring speed of 1000 rpm. The Twister was removed from the sample vial and gently dried using a lint-free paper cloth and placed in a TDU liner, which was placed in the MPS sample tray for subsequent thermal desorption and GC-MS/MS analysis.

Conclusions

Dr. Vas and his colleagues succeeded in developing and validating an SBSE-TD-GC-MS/MS based method for quantification of TCA, TCP, TBA and TBP in solid pharmaceutical products [3].

The method was validated as a standard addition method for the analysis of pharmaceutical products for the above mentioned target analytes, relevant to off flavor incidents. The validated range is 100-1000 pg/tablet for halogenated anisoles and 2500-10000 pg/tablet for halogenated phenols. Detection limits (absolute amounts) for TCA: 4 pg; TCP: 286 pg; TBA: 9 pg; TBP: 371 pg.

Recovery based on 100 pg of the halogenated anisoles and 2500 pg of the halogenated phenols added; recovery ranges determined in four different solid dosage formulations for TCA: 79-97 %; TCP: 67-89 %; TBA: 68-76 %; TBP: 56-72 %

Precision data for repeat measurements on the same sample performed by the same user on the same instrument on the same day using deuterated TBA (d5-TBA) as internal standard gave the following relative standard deviations (RSD) in percent for TCA: 6.2-11.3 %; TCP 3,2-12,9 %; TBA: 3.1-11.0 %; TBP: 6.5–15.6 %.

Sources

[1] Timothy B. Rowe, Thomas E. Macrini, and Zhe-Xi Luo, Fossil Evidence on Origin of the Mammalian Brain, Science 20 (2011) 955-957

[2] H. Rudy, Efficient and sensitive determination of corkiness and other off-flavours in wine, LCGC The Column, Vol. 10, Jan. 2014.

[3] Jiun-Tang Huang, Lori Alquier, Joyce P. Kaisa, Gail Reed, Timothy Gilmore, and Gyorgy Vas, Method development and validation for the determination of 2,4,6-tribromoanisole, 2,4,6-tribromophenol, 2,4,6-trichloroanisole, and 2,4,6-trichlorophenol in various drug products using stir bar sorptive extraction and gas chromatography-tandem mass spectrometry detection, Journal of Chromatography A 1262 (2012) 196-204.

Wondrous Vanilla

Vanilla isn't just vanilla or even plain vanilla. There is a whole plethora of vanilla types and many different ways to prepare vanilla pods. Their characteristic flavor patterns aren't only determined by vanillin, the main known flavor compound, but also by a large number of other flavor compounds, many of which have not yet been identified. Each type of vanilla has its own characteristics. In order to determine the origin of vanilla, and for quality control purposes, laboratories typically rely on Headspace or Thermal Desorption techniques used in combination with GC/MS. This article describes interesting aspects of the analysis of vanilla pods and vanilla extracts and reports on newly discovered vanilla flavor compounds.

No other spice or flavor ingredient is as widely used and appreciated as vanilla. In spite of the expression "plain vanilla", vanilla is the king of flavors and it is not only used in food and sweets, but also in perfumes, cosmetics and pharmaceutical products. Vanilla is said to soothe the soul and reinvigorate the body explaining its widespread use in aroma therapy. Recent research reports indicate that vanillin, the key flavor compound in vanilla, may have cancer inhibiting properties. The ancient Aztecs considered vanilla the "food of the gods", today it is among the most important and most widely loved spices in the world. One of the reasons why vanilla is so widely used is because key flavor compounds can be synthesized from cheap ingredients such as paper pulp. Real vanilla, on the other hand, is very expensive with prices reaching as high as $ 80,000 per ton [1]. However, the amount of naturally grown and fermented vanilla does not even come close to satisfying world demand. With prices of the real thing so high, quality and authenticity, i.e. origin and potential adulteration must be carefully monitored. Traditionally this job has been the responsibility of taste and flavor panels, but increasingly, chemical analysis has come into play.

Producers of food and other products that contain vanillin as a flavor ingredient can rely on standard chemical analysis techniques for quality control. The analysis of real vanilla, however, can present a challenge. Due to the complexity of the chemistry and fermentation processes involved, it typically requires more than one analytical technique to provide reliable results. Stephen J. Toth, Ph.D. knows this better than most people – and from firsthand experience. Over the course of his work on his doctoral dissertation: "Comparison and integration of analytical methods for the characterization of vanilla chemistry" [1] at the State University of New Jersey, the scientist strove to find a unified analytical method for the determination of volatile and semi-volatile organic compounds (VOCs and SVOCs) in vanilla pods and extracts. The solution he found was to use two techniques: 1) Liquid chromatography (LC); and 2) Gas chromatography (GC) in combination with headspace and thermal desorption.

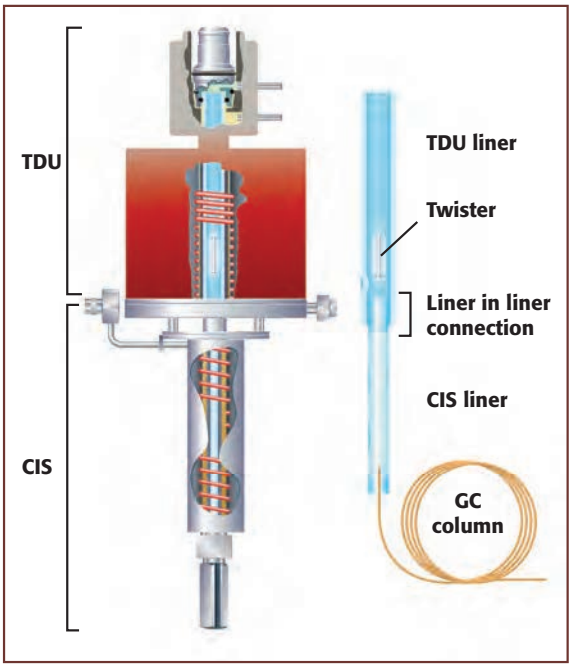
Determination of vanilla flavor components

For the determination of semi-volatile or non-volatile flavor compounds in vanilla, such as vanillin, 4-hydroxy benzaldehyde, vanillic acid and 4-hydroxy benzoic acid, HPLC is the separation method of choice. In his dissertation, Dr. Toth listed as reference a large number of journal papers based on HPLC methods, mainly for the analysis of vanilla extracts, which served as reference points for his method development work. The separation was ultimately performed using much shorter UHPLC columns, which are based on smaller particle size packing. As a result, the analysis time for vanillin and related phenolic compounds was cut from 13.45 to 1.86 minutes, a seven-fold improvement in throughput. In addition, the amount of acetonitrile used was reduced by about two thirds. Still, for the wider range of more volatile compounds that are responsible for the bulk of vanilla's flavor, including many unknown compounds, HPLC is not the technique of choice. For these, gas chromatography combined with mass spectrometry (GC/MS) must be used – preferably combined with headspace (HS), HS-SPME or other sample introduction and analyte concentration techniques.

To determine which technique was best suited for the analysis, the scientist compared results from the following: Solid Phase Micro Extraction (SPME), Headspace Sorptive Extraction (HSSE) using the GERSTEL Twister, and direct thermal desorption of deep

A brief introduction to vanilla

Vanilla belongs to the orchid family. Vanilla grows in tropical regions and there are more than one hundred different species of vanilla, only two of which are commercially relevant. Vanilla Planifolia and Vanilla Tahitensis are the only orchid species to produce a commercially useful fruit. Originally from Mexico, vanilla is mainly grown in Indonesia and Madagascar today. Most of us wouldn't recognize a vanilla plant or its fruit. The green odorless bitter tasting vanilla fruit is nothing like the vanilla pods we are exposed to in high-end food stores. Over the course of a five month complex and intricate fermentation process, the fruit is transformed into dark brown, extremely tasty and flavorful vanilla pods. Among other compounds, vanillin is formed by hydrolysis of glucovanillin. Controlling the fermentation process is key to producing the right flavor profile, the right taste and an overall quality product. The fermented vanilla is marketed in two different forms: As an extract in alcohol solution (extraction grade) and unprocessed (gourmet grade). A high quality vanilla pod is characterized by highly pleasant flavor and taste, moisture content of 18 – 25 %, and dark chocolate-like color. The surface must be oily and free from defects and mold. The vanillin content should be greater than 2 % as a key indicator to high quality, but this is not always the decisive factor: Many vanilla types have excellent overall taste and flavor even though their vanillin content is lower than 2 %. This leads to the conclusion that other flavor compounds contribute significantly to the overall flavor and taste [1].



Thermal desorption of the Twister in the TDU followed by cryofocusing of the analytes in the CIS and temperature programmed transfer to the GC column.

frozen ground vanilla pods. The flavor compound profiles of two bourbon vanilla pods were investigated. One was a perfect pod of good quality and the other had been rejected by a customer as unacceptable due to an "alcohol" off flavor, thought to be caused by bacterial degradation products, including guaiacol formed by degradation of vanillin under anaerobic conditions.

Solid Phase Micro Extraction (SPME)

In his literature searches, Toth found a large number of articles reporting on the analysis of vanilla extracts based on the SPME technique, especially when it came to polar compounds in alcohol extracts. He ascribed this to the multitude of SPME phases available, ease of automation as well as fast desorption of the concentrated analytes in the GC inlet. SPME has proven itself to be selective, efficient and highly useful in practical analysis work when it comes to extraction of volatile compounds from the headspace phase. The main shortcoming of SPME lies in the limited phase volume (0.5 µL), limiting the sorptive capacity and thus the sensitivity of the technique. Nevertheless, SPME was used to extract 35 compounds from vanilla, including contaminants from packaging as well as eight new compounds, which hadn't previously been identified.

Headspace Sorptive Extraction (HSSE)

Headspace Stir Bar Sorptive Extraction (HSSE) is an add-on development of Stir Bar Sorptive Extraction (SBSE). HSSE has been used for flavor analysis work involving a large number of different products [2] and it is based on using the GERSTEL Twister as a passive sampler in the headspace of a sample. In standard SBSE, the Twister extracts analytes from a liquid sample while actively stirring it; in HSSE, the Twister is positioned in the headspace above the sample from which it indirectly extracts volatile compounds. After equilibrium has been established between the liquid, headspace and Twister sorbent phases, the Twister is removed and transferred to the MultiPurpose Sampler (MPS) sample tray. From there, it is automatically transferred to the Thermal Desorption Unit (TDU) for thermal desorption and transfer of the analytes to the GC column. Due to the much higher phase volume of the sorbent (Twister: 125µL / SPME: 0.5 µL), the HSSE technique is exceptionally efficient in extracting medium to non-polar compounds. An EG-Silicone Twister is now also available, which enables efficient extraction of polar compounds such as phenols and other aromatic alcohols. Using HSSE, a total of 19 previously unknown compounds were found and identified.

Dynamic Headspace (DHS)

Dynamic Headspace, as the name implies, is not a static technique relying on equilibrium between phases, but rather a dynamic technique relying on driving analytes out of the headspace, and thus indirectly out of the sample, using carrier gas. Purged analytes are trapped and concentrated on an adsorbent trap at the purge outlet. In this case, Tenax TA adsorbent was used in the trap. As opposed to SPME and HSSE, Tenax TA showed no specific affinity for individual compound classes, but rather concentrated analytes over a wide polarity range. However, Toth found that compounds with three or less carbon atoms generally were not as efficiently trapped. Using DHS, he was able to extract and identify 24 compounds from the high quality vanilla, including 10 compounds that had not yet been found in vanilla.

Direct Thermal Desorption (DTD)

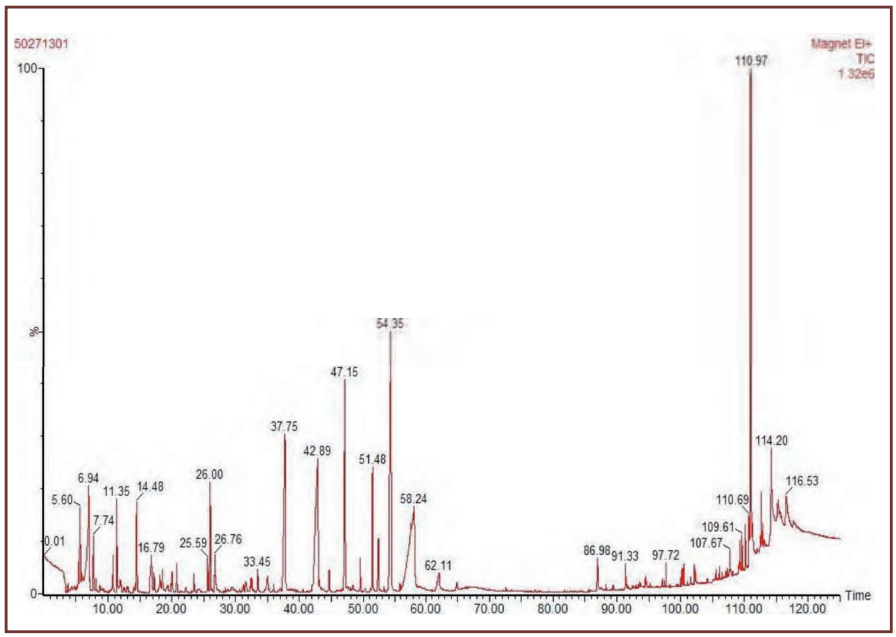
For direct thermal desorption, the sample was placed in a suitable inert glass tube (TDU-liner) between two plugs of glass wool and placed in the MPS sample tray. The TDU liner was transferred to the TDU by the MPS. Thermal desorption/thermal extraction was performed using the following temperature program: Initial temperature 30 °C – 60 °C/min – 275 °C.

Desorbed analytes were trapped in the GERSTEL Cooled Injection System (CIS) PTV-type inlet and transferred to the GC column using a temperature program. Direct thermal desorption using the TDU-GC/MS method enabled Toth to identify 74 compounds in both the "good" and "bad" vanilla samples. After further work, he found another 30 flavor compounds that had not yet been reported in vanilla pods. The most remarkable difference between the "good" and "bad" vanilla was indeed in the vanillin concentration: The "good" sample contained 1.2 %, the "bad" only 0.1 %. In the accepted "good" vanilla, Toth further found high concentrations of acetic acid, 2-methoxy phenol, hydroxyl dihydromaltol, 5-(hydroxymethyl) furan-2-carbaldehyde, 4-hydroxybenzaldehyde, hexadecanoic acid and 1-octadecanol. Toth reports: "The list of compounds identified in the good bourbon vanilla pod correlates well with previously reported data from the literature". Compounds, which he was the first to report in vanilla pods, included acetone, 2-methyl propanal, 3-hydroxy-3-pentene-2-one, 2(5H)-furanone, 2-hydroxy-2-cyclopentene-1-one, 4-hydroxy-5-methyl-3(2H)-furanone, furan-2-carboxylic acid (2-furoic acid), lilial acid, 4-(4-hydroxyphenyl)-3-buten-2-one, 4-(4-hydroxy-3-methoxyphenyl)-3-butene-2-one (E), two isomers of vanillin glyceryl acetal, 1-octadecanol, ethyl heptadecanoate, ethyl octadecanoate, z-12-pentacosene and z-14-nonacosene.

In the "bad" vanilla pods, Toth identified high concentrations of compounds such as: 2-methoxy phenol, 2-methoxy-4-methyl-phenol, hexadecanoic acid and 1-octadecanol.

Among the biggest differences uncovered by direct thermal desorption of the "good" and "bad" bourbon vanilla pods were the loss of vanillin, increased concentrations of 2-methoxy-4-methyl phenol and 2-methoxy phenol as well as the loss of hydroxyl dihydro maltol and hydroxymethyl furfural, Toth writes. Unlike the results from the analyses performed by SPME, HSSE, and DHS, the direct thermal desorption results didn't include fusel alcohols, even though the rejected vanilla products did contain these compounds at different concentration levels. The presence of fusel alcohols is in itself a strong indication that bacterial degradation has taken place.

Even though each technique had clear strengths and weakness, they complemented each other well. When combined, they serve to provide a comprehensive picture of the diverse chemical landscape of a complex product such as vanilla. As a flavor analyst, being able to draw on the whole range of headspace techniques: SPME, HSSE, Dynamic Headspace (DHS) and direct thermal desorption (DTD) provides a critical advantage in terms of being able to cover all analytes while using solvent-free and highly sensitive extraction techniques. The flavor chemist's efficiency as well as the resulting data is greatly improved when these techniques are automated, and the ultimate enhancement is provided by the GERSTEL MultiPurpose Sampler (MPS), which automates all of these techniques on a single platform.



Total Ion Chromatogram of a Tahitian Vanilla resulting from DTD-(TDU)-GC/MS analysis. The following compounds were found: 2-(5H)-furanone, 2-hydroxy-2-cyclopentene-1-one, 2-acetyl-2-hydroxy-gamma-butyrolactone, 3,5-dihydroxy-2-methylpyrane-4-one, 3-phenyl-2-propenoic acid, 4-hydroxy-2-methoxy cinnamic aldehyde, 4-(4-hydroxy-3-methoxyphenyl)-3-butene-2-one (E), 2 isomers of 2-(4-hydroxy-3-methoxyphenyl)-1,3-dioxane-5-ol, kauren and z-12-pentacosene.

Sources

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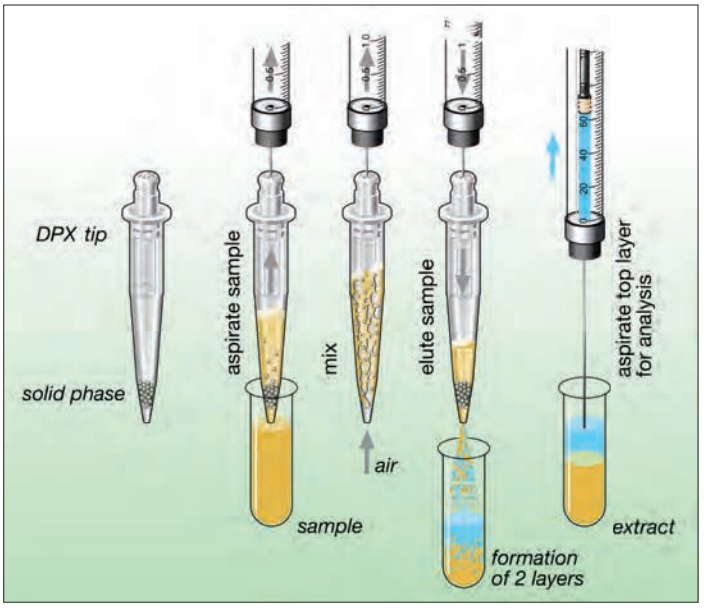
[2] Flavor, Fragrance, and Odor Analysis, 2. Edition, CRC Press, Taylor & Francis Group 2012

Toxicology

Pain Management (PM) drugs in urine – can the analysis be fully automated?

A lot of useful information can be extracted from urine regarding medication taken and metabolism of drugs in the human body. The technique most often used is enzymatic hydrolysis of metabolites followed by SPE clean-up and HPLC-MS/MS determination. When performed manually, the sample preparation is labor intensive and time consuming. If hydrolysis and clean-up are automated and dispersive SPE (DPX) is chosen instead of standard SPE, the process is accelerated as shown in the following example.

The amount of work required is significant whenever toxicologists and clinical chemists want to determine the concentration of active pharmaceutical ingredients (APIs) - or of their metabolites - in urine. One of the time-intensive steps required is the hydrolysis of conjugated analytes to their original form. This transformation is typically performed enzymatically, for example, using β-glucuronidase (GUSB). To ensure that the hydrolysis reaction is complete and reproducible, control, monitoring and optimization of various parameters is needed. Among these are the pH value, the temperature and the length of the hydrolysis period, which varies from enzyme to enzyme. These factors have a profound impact on the quality of the analysis results. The same is the case for matrix compounds, present in significant amounts in urine. In order to reach the very low limits of detection required for monitoring of API residues and metabolites, interfering compounds from the matrix, or those generated during the hydrolysis process, must be eliminated. Typically, this is done using a suitable extraction technique such as solid phase extraction (SPE), which is also widely used in forensic analysis. Performing standard, cartridge based SPE has a number of drawbacks when used for this type analysis. It uses relatively large amounts of costly solvent, the solvent and sample elution need to be precisely controlled, increasing the risk of error and making the process slow, and the resulting sample dilution increases detection limits.



Graphical representation of the automated DPX urine cleanup process.

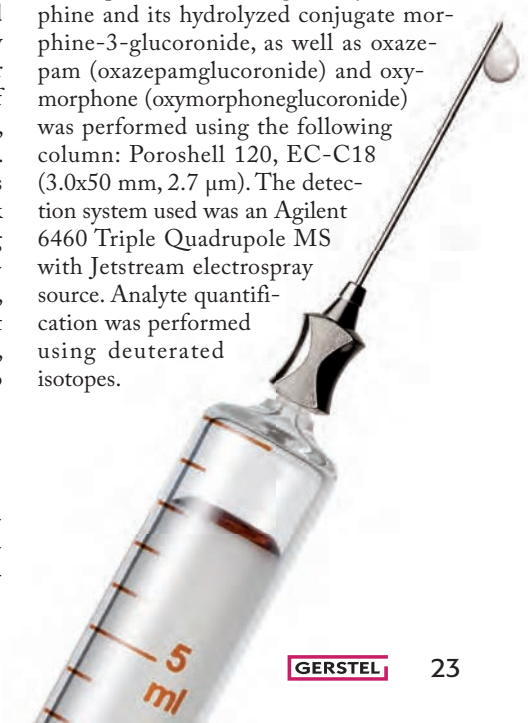
Attractive alternative to standard SPE

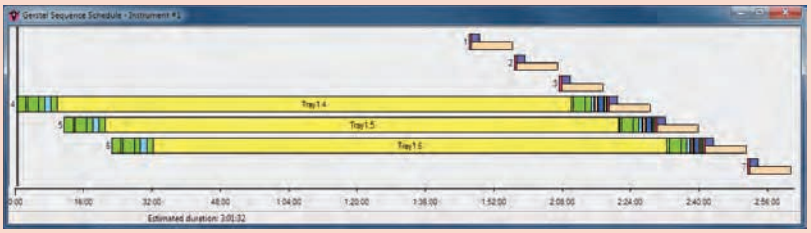
When he was initially searching for a more efficient solid phase extraction method, William E. Brewer from the University of South Carolina developed Disposable Pipette Extraction (DPX), a dispersive SPE (dSPE) technique. Instead of the sorbent being present as a packed bed, in DPX the sorbent is a loose powder contained inside a standard disposable pipette tip by fixed screens at the top and bottom of the tip. Sample is aspirated into the DPX tip only, eliminating both the risk of sample to sample carry-over and the need for extensive washing of syringes used in systems based on standard SPE cartridges. With the sample-powder mix inside the tip, air is aspirated leading to turbulent mixing of the phases resulting in highly efficient extraction. Typically, the remaining sample is discharged and the concentrated analytes eluted with a small volume of solvent into an empty clean autosampler vial followed by LC/MS or GC/MS determination. Key differentiators of DPX are: Fast extraction; high recovery rates, and the very small amounts of solvent used. Reducing solvent use in the laboratory brings many benefits ranging from improved work environment to reduced cost for purchasing and disposing of often toxic solvents. "A further key benefit is the ease of automation", says Fred D. Foster, Application Scientist at GERSTEL, Inc. near Baltimore, Maryland, "and this includes automated introduction to the LC/MS analysis system."

Wanted: Complete automation

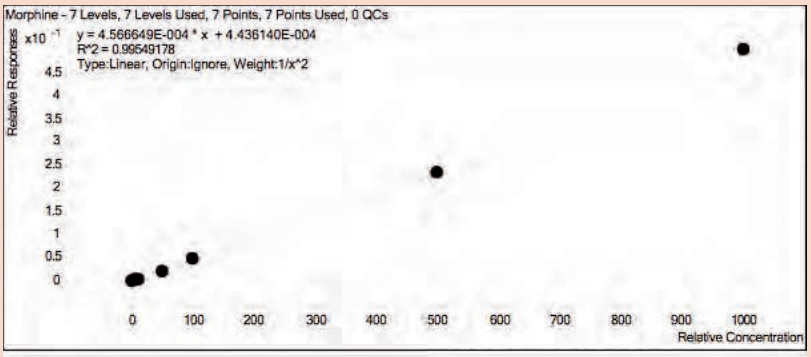
At GERSTEL, Inc. Fred Foster and his colleagues have long been focused on automating the last key step on the way to a completely automated solution: Hydrolysis of the conjugates formed during drug metabolism in order to quantify the total amount of drug taken. They used a Dual Head version of the GERSTEL Multi-Purpose Sampler (MPS). This system uses one head to perform the DPX based sample extraction and clean-up, while the second head performs the injection into the LC/MS system. The Dual Head configuration enables optimum sample preparation and injection time of less than 7.5 minutes per sample following the automated hydrolysis [1]. Fred Foster arrives at the following conclusion after finishing and evaluating the project: "We can now offer a combined automated system, which performs high throughput urine analysis, including enzymatic hydrolysis, extraction and LC-MS/MS determination." The manual work required is minimal: A 1 mL sample of urine is manually pipetted into an autosampler vial. The vial is capped and placed in the autosampler tray. All further steps in the sample preparation and introduction process are controlled by the MAESTRO software, fully automated.

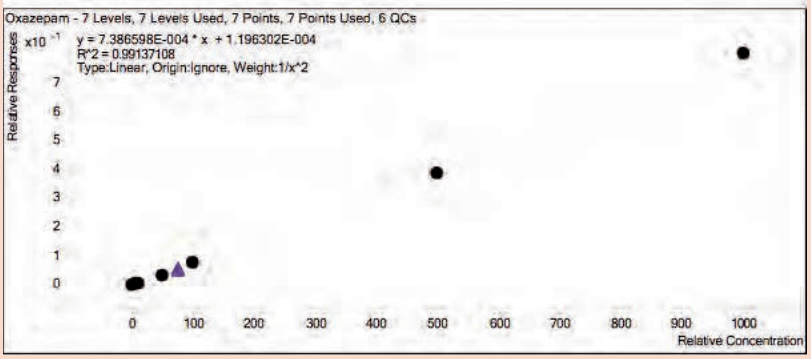
Separation of the target analytes: Morphine and its hydrolyzed conjugate morphine-3-glucoronide, as well as oxazepam (oxazepamglucoronide) and oxymorphone (oxymorphoneglucoronide) was performed using the following column: Poroshell 120, EC-C18 (3.0x50 mm, 2.7 µm). The detection system used was an Agilent 6460 Triple Quadrupole MS with Jetstream electrospray source. Analyte quantification was performed using deuterated isotopes.



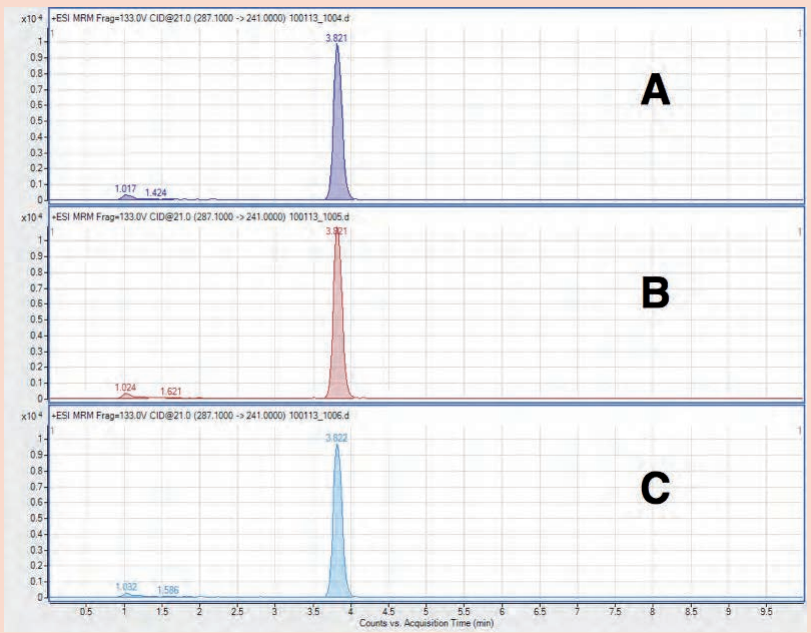


MAESTRO Prep Sequence Scheduler for the optimization of the automated β-glucuronidase hydrolysis procedure using 0.66M acetate buffers at different pH.





Calibration curves for Morphine and Oxazepam resulting from automated hydrolysis of Morphine-3-glucuronide and Oxazepam glucuronide, DPX clean-up, and LC/MS/MS determination.



Stacked view of Oxazepam peaks resulting from pH variation experiments. No significant difference was found when the 0.66M acetate buffer pH was varied between 4.0 (A), 4.5 (B), and 5.0 (C).

Putting the automated system to the test

In order to establish that the enzymatic hydrolysis of urine samples using the typical β-glucuronidase procedure could be automated successfully, triplicate urine samples spiked at a concentration of 1000ng/mL with Oxazepam glucuronide were hydrolyzed both manually and using the automated hydrolysis procedure. Following hydrolysis, all samples were extracted and analyzed using the DPX-LC/MS/MS procedure. The results for the manual and automated procedures matched well with only a 4% difference between the two sets of results.

The automated β-glucuronidase hydrolysis procedure was then compared to a typical acid hydrolysis procedure in which equal parts concentrated hydrochloric acid were added to spiked urine samples containing either Oxymorphone-3-β-D-glucuronide or Oxazepam glucuronide at 1000ng/mL and then allowed to incubate at 100° C for 90 minutes. After cooling to room temperature, the pH of these samples was adjusted to 4 using dilute ammonium hydroxide prior to extraction along with the automated β-glucuronidase hydrolyzed sample group using the automated DPX-LC/MS/MS procedure. The final volumes of the samples being compared were adjusted prior to extraction, in order to ensure that the final concentrations would be equivalent. The native Oxymorphone and Oxazepam concentrations were found to be lower when the acid hydrolysis procedure was used. Since no response was observed when monitoring for Oxymorphone-3-β-D-glucuronide and Oxazepam glucuronide, it is believed that the lack of response may be due to either further degradation of the native analytes or interference with their ionization rather than incomplete hydrolysis when using the acid hydrolysis procedure.

One of the benefits of automation is the ease with which designed experiments can be performed in order to quickly optimize or compare various steps involved in the manual procedures being automated. An examination of the automated β-glucuronidase hydrolysis procedure using 0.66M acetate buffers of different pH was easily set up in MAESTRO and performed by the MPS, the MAESTRO Prep Sequence Scheduler for the experiment is shown in the box on this page. A stacked view of mass chromatograms resulting from Oxazepam determinations is also shown; as can be seen, no significant difference was found when changing the pH of the 0.66 M acetate buffer from 4.0 (A) to 4.5 (B) or to 5.0 (C).

In order to ensure that the automated hydrolysis procedure was complete and could

MultiPurpose Sampler (MPS XL) with GERSTEL DPX Option used for the automated hydrolysis and DPX-LC/MS/MS method shown mounted on top of an Agilent 6460 LC-MS/MS system without adding to the lab bench footprint.

be used within an automated DPX-LC/MS/MS method for the quantitation of analytes, standards and QC samples in urine were prepared using the glucuronide conjugated analytes Morphine-3-β-D-glucuronide or Oxazepam glucuronide and then both the native and the conjugated forms of the analytes were monitored using the previously described LC/MS/MS method. In all cases, the absence of detected response for the glucuronide conjugated analytes proved the successful complete hydrolysis of the urine sample being analyzed.

The accuracy and precision achieved for Morphine and Oxazepam using the complete automated hydrolysis-DPX- LC/MS/MS method were determined by extracting replicate (n=6) QC samples at 75 ng/mL concentrations. Accuracy data averaged 102 % for Morphine and 96.3 % for Oxazepam and the precision (% CV) was 3.52 % for Morphine and 4.70 % for Oxazepam.

Representative calibration curves for Morphine and Oxazepam are shown on the preceding page. Regression analysis for both analytes resulted in R^2 values of 0.99 or greater. The complete automated process resulted in linear calibration curves with R^2 values 0.99 or greater achieved for glucuronide conjugated analytes with LOQs of 1 ng/mL for both Morphine and Oxazepam.

Automation

Automated DPX Prep Sequence
(Cleanup procedure).

1. Aspirate 750 µL acetonitrile from the fast wash station using the 2.5 mL DPX syringe.
2. Pick up DPX tip (patented sorbent: DPX-RP-S) from the DPX Tray.
3. Add 500 µL acetonitrile through the DPX tip into the urine sample on the MPS tray.
4. Wait for 6 seconds to ensure complete wetting of the DPX sorbent with acetonitrile.
5. Aspirate the complete sample; mix sample and sorbent turbulently by aspirating 1400 µL of air into the DPX tip.
6. After an equilibration period of 5 sec: Discharge the content of the DPX tip back into the vial on the tray.
7. Bring the DPX tip to the "PipWaste" position and discard it.
8. Transfer 250 µL of the upper liquid layer in the vial to a clean 2 mL autosampler vial; Cap the vial.
9. Dilute the extract by adding 250 µL of formic acid 0.05% in water into the sample vial.
10. Inject 25 µL of the diluted sample into the HPLC injection valve.

Method parameters

Analysis conditions LC

Mobile Phase:	A: 5 mM ammonium formate in water with 0.05% formic acid B: 0.05% formic acid in methanol
LC Pump conditions:	Isocratic, 50:50 (A:B) at a flow of 0.300 mL/min
Run time:	10 Minutes
Injection volume:	2 µL (loop overflow)
Column temperature:	55 °C

Analysis conditions MS

Operating mode:	Electrospray, positive mode + Agilent Jet stream
Gas temperature:	350 °C
Gas flow (N2):	5 L/min
Nebulizer gas pressure:	35 psi
Sheath gas temperature:	250 °C
Sheath gas flow:	11 L/min
Capillary voltage:	4000 V
Nozzle voltage:	500 V

Conclusion and outlook

The automated system performed well; Fred Foster and his colleagues were able to demonstrate that the described enzymatic hydrolysis and subsequent DPX cleanup methods were successfully automated for glucuronide conjugated analytes in urine using the dual head GERSTEL MPS autosampler and sample preparation robot. Analytes were rapidly and reproducibly isolated from hydrolyzed urine samples and subsequently determined using the described automated DPX cleanup procedure coupled with introduction to LC/MS/MS based on the Agilent 6460 Triple Quadrupole Mass Spectrometer.

The automated hydrolysis method described here can be combined with other sample preparation techniques such as SPE, liquid/liquid extraction, centrifugation, and evaporative concentration etc.

This research is currently being expanded to facilitate the hydrolysis procedure in smaller liquid handling containers, such as microtiter and deepwell plates, with automated heating and shaking included. In addition to this, other enzymatic reaction possibilities (e.g., Tryptic Digestion) will be evaluated for full automation with the GERSTEL MPS system.

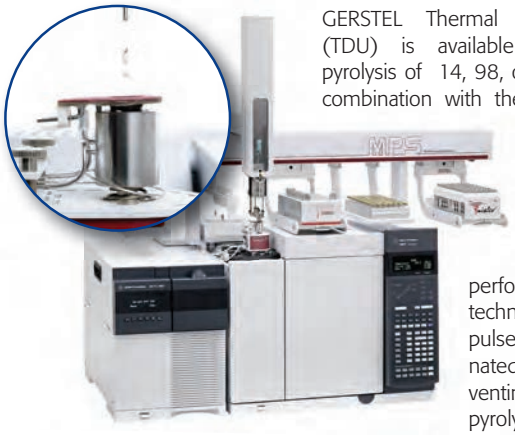
Literature

[1] F. D. Foster, J. R. Stuff, E. A. Pfannkoch, W. E. Brewer. Automated Hydrolysis, DPX Extraction and LC/MS/MS Analysis of Pain Management Drugs from Urine, GERSTEL AppNote 1/2014

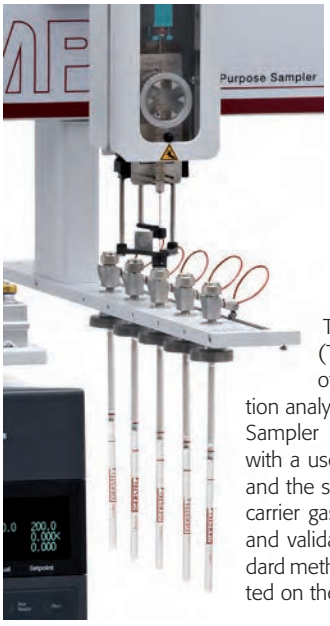
Vortex/Shaker option for the MPS

The Vortex and shaker option (ᵐVORX) for the GERSTEL MultiPurpose Sampler (MPS) performs vortexing and agitation at up to 3,000 rpm, speeding up sample preparation steps such as liquid-liquid extraction, dissolution, and homogenization. The ᵐVORX performs efficient simultaneous vortex mixing of various vial sizes and 96 well plates. The MPS automatically loads samples placed in 2, 4 and 10 mL vials. A heating option for 96 well micro-titer plates is also available. Mixing movements are finely controlled using linear motors and are restricted to the horizontal plane, allowing even the most sensitive samples to be mixed efficiently without over-agitation. Automated operation under MAESTRO software control enables reliable and flexible sample processing, the PrepAhead functionality ensures best possible productivity and throughput.

Automated Pyrolysis Option



The GERSTEL PYRO module for the GERSTEL Thermal Desorption Unit (TDU) is available for automated pyrolysis of 14, 98, or 196 samples in combination with the GERSTEL MultiPurpose Sampler (MPS). PYRO enables pyrolysis of solids and liquids at up to 1000 °C. PYRO performs the following techniques: Standard pulsed pyrolysis; fractionated pyrolysis; solvent venting combined with pyrolysis; Evolved Gas Analysis (EGA); derivatization combined with pyrolysis. Thermal desorption prior to pyrolysis enables the determination of volatile compounds and provides a clean pyrolysis chromatogram that is easy to interpret. Solvent venting can remove water prior to pyrolysis. PYRO is controlled using the MAESTRO software or integrated with the GC/MS software.



Automated spiking of standard tubes for thermal desorption (TDS/TDU)

The GERSTEL Tube Spiking System (TSS) enables automated generation of standard tubes for Thermal Desorption analysis. Using the GERSTEL MultiPurpose Sampler (MPS), adsorbent tubes are spiked with a user defined volume of liquid standard and the solvent purged with a defined flow of carrier gas as required for method calibration and validation according to international standard methods. Multiple TSS units can be mounted on the MPS for higher throughput.

Multi-Position Evaporation Station (ᵐVAP)

A six-position evaporation station (ᵐVAP) is available for the GERSTEL MultiPurpose Sampler (MPS). Samples are concentrated at user defined temperature and vacuum, enabling significantly improved limits of detection. The MPS automatically loads samples placed in 2 mL, 4 mL or 10 mL vials. Solvent exchange to a GC or HPLC compatible solvent can be performed for improved chromatography. ᵐVAP can be used in a stand-alone WorkStation in combination with SPE, Dispersive SPE (DPX) or liquid/liquid extraction to evaporate solvent from extracts. Configuration for injection into GC/MS or LC/MS is also possible. Every step is controlled by mouse-click using the MAESTRO PrepBuilder. Just one method and/or one sequence table is needed for the entire process including GC/MS or LC/MS analysis.

Automated Filtration Option

The Filtration option for the GERSTEL MultiPurpose Sampler (MPS) enables efficient automated clean-up of up to 98 samples or extracts using standard filters with Luer® fittings. Filtration can be combined with other sample preparation steps and/or with introduction to an LC-MS/MS- or GC/MS system. Without adding to the laboratory workload, filtration can significantly improve the reliability of the analysis and of the analysis system. Depending on the filter size used, up to 98 filters can be placed in the MPS tray. Liquid transfer is performed with exact control of flow and volume for highly reproducible results. MAESTRO software offers simple and efficient control by mouse-click, requiring no macro programming.

Microwave Prep

The sample preparation portfolio of the GERSTEL MultiPurpose Sampler MPS has been expanded to include advanced microwave technology for accelerated solvent extraction and rapid chemical reactions. A recent application example is automated saponification of fats in food in combination with esterification and FAME analysis by GC. The MPS including microwave can be coupled directly to a GC/MS or LC/MS system or used in stand-alone mode.

TDS 3C: Thermal desorption the cooler way

The GERSTEL Thermal Desorption System (TDS) is an accepted standard system used world-wide by leading companies to perform standardized test methods in the automotive, semiconductor and Toy Safety fields and widely in the food, flavor and fragrance and packaging industries among many others. The TDS 3C represents the latest addition to the extensive GERSTEL thermal desorption product line. It can be cooled using a cryostatic cooling device for simpler handling when only moderate trap temperatures are required.

Online SPE system: SPE^{XOS}

GERSTEL SPE^{XOS} performs online SPE with automated cartridge exchange. SPE^{XOS} uses small cartridges, which are inserted directly into the HPLC mobile phase for elution resulting in quantitative transfer of analytes to the HPLC column. Much smaller sample sizes can be used to reach required detection limits and much less solvent is needed, for example, for analyte elution, increasing the concentration factor and reducing both cost and environmental impact.

In combination with the GERSTEL MPS and MAESTRO software, liquid sample preparation, clean-up and LC-MS/MS analysis can be performed in one integrated system operated with one integrated sequence table. The Prep-Ahead functionality with multi-sample overlap ensures maximum efficiency and throughput for the complete system, including the LC-MS/MS.

GERSTEL MPS robotic^{PRO}

MPS robotic^{PRO}, the newest member of the GERSTEL MultiPurpose Sampler (MPS) family, is a highly efficient GC/MS autosampler, optimized to hold a significantly larger number of samples for higher throughput. MPS robotic^{PRO} provides fast, reliable and highly accurate processing of complex tasks. Tools can be exchanged automatically, enabling automated change between Liquid Injection, Headspace, and SPME. MPS robotic^{PRO} is controlled by the GERSTEL MAESTRO software enabling PrepAhead productivity under integrated GC/MS system control. Priority samples can be added at any time without stopping the ongoing analysis sequence. Fast and error-free set-up of the daily analysis sequence is performed using the intelligent sequence editor functions or by importing it directly from your LIMS system.

More than one Twister needed: Move up to the Twicester

A cooperation between GERSTEL K.K., Tokyo, Japan and the Research Institute for Chromatography (RIC) in Belgium has produced the Multi-Stir Bar Sorptive Extraction (^{m}SBSE) technique. ^{m}SBSE has been used successfully for the determination of a variety of flavor compounds in aqueous matrices such as green tea. Key elements of the technique are to use PDMS and EG-Silicone Twisters simultaneously in one sample vial and to desorb these together in the TDU for combined and comprehensive analysis. While the PDMS Twister stirs the sample and extracts non polar to medium polarity analytes, one or more EG-Silicone Twisters are kept stationary at the vial wall by a special holder. The EG-Silicone Twisters extract polar analytes, especially compounds with -OH or -NH functional groups. The Twicester technique enables the determination of a wide range of analytes of different polarities in one extraction step and one GC/MS run.

Controller C200

The compact GERSTEL C200 controller offers heating and cooling control including temperature ramps for GERSTEL modules such as the Cooled Injection System (CIS) PTV-type GC inlet, the Cryo Trap System (CTS) for multidimensional GC, the GERSTEL Thermal Extractor (TE 2), as well as the market leading GERSTEL Olfactory Detection Port (ODP). Multiple cycles with temperature ramps can be performed for highly efficient conditioning of up to 50 GERSTEL Twisters or 10 adsorbent tubes in the GERSTEL Tube Conditioner (TC). The C200 can be operated standalone using the scroll wheel at the front or through the GERSTEL MAESTRO software. The system status is clearly indicated through the colored light in the scroll wheel.

Preview - coming up in the next issue

Focus on flavor & fragrance analysis and product safety

Pleasant flavors and fragrances applied to products can improve consumer acceptance and increase consumer binding, improving sales. This makes it easy to explain the increased use of flavors and fragrances in consumer products, food and beverages. When applied to car interiors, fragrances can enhance the feel of the drive supposedly making it a better, maybe even a more relaxed experience. Little wonder then that industry expends considerable amounts of resources to identify flavor compounds with good properties and to extract and reproduce them in the laboratory. It is equally clear that the human nose plays a central role in the final assessment of the quality of flavors and fragrances for any practical application. To match the highly sensitive human nose, highly sensitive chemical analysis is required in this field in order to document and control the quality of odorized products. Especially gas chromatography in combination with mass selective detection and olfactory detection GC-MS/ODP is a key tool. How else would one be able to sniff out and provide reliable quantitative results on odors and their sources? Incidentally, this applies not only to pleasant odors, but certainly equally to off-odors, compounds that smell unpleasant or even stink making consumers reject a product. In the next GERSTEL Solutions worldwide Magazine, we shed light on flavor and fragrance analysis as well as on the determination of off-odors. In addition, we report on concentration techniques used to determine pesticides without the risk of carry-over as well as on the efficient and sensitive determination of mycotoxins in food.

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45473 Mülheim an der Ruhr, Germany

Editorial director
Guido Deussing
Uhlandstrasse 16
41464 Neuss, Germany
guido.deussing@pressetextkom.de

Scientific advisory board
Dr. Eike Kleine-Benne
eike_kleine-benne@gerstel.de
Dr. Oliver Lerch
oliver_lerch@gerstel.de
Dr. Malte Reimold
malte_reimold@gerstel.de

Translation and editing
Kaj Petersen
kaj_petersen@gerstel.com

Design
Paura Design, Hagen, Germany
www.paura.de

GERSTEL worldwide solutions

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GERSTEL GLOBAL ANALYTICAL SOLUTIONS

GERSTEL, Inc., USA
+1 410 - 247 5885
sales@gerstelus.com

GERSTEL GmbH & Co. KG, Germany
+49 208 - 7 65 03-0
gerstel@gerstel.com

GERSTEL K.K., Japan
+81 3 57 31 53 21
info@gerstel.co.jp

GERSTEL BRASIL
+55 11 5665 8931
gerstel-brasil@gerstel.com

GERSTEL AG, Switzerland
+41 41 - 9 21 97 23
swiss@ch.gerstel.com

GERSTEL LLP, Singapore
+65 6622 5486
sea@gerstel.com

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