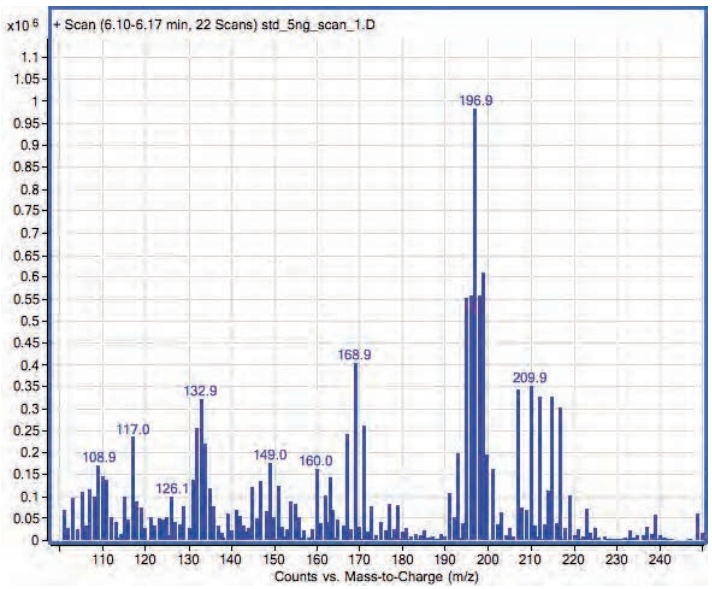
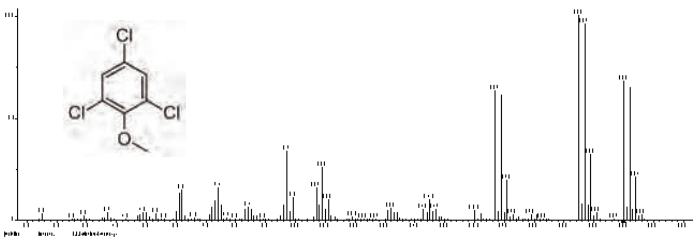
"Corked" pills – no thanks!

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).

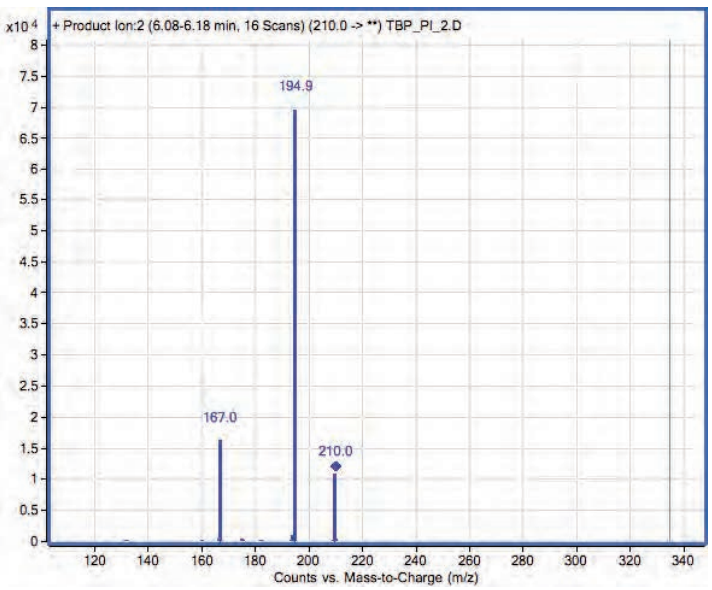
When it comes to quality control, the devices given to us by Mother Nature are hard to beat. Anything we ingest passes through several sophisticated anatomically based inspection steps. First a visual inspection, then a sensory evaluation of "mouth feel" combined with the taste sensed on the tongue and 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.