



Flavor, Fragrance, and Odor Analysis

A new Twist: Free acids and phenols

Sensitive SBSE determination of free acids and phenols - along with the usual suspects

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Stir Bar Sorptive Extraction (SBSE) and Headspace Sorptive Extraction (HSSE) have regularly been applied successfully to the analysis of odor-active chemicals in a wide variety of sample matrices at concentrations as low as ultra-trace levels. The addition of the Ethylene Glycol-Silicone (EG-Silicone) Twister® has significantly enhanced the usefulness of these techniques by enabling the efficient extraction of polar hydrogen bond donors like phenols and carboxylic acids – important odor and flavor compounds. Various SBSE and HSSE extraction techniques using different combinations of PDMS and EG-Silicone Twisters were used to analyze samples discussed in this ar-

article. Versatile application of Twister technology combined with Thermal desorption – GC/MS and peak deconvolu-

tion are adding up to a significant analytical tool-kit to assist the flavor chemist in off-flavor and mal-odor elucidation, detecting both non-polar and polar analytes spanning a wide range of volatilities and concentrations.

Examples presented in this article include the determination of off odors in Beer, Pretzels, and Casein, a widely used food ingredient. Last but not least, axillary off odors were determined in well-worn T-shirts to determine the odor-reducing impact of antibacterial fabrics.

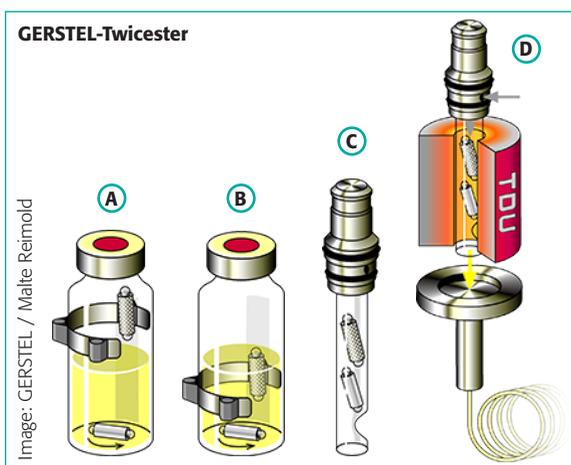


Figure 1: Schematic diagram of the multi stir bar extraction process using Twicester® (A) Magnetic positioning of up to three Twisters using Twicester Showing 1 Twister in HS & 1 stirring (B) SBSE using two Twisters submerged in liquid (C) Transfer of Twisters to TDU Tube (D) Simultaneous thermal desorption of the Twisters, cryofocusing in the CIS and GC/MS analysis.

A simple, solvent free, quantitative, and sensitive analytical method for studying off-flavor development in beer based on SBSE using the GERSTEL PDMS Twister has previously been published [1]. Beer contains dozens of odor active chemicals in concentrations in the parts per billion range. Developing an analytical technique to cover such a wide range of volatiles and concentration levels is challenging. In the work presented here, recently developed SBSE techniques were combined with gas chromatography-time of flight mass spectrometry (GC-TOFMS) incorporating peak deconvolution software.

Beer off-flavor chemicals

The aim was to determine which approach would be most appropriate for detecting low levels of potential off-flavor chemicals in aged beer. Four Twister methods were used to analyze Blue Moon beer, a Belgian-style wheat ale. Ten milliliters of beer were extracted in all cases:

- One PDMS Twister (1 cm x 0.5 mm) submerged in the beer inside a sealed 20 mL vial, stirring at 900 rpm for 2 hours;
- Same as (a) with a second PDMS Twister submerged in the beer, attached to the inside of the vial using a GERSTEL Twicester® magnet clip as seen in Figure 1;
- Sequential SBSE with two PDMS Twisters (1 cm x 0.5 mm) stirred in sample for 1hr [2], followed by salt addition (20%) and stirring for an additional hour; and finally

- Extracting the beer sample with one immersed PDMS Twister used for stirring and one EG-Silicone Twister attached to the inside of the vial submerged in the beer using Twicester®.

Our results show that using the combination of the PDMS and EG-Silicone Twisters enabled significantly more sensitive determination of polar compounds including carboxylic acids compared with the other methods. In figure 2, peak areas for fourteen odor-active compounds are shown (normalized to PDMS + EG-Silicone results) for the four SBSE methods used on the same Blue Moon beer. For all but one of the compounds, (2-methyl-2-pentenoic acid), the PDMS + EG-Silicone method provided the highest recovery. Sequential SBSE was the second most favorable. The higher recoveries for carboxylic acids with the PDMS + EG-Silicone method make the Twister combination method an excellent choice for the determination of this important difficult-to-detect class of compounds. The compounds in question are: 4-vinyl guaiacol

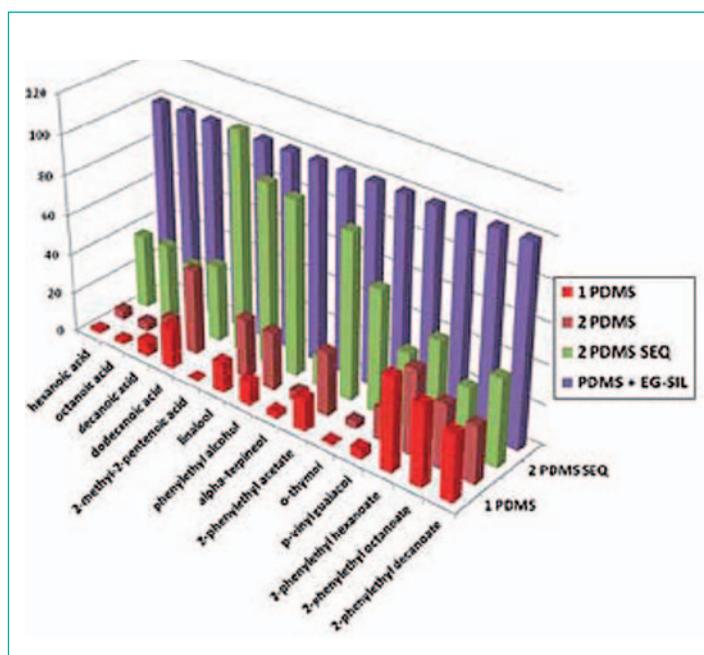


Figure 2: Comparison of four SBSE extraction techniques for odor-active chemicals in beer (normalized to PDMS + EG-Silicone peak areas).

(clove flavor); linalool (floral); phenylethyl alcohol (rose); *alpha*-terpineol (pine, woody); 2-phenylethyl acetate (floral, honey); *o*-thymol (woody, camphor); and 2-phenylethyl esters of hexanoic, octanoic, and decanoic acids.

Axillary malodors in clothing fabrics

An understanding of human body odor chemicals may be desirable for many reasons. For example, knowledge of the composition of VOC metabolite excretions may be a useful health-related diagnostic tool. Our studies were undertaken to determine the ability of new fabrics to minimize axillary malodors.

Instrumentation

The thermal desorption-gas chromatography/mass spectrometry (TD-GC/MS) analysis was performed using a GERSTEL Thermal Desorption Unit (TDU) combined with a MultiPurpose Sampler (MPS) and a GERSTEL Cooled Injection System (CIS 4) programmed temperature vaporization (PTV) type inlet. Two GC-MS systems were used: an Agilent 7890A GC with an Agilent 5973B MSD and a GC-TOF/MS system.

GC parameters

Column: DB-5MS (30 m x 0.25 mm x 0.25 μm).
 Temperature program: 40 °C (3 min), 10 °C/min to 270 °C (10 min).
 Carrier gas flow: 1.0 mL/min, splitless.
 The same GC conditions were used for DHS and SPME.
 The same column type and GC parameters were used on both the Agilent and Leco systems.

Thermal desorption parameters

GERSTEL CIS 4: PTV Solvent Vent mode at a flow of 50 mL/min. Initial temperature -100 °C (0.50 min) ramped to 280 °C (3.0 min). GERSTEL TDU: Initial temperature 30 °C (0.40 min) 60 °C/min to 280 °C (4.00 min hold time) when only PDMS Twisters were used. A final temperature of 220 °C (4.0 min hold time) was set when EG-Silicone Twisters were used. TDU transfer line temperature: 300 °C.

Materials

GERSTEL Twisters used were PDMS 1 cm x 0.5 mm; PDMS 2 cm x 0.5 mm; and EG-Silicone Twister. A GERSTEL Twicester® magnetic clip was used to hold multiple Twisters inside the sample vial.

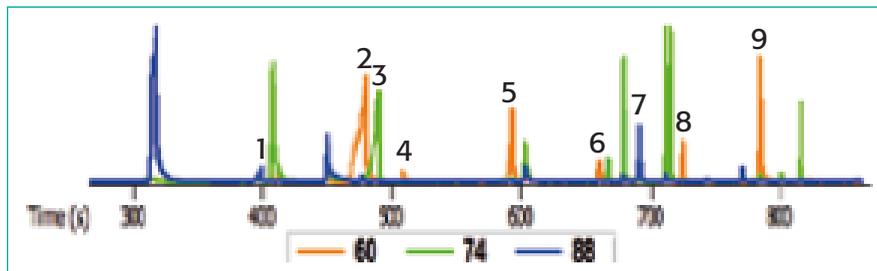


Figure 3: Chromatogram of cotton shirt worn during exercise and analyzed by an HSSE dual Twister method (1 PDMS + 1 EG-Silicone). Masses plotted for 60, 74 and 88 amu. Peak identities are: (1) isobutanoic acid; (2) 3-methyl butanoic acid; (3) 2-methyl butanoic acid; (4) pentanoic acid; (5) hexanoic acid; (6) heptanoic acid; (7) 2-ethyl hexanoic acid; (8) octanoic acid; (9) nonanoic acid. None of these axillary malodor chemicals were present in the same shirt prior to exposure to an exercising person.

Malodors form various sites on the body when natural secretions are converted to volatile odorous chemicals through microbial activity. Short chain volatile fatty acids VFAs (C_2 - C_5) are the primary axillary malodors formed; 16-androstene steroids and thioalcohols have also been identified as contributors along with 3-Methyl-2-hexenoic acid (3M2H) [3]. Following the successful combined PDMS and EG-Silicone Twister extraction of volatile organic acids from beer, the same Twister combination was used for analyzing functional T-shirts treated with antimicrobial chemicals and contaminated with perspiration to monitor production of various axillary malodor chemicals.

Experimental

The combination Twicester® approach was investigated using HSSE. One gram of soiled fabric was suspended from a paper clip protruding through the septum of a 40 mL glass vial. A Teflon coated micro-stir bar was placed at the bottom of the vial (to agitate the air in the vial) and one 1 cm x 0.5 mm PDMS Twister and one EG-Silicone Twister were attached to the sides of the vial with magnetic clips (GERSTEL Twicester®). The Teflon stir bar was stirred at 800 rpm, and the vial was thermostated at 50°C. Extraction was conducted for 2 h. The two Twisters were removed from the vial and desorbed in the GERSTEL TDU using the method parameters previously described for the beer analysis except with a maximum desorption temperature of 220 °C (instead of 280 °C) to protect the EG-Silicone Twister from thermal decomposition.

Results and discussion

Several volatile acids were detected at low ppb ($\mu\text{g/L}$) levels from the sweat residue on worn T-shirts. These included acetic acid, propanoic acid, isobutanoic acid, 3-methyl butanoic acid, 2-methyl butanoic acid, pentanoic acid, hexanoic acid, 3-methyl-2-hexenoic acid, octanoic acid, and

nonanoic acid. In addition to these acids, several pyrazines were detected along with 2-nonenal, an unsaturated aldehyde with an unpleasant greasy and grassy odor, which was primarily found in subjects 40 years old or older. Results from one study indicate that 2-nonenal is generated by the oxidative degradation of omega-7 unsaturated fatty acids and suggest that 2-nonenal may be involved in the age-related change of body odor [4]. According to the study, the change of the monounsaturated fatty acid composition of skin surface lipids and the increase of lipid peroxides associated with aging may be involved in the formation of this characteristic odor component.

Table I shows results for unsoiled control cotton shirt samples spiked with five levels of various volatile acids and 2-nonenal ranging from 10 to 500 $\mu\text{g/L}$. Excellent least square correlation coefficient and detection limits are demonstrated for all acids and 2-nonenal. Figure 3 shows a chromatogram of axillary malodor volatile acids extracted from a soiled shirt by HSSE with PDMS and EG-Silicone Twisters.

A separate SBSE method was developed to determine androstenone, in which the PDMS Twister was desorbed at 270 °C for 5 min (instead of 220 °C for 5 min used for the EG-Silicone Twister-based work). It is worth remarking that Soini et al. [5] developed a novel tool for using a PDMS Twister to extract volatile organic malodorants directly from the skin. The stir bar, which is attached to a miniaturized holder that looks like a miniature paint roller, is rolled over a determined area of a human skin while collecting a representative sample of VOCs from the skin surface.

Table I: Calibration curve results for five concentrations of axillary malodorant standards in the 10 to 500 ppb ($\mu\text{g/L}$) range analyzed by HSSE method with 1 PDMS + 1 EG-Sil Twister. XIC = extracted ion mode; SIM = selected ion mode.

Standard	Mass Ion	Detection Limit (ppb)	Linear Least Squares Correlation Coefficient	Analytical Method
butyric acid	60 (XIC)	5	0.9921	HSSE
isovaleric acid	60 (XIC)	5	0.9731	HSSE
pentanoic acid	60 (XIC)	5	0.9737	HSSE
hexanoic acid	60 (XIC)	5	0.9972	HSSE
octanoic acid	60 (XIC)	10	0.9911	HSSE
2-methylbutanoic acid	74 (XIC)	5	0.9655	HSSE
3-methyl-2-hexenoic acid	113 (XIC)	10	0.9901	HSSE
2-nonenal	70 (XIC)	5	0.9900	HSSE
Androstenone	272 (SIM)	0.4	0.9914	SBSE

Complaint Casein Powders with Musty Off-Flavor

Due to its flavor stability and functional properties, casein powder is commonly used in cheese analogues, bakery products, meat products, confectionery products, desserts, nondairy coffee creamers, and beverages. Several complaint casein powders from various international food and beverage companies were received in our laboratory. These had extreme musty taints and were implicated as causes of expensive product recalls involving cereal bars, nutritional beverages, a chocolate-flavored beverage, and nondairy coffee creamers in the U.S.

Musty off-flavors are commonly associated with microbial metabolites such as trihaloanisoles, geosmin (trans-1,10-dimethyl-trans-9-decalol), 2-methylisoborneol, and 2-isopropyl-3-methoxy pyrazine. These compounds have such low odor detection thresholds that they must be detected in the ng/L range making determination challenging (2,4,6-trichloroanisole odor threshold: 0.15-2.0 ng/L; geosmin: 1-10 ng/L). These compounds have relatively large log Ko/w values, however, allowing PDMS Twisters to extract them very efficiently from an aqueous sample or aqueous suspension of a solid sample. Indeed, SBSE GC-TOFMS was found to be a sensitive and simple technique for the determination of these compounds in casein powders.

Experimental

1 g of casein + 25 mL water was stirred 30 min at room temperature with a Teflon coated stir bar, which was then replaced with a 2 cm x 0.5 mm PDMS Twister and stirred for three hours. The Twisters were thermally desorbed in a GERSTEL TDU using the parameters previously indicated.

Results and discussion

The primary musty off-flavor chemicals found in the casein were trichloroanisole isomers formed by microbial methylation of 2,4,6-trichlorophenol, a common ingredient in fungicides, pesticides, and wood preservatives. In extracted ion chromatograms at 212, 195, 196 and 161 amu, haloanisole peaks were readily apparent. The major haloanisoles identified were 2,4-dichloroanisole, 2,4,6-trichloroanisole (at 7 µg/L), and 2,3,6-trichloroanisole contaminated with coeluters.

In resolving the product recall lawsuits, it was important to provide supporting evidence that the substrate 2,4,6-trichlorophenol was also present in the complaint caseins. The source of the trichlorophenol was found to be the wooden shipping pallets that the bags of casein were stacked on during overseas shipment.

Interestingly, testing of additional complaint casein samples revealed the presence of 2,4,6-tribromoanisole (2,4,6-TBA), the source of which was identified as the slip sheets used when stacking the bags of casein during transport. These sheets were manufactured from recycled plastic, mainly high-density polyethylene, contaminated with tribromophenol (TBP), likely stemming from the use of organobromine flame retardants, such as polybrominated diphenyl ethers (PBDEs).

Standard calibration curves for 2,4,6-TBA demonstrated correlation coefficients (R² values) greater than 0.99. For TBA testing, samples were spiked with deuterated TBA at 133 µg/L. Stable Isotope Dilution Analysis (SIDA) is arguably one of the most accurate quantitation techniques for the determination of organic compounds by GC-MS.

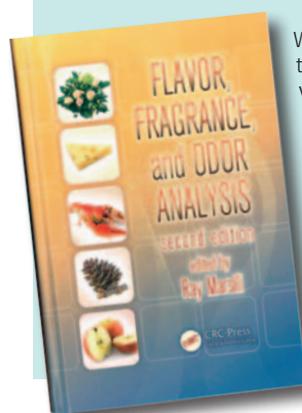
Conclusion

The application examples presented in this article demonstrate that SBSE and HSSE have been successfully extended to a wide variety of sample matrices. The new polar phase EG-Silicone Twister has significantly extended the application potential of SBSE and HSSE to include analytes that were difficult to extract at high recoveries with PDMS. Twister technology combined with thermal desorption-GCMS can be applied to a broad spectrum of sample types using SBSE and HSSE, making the Twister a significant analytical tool to assist the flavor chemist in off-flavor and malodor elucidation of a wide profile of non-polar and polar analytes covering a broad range of analyte volatilities and concentrations.

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Suggested reading



Written from a practical perspective, the second edition of Flavor, Fragrance, and Odor Analysis highlights the powerful SBSE technique and emphasizes the range of applications available.

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