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Analysis of Beverage Samples using Thin Film Solid Phase Microextraction (TF-SPME) and Thermal Desorption GC/MS

John R. Stuff[1], Jackie A. Whitecavage[1], Jonathan J. Grandy[2], and Janusz Pawliszyn[2]

[1]GERSTEL, Inc., 701 Digital Drive, Suite J, Linthicum, MD 21090, USA
[2]University of Waterloo, Department of Chemistry, 200 University Avenue, Waterloo, ON, N2L 3G1, Canada

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Abstract
Alcoholic and non-alcoholic beverages are enjoyed by millions of people around the globe. The aroma and flavor profiles of each product are unique and made up from a variety of semi-volatile and volatile compounds including aldehydes, ketones, acids, alcohols, terpenes, esters along with various trace level compounds. Monitoring of these compounds is a necessity for beverage manufacturers in order to ensure consistent product quality. Analysis may also be necessary due to off flavor or odor complaints. The wide range of concentrations, polarities, and functional groups used in the composition of a flavor/aroma profile can make the analysis of the sample difficult. Techniques which are simple, use little or no solvent, and encompass a wide range of analytes are desirable.

Thin Film Solid Phase Micro-Extraction (TF-SPME) is an extension of regular SPME. TF-SPME is more sensitive than regular SPME due to the increased surface area and phase volume, both of which lead to improved analyte recovery. The TF-SPME device is a 20 mm x 4.8 mm carbon mesh sheet impregnated with a sorptive phase. The TF-SPME devices can be used in headspace or immersion mode. In headspace mode, the TF-SPME device is suspended above a solid or liquid sample in an enclosed vial. In immersive mode, it is placed directly in a liquid sample. In both cases, the sample is agitated by stirring. After extraction, the devices are blotted dry and placed in an empty thermal desorption tube. They are analyzed by thermal desorption GC/MS, a technique that is simple to use and requires no solvent.

The work presented here demonstrates the application of TF-SPME to the determination of aroma and flavor components in alcoholic and non-alcoholic beverages.

Introduction
In this study, Thin Film Solid Phase Micro-Extraction (TF-SPME) with a divinylbenzene/polydimethylsiloxane coating (DVB/PDMS) is used to extract flavor components from a variety of beverage samples. Monitoring of key flavor compounds is important for quality control. Also, identification of off flavor notes can help pinpoint product defects.

Experimental

Instrumentation

GERSTEL LabWorks Platform on Agilent 7890 GC/5977B Inert plus MSD (Agilent Technologies, Inc.).

Analysis Conditions LabWorks Platform

SPME	PDMS/DVB; 65 µm, 23 gauge, 1 cm	
TF-SPME	PDMS/DVB	
TDU 2	splitless	
CIS 4	solvent vent (60 mL/min)	
	split 30 mL/min	
	SPME:	250 °C isothermal
	TF-SPME:	-120 °C (0.2 min), 12°C/s to 275 °C (3min)

Analysis Conditions GC

Agilent 7890 GC

Pneumatics	He, constant flow, 1 mL/min	
Column	30 m DB-5 MS UI (Agilent)	
	d_i = 0.25 mm	d_f = 0.25 µm
Oven	40 °C (1 min), 10 or 15 °C/min to 80 °C (3 min)	

Analysis Conditions MS

Agilent 5977B

MSD	full scan, 40-350 amu

Sample Preparation

All samples were purchased at a local store.

Headspace TF-SPME

A 5 mL aliquot of undiluted sample was pipetted into a 20 mL screw capped vial and a stir bar was added. A TF-SPME device was suspended in the headspace of the vial and the vial was capped and placed on a GERSTEL Twister 20 stir plate. The sample was extracted for 60 minutes while being stirred at 1000 rpm. Up to 20 samples can be extracted simultaneously. After extraction, the TF-SPME device was removed and was placed in an empty TDU tube with a glass wool plug at the base, which was placed in a 40 position Twister rack on the MPS robotic for automated analysis.

Immersion TF-SPME

A 10 mL aliquot of the undiluted sample was pipetted into a 10 mL screw capped vial. A GERSTEL Twister stir bar (10 mm x 0.50 mm) was added. A TF-SPME device was suspended in the vial and the vial was placed on a GERSTEL Twister 20 stir plate. Up to 20 samples can be extracted simultaneously. The sample was extracted for 60 minutes while being stirred at 1000 rpm. After extraction, the TF-SPME device was removed, dried, and was placed in an empty TDU tube with a glass wool plug at the base. The TDU tube was sealed with a transport adapter and placed in a 40 position Twister rack on the MPS robotic for automated analysis.

Results and Discussion

The instrumentation used for this analysis is the GERSTEL LabWorks Platform, which is perfectly suited for GC/MS sample introduction in combination with Thin Film SPME (TF-SPME). Figure 1 shows an assembled TF-SPME device with holder inside a 10 mL vial. A stir bar was added to agitate the sample. Following sample extraction, the device is dried and placed in a thermal desorption tube. Figure 2 shows a TF-SPME device placed inside a TDU tube. Several examples of the analysis of beverage samples are shown in the following.

Figure 1: TF-SPME device in 10 mL vial.

Figure 2: TF-SPME device in TDU tube.

Figure 3 shows a stacked view of Total Ion Chromatograms (TICs) of a white wine, blended from Sauvignon Blanc and Gewurztraminer varietals, extracted using a conventional SPME fiber (top) and TF-SPME device (bottom). The TF-SPME device shows a much higher response relative to the fiber with the same phase, especially for compounds eluting early in the chromatogram. Several classes of compounds are identified in the figure. These include alcohols and ethyl esters. A Sauvignon Blanc wine imparts citrus, grass, herbal and mineral flavors. Gewurztraminer flavors and aromas include floral, fruity and spicy. The alcohols and ethyl esters identified impart the floral and fruity flavors which are found in this type of wine.

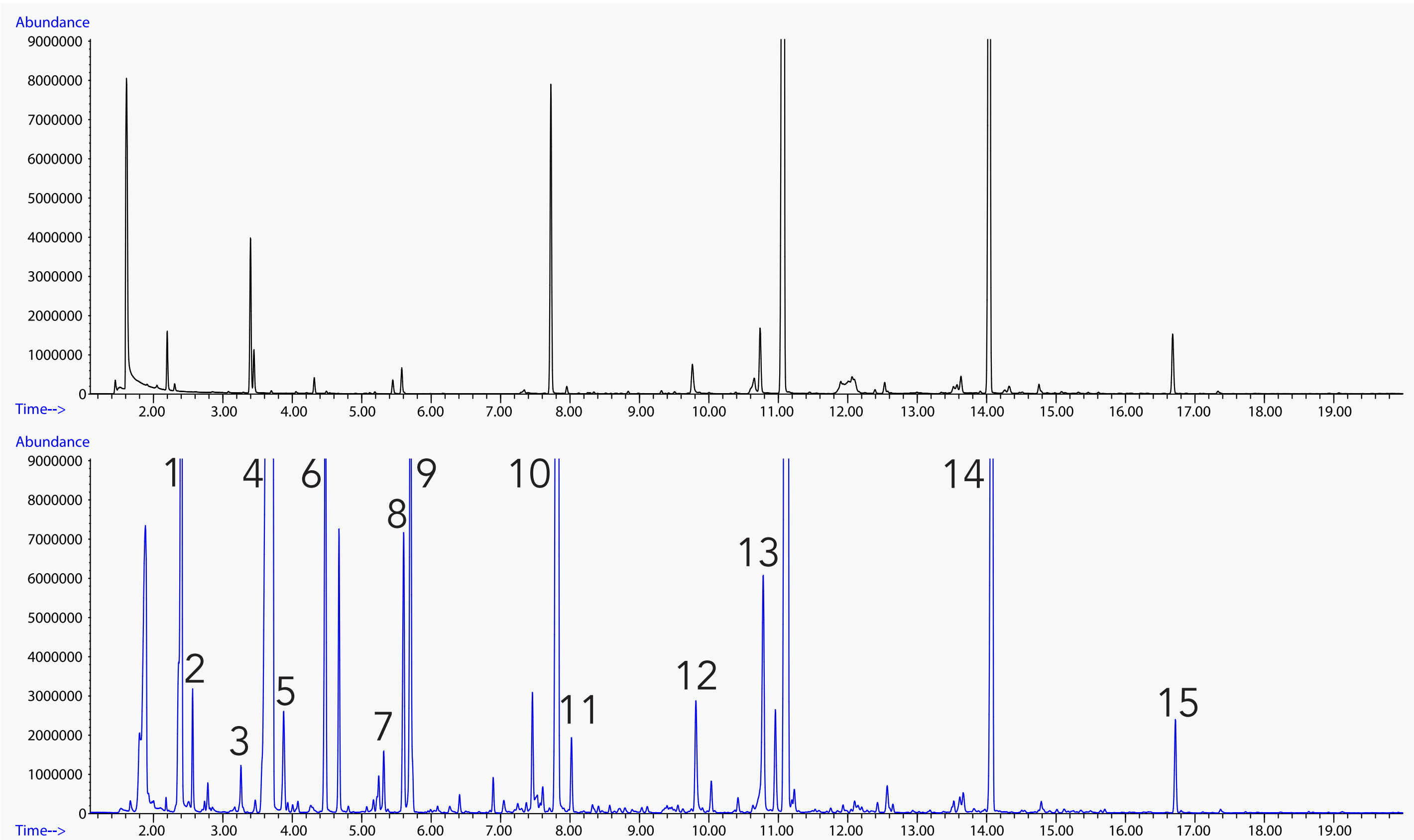

Figure 3: Stacked view of TICs for a Sauvignon Blanc and Gewurztraminer blend wine using SPME (top) and TF-SPME (bottom).

Table 1: List of compounds for figure 3.

Peak #	Compound	Log $K_{o/w}$	Peak #	Compound	Log $K_{o/w}$
1	Ethyl acetate	0.86	9	Isoamyl acetate	2.26
2	2-Methyl-1-propanol	0.77	10	Ethyl hexanoate	2.83
3	Ethyl propanoate	1.36	11	Hexyl acetate	2.83
4	3-Methyl-1-butanol	1.26	12	Phenylethyl alcohol	1.57
5	Ethyl isobutyrate	1.77	13	Diethyl succinate	1.39
6	Ethyl butyrate	1.85	14	Ethyl decanoate	4.79
7	Ethyl isovalerate	2.26	15	Ethyl dodecanoate	5.78
8	1-Hexanol	1.82			

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Figure 4 shows the TIC of a cocoa flavored coffee sample extracted by TF-SPME in headspace mode. The numbers in the figure correspond to the compounds in table 2. Ten compounds with Log $K_{o/w}$s ranging between 0.16 - 2.13 were identified. Some of these compounds include pyridine and pyrazines, which contribute to the characteristic aroma and flavor in coffee. Methyl pyrazine imparts a nutty odor and nutty roasted flavor, while 2,6-dimethyl pyrazine imparts a chocolate odor and nutty flavor. Trimethyl pyrazine imparts a nutty odor and musty flavor. Compounds with Log Kow less than 2 are normally difficult to extract with a 100% PDMS device. Using the PDMS/DVB TF-SPME device, these compounds are easily detected.

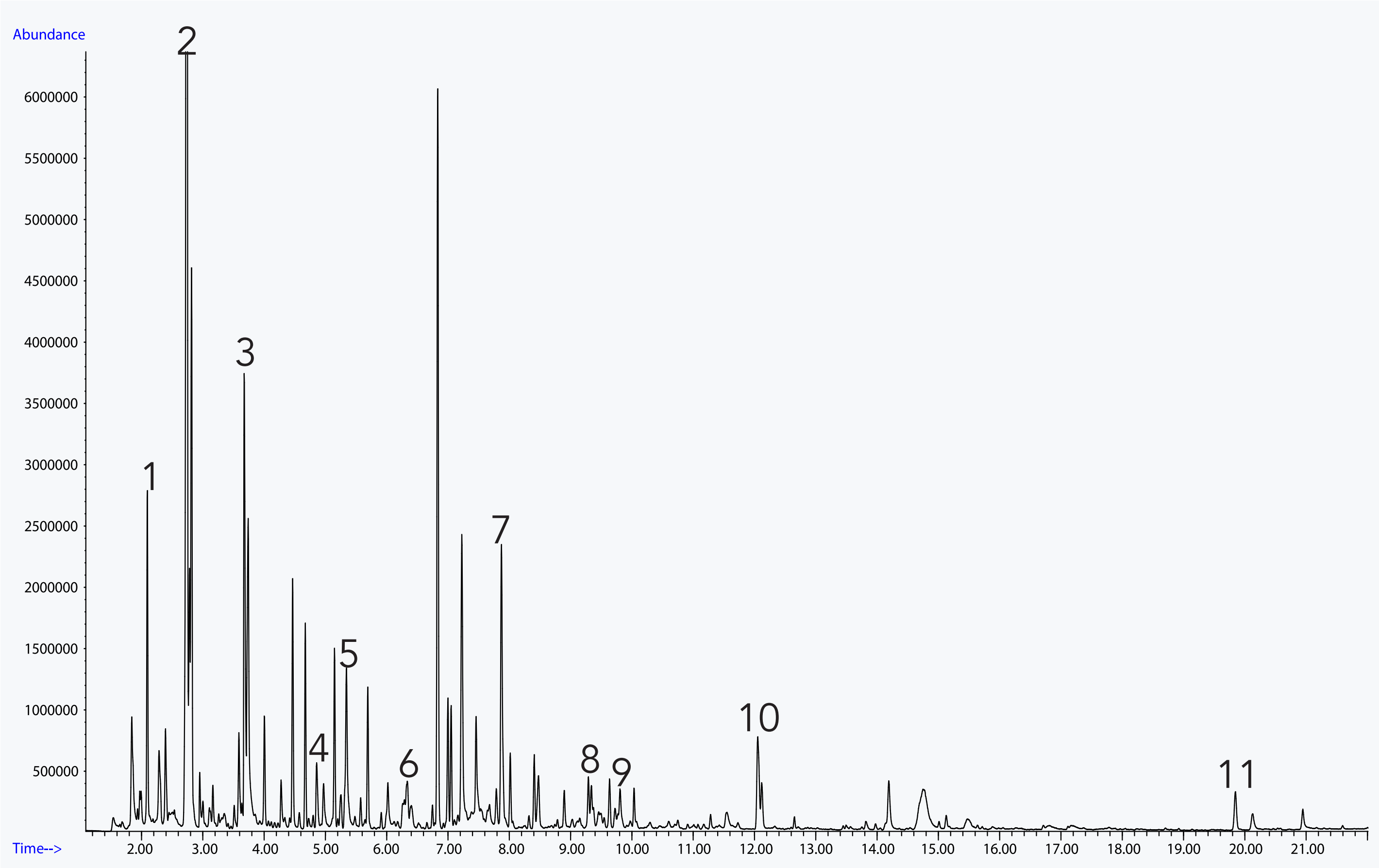

Figure 4: TIC resulting from headspace TF-SPME extraction of cocoa flavored coffee.

Table 2: List of compounds for figure 4.

Peak #	Compound	Log K_{ow}	Peak #	Compound	Log K_{ow}
1	2-Methyl propanal	0.74	7	Trimethyl pyrazine	1.58
2	3-Methyl butanal	1.23	8	Tetramethyl pyrazines	2.13
3	Pyridine	0.8	9	Phenylethyl alcohol	1.57
4	Methyl pyrazine	0.49	10	γ-Octalactone	1.59
5	2-Furanmethanol	0.45	11	Caffeine	0.16
6	2,6-Dimethyl pyrazine	1.03			

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Figure 5 shows the TIC of a red wine sample extracted by TF-SPME in headspace mode. Table 3 lists the compounds identified in the figure. Compounds contributing to the aroma and flavor in red wine with log $K_{o/w}$'s ranging between -0.36 - 2.79 are listed in the table. The TF-SPME device does an excellent job, extracting a wide range of compounds. Some with very low log $K_{o/w}$ values are important flavor compounds, such as 2,3-butanediol.

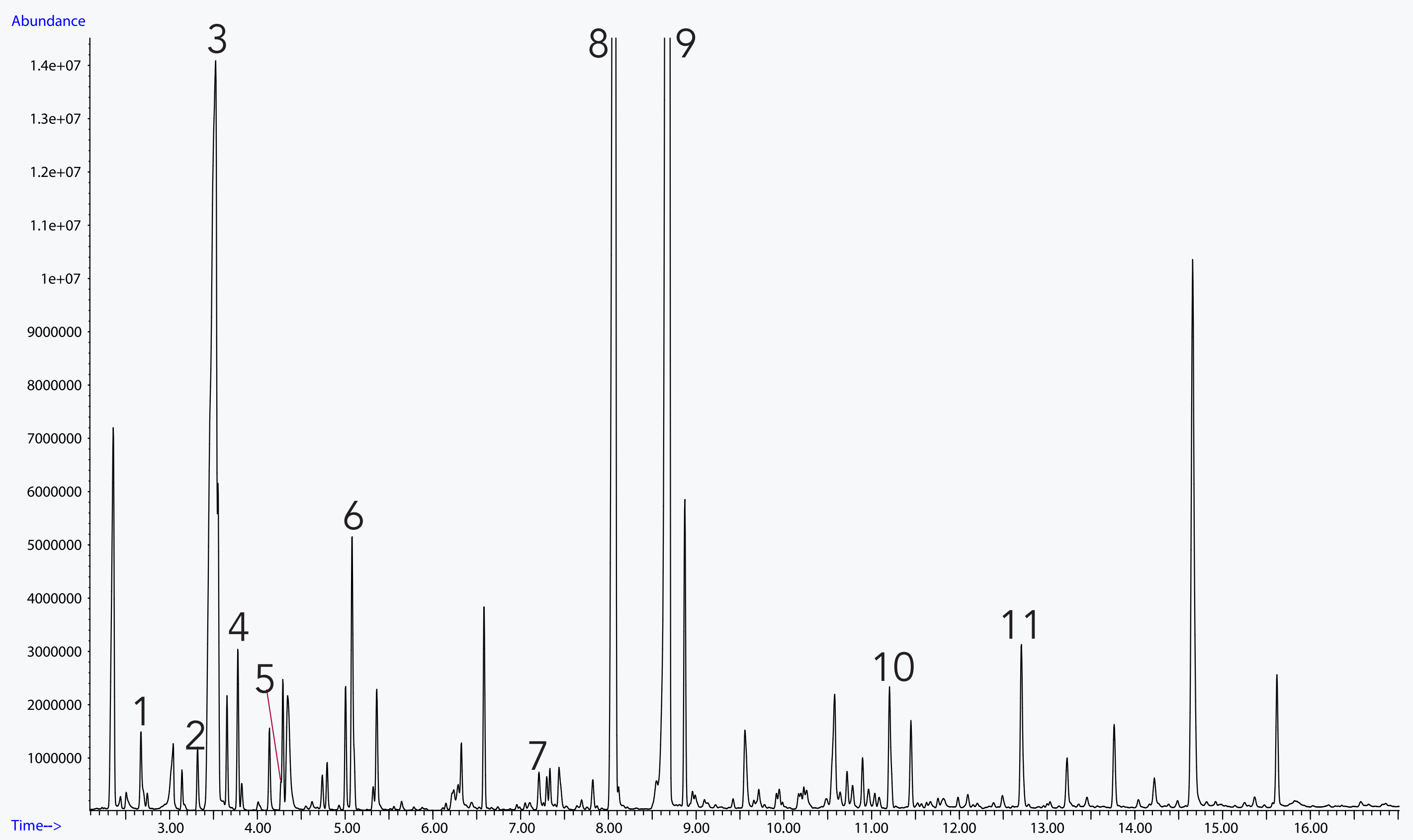

Figure 5: TIC resulting from headspace TF-SPME extraction of a red wine.

Table 3: List of compounds for figure 5.

Peak #	Compound	Log $K_{o/w}$	Peak #	Compound	Log $K_{o/w}$
1	3-Methylbutanal	1.23	7	Benzeneacetaldehyde	1.54
2	Ethylpropionate	1.36	8	Phenylethyl alcohol	1.57
3	3-Methyl-1-butanol	1.26	9	Diethyl succinate	1.39
4	Ethyl isobutyrate	1.77	10	Ethyl-3-methylbutyl succinate	2.79
5	2,3-Butanediol	-0.36	11	Ethyl vanillate	2.31
6	Isoamyl acetate	2.26			

Figure 6 shows the TIC of a breakfast blend coffee sample extracted using immersion TF-SPME. Several classes of flavor compounds, including alcohols, aromatics and phenols, are listed in table 4. These compounds are important contributors to the aroma and flavors in coffee. For this analysis, a GERSTEL PDMS Twister was used to agitate the sample, the additional stationary phase helped to increase the extraction efficiency. The Twister and TF-SPME device were desorbed in the same TDU tube resulting in a combined GC/MS run.

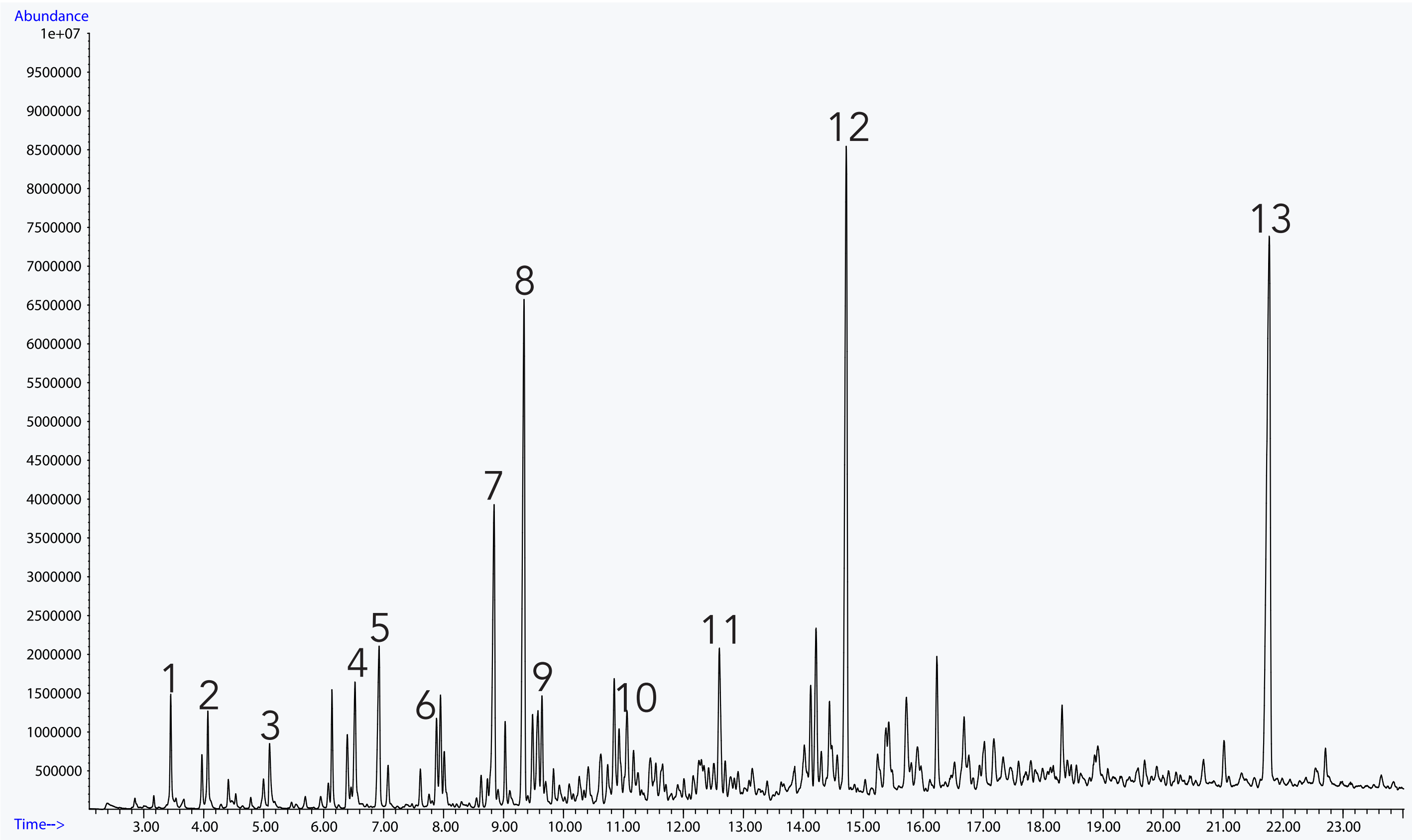

Figure 6: TIC resulting from immersion extraction of a breakfast blend coffee, using a combination of TF-SPME and SBSE.

Table 4: List of compounds for figure 6.

Peak #	Compound	Log $K_{o/w}$	Peak #	Compound	Log $K_{o/w}$
1	3-Methylfuran	1.91	8	Furfuryl acetate	1.45
2	2-Methylbutanal	1.23	9	2-Ethyl-6-methylpyrazine	1.53
3	Pyridine	0.8	10	2-Methoxyphenol	1.34
4	Furfural	0.83	11	1-Furfurylpyrrole	2.5
5	2-Furanmethanol	0.45	12	2-Methoxy-4-vinylphenol	2.24
6	1-(2-Furanyl)-ethanone	0.8	13	Caffeine	0.16
7	5-Methyl-2-furaldehyde	1.38			

Figure 7 shows the TIC of a lemon lime soda sample extracted by immersion TF-SPME, again using a GERSTEL Twister for agitation. Furfural and 2-furanmethanol, both compounds with Log $K_{o/w}$ <1.0, were identified. These compounds impart flavors described as brown, burnt, and caramellic. Other compounds contributing to the aroma and flavor in the sample are also labeled in the figure.

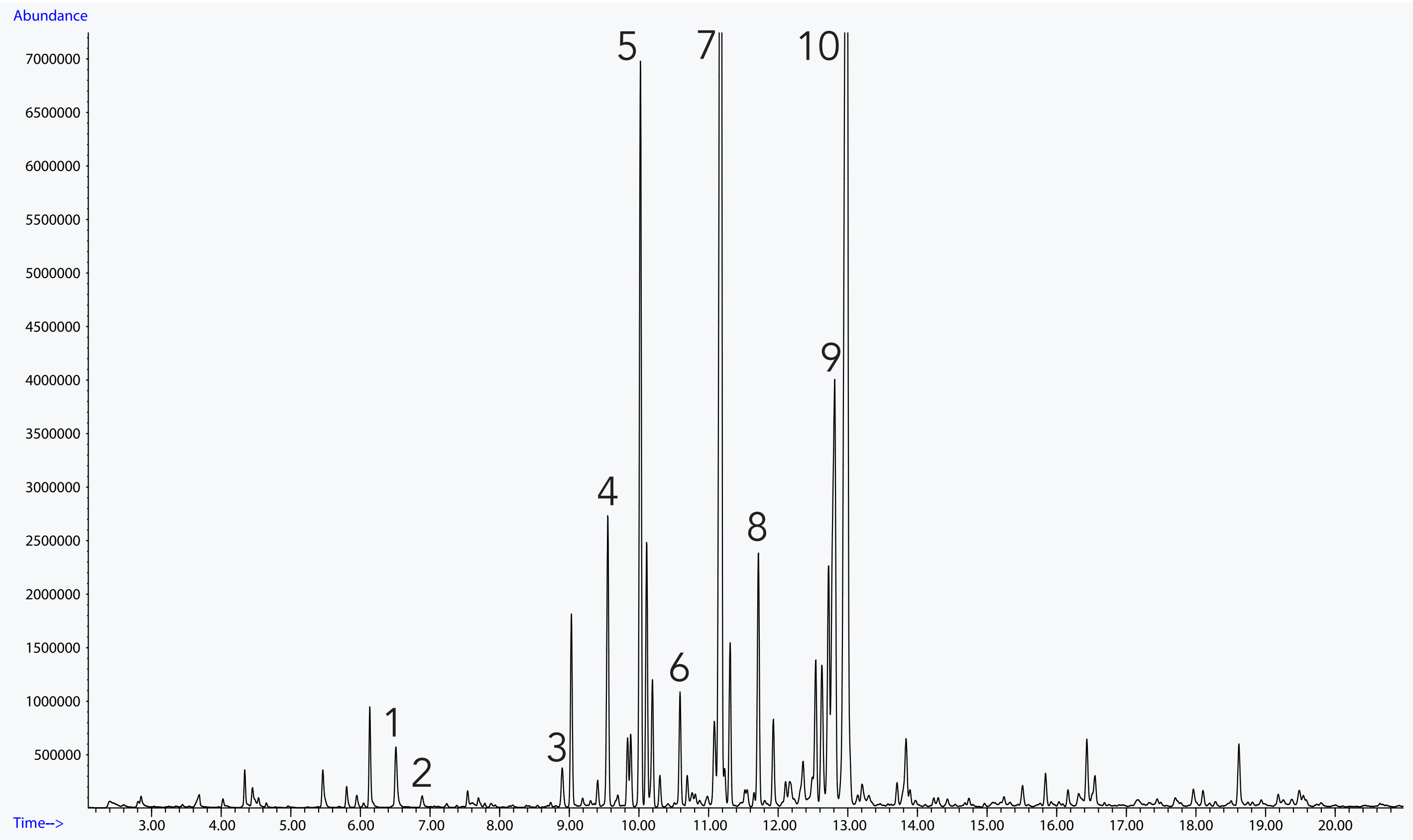

Figure 7: TIC resulting from immersion extraction of a lemon lime soda, using a combination of TF-SPME and SBSE.

Table 5: List of compounds for figure 7.

Peak #	Compound	Log $K_{o/w}$	Peak #	Compound	Log $K_{o/w}$
1	Furfural	0.83	6	γ-Terpinene	4.75
2	2-Furanmethanol	0.45	7	p-Cymenene	3.99
3	Benzaldehyde	1.71	8	Fenchol	2.85
4	Octanal	2.78	9	p-Cymene-1-ol	2.49
5	p-Cymene	4	10	α-Terpineol	3.33

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Figure 8 shows the TIC of a mixed berry sports drink extracted by immersion TF-SPME. Several classes of compounds, including aldehydes, ethyl and methyl esters and ketones are identified in the chromatogram. Some of the flavor notes associated with these compounds are berry, citrus, cinnamyl and fruity.

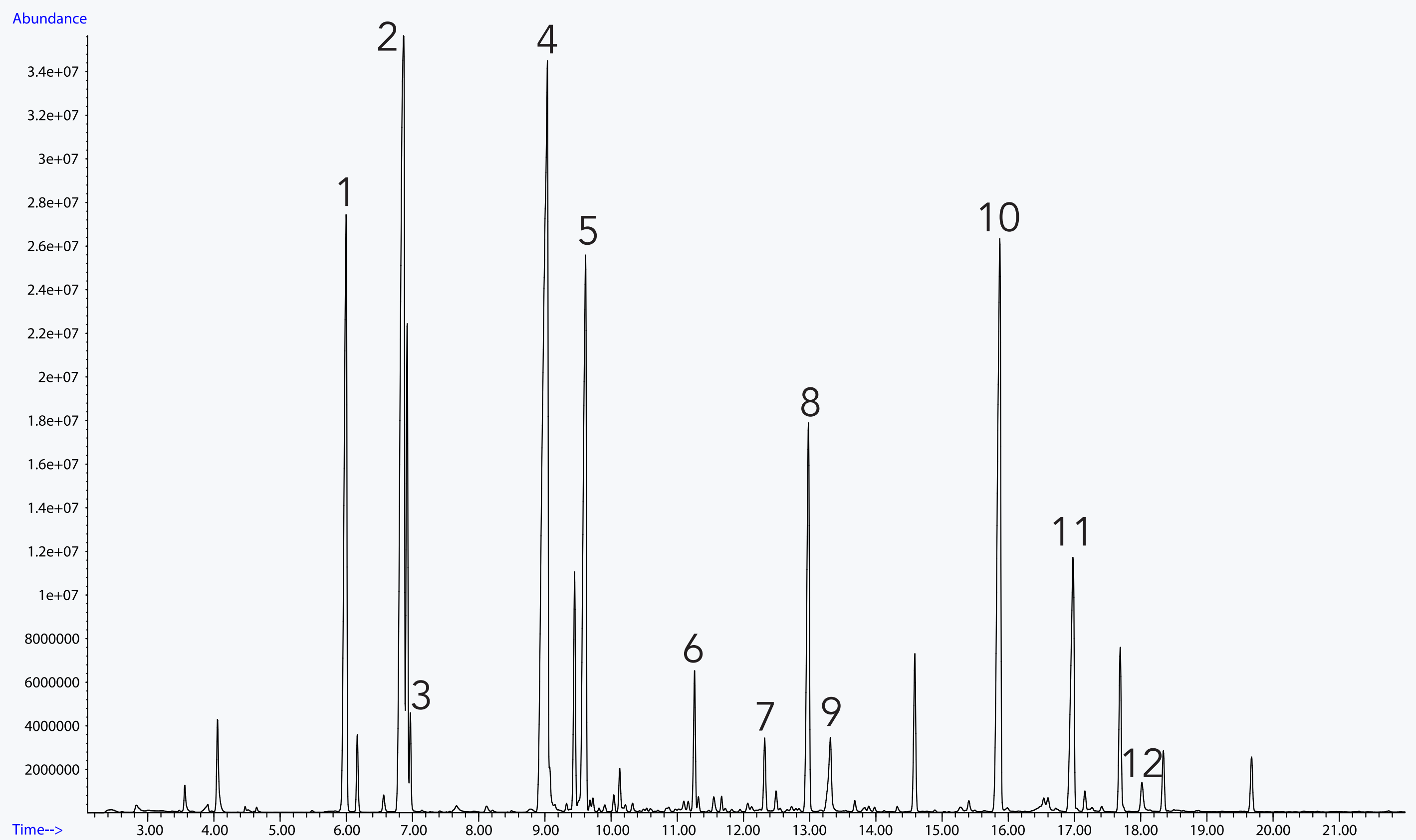

Figure 8: TIC resulting from immersion extraction of a mixed berry sports drink.

Table 6: List of compounds for figure 8.

Peak #	Compound	Log $K_{o/w}$	Peak #	Compound	Log $K_{o/w}$
1	Ethyl butyrate	1.85	7	Benzyl acetate	2.08
2	Ethyl 2-methylbutyrate	2.26	8	α-Terpineol	3.33
3	3-Hexen-1-ol	1.61	9	5-(Hydroxymethyl) furfural	-0.09
4	Benzaldehyde	1.71	10	Methyl cinnamate	2.36
5	cis-3-Hexenyl acetate	2.61	11	γ-Decanolactone	2.57
6	Linaool	3.38	12	Raspberry ketone	1.48

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Conclusions

TF-SPME shows good ability to extract a wide range of polar compounds, flavor constituents and other compound groups found in alcoholic beverages, coffees, soda and sports drinks. This results in good sensitivity of the overall analysis. A TF-SPME device can be combined with a GERSTEL Twister stir bar for agitation and enhanced extraction efficiency. This approach could be readily used for quality control or for troubleshooting when off–flavors are found in these sample types.