LabWorks APPNOTE

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Comparison of Four Immersion Extraction Techniques Using the GERSTEL Twister® and TF-SPME Devices to Determine Analytes Found in Hard Seltzer Flavors

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Keywords

Hard seltzer, flavor profile, Twister®, TF-SPME, GC/MS, LabWorks Platform

Abstract

In this study, four immersion extraction techniques using the GERSTEL PDMS Twister® and PDMS/HLB TF-SPME devices were compared for their efficacy in extracting a broad range of flavor compounds in hard seltzer beverages. A variety of hard seltzer flavors were analyzed including black cherry, watermelon, and grapefruit. Relevant flavor analyte peak areas were integrated at their respective base peak ions. Peak areas were compared between extraction techniques to determine which method was most efficient in recovering flavor analytes found in the three flavored hard seltzers. The Log $K_{O/W}$ values of the compounds found in the seltzers ranged from 0.33 to 4.57. The GERSTEL Cooled Injection System (CIS 4) PTV type GC inlet is used in combination with the Thermal Desorption Unit (TDU 2) for the automated desorption of the extraction devices. This technique allows for simultaneous desorption of the extraction devices in one TDU 2 tube thus eliminating the need for stacking multiple samples to increase mass on column. It was found that simultaneous extraction provided the best results, extracting and identifying 120 compounds.

Introduction

Hard seltzers have become extremely popular in recent years. The craze can be attributed to their gluten-free nature, low sugar, and low-calorie content. The beverage comes in a variety of mixes like lemonade, fruit punch and tea, and can be produced in several flavors like pineapple, lime, and mixed berry. To produce these various flavor profiles in the hard seltzer matrix, the appropriate compound(s) must be incorporated into the formulation to yield an accurate representation of the true fruit flavor. The compounds used in these flavorings can range from non-polar to polar, therefore, any technique used to extract them, must be able to efficiently extract a wide range of analytes. Flavor analysis of these types of samples are of interest for quality control, competitive analysis, customer complaints and product authentication.

One potential issue for extraction devices containing sorptive particles is capacity. As compounds compete for sites on the sorptive phase, displacement effects become prominent and the extraction efficiency is reduced. A possible solution to this issue is to first expose the sample to a non-polar phase, like polydimethylsiloxane (PDMS), to extract non-polar analytes, followed by a second extraction using a polar sorptive phase, like hydrophilic lipophilic balanced (HLB), for more polar analytes. This is known as a sequential extraction technique.

This work aims to compare a sequential extraction, simultaneous extraction, individual extraction with the Twister®, and individual extraction with the TF-SPME device using three different hard seltzer flavors. Immersion was used for all extractions. Peak areas were determined for each technique and compared to determine whether the signal intensity correlated with the polarity of the flavor analytes of interest.

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Experimental

Instrumentation

GERSTEL LabWorks Platform with Agilent 8890 GC and 5977B Inert plus MSD.

Analysis Conditions

LabWorks Platform

TF-SPME	PDMS/HLB
Twister	PDMS
TDU 2	solvent vent/dry purge vent time 3.33 min 40 °C, 60 °C/min to 60 °C (3 min), 400 °C/min to 250 °C (5 min)
CIS 4	solvent vent (50 mL/min), split 100:1 -120 °C (0.2 min), 12 °C/sec to 280 °C (3 min)

Agilent 8890 GC

Column	30 m Rxi-5 Sil MS (Restek) d_i=0.25 mm, d_f=0.25 µm
Pneumatics	He, P_i = 7.1 psi Constant Flow 1.0 mL/min
Oven	40 °C (2 min), 10 °C/min to 280 °C (3 min)

Agilent 5977 MSD

full scan	40 – 350 amu

Sample preparation

Three hard seltzer varieties were purchased from a local liquor store. The three flavors used for this study were black cherry, watermelon, and grapefruit. A 1 mL aliquot of each hard seltzer flavor was pipetted into a 10 mL screw-capped vial and filled to its total volume with 9 mL of bottled water. Four vials of each 1:10 hard seltzer dilution were prepared for sequential, simultaneous, and individual TF-SPME and Twister® extractions respectively.

Extractions were performed on a 20 position GERSTEL Twister® stir plate at 1100 rpm at room temperature. The sequential extraction was conducted by first immersing the Twister® into the sample solution and stirring for 90 minutes. After 90 minutes, the vial was taken to the freezer and stored for 15 minutes to prevent the loss of volatile analytes. Then, the Twister® was removed, rinsed with bottled water, and dried with a lint free wipe. The TF-SPME device was suspended on a TF-SPME holder, a non-coated magnetic stir bar was immersed for stirring, and the sample was extracted for 90 minutes. The device was rinsed and dried for analysis.

For the simultaneous extraction, the GERSTEL Twister® and TF-SPME devices were immersed in the sample at the same time. The sample was extracted for 90 minutes at a speed of 1100 rpm. The sample was not put in the freezer post-extraction. The TF-SPME and Twister® were rinsed and dried, then placed together in an empty TDU tube for desorption.

Single technique extractions were performed by immersing the device in question to extract the hard seltzer analytes. For the TF-SPME extraction, a non-coated magnetic stir bar was used for stirring and the device was held in place with a holder. For the Twister® extraction, the device was immersed. Both extractions occurred for 90 minutes. Each device was separately rinsed and dried.

The devices used for the sequential extraction were placed together in an empty TDU tube for simultaneous desorption. The devices used for the simultaneous extraction were also placed together in an empty TDU tube for desorption. The Twister® and TF-SPME from individual extractions were placed in separate TDU tubes for desorption.

The TDU tubes were loaded onto the MPS Robotic VT-40t tray. Samples were vented at 60 °C for 3 minutes under a 50 mL/min helium flow in the TDU. The temperature was then increased to 250 °C and held for 5 minutes to perform the thermal desorption. Analytes were trapped in the CIS 4 inlet at -120 °C on a glass bead filled liner. When desorption was complete, analytes were transferred to the GC column by heating the inlet to 280 °C with a 100:1 split.

Results and Discussion

Figure 1 shows the stacked view of total ion chromatograms (TICs) obtained for the black cherry hard seltzer using all four extraction technique combinations with the PDMS/HLB TF-SPME device and PDMS Twister®. For this sample, a very simple chromatographic profile is seen. The main compound in the chromatogram is benzaldehyde. Benzaldehyde's organoleptic properties are described as maraschino cherry and almond. The other major component seen in the chromatogram is sorbic acid, a preservative. The individual PDMS/HLB TF-SPME device extracted both compounds, whereas the Twister® only extracted benzaldehyde, and with much lower signal. The simultaneous and sequential extractions yielded the best detection of benzaldehyde and sorbic acid. Table 1 lists the analytes' respective Log $K_{O/W}$ values and peak areas normalized to the simultaneous extraction.

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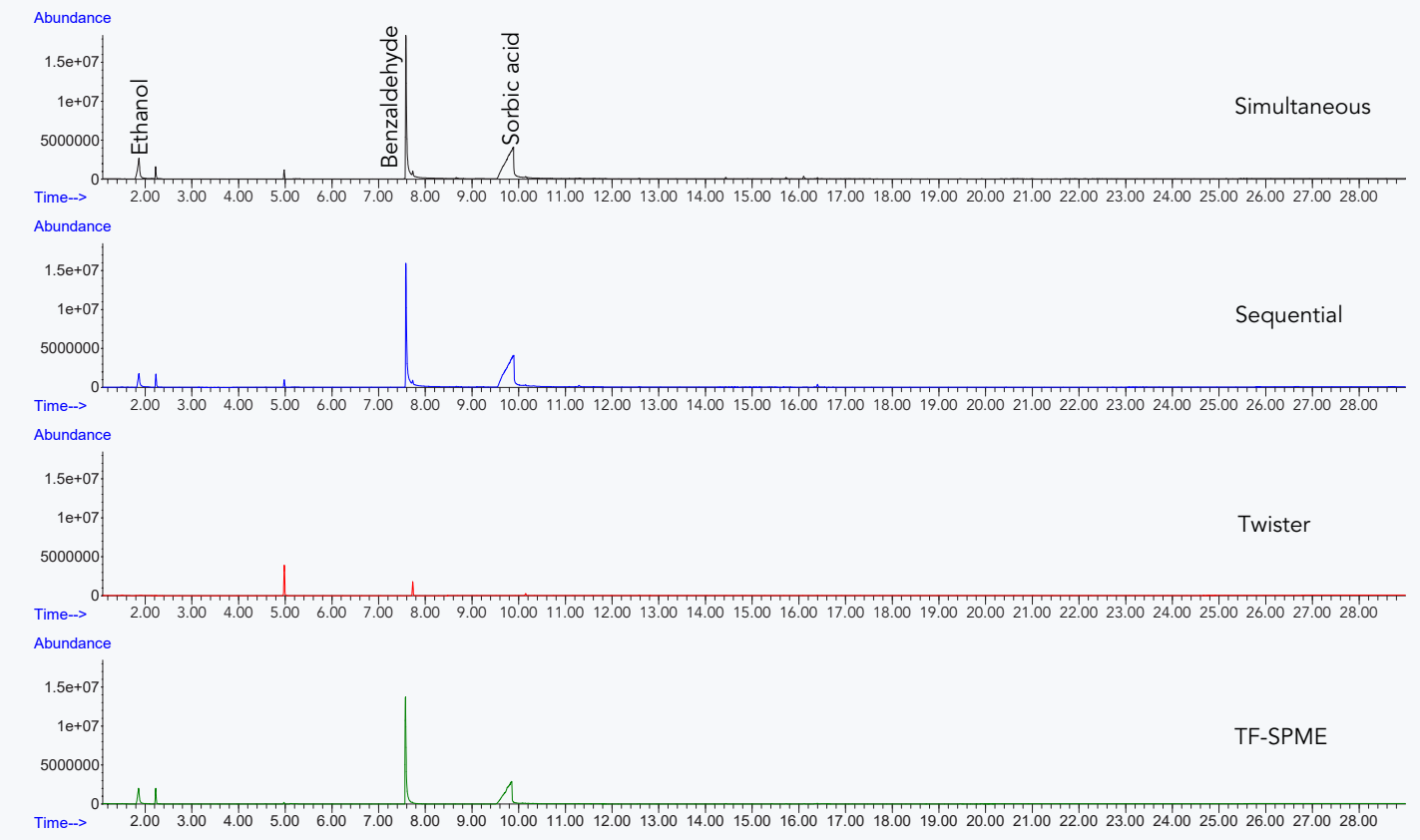

Figure 1: Stacked view of total ion chromatograms obtained for the black cherry hard seltzer using the simultaneous (top), sequential (top middle), Twister® stir bar (bottom middle), and PDMS/HLB TF-SPME extraction techniques (bottom).

Table 1: Black cherry analytes' Log $K_{O/W}$ values and relative area counts normalized to the simultaneous extraction.

Compound	Log $K_{O/W}$	m/z	Simultaneous	Sequential	TF-SPME	Twister®
Benzaldehyde	1.48	106	100	80.5	76.9	2.3
Sorbic acid	1.33	97	100	104.2	92.7	0.0

Figure 2 shows the stacked view of TICs obtained for the watermelon hard seltzer using all four extraction techniques. The compounds contributing to the fruity and green notes of the watermelon flavor are ethyl butanoate, ethyl-2-methylbutanoate, isoamyl acetate, ethyl hexanoate, methyl cinnamate, butyl acetate, 6-methyl-5-hepten-2-one, and benzyl alcohol. Table 2 shows the relative peak intensities for the four extraction techniques. The simultaneous extraction shows the best results of the four techniques, especially for methyl cinnamate, benzyl alcohol, 6-methyl-5-hepten-2-one and γ-decalactone found in the sample.

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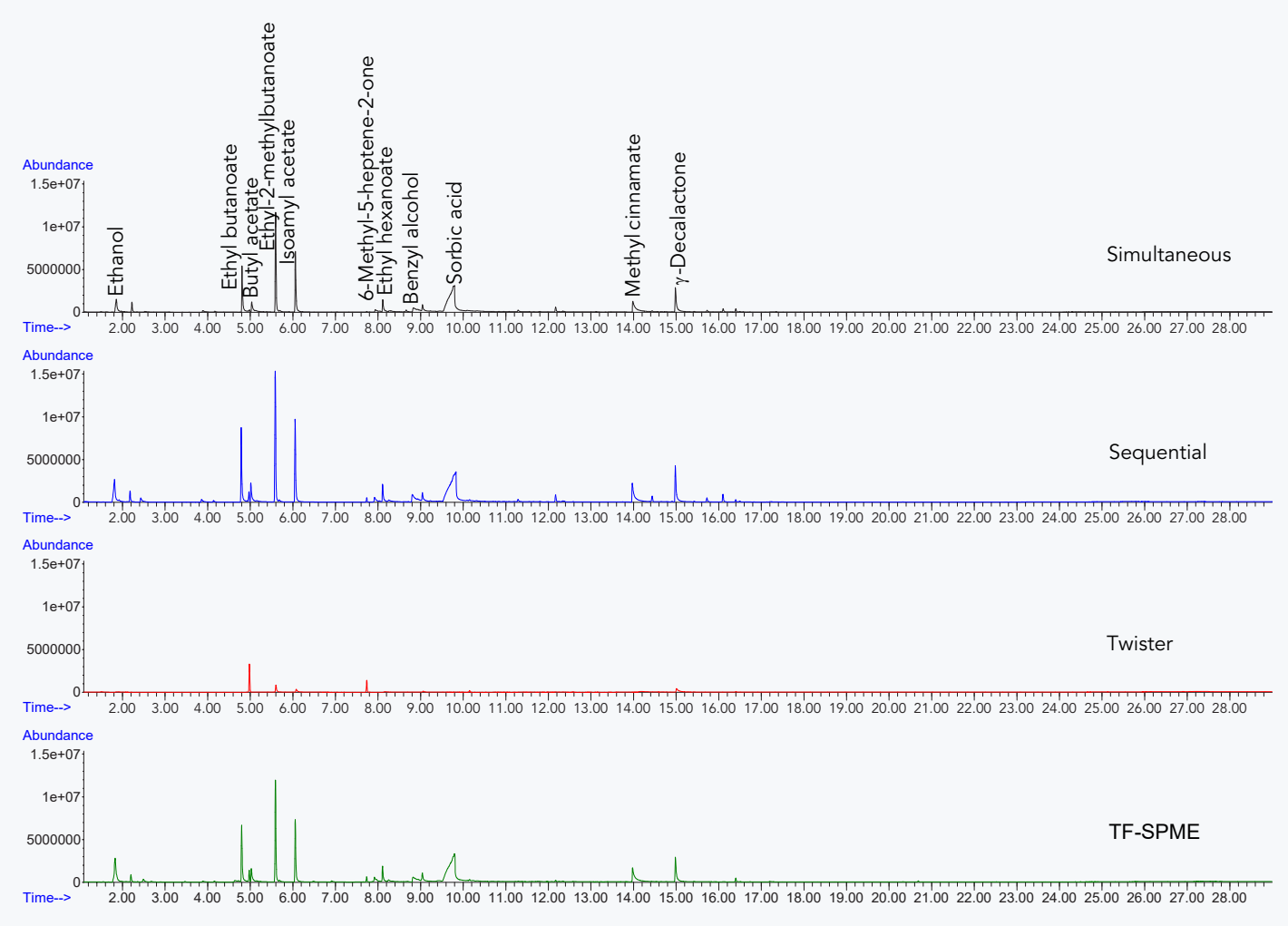

Figure 2: Stacked view of total ion chromatograms obtained for the watermelon hard seltzer using the simultaneous (top), sequential (top middle), Twister® stir bar (bottom middle), and PDMS/HLB TF-SPME extraction techniques (bottom).

Table 2: Watermelon analytes' Log $K_{O/W}$ values and relative area counts normalized to the simultaneous extraction.

Compound	Log $K_{O/W}$	m/z	Simultaneous	Sequential	TF-SPME	Twister®
Ethyl butanoate	1.85	71	100	76.0	63.0	0.0
Butyl acetate	1.78	43	100	71.6	51.2	0.2
Ethyl-2-methylbutanoate	2.26	57	100	77.0	74.6	7.5
Isoamyl acetate	2.25	43	100	78.3	75.2	7.1
6-Methyl-5-hepten-2-one	2.06	43	100	18.4	10.1	0.0
Ethyl hexanoate	2.83	88	100	90.9	80.2	14.6
Benzyl alcohol	1.10	79	100	62.8	61.0	0.0
Sorbic acid	1.33	97	100	92.3	81.3	0.0
Methyl cinnamate	2.62	131	100	87.4	80.8	16.8
γ-Decalactone	2.72	85	100	72.7	78.3	21.4

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Figure 3 shows the stacked view of TICs obtained for the grapefruit hard seltzer using all four extraction techniques. The main compounds that make up grapefruit flavoring are limonene, linalool, α-terpineol, γ-decalactone, triethyl citrate, and nootkatone, which contribute citrus-orange, woody-lavender, woody-citrus, creamy, wine-like, and direct grapefruit qualities, respectively. Other contributing flavor compounds identified in this study included ethyl butanoate, β-myrcene, octanal, γ-terpinene, β-terpineol, and decanal. These are all characteristic of more floral, herbaceous, fruity/citrus, and earthy notes and were present at lower signal intensities. The simultaneous and sequential extractions show the best results of the four techniques for this sample. The simultaneous extraction shows better results for ethyl butanoate, octanal and decanal.

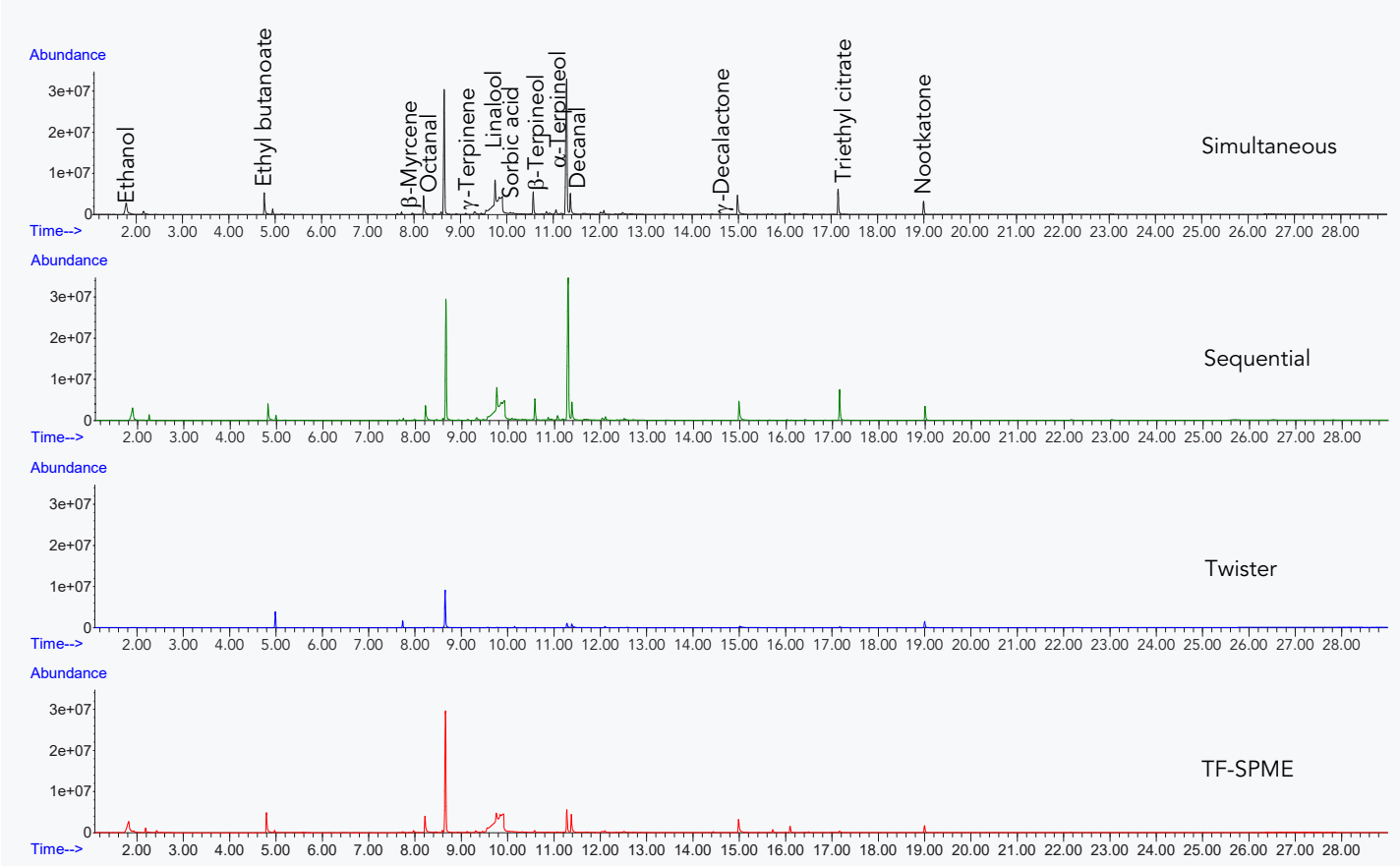

Figure 3: Stacked view of total ion chromatograms obtained for the grapefruit hard seltzer using the simultaneous (top), sequential (top middle), Twister® stir bar (bottom middle), and PDMS/HLB TF-SPME extraction techniques (bottom).

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Table 3: Grapefruit analytes' Log $K_{O/W}$ values and relative area counts normalized to the simultaneous extraction.

Compound	Log $K_{O/W}$	m/z	Simultaneous	Sequential	TF-SPME	Twister®
Ethyl butanoate	1.85	71	100	78.7	92.5	0.5
β-Myrcene	4.17	93	100	90.3	93.3	12.6
Octanal	2.78	41	100	85.2	85.5	4.7
Limonene	4.57	68	100	93.4	96.9	27.9
γ-Terpinene	4.50	93	100	94.2	92.4	24.4
Sorbic acid	1.33	97	100	102.9	98.8	0.0
Linalool	2.97	71	100	94.5	34.1	2.5
β-Terpineol	3.41	71	100	100.9	9.2	1.6
α-Terpineol	2.98	59	100	101.2	14.4	3.5
Decanal	3.76	57	100	86.5	90.0	25.2
γ-Decalactone	2.72	85	100	99.6	72	16.6
Triethyl citrate	0.33	157	100	105.7	5.2	4.1
Nootkatone	3.84	147	100	101.2	52.7	46.6

The simultaneous extraction produced the best results for the watermelon and cherry flavored seltzers. For the grapefruit flavor, the simultaneous and sequential extractions provided near equivalent results.

The sequential extraction did not show a vast improvement over simultaneous extraction for this sample type, so displacement was not a contributing factor. This may be due to the high phase volume of the Twister® (24 µL) relative to the TF-SPME device (9 µL). The increased surface area on the TF-SPME device allows for better interaction of the analytes with the sorbent. Additionally, the PDMS TF-SPME device with HLB particles enhances the extraction of analytes with a wide range of Log $K_{O/W}$ values. An additional advantage of simultaneous extraction over sequential extraction is that the extraction time is cut in half and eliminates the need for cooling the sample between immersions of extraction devices.

The above work was intended to show a comparison of the four techniques using TF-SPME and Twister® for extracting the more abundant flavor compounds in these sample types. For a more complete profile, an undiluted sample with a lower split transfer can be used. Figure 4 shows a simultaneous extraction of the watermelon flavored hard seltzer, undiluted with a split transfer of 20:1 from the inlet to the column. Agilent Technologies Unknowns Analysis software was used for deconvolution and peak matching. The minimum match factor was set to 80. The peaks identified in the chromatogram are summarized in Table 4. Simultaneous extraction with a PDMS Twister and PDMS/HLB TF-SPME device yielded 120 compounds detected in the undiluted sample.

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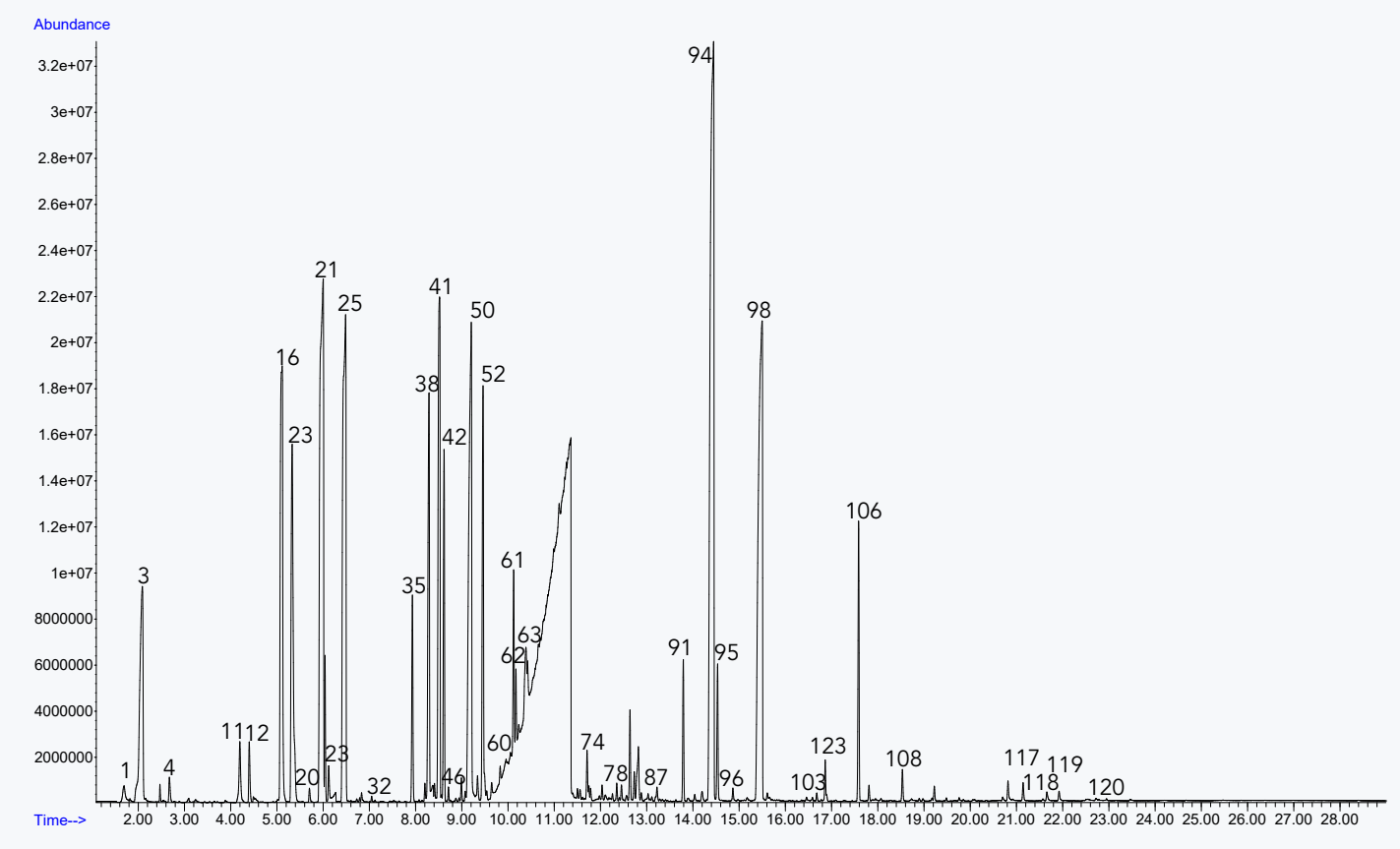

Figure 4: Total ion chromatogram obtained for the undiluted watermelon hard seltzer using the simultaneous extraction technique with a 20:1 split transfer.

Table 4: Compounds detected in the undiluted watermelon hard seltzer using a 20:1 split transfer with respective retention times and match factors listed.

Peak #	Component RT	Compound Name	Match Factor
1	1.69	(2-Aziridinylethyl) amine	80.36
2	1.82	1-Propene, 2-methyl-	97.37
3	2.09	Ethanol	99.36
4	2.67	Ethyl Acetate	99.08
5	2.89	1-Propanol, 2-methyl-	91.43
6	3.09	Acetic acid	99.14
7	3.11	Fumaronitrile	80.61
8	3.24	1-Butanol	98.04
9	3.65	2-Propanone, 1-hydroxy-	97.49
10	3.71	Propanoic acid, ethyl ester	89.48
11	4.20	1-Butanol, 3-methyl-	99.74
12	4.40	Propanoic acid, 2-methyl-, ethyl ester	99.70
13	4.49	(S)-(+)-1,2-Propanediol	95.02
14	4.54	Spiro [2,4] hepta-4,6-diene	87.12

Peak #	Component RT	Compound Name	Match Factor
15	4.58	Propanoic acid, 2-methyl-	84.09
16	5.11	Butanoic acid, ethyl ester	94.00
17	5.33	Acetic acid, butyl ester	99.21
18	5.38	Methylal	86.08
19	5.56	1-Methylallyl acetate	85.94
20	5.70	Furfural	99.15
21	6.00	Butanoic acid, 2-methyl-, ethyl ester	93.98
22	6.04	Butanoic acid, 3-methyl-, ethyl ester	98.04
23	6.12	3-Hexen-1-ol, (Z)-	98.88
24	6.27	Butanoic acid, 2-methyl-	97.02
25	6.49	1-Butanol, 3-methyl-, acetate	99.13
26	6.60	Butanoic acid, 2-methyl-, 1-methyleth-yl ester	94.04
27	6.66	2-Heptanone	82.13

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Table 4 (cont.): Compounds detected in the undiluted watermelon hard seltzer using a 20:1 split transfer with respective retention times and match factors listed.

Peak #	Component RT	Compound Name	Match Factor
28	6.73	Styrene	97.81
29	6.75	Benzene, 1,3-dimethyl-	82.71
30	6.79	Butanoic acid, propyl ester	96.73
31	6.83	Pentanoic acid, ethyl ester	96.53
32	7.05	Acetic acid, pentyl ester	97.62
33	7.29	1,2-Cyclopentanedione	82.52
34	7.49	2-Propenoic acid, 2-methyl-, 2-methyl-propyl ester	90.67
35	7.93	Benzaldehyde	99.50
36	8.08	2H-Pyran, 2-ethenyltetrahydro-2,6,6-trimethyl-	89.40
37	8.14	Hexanoic acid	92.29
38	8.29	5-Hepten-2-one, 6-methyl-	97.93
39	8.38	Hexanoic acid	91.22
40	8.41	trans, trans-3,5-Heptadien-2-one	95.16
41	8.51	Hexanoic acid, ethyl ester	99.30
42	8.56	3-Hexen-1-ol, acetate, (Z)-	87.91
43	8.57	Benzene ethanol, β-ethenyl-	80.32
44	8.62	3-Hexen-1-ol, acetate, (E)-	98.96
45	8.67	1,3,8-p-Menthatriene	81.36
46	8.71	Acetic acid, hexyl ester	98.53
47	8.87	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	92.09
48	8.99	o-Cymene	98.75
49	9.07	D-Limonene	95.55
50	9.20	Benzyl alcohol	98.82
51	9.34	Cyclohexanone, 2-(1-methylethyl)-	87.06
52	9.46	Butanoic acid, 3-methylbutyl ester	97.51
53	9.54	γ-Terpinene	89.69
54	9.65	5-Heptenal, 2,6-dimethyl-	80.95
55	9.67	Acetophenone	91.66
56	9.71	1,3-Benzenediol, O, O'-di(2-methylbenzoyl)-	83.77
57	9.83	Hexanoic acid, 2-propenyl ester	93.89
58	9.89	2-Furancarboxylic acid, hydrazide	84.34
59	9.96	Benzene, 1-ethenyl-4-ethyl-	90.10
60	10.12	Sorbic acid	98.03
61	10.17	Linalool	97.26
62	10.23	Nonanal	89.58
63	10.39	Maltol	92.42
64	10.42	Phenylethyl Alcohol	96.54
65	10.44	Naphthalene, 1,2-dihydro-	88.71
66	11.11	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, trans-	82.50
67	11.26	l-Menthone	80.00
68	11.29	Benzoic acid, ethyl ester	89.55
69	11.34	p-Mentha-1,5-dien-8-ol	86.41
70	11.50	Terpinen-4-ol	83.81
71	11.56	Benzenemethanol, α, α,4-trimethyl-	95.16
72	11.61	Octanoic acid, ethyl ester	88.51
73	11.66	Methyl salicylate	83.56
74	11.71	L-α-Terpineol	94.16
75	11.75	Octanoic acid	96.10
76	11.79	Decanal	87.68
77	12.10	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	87.33
78	12.35	Pulegone	97.30
79	12.41	Isopentyl hexanoate	89.24
80	12.45	Geraniol	97.56
81	12.51	Benzenebutanal	80.24
82	12.80	Nonanoic acid	85.26
83	13.00	Estragole	82.37
84	13.03	2-Undecanone	95.07
85	13.03	Carbonic acid, (1R)- (-)-menthyl butyl ester	80.31
86	13.11	Propanoic acid, 2-methyl-, phenylmethyl ester	86.66
87	13.22	2-Propenoic acid, 3-phenyl-, methyl ester, (E)-	97.41
88	13.22	2-Propenoic acid, 3-phenyl-, methyl ester	97.31
89	13.28	1-Nonen-3-one, 1-phenyl-	81.62
90	13.34	2-Ethyl-4-methylanisole	85.28
91	13.79	Butanoic acid, phenylmethyl ester	99.23
92	14.04	n-Decanoic acid	93.11
93	14.20	Geranyl acetate	96.84
94	14.45	2-Propenoic acid, 3-phenyl-, methyl ester	96.65
95	14.53	Vanillin	98.94
96	14.87	α-Ionone	95.92

Table 4 (cont.): Compounds detected in the undiluted watermelon hard seltzer using a 20:1 split transfer with respective retention times and match factors listed.

Peak #	Component RT	Compound Name	Match Factor
97	15.42	2-Propenoic acid, 3-phenyl-, ethyl ester	87.80
98	15.49	2(3H)-Furanone, 5-hexyldihydro-	97.28
99	15.61	trans-β-Ionone	81.19
100	16.46	Dodecanoic acid	94.41
101	16.56	2,4-Pentanedione, 3-phenyl-	83.39
102	16.63	1-Pentanone, 1-phenyl-	88.76
103	16.68	2(3H)-Furanone, 5-heptyldihydro-	98.11
104	16.86	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	98.67
105	17.45	Benzophenone	88.86
106	17.59	Cinnamaldehyde, α-pentyl-	98.58
107	18.07	α-Bisabolol	87.09
108	18.53	2H-1-Benzopyran-2-one, 7-methoxy-	98.35
109	18.70	Octanal, 2-(phenylmethylene)-	88.52
110	18.73	Tetradecanoic acid	88.61
111	18.97	Benzyl Benzoate	84.12
112	19.85	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	91.94
113	20.05	1-Undecene, 8-methyl-	83.64
114	20.08	4-Benzyloxybenzoic acid	82.22
115	20.70	Dehydro-cohumulinic acid	91.10
116	20.81	Dibutyl phthalate	88.96
117	21.14	2H-1-Benzopyran-2-one, 5,7-dimethoxy-	98.58
118	21.66	3-Cyclopenten-1-one, 3-hydroxy-2-(1-hydroxy-3-methylbutylidene)-5-(3-methyl-2-butenylidene)-	88.38
119	21.92	7H-Furo[3,2-g][1] benzopyran-7-one, 4-methoxy-	96.84
120	22.95	Hexadecanoic acid, butyl ester	91.55

Conclusions

This study shows the usefulness of simultaneously employing two extraction devices with different sorbents in a sample for the recovery of a broad range of flavor analytes found in hard seltzers. While extraction efficiency depends on a variety of factors, including concentration and matrix effects, the simultaneous extraction technique covered the widest polarity range and achieved the best signal detection in comparison to the other three techniques used. Simultaneous extraction provides the highest phase volume and surface area available in the sample thus generating higher extraction efficiencies and lower limits of detection. Simultaneous extraction also allows for simultaneous desorption of both extraction devices in the TDU 2, reducing sample run time and eliminating the need for multiple tube stacking and trapping of analytes.