APPNOTE

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Identification and Determination of Flavor Compounds in Dietary Supplements using Thermal Desorption GC-MS with Automated Introduction of Standards and Retention Time Index Mix

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Keywords

Dietary supplements, flavor compounds, retention indices, thermal desorption, gas chromatography, mass spectrometry

Abstract

The dietary supplements market is valued at over 150 billion dollars (U.S.) worldwide. Dietary supplements are manufactured products that come in many forms, such as pills, capsules, powders, liquids, or tablets. They are used to supplement a person's diet. The nutrients can be synthetic or extracted from natural sources. Many of these products contain flavoring agents, making them more palatable.

This document outlines a methodology for the determination of flavor compounds in dietary supplements using stir bar sorptive extraction. Quantitation was accomplished by automating the spiking of Tenax-TA® filled sorbent tubes. A retention index standard was also introduced using automated spiking. The GERSTEL Internal Standard/Dry Purge Plus (ISDP+) module is a station that attaches to the MPS rail and is used for automated spiking of standards, internal standards, or a retention index mix. The ISDP+ can also be used to dry purge sorbent tubes where excess water or solvent may adversely affect the trapping and transfer of analytes from the adsorbent tube to the GC column.

Introduction

Fragrance/flavor compounds are added to a wide variety of products, including scented candles, laundry detergents, dietary supplements, beverages, and air fresheners.

Identification and quantitation of flavor and off-flavor compounds in these product types are important for quality control, competitive analysis, and customer complaint samples.

This work demonstrates the identification and quantitation of flavor compounds in dietary supplements using automated spiking of a retention index standard and the creation of calibration curves using the GERSTEL ISDP+.

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Experimental

Instrumentation

GERSTEL MPS Robotic sampler with TDU 3.5+ option, GERSTEL CIS 4 Cooled Injection System with liquid nitrogen cooling option, GERSTEL ISDP+, and Agilent 8890 GC/7000E TQ MS.

Analysis Conditions

TD 3.5+	splitless
	50 °C (0 min); 200 °C/min; 280 °C (3 min)
CIS 4	Tenax TA filled liner
	solvent vent (50 mL/min)
	split 50:1
	10 °C (0 min); 12 °C/sec; 280 °C (3 min)

Analysis Conditions Agilent 8890 GC / 7000E TQ MS

Column	30 m HP-5MS UI (Agilent)
	d_i=0.25 mm, d_f=0.25 µm
Pneumatics	constant flow 1 mL/min
Oven	40 °C (1 min); 15 °C/min; 280 °C (3 min)
MS1 Scan	45-300 amu

Sample Description

Diesel range organic (DRO) standard (Restek, 31214). Benzene, toluene, ethylbenzene, xylene (BTEX) standard (Restek, 30213). Flavored dietary supplements were purchased from a local store. The flavors were elderberry, berry, and raspberry.

Sample Preparation

The powdered supplements were dissolved in 150 mL of water. One milliliter of the solution was added to 9 mL of water in a 10 mL vial. A GERSTEL Twister was added to the vial. The solution was stirred for one hour at 1200 rpm. The Twister was removed, rinsed in water, and dried on a lab tissue. It was then placed in an empty TD vial and placed on the TD tray on the MPS rail.

Standard Preparation

Standards of methyl butyrate and linalool were prepared in methanol. A four-point calibration curve was prepared in the range of 10-1000 ng on the tube using the ISDP+ and Tenax-TA® tubes. The DRO mix was diluted in methanol to a concentration of 10 µg/mL. Heptane, octane, and nonane were added to the DRO mix to prepare the retention index standard. The BTEX mix was diluted in methanol to a concentration of 10 µg/mL.

The ISDP+ was used to automate the addition of the calibration standards, DRO mix and retention index standard to the Tenax-TA® tubes.

Sample Introduction

The Twisters and Tenax-TA tubes were placed in a VT-40t tray on the autosampler. The standard was desorbed in splitless mode under a 50 mL/min helium flow @ 280 °C for 3.0 minutes. Analytes were trapped in the CIS inlet at 10 °C on a Tenax-TA liner. When desorption was complete, analytes were transferred to the column in split mode (50:1) by heating the inlet rapidly to 280 °C.

Results and Discussion

Figure 1 shows a picture of a TD 3.5+ with ISDP+. The gripper on the MPS universal syringe tool moves the thermal desorption tubes to and from the tray, ISDP+, and thermal desorption unit.

Figure 1: Picture of GERSTEL TD 3.5+ with ISDP+.

The ISDP+ unit is controlled through the GERSTEL Maestro software using a preplet or PrepSequence. A Maestro PrepSequence was used in this study. Figure 2 shows an example of a PrepSequence for adding a liquid standard to a tube, followed by injection of the tube in the thermal desorption unit.

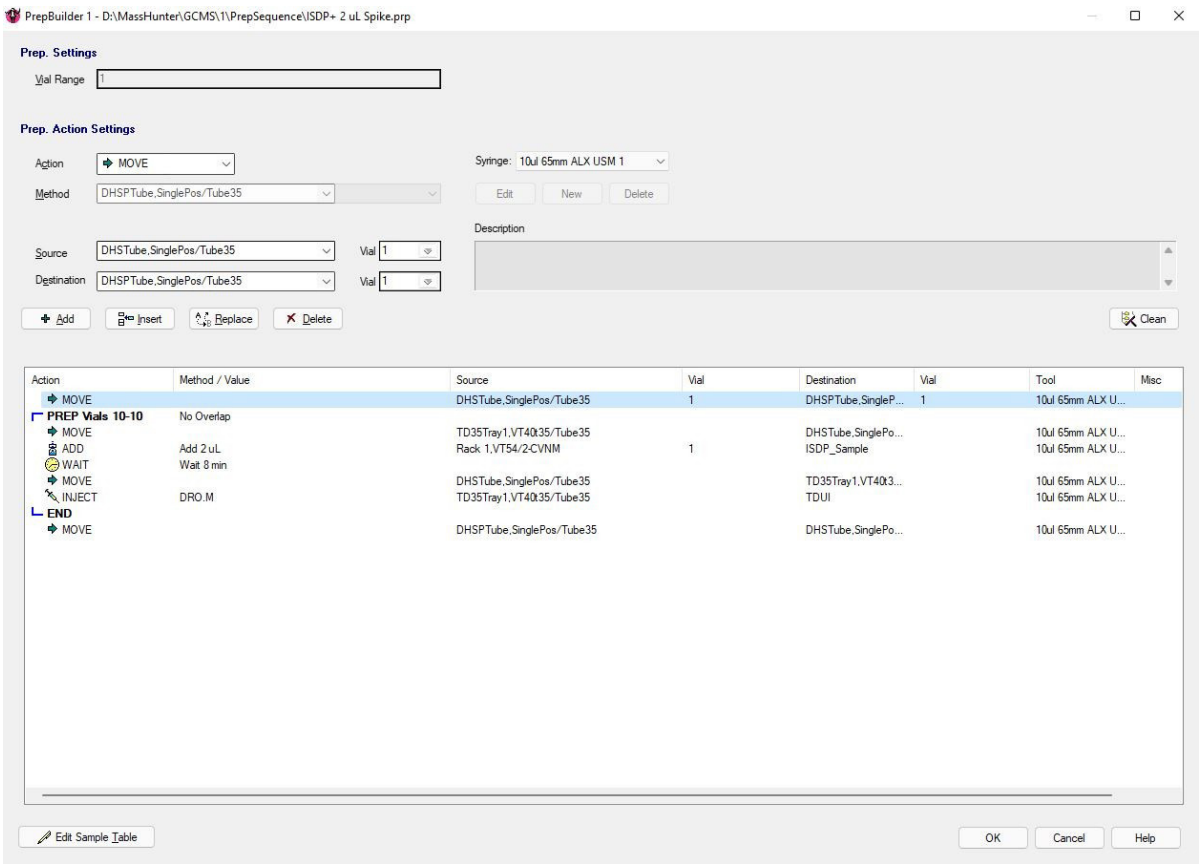

Figure 2: PrepSequence for tube spiking with ISDP⁺.

The BTEX mix was used to evaluate the precision between manual spiking and automated spiking with the ISDP⁺. The ISDP⁺ conditions were: incubator 150 °C, transfer heater 150 °C, flow 50 mL/min (3 min). For the manual spiked tubes, two microliters were spiked onto the fritted end of the tube. Dry nitrogen was purged through the tube for 3 minutes at 50 mL/min.

Table 1 shows the results for the manual versus automated spikes. The recovery was determined by dividing the ISDP⁺ peak area by the manual spike peak area and multiplying by 100. The recoveries were good for all components of the standard. The precision was also better for all analytes using automated spiking.

Table 1: Precision (%) and recoveries for manual and automated spiking.

	Benzene	Toluene	Ethylbenzene	Xylene	Xylene
Manual	5.3	5.5	5.0	4.6	5.1
ISDP⁺	2.5	0.68	0.58	0.61	0.60
Recovery	110	100	97.9	98.6	97.3

The DRO mix was used to examine the effects of the ISDP⁺ settings on recovery for a range of boiling point compounds. The recovery was calculated versus a manually spiked Tenax-TA® tube. The ISDP⁺ flow was set to 100 mL/min for all samples. Table 2 shows the recovery of the analytes in the DRO mix for four sets of ISDP⁺ conditions. The temperatures refer to the incubator/transfer heater settings.

For C10-C20, the recoveries are above 70% for all four sets of conditions. The recoveries for C21-C25 are low for the 150/150 (3 min) conditions. The recoveries for these compounds improve slightly when the ISDP⁺ incubator temperature is set at the maximum value of 200 °C. At 200/150 (5 min), the recoveries for C21-C25 are all above 70%. Increasing the purge time to 8 minutes do not substantially improve the recoveries for C21-C25.

Table 2: Percent recoveries for n-alkanes as a function of ISDP+ settings.

Temps >	150/150	200/150	200/150	200/150
Purge Time >	3 min	3 min	5 min	8 min
Carbon #				
10	97.0	91.2	103	104
11	97.6	92.0	103	104
12	95.4	94.8	103	103
13	95.3	95.6	102	104
14	95.8	98.9	104	104
15	94.4	98.6	103	103
16	95.3	100	102	103
17	97.7	102	105	106
18	99.1	108	112	112
19	89.9	102	109	111
20	72.1	85.0	96.7	98.1
21	56.2	71.0	85.8	88.4
22	43.5	59.0	79.8	81.8
23	34.9	51.8	75.7	75.0
24	30.8	46.7	73.3	73.6
25	29.4	45.8	72.0	78.5

The precision for n=5 spikes with a purge flow of 100 mL/min, and the 200/150 (5 min) conditions was measured. The results are shown in Table 3. The precision is good for all analytes (range 2.10-16.3) but increases as the recoveries decrease, as one would expect.

Table 3: Precision for n=5 replicate spikes of DRO mix.

Carbon #	Precision	Carbon #	Precision
10	2.87	18	3.20
11	3.50	19	3.46
12	3.37	20	4.65
13	2.10	21	6.19
14	2.95	22	8.89
15	2.42	23	11.6
16	2.18	24	12.3
17	3.09	25	16.3

Analysis and Quantitation of Flavored Supplements

Figure 3 shows the total ion chromatogram for the Twister extraction of the raspberry-flavored sample. The largest peaks in the chromatogram are α- and β-ionone and 3-hexen-1-ol, acetate. These compounds exhibit, sweet- floral, floral-fruity-berry, and green-fruity note, respectively. Raspberry ketone is another important flavor compound seen in the chromatogram, along with various esters.

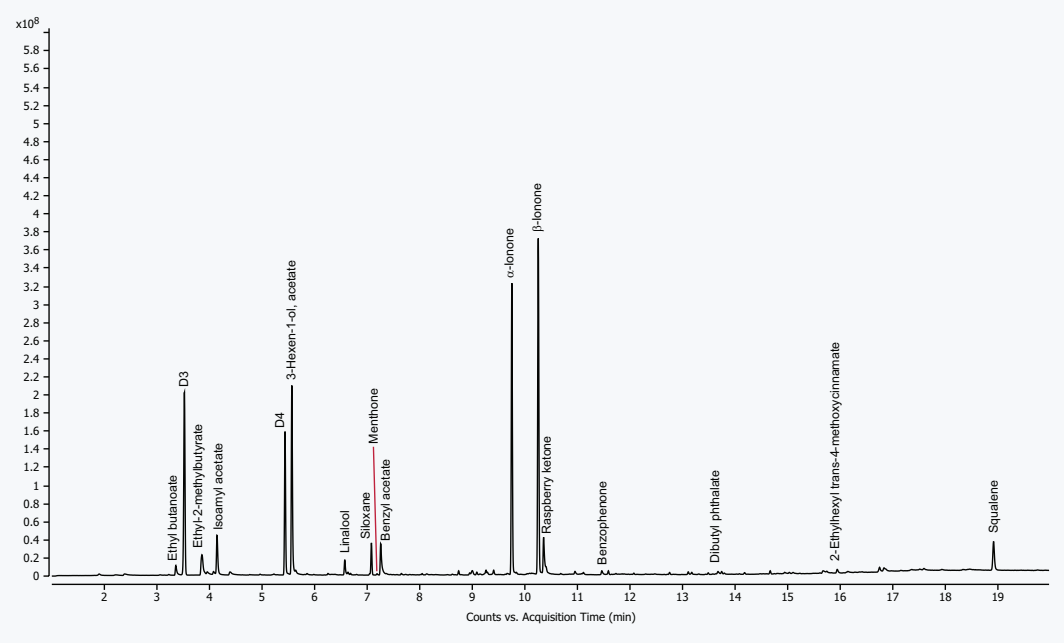

Figure 3: Total ion chromatogram for raspberry flavor sample.

Figure 4 shows the total ion chromatogram for the Twister extraction of the berry-flavored sample. The largest peak in the chromatogram is methyl cinnamate, which gives a sweet strawberry note. Other compounds include γ-decalactone (fruit-coconut), δ-dodecalactone (sweet-peach) exhibit, α-curcumene (herbal) and zingerone (sweet-spicy). Other esters are identified in the chromatogram.

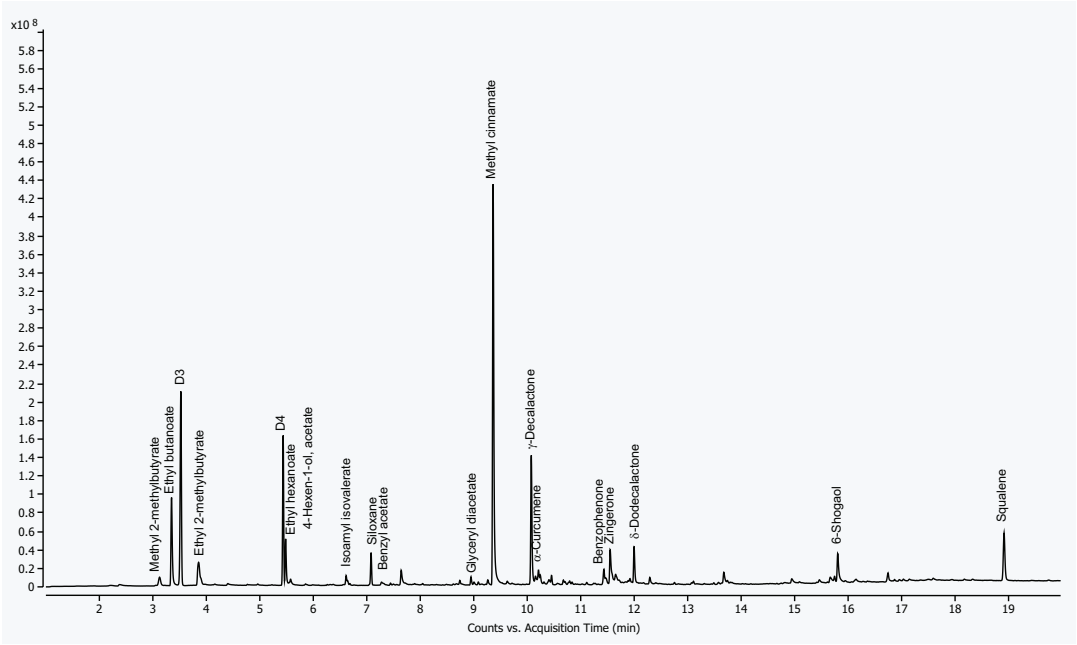

Figure 4: Total ion chromatogram for berry flavor sample.

Figure 5 shows the total ion chromatogram for the Twister extraction of the elderberry-flavored sample. The largest peaks in the chromatogram are isoamyl isovalerate (sweet-fruity) and ethyl 2-methylbutyrate (fruity-apple). Other compounds present include α- and β-ionone, raspberry ketone, linalool, methyl cinnamate, and other esters.

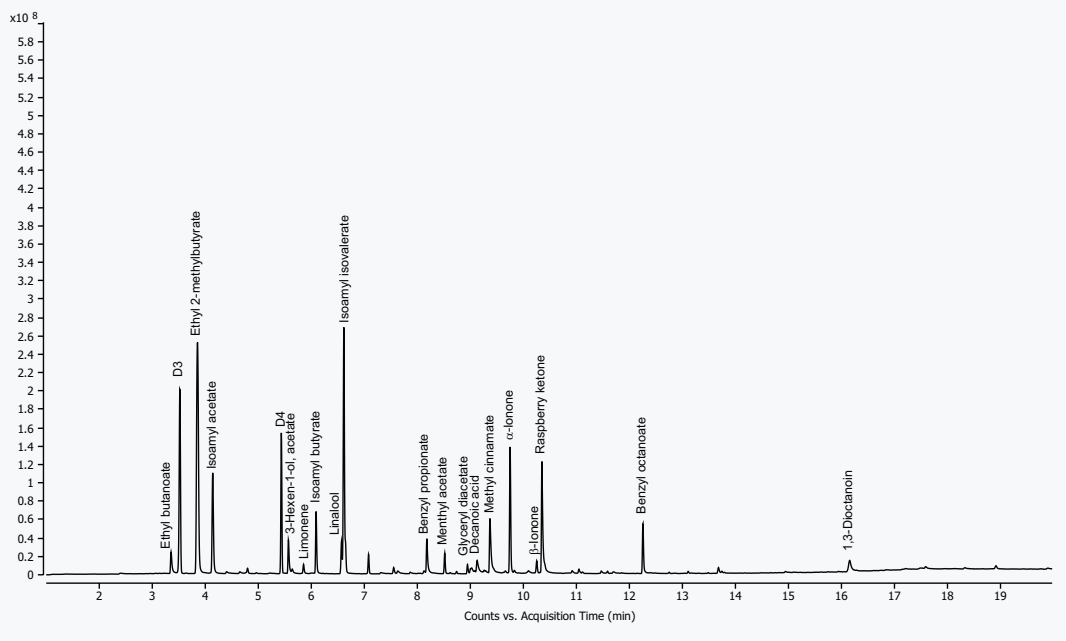

Figure 5: Total ion chromatogram for elderberry flavor sample.

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The ISDP+ was used to prepare calibration curves, spiked onto Tenax-TA® tubes, for linalool and ethyl butyrate. One microliter of each standard, in methanol, was spiked onto a tube. Figure 6 shows the resulting curve for ethyl butyrate. The curves exhibited excellent linearity with r^2 values of 0.9998 and 0.9991 for ethyl butyrate and linalool, respectively. The curves were used to quantify these two compounds in each of the samples. The elderberry sample was run in triplicate. The results are shown in Table 4. The results were corrected for the predicted percent recovery by Twister based on the log $K_{o/w}$ for each compound: Ethyl butyrate (15%) and linalool (85%). The results show the berry-flavored sample had the highest level of ethyl butyrate but no linalool. The elderberry-flavored sample had the highest level of linalool.

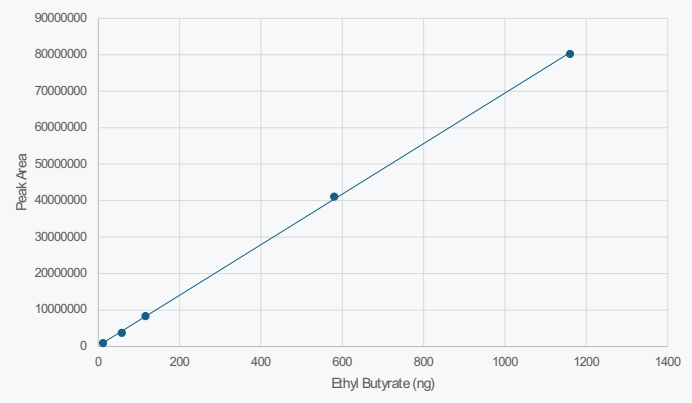

Figure 6: Calibration curve for ethyl butyrate.

Table 4: Quantitative results for ethyl butyrate and linalool (ng/mL).

Sample	Ethyl Butyrate	Linalool
Raspberry	42.3	3.66
Berry	331	0.00
Elderberry (Ave. n=3)	87.0	8.06

Retention Indices

The spike of the C7-C25 n-alkane mix was automated using the ISDP+. The retention times of the n-alkanes were used to generate retention indices for the compounds identified in the three flavored samples. Retention indices are commonly used to complement mass spectral data for the identification of flavor/fragrance compounds, as many of these compounds are isobaric. The results are shown in Table 5 for the compounds identified in the samples. The agreement between the experimental and literature values is good.

Table 5: Retention time/indices for identified flavor compounds.

Analyte	Retention Time	Elderberry	Berry	Raspberry	Experimental RI	Literature RI[1]
Methyl 2-Methylbutyrate	3.1298		X		785	780
Ethyl butanoate	3.3592	X	X	X	801	803
Ethyl 2-methylbutyrate	3.8586	X	X	X	851	850
Isoamyl acetate	4.1451	X		X	878	876
Ethyl hexanoate	5.4878		X		998	996
3-Hexen-1-ol, acetate	5.5742	X	X	X	1007	1005
Limonene	5.8593	X			1035	1031
Isoamyl butyrate	6.096	X			1057	1054
Linalool	6.5782	X		X	1101	1098
Isoamyl valerate	6.6198	X	X		1105	1105
Menthone	7.1935			X	1163	1154
Benzyl acetate	7.2818		X	X	1172	1170
Benzyl propionate	8.1862	X			1265	1257
Menthyl acetate	8.5238	X			1299	1294
Decanoic acid	9.1344	X			1368	1387
Methyl cinnamate	9.3765	X	X		1394	1392

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Table 5 (cont.): Retention time/indices for identified flavor compounds.

Analyte	Retention Time	Elderberry	Berry	Raspberry	Experimental RI	Literature RI[1]
α-Ionone	9.7578	X		X	1439	1427
γ-Decalactone	10.0829		X		1477	1470
α-Curcumene	10.2179		X		1493	1483
β-Ionone	10.2619	X		X	1498	1493
Raspberry ketone	10.3579	X		X	1510	1498
Benzophenone	11.4717		X		1651	1635
Zingerone	11.5588		X		1663	1656
δ-Dodecalactone	12.0056		X		1722	1718
Benzyloctanoate	12.2637	X			1757	1726[2]
6-Shogaol	15.8154		X		2333	2294[2]
2-Ethylhexyl trans-methoxy-cinnamate	15.9513			X	2352	2339
1,3-Dioctanoin	16.1654	X			2380	2376

1 = NIST WebBook

2 = DB-1 Column

Conclusion

This study demonstrates the use of the GERSTEL TD 3.5+ with ISDP+. The IDSP+ can be used to automatically add both liquid and gas standards to a thermal desorption tube. The ISDP+ can be used to add internal standards or retention time standards to a tube. It can also create calibration curves, which can be used for quantitation of air samples, TF-SPME and Twister extracts, or samples analyzed by direct thermal extraction.