Delivering the Right Results

Methods for Profiling Gin for Essential Oil Components with GC-TOFMS and GCxGC-TOFMS

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INTRODUCTION

The detection of minor components in gin is important in the evaluation of the flavor composition and/or the geographic origin of the product. In this investigation we demonstrate the use of a single injection for the identification of flavor components in a set of six gin samples. Neat samples were examined by injection without further preparation and analyzed by both gas chromatography time-of-flight mass spectrometry (GC-TOFMS) and comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GCxGC-TOFMS). Analysis by GC-TOFMS has the advantage of being simple, fast, and sensitive to a full range of analytes. Analysis by GCxGC-TOFMS allows for the detection of trace level analytes that may not be seen using other methods. Chromatographic peaks generated in high-speed GC and GCxGC separations are often quite narrow and require the use of a relatively fast detector in order to be fully characterized. The high acquisition speed of the TOFMS allows for the characterization of these narrow peaks and also allows for spectral deconvolution of overlapping chromatographic peaks. The mass spectra were automatically compared against the NIST and Terpene[1] libraries for peak identification.

SAMPLE

By definition, all gin samples are derived from the juniper berry[2]. Gin is made through the distillation of the juniper berry or by adding juniper berry oil to a refined spirit. Many gins also contain additional essential oils such as citrus oils in order to enhance their flavor. In this study, a set of six gin samples was studied and compared to a juniper oil standard. Initial data review was done on a GC-TOFMS analysis of juniper oil. The identified compounds in the juniper oil were then used to build a reference against which all other gin samples were compared. This was done through the use of the "Reference" feature in ChromaTOF® which allows for the automated comparison of chromatograms against a standard based on retention time, a user-defined s/n threshold and spectral similarity. Once the comparison was made on the one-dimensional data, the two-dimensional chromatograms were compared to their one-dimensional counterparts. The one-dimensional data shows a good correlation between the juniper oil standard and the gin samples. The two-dimensional chromatograms show enhanced selectivity and separation of target compounds in addition to increased s/n for target analytes and an overall increase in the total number of compounds identified.

EXPERIMENTAL

GC-TOFMS Analysis

Sample Introduction:
Agilent Autosampler with a 5 L syringe
Injection: 1 µL (splitless)

GC: Agilent 6890 Gas Chromatograph
Inlet: split/splitless; 275°C
Carrier Gas: He at 1.5 mL/min
Column: 10 m x 0.18 mm x 0.20 m Rtx-5
GC Oven: 60°C (1 min hold) programmed to 250°C at 20°C/min (2 min hold)
MS Transfer Line: 250°C

MS: LECO TruTOF® HT
Ionization: EI at -70 eV
Ion Source Temperature: 300°C
Spectral Acquisition Rate: 10 spectra/s
Acquired Mass Range: 35-500
Acquisition Delay: 60 s

Instrument Control and Data Review:
ChromaTOF optimized for TruTOF HT

GCxGC-TOFMS Analysis

Sample Introduction:
Agilent Autosampler with a 10 L syringe
Injection: 1 µL (splitless)

GC: Agilent 6890 Gas Chromatograph
Inlet: split/splitless; 275°C
Carrier Gas: He at 0.6 mL/min
Primary Column: 10 m x 0.18 mm x 0.18 m DB-5
Secondary Column: 1 m x 0.10 mm x 0.10 m BPX-50
Primary Oven: 80°C (2 min hold) programmed to 225°C at 5°C/min (3 min hold)
Secondary Oven: 5°C offset from the primary oven
Thermal Modulator: 30°C offset from the primary oven
Modulation Period: 5 s
MS Transfer Line: 250°C

MS: LECO Pegasus® 4D
Ionization: EI at -70 eV
Ion Source Temperature: 200°C
Spectral Acquisition Rate: 150 spectra/s
Acquired Mass Range: 35-350
Acquisition Delay: 100 s

Instrument Control and Data Review:
ChromaTOF optimized for Pegasus 4D

TruTOF HT

Pegasus 4D

RESULTS AND DISCUSSION

Name	m/z	R.T. (s)	Area	Height	Quant S/N
Tricyclene	92	75.5	57473	5200.2	39.685
Bicyclo[3.1.0]hexane, 4-methyl-1-(1-methylethyl)-,	93	74.4	1959065	131342	597.86
Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, (α)-	93	77.8	33652552	1138926	51810
Camphene	93	83.6	2389034	187616	854.02
THUJA-2,4(10)-DIENE	91	86	112931	8039.7	32.071
Sabinene	91	94.4	9498668	602063	2405.7
α-Myrcene	70	95.7	144753	12087	105.74
Myrcene	93	102.3	11399964	1006980	4583.7
Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, (α)-	93	108	488571	36443	165.88
α-Phyrene	93	110.9	13685482	1235255	5625.1
Terpilene	121	113.8	661487	59095	453.59
m-Cymene	119	117.6	7582463	624567	4314.8
Limonene	93	119.8	27382554	2723438	12397
1,4-p-Menthadiene	93	133.3	1573102	155603	708.29
2-Carene	136	146.9	726087	74374	831.21
Linalool	71	152.5	196919	16583	127.21
Isothujone	81	155.1	180728	16374	84.108
Isothujone	110	159.9	55118	5415	66.799
L-pinocarveol	92	169.9	111423	9250.7	70.597
Verbenol	109	172.8	168967	15462	111.08
L-borneol	95	182.2	204543	19321	114.4
4-Terpineol	71	187.1	2812377	290653	2229.7
p-Cymen-8-ol	135	190.8	319814	28037	249.4
p-menth-1-en-8-ol	59	193.1	749486	73879	621.55
Myrtenal	79	195.7	186120	15065	70.696
Verbenone	107	201.5	80250	7483.1	47.071
Methyl thymol ether	149	212	64504	6687.6	90.234

Name	m/z	R.T. (s)	Area	Height	Quant S/N
Carvone	82	216.1	72061	6405.9	53.978
Trans-Ascaridol glycol	109	226.8	185412	15893	114.18
Isobornyl acetate	95	233.8	4237546	373261	2102.2
α-Cubebene	161	258.6	283984	29632	409.83
Copaene	161	268.9	236946	24542	339.44
b-Elemene	68	275.2	537508	54307	437.36
Isoledene	204	279.2	37609	3664.2	75.297
Junipene	161	280.5	56740	5236.2	72.421
α-Cedrene	119	283	69444	6729.7	46.492
Caryophyllene	133	285.8	897059	91476	887.05
t-Elemene	121	290.9	353050	32449	249.07
α-Caryophyllene	93	298.6	2312363	215716	981.93
α-Muurolene	189	306.9	61758	4584.3	83.393
Germacrene D	120	308.8	347661	32675	375.22
α-Selinene	189	310.7	26635	2699.4	49.977
Valencene	189	313.9	101620	9584.7	177.45
α-Muurolene	105	315.8	434722	41766	252.8
γ-Cadinene	161	320.8	429393	40164	555.5
δ-Cadinene	161	324.1	1069865	106167	1468.4
trans-Cadino-1,4-diene	119	327.1	69537	5567.6	38.464
α-Cadinene	105	329.1	146926	11425	69.153
Elemol	59	333.2	107987	10184	85.678
Germacrene B	121	336.1	638603	61037	468.5
(-)-Spathulenol	205	343.4	44859	4205.5	86.652
Caryophyllene oxide	96	345.3	61692	5395.8	58.507
α-Cadinol	95	369.3	155847	12483	75.099
Benzyl cinnamate	192	499.8	137479	8578.9	192.17

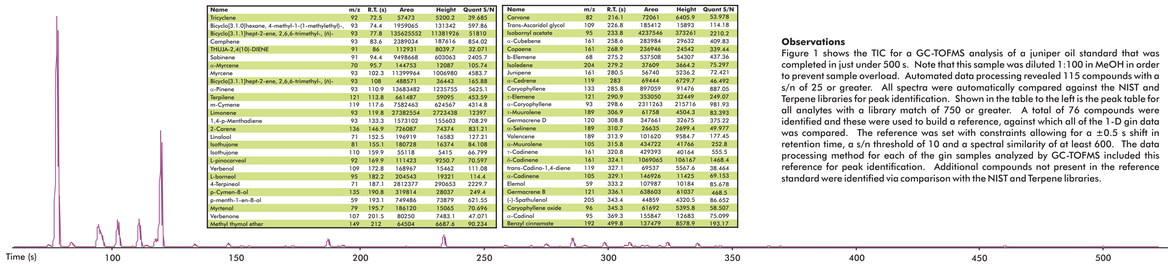

Figure 1. Total Ion Chromatogram (TIC) and peak table for the analysis of Juniper Oil.

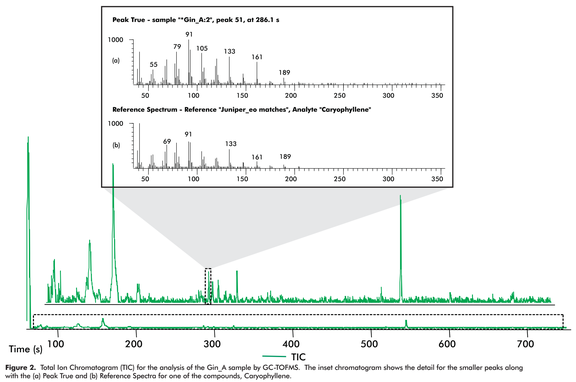

Figure 2. Total Ion Chromatogram (TIC) for the analysis of the Gin_A sample by GC-TOFMS. The inset chromatogram shows the detail for the smaller peaks along with the (a) Peak True and (b) Reference Spectra for one of the compounds, Caryophyllene.

Observations

Figure 2 shows a TIC for the analysis of a commercially-available gin sample. The large peak at the beginning of the chromatogram is Ethanol. The inset shows the smaller peaks in detail. Shown above the chromatogram are the (a) Peak True and (b) Reference spectra for one of the analytes, Caryophyllene. The Peak True spectrum contains all of the ions associated with a particular compound following the application of the Deconvolution algorithm. The peak true spectrum was then compared against the reference standard in order to identify the analyte as Caryophyllene. In this case, the Reference Spectrum is the Caryophyllene Peak True spectrum from the Juniper Oil chromatogram shown in Figure 1.

A total of 17 compounds that were present in the Juniper Oil standard were identified in the "Gin_A" standard. An additional 43 compounds were identified with a s/n greater than 25. Of those compounds, 22 were identified following comparison with the standard NIST and Terpene libraries.

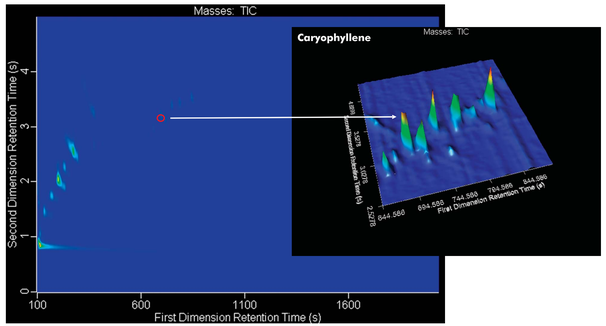

Figure 3. Total Ion Chromatogram (TIC) for the analysis of the Gin_A sample by GCxGC-TOFMS. The inset surface plot shows the detail for a group of smaller peaks. The Caryophyllene peak is highlighted in both the contour and surface plots.

Observations

Figure 3 shows a TIC for the analysis of a commercially-available gin sample using GCxGC-TOFMS. The retention time on the first column is displayed on the x-axis and the retention time on the second column is displayed on the y-axis. The inset shows a close-up view of a region of low-level peaks displayed as a contour plot. The red circle shows the location of the Caryophyllene peak on the surface plot and the arrow is pointing at the same peak on the contour plot. Note that in the GC chromatogram shown in Figure 2, Caryophyllene (m/z 133) had a s/n of 91.00. The same peak in the GCxGC chromatogram in Figure 3 has a s/n of 476.54. This large increase in s/n is due to the band focusing that occurs in the thermal modulator in a GCxGC system. Wide bands that elute off of the first column are sliced and focused prior to injection onto the second column resulting in tall, narrow peaks. This is what allows for the increased detectability commonly associated with GCxGC analyses. The same gin sample was analyzed on the Pegasus GCxGC-TOFMS system with the thermal modulator turned off. This experiment gave a s/n for the Caryophyllene peak of 94.305 which is similar to that observed on the TruTOF, and it indicates that the dramatic increase in s/n is due to the chromatography and not the mass spectrometer.

The 17 compounds from the Juniper Oil standard that were identified in the GC chromatogram were also identified in the GCxGC chromatogram. As expected, significant increases in the s/n values were observed for all compounds in addition to an increase in the overall number of peaks detected and identified.

Observations

Figure 1 shows the TIC for a GC-TOFMS analysis of a juniper oil standard that was completed in just under 500 s. Note that this sample was diluted 1:100 in MeOH in order to prevent sample overload. Automated data processing revealed 115 compounds with a s/n of 25 or greater. All spectra were automatically compared against the NIST and Terpene libraries for peak identification. Shown in the table to the left is the peak table for all analytes with a library match of 750 or greater. A total of 76 compounds were identified and these were used to build a reference, against which all of the 1-D gin data was compared. The reference was set with constraints allowing for a ±0.5 s shift in retention time, a s/n threshold of 10 and a spectral similarity of at least 600. The data processing method for each of the gin samples analyzed by GC-TOFMS included this reference for peak identification. Additional compounds not present in the reference standard were identified via comparison with the NIST and Terpene libraries.

SPECTRAL DECONVOLUTION

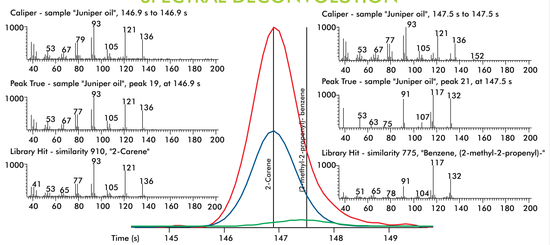

Figure 4. Example of spectral deconvolution for the identification of two overlapping components in the Juniper Oil sample.

Observations

Figure 4 shows an example of spectral deconvolution observed in the analysis of the juniper oil standard. The TIC (red) appears to show the presence of a single chromatographic peak. However, by plotting the extracted ions (m/z 93 and 177) it is clear that there are in fact two analytes present. The spectra on either side of the chromatogram are for the two overlapping analytes. The Caliper spectra show all ions present at the peak apex. As a result of the overlap between the two compounds, ions for both are present in each caliper spectrum. Following deconvolution, the Peak True spectrum is generated which contains ions specific to that compound. Note the presence of m/z 121 and 136 in the caliper spectrum on the right. These are both due to the overlap between the two compounds. Following deconvolution, the Peak True spectrum on the right no longer contains these ions. The Peak True spectra were automatically compared against library spectra in order to identify the compounds as 2-Carene and (2-methyl-2-propenyl)-Benzene with similarity rankings of 910 and 775 respectively.

COMPARISON OF SAMPLES

Compound	Gin_A		Gin_B		Gin_C		Gin_D		Gin_E		Gin_F	
	m/z	GC	GC	GCxGC	GC	GCxGC	GC	GCxGC	GC	GCxGC	GC	GCxGC
α-Cubebene	161		17.14	36.98								
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	81	10.37	399.46	11.82	70.47		15.00	319.34			10.18	228.51
Isoledene	161	28.45	144.54				30.63	59.84			30.68	38.86
Caryophyllene	133	91.00	716.59	18.12	98.69		137.75	272.97			179.65	427.18
g-Elemene	121	109.14	806.41	36.92	216.14		249.96	742.49				
α-Caryophyllene	93	150.65	2166.20	42.59	510.62		212.81	1311.90			295.39	1416.00
(+)-Epi-bicyclosesquiphellandrene	161										40.33	51.51
Germacrene D	161	148.36	669.50	41.52	165.02		424.57	865.34			360.99	718.37
α-Selinene	93						12.03	66.17				
α-Muurolene	105	44.36	572.71				60.52	305.16			56.51	247.48
t-Cadinene	161	78.35	281.76				88.45	152.00				
d-Cadinene	161	368.27	1170.50	66.24	177.94		482.03	626.95	135.52	256.27	305.23	328.56
α-Calacorene	157	16.89	41.34									
1-epi-Cubenol	119	11.28	80.44				12.97	168.23				
Caryophyllene oxide	79	14.84	96.99									
α-Cadinol	95	39.67	107.78				31.39	47.73	86.23	238.26		
b-Bisabolenol	69	21.56	110.78	20.85	77.60		21.28	104.80				
Limonene	68	539.61	23448		104.84	11.58		205.7	2131.3	38.509	809.2	
Linalool	71	1142.6	13627	540.97	3930	192.8	1651.1	2057.5	12297	2413.5	17469	1843.9	13160
Verbenyl Ethyl Ether	100	76.801	545.88	53.287	234.17	3		36.711	61.912	43.3	175.12		21.275
L-Camphor	95	56.698	642.31	29.238	192.34		92.006	141.91	632.86	149.33	732.59	148.26	718.84
Total # of peaks detected		40	667	34	356	27	300	63	225	129	773	67	421

Table 1. Comparison of the s/n values observed for a select group of compounds in 6 different gin samples analyzed by GC-TOFMS and GCxGC-TOFMS.

Observations

Table 1 shows a comparison of each of the six gin samples analyzed by GC-TOFMS and GCxGC-TOFMS. The top part of the table shows a select group of compounds that were identified based on the reference comparison to juniper oil. The bottom set of compounds were not in the juniper oil, but seemed to be common among the gin samples and are likely flavor additives. As expected, there was a significant increase in the s/n values observed using GCxGC-TOFMS. Gin sample C did not have any similarities to the juniper oil standard. Gin sample E did not have many similarities to the juniper oil standard either, but contained many other essential oil components which was expected as this was labeled as a botanical gin.

CONCLUSIONS

This study showed a comparison of GC and GCxGC experiments for the screening of gin samples for their flavor components. Liquid injections were done of the neat samples with no sample preparation required. Automated data processing revealed a large number of compounds in samples analyzed by both methods. The use of the Reference feature in ChromaTOF allows for the fast and easy comparison of a group of samples to a reference sample, which in this case was the Juniper Oil. The fast detection offered by the TOFMS makes spectral deconvolution possible and allows for the detection and identification of analytes that may not be chromatographically resolved. As expected, a significant increase in s/n was observed in the GCxGC results relative to the GC results; however, the GC analysis revealed a large amount of information about the samples as well.

References:
1. The Terpene Library contains mass spectra of essential oil components compiled by Robert P. Adams, Baylor University Plant Biotechnology Center.
2. E.M. Hansen et al. J. Chromatogr. A 1134 (2007) 281-290