Analysis of Sagebrush Aromas by Headspace SPME-GCxGC-TOFMS and Utilization of Variable Modulation in the Second Dimension Separation

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1. Introduction

The common sagebrush (A. *tridentata*), also called big sagebrush, is a silvery-gray low shrub with a pungent odor of sage. Sagebrush is particularly abundant in arid regions of western North America. It is one of the most common shrubs of the West, where it is important as a forage plant on many cattle ranges and is often indicative of good soil. This species has been employed as a domestic remedy and tonic. The seeds were used for food by Native Americans. The wood ignites easily and burns well making it valuable for starting fires by friction. It is native to both Siberia and North America, from Alaska to Texas.

This novel food/flavor/fragrance application was conducted to show enhanced characterization of the aroma components released from sagebrush using headspace solid phase microextraction (SPME). Analysis of this very complex sample illustrates the advantages of GCxGC-TOFMS. The experimental method utilized comprehensive two-dimensional gas chromatography as well as variable modulation to maximize peak capacity, and chromatographic resolution for this complex sample. Tiime-of-flight mass spectrometry (TOFMS) detection provides the fast data acquisition and data density required to fully characterize complex matrices such as sagebrush volatile aromatics. Data processing was accomplished using ChromaTOF® software. ChromaTOF used automated peak find algorithms and mass spectral similarity searches to detect and identify the volatile components present.

2. Experimental Conditions

Fresh sagebrush was collected in the high desert region of northeastern Utah, placed in plastic containers and then frozen at -20°C for preservation of volatiles. Samples were prepared by placing 1 gram of sagebrush leaves in a 20 mL glass headspace sample vial and sealing with a septum cap. An automated headspace extraction was made in ChromaTOF software with the Gerstel MPS2 autosampler/prepstation. The headspace extraction was conducted at 35°C for 30 min with a 2 cm, 50/30 µm, divinylbenzene/carboxen/polydimethyl-siloxane SPME fiber (Supelco, Inc., Bellefonte, PA). Fiber desorption and sample injection followed immediately after headspace SPME extraction. A split injection of 25:1 was used for this analysis.

A LECO Pegasus® 4D time-of-flight mass spectrometer (LECO, St. Joseph, MI) was used to generate the GCxGC-TOFMS results. The Pegasus 4D instrument was equipped with an Agilent 7890 gas chromatograph (Agilent Technologies, Palo Alto, CA) featuring a two stage cryogenic thermal modulator and a secondary oven (LECO). LECO ChromaTOF® software was used for all acquisition control and data processing. A 30 m x 0.25 mm x 0.25 µm film thickness, Rxi-5Sil-MS GC capillary column (Restek Corp., Bellefonte, PA), was used as the primary column for the GCxGC-TOFMS analysis. In the GCxGC configuration, a second column 1.2 m x 0.10 mm id. x 0.10 µm film thickness, BPX-50 (SGE Analytical Science, Austin, TX) was placed inside the secondary GC oven after the thermal modulator. The helium carrier gas flow rate was set to 1.0 mL/min at constant flow. The primary column was programmed with an initial temperature of 70°C for 0.50 min then ramped at 4.0°C/min to 285°C for 1.0 min. The secondary column temperature program was set to an initial temperature of 75°C for 0.50 min then ramped at 4.0°C/min to 290°C for 1.0 min. The thermal modulator was set to +20°C relative to the primary oven and variable modulation periods of 3, 4, and 5 seconds were used. The GCxGC-TOFMS analysis total runtime was 55.25 minutes. The MS mass range was 45-400 m/z with an acquisition rate of 200 spectra/second. The ion source chamber was set to 230°C.

Delivering the Right Results

3. Results

A total of 1748 peaks were detected by this headspace SPME-GCxGC-TOFMS analysis of sagebrush volatiles. Figure 1 displays the two-dimensional contour plot chromatogram with peak markers for this analysis. The library searched mass spectra produced 741 compounds with similarity matches greater than or equal to 70%. The Contour plot chromatogram in figure 1 clearly shows the ability of GCxGC coupled with variable modulation to maximize separation and resolution capabilities that effectively characterize extremely complex samples.

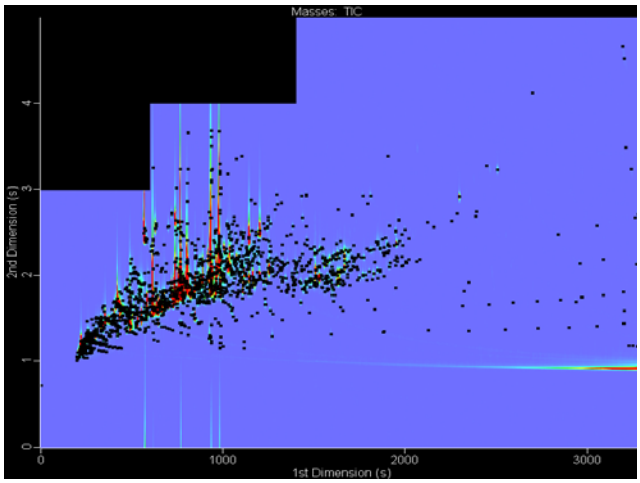

Figure 1. This contour plot shows the automated headspace SPME-GCxGC-TOFMS analysis of a 1 g sample of fresh sagebrush heated to 35°C. A total of 1748 peaks were found with a S/N ratio of at least 50. The library search results identified 741 compounds with a match similarity of at least 70%.

Using Variable Modulation for Optimized GCxGC Method Development

The contour plot in Figure 1 illustrates the application of variable modulation periods showing the second dimension time periods of 3, 4, and 5 seconds respectively. The variable modulation capability allows the chromatographer to incrementally increase the modulation period only as needed. Notice in Figure 1 the second dimension modulation period was increased from 3 to 4 seconds at the 1st dimension retention time of 600 seconds. Lengthening the 2nd dimension modulation period to 4 seconds from 600 to 1400 seconds as well as an incremental increase of the hot pulse time from 0.5 to 0.6 seconds facilitated optimization of the peak capacity and chromatographic resolution in this data rich region of the GCXGC analysis. The third modulation period of 5 seconds with an increased hot pulse time of 0.7 seconds begins at 1400 seconds to the end of the run. The increased modulation period and hot pulse time allowed higher boiling components to elute within the modulation period therefore avoiding any analyte "wrap around".

Table I. Table I shows the modulation timing section in ChromaTOF software. The headers found in the table allow the user to set the modulation start and end time in seconds. The modulation periods and the hot pulse times can be varied to optimize chromatographic resolving power and peak capacity for the GCxGC separation.

Modulation Timing: For 1D GC set second dimension time to 0

#	Start	End	Modulation Period (s)	Hot Pulse Time	Cool Time Between Stages
1	Start of Run	600 s	3.00	0.50	1.00
2	600 s	1400 s	4.00	0.60	1.40
3*	1400 s	End of Run	5.00	0.70	1.80

Enhanced Peak Capacity and Resolving Power of GCxGC

The example in Figure 2 shows the separation and resolution of 7 different analytes that would not be possible by a single dimension separation. Components of widely varying concentrations were separated without difficulty by comprehensive two-dimensional chromatography. These components would otherwise be buried in heavy sample matrices. The yellow and pink arrows represent two sets of components that are totally coeluted by their 1st dimension retention time yet are resolved completely by the 2nd dimension separation.

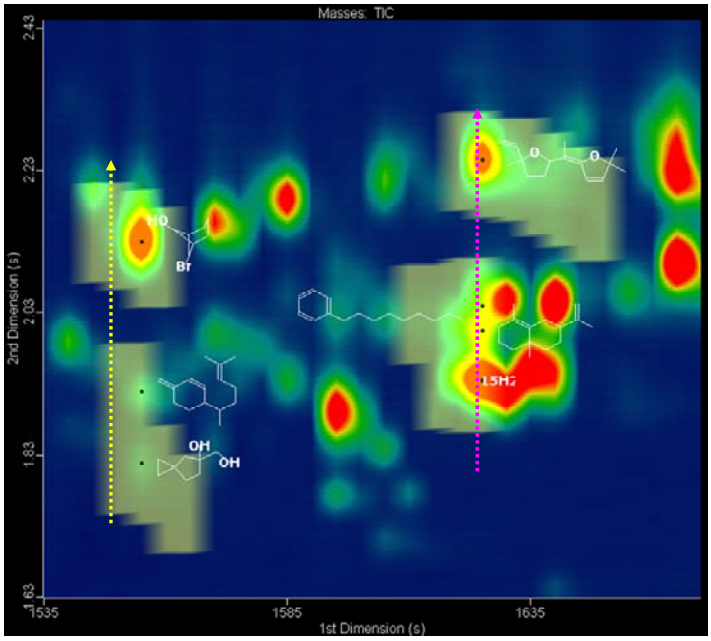

Figure 2. The contour plot in Figure 2 demonstrates the resolving power of comprehensive two-dimensional GCxGC. The yellow arrow at 1555s 1st dimension retention time and the pink arrow at 1625s 1st dimension retention time show 7 components identified with their chemical structures. The components are separated and resolved in the 2nd dimension by approximately 420 milliseconds.

Table II. Peak table for the analytes identified in the Figure 2 example which illustrates the enhanced peak capacity of GCxGC.

Peak #	Name	R.T. (s)	Area	Height	UniqueMass	Similarity	S/N	Library
1513	Spiro[2.4]heptane-5-methanol, 5-hydroxy-	1555 , 1.820	43287	2998.3	111	747	361.98	mainlib
1514	á-SESQUIPHELLANDRENE	1555 , 1.920	45993	4018.2	69	813	257.07	Wiley7
1515	Bicyclo[3.1.1]heptan-endo-6-ol, syn-7-bromo-	1555 , 2.130	544096	38778	111	802	5129.5	mainlib
1567	ç-CURCUMENE	1625 , 1.935	497415	35679	93	816	2781.5	Wiley7
1568	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	1625 , 2.005	69712	5523	189	780	669.65	mainlib
1569	BENZENE, NONYL-	1625 , 2.040	5412.7	441.06	128	601	62.401	Wiley7
1570	Davana ether	1625 , 2.245	462044	34423	109	834	3134.3	mainlib

The peak table shown in Table II highlights the increased peak capacity and enhanced detectability available using GCxGC-TOFMS. The 7 analytes identified in this example show significant variations in concentrations by their S/N and peak areas. This example illustrates the advantages of GCXGC-TOFMS to identify and detect even trace components in complex matrices of varying concentrations.

4. Conclusions

This novel food/flavor/fragrance application demonstrates that headspace SPME GCxGC-TOFMS analysis of sagebrush provides enhanced chromatographic separation which facilitates volatile component characterization. This work illustrates the ability of GCxGC-TOFMS to provide enhanced analyte detectability for complex volatile matrices such as sagebrush. The use of variable modulation to optimize GCxGC method development was also shown.

5. References

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