LabWorks APPNOTE

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Static Headspace combined with Hot Injection and Trapping (HIT-HS) for the Analysis of Flavor Volatiles in Kombucha

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Keywords

LabWorks, Kombucha, static headspace, trapping, HIT-HS, gas chromatography, mass spectrometry

Abstract

Static headspace analysis is often considered to be useful only when the analytes of interest are present in high concentrations. Although techniques like solid phase microextraction (SPME) and dynamic headspace (DHS) have better extraction efficiencies, due to advances in mass spectrometry design, VOCs can now be detected in the parts per billion range using static headspace. Static headspace is routinely used for blood alcohol determination and for the analysis of residual solvents in pharmaceuticals. Method development is fast and simple, usually with little to no sample preparation.

Trapping for static headspace injection offers several improvements over simple injection into the GC inlet. Analyte focusing after headspace injection can help to sharpen peaks and reach improved limits of detection for early eluting compounds, which tend to not focus well on the head of the GC column. Trapping also enables analyte stacking over multiple injections from one or more vials. This leads to more mass on column and a further lowering of detection limits.

This application note demonstrates the application of the hot injection and trapping headspace technique (HIT-HS) for determination of volatile compounds in flavored kombucha tea.

Introduction

The GERSTEL Labworks Platform enables static headspace injection into the TDU 2 combined with subsequent trapping in the CIS 4 inlet. A single injection or multiple injections from a single vial can be made. The Maestro software also allows for single or multiple injections from multiple vials for improved limits of determination.

Kombucha is a fermented tea which is purported to have health benefits. It has become popular in the US over the past several years. Spices, juice, sugar, fruit or other flavorings are added to the drink to enhance its taste. Static headspace can be used to monitor the flavor profile for quality control or to look for off odors which may have formed.

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Experimental

Instrumentation

GERSTEL Labworks Platform on Agilent® GC-MSD.

Analysis Conditions LabWorks Platform

Static Headspace

Incubation temperature	40 °C
Incubation time	20 min
Injection volume	2 mL/injection

Thermal Desorption TDU 2

Pneumatics mode	splitless
Temperature	250 °C isothermal

CIS 4 Inlet

Pneumatics mode	solvent venting, splitless
Vent flow	50 mL/min until 1.0 min
Split flow	20 mL/min @ 1.01 min
Temperature	10 °C (1 min); 12 °C/sec to 280 °C (5 min)

Analysis Conditions GC

GC Agilent 7890

Column	30 m DB-5MS UI (Agilent), d_i=0.25 mm, d_f=0.25 µm
Pneumatics	He; P_i = 7.1 psi constant flow, 1.0 mL/min
Temperature	40 °C (2 min); 20 °C/min to 280 °C (2 min)

Analysis Conditions MS

MSD Agilent 5977A

Scan	40 to 450 amu

Sample Preparation

A five gram sample of a berry, lemon, ginger flavored kombucha was weighed into a 20 mL screw cap vial.

Results & Discussion

Figure 1 shows a HIT-HS injection into the TDU 2. The transport adapters for the TDU tubes used for this technique contain a septum, which allows for liquid, static headspace or solid phase microextraction (SPME) injections into the TDU 2. This technique can also be used with the TD 3.5+ thermal desorption unit.

Figure 1: Static headspace injection into the TDU 2 in HIT-HS mode.

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Figure 2 shows the resulting chromatogram from a single 2 mL HIT-HS injection of kombucha tea headspace. The two largest peaks are limonene and γ-terpinene, which provide the lemon/citrus notes. Ethyl acetate and isoamyl acetate are contributors to the berry flavor. Other minor terpenes are present in the chromatogram. The chromatogram shows a good peak shape for ethyl acetate due to refocusing in the CIS 4.

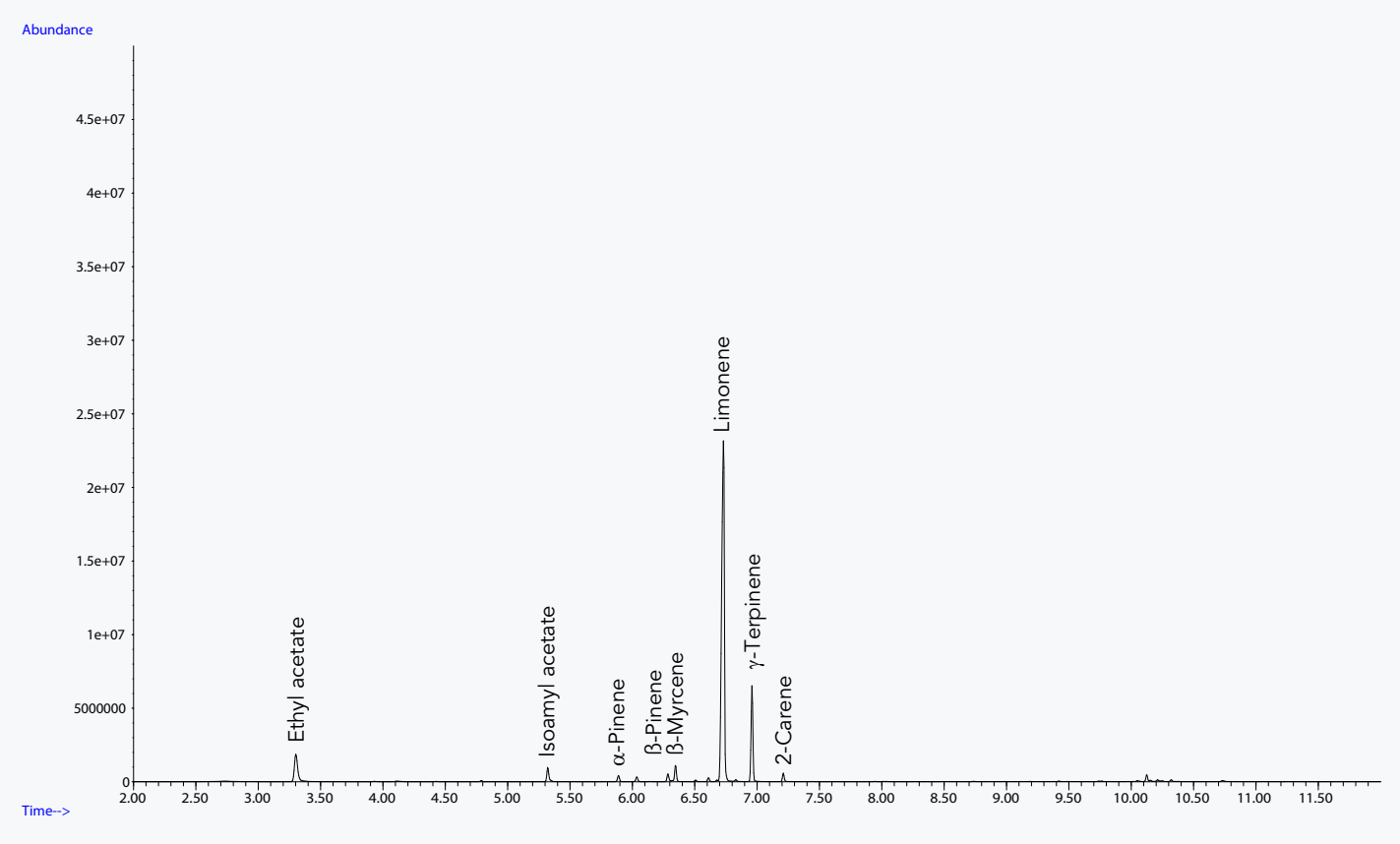

Figure 2: Chromatogram resulting from a single 2 mL injection in HIT-HS mode.

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Figure 3 shows two HIT-HS chromatograms. The upper chromatogram shows the injection of three 2 mL injections from the same headspace vial. The three injections were stacked in the CIS 4 inlet. This is easily accomplished in the Maestro software using the Multiple Headspace Static Enrichment (MHSE) option in the software. It allows the user to select multiple injections, the ability to set a delay time between injections to re-equilibrate the sample, and the ability to pressurize the vial in between injections, so as to avoid creating a vacuum in the vial through the repeated sampling steps. The start signal is sent to the GC after the last injection.

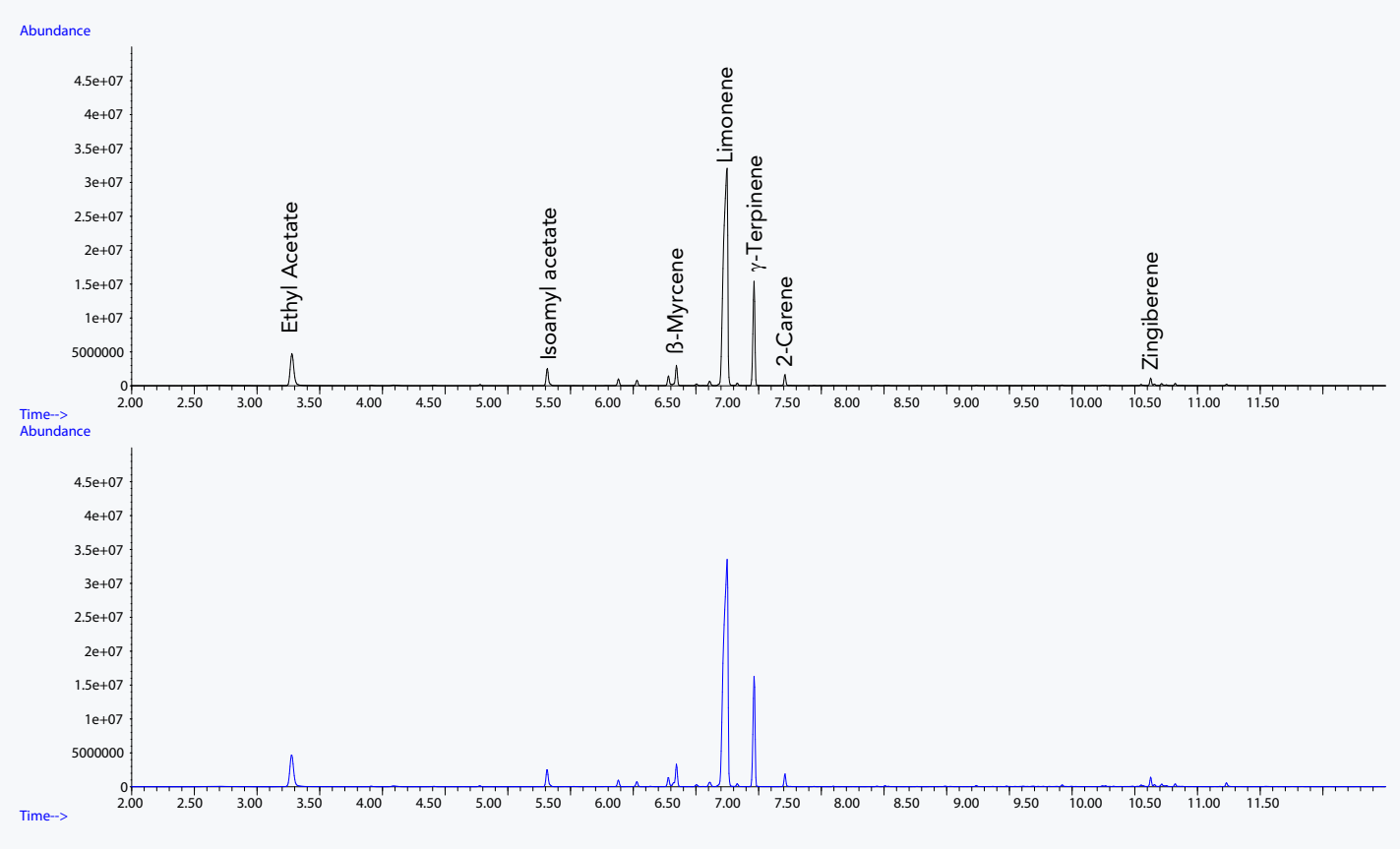

Figure 3: Stacked view of chromatograms resulting from 3 injections from a single vial (top) and 3 injections from separate vials (bottom) using HIT-HS.

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The bottom chromatogram of figure 3 shows the results from stacking three 2 mL injections from separate vials in the inlet. This is accomplished using the prep functions in Maestro. The PrepBuilder function in Maestro enables simple set up of sample preparation steps. Comparison of the two chromatograms shows that for this sample type and for the volatiles in question, no distinct benefit was gained from injecting from multiple separate samples. Both approaches loaded a total of 6 mL headspace volume into the inlet with similar responses. When comparing these chromatograms to the one in figure 2, one can clearly see the increase in signal achieved when a larger volume of sample headspace is injected combined with analyte trapping in the inlet. Figure 4 shows the resulting chromatogram when a total volume of 18 mL headspace is injected in HIT-HS mode using three successive 2 mL injections from each of three separate vials. The increase in signal is evident when compared to the other chromatograms, and is very clearly seen for the sesquiterpenes between 9.5-11.5 minutes. Zingiberene and Bisabolene are two of the main components responsible for the ginger flavor. Other compounds that can be determined based on the increased sample volume are 2-methyl butanal (chocolate, nutty), 3-methyl butanal (fruity, green, chocolate), ethyl propanoate (fruity, sweet, winey), isobutyl acetate (fruity, apple, banana, terpinen-4-ol (pepper, woody), α-terpineol (citrus, woody, lemon) and 2-phenethyl acetate (sweet, honey, floral). All these compounds contribute to the beverage flavor profile.

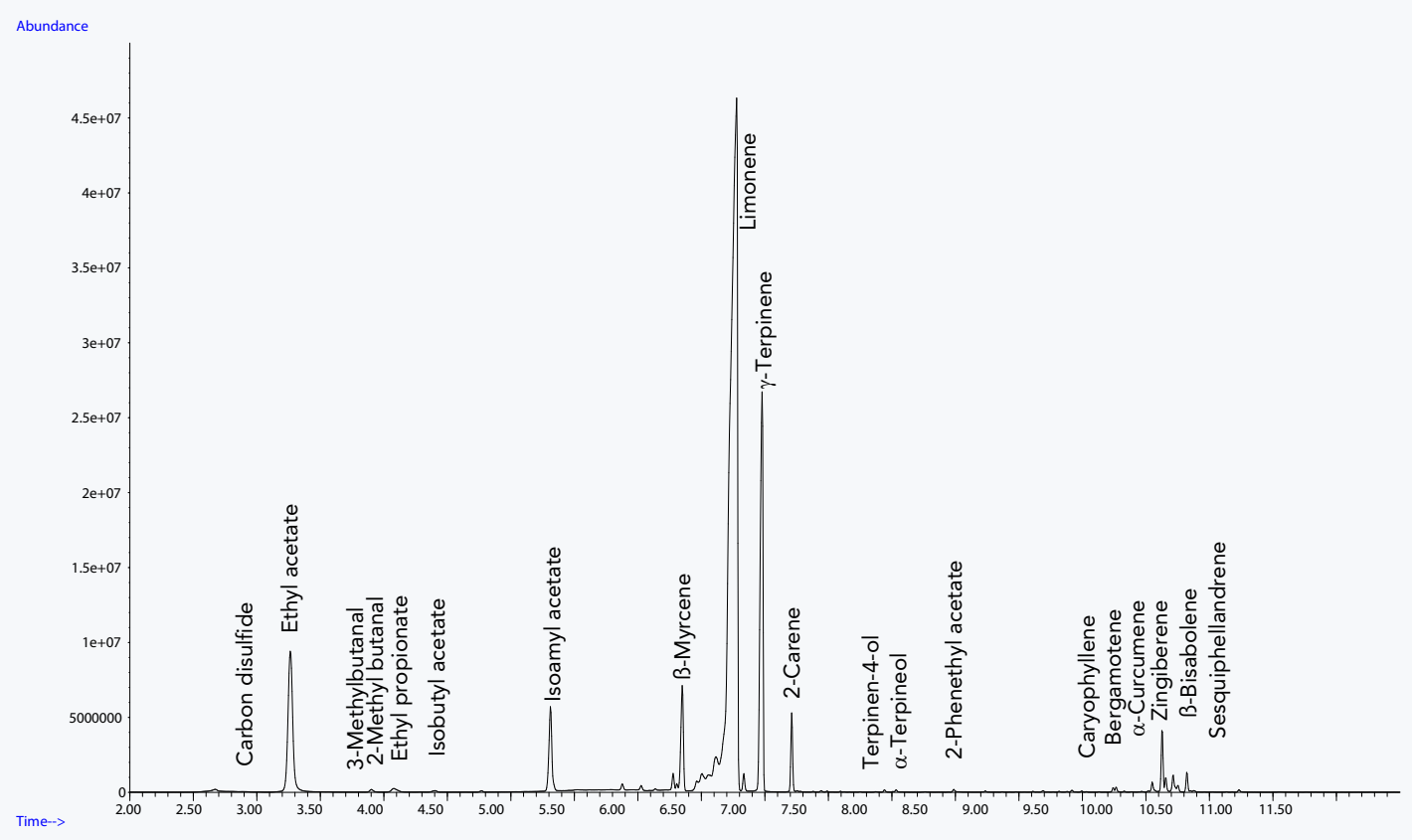

Figure 4: Stacked injection of nine 2 mL headspace volumes from 3 separate vials using HIT-HS (total volume injected: 18 mL).

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Table 1 shows peak area ratios for selected compounds identified in the chromatograms. The volume ratio for the four injection modes is 1:3:3:9. Beta-myrcene and zingiberene show nearly linear correlation between peak area and injected volume. The other compounds show a slight decrease from ideal behavior for the 18 mL injection, though the area counts increase for all.

Table 1: Area Ratio Comparison

		Name →	CS_2	Ethyl Acetate	Isoamyl Acetate	Beta Myrcene	Gamma Terpinene	Zingiberene
		R.T. →	2.90	3.29	5.32	6.34	6.95	10.13
		m/z →	62	43	43	93	93	119
Number of Injections	Number of Vials	Total Volume [mL]	Area Ratio	Area Ratio	Area Ratio	Area Ratio	Area Ratio	Area Ratio
1	1	2	1.0	1.0	1.0	1.0	1.0	1.0
3	1	6	3.2	2.9	2.9	2.8	2.5	2.7
1	3	6	2.5	2.9	3.0	3.2	2.8	2.9
3	3	18	5.3	7.0	7.7	8.6	6.0	9.9

Conclusions

Static headspace can be used for the determination of volatile flavor compounds in beverages. Method development is simple with little to no sample preparation. Static headspace injections are "clean" relative to direct liquid, higher boiling compounds and matrix are eliminated in the headspace sampling process, reducing instrument maintenance requirements. Early eluting peaks can benefit from trapping prior to release onto the column resulting in narrower peaks and lower detection limits. The GERSTEL Maestro software allows for stacking multiple injections from a single vial or multiple injections from multiple vials into the CIS 4, resulting in much lower detection limits. Injecting into a hot thermal desorption unit eliminates any needle discrimination that might occur when injecting into a cool inlet, good recovery is ensured even for higher boiling compounds. The GERSTEL LabWorks Platform thermal desorber and CIS 4 inlet trap are connected directly without the use of a valve or transfer line, reducing or eliminating loss of analytes and ensuring that correct results can be achieved.