Application Note

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Analysis of Flavor Compounds in Beer using the Integrated Agilent 8697 Headspace Sampler with the Agilent 8890 GC System

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Abstract

This application note describes the determination of 15 volatile flavor compounds in complex beer matrices using an Agilent 8697 headspace sampler integrated with the Agilent 8890 GC system. The inertness of the whole system ensured that the GC method achieved excellent performance in terms of linearity, repeatability, and high sensitivity, with no analyte loss. Correlation coefficients (R^2) were ≥0.997 for all 13 alcohol, aldehyde, and ester compounds and the percent relative standard deviation (%RSD) of the concentration results from multiple measurements of the compounds was ≤4.5%. For the two diketones analyzed in the study, the R^2 values were better than 0.999, and the concentration %RSD was ≤1.7%.

Introduction

Beer is a popular drink in many parts of the world. The taste and flavor of beer are mainly determined by volatile flavor compounds that are produced as by-products during fermentation. Although the concentration of compounds that affect the flavor of beer is low in the final product, the compounds are complex, varied, and many hundreds have been identified. Flavor compounds include alcohols, esters, acids, aldehydes, ketones, sulfides, phenols, etc.[1,2] The combination of these compounds gives each beer its unique taste and flavor. However, the balance and concentration of the compounds needs to be controlled to prevent the beer from tasting or smelling "off". To control the flavor of each batch, beer producers typically use gas chromatography (GC) with flame ionization detection (FID) or an Electron Capture Detector (ECD) to detect volatile flavor substances. The application is part of the quality control (QC) testing program of beer to ensure consistency between batches. Breweries therefore require easy to use, reliable, and productive GC methods with good specificity and sensitivity for the routine analysis of beer.

In this study, an Agilent 8697 headspace sampler integrated with the Agilent 8890 GC was used to characterize two groups of flavor compounds in beer. Alcohols, aldehydes, and esters were measured separately from diketones, including butanedione and 2,3-pentanedione. The method was evaluated for linearity, sensitivity, repeatability, peak shape, and resolution of each compound.

Experimental

Instrumentation

The 8890 GC was configured with an FID and an ECD. All the analytes were introduced into the split/splitless (SSL) inlet of the 8890 GC by the 8697 headspace sampler. As the 8697 headspace sampler is fully integrated with the 8890, the method was managed from a single interface, simplifying operation of the method. An Agilent HP-INNOWax column was used for separation of the alcohols, aldehydes, and esters analysis, and all the sample components were detected by FID. An Agilent DB-5 column with thick film was used to provide better resolution and peak shape for the separation of butanedione and 2,3-pentanedione. The ECD was used due to its higher selectivity and sensitivity for the measurement of ketones. Tables 1, 2, and 3 show the instruments and conditions used.

Chemicals and sample preparation

Single standards of the 15 flavor compounds were bought from ANPEL Laboratory Technologies (Shanghai) Inc. (China). Two groups of mixed standard stock solutions were prepared in ethanol. Group 1 contained the alcohols, aldehydes, and esters and the two ketones were included in group 2.

Table 1. Agilent 8697 headspace sampler operating conditions for the introduction of volatile flavor compounds in beer to the GC.

Parameter	Value
Loop Size	1 mL
Pressurization Gas	Nitrogen
Oven Temperature	70 °C
Loop Temperature	70 °C
Transfer Line Temperature	100 °C
Vial Equilibration Time	30 min
Injection Duration	0.5 min
Vial Size	20 mL
Fill Pressure	15 psi
Loop Final Pressure	4 psi
Vial Shaking	Level 8

Table 2. GC method conditions for the separation of alcohols, aldehydes, and esters.

Parameter	Value
GC System	Agilent 8890 GC/FID
Inlet	Split/splitless, 250 °C; split ratio 5:1; Liner: straight, deactivated, 2 mm id (p/n 5181-8818)
Column	Agilent HP-INNOWax, 60 m × 0.32 mm, 0.5 µm (p/n 19091N-216I)
Carrier	Nitrogen, 3 mL/min, constant flow
Oven	38 °C (1 min), then 10 °C/min to 135 °C, then 5 °C/min to 150 °C, then 10 °C/min to 180 °C (5 min)
FID	250 °C; hydrogen: 40 mL/min; air: 400 mL/min; make up gas (N_2): 25 mL/min

Table 3. GC method conditions for the separation of butanedione and 2,3-pentanedione.

Parameter	Value
GC System	Agilent 8890 GC/ECD
Inlet	Split/splitless, 150 °C; split ratio 1:1; Liner: straight, deactivated, 2 mm id (p/n 5181-8818)
Column	Agilent DB-5, 60 m × 0.53 mm, 5 µm (p/n 125-5065)
Carrier	Nitrogen, 10 mL/min, constant flow
Oven	45 °C (2 min), then 10 °C/min to 150 °C (5 min)
ECD	150 °C; make up gas (N_2): 30 mL/min

Group 1 calibration solution: several headspace vials containing 3 g sodium chloride and 10 mL of the 5% ethanol water solution were spiked with varying amounts of stock and intermediate stock solution to achieve the required levels. Adding sodium chloride to the calibration solution improved the sensitivity of the measurements. Table A1 in Appendix A shows the concentrations of the different calibration range for each compound.

Group 2 calibration solution: six headspace vials were prepared at 5, 10, 25, 50, 100, and 200 µg/L by spiking varying amounts of the stock solution in 10 mL of the 5% ethanol water solution.

Two commercial beer samples (samples 1 and 2) were bought from a local supermarket for the recovery test.

Results and discussion

Alcohols, aldehydes, and esters analysis

Chromatogram

The 8890 GC with 8697 headspace sampler and FID was used for the analysis of alcohols, aldehydes, and esters in beer. Figure 1 shows the chromatogram of the 13 target compounds at a concentration of Level 7 (see Table A1 in Appendix A). The amount of each compound in beer varies with different type or brand of beer, thus, the linear range of each compound in this study was based on a typical amount.[1] Also, different compounds have different responses at the FID, so the peak area of each compound can vary greatly at the same compound-concentration level. Sharp, well-resolved peaks were obtained for most compounds using the 60 m × 0.32 mm, 0.5 µm HP-INNOWax column. Methanol (peak 6) is strongly polar and is prone to tailing, so the peak shape was not as sharp as the other compounds.

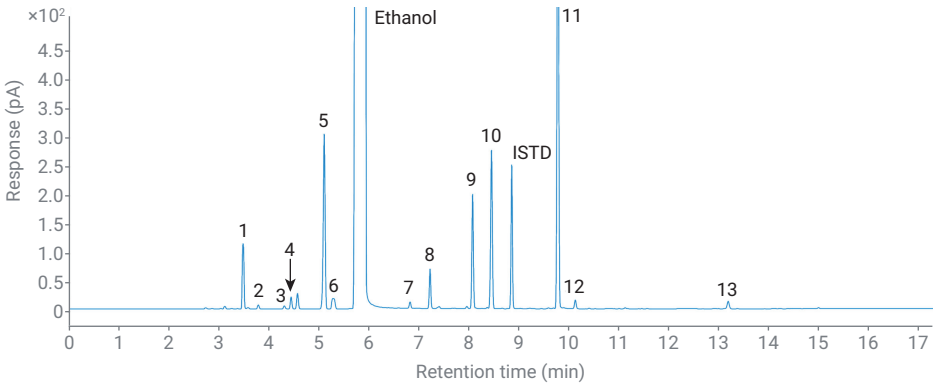

Figure 1. GC/FID chromatogram of target compounds (at the concentration of L7) in 5% ethanol water solution using an Agilent HP-INNOWax column.

Linearity, repeatability, and limit of detection evaluation

The figures of merit for alcohols, aldehydes, and esters are shown in Table 4. The correlation coefficients (R^2) were ≥0.999 for all compounds apart from ethyl formate (R^2 = 0.997). Ethyl formate tends to react with water at room temperature, so the solution needs to be freshly prepared. Also, hydrolysis of ethyl formate is likely to occur during the long sequences needed for the linearity and repeatability tests. However, acceptable results were obtained for ethyl formate, as shown in Table 4. Repeatability was tested using six injections of the standard mixture at concentration level 4. The concentration %RSD from the six measurements was ≤2.5% for all compounds except ethyl formate (%RSD at 4.5%), as shown in Table 4 and Figure 2. The concentration of the lowest calibration point of the standard solution was used to calculate the limits of detection (LODs), based on the signal-to-noise ratio (S/N), Table 4.

Table 4. R^2, RSDs, and LODs for 13 target compounds.

Number	Name	Linearity Range	R^2	Conc. RSD% (n = 6)	LOD (µg/L)
1	Aldehyde	0.1 to 50 µg/mL	0.9999	1.2	10
2	Dimethyl sulfide	1 to 500 µg/L	0.9997	1.6	0.4
3	Isobutyraldehyde	5 to 500 µg/L	0.9999	2.1	2
4	Ethyl formate	0.1 to 50 µg/mL	0.997	4.5	30
5	Ethyl acetate	0.1 to 50 µg/mL	0.9997	2.5	0.5
6	Methanol	0.4 to 200 µg/mL	0.9999	2	110
7	Isobutyl acetate	1 to 500 µg/L	0.9999	3.4	0.3
8	Propyl alcohol	0.1 to 50 µg/mL	0.9999	1.2	20
9	Isobutanol	0.1 to 50 µg/mL	0.9999	0.6	5
10	Isoamyl acetate	0.02 to 10 µg/mL	0.9999	1.3	1
11	Isoamylol	0.4 to 200 µg/mL	0.9998	0.7	3
12	Ethyl hexanoate	5 to 500 µg/L	0.9998	2.4	1
13	Ethyl caprylate	5 to 500 µg/L	0.9998	2.4	1

Butanedione and 2,3-pentanedione analysis

Chromatogram

The 8890 GC with 8697 headspace sampler and ECD was used for the analysis of butanedione and 2,3-pentanedione in beer. Figure 3 shows the chromatogram of the two target compounds at a concentration of 50 µg/L in a beer sample. Peaks were well resolved on the 60 m × 0.53 mm, 5 µm DB-5 column. The peak shape for both butanedione and 2,3-pentanedione were sharp, and there was no obvious interference from other compounds in the beer.

Parameter optimization

The response mechanism of ECD is complex.[3] Temperature plays an important role in the sensitivity performance of the detector so it should be strictly controlled. As Figure 4 shows, the response of butanedione and 2,3-pentanedione decreases with an increase of the ECD temperature. However, if the temperature is too low, ECD is vulnerable to contamination. Considering both the sensitivity and contamination, 150 °C was selected as the ECD working temperature in this study.

Linearity, repeatability, and limit of detection evaluation

Linearity, repeatability, and LOD data for butanedione and 2,3-pentanedione is shown in Table 5. The R^2 values were better than 0.999 over the calibration range of 5 to 200 µg/L. Repeatability was calculated by determining the %RSD from seven replicate injections at a concentration of 25 µg/L. The concentration %RSD was ≤1.66% for both compounds. A concentration of 5 µg/L standard solution was used to calculate the LODs based on the S/N.

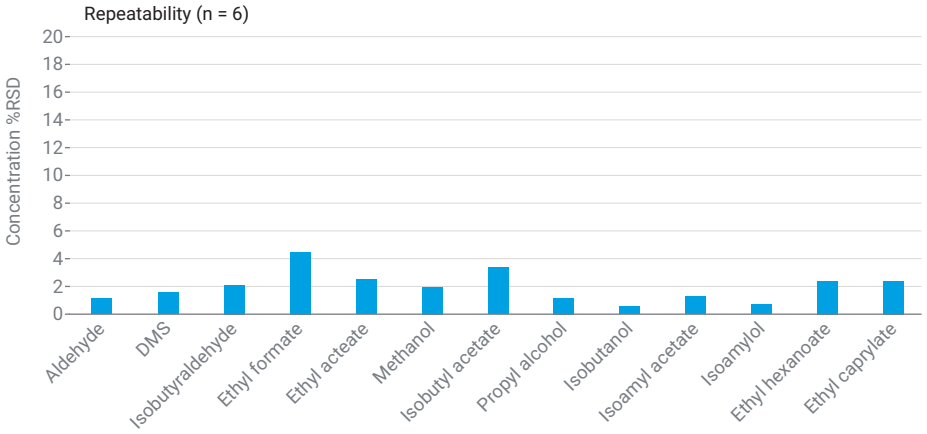

Figure 2. Conc %RSD results for the 13 compounds.

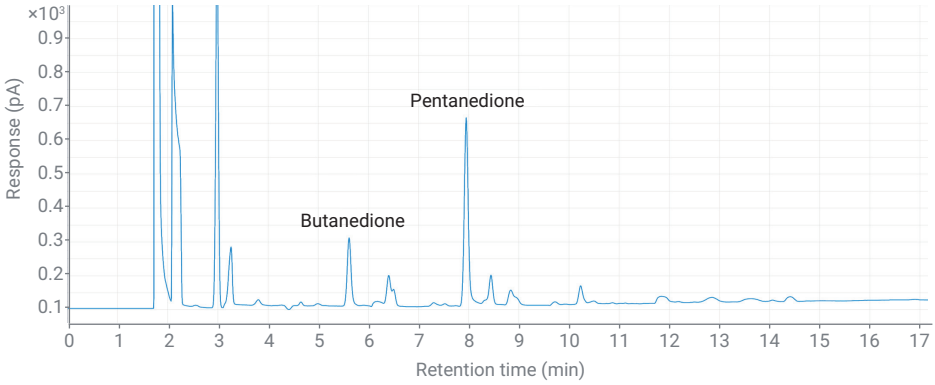

Figure 3. GC/ECD chromatogram of 50 µg/L butanedione and 2,3-pentanedione in beer.

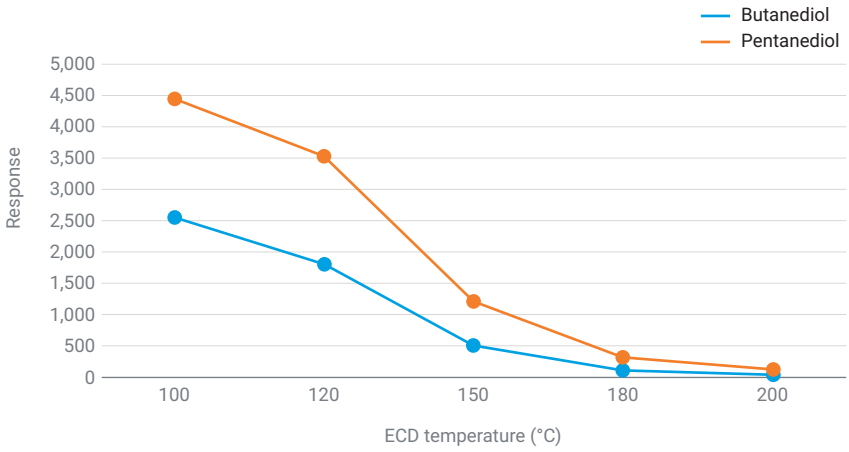

Figure 4. Response versus different ECD temperature for butanedione and 2,3-pentanedione.

Table 5. R^2, RSDs, and LODs for diketones.

Number	Name	R^2	%RSD (25 µg/L, n = 7)	LOD (µg/L)
1	Butanedione	1	1.4	0.2
2	2,3-pentanedione	0.9996	1.7	0.08

Recovery evaluation

To validate the method performance in beer samples, recoveries for butanedione and 2,3-pentanedione were evaluated by analyzing unspiked and spiked samples of the two commercial beers, samples 1 and 2. The average measured concentrations of the two compounds in both beer samples are shown in Table 6. Sample 1 contained double the amount of both butanedione and 2,3-pentanedione than sample 2, showing that the concentration of these compounds varies between different types of beer. The averaged recoveries for butanedione and 2,3-pentanedione were obtained from samples 1 and 2 spiked at 25 µg/L with three replicates. The recoveries ranged from 77.2 to 124.4%, as shown in Table 6.

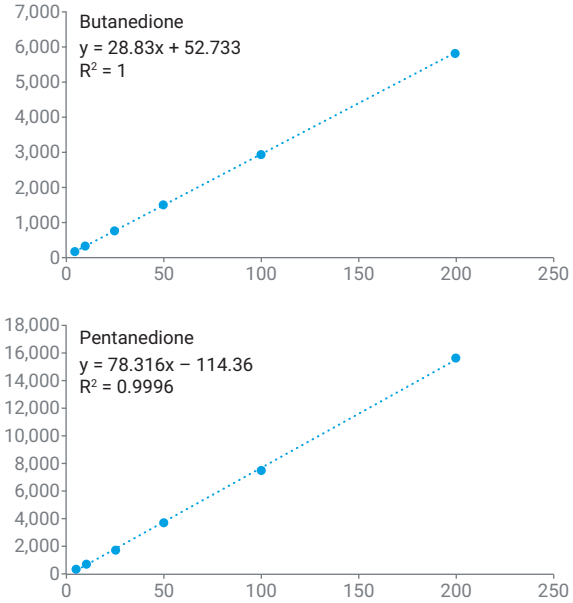

Figure 5. Calibration of butanedione and 2,3-pentanedione from 5 to 200 µg/L.

Table 6. Results of spike recovery of 25 µg/L ketone standard added to the two beer samples.

Name	Sample 1				Sample 2			
	Background (µg/L)	Calculated Conc. (µg/L)	Corrected Conc. (µg/L)	Average Recovery (%)	Background (µg/L)	Calculated Conc. (µg/L)	Corrected Conc. (µg/L)	Average Recovery (%)
Butanedione	10.6	29.9	19.3	77.2	4.7	24.5	19.8	79.2
2,3-Pentanedione	10.0	41.1	31.1	124.4	5.0	36	31.0	124.0

Conclusion

The taste and smell of beer are fundamental to the popularity of the product with consumers, so brewers aim for consistency of flavor between batches. This study has demonstrated that the Agilent 8697 headspace and 8890 GC configured with an FID and an ECD system can determine 15 different flavor compounds in beer. An Agilent HP-INNOWax was used for separation for alcohols, aldehydes, and esters on FID, while a DB-5 column was used for diketones analysis on ECD. Both methods achieved excellent linearity, repeatability, and sensitivity for the two groups of flavor substances, demonstrating the applicability of the method for the routine QA/QC analysis of beer.

References

1. Zhi -pei Wang, *et al.* Analysis and Evaluation of Aroma Volatiles in Beer, *Liquor-making Science & Technology* **2001**.
2. Yunfei Bai; Hao Wang; Shun Na, Determination of Diacetyl (Butanedione)& Pentanedione in Beer by HS-GC; *Agilent Technologies application note*, publication number 5991-1563EN, **2012**.
3. Guowang, Xu. Practical Gas Chromatography **2004**.

Appendix A

Table A1. Concentration of each compound at the different calibration levels (L1 to L9).

No.	Name	Concentration									Unit
		L1	L2	L3	L4	L5	L6	L7	L8	L9	
1	Aldehyde	0.1	0.2	0.5	1	2	5	10	20	50	µg/mL
2	Dimethyl sulfide	1	2	5	10	20	50	100	200	500	µg/L
3	Isobutyraldehyde	NA	NA	5	10	20	50	100	200	500	µg/L
4	Ethyl formate	0.1	0.2	0.5	1	2	5	10	20	50	µg/mL
5	Ethyl acetate	0.1	0.2	0.5	1	2	5	10	20	50	µg/mL
6	Methanol	0.4	0.8	2	4	8	20	40	80	200	µg/mL
7	Isobutyl acetate	1	2	5	10	20	50	100	200	500	µg/L
8	Propyl alcohol	0.1	0.2	0.5	1	2	5	10	20	50	µg/mL
9	Isobutanol	0.1	0.2	0.5	1	2	5	10	20	50	µg/mL
10	Isoamyl acetate	0.02	0.04	0.1	0.2	0.4	1	2	4	10	µg/mL
11	Isoamylol	0.4	0.8	2	4	8	20	40	80	200	µg/mL
12	Ethyl hexanoate	NA	NA	5	10	20	50	100	200	500	µg/L
13	Ethyl caprylate	NA	NA	5	10	20	50	100	200	500	µg/L
14	Butanedione	5	10	25	50	100	200	NA	NA	NA	µg/L
15	2,3-pentanedione	5	10	25	50	100	200	NA	NA	NA	µg/L

NA indicates that the concentration level was not used in the calculation of linearity.

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