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Application News

No. GC-2008

Gas Chromatography

Simultaneous Analysis of Vicinal Diketones, Volatile Compounds and Ethanol Content in Beer with a Single Headspace Injection (Shimadzu Beer 3-in-1 Analyzer)

■ Introduction

The chemistry of beer brewing is quite complex. Numerous compounds are involved, some contributing to desired flavors, while others could ruin the product. The fermentation process as well as the quality of the product can be monitored through analysis of a few of such compounds using gas chromatography (GC), as described in the standard methods from American Society of Brewing Chemist (ASBC). Specifically, two vicinal diketones (VDKs), diacetyl (2,3-butatnedione) and 2,3-pentanedione, give beer the unwanted buttery flavor and their concentrations decrease as fermentation progresses. The level of these two VDKs can be monitored by GC (ASBC Beer-25) to indicate the completion of the fermentation process. Furthermore, volatile compounds, such as esters, aldehydes, and alcohols, are formed through fermentation and give each type of beer its characteristic flavor. Like the VDKs, concentrations of these volatile organic compounds (VOCs) can be determined by GC (ASBC Beer-48).

Finally, GC can be used to measure the ethanol content (ASBC Beer-4), which is required by legal organizations such as USA's Alcohol and Tobacco Tax and Trade Bureau. To simplify the analysis procedures, we designed a new GC system to provide the brewers with an innovative, easy solution: the Shimadzu Beer 3-in-1 Analyzer. This new system is capable of simultaneously quantifying the above three categories of compounds with only one sample injection. Moreover, nitrogen is used as the carrier gas and the makeup gas, making this Shimadzu analyzer a cost-effective, time-saving solution for craft beer brewers.

■ Samples and Analytical Conditions/Experimental

The compounds analyzed in this study are shown in Table 1 below.

Table 1: List of compounds

Peak no.	Name	Compound Category	Detector
1	Acetaldehyde	VOC	FID
2	Ethyl acetate	VOC	
3	Ethanol	Ethanol	
4	*n*-Propanol	Internal standard (IS1) for Ethanol	
5	*iso*-Amyl acetate	VOC	
6	*n*-Butanol	Internal standard (IS2) for VOC	
7	*iso*-Amyl alcohol	VOC	
8	2,3-butanedione	VDK	ECD
9	2,3-pentanedione	VDK	
10	2,3-hexanedione	Internal standard (IS3) for VDK	

The acidified $CuSO_4$ solution, VDK stock solution, VOC stock solution and the internal standard (IS) stock solutions were prepared according to ASBC Beer-25 and ASBC Beer-48 methods. Because VOC and VDK standards are diluted with 5% ethanol, these standards cannot be used to perform a multi-point calibration for ethanol concentration determination. Therefore, two sets of calibration must be carried out.

The ethanol standards (2, 4, 8 and 10 %) were prepared by serial dilution from 200-proof ethanol with deionized water to specified concentrations. The VOC and VDK calibration standards are combined to simplify analysis. The concentrations of calibration standards used for VOC/VDK analysis are shown in Table 2 below.

Table 2: Concentrations of each analyte in VOC/VDK calibration standards. 5% ethanol was used as diluent to make the VDK/VOC calibration standard.

Compound	Std 1	Std 2	Std 3	Std 4	Std 5
Acetaldehyde	0.8 ppm	3.9 ppm	7.9 ppm	19.7 ppm	39.4 ppm
Ethyl acetate	0.9 ppm	4.5 ppm	9.1 ppm	22.6 ppm	45.3 ppm
iso-Amyl acetate	0.09 ppm	0.4 ppm	0.9 ppm	2.2 ppm	4.4 ppm
iso-Amyl alcohol	3.2 ppm	16.2 ppm	32.4 ppm	81 ppm	162 ppm
2,3-Butanedione	5 ppb	25 ppb	50 ppb	125 ppb	250 ppb
2,3-Pentanedione	5 ppb	25 ppb	50 ppb	125 ppb	250 ppb

The 3-in-1 internal standard (Tri-IS) was prepared by diluting 2,3-hexanedione ISTD stock solution and *n*-butanol into *n*-propanol.

Aliquots for analyses were prepared by mixing 5 mL of sample (beer, standard or blank) with 250 µL of Tri-IS and 250 µL acidified $CuSO_4$ solution in 20 mL headspace vials, which were then sealed with caps with PTFE/silicone septa and swirled by hand for 30 sec. Deionized water was used as blank solution for Ethanol analysis and 5% Ethanol was used as the blank solution for VOC/VDK analysis.

A Shimadzu GC-2014 with a split/splitless injector equipped with a 2-way capillary column adaptor, FID and ECD detector, and an HS-10 static headspace autosampler was configured, as shown in Figure 1, and used for analysis of ethanol, volatile compounds and diacetyls according to methods ASBC-4, -48 and -25. Analysis conditions are outlined in Table 2 below.

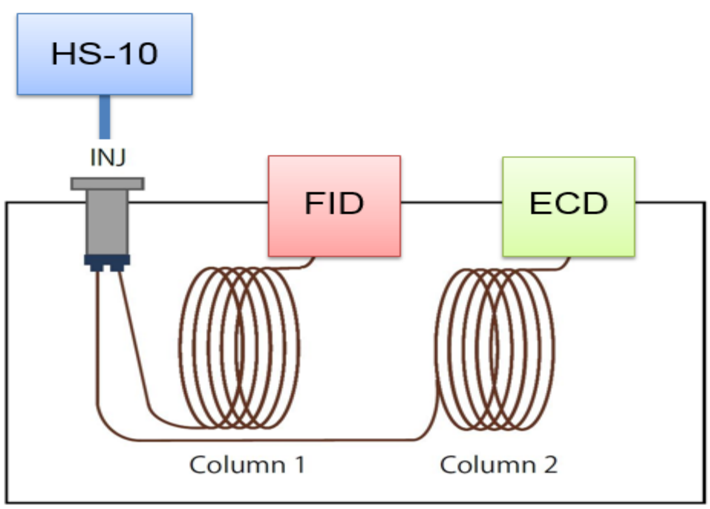


Figure 1: Schematic drawing of the dual column configuration for the Shimadzu Beer 3-in-1 Analyzer.

Table 2: Instrument Configuration and Analysis Conditions

GC system	Shimadzu GC-2014 with SPL (equipped with 2-way capillary column adaptor), FID and ECD
Column	SH-Stabilwax, 30m x 0.32mm x 1.0µm (line 1, to FID) SH-Rxi5Sil-MS, 30m x 0.32mm x 1.0µm (line 2, to ECD)
Injector Mode	Split at 1:20 (effluent split equally between two columns)
Injection Volume	1mL headspace
Carrier Gas	Nitrogen
Flow mode	Constant linear velocity of 35 cm/sec
Column Temp	40°C, 2.5 min – 10°C/min – 170°C, 4 min
Injection Port Temp	120°C
FID Temp and Gas	200°C, H_2 at 40 mL/min, Air at 400 mL/min, N_2 makeup at 30 mL/min
ECD Temp and Current	200°C, 1.5nA, N_2 makeup at 12mL/min
GC run time	19.5 min

HS system	Shimadzu HS-10 static headspace autosampler
Vial Equilibration	45 min at 60°C
Sample Pathway and Transfer Line Temp	100°C
Vial Pressurization	100 kPa with nitrogen

■ Results and Discussion

Resolution of the Components

VOC and VDK standards containing indicated compounds in 5% ethanol were added to 20mL headspace vials with a 3-in-1 internal standard (Tri-IS) solution. The vials were sealed, mixed and analyzed using Shimadzu HS-10 headspace autosampler and GC-2014 as described in the methods.

Representative chromatograms on FID and ECD are shown in Figures 2 and 3. All compounds were well resolved in under 20 minutes.

1) *FID channel, Volatile Flavor Compounds and Ethanol*

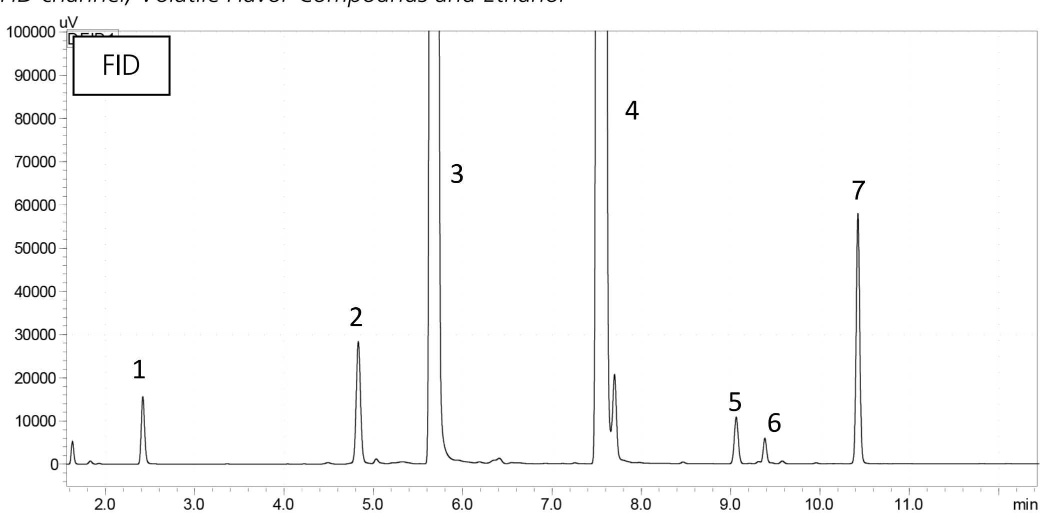


Figure 2: Chromatogram of VDK/VOC calibration standard in FID channel. Compounds of interest: ***1.*** Acetaldehyde ***2.*** Ethyl acetate ***3.*** Ethanol ***4.*** *n*-Propanol (ISTD for Ethanol) ***5.*** *iso*-Amyl acetate ***6.*** *n*-Butanol (ISTD for VOCs) ***7.*** *iso*-Amyl alcohol.

2) ECD channel, Diacetyl and 2,3-pentanedione

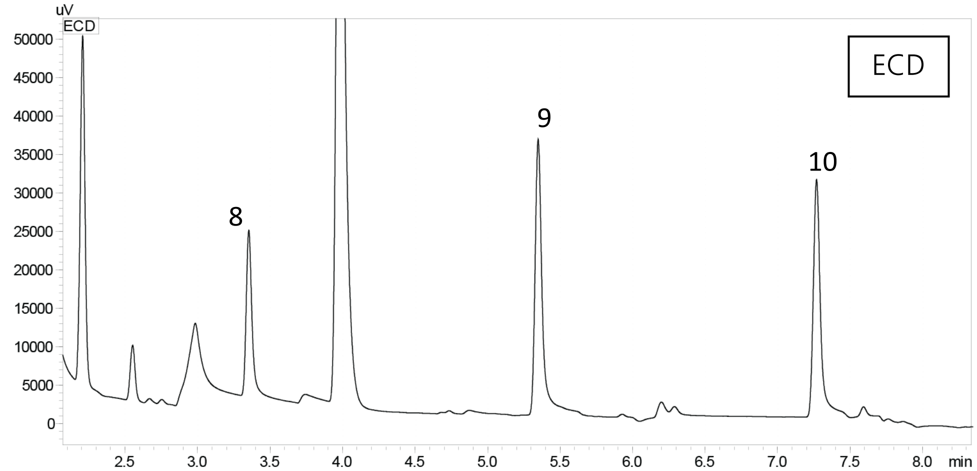


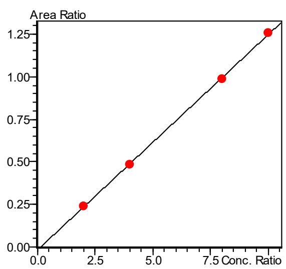
Figure 3: Chromatogram of VDK/VOC calibration standard in ECD channel. Compounds of interest: ***8.*** Diacetyl (2,3-butanedione) ***9.*** 2,3-pentanedione ***10.*** 2,3-hexanedione (ISTD for VDKs).

Calibration Curves

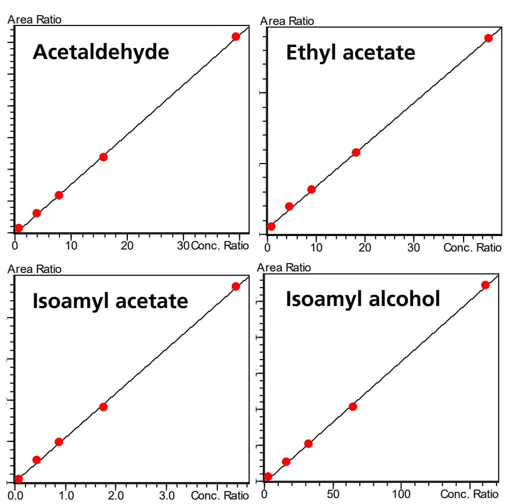
The standards containing VOC and VDK compounds were prepared as described in the methods. Internal standard quantification methods were used, and the calibration curves were fitted with linear regression without forcing through zero.

Calibration curves for each analyte are shown in Figure 4 and the coefficient of determination (r^2) values are shown in Table 3.

1) FID, Ethanol



2) FID, VOCs



3) ECD, VDKs

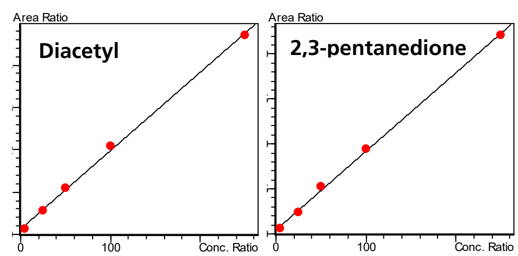


Figure 4: Four-point calibration curve for ethanol and five-point calibration curves for VOCs and VDKs. Concentrations of analytes and internal standards were as described in methods.

Table 3: Coefficient of determination (r^2) of the calibration curves.

Compounds	r^2 value
Acetaldeyde	0.999
Ethyl acetate	0.999
iso-Amyl acetate	0.999
iso-Amyl alcohol	0.999
2,3-butanedione (diacetyl)	0.998
2,3-pentanedione	0.998
Ethanol	1.000

VOC, VDK and Ethanol concentration in Beer samples

Next, three commercial beer samples were analyzed for ethanol content, VOC and VDK concentrations. Each beer was chilled, degassed, and added to 20mL headspace vials along with Tri-IS solution.

The vials were sealed, mixed and analyzed as described. The chromatograms are shown in Figures 5 and 6, and the quantification results were shown in Table 4.

1) *FID channel, Volatile Flavor Compounds and Ethanol*

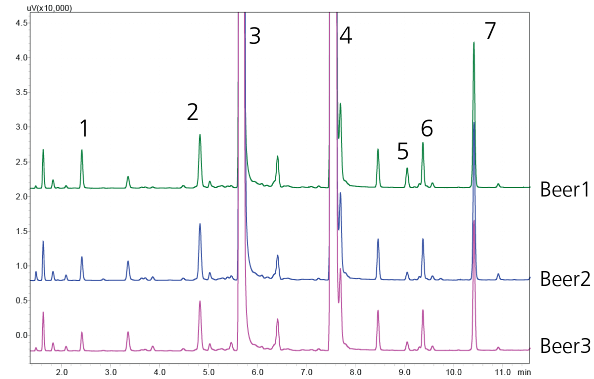


Figure 5: Chromatograms of VOCs in three different beer samples. Compound of interest: ***1.*** Acetaldehyde ***2.*** Ethyl acetate ***3.*** Ethanol ***4.*** *n*-Propanol (ISTD for Ethanol) ***5.*** *iso*-Amyl acetate ***6.*** *n*-Butanol (ISTD for VOCs) ***7.*** *iso*-Amyl alcohol.

2) *ECD channel, Diacetyl and 2,3-pentanedione*

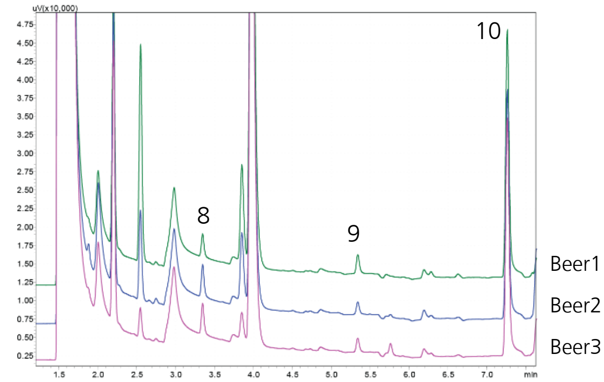


Figure 6: Chromatograms of VDKs in three different beer samples. Compound of interest: ***8.*** Diacetyl (2,3-butanedione) ***9.*** 2,3-pentanedione ***10.*** 2,3-hexanedione (ISTD for VDKs).

Table 4: Concentrations of various analytes in beer samples. Results shown are average of duplicate assays.

	Beer 1	Beer 2	Beer 3
Acetaldehyde	12.771 ppm	8.507 ppm	6.622 ppm
Ethyl acetate	10.598 ppm	12.007 ppm	10.694 ppm
Isoamyl acetate	1.003 ppm	0.392 ppm	0.457 ppm
Isoamyl alcohol	54.868 ppm	61.672 ppm	52.492 ppm
Ethanol (ABV)	4.1 percent	5.9 percent	4.3 percent
2,3-butandione	24.928 ppb	37.410 ppb	35.459 ppb
2,3-pentanedione	9.386 ppb	5.386 ppb	6.659 ppb

Comparing direct injection method and headspace method for determining ethanol content

The ASBC method Beer-4 calls for determining ethanol content (ABV) by direct injection of liquid beer into GC. In order to simplify the assay, we measured ethanol concentrations using headspace samples (above). To make sure that using headspace samples suffer no adverse effect, we compared the results obtained using headspace injection above (Beer 3-in-1 method) with the one obtained by direct liquid injection (ASBC method Beer-4).

We then compared the measured concentrations to what was on the bottle/can label. As shown in Table 5 below, while both methods produced similar results, the ethanol concentration obtained by the Beer 3-in-1 method in fact matched the labeled ABV more closely.

Table 5: Comparison of ethanol concentrations determined by liquid injection vs. headspace injection method. Results shown are average of duplicate or triplicate assays.

Beer sample	Conc. measured by liquid Injection (%)	Conc. measured by headspace injection (%)	Labeled ABV (%)
a	3.9	4.1	4.1
b	9.1	9.0	9
c	7.2	7.3	7.25
d	4.8	5.0	5

Repeatability

Repeatability of quantification of compounds in 3-in-1 analysis was also examined, and the relative standard deviation (RSD) were under 3% for ethanol, under 5% for VDKs and under 8% for flavor compounds (VOCs) (Table 6).

Table 6. Relative standard deviation (RSD, %) for replicate analysis of beer sample 1 (*n*=6).

Compound	RSD for Ret. Time (%)	RSD for concentration (%)
Acetaldehyde	0.004	2.766
Ethyl acetate	0.006	5.981
Isoamyl acetate	0.006	7.686
Isoamyl alcohol	0.006	2.878
Ethanol (ABV)	0.011	2.542
2,3-butandione	0.010	4.008
2,3-pentanedione	0.007	4.453

Peak shape of VKDs on different columns

In the study above, diacetyl and 2,3-pentanedione were analyzed on a Rxi-5Sil MS column, simultaneously with VOC compounds on a Stabilwax column. Some peak tailing was observed for VDK compounds on Rxi-5Sil MS column. Given the structure of VDKs, it might be expected that the peak shape would improve on a polar column. Therefore, VDKs were also analyzed on the Stabilwax using the same method. While there is a slight improvement on peak symmetry (Table 7), 2,3-pentanedione is eluting very closely to a contaminating peak in the blank, making it hard to quantify this compound at low concentrations (Figure 7).

It is possible that oven program may be adjusted to improve the resolution, although the run time may become longer. This will require further investigation.

Table 7. Peak tailing factors for VDKs on Rxi-5Sil MS or Stabilwax column.

	Rxi-5Sil MS	Stabilwax
2,3-butandione	1.406	1.298
2,3-pentanedione	2.242	1.075

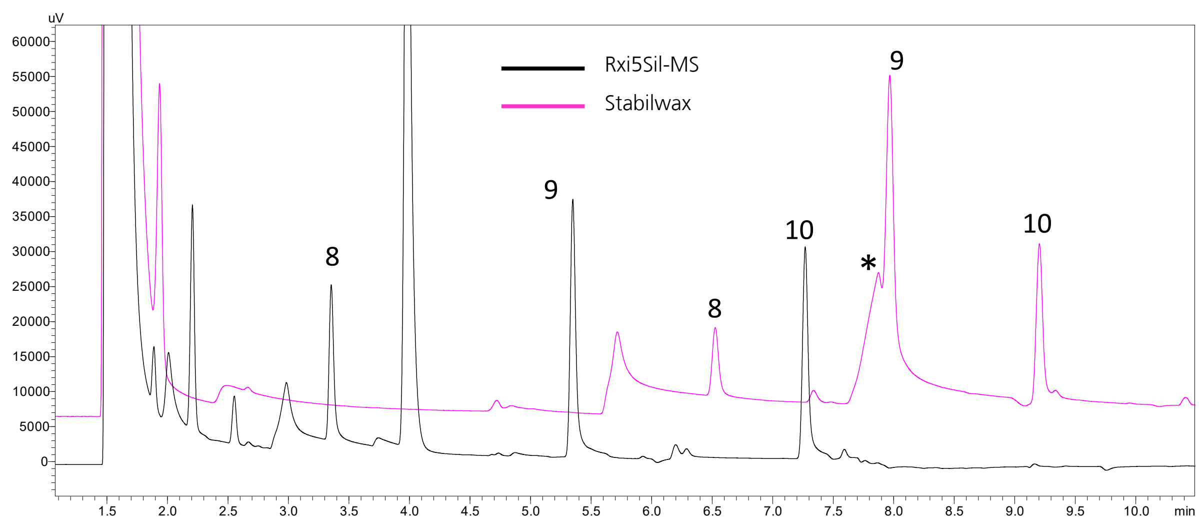


Figure 7: Chromatogram of VDK standard in ECD channel on either Rxi-5Sil MS or Stabilwax column. Compounds of interest: ***8.*** Diacetyl (2,3-butanedione) ***9.*** 2,3-pentanedione ***10.*** 2,3-hexanedione (ISTD for VDKs). * denotes a non-analyte peak present in blank.

■ Conclusion

In this study, analysis of beer volatiles, diacetyl and ethanol content was carried out using the Shimadzu Beer 3-in-1 Analyzer. Traditionally, analyses of these three types of compounds has been performed separately using different samples and analysis methods. We demonstrated that by using the HS-10 static headspace autosampler and GC-2014 gas chromatograph with a 2-way column adaptor, beer samples were easily divided between two analytical lines, allowing simultaneous determination of the concentration of three types of compounds with a single injection.

Moreover, nitrogen was used as both carrier gas and ECD makeup gas, making this analyzer an efficient and cost-effective choice for beer brewers. This approach streamlines beer analysis, providing exceptional multi-component beer analysis in an easy-to-use, single method.

■ References

- American Society of Brewing Chemists Methods of Analysis Beer-4, *Alcohol*, 2004, Amer. Soc. Brewing Chem., St. Paul, MN. doi: 10.1094/ASBCMOA-Beer-4
- American Society of Brewing Chemists Methods of Analysis Beer-25, *Diacetyl*, 2014, Amer. Soc. Brewing Chem., St. Paul, MN. doi: 10.1094/ASBCMOA-Beer-25
- American Society of Brewing Chemists Methods of Analysis Beer-48, *Headspace Gas Chromatography–Flame Ionization Detection Analysis of Beer Volatiles*, 2012, Amer. Soc. Brewing Chem., St. Paul, MN. doi: 10.1094/ASBCMOA-Beer-48

■ Consumables

Part Number	Description	Unit	Instrument
221-76650-01	Septa, Green, Premium Low Bleed	Pk of 25	GC-2014
221-76863-73	Inlet liner for HS-10, 1.2mm ID	each	
221-70162-92	FID jet for GC-2014	each	
221-32126-05	Graphite Ferrule 0.5mm	Pk of 10	
220-97331-16	20mL HS vials with screw caps and Silicone/PTFE septa	Pk of 100	HS-10
220-97331-53	20mL HS vials with crimp caps and Silicone/PTFE septa	Pk of 100	
221-76863-01	HS-10 needle	each	
227-36041-01	SH-Rxi-5Sil MS Capillary Column, 30 x 0.32 x 1.0	each	Column
227-36252-01	SH-Stabilwax Capillary Column, 30x 0.32 x 1.0	each	

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