Determination of Hop Aroma Compounds in Beer using Headspace-Solid Phase Microextraction coupled with Gas Chromatography and Mass Spectrometry (HS/SPME/GC/MS): BCOJ Collaborative Work

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ABSTRACT

Hop aroma compounds, such as *β*-myrcene, linalool, and geraniol, play an important role as indexes for the evaluation of beer flavor and process control. In addition, the increasing diversity of consumer preferences in recent years has intensified the demand for an analytical method to detect the hop aroma compounds. In general, a typical beer sample consists of various compounds with diverse physical and chemical properties in low concentration, which impedes their accurate detection through traditional methods. As a result, no such methods have been proposed for inclusion in the *Methods of Analysis of BCOJ*. Therefore, we proposed a method for the simultaneous analysis of the low-concentration hop aroma compounds in beer through HS/SPME/GC/MS in the SIM mode as one of the potential *Methods of Analysis of BCOJ* and evaluated its repeatability and reproducibility.

It was concluded that the method can determine hop aroma compounds in beer, low-malt beer (Happou-Shu), and beer-based alcoholic beverages (third-category beer). The BCOJ Analysis Committee recommends that the validated method be adopted for inclusion in the *Methods of Analysis of BCOJ*.

PROCEDURE

Number of laboratories	12
Sample	5pairs (A1/A2, B1/B2, C1/C2, D1/D2, E1/E2)
Mixed standard solution	16 mg/L *β*-myrcene, 20 mg/L linalool, 6 mg/L geraniol, in ethanol
Internal standard solutin	10 mg/L benzyl acetate in 10% ethanol

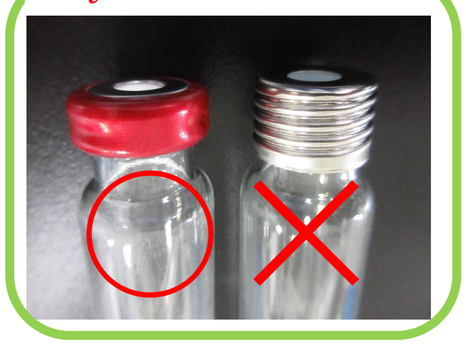
Sample Preparation for Standard Addition Method

20 ml SPME vials containing 3 g NaCl

- 8mL of sample
- 100 µL internal standard solution
- Mixed standard solution as listed in Table1.

Vial capped Immediately

Key Point 1



Use crimp-type caps for airtight vials

Key Point 2

Perform a series of steps in quick succession, one sample at a time.

Table 1. Concentration of various compounds added at each level (µg/L)

Name	sample	level 1	level 2	level 3	level 4	level 5	level 6
β-myrcene	-	10	20	30	40	60	80
linalool	-	12.5	25	37.5	50	75	100
geraniol	-	3.8	7.5	11.3	15	22.5	30

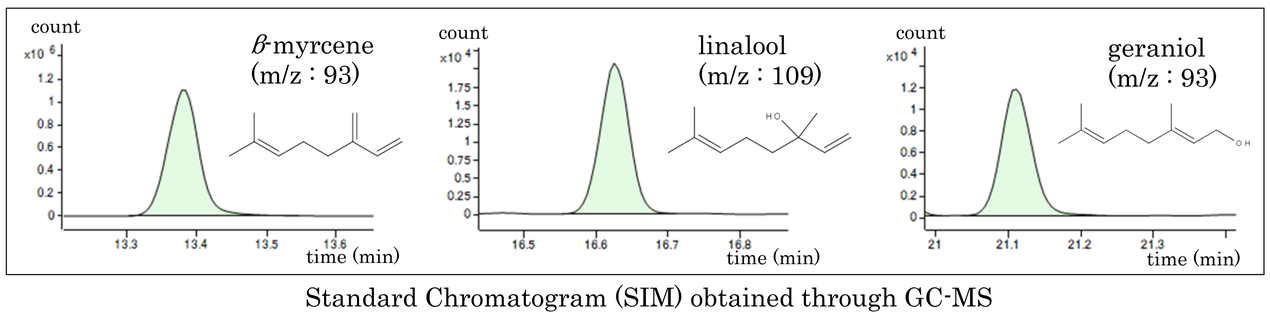
HS/SPME/GC/MS Conditions

Table 2. Autosampler Conditions

Auto Sampler	Combi PAL or equivalent
SPME Fiber	PDMS 100 µm or equivalent
Extraction	40°C, Agitator Speed; 500rpm, Agitator On Time 20 s, Agitator Off Time 2 s
Adsorption time	15 min
Desorption time	3 min
Fiber conditioning	Post 10 min 250°C, Preheat for 30 minutes before measurement

Table 3. GC-MS Conditions

Column	HP-1MS 30 m ×0.25 mm ×1 µm or equivalent
Inlet	Inlet Temperature; 260°C, SPME Liner (0.75 mm id) or equivalent
Purge flow	20 mL/min, 3 min,
Career Gas	He, Flow; 0.9 mL/min (Constant Flow)
Oven Temperature	50°C (1min) - 250°C (5°C/min) - 1min
Transferline	250°C
SIM Parameter	*β*-myrcene; Target (m/z)=93, Qualifier (m/z)=69
	linalool; Target (m/z)=109, Qualifier (m/z)=121
	geraniol; Target (m/z)=93, Qualifier (m/z)=69
	benzyl acetate; Target (m/z)=108, Qualifier (m/z)=91

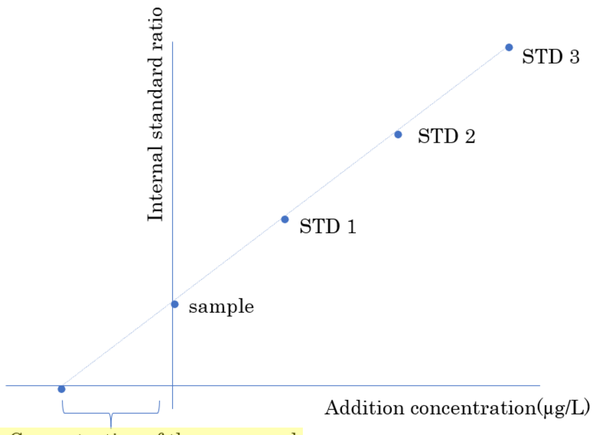


Standard Chromatogram (SIM) obtained through GC-MS

Method for Quantitative Calculations

Internal standard ratio = Peak area of each aroma compound / Peak area of benzyl acetate

Concentration (µg/L) = Intercept of vertical axis and calibration curve / Calibration curve slope (F)



Determination by the standard addition method

Calibration curve design

Calibration points for the corresponding compounds in each sample were selected at the levels shown in Table 1, equally spaced and with a maximum added concentration of approximately three times the expected concentration of the sample.

Statistical Analysis

The data acquired from one of the laboratories were not considered as the appropriate conditions for data collection were not maintained. Statistical analysis was performed on the data collected from the remaining 11 laboratories. The results were processed according to the JIS Z 8401:1999 guidelines (1), and the statistical analysis of the processed data was performed according to the JIS Z 8402-2:1999 (2) and AOACI (3) guidelines. The samples were checked for outliers using Mandel's h and k statistics and the Cochran and Grubbs outlier test.

RESULTS AND DISCUSSION

The range of the calculated analytical values is as follows: The relative standard deviation of repeatability (RSDr) ranged from 4.8%–20.3%, 3.7%–5.2%, and 5.7%–9.6% for *β*-myrcene, linalool, and geraniol, respectively; while the repeatability at 95% probability (r_{95}) ranged from 1.5–15.3 µg/L, 2.7–6.2 µg/L, and 0.7–2.1 µg/L for *β*-myrcene, linalool, and geraniol, respectively; The relative standard deviation of reproducibility (RSD_R) ranged from 11.0%–41.2%, 8.1%–11.6%, and 12.7%–25.8% for *β*-myrcene, linalool, and geraniol, respectively; while the reproducibility at 95% probability (R_{95}) ranged from 3.4–22.8 µg/L, 5.6–15.6 µg/L, and 2.7–4.3 µg/L for *β*-myrcene, linalool, and geraniol, respectively. These results were deemed acceptable. As is evident from the data, the statistical results for *β*-myrcene were slightly inferior to that of linalool and geraniol. This could be attributed to the highly volatile nature of *β*-myrcene, which can escape during sample preparation. Therefore, proper sample preparation is important to stabilize the analytical values.

It was concluded that the HS/SPME/GC/MS method can determine hop aroma compounds in beer, low-malt beer, and beer-based alcoholic beverages. The BCOJ analysis committee recommended the adoption of the validated method for inclusion in the *Methods of Analysis of BCOJ*.

Table 4. Statistical summary of results based on HS/SPME/GC/MS of *β*-myrcene

	Sample Pair A1/A2	Sample Pair B1/B2	Sample Pair C1/C2	Sample Pair D1/D2	Sample Pair E1/E2
Number of Laboratories	10	9	9	9	11
Grand mean (m)	34.8	10.9	32.7	16.2	18.6
Repeatability Standard Deviation (s_r)	5.5	0.5	1.8	1.9	3.8
Relative Repeatability Standard Deviation (RSD_r, %)	15.7	4.8	5.5	12.0	20.3
Repeatability Limit(r_{95})	15.3	1.5	5.0	5.4	10.6
Predicted Relative Repeatability Standard Deviation ($PRSD_r$, %)	14.7	14.7	14.7	14.7	14.7
HorRat$_r$ ($RSD_r/PRSD_r$)[a]	1.1	0.3[b]	0.4[b]	0.8	1.4
Reproducibility Standard Deviation (S_R)	6.7	1.2	8.1	2.9	7.7
Relative Reproducibility Standard Deviation (RSD_R, %)	19.3	11.0	24.9	18.2	41.2
Reproducibility Limit (R_{95})	18.8	3.4	22.8	8.2	21.5
Predicted Relative Reproducibility Standard Deviation ($PRSD_R$, %)	22.0	22.0	22.0	22.0	22.0
HorRat$_R$ ($RSD_R/PRSD_R$)[a]	0.9	0.5	1.1	0.8	1.9

a According to AOAC International Guidelines, HorRat values should be between 0.5 and 2.0 (3).
b The accuracy of the data was confirmed although HorRat values were <0.5.

Table 5. Statistical summary of results based on HS/SPME/GC/MS of linalool

	Sample Pair A1/A2	Sample Pair B1/B2	Sample Pair C1/C2	Sample Pair D1/D2	Sample Pair E1/E2
Number of Laboratories	10	11	11	11	10
Grand mean (m)	27.8	54.3	30.1	34.5	24.5
Repeatability Standard Deviation (s_r)	1.4	2.2	1.1	1.8	1.0
Relative Repeatability Standard Deviation (RSD_r, %)	5.2	4.1	3.7	5.2	3.9
Repeatability Limit(r_{95})	4.0	6.2	3.1	5.0	2.7
Predicted Relative Repeatability Standard Deviation ($PRSD_r$, %)	14.7	14.7	14.7	14.7	14.7
HorRat$_r$ ($RSD_r/PRSD_r$)[a]	0.4[b]	0.3[b]	0.2[b]	0.4[b]	0.3[b]
Reproducibility Standard Deviation (S_R)	2.4	5.6	3.5	3.4	2.0
Relative Reproducibility Standard Deviation (RSD_R, %)	8.7	10.3	11.6	9.9	8.1
Reproducibility Limit (R_{95})	6.8	15.6	9.8	9.6	5.6
Predicted Relative Reproducibility Standard Deviation ($PRSD_R$, %)	22.0	22.0	22.0	22.0	22.0
HorRat$_R$ ($RSD_R/PRSD_R$)[a]	0.4[b]	0.5	0.5	0.5	0.4[b]

a According to AOAC International Guidelines, HorRat values should be between 0.5 and 2.0 (3).
b The accuracy of the data was confirmed although HorRat values were <0.5.

Table 6. Statistical summary of results based on HS/SPME/GC/MS of geraniol

	Sample Pair A1/A2	Sample Pair B1/B2	Sample Pair C1/C2	Sample Pair D1/D2	Sample Pair E1/E2
Number of Laboratories	11	11	10	11	11
Grand mean (m)	6.1	4.6	7.6	10.9	5.9
Repeatability Standard Deviation (s_r)	0.6	0.3	0.5	0.8	0.6
Relative Repeatability Standard Deviation (RSD_r, %)	9.5	5.7	6.2	6.9	9.6
Repeatability Limit(r_{95})	1.6	0.7	1.3	2.1	1.6
Predicted Relative Repeatability Standard Deviation ($PRSD_r$, %)	14.7	14.7	14.7	14.7	14.7
HorRat$_r$ ($RSD_r/PRSD_r$)[a]	0.6	0.4[b]	0.4[b]	0.5	0.7
Reproducibility Standard Deviation (S_R)	1.0	1.2	1.0	1.5	1.0
Relative Reproducibility Standard Deviation (RSD_R, %)	16.7	25.8	12.7	14.2	17.1
Reproducibility Limit (R_{95})	2.9	3.3	2.7	4.3	2.8
Predicted Relative Reproducibility Standard Deviation ($PRSD_R$, %)	22.0	22.0	22.0	22.0	22.0
HorRat$_R$ ($RSD_R/PRSD_R$)[a]	0.8	1.2	0.6	0.6	0.8

a According to AOAC International Guidelines, HorRat values should be between 0.5 and 2.0 (3).
b The accuracy of the data was confirmed although HorRat values were <0.5.

CONCLUSIONS

1. The HS/SPME/GC/MS method can determine hop aroma compounds in beer, low-malt beer, and beer-based alcoholic beverages.
2. The BCOJ Analysis Committee recommends adopting the HS/SPME/GC/MS method for inclusion in the *Methods of Analysis of BCOJ*.

LITERATURE CITED

(1)Japanese Standards Association. Rules for rounding off of numerical values (Z 8401). *JIS Handbook 57: Quality Control 2014*. Japanese Standards Association, Tokyo, Japan. Pp. 1539-1540, 2014.

(2) Japanese Standards Association. Accuracy (trueness and precision) of measurement methods and results. Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method (Z 8402-2). *JIS Handbook 57: Quality Control 2014*. Japanese Standards Association, Tokyo, Japan. Pp. 363-395, 2014.

(3) AOACI. Guidelines for collaborative study procedures to validate characteristics of a method of analysis. *Official Methods of Analysis of AOAC International* (Appendix D), 18th ed. The Association, Gaithersburg, MD, 2005.

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