Headspace Gas Chromatography-Flame Ionization Detector Analysis for Beer Volatiles

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CONCLUSIONS

1. Repeatability coefficients of variation for the determination of acetaldehyde by headspace GC-FID ranged from 4.9 to 17% and were judged acceptable.
2. Reproducibility coefficients of variation for the determination of acetaldehyde by headspace GC-FID ranged from 7.3 to 22% and were judged unacceptable.
3. Repeatability and reproducibility coefficients of variation for the determination of ethyl acetate by headspace GC-FID ranged from 3.6 to 5.4% and from 8.0 to 10%, respectively, and were judged acceptable.
4. Repeatability coefficients of variation for the determination of isoamyl acetate by headspace GC-FID ranged from 3.5 to 7.3% and were judged acceptable.
5. Reproducibility coefficients of variation for the determination of isoamyl acetate by headspace GC-FID ranged from 11 to 24% and were judged unacceptable.
6. Repeatability and reproducibility coefficients of variation for the determination of isoamyl alcohol by headspace GC-FID ranged from 2.6 to 4.2% and from 6.9 to 8.1%, respectively, and were judged acceptable.

RECOMMENDATIONS

1. The subcommittee recommends accepting the method as a provisional method based on the acceptable repeatability but unacceptable reproducibility and include in the provisional section of *Methods of Analysis*.
2. Discharge the subcommittee.

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This was the third year of this subcommittee's existence. Based on polling by the subcommittee for Coordination of New and Alternative Methods of Analysis (1), this subcommittee was formed to evaluate the applicability of headspace gas chromatography-flame ionization detector (GC-FID) analysis for the determination of volatile organic compounds in beer. In the first year,

TABLE I
Acetaldehyde (mg/L) in Beer by Headspace Gas Chromatography- Flame Ionization Detector

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**
1	2.5	2.1	4.2	4.8	2.2	2.3
2	2.7	2.1	4.5	5.1	1.6	2.9
3	3.5[a]	3.1[a]	4.9[a]	5.6[a]	2.6[a]	4.4[a]
4	2.4	2.2	3.5	3.6	2.3	3.2
5	2.4	2.1	3.5	4.4	2.4	4.1
6	2.6	2.3	3.7	4.2	1.6	2.8
7	2.7	2.6	4.1	5.0	1.3	3.0
8[b]	…	…	…	…	…	…
Mean[c]	2.55	2.24	3.90	4.52	1.92	3.06
Grand mean[c]	2.39		4.21		2.49	

[a] Outlier at $P \leq 0.05$ based on totals and/or differences (1).
[b] Data excluded due to known deviation from protocol.
[c] Calculated excluding outliers.

TABLE II
Ethyl Acetate (mg/L) in Beer by Headspace Gas Chromatography- Flame Ionization Detector

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**
1	20.3	18.8	34.0[a]	33.6[a]	12.6	15.7
2	24.0[a]	24.9[a]	35.7[a]	35.0[a]	16.3[a]	19.0[a]
3	18.9	19.5	27.1	26.5	12.7	14.9
4	17.2	16.7	21.2	20.0	10.7	12.4
5	16.9	16.5	23.7	24.8	11.1	13.5
6	17.4	17.1	23.7	24.1	11.4	13.1
7	15.9	18.4	24.8	26.7	13.0	14.0
8[b]	…	…	…	…	…	…
Mean[c]	17.77	17.82	24.10	24.41	11.92	13.92
Grand mean[c]	17.80		24.25		12.92	

[a] Outlier at $P \leq 0.05$ based on totals and/or differences (1).
[b] Data excluded due to known deviation from protocol.
[c] Calculated excluding outliers.

TABLE III
Isoamyl Acetate (mg/L) in Beer by Headspace Gas Chromatography- Flame Ionization Detector

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**
1	2.2	2.1	4.2[a]	4.4[a]	1.0	1.4
2	2.3	2.5	3.6	3.7	1.1	1.4
3	2.1	2.4	3.3	3.5	1.1	1.3
4	1.9	1.9	2.5	2.5	0.9	1.0
5	1.3[a]	1.4[a]	2.0	2.2	0.7[a]	0.8[a]
6	1.4[a]	1.5[a]	2.0	2.2	0.7[a]	0.8[a]
7	1.7	2.1	2.8	3.2	1.0	1.1
8[b]	…	…	…	…	…	…
Mean[c]	2.05	2.21	2.70	2.87	1.02	1.24
Grand mean[c]	2.13		2.79		1.13	

[a] Outlier at $P \leq 0.05$ based on totals and/or differences (1).
[b] Data excluded due to known deviation from protocol.
[c] Calculated excluding outliers.

TABLE IV
Isoamyl Alcohol (mg/L) in Beer by Headspace Gas Chromatography- Flame Ionization Detector

	Sample Pair		Sample Pair		Sample Pair	
Collaborator	**A**	**B**	**C**	**D**	**E**	**F**
1	49.1	48.0	58.2	60.0	48.9	56.7
2	57.3	60.1	61.0	62.3	60.4	65.4
3	54.5	58.4	58.7	61.4	61.1	65.3
4	51.9	51.0	49.2	49.6	53.2	58.0
5	56.5	55.5	56.9	62.0	58.0	63.3
6	54.7	53.6	54.6	57.3	56.3	59.6
7	51.7	58.6	57.7	64.0	66.3	66.4
8[a]	…	…	…	…	…	…
Mean	53.68	55.02	56.62	59.52	57.82	62.10
Grand mean	54.35		58.07		59.96	

[a] Data excluded due to known deviation from protocol.

collaborative analysis showed unacceptable repeatability for two of four compounds tested and unacceptable reproducibility for three of four compounds tested (2). Following ruggedness analysis, minor modifications were made to the sampling portion of the collaborative protocol. In the second year, collaborative analysis showed acceptable repeatability for all compounds tested but unacceptable reproducibility for three of four compounds tested (3). In the third year, a check sample containing known concentrations of the four tested compounds was provided along with the beer samples to provide additional information on potential bias due to calibration standard preparation.

PROCEDURE

Three sample pairs of commercial beers were sent to each collaborator. Each pair was of the same brand but from different production times. All sample pairs were commercially available lager beers selected to cover a range of volatile concentrations. Calibration was accomplished by standard additions of volatiles with 1-butanol as an internal standard. Results were evaluated using the Youden unit block design (4).

RESULTS AND DISCUSSION

Results from eight collaborators were received for the three sample pairs. Results for one collaborator were excluded prior to statistical analyses because of known deviations from the prescribed experimental protocol. Therefore, seven data sets were used for statistical analysis. Data for acetaldehyde, ethyl acetate, isoamyl acetate, and isoamyl alcohol are presented in Tables I through IV, respectively. Outliers were identified using Dixon's ratio test (4). The statistical summary of the volatile data are shown in Table V.

The repeatability coefficients of variation were judged acceptable for all compounds tested with the exception of acetaldehyde in sample set E/F. With inclusion of sample set (E/F) for acetaldehyde, the repeatability coefficient was 4.9 to 17%. The repeatability coefficients of variation for ethyl acetate, isoamyl acetate, and isoamyl alcohol ranged from 3.6 to 5.4%, 3.5 to 7.3%, and 2.6 to 4.2%, respectively.

The reproducibility coefficients of variation were judged acceptable for two of four compounds tested. The reproducibility coefficients of variation for ethyl acetate and isoamyl alcohol ranged from 8.0 to 10%, and 6.9 to 8.1%, respectively and were judged acceptable. The reproducibility coefficients of variation for acetaldehyde and isoamyl acetate ranged from 7.3 to 22% and 11 to 24%, respectively and were judged unacceptable.

A check sample prepared at known concentrations for all target compounds in a 5% by volume ethanol/water solution was provided with collaborative samples. The data for the check sample (see Table VI) was evaluated to look for differences in collaborators calibration curves for each compound. For example, the results for ethyl acetate from Collaborator 2 are all biased high in relation to the rest of the data set indicating that the prepared standard and/or calibration curve used for quantification of beer samples may have been biased. The check standard result for ethyl acetate from Collaborator 2 also shows a high bias (121%) which confirms the potential bias.

The current data shows acceptable repeatability and marginal reproducibility. The difference in the inter-laboratory results is

TABLE V
Statistical Summary of Results[a]

				Repeatability			Reproducibility		
Compound	**Sample Pair**	**# of Labs**	**Grand Mean**	S_r	cv_r	r_{95}	S_R	cv_R	R_{95}
Acetaldehyde									
	A–B	6	2.39	0.12	4.9	0.33	0.17	7.3	0.49
	C–D	6	4.21	0.21	5.1	0.60	0.52	12	1.44
	E–F	6	2.49	0.42	17	1.17	0.54	22	1.50
Ethyl acetate									
	A–B	6	17.80	0.97	5.4	2.71	1.42	8.0	3.97
	C–D	5	24.25	0.88	3.6	2.46	2.43	10	6.81
	E–F	6	12.92	0.50	3.9	1.39	1.09	8.4	3.10
Isoamyl acetate									
	A–B	5	2.13	0.16	7.3	0.44	0.25	12	0.70
	C–D	6	2.79	0.10	3.5	0.27	0.67	24	1.87
	E–F	5	1.13	0.05	4.5	0.14	0.13	11	0.36
Isoamyl alcohol									
	A–B	7	54.35	2.26	4.2	6.32	3.75	6.9	10.49
	C–D	7	58.07	1.48	2.6	4.16	4.35	7.5	12.19
	E–F	7	59.96	1.70	2.8	4.77	4.88	8.1	13.66

[a] All calculations were made based on Tables I through IV.

TABLE VI
Check Sample Results (Known Solution)

Collaborator	**Acetaldehyde (mg/L)**	**Ethyl Acetate (mg/L)**	**Isoamyl Acetate (mg/L)**	**Isoamyl Alcohol (mg/L)**
1	4.3	8.3	0.84	35.4
2	4.8	9.7	0.79	37.7
3	5.4	8.5	0.86	40.0
4	3.7	7.7	0.76	38.6
5	4.2	8.7	0.70	40.9
6	4.0	7.3	0.57	35.9
7	5.3	8.4	0.84	43.0
Certified concentration	4.0	8.0	0.81	40.0

most likely attributable to difficulties in the preparation of calibration standards or unfamiliarity in handling volatile chemicals. Analysts must use care in dealing with the volatile chemicals as the compounds can evaporate (i.e., volatilize) quickly when opened. Standard preparations should be performed by a trained analyst and be performed both accurately and quickly to reduce potential errors. It is recommended to include the use of a second-source or certified reference material check standard to establish the accuracy of the calibration curve for each compound prior to analysis and validate the standard preparation.

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