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Determination of DMS and other volatile compounds in beer by headspace capillary gas chromatography and flame ionisation detection

Submitted on behalf of the Analysis Committee of the European Brewery Convention

The determination of beer volatiles by the headspace capillary gas chromatography with flame ionisation detection method of the Institute of Brewing Analysis Committee has been collaboratively tested by the members of the Analysis Committee of the European Brewery Convention and some members of the American Society of Brewing Chemists. Determined analytes were acetaldehyde, dimethylsulphide, ethylacetate, n-propanol, isobutanol, isoamylalcohols and isoamylacetates. The internal standards used were ethylmethyl sulphide and n-butanol. After three preliminary collaborative trials, a last fourth one was performed on three beer samples in 15 laboratories. Repeatability (r_{95}) and reproducibility (R_{95}) values were obtained for 12 laboratories. Assuming that too many members did not follow exactly the proposed method and judging that reproducibility values were poor, it was decided to not include the method in the Analytica EBC as an official method, but to include it as a guideline method with the aforementioned notice.

BC 03 Chemistry

(Descriptors: Collaborative trial, beer, gas chromatography, demethylsulphide, ethylacetate, n-propanol, isobutanol, isoamylalcohol, isoamylacetate.

Descriptoren: Ringanalyse, Bier, Gaschromatographie, Dimethylsulfide, Ethylacetat, N-Propanol, Isobutanol, Isoamylalkohol, Isoamylacetat).

1 Introduction

There is no current recommended method of the European Brewery Convention for determining the main organic volatile compounds in beer. In 1991, the Institute of Brewing published in its Recommended Methods of Analysis the determination of lower boiling point volatile compounds in beer by headspace gas chromatography (1). No precision values were given on the method. In order to assess to suitability of this method for the analysis of acetaldehyde, ethylacetate, n-propanol, isobutanol, isoamylalcohol and isoamylacetate, the Analysis Committee of the EBC decided to collaboratively test the IOB method and to include in the analytes the dimethylsulphide.

2 Experimental

The organisation of the collaborative trials and the statistical treatment of the data were aimed to be carried out according to the procedures given in the International Standard ISO 5725 (2). A uniform design was employed and four times three samples of beer covering the approximate usual values of the analytes were distributed to the participating laboratories. It was asked to the participants to change the internal standard in the method. 3-Heptanone has been replaced by n-butanol at a concentration of 1,6 mg/l and ethylmethylsulphide at a concentration of 100 µg/l. It appeared after the collaborative testings that a 10 times higher concentration of n-butanol would have been better. Fifteen laboratories were involved in this fourth collaborative trial.

Table 1 Sample results for acetaldehyde concentrations in milligrammes/ litre

Laboratory	A		B		C	
1	4.4*	8.8*	7.3*	19*	5.1***	18.6***
2	0.7	0.7	3.3	3.2	2.5	2.3
3	7.1	6.9	13	15	14**	14**
4	6.8	5.9	3.5*	9.2*	4.8	5.6
5	1.1	1.4	3.3	3.0	2.8	2.7
6	1.5	1.5	2.9	2.5	2.2	2.1
7	2.8	2.9	4.4	4.5	3.6	3.5
8	0.3	0.3	1.3	1.3	0.9	1.1
9	1.1	1.7	3.2	3.1	2.9	3.0
10	0.8	1.0	2.2	2.1	1.9	2.0
11	1.6	1.9	3.1	3.5	2.1	2.2
12	2.7	2.9	4.1	4.1	3.5	3.8
13	(–)		(–)		(–)	
14	(–)		(–)		(–)	
15	(5.3)		(8.3)		(6.4)	
Mean****	2.4	2.5	4.1	4.2	2.7	2.8
Grand Mean****	2.4		4.2		2.8	

* Outlier at p = 0,01 based on differences
** Outlier at p = 0,01 based on totals
*** Outlier at p = 0,01 based on totals and differences
**** Calculated excluding the outlier(s)
( ) Not taken into account because only one result

Table 2 Sample results for dimethylsulphide concentrations in microgrammes/litre

Laboratory	A		B		C	
1	34*	9*	25*	53*	31	16
2	84	76	45	46	48	49
3	54	63	31	32	33	35
4	43	44	14*	50*	25	13
5	66	68	36	36	37	38
6	81	81	44	44	46	46
7	43	40	30	28	27	27
8	58	77	35*	22*	51	41
9	87	81	48	47	51	56
10	69	66	47	45	46	48
11	73	108	67	67	71	70
13	(43)		(25)		(18)	
14	(–)		(–)		(–)	
15	(237)		(225)		(160)	
Mean****	63	65	44	43	42	40
Grand Mean****	64		43		41	

* Outlier at p = 0,01 based on differences
**** Calculated excluding the outlier(s)
( ) Not taken into account because only one result

Table 3 Sample results for ethylacetate concentrations in milligrammes/litre

Laboratory	A		B		C	
1	16*	3.3*	10*	4.6*	19*	2.7*
2	19	19	13	12	22	22
3	25	27	15	16	35	3
4	9.5	9.5	5.8	5.9	11	9.4
5	15	14	8.9	9.2	16	16
6	19	19	13	12	22	21
7	18	18	11	11	20	20
8	10	11	7.1	7.8	11	12
9	19	19	12	11	21	21
10	14	13	8.1	8.8	15	15
11	18	17	11	11	20	20
12	15	16	11	11	18	20
13	(17)		(14)		(25)	
14	(16 ± 0.3)		(10 ± 0.2)		(17 ± 0.3)	
15	(18)		(11)		(20)	
Mean****	17	17	11	11	19	19
Grand Mean****	17		11		19	

* Outlier at p = 0,01 based on differences
**** Calculated excluding the outlier(s)
( ) Not taken into account because only one result

Table 4 Sample results for n-propanol concentrations in milligrammes/litre

Laboratory	A		B		C	
1	9.9*	1.7*	9.1*	5.5*	8.6*	2.5*
2	10	10	11	11	9.9	9.6
3	19	20	17	18	18	18
4	4.7	3.4	3.9	6.7	6.2	5.7
5	7.1	7.3	7.8	7.2	7.5	7.6
6	12	12	11	11	11	11
7	8.0	8.2	8.2	8.2	7.1	7.1
8	7.4	7.4	4.0	7.6	4.8	4.1
9	15	12	11	12	9.8	9.8
10	8.8	9.1	7.6	9.5	7.9	7.9
11	11	10	9.7	9.5	9.1	9.1
12	12	12	11	11	10	12
13	(8.8)		(9.8)		(10.7)	
14	(51 ± 4)		(11 ± 0.8)		(11 ± 0.8)	
15	(13)		(11)		(11)	
Mean****	11	10	9.3	9.9	9.2	9.3
Grand Mean****	10		9.6		9.2	

* Outlier at p = 0,01 based on differences
**** Calculated excluding the outlier(s)
( ) Not taken into account because only one result

Table 5 Sample results for isobutanol concentrations in milligrammes/litre

Laboratory	A		B		C	
1	6.3	3.1	7.3*	2.5*	7.4*	1.8*
2	7.2	6.7	9.0	8.6	8.6	8.6
3	11	12	13	13	14	15
4	4.3	4.7	5.7	5.5	5.1	5.7
5	7.4	8.0	8.8	8.8	8.8	8.7
6	8.2	8.0	9.2	9.1	9.5	9.1
7	7.0	7.2	8.2	8.3	8.3	8.1
8	5.7	5.9	6.5	7.1	6.9	6.9
9	9.4	7.7	9.4	9.3	9.0	9.0
10	6.0	5.2	6.5	6.4	6.6	6.5
11	7.0	6.6	8.4	8.2	8.1	8.3
12	7.4	7.4	8.7	8.4	8.5	9.0
13	(7.4)		(8.3)		(9.5)	
14	(8.4 ± 0.2)		(7.6 ± 0.2)		(7.8 ± 0.2)	
15	(7)		(8)		(8)	
Mean****	7.2	6.9	8.5	8.4	8.5	8.6
Grand Mean****	7.1		8.5		8.6	

* Outlier at p = 0,01 based on differences
**** Calculated excluding the outlier(s)
( ) Not taken into account because only one result

3 Results and discussion

The raw data such as they were received from the fifteen laboratories are presented in tables 1 to 7. Three laboratories reported single results which were not taken into account in the statistical treatments and four laboratories recognized to not have fully respected the method to test. A statistical treatment was nevertheless performed and the results are summarized in tables 8 to 14. The precision values in terms of repeatability (r_{95}) and reproducibility (R_{95}) were calculated excluding the outliers.

Table 6 Sample results for isoamylacetate concentrations in milligrammes/litre

Laboratory	A		B		C	
1	0.9***	5.5***	0.4***	6.8***	1.7*	6.0*
2	1.1	1.1	0.6	0.5	2.1	2.1
3	1.4	1.5	0.6	0.7	3.1	3.1
4	0.4	0.4	0.2	0.2	0.6	0.7
5	0.5	0.3	0.9	0.8	0.5	0.4
6	1.1	1.0	0.5	0.5	2.1	2.1
7	0.9	0.9	0.5	0.5	1.7	1.6
8	0.8	0.7	0.2	0.3	0.8	1.0
9	1.5	1.2	0.4	0.5	2.0	2.0
10	0.6	0.5	0.3	0.3	1.2	1.2
11	1.1	1.0	0.5	0.5	2.0	2.0
12	0.8	0.8	0.5	0.4	1.4	1.6
13	(0.7)		(0.4)	(1.4)		
14	(0.9 ± 0.04)		(0.4 ± 0.02)		(1.3 ± 0.06)	
15	(0.6)		(0.5)	(1.8)		
Mean****	0.9	0.9	0.5	0.5	1.6	1.6
Grand Mean****	0.9		0.5		1.6	

* Outlier at p = 0,01 based on differences
*** Outlier at p = 0,01 based on differences and totals
**** Calculated excluding the outlier(s)
( ) Not taken into account because only one result

Table 7 Sample results for isoamylalcohol concentrations in milligrammes/litre

Laboratory	A		B		C	
1	56*	10*	47*	25*	61*	17*
2	56	56	53	51	65	65
3	92	99	83**	86**	109**	112**
4	24	26	21	21	28	29
5	52	53	44	46	56	56
6	63	63	56	56	71	69
7	54	55	48	48	60	61
8	60	61	48	51	64	66
9	63	60	54	54	67	67
10	48	44	39	43	51	53
11	59	54	49	49	62	61
12	53	53	50	48	59	65
13	(42)		(41)		(55)	
14	(60 ± 0.7)		(48 ± 0.6)		(63 ± 0.8)	
15	(36)		(50)		(64)	
Mean****	57	57	46	47	59	59
Grand Mean****	57		46		59	

* Outlier at p = 0,01 based on differences
** Outlier at p = 0,01 based on totals
**** Calculated excluding the outlier(s)
( ) Not taken into account because only one result

Table 8 Summary of the statistical treatment for acetaldehyde

Sample pairs	N° of labs	Grand Mean	Repeatability			Reproducibility		
			Sr	cvr	r_{95}	SR	cvR	R_{95}
A	11	2.4	0.27	11.04	0.75	2.25	92.31	6.30
B	10	4.2	0.48	11.66	1.36	3.69	86.38	10.05
C	10	2.8	0.20	7.35	0.57	1.17	41.99	3.26

Table 9 Summary of the statistical treatment for dimethyl-sulphide

Sample pairs	N° of labs	Grand Mean	Repeatability			Reproducibility		
			Sr	cvr	r_{95}	SR	cvR	R_{95}
A	11	64	10.90	17.06	30.51	22.44	35.14	62.84
B	8	43	0.84	1.94	2.35	11.90	27.48	33.32
C	11	41	4.68	11.39	13.11	15.25	37.07	42.70

Table 10 Summary of the statistical treatment for ethylacetate

Sample pairs	N° of labs	Grand Mean	Repeatability			Reproducibility		
			Sr	cvr	r_{95}	SR	cvR	R_{95}
A	11	17	0.67	4.03	1.87	4.62	27.92	12.94
B	11	11	0.50	4.79	1.41	2.71	25.71	7.58
C	11	19	0.79	4.11	2.20	6.39	33.42	17.88

Table 11 Summary of the statistical treatment for n-propanol

Sample pairs	N° of labs	Grand Mean	Repeatability			Reproducibility		
			Sr	cvr	r_{95}	SR	cvR	R_{95}
A	11	10	0.76	7.38	2.13	4.09	39.65	11.45
B	11	9.6	0.72	7.51	2.02	3.69	37.40	10.01
C	11	9.2	0.49	5.31	1.37	3.57	38.70	10.00

Table 12 Summary of the statistical treatment for isobutanol

Sample pairs	N° of labs	Grand Mean	Repeatability			Reproducibility		
			Sr	cvr	r_{95}	SR	cvR	R_{95}
A	12	7.1	0.80	11.38	2.25	1.98	28.02	5.54
B	11	8.5	0.19	2.19	0.52	1.93	22.84	5.41
C	11	8.6	0.29	3.37	0.81	2.31	27.04	6.48

Table 13 Summary of the statistical treatment for isoamylacetate

Sample pairs	N° of labs	Grand Mean	Repeatability			Reproducibility		
			Sr	cvr	r_{95}	SR	cvR	R_{95}
A	11	0.9	0.08	8.76	0.22	0.36	40.07	1.00
B	11	0.5	0.05	11.59	0.15	0.19	39.68	0.53
C	11	1.6	0.07	4.45	0.20	0.77	48.21	2.17

Table 14 Summary of the statistical treatment for isoamylalcohol

Sample pairs	N° of labs	Grand Mean	Repeatability			Reproducibility		
			Sr	cvr	r_{95}	SR	cvR	R_{95}
A	11	57	2.30	4.06	6.45	16.63	29.31	46.56
B	10	46	1.38	2.98	3.88	9.95	21.43	27.87
C	10	59	1.54	2.63	4.32	11.89	20.24	33.30

Repeatability values are acceptable for all the determined analytes but reproducibility values are in all cases judged to be inacceptable except for isoamylalcohol.

4 Conclusions

Considering the poor precision values obtained in this collaborative trial and particularly the inacceptable reproducibility values for all the determined analytes, the Analysis Committee of the EBC judged not to accept this method of determination of DMS and other volatile compounds in beer by capillary gas chromatography and flame ionisation detection as an official method.

Nevertheless, due to the utility of the method, the Committee decided to include it in the Analytica EBC as a guideline method.

5 Zusammenfassung

Dupire, S.: Bestimmung von DMS und anderen flüchtigen Verbindungen in Bier durch Headspace-Analyse mittels Kapillargaschromatographie sowie durch Flammenionisations-Nachweis — Monatsschrift für Brauwissenschaft 53, Nr. 1/2, 8 – 11, 2000

BC 03 Chemie

Die Bestimmung flüchtiger Substanzen in Bier durch Headspace-Analyse mittels Kapillargaschromatographie in Verbindung mit dem Flammenionisations-Nachweisverfahren des Analysenkomitees des Institute of Brewing wurde in einer Ringanalyse von den Mitgliedern des Analysenausschusses des European Brewery Convention sowie einigen Mitgliedern der American Society of Brewing Chemists untersucht. Die bestimmten Analyte waren Acetaldehyd, Dimethylsulfid, Ethylacetat, n-Propanol, Isobutanol, Isoamylalkohole und Isoamylacetate. Als interne Standards wurden Ethylmethylsulfid und n-Butanol verwendet. Nach drei vorausgehenden Ringversuchen wurde schließlich in 15 Labors an drei Bierproben noch ein viertes Experiment durchgeführt. Die Werte für Wiederholbarkeit ($r_{95)}$ und Reproduzierbarbeit (R_{95}) wurden in 12 Labors gewonnen. In der Annahme, daß zu viele Mitglieder nicht genau das vorgeschlagene Verfahren angewandt haben, und aufgrund der Beurteilung, daß die Werte für die Reproduzierbarkeit (R_{95}) niedrig waren, wurde beschlossen, die Methode nicht als offizielle Methode in die Analytica EBC aufzunehmen, sondern vielmehr als Orientierungsverfahren (Hilfsmethode) mit dem obengenannten Hinweis.

Dupire, S.: Détermination du DMS et autres composés volatils dans la bière par chromatographie en phase gazeuse dans l'espace de tête et détection par ionisation de flamme — Monatsschrift für Brauwissenschaft 53, Nr. 1/2, 8 – 11, 2000

BC 03 Chimie

La détermination de volatils de la bière par chromatographie en phase gazeuse dans l'espace de tête avec la méthode de détection par ionisation de flamme du Comité des Analyses de l'Institute of Brewing a été évaluée par les membres du Comité des Analyses de l'European Brewery Convention et par quelques membres de l'American Society of Brewing Chemists. Les composés analysés étaient l'acétaldéhyde, le diméthylsulfure, l'acétate d'éthyle, le n-propanol, les alcools isoamyliques et les acétates d'isoamyle. Les étalons internes utilisés étaient l'éthylméthyl-sulfure et le n-butanol. Après trois essais collaboratifs préliminaires, un quatrième dernier essai a été effectué sur trois bières dans 15 laboratories. La répétabilité (r_{95}) et la rproductibilité (R_{95}) ont été obtenues par 12 laboratories. Compte tenu que trop de membres n'ont pas suivi exactement la méthode proposée et que les valeurs de reproductibilité ont été jugées insuffisantes, il a été décidé de ne pas inclure la méthode dans l'Analytica EBC ent tant que méthode officielle mais plutôt comme méthode guide avec la notice mentionnée ci-dessus.

6 References

1. Institute of Brewing, Recommendes Methods of Analysis, method 9.32, 1997.
2. International Standard Organisation, Precision of test methods – determination of repeatability and reproducibility for a standard test method by inter-laboratoy tests, ISO 5725, 1966.
3. Methods of Analysis of the ASBC, Statistical Analysis, Method 4, 1 – 8, 1992.