N-Nitrosamines in Malt and Beer

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CONCLUSIONS

The data obtained on the preliminary collaborative study warrant continued evaluation of both beer methods. Beer Methods I (distillation followed by dichloromethane extraction of the distillate) and II (direct dichloromethane extraction) did not give statistically different results when the same calculation method was used. Neither method showed consistently better precision than the other. Calculation methods 2 and 3, which utilize the internal standard to calculate *N*-nitrosodimethylamine (NDMA) concentrations, gave consistently higher average results than did calculation method 1. The calculation methods did not, however, give statistically different results. Coefficients of variation for combined laboratory errors were, generally, not excessive for a preliminary collaboration.

RECOMMENDATIONS

1. Additional collaborative investigations should be postponed for several months to allow all collaborators more time to complete their studies and to submit data.
2. The collaborative study should be repeated for both beer methods and should be started for a third beer method involving the Preptube™[1].
3. The collaborative study should be repeated for at least one and possibly both malt methods, with the choice of methods to be determined after evaluation of the preliminary collaborative malt data.

This subcommittee was organized in late 1979 and held its first meeting January 18, 1980. Subcommittee members attending the meeting included representatives from U.S. and Canadian government laboratories, commercial testing laboratories, and malting, brewing, and distilling industry laboratories.

Numerous methods and method modifications were proposed for the determination of *N*-nitrosodimethylamine (NDMA) in malt and beer. While most collaborators agreed on methods for the gas-chromatographic separation and detection of NDMA, they expressed a considerable amount of disagreement regarding techniques for isolating NDMA from malt and beer. In general, the proposed isolation procedures could be divided into two approaches: 1) direct extraction of beer or an aqueous extract of malt with dichloromethane, and 2) distillation of beer or a malt/water slurry followed by extraction of the distillate with dichloromethane. Additionally, collaborators disagreed on the need for using sulfamic acid during sample preparation to prevent nitrosation and on the methods for determining recoveries of NDMA.

Because of the lack of agreement on analytical details and the relatively short time before the next Annual Meeting, the subcommittee was assigned to perform a preliminary evaluation of four methods, two each for malt and beer, and to evaluate the use of sulfamic acid in conjunction with the malt methods.

[1] Available from Thermo Electron Corp., 115 Second Ave., Waltham, MA 01254.

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PROCEDURE

Four pairs of test beers and three pairs of test malts were sent to each collaborator. The beers were prepared in a pilot brewery and were "spiked" to achieve concentrations of NDMA ranging from approximately 0.2 to 10 μg/L. The malts were obtained from brewery blends and were estimated, by previous analysis, to contain NDMA ranging from less than 1 to 20 μg/kg. This design conforms to the Youden Unit Block method (3).

Collaborators were also sent standard solutions containing NDMA and *N*-nitrosodipropylamine (NDPA) at certified concentrations in ethanol. The NDPA was used as an internal standard.

The collaborators were requested to familiarize themselves with each method before analyzing the test samples. The methods to be evaluated were: Beer I, distillation of beer followed by dichloromethane extraction of the distillate; Beer II, dichloromethane extraction of beer; Malt I, dichloromethane extraction of a 70°C aqueous extract of malt; Malt II, vacuum distillation of a malt/water slurry followed by dichloromethane extraction of the distillate.

For all of the methods, the dichloromethane is concentrated to a small volume and an aliquot is injected into a gas chromatograph equipped with either a Hall™ electrolytic conductivity detector modified for nitrosamine analysis or with a Thermal Energy Analyzer. Several methods of calibration and calculation were to be evaluated. For this preliminary study, sulfamic acid was to be used by all collaborators for the Malt II method. Several collaborators were to perform the Malt I method both with and without sulfamic acid, and the remaining collaborators were to perform it without this compound.

For both beer methods, the internal standard, NDPA, was added to the beers before distillation or direct extraction. For Beer Method I, two calculation methods were employed. For calculation method 1, a standard curve was prepared by direct injection of NDMA standards of varying concentration into the gas chromatograph followed by measurement of the NDMA peak height (or area). A comparison of the NDMA peak height from the chromatograms of the beer distillate extracts to those of the standards allowed calculation of the concentration of NDMA in the beers.

For calculation method 2, a solution of NDPA internal standard was injected into the gas chromatograph several times, and the resulting peak values were averaged. The average value was compared to the peak height of NDPA on each of the test beer chromatograms to obtain a percent recovery of NDPA from beer. The value obtained using calculation method 1 was then adjusted based on the percent recovery of NDPA and this value was reported for calculation method 2.

Calculation methods 1 and 2 were also used for Beer Method II. Additionally, a third calculation method was used by some collaborators to calculate the results of Beer Method II. This calculation involved preparing a standard curve by additions of varying amounts of NDMA to aliquots of the same beer before extraction with dichloromethane. The ratio of the peak height (or area) of the NDMA peak to the peak height of the NDPA internal standard peak from each chromatogram was used for plotting the standard curve. The ratio of the NDMA peak to the NDPA peak from the test beer chromatograms was then used to determine the NDMA concentration in the beer.

RESULTS AND DISCUSSION

Nine collaborators submitted results for the beer methods, but only two for the malt methods. Only the results reported for the beer methods will be discussed at this time.

TABLE I
***N*-Nitrosodimethylamine in Beer[a]—Method I: Distillation**

	Samples											
	Calculation Method 1[b]						Calculation Method 2[c]					
Collaborator	A	B	C	D	E	F	A	B	C	D	E	F
1	2.0	2.2	6.0	6.3	8.7	10.0	2.0	2.3	6.5	6.6	9.1	10.0
2	2.2	2.6	5.4	5.7	9.0	10.2	2.1	2.6	5.3	5.7	8.8	10.0
3	1.4	2.1	3.9	4.7	6.6	8.4	2.0	2.6	4.8	5.6	8.3	9.6
4	1.9	2.5	5.4	6.3	9.3	9.6	2.1	2.9	5.9	6.9	10.4	12.0
5	1.9	2.6	4.7	5.7	8.6	9.3	2.2	2.7	5.7	6.3	9.7	10.7
6	1.7	1.7	3.3	3.7	5.4	7.8	2.1	2.4	5.6	4.9	9.2	11.2
7	1.8	2.3	5.1	5.2	8.6	8.8	1.7	2.1	4.5	5.0	7.5	8.9
8	...	...	...	...	...	...	...	...	...	...	...	...
9	1.7	2.2	4.6	4.7	7.1	7.7	1.9	2.4	5.6	5.5	8.2	8.5
Mean	1.82	2.28	4.80	5.29	7.91	8.98	2.01	2.50	5.49	5.81	8.90	10.11
Grand Mean	2.05		5.04		8.44		2.26		5.65		9.51	

[a] Values are μg/kg.
[b] External standard curve.
[c] Results from calculation method 1 corrected on the basis of recovery of internal standard.

TABLE II
***N*-Nitrosodimethylamine (NDMA) in Beer[a]—Method II: Direct Dichloromethane Extraction**

	Samples																	
	Calculation Method 1[b]						Calculation Method 2[c]						Calculation Method 3[d]					
Collaborator	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
1	1.7	2.3	5.1	5.3	8.0	9.0	1.8	2.3	5.4	5.5	8.2	9.0	2.0	2.6	6.5	6.5	9.5	10.0
2	1.8	2.2	5.4	5.2	8.6	8.8	2.1	2.6	6.2	6.4	11.4	12.4	...	...	...	...	...	...
3	1.6	2.1	4.5	4.9	5.7	6.1	2.3	2.6	5.5	6.1	8.4	9.5	2.3	2.6	5.2	6.5	9.4	10.1
4	2.0	2.4	5.1	6.1	9.3	10.7	2.1	2.5	5.6	7.1	10.1	11.2	1.9	2.1	4.6	5.9	9.0	9.3
5	1.5	2.4	4.8	5.3	8.1	8.9	2.1	2.7	5.8	6.1	9.7	10.5	...	...	...	...	...	...
6	2.2	2.4	5.3	5.1	8.4	8.9	2.3	2.7	6.4	6.7	10.5	11.6	2.0	2.3	5.6	5.8	9.1	10.0
7	1.7	2.1	4.4	5.3	6.6	7.1	1.6	2.0	4.3[e]	5.2	7.3	8.0	1.9	2.4	5.0	6.0	8.5	9.3
8	2.1	3.3[e]	5.4	5.3	7.6	9.6	2.0	2.6	5.3	5.6	8.8	10.1	3.2[e]	3.8[e]	7.6	8.4[e]	10.1	10.0
9	2.2	2.6	5.6	6.0	8.8	10.2	2.1	2.5	5.8	5.8	9.0	10.3	2.2	2.6	6.0	6.0	9.1	10.6
Mean[f]	1.84	2.31	5.07	5.39	7.90	8.81	2.04	2.50	5.75	6.16	9.27	10.29	2.05	2.43	5.48	6.11	9.24	9.90
Grand Mean[f]	2.08		5.23		8.36		2.27		5.96		9.78		2.24		5.80		9.57	

[a] Values are μg/L.
[b] External standard curve.
[c] Results from calculation method 1 corrected on the basis of recovery of internal standard.
[d] Standard addition curve based on ratio of NDMA to internal standard.
[e] Outlier according to Dixon's test, $P = 0.05$ (1).
[f] Means do not include outliers.

TABLE III
Statistical Summary for Sample Pair AB

Method	Calculation Method	No. of Labs.	Grand Mean[a]	Error			c.v.[d] (%)	Calculated F[b]	Critical F[c]
				Within-Lab.[b]	Between-Lab.[b]	Combined[c]			
Beer I	1	8	2.05	0.17	0.21	0.27	13.2	3.90	3.79
	2	8	2.26	0.12	0.17	0.21	9.2	5.45	3.79
Beer II	1	8	2.08	0.15	0.17	0.22	10.8	3.80	3.79
	2	9	2.27	0.07	0.21	0.22	9.9	18.51	3.44
	3	6	2.24	0.10	0.15	0.19	8.3	5.43	5.05

[a] Grand Mean = GM = $(\bar{A} + \bar{B})/2$.
[b] Calculated per Youden and Steiner (3).
[c] Combined error (S_c) calculated from within-lab. error (S_r) and between-lab. error (S_b); $S_c = \sqrt{S_r^2 + S_b^2}$.
[d] Coefficient of variation of S_c = c.v. = $100(S_c/GM)$.
[e] Critical F from tables of F distribution (2) at $P = 0.05$.

TABLE IV
Statistical Summary for Sample Pair C, D

Method	Calculation Method	No. of Labs.	Grand Mean[a]	Error			c.v. (%)	Calculated F	Critical F
				Within-Lab.	Between-Lab.	Combined			
Beer I	1	8	5.04	0.25	0.85	0.89	17.5	23.14	3.79
	2	8	5.65	0.38	0.56	0.68	12.0	5.20	3.79
Beer II	1	9	5.23	0.31	0.26	0.41	7.8	2.39	3.44
	2	8	5.96	0.33	0.34	0.48	8.0	3.03	3.79
	3	6	5.80	0.45	0.29	0.54	9.2	1.86	5.05

[a] $(\overline{C} + \overline{D})/2$. Refer to footnotes of Table III.

TABLE V
Statistical Summary for Sample Pair E, F

Method	Calculation Method	No. of Labs.	Grand Mean[a]	Error			c.v. (%)	Calculated F	Critical F
				Within-Lab.	Between-Lab.	Combined			
Beer I	1	8	8.44	0.54	1.06	1.19	14.1	8.70	3.79
	2	8	9.51	0.36	0.98	1.04	11.0	16.09	3.79
Beer II	1	9	8.36	0.42	1.22	1.29	15.4	18.10	3.44
	2	9	9.78	0.15	1.30	1.31	13.4	146.03	3.44
	3	7	9.57	0.36	0.32	0.48	5.0	2.63	4.28

[a] $(\overline{E} + \overline{F})/2$. Refer to footnotes of Table III.

The results reported by collaborators are tabulated in Tables I and II and statistical summaries for each sample pair are given in Tables III, IV, and V. Beer samples G and H were not "spiked" with NDMA, and all collaborators reported either very low values or levels less than the limit of detectability. These data could not be statistically evaluated and are, therefore, not tabulated in this report. Calculation methods 2 and 3 resulted in higher average values than did calculation method 1. The calculation methods did not, however, give statistically different results as determined by a t test ($P = 0.05$). Although in most cases the F ratios were statistically significant, indicating the presence of between-laboratory error, the magnitude of the errors was not excessive. A comparison of the errors for Methods I and II using any particular calculation technique did not indicate that either method was consistently more precise than the other. This was also true when the various calculation methods were compared.

Comments from collaborators indicated a preference for Method II, primarily because it was less time-consuming even though centrifugation was usually required to break the emulsions. Several collaborators recommended a gentle rocking motion to avoid emulsion formation. One collaborator felt Method I was definitely superior to Method II because of the emulsion problem with Method II. Numerous collaborators recommended evaluation of the Preptube method. The latter could not be evaluated initially because Preptubes were temporarily unavailable from the supplier.

LITERATURE CITED

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