

Report of ASBC Subcommittee on *N*-Nitrosamines in Malt and Beer for 1979–1980, Part II^{1,2}

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Key words: *Dichloromethane extraction, Distillation, Hall™ electrolytic conductivity detector, N-Nitrosodimethylamine, Thermal Energy Analyzer™.*

The decision to plan and embark on a collaborative study to evaluate methods for the determination of *N*-nitrosamines in malt and beer was made in late 1979. Accordingly, this subcommittee was not able to formulate and expedite its program until early 1980, less than four months before the 46th Annual Meeting of the Society.

Data available on malt analysis were inadequate to report at the Annual Meeting. However, more than half of the collaborators were able to report on beer analysis. Because of the intense interest in the topic, a preliminary report covering only the available beer data was presented (1).

The planned 1979–1980 collaborative study was completed, with 15 collaborators actively participating, and this supplemental report was prepared to include the malt data and update the beer data.

CONCLUSIONS

The data obtained from the collaborative study warrant continued evaluation of both the malt and beer methods. Beer

¹ This report supplements, as well as completes, the preliminary report (1).

² Published for the Society at the request of the ASBC Technical Committee.

Methods I, distillation followed by extraction, and II, direct extraction, did not give significantly different results, nor was either method consistently more precise. The results using calculation methods 2 and 3, which utilize an internal standard to calculate *N*-nitrosodimethylamine (NDMA) concentrations, were significantly different from the results of calculation method 1 and were consistently higher and more accurate.

Malt Methods I, extraction of an aqueous extract of ground malt, and II, vacuum distillation of a ground malt/water slurry followed by extraction of the distillate, gave significantly different results for the C/D pair but not for the A/B pair. Neither malt method gave consistently better precision. The results from calculation method 2, which utilizes the internal standard, were significantly different from the results of calculation method 1 and were consistently higher.

RECOMMENDATIONS

1. The collaborative study should be repeated for both malt and both beer methods.
2. A third method, involving the Preptube™, should be evaluated for both malt and beer.
3. Emphasis should be placed on decreasing the detection limits of the methods.

PROCEDURE

Details for the design and implementation of the collaborative study were reported previously (1).

TABLE I
N-Nitrosodimethylamine (NDMA) in Beer^a—Method I: Distillation

Collaborator	Samples												
	Calculation Method 1 ^b						Calculation Method 2 ^c						
	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	
10	2.1	2.6	5.6	5.7	8.3	9.9	2.4	2.9	6.6	6.6	9.2	11.3	
11	2.1	2.5	5.5	5.9	9.1	9.8	2.8 ^d	3.2	7.5	7.5	10.7	12.2	
12	1.5	2.0	7.6 ^d	5.5	8.3	10.4	2.0	2.6	8.4	6.0	9.9	11.7	
13	1.8	1.8	5.2	4.4	7.8	8.0	2.0	2.0	5.3	5.3	7.2	8.0	
14	1.5	1.4	4.2	4.9	6.0	7.8	1.7	2.1	6.2	5.0	6.2	9.8	
Mean ^e	1.82	2.19	4.91	5.27	7.91	9.05	2.02	2.47	5.99	5.92	8.80	10.30	
NDMA added	2.06	2.53	5.62	5.97	9.18	10.33	2.06	2.53	5.62	5.97	9.18	10.33	
Percent NDMA recovered	88.3	86.6	87.4	88.3	86.2	87.6	98.1	97.6	106.6	99.2	95.9	99.7	
Grand mean ^e	2.00		5.09			8.48			2.24		5.95		9.55

^a Values are $\mu\text{g}/\text{kg}$.

^b External standard curve.

^c Results from calculation method 1 corrected on the basis of recovery of internal standard.

^d Outlier according to Dixon's test, $P = 0.05$ (2).

^e Means do not include outliers.

The methods under evaluation were: Beer Method I, distillation followed by dichloromethane extraction of the distillate; Beer Method II, direct dichloromethane extraction; Malt Method I, dichloromethane extraction of an aqueous extract (70° for 1 hr) of ground malt; and Malt Method II, vacuum distillation of a ground malt/water slurry followed by dichloromethane extraction of the distillate. Two calculation/calibration methods were used for both

the malt and beer methods. These were calculation method 1, external standard curve, and calculation method 2, correction of the results from calculation 1 based on internal standard recovery. Additionally, a third calculation/calibration method was used for Beer Method II. This method employed a standard addition curve, and calculation was based on the ratio of NDMA to the internal standard, thus correcting for the internal standard recovery.

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

Collaborator	Samples																	
	Calculation Method 1 ^b						Calculation Method 2 ^c						Calculation Method 3 ^d					
	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	2.6	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	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	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
10
11	2.0	2.3	5.5	6.0	8.9	9.6	2.6	3.2	6.3	7.1	10.5	12.2	2.1	2.7	6.1	6.8	10.4	12.1
12	1.9	2.6	9.1 ^f	6.2	9.3	10.7	2.3	2.8	9.9 ^e	6.8	9.9	12.3	2.2	2.6	5.2	5.9	5.9 ^e	11.1
13	1.7	2.0	4.4	4.7	8.1	7.1	1.7	2.0	4.4	5.1	8.1	7.3
14
Mean ^f	1.85	2.31	5.05	5.38	8.12	8.89	2.08	2.54	5.55	6.06	9.32	10.37	2.11	2.49	5.52	6.18	9.39	10.18
NDMA added	2.08	2.55	5.67	6.02	9.26	10.42	2.08	2.55	5.67	6.02	9.26	10.42	2.08	2.55	5.67	6.02	9.26	10.42
Percent NDMA recovered	88.9	90.6	89.1	89.4	87.7	85.3	100.0	99.6	97.9	100.7	100.6	99.5	101.4	97.6	97.4	102.7	101.4	97.7
Grand mean ^f	2.08		5.21		8.50		2.31		5.80		9.85		2.30		5.85		9.78	

^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, $P = 0.05$ (2).

^f Means do not include outliers.

TABLE III
Statistical Summary for Beer Samples

Sample Pair	Beer 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					
AB	I	1	13	2.00	0.190	0.263	0.324	16.2	4.85	2.69		
		2	12	2.24	0.140	0.212	0.254	11.3	5.62	2.82		
		II	1	11	2.08	0.142	0.163	0.216	10.4	3.62	2.98	
	II	2	12	2.31	0.077	0.298	0.308	13.3	31.30	2.82		
		3	8	2.30	0.145	0.161	0.217	9.4	3.46	3.79		
		CD	I	1	12	5.09	0.342	0.719	0.796	15.7	9.86	2.82
	II	2	13	5.95	0.647	0.698	0.951	16.0	3.33	2.69		
		1	11	5.21	0.283	0.356	0.455	8.7	4.16	2.98		
		2	11	5.80	0.310	0.626	0.698	12.0	9.18	2.98		
	3	3	8	5.85	0.380	0.358	0.522	8.9	2.78	3.79		
		EF	I	1	13	8.48	0.531	1.004	1.136	13.4	8.15	2.69
		2	13	9.55	0.570	1.195	1.324	13.9	9.78	2.69		
	II	1	12	8.50	0.544	1.160	1.281	15.1	10.10	2.82		
		2	12	9.85	0.524	1.371	1.468	14.9	14.68	2.82		
		3	8	9.78	0.420	0.638	0.764	7.8	5.61	3.79		

^a Grand mean = $GM = (\bar{A} + \bar{B})/2, (\bar{C} + \bar{D})/2, \text{ or } (\bar{E} + \bar{F})/2$.

^b Calculated per Youden and Steiner (4).

^c Combined error (S_c) calculated from within-lab. error (S_w) and between-lab. error (S_b); $S_c = \sqrt{S_w^2 + S_b^2}$.

^d Coefficient of variation of $S_c = c.v. = 100(S_c/GM)$.

^e Critical F from tables of F distribution (3) at $P = 0.05$.

Four pairs of test beers, labeled A through H, were sent to each collaborator. The beers labeled G and H were identical to each other and to the beer that was used as a base for "spiking" with NDMA to achieve addition levels of 2.08, 2.55, 5.67, 6.02, 9.26 and 10.42 $\mu\text{g/L}$ for beers A through F, respectively.

Three pairs of test malts, labeled A through F, were sent to each collaborator. The malts were obtained from normal brewery blends but selection was based on preliminary testing by several collaborators.

RESULTS AND DISCUSSION

Beer. The results obtained by collaborators on the beer samples are reported in Tables I and II. The results obtained on unspiked samples G and H are not reported because most collaborators reported undetectable NDMA concentrations for these beers. Reported limits of detectability ranged from <0.05 to $0.2 \mu\text{g/L}$. Therefore, because beers G and H contained less than detectable levels of NDMA, the assumptions were made that the base beer was

TABLE IV
N-Nitrosodimethylamine in Malt^a—Method I:
Dichloromethane Extraction of Aqueous Extract^b

Collaborator	Samples							
	Calculation Method 1 ^c				Calculation Method 2 ^d			
	A	B	C	D	A	B	C	D
1	9.2	14.0	3.0	2.4	10.0	15.0	3.0	3.4
3	8.8	14.4	2.9	2.7	12.4	20.0	3.7	3.5
4	9.3	16.2	1.8	2.2	9.4	17.0	1.9	2.2
5	11.1	14.8	1.9	2.4	12.9	18.7	2.2	2.9
6	7.9	12.4	1.6	1.2	10.9	17.5	2.4	1.6
7	9.1	12.6	2.5	1.9	9.1	13.3	2.2	2.3
8	8.5	13.6	3.4	2.6	8.5	11.3	2.7	2.5
9	12.0	15.9	2.0	2.2	12.0	18.4	2.4	2.4
10
11	9.5	17.7	1.8	3.8	11.1	19.4	2.1	4.3
12	10.3	14.0	1.8	1.5	11.8	17.1	2.2	2.2
15	6.7	13.7	1.1	2.4	9.0	16.8	1.4	2.6
Mean	9.31	14.48	2.16	2.30	10.64	16.77	2.38	2.72
Grand mean	11.90		2.23		13.71		2.55	

^a Values are $\mu\text{g/kg}$.

^b 70°C aqueous extract (1 hr) of ground malt.

^c External standard curve.

^d Results from calculation method 1 corrected on the basis of recovery of internal standard.

negligible in NDMA content and that the addition levels were the actual levels in beers A–F.

The *t*-test showed significant differences ($P = 0.05$) between actual levels of NDMA in beer samples and levels found by calculation method 1. No significant differences were found between actual levels and mean results for calculation methods 2 and 3. Tables I and II show these comparisons and demonstrate the excellent accuracy obtained on both methods by using either calculation method 2 or 3.

Summaries of statistical evaluations on the beer methods are reported in Table III. A paired *t*-test showed no significant difference ($P = 0.05$) between the two methods, ie, distillation and direct extraction. Calculation methods 2 and 3, however, were significantly different from calculation 1, with the latter giving consistently lower results.

No significant differences ($P = 0.05$) were found between combined-laboratory variances when the two methods were compared using any one calculation method. Nor were significant

TABLE V
N-Nitrosodimethylamine in Malt^a—Method II: Vacuum Distillation^b

Collaborator	Samples							
	Calculation Method 1 ^c				Calculation Method 2 ^d			
	A	B	C	D	A	B	C	D
1	5.4	7.4	1.3	1.3	6.4	8.6 ^e	1.7	1.6
3	7.5	13.5	1.5	1.6	9.4	16.9	1.7	1.9
4	8.4	16.5	1.5	1.3	10.1	19.2	1.7	1.7
5 ^f	10.8	18.1	1.8	2.5	11.4	19.8	2.0	2.7
6	7.4	7.4	0.6 ^e	0.9	19.9	19.8	0.9	1.1
7	10.3	13.3	1.8	1.5	17.2	26.9 ^e	3.7	4.9 ^e
8
9	7.5	16.6	1.5	1.7	10.2	17.1	1.9	2.1
10	10.6	15.5	1.9	1.9	11.3	16.3	1.9	2.0
11	10.4	15.4	2.1	2.3	11.9	18.0	2.8	2.7
12	9.3	16.8	1.7	2.2	11.2	20.4	2.3	2.6
Mean ^g	8.76	14.02	1.68	1.81	11.92	18.44	1.88	2.04
Grand mean ^g	11.39		1.74		15.18		1.96	

^a Values are $\mu\text{g/kg}$.

^b Ground malt and water slurry.

^c External standard curve.

^d Results from calculation method 1 corrected on the basis of recovery of internal standard.

^e Outlier according to Dixon's test, $P = 0.05$ (2).

^f Collaborator 5 used a modification of Malt Method 11.

^g Means do not include outliers.

TABLE VI
Statistical Summary for Malt Samples

Sample Pair	Malt 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			
AB	I	1	11	11.90	1.118	1.034	1.523	12.8	2.71	2.98
		2	11	13.71	1.204	1.786	2.154	15.7	5.40	2.98
	II	1	10	11.39	2.084	2.172	3.010	26.4	3.17	3.18
		2	8	15.18	2.151	1.458	2.598	17.1	1.92	3.79
CD	I	1	11	2.23	0.616	0.300	0.685	30.7	1.47	2.98
		2	11	2.55	0.570	0.376	0.683	26.8	1.87	2.98
	II	1	9	1.74	0.224	0.279	0.357	20.5	4.11	3.44
		2	9	1.96	0.173	0.501	0.530	27.0	17.74	3.44

^a Grand mean = $GM = (\bar{A} + \bar{B})/2$ or $(\bar{C} + \bar{D})/2$.

^b Calculated per Youden and Steiner (4).

^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 (3) at $P = 0.05$.

differences found between calculation method variances except for the E/F sample pair, which showed a significantly lower combined laboratory variance when calculation method 3 was used. This probably has no practical significance because it occurred with only one sample pair.

Malt. The results reported by collaborators on malt samples are listed in Tables IV and V. The data reported for malts E and F are not included because most collaborators reported "none detectable" or less than certain detection limits for both samples. Summaries of the statistical evaluations are reported in Table VI.

A paired *t*-test showed a statistically significant difference ($P = 0.05$) between the results of the two malt methods, ie, direct extraction of an aqueous extract and vacuum distillation of a malt/water slurry, for sample pair C/D but not for sample pair A/B. A significant difference was also found between the two calculation methods, with calculation method 2, which uses a correction for internal standard recovery, giving higher results.

Comparison of combined laboratory variances for the two malt methods showed a significant difference when calculation method I was used for both sample pairs. However, because Malt Method I gave a lower variance with the A/B pair and Malt Method II gave a

lower variance with the C/D pair, this probably has no practical significance. No significant differences were found between combined laboratory variances when the calculation methods were compared.

General. Based on a comparison with the amounts of NDMA added to the beers, the results of both beer methods showed excellent accuracy when calculation methods 2 and 3 were employed. This implies that calculation method 2 or 3 would also be more accurate for malt than would calculation method 1.

No significant differences were found between the two detection methods, the Hall™ electrolytic conductivity detector and the Thermal energy Analyzer™.

LITERATURE CITED

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