

FOOD ADDITIVES

Gas Chromatographic-Chemiluminescence Method for Determination of Volatile *N*-Nitrosamines in Minced Fish-Meat and Surimi-Meat Frankfurters: Collaborative Study

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A collaborative study was carried out on a solid-phase extraction method for separating volatile *N*-nitrosamines, particularly *N*-nitrosodimethylamine (NDMA), from combination minced fish or surimi-meat frankfurters with detection by gas chromatography-chemiluminescence (thermal energy analyzer). The results from the 10 collaborators were evaluated using the most recent AOAC guidelines for determining outliers and for the analysis of variance. For NDMA, repeatability standard deviations, s_r , ranged from 0.56 to 2.25; repeatability relative standard deviations, RSD_r , ranged from 8.9 to 11.5%. Reproducibility standard deviations, s_R , for NDMA ranged from 1.40 to 6.49, and reproducibility relative standard deviations, RSD_R , ranged from 24.2 to 28.9%. Our data compared favorably to the reproducibility (RSD_R) curve of Horwitz. The method has been adopted official first action by AOAC.

An earlier study to determine volatile *N*-nitrosamines in frankfurters that contained minced fish or surimi, using the then available methodology that was applicable to cured meat products, resulted in inaccurate values caused by artifactual *N*-nitrosodimethylamine (NDMA) formation, particularly in franks that contained unwashed Alaska pollock mince (1). This was undoubtedly caused by the simultaneous presence of nitrite and high concentrations of dimethylamine and its precursors in the fish. Methods typically used for determining NDMA in fish without nitrite were also not applicable (1-3). Even the use of known nitrosation inhibitors, sodium ascorbate and alpha-tocopherol, with currently available methods was not effective in completely eliminating artifactual nitrosamine formation (1). The Eastern Regional Research Center dry column, solid-phase extraction method, primarily designed to measure *N*-nitrosopyrrolidine (NPYR) in fried bacon (4), and a similar method to determine NDMA in nonfat dry milk (5) and beer (6), were not suitable for this sample type. However, we developed a new method, based on a principle similar to these methods, but using 2 columns.

We reported the development and preliminary evaluation

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The report has been evaluated by the General Referee and the Committee Statistician and reviewed by the Committee on Foods I. The method was approved interim official first action by the Chairman of the Official Methods Board and was adopted official first action at the 104th AOAC Annual International Meeting, September 9-13, 1990, at New Orleans, LA. Association actions will be published in "Changes in Official Methods of Analysis" (1991) *J. Assoc. Off. Anal. Chem.* 74, January/February issue.

The mention of firm names or trade products does not imply endorsement or recommendation by the U.S. Department of Agriculture over other firms or similar products not mentioned.

of the ruggedness, reliability, and precision of this rapid, distillation-free, dry column chromatographic method for volatile *N*-nitrosamines, particularly NDMA, in combination minced fish-meat frankfurters (7). The satisfactory performance of this method, particularly for NDMA, the major nitrosamine present in this product type, indicated its potential usefulness as an accurate in-laboratory screening procedure suitable for both research and regulatory monitoring of cured, combination meat and fish products. Because this method was previously tested for artifact formation (7), and found not to result in artifactual formation of *N*-nitrosamines, we therefore carried out a multiple laboratory collaborative study to determine the accuracy and precision of the method within and among laboratories. The results of this study are reported here.

Collaborative Study

Sixteen federal, industrial, and university laboratories familiar with the determination of volatile nitrosamines in cured meat products were invited to participate in the collaborative study. Two different sets of practice samples, consisting of 15% minced fish-meat frankfurters containing 0, 3, or 5 ppb NDMA, NPYR, and NMOR, were sent to each laboratory to check for compliance with the protocol before the start of the main study. After the first set, 6 laboratories dropped out of the study because of difficulties with their thermal energy analyzer, poor results, or insufficient time available to complete the entire study. The remaining 10 collaborators in this study operated independently, and each laboratory was requested to use the same analyst for the full study.

Each collaborator received 16 cans that were duplicates of 8 samples of 15% minced fish or surimi-meat frankfurters, coded so that the duplicates were unknown to the analysts. All samples were ground twice through a 3 mm plate, mixed thoroughly, and 10.0 ± 0.1 g aliquots were placed in aluminum cans. Six samples, originally free of nitrosamines, were spiked to obtain target levels of 5, 10, and 15 ppb of each nitrosamine, NDMA, NPYR, NMOR in each sample; one sample had only naturally incurred NDMA, and one sample, containing no detectable nitrosamines, was used as the zero control, designed to detect cross contamination. The collaborative study samples were identified as follows: Sample 1, menhaden mince, spiked at 5 ppb; Sample 2, menhaden mince, spiked at 15 ppb; Sample 3, menhaden surimi, spiked at 10 ppb; Sample 4, pollack surimi, zero control; Sample 5, pollack mince, spiked at 10 ppb; Sample 6, pollack surimi, spiked at 5 ppb; Sample 7, pollack surimi, spiked at 15 ppb; Sample 8, red hake mince, normally incurred.

The cans were vacuum-sealed and stored in a freezer at -30°C for 24 h. The frozen samples and the nitrosamine

standards were packed in Styrofoam shipping containers with Insul-Ice singles to maintain temperature, and shipped by air freight to each collaborating laboratory. The collaborators were instructed to let the samples thaw in a refrigerator and to prepare working concentrations of the nitrosamine standards in methylene chloride just before starting the analyses. The analysts were requested to return the results and the chromatograms to our laboratory after the analyses were completed.

N-Nitrosamines in Minced Fish-Meat and Surimi-Meat Frankfurters

Gas Chromatographic-Thermal Energy Analyzer Method First Action

(Applicable to determination of >5 ppb NDMA, NPYR, and NMOR in product)

(Caution: N-Nitrosamines are potential carcinogens. Handle these materials with great care.)

Method Performance:

NDMA spiked at 5 ppb

$s_r = 0.56$; $s_R = 1.40$; $RSD_r = 11.5\%$; $RSD_R = 28.9\%$

NDMA spiked at 10 ppb

$s_r = 0.98$; $s_R = 2.19$; $RSD_r = 10.9\%$; $RSD_R = 24.2\%$

NDMA spiked at 15 ppb

$s_r = 1.19$; $s_R = 3.87$; $RSD_r = 8.9\%$; $RSD_R = 28.9\%$

NDMA naturally incurred (ca 23 ppb)

$s_r = 2.25$; $s_R = 6.49$; $RSD_r = 9.8\%$; $RSD_R = 28.2\%$

NPYR spiked at 5 ppb

$s_r = 0.48$; $s_R = 1.12$; $RSD_r = 12.5\%$; $RSD_R = 29.5\%$

NPYR spiked at 10 ppb

$s_r = 0.97$; $s_R = 2.00$; $RSD_r = 12.0\%$; $RSD_R = 24.7\%$

NPYR spiked at 15 ppb

$s_r = 1.06$; $s_R = 3.17$; $RSD_r = 8.9\%$; $RSD_R = 26.5\%$

NMOR spiked at 5 ppb

$s_r = 0.55$; $s_R = 1.18$; $RSD_r = 13.3\%$; $RSD_R = 28.5\%$

NMOR spiked at 10 ppb

$s_r = 1.03$; $s_R = 2.12$; $RSD_r = 12.1\%$; $RSD_R = 24.9\%$

NMOR spiked at 15 ppb

$s_r = 1.26$; $s_R = 3.11$; $RSD_r = 10.1\%$; $RSD_R = 25.0\%$

A. Principle

Volatile nitrosamines and amines are eluted with pentane- CH_2Cl_2 from column containing mixture of Celite, anhydrous Na_2SO_4 , and sample, leaving insoluble nitrite on column. Eluate passes through second column containing acid Celite to remove amines. Nitrosamines are eluted from acid Celite column with CH_2Cl_2 , eluate is concentrated, and nitrosamines are determined by gas chromatography with thermal energy analyzer detector (chemiluminescence detector specific for nitric oxide). Minimum detectable level of NDMA, NPYR, and NMOR in study of this method was 0.2 ppb.

B. Apparatus

(a) *Mortar and pestle*.—Glass, 473 mL (16 oz; Thomas Scientific).

(b) *Chromatographic column*.—Glass, 350 × 32 mm id, with 60 × 6 mm id drip tip (prepared by glassblower).

(c) *Tamping rod*.—Glass, 450 mm long with 12 mm diameter disk at end (prepared by glassblower).

(d) *Evaporative concentrator*.—Kuderna—Danish (K-

D) 250 mL; 4 mL graduated concentrator tube; Snyder (3-section) and micro-Snyder distilling columns (Kontes Glass Co.).

(e) *Gas chromatograph-thermal energy analyzer (GC-TEA)*.—Gas chromatograph (Shimadzu Mini 3) interfaced to thermal energy analyzer (Model 502, Thermedics, Inc., Woburn, MA). Operating conditions: 3 m × 2.6 mm id glass column packed with 15% Carbowax 20M-TPA on 60–80 mesh Gas-Chrom P; He carrier gas 35 mL/min; column programmed from 120 to 220° at 4°/min; injector 200°; TEA furnace 450°; TEA vacuum 1.0 mm; liquid N_2 cold trap.

C. Reagents

(a) *Celite 545*.—Not acid-washed (Fisher Scientific). Run reagent blank before start of sample analysis, particularly if new lot of Celite is used. If interfering chromatographic peaks are found, prewash Celite twice with CH_2Cl_2 , then dry 4 h in 120° vacuum oven before use.

(b) *Phosphoric acid*.—6N. Before use, extract once with equal volume of CH_2Cl_2 to remove potential contaminants.

(c) *Sodium sulfate*.—Anhydrous, granular Na_2SO_4 (Mallinckrodt No. 8024).

(d) *Methylene chloride and pentane*.—Distilled-in-glass grade.

(e) *Nitrosamine standards*.—NDMA, NPYR, and NMOR are commercially available. NAZET was synthesized as follows: To an equimolar solution of azetidine and acetic acid, slowly add 2-fold excess of aqueous solution of NaNO_2 . Reflux mixture for 2 h, then distill. Saturate aqueous distillate with K_2CO_3 , then extract with CH_2Cl_2 . Dry CH_2Cl_2 extract over anhydrous Na_2SO_4 , then remove solvent on vacuum rotary evaporator. NAZET is pale yellow oil. Confirm identity of NAZET by mass spectrometry.

(f) *GC-TEA working standard solution*.—Prepare stock solution of 1.0 μg each of NDMA, NAZET, NPYR, and NMOR/mL isooctane. Dilute to 0.10 $\mu\text{g}/\text{mL}$ with CH_2Cl_2 before use.

(g) *N-Nitrosoazetidine internal standard solution*.—Prepare stock solution of 1.0 μg NAZET/mL isooctane. Dilute to 0.10 $\mu\text{g}/\text{mL}$ with CH_2Cl_2 before use.

D. Determination

Weigh 10 g Celite into 250 mL beaker. Add 10 mL 6N phosphoric acid in ca 3 mL portions and stir Celite with small glass rod until mixture is fluffy and uniform in texture. Pour acid Celite through powder funnel into glass chromatographic column containing glass wool plug at bottom. Insert tamping rod through Celite bed and tamp from bottom up to final height of ca 25 mm. Add 25 g anhydrous Na_2SO_4 to top of column.

Grind sample twice through $\frac{1}{8}$ in. plate. Accurately weigh 10.0 ± 0.1 g ground sample into mortar. Use 1.0 mL transfer pipet to add 1.0 mL internal standard solution (equivalent to 10 ppb) to mortar. Add 25 g anhydrous Na_2SO_4 to mortar and mix with pestle for ca 15 s. Then add 20 g Celite and again mix using pestle for ca 15–20 s, or until Celite is thoroughly mixed with sample and Na_2SO_4 . Grind entire mixture using moderate pressure for ca 1 min, then quantitatively transfer mixture to second glass column containing glass wool plug; tamp mixture in similar manner to that used for first column, to final height of ca 75 mm. Add 25 g anhydrous Na_2SO_4 to top of this column.

Table 1. Collaborative results for determination of *N*-nitrosodimethylamine (ppb) in minced fish or surimi-meat frankfurters (uncorrected)^a

Lab.	Sample							
	1	2	3	4	5	6	7	8
A	6.80	—	10.30	0.00	—	4.80	—	—
	6.70	—	10.00	0.00	—	4.60	—	—
B	6.50	14.70	10.00	0.00	11.00	4.10	16.10	26.00
	4.70	15.50	9.70	0.00	10.40	5.00	16.30	25.80
C	7.11	14.98	9.25	1.13 ^b	8.63	4.07	14.58	25.21
	6.08	13.88	7.19	0.73 ^b	9.61	3.71	13.00	24.16
D	4.43	9.48	7.04	0.00	6.96	2.43	9.04	15.22
	4.50	10.20	7.86	0.00	7.64	2.57	11.07	18.50
E	3.88	9.03	6.81	0.00	7.31	2.63	9.58	17.08
	—	9.06	4.38	0.00	5.56	3.31	9.05	15.50
F	8.24	21.10	12.40	0.00	13.60	4.56	17.50	33.20
	10.10	22.00	12.40	0.00	11.90	4.56	17.10	34.80
G	6.01	15.89	10.00	0.00	9.86	4.20	14.00	24.50
	6.73	17.05	9.86	0.00	11.67	4.36	14.00	27.80
H	6.33	16.10	9.50	0.00	9.80	3.80	13.10	23.70
	6.20	13.60	9.00	0.00	9.00	4.20	14.40	25.30
I	3.90	11.80	8.70	0.00	8.10	3.10	8.00	19.50
	4.50	7.10	6.20	0.00	6.20	2.60	10.00	12.40
Average	6.04	13.84	8.92	0.10	9.20	3.81	12.93	23.04
				(0.00) ^c				
<i>s_r</i>	0.74	1.43	0.99	0.09	0.98	0.32	0.89	2.25
				(0.00)				
<i>s_R</i>	1.63	4.39	2.10	0.32	2.27	0.86	3.21	6.49
				(0.00)				
RSD _r , %	12.19	10.35	11.06	91.24	10.61	8.52	6.92	9.75
				(0.00)				
RSD _R , %	27.03	31.70	23.57	306.86	24.69	22.59	24.80	28.15
				(0.00)				

^a See text for discussion of missing data.

^b Outliers, see text.

^c Values without laboratory C results are in parentheses.

Place column containing sample mixture directly over column containing acid Celite such that solvent flows through sample-containing column directly into acid Celite column. Rinse mortar, pestle, and tamping rod with 15 mL pentane-CH₂Cl₂ (95 + 5) solvent and add rinse to top of column that contains sample. Immediately add additional 160 mL of same solvent to column. Collect eluate that passes through both columns in 125 mL Erlenmeyer flask. When solvent from top column stops dripping, remove top column and discard contents. Also discard contents of 125 mL Erlenmeyer flask. Add 125 mL CH₂Cl₂ to remaining column and collect eluate in 250 mL K-D flask equipped with 4 mL concentrator tube. When column stops dripping, remove K-D flask, add 2 small boiling chips to flask, attach 3-section Snyder column, and concentrate eluate to ca 4 mL on steam bath. After cooling, continue concentration (add new boiling chip) to 1.0 mL with micro-Snyder column and 70° water bath. (Note: Room temperature should be <24° during sample analysis. Do not concentrate sample under stream of nitrogen; nitrosamines will be lost.)

Manually inject 9.0 μL GC-TEA standard solution at lowest attenuation that yields signal at least 1/3 full-scale TEA response, and measure peak heights. Repeat injection to ensure good reproducibility of retention time and response. Inject 9.0 μL sample solution, and measure peak heights. For each injection, calculate nitrosamine concentration in ppb (μg/kg), as follows:

$$N\text{-Nitrosamine, ppb} = (R/R') \times [(C \times V)]/W \times 1000$$

where *R* and *R'* = peak height of nitrosamine in sample and standard, respectively; *C* = nitrosamine concentration in standard solution = 0.10 μg/mL; *W* = sample weight, g; *V* = final volume of sample solution = 1.0 mL. Report data to nearest 0.1 ppb. Calculate recovery of NAZET internal standard as follows:

$$\text{NAZET, \%} = (R_1/R'_1) \times 100$$

where *R*₁ and *R'*₁ = peak height of NAZET in sample and standard, respectively.

If NAZET recovery is <60%, results for other nitrosamines (NDMA, NPYR, and NMOR) are unacceptable.

Ref.: JAOAC 73, November/December issue (1990).

CAS-62-75-9 (NDMA)

CAS-930-55-2 (NPYR)

CAS-59-89-2 (NMOR)

Results

The overall results of this study were treated following the most recent procedures for determining outliers and for analysis of variance as outlined by AOAC (8).

After an initial audit of the data reported by the 10 collaborators, all the results from one collaborator were determined to be invalid because the laboratory reported deviating from

Table 2. Collaborative results for determination of *N*-nitrosopyrrolidine (ppb) in minced fish or surimi-meat frankfurters (uncorrected)^a

Lab.	Sample							
	1	2	3	4	5	6	7	8
A	5.20	—	10.10	0.00	—	4.20	—	—
	4.60	—	9.40	0.00	—	4.50	—	—
B	4.30	16.90	8.20	0.00	10.20	4.10	17.00	0.00
	4.30	14.60	10.00	0.00	10.00	5.00	15.50	0.00
C	3.89	9.43	5.46	0.00	6.63	2.15	9.55	0.00
	2.83	8.20	4.42	0.00	5.60	2.15	9.20	0.00
D	3.94	11.84	9.21	0.00	8.68	3.94	11.84	0.00
	4.09	9.53	7.50	0.00	7.95	3.64	11.40	0.00
E	3.11	10.26	6.67	0.00	7.11	2.31	10.25	0.00
	—	10.67	5.56	0.00	7.56	4.22	11.11	0.00
F	5.53	15.50	10.20	0.00	11.10	3.97	15.30	0.00
	5.52	16.80	9.70	0.00	10.00	3.71	13.80	0.00
G	5.00	14.40	9.22	0.00	8.71	4.00	12.90	0.00
	4.76	13.77	8.79	0.00	10.38	4.85	12.30	0.00
H	5.07	13.05	9.20	0.00	8.90	3.80	13.10	0.00
	4.63	13.00	8.30	0.00	8.90	3.30	13.60	0.00
I	2.20	8.00	6.70	0.00	6.60	1.10	6.20	0.00
	1.90	5.40	3.80	0.00	4.40	1.60	9.20	0.00
Average	4.17	11.96	7.91	0.00	8.30	3.47	12.02	0.00
s _r	0.34	1.15	1.02	0.00	0.82	0.57	0.97	0.00
s _R	1.09	3.46	2.07	0.00	1.93	1.16	2.86	0.00
RSD _r , %	8.15	9.62	12.85	0.00	9.85	16.52	8.10	0.00
RSD _R , %	26.21	28.90	26.18	0.00	23.28	33.28	23.82	0.00

^a See text for discussion of missing data.

the protocol. A second collaborator's results for samples 2, 5, 7, and 8 were determined to be invalid because of contamination problems. One of the duplicates from sample 1, laboratory E, was accidentally lost prior to GC-TEA analysis. The

resulting set of valid data values consisted of responses for NDMA, NPYR, NMOR, and NAZET for the 8 samples under study from either 8 or 9 collaborators. These are listed in Tables 1-4.

Table 3. Collaborative results for determination of *N*-nitrosomorpholine (ppb) in minced fish or surimi-meat frankfurters (uncorrected)^a

Lab.	Sample							
	1	2	3	4	5	6	7	8
A	6.00	—	11.80	0.00	—	5.10	—	—
	5.60	—	10.60	0.00	—	5.70	—	—
B	4.30	15.90	8.90	0.00	10.50	4.80	16.30	0.00
	4.80	14.80	10.90	0.00	10.90	5.00	16.80	0.00
C	4.37	10.88	6.10	0.00	7.67	2.52	10.75	0.00
	3.34	9.28	5.11	0.00	6.34	2.30	10.46	0.00
D	4.67	15.33	10.33	0.00	10.00	5.00	14.67	0.00
	5.00	10.60	9.41	0.00	9.71	4.12	13.20	0.00
E	3.53	9.67	7.06	0.00	7.35	2.33	10.67	0.00
	—	9.39	5.29	0.00	7.94	4.41	10.88	0.00
F	5.14	14.90	10.30	0.00	9.63	3.94	15.10	0.00
	5.67	15.50	9.20	0.00	9.16	3.75	13.00	0.00
G	4.95	14.41	9.68	0.00	9.15	4.41	13.44	0.00
	5.38	14.55	9.14	0.00	10.61	4.35	13.66	0.00
H	5.13	13.80	9.00	0.00	9.30	3.70	13.10	0.00
	4.60	13.90	7.90	0.00	8.90	3.30	14.50	0.00
I	3.00	8.50	6.80	0.00	6.90	1.40	6.50	0.00
	1.70	5.10	3.60	0.00	4.10	2.10	8.40	0.00
Average	4.54	12.28	8.40	0.00	8.64	3.79	12.59	0.00
s _r	0.50	1.54	1.14	0.00	0.89	0.59	0.89	0.00
s _R	1.10	3.31	2.33	0.00	1.86	1.25	2.89	0.00
RSD _r , %	11.04	12.58	13.57	0.00	10.32	15.53	7.04	0.00
RSD _R , %	24.25	26.92	27.71	0.00	21.60	32.95	22.95	0.00

Table 4. Collaborative results for recovery (%) of *N*-nitrosoazetidine internal standard^a

Lab.	Sample							
	1	2	3	4	5	6	7	8
A	100.0	—	95.0	95.0	—	92.0	—	—
	101.0	—	97.0	94.0	—	111.0	—	—
B	94.6	100.0	97.3	87.2	94.6	94.6	100.0	92.3
	73.7	91.9	94.7	92.1	94.6	100.0	97.4	100.0
C	99.0	97.0	94.0	92.0	92.0	89.0	97.0	102.0
	94.0	93.0	87.0	85.0	93.0	87.0	91.0	89.0
D	97.4	73.7	86.8	81.6	89.5	76.3	78.9	89.5
	85.7	92.5	71.4	100.0	81.0	81.0	83.3	81.0
E	73.8	83.3	88.1	89.0	85.7	71.4	88.1	91.0
	—	90.5	90.5	87.0	85.7	97.6	90.5	81.0
F	88.0	91.0	103.0	102.0	95.0	94.0	102.0	105.0
	98.0	89.0	98.0	97.0	80.0	92.0	80.0	92.0
G	81.5	89.2	80.3	80.0	80.3	92.2	89.1	78.8
	83.3	92.5	80.0	81.5	91.0	86.1	84.4	84.6
H	102.8	102.5	89.1	88.5	96.3	95.5	96.2	94.2
	96.7	94.1	99.3	88.5	95.5	90.8	89.9	99.2
I	99.2	95.6	95.6	95.0	97.4	91.6	89.6	86.7
	95.5	83.3	98.2	87.4	101.0	92.4	101.0	80.6
Average	92.0	91.2	91.4	90.2	90.8	90.8	91.2	90.4
s _r	6.9	6.7	4.9	5.3	5.2	8.1	6.8	6.5
s _R	9.3	6.9	8.2	6.3	6.6	8.8	7.3	8.3
RSD _r , %	7.5	7.4	5.4	5.9	5.7	8.9	7.5	7.1
RSD _R , %	10.1	7.6	9.0	7.0	7.2	9.7	8.0	9.1

^a See text for discussion of missing data.

The valid data values, on a sample-by-sample basis, were then submitted to the sequence of Cochran, Grubbs, and pair Grubbs outlier tests. This was done separately for each response (NDMA, NPYR, NMOR, NAZET). As a result, data for sample 4 from collaborator C were determined to be outliers for NDMA. Performance parameters for NDMA

were determined by analysis of variance of the data in Table 1 on a sample-by-sample basis. The resulting values for repeatability and reproducibility standard deviations, s_r and s_R, and relative standard deviations, RSD_r and RSD_R, for the individual samples are shown in Table 1. The parameters for sample 4 were calculated with and without the outliers from

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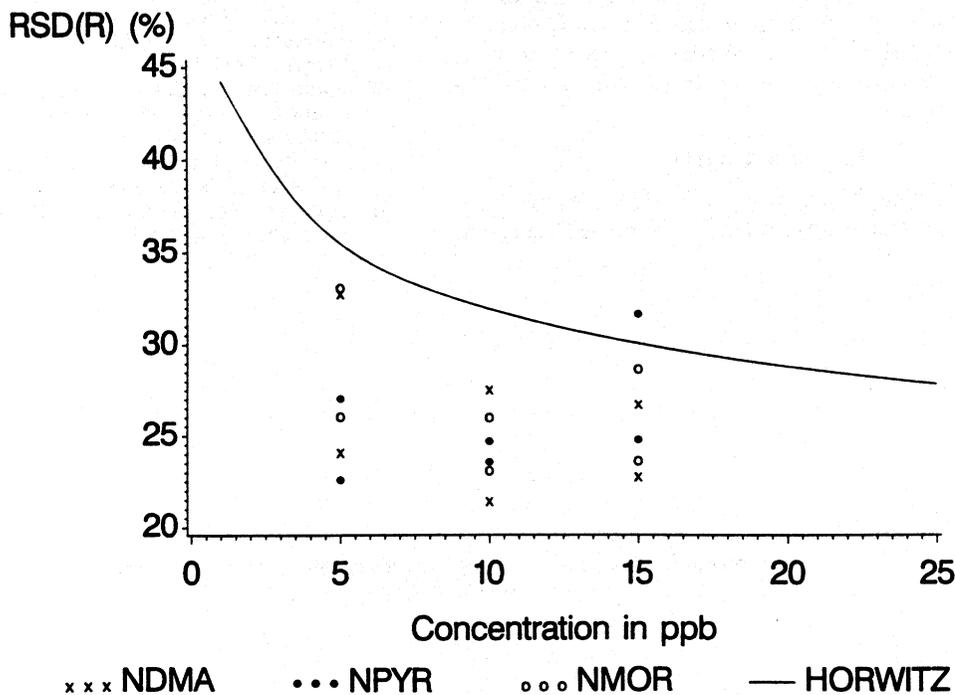


Figure 1. Comparison of RSD_R values obtained in present study with values obtained in typical procedures for determining analytes at similar concentration levels, as described by Horwitz et al. (9).

collaborator C. Results from the other collaborators for sample 4, the zero control, show that there is no evidence of artifacts in the determination of NDMA. Values of s_r for the remaining samples ranged from 0.32 to 2.25; RSD_r values ranged from 6.9 to 12.2%. s_R values ranged from 0.9 to 6.5, and RSD_R values ranged from 22.6 to 31.7%. Estimates of repeatability and reproducibility of NDMA for the spiked samples agreed well with that for sample 8, which had normally incurred NDMA.

Performance parameters for NPYR and NMOR were similarly calculated from the data in Tables 2 and 3, respectively. The resulting values of the individual samples for repeatability and reproducibility parameters are shown in each table. The parameter estimates for samples 4 and 8 showed no evidence of false positives for either NPYR or NMOR. The estimates for the remaining samples covered the following ranges: NPYR; s_r , 0.34–1.15; s_R , 1.2–3.5; RSD_r , 8.1–16.5%; RSD_R , 23.3–33.3%, and NMOR; s_r , 0.5–1.5; s_R , 1.1–3.3; RSD_r , 7.0–15.5%; RSD_R , 21.6–33.0%. RSD_R values for NDMA, NPYR, and NMOR compare favorably with those from typical procedures for determining analytes at the concentrations under study as shown by comparing the former with the RSD_R curve of Horwitz et al. (9, 10) (Figure 1).

Finally the performance parameters for the NAZET internal standard were calculated; the results are shown in Table 4. If the recovery of this nitrosamine is <60%, the results for the other nitrosamines (NDMA, NPYR, NMOR) would be considered unacceptable.

The minimum detectable level of NDMA using this method was 0.2 ppb. We have observed a linear response for levels of NDMA from 0.2 to 50.0 ppb and, on the basis of similar methods, we expect that linearity can be extended to 1.0 ppm or more.

Recommendation

We recommend that the dry, solid-phase extraction column screening method be adopted official first action for the determination of volatile *N*-nitrosamines, particularly NDMA, in combination fish-meat frankfurters on the basis of its simplicity, good recovery, freedom from artifacts, and acceptable precision.

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REFERENCES

- (1) Brooker, J. R. (1985) *An Evaluation of Fish Meat as an Ingredient in Hot Dogs—Summary Report*, National Marine Fisheries Service, Washington, DC
- (2) Yamamoto, M., Iwata, R., Ishiwata, H., Yamada, T., & Tanimura, A. (1984) *Food Chem. Toxicol.* **22**, 61–64
- (3) Sen, N. P., Tessier, L., Seaman, S. W., & Baddoo, P. A. (1985) *J. Agric. Food Chem.* **33**, 264–268
- (4) Pensabene, J. W., Miller, A. J., Greenfield, E. L., & Fiddler, W. (1982) *J. Assoc. Off. Anal. Chem.* **65**, 151–156
- (5) Sen, N. P., Seaman, S., & Karpinsky, K. (1984) *J. Assoc. Off. Anal. Chem.* **67**, 232–236
- (6) Havery, D. C., Perfetti, G. A., & Fazio, T. (1984) *J. Assoc. Off. Anal. Chem.* **70**, 91–95
- (7) Pensabene, J. W., & Fiddler, W. (1988) *J. Assoc. Off. Anal. Chem.* **71**, 839–843
- (8) "Guidelines for Collaborative Study Procedure to Validate Characteristics of a Method of Analysis" (1988) *J. Assoc. Off. Anal. Chem.* **71**, 161–173
- (9) Horwitz, W., Kamps, L. R., & Boyer, K. W. (1980) *J. Assoc. Off. Anal. Chem.* **63**, 1344–1354
- (10) "Committee on Interlaboratory Studies" (1987) *J. Assoc. Off. Anal. Chem.* **70**, 352–356