

## FOOD ADDITIVES

### Determination of Nitrite in Cured Meats by Ion-Exclusion Chromatography with Electrochemical Detection

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**A rapid liquid chromatographic (LC) method was developed for a sensitive determination of nitrite in cured meats, using ion-exclusion chromatographic separation and electrochemical detection (IEC-EC). The current AOAC colorimetric method requires 2 h shaking in a steam bath to eliminate interference from reducing compounds such as ascorbic acid. In the present method, nitrite was analyzed in the presence of ascorbic acid without interference, and the extraction time was reduced to 1 min. The extracted nitrite was determined by ion chromatography using anion-exclusion/HS column and amperometric detector equipped with platinum or glassy carbon electrode operating at +1.0 V vs Ag/AgCl reference electrode. The detection limit was 1 ppb as  $\text{NO}_2^-$ . The recoveries of 50 ppm nitrite added to frankfurter and meat stick were 103 and 99.6%, respectively, with relative standard deviations less than 4%. The high speed, sensitivity, and selectivity make the new method a useful alternative to the AOAC colorimetric method.**

Nitrite in foods is of concern because it can induce methaemoglobinemia and react with secondary and tertiary amines, forming carcinogenic nitrosamines (1). Even though saliva is the major source of human exposure to nitrite (8.6 mg daily), a significant amount of nitrite is consumed through cured meats (2.4 mg daily) in the United States (1). Most countries impose limits on the use of nitrite in cured meats (2).

In the current AOAC method, nitrite is extracted from comminuted meats with 80°C water by shaking for 2 h in a steam bath and is determined colorimetrically following diazotization of sulfanilamide and coupling with *N*-(1-naphthyl)-ethylenediamine dihydrochloride (3). The 2 h extraction at high temperature is required to eliminate interfering compounds such as ascorbic acid and erythorbic acid, which are widely used in cured meats (4). This lengthy extraction is unnecessary if nitrite is separated from such interfering compounds before determination.

A number of researchers have reported analysis of nitrite in foods by using either ion-interaction (5-7) or ion-exchange (8-10) chromatographic separation with UV detection. Unfortunately, nitrite is inadequately separated from other food components in ion-interaction or ion-exchange chromatography. For example, de Kleijn and Hoven (6) had to use 240 nm (instead of 210 nm) for detection, at the expense of sensitivity, to obtain a clean chromatogram from meats.

Recently, Kim and Kim (11) noted several advantages of the IEC-EC system for the determination of weak acids in foods such as sulfite, ascorbic acid, and nitrite. Tanaka (12) first used ion-exclusion chromatography for nitrite, using UV detection at 210 nm. Electrochemical (amperometric)

detection offers high specificity because only selected compounds are oxidized at the typical operating potential of an amperometric detector (between +0.4 and +1.2 V). The advantage of electrochemical detection for nitrite was first noted in 1982 by Wheals (13). Kordorouba and Pelletier (14) demonstrated that high selectivity and sensitivity for analysis of nitrite in meat products can be achieved by ion-exchange chromatography with electrochemical detection. They reported a detection limit of 1 ppb  $\text{NO}_2^-$ . Kim and Kim (15) reported extremely high selectivity and sensitivity of the IEC-EC method for determination of nitrite in drinking water and environmental samples. In this paper, we present results which demonstrate that a rapid, accurate, and sensitive analysis of nitrite in cured meats is possible using the IEC-EC method.

#### Experimental

##### Reagents

(a) *Nitrite standard solutions*.—1000 ppm  $\text{NO}_2^-$  stock solution: Dissolve 150 mg sodium nitrite (Kodak Chemical, Rochester, NY) in 100 mL deionized water. Stock solution is stable for several weeks in the refrigerator. Working solutions: 10, 1.0, 0.3, and 0.1 ppm. Prepare daily by diluting stock solution successively with deionized water.

(b) *Sulfanilamide reagent*.—Dissolve 0.5 g sulfanilamide (Pfaltz & Bauer, Waterbury, CT) in 150 mL 15% HOAc. Filter and store in brown glass bottle. Stable for several weeks at room temperature.

(c) *NED reagent*.—Dissolve 0.2 g *N*-(1-naphthyl)-ethylenediamine dihydrochloride (Pfaltz & Bauer) in 150 mL 15% HOAc. Filter and store in brown glass bottle. Store for several weeks at room temperature.

(d) *Eluant*.—0.2M sulfuric acid stock solution: Prepare by adding 9.8 mL concentrated sulfuric acid to ca 400 mL deionized water and bringing volume to 500 mL. Prepare 5mM sulfuric acid eluant by mixing 10 mL stock solution with 390 mL deionized water. Degas under vacuum.

##### Apparatus

(a) *Homogenizing tube*.—100 mL Pyrex centrifuge tube.

(b) *Homogenizer*.—Polytron (Brinkmann Instruments, Westbury, NY), or equivalent.

(c) *Shaker bath*.—Temperature-controlled shaker bath (Lab-Line Instruments, Melrose Park, IL).

(d) *Membrane filter*.—0.45  $\mu\text{m}$  Nylon 66 filter (Alltech Associates, Deerfield, IL). Prewash filters with deionized water to remove traces of nitrite often found in membrane filters.

(e) *Ion chromatography system*.—(Wescan Instruments, Deerfield, IL). Equipped with anion exclusion Ion-Guard cartridge, anion-exclusion/HS column (4.6  $\times$  100 mm), Rheodyne injector with 20  $\mu\text{L}$  loop, Model 271 electrochemi-

cal detector with Pt working electrode, and Model 4270 computing integrator (Spectra-Physics, San Jose, CA). Similar system with Waters (Waters Chromatography Division, Milford, MA) Model 510 pump, Wescan anion-exclusion Ion-Guard cartridge, and HS column and Waters Model 460 electrochemical detector with glassy carbon electrode was also used.

(f) *Spectrophotometer*.—Spectronic 1201 (Milton Roy, Rochester, NY).

#### Sample Extraction

Cured meat products were purchased from a local supermarket and comminuted with a food chopper to produce homogeneous samples. Four replicate 1 g portions of the homogenized meat sample were weighed into 100 mL homogenizing tubes. To each tube, 49 mL deionized water at room temperature was added and the sample was homogenized 1 min with Polytron at setting of 6 (Extraction A). Four replicate extractions were repeated using deionized water heated to 80°C (Extraction B). Another set of the same 4 replicate extractions was carried out using the AOAC method of extraction in a shaker bath at 80°C for 2 h (Extraction C). The final volume in Extraction C was brought to 50 mL with deionized water to compensate for evaporation of water. The aqueous phase of the extract was filtered, after centrifugation if necessary, through a 0.45  $\mu\text{m}$  membrane filter for analysis.

#### Determination

(a) *IEC-EC method*: Detector voltage for either Pt electrode or glassy carbon electrode was set at 1.0 V vs Ag/AgCl reference electrode (15). Using isocratic conditions, the entire system was equilibrated with mobile phase until steady baseline was obtained at a flow rate of 0.8 mL/min and chart speed of 1 cm/min. Twenty  $\mu\text{L}$  portions of 0.1, 0.3, and 1.0 ppm working standard were injected and attenuation on the integrator that yielded about half the full deflection for each concentration was determined. Each sample extract and appropriate working standard (0.1 ppm for meats containing less than 5 ppm nitrite, 0.3 ppm for meats with 10–15 ppm, 1.0 ppm for 50 ppm spiking experiment) were injected. Nitrite concentration was calculated by comparing peak height with the standard.

(b) *AOAC colorimetric method*: Nitrite in each extract was determined by the AOAC colorimetric method (3).

#### Recovery Study

Six replicate portions of 1 g chopped meat (frankfurter and a tough meat sample, bacon and beef stick) were weighed into homogenizing tubes and 5 mL deionized water was added to each tube for 0 ppm spike experiment. After 10 min at room temperature, 44 mL hot water (80°C) was added and the mixture was homogenized for 1 min with Polytron. The procedure was repeated using 5 mL 10 ppm standard instead of deionized water for 50 ppm spike experiment. The extracted nitrite was determined, after filtration, by the IEC-EC method and the colorimetric method using 0.1 ppm and 1.0 ppm standard for unspiked and spiked samples, respectively. The mixtures, with and without added nitrite, were also shaken for 2 h in 80°C shaker bath and the extracted nitrite was determined by the colorimetric method. The recovery was calculated as the difference between measurements with and without added nitrite.

## Results and Discussion

### Interference by Ascorbic Acid

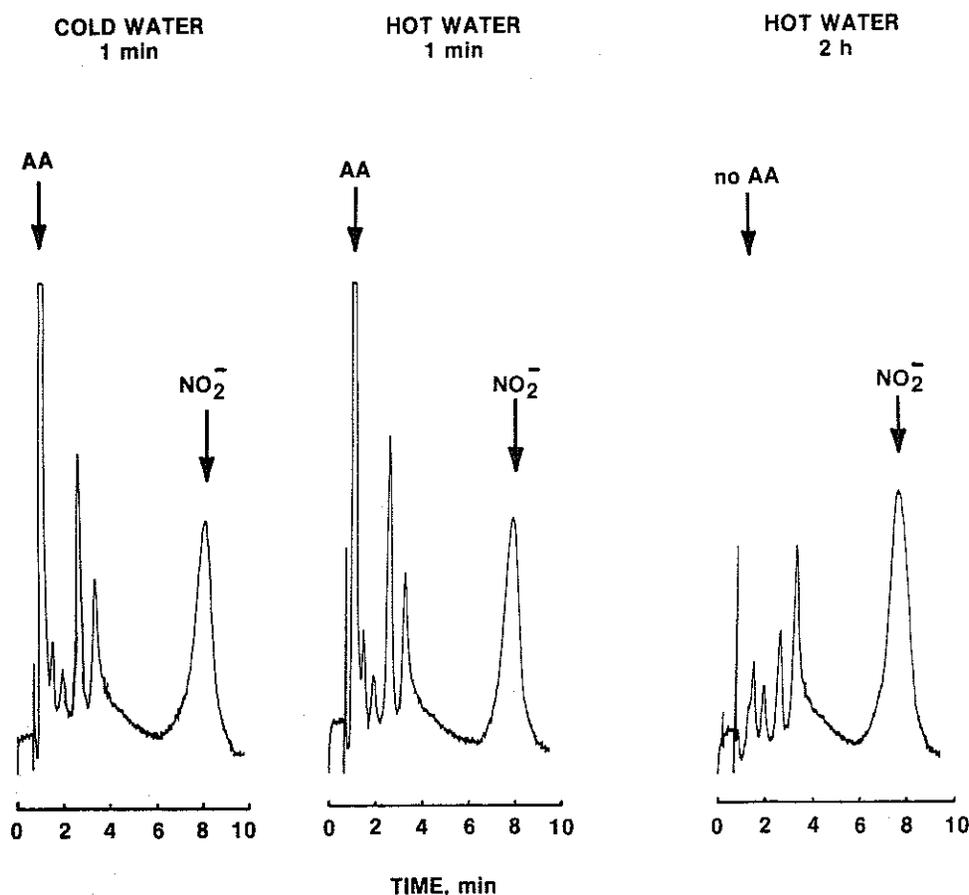
Two major drawbacks of the colorimetric method are interference and the lack of speed. Interference in cured meats is caused primarily by added reducing compounds such as ascorbic acid or erythorbic acid (16, 17). The 2 h extraction in the steam bath is required to oxidize these compounds. In fact, the 2 h extraction does not guarantee complete oxidation of these reductants. Nicholas and Fox (16) showed that, even if 2 h heating is used, higher nitrite concentration is obtained with certain samples if the water-to-meat ratio is increased above the AOAC recommended 100. They suggested that higher dissolved oxygen/reductant ratio leads to more complete oxidation of the interfering compounds. The nitrite concentration vs dilution curve showed a maximum slope around the AOAC dilution. Therefore, the AOAC method is subject to uncertainty if the initial concentration of the reducing compounds is high.

Kim and Kim (11) showed that nitrite is eluted much later than ascorbic acid from an anion-exclusion chromatographic column and detected amperometrically with a high sensitivity. Therefore, it was expected that a rapid and accurate determination of nitrite by the IEC-EC system should be possible in the presence of interfering compounds if nitrite could be extracted efficiently by a rapid homogenization.

The chromatograms in Figure 1, obtained using a Pt working electrode, show nitrite peaks corresponding to approximately 0.25 ppm following 1 min extraction and 0.28 ppm following 2 h shaking. A similar chromatogram was also obtained using a glassy carbon electrode. The retention time for nitrite was 8 min when the flow rate of the 5 mM sulfuric acid eluant was 0.8 mL/min. The chromatograms show several peaks including ascorbic acid (AA) in the earlier portion of the chromatogram.

Table 1 shows the comparison of results obtained from imported Polish ham by the IEC-EC method using Wescan electrochemical detector with Pt electrode and by the colorimetric method. The results by the IEC-EC method following 3 different extraction methods are in reasonably good agreement. Practically the same results were obtained after 1 min extraction with either cold water (Extraction A) or hot water (Extraction B). Results by 2 h shaking (Extraction C)/IEC-EC method were approximately 10% higher than those by the 1 min extraction/IEC-EC methods. The difference might be due to experimental errors, sample inhomogeneity, or slightly higher efficacy of Extraction C.

On the other hand, much lower results were obtained by the colorimetric method after 1 min extraction. The ham sample used indicated the presence of ascorbic acid on the label. The chromatograms obtained after 1 min extraction showed a strong signal corresponding to ascorbic acid (Figure 1; left, middle). The extract was diluted 10-fold and injected for quantitation of ascorbic acid. Ascorbic acid was determined with the detector voltage at 1.0 V instead of re-equilibrating the system at 0.6 V (18) or 0.8 V (19). The concentration of ascorbic acid in the ham determined by Extraction A/IEC-EC method was 643 mg/kg. A similar result was obtained by Extraction B. Clearly, ascorbic acid present at approximately 13 ppm after 50-fold dilution introduced a negative bias of 68–73% (4.0 or 3.3 ppm vs 12.4 ppm) to nitrite present at approximately 0.25 ppm in the extract when the colorimetric method was used (Table 1). Usher and



**Figure 1.** Ion-exclusion chromatograms of nitrite in ham, following 3 different extraction procedures. Presence of ascorbic acid in chromatograms is indicated by AA. Detector voltage on Pt working electrode is 1.0 V vs Ag/AgCl reference electrode. Detector current for the nitrite peak is ca 24 nA.

Telling (17) showed that 16 ppm ascorbic acid decreased the recovery of 0.5 ppm nitrite by 72%.

After 2 h shaking at 80°C, no residual ascorbic acid was observed in the extract by the IEC-EC method (Figure 1, right). Consequently, the same results within experimental errors were obtained by the IEC-EC method following Extraction A, B or C, and by the AOAC (Extraction C/colorimetric) method. The results clearly indicate that ascorbic acid interferes with nitrite by the colorimetric method and that 2 h heating is required to eliminate ascorbic acid. The

results in Table 1 also demonstrate that accurate determination of nitrite in the presence of reducing compounds is possible using the rapid 1 min extraction/IEC-EC method. The average relative standard deviation of the IEC-EC analyses in conjunction with 3 extraction procedures was 4.8%. Using the IEC-EC method, one does not need to be concerned about incomplete oxidation of the reductants after 2 h heating even if the initial level of the reductants is high.

#### Extraction Efficacy

Since most meat products have a high fat content, 1 min extraction with hot water seemed to be more convenient than with cold water. When cold water was used, fat from the meat samples collected on the Polytron and had to be removed after each extraction. Moreover, when extracting nitrite from tough meat products, hot water facilitated the breakdown of the meat and increased the extraction efficiency. For example, 12.3 ppm nitrite was observed from a pepperoni stick by Extraction A/IEC-EC method and 14.8 ppm was observed by Extraction B/IEC-EC method. Extraction C/IEC-EC method yielded only 7.8 ppm indicating that shaking for 2 h instead of homogenizing at high speed is insufficient for tough meats. For certain meats, soaking in hot water for 10–30 min before homogenizing may facilitate extraction of the nitrite. When several samples are analyzed, it is recommended that the weighed samples be soaked in hot water while more samples are weighed. Hot water from the

**Table 1.** Comparison of nitrite (ppm) in ham determined by IEC-EC method and colorimetric method, following different extraction procedures

Extraction method	IEC-EC method			Colorimetric method		
	Mean <sup>a</sup>	SD	RSD, %	Mean <sup>a</sup>	SD	RSD, %
(A) Cold water 1 min, Polytron	12.3	0.7	5.7	3.3	0.4	12.1
(B) Hot water 1 min, Polytron	12.7	0.2	1.6	4.0	0.7	14.9
(C) Hot water 2 h, shaking	13.9	1.0	7.1	12.4 <sup>b</sup>	1.2	9.7

<sup>a</sup> Average of 4 determinations.

<sup>b</sup> AOAC method.

**Table 2. Recovery of added nitrite from frankfurter and meat stick by 3 methods**

Spike, ppm	1 min extraction						2 h shaking, colorimetric method (AOAC)		
	IEC-EC method			Colorimetric method			Mean, <sup>a</sup> ppm	SD	RSD, %
	Mean, <sup>a</sup> ppm	SD	RSD, %	Mean, <sup>a</sup> ppm	SD	RSD, %			
<b>Frankfurter</b>									
0	7.3	0.5	6.8	7.2	0.5	6.9	9.0	0.3	3.3
50	58.7	1.1	1.9	54.6	0.8	1.5	58.5	1.2	2.1
Rec.	51.4	1.2	2.3	47.4	0.9	1.9	49.5	1.2	2.4
Rec., %	102.8			94.8			99.0		
<b>Bacon and beef stick</b>									
0	2.4	0.3	12.5	1.1	0.4	36.4	2.5	0.6	24.0
50	52.2	1.6	3.1	46.3	0.8	1.7	37.9	4.0	10.6
Rec.	49.8	1.6	3.2	45.2	0.9	2.0	35.4	4.0	11.3
Rec., %	99.6			90.4			70.8		

<sup>a</sup> Average of 6 determinations.

tap could be used after making sure that nitrite is absent. A small amount of nitrite is occasionally observed from membrane filters. The filter should be washed by passing several mL volumes of deionized water and the absence of nitrite in the final wash should be checked.

#### Recovery of Added Nitrite

The recovery of added nitrite from meats was investigated at the 50 ppm level, which is somewhat lower than the maximum permissible nitrite concentration in most cured meats in many countries (2). The recovery was measured by 3 methods: IEC-EC method using Waters electrochemical detector with glassy carbon electrode following 1 min extraction, colorimetric method following 1 min extraction, and colorimetric method following 2 h shaking (AOAC method). The recovery of 50 ppm added nitrite from frankfurter was satisfactory by all 3 methods (Table 2). The recovery from bacon and beef stick following 1 min extraction was 99.6% (RSD, 3.2%) and 90.4% (RSD, 2.0%) by the IEC-EC method and the colorimetric method, respectively. Nevertheless, the recovery by the AOAC method was 70.8% (RSD, 11.3%). This incomplete recovery was reproducible. No ascorbic acid was observed in the extract after 2 h shaking. It appears that 2 h shaking does not effectively extract all nitrite from tough meats. This observation is consistent with the incomplete extraction of nitrite from a pepperoni stick mentioned above. Overall, the recovery by the IEC-EC method was satisfactory for both regular and tough cured meats.

#### Linearity, Sensitivity, and Stability

The detector response was linear up to 10 ppm nitrite when a Pt working electrode, a high-speed column, and 20  $\mu$ L injection volume were used. The typical detection limit of nitrite by amperometric detection is approximately 1 ppb under optimal conditions (14, 15, 20), which is about one-tenth of that obtained by the conductivity or UV detection or by the AOAC colorimetric method. A similar detector current was observed from a standard nitrite solution using both Wescan and Waters electrochemical detector. Therefore, the signal-to-noise ratio was determined primarily by the detector noise. Nitrite at 1 ppb concentration was routinely detected especially with the Waters electrochemical detector, which is equipped with a noise filter.

Nitrite in the extract was stable for several hours. The

detector current for a nitrite standard was stable within 10% over several hours for both Pt and glassy carbon electrode. Therefore, automated analysis of nitrite in the extract should be feasible. The present method is expedient, sensitive, and reliable. It is also a reliable method for simultaneous determination of nitrite and ascorbic acid/erythorbic acid in cured meat products.

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