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DETERMINATION OF NITRITE AND NITRATE BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING ON-LINE POST-COLUMN PHOTOLYSIS WITH ULTRAVIOLET ABSORBANCE AND ELECTROCHEMICAL DETECTION

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SUMMARY

Ion-pair (paired-ion) reversed-phase HPLC has proved to be an effective technique for the analysis of inorganic anions. Also referred to as ion-interaction chromatography, it has been used with conductimetric, ultraviolet (UV) absorbance, refractive index, and electrochemical detection. Nitrite and nitrate are amenable to UV detection at wavelengths of approximately 240 nm and below. In addition, nitrite is readily oxidized at potentials of +0.9 V or higher at a glassy carbon electrode. While nitrate is electrochemically unreactive under these conditions, it will undergo photolysis, with the conversion product (in all likelihood the nitrite anion) generating an oxidative signal.

This paper describes the use of a Teflon™ knitted open tubular (KOT) reactor, which when wrapped around a UV source, provides a means of continuous, on-line photolysis. This derivatization step, combined with high-performance liquid chromatography, permits the determination of both nitrite and nitrate using oxidative amperometric detection. We have applied this technique to a number of samples (cured meats, smoked and fresh salmon, smoked cod, spiked water solutions) and have also obtained comparative data for nitrite by UV and direct (*i.e.* non-photolytic) oxidative electrochemical detection, as well as by a standard spectrophotometric procedure.

INTRODUCTION

Food additives, both direct and indirect, are permitted at levels which are set forth in United States Code of Federal Regulations (Part 172). It is the responsibility of the Food and Drug Administration to monitor the food supply for such additives. Nitrite and nitrate are typically determined in products such as cured meats and smoked fish using a spectrophotometric procedure[1]. Nitrite (or nitrate, following its reduction to nitrite) is reacted with sulfanilamide, forming a diazonium salt, which is in

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turn combined with N-(1-naphthyl)ethylenediamine to produce a colored solution. Quantitation is accomplished by comparison of the sample absorbance with a standard curve.

We hoped to develop an alternative chromatographic procedure which would be sensitive, selective, and broadly applicable. We chose to employ ion-interaction chromatography for the separation of nitrite and nitrate, since previous experience with this technique had proved very positive[2]. Numerous articles have appeared in recent years describing the use of this scheme as an alternative to conventional ion chromatography, as first described by Small *et al.*[3]. Moreover, nitrite and nitrate, specifically, have been the focus of a number of papers in which high-performance liquid chromatography (HPLC) has been employed. Typically, single column ion exchange or ion-interaction chromatography was used and detection techniques most commonly reported were UV absorbance and electrochemical detection (ED) (oxidative, amperometric)[4–15].

With a sample matrix as complex as that presented by cured meats, sample preparation or clean-up is an obstacle which can pose serious problems. A comprehensive discussion of this subject (with specific reference to the colorimetric analysis of nitrite in frankfurters) has been presented by Fiddler and Fox[16]. Those authors cautioned against the use of commonly used chemical treatments, *e.g.* the addition of protein precipitants, such as Carrez I (zinc acetate) and Carrez II (potassium ferrocyanide). Instead, they recommended the digestion procedure which has been incorporated in the Association of Official Analytical Chemists' (AOAC) Official Methods of Analysis[1]. We decided to adopt the AOAC procedure for our work. Although the resulting filtered aqueous extracts can yield rather complex chromatograms, reliable quantitation of nitrite and nitrate has usually been possible.

In summary then, the fundamental components of the overall analytical methodology consist of sample extraction (followed by an optional solid phase extraction clean-up), ion-interaction reversed-phase HPLC for the separation of nitrite and nitrate, UV absorbance detection for quantitation of both species, on-line, post-column photolysis for ED of both species, and ED without photolysis for the quantitation of nitrite. The applications described herein include: (1) single-blind spiked water samples, (2) incurred and spiked levels in smoked salmon and cod, and (3) incurred and spiked levels in cured meat products with comparison to AOAC methodology.

EXPERIMENTAL

Apparatus

A modular HPLC system was assembled, using as components, a Waters Chromatography Division/Millipore (Milford, MA, U.S.A.) Model 6000A solvent delivery system, either a Rheodyne (Cotati, CA, U.S.A.) Model 7125 syringe loading injector or a Waters/Millipore WISP 710B autosampler, and Spectra-Physics (San Jose, CA, U.S.A.) Model SP 4270 recording integrators. Detectors employed were the Kratos (Ramsey, NJ, U.S.A.) Spectroflow 757 variable-wavelength UV detector and the Bioanalytical Systems (West Lafayette, IN, U.S.A.) electrochemical detector, consisting of two Model LC-4B amperometric controllers and a Model TL-5A thin-layer transducer. Specific sub-components of the Model TL-5A included a Model

RE-1 Ag/AgCl reference electrode, a Model TG-5M 0.127-mm gasket, and dual glassy carbon electrodes. The chromatographic column used was an Alltech Assoc. (Deerfield, IL, U.S.A.) Econosil (Cat. No. 60148) C_{18} reversed-phase column, 250 mm × 4.6 mm I.D.

The on-line, post-column photolytic reactor has been described elsewhere in detail[17,18]. The reactor consists of a knitted open tubular reaction coil (total path length approximately 10 m), constructed from 0.5 mm I.D. Teflon tubing (Rainin Instruments, Woburn, MA, U.S.A.), which is in turn wound about a mercury lamp. The UV source and the knitted open tubular (KOT) reactor are placed in a covered stainless-steel vessel (kettle). The kettle's lid is fitted with a glass tube (finger) which accommodates the lamp and KOT reactor and allows the reactor to be immersed in an ice–water bath. The kettle and lamp are commercially available, as a unit, from Photronix (Medway, MA, U.S.A.) as the Model 816 H.P.L.C. Reservoir.

Mobile phase

Preliminary separations of nitrite and nitrate were carried out using a mobile phase which consisted of 0.005 *M* (5 m*M*) tetrabutylammonium hydrogensulfate (TBAHS) dissolved in a methanol–phosphate buffer (10:90). The composition of the phosphate buffer was 0.025 *M* each of potassium dihydrogenphosphate and disodium hydrogenphosphate. The final pH of the mobile phase was adjusted to 6.8 with phosphoric acid. Prior to use, the mobile phase was routinely aspirated through a Rainin Instruments 0.45 μm pore size membrane filter (Nylon 66) and degassed under vacuum or by sonication.

Two other ion-pair reagents were also investigated, *viz.*, octyltriethylammonium phosphate and dodecyltriethylammonium phosphate (Q8 and Q12, Regis Chemical Co., Morton Grove, IL, U.S.A.). Greater retention and resolution could be achieved for nitrite and nitrate using these two reagents. The only disadvantage associated with them was noticeably longer equilibration times for the chromatographic system. In fact, most sample analyses were carried out with the Q12-based mobile phase because of the complexity of many of the sample matrices, which frequently proved intractably resistant to sample clean-up. They were incorporated in the methanol–phosphate buffer mobile phase at the 2–5 m*M* levels and the pH was again controlled at 6.8 using phosphoric acid.

Chemicals

Deionized, distilled water and HPLC-grade methanol (EM Science, Cherry Hill, NJ, U.S.A.) were used to prepare the mobile phases. Ion-pairing reagents of the highest available purity were obtained from Fluka (Hauppauge, NY, U.S.A.), in the case of tetrabutylammonium hydrogensulfate, and from Regis Chemical Co. or Alltech Assoc. in the case of octyltriethylammonium (Q8) and dodecyltriethylammonium (Q12) phosphate. Standard reagents of sodium nitrate and sodium nitrite were purchased from Aldrich (Milwaukee, WI, U.S.A.) and Baker (Phillipsburg, NJ, U.S.A.), respectively.

Procedures

Authentic mixtures (aqueous solutions) of sodium nitrate and sodium nitrite were prepared and analyzed in accordance with a single blind protocol. Determina-

tions of nitrite and nitrate were performed concomitantly using sequential UV detection and post-column, photolytic ED. The UV detector was placed prior to and in series with the post-column KOT reactor, which was in turn connected to the electrochemical detector (dual, parallel-configured, glassy carbon electrodes) cell. Six studies were conducted, three with both species (*i.e.* nitrite, nitrate) present, two with only one or the other present, and one with neither present. Initial dilutions were made with deionized, distilled water, with subsequent dilutions being made with mobile phase.

Additionally, a number of samples of smoked or fresh fish (salmon, cod), as well as cured meat products (beef/pork bologna or hot dogs, turkey bologna) were analyzed. In these cases, the sample preparation followed was that described in the previously cited standard procedure[1]. Final analysis was carried out by HPLC. Recovery data were obtained by spiking separate portions of these samples (in a single blind format, when possible) and repeating the analysis. All filtrations were performed using either nylon membrane filters or filter paper known to be free of nitrite or nitrate. Membrane filters manufactured from mixed esters of cellulose acetate and nitrate are not suitable, as nitrate will be leached from them during the filtration process.

In some cases an optional solid phase extraction clean-up step was employed. The use of this technique for HPLC determinations of nitrite and nitrate has been previously reported. Osterloh and Goldfield[7] evaluated anion-exchange versions of the solid-phase extraction cartridges, but encountered difficulty achieving reproducible recoveries of each analyte. Jackson *et al.*[6] employed C_{18} cartridges with apparent success to clean-up cheese, meat and vegetable samples. However, Wootton *et al.*[13], in examining an impressive variety of foodstuffs, found the C_{18} cartridges to be of only limited utility. Our experience was largely in line with that of Wootton's group and Osterloh and Goldfield. The only cartridge we found to be of even limited utility was the cyano bonded phase variety. Unfortunately it could not be applied to a wide range of samples.

RESULTS AND DISCUSSION

Optimization of UV and electrochemical detection

Both nitrite and nitrate exhibit significant UV absorptivity at wavelengths of roughly 240 nm and below. Our choice of 220 nm as a detection wavelength simply represents a compromise between signal response for the two analytes and background noise levels. In addition, nitrite possesses intrinsic electrochemical activity (oxidative) at a glassy carbon surface. While nitrate generates no such response under these conditions, a study evaluating the electrochemical activity of a number of inorganic anions, both with and without photolysis, clearly indicated that an oxidative response could be photolytically induced in the nitrate anion[18]. There is evidence from chemical literature to support the assumed photoreductive generation of nitrite from nitrate under solution conditions comparable to those described herein[19,20]. Additional confirmation has been provided by the batch irradiation of nitrate and the subsequent chromatographic identification of the nitrite generated, as well as by the dual electrode response ratio for nitrate which has undergone on-line, post-column photolysis.

Hydrodynamic voltammograms of nitrite indicated that an oxidative response is produced at potentials of +0.8 V (*vs.* Ag/AgCl) on a glassy carbon surface. For analytical use, working potentials of +1.10 V (W1) and +1.00 V (W2) were chosen.

Residence time in the post-column reactor

The theoretical basis for, and the practical considerations involved in, the use and construction of KOT reactors have been discussed elsewhere[21–23]. In essence, a crocheting technique is used to produce a tube which is coiled in three dimensions. The resulting geometry enables flow to occur through a considerable length of tubing without the creation of drastic band broadening.

For our work we used a KOT reactor constructed from a 10 m × 0.5 mm I.D. Teflon tubing. This corresponds to a nominal coil volume of approximately 2 ml. Using a pump (Waters 6000A) as a source of metered flow, we determined the actual volume to be 2.3 ml. The residence time can be controlled by varying the flow-rate. Moreover, an optimum value can be determined by evaluating the response for nitrate with photolysis as opposed to the response for nitrite without photolysis (flow injection). With increasing flow-rates, the area response for nitrite with the lamp turned off decreased. For nitrate, with the lamp turned on, the same general trend was observed. The fact that signal response decreases with increasing flow-rate is indicative of the fact that the thin-layer cell design of the amperometric detector results in concentration-sensitive behaviour (as opposed to mass-sensitive). Presumably mass transfer of the analyte(s) through the diffusion layer (and to the surface of the electrode itself) is adversely affected by increased flow. However, when the second set of data was normalized for any decrease in signal response due simply to changes in flow, it was the case that signal response was maximized at flow-rates between 0.6 and 1.0 ml/min. The maximum response observed corresponded to a residence time of 2.8 min (0.8 ml/min flow-rate). The degree of photoconversion of nitrate to nitrite was determined (once again via flow injection). Under optimum conditions we estimated the conversion efficiency to be 85–90%.

It was our usual practice to acquire simultaneous (sequential) signals from both the UV and the amperometric detector. Residence time for the analytes in the UV detector's flow cell is very brief. Were any detector-induced photolysis to occur, this would be apparent in the electrochemical chromatogram as a peak with a retention time corresponding to nitrate (and attributable, of course, to any photoconversion of nitrate to nitrite). No such peak was ever observed. We feel that our findings are in all probability applicable to other commercially available detectors. Nonetheless it would be prudent to establish this on a case-by-case basis.

Effect of pH on photolysis of nitrate

A brief study on the influence of eluent pH upon the photolytic conversion of nitrate revealed that at a pH of 7, a stable response was produced. At a pH of 5, roughly a 40% reduction in response was encountered. At a pH of 3, no photolytically induced response for nitrate could be detected. For this reason we used buffered mobile phases with a pH adjusted to 6.8.

Our primary concern regarding mobile phase pH was with respect to its effect upon the photoconversion of nitrate to nitrite and its influence on the stability of the nitrite anion itself, which is threatened at lower pH levels. Chromatography is also influenced by pH, but this was of secondary concern. Previous studies have demonstrated ion-interaction chromatographic separations of nitrite and nitrate are readily achieved in a pH range of roughly 4 to 7[24,25].

Linearity of response, current response ratios

The linearity of response for nitrite was evaluated using UV detection, ED without photolysis (lamp off), and ED with photolysis (lamp on). In the case of nitrate, UV detection and lamp on ED were used (nitrate shows no response via ED with the lamp off). ED was carried out using parallel dual electrodes maintained at +1.10 V and +1.00 V, respectively. The results are summarized in Table I.

For each analyte, linear responses were obtained for all detection schemes. A minimum detection level of 50 ppb is conservatively proposed for each analyte using either ED or UV detection. Solutions as low as 5–10 ppb yielded responses, but the signals began to deviate from linearity. Our findings are in general agreement with detection limits recently published by Schroeder[15], who also employed reversed-phase HPLC, along with UV detection at 210 nm, for the determination of nitrate alone. It should be noted that the "lamp-on" and "lamp-off" slope values for nitrite at a particular potential (*e.g.* see Table I) are not necessarily comparable. In this case the two sets of experiments were performed on different days and electrode response can vary from day-to-day.

The reproducibility of injection for standard solutions of nitrate or nitrite by either detection technique was very good, with relative standard deviations of less than one percent being typical.

By using a parallel-configured dual-electrode transducer and maintaining the electrodes at a voltage differential of 100–150 mV, it is possible to obtain current response ratios, *i.e.* i_1/i_2. These ratios will ideally lend an additional degree of selectivity for the electrochemical detection scheme, since they should be characteristic

TABLE I

LINEARITY OF RESPONSE FOR NITRITE AND NITRATE BY UV ABSORBANCE AND ELECTROCHEMICAL DETECTION (WITH AND WITHOUT PHOTOLYSIS)

A = absorbance units, i = current in nA. Concentrations expressed as ppm. Chromatographic conditions: Alltech Assoc. Econosil C_{18} column, 250 mm × 4.6 mm I.D.; mobile phase: 5 mM TBAHS dissolved in methanol–phosphate buffer (0.025 M each of potassium dihydrogenphosphate and disodium hydrogenphosphate) (10:90); flow-rate: 0.8 ml/min; injection volume: 200 μl; nitrite: 0.005400–10.80 ppm, nitrate: 0.005125–10.25 ppm (220 nm); nitrite: 0.005400–1.080 ppm (ED, lamp off); nitrite: 0.05400–1.080 ppm, nitrate: 0.05125–1.025 ppm (ED, lamp on).

Anion	*Detection*	*Equation line*	*Correlation coefficient, r*
Nitrite	UV (220 nm)	$A = 0.04100[NO_2^-] + 0.00139$	0.9998
Nitrate	UV (220 nm)	$A = 0.0233[NO_3^-] + 0.00115$	0.9999
Nitrite	ED (lamp on) (+1.10 V)	$i = 465[NO_2^-] + 2.23$	0.9999
Nitrite	ED (lamp on) (+1.00 V)	$i = 325[NO_2^-] + 0.718$	0.9999
Nitrite	ED (lamp off) (+1.10 V)	$i = 195[NO_2^-] + 2.81$	0.9999
Nitrite	ED (lamp off) (+1.00 V)	$i = 425[NO_2^-] + 1.90$	0.9999
Nitrate	ED (lamp on) (+1.10 V)	$i = 290[NO_3^-] + 12.3$	0.9997
Nitrate	ED (lamp on) (+1.00 V)	$i = 205[NO_3^-] + 7.62$	0.9998

of a particular analyte. In Table II the stability of the current response ratio for nitrite and nitrate, under photolytic conditions and over a range of concentrations, is summarized. The ratios for both species display reasonable stability over a 20-fold concentration range. Moreover, the actual values for nitrite and nitrate are in very close agreement, serving to substantiate the hypothesis that nitrate is indeed photolyzed to nitrite.

Photolytic conversion of nitrate to nitrite

In addition to relying on the comparison of current response ratios (for the two peaks with retention times corresponding to nitrite and nitrate) as evidence of the fact that nitrate was indeed being photolytically reduced to nitrite, we also subjected a solution of sodium nitrate standard (5 ppm) to batch irradiation (3 min). This irradiated solution was then analyzed chromatographically. Whereas the unphotolyzed solution revealed only the single peak corresponding to nitrate (UV detection), the photolyzed solution showed two peaks, one with a retention time matching nitrate, and a second peak matching nitrite. By amperometric oxidative detection, a peak was observed for nitrite in the photolyzed solution, while for the unphotolyzed solution, no peaks were observed (nitrate is unreactive in the absence of photolysis). Current response ratios obtained by chromatographing a standard solution of nitrite did indeed compare favorably with those obtained from the photolyzed solution of nitrate [$i_1/i_2 = 2.43$, relative standard deviation (R.S.D.) = 0.82% ($n = 3$) for the photolyzed solution as opposed to $i_1/i_2 = 2.46$, R.S.D. = 0.62% ($n = 3$) for the nitrite standard]. The fact that the current ratios obtained in this study differ significantly from those presented in Table II is again illustrative of the fact that the current generated by a particular glassy carbon electrode is dependent upon a number of factors, such as the age and surface condition of the electrode. It is not at all unreasonable to observe different current ratios for essentially similar experiments conducted at separate intervals. The key issue is that standard and sample current ratios, obtained concomitantly, do in fact correspond closely.

Interestingly, when a solution of sodium nitrite (2 ppm) was photolyzed (again for 3 min), there was a partial conversion of nitrite to nitrate. The nitrite response was reduced by approximately 30% under both UV detection and ED. In bulk aqueous

TABLE II

CURRENT RESPONSE RATIOS (i_1/i_2) FOR NITRITE AND NITRATE (PHOTOLYZED) AS A FUNCTION OF CONCENTRATION

Chromatographic conditions as Table I. $W_1 = +1.10$ V, $W_2 = +1.00$ V *vs.* Ag/AgCl.

Anion	*Concentration (ppm)*	i_1/i_2	*RSD (%) (n = 3)*
Nitrite	1.080	1.43	0.43
	0.5400	1.44	0.15
	0.1080	1.46	1.20
	0.05400	1.48	2.70
Nitrate	1.025	1.42	0.43
	0.5120	1.43	0.63
	0.1025	1.46	0.79
	0.05120	1.48	2.69

solutions, it would thus appear that the photolytic conversion of nitrate to nitrite involves an equilibrium reaction. In fact, the reduction in the nitrite peak was very nearly stoichiometric. The unphotolyzed solution of 0.0300 μM nitrite yielded, after photolysis, a chromatogram with a nitrite peak corresponding to 0.0225 μM nitrite and a nitrate peak corresponding to 0.00774 μM nitrate (0.0225 + 0.00774 = 0.0302). This is also most likely the case when the solution is a buffered methanolic mobile phase under conditions of flow. One of the earliest studies we performed was a comparison of "lamp-on" and "lamp-off" response for nitrite under actual analytical conditions of chromatographic flow and post-column photolysis. A reduction in the nitrite response of between 15 and 20% was observed.

Method validation

The accuracy of the analytical methodology was initially assessed through the use of authentic aqueous solutions of sodium nitrate and sodium nitrite which were prepared and analyzed according to a single blind protocol. Analyses were performed using essentially simultaneous UV detection and post-column photolytic ED. The UV detector was placed in series with, and prior to, the post-column reactor, which was then connected to the amperometric detector. Six samples were analyzed, three containing both analytes, one containing neither analyte, and two containing only one or the other of the analytes. No false positive results were obtained. The amounts determined, expressed as a percentage of the actual weight of analyte added, are summarized in Table III. For sodium nitrite, they ranged from 98.4% to 99.9% (UV, 220 nm), from 97.9% to 100.6% (ED, W_1 = +1.10 V), and from 97.7% to 100.4% (ED, W_2 = +1.00 V). For sodium nitrate the corresponding figures are 98.7% to 101.0% (220 nm), 97.8% to 99.9% (+1.10 V), and 97.6% to 99.8% (+1.00 V).

TABLE III

SUMMARY OF ANALYSES: AUTHENTIC SOLUTIONS OF NITRITE AND NITRATE (SINGLE BLIND SPIKES) BY UV AND PHOTOLYTIC ED

Chromatographic conditions: see Table I. Figures in parentheses are average calculated amounts of sodium nitrite and sodium nitrate expressed as a percentage of the actual amount added. The amounts themselves represent weight (mg) of standard in original solutions. N.D. = not determined.

Sample	*$NaNO_2$ (mg)*	*$NaNO_3$ (mg)*	*Average calculated values (n = 3)*					
			$NaNO_2$			*$NaNO_3$*		
			220 nm	*+1.10 V*	*+1.00 V*	*220 nm*	*+1.10 V*	*+1.00 V*
A	0	123.4	N.D.	N.D.	N.D.	124.6 (101.0)	122.8 (99.5)	121.2 (98.2)
B	158.9	138.9	158.8 (99.9)	159.9 (100.6)	159.6 (100.4)	139.0 (100.1)	138.7 (99.9)	138.3 (99.6)
C	176.9	124.9	174.1 (98.4)	173.1 (97.9)	172.8 (97.7)	123.3 (98.7)	122.2 (97.8)	121.9 (97.6)
D	149.1	136.8	147.6 (99.0)	148.5 (99.6)	148.4 (99.5)	135.8 (99.3)	136.5 (99.8)	136.5 (99.8)
E	147.1	0	144.9 (98.5)	144.6 (98.3)	144.3 (98.1)	N.D.	N.D.	N.D.

Actual samples were also analyzed in order to more fully assess the suitability of the method. Tables IV and V represent the results of two separate sets of analyses. The first involved the analysis of bologna and frankfurters, while the second involved only bologna. In each case a single blind comparison was carried out with respect to the official colorimetric procedure[1]. In the case of the second study, involving only bologna, and in which all HPLC analyses were completed within the same day, good agreement was seen between the AOAC results and the HPLC–ED (lamp on, as well as

TABLE IV

DETERMINATION OF NITRITE BY COLORIMETRIC* PROCEDURE AND BY HPLC WITH UV DETECTION AND ED** (SINGLE BLIND STUDY)

Value in parenthesis is relative standard deviation.

	AOAC	*Nitrite (as $NaNO_2$) (ppm in product)*				
		UV (220 nm)	*ED (lamp off)*		*ED (lamp on)****	
			+1.10 V	*+1.00 V*	*+1.10 V*	*+1.00 V*
Bologna (turkey)	66.3	74.6 (1.47)	75.3 (0.86)	75.4 (0.93)	67.9 (1.15)	67.6 (2.02)
Frankfurter (beef/pork)	34.4	39.0 (0.90)	39.2 (0.78)	39.2 (0.78)	29.8 (1.27)	29.9 (1.72)
Bologna§ (turkey)	18.0		18.0 (0.58)	17.9 (0.62)	18.0 (0.76)	18.3 (1.78)

* AOAC 14th ed., sections 24.044 and 24.045, single determination.

** Chromatographic conditions: same as in Table I except for the mobile phase: methanol–water (25:75), 2 m*M* Q12, 0.025 *M* each potassium dihydrogenphosphate and disodium hydrogenphosphate.

*** Determination performed on following day (frozen storage of extract overnight).

§ Same day determination; matrix interference prevented UV determination.

TABLE V

DETERMINATION OF NITRATE BY HPLC WITH UV DETECTION AND PHOTOLYTIC ED

Chromatographic conditions: see Table IV. Values in parenthesis are relative standard deviations.

	Nitrate (as $NaNO_3$) (ppm in product)		
	UV (220 nm)	*ED (lamp on)**	
		+1.10 V	*+1.00 V*
Bologna (turkey)	144 (3.82)	133 (3.91)	132 (1.75)
Frankfurter (beef/pork)	79.8 (1.26)	66.2 (5.83)	65.2 (6.03)
Bologna (turkey)**	122 (0.83)	130 (0.67)	135 (1.00)

* Determination performed on following day (frozen storage of extract overnight).

** Same day determination for UV and ED.

lamp off). Matrix interferences unfortunately prevented accurate quantitation of nitrite by UV detection (Fig. 1). Nitrite values obtained in the first study exhibit less than ideal correlation, and this may in large part be due to the fact that the HPLC data could not all be generated on the same day. In Table V, the values for nitrate are those obtained by the two separate detection schemes for HPLC, *i.e.*, UV detection and ED (lamp on) at +1.10 V and +1.00 V (Figs. 2 and 3). In this study same-day results ranged from 122–135 ppm. While internal agreement could be better, these data are none the less encouraging as a first attempt.

Table VI summarizes similar data, this time for smoked cod and salmon. Since intrinsic levels of nitrite and nitrate in fish products were routinely found to be either

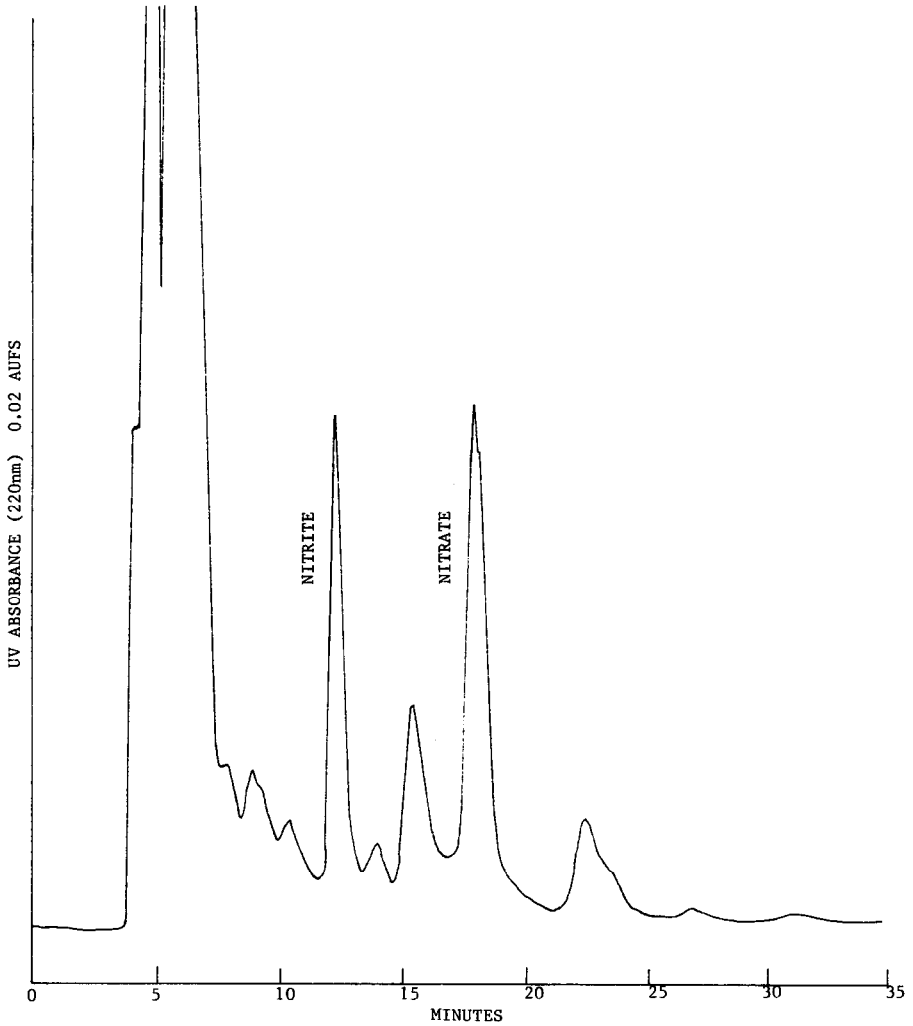

Fig. 1. Chromatogram of bologna extract (5 g/500 ml water) with UV detection at 220 nm (100 μl injection). Alltech Econosil C_{18} column, 250 mm × 4.6 mm I.D., mobile phase: 2 m*M* dodecyltriethylammonium phosphate in methanol–phosphate buffer, 0.8 ml/min flow-rate.

very low or non-existent, the essential point being conveyed here is that the spiked recoveries (one of which was conducted in triplicate via a single blind protocol) were, as a rule, quite good.

We are encouraged by the results which we have thus far obtained. Certainly in the case of nitrite, which may be detected electrochemically without photolysis, sample matrix problems are generally minimal, and the overall technique is probably applicable to real samples without further modification or development. For nitrate, which must be detected either by UV or photolytic ED, matrix interferences are more of a problem. Additional work in the area of sample clean-up, ideally through the use of solid-phase extraction, will hopefully resolve any deficiencies. But, even as the method now stands, with essentially no clean-up subsequent to aqueous extraction, it is capable of detecting nitrate with no difficulty at levels of regulatory concern (500 ppm in the product/5 ppm in the sample extract). The ability to screen products for levels of both nitrite and nitrate with a single injection is certainly very desirable.

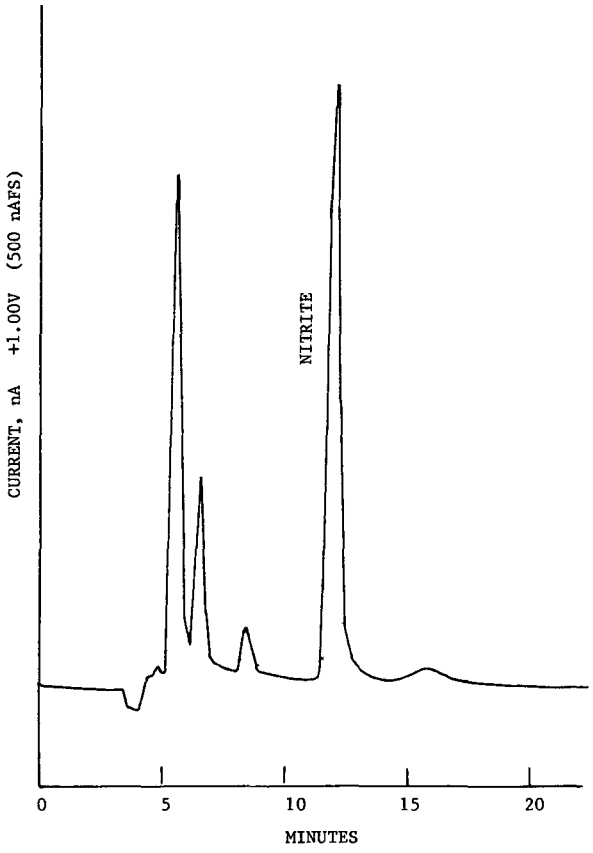

Fig. 2. Chromatogram of bologna extract (5 g/500 ml water) with ED (100 μl injection). Alltech Econosil C_{18} column, 250 mm × 4.6 mm I.D. mobile phase: 2 m*M* dodecyltriethylammonium phosphate in methanol–phosphate buffer, 0.8 ml/min flow-rate.

Finally, it should be indicated that the photoreduction of nitrate to nitrite as an analytical approach appears to have more widespread, perhaps general, applicability to many inorganic, oxidized anions. We have demonstrated the photoreductive-oxidative ED of already oxidized halogen anions, such as iodate–periodate, chlorate–perchlorate, and bromate–perbromate[26]. In as yet unpublished work, we have shown by flow injection–post-column photolytic methods that anions such chromate, permanganate, bicarbonate, hydrogenphosphate, dihydrogenphosphate, benzoate, and others, can all be photoreduced, to varying degrees, and detected at reasonable working potentials, thus far with glassy carbon electrodes[27]. Additional work is underway to demonstrate the full extent of applicability of this newer analytical approach for inorganic and organic, oxidized, anionic species.

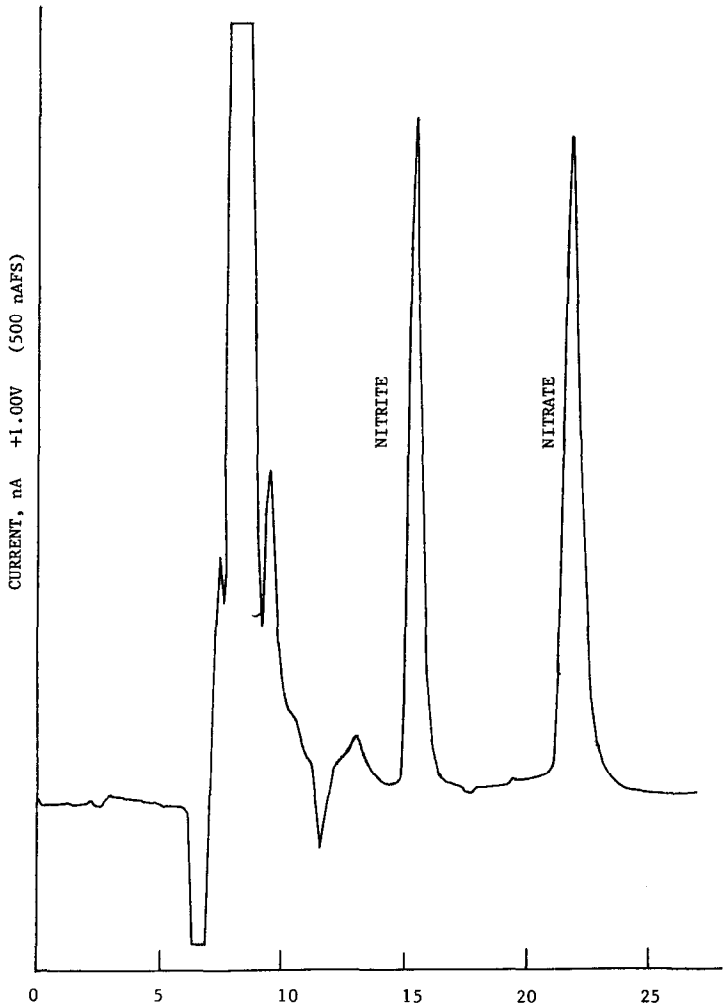

Fig. 3. Chromatogram of bologna extract (5 g/500 ml water) with photolytic ED (100 μl injection). Alltech Econosil C_{18} column, 250 mm × 4.6 mm I.D., mobile phase: 2 m*M* dodecyltriethylammonium phosphate in methanol–phosphate buffer, 0.8 ml/min flow-rate.

TABLE VI

RECOVERIES OF ADDED NITRITE AND NITRATE (FROM SMOKED SALMON, COD) FOR HPLC PROCEDURE WITH UV DETECTION AND ED

N.D. = not detected in product (could have been seen, if present, under these analytical conditions). – = cannot be detected in the absence of irradiation (under lamp off conditions).

Sample	*Detection*	*$NaNO_2$ (ppm)*	*Recovery (%)*	*$NaNO_3$ (ppm)*	*Recovery (%)*
Salmon	UV (220 nm)	N.D.	97.4	N.D.	95.9
	W_1 = +1.05 V (lamp off)	N.D.	94.2	–	
	W_2 = +0.95 V (lamp off)	N.D.	96.5	–	
	W_1 = +1.05 V (lamp on)	N.D.	112.5	N.D.	111.2
	W_2 = +0.95 V (lamp on)	N.D.	113.3	N.D.	112.4
	UV (220 nm)*	N.D.	108.7	N.D.	104.9
	UV (220 nm)**	N.D.	104.0, 101.0, 99.8***	44	103.5, 95.5, 101.1***
Cod§	UV (220 nm)	N.D.	105.6	N.D.	103.8
	W_1 = +1.10 V (lamp off)	N.D.	101.4	–	
	W_2 = +1.00 V (lamp off)	N.D.	101.1	–	

* Solid phase extraction (SPE) as clean-up, using Analytichem Cyano cartridge.
** SPE with Waters C18 Sep-Pak.
*** Triplicate single blind spikes of product. Chromatographic conditions: see Table IV.
§ SPE with Baker Cyano cartridge.

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