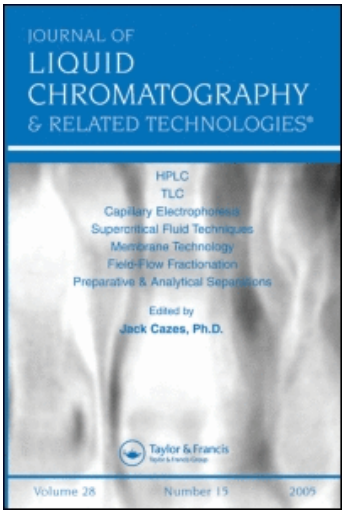

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HIGH PERFORMANCE ION CHROMATOGRAPHY DETERMINATION OF NITRITE AND NITRATE IN FOODSTUFFS

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ABSTRACT

An alternative chromatographic method has been developed to selective and simultaneous detection of nitrite and nitrate in the presence of chloride, based on the use of an anion-exchange polymethacrylate column with 5mM phosphate buffer as eluent and ultraviolet absorbance at 214 nm as detection procedure. The detection limit was 0.5 µg/ml for both anions. This method was shown to be applicable to the analysis of several foodstuffs.

INTRODUCTION

The determination of nitrite and nitrate in foodstuffs has become increasingly important because of concern over excesive human dietary intake of these chemical products.

Toxicity of nitrites, specially in relation to nitrosamine production has been well established (1,2); concerning nitrate levels, their effect on children must be studied with special regard ,because food intake containing high quantities of nitrates (IDA:0-0.5 mg/kg according to FAO/WHO) can lead to methemoglobinemia (3,4).

On the other hand, nitrites and nitrates can be found in a wide range of both natural and processed foods because of the general usage of nitrogenous fertilizers in the agricultural industry (5,6).

Most traditional methods which have been developed are based on spectrophotometric procedures which are time-consuming, sensitivity is relatively poor and can be unreliable for some samples (7,8).

HPLC techniques have emerged as alternative procedures for the determination of nitrate and nitrite. Methods involving precolumn derivatization usually have as main drawback the fact that nitrates can only be determined after their reduction to nitrites (9-11). Suppressed ion-chromatography is perhaps an obvious alternative, but complications arise with the oxidation of nitrites into nitrates in the acidic conditions used on the suppressor column (12).

Various authors make use of ion-exchange chromatography with UV detection (13-17), however the sensitivity is rather low when using high-capacity anion-exchange columns with sodium perchlorate as eluent. High-capacity polymethacrylate gel anion-exchange with dipotassium phosphate as eluent and 215 nm UV detection, render good results in meat products.

Once the above-mentioned chromatographic procedures were evaluated, we chose an anion-exchange with 214 nm UV detection as our working method with a pH 6.5 phosphate buffer as eluent, considering that this is the most adequate technique in the determination of the aforementioned anions in products having

chlorides, being the case of the most of the samples analysed in our laboratory.

MATERIALS AND METHODS

Reagents

All the reagents used were of analytic grade; organic solvents of high purity grade for HPLC; water was Milli-Q (Millipore-Waters) deionized. Standard reagents of sodium nitrite and potassium nitrate were purchased from Merck.

Equipment

Ionic chromatography system (Millipore-Waters) composed of a Universal Injector (U6K); Variable UV Detector (490E); Powerline System (600E); Data Module Integrator (745) and Anionic column IC-Pak.

Samples

Analyzed samples were purchased from food stores after a sampling carried out by Food Health Department in different Regional Communities.

Method

Sample preparation follows the method of Fudge and Truman (18). The purification was carried out by filtering 2-3 ml of extract through 0.45 μm membrane filter (Millex HV, Millipore). This solution was applied to a Sep-Pak C18 cartridge (Millipore-Waters) which was pretreated with 5 ml of methanol and 5 ml of water. Eluates aliquots of 20 μl were injected into the chromatograph.

High Performance Ion Chromatography was carried out under the following conditions: UV detection at 214 nm (1 AUFS);

eluent phosphate buffer (5mM) to pH 6.5 (Na_2HPO_4/KH_2PO_4 1/1); flow rate:1.2 ml/min.

Linearity and sensitivity of the detector were calculated from a series of standard solutions from 0.5 to 100 µg/ml.

RESULTS AND DISCUSSION

Conductivity detection methods are suitable for the determination of nitrate and nitrite (19-20), but in samples containing chloride, the nitrite couldn't be correctly quantified because resulting chloride peak masked the nitrite peak. Figure 1 shows the chromatograms of Cl^-,NO_2^-, and NO_3 anions, obtained with conductivity detection and borate/gluconate eluent.

The UV-absorbance detection method has obvious potential for the determination of nitrite and nitrate, because both have appreciable absorptivities at 214 nm whereas chloride has no significant absorbance. This is why we chose this wavelength as a suitable compromise between sensitivity and interference from other compounds.

Because nitrite is susceptible to oxidation at pH values less than 5 and will not be retained on an ion-exchange column near or below its pKa, we chose phosphate 5mM as eluent as it has sufficient buffer capacity at pH 6.5 and is an effective anion in displacing nitrate and nitrite.

The chromatogram of a standard solution containing 10 µg/ml of sodium nitrite and potassium nitrate, recorded at 214 nm using 5mM phosphate as eluent is shown in Figure 2.

A linear regression analysis of the relationship between peak area versus amounts of standards was carried out within the range 0.5-100 µg/ml. The results obtained were : y=

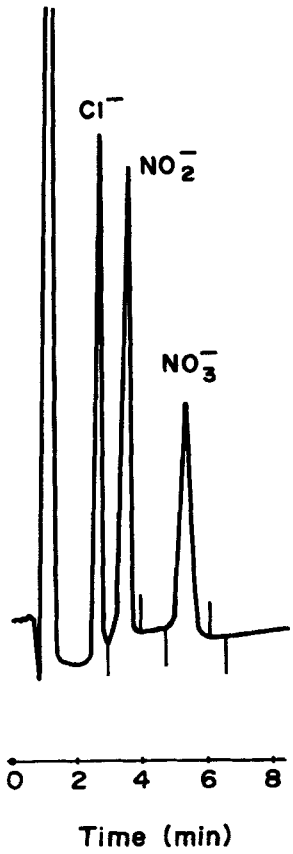

FIGURE 1.- Chromatogram of a mixture of chloride, nitrite and nitrate standards (2 μg/ml, 4 μg/ml and 4 μg/ml respectively). Conditions: Waters IC-Pak anion column with Sodium Borate/Gluconate pH=8.5 eluent; conductivity detection; flow-rate: 1.2 ml/min; injection volume: 100 μl.

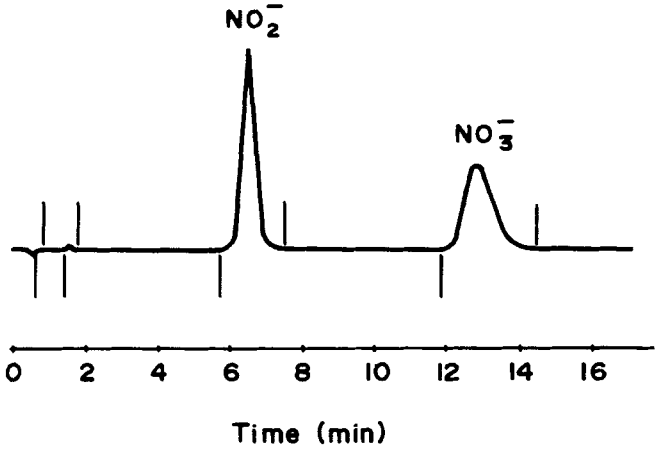

FIGURE 2.- Separation of nitrite (10 μg/ml) and nitrate (10 μg/ml) in standards, using the proposed chromatographic procedure. Conditions: Waters IC-Pak anion column; 5mM phosphate pH 6.5 eluent; UV detection at 214 nm (1 AUF); flow-rate: 1.2 ml/min; injection volume: 20 μl.

TABLE 1

Percentage Recovery of Nitrite and Nitrate from Chopped-Pork after Extraction and HPIC Analysis.

NITRITE			NITRATE		
Amount added (μg/g)	Amount recovered (μg/g)	Recovery (%)	Amount added (μg/g)	Amount recovered (μg/g)	Recovery (%)
2	1.92	96.05	2	2.37	118.70
10	8.34	83.45	10	9.70	97.00
50	47.54	95.05	50	47.14	94.30
100	85.98	85.95	100	84.12	84.10
Mean=90.1%;n=16;S.D=21.8%			Mean=98.5%;n=16;S.D=21.17%		

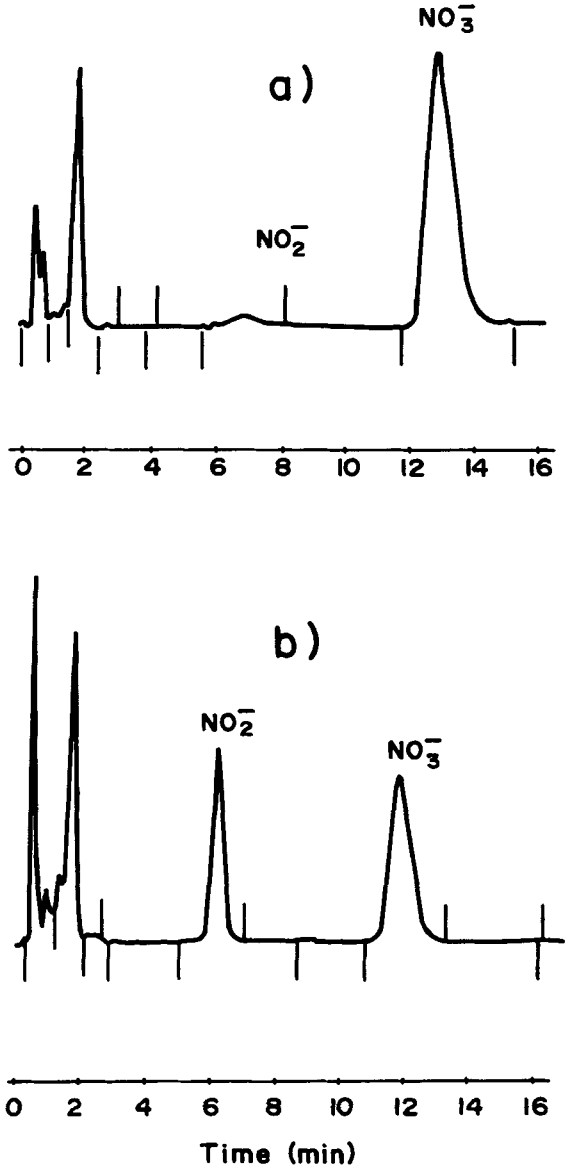

FIGURE 3.- Chromatogram obtained with: a) canned vegetable (asparragus); b) chopped-pork, using the proposed chromatographic method.

TABLE 2

Nitrite Content in Commercial Samples (μg/g of sodium nitrite).

Sample (type)	n	Mean (μg/g)	S.D. (%)	Range (μg/g)	N.D. (%)
Infant Foods	90	21	9	N.D-30	95.50
Vegetable Canned Foods	276	20.4	6.7	N.D-35	92.02
Marmalades	238	14.1	8.6	N.D-40	95.37
Syruped Canned Fruit	81	--	--	--	100
Cured Meat	28	36.3	23.3	N.D-80	78.60

N.D= not-detected

2.21x-0.25 for nitrite and y= 2.03x+0.12 for nitrate, with correlation coefficients of 0.999 and 1.000 respectively.

The detection limit was 0.5 μg/ml for both anions under the chromatographic conditions described, although it could be lower when operating at higher sensitivity.

The mean recoveries of standards in the range 2-100 μg/ml were 102% ($\tilde{O}$ =3.56; n=8) for nitrite and 97.4% ($\tilde{O}$ =3.8; n=8) for nitrate.

Recovery studies were performed on chopped-pork sample by adding known quantities of nitrate and nitrite to the sample solution prior to the initial homogenization step. The results given in Table 1, indicate that satisfactory recoveries were achieved for the sample tested.

Some typical chromatograms obtained with samples of canned vegetables (asparragus) and chopped-pork are presented in Figure 3.

TABLE 3

Nitrate Content in Commercial Samples (μg/g of potassium nitrate).

Sample (type)	n	Mean (μg/g)	S.D. (%)	Range (μg/g)	N.D. (%)
Infant Foods	90	62.7	127.9	N.D-325	26.6
Vegetable Canned Foods	276	88.2	195.1	N.D-1400	50.9
Marmalades	236	70.6	77.6	N.D-715	41.9
Syruped Canned Fruit	81	44.3	32.8	N.D-140	65.4
Cured Meat	28	56.8	66.5	N.D-315	25.0

N.D.= not-detected

A total of 710 different commercial food samples were analyzed: 90 infant food products (whose principal component were vegetables); 275 vegetable canned foods (asparragus, artichokes, chards, peas and tomatoes); 236 jams and marmalades; 81 syruped canned fruit and 28 cured meats (chopped-pork and sausages).

Nitrite contents are shown on Table 2, where we can appreciate that the maximum amount reached was 80 μg/g and that for a high percentage of samples nitrite contents were not detected under the proposed chromatographic conditions.

Nitrate results are quite different (Table 3). In infant foods, 26.6% of the samples did not present any detectable level, being 62.7±127.9 μg/g the mean value of positive samples.

In vegetable canned foods, the percentage of non-detectable samples was as high as 50.9% (specially with respect

to canned tomato). The average value was 88.2±195.1 μg/g, although in some cases we observed levels up to 1400 μg/g of nitrate (chards).

In jams and marmalades results were very similar: 41.9% of analyzed samples were non-detectable, with a range between 10 and 715 μg/g in positive samples.

The highest percentage in non-detectable samples was found in syruped canned fruit (65.4%), with a mean value of 44.3±32.8 μg/g.

In cured meats, only 25% of the samples presented non-detectable levels of nitrates; the range was betwen 10 and 315 μg/g.

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