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DETERMINATION OF NITRATE AND BROMIDE IN FOODSTUFFS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The determination of nitrate and bromide directly from aqueous extracts by high-performance liquid chromatography on an amino phase chemically bonded to silica gel is described. In comparison to other methods, this procedure needs less clean-up, no derivatization and is faster. The detection limit varies between 0.05 ppm and 10 ppm depending on the food matrix.

INTRODUCTION

It is well known that nitrite in food and water can cause serious health problems to humans such as methemoglobinemia of infants, and potential formation of carcinogenic nitrosamines under certain conditions. Nitrate is not as toxic as nitrite although it is prevalent in the environment. Ingested nitrate can be converted into nitrite by bacteria and its content in food is thus as important as that of nitrite. An extensive review of this topic was published recently[1,2].

Since some tap-water contains as much as 100 ppm nitrate and since certain foods, especially some vegetables, contain relatively high concentrations of nitrate, there is great interest in the analysis of nitrate and, to a lesser extent, nitrite. The main approaches employed so far are: spectroscopy of the nitro compound (nitrate)[3,4] or azo dye (nitrite)[5–9]; potentiometry using a nitrate-selective electrode[10–12]; thin-layer chromatography (TLC)[10] or gas chromatography (GC)[10,13] of the nitro compounds. Recently, high-performance liquid chromatographic (HPLC) methods were described which employed columns of the ion exchanger Permaphase AAX[14] or ECTEOLA-cellulose[15] together with an UV detector. Davenport and Johnson[16] used Amberlite as stationary phase and a cadmium disk electrode as electrochemical detector.

During the development of the separation of nitrate and nitrite on an amino phase chemically bonded on silica, several potentially interfering ions were tested. Kamiura and Tanaka[14] mentioned the liquid chromatographic behaviour of bromide, nitrate and nitrite on an anion exchanger. Since bromide does not interfere and can even be well separated in the same isocratic HPLC system, the present method can also be used to determine this ion. Methyl bromide is used to fumigate various

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dried foods such as cereals, nuts and mushrooms, or to sterilize the soil in greenhouses. In this way, nitrogen or sulphur containing proteins are methylated and inorganic bromide may be found as a residue. Several methods of determining bromide have been published, *e.g.* titrimetry[17], colorimetry[18] and GC[19,20].

The present HPLC method has the advantage of simplicity since no or little sample treatment is necessary: a simple aqueous extract of solid or fluid samples such as tap-water, fruit or vegetable juices can be analysed directly. No derivatization step is necessary, and the selectivity for nitrate seems to be much better than with the published methods, other than chromatography. Furthermore, the absorbents used are commercially available as either prepacked columns or as bulk material.

EXPERIMENTAL

Materials and chemicals

When monitoring nitrate and nitrite, doubly distilled water was always used to avoid contamination with these ions. The following stock solutions were prepared: Carrez-I solution, 15 g $K_4Fe(CN)_6$ (No. 4984; Merck, Darmstadt, G.F.R.) dissolved in 100 ml water; Carrez-II solution, 30 g $ZnSO_4 \cdot 7\ H_2O$ (Merck 8883) dissolved in 100 ml water; 6.2 g boric acid (Merck 165) dissolved in water to give 100 ml solution; *ca.* 8.5% phosphoric acid, one part conc. H_3PO_4 (Merck 573) diluted with nine parts of water; ionic strength adjuster (ISA), 26.4 g $(NH_4)_2SO_4$ (Merck 1217) dissolved in water to a volume of 100 ml.

Standard solutions

For HPLC 81.5 mg KNO_3 (Merck 5063), 75.0 mg $NaNO_2$ (Merck 6549) and 74.5 mg KBr (Merck 4905) were dissolved in water and made up to 1 l to give a solution 50 mg/l in each ion. For the potentiometric method, a stock solution of 16.3 g/l KNO_3 (Merck 5063) corresponding to 10 g/l NO_3^- was prepared and preserved with 10 ml boric acid solution. Working standards of 10, 100 and 500 mg/l were prepared by dilution of this stock solution.

Instrumental

A Type PT 45-50 Polytron mixer (Kinematica, Kriens, Switzerland) was used.

HPLC. A steel column (30 cm × 3.9 mm I.D.) was packed with 3.5 g LiChrosorb-NH_2, 10 μm (Merck 9331), or μBondapak NH_2 (Waters Assoc.) using 35 ml of methanol–glycerol (2:1) at 10,000 p.s.i. and a flow-rate of 20 ml/min. The column was connected to a WISP autosampler (Waters Assoc., Milford, MA, U.S.A.), a Model 100 pump (Altex, Berkeley, CA, U.S.A.) and a Uvikon LCD 725 UV detector (Kontron, Zürich, Switzerland) used at 210 nm. Integration was performed with the SP 4000 multichannel data system (Spectra-Physics, Santa Clara, CA, U.S.A.).

With the exception of tap-water, all samples were filtered with disposable 0.45-μm filters (Millex SLHA 025 OS; Millipore, Bedford, MA, U.S.A.). The mobile phase comprised 10 g KH_2PO_4 (Merck 4873) dissolved in 1 l water, adjusted to pH 3.0 (pH-meter) with *ca.* 5.5 ml phosphoric acid. It was filtered with a 0.45-μm filter (Type BA 85/20; Schleicher & Schüll, Feldbach, Switzerland) prior to use.

Potentiometry. An Orion Model 92-07 nitrate ion electrode and a double-

junction reference electrode (Orion Model 90-02) were used in conjunction with an Orion Model 907 specific ion meter.

Sample treatment

General procedure. Tap-water, juices or other aqueous foods can be injected directly after removing particulate matter with a Millex filter. Inhomogeneous aqueous samples were diluted 3–10 times with water (depending on the nitrate/nitrite content), homogenized with a Polytron mixer, heated for 30 min at 60–80°C and filtered. Proteins can be precipitated with 2–10 ml Carrez solutions.

The amount of nitrate in freshly prepared salad homogenate and whey decreased considerably during storage owing to its conversion into nitrite. For this reason it is advisable to heat the sample as described above or to add boric acid and store the sample at −18°C, unless it is analysed immediately.

Nitrate in cheese. A 30-g amount of cheese was ground and homogenized with 60 ml water for 7 min with a Polytron mixer. The sample was quantitatively transferred to a 250-ml measuring flask and made up to volume with water. A 100-ml part of this homogeneous mixture was filtered (Schleicher & Schüll No. 595½) and 2 ml of each Carrez solution were added, precipitating the proteins. After 30 min the supernatant was centrifuged for 10 min at *ca.* 1200 *g* and filtered with a Millex filter.

Nitrate in whey. A 50-ml volume of whey was treated with 2 ml of each Carrez solution as described above.

Nitrate in salads and vegetables. A representative sample was homogenized without solvents in a mixer to give 60 g of pulp. This was then diluted and extracted with 200 ml water using a Polytron mixer for 7 min. The resulting mixture was allowed to stand for 30 min at 60–80°C before adding 2–10 ml of each Carrez solution and water. The 300 g of slurry obtained were filtered and used for analysis either by HPLC, potentiometry or spectroscopy.

Bromide in flour and rice. A 10-g sample was suspended in 50 ml water and extracted for 15 min in an ultrasonic bath (flour) or with a magnetic stirrer (rice). After centrifuging (10 min, *ca.* 1200 *g*), 4.5 ml of the clear extract were treated with 0.25 ml of each Carrez solution for 5 min to precipitate proteins. This mixture was centrifuged as above and filtered with a Millex filter.

Comparison of procedures

Nitrate can also be determined as nitroxylenol by spectroscopy as outlined in ref. 3 or by TLC as proposed by Müller and Siepe[10]. In contrast to the method described in ref. 3 where steam distillation was used to separate the reaction product nitroxylenol, we extracted this compound with 5 ml toluene. The organic layer was re-extracted with 2 ml 0.1 *M* NaOH and measured at 430 nm in a spectrophotometer against 0.1 *M* NaOH. For the potentiometric determination according to ref. 11 the standard addition method was used. The slope of the electrode response was checked before and after each series of measurements (range: 10–100 mg/l). A 1-ml volume of ISA was added to each 50 ml of standard and sample solutions. Bromide analysis was verified by GC according to ref. 20.

RESULTS AND DISCUSSION

Fig. 1 shows a chromatogram of a standard containing all three ions. With this system, contents of 0.05 ppm nitrate or nitrite in drinking water can be detected if 200-μl samples are injected (about 10 ng of each compound gives a UV reading of three times the noise).

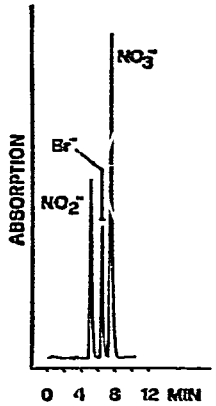

Fig. 1. Chromatogram of a mixed standard (10 μl, 0.33 μg of each compound). Flow-rate, 1 ml/min; pressure, 800 p.s.i.

The content of nitrate in cheese can easily be determined as demonstrated in Fig. 2. Nitrite cannot be detected since this ion is eluted in the back of the solvent front. The lower limit of detection is 2–5 ppm depending on the cheese. Since May 1980, the use of nitrate in cheese has been prohibited in Switzerland. It is known that the content of nitrate added during manufacture decreases quite rapidly during the ripening stage. In order to monitor nitrate levels it is much easier to check the whey on the first day of production. Fig. 3 shows chromatograms of unspiked and spiked

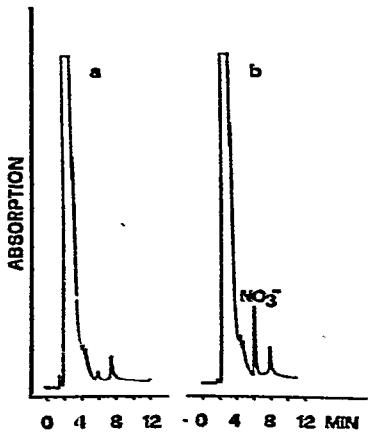

Fig. 2. Chromatograms of 10 μl of cheese extract: a, unspiked (*ca.* 2 ppm nitrate); b, spiked with 67 ppm nitrate.

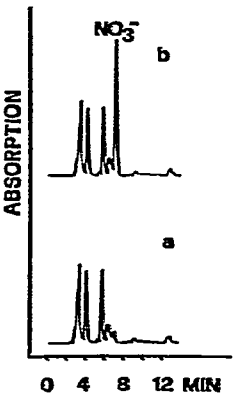

Fig. 3. Chromatograms of whey extract spiked with 50 ppm nitrate (b) and unspiked (a).

extracts of whey. Considering an addition of at least 10 g nitrate per 100 l milk for cheese production, a detection limit of 5 ppm is sufficient.

The nitrate content in various vegetables can be very high as shown in Figs. 4 and 5: salad vegetables such as cabbage-lettuce can contain as much as about 2000 ppm nitrate. When planted in greenhouses, such vegetables can also contain considerable amounts of bromide[20] if the soil is treated with methyl bromide. The chromatogram of a spiked salad extract can be seen in Fig. 5b; the unfortified sample shows no bromide peak (Fig. 5a).

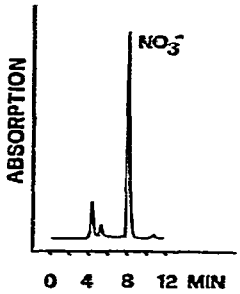

Fig. 4. Chromatogram of mangold extract containing 153 ppm nitrate.

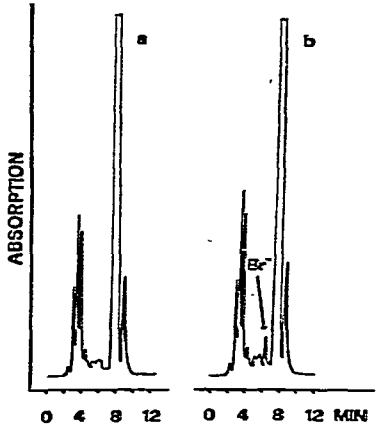

Fig. 5. Chromatograms of lettuce extract: a, unspiked; b, spiked with 40 ppm bromide. The large peak after bromide is due to nitrate.

Further research was done to compare the HPLC method with other methods of nitrate determination. Lettuce salad was chosen as representative of a high natural content of nitrate, together with tap-water and whey (Table I). As can be seen, TLC as well as spectroscopy can be used to verify the presence of nitrate found by the simple HPLC procedure.

The nitrate contents determined by potentiometry were 15–20% higher than those obtained by the other methods. Replacing the ISA, $(NH_4)_2SO_4$, by $Al_2(SO_4)_3$ as proposed in ref. 12, significantly decreased the differences between the potentiometric and HPLC methods (−2 to +15%) as shown in Table II. An advantage of the potentiometric approach is that only simple instrumentation is needed. However, considering the consistently higher values obtained by the potentiometric method and comparing the reproducibilities, HPLC or spectroscopy is preferred. The clean-up step for the spectrophotometric determination of nitrate as nitroxylenol can be done either by steam distillation as described in ref. 3 or by extraction of the nitro-compound with toluene (see *Comparison of procedures*). The latter procedure seems to be easier.

A number of tap-water samples were compared using this HPLC method and the official procedure in ref. 4. Table III shows the good agreement between the two different determinations.

TABLE I

COMPARISON OF FOUR METHODS OF DETERMINING NITRATE

Values in ppm.

Sample	*HPLC*	*Nitroxylenol*			*Potentiometry***
		TLC[10]	*Spectroscopy (distillation)*[3]	*Spectroscopy (extraction)**	
Salad 1	2430	2040	2145	2410	2800
2	2040	2010	2060	2050	2390
3	2840	3080	2770	2840	3300
4	2540	2330	2400	2200	2930
5	1880	2060	1920	1960	2250
6	1900	1740	2100	1980	2230
Tap-water	7	7	—	—	—
Whey (spiked with 10 ppm NO_3^-)	10	8	—	—	—

* Modified version of method in ref. 3 as described in the text.

** With $(NH_4)_2SO_4$ as ISA.

TABLE II

NITRATE CONTENT OF SALAD SAMPLES, DETERMINED BY HPLC AND POTENTIOMETRY

Values in ppm.

Sample	*HPLC*	*Potentiometry**
Salad 1	2600	2800
2	2350	2370
3	2780	3280
4	2640	2920
5	2280	2340
6	2210	2170

* With $Al_2(SO_4)_3$ as ISA.

TABLE III

COMPARISON OF SPECTROSCOPIC AND HPLC DETERMINATIONS OF NITRATE IN TAP-WATER

Values in ppm. Two determinations (I, II) were made in each case.

Sample No.	*HPLC method*			*Spectroscopic method*		
	I	*II*	$\bar{X}$	*I*	*II*	$\bar{X}$
1	38.5	38.9	38.7	39.2	38.6	38.9
2	24.2	24.0	24.1	23.3	23.3	23.3
3	31.5	31.5	31.5	30.6	31.3	31.0
4	30.6	30.4	30.5	30.6	30.6	30.6
5	29.4	29.5	29.5	29.6	29.7	29.7
6	43.5	43.6	43.6	43.1	43.2	43.2
7	38.3	38.3	38.3	37.7	38.1	37.9
8	42.7	42.9	42.8	41.7	41.1	41.4
9	39.3	39.3	39.3	38.6	38.6	38.6
10	32.5	32.6	32.6	32.1	31.8	32.0

Nitrite could not be detected with certainty in the analysed vegetables. However, it is possible that the nitrite peak, depending on the food matrix is either poorly separated from interfering peaks or appears as a shoulder to the steep solvent peak. For this reason no general approach can be given at present; but it may well be that nitrite can be determined in other foodstuffs such as meat products by using an appropriate clean-up step. The recoveries for the nitrate determination in whey cheese and tap-water are between 90% and 100% if spiked with 50 ppm.

As mentioned before, methyl bromide is used to fumigate dried agricultural products such as cereals. A broad spectrum of flours has been analysed for residual bromide. The chromatogram of a simple aqueous extract of a fumigated wheat flour is shown in Fig. 6. The sensitivity is high enough to monitor the Swiss limit of 50 ppm bromide in cereals. Untreated flour samples show no bromide peak. The recovery in dry raisins, wheat flour, rice and salad spiked at a level of 50 ppm varies between 80% and 90%. Preliminary bromide analyses of dehydrated mushrooms were unsuccessful; aqueous extracts were not clean enough.

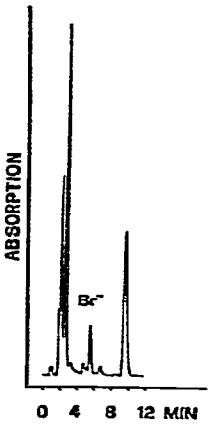

Fig. 6. Chromatogram of aqueous extract of wheat flour treated with methyl bromide. Bromide concentration found, 51 ppm.

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REFERENCES

1 R. Biedermann, D. Leu and W. Vogelsanger, *Deut. Lebensm.-Rundsch.*, 76 (1980) 149.
2 R. Biedermann, D. Leu and W. Vogelsanger, *Deut. Lebensm.-Rundsch.*, 76 (1980) 198.
3 A. Grob, *Schweiz. Lebensmittelbuch*, EDMZ, Berne, 1972, Ch. 25 A, p. 9.
4 E. Wieser, *Schweiz. Lebensmittelbuch*, EDMZ, Berne, 1972, Ch. 27 A, p. 31.
5 W. Davison and C. Woof, *Analyst (London)*, 103 (1978) 403.
6 J. E. Hamilton, *J. Ass. Offic. Anal. Chem.*, 59 (1976) 284.
7 R. Fawcett, D. A. Tame and T. E. Johnson, *J. Assoc. Publ. Analysts*, 14 (1976) 23.

8 E. Wieser, *Schweiz. Lebensmittelbuch*, EDMZ, Berne, 1972, Ch. 27 A, p. 30.
9 W. Ritter, *Schweiz. Lebensmittelbuch*, EDMZ, Berne, 1970, Ch. 5, p. 22.
10 H. Müller and V. Siepe, *Deut. Lebensm.-Rundsch.*, 75 (1979) 175.
11 *Instruction Manual Orion Model 901*, Orion Res. Inc., Cambridge, MA 02139.
12 *Analytischer Methodenführer 1979*, Orion Res. Inc., Cambridge, MA, 1977.
13 D. J. Glover and J. C. Hoffsommer, *J. Chromatogr.*, 94 (1974) 334.
14 T. Kamiura and M. Tanaka, *Anal. Chim. Acta*, 110 (1979) 117.
15 R. G. Gerritse, *J. Chromatogr.*, 171 (1979) 527.
16 R. J. Davenport and D. C. Johnson, *Anal. Chem.*, 46 (1974) 1971.
17 M. Rohrlich, in J. Schormüller (Editor), *Handbuch der Lebensmittelchemie*, Vol. V/1, Springer, Berlin, 1967, p. 63.
18 M. Feuersenger and G. Müller, *Deut. Lebensm.-Rundsch.*, 59 (1963) 69.
19 T. Stijve, *Deut. Lebensm.-Rundsch.*, 73 (1973) 321.
20 J. C. Cabanis and M. T. Cabanis, *Ann. Falsif. Expert. Chim.*, 72 (1979) 519.