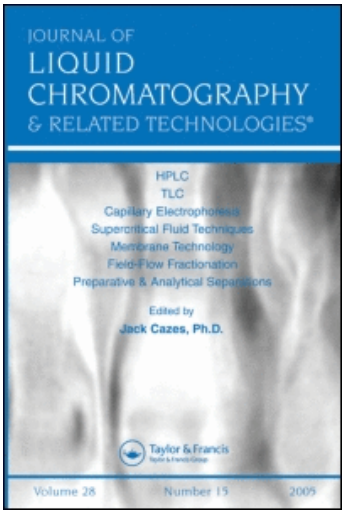

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DETERMINATION OF NITRATE AND NITRITE IN TAP WATER AND VEGETABLES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A method is presented for the simultaneous determination of nitrate and nitrite by high performance liquid chromatography (HPLC) with an anion exchange (Partisil SAX) column, a 0.030 M potassium dihydrogen orthophosphate/phosphoric acid buffer of pH 3.5 mobile phase and UV detector. Without using a preconcentration system the detection limits are 0.2 ng for nitrate and 1 ng for nitrite. A suitable extraction procedure has been established for its application to the analysis of tap water, lettuce and apple tree leaves. The reproducibility of the method, calculated as the relative standard deviations in the optimum range, is always less than 2% for nitrates and 5% for nitrites.

INTRODUCTION

The abundant use of chemical fertilizers, animal manure, compost, etc. in agriculture and horticulture has to be considered as the most important reason for the presence of

high concentrations of nitrates in vegetables (1). On one hand, these high nitrogen contents in leaves of apple trees (2) can rise the susceptibility to various parasites such as scab (*Venturia inaequelis* (Cke.) Wint.), canker (*Nectria galligena* Bres.) and european spider red mite (*Panonychus ulmi* Koch.) (3).

On the other hand, it is generally accepted that, except for the possible production of changes in the thyroid, nitrate itself is not toxic. However, nitrate can be reduced to nitrite, most importantly by bacterial action during the storage of nitrate containing food (4) or in the human body (5) during digestion.

Nitrite can then react under certain conditions with secondary and tertiary amines and amides to form the N-nitroso compounds (6), most of which so far tested in laboratory animals have been found to be carcinogenic. Also, nitrite formed by the reduction of nitrate can react with haemoglobin to form methaemoglobin which impairs the capacity of the bloodstream to carry oxygen (7). Problems of this nature have occurred very occasionally in babies.

There are many approaches to nitrate and nitrite determination: spectrophotometric techniques and potentiometry using a nitrate selective electrode are widely used (8-12). These methods, however, do not allow the simultaneous determination of both anions and have limitations in terms of detection limits and interference by a variety of ions. Gas chromatography after derivatization can also be used in nitrate determination (13).

Several authors have reported successful nitrate analysis by ion chromatography (14-16) using an anion-exchange column and conductivity detector. The precision, except for some exceptions (17), is limited.

Anion-exchange high performance liquid chromatography together with an UV detector is also able to determine nitrate and nitrite in one step, without derivatization (18-20).

The objective of the present work was to develop an alternative method for the simultaneous determination of nitrate and nitrite so as to establish a suitable extraction procedure for its application to the analysis of tap-water, lettuce and apple tree leaves.

EXPERIMENTAL

Chemicals

Potassium nitrate and sodium nitrite were purchased from Sigma (St Louis, MO, U.S.A.). Potassium dihydrogen phosphate and all other chemicals used to prepare buffer or samples were analytical reagent grade and supplied by Merck (Darmstad, Germany). The methanol used was HPLC grade and was employed as supplied by Romil (Loughborough, Leics, U.K.). High purity water was produced with a Millipore Milli-Q system (Mildford, MA, U.S.A.).

Equipment

A Hewlett-Packard HPLC system was used, comprising of a HP 1090 pump, a Hewlett Packard 79881A filter photometric detector, a HP 85B personal computer, a HP 3390A integrator and a Rheodyne 7010 injection valve with a 20 µL sample loop.

The stainless steel column used was a Partisil SAX (250x4.6 mm i.d., 10 µm).

An ultrasonic bath, a vibromatic stirrer and a centrifuge (all three from Selecta, Spain) were used.

For pH measurements, a PW 9422 Philips pHmeter equipped with a combined glass-Ag/AgCl electrode was employed.

Chromatographic conditions

The mobile phase was 0.030M potassium dihydrogen orthophosphate/phosphoric acid buffer of pH 3.5 and was

pumped at a flow-rate of 1.5 mL/min. Before being used the solution was vacuum-filtered through a 0.22 µm Nylon filter and degassed with helium.

Detection was performed at 210 nm. The chromatographic experiments were carried out at room temperature (20±2°C).

Standard solutions

Individual stock solutions of nitrate and nitrite were prepared in ultrapurified water to provide a concentration of 100 ppm. For the nitrite solution it was necessary to add 5 mL of NH_4Cl/NH_3 0.2M buffer of pH 9 to avoid its oxidation. These solutions were stored below 4°C.

Standard solutions were prepared by appropiate dilution of the stock solution, always maintaining the basicity required, and filtered through a 0.45 µm membrane (Millex-HV_{13}, Millipore) before being injected into the system.

Sample treatment

Tap-water can be injected directly after removing particulate matter with a Millex filter. For lettuce extracts, the alkaline extraction method described by Sen and Donaldson (21), with some modifications in the reactive concentration, was used: 50 g of several inside and outside leaves of lettuce were washed and cut in pieces less than 1 cm. This sample was homogenized without solvents in a Polytron mixer for 5 minutes. 1 g of a representative sample was then diluted and extracted with 30 mL water and 0.5 mL 10% NaOH. The resulting mixture was allowed to stand for 15 minutes at 50-60°C before adding 4.5 mL NaOH and 6 mL 30% $ZnSO_4$ (Carrez II), the slurry being kept another 15 minutes at 50-60°C. The mixture was cooled to room temperature, centrifuged, washing twice with alkaline water and diluted to 100 mL water. Extracts were filtered before analysis through a 0.22 µm filter. This step is very important for preventing the column from clogging.

For the apple tree leaves, the method described by Schuster and Lee (22), with some modification, was used: 0.5 g of liofilized leaves was diluted and extracted with 10 mL water, 20 mL 2% borax, 5 mL 15% $K_4Fe(CN)_6$ (Carrez I) and 5 mL 30% $ZnSO_4$ (Carrez II). The resulting mixture was heated and centrifuged as above and diluted to 50 mL water. An aliquot was filtered through a 0.22 µm filter and cleaned-up through a solid-phase extraction C_{18} cartridge used to retain several interference compounds. It was then injected into the chromatographic system.

RESULTS

Standards

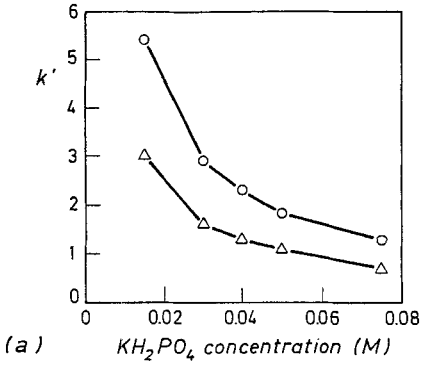
The relative retention times of the investigated anions are dependent on pH and eluent concentration.

The results obtained (Figure 1.a) show that the capacity factor, k', decreases when the ionic strength increases due to the competitiveness between the ions used to establish the ionic strength and the nitrate and nitrite anions. Figure 1.b shows the variation of the retention with pH. As can be seen, a rise in pH causes an increase in the nitrite capacity factor, because the anion nitrite concentration increases (pK_a = 3.4) with pH.

The optimum mobile phase flow-rate, tested between 0.8 and 1.8 mL/min, was found to be 1.5 mL/min.

An optimal resolution in all cases allows us to choose a concentration of 0.030M KH_2PO_4 and pH 3.5 in order to avoid any overlapping of the nitrite peak with the elution front that presumably could appear in some vegetable samples, without prolonging the analysis time, since both anions are eluted in less that 8 minutes.

Figure 2 shows a chromatogram of a standard containing both ions. With this system, the detection limits for both



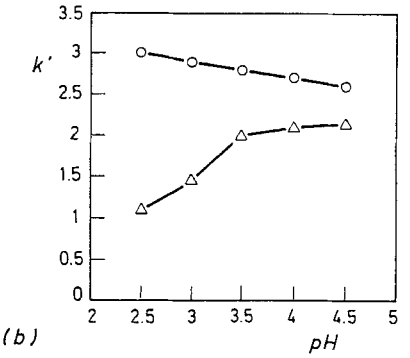


Figure 1:

Capacity factor variation of nitrate and nitrite with: a) ionic strength and b) pH. Column: Partisil SAX (250x4.6 mm i.d., 10 µm). Mobile phase: 0.03M KH_2PO_4/H_3PO_4, pH 3.5. Flow-rate: 1.5 $mL.min^{-1}$. $\lambda_{det.}$ = 210 nm.

ions - based on a signal-to-noise of 3:1 - were 0.2 ng for nitrate and 1 ng for nitrite.

Samples

Examples of the application of the described procedure for the determination of nitrate and nitrite in samples of

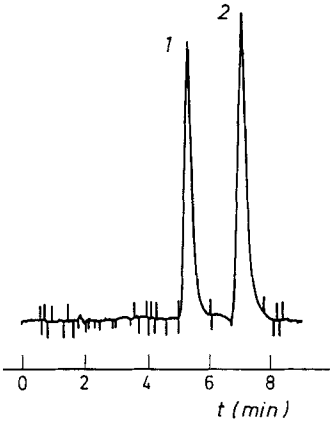


Figure 2:

Chromatogram of a standard solution containing 2 ppm of each anion. Column: Partisil SAX (250x4.6 mm i.d., 10 µm). Mobile phase: 0.03M KH_2PO_4/H_3PO_4, pH 3.5. Flow-rate: 1.5 mL.min^{-1}. $\lambda_{det.}$ = 210 nm. Peaks: 1) NO_2^-, 2) NO_3^-.

tap-water, lettuce and apple tree leaves are shown in Figure 3. The samples peaks were identified by comparing the relative retention times of each one with those of the standard reference anions. Nitrite could not be detected in the analysed lettuce extracts nor the tap-water.

The quantification of nitrate and nitrite was achieved by using the external standard method. Standards were injected and the integrator response factors were computed. These were then stored in the integrator for the computation of unknown concentrations. Injection volumes of 20 µL were employed for all quantitative evaluations and the amount of anions was directly obtained from the data module. The calibration curves constructed from the peak area versus anion concentrations were linear (r = 0.9999) from the determination limit to at least 80 ppm for NO_3^- and 40 ppm for NO_2^-. Recalibration was performed regularly.

To study the accuracy of the method, recovery experiments were performed. Known amount of each anion were

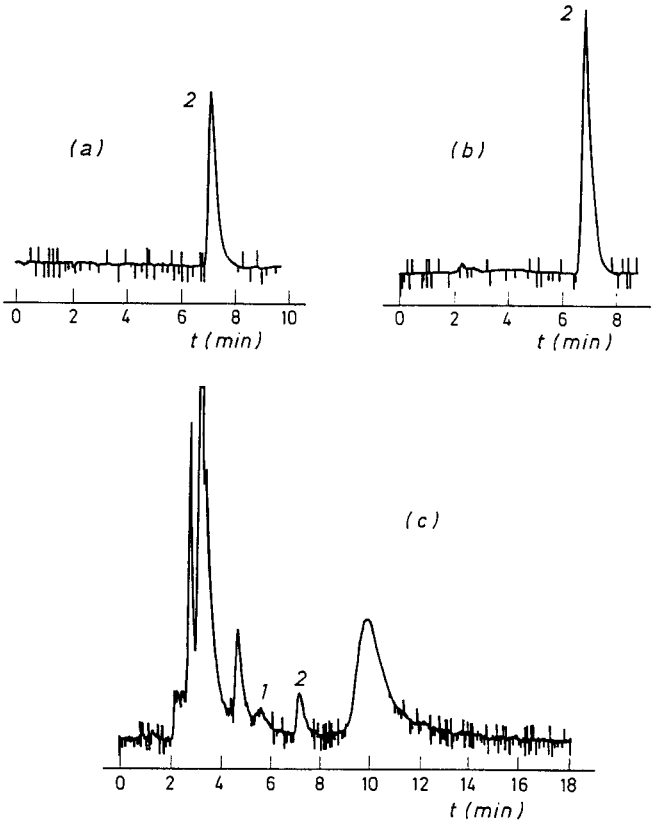


Figure 3:

Typical chromatograms of a) tap-water, b) lettuce extract and c) apple tree leaves extract. Column and chromatographic conditions as in Figure 2. For peak identification see also Figure 2.

added to a variety of samples and the resulting spiked samples were subjected to the entire analytical sequence. Each solute was spiked at three different concentrations and recoveries were calculated based on the difference between the total amount determined in the spiked samples and the amount observed in the non-spiked samples. All analyses were carried out in triplicate. The recoveries obtained for tap-

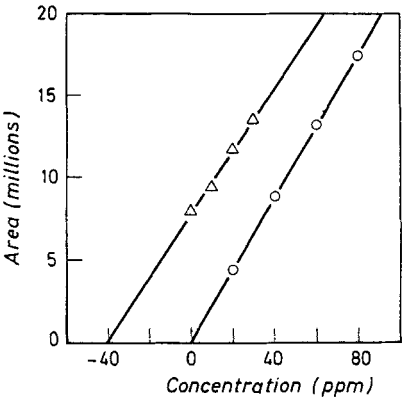


Figure 4:

Calibration graph with standards and the standard additions graph with a lettuce sample for NO_3^-.

TABLE 1

Sample	Nitrate	Nitrite
Tap-water (mg/L)	2.58±0.01	n.d.
Lettuce (mg/Kg fresh weight)	4.100±300	n.d.
Apple tree leaves (mg/Kg fresh weight)	18.2±0.4	8.9±0.4

n.d.: not detected

water samples, which ranged between 98.0 and 100.5%, testify to the accuracy of the proposed method for this type of sample.

In the lettuce extracts and the apple tree leaves samples, the matrix effects were present, as can be seen in Figure 4 and 5 which present the calibration graphs with standards and the standard additions graphs. There are different slopes in the calibration lines, which explain the

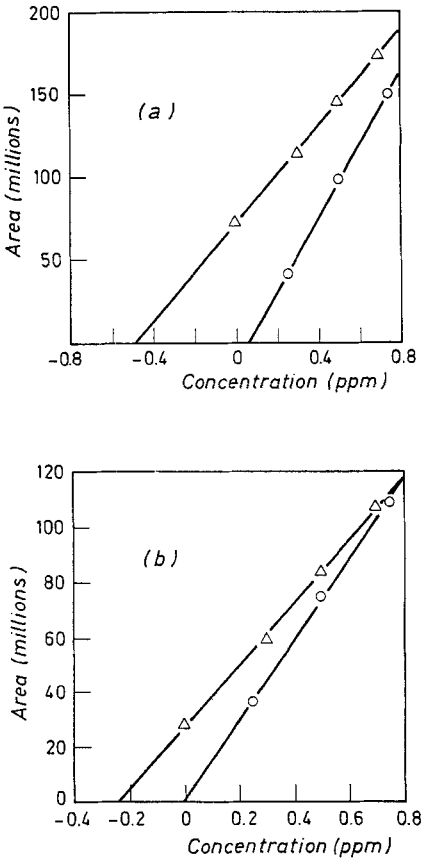


Figure 5:

Calibration graph with standards and standard additions graph with an apple tree leaves sample for a) NO_3^- and b) NO_2^-.

increased variability obtained by the recovery data (69%-87% for nitrate in lettuces and 63%-65% for nitrate and 70%-77% for nitrite in apple tree leaves). In both cases the determination of nitrate and nitrite was carried out by using the standard addition method.

The precision of the method was investigated using three different pool sample and by analysing each one in

triplicate. The variation coeficient was always less than 2% for nitrates and 5% for nitrites.

The results of some determinations are given in Table I. As can be seen, the nitrate values in the lettuce extracts show considerable variations according to their origin.

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