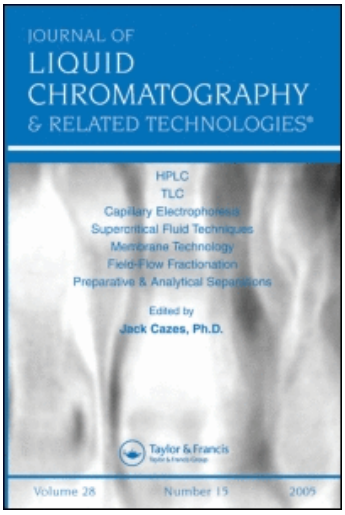


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

SIMULTANEOUS DETERMINATION OF NITRITE AND NITRATE IN DRINKING WATER AND HUMAN SERUM BY HIGH PERFORMANCE ANION-EXCHANGE CHROMATOGRAPHY AND UV DETECTION

I. N. Papadoyannis[a]; V. F. Samanidou[a]; Ch. C. Nitsos[a]
[a] Laboratory of Analytical Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki, Greece

Online publication date: 13 January 2005

J. LIQ. CHROM. & REL. TECHNOL., 22(13), 2023–2041 (1999)

SIMULTANEOUS DETERMINATION OF NITRITE AND NITRATE IN DRINKING WATER AND HUMAN SERUM BY HIGH PERFORMANCE ANION-EXCHANGE CHROMATOGRAPHY AND UV DETECTION

I. N. Papadoyannis, V. F. Samanidou, Ch. C. Nitsos

Laboratory of Analytical Chemistry
Department of Chemistry
Aristotle University of Thessaloniki
GR-540 06 Thessaloniki, Greece

ABSTRACT

A rapid, accurate, and sensitive method has been developed for the simultaneous determination of nitrite and nitrate. A low capacity strong anion exchange PRP-X100 Hamilton, 150 x 4.1 mm, 10 μm, with exchange capacity 0.19 ± 0.02 meq/g, analytical column was used, with a mixture of 0.1 M NaCl-CH_3OH, at a volume ratio 45:55. The flow rate of 2 mL/min, lead to a pressure of 150 kg/cm^2. Detection was performed with a variable wavelength UV-visible detector, at 230 nm, resulting in detection limits of 0.1 ng and 0.2 ng for nitrite and nitrate, respectively, per 20 μL injection. For the quantitative determination, iodide was used as internal standard, at a concentration of 7.0 ng/μL. A rectilinear relationship was observed up to 12 ng/μL for nitrite and up to 25 ng/μL for nitrate. Analysis time was less than 6 min. The statistical evaluation of the method was examined performing intra-day (n=8) and inter-day calibration (n=8) and was found to be satisfactory, with high accuracy and precision results.

The method was applied to the direct determination of nitrite and nitrate in drinking water (tap, table, and ground water) and in biological fluids (human serum). Solid phase extraction was tested for sample clean-up and analyte retention using SAX Lichrolut Merck cartridges. Low recovery of nitrite ca. 70% and high recovery of nitrate, approximately 130%, indicated the partial oxidation of nitrite during sample preparation. Solid phase extraction of solutions containing solely nitrite proved that a 40% oxidation is taking place, while the solid phase extraction of spiked serum samples yielded a 30% oxidation of nitrite anions. Therefore, a sample pre-treatment assay avoiding the solid phase extraction technique was applied, leading to approximately 80% recovery of nitrite and 120% recovery of nitrate.

The pre-treatment involves protein precipitation, using organic solvent and centrifugation of the sample. Endogenous compounds of human serum did not interfere in both cases, after SPE or without SPE. Total nitrogen recovery appeared to be quite the same, 98.6% and 98.0% respectively. Ascorbic acid used as anti-oxidant did not prevent the oxidation of nitrite.

INTRODUCTION

Nitrites and nitrates can be found in a wide range of both natural and processed foods, because of the general usage of nitrogenous fertilisers, in the agricultural industry.

Nitrite used as a meat preservative may play a significant role in carcinogenesis. The in vivo interaction of nitrite with amines or amides can result in the formation of nitrosamines. The carcinogenicity of nitrosamines in animals, has been tested and demonstrated. Cancer could be induced at specific sites in animals, by nitrosamines and nitrosamides, as well as, by nitrite administered together, with the corresponding amine or amide. Therefore, it is important to determine the nitrite concentration in foods for humans and animals.[1]

Nitrate itself, is not as toxic as nitrite, although it is prevalent in the environment. However, nitrate can be reduced to nitrite, mainly by bacterial action, during the storage of food containing nitrate, or in the human body during digestion. Nitrite can then react, under certain conditions, with secondary and tertiary amines and amides, to form the N-nitroso compounds most of which, as mentioned above, have been found to be carcinogenic.[2,3]

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. Problems of this nature have occurred very occasionally in babies.[2]

Additionally, serum levels of nitrite and nitrate, the degradation products of nitric oxide, are increased in patients with *Plasmodium falciparum* and *Plasmodium vivax* malaria and may be indicators for the severity of disease.[4] Therefore, the determination of the nitrite and nitrate ions, in biological fluids, is of great importance.

There are many approaches to nitrate and nitrite determination: spectrophotometric techniques and potentiometry, using a nitrate selective electrode, are widely used. 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. Additionally, the traditional methods are time-consuming, have relatively poor sensitivity, and can be unreliable for some samples.[2,5,6]

Chromatographic techniques have also been involved in the determination of nitrite and nitrate, including gas chromatography, HPLC and ion chromatography, using an anion exchange column, with conductivity detector or electrochemical detection. Gas chromatography, after derivatization can also be used in nitrate determination.[1,2,6-8]

HPLC techniques have emerged, as alternative procedures, for the determination of nitrite and nitrate. Methods involving pre-column derivatization, usually, have as a main drawback, the fact that nitrates can only be determined, after their reduction to nitrites.[5,9]

Two on-line post column reactions have also been used, involving nitrate reduction to nitrite, on a copper plated, cadmium filled column, and diazotization-coupling reaction, between nitrite and the Griess reagent. The absorbance chromophore was read at 540 nm.[5]

Some authors use high capacity anion exchange columns, with sodium perchlorate as eluent, however the sensitivity is rather low. Detection is achieved mostly in the range 210-215 nm, when using UV detection, with the problem of baseline unstability.[5]

The problem with analysing nitrate and nitrite in biological samples, by high performance ion chromatography, is the high concentration of chloride ions, which interferes with the determination of nitrite, due to lack of resolution and column saturation. The addition of silver reagents or silver-loaded cation exchange resins, leads to reaction, with the chloride and to its elimination as an interfering compound. However, it is reported, that the silver chloride

precipitation process, causes a substantial reduction in the performance of ion exchange columns, used for anions, with insoluble silver salts. Two solid phase extraction columns C_{18} and IC-Ag^{+}, connected in series, eliminate not only the chloride ions, but also purify the sample.[1]

For the determination of inorganic anions in drinking water, a number of chromatographic methods appear in literature, using various detection techniques. Among these methods, conductometric detection remains the mainstay of HPIC. Tap and table water can be injected directly after removing particulate matter, to prevent the column from clogging.[2]

In a previous work, the authors of this paper applied non-suppressed single column ion chromatography to the determination of drinking water, since conductivity detection was the most appropriate, as non UV absorbing anions were not present. In the case, however, of the detection of UV absorbing ions, the spectrophotometric detection technique is more sensitive, leading to lower detection limits.[10]

The direct determination of nitrite and nitrate in serum, by ion chromatography, has been proposed, recently, after dilution (1+3) and centrifugation through a Centricon filter.[11]

Solid phase extraction protocol, with a combination of C_{18} and anion exchange column has been introduced, using methansulfonic acid sodium salts, for the ion elution from serum samples.[4]

Anion exchange HPLC, together with a UV detector, is also able to determine nitrate and nitrite, in one step without derivatization. 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 drinking water (tap, table, and ground water) and biological fluids (human blood serum).

The accuracy of the developed HPLC methodology was established, by the spiked recovery approach. To study the accuracy of the method, recovery experiments are performed. Known amounts of each anion were added to a variety of samples and the resulting spiked samples were subjected to the entire analytical sequence. The determination of nitrate and nitrite was carried out by using the standard addition method.

The developed method is characterised by high sensitivity, accuracy, and precision. Short analysis time enables the application to routine sample analyses. Among the advantages of the method, low limits of detection and small sample volumes required are included.

No interference was observed in the determination of the ions in drinking water. No endogenous interference was noticed, in the determination of the nitrite and nitrate ions in blood serum. Though a partial oxidation of nitrite to nitrate is taking place during sample pre-treatment, the total nitrogen recovery in blood serum is approximately 98%, either using SPE or not, ensuring the high recovery rates of nitrogen containing ions, from biological fluids.

EXPERIMENTAL

Equipment/Instrumentation

A Shimadzu (Kyoto, Japan) LC-9A pump was used to deliver the mobile phase to the analytical column, a low capacity strong anion exchange PRP-X100 Hamilton, 150 x 4.1 mm, 10 μm, with exchange capacity 0.19 ± 0.02 meq/g.

Sample injection was performed via a Rheodyne 7125 injection valve (Rheodyne, Cotati California, U.S.A), with a 20 mL loop.

Detection was achieved by an SSI 500 UV-Vis detector (SSI, State College, PA, U.S.A.), at a wavelength of 230 nm and a sensitivity setting of 0.002 AUFS.

A Hewlett-Packard (Avondale, PA, U.S.A.) HP 3396 Series II integrator was used, for quantitative determination of eluted peaks.

A glass vacuum-filtration apparatus obtained from Alltech Associates, was employed for the filtration of the buffer solution, using 0.2 μm membrane filters, obtained from Schleicher and Schuell (Dassel, Germany).

Degassing of solvents was achieved by sonication in a Transonic 460/H Ultrasonic bath (Elma, Germany) prior to use. A Glass-col, Terre Haute 47802 small vortexer and a Hermle centrifuge, model Z 230 (B. Hermle, Gosheim, Germany) were employed for the sample pre-treatment.

The SPE assay was performed on a Vac-Elut vacuum manifold column processor, purchased from Analytichem International, a division of Varian (Harbor City, USA).

All evaporations were performed, with a 9-port Reacti-Vap evaporator (Pierce, Rockford, IL, USA). All computations were achieved using a VIP 312 computer.

Reagents and Chemicals

Potassium iodide, sodium chloride, sodium nitrate, and sodium nitrite pro analysi grade were purchased from Merck (Darmstadt, Germany). L(+) ascorbic acid pro analysi grade was also from Merck.

HPLC grade methanol and acetonitrile were obtained from Lab-Scan (Dublin, Ireland). Bis de-ionised water was used throughout analysis.

Solid phase extraction cartridges SAX Lichrolut were from Merck.

Preparation of Stock and Standard Solutions

Aqueous stock solutions of nitrite and nitrate ions, at concentrations of 100 mg/L were prepared and stored refrigerated at 4°C. These solutions were found to be stable throughout experimental analyses.

Working aqueous solutions were prepared from stocks at concentrations: 0.1, 0.25, 0.5, 1.0, 2.0, 3.0, 5.0, 7.0, 10.0, 12.0, 15.0, and 25.0 ng/μL.

Iodide solution at a concentration of 7.0 mg/L was added as internal standard.

Chromatography

Peak height of nitrite and nitrate ions were measured and the ratios to internal standard, iodide, were compared to that of the calibration standard. Chromatographic separations were performed at room temperature.

RESULTS AND DISCUSSION

The chromatographic system for determining nitrite and nitrate ions was chosen among others, regarding the elution time and eluent consistence. A variety of binary mixtures of organic modifiers (methanol and acetonitrile), with NaCl, in several ratios was tested, to result in optimum chromatographic system.

The final mobile phase was chosen in terms of peak shape, column efficiency, chromatographic analysis time, selectivity, and resolution. Inlet pressure observed with the eluent system, at a flow rate 2 mL/min, was 150 kg/cm^2. The use of acetonitrile lead to an increase of analysis time, which enhanced the analysis cost.

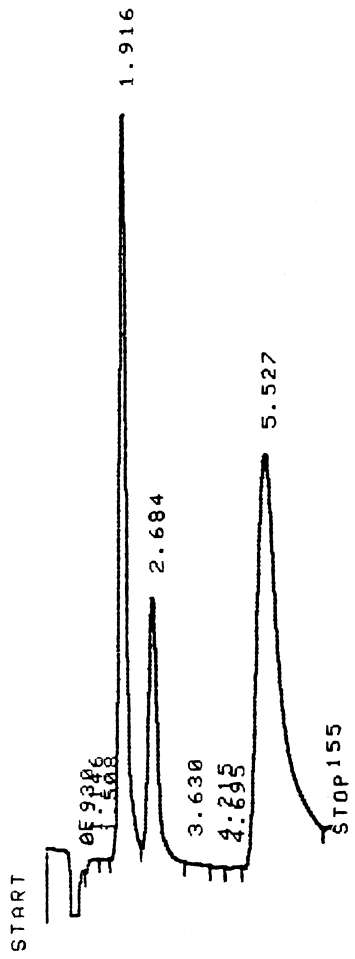


Figure 1. Chromatogram of standard solutions of nitrite (1.916 min.), nitrate (2.684 min.) and internal standard iodide (5.527 min.). Chromatographic conditions are described in Experimental.

The optimum mobile phase is consisted of 0.1M NaCl: CH_3OH (45:55 v/v). Figure 1 shows the chromatogram obtained using the conditions described in text. Resolution factors, 1.08 for nitrite-nitrate and 2.37 for nitrate-iodide, indicate sufficient separation.

Table 1

Calibration Data for Nitrite and Nitrate Determination with Iodide as Internal Standard

Parameter	Value	NO_2^-	NO_3^-
Concentration range	ng/μL	0.1 - 12.0	0.1 - 25.0
Slope	AU/ng	0.335719 + 0.005689	0.120572 ± 0.001677
Intercept		0.065220 ± 0.020539	0.010213 ± 0.004564
Correlation coefficient		0.99914	0.99913
Detection limit	ng	0.1	0.2

Analytical Variables

Optimised chromatographic conditions were set and the statistical evaluation of the proposed method was performed according to the following parameters:

- Calibration data and analysis time.
- Working range and detectability.
- Precision and accuracy.
- Real sample analysis.
- Solid-phase extraction.

Calibration Data and Analysis Time

The sample analysis time of nitrite and nitrate ions, in the proposed method, is approximately 6 min. Calibration of the method was performed by injection of standards, covering the entire working range. Ten concentrations were used in the range 0.1-12.0 ng/μL and 0.1-25.0 ng/μL, for nitrite and nitrate ions respectively. The sensitivity setting of the UV-VIS detector was adjusted at 0.002 AUFS. Each sample was injected six times. Linear correlation between absolute injected amount or concentration and peak height ratio, with iodide, as internal standard, at a concentration of 7.0 ng/μL was observed. The results of the statistical treatment of calibration data are summarised in Table 1.

Table 2

Within-Day Precision and Accuracy for Nitrite and Nitrate Determination*

Amount of Nitrite/ Nitrate Added (ng)	Amount of Nitrite Found (ng)	SD (ng)	RSD (%)	Recovery (%)	Amount of Nitrate Found (ng)	SD (ng)	RSD (%)	Recovery (%)
40	41.76	0.18	0.43	104.4	42.38	0.11	0.26	106.0
60	59.65	4.45	7.46	99.4	61.84	2.66	4.30	103.1
90	92.42	1.52	1.64	102.7	93.17	2.05	2.20	103.5
120	122.30	4.07	3.33	101.9	109.42	2.12	1.94	91.2

* n = 8.

Working Range and Detectability

The upper limit was found to be 12 and 25 mg/L, for nitrite and nitrate ions respectively, while limit of detection, calculated as a three fold signal to noise ratio, at the baseline (S/N=3), was found to be 0.1 ng and 0.2 ng, for nitrite and nitrate ions respectively, when 20 μL of the sample were injected onto the column. Limit of quantitation was found to be 2 ng for both ions.

Iodide was selected among other UV absorbing anions, as not being present, neither in drinking water, nor in biological fluids.

Precision and Accuracy

Method validation regarding reproducibility, was achieved, by replicate injections of standard solutions, at low and high concentration levels, where peak heights were measured, in comparison to the peak height of the internal standard. Statistical evaluation revealed relative standard deviations, at different values for eight injections. Results are shown in Table 2. Long term stability studies were conducted during routine operation of the system over a period of eight consecutive days. Mean values of five injections are presented in Table 3.

Solid Phase Extraction of Nitrite and Nitrate Ions

Strong anion exchange Lichrolut (Merck) cartridges were used for nitrite and nitrate pre-concentration or sample clean up procedure.

Table 3

Day-to-Day Precision and Accuracy for Nitrite and Nitrate Determination over a Period of 8 Consecutive Days

Amount of Nitrite/ Nitrate Added (ng)	Amount of Nitrite Found (ng)	SD (ng)	RSD (%)	Recovery (%)	Amount of Nitrate Found (ng)	SD (ng)	RSD (%)	Recovery (%)
40	42.95	4.04	9.41	107.4	42.25	4.31	10.20	105.6
60	60.14	2.55	4.24	100.2	62.53	4.58	7.32	104.2
80	82.47	1.71	2.07	103.1	83.59	2.01	2.40	104.5
100	101.33	1.61	1.59	101.3	102.77	6.20	6.03	102.8

Table 4

Recovery of Nitrite and Nitrate Ions After SPE Using SAX Licrolut Cartridges

Amount of Nitrite/ Nitrate Added (ng)	Amount of Nitrite Found (ng)	SD (n=5) (ng)	RSD (%)	Recovery (%)	Amount of Nitrate Found (ng)	SD (n=5) (ng)	RSD (%)	Recovery (%)
20	12.4	0.4	3.22	62.0	25.8	1.6	6.20	129.0
40	26.1	0.6	2.30	65.2	52.8	2.8	5.30	132.0
60	40.3	1.1	2.73	67.2	76.6	4.0	5.22	127.7
80	53.0	3.1	5.85	66.2	100.0	3.1	3.10	125.0
100	68.8	2.4	3.49	68.8	134.8	2.9	2.15	134.8
	Mean Recovery			**65.9%**		**Mean Recovery**		**129.7%**

Cartridges were conditioned with 3 mL of CH_3OH and 3 mL of NaCl 0.05 M. An aliquot of 500 µl of standard solution was applied to the cartridge and nitrite and nitrate were eluted, by flushing the cartridge with 1mL of a mixture consisted of 0.5 M NaCl - CH_3OH 70-30% v/v. Then the sample was either injected onto the column, or evaporated to dryness, under gentle nitrogen stream, in a 45°C water bath and diluted to 500 µL internal standard solution.

Extraction efficiency was calculated by extracting standard solutions of nitrite and nitrate ions, at five different amount levels i.e. 1.0, 2.0, 3.0, 4.0, and 5.0 ng/µL.

Table 5

Nitrate Determination in Drinking Water*

Water Sample	Nitrate Found ± SD (ng/μL)	Labelled Amount of Nitrate (μg/μL)
Tap water	14.1 ± 1.0	
Tap water	5.7 ± 0.3	7 - 10[a]
Table water	5.4 ± 0.4	1.2
Table water	33.2 ± 2.6	36.4
Table water	5.1 ± 0.2	2.5
Ground water	30.2 ± 1.8	33.7[b]
Ground water	25.0 ± 0.8	25.4[b]
Ground water	161.7 ± 10.2	178.0[b]
Ground water	59.6 ± 4.6	58.2[b]
Ground water	106.8 ± 4.9	99.4[b]
Ground water	7.5 ± 0.5	5.5[b]

[a] The value provided by the state laboratory.
[b] The values are provided by other laboratories as cross analysis study, using single column ion chromatography.

Regression equations after SPE are:

Y = (-0.067070 ± 0.079667) + (0.175800 ± 0.019514) X, where X= ng/μL NO_3^-,
Correlation coefficient R= 0.99390.

Y = (0.055200 ± 0.035446) + (0.173100 ± 0.010687) X, where X= ng/μL NO_2^-,
Correlation coefficient R= 0.99433.

Recovery of compounds was calculated by comparing peak height ratios, against internal standard, with those obtained for not extracted solutions. Results obtained are presented in Table 4.

The low recovery of nitrite and high recovery of nitrate imply a partial oxidation of the former to the latter, at a percentage of 30 %. However, total nitrogen recovery stands at high percent values: 93.6 ± 2.5 %.

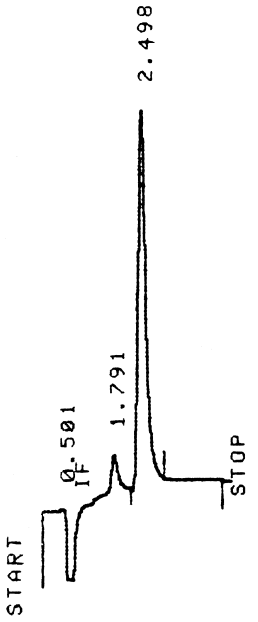


Figure 2. Chromatogram of drinking water sample.

The oxidation of nitrite to nitrate was proved by extracting a nitrite sample under the conditions described above. While nitrate ions were absent in this sample, a nitrate peak appeared in the chromatogram. The addition of ascorbic acid as anti-oxidant, did not prevent the oxidation.

Real Sample Analysis

Drinking Water

The nitrite and nitrate content of drinking water samples were determined after filtration of the sample and direct injection without further treatment.

Results of the analysis of tap, table, and ground water samples are given in Table 5, in comparison to the labelled or known amounts. A sample chromatogram is illustrated in Figure 2.

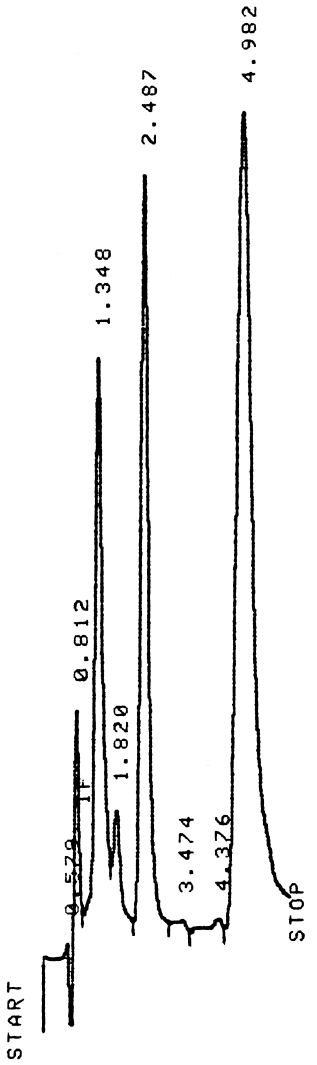


Figure 3. Chromatogram of spiked blood serum after SPE. Nitrite (1.820 min.), nitrate (2.487 min.), and internal standard iodide (4.982 min.). Chromatographic conditions are described in text.

Table 6

Recovery of Nitrite and Nitrate Anions from Human Blood Serum after SPE

Nitrite Added (ng)	Nitrite Found ± SD (ng)	Recovery (%)	Nitrate Added (ng)	Nitrate Found ± SD (ng)	Recovery (%)
40	28.7 ± 2.0	71.8	40	53.1 ± 4.3	132.8
80	57.2 ± 4.6	71.5	80	112.4 ± 5.9	140.5
100	71.8 ± 5.1	71.8	100	133.6 ± 6.4	133.6
140	101.1 ± 6.9	72.2	140	177.8 ± 10.7	127.0
Mean Recovery (%)		**71.8**	**Mean Recovery (%)**		**133.5**

Human Blood Serum

After SPE

Aliquots of 100 μL of human blood serum were treated with 200 μL of acetonitrile, to precipitate proteins.

After 2 min. vortex mixing, 100 μL of mixed standard solution were added to the sample at concentrations: 2.0, 3.0, 5.0, and 7.0 ng/μL. The concentration of iodide was 7.0 ng/μL.

The sample was subsequently centrifuged at 4000 rpm for 15 min and the supernatant was transferred to the SPE cartridge, after removal of organic solvent under gentle nitrogen stream.

The sample was subsequently treated according to the procedure described under solid-phase extraction paragraph. Figure 3 presents a human blood serum chromatogram of nitrite and nitrate after SPE.

Regression equations obtained are:

$Y= (0.004050 \pm 0.005671) + (0.03495 \pm 0.001301) X$, where X= ng/μL NO_3^-.
Correlation coefficient R= 0.99793.

$Y= (0.042567 \pm 0.047259) + (0.096317 \pm 0.011297) X$, where X= ng/μL NO_2^-.
Correlation coefficient R= 0.98652.

Table 7

Recovery of Nitrite and Nitrate Anions from Human Blood Serum Without SPE

Nitrite Added (ng)	Nitrite Found ± SD (ng)	Recovery (%)	Nitrate Added (ng)	Nitrate Found ± SD (ng)	Recovery (%)
20	15.9 ± 0.9	79.5	20	24.2 ± 1.3	121.0
40	35.0 ± 1.7	87.5	40	47.6 ± 4.2	119.0
60	51.0 ± 4.4	85.0	60	72.9 ± 3.5	121.5
80	60.2 ± 2.3	75.2	80	96.2 ± 4.1	120.2
140	101.4 ± 3.1	72.4	140	176.1 ± 11.1	125.8
	Mean Recovery (%)	**79.9**		**Mean Recovery (%)**	**121.5**

Recovery results of nitrite and nitrate from human serum after SPE are presented in Table 6. It is obvious that the same phenomenon, as described under the SPE paragraph, was noticed for serum samples, as well. Namely a 30 % oxidation of nitrite to nitrate took place, while the total nitrogen recovery was 98.6 ± 2.2%.

Without SPE

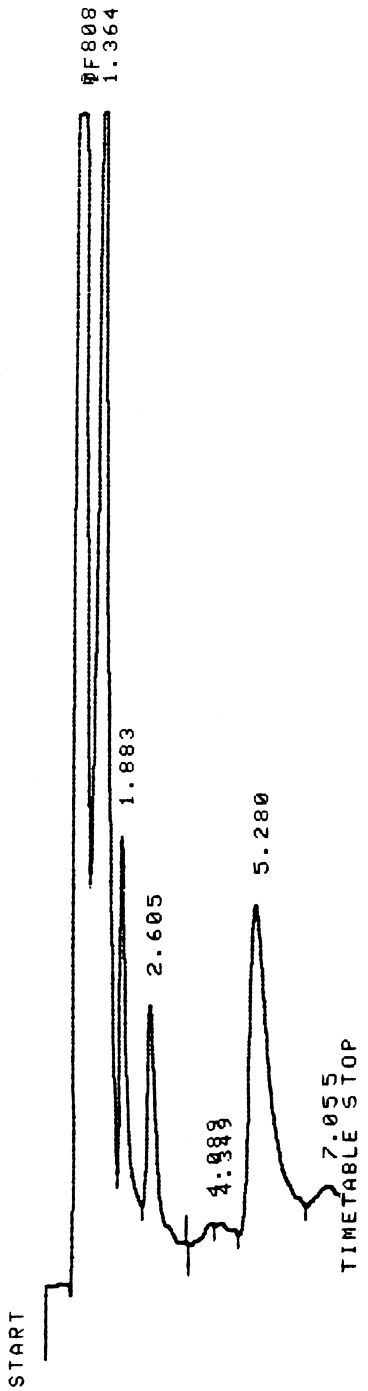
The next step was to develop a method, for blood serum pre-treatment, avoiding SPE, by analysing the samples directly, after protein precipitation.

For this purpose, 100 μL of mixed standard solution and 250 μL of acetonitrile, were added to 50 μL of serum. After 2 min. Vortex mixing and 15 min. centrifugation at 4000 rpm., the supernatant was evaporated to dryness and reconstituted to volume with the internal standard solution.

Solutions at five different concentration levels, 1.0, 2.0, 3.0, 4.0, and 7.0 ng/μL, were used and five repeated measurements were made. The mean values of the peak height ratios of nitrite and nitrate to internal standard were used for quantitation. Results are presented in Table 7.

Regression equations obtained are:

$Y = (0.097057 \pm 0.015491) + (0.043425 \pm 0.003897) X$, where X= ng/μL NO_3^-,
Correlation coefficient R= 0.98813



Y= (0.085226 ± 0.006251) + (0.063916 ± 0.001501) X, where X= ng/μLNO_2^-.
Correlation coefficient R= 0.99890

As shown in Table 7 a partial oxidation, approximately 20%, of nitrite to nitrate, is noticed, even avoiding SPE pre-treatment, while the total nitrogen recovery was at the same level, 98.0 ± 2.9%. A sample chromatogram is illustrated in Figure 4.

CONCLUSIONS

With the proposed method a satisfactory separation of the analytes, extended linear range and a rapid analysis time is achieved.

At the retention times of the analytes, no interference from endogenous compounds were found in chromatograms of drinking water samples or from extracted and not extracted spiked human blood serum, as can be seen in Figures 2, 3, and 4, respectively.

High performance anion exchange liquid chromatography, in conjunction with a specific mobile phase of NaCl-CH_3OH, appears to represent a powerful and sensitive method for the simultaneous separation and detection of nitrite and nitrate ions.

Peak height ratios of nitrite and nitrate relative to internal standard were linearly related to concentrations ranging from 0.1 to 12.0 ng/μL for nitrite and 0.1 to 25.0 ng/μL for nitrate or 2-240 ng and 2-500 ng respectively injected onto the column.

The detection limit defined as the quantity producing a signal-to noise ratio of 3 (in terms of peak height) are 0.1 ng for nitrite and 0.2 ng for nitrate, when 20 ng were injected on column.

In order to assess the precision and accuracy of the proposed analytical method, with-in assay variation was estimated by calculating the mean and standard deviation of standard solutions of the ions, at four concentration levels, with internal standard, injected 8 times each, within the same day and 5 times per day over a period of 8 days. Thus, within-day repeatability and between-day reproducibility were evaluated.

Figure 4 (left). Chromatogram of spiked blood serum without SPE. Nitrite (1.883 min.), nitrate (2.605 min.) and internal standard iodide (5.280 min.). Chromatographic conditions are described in text.

For nitrite the RSD values and relative recoveries ranged from 0.43 to 7.46% and from 99.4 to 104.4% in within day assay and from 1.59 to 9.41% RSD and 100.2 to 107.4% recovery in day-to-day assay.

For nitrate ions the respective values range from 0.26 to 4.30% and 91.2 to 106.0% in within day assay and from 2.40 to 10.20% and 102.8 to 105.6% in day to day assay.

The accuracy of the proposed HPLC methodology in biological fluids, was established by the spiked recovery approach. The recoveries for both anions were performed by spiking standards of the nitrite and nitrate ions into human control serum.

The proposed method provides also a precise and accurate determination of nitrite and nitrate in drinking water and in human blood serum samples. It allows the determination of the aforementioned anions in the presence of chloride.

Total nitrogen recovery from spiked serum samples was 98%, either by applying SPE or not, while a 30% oxidation of nitrite to nitrate was noticed by the first pre-treatment and 20% by the latter.

This approach offers the advantages of rapid simultaneous chromatographic detection, determination, and quantification of nitrite and nitrate ions at ambient temperature. Analysis time is approximately 6 min.

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Received October 13, 1998
Accepted December 21, 1998
Manuscript 4931

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