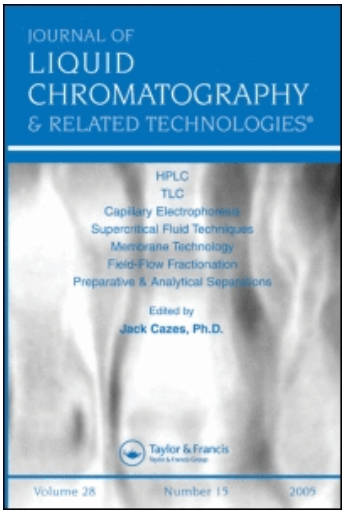

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SOLID-PHASE EXTRACTION PROCEDURE FOR THE CLEAN-UP OF URINE AND GASTRIC JUICE SPECIMENS FOR NITRITE AND NITRATE ANALYSIS BY ION CHROMATOGRAPHY

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ABSTRACT

A procedure was developed for the clean-up of gastric juice and urine specimens for nitrite and nitrate analysis by ion chromatography. After dilution with deionized water, the sample is passed through two solid-phase extraction columns (C_{18} and IC-Ag^+) connected in series. Nitrite and nitrate are eluted off the column and the sample is ready for analysis. This treatment of the samples eliminates any particles, organics and interfering chloride ions.

INTRODUCTION

Nitrite, 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 (1). The carcinogenicity of nitrosamines in animals has been demonstrated; cancer could be induced at specific sites in animals by nitrosamines and nitrosamides as well as by nitrite administered together with

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the corresponding amine or amide (1,2). Therefore, it is important to determine the nitrite concentration in foods for humans and animals. The amount of nitrite ingested by humans may also be determined by analyzing the serum, gastric juice and urine. The determination of nitrite and nitrate has been accomplished by colorimetry (3-8), ion specific electrode (3,9), chemiluminescence (10), gas chromatography (11,12), HPLC (13,14), and ion chromatography (15-21).

The problem with analyzing for 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 (21). The addition of silver reagents or silver-loaded cation exchange resins will react with the chloride and leads 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 (21).

The present study reports on the use of a solid-phase extraction procedure which eliminates not only the chloride ions but also purifies the sample and makes it suitable for direct injection and subsequent analysis by HPIC.

EXPERIMENTAL

Materials

A standard anion solution with F^-, Cl^-, NO_3^-, PO_4^{3-}, SO_4^{2-} as their sodium salts, was purchased from Dionex. Sodium nitrite, sodium carbonate and sodium bicarbonate were purchased from Fisher Scientific. The sodium nitrite was weighed on a Fisher Scientific XA-200DS analytical balance, and added to the anion/salt solution. The solution was diluted to appropriate concentrations with deionized water in a volumetric flask.

Apparatus and Method

Ion chromatography was performed with a Dionex ion chromatograph Series 4000i equipped with a 50 μL loop, Dionex AMMS-11 anion membrane suppressor, Dionex IonPac AS5 column, AG5 guard column, and conductivity detection (30 μS).

HPLC conditions: Mobile phase: 2.1 mM sodium bicarbonate/1.7 mM sodium carbonate

Flow rate: 1.0 mL/min

Regenerant: 700 μL H_2SO_4/L deionized water

All chromatograms were integrated using a Hewlett Packard 3392A integrator.

Sample Preparation and Extraction

A 200 mg C_{18} Burdick and Jackson solid-phase extraction SPE column was prepared by washing with 3 mL MeOH followed by 6 mL H_2O. The C_{18} solid-phase extraction (SPE) column was then connected in series to an Alltech IC-Ag^+ SPE column (300 mg), and washed with 6 mL H_2O. The biological samples were prepared as follows: 500 μL urine and 1 mL of gastric juice were diluted each to 3 mL with deionized water. 3 mL of sample was passed through both columns. The first eluting mL was discarded and the second mL was collected for IC analysis. 50 μL of the extracted sample was then injected onto the ion exchange column.

RESULTS AND DISCUSSION

Solid-phase extraction is a simple and efficient procedure for sample preparation and subsequent analysis. By selecting the appropriate experimental conditions, eluting solvent and column, the analyst can elect to either elute the analytes of interest and retain the undesirable compounds or retain the analyte of interest and allow the undesirable compounds to pass through. The analyte of interest is then eluted into a clean container by eluting off the column with an appropriate solvent. In this study, the first procedure was selected since only one step is required. Clean-up of the human urine and gastric juice specimen was carried out by first diluting each of the samples with distilled deionized water as specified in the experimental section. The sample was then passed through the C_{18} solid-phase extraction (SPE) column which was connected in series to the IC-Ag^+ column. The C_{18} SPE column is used as a filter (to remove any particles) and to remove undesirable organic and biomolecules. The IC-Ag^+ removes the

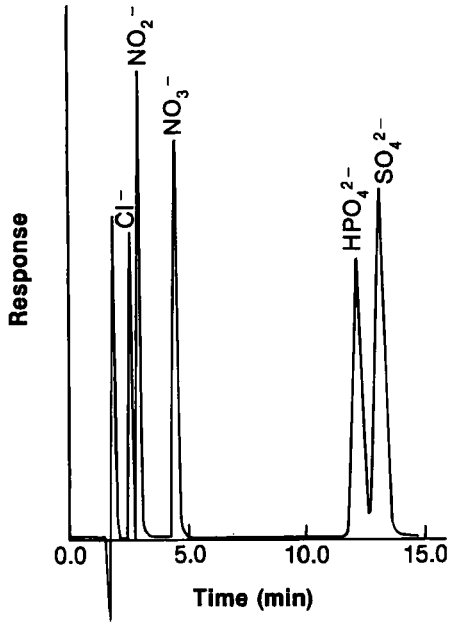

FIGURE 1. A chromatogram of a standard mixture of ions obtained on an ion chromatograph equipped with a 50 μl loop, anion membrane suppressor, IonPac AS5 column (4 x 250 mm), AG5 guard column and a conductivity detector. The mobile phase was made of 2.1 mM sodium bicarbonate and 1.7 mM sodium carbonate. Flow rate was set at 1 ml/min.

chloride ions. This effective and simple procedure takes on the average about 2 min/sample when working with a set of six samples at a time.

As far as recovery of the ions of interest is concerned our results show that a recovery above 98% was achieved for nitrite and nitrate.

Figure 1 shows the chromatogram of a standard solution containing chloride, nitrite, nitrate, phosphate and sulphate. Bromide eluted between chloride and phosphate. Figure 2 is the chromatogram of a 50 μl injection of diluted urine (1:6) with water. Note that the chloride peak interferes with nitrite peak. Figure 3 shows the chromatogram of the same urine specimen after clean-up. Note that the chloride peak was eliminated from the chromatogram. Figure 4 is a

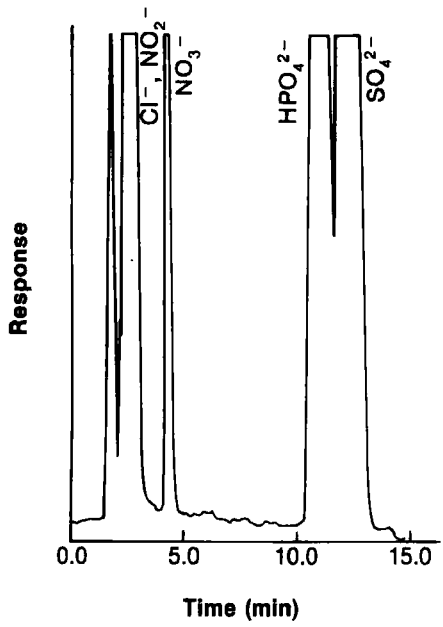

FIGURE 2. Chromatogram of untreated urine sample. Experimental conditions as in figure 1.

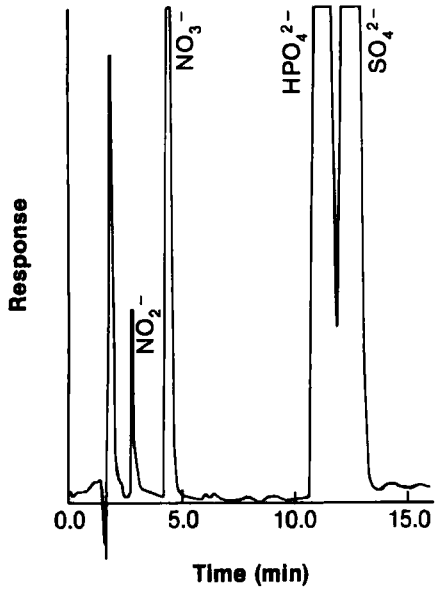

FIGURE 3. Chromatogram of treated urine sample. Experimental conditions as in figure 1.

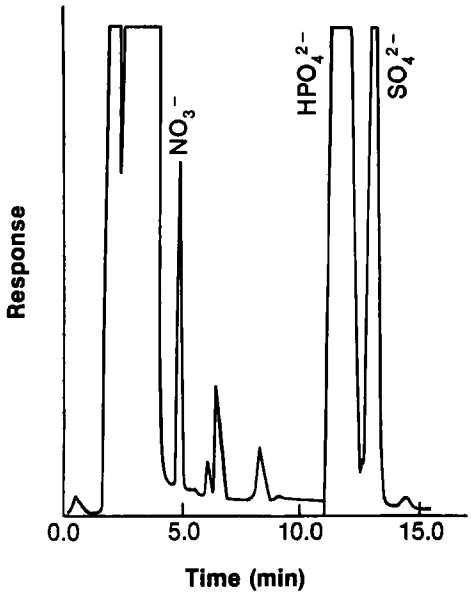

FIGURE 4. Chromatogram of untreated gastric juice sample. Experimental conditions as in figure 1.

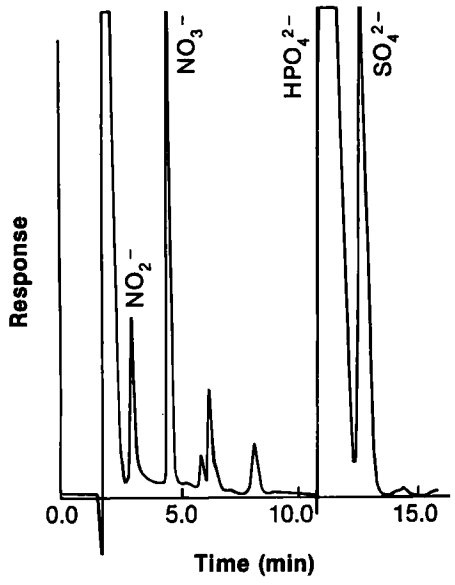

FIGURE 5. Chromatogram of treated gastric juice sample. Experimental conditions as in figure 1.

chromatogram of a diluted gastric juice sample. Here, as in urine, the chloride peak is very large and overwhelms both the fluoride peak (before it) and the nitrite peak. When the specimen was passed through the C_{18} and Ag^{+} SPE columns the chloride peak was eliminated and nitrite can be quantified, figure 5.

The clean-up procedure for urine and gastric juice specimens which eliminates organics, particulates and the interfering chloride peak is very effective. Approximately five hundred samples of urine and gastric juice were analyzed using the same analytical column before band broadening was observed. It is simpler and faster than the procedure described by Lippsmeyer et al. (21), it does not require the use of a centrifuge and uses minimal amounts of organic solvents for sample preparation and clean-up. The clean-up procedure can also be used for the analysis of other ions in biological samples.

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