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Determination of inorganic anions in the whole blood by ion chromatography

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ABSTRACT

A fast, precise, and accurate method that can simultaneously determine 7 anions in whole blood was established by on line dialysis-double suppression ion chromatography. Performance parameters which could affect the determination of anions were optimized, including the selection of protein precipitant in samples, the amount of filtrate discarded, selection of eluent flow rate, influence of the Ag-Na column on experimental results, influence of ethylenediamines on ClO_2^-, and investigation of nitrogen drying. Finally, 3.6 mmol/L sodium carbonate was selected as eluent, with a flow rate of 0.8 mL/min, to separate the 7 anions. Blood and alcohol (v/v, 1:4) were used to precipitate the proteins in blood. The 7 anions reached an adequate recovery rate when the first 2 mL of filtrate from the C18 column was discarded. The recovery rate at LLOQ, low, medium, and high concentrations was 80–120%. The correlation coefficients (r^2) of the calibration curves of the targeted anions ranged from 0.9975 to 0.9998. The limit of detection (LOD) was 0.309–7.71 μg/L. This method has simple pretreatment, high accuracy, and good reproducibility and selectivity, and is suitable for the separation and determination of anions in blood.

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1. Introduction

Biological fluids contain various inorganic anions, the intake of which is mainly through external sources such as water, vegetables, and meat. Inorganic anions have certain functions in the human body. Fluoride ions can prevent dental caries. Nitrates and nitrites are the precursors and metabolites of nitric oxide (NO), which exhibits a protective effect on the cardiovascular system [1]. Bromides are used in drugs for the treatment of epilepsy [2]. Sulfate radicals play important roles in the growth, development, and metabolism of human and other mammals [3]. Chlorite and chlorate are mainly utilized in industry, and both are the by-products of chlorine dioxide disinfection. Chlorite is used primarily in the bleaching of paper pulp, paper, and textiles [4]. Chlorate is employed to reduce the amount of some Gram-negative bacteria in livestock industry [5]. But there are no defined concentrations of these anions in normal blood.

Too high a concentration of inorganic anions in the body can have adverse effects. Excessive fluoride can induce apoptosis, resulting in fluorosis of bone and teeth [6]. Chlorite, chlorate, nitrite are oxidants in the body [1,4,7], and when the concentrations exceed a certain level, red blood cells will rupture and hemoglobin will be oxidized to methemoglobin, which has a reduced oxygen-carrying capacity. Bromide ion is a marker for death from drowning in seawater [8]. Besides, in forensic analysis, some cases are associated with the excess of anionic [9]. Therefore, the determination of various anions is helpful in blood disease's diagnosis and forensic science.

Ion chromatography is widely used in determining inorganic anions, and it has advantages for ion analysis including simultaneous determination of many anions in a single chromatography run, high sensitivity, high selectivity in samples with complex matrices and so on [10]. It has been shown that ion chromatography can be used for the determination of iodides in healthy and pathological human thyroids [11] and in urine [12] and serum samples [13], besides for the determination of fluoride [14], chloride, nitrate, phosphate and sulfate in serum [15]. Early in 1989 [16], studies reported that ion chromatography could determine nitrite, sulfate, bromide, and nitrates in serum, using ultrafilter-paper for the pretreatment of blood. Some studies showed that ultrafiltration may be a better method to remove proteins in serum [17], but it should be careful when determining nitrates due to nitrate ions released from filter membrane [18,19]. In recent years, ion chromatography has made many advances in hyphenation technique of IC with

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mass spectrometry (IC-MS) and capillary IC technique and two-dimensional IC technique [20]. On line dialysis-double suppression ion chromatography is an analytical method for the separation of low-molecular-matter from the polymer by using dialysis membrane, and the sample enters the chromatographic column for separation after chemical inhibition and carbon dioxide inhibition.

One of the key problems in the determination of biological samples is the method of sample pre-treatment in order to avoid trace element contamination and ensure the quality of the data acquired [21,22]. Protein precipitation (PP), solid phase extraction (SPE) and liquid-liquid extraction are commonly used in sample preparations. However, PP and SPE are prior to liquid-liquid extraction because of high extraction recovery. PP is easy to operate and costly, but it may lack the efficiency for chromatography detection which SPE can offer [23]. SPE is based on the partition of the analyte between a solid phase which is usually a sorbent held in a column and a liquid phase which is a sample matrix or a solvent with analytes [24]. SPE is widely used in separating analytes from the matrix, whereas the major limitation is that it can rarely be used in complex samples such as the whole blood. Sample viscosity may be the mainly reason that it can blot the sorbent [25]. For whole blood, it needs certain treatment to become relatively clean in order to pass SPE column. So we integrate PP with SPE to cleaning up endogenous interference in biological samples. The blood sample is very complex. Some samples' plasma or serum is unobtainable [9], so the whole blood analysis is extremely significant.

In this study, we will establish an accurate and convenient method to perform qualitative and quantitative analysis in inorganic anions in whole blood by ion chromatography. We use ethanol to precipitate proteins in whole blood to further remove proteins, fats and other compounds during the process of sample pretreatment. Chromatography conditions were optimized. The optimized method was applied to determine fluoride, chlorite, chlorate, nitrite, nitrate, bromide and sulfate in blood.

2. Material and methods

2.1. Chemicals

Standard solutions of F^-, SO_4^{2-}, NO_2^-, NO_3^-, Br^-, ClO_2^-, ClO_3- (1000 mg/L, NSI, America), ethanol (analytically pure, Guangdong Province Chemical Reagent Engineering Technology Research and Development Center), ethylenediamines (analytically pure, Shanghai Runjie Chemical Reagent Co., Ltd.), methanol and sulfuric acid (analytically pure, Guangzhou Chemical Reagent), and sodium carbonate (analytically pure, Guangdong Province Chemical Reagent Engineering Technology and Development Center) were used.

2.2. Instruments

A Metrohm-861 double-suppression ion chromatograph (Metrohm AG), IC Net 2.3 chromatographic work station, a Metrohm-813 autosampler, an 853 CO_2 suppressor, and a MSMII chemical suppressed conductivity detector were used to analyze the samples. A CT15RT high speed freezing centrifuge (Shanghai Techcomp Instrument Ltd.) was used to treat samples. A SHZ-IIIA circulating water-type vacuum pump (Gongyi Yuhua Instrument Co., Ltd.) with eluent, a CP2250 1/10 million electronic balance (Sartorius Scientific Instruments(Beijing) Co., Ltd), a MTN-2800 W sample concentrator (Tianjin Automatic Science Instrument Co., Ltd.), a Biotage 48 hole positive voltage extraction device (Biotage Trading (Shanghai) Co., Ltd.,) and a numeric control ultrasound cleaner (Kun Shan Ultrasonic Instruments Co., Ltd.) were also used.

2.3. Preparation of standard solutions

A standard solution of F^- (1000 mg/L) was used to prepare a series of standard solutions at the concentrations of 0.2, 1, 2, 5, 10, 20, and 40 mg/L. A standard solution of SO_4^{2-} (1000 mg/L) was used to prepare a series of standard solutions at the concentrations of 1, 2, 5, 10, 20, and 40 mg/L. Standard solutions of NO_2^-, NO_3^-, Br^-, ClO_2^-, ClO_3- (all, 1000 mg/L) were all used to prepare a series of standard solutions at the concentrations of 0.2, 0.4, 1, 2, 4, and 8 mg/L.

2.4. Preparation of eluent

First, 0.3816 g of sodium carbonate (dried for 2 h at the temperature of 105° and kept in the dryer) was accurately weighted and placed in a 1000 mL volumetric flask, and then was diluted to volume with deionized water. After shaking it well, it was filtered and degassed by vacuum.

2.5. Preparation of sulfuric acid regenerated liquid (50 mmol/L)

First, 3 mL of sulfuric acid was correctly absorbed and was placed in a 1000 mL volumetric flask, and then was diluted to volume with deionized water followed by shaking.

2.6. Pretreatment of samples

2.6.1. C18 column activation

The CNWBOND HC-C18 column (Shanghai ANPEL silica matrix, 3 mL, Scientific Instrument Co., Ltd.) was firstly activated with 5 mL of methanol and 10 mL of deionized water followed by standing for 10 min, according to the product instructions.

2.6.2. Blood treatment

The whole blood was stored in -4° before analysis and it can be stored about one week in -4° before final analysis. First, 0.8 mL of blood was obtained. Then, 3.2 mL of ethyl alcohol and 4 mL of deionized water were added. The specific order and amount are shown in Table 1. An adjustable turbine mixer (Guangzhou Shenhua Biological Technology Co., Ltd., China) was used to shake the liquids to uniformity, followed by centrifugation for 10 min at 13,000 rpm/min at 20°. Supernatants passed through the activated C18 column and 0.22 μm organic and inorganic filters (Shanghai ANPEL Scientific Instrument Co. Ltd.). After discarding the first 2 mL of liquid, the remaining liquids were collected for analysis. According to some papers [9], nitrite can react with oxyhemoglobin to form methemoglobin and nitrate. Once we received the blood sample and precipitate the proteins especially oxyhemoglobin, thus the nitrite can be as stable as nitrates. Our studies have been approved by the Ethics Committee of Southern Medical University and blood specimens were collected according to the International Standardization Organization for manual sampling of blood.

2.7. Quality control program

According to the Guidance for Industry Bioanalytical Method Validation [26], we validated the proposed IC technique for the analysis of anions in whole blood. Various parameters were determined and each experiment was carried out in at least triplicates. The quality control validation parameters investigated were the limit of detection (LOD), lower limit of quantification (LLOQ), linearity, precision, accuracy and stability. LOD was defined by three times of signal to noise. The lowest concentration of analytes on the calibration curve was employed to define LLOQ. And the analyte response at the LLOQ was at least five times the response compared to blank response and analyte peaks were identifiable, discrete,

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