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Determination of several sugars in serum by high-performance anion-exchange chromatography with pulsed amperometric detection

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Abstract

In this paper, a sensitive, simple and direct method for simultaneous determination of glucose, ribose, isomaltose and maltose in serum sample by high-performance anion-exchange chromatography coupled with integrated pulsed amperometric detection was developed. The four target analytes were easily and completely separated on an anion-exchange column at a flow-rate of 0.25 mL/min by binary step gradient elution in about 16 min and the two eluents were deionized water and 500 mM sodium hydroxide, respectively. The separated four analytes were detected directly by using a gold electrode and quadruple-potential waveform integrated pulsed amperometry without derivatization. Under the optimized conditions, when the injection volume was 25 μL, the detection limits (signal-to-noise ratio equal to 3) for glucose, ribose, isomaltose and maltose were 0.92, 7.50, 12.9 and 10.3 ng/mL, respectively. The calibration graphs of peak area for the four analytes were linear over two to three orders of magnitude with correlation coefficients greater than 0.998. R.S.D. of peak areas of the four analytes for five determinations were no more than 5.6%. The analytical method had been applied to the determination of glucose, ribose, isomaltose and maltose in real serum samples and good results with low relative standard deviation not more than 5.3% were obtained. The accuracy of the proposed method was tested by recovery measurements on spiked samples and good recovery results (98.1–107.9%) were obtained.

Keywords: Sugar; Serum; High-performance anion-exchange chromatography; Pulsed amperometric detection

1. Introduction

Sugars are among the most abundant organic compounds in the biosphere and they play very important roles in many life science systems. So the determination of them is very important in life science, food science, medical science, and agricultural science. The analysis of some sugars in serum has long been essential for the diagnosis of certain disease states and the effects of certain drugs. For example, monitoring blood glucose concentration levels is helpful to the control and treatment of diabetes, and the colorimetric assay of blood glucose has become a routine test in medical treatment. Ribose is a component of RNA, DNA, ATP, and it is required for the biosynthesis of these important biomolecules. In many human being and animal tissues and body fluids including blood, endogenous ribose is available from glucose via the hexose monophosphate shunt, so it is very necessary to develop simple, sensitive and reliable method for the determination of ribose in the life and medical science research. In recent years, it is showed that the maltose can act as an effective component in intravenous injections for the diabetes patients and it can replace glucose in some cases in medical treatment practice. After injection, the maltose undergoes a series of metabolic process passing through glucose intermediate in human body, therefore, it is also very important to monitor the concentration levels of maltose and related other compounds in blood for the medical research and medical treatment practice. Of the various determination methods available nowadays for the above-mentioned sugars, although simple colorimetric assay based on enzyme reactions and various enzyme sensors are the most often used

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methods, these methods cannot simultaneously determine multiple compounds [1–7]. In some cases, for some special reasons, it is very essential to get confirmatory information by using more specific and confirmatory analytical technique such as chromatographic methods. HPLC with refractive index detection has been widely used in sugar analysis field, but is limited by poor sensitivity and specificity [8,9]. The application of HPLC coupled with postcolumn derivatization and UV–vis detection in sugar analysis is impaired by the complicated and time-consuming experimental process and poor sensitivity [10,11]. Therefore, sensitive, selective and simple analytical methods that are suitable to direct and simultaneous determination of multiple sugars are in great demand. Recently, because of its advantages of high sensitivity, simplicity, organic solvent freedom and no need derivatization, high-performance anion-exchange chromatography at acidity higher than pH 13 in conjunction with integrated pulsed amperometric detection has been applied to analyze carbohydrates, amino acids, some life related compounds and some antibiotics in many different kinds of samples [12–17]. But this relatively new analytical method only has a very limited application in the analysis of sugars in blood samples. For example, Cox et al. [18] developed a method for the determination of mannitol and lactulose in serum for the small intestinal permeability study. In the Dionex Application Update 125 obtained from dionex website (http://wwwl.dionex.com/), a method for the determination of glucose and xylose in serum was recommended. However, up to now, there is no report of direct and simultaneous determination of glucose, ribose and maltose in serum sample by high-performance anion-exchange chromatography (HPAEC) separation and pulsed amperometric detection (PAD).

In this paper, we present a sensitive, selective, simple and time-saving analytical method for direct and simultaneous determination of glucose, ribose, isomaltose and maltose in serum sample by HPAEC and PAD. (In view of the fact that maltose related reagents or products often contain isomaltose impurity, our research targets also include isomaltose.) We have developed the new gradient elution conditions for separation of the four target analytes. Under the optimized conditions, the four target analytes were easily and completely separated on an anion-exchange column at a flow-rate of 0.25 ml/min by binary step gradient elution in about 16 min, and then they were detected directly by using quadruple-potential waveform pulsed amperometry on a gold electrode without derivatization. The analytical method had been successfully used to the determination of glucose, ribose, isomaltose and maltose in real serum samples.

2. Experimental

2.1. Reagents

To prepare the standard solutions, sample solutions and the mobile phase, 18 MΩ purified water produced with a laboratory water purification system (Barnstead, IA, USA) was used throughout the experiments. D-Glucose, ribose, maltose, isomaltose and sodium azide were purchased from Sigma–Aldrich (St. Louis, MO, USA) and were used as received. The single component stock solutions of the four sugars with concentration of 1 mg/mL were prepared by dissolving suitable amount of sugar in 20 mg/L sodium azide diluent. The injected standard mixture solutions of the four sugars were made by diluting the four corresponding aliquots of single component stock solutions of sugars with an aqueous diluent containing 20 mg/L sodium azide. Guarantee grade reagent sodium hydroxide used to prepare mobile phase was purchased from Beijing Chemicals Corporation, Beijing, China. The 50% sodium hydroxide concentrated solution was prepared by dissolved suitable amount of solid sodium hydroxide into equal amounts of purified water. To precipitate as more amounts of sodium carbonate as possible before use, this solution was left undisturbed for at least 24 h. The separation of the four target analytes was obtained on an anion-exchange column by binary step gradient elution. Mobile phase I was 18 MΩ purified water vacuum filtered through a 0.2 μm nylon filter. Mobile phase II, a 500 mM sodium hydroxide solution, was prepared by diluting an aliquot of 50% sodium hydroxide concentrated solution. All of the mobile phases were kept under nitrogen to prevent contamination by atmospheric carbon dioxide.

2.2. Chromatographic conditions

The chromatography system used in our experiments was a DX-600 IC system (Dionex, Sunnyvale, CA, USA). This system consisted of a GS50 gradient pump with on-line degas, an AS50 thermal compartment with 25 μL injection loop, an AS50 autosampler, and an ED50 electrochemical detector equipped with a thin-layer type amperometric cell. The cell comprised a gold working electrode with about 1 mm diameter, a glass and Ag/AgCl combination reference electrode (Dionex) and a titanium counter electrode consisting of the cell body. The chromatographic separation of the four sugars was performed on an CarboPac PA10 analytical column (250 mm × 2 mm i.d. Dionex) and an CarboPac PA10 guard column (40 mm × 2 mm i.d. Dionex) at a flow-rate of 0.25 mL/min, both columns were packed with an identical microporous, polymeric anion-exchange material and they were installed in the thermal compartment at a controlled temperature of 30 °C. The sample injection volume was 25 μL. The gradient elution was performed with water and 500 mM sodium hydroxide mobile phases using a binary step gradient elution shown in Table 1. The four target sugars were detected directly by using quadruple-potential waveform pulsed amperometry without derivatization on a gold electrode and the detection conditions are given in Table 2. A personal computer equipped with a Chromeleon 6.5 chromatography software (Dionex) was used to acquire and process chromatographic data. Peak area was used as the analytical measurement.