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Synthesis of silica-based benzeneboronic acid affinity materials and application as pre-column in coupled-column high-performance liquid chromatography

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Abstract

Three silica-based benzeneboronic acid affinity materials were synthesized by using an *m*-aminobenzeneboronic acid as the ligand and using three different spacer arms. Under high-pressure, three affinity pre-columns were packed with these materials and the retention of every affinity pre-column with 11 urinary nucleosides was studied. With different spacer arms of boronic acid-substituted silica materials, the absorption to vicinal alcohols (*cis*-diols) and stability under acidic elution conditions are of great difference. Coupled-column liquid chromatographic methods for the direct analysis of urinary nucleosides were respectively established. As a result, two of three affinity pre-columns showed good chromatographic property in the on-line analysis of urinary nucleosides. The coupled-column system including pre-column I is the best with excellent linearity ($R^2 > 0.995$), good recoveries (85.6–96.9%) and reproducibility (R.S.D.: 1.01–4.02%). The pre-column I could at least endure 150 repetitive injections of a 100 μL urinary sample.

Keywords: Benzeneboronic acid-substituted silica; Coupled-column; High-performance affinity chromatography; High-performance liquid chromatography; Direct analysis; Urinary nucleosides

1. Introduction

The ever-increasing number of samples, together with pressure for higher laboratory efficiency, requires the development of high-throughput, fully automated analytical methods [1]. Despite its high-resolution power, sensitivity, precision and practicability, high-performance liquid chromatographic (HPLC) analysis of biological samples like blood, serum, plasma, urine, milk, lymphatic fluids, liquor, feces or tissue homogenates is restricted by the pretreatment and processing of such highly complex matrices. Often the sample preparation represents the weakest chain segment in an analytical HPLC procedure as it still involves many elaborate, manual and thus error-prone and time consuming work-up steps. Thus, the goal in bioanalytical sample processing should be a rapid and, if possible, an automated, HPLC integrated procedure that exhibits a high selectivity to the analyte and tolerates the direct injection of a biological sample. In many cases, analytes are present in too small amounts and biological samples are too complex or incompatible with conventional HPLC phase systems to permit an analysis by direct injection into an analytical column [2]. Thus, simplification of such multicomponent mixtures as well as analyte enrichment is needed prior to analysis. In general, this is gotten by prefractionation or class separation and preconcentration steps. But, in the HPLC analysis of low-molecular-mass compounds (metabolites and drugs) from biological fluids, macromolecular matrix components present a challenge for sample preparation: proteins and nucleic acids have to be removed before chromatographic separation [3].

To solve these problems, direct injection supports and on-line chromatographic techniques have been developed in HPLC. In principle, these techniques always use two or more columns that are connected in parallel or in series and thus allow the selective prefractionation and subsequent analysis of the target compounds in deproteinized biological fluids [1–3].

According to this principle, an on-line multidimensional high-performance affinity chromatography (HPAC)–reversed-phase high-performance liquid chromatography (HPLC)

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method for the group selective separation and quantification of ribonucleosides in human urine and ultrafiltrated human serum was developed by Boos et al. [3]. This was realized using a laboratory-prepared boronic acid-substituted silica pre-column coupled to a reversed-phase column by a switching technique [3,4]. We had already established this kind system to determine 14 urinary nucleosides by direct injection of urinary samples on the boronic acid-substituted silica pre-column from Boos [5]. The high selectivity and capacity as well as the protein-eliminating property of this boronic acid-substituted silica material formed the basis for a successful application of the HPAC–HPLC method. Except for nucleosides, boronic acid can purify many important biomolecules, such as nucleotidyl peptides, catechols, sugars, glycosylated protein, some specific RNA and some specific proteases by interaction of boronic acid with vicinal alcohols (*cis*-diols) [6]. For further study, we have synthesized some silica-based boronic acid affinity materials.

In this paper, three boronic acid-substituted silica materials were synthesized by using an *m*-aminobenzeneboronic acid as the ligand and using three different spacer arms, then, were packed into the same small stainless-steel column under high pressure as the affinity pre-columns. And the affinity behavior of the pre-columns with 11 urinary nucleosides was studied. Two of three affinity pre-columns showed good chromatographic property in on-line coupled-column HPLC method for the direct analysis of urinary nucleosides.

2. Experimental

2.1. Chemicals and reagents

LiChrosorb Si 100, 5 μm, was obtained from E. Merck (Darmstadt, F. R. G). *m*-Aminobenzeneboronic acid hemisulphate, γ-chloropropyltrimethoxysilane, γ-glycidoxypropyltrimethoxysilane, carbonyldiimidazole and dioxane were obtained from Sigma (St. Louis, MO, USA).

The following 11 nucleoside standards including the internal standard 8-bromoguanosine hydrate (Br8G) were obtained from Sigma (St. Louis, MO, USA): pseudouridine (Pseu), cytidine (C), uridine (U), 1-methyladenosine (m1A), 1-methylinosine (m1I), 1-methylguanosine (m1G), N^4-acetylcytidine (ac4C), N^2-methylguanosine (m2G), adenosine (A), N^2,N^2-methylguanosine (m22G) and N^6-methyladenosine (m6A). Methanol (MeOH) was HPLC-grade purchased from Tedia (Fairfield, OH, USA). Ammonium acetate (NH_4OAc), ammonia ($NH_3{\cdot}H_2O$) and potassium dihydrogenphosphate (KH_2PO_4) were all analytical reagents obtained from Shenyang Federation Reagent Factory (Shenyang, Liaoning, China). Water was deionized and purified by a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Equipment

The HPLC system consisted of three Shimadzu LC-10ATVP pumps (Kyoto, Japan), an autoinjector model SIL 10ADVP, a SPD-10AVP UV–vis detector, set at 260 nm and a SCL 10AVP interface. An electric six-port valve (Rheodyne, USA) was used for the automated column switching. Valve switching and data acquisition were performed on Shimadzu Class-VP Version 6.10 software. Schematic diagram of the coupled-column HPLC system was the same as Fig. 1 in [5]. The column 1 (35 mm × 4.6 mm i.d.) was one of three laboratory-prepared boronic acid-substituted silica columns (pre-columns I, II or III). The column 2 (250 mm × 4.6 mm i.d.) was packed with 5 μm Hypersil ODS_2 (Elite, Dalian, China). For efficient on-line clean-up and concentration of 11 nucleosides from urine samples, the mobile phases applied consisted of 0.25 mol L^{-1} ammonium acetate (respectively pH 7.50, 7.95 or 8.50) on column 1, and of 25 mmol L^{-1} potassium dihydrogen phosphate

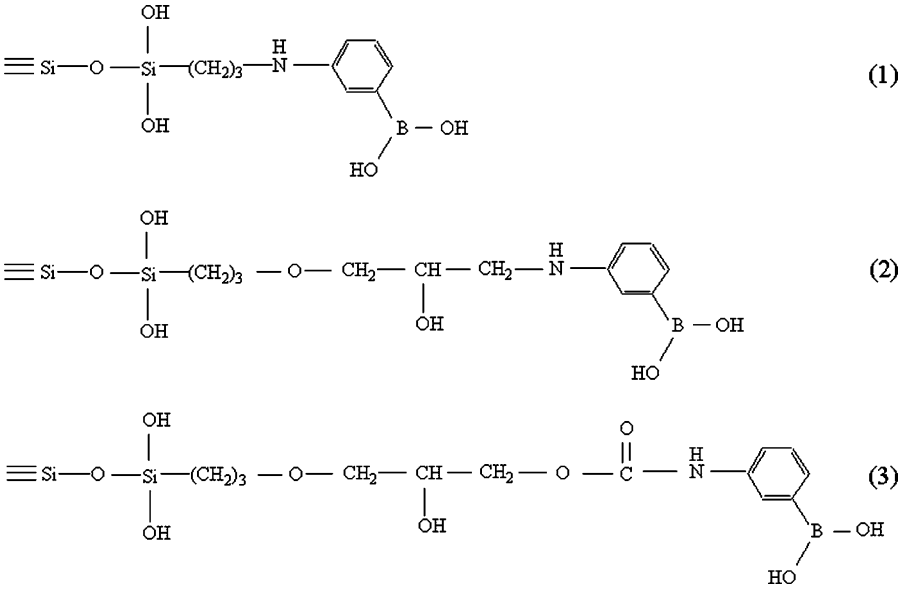

Fig. 1. Structures of boronic acid-substituted silica I (1), boronic acid-substituted silica II (2) and boronic acid-substituted silica III (3) of pre-columns I–III, respectively.