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In-tube solid phase microextraction using a β-cyclodextrin coated capillary coupled to high performance liquid chromatography for determination of non-steroidal anti-inflammatory drugs in urine samples

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Abstract

A configuration of in-tube solid-phase microextraction (SPME) coupled to HPLC was constructed by using a pump and a six-port valve combined with a PEEK tube as the pre-extraction segment. The extraction capillary was fixed directly on the HPLC six-port valve to substitute for the sample loop. The whole system could be handled easily to perform accurate on-line extraction, and the possible inaccurate quantification caused by sample/mobile phase mixing when using an autosampler could be eliminated.

A β-cyclodextrin coated capillary, prepared by sol–gel method, was used as the extraction capillary for in-tube SPME. Three non-steroidal anti-inflammatory drugs, ketoprofen, fenbufen and ibuprofen, were employed to evaluate the extraction performance of the capillary. After optimizing the extraction conditions, satisfactory extraction efficiency was obtained and detection limits for ketoprofen, fenbufen and ibuprofen in diluted urine samples were 38, 18 and 28 ng/mL, respectively. The extraction reproducibility was evaluated with intra-day and inter-day precision, and the R.S.D.s obtained were lower than 4.9 and 6.9%, respectively. The capillary was proved to be reusable and the extraction efficiency did not decrease after 250 extractions.

Keywords: In-tube solid-phase microextraction; β-Cyclodextrin coated capillary; Non-steroidal anti-inflammatory drugs; SPME-HPLC

1. Introduction

The main purpose of sample pretreatment methods are sample clean-up and analyte enrichment, which will lead to higher sensitivity and better accuracy of analysis. In coping with samples in complicated matrices or analytes at low concentrations, such as biological and environmental samples, traditional treatment methods such as liquid–liquid extraction (LLE), protein precipitation and so on, cannot satisfy all the analytical requirements. This situation has propelled the development of new sample pretreatment techniques, such as solid-phase extraction (SPE), solid-phase microextraction (SPME), membrane extraction, microwave assisted extraction, etc.

SPME was introduced by Arthur and Pawliszyn in the beginning of 1990s [1] and was combined with HPLC in 1995 [2], providing an alternative choice for sample clean up and enrichment for the analysis of non-volatile analytes. SPME has gained wide acceptance due to its better sensitivity compared to direct injection, adequate extraction reproducibility and commercial availability of SPME devices for combining with GC, LC, and CE [3,4]. The original forms of SPME-HPLC and the automated form of in-tube SPME-HPLC, introduced at 1997 [5], have been widely used since then in the analysis of environmental and biological samples and determination of food and drug samples. Its applications were reviewed recently by Kataoka [6].

In-tube SPME-HPLC can be automated by the modification of a commercial LC autosampler into an automated extraction device [5]. It provides shorter sample analysis time, more accurate quantification and better reproducibility and thus has good potential for routine analysis. However, little improvement was made since the device was first described. An inherent systematic error of this configuration was demonstrated when coping with analytes in given matrix by Raghani and Schultz recently [7]. In that system, the

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six-port valve is allowed to be situated at the LOAD position during extraction, and there is residual mobile phase contained in both the pre-extraction, including the sample loop, metering valve, tube prior to the metering valve, and the extraction segment. When the sample solution is drawn, traces of analyte migrate to these segments causing contamination of the mobile phase that resides inside the segments. Thus the concentration of analyte cumulatively increases as a result of sample/mobile phase mixing as the number of draw/eject steps increase [7]. This will result in inaccurate quantitative information and overestimation of the limit of detection. Raghani and Schultz demonstrated this by substituting the extraction capillary with an inert stainless steel tube and obvious analyte enrichment was found when increasing the draw/eject cycles. As to the solution of the problem, they suggested the insertion of an air plug into the extraction step to minimize the sample/mobile phase mixing. However, the attempt to construct other forms of the in-tube SPME-HPLC configurations that satisfy the same extraction requirement could be taken.

Common coatings applied in SPME are PDMS, PDMS-DVB, PA and CAR-TPR, which are mainly transplanted from GC capillary coatings. In the past several years, the polypyrrole coating, introduced by Pawliszyn and co-workers, has been employed in a wide range of research works [8–14]. Besides, biocompatible restricted-access materials (RAMs) [15,16], and chemical bonded silica monolith [17] were also introduced. However, the development of further coatings for SPME would be useful.

β-Cyclodextrin has been widely used in separation and analytical chemistry due to its abilities to form inclusion complexes with certain analytes. As to non-steroidal anti-inflammatory drugs (NSAIDs), the research on complex formation [18,19] and chromatographic separations [20,21] involving β-cyclodextrin have been reported, which provides the possibility of using β-cyclodextrin coating for extraction. However, the use of β-cyclodextrin in SPME-HPLC has not been reported as yet. It is expected that satisfactory extraction efficiency towards these analytes could be obtained with β-cyclodextrin contained capillary coating.

In the present paper, we describe a configuration of in-tube SPME-HPLC. A capillary coated with β-cyclodextrin using a sol–gel method was employed for the determination of three non-steroidal anti-inflammatory drugs. The results indicate that this system can be used for quantitative analysis and has the potentiality for extraction of real biological samples.

2. Experimental

2.1. Chemicals and reagent

The three investigated NSAIDs were ketoprofen (KEP) [2-(3-benzoylphenyl)propionic acid], fenbufen (FEP) [3-(4-biphenylylcarbonyl)propionic acid], and ibuprofen (IBP) [2-(4-isobutylphenyl)propionic acid], obtained from Pharmacy Administration of Hubei Province (Wuhan, China). The stock solution of the drugs was 1 mg/mL, prepared in methanol, with which the sample solution was spiked to a certain concentration. Sodium acetate (NaAc), sodium chloride (NaCl), methanol and other solvent were purchased from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China), and were of analytical grade.

β-Cyclodextrin was purchased from Sigma (St. Louis, MO, USA); 3-glycidoxypropyltrimethoxysilane (KH-560) and *n*-tetraethoxylsilane (TEOS) (95%) were obtained from Chemical Plant of Wuhan University (Wuhan, China).

2.2. In-tube SPME-HPLC instrument

The configuration of the in-tube SPME-HPLC is shown in Fig. 1. The whole system consisted of the pre-extraction segment, which included a Shimadzu LC-4A six-port valve (valve 1), a Shimadzu LC-6A pump (pump A) (Shimadzu, Tokyo, Japan) and a PEEK tube (0.03 in. i.d., 575 μL total volume), and the analytical segment, which included a Shimadzu LC-10AT pump (pump B) (Shimadzu, Tokyo, Japan), Rheodyne 7125 six-port valve (valve 2) with a 20 μL loop (Cotati, CA, USA) and a Shimadzu SPD-10A UV detector (Shimadzu, Tokyo, Japan). Valve 1 and valve 2 were connected by a stainless steel tube.

The analytical column was 150 mm × 4.6 mm i.d., packed with Kromasil ODS (5 μm),which was purchased from Eka Chemicals (Bohus, Sweden). The optimized mobile phase was 70% methanol and 30% 0.025 mol/L NaAc buffer solution with the pH adjusted to 5.0. The flow rate of the mobile phase was kept at 1 mL/min, and detection was performed at 223 nm with the UV detector for all the analytes.

2.3. Preparation of β-cyclodextrin coated capillary

The inner surface of the capillary was coated with β-cyclodextrin by a sol–gel method in order to obtain relatively high β-cyclodextrin content. The fused silica capillary (60 cm × 0.25 mm, i.d.), obtained from Yongnian Fiber Plant (Hebei, China), was first activated by 1 mol/L NaOH and then 1 mol/L HCl. After rinsing with double distilled water, it was dried at 160 °C under N_2 flow for several hours.

0.1 mL of TEOS was added to 0.1 mL of 0.01 mol/L HCl and the mixture was allowed to stir at 60 °C in water bath until a homogenous solution (A) was obtained. KH-560 derived β-cyclodextrin (synthesized according to reference [22]) 0.05 g was dissolved in a solution of 0.5 mL of 0.01 mol/L HCl and 0.3 mL of acetonitrile, and the whole mixture (B) was stirred to thoroughly dissolve the materials. Then A and B were mixed, and the whole mixture was stirred at room temperature for 5 min before centrifugation. The supernatant was used for coating. The solution was first allowed to fill capillary and left static for 20 min, and then it was driven out slowly with the aid of N_2, followed