Journal of Chromatography A, 1485 (2017) 24–31

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Urea-formaldehyde monolithic column for hydrophilic in-tube solid-phase microextraction of aminoglycosides

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ARTICLE INFO

Article history:
Received 4 November 2016
Received in revised form 9 January 2017
Accepted 9 January 2017
Available online 10 January 2017

Keywords:
Aminoglycosides
Evaporative light scattering detector
Hydrophilic interaction
In-tube solid-phase microextraction
Urea-formaldehyde monolithic column

ABSTRACT

A novel urea-formaldehyde (UF) monolithic column has been developed and exploited as a sorbent for hydrophilic in-tube solid-phase microextraction (in-tube SPME) of aminoglycosides (AGs). Because of the innate hydrophilicity, UF monolith showed high extraction efficiency towards these hydrophilic analytes. The adsorption capacities for target compounds dissolved in water/ACN (1:1, v/v) were in the range of 5.18–7.36 μg/cm. Due to the lack of a chromophore, evaporative light scattering detector (ELSD) was selected as the detector for AGs, and coupled with the online in-tube SPME-HPLC system. Several factors of the online system, such as trifluoroacetic acid (TFA) and ACN percentage in the sampling solution, ionic strength in the sample solution, elution volume, sampling and elution flow rate, were optimized with respect to the extraction efficiencies. Under the optimized conditions, the limits of detection (LODs) of streptomycin, tobramycin and neomycin were discovered in the range of 3.0–5.2 μg/kg. The recoveries were ranged from 82.1 to 96.7% with relative standard deviations (RSDs) of 2.3–5.1% (n = 4) at spiking levels of 50, 200 and 500 μg/kg, respectively. The excellent applicability of the UF monolithic column was examined by the determination of streptomycin in practical tilapia samples, which showed the potential advantages for the analysis of polar analytes in complicated samples.

1. Introduction

Aminoglycosides (AGs) are broad-spectrum antibiotics used to treat Gram-negative bacterial infections, and they are also used in food-producing animals to perform as growth promoters [1,2]. AGs possess ototoxicity and nephrotoxicity which, due to their low cost, do not hinder the widespread use of AGs in therapeutic and veterinary applications [3,4]. Due to their high affinity to tissue, AGs result in a high residue level and prolonged withdrawal times [5]. Thus, it is imperative to monitor AGs residues in food for control and monitoring purposes. EC Regulation 37/2010 establishes maximum residue limits (MRLs) for some AGs in different samples. For example, MRLs for streptomycin and neomycin in the muscle of different animal species have been set in the concentration of 500 μg/kg.

Since AGs are frequently used in various applications, the residues could be found in different samples, such as body fluids and tissues, food of animal origin and environmental samples. Considering these sample matrices, liquid chromatographic (LC) methods, including reserved-phase liquid chromatography (RPLC) [6,7], ion-pair liquid chromatography [8,9], and hydrophilic interaction chromatography (HILIC) [10,11] have been utilized as the suitable methods for the analysis of AGs. With respect to these LC-based methods, there is a challenge that because of the lack of a chromophore, UV or fluorescence detection could not be used, unless at a wavelength of 195 nm, which is not applicable in complex samples [3]. Alternative detection technologies for LC, such as evaporative light scattering detection (ELSD) [12], pulsed electrochemical detector (PED) [13], refractive index detection (RI) [14], extreme UV absorption method, charged aerosol detector (CAD) [15] and mass spectrometry (MS) [16–18], are used for their determination. However, RI is greatly affected by the mobile phases and the temperature, which is not sensitive enough and need to be

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http://dx.doi.org/10.1016/j.chroma.2017.01.027
0021-9673/

balanced for a long time. Extreme UV absorption method requires the hyperpure mobile phases. CAD and PED are rare detections, which are limited by the lacking of highly trained personnel. MS is too expensive to be widely applied, especially in poor economies and developing countries. Among these detection technologies, for its low running cost and wide application field, ELSD is commonly used in detecting the analytes which have lower volatility than mobile phase. Given that ELSD is not as sensitive as MS, to meet requires of the standards, high-efficiency extraction of AGs is indispensable for their valid determination.

Currently, several extraction methods, such as solid phase extraction (SPE) [12,16–23] and matrix solid phase dispersion [24] have been employed for the extraction of AGs. Among them, SPE has been a well-established technique for treating different kinds of samples. Several kinds of SPE sorbents (e.g. weak cation exchange (WCX) [12,21,23], mixed mode of cation exchange/reversed phase [20] and Oasis HLB [18]) have been proposed to extract AGs. Due to the strong polarity of AGs, hydrophilic sorbents for AGs were found to be effective for extraction [25]. In-tube solid-phase microextraction (in-tube SPME), which addresses the need to facilitate rapid and efficient sample pretreatment, was first developed by Pawliszyn et al. [26,27]. Compared with SPE, in-tube SPME possesses the advantages of solvent-free, small sample volume, simplicity, and easy automation. In the last decade, monolith-based in-tube SPME has been proposed and characterized by utilizing monolithic column as extraction media or sorbent [28–31]. The extraction efficiency of monolith-based in-tube SPME is superior to the previous in-tube SPME, for which coated capillaries were employed as extraction media. Owing to its merits of high phase ratio, fast mass transfer and diverse surface functionalization, monolithic column has been considered as the promising sorbents for in-tube SPME and attracted tremendous attention [32–34]. Recently, hydrophilic monolithic column has been developed as sorbent for hydrophilic in-tube SPME of cyromazine and melamine [35]. So far, the report on the development of newly hydrophilic monolithic column for hydrophilic in-tube SPME of AGs is still rare.

More recently, we have proposed a facile way for rapidly fabricating a functionalized urea-formaldehyde (UF) monolithic column, which demonstrated highly hydrophilic retention when ACN percentage in the mobile phase exceeded 20% [36]. In this work, a simple urea-formaldehyde monolithic column was fabricated within a PTFE tube by rapid polycondensation. So far as we aware, this is the first report on developing a UF monolithic column for hydrophilic in-tube SPME. This UF monolithic column was characterized and online coupled with HPLC-ELSD to enhance sensitivities for detection of AGs. Several factors of the online system were optimized systematically. The proposed UF-monolith-based in-tube SPME was successfully applied for the microextraction of AGs in tilapia samples.

2. Experimental

2.1. Chemicals and materials

Streptomycin sulfate, neomycin sulfate and tobramycin were purchased from J&K Chemical (Beijing, China). Urea was supplied by Acros (New Jersey, USA). Formaldehyde solution, trifluoroacetic acid (TFA), trichloroacetic acid (TCA), sodium hydroxide, EDTA and hydrochloric acid were obtained from Shanghai Zhanyun Chemical LTD (Shanghai, China). Acetonitrile (ACN), *n*-hexane and methanol (Chemical Reagent Corporation, Shanghai, China) were of HPLC grade. Deionized water was obtained by using a Millipore Milli-Q purification system (Milford, MA, USA). Polytetrafluoroethylene (PTFE) tubes (750 μm i.d.) were obtained from Unimicro Technologies (Shanghai, China).

2.2. Instrumentation and analytical conditions

The in-tube SPME-HPLC-ELSD system consisted of the pre-extraction segment, which included a Rheodyne 7725i six-port valve (valve 1), a LC-10AD pump (pump A) (Shimadzu, Kyoto, Japan) and a PEEK tube (0.03 in. i.d., 0.5 mL total volume), and the analytical segment, which included a LC-10AD pump (pump B) (Shimadzu, Kyoto, Japan), a VICI six-port valve (valve 2) with 10 cm UF polymeric monolithic column and an Alltech 3300 evaporative light scattering detector (ELSD) (Grace Alltech, Illinois, USA). The online in-tube SPME-HPLC-ELSD manipulation was referred to our previous work [37] with some modifications.

Before extraction, valves 1 & 2 were initially set at LOAD positions. The sampling solution (0.2% TFA solution containing 50% (v/v) ACN) was driven by pump A to flow through the monolithic column for conditioning at 0.2 mL/min. The mobile phase was driven by pump B directly through the analytical column to obtain a stable baseline for chromatographic separation. Meanwhile, the PEEK loop was filled with the sample solution using a syringe.

When extraction began, valve 1 was directed towards INJECT position for a given time and returned to LOAD position immediately to perform extraction. The sampling solution was kept to flow through the monolithic column for 90 s in order to eliminate the residual sample solution and reduce the interference.

Then, the extracted analytes were desorbed from the monolithic column by the mobile phase at a flow rate of 0.1 mL/min by simply switching the valve 2 to the INJECT position. When extraction had finished, valve 2 was switched to the LOAD position, and followed by adjusting the flow rate of the mobile phase to 0.7 mL/min for separation.

A Syncronis 5u C18 chromatographic column (250 × 4.6 mm) from Thermo (Boston, USA) was used for the separation. Experimental conditions for the online in-tube SPME-HPLC-ELSD method were optimized as followed: the mobile phase for HPLC separation was 0.2% TFA solution/ACN (90:10, v/v) at a flow rate of 0.7 mL/min; sample volume was 0.5 mL; column temperature was 40 °C; ELSD was maintained at 60 °C with a flow of N_2 stream set at 1.5 L/min; detector gain for ELSD was 16. In addition, SEM images were obtained with an XL30E scanning electron microscope (Philips, Netherlands). The refrigerated centrifuge was purchased from Thermo Scientific (Boston, USA).

2.3. Preparation of urea-formaldehyde monoliths

A PTFE tube was treated and rinsed with methanol, then dried by nitrogen stream. The reaction mixture was consisted of 1 g/mL urea (450 mg), formaldehyde solution (550 mg), 0.1 mol/L HCl solution (100 mg), which was followed our previous work with some modification [36]. The mixture was allowed to fill the tube. The tube was sealed at both ends with rubbers immediately and submerged into a thermostatic bath at 80 °C for 10 min. After that, the obtained monolithic column was rinsed using a μHPLC pump with water for 1 h, then methanol for 2 h to remove the residues. Finally, the monolithic column was cut to an appropriate length for use.

2.4. Sample preparation

Tilapia was purchased from the local supermarket. Tilapia sample was pretreated according to previous work with some modification [38]. In brief, tilapia muscle samples were homogenized using a food blender and a 10 g sample was transferred to a 50 mL centrifuge tube. Then, 200 μL of 150 mmol/L EDTA was added to each tube. The samples were allowed to stand for 10 min. A volume of 10 mL of 15% TCA solution was added and the tubes were mixed in a shaker for 20 min. The mixture was centrifuged at 4 °C with 8400 r/min for 20 min to remove protein from the matrix. After that,