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On-line coupling of hydrophilic ionic liquids-based polymer monolith microextraction to capillary liquid chromatography with amperometric detection: An ultrasensitive residue analysis method for glycopeptide antibiotics☆

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ABSTRACT

A hydrophilic ionic liquids based polymer monolith microextraction (PMME) column (poly(ionic liquid-co-hydroxyethyl methacrylate-co-ethylene dimethacrylate); poly(IL-co-HEMA-co-EDMA)) was prepared for the first time in a capillary and utilized in PMME for the enrichment of glycopeptide antibiotics (GAs), followed by the online coupling analysis of capillary liquid chromatography with amperometric detection (cLC-AD). The prepared monolith exhibited large through pores and good storage stability, as well as a selective extraction machanism for GAs that was attributed to the hydrogen bonding, hydrophilic, electrostatic and π-π interactions between GAs and the imidazolium cations or hydroxyl groups on the surface of absorbent. Several experimental parameters, such as sample flow rate, composition of eluent and solvent desorption conditions, were examined to improve the extraction efficiency of PMME. Under the optimal conditions, the proposed PMME-cLC-AD method provides detection limits (S/N = 3) of 1.0–8.0 μg L^{-1} for three GAs, which are 1000-fold lower than those obtained by cLC-AD, with a wide linear range of 10.0–12000.0 μg L^{-1}. It was successfully applied for the analysis of GAs residues in feed samples with good recoveries (80.3–119.1%) and satisfied intra-day/inter-day precision (<10%). Compared with LC-3Q-MS method, the proposed online approach has the merits of simple, low cost, smaller matrix interference and environmental friendly, which is demonstrated to be a feasible tool for residue analysis of peptide antibiotics in food safety application.

1. Introduction

During the past two decades, the problem of antibiotic residue and resistance has become an increasing concern in areas of food safety and human health. Glycopeptide antibiotics (GAs), a class of glycosylated non-ribosomal peptides of microbial origin, have been used largely as anti-gram-positive cocci drug or antibiotic growth promoters in animals. In particular, vancomycin and teicoplanin are used to be the defense line for cases of methicillin-resistant *Staphylococcus aureus* (MRSA). However, clear evidences showed that antibiotic-resistant bacteria and antibiotic residues, which are related to the overuse of GAs in animal feeds, can transfer from animal to human via the food chain and impact possible risk on human health [1,2]. Due to their toxicity, many countries have set regulatory limits for GAs residues in animal-derived food [3]. For example, USA and China have restricted the addition of vancomycin in animal foodstuff. According to the Japan regulation, the maximum residue level of vancomycin in milk is 10 μg/kg. The sensitive analysis and monitoring of residual GAs in animal-derived food are therefore of highest concern.

Several methods have been developed for GAs analysis, primarily in clinical body fluids, including liquid chromatography [4,5], spectrophotometric methods [6], immunologic method [7], sensor [8] and micellar electrokinetic chromatography [9]. LC coupled to MS or UV detector is the most commonly utilized instrumental method for GAs residue analysis in animal-derived food [10].

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Although LC–MS has better capability in identification and sensitive detection of multiple GAs than LC–UV, this method requires expensive instruments, highly usage costs and trained personnels, thus it is more suitable for the confirmation purpose. Compared with LC, capillary LC (cLC) using a smaller column offers several advantages for the high-efficiency separation of complicated sample, including less sample and solvent consumption, taller peaks and lower detection limits. cLC can be effectively conjuncted with a highly sensitive detector such as electrochemical or laser-induced fluorescence detector, which closely matches the drastically reduced column dimensions [11]. This coupling technique represents a promising field for both improving separation and detection sensitivity [12,13], and has been widely applied in the analysis of environmental and biological samples [14,15].

The residue analysis of GAs in animal-derived samples is a challenging task, due to the complex matrix and their very low residual levels. Prior to LC analysis, sample pretreatment method such as solid phase extraction is usually required for reducing the matrix interference and improving the detection sensitivity of trace targets [16], but it is time-consuming, cumbersome and still an off-line method. Therefore, the development of efficient and rapid online sample pretreatment methods for GAs analysis is in great demand.

Miniaturized sorbent-phase extraction, typically solid phase microextraction (SPME), is one of the most powerful and economic sample pretreatment techniques. It has the merits of simple, low sample and solvent consumption, fast and easy online operation. As an alternative of fiber-based SPME, polymer monolith microextraction (PMME) uses organic monolithic materials as the biocompatible extraction sorbent, greatly improving the lacks of the extraction capacity and sorbent stability in conventional fiber- or coating-based SPME [17–19]. Additionally, the on-line coupling of PMME with LC, cLC and CEC or ICP-MS avoids the loss of analytes and the troublesome off-line operation, thus ensuring the sensitivity, accuracy and speed for analysis of organic pollutants, inorganic ions, biological molecules, nanoparticles or drugs in various sample matrices [20–24]. Owing to their unique bimodal meso/macroscopic porous structures, ploymer monoliths can provide fast and efficient dynamic mass transfer, low backpressure, and high surface area for sample loading and washing in extraction [25]. However, in the case of real sample application, the most popular methacrylate-based monoliths [26] in smaller devices tend to shrink or swell during the organic solvents treatment procedures, resulting in poor mechanical stability and reduced extraction efficiency. These problems may be minimized by grafting the monoliths to the capillary or tubing walls and further derivatization. Recently, great efforts have been focused on the incorporation of polymer monoliths with various functional materials, including carbon nanomaterials [27], metal-organic frameworks [28], nanometallic particles and oxides [29,30], molecularly imprinted polymers [31] and ionic liquids (ILs) [32,33]. Because of their unique physicochemical properties of low vapor pressure, good solubility and designability, ILs have been widely used as buffer additives, modifiers and covalently bound monomers in chromatographic and microseparation fields to offer better separation capability for different analytes [34–40]. The incorporation of ILs in monoliths demonstrated the multiple interaction mechanisms (eg, electrostatic, π-π interaction, hydrogen bonding, etc.), and the improved mechanical stability as well.

In this work, a new hyphenated method for on-line enrichment and sensitive analysis of GAs was established by coupling PMME to cLC with amperometric detection (AD). For the purpose of effective extraction, a hydrophilic imidazolium ionic liquids based polymer monolithic capillary microextraction column poly(IL-co-HEMA-co-EDMA) was prepared by utilizing ethylene dimethacrylate (EDMA) as the cross-linker and binary polar monomers of hydroxyethyl methacrylate (HEMA) and hydrophilic 1-butyl-3-vinylimidazolium bromide ([VBIM][Br]). The effect of polymerization conditions on the porous structure and the permeability of monolith were investigated and characterized. The experimental conditions for PMME on-line extraction and cLC-AD analysis were optimized, and the quantitative parameters were examined for method validation. To demonstrate the capability for antibiotics residue analysis, the proposed method was applied for the analysis of GAs in animal feeds samples, and a comparative experiment with the official method (LC-3Q-MS) was executed.

2. Material and methods

2.1. Instrumentation

A laboratory-constructed coupling system of a Trisep™-2100 cLC (Unimicro, Pleasanton, USA) with an LC-3D amperometric detector (BAS, USA) [15] was used for cLC-AD analysis, which consists of a LC pump, a microfluid manipulation module (including a six-port injector), a packed cyano capillary column (50 μm I.D. × 375 μm O.D., 3 μm; Unimicro, Shanghai) and a constant-potential amperometric detector with end-column detection cell. The current flowed through the working electrode (300 μm diameter carbon disc, vs. Ag/AgCl reference electrode) and platinum auxiliary electrode was recorded with a HW-2000 chromatographic workstation (Qianpu Software, Shanghai, China). A PMME unit composed of a KDS101 syringe infusion pump (KD Scientific, USA), a LC pump A (Unimicro, Shanghai), a six-channel multifunctional valve (Valco Instruments Co. Inc, USA) and a capillary PMME monolith, was used in combination with the cLC-AD system for online enrichment of targets.

Structural and electrochemical characterization apparatuses employed in this study include a Thermo Nicolet 6700 Fourier transform infrared spectrometer (Thermo Fisher, USA), a Nova NanoSEM230 field emission scanning electron microscope (FEI, Holland) and a CHI 660 electrochemical workstation (Shanghai CH Instruments, China). BF 2000 Nitrogen blowing concentrator (Bafang Century, Bijing, China) was used for sample concentration. LC-3Q-MS (Thermo Fisher, USA) was used for the comparative study.

2.2. Chemicals and reagents

Vancomycin was purchased from Sigma-Aldrich (St. Louis, MO, USA). Norvancomycin and teicoplanin were purchased from the national institute for the control of pharmaceutical and biological products (Beijing, China). 1-butyl-3-vinylimidazolium bromide ([VBIM][Br]) (98%) (Chengjie Chemical, Shanghai, China) and hydroxyethyl methacrylate (HEMA) (Jingchun, Shanghai, China; 98%) were selected as monomers. Dodecanol (Fuchen Chemical, Tianjin, China) and cyclohexanol (≥98.5%; Aladdin, Shanghai, China) were used as porogens. Ethylene dimethacrylate (EDMA) (98%) and 3-methacryloxypropyltrimethoxysilane (γ-MAPS) (99%) were purchased from J&K Scientific (Beijing, China). 2,2′-Azobis(2-methylpropionitrile) (AIBN) as initiator was purchased from Shanghai No.4 Reagent Factory (Shanghai, China). Methanol (MeOH) and acetonitrile (ACN) of HPLC grade (Sinopharm, Shanghai, China) were used without further purification. All other reagents are analytical pure grade or better. Milli-Q ultra pure water (18.2 MΩ·cm, Millipore, Bedford, MA, USA) was used throughout the experiments. A HyperSep SCX solid phase extraction cartridges (200 mg/6 mL) was purchased from Shanghai Mailong Technology Co., Ltd. (Shanghai, China).

All glycopeptide antibiotics stock solutions were prepared individually in ultrapure water at a concentration of 1.0×10^4 mg L^{-1} and stored at 4 °C in darkness. A standard working solution was