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Covalent triazine framework-1 as adsorbent for inline solid phase extraction-high performance liquid chromatographic analysis of trace nitroimidazoles in porcine liver and environmental waters

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ABSTRACT

In this study, covalent triazine framework-1 (CTF-1) was adopted as solid phase extraction (SPE) sorbents, and a method of SPE inline coupled with high performance liquid chromatography-ultraviolet (HPLC-UV) detection was developed for trace analysis of three nitroimidazolaes (including metronidazole, ronidazole and dimetridazole) in porcine liver and environmental water samples. CTF-1 has rich π-electron and N containing triazine, thus can form π–π interaction and intermolecular hydrogen bond with three target polar nitroimidazoles, resulting in high extraction efficiency (87%–98%). Besides, CTF-1 has large specific area, which benefits rapid mass transfer and low column pressure, leading to fast adsorption/desorption dynamics. Several parameters affecting inline SPE including pH, sample flow rate, sample volume, desorption reagents, elution flow rate, elution volume, and ionic strength were investigated. Under the optimal experimental conditions, the limits of detection (S/N = 3) were found to be in the range of 0.11-0.13 μg/L. The enrichment factors (EFs) ranged from 52 to 59 fold (theoretical EF was 60-fold). The relative standard deviations were in the range of 4.3–9.4% (n = 7, c = 1 μg/L), and the linear range was 0.5–500 μg/L for three target analytes. The sample throughput is 7/h. The proposed method was successfully applied to the analysis of nitroimidazoles in porcine liver and environmental water samples with good recoveries for the spiked samples.

1. Introduction

Metronidazole (MNZ), ronidazole (RNZ) and dimetridazole (DMZ) are typical antiprotozoal and antibacterial agents that are widely used to prevent and treat histomoniasis and coccidiosis in poultry and fishery. However, nitroimidazoles and their metabolites are found to have mutagenicity, genotoxicity, and carcinogenicity [1]. To prevent these compounds from entering environment and eventually intaking by human, their use in food animals has been banned by European Union, USA and China. In China, the maximum residue limits (MRLs) of DMZ and MNZ in porcine and poultry muscle, kidney, and liver are 5 and 50 μg/kg, respectively [2]. The presence of antibiotics such as MNZ in sewage and natural water has been already reported [3,4]. The environmental risks caused by antibiotics have been a serious concern for the public, and more researches in this area are required to get a fully understanding of their presence and concentration levels in ecosystems. Thus, analytical methodology for detection and quantification of nitroimidazoles in the environment is highly demanded in order to evaluate the exposition of the environment to nitroimidazoles and their risks.

Many analytical techniques have been applied for the analysis of trace nitroimidazoles in environmental samples including gas chromatography (GC) [5,6], high performance liquid chromatography (HPLC) [7–9] and capillary electrophoresis (CE) [10,11]. Among them, HPLC is the most popular analytical technique for nitroimidazoles due to its good resolution, high reproducibility, and easy inline operation. However, nitroimidazoles in environment samples are at trace level, and the matrix in the real-world sample is quite complicated; therefore, a suitable sample pretreatment technique to remove sample matrix and enrich analytes before HPLC analysis is highly demanded.

Different sample treatment techniques have been employed for the determination of nitroimidazoles in a great variety of matrices such as food, environmental and biological samples [11–19]. Among them, solid phase extraction (SPE) has been one of the

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most commonly employed techniques [8,14,17–19] due to its simple operation, high enrichment factor, rapid phase separation, and the ability to combine with different detection techniques. Generally, SPE could be operated in offline or inline mode. However, in order to improve sample throughput, minimize manual labor, and reduce the exposure to hazardous solvents, the automated and time effective inline SPE system has become increasingly popular [20].

It should be pointed out that the adsorbents used in SPE play a great role on the extraction performance [21]. The ideal SPE adsorbent should possess certain properties such as high selectivity to the interest analytes, fast adsorption/desorption dynamics, larger surface area, high adsorption capacity and high stability. Some commercial sorbents have been applied in the analysis of nitroimidazoles, these include mixed cation exchange sorbent (MCX) (Milford, MA, USA) [14], hydrophile lipophile balance sorbent (HLB) (Milford, MA, USA) [22] and strong cation exchange sorbent (SCX) (Milford, MA, USA) [8]. However, large volume of organic solvent is needed to completely elute the targets from the column and an evaporation step is usually required to reduce the volume of eluent in order to increase the sensitivity. Thus, the development of novel SPE adsorbents with higher extraction efficiency and faster extraction kinetics is still necessary.

Covalent triazine framework-1 (CTFs-1) [23] is a novel class of porous materials constructed from terephthalonitrile by ionothermal synthesis. It possesses good properties, such as good thermal/chemical stability and large surface area, and has been successfully applied in catalysis [24] and gas separation [25]. The π-conjugated system in CTF-1 could be as big as the frameworks itself, which could form strong π–π interaction with aromatic compounds. This implies that CTF-1 can be an effective adsorbent for aromatic compounds. However, the investigation and application of CTF-1 as the adsorbent is at the initial stage. Zhang et al. [26] synthesized magnetic porous covalent triazine framework composites, which exhibited high adsorption capacity (291 mg/g) for methyl orange. Asamanjoy et al. [27] studied the adsorption behavior of surfactants on CTF-1, and the adsorption capacity for alkyl polyglycolether surfactants was as high as 4 g/g.

Considering that nitroimidazoles compounds contain an aromatic heterocyclic imidazole ring, while triazine is an aromatic heterocyclic ring that contains N atom and large π-electron structure, CTF-1 is expected to be an efficient SPE adsorbent for nitroimidazoles based on the possible interaction including π-π interaction and intermolecular hydrogen bond. Thus, the purpose of this work is to develop a new method of CTF-1 based inline-SPE-HPLC-UV for the determination of nitroimidazoles in environmental samples. The experimental parameters affecting the extraction efficiencies of target nitroimidazoles by SPE such as pH, sample flow rate, sample volume, desorption reagents, elution flow rate, elution volume, and ionic strength were studied, and the analytical performance of the proposed method was evaluated. The developed method was finally applied to the analysis of nitroimidazoles in environmental water and porcine liver samples for validation.

2. Experimental

2.1. Reagents and standards

Terephthalonitrile and zinc chloride ($ZnCl_2$) were purchased from TCI Development Co., Ltd. (Shanghai, China). Methanol, ethanol, acetone, acetonitrile, tetrahydrofuran (THF), and sodium chloride (NaCl) were purchased from China Medicine (group) Shanghai Chemical Reagent Corporation (Shanghai, China). Solid reagents and all solvents used in this work were of analytical grade. High purity water obtained by a Milli-Q water purification system (18.25 MΩ cm, Millipore, Molsheim, France) was used throughout the whole experiments.

MNZ, RNZ and DMZ were purchased from J&K Acros Organics (New Jersey, USA). The chemical structures, log*Ko/w* and p*Ka* values of the target nitroimidazoles are shown in Table S1. Each standard stock solution (1 mg/mL) of nitroimidazoles was prepared in methanol, and a mixed standard solution containing 0.1 mg/mL of each nitroimidazoles was also prepared in methanol. All standard stock solutions were stored at 4 °C in the refrigerator.

2.2. Instrumentation

An Agilent 1100 HPLC (Agilent Technologies, Waldbronn, Germany) equipped with a degassing device, a quaternary pump, a 20-μL sample loop, and a variable wavelength UV–vis detector was used for separation and detection of nitroimidazoles. A reversed phase-C_{18} column (250 mm × 4.6 mm, 5 μm, Merck KGaA, Germany) was used for the separation of target nitroimidazoles, and a mixture of CH_3CN and H_2O at a volume ratio of 22:78 (v/v) was employed as the mobile phase at a flow rate of 1 mL/min. UV detection was performed at 320 nm.

A flow injection (FI) analysis processor (FIA-3110, Titan Instrument Co. Ltd., Beijing, China) and a self-made PTFE micro-column (20 mm × 2.0 mm i.d.) packed with CTF-1 were used in the inline SPE process.

Powder X-ray diffraction (PXRD) data was obtained by Panalytical EMPYREA diffractometer with CuKα1 (λ = 1.54056 Å) radiation operated at 40 kV and 40 mA. The nitrogen adsorption and desorption isotherms were measured at 77 K using a NOVA 4200e surface area size analyzer.

2.3. Synthesis of CTF-1

CTF-1 was synthesized according to the literature [23] by cyclotrimerization of 1,4-dicyanobenzene in molten $ZnCl_2$ at 400 °C. Typically, 0.52 g terephthalonitrile and 0.48 g $ZnCl_2$ were transferred into a pyrex ampoule under an inert atmosphere. The ampoules were evacuated, sealed and maintained at 400 °C for 40 h. The products were ground with a sieve of 200–500 mesh (particle size in the range of 30–70 μm), and then washed thoroughly with water, HCl, and THF sequentially.

2.4. Column preparation

20 milligrams of dried powder CTF-1 were filled into a PTFE micro-column. Some cotton wool was used on both ends to prevent packing losses. Before use, MeOH and H_2O were passed sequentially through the column in order to clean and condition the CTF-1 packed micro-column.

2.5. General inline SPE procedure

30 mL of sample solution was pumped through the micro-column at a flow rate of 2 mL/min. The adsorbed analytes were eluted with the 0.5 mL of MeOH at a flow rate of 1 mL/min. An inline SPE-HPLC-UV system is illustrated in Fig. 1. An FI system, consisting of pumps (A, B and C), an eight-way valve and a six-port valve, was used for inline coupling SPE and HPLC-UV, and two SPE columns were parallelly placed in the FI system. As listed in Table 1, the designed programs included several steps. Initially, the FI valve was in position 0 and the six-port valve was in INJECT position. In step 1 (shown in Fig. 1a), pump A and pump B pumped sample solution for 15 min into the SPE column 1 (C1) and SPE column 2 (C2), respectively, for the sample loading. At step 2, the FI valve was switched to position 1. C1 started elution, and the eluent was