Journal of Chromatography B, 1007 (2015) 127–131

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Short communication

Synthesis of monodisperse molecularly imprinted microspheres with multi-recognition ability via precipitation polymerization for the selective extraction of cyromazine, melamine, triamterene and trimethoprim

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ARTICLE INFO

Article history:
Received 3 August 2015
Received in revised form 4 November 2015
Accepted 8 November 2015
Available online 12 November 2015

Keywords:
Molecularly imprinted polymers
Precipitation polymerization
Pseudo template
Multi-recognition

ABSTRACT

Through precipitation polymerization, three monodisperse molecularly imprinted polymers (MIPs) containing imprints of 2,4-diamino-6-methyl-1,3,5-triazine (DM), cyromazine (CY) or trimethoprim (TM), were synthesized using methacrylic acid as functional monomer, divinylbenzene as cross-linker, and a mixture of acetonitrile–toluene (90/10, v/v) as porogen. The morphology and selectivity of the MIPs were characterized and compared systematically. The MIPs had the best specific binding in pure acetonitrile, and the data of adsorption experiment were fitted well with Langmuir and Freundlich model. In addition, DM-MIPs showed the excellent binding and multi-recognition capability for CY, melamine (ME), triamterene (TA) and TM, and the binding capacity were 7.18, 7.56, 5.66 and 5.45 μmol/g, respectively. Due to the pseudo template and the ability of multi-recognition, DM-MIPs as sorbent material could avoid the effect of template leakage on quantitative analysis. Therefore, DM-MIPs were used as a solid-phase extraction material to enrich ME, CY, TA and TM from different bio-matrix samples for high performance liquid chromatography analysis. Under the optimized conditions, the recoveries of three spiked levels in different bio-matrix samples were ranged from 80.9% to 91.5% with RSD ≤ 4.2 ($n = 3$).

1. Introduction

Cyromazine (CY, *N*-cyclopropyl-1,3,5-triazine-2,4,6-triamine) is an inset growth regulator as an acaricide and an insecticide. It is a cyclopropyl derivative of melamine (ME, 2,4,6-triamine-1,3,5-triazine) and can be metabolized into ME. ME was illegally added to animal-derived food to falsely enhance protein levels [1]. Triamterene (TA, 6-phenylpteridine-2,4,7-triamino) is a potassium-sparing diuretic for the treat of hypertension and edema, and banned for sport. The World Anti-Doping Agency and International Olympic Committee have required that the concentration of TA in urine is no more than 0.2 μg/mL [2]. Trimethoprim (TM, 5-(3,4,5-trimethoxybenzyl) pyrimidine-2,4-diamine) is a bacteriostatic antibiotic, which is mainly used in the prevention and treatment of urinary tract infections. The analysis of TM in serum is important in the infected patients [3]. Therefore, it is important to have efficient methods for determination of CY, ME, TA and TM in different samples.

Molecularly imprinted polymers (MIPs) with higher selectivity are increasingly applied to pre-concentration of bio-matrix samples [4]. In the preparation of MIPs, an excess of cross-linker was universally used to obtain the specific rigid binding cavities. The result is the imprinted sites not only located at the surface, but also the interior area. The interior template is difficult to completely elute, therefore, the potential risk of template leakage should not be neglected [5]. The imprinting based on pseudo template has been regarded as the most effective strategy to overcome the problem of template leakage, since the approach allows the structural analogue of the target analyte as template. The pseudo template MIPs for TM, TA, CY and ME have been successfully synthesized and applied in enrichment of different samples, respectively [6–8]. These compounds all included the same fragment (2,4-diamino pyrimidinyl, Fig. S1 in ESM). If pseudo template including the same fragment was used to prepare MIPs, they could possess the

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http://dx.doi.org/10.1016/j.jchromb.2015.11.009
1570-0232/

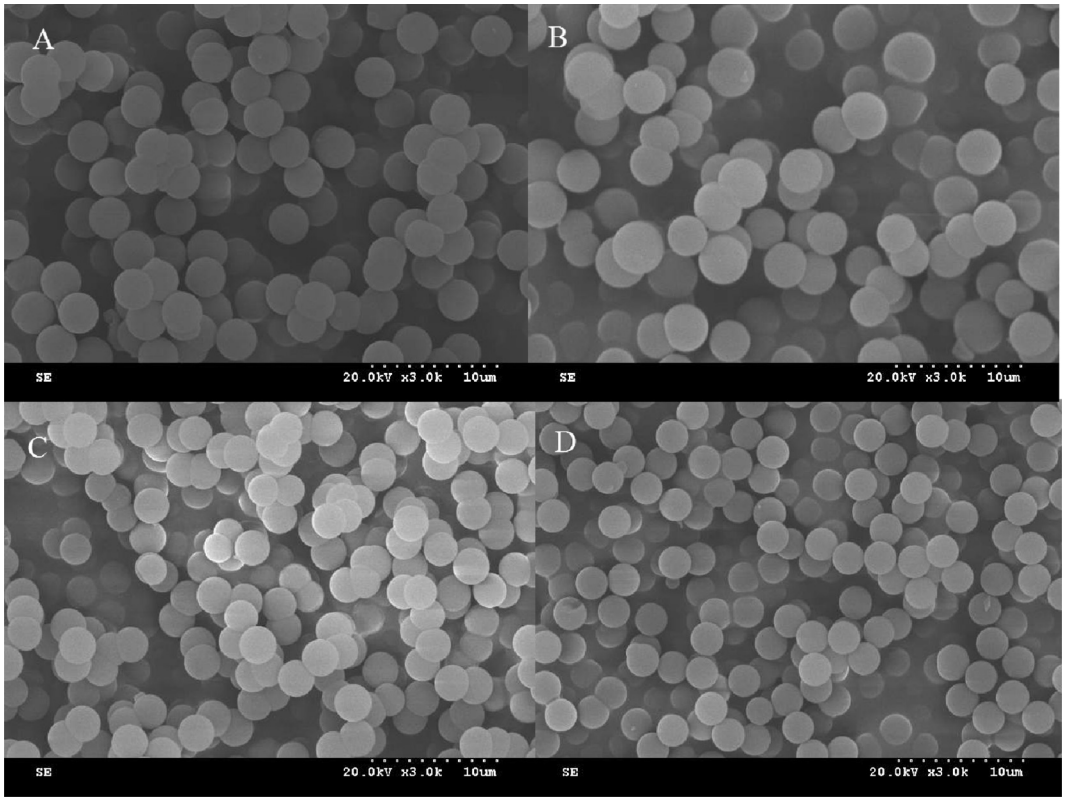

Fig. 1. SEM images of the TM-MIPs (A), CY-MIPs (B), DM-MIPs (C) and NIPs (D).

multi-recognition for these compounds. In our previous report, multi-recognition monolithic MIPs were prepared by in situ polymerization, and the retention mechanism was studied in detail [9]. MIPs synthesized by this method have to be ground and sieved to deliver particles of irregular shape for the intended application, which is low yield and time-consuming process. Precipitation polymerization has been used to prepare MIPs beads, because there is no need to add polar solvents and surfactants to the reaction system, and the prepared MIPs beads are monodisperse while retaining high selectivity [10].

In this work, three MIPs beads were prepared through precipitation polymerization method using 2,4-diamino-6-methyl-1,3,5-triazine (DM), CY and TM as template molecule, respectively. A detailed comparison for equilibrium binding properties of three resulting MIPs was performed to examine the multi-recognition capability for CY, ME, TA and TM. Finally, the multi-recognition DM-MIPs beads were used in MIPs solid-phase extraction (MISPE) protocols for selective extraction these analytes from different biomatrix samples, respectively.

2. Experimental

2.1. Reagents and chemicals

2,4-Diamino-6-methyl-1,3,5-triazine (DM, catalog number 407801), cyromazine (CY, catalog number 1161203), melamine (ME, catalog number M2659), triamterene (TA, catalog number 1680007), trimethoprim (TM, catalog number 92131), divinylbenzene (DVB, catalog number 414565), were purchased from Sigma–Aldrich (St. Louis, MO, USA). Methacrylic acid (MAA), toluene, acetic acid, and 2,2′-azobisisobutyronitrile (AIBN) were obtained from Kermel Chemical Reagents Development Center (Tianjin. China). Acetonitrile (ACN) and methanol (MeOH) were from Tianjin Biaoshiqi Chemical Reagent (HPLC grade, Tianjin, China). All other reagents were analytical or HPLC grade from Tianjin Chemical Reagent Ltd. Co. (Tianjin, China).

2.2. Instruments

The chromatographic analysis was carried out using Angilent 1100 series chromatographic system (Angilent Technologies, Palo Alto, CA, USA). Data processing was performed with an HPCORE workstation. TA, DM, CY and ME were analyzed on a BaseLine SCX column (150 × 4.6 mm I.D., 4 μm, Tianjin BaseLine Chromtech Research Center, Tianjin, China). For TA, the mobile phase was MeOH-acetate buffer (20 mM NaAc, pH 4.0) (60:40, v/v), and detection wavelength was set at 367 nm. For DM, ME and CY, the mobile phase was MeOH-buffer (50 mM KH_2PO_4, pH 3.0) (25:75, v/v), and detection wavelength was 214 nm. The column for TM analysis was an Aqua C18 column (150 × 4.6 mm, I.D., 5 μm, Phenomenex, Torrance, USA) using MeCN–water–triethylamine (adjusted to pH 6.4 by NaOH, 200:799:1, v/v/v) as mobile phase with detection wavelength of 270 nm. The flow rate was 1.0 mL min^{-1}, and the injection volume was 20 μL.

2.3. Synthesis of MIPs and NIPs

The MIPs were prepared via precipitation polymerization according to previous report [11]. Template (0.8 mmol), MAA (8.0 mmol), DVB (32 mmol), ACN (90 mL), toluene (10 mL) and AIBN (80.0 mg) were added to a round-bottom flask (250 mL) successively. A clear solution was obtained after stirring at ambient temperature for 30 min. The reaction mixture was degassed with N_2 for 10 min, sealed and rotated slowly using a magnet rotor. Reaction temperature was raised form 25 to 60 °C for 1 h and then kept at 60 °C for 23 h. The polymer particles were washed with acetic acid/methanol (1:9, v/v), methanol stepwise by Soxhlet extraction.