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Selective extraction of methenamine from chicken eggs using molecularly imprinted polymers and LC-MS/MS confirmation

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ABSTRACT

Molecularly imprinted polymers (MIPs) were synthesized as the selective sorbents for solid phase extraction (SPE), which was proposed for the determination of methenamine in chicken eggs by high performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS). The MIPs were prepared using methenamine as the template molecule, methacrylic acid (MAA) as the functional monomer and ethylene glycol dimethacrylate (EGDMA) as the cross-linker. The scanning electron micrograph was applied to characterize the morphologies of MIPs and non-imprinted polymers (NIPs), and the static and dynamic adsorption experiment were studied. The compositions and volume of the loading and eluting solvent were optimized. Linear calibration curve was obtained by chicken egg matrix-matched standard, within the range of 1.0–50.0 μg L^{-1} ($R^2 = 0.9983$). The limit of detection (LOD) and limit of quantification (LOQ) were 0.6 μg kg^{-1} and 2.0 μg kg^{-1}, respectively. Recoveries of methenamine in chicken eggs samples were in the range of 88.0%–112.8%. The relative standard deviation (RSD) of intra-day assay ranged from 2.3% to 8.7%, and inter-day assay ranged from 4.8% to 10.9%.

1. Introduction

Methenamine is an organic compound that is usually used for the urinary tract infection therapy (Musher & Griffith, 1974). In the acidic environment, methenamine may decompose into formaldehyde and ammonia. The urinary tract infection treatment mechanism by methenamine is attributed to formaldehyde (Ariens, Hanselaar, Henderson, & Simonis, 1982). Because it is economical and practical, methenamine is also utilized to reduce disease in poultry farming, for instance treatment of coccidiosis in chicken (Yuan, 1985). This practice may lead to veterinary drug residues exist in agricultural and sideline products. However, veterinary drug residues may cause human allergic reactions or antibiotic resistance. Moreover, formaldehyde that was from methenamine has carcinogenicity, acute and chronic toxicity (Ariens et al., 1982). Methenamine is listed as the possible illegal added non-edible substances and abusive food additives (IV) by Chinese Ministry of Health (2010). Nowadays, researchers begin to concern about the potential harm of methenamine as veterinary drug residue in animal-derived food. It may endanger the public health and safety. In view of this situation, it is necessary to develop a reliable method to determine the methenamine in food.

Determination methods for methenamine are mainly concentrated in the medical field (Mirza, George, Bodenmiller, & Belanich, 1998; Pavitrapok & Williams, 2006). In recent years, some researchers have poured attention to analyze methenamine in the food field. As an example, methenamine was extracted from cheese, and analyzed by LC-MS/MS after centrifugation and membrane filtration (Fuselli et al., 2012). Because the matrix effects for methenamine are obvious in mass spectrometry, some improved methods have been used to compensate the matrix effects, such as the combination of isotope dilution mass spectrometry technique and GC-MS/MS to determine methenamine in dairy products (Xu et al., 2015); a QuEChERS method with stable isotope internal standard and HPLC-MS/MS was applied to analyze methenamine residues in edible animal tissues (Xu, Zhang, Duhoranimana, Zhang, & Shu, 2016). In general, reference material labeled with isotope is expensive. In view of the popularization, the more economical

Abbreviations: ACN, acetonitrile; AIBN, 2,2′-azobisisobutyronitrile; EGDMA, ethylene glycol dimethacrylate; LC-MS/MS, liquid chromatography coupled with tandem mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; MAA, methacrylic acid; MIPs, molecularly imprinted polymers; NIPs, non-imprinted polymers; PDA, Photo-Diode Array; RSD, relative standard deviation; SEM, scanning electron microscopy; SPE, solid phase extraction.

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approach needs to be developed. More recently, molecular imprinting technique has been used for enrichment and separation of target compounds (Figueiredo, Erny, Santos, & Alves, 2016; Ji et al., 2015; Turiel & Martin-Esteban, 2010). Molecularly imprinted polymers (MIPs) have specific binding sites for a template molecule (Garcia et al., 2015; Tiwari & Prasad, 2015; W.; Yang et al., 2016), which are synthesized by the copolymerization of functional monomers and cross-linkers in the presence of the template molecule (Alenazi, Manthorpe, & Lai, 2015; Martin-Esteban, 2013). Therefore, MIPs are often used as selective sorbents in the SPE (Chrzanowska, Poliwoda, & Wieczorek, 2015; Liu et al., 2015; Xiao, Yan, Xu, & Li, 2015), and target compounds can be efficiently extracted and purified from various matrices (Miao et al., 2015; Pardeshi, Dhodapkar, & Kumar, 2014; K. J.; Tang et al., 2014).

Therefore, the aim of this study was to synthesize specific MIPs for methenamine, and develop a MIP-SPE method to analyze methenamine in chicken eggs samples. The scanning electron microscopy (SEM) was generally applied to characterize the properties of MIPs and non-imprinted polymers (NIPs) (Ji et al., 2014; Ma et al., 2015). Moreover, the binding capacity of MIPs and NIPs for methenamine was systematically investigated. Additionally, the solvent for loading and eluting was optimized. The method validation such as linearity, accuracy, precision LOD and LOQ were evaluated.

2. Materials and methods

2.1. Reagents and materials

The reagents were analytical reagent grade unless otherwise specified. Methenamine and melamine (purity $\geq$ 99%) were obtained from Sigma-Aldrich (St. Louis, USA). HPLC grade acetonitrile (ACN), methanol and ethanol were from Merck KGaA (Darmstadt, Germany). 2,2′-Azobisisobutyronitrile (AIBN), methacrylic acid (MAA) and ethylene glycol dimethacrylate (EGDMA) were from the ANPEL Scientific Instrument Co., Ltd (Shanghai, China). Ammonia hydroxide and acetic acid glacial were obtained from Chongqing Chuandong Chemical Co. (Chongqing, China). Ultrapure water was produced by a Biogen Ultrapure Water Type1 (Weston, USA). Organic phase needle type filters (0.22 μm nylon), 3 mL empty polypropylene SPE cartridges and 20 μm polyethylene frits were from ANPEL Scientific Instrument Co., Ltd (Shanghai, China). Strata-X-C, Strata-WCX and Strata-SCX solid phase extraction cartridges were obtained from Phenomenex (Torrance, USA). Standard stock solutions of methenamine (1000 mg L^{-1}) were respectively prepared in ACN. Standard working solutions were prepared from the stock solution by serial dilution. All solutions were stored in the dark at −20 °C.

2.2. LC and LC-MS/MS condition

Liquid chromatographic analysis was performed using Waters ACQUITY UPLC (Milford, USA). Separations were carried out with a Phenomenex Luna HILIC (100 mm × 2.0 mm I.D., 3 μm; Torrance, USA) analytical column, which was maintained at 40 °C. Methenamine was detected at $\lambda = 210$ nm by Photo-Diode Array (PDA) detector. The mobile phase consisted of 5.0 mmol L^{-1} of ammonium acetate in water (solvent A) and ACN (solvent B). The flow-rate and injection volume was 0.8 mL min^{-1} and 5.0 μL respectively. The elution program was: 0–10 min (70%) B. Liquid chromatographic analysis was used for adsorption experiments.

The LC-MS/MS system was consisted of a Shimadzu Prominence UFLC system (Kyoto, Japan) and API 3200 triple quadrupole mass spectrometer (AB SCIEX Corp., Framingham, USA) equipped with an electrospray ionization (ESI) source. Methenamine was analyzed in multiple reactions monitoring (MRM) acquisition mode through positive monitoring mode. The ion pair 141 > 112 was quantitative, and 141 > 85 was qualitative ion pair. Other measurement conditions referred to literature (Xu et al., 2016). LC-MS/MS was used for determination of methenamine in chicken eggs samples.

2.3. Preparation of MIPs

For the preparation of MIPs, the template (methenamine, 1.0 mmol) and the functional monomer (MAA, 4.0 mmol) were dissolved with 20 mL solvent (water/methanol, 1:10, v/v) in a 100 mL borosilicate glass bottle, equipped with a rubber cap. Then, the cross-linker (EGDMA, 20.0 mmol) and initiator (AIBN, 0.25 mmol) were added. This mixture was sonicated and saturated with nitrogen for 10 min, respectively. Next, the bottle was sealed and placed in a water bath at 60 °C for 24 h. After polymerization, the monolith was crushed and ground, then sieved using acetone to deliver the particles with size dimensions between 38 μm and 74 μm (200–400 mesh) (L. M. He et al., 2009). Following the polymers particles were washed with 300 mL of methanol containing 10% acetic acid using a Soxhlet apparatus for 48 h to be free of the residual reagent and template molecules. The effluent was analyzed by UPLC until methenamine could not be detected. Next, the products were washed with 300 mL of methanol for 24 h and dried under vacuum at 60 °C for 10 h. The non-imprinted polymers (NIPs) were prepared in an identical manner but without the addition of the template methenamine. The morphological characteristics of MIPs and NIPs were observed with a SWPRATM55 scanning electron microscope (Carl Zeiss, AG, Aalen, Germany).

2.4. Selectivity study

The selectivity of the MIPs and NIPs were investigated with methenamine as the structural template, and melamine as the reference compound. The selective binding experiment referred to literature (D. He et al., 2014; Ji et al., 2014). The experiment was applied by adding 50.0 mg MIPs or NIPs into a glass tube which contains 2.0 mL of the standard solution of methenamine and melamine (100 mg L^{-1} in ACN), respectively. The mixture was equilibrated for 6 h, and then the residual concentration of methenamine and melamine were analyzed by UPLC after centrifugation and filtration.

2.5. Adsorption experiments

Static and dynamic adsorption experiments were applied to investigate the binding capacity of the MIPs and NIPs, and the methodology referred to Yan et al. (2012). An aliquot of 50 mg of MIPs or NIPs particle was respectively added in 10 mL glass tube containing 2.0 mL of methenamine solutions with various concentrations (50–400 mg L^{-1} in ACN). Then it was slightly shaken in a table concentrator for 6 h. The solutions were filtered by organic phase needle type filters. Final methenamine concentrations were analyzed by UPLC. Dynamic adsorption experiment was the same as the static adsorption experiment except different times (1, 2, 5, 10, 20, 30, 45 and 60 min) at the constant concentration (100 mg L^{-1} in ACN). The maximum binding quantity (Q_{max}) and dissociation constant (K_d) were estimated with the Scatchard equation: $Q/C_e = (Q_{max} - Q)/K_d$. Then the adsorption quantity (Q) was calculated by subtracting the equalized concentration (C_e) from the initial concentration. The Scatchard plot was analyzed by reference to D. He et al. (2014).