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Molecularly imprinted polymer for extraction of patulin in apple juice samples

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ABSTRACT

A new molecularly imprinted polymer (MIP) was prepared in a two-step process. First, SiO_2-γ-MPTS was obtained by mixing γ-methacryloxypropyltrimethoxysilane (γ-MPTS) and tetraethoxysilane (TEOS). Second, SiO_2-γ-MPTS was polymerized in the presence of patulin (PAT) as a template, maleic acid (MA) as a functional monomer, ethylene glycol dimethacrylate (EGDMA) as a cross linker, 2,2-azobis- (2-methylpropionitrile) (AIBN) as a precursor and acetonitrile as a porogen solvent. The prepared sorbent was successfully applied to selective solid phase extraction (SPE) of PAT in containing apple juices and the MIP@SPE method was validated. The optimum conditions for PAT extraction using the novel MIP@SPE were: 50 mg mass of adsorbent, sodium bicarbonate with (1%) acetic acid as washing solvent and 5 mL acetonitrile as eluting solvent. The developed MIP@SPE method had high selectivity and good affinity toward PAT and the recoveries of the target analyte were in the range of 82–98% with a precision of 3.03%–3.83% (RSD). In addition, a good linearity ($r^2 > 0.99$) within the range 0.1–10 mg L^{-1} and a low LOD (8.6 μg L^{-1}) and LOQ (28.6 μg L^{-1}) were obtained. The test of reusability showed good results for at least 6 cycles.

1. Introduction

Patulin (PAT) (α, β-unsaturated-γ-lactone) is a toxic mycotoxin produced by numerous species of filamentous fungi belonging to the genera *Penicillium*, *Aspergillus* and *Byssochlamys* (Al Wright, 2015; Puel, Galtier, & Oswald, 2010). The former genera and particularly *Penicillium expansum* is by far the most worrisome species, commonly associated with patulin contamination in fruit- and vegetable-based products, notably apples (Tannous et al., 2015). The systematic toxicity evaluations revealed that acute, sub-acute and chronic health risks of patulin consumption include, among others, agitation, convulsions, dyspnea, pulmonary congestion, ulceration, oedema, hyperemia, intestinal inflammation, epithelial cell degeneration, immunotoxic, neurotoxic, hepatotoxic, genotoxic, teratogenic and carcinogenic effects (Moake, Padilla-Zakour, & Worobo, 2005; Puel et al., 2010; Song et al., 2014). Given the health risks posed by PAT, governments have established regulatory guidelines for maximal levels permitted in fruits and fruit products. In the United States, the maximum acceptable level of PAT was set at 50 ppb (Food and Drug Administration, 2005). The European Commission regulations (European Commission Reg. 1881/2006) set the maximum limits of PAT equal to 50 ppb for fruit juices and derived products, 25 ppb for solid apple products and 10 ppb for juices and foods aimed for babies and young infants.

Miscellaneous analytical methods have been developed for determination of patulin in apple matrices, (Iha, Souza, & Sabino, 2009; Maragos, Busman, Ma, & Bobell, 2015; Shephard & Leggott, 2000; Wang & Yang, 2003; Zhou, 2001). These methods were based on gas chromatography (GC), Thin Layer Chromatography (TLC) and particularly Liquid Chromatography with UV detector (LC-UV) or Mass Spectrometry (LC-MS) (Boonzaaijer, Bobeldijk, & Van Osenbruggen, 2005; Kharandi, Babri, & Azad, 2013). Among

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these techniques, HPLC-UV is the well suited for PAT determination and has been validated as an AOAC international official method (Trucksess, 2005). Meanwhile, sample preparation and clean-up processes prior to the analysis are mandatory in the analytical procedures. In the particular case of PAT, liquid-liquid extraction (LLE) and solid phase extraction (SPE) are universally used for its extraction (Turner, McNabb, Harwood, Selwood, & Boundy, 2015). Molecularly imprinted polymers (MIPs) based-SPE (MIP@SPE) as a novel technique has been recently used for extraction of mycotoxins from various food matrices (Szumski, Grzywiński, Prus, & Buszewski, 2014). This emergent process which is proven to be selective, stable and more effective is based on their ability to recognise template molecules with high affinity and selectivity (Capriotti et al., 2010). They are cross linked functional polymers synthesized in the presence of the target analyte known as template, which after removal leads to formation of specific cavity complementary to the template molecule (Vidal et al., 2013). In the case of PAT, papers dealing with this approach are scarce (Khorrami & Taherkhani, 2011; Zhao, Jia, Yu, & Sun, 2011). In these studies, a MIP-based on oxindole (structurally analogue of patulin) was synthesized with methacrylic acid and ethylene glycol dimethacrylate (EGDMA) was successfully applied for MIP@SPE of patulin in contaminated apple juice (Khorrami & Taherkhani, 2011). In the other study, a selective sorbent for PAT extraction and clean-up was produced by using EGDMA and 4,4'- azobis(4-cyanopentanoic acid) grafted on silica gel and applied to SPE for the extraction of patulin in apple juice and other related products (Zhao et al., 2011). Despite that both MIP proved their efficiency to extract PAT, attempt to synthesise new material with high efficiency and selectivity toward PAT is highly desirable. Consequently, the objectives of the present work were to develop a novel extraction procedure using a molecularly imprinted polymer as thin films of methacrylic and maleic acids copolymers based onto silica gel, and to evaluate the efficiency and selectivity of the novel MIP@SPE in extracting and preconcentration of PAT in apple juice.

2. Materials and methods

2.1. Chemicals and reagents

Patulin (PAT) standard (purity $\geq$ 98%) was purchased from AG Scientific (AG Scientific Inc, San Diego, CA, USA). 5-(hydroxyméthyl) furfural (HMF) (purity $\geq$ 95%) was obtained from TCI (Tokyo Chemical Industry CO. LTD). All solvents acetonitrile (MeCN), ethyl acetate (EtOAc), hexane (HA), acetone (Ac) of HPLC grade were obtained from Fisher Scientific (Illkirch-Graffenstaden, France). Acetic acid and sodium carbonate were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Deionized water was prepared in a Milli-Q ultrapure water system (Bedford, MA, USA).

2.2. Standard solutions

Stock standard solution of PAT (500 μg mL^{-1}) was prepared in water/acetonitrile (90:10 v/v) and working solutions of 0.1–10 μg mL^{-1} were obtained by successive dilutions in water/acetonitrile Fig. 1. HMF stock standard solution was prepared by dissolving 14 mg of pure HMF in 100 mL of deionized water. Working standard solutions were prepared by appropriate dilution of this solution with water. Stock standard and working standard solutions were stored at 4 °C until used.

2.3. Sample preparation

Samples of apple juice, fruit juice containing apple and apple were obtained from local market. Each sample (1 g or 1 mL, depending on the physical state) was mixture with 1 mL of water using vortex agitation and ultrasounds (10 min) then centrifuged at 5000 tr/min for 5 min. The supernatant was then diluted with water and stored at 4 °C before analysis.

2.4. Preparation of MIP

Two-step preparations of SiO_2maleicpolymer@MIP were adopted as described elsewhere (Anene, Kalfat, Chevalier, & Hbaieb, 2016). At first, SiO_2-γ-MPTS was obtained by mixing γ-methacryloxypropyltrimethoxysilane (γ-MPTS) and tetraethoxysilane (TEOS). The molar ratio of γ-MPTS to TEOS in the final solution was kept at 1:4, and the reaction mixture was left overnight at 80 °C in an oil bath. Then the polymer was obtained by centrifugation and washing with ethanol. In the second step, the dried silica SiO_2-γ-MPTS and 0.01 mmol of PAT (template), 20 mmol maleic acid (MA) were dispersed in acetonitrile (10 mL) either the cross-linker EGDMA (20 mmol) and AIBN (0.2 mmol) as precursor were added into the solution. Polymerization was performed by heating at 60 °C for 6 h. Thereafter, the polymer SiO_2maleicpolymer@PAT was filtered and washed with acetonitrile and stored under vacuum for subsequent uses. The template was removed by repeated extractions with ethyl acetate for 8 h and methanol-acetic acid (90:10, v/v) in a Soxhlet apparatus. The prepared polymer was dried under reduced pressure and stored at room temperature prior to use. For comparison, non-imprinted polymer (SiO_2maleicpolymer@NIP) was also prepared by using the same procedures and conditions but without PAT.

2.5. MIP@SPE method

The MIP@SPE columns were prepared by packing 50 mg of the SiO_2maleicpolymer@MIP or SiO_2maleicpolymer@NIP into 3 mL SPE cartridges. The polymers in the cartridges were secured by polyethylene frits at the top and the bottom. The cartridges were conditioned with 6 mL methanol, and then they were loaded with the working standard solutions and apple juices spiked with PAT (0.5 μg mL^{-1}) at 0.5 mL min^{-1} flow rate. After loading, the columns were washed with 4 mL of Na_2CO_3 solution (1%, w/v) at 1 mL min^{-1} flow rate flowed by 2 mL of acetic acid solution in water (1%,v/v), and then eluted with 5 mL acetonitrile containing few drops of acetic acid. Finally, all of the fractions from loading, washing and elution steps were collected and then evaporated to dryness at 40 °C under a stream of nitrogen gas. The residues were dissolved in water for HPLC analysis.

2.6. HPLC-DAD analysis

Liquid chromatography was performed on an Agilent Technologies instrument with an 1100 series quaternary pump, an autosampler, and a diode array detector linked to an HP-ChemStation data handling system (Agilent Technologies, Palo Alto, CA, USA). The separation of PAT was achieved on a Nucleosil C18 column (250 × 4 mm, i.d, 5 μm, Macherey-Nagel, Düren, Germany). The separation was performed in the isocratic mode using water and acetonitrile (90:10, v/v) at a flow rate of 1 mL min^{-1}, and the DAD detector was monitored at 276 nm Wavelength. The quantity of patulin (μg mL^{-1}) was determined by using a calibration curve, correlating the peak area with the concentration.

2.7. Method validation

The developed method was fully validated according to XPT 90–210 and Eurachem-Citac guidelines (XPT, 1999; Eurachem-Citac, 2000). The performance characteristics of this method were