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Analytical Methods

Pipette-tip solid-phase extraction using poly(1-vinylimidazole-*co*-trimethylolpropane trimethacrylate) as a new molecularly imprinted polymer in the determination of avermectins and milbemycins in fruit juice and water samples

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ABSTRACT

A simple HPLC method was developed for the determination of abamectin (ABA), eprinomectin (EPR), and moxidectin (MOX). Pipette-tip molecularly imprinted polymer solid-phase extraction (PT–MIP–SPE) using poly (1-vinylimidazole-*co*-trimethylolpropane trimethacrylate) as a selective adsorbent material was studied in detail, including the washing solvent, type and volume of eluent, pH, quantity of adsorbent material and sample volume. The performance criteria for linearity, sensitivity, precision, accuracy, recovery, robustness and stability have been assessed and were within the recommended guidelines. The mean extraction recoveries/relative standard deviation for ABA 1b, EPR, ABA 1a and MOX were 98.77 ± 3.82%, 88.19 ± 2.57%, 110.54 ± 1.52% and 100.42 ± 0.59%, respectively. Finally, the results proved that PT–MIP–SPE coupled to HPLC–UV is an economical, simple and easy-to-perform technique, and presented a high potential for extraction of macrocyclic lactones in mineral water and grape and juice samples.

1. Introduction

The avermectins (AVM) and milbemycins (MBM) are macrocyclic lactones, consisting of a 16-membered heterocyclic ring, derived from the fermentation of bacteria belonging to the family of actinomycetes (*Streptomyces avermitilis* and *Streptomyces cyanogriseus*, respectively) found in soil. The AVM were isolated in the 1970s from a soil sample in Japan. These compounds have anthelmintic properties, being widely used in veterinary and human medicine and in agriculture as pesticides. The AVM have an incredible ability to act as 'endectocides' in both ecto- (ticks, bernes, scabies) and endoparasites (worms). Although the mechanism of action is still not fully understood, researchers suggest that they induce neurotoxicity within invertebrates and vertebrates through the direct activation or potentiation of chloride channels of the gamma-aminobutyric acid neurotransmitter and may also act on glutamate receptors, resulting in paralysis and eventual death of the parasite (Diserens & Henzelin, 1999; Hodoscek et al., 2008; Li, et al., 2014; Raftery & Volz, 2015; Valenzuela, Popa, Redondo, & Mañes, 2001).

Abamectin (ABA) and eprinomectin (EPR) are examples of the AVM class while moxidectin (MOX) belongs to the MBM class. ABA is the generic name given to commercialization of the mixture of two homologs of AVM, which contains not less than 80% AVM B1a and not more than 20% AVM B1b. ABA B1a differs from ABA B1b by a single methylene group at the C-25 position, i.e. ABA B1a has a secondary butyl substituent and ABA B1b an isopropyl substituent (Danaher, Howells, Crooks, Cerkvenik-Flajs, & O'Keeffe, 2006; Hernández-Borges, Ravelo-Pérez, Hernández-Suárez, Carnero, & Rodríguez-Delgado, 2007). EPR and MOX are semisynthetic compounds, derived from ABA and ivermectin, respectively (Fig. S1) (Alvinerie, Sutra, Galtier, & Mage, 1999; Chen, Hung, & Fleckenstein, 2002).

Pesticides are widely used in agriculture, primarily for pest control, thus improving crop yields. There is a need to monitor pesticide residues, assuring the consumer that the product is safe for consumption and that it does not harm the environment. Applications of pesticides should be carried out in accordance with good agricultural practice (Portolés, Mol, Sancho, López, & Hernáandez, 2014). The recurrent use of AVM in agriculture and livestock is a notable entry point for the spread of these compounds into the environment. Once in the soil, such compounds may be potentially transported to aquatic systems, which may be detrimental because the AVM are insoluble in water. Another

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important route of entry of AVM into the environment is their direct use in aquaculture (Dionisio & Rath, 2016; Kolar, Erzen, Hogerwerf, & Van Gestel, 2008).

The maximum residue limits (MRLs) established by the European Union (EU) for AVM are 10–50 g/kg tolerance for food and food of plant origin. In the case of food of animal origin, including aquaculture products, the tolerance levels recommended by the European Medicines Agency (EMEA) is 10–150 g/kg for widely used AVM such as emamectin benzoate, ivermectin and doramectin (Hernando, Suárez-Barcena, Bueno, Garcia-Reyes, & Fernández-Alba, 2007). The Codex Committee on Pesticide Residues under the Joint FAO/WHO Food Standards Program established MRLs for AVM of 0.01–0.02 mg/kg for fruits and tomatoes. The MRL set by the Spanish Government for citrus fruits is 0.01 mg/kg (Valenzuela, et al., 2001).

The high number of different active substances found in pesticides and the low levels of concentration allowed by the current legislation for the presence of interferents in the samples makes the analysis of pesticide residues a challenging task for the analytical process (Portolés et al., 2014). For the detection of the residues of AVM in environmental and food samples, different analytical approaches have been used, such as thin layer chromatography, immunochemical analysis, gas chromatography and high-performance liquid chromatography (HPLC), coupled to a fluorimetric detector with a pre-column with a derivative of AVM with trifluoroacetic anhydride and methylimidazole. Methods involving the detection by mass spectrometry and liquid/gas-tandem chromatography are also widely used (Antonian, DeMontigny & Wislocki, 1998; Furlani et al., 2015; Giannetti, et al., 2011; Hou, et al., 2006; Krogh et al., 2008; Li, et al., 2014; Pimentel-Trapero, Sonseca-Yepes, Moreira-Romero, & Hernández-Carrasquilla, 2016; Pozo, Marin, Sancho, & Hernández, 2003; Sheridan & Desjardins, 2006). As the analyte is present at low concentrations in the matrix, which is generally incompatible with the analytical system, it is necessary to pre-concentrate and prepare the samples. An ideal sample preparation should meet these main characteristics: elimination of matrix interferents, minimum sample loss, analyte pre-concentration, good recovery, compatibility with the detection technique, simplicity, robustness, reproducibility, quickness and low cost (Ansari & Karimi, 2017a). Pipette-tip solid-phase extraction (PT–SPE) is a simple miniaturization of SPE, being quite efficient for extraction and/or pre-concentration of analytes, especially in complex matrices (Hasegawa et al., 2007; Shen et al., 2013). This promising technique is less expensive, easier to operate and consumes a lower quantity of solvents and samples than conventional SPE cartridges, combining flexibility, ease and speed of use and providing excellent recovery, high reproducibility and versatility (Andrade, Silva, Pereira, & Borges, 2015; da Silva et al., 2017; de Oliveira et al., 2016; Hasegawa et al. 2007; Luo et al., 2016; Shen et al., 2013; Silva, Mano, Pereira, Figueiredo, & Borges, 2016). Molecularly imprinted polymers (MIP) are synthetic polymers used as adsorbents, which have specific recognition sites, stereochemically shaped by a template (analyte). The MIP synthesis step occurs after the formation of a complex between the functional monomers and template. In this way, the terminations of the functional monomer ligands are positioned at points complementary to those from the template, allowing the formation of bonds and, consequently, a remarkable enantioselective capacity. In this way, an adsorbent material with selective affinity is obtained for an analyte or an analyte group (Ansari & Karimi, 2017b; Lima, Vieira, Martins, Borges, & Figueiredo, 2016).

The aim of this work was to perform the synthesis, characterization and application of a MIP based on poly(1-vinylimidazole-*co*-trimethylolpropane trimethacrylate) (1-VI-*co*-TMPTMA). The VI-*co*-TMPTMA was characterized by Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and thermogravimetric analysis (TGA). This selective material was used as an adsorbent in pipette-tip molecularly imprinted polymer solid-phase extraction (PT–MIP–SPE) to pre-concentrate and extract ABA 1b, EPR, ABA 1a and MOX. The target analytes were chosen based on their priority of use and considering the EU legislation, water samples and fruit juices were considered relevant matrices to demonstrate the applicability of the method for monitoring these residues. Relevant parameters influencing the sample preparation were also studied in detail to obtain maximum extraction efficiency and sensitivity. Finally, using the new VI-*co*-TMPTMA as an adsorbent, a PT–MIP–SPE coupled to the HPLC–UV method was successfully applied to the determination of AVM and MBM in mineral water and grape juice samples.

2. Experimental

2.1. Solvents and reagents

Analytical standards were ABA (1.99% ABA 1b + 95.73% ABA 1a) obtained from Hebey Veyong, EPR (95%) from Dr. Ehrenstorfer GmbH and MOX (98.96%) from Zhejiang. All solvents are HPLC grade and were purchased from J.T. Baker® (Mexico City, MX, Mexico): acetonitrile, methanol, tetrahydrofuran (THF) and hexane. The water was distilled and purified using a Millipore Milli-Q Plus® system (Bedford, MA, USA). The reagents used in the synthesis of the MIP and non-imprinted polymer (NIP) were obtained from Sigma-Aldrich (St Louis, MO, USA): 1-vinylimidazole (1-VI), trimethylolpropane trimethacrylate (TMPTMA), 2,2′ azo-bis-isobutyronitrile (AIBN) and acetic acid. The dimethyl sulfoxide (DMSO) was obtained from Vetec® (Rio de Janeiro, RJ, Brazil).

2.2. Instrumentation and chromatographic separation

The analyses of the standards and samples were performed on HPLC Agilent® Model 1220 (G4286B) (Santa Clara, CA, USA). The data were collected and analysed by Agilent OpenLAB Chromatography Data System software (Santa Clara, CA, USA). An analytical Phenomenex® Gemini C18 column (150 mm × 4.60 mm, 5 µm) (Torrance, CA, USA) was used for the chromatographic separation of the AVM and MBM. The mobile phase consisted of acetonitrile:methanol:water (55:27:18, *v/v/v*), temperature at 25 °C, flow rate at 1.2 mL min^{-1}, 20 µL injection volume and detection at 250 nm. All solvents used in the mobile phase were degassed using an ultrasound Unique Ultracleaner USC800 (Indaiatuba, SP, Brazil). The centrifuge employed in the preparation of grape juice samples and polymer synthesis was a Clinical Centrifuge Centribio/Daiki 80-2B (Ramos, RJ, Brazil).

2.3. MIP and NIP synthesis

The synthesis of 1-VI-*co*-TMPTMA was performed in a 100-mL amber glass with a lid. First, 0.8 mmol ABA (template) were placed in the flask together with 15 mL solution of acetonitrile:DMSO (9:1, *v/v*). The mixture was sonicated for 1 min until solubilization. Thereafter, 54.5 mmol of 1-VI was added and stirred for 10 min in an ice bath. Then, 22.3 mmol of TMPTMA and 1.5 mmol of AIBN were added. This solution was sonicated for 2 min. Finally, the flask was closed, sealed and placed in an oven at 60 °C for 24 h. Then, the material was triturated, and the template was removed by washing the material with 30 portions of 50 mL methanol:acetic acid (9:1, *v/v*). Next, two portions of 50 mL of methanol:ultrapure water (1:1, v/v) was added to the flask and ultrasonicated for 10 min to remove all other reagents remaining from the synthesis and the acetic acid. After the last washing, the washing solution was analysed using HPLC–DAD to ensure full removal of ABA from the MIP. For the NIP synthesis, the same amount and the same protocols and solutions were used, except the template.

2.4. MIP and NIP characterizations

TGA were performed to evaluate the thermal stability of MIP and NIP and were carried out in a thermocouple (2950 Instrument of Thermal Analysis, TA Instruments, New Castle, DE, USA) using an