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Synthesis and application of molecularly imprinted polymers for the selective extraction of organophosphorus pesticides from vegetable oils[☆]

Sara Boulanouar[a], Audrey Combès[a], Sakina Mezzache[b], Valérie Pichon[a,c,*]

[a] Dept of Analytical, Bioanalytical Sciences and Miniaturization (LSABM) – UMR CBI 8231 (CNRS-ESPCI), ESPCI, PSL Research University, 10 rue Vauquelin, 75 231 Paris Cedex 05, France
[b] L'Oréal Research and Innovation, 1 avenue Eugène Schueller BP22, 93601 Aulnay sous Bois, France
[c] Sorbonne Universités, UPMC, 4 Place Jussieu, 75005 Paris, France

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ABSTRACT

The increasing use of pesticides in agriculture causes environmental issues and possible serious health risks to humans and animals. Their determination at trace concentrations in vegetable oils constitutes a significant analytical challenge. Therefore, their analysis often requires both an extraction and a purification step prior to separation with liquid chromatography (LC) and mass spectrometry (MS) detection. This work aimed at developing sorbents that are able to selectively extract from vegetable oil samples several organophosphorus (OPs) pesticides presenting a wide range of physico-chemical properties. Therefore, different conditions were screened to prepare molecularly imprinted polymers (MIPs) by a non-covalent approach. The selectivity of the resulting polymers was evaluated by studying the OPs retention in pure media on both MIPs and non-imprinted polymers (NIP) used as control. The most promising MIP sorbent was obtained using monocrotophos (MCP) as the template, methacrylic acid (MAA) as the monomer and ethylene glycol dimethacrylate (EGDMA) as the cross-linker with a molar ratio of 1/4/20 respectively. The repeatability of the extraction procedure and of the synthesis procedure was demonstrated in pure media. The capacity of this MIP was 1 mg/g for malathion. This MIP was also able to selectively extract three OPs from almond oil by applying the optimized SPE procedure. Recoveries were between 73 and 99% with SD values between 4 and 6% in this oil sample. The calculated LOQs (between 0.3 and 2 μg/kg) in almond seeds with a SD between 0.1 and 0.4 μg/kg were lower than the Maximum Residue Levels (MRLs) established for the corresponding compounds in almond seed.

1. Introduction

Vegetable oils occupy a large place among food products and their nutritional or health contribution does no need demonstration. Their constituents play a very important role in human health. In addition, their beneficial properties in cosmetics have been known since antiquity by nourishing, protecting and moisturizing the skin. However, pesticides used in agriculture may possibly be found in vegetable oils. The EU harmonization of the pesticides Maximum Residue Levels (MRLs) within Regulation 396/2005 has led to specific MRLs being set on raw materials (oil seeds and oil fruit), but not on processed products. A processing factor was proposed by FEDIOL (vegetable oil and protein meal industry association), to define the limits allowed in the processed products such as vegetable oils, fats and meals. To reach the MRLs values that are established at 10 μg/kg by EU for pesticides as general default for food or feed constitutes a significant analytical challenge for the safe use of such oils.

Organophosphorus (OPs) compounds constitute an important class of pesticides whose toxicity arises from the inhibition of the acetylcholinesterase enzyme. They exhibit a wide range of physico-chemical properties thus rendering their determination in complex oil samples particularly difficult. Their analysis often requires a previous extraction step using gel permeation [1] or a liquid–liquid extraction (LLE) step [2] that is nowadays usually followed by a purification step by dispersive solid-phase (dSPE) extraction, *i.e.* a global QuEChERS-based procedure adapted for fatty matrices [3–5]. Primary secondary amine (PSA), octadecylsilica (C18) and

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[*] Corresponding author at: LSABM-ESPCI, 10 rue Vauquelin, 75005 Paris, France.
E-mail address: valerie.pichon@espci.fr (V. Pichon).

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graphitized carbon black (GCB) are the three most commonly used sorbents for QuEChERS. However, their amount and their proportion when they are used in combination must be optimized to reach the most powerful clean-up effect without affecting the extraction recovery of the target analytes [5]. Indeed, it was recently shown that the addition of GCB to PSA/C18 was efficient for trapping oil components and their removal from the extract but also affects the extraction recovery for some compounds [3].

These drawbacks led to the recent development of molecularly imprinted polymers (MIPs). These synthetic polymeric materials possess specific cavities designed for a template molecule involving a retention mechanism based on molecular recognition. The MIPs have been already successfully used in several fields, such as sensors, organic synthesis and separation of enantiomers [6–9]. The first application of a MIP as SPE sorbent was carried out by Sellergren et al. in 1994 for extracting pentamidine present at low concentrations in urine [10]. The principle of selective extraction on a MIP is the same as for a conventional SPE sorbent. After a conditioning step, the sample is percolated through the MIP and a washing step removes the interfering compounds. The desorption of analytes is achieved by percolating a solvent able to develop interactions with the sorbent in order to desorb the analytes retained on the MIP. Several MIPs dedicated to the selective extraction of mycotoxins, drugs, pollutants or steroids are now commercially available.

The development of MIPs for the extraction of OPs has been largely reported these last years. MIPs were prepared as particles to be used in cartridges between two frits as SPE sorbent [11–26] or as dispersive sorbent for dSPE [27–31] and for matrix solid-phase dispersion (MSPD) [32–34] or as a thin film in solid-phase microextraction (SPME) [35–37] or in stir bar sorption extraction (SBSE) [38]. They were applied to the selective extraction of OPs from vegetable extracts (cucumber, lettuce, apple, pear. . .) and environmental samples such as waters and soil extracts.

In the common approach, the synthesis of MIPs involves first the complexation of a template molecule with functional monomers through non-covalent bonds in a porogenic solvent, followed by polymerization of these monomers around the template with the help of a cross-linker in the presence of an initiator. The choice of the chemical reagents used for the synthesis of the MIP must be judicious in order to really create specific cavities designed for the template molecule. In 85% of the reported works, MIP for OPs were produced in fixed conditions without optimizing the nature and the ratio of the reagents. The target OP was taken as template molecule, methacrylic acid as monomer, ethylene glycol dimethacrylate as cross-linker in a non protic solvent (mainly acetonitrile, dichloromethane and chloroform). The effect of the template was studied only once for the development of an MIP for dimethoate and its metabolite omethoate showing that the metabolite was better adapted for the trapping of both molecules [19]. A few studies described the synthesis of an MIP by varying the nature of the monomer [11,19,28,30,31] and/or the porogen [11,25,30,31] or the template/monomer ratio [24]. In some studies, the choice of the monomer for a given template resulted from studies by molecular modeling and computational design [17,19,23]. Once, the MIP synthesized, its selectivity was mainly evaluated by binding experiments or retention studies in pure media. These evaluations were achieved by comparing results using the MIP with results obtained using a non-imprinted polymer (NIP) that is prepared in the same conditions as MIP but in the absence of template. In most reported works, these studies were carried out using up to three OPs including the OP used as template. This comparison between MIP and NIP achieved in spiked pure media allows to put in evidence the presence of cavities in the MIP and is also useful to optimize the extraction procedure that must give rise to high extraction recovery on the MIP and low one on the NIP [8]. Except for one reported work [21], the conditions of extraction finalized in pure media were applied to real samples [11,17–20,22–27,38,39] without a control of the selectivity by using the NIP or without reoptimization of the extraction conditions to circumvent matrix effects as already reported [8]. These matrix effects were well illustrated by Sanagi et al. who reported recoveries obtained in pure media and in real samples after applying the same extraction procedure on MIP and on NIP [21]. While recovery of extraction for quinalphos in pure media was 92.3% and 43.9% for MIP and NIP respectively, the recovery was 99% and 64.8%, respectively for a real sample, thus illustrating the effect of the matrix components that increase the retention on both sorbents and induce consequently a loss of selectivity. At last, very few works reported the application of MIPs for the selective extraction of OPs from vegetable oils. These works were carried out by Bakas et al. who studied the extraction of methidathion [17], dimethoate [19] and fenthion [23] from olive oil samples.

The objectives of this work were to prepare a MIP able to extract from vegetable oil the maximum number of OPs that were selected by taking into account the risk of their occurrence in such samples. For this, different conditions of synthesis were screened by varying the nature of the template, of the monomer and of the porogenic solvent in order to find the conditions of synthesis of a MIP able to selectively trap the largest number of OPs from vegetable oils The MIP resulting in best selectivity for five OPs was studied more in detail by investigating its behavior towards ten OPs from pure media but also from vegetable oils whose content may affect the recoveries on the MIP. At last, to highlight the potential of the developed MIP, a comparison with results obtained while applying C18 silica to an almond oil extract was performed.

2. Materials and methods

2.1. Chemicals

HPLC-grade acetonitrile (ACN), methanol (MeOH), dichloromethane (DCM) and toluene were supplied by Carlo Erba (Val de Reuil, France). High purity water was dispensed by a Milli-Q purification system (Millipore, Saint Quentin en Yvelines, France).

Certified reference material: dimethoate (DMT) 98%, fenthion sulfoxide (FSX) 99%, fenthion sulfone (FSN) 99%, methidathion (MTH) 98%, malathion (MAL) 99%, fenitrothion (FNT) 98%, diazinon (DIZ) 98%, pirimiphos methyl (PIM) 99.5%, fenthion (FEN)99% and chlorpyrifos-ethyl (CLE) 99.5% were supplied by Cluzeau Info Labo (Saint-Foy-La-Grande, France). Individual stock solutions from each OP were made at a concentration of 100 mg/L in ACN. A stock solution mixture containing 5 mg/L of each OP was prepared in ACN and stored at 4 °C until further use.

Parathion ethyl (PE), monocrotophos (MCP), fenamiphos (FEM), 2-trifluoromethyl acrylic acid (TFMA) 98%, acetonitrile anhydrous 99.8%, ammonium acetate for HPLC 99.0% (AC), *n*-hexane, methacrylic acid (MAA) and ethylene glycol dimethacrylate (EGDMA) were supplied by Sigma-Aldrich (Saint Quentin Fallavier, France). Washed EGDMA and MAA were distilled under vacuum in order to remove inhibitors. Azo-N,N′-bis-isobutyronitrile (AIBN) was purchased from Acros Organics (Noisy-le-Grand, France). Acetic and formic acids (AA and FA respectively) were purchased from VWR (Fontenay-sous-Bois, France).

2.2. Apparatus and analytical conditions

The LC–MS/MS analyses were performed using a liquid chromatograph (UltiMate 3000®, Thermo Scientific, Illkirch, France) coupled with Triple Stage Quadrupole Mass Spectrometer (TSQ