Synthesis of molecularly imprinted nanoparticles for selective exposure assessment of permethrin: optimization by response surface methodology

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Abstract

Background Extensive use of high-efficiency pyrethroid pesticides as pest-control agents lead to remarkable adsorption and release of these materials in soil and aquatic environment which could have serious adverse effects on water and food chain quality as well as human health. In this study, a molecularly imprinted polymer was synthesized and used as a selective sorbent in the sample preparation procedure in order to facilitate sensitive and quantitative exposure assessment of insecticide permethrin.

Methods Molecular imprinted nanoparticles were prepared by precipitation polymerization technique using 1:4:20 mmol ratio of the template, functional monomer, and cross-linker, respectively, as well as 80 mL of chloroform as progen solvent. The obtained nanoparticles were characterized by field emission scanning electron microscopy (FESEM) and Fourier transform infrared spectrometry (FT-IR). The optimization of critical variables in the MISPE process was done using the central composite design (CCD) of the response surface methodology.

Results Quadratic regression models were developed to correlate the response and independent variables and the analysis of variance (ANOVA) verified the excellent fitting of proposed models for experimental data. Optimum conditions for the highest MISPE yield were selected as follow: sorbent mass of 7.71 mg, sample pH 5.58 and 5.68 for cis and trans-permethrin, respectively, sample flow rate of 0.6 mL/min, as well as 5 and 3.94 mL of methanol/acetic acid at the flow rate of 2 mL/min as elution solvents for cis and trans-permethrin, respectively. Under optimized conditions, the linear range was obtained 20–120 µg/L ($R^2 = 0.99$) and the detection limits were 5.51 and 5.72 µg/L for cis and trans-permethrin, respectively. Analysis of real samples demonstrated the high extraction efficiency of designed protocol ranging from 93.01 to 97.14 with the relative standard deviation (RSD) less than 4.51%.

Conclusions The satisfactory results confirmed the reliability and efficiency of the proposed method for trace analysis of permethrin isomers in biological and environmental samples.

Keywords Permethrin · Molecularly imprinted polymer · Solid phase extraction · Response surface methodology · Central Composite design · High performance liquid chromatography

Introduction

Despite the beneficial effects of pesticides for controlling pests and improving the quality of agricultural products, extensive

use of these toxic materials can cause different adverse effects on non-target organisms including human. Direct and indirect exposures to these compounds via different occupational and environmental pathways may result in acute and chronic disorders [1–3].

Permethrin (3-Phenoxybenzyl-cis,trans-(1RS)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) is one of the widely used pyrethroid insecticides for elimination of the insects dangerous to agriculture and human lives [4–6]. Similar to the organochlorine pesticide DDT, permethrin acts by interfering with sodium channels to disrupt the function of neurons in the nervous system [7, 8]. Pyrethroid insecticides, including permethrin, are lipophilic compounds that appear in a very low concentration level in aquatic environments due to

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their low water solubility [9, 10]. A guideline value of 20 µg/L has recommended for permethrin in drinking water by the World Health Organization (WHO) [11]. Exposure to these compounds and their residuals in water and food through inhalation, skin contact, and oral ways may lead to the rapid distribution of them in various human organs [12–15]. According to the US Environmental Protection Agency (EPA) classification, permethrin is placed in the possible human carcinogens group [9]. In addition, some evidence-based toxicological studies have proven the association of permethrin exposure with the genotoxicity, lymphocyte DNA damage, as well as the immune system suppression, endocrine disruption, lymph node, spleen damage, inhibition of gap-junctional intercellular communications, and carcinogenesis [16–22].

Considering the above-mentioned facts, development of selective and sensitive analysis methods for environmental and biological monitoring of pesticides concentration is of great importance.

In recent years, various analytical methods have been reported for determination of pyrethroids, such as spectrophotometry [23], immunoassay [24], electrophoresis [25], electrochromatography [26], gas chromatography (GC) [27], liquid chromatography (LC) [28], GC–MS [29], and LC–MS [30]. However, due to the complexity of environmental and biological samples as well as the importance of determining ultra-low levels of analytes, a selective and sensitive sample preparation step prior to detection is required when using mentioned analytical methods [31, 32].

The drawbacks of traditional sample preparation techniques have actuated a growing tendency towards new, inexpensive, and quite fast approaches. In this regard, several novel techniques such as solid phase extraction (SPE) [33, 34], solid-phase microextraction (SPME) [35, 36], matrix solid-phase dispersion (MSPD) [37, 38], stir bar sorption extraction (SBSE) [39, 40], supercritical fluid extraction (SFE) [41], accelerated solvent extraction (ASE) [42, 43] and liquid-phase microextraction (LPME) [44, 45] have been developed in order to improve organic material extraction throughput in terms of time, solvent consumption, reproducibility and enrichment factor (EF). Despite the benefits of these methods, all of them suffer from lack of selectivity limiting their application in large-scale analyses. Thus, in recent years, researchers have conducted some efforts to improve the selectivity of conventional sorbents, leading to the synthesis of tailor-made materials based on the antibody-antigen mechanism, so-called molecular imprinted polymers (MIPs) [46].

The MIPs are artificially synthesized receptors with three dimensional polymeric adsorption sites imprinted in the same size and shape with the target analyte (template molecule) [47, 48]. In addition to remarkable advantageous exhibited by MIPs, such as physicochemical stability in a wide range of pH, temperature and solvents as well as predetermined recognition ability, repeated operations without loss of activity, low

cost and ease of preparation, their unique and excellent affinity and selectivity make them attractive to use for selective and sensitive sample clean-up of a wide range of analytes including drugs, pesticides, peptides, viruses, immunoglobulins, and erythrocytes in complicated matrices [49–51].

Determination and quantification of pyrethroid pesticides by the application of MIP technology have been reported in some studies in recent years. Vonderheide et al. used the commercial molecular imprinted polymer (SupelMIP Pyrethroids) coupled with isotope dilution gas chromatography-ion trap mass spectrometry for analysis of permethrin isomers in composite diet samples. The detection limit of the method was reported 3.4 µg/kg and 3.1 µg/kg for *cis* and *trans*-permethrin, respectively [52]. In another attempt for the enrichment and determination of six pyrethroid insecticides in aquaculture seawater samples, Shi et al. prepared two types of MIPs using deltamethrin and cypermethrin as template molecules. Higher recovery of the intended pesticides was achieved using the cypermethrin-MIP (86.4–96.0%) with RSD values ranging from 2.4 to 7.8%. The detection limits of the method were also reported in the range of 16.6–37.0 ng/L [53]. Development of magnetic molecularly imprinted polymers for the separation of permethrin and three other pyrethroid pesticides was reported by Ma et al. [54]. In this study, cyhalothrin was used as template molecule and the important parameters affecting the extraction of the analytes were investigated and optimized. The LOD of permethrin was reported 0.0068 mg/kg.

Improvement of the functional properties of MIPs through the optimization of critical parameters are of great importance in order to achieve the desired extraction recoveries. In the synthesis step, feasible strategies such as suspension, multi-step swelling, in-situ, and precipitation polymerization have been developed in order to control the characteristics of the adsorbent particles [55]. Among these techniques, precipitation polymerization can provide monodisperse MIP nanoparticles without the use of any surfactant or stabilizer [56]. Producing a nanostructure adsorbent provides higher surface-area-to-volume ratios and faster equilibration with the analyte [57] which lead to the enhancement of the binding capacity and also higher yields by the use of much less adsorbent [58]. In addition, due to the drawbacks of traditional one-variable-at-a-time optimization methods, recent studies [59–62] have considered the alternative chemometric approaches such as response surface methodology (RSM) for investigation of the interactions between operational variables as well as their simultaneous effects on the extraction efficiency in a *n*-dimensional matrix.

The present study aimed to combine the features of molecularly imprinted polymer nanoparticles with response surface methodology as a powerful approach to facilitating selective and sensitive quantification of permethrin isomers in urine as a biological sample.

Experimental

Chemicals

Permethrin analytical standard (a mixture of *cis* and *trans* isomers) was purchased from Sigma-Aldrich (Germany). Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA), and 2, 2 azobisisobutyronitrile (AIBN) were obtained from Merck (Germany). Analytical grade solvents (chloroform, acetonitrile, acetic acid, hydrochloric acid 37%) and also sodium hydroxide pellets and buffer solutions (pHs 4, 7 and 11) were purchased from Merck (Germany), Double distilled water was provided by Purite purification system.

Instruments and analytical conditions

Analyses were performed using a high-performance liquid chromatography system from the Knauer Company (Germany) equipped with a K-1001 series high-pressure pump, a K-2006 ultraviolet detector and VS injection valve with a 20 μL loop. The chromatographic separation was carried out on a reverse phase Prontosil 120–5-C18 column (150 $\times$ 4.6 mm, Germany), using isocratic acetonitrile/water mobile phase composition (80:20, v/v) at a flow rate of 1.5 mL min^{-1} and finally measured at a wavelength of 200 nm. Other equipment including micro sampler (Socorex, Germany), digital scale (Sartorius, Germany), ultrasonic bath (Sono, Swiss), magnetic stirrer hotplate (Chiltem, USA), nitrogen supply system, digital thermometer (TP3001, China), reactor heater system (Mettler, Germany), Soxhlet extractor (Duran, Germany), digital pH meter (Metrohm, Switzerland), vacuum manifold (Tajhizteb, Iran) equipped with a vacuum pump were used for the preparation of MIP and performing the MISPE procedure. Investigation of the morphological characteristics of MIP nanoparticles was performed using field emission scanning electron microscopy (FE-SEM, Tescan, MIRA II, Czech Republic). Chemical structure and binding properties of imprinted and non-imprinted polymeric nanoparticles were studied by Fourier transform infrared spectrometer (FT-IR, Nicolet, Magna-IR 550, USA).

Preparation of MIP

The spherical MIP nanoparticles were synthesized by precipitation polymerization technique as follow: 1 mmol of permethrin (template) dissolved in 80 mL of chloroform was mixed with 4 mmol of methacrylic acid (functional monomer) for 40 min. Subsequently, cross-linking agent EGDMA (20 mmol) and initiator AIBN (40 mg) were added to the reaction flask. In order to remove oxygen, the solution was purged with nitrogen for 7 min and then sealed under this atmosphere. The polymerization process was carried out in an oil bath at 55 $^{\circ}\text{C}$ for 18 h under stirring at 80 rpm. By the

end of polymerization process, the template molecules were extracted in a Soxhlet apparatus using methanol/acetic acid (90:10, v/v) for 44 h. Finally, the polymer particles were dried in an oven at 50 $^{\circ}\text{C}$. The corresponding non-imprinted polymer (NIP) was similarly prepared except for the absence of the template molecule.

Design of experiments

In order to reach the desired efficiency in the MISPE procedure, identification and improvement of the effective recognition elements are essential. The conventional optimization methods such as one-variable-at-a-time involve many time-consuming experimental runs which ignore the interaction effects between operational parameters. Therefore, in order to overcome such problems, chemometric approaches such as response surface methodology (RSM) based on the statistical design of experiments (DOEs) have been increasingly used for the optimization of analytical methods in recent years. In this regard, the present study applied a full factorial (2^n) central composite design (CCD) of orthogonal type with 9 central and 6 axial points ($\alpha = 1.682$) via R 3.4.1 statistical software package for the response surface modeling and optimization of the adsorption and desorption of permethrin isomers in MISPE process. In the proposed method, six parameters with their respective ranges (Table 1), including sorbent mass, pH and flow rate of the sample, volume and flow rate of elution solvent and also the amount of acid in the composition of elution solvent were taken as independent input variables based on preliminary studies and experiments. The linear, quadratic and interaction effects of these operational parameters on the response variables were investigated through the analysis of variance (ANOVA). In this respect, among the ANOVA indicators, higher F-value which is a measurement of variance of data about the mean, based on the ratio of mean square of group variance due to error as well as lower *P* value (less than 0.05) demonstrate the significance of response surface models. On the other hand, closer to 1 determination coefficients (R^2 and adjusted R^2) are considered as an indicator of the models' quality of fit. The lack-of-fit is also calculated as another ANOVA estimator in which a higher than 0.05 values implies the adequacy of models to describe the observed data [63, 64].

Experimental procedure of MISPE

Based on the conditions set in two separate 29-run experiments for adsorption and desorption of permethrin isomers, MISPE cartridges (length of 65 mm and i.d., 5 mm) were prepared in each step by packing a certain amount of MIP in a 1 mL empty polypropylene tube between two frits. Afterward, preconditioning of the prepared cartridges was done using 1 mL methanol followed by 1 mL double distilled

Table 1 Operational range and levels of independent variables for experimental design

MISPE ^a phase	Input variables	Unit	Symbol	Levels	
				Lower	Upper
Adsorption	Sample pH	—	x ₁	3	11
	Sorbent mass	mg	x ₂	1	10
	Sample flow-rate	mL/min	x ₃	0.5	2
Desorption	Elution solvent volume	mL	x ₁	1	5
	Amount of acid in elution solvent	%, v/v	x ₂	1	10
	Elution flow-rate	mL/min	x ₃	0.5	2

^a Molecular imprinted solid phase extraction

water at a flow of 1 mL/min. Then, a 30 mL volume of 5 ppm permethrin standard was loaded onto the column at defined pH and flow rate. The extracted solution was injected into HPLC for the determination of residual permethrin at a wavelength of 200 nm. Eventually, the adsorption efficiency of MIP was calculated by the following equation [31], where C_a and C_b are the concentration of permethrin isomers in the solution before and after adsorption, respectively.

$$\text{Adsorption efficiency (\%)} = (C_a - C_b / C_a) \times 100$$

In desorption experiments, after loading of samples under the optimum conditions achieved for adsorption variables, the cartridges were washed using 2 mL of deionized water. Subsequently, the extraction of permethrin isomers was done using a certain volume of methanol/acetic acid passed through the cartridge at the desired flow rate. The elution solvent was directly analyzed by HPLC using a working wavelength of 200 nm. The percentage of extracted permethrin was calculated through the following equation [65]:

$$\text{Extraction efficiency (\%)} = (C_c / C_d) \times 100$$

Where C_c and C_d are the permethrin concentration in loading solution and eluent, respectively.

Preparation of solutions

The stock solution (1000 mg/L) was prepared by dissolving 25 mg of permethrin in 25 mL methanol and kept in the refrigerator. Various concentrations of working solutions were prepared daily by diluting the stock solution with deionized water. In order to assess the applicability of the proposed MISPE method for determination of permethrin isomers in real sample, unexposed urine samples diluted at the ratio of 1:5 with deionized water were considered as biological ones and spiked with four levels of permethrin after pH adjustment.

Results and discussion

Characterization of synthesized MIP nanoparticles

The morphology and size of prepared MIP particles were studied by field emission scanning electron microscopy (FESEM). As Fig. 1 indicates, rough and porous surface MIP spherical particles with diameter distribution from 12.4 to 21.2 nm were obtained by employing the precipitation polymerization technique. In addition, FT-IR spectroscopic analysis was performed to investigate the chemical structure of MIP before and after template molecule removal. As it can be seen in Fig. 2a and b, there are the similar characteristic bands related to leached and unleached polymers. The bands related to leached polymer indicate a shift toward lower frequencies, confirming the presence of the different intermolecular bonding between template and molecules of MAA and EGDMA during the polymerization process. The carboxylic acid O-H bands in leached and unleached MIPs are 2989 and 2990 cm^{-1} , respectively. The peaks appeared at 1731 and 1732 cm^{-1} may be attributed to the stretching vibrations of C=O of MAA or EGDMA. The peaks positioned beyond 1500 cm^{-1} , so called the fingerprint region, are complicated to interpret due to bands overlapping.

Optimization of the MISPE procedure

The complete CCD matrices as well as the achieved response values (permethrin adsorption and desorption efficiencies (%)) obtained from each set of 29-run experimental studies can be seen in Tables 2 and 3. The correlations of input variables and responses were investigated by developing a second order polynomial model with main, quadratic and interaction terms based on the following empirical equation:

$$\hat{R} = b_0 + \sum_{i=1}^n b_i x_i + \sum_{i=1}^n b_{ii} x_i^2 + \sum_{i < j}^n b_{ij} x_i x_j + \varepsilon$$

Fig. 1 FE-SEM image of prepared MIP nanoparticles

Where $\hat{R}$ is the predicted response of the process, x_i refers to the coded levels of the factors (independent or control variables), b_0 , b_i , b_{ii} , b_{ij} are the regression coefficients, and ε is the statistical error.

The adequacy of the fitted RS models was verified through analysis of variance (ANOVA), where the statistical significance of the models was represented by P value, R^2 and adjusted R^2 indicators. According to the results in Tables 4 and 5, the coefficients of multiple determinations, R^2 , were so close to 1 in both developed models. These desirable findings imply that more than 97.76 and 91.92% of data deviations can be explained by adsorption and desorption models, respectively. In addition, the agreement of predicted R^2 values with the adjusted coefficient of determination, R^2_{adj} , means that the proposed empirical models properly explain

the relationship between independent variables and responses. The statistical significance of the models was also evaluated by the calculated lack-of-fit and P -values of the model terms. The lack-of-fit (LOF) measures the models' disability to represent the data in the experimental domain at points which are not included in the regression. As indicated in Tables 4 and 5, significant P -values and non-significant values of LOF prove the validity of models for explaining the adsorption and desorption of permethrin isomers in the MISPE procedure.

Finally, the empirical models were conducted by applying multiple regression analysis for the experimental data. Prediction of the efficiency for retention and recovery of permethrin isomers through MISPE procedure could be

Fig. 2 FT-IR spectras of washed (a) and unwashed (b) MIPs

Table 2 Central composite design matrix and the obtained results in adsorption step

Run	X ₁	X ₂	X ₃	Cis-permethrin retention (%) (Mean±SD ^a)	Trans-permethrin retention (%) (Mean ± SD)
1	7.00	5.50	1.25	86.01 ± 0.82	86.83 ± 0.86
2	7.00	5.50	1.25	83.72 ± 1.06	82.28 ± 1.14
3	7.00	5.50	1.25	88.37 ± 0.27	87.96 ± 1.44
4	7.00	5.50	1.25	87.95 ± 0.77	87.81 ± 1.33
5	7.00	5.50	1.25	84.05 ± 0.7	81.69 ± 0.55
6	4.62	8.18	0.80	99.19 ± 0.44	99.93 ± 0.38
7	7.00	5.50	1.25	82.75 ± 0.51	81.68 ± 1.91
8	7.00	5.50	1.25	80.72 ± 0.24	80.9 ± 0.51
9	7.00	5.50	1.25	84.45 ± 0.44	84.18 ± 0.64
10	4.62	2.82	1.70	79.26 ± 0.43	77.71 ± 0.68
11	9.38	2.82	0.80	54.43 ± 1.6	51.37 ± 0.94
12	4.62	2.82	0.80	81.92 ± 1.5	81.94 ± 0.4
13	9.38	8.18	0.80	81.38 ± 0.15	80.77 ± 0.6
14	9.38	2.82	1.70	31 ± 1.48	28.63 ± 3.79
15	4.62	8.18	1.70	92.72 ± 0.24	91.11 ± 0.6
16	9.38	8.18	1.70	61.74 ± 1.81	60.87 ± 2.18
17	7.00	5.50	1.25	88.84 ± 0.95	88.26 ± 1.27
18	11.00	5.50	1.25	37.63 ± 0.19	35.87 ± 0.8
19	7.00	5.50	1.25	88.29 ± 0.75	88.31 ± 1.01
20	7.00	5.50	1.25	82.79 ± 0.87	81.04 ± 0.92
21	3.00	5.50	1.25	94.96 ± 0.39	95.04 ± 0.46
22	7.00	5.50	0.50	90.37 ± 0.63	88.98 ± 1
23	7.00	10.00	1.25	89.88 ± 0.39	89.76 ± 0.74
24	7.00	5.50	1.25	85.17 ± 0.6	85.01 ± 0.2
25	7.00	5.50	1.25	86.48 ± 1.47	86.77 ± 1.22
26	7.00	5.50	1.25	84.89 ± 0.26	83.11 ± 1.48
27	7.00	5.50	1.25	85.12 ± 0.7	83.65 ± 1.48
28	7.00	1.00	1.25	41.71 ± 0.96	38.81 ± 3.65
29	7.00	5.50	2.00	69.09 ± 2.45	67.83 ± 1.79

^a SD, standard deviation for $n = 3$

expressed by the following equations based on the significant regression coefficients ($P < 0.05$):

Adsorption step:

Cis permethrin

$$\hat{Y} = 20.76 + 10.39x_1 + 10.58x_2 + 30.53x_3 - 1.07x_1^2 - 0.87x_2^2 - 6.81x_3^2 + 0.52x_1x_2 - 3.99x_1x_3$$

Trans permethrin

$$\hat{Y} = 24.81 + 9.26x_1 + 10.81x_2 + 29.06x_3 - 1.08x_1^2 - 0.91x_2^2 - 7.87x_3^2 + 0.59x_1x_2 - 3.48x_1x_3$$

Desorption step:

Cis permethrin

$$\hat{Y} = 84.3 - 11.73x_1 + 1.33x_1^2 + 0.23x_2^2 + 5.4x_3^2$$

Trans permethrin

$$\hat{Y} = 47.24 - 8.4x_1 + 3.32x_2 + 33.99x_3 - 0.7x_1^2 - 7.01x_3^2 + 0.69x_1x_2 + 2.5x_1x_3 - 1.18x_2x_3$$

Where $\hat{Y}$ denotes the predicted permethrin isomers adsorption/desorption efficiency (%) and x_1 , x_2 and x_3 are the coded values of the independent variables.

Table 3 Central composite design matrix and the obtained results in desorption step

Run	X ₁	X ₂	X ₃	Cis-permethrin recovery (%) (Mean±SD ^a)	Trance-permethrin recovery (%) (Mean ± SD)
1	3.00	5.50	1.25	78.63 ± 1.17	80.41 ± 1.38
2	1.81	2.82	0.80	71.23 ± 2.31	65.28 ± 0.98
3	3.00	5.50	1.25	80.30 ± 0.81	73.19 ± 0.49
4	4.19	8.18	1.70	91.81 ± 0.48	89.24 ± 1.28
5	3.00	5.50	1.25	72.72 ± 1.44	76.39 ± 1.19
6	3.00	5.50	1.25	80.61 ± 0.73	81.08 ± 0.88
7	3.00	5.50	1.25	73.67 ± 0.94	81.14 ± 1.09
8	4.19	8.18	0.80	75.08 ± 0.35	72.85 ± 1.98
9	1.81	8.18	1.70	98.97 ± 0.74	98.54 ± 0.13
10	3.00	5.50	1.25	77.94 ± 0.38	77.87 ± 0.59
11	4.19	2.82	1.70	74.28 ± 0.71	66.1 ± 0.82
12	3.00	5.50	1.25	73.94 ± 2.48	80.54 ± 1.80
13	3.00	5.50	1.25	76.31 ± 0.61	77.41 ± 0.68
14	1.81	8.18	0.80	88.42 ± 0.50	85.17 ± 1.76
15	3.00	5.50	1.25	77.19 ± 2.03	79.21 ± 2.28
16	1.81	2.82	1.70	87.74 ± 1.68	82.01 ± 2.51
17	4.19	2.82	0.80	68.76 ± 0.36	41.76 ± 1.25
18	3.00	5.50	2.00	89.13 ± 0.50	86.08 ± 0.53
19	3.00	5.50	1.25	74.53 ± 1.36	79.61 ± 1.38
20	3.00	5.50	1.25	78.11 ± 1.45	76.64 ± 1.13
21	5.00	5.50	1.25	74.1 ± 1.68	67.56 ± 0.26
22	3.00	5.50	1.25	72.89 ± 1.15	78.52 ± 1.06
23	3.00	10.00	1.25	94.56 ± 2.51	93.93 ± 3.01
24	3.00	1.00	1.25	69.1 ± 2.65	62.1 ± 1.20
25	3.00	5.50	1.25	78.43 ± 0.98	78.79 ± 0.99
26	3.00	5.50	0.50	69.68 ± 0.84	64.97 ± 0.43
27	3.00	5.50	1.25	75.26 ± 0.91	77.12 ± 0.75
28	3.00	5.50	1.25	79.03 ± 1.20	79.42 ± 1.34
29	1.00	5.50	1.25	88.57 ± 2.73	85.78 ± 0.83

^a SD, standard deviation for n = 3

Analysis of response surface

The interactive relationship of each pair of independent variables upon the achieved responses was represented graphically by 2D contour plots. Sample pH is one of the most important variables affecting the chemical structure of the target analytes and the quality of hydrogen bonding between MIP functional groups and the desired compounds. The interactive relationship of sample pH and the other independent variables for the adsorption efficiency of permethrin isomers has been depicted in Fig. 3a and b. As can be seen, higher adsorption yield of both permethrin isomers was obtained by decreasing the sample pH from 10 to 4. In addition to the stability of permethrin molecular form in a neutral to mid-acidic range, the enhancement of adsorption efficiency was predictable in acidic sample pH range due to the nature of hydrogen

bonding. At higher pH values, hydrolysis reaction suppresses the capability of sorbent's functional groups for effective bonding with permethrin molecules which decreases the adsorption yield.

Figure 3a and c indicates the combined effect of the sorbent mass with sample pH and flow-rate on the adsorption of permethrin isomers. As can be observed in Fig. 3a, increasing the sorbent mass up to 8 mg, while the sample pH is about 4, causes more than 95% of permethrin to be adsorbed by the MISPE column. It is also clear from Fig. 3c that in neutral sample pH, increasing the sorbent mass can only lead to the maximum adsorption efficiency when lower sample loading flow-rate is applied. As it has been observed in similar studies [66, 67], better adsorption yields in higher amounts of the sorbent can be attributed to the increase of specific binding sites and adsorption capacity in higher amounts of adsorbent.

Table 4 Analysis of variance (ANOVA) for the fitted quadratic polynomial model (adsorption step)

Model term	Df ^a	Cis-permethrin				Trans-permethrin			
		Sum of squares	Mean square	F-value	P- value	Sum of squares	Mean square	F-value	P- value
x ₁	1	3575	3575	429.665	<0.0001	3825	3825	366.277	<0.0001
x ₂	1	2102	2102	252.673	<0.0001	2338	2338	223.925	<0.0001
x ₃	1	567	567	68.138	<0.0001	610	610	58.398	<0.0001
x ₁ ²	1	811	811	97.497	<0.0001	831	831	79.583	<0.0001
x ₂ ²	1	713	713	85.724	<0.0001	780	780	74.67	<0.0001
x ₃ ²	1	32	32	3.819	0.065549	42	42	4.063	0.05819
x ₁ :x ₂	1	91	91	10.92	0.003727	114	114	10.953	0.00369
x ₁ :x ₃	1	144	144	17.307	0.000532	109	109	10.48	0.00434
x ₂ :x ₃	1	0	0	0	0.99807	0	0	0.037	0.8502
Residuals	19	158	8	—	—	198	10	—	—
Lack of fit	5	79.5	15.90	2.8318	0.056971	87.8	17.56	2.2220	0.10987
Multiple R ² = 0.9807; Adjusted R ² = 0.9716						Multiple R ² = 0.9776; Adjusted R ² = 0.967			

^a Df, degree of freedom

It is worth mentioning that, synthesis of the MIP in nano-scaled particles provides much more specific binding sites per mass unit of adsorbent, resulting in higher adsorption and recycling efficiencies.

The effect of sample flow-rate (0.5–2 mL/min) on permethrin adsorption was also investigated in correlation with the other factors. As illustrated in Fig. 3b and c, similar to some other studies [68, 69], a minimum to the average value of initially predicted range for the sample flow-rate provides the opportunity required for the adsorption of more than 90% of the analyte especially at lower sample pH. However, from the interactional relation of sample flow-rate and pH, it is

also deduced that the pace of experiments could be enhanced through applying lower pH values.

As mentioned previously, the interactional effect of three variables including volume, concentration, and flow-rate of elution solvent was studied on the desorption process of permethrin. As Fig. 4a and d indicates, increasing the amount of acetic acid in elution solvent composition can raise the desorption efficiency up to maximum and reduce the amount of required solvent. Also, as it has been outlined in Fig. 4b, c, e, and f, reducing the elution solvent volume containing an appropriate amount of acid (5.5–10%, v/v) provides satisfactory desorption yield in higher flow-rate of elution. So, beside

Table 5 Analysis of Variance (ANOVA) for the fitted quadratic polynomial model (desorption step)

Model term	Df ^a	Cis-permethrin				Trans-permethrin			
		Sum of squares	Mean square	F-value	P- value	Sum of squares	Mean square	F-value	P- value
x ₁	1	270.4	270.4	36.181	<0.0001	615.7	615.7	95.025	<0.0001
x ₂	1	662	662	88.59	<0.0001	1522.3	1522.3	234.942	<0.0001
x ₃	1	492.6	492.6	65.92	<0.0001	828	828	127.792	<0.0001
x ₁ ²	1	80.2	80.2	10.728	0.00398	21.9	21.9	3.384	0.0815
x ₂ ²	1	78.3	78.3	10.484	0.00433	6	6	0.931	0.3467
x ₃ ²	1	23.5	23.5	3.15	0.09194	31.9	31.9	4.919	0.039
x ₁ :x ₂	1	2.6	2.6	0.349	0.56145	39.6	39.6	6.119	0.023
x ₁ :x ₃	1	2.9	2.9	0.387	0.54128	14.1	14.1	2.18	0.1562
x ₂ :x ₃	1	3.4	3.4	0.461	0.50533	16	16	2.468	0.1327
Residuals	19	142	7.5	—	—	123.1	6.5	—	—
Lack of fit	5	43.79	8.76	1.2488	0.338742	59.41	11.88	2.6116	0.07185
Multiple R ² = 0.9192; Adjusted R ² = 0.881						Multiple R ² = 0.9618; Adjusted R ² = 0.9436			

^a Df, degree of freedom

Fig. 3 Response surface contour plots for the adsorption yield of permethrin isomers (cis and trans) as a function of mutual interaction between: **a** sample pH and sorbent mass; **b** sample pH and sample flow-rate; **c** sorbent mass and sample flow-rate

the prominent impact of weak acids such as acetic acid in breaking hydrogen bonds and improvement of desorption efficiency [70], which also has observed in some other cases, it seems that higher flow rate of elution solvent can also result in a more appropriate extraction [31, 71]. Eventually, based on the designed regressional models, the overall optimum experimental conditions of the MISPE process were calculated via Microsoft Excel 2013 Solver add-in considering the primary operational ranges (Table 1) as well as the desired efficiency for the adsorption and desorption steps. The optimum operating conditions were selected as follow: a sorbent mass of 7.71 mg, sample pH 5.58 and 5.68 for cis and trans-permethrin, respectively, and sample flow rate of 0.6 mL/min. Also, 5 mL and 3.94 mL of methanol/acetic acid at the ratios of 91:9 and 90:10 (v/v, %) and at the flow rate of 2 mL/min were chosen as the elution solvents for extraction of cis and trans isomers, respectively.

Comparison of the NIP and MIP

Under the optimized conditions, binding experiments were performed in order to compare the capability of prepared MIP and NIP for the adsorption of permethrin isomers. For this purpose, 20 mL of 5 mg/L permethrin solutions were applied for the adsorption experiments ($n=3$) and the adsorption efficiencies were calculated for the imprinted and non-imprinted nano-particles. For both permethrin isomers, significant difference was observed between the adsorption efficiencies of MIP cis: $96.7 \pm 0.24\%$, trans: $96.88 \pm 0.57\%$ and NIP (cis: $37.29 \pm 5.18\%$, trans: $37.45 \pm 6.67\%$), demonstrating the appropriate forming of selective adsorption sites in the MIP structure.

Effect of the interferences

To confirm the selectivity of the method, the extraction recovery of permethrin isomers was evaluated in the presence of three commonly used pesticides. In this regard, different concentrations of a combined metribuzin, diazinon, and malathion samples were added to four vials containing 30 ml of 50 ppb permethrin solution. Subsequently, the optimized MISPE procedure was applied for sample cleanup. The extraction efficiencies of more than 92 and 94% were obtained for both permethrin isomers in the presence of 50 and 100-fold of mentioned

Fig. 4 Response surface contour plots for the adsorption yield of cis (**a–c**) and trans-permethrin (**d–f**) as a function of mutual interaction between: **a** and **d** elution solvent volume and the amount of acid in elution solvent; **b**

and **e** elution solvent volume and elution flow rate; **c** and **f** the amount of acid in elution solvent and elution flow rate

pesticides, respectively, indicating the high potential of prepared nanoparticles for selective recognition of permethrin in presence of matrix interferences.

Method validation

After successful application of the proposed method under the optimal conditions for the adsorption and desorption of permethrin isomers, the developed MISPE technique was used to plot two calibration curves in the ranges of 20 to 120 and 50 to 110 µg/L of permethrin isomers in water and urine samples, respectively. A linear relationship was observed between permethrin concentration and chromatogram peaks area ($y = 0.302x + 0.969$; $R^2 = 0.9975$ and $y = 0.215x + 0.146$; $R^2 = 0.9908$ for cis and trans-permethrin in water sample, respectively, as well as $y = 0.297x + 1.501$; $R^2 = 0.9992$ and $y = 0.214x + 0.566$; $R^2 = 0.99$ for cis and trans-permethrin in urine sample, respectively). The limits of detection (LOD) and the limits of quantification (LOQ) were also calculated (Table 6) based on the following equations [3]:

$$\text{LOD} = \frac{3 S_b}{m} \quad \text{LOQ} = \frac{10 S_b}{m}$$

Where, S_b is the standard deviation of the blank and m is the slope of calibration graph.

The reproducibility of the proposed method was also assessed by performing six consecutive experiments for a single permethrin concentration (100 µg/L) during one day. The relative standard deviations (RSDs) of 2.19 and 4.4% were obtained for cis and trans-permethrin samples, respectively, which shows the satisfactory performance of the optimized MISPE method for determination of permethrin isomers.

Real sample analysis

In order to investigate the performance of the developed protocol towards the determination of trace permethrin residue in

Table 6 LOD and LOQ of the proposed method for cis and trans-permethrin in water and urine samples

Analyte	LOD (µg/L)		LOQ (µg/L)	
	Water	Urine	Water	Urine
Cis-permethrin	5.51	12.22	18.37	40.74
Trans-permethrin	5.72	13.17	19.34	43.92

Table 7 Recovery of permethrin isomers extracted from spiked urine samples under optimal MISPE condition

Concentration of analyte (ppb ^a)	Cis-permethrin		Trans-permethrin	
	Relative recovery (%)	RSD ^b (%) (n = 3)	Relative recovery (%)	RSD (%) (n = 3)
50	94.05 ± 3.90	4.14	94.58 ± 4.27	4.51
70	93.01 ± 0.86	0.92	93.10 ± 0.007	0.0075
90	94.64 ± 2.74	2.89	94.10 ± 3.11	3.3
110	97.14 ± 3.90	4.01	96.26 ± 2.73	2.83

^a ppb, Part per billion^b RSD, Relative standard deviation

real samples, four concentration levels of permethrin isomers were spiked in 10 mL of diluted urine samples as mentioned in “[Preparation of solutions](#)” section. The optimized MISPE procedure was successfully applied for the determination of permethrin isomers in urine samples. The obtained results presented in Table 7 depict desirable extraction recovery of the target analytes, confirming the high potential of the synthesized MIP for sensitive and selective biological monitoring of permethrin.

Comparison of the present study with literatures

The proposed method for determination of permethrin isomers in urine sample was compared with the other attempts for quantitative analysis of various analyses using molecularly imprinted nanoparticles. As it can be seen in Table 8, the satisfactory recovery and sensitivity, which was achieved by the considerably lower amount of sorbent mass are clearly comparable with other studies and in some cases, the proposed method is superior to the other studies.

Conclusions

In this work, MISPE technique was developed for selective extraction and preconcentration of permethrin samples prior to determination by high performance liquid chromatography. MIP nanoparticles were synthesized using precipitation polymerization technique and the critical variables affecting the efficiency were optimized by means of response surface modeling approach. Finally the designed MISPE-HPLC approach was used for cleanup of urine samples. The satisfactory results confirmed the reliability and efficiency of the proposed method for trace analysis of permethrin isomers in biological samples. Compared to the conventional sample preparation techniques, MISPE-HPLC suggests an inexpensive and easy to operation method with lower consumption of organic solvents and higher extraction efficiency that made it a promising choice for fast and reliable determination of pesticides in complex biological and environmental matrices with no special sample pre-treatment prior to the analysis.

Table 8 Comparison of the proposed method with literatures

Target analytes	Synthesis method	Sample	Applied sorbent mass (mg)	Recovery (%)	LOD (µg/L)	Ref.
Diazinon	Ultrasonication-assisted encapsulation	Tap water	–	98.2–105.4	38.6	[72]
Carbaryl	Precipitation	River and tap water	9.42	97–112	33	[73]
2,4-dichlorophenoxyacetic acid	Precipitation	Water and urine	50	97.8–99	1.2–1.8	[31]
Metribuzin	Precipitation	Human urine	25	93.82–97.84	5.75	[74]
Hippuric acid	Sol-gel	Urine	200	88–104	0.15	[75]
Celecoxib	Precipitation	Human plasma	250	96	0.08	[76]
17β-estradiol	Surface molecular imprinting	Milk	100	88.9–92.1	0.01	[77]
Nicotinamide	Surface molecular imprinting	Urine and milk	2	95.85–101.27	1.96	[78]
Permethrin	Precipitation	Human urine	7.71	93.01–97.14	5.51 and 5.72	This study

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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