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Amira Anene, Yves Chevalier, Souhaira Hbaieb. Molecularly Imprinted Polyaniline as Solid-Phase Material for the Extraction of UV Filters From Waters. *SEPARATION SCIENCE PLUS*, 2024, 7 (9), pp.e202400104. 10.1002/sscp.202400104 . hal-04688043

HAL Id: hal-04688043

<https://hal.science/hal-04688043>

Submitted on 4 Sep 2024

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Molecularly Imprinted Polyaniline as Solid Phase Material for the Extraction of UV Filters from Waters

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Highlights

- ▶ Development of novel PANI@MIP-SPE procedure for maximum retention of benzophenone-4.
- ▶ High selectivity and good affinity of PANI@MIP-SPE method toward benzophenone-4.
- ▶ Successful application of PANI@MIP-SPE procedure for determination of benzophenone-4 in water samples.

Abstract

Solid-phase extraction (SPE) using a polyaniline molecularly imprinted polymer (PANI@MIP) is optimized for the extraction of benzophenone-4 (BP4) from aqueous matrices. Firstly, the three main variables influencing the washing step of the SPE method: type, composition, volume and percolation flow rate of the washing solvent were investigated. The optimal conditions for the washing step using the new PANI@MIP-SPE for maximum retention of BP4 and elimination of interfering molecules (BP3 and BP) were determined as follows: 1.25 mL acetonitrile/acetic acid/hexane (45/05/50 v/v/v) as wash solvent at a flow rate of 1 mL·min⁻¹. A multiple linear regression model was validated using Student's *t*-test to identify the most significant variables affecting this step, including the type and volume of each eluent solvent and the volume of the ternary mixture solvents. The reliability of the model was further confirmed by analysis of variance (ANOVA) and Fisher's F test at $\alpha = 0.05$. The extraction recoveries of the target analyte exceeded 92% over the range of concentrations (from 0.1 mg·L⁻¹ to 20 mg·L⁻¹), and the maximum retention capacity of PANI@MIP-SPE was 600 µg·g⁻¹, in contrast to 50 µg·g⁻¹ for PANI@NIP-SPE.

Keywords: Benzophenone-4; Solid phase extraction, molecularly imprinted polyaniline, selective extraction; experimental design.

Introduction

Water pollution is an ongoing threat to public health, with a wide range of persistent organic pollutants, including personal care products (PCPs), being released at high concentrations, resulting in their detection in water [1–3]. The UV filter BP4 (2-hydroxy-4-methoxybenzophenone-5-sulphonic acid) is widely used in sunscreens and as an anti-UV agent in various products such as cosmetics, beauty creams, shower gels, hairsprays, shampoos, plastics and agricultural products [4]. Due to its widespread use, chemical stability and high water solubility, BP4 is increasingly detected in surface waters including rivers, oceans, treated effluent from swimming pools, recreational ponds, and sewage [5–7]. Its potential endocrine disrupting effects is a concern to public health and aquatic life. There is a lack of suitable analysis methods for BP4, so that the aim of this study was to develop a method for quantitative analysis and monitoring of it. Until recently, gas chromatography coupled to mass spectrometry (GC-MS) has been the predominant technique for the determination of benzophenone UV filters in aqueous environmental matrices [8]. Unfortunately, GC analysis requires a derivatization into volatile compounds by reacting the phenolic hydroxyls with acetic anhydride or a silylation reagent [9], which is not suitable for BP4 that contains a sulphonic group attached to the aromatic ring. Liquid chromatography (LC) overcomes these limitations but requires sample pretreatment by liquid-liquid extraction or solid-phase extraction (SPE) for a clean-up and concentration of the analyte that improves the detection. However, conventional SPE sorbents (C8, Oasis HLB, Oasis MCX, StrataX and C18) retain analytes through non-specific interactions, leading to partial co-extraction of interfering substances [10,11]. Therefore, new SPE sorbents with high selectivity, such as molecular imprinting polymers (MIPs) [12], are desirable for the purification and concentration of molecules in complex environmental samples. A new concept of PIP for selective extraction of BP4 has been disclosed using silica stationary phases designed for HPLC grafted with polyaniline [13,14]. The original idea is using the rigid polyconjugated polyaniline under its cationic emeraldine salt. The purpose of the present work is the implementation of these PANI@MIP materials in SPE devices for purification and concentration of BP4. The extraction efficiency of the PANI@MIP SPE devices can be influenced by certain parameters, such as the type, volume and flow rate of the washing solvent and the type and volume of the eluent. The influence of these parameters on the performance of the PANI@MIP-SPE procedure was simultaneously optimized (multivariate optimization) using experimental design (ED).

2. Experimental section

2.1. Chemicals and reagents

Benzophenone-4 (BP4), benzophenone-3 (BP3) and benzophenone (BP) standards were purchased from TCI-EP. Aniline, N-[3-(trimethoxysilyl)propyl]aniline (APTMS) and diisopropylethylamine (DIEA) were purchased from Sigma-Aldrich. All solvents, including HPLC grade acetonitrile, ethanol, methanol, acetone and hexane, were purchased from Sigma-Aldrich and used as received. Acetic acid was purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Deionized water was prepared using a Milli-Q ultrapure water system (Bedford, MA, USA).

2.2. Standard solutions

Stock solutions ($1 \text{ mg}\cdot\text{mL}^{-1}$) of each benzophenone-4, benzophenone-3 and benzophenone were prepared in ethanol. Working solutions ranging from 0.1 to $120 \text{ mg}\cdot\text{L}^{-1}$ were obtained by successive dilutions in ethanol (0.6%)/acetate buffer solution (1 mM, pH 4). Both stock and working standard solutions were stored in a refrigerator at 5°C .

2.3. Preparation of PANI@MIP

A two-step preparation method for PANI@MIP was used following our previous works [13,14]. First, the silica support was modified with an organosilane containing aniline, and the latter was copolymerized with aniline in a second step. 5 g of dried silica dispersed in 200 mL of anhydrous toluene containing 4 g of APTMS and 4 g of DIEA was heated at reflux under a nitrogen atmosphere for 12 h, rinsed with 30 mL of THF, and dried overnight at 60°C . 500 mg of modified silica was dispersed in 100 mL of aqueous HCl solution at pH 4, in the presence of (2 mmol) of aniline monomer and (0.4 mmol) of BP4. The reaction medium was cooled to 0°C and 22.8 mg (0.1 mmol) of ammonium persulfate (APS) in 2 mL of water was added dropwise to the solution. The reaction was run at 0°C for 8 h. The resulting material was isolated by filtration, subsequently washed repeatedly with aqueous ammonia solution (0.3 M) and with methanol in a Soxhlet extractor. The polyaniline imprinted polymer (PANI@MIP) was then dried under vacuum at 40°C for 24 h and stored under vacuum.

2.4. PANI@MIP-SPE method

The PANI@MIP SPE columns were assembled by packing 50 mg of PANI@MIP into SPE cartridges. To ensure proper compaction of the material, the polymers in the cartridges were secured by polyethylene frits at the top and bottom. The cartridges were conditioned with 1 mL ethanol followed by 1 mL acetate buffer solution (1 mM, pH 4) and then loaded with the working solutions or spiked with $2 \text{ mg}\cdot\text{L}^{-1}$ BP4, BP3 and BP in tap water at a flow rate

of $0.5 \text{ mL}\cdot\text{min}^{-1}$. The columns were then washed with 1.25 mL of an (acetonitrile/acetic acid/hexane) mixture (45/05/50 v/v/v) at a flow rate of $1 \text{ mL}\cdot\text{min}^{-1}$ and finally eluted with 3 mL of a (methanol/acetone) mixture (50/50 v/v). All the fractions collected were then evaporated to dryness at $35 \text{ }^\circ\text{C}$ under a stream of nitrogen gas. The dry residues were then dissolved in ethanol for their HPLC analysis. The principle of extraction in SPE is summarized in [Figure 1](#).

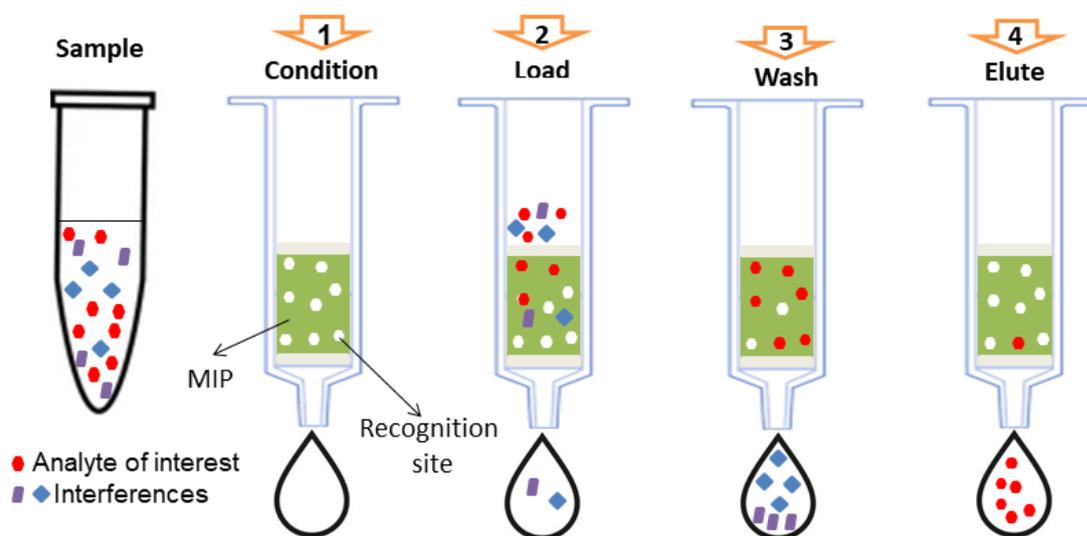


Figure 1. General protocol for solid phase extraction.

2.5. Chromatographic conditions

A high performance liquid chromatography (HPLC) system, Agilent 1100, equipped with a DAD detector, two pumps and an automatic injector with a six-port injection valve, was used for the identification and quantification of (BP4, BP3 and BP) in aqueous solutions. A Chemstation LC software workstation was used for data processing. The method has been optimized regarding the temperature and type of mobile phase. The optimized HPLC operating conditions included a C18 reversed-phase column ($250 \text{ mm} \times 3 \text{ mm i.d.}, 5 \mu\text{m}$) and a solvent mixture (methanol: ammonium acetate buffer) at pH 6.9 as mobile phase, with a flow rate of $0.5 \text{ mL}\cdot\text{min}^{-1}$ and an injection volume of $20 \mu\text{L}$. The oven temperature was set at $35 \text{ }^\circ\text{C}$ and the BPs were detected at a wavelength of 286 nm. Separation of the UV filters was achieved in less than 30 min ([Figure 2](#)).

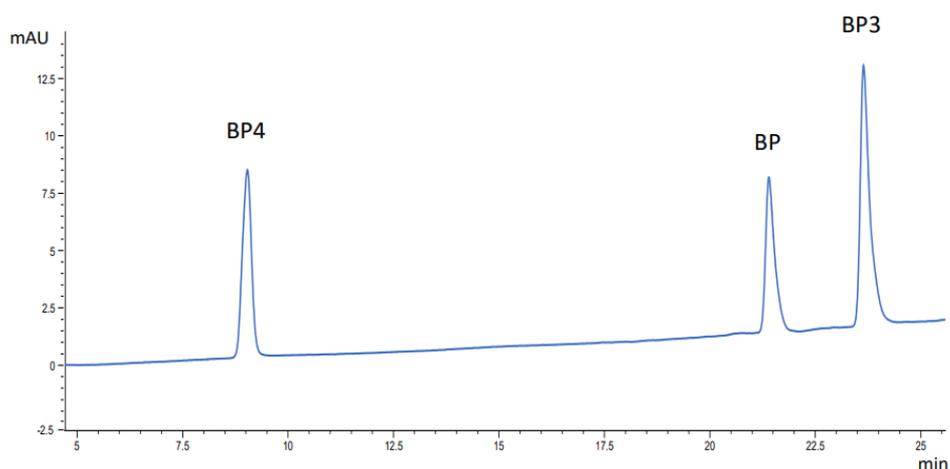


Figure 2. Chromatograms of a mixture of BP4, BP3 and BP compounds. The concentration of each molecule in the mixture was $0.1 \text{ mg}\cdot\text{L}^{-1}$.

The calibration curve of (BP4, BP3 and BP) were linear within the concentration range of 0.1 to $120 \text{ mg}\cdot\text{L}^{-1}$ in ethanol (0.6%)/acetate buffer solution (1 mM , pH 4). The average linear regression equation of the curve were $y = 64.315x - 4.6037$ ($r^2 = 0.9996$, $F = 53894$) for BP4, $y = 89.025x + 13.591$ ($r^2 = 0.9991$, $F = 54494$) for BP3 and $y = 54.106x + 7.0012$ ($r^2 = 0.9992$, $F = 52894$) for BP. This is considered acceptable as the linear regression was constructed with 25 different injections at different calibration levels. The limits of detection and quantification (LOD and LOQ) were estimated from the background noise of the analytical blank using the graphical method. The calculated LOD and LOQ were lower for BP4 than for BP3 and BP (Table 1).

Table 1. Summary of linearity, LOD, LOQ, inter-day precision and intra-day precision.

	BP4	BP3	BP
Linearity (r^2)	0.9996	0.9991	0.9992
LOD ($\text{mg}\cdot\text{L}^{-1}$)	0.010	0.004	0.008
LOQ ($\text{mg}\cdot\text{L}^{-1}$)	0.036	0.015	0.027
Inter-day precision (RSD, %, $n = 10$)	3.8	4.2	4.2
Intra-day precision (RSD, %, $p = 4$)	2.9	3.0	3.0

2.6. Capacity and selectivity of PANI@MIP-SPE method

The capacity of the PANI@MIP polymer was evaluated by passing different concentrations of BP4 (0.1 to 120 mg·L⁻¹) through spiked tap water using PANI@MIP-SPE cartridges according to the developed method. The selectivity of the PANI@MIP-SPE method was evaluated based on the retention of BP4 and its structurally related compounds (BP3 and BP) from 2 μmol·L⁻¹ spiked tap water. The amount of analytes percolating through PANI@MIP-SPE or PANI@NIP-SPE cartridges was determined in wash and elution fractions.

3. Results and discussion

3.1. Coating of imprinted polyaniline to silica surface

The two-step preparation of PANI@MIP described in previous work [13,14] is shown in Figure 3. The classical polymerization process followed a chemical oxidative condensation of the anchored aniline moieties and the aniline monomers. This resulted in the formation of a PANI layer on the surface of SiO₂ in the presence of BP4. PANI@MIP was subsequently obtained after extraction of BP4, leaving recognition cavities complementary in shape, size and functional groups to the template molecules.

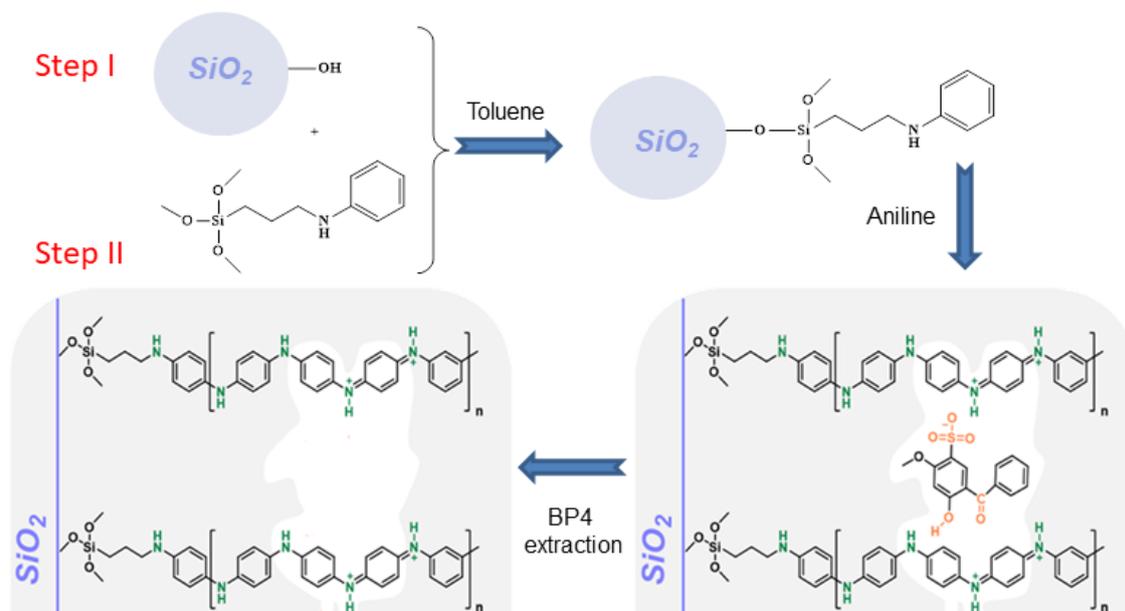


Figure 3. Two-step synthesis scheme of molecularly imprinted polyaniline. The first step is the grafting of aniline onto the silica surface and the second step is the copolymerization of free and grafted aniline.

3.2. Mass of adsorbent and binding capacity

A novel procedure was optimized for the development of PANI@MIP-SPE, ensuring efficient and selective extraction of BP4. The ability of the PANI@MIP-SPE to selectively extract BP4 was evaluated at different adsorbent masses. The amount of adsorbent in the SPE cartridge plays a crucial role in the extraction recovery of the target molecule. Too much adsorbent mass can lead to incomplete elution of the analyte, requiring a large elution volume, while too little adsorbent mass can lead to premature elution. Therefore, different amounts of PANI@MIP material (30, 50, 80 and 100 mg) were used to optimize the amount of PANI@MIP material. Too low recoveries (< 70%) of the interfering molecules BP3 and BP and (90%) of BP4 were obtained with 30 mg of PANI@MIP adsorbent, suggesting rapid saturation of the recognition sites and release of unbound PBs molecules during the percolation and washing steps. Increasing the mass of the adsorbent to 50, 80 and 100 mg significantly improved the retention of interfering PBs, with a maximum recovery above 99%, indicating that the increased number of binding sites leads to better binding of PBs molecules. As 50 mg PANI@MIP showed a high capacity for BP4, this mass was selected for the optimization of the PANI@MIP SPE solid-liquid extraction protocol.

3.3. Effect of washing solvent on benzophenone-4 recovery

The competitive molecules (BP3 and BP) weakly retained by the non-specific sites were eliminated with different solvents, while the target molecule BP4 remained adsorbed on PANI@MIP during the PANI@MIP-SPE analysis. For this purpose, water spiked with BP3, BP and BP4 was loaded onto the developed PANI@MIP-SPE at a flow rate of $0.5 \text{ mL}\cdot\text{min}^{-1}$ and washed with acetonitrile/acetic acid/hexane mixture. Indeed, the acetonitrile/acetic acid mixture is able to break the non-specific hydrogen bonds while keeping the BP4 molecules attached to selective molecular sites of the PANI@MIP material. The dissociating nature ($\epsilon_r = 37.5$) of acetonitrile weakens the hydrogen bonds between the interfering molecules and the imprinted polymer [15], while the acetic acid, a hydrogen bond donor-acceptor, interacts with the functional groups of the MIP material. The hexane low eluting solvent was used to elute non-polar interfering compounds. After the washing step of the SPE method, three factors that could affect the retention of BP4 and the elution of interfering molecules (BP, BP3) were considered: the composition of the washing solvent (F_1), the percolated volume (F_2) and the percolation flow rate (F_3). A full two-level factorial design was used to explore the functional relationship between a response of interest "Y" and the F_i independent variables coded as X_i (-1, 0, +1). This design allows for the study of k factors at two levels,

containing all possible combinations of k factors at their two levels giving 2^k experiments [16]. The total number of trials required for this study is therefore 8 (2^3). To statistically evaluate the significance of the main effects and the effects of the interaction, four points were made in the center of the study area. These factors were represented by +1 for high level (F_1 : (acetonitrile/acetic acid/hexane) (97/03/0 v/v/v), F_2 : 0.5 mL, F_3 : 0.5 mL·min⁻¹), 0 for central level (F_1 : (acetonitrile/acetic acid/hexane) (47/03/50 v/v/v), F_2 : 1.25 mL, F_3 : 1 mL min⁻¹) and -1 for low level (F_1 : (acetonitrile/acetic acid/hexane) (0/0/100 v/v/v), F_2 : 2 mL, F_3 : 1.5 mL·min⁻¹). For mathematical modeling, a first order polynomial model was applied and the predicted response Y was calculated using the following equation:

$$Y = a_0 + \sum_i a_i X_i + \sum_{i,j, i \neq j} a_{ij} X_i X_j + \sum_i a_{ii} X_i^2 \quad (\text{Eq. 1})$$

a_0 represents the intercept coefficient; a_i ($i = 1, 2, 3$) are the linear coefficients; a_{ii} ($i = 1, 2, 3$) are the squared coefficients; a_{ij} ($i = 1, 2, 3; j = 1, 2, 3$) interaction coefficients.

The significance of the coefficients was tested using Student's t -test. The critical value of t is $t_{(3, 0.05)} = 3.182$ for a significance level $\alpha = 0.05$. Table 2 reports the twelve experiments built by screening the 2 levels for the 3 factors (X_1 to X_3) as well as the 3 responses (Y_1 to Y_3) of the extraction recovery of each compound BP4, BP3 and BP.

Table 2. Experimental matrix and observed values of responses (Y_1 , Y_2 and Y_3).

Experiment number	Actual variables			Response (Y_1)	Response (Y_2)	Response (Y_3)
	F_1 (mL)	F_2 (mL)	F_3 (mL)	Recovery of BP4 (%)	Recovery of BP3 (%)	Recovery of BP (%)
1	0	0.5	0.5	ND	72.4	75.30
2	100	0.5	0.5	14.2	69.9	76.00
3	0	2.0	0.5	ND	79.0	89.00
4	100	2.0	0.5	17.5	76.3	82.60
5	0	0.5	1.5	ND	62.5	67.40
6	100	0.5	1.5	7.4	61.6	62.40
7	0	2.0	1.5	ND	70.9	73.60
8	100	2.0	1.5	10.6	62.7	67.10
9	50	1.25	1.0	0.73	99.6	99.10
10	50	1.25	1.0	0.81	98.7	99.50
11	50	1.25	1.0	0.76	99.4	99.24
12	50	1.25	1.0	0.86	99.7	99.21

ND: not detectable

The effects of all the factors and their interactions on the three responses, as well as the statistical values of the Student's *t*-test, are summarized in the [Table 3](#).

Table 3. Factors and interactions and their significance on the responses (*i* = 1, 2, 3).

Factors and their interactions	Coefficient names	Coefficient values	Standard deviation	<i>t</i> -Student
Response 1				
F_1	a_1	6.212	0.020	307.439
F_2	a_2	0.812	0.020	40.208
F_3	a_3	-1.712	0.020	-84.746
Interaction 1-2	a_{12}	0.812	0.020	40.208
Interaction 1-3	a_{13}	-1.712	0.020	-84.746
Interaction 2-3	a_{23}	-0.012	0.020	-0.618
Response 2				
F_1	b_1	-1.787	0.159	-11.212
F_2	b_2	2.812	0.159	17.641
F_3	b_3	-4.987	0.159	-31.284
Interaction 1-2	b_{12}	-0.937	0.159	-5.880
Interaction 1-3	b_{13}	-0.487	0.159	-3.057
Interaction 2-3	b_{23}	-0.437	0.159	-2.744
Response 3				
F_1	c_1	-2.237	0.059	-37.362
F_2	c_2	3.987	0.059	66.584
F_3	c_3	-6.462	0.059	-107.911
Interaction 1-2	c_{12}	-0.987	0.059	-16.489
Interaction 1-3	c_{13}	-0.637	0.059	-10.645
Interaction 2-3	c_{23}	-1.262	0.059	-21.081

According to the results shown in [Table 3](#) related to Response 1, the effects of the composition of the washing solvent (F_1), its volume (F_2) and the percolation flow rate (F_3) are statistically significant. The positive sign of the values of the coefficients related to the factors F_1 and F_2 shows that the percentage of extraction of BP4 increases when the volume of the washing solvent is composed of 100% ACN-AcOH. On the other hand, an increase in the percolation rate (F_3) of the washing solvent causes a decrease of the response. The optimal condition maintaining the BP4 molecules adsorbed on the PANI@MIP material and eluting the interfering molecules (BP3, BP) involve using a low volume of hexane and a high percolation flow rate (experiment 5, [Table 2](#)). The *t* values given in [Table 3](#) show that two interactions have a statistically significant effect, interactions 1-2 and 1-3 referring to the interactions of the washing solvent composition with its volume the percolation flow

rate. Table 3 for Response 2 shows that the three factors F_1 , F_2 and F_3 have a significant effect on the percentage extraction of BP3. The factors b_1 and b_3 are negative, indicating that the extraction of BP3 decreases when these F_1 and F_3 increase. The effect of the factor is positive. The Response 2 increases when F_2 increases (b_2 is positive). The optimal condition for removal of BP3 is only hexane as a washing solvent with a low percolation flow rate (experiment 3, Table 2). The interaction between the composition of the washing solvent and its volume has an influence on the extraction of BP3. Table 3 for Response 3 shows similar results to these obtained with Response 2 in terms of the main effects of factors F_1 , F_2 and F_3 and to the significance of their coefficients. This was quite expected, since the two interfering molecules (BP3, BP) are structural analogues. However, in this case all interactions (1-2, 1-3 and 2-3) are significant. As obtained with Response 2, the optimal condition for extraction of BP is using hexane as the washing solvent and a low percolation flow rate (experiment 3, Table 2). In this study, optimal multi-criteria experiments were adjusted in order to maintain BP4 onto PANI@MIP and to elute interfering molecules (BP3 and BP) and therefore to manage the compromise between the three responses. In this specific case, experiments at the center of the field of study (experiments 9, 10, 11 and 12, Table 2) were retained and repeated four times. Indeed, in these experiments, a negligible elution of BP4 (0.79 ± 0.1) and an almost total extraction of both interfering molecules BP3 (99.3 ± 0.7) and BP (99.2 ± 0.2) was achieved.

In the further study, to optimize the conditions of the elution step, a mixture of ACN and AcOH/hexane (50/50 v/v) was chosen as washing solvent with a volume of 1.25 mL and a percolation flow rate of $1 \text{ mL}\cdot\text{min}^{-1}$.

3.4. Optimization of elution step

The elution step of breaking the interactions between the recognition sites of PANI@MIP and BP4. Once the interfering molecules have been eliminated, the BP4 molecules can be eluted with a solvent of higher eluting power to eliminate the specific interactions between the BP4 molecules and the PANI polymer, which are mainly of electrostatic origin and hydrogen bonding [13]. In order to increase BP4 recovery, an appropriate choice of eluting solvent is required. In this study, two eluting organic solvents including methanol and acetone were evaluated for BP4 recovery. An aqueous solution of sodium hydroxide (0.5 M) was also tested so as to eliminate the ionic interactions between the template and the PANI polymer. At the same time, mixtures of these solvents were used to determine if the recovery of BP4 molecules could be increased. During this step, the percolation flow rate was kept at

0.5 mL·min⁻¹. The effects of these different solvents on the recovery of BP4 were studied using the mixture design method for a volume of 3 mL and 6 mL of solvent. The response depends on the ratio of each species and not on their amount. In the case of the ternary mixture studied, the compositions X_A , X_B and X_C of the solvents A: methanol, B: acetone and C: aqueous sodium hydroxide solution (0.5 M) were related as follows:

$$X_A + X_B + X_C = 1 \text{ with } 0 \leq X_A, X_B, X_C \leq 1 \quad (\text{Eq. 2})$$

For responses $Y(3 \text{ mL})$ and $Y(6 \text{ mL})$, augmented centered design was applied and allowed to use the following model form:

$$Y = z_A X_A + z_B X_B + z_C X_C + z_{AB} X_A X_B + z_{AC} X_A X_C + z_{BC} X_B X_C + z_{ABC} X_A X_B X_C \quad (\text{Eq. 3})$$

where z_i are the coefficients of the model determined from experiments.

A three-variable Box-Behnken design with 10 experiments was carried out to estimate the model coefficients. As for the experimental design, the significance of the coefficients of the mathematical model was verified by the Student's t -test. The Student's table for $\alpha = 5\%$ and $\nu = 10 - 7 = 3$ gives $t_{(3, 0.05)} = 3.182$. However, for mixing design models, no attempt should be made to interpret the effect of a factor variation on the response from positive or negative coefficient values [15]. Therefore, the determination of the significant factors affecting the recovery of BP4 extraction was followed by the application of analysis of variance (ANOVA) and Fisher's F-test based on the comparison of the variance of the fitted model and the residual variance. The experimental conditions of the Box-Behnken design are presented in Table 4.

Table 4. Experimental matrix for elution step ($V = 3 \text{ mL}$) and ($V = 6 \text{ mL}$).

Experiment number	Real values			Response: BP4 (%) for each volume of eluting solvent	
	P_A	P_B	P_C	$V = 3 \text{ mL}$	$V = 6 \text{ mL}$
1	3	0	0	67.2	68.9
2	0	3	0	68.5	70.2
3	0	0	3	3.9	7.2
4	1.5	1.5	0	99.1	99.5
5	1.5	0	1.5	35.5	46.2
6	0	1.5	1.5	46.1	59.7
7	1	1	1	60.3	68.5
8	2	0.5	0.5	63.2	75.1
9	0.5	2	0.5	72.1	73.3
10	0.5	0.5	2	30.0	41.3

P_A : Volume of methanol (mL); P_B : Volume of acetone (mL); P_C : Volume of aqueous sodium hydroxide solution (mL).

As shown in Table 5, based on to a volume of 3 mL of elution solvent, the factors P_A (methanol), P_B (acetone) and the P_AP_B interaction are significant. The P_C factor, which relates to the proportion of aqueous sodium hydroxide solution, and its interaction with the other solvents have no significant effect on the recovery of BP4. It seems that the contact time of the base (NaOH) with the PANI material was very short to allow the de-doping of all the sites of the polyaniline emeraldine salt (PANI-ES). Indeed, the number of protonated sites of PANI-ES gradually decreases as de-doping progresses [17]. It has been found that the optimum de-doping time of polyaniline by aqueous sodium hydroxide is one hour [17]. For a volume of 6 mL of elution solvent, the factors X_A (methanol), X_B (acetone) and the interactions X_AX_B , X_BX_C are significant (Table 5). Therefore, a multi-linear regression gives the following models for the two volumes of elution solvent as:

$$Y_{(3 \text{ mL})} = 22.15X_A + 23.04X_B + 8.19X_AX_B \quad (\text{Eq. 4})$$

$$Y_{(6 \text{ mL})} = 22.28X_A + 22.11X_B + 7.53X_AX_B + 5.24X_BX_C \quad (\text{Eq. 5})$$

Table 5. Significance of main effects and interaction effects for $V = 3 \text{ mL}$ and $V = 6 \text{ mL}$ of elution solvent.

Variables	Coefficient values	t -Student	Results
Volume of the eluting solvent ($V = 3 \text{ mL}$), $t_{(3, 0.05)} = 3.182$			
X_A	$d_A = 65.990$	$t_A = 22.15$	Significant
X_B	$d_B = 68.636$	$t_B = 23.04$	Significant
X_C	$d_C = 4.209$	$t_C = 1.41$	Non-significant
X_AX_B	$d_{AB} = 122.851$	$t_{AB} = 8.19$	Significant
X_AX_C	$d_{AC} = -2.003$	$t_{AC} = -0.13$	Non significant
X_BX_C	$d_{BC} = 40.489$	$t_{BC} = 2.7$	Non significant
Volume of the eluting solvent ($V = 6 \text{ mL}$), $t_{(3, 0.05)} = 3.182$			
X_A	$e_A = 69.335$	$t_A = 22.28$	Significant
X_B	$e_B = 68.798$	$t_B = 22.11$	Significant
X_C	$e_C = 7.644$	$t_C = 2.46$	Non-significant
X_AX_B	$e_{AB} = 117.864$	$t_{AB} = 7.53$	Significant
X_AX_C	$e_{AC} = 34.358$	$t_{AC} = 2.19$	Non significant
X_BX_C	$e_{BC} = 82.083$	$t_{BC} = 5.24$	Significant

The fitted model was evaluated using Fisher's test, which consists of comparing the variance attributable to the regression with the residual variance. The ANOVA was performed to evaluate the significance of the linear regression fit of the experimental data (Table 6). The value of F_{observed} should be larger than the critical value at a level of confidence equal to the 95% ($F_{0.05, df_R, df_r}$) for the regression.

Table 6. ANOVA of the regression model for $V_{\text{elution}} = 3 \text{ mL}$ and 6 mL .

Variation	SSD	df	MSS	F_{observed}
Volume of the eluting solvent ($V = 3 \text{ mL}$), $F_{(0.05, df_R, df_i)} = F_{(0.05, 2, 7)} = 4.74$				
Regression	6248	2	3124	199
Residual	110	7	15.7	-
Total	6358	9	-	-
Volume of the eluting solvent ($V = 6 \text{ mL}$), $F_{(0.05, df_R, df_i)} = F_{(0.05, 3, 6)} = 4.76$				
Regression	5442	3	1814	113
Residual	96	6	16.1	-
Total	5539	9	-	-

The ANOVA of the regression model showed that all its coefficients have a significant effect on the recovery rate of the BP4 (Table 6). Response surface and isoresponse curves were constructed for 3 mL and 6 mL volumes of elution solvent in order to determine the optimum values for the proportions of methanol, acetone and aqueous sodium hydroxide solution (0.5 M). The evolution of the amount of interest as a function of the proportion of each solvent is shown in Figures 4 and 5.

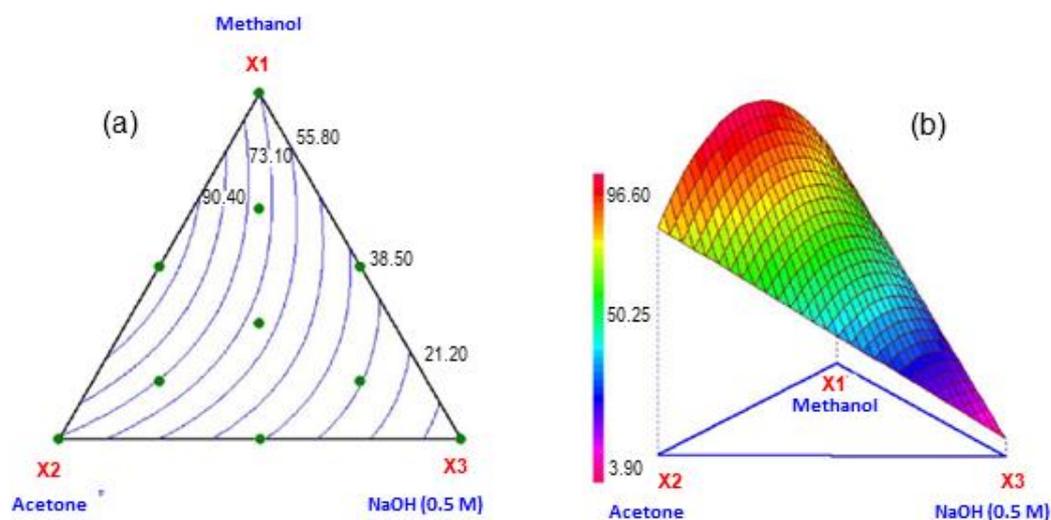


Figure 4. Isoresponse curve (a) and response surface (b), for recovery of BP4 using $V_{\text{elution}} = 3 \text{ mL}$.

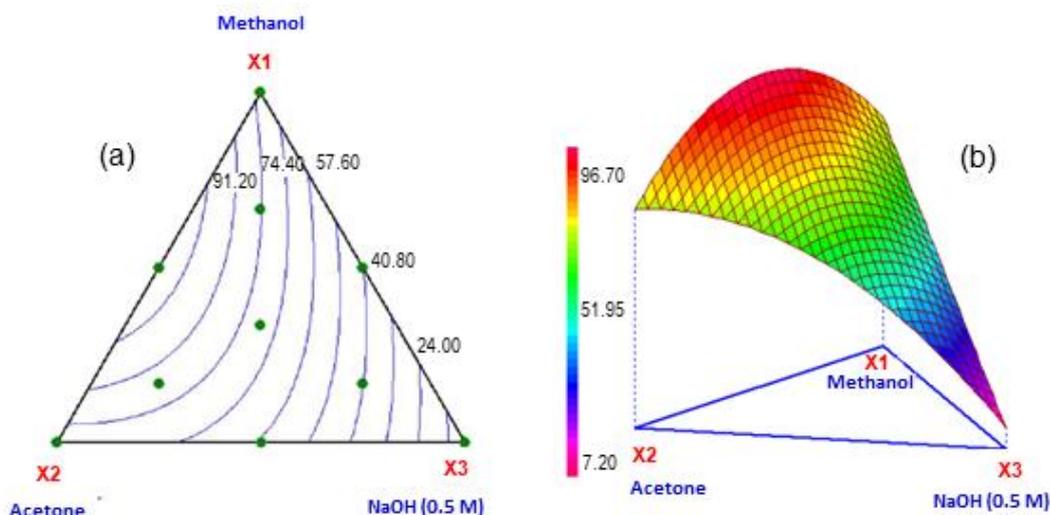


Figure 5. Isoresponse curve (a) and response surface (b), for recovery of BP4 using $V_{elution} = 6$ mL.

The isoresponse and response surface diagrams show that the recovery of BP4 becomes higher when the proportions of methanol and acetone increase. The maximum recovery yield was about 96.6% for the two volumes (3 mL and 6 mL) of a mixture of methanol/acetone (50/50 v/v), which is in agreement with the experimental results. This result suggests that increasing the volume of eluting solvent does not affect the extraction yield of BP4. Therefore, a volume of 3 mL of eluting solvent was chosen for further work.

3.5. Performance evaluation of PANI@MIP-SPE procedure to real sample

The optimized PANI@MIP-SPE method was applied for the extraction of BP4 in tap water. Indeed, matrix effects do influence the accuracy of analyses [18]. Samples were collected at two points in the drinking water distribution network. A recovery study of BP4 was carried out on non-spiked and spiked aqueous samples at three different concentration levels (from $0.1 \text{ mg}\cdot\text{L}^{-1}$ to $120 \text{ mg}\cdot\text{L}^{-1}$) according to the procedure described in the experimental section. No trace of BP4 was detected in the water samples. Recovery rates were calculated using the following equation [19]:

$$\text{Recovery (\%)} = (C_{doped} - C_{non-doped}) \frac{100}{C_{addition}} \quad (\text{Eq. 6})$$

where $C_{non-doped}$ is the concentration of BP4 determined in the non-spiked aqueous sample; C_{doped} is the concentration of BP4 determined in the spiked aqueous sample and $C_{addition}$ is the concentration of introduced BP4 in the aqueous sample.

The retention capacity of the PANI@MIP-SPE was composed of two linear parts. In the first part, the amount retained increased proportionally to the amount initially percolated, corresponding to excellent recoveries of BP4 for samples at concentration levels (from $0.1 \cdot \text{mg L}^{-1}$ to $20 \text{ mg} \cdot \text{L}^{-1}$) varying from 99% to 92% (Figure 6). Above a given amount of BP4, a second linear part with a lower slope was observed, indicating the saturation of the molecular imprints in the PANI@MIP sorbent. Therefore, the extraction yield progressively decreased down to 32%. In fact, some further retention persisted once all the cavities were filled with BP4 due to non-specific interactions. On the other hand, the curve corresponding to the PANI@NIP SPE was linear and the slope was the same as in the second part of PANI@MIP SPE because the same non-specific interactions were active. Most of the loaded BP4 was lost in the wash fraction of PANI@NIP-SPE. The maximum binding capacity of PANI@MIP-SPE was found to be $600 \mu\text{g} \cdot \text{g}^{-1}$ compared to $50 \mu\text{g} \cdot \text{g}^{-1}$ for PANI@NIP-SPE. Taken together, these results indicate that the MIP material has a good affinity and high retention of BP4.

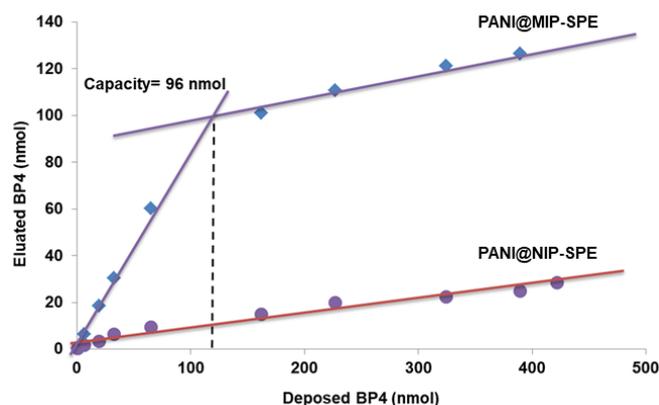


Figure 6. Retention capacities of BP4 using PANI@MIP-SPE and PANI@NIP-SPE methods.

To ensure that the retention of BP4 provided by the PANI@MIP material is indeed specific, due to the presence of the recognition sites, comparative experiments were carried out in an aqueous solution containing mixtures of BP4 and its structural analogs BP3 and BP with PANI@NIP material during the washing and elution steps of the SPE process. As shown in Figure 7, PANI@MIP and PANI@NIP had a much higher recovery rate for BP4 than for its analogs. The good recognition selectivity and binding affinity for BP4 molecules is related to the imprinting effect, and the recognition of the PANI@MIP material is related to the structure, molecular size and memory of specific functional groups [20–22]. All these results clearly showed that the PANI@MIP-SPE method is suitable for the determination of benzophenone-4 in aqueous samples.

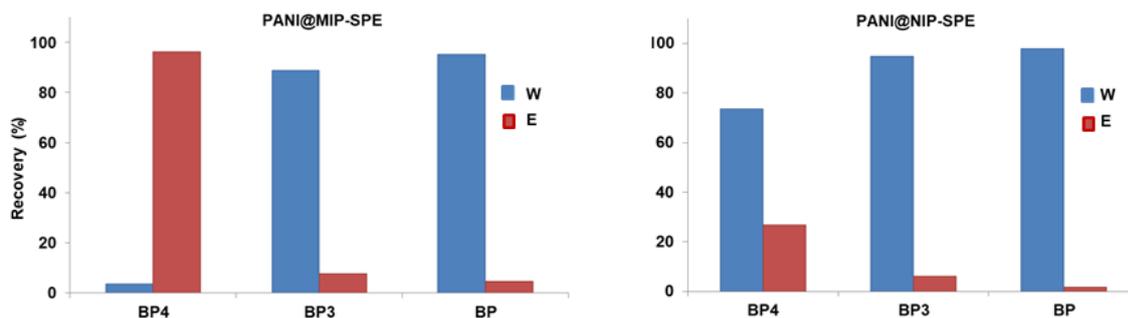


Figure 7. Washing and elution profiles obtained with PANI@MIP-SPE and PANI@NIP-SPE after percolation of 5 mL drinking water spiked with 2 mg·L⁻¹ of BP4, BP3 and BP.

PANI@MIP-SPE is comparable to other methods in the literature, for example, an optimized solid-phase extraction of parabens and BPs using commercial C18 silica phases shows extraction yields ranging from 65% to 100%. However, their method primarily focuses on extraction without the elimination of interfering substances [23]. Using a cationic polymer, PANI@MIP, achieves comparable results with extraction recoveries close to 100% and effective elimination of interferents like BP3 and BP of molecularly imprinted polymer particles using a 4-vinylpyridine monomer, demonstrating similar efficiency and robustness, ensuring accurate and reliable analysis in complex environmental samples [24].

4. Conclusion

Molecularly imprinted polyaniline material was used as the adsorbent of the PANI@MIP-SPE method. Various parameters including adsorbent mass, type, composition and amount of the wash and elution solvents were optimized for the selective and quantitative extraction of the benzophenone-4 UV filter. The influence of these parameters was investigated using a full factorial (2³) design of experiments for the washing step. The optimal composition of the elution solvent was obtained by an augmented simplex centroid mixture design. The best results were obtained using 50 mg of adsorbent, 1.25 mL acetonitrile/acetic acid/hexane (45/5/50 v/v) as wash solvent and 3 mL methanol/acetone mixture (50/50 v/v) as elution solvent at a flow rate of 1 mL·min⁻¹. The recoveries of BP4 from spiked aqueous solution with 2 mg·L⁻¹, 5 mg·L⁻¹ and 10 mg L⁻¹ BP4 are in the range of 99–93% with relative standard deviations ranging from 3.0% to 5.1%. The chemometric method allowed the fast optimization of the SPE process using a minimum number of experiments. The PANI@MIP-SPE extraction material is well-suited for the high yield extraction and purification of BP4.

Acknowledgments. This work was supported by the “PHC Utique” program for French-Tunisian cooperation (project number 19G1204).

Funding. The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Notes. The authors declare no competing financial interest.

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