Determination of benzomercaptans in environmental complex samples by combining zeolitic imidazolate framework-8-based solid-phase extraction and high-performance liquid chromatography with UV detection


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Abstract

In this work, the synthesis of zeolitic imidazolate framework-8 (ZIF-8) crystals and their subsequent application as effective sorbents for extraction and preconcentration of several benzomercaptans from environmental complex samples is described. These materials were prepared by solvothermal approach varying the concentration of n-butylamine modulator to modify the surface of the metal-organic framework. The resulting materials were characterized by scanning and transmission electron microscopy, powder X-ray diffraction and Fourier transform infrared spectroscopy. The ZIF-8 material that gave the best features was selected as extractive phase and the influence of various parameters (sample pH and elution solvent composition, among others) on the extraction efficiency of target compounds were investigated. Under the optimal conditions of the method, the tested analytes (2-mercaptobenzothiazole, 2-mercaptobenzoxazole and 2-mercapto-6-nitrobenzothiazole) were retained and eluted quantitatively with alkaline 50:50 (v:v) methanol-water mixture. Using the proposed method, low limits of detection, in the range of 16-21 ng L⁻¹ for aqueous samples and 0.4-0.5 µg kg⁻¹ for soil samples, were achieved whereas the precision (expressed as relative standard deviation) was lower than 7%. The resulting solid-phase extraction protocol, using the zeolitic material as sorbent, was combined with liquid chromatography and ultraviolet-vis detector and successfully applied to determine traces of these organic pollutants in environmental samples.

Keywords: Metal-organic frameworks; environmental pollutants; soil and aqueous samples; solid-phase extraction; sorbent; sample preparation
1. Introduction

Metal-organic frameworks (MOFs) have aroused a great interest over the last decades. These coordination polymers are a class of highly porous materials composed of metal ions or clusters linked by organic ligands. Their crystalline structures, including their pore sizes and surfaces, can be controlled by a careful selection of their precursors. MOFs are characterized by a large specific surface area and intrinsic porosity, as well as profuse active sites [1]. Many applications have been proposed for this type of materials, and surely, their number will grow in the coming years. Some of those applications include gas/electrical storage and catalysis [2], removal of contaminants in air or water by adsorption and separation [3-4], sensors [5] and sorbents for sample preparation [6]. In particular, zeolitic imidazolate frameworks (ZIFs) are a subfamily of MOFs consisting of Zn or Co metal ions tetrahedrally coordinated by anionic imidazolate ligands [7, 8]. ZIF-8 [Zn(2-methylimidazolate)$_2$] has been widely used due to its easy synthesis, high thermal and chemical stabilities [9, 10], great pore volume and surface area [9]. ZIF-8 shows a very large adsorption capacity, which is attributed to its high porosity and the different ways in which it can interact with adsorbates, such as π-stacking, hydrogen bonding and ionic interactions [11, 12]. Such good features have become this material in one of the most attractive MOFs for the extraction and preconcentration of analytes. Related to this, solid-phase microextraction (SPME) [6] has been commonly reported as a suitable sample preparation technique in the extraction of amines [13], polycyclic aromatic hydrocarbons [14], BTEX [15] and fluoroquinolones [16] from environmental water samples. Despite its advantages, SPME has certain disadvantages such as the limited fiber lifetime, its fragility and its relatively high cost. In this sense, other alternatives based on conventional solid-phase extraction (SPE) format (syringe/cartridges) [17], magnetic SPE [18] or the use of membrane as support medium [19-21] have been developed. However, the preparation of homogenous ZIF-8 membranes is a difficult task presenting some drawbacks such as possible sedimentation phenomena of MOF crystals during the preparation stage, the formation of bubbles during stirring (poor reproducibility) and limited reusability (blockage of pores of membrane). Besides, in most of these works, the selectivity of sorbent has been attributed to the hydrophobic and π-interactions of solutes with the 2-methylimidazole ligands instead of taking advantages of their tunable pore size and feasible chemical functionalization [22].

The pollution of the environment by organic compounds is always of great concern due to the toxicity risks for aquatic organisms and human posed by their occurrence and
increasing concentration in water bodies. Among organic pollutants, benzomercaptans, such as 2-mercaptobenzothiazole (MBT) and 2-mercaptobenzoxazole (MBO), deserve special attention due to their broad use in many industrial processes. These compounds are commonly used as vulcanization accelerators in the rubber industry, as biocorrosion inhibitors, as coating agents of metallic surfaces, and as biocides in medical applications [23-24]. As consequence, such compounds have been found in different environmental compartments such as surface waters, wastewater effluents, sewage treatment plants, soils, roadsides, etc. These poorly biodegradable pollutants may cause allergic reactions, induce tumours, and be toxic to aquatic organisms [25-26]. According to the NSF International Standard/American National Standard (NSF/ANSI 60-2016), the drinking water criteria (Annex C) for MBT is maximum contamination levels or maximum allowable concentration, respectively of total allowable concentration is 20 µg L⁻¹, single product allowable concentration is 2 µg L⁻¹ [27]. In order to reach these low concentrations, determination techniques mainly based on LC combined with mass spectrometry detectors have been reported [28-31]. In any case, sample preparation techniques are required in order to reduce matrix interferences and to improve sensitivity to benzomercaptans in environmental complex samples, being SPE the most common technique used for extraction and preconcentration of these kind of analytes [23, 24, 28, 30, 31]. However, SPE shows some disadvantages, such as limited selectivity and poor reusability.

In order to overcome the above-mentioned drawbacks, there is a recent interest concerned to functionalized MOFs, which are obtained by incorporation of ligands including additional functional groups (–OH, –NH₂, …). Particularly, amine-functionalized MOFs have mainly attracted attention since they improved MOF water stability and provided the possibility of forming hydrogen bonds and even electron transfer capability towards analytes [32]. Recently, these materials have applied for capturing of several polar guests from different matrices. Thus, NH₂-MIL-101(Fe) and NH₂-MIL-53(Al) have been used for the removal of imidacloprid in water [33] and to selectively recognize tetracyclines from milk [34], respectively. Also, amino-functionalized UiO-66 combined with magnetic Fe₃O₄ microspheres has been used for the selective extraction of bisphenols in river water samples [35]. In these works, the introduction of amino moieties is accomplished by one-pot solvothermal synthesis MOFs, which has the advantage of avoiding the complicated steps required in post-modification approach. Alternatively, amino moieties can be incorporated through the addition of organic amines as modulators.
in the synthesis mixture of MOF [22]. However, this approach has not been explored in literature with sample treatment purposes.

In this work, amine-modulated ZIF-8 (nano)crystals (NH$_2$-ZIF-8) was used as sorbent for the extraction of these target compounds (MBO, MBT and its nitro derivative) in environmental samples. The incorporation of n-butylamine molecules to the framework structure resulted appropriate to tailor the surface properties improving the selectivity of the extraction of these polar compounds. Although this type of material has been previously used in hydrophobic compounds in dispersive mode with sophisticated technique (GC-MS) [36], its application to hydrophilic compounds in cartridge format with accessible instrumentation (LC-UV) has not been explored. Taking into account the key role of butylamine (BA) as modulator, in this work, its content on crystal structure and posterior interaction with analytes was studied. The resulting MOF that provided the best features was selected as SPE sorbent and parameters affecting the extraction process such sample pH and eluent solvent composition (among others) were investigated.

Finally, the applicability of the method for the analysis of these compounds by HPLC-UV was evaluated in environmental samples obtained from different sources.

2. Experimental section

2.1. Reagents and materials

Zinc nitrate hexahydrate, 2-methylimidazole (Hmim), n-butylamine (BA), ethylamine and hexylamine were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Sodium hydroxide was obtained from Panreac Química SA (Barcelona, Spain). Acetonitrile (ACN), methanol (MeOH), acetic acid (HAcO) and hydrochloric acid were from VWR International Eurolab (Barcelona, Spain). The benzomercaptans selected in this work were: 2-mercaptobenzoxazole (MBO), 2-mercaptobenzothiazole (MBT) and 6-nitrobenzo[d]thiazole-2-thiol (NMBT), all purchased from Sigma-Aldrich. The structures of these compounds are given in Table S1. Deionized water was prepared in Crystal B30 EDI Adrona deionizer (Riga, Latvia). All other reagents were of analytical grade unless otherwise stated. 1 mL empty propylene disposable SPE cartridge and frits (1/16′, 20 μm) were from Análisis Vínicos (Tomelloso, Spain). Individual standard solutions of benzomercaptans (1000 mg L$^{-1}$) were prepared in MeOH and stored at 4 °C until its use. Working standard solutions were done daily by appropriate dilution from these stock solutions.
2.2. Instrumentation

Scanning electron microscopy (SEM) images were obtained with a Hitachi S-4800 electron microscope (Ibaraki, Japan) equipped with a retrodispersive electron detector and an energy dispersive spectrometer (EDAX Genesis 4000). A transmission electron microscope (TEM) coupled to a digital camera AMT RX80 model JEM-1010 JEOL (Akishima, Japan) was used to obtain the transmission electron micrographs. Adsorption-desorption isotherms of nitrogen were registered in a S5 Micromeritics ASAP2020 instrument (Norcross, USA) at 77 K. The Brauner-Emmet-Teller (BET) model was used to calculate the specific surface area using the low-pressure range. Powder X-ray Diffraction (p-XRD) patterns were obtained in a D8 Advance A25 diffractometer (Bruker). Attenuated total reflection Fourier-transform infrared (FT-IR) spectra of powdered materials were registered with a DuraSampIR II accessory from Smiths Detection Inc. (Warrington, UK) equipped with a nine reflection diamond/ZnSe DuraDisk plate, installed on a Bruker FT-IR spectrometer (Bremen, Germany) model Tensor 27. The zeta potential measurements were performed with Zetasizer Nano ZS equipment (Malvern Instruments, Malvern, UK). NH$_2$-ZIF-8-BA 1 was dispersed in distilled water at a concentration of 1 g L$^{-1}$ and sonicated during 1 min. Measurements were carried out per triplicate at 25 °C. The final zeta potential value was estimated from the particle mobility using the Smoluchowski model.

Chromatographic separation of standards and samples was performed on a 1260 Infinity II HPLC from Agilent Technologies (Waldbronn, Germany) with a UV-Vis diode-array detector. The separation conditions of benzomercaptans were adapted from Parham et al. [30]. The chromatographic column was a Kromasil C18 (250 × 4.0 mm, 5 µm particle size, Análisis Vínicos). Separation was done under gradient elution mode (at flow rate of 1.0 mL min$^{-1}$) using as solvents: (A) water and (B) ACN containing both 0.1% (v/v) of HAcO. The gradient elution and the detection wavelength programs are given in supplementary information (see Table S2).

2.3. Synthesis of ZIF-8 crystals

ZIF-8 materials were synthesized following protocols reported in the literature by Cravillon et al. [22, 37]. To obtain ZIF-8 (nano)crystals (without modulator), two methanolic solutions were needed. Solution (A) composed of Zn(NO$_3$)$_2$·6H$_2$O (9.87 mmol) in MeOH (200 mL) and solution (B) of 2-methylimidazole (79.04 mmol) in MeOH (200 mL). The later solution was added to the former under stirring. After keeping at
room temperature for 24 h, a white dispersion of ZIF-8 was formed, and separated by centrifugation at 6000 rpm for 10 min. Then, the resulting solid was washed with MeOH three times and dried at 40°C overnight. On the other hand, ZIF-8 nanocrystals using BA (NH2-ZIF-8-BA) as modulator agent were prepared as given elsewhere [22]. A ligand solution (B) (9.874 mmol of Hmim in 50 mL MeOH) including the modulator agent (9.874 mmol of n-BA) was poured slowly into a solution (A) containing the metallic cation (2.469 mmol of Zn(NO3)2·6H2O in 50 mL of MeOH) under stirring and left at room temperature for 24 h. The resulting white solid (namely, NH2-ZIF-8-BA 1) was recovered and washed with the same protocol that is described above for ZIF-8 (nano)crystals. Also, different molar contents of BA as modulator (5 and 20 mmol, NH2-ZIF-8-BA 2 and 3, respectively) were tested by keeping constant the molar ratio of other starting reagents. Additionally, other alkyl amines (ethyl- and hexylamine) were investigated as modulator agent (at 10 mmol) following the abovementioned protocol for NH2-ZIF-8-BA materials.

2.4. Sample collection and treatment
The sampling of water was performed from different locations in Valencia. Approximately, 500 mL were collected in dark containers and refrigerated at 4°C until their use.
Several soil samples from different sources were collected, air-dried at room temperature, crushed and then sieved through a 1 mm sieve. These samples were stored on polypropylene bags in darkness at 25°C. The pH, humidity and electrical conductivity of soil samples were measured following ISO rules [38]. pH and electrical conductivity were measured in a 1:5 (soil:deionized water) extract shaken for 5 min and measured after 2 h. For the determination of humidity, 1 g of each sample was heated at 70°C overnight until constant weight. These soil parameters are listed in Table S3. The extraction of benzomercaptans from soil samples was adapted from Zhang et al [39], as follows.
Briefly, 0.1 g approx. of each sample was weighed and spiked at 500 µg kg⁻¹ with the investigated pollutants. After vortex-assisted and ultrasonic homogenization, the samples were allowed to stand with the analytes for 2 h. Then, 5 mL of MeOH were added, shaken during 20 min and centrifuged at 6000 rpm for 3 min. The supernatant was collected and the extraction procedure was repeated twice. All methanolic fractions were combined and evaporated at low pressure up to small volume (ca. 100 μL) and reconstituted up to 5 mL with water before application of SPE protocol described below.
2.5. Extraction procedure

A SPE protocol using the developed materials as sorbents was applied to the extraction of aromatic benzomercaptans in environmental samples. For the preparation of SPE devices, 20 mg of the metal-organic framework was packed between two frits into 1 mL empty polypropylene cartridges. Prior to loading, the sewage waters were sonicated and centrifuged, and the extraction was carried out following the protocol described above. The material was firstly conditioned with MeOH (500 μL) and water (500 μL), and after that, a known volume of standard solution or sample (5 mL and 25 mL for soil and water samples, respectively) was passed through the cartridge. The sorbent was finally washed with water (500 μL). The retained analytes were later eluted with 250 μL of 50% (v/v) MeOH containing 0.5 mol L\(^{-1}\) NaOH. The extraction units were regenerated with water (10 mL) in order to eliminate the NaOH excess. Prior to HPLC injection, the elution fraction was properly neutralized with hydrochloric acid.

3. Results and discussion

3.1. Preliminary considerations

As it was mentioned in the Introduction, the selection of the adequate MOF as SPE sorbent is a key aspect since it strongly influences on the selective retention and the way of interaction with the target analytes. In this sense, ZIF-8 material, due to its pore size and the kind of interactions it can establish with the analytes, has advantageous features making it a good SPE phase candidate. As shown in Fig. S1, the molecular sizes of MBO and MBT are smaller than the pore size of ZIF-8 (3.4 Å) [40, 41]; whereas for NMBT, a partial embedding process may happen into ZIF-8 structure.

Regarding the interaction forces involved, there are different possible interaction modes between this MOF and benzomercaptans: i) hydrophobic effects and π-interaction between the imidazole rings of ZIF-8 and the aromatic moieties of these analytes (see structures in Fig. S1); ii) hydrogen bonding between NH groups in ZIF-8, and certain hydrogen-bond accepting groups (e.g. ether, organo sulfurs, nitro, among others) present in the analytes (Fig. S1) thus enhancing the hydrophilic binding and improving the selectivity; and iii) electrostatic interaction between ZIF-8 and benzomercaptan molecules. In this sense, the surface charge of ZIF-8 nanocrystals and the ionization degree of benzomercaptans (see Table S1) should be considered (as we discussed below).

Additionally, other potential contributions due to the amine moiety such as cation-π
interaction between aromatic ring and amino protonated ZIF-8 structure can occur [42], which have been previously reported with other amino functionalized MOFs and compounds with structural similarities [43]. In this regard, the synergetic contribution of BA modulator of the NH$_2$-ZIF-8-BA in these last interactions can be explained by its presence onto the surface of ZIF-8 crystals as previously demonstrated [36]. Indeed, the use of modulators during MOF synthesis has proven to be a promising strategy through which the internal MOF structure (crystal size and morphology), and outer surface functionalities, can be controlled by using monodentate ligands [44]. The modulators then compete with conventional multidentate ligands for coordination to the metal cations [45, 46]. In order to study the influence of BA upon the crystal growth of ZIF-8 and other features, several materials were synthesized varying its molar ratio. Taking into account all these considerations, the ability of these synthesized materials as SPE phases is here evaluated (see Table 1).

3.2. Characterization of material

The morphology and crystal size of the prepared materials (ZIF-8 and NH$_2$-ZIF-8 with different modulators) were investigated by SEM and TEM analysis, respectively. Fig. 1A and B shows the SEM micrograph of the ZIF-8 (without modulator) and NH$_2$-ZIF-8-BA 1 (corresponding to ca. 10 mmol of BA) materials, respectively. In both images, a polyhedral shape of the nanocrystals was evidenced, which is in agreement with the typical form of ZIF-8 nanocrystals [40, 41]. Also, SEM images of MOFs prepared at other BA contents were taken (Fig. S2). As it can be seen, the morphology did not change substantially with modulator content. The TEM images of the resulting materials for this set of experiments are summarized in Fig. S2, and the mean sizes of the crystals are given in Table 1. As it can be seen, an increase of the concentration of BA modulator led to the increased mean size of the resulting crystals. The tendency observed for BA-modulated MOFs is consistent with that reported in previous studies focused on the addition of monocarboxylic acid modulators [44, 47]. These results can be explained taking into account that modulating ligands can tailor crystal nucleation and growth via both coordination and deprotonation equilibria [22]. Thus, the addition of a basic ligand (L) as BA (pK$_a$ value in aqueous media is 10.7) can deprotonate the [Zn(Hmim)$_m$L$_n$] species, and thus producing an acceleration of rate of ligand exchange reactions, resulting in a higher nucleation rate and therefore in a smaller final crystal size. When a low amount of BA is added a, a large number of nuclei were formed and rapidly grow at the same time.
affording smaller crystals. However, higher concentration of BA provide a slow nucleation (fewer nuclei) of the MOF giving as result larger crystals. This is due to this additive starts to have a relevant role as a competitive ligand in the complex formation.

On the other hand, in absence of modulating ligand (BA), there is not a deprotonation reaction, which led to small concentration of [Zn(Hmim)$_m$L$_n$] species decreasing the nucleation rate, and consequently in a large final crystal size.

Additionally, energy dispersive X-ray spectroscopy (EDX) of the synthesized materials were performed. As shown in Table 1, nitrogen content increased with increasing BA content, which confirmed not only the presence of BA onto the surface of ZIF-8 crystals but also the possibility of tailoring the loading of surface amine moieties.

To determine the surface area, nitrogen adsorption/desorption isotherms of the synthesized materials were performed. They exhibited typical Type I isotherms (a representative example is depicted in Fig. S3), which are indicative of their microporous nature. The specific surface area, calculated using the BET equation, are shown in the Table 1. As it can be observed, high surface areas (> 1000 m$^2$/g) were obtained from BA contents of 10 mmol or higher.

Furthermore, X-ray powder diffraction patterns, depicted in Fig. S4, were in good agreement with the simulated one, thus indicating that the current materials were successfully prepared. Also, FT-IR spectra of ZIF-8 materials were taken (Fig. S5). All the spectra display the typical bands of the ZIF-8 MOF [36, 40], corroborating their successful preparation. In addition, a small band close to 2900 cm$^{-1}$ appeared in the NH$_2$-ZIF-8-BA, which can be assigned to the aliphatic stretching C–H of BA.

Next, the synthesized MOFs were evaluated as SPE sorbents in order to study the influence of modulation and choose the phase that provided the highest extraction efficiency. As shown in Table 1, the NH$_2$-ZIF-8-BA 1 showed high recoveries jointly with exposed surfaces having enough amino moieties for the selective capture of analytes.

Additionally, this material was superior to that synthesized without modulator, which clearly demonstrated the important contribution of the amount of attached BA onto ZIF-8 crystals in the extraction efficiency of these pollutants. It is evident from the previous discussion and experimental results that amine group of BA modulator plays a key role in the crystal growth of MOF as well as in his subsequent retention properties. For this purpose, other organic amines (ethyl and hexyl) with similar basicity (pK$_a$ values ranged between 10.6-10.9) were studied as modulators using the same conditions of NH$_2$-ZIF-8-BA 1. The characterization of the resulting materials gave mean sizes of the crystals.
similar to those obtained with BA modulated material and surface area values ranged between 1150 and 1324 m² g⁻¹. Both materials were tested as SPE sorbents, giving recoveries up to 90%. These results suggested that the alkyl chain has not a relevant effect, whereas the introduction of amine in the final framework is an important issue to improve the retention performance. At sight of these results, NH₂-ZIF-8-BA 1 was selected for further studies.

3.3. Optimization of SPE protocol

In order to obtain the maximal extraction performance, various parameters such as sample pH value, elution solvent composition and volume were investigated. During optimization of SPE conditions, an aqueous solution containing 250 µg L⁻¹ of each benzomercaptan was employed as a test mixture. Sample pH is an important variable that influences the extraction performance since it affects the ZIF-8 charge surface as well as the analyte charge state. According to previous reports, ZIF-8 material is charged positively in the pH range of 2-10 due to its imidazole moieties [48, 49]. In any case, zeta potential measurements of the selected material were performed at loading/elution conditions (Fig. S6). Thus, the positive value of this parameter at loading step (pH close to 7.0) implied a positive surface charge of MOF nanoparticles, whereas that it reversed from positive to negative when eluting solution was tested (pH > 12). These results were consistent with the above-mentioned studies.

On the other hand, the heterocyclic benzomercaptans may exist as thione and/or thiol forms, and the thiol form can be further ionized in aqueous solutions (see pKₐ values in Table S1). In this study, the effect of the sample pH value was evaluated from 5 to 9 (data not shown), by considering aspects such as the structural integrity of this framework (pH > 4) [50] as well as the usual pH range found in surface water systems (6.5 to 8.5). Recoveries in the tested pH range were almost constant for each benzomercaptan. These results can be explained as follows. On the one side, the existence of electrostatic interactions can be present taking into account the surface charge of MOF and the ionization state of benzomercaptans. Particularly, when the solution pH was below 6.0, these compounds are predominantly as neutral (molecular) species, while the surface of ZIF-8 remained positive, which made the electrostatic attraction between them become weak. When solution pH increased from 6.0 to 8.5, the anionic form of benzomercaptans begin to have greater significance due to progressive deprotonation of these molecules (see pKₐ values), and the electrostatic attraction between these solutes and ZIF-8 became
a main factor in the interaction mechanism. Nevertheless, the recovery values of target compounds on ZIF-8 along the tested pH range remained nearly constant. This fact suggested that the factor controlling the retention of probe compounds was not just electrostatic interaction, and there should be other modes of action that influence on the adsorption process (such as π–stacking and hydrogen bonding interactions). Therefore, no pH adjustment was required in sample solutions.

The selection of an appropriate eluting solvent is of major concern for the optimization of the SPE process. To obtain the highest eluting efficiencies, several eluting solvents were investigated (see Fig. 2, left part). Desorption solvents as pure MeOH gave very low recovery values of analytes (< 8%). As can be observed, when MeOH-water mixtures (50/50, v/v) were used, higher recoveries were obtained (up to 29%), which subsequently increased up to around 79% when using mixtures of MeOH-water basified with 0.1 M NaOH. This increment can be explained taking into account the surface charge of MOF and the ionization state of benzomercaptans. As it was mentioned before, the point of zero charge for ZIF-8 is approximately at pH 9.8 [48, 49]. Consequently, at pH above this value, an electrostatic repulsion between the negatively charged ZIF-8 surfaces and the benzomercaptan anions is produced, which favors the desorption of these analytes from the sorbent. Consequently, the content of NaOH (from 0.1 to 0.5 M) in MeOH-water mixtures was optimized. It was found that a content of 0.5 M NaOH gave recoveries ranging between 97 and 108%. So, as in agreement with previous studies [50], the MOF structure was stable at this pH, a 0.5 M NaOH concentration was selected for further studies.

The volume of eluent can affect not only the elution efficiency, but also the concentration of the analytes in the eluent. Therefore, the effect of the solvent volume (100-1000 µL) was also investigated (Fig. S7). As can be seen, recoveries of analytes higher than 87.3% (< 6.1% relative standard deviation, RSDs) were obtained for 250 µL, and this value was selected for further experiments.

Another essential parameter in a SPE protocol, especially to monitor trace pollutants in environmental analysis, is the breakthrough volume. Thus, the effect of sample volume was evaluated using the NH$_2$-ZIF-8 as sorbent. Several volumes (in the range 1-100 mL) were loaded by keeping constant the total amount of each pollutant (500 ng) (see Fig. 2, right part). As can be seen, the extraction recoveries of all the benzomercaptans were higher than 80% up to 50 mL, however, higher volumes caused a diminution in the extraction efficiency. Consequently, considering the minimum elution volume found (250
µL) and the maximum sample loading volume (25 mL) with average recoveries values ca. 100%, a preconcentration factor of 100 was estimated. Finally, the reusability of sorbent was also evaluated, and the obtained results indicated that the MOF could be reused at least 10 times without significant decrease of efficiency extraction (between 87-104% with RSD below to 8%).

3.4. Figures of merit and application to real samples

The developed SPE procedure using NH$_2$-ZIF-8-BA 1 as sorbent, combined with HPLC-UV detection, was validated in terms of linearity, sensitivity and precision under the previously optimized experimental conditions. The obtained results are summarized in Table 2. As can be seen from this table, a good linearity range ($r > 0.998$) was observed in the chromatographic dynamic range of 7-2500 µg L$^{-1}$ for all analytes. The limits of detection (LOD) and quantification (LOQ) (after applying the extraction protocol) were experimentally obtained as the concentration of the analyte that provided a signal-to-noise ratio (S/N) of 3 and 10, respectively. Thus, the LODs for aqueous samples varied from 16 ng L$^{-1}$ to 21 ng L$^{-1}$, whereas the LOQs were in the range 52 ng L$^{-1}$ and 69 ng L$^{-1}$. In the case of soil samples, the LOD and LOQ values ranged 0.4-0.5 µg kg$^{-1}$ and 1.3-1.7 µg kg$^{-1}$, respectively.

The precision of the method (intra and inter-units), expressed as relative standard deviation (RSD, %) was also determined from standard solutions at a concentration level of 250 µg L$^{-1}$ of each analyte and subjected to the SPE protocol. RSD values comprised between 2.0 and 7.0% were found.

To study the applicability of the developed SPE-HPLC-UV method using NH$_2$-ZIF-8-BA 1 as sorbent, the developed method was applied to monitor the target analytes in environmental complex matrices. The samples were analyzed in order to find any potential presence of the analytes. The results (see Table 3) showed that none of the target benzomercaptan was detected in the nonspiked real samples. Next, validation samples were prepared using these samples fortified with the three target compounds at concentration level of 5-500 µg L$^{-1}$. As shown in Table 3, the recoveries of analytes were satisfactory, ranging between 74 and 117%. In order to discard matrix effect in sewage water, particularly for that one from Gandía (influent), standard addition calibration curves were done. Several spiked samples at different levels (25-100 µg L$^{-1}$) were percolated through cartridges and the slopes obtained in the resulting calibration curves were not statistical different from those found with the external calibration method.
(confidence level of 95%) As representative examples, Fig. 3 (left) shows the chromatograms of a water sample (from influent sewage water) spiked with the analytes without and with SPE pretreatment. On the other hand, the right part of Fig. 3 illustrates the eluted fraction corresponding to a blank soil sample and that spiked with the analytes. As observed, an effective enrichment of analytes of interest in both matrices was evidenced, which demonstrated the performance of the synthesized sorbent to be used as preconcentration purposes in complex samples.

3.5. Comparison with other commercial sorbents and extraction methodologies

Next, a comparison in terms of extraction performance with one commercial sorbent (silica gel) commonly used for the extraction of benzomercaptans [51, 52] was made. This generic sorbent gave retention values below 50% (see Fig. S8), which underlines the convenience of using our synthesized sorbent. Finally, the developed material was compared with other extraction sorbents of target analytes in environmental samples (see Table 4). Regarding aqueous samples analysis, the recovery values obtained were quite similar to those found in most reported studies; however, in certain works [23, 28] these values were lower than 65%. Regarding the LODs, our values were better than those described by Parham et al. [24], and similar to those reported in refs. [28, 30, 31] using a sophisticated and high-cost MS detector. Other advantage found is the less amount (20 mg) of sorbent required compared with single-use commercial SPE sorbents (commonly up to 150-500 mg) [28, 30, 31]. This amount was also similar to a nanomaterial-based method using CuNPs [23, 24] probably due to surface area-to-volume ratio; however, its reusability was quite lower than that found in the present study. Likewise, in soil monitoring, the present method gave higher recoveries than those described in literature [53, 54]. Concerning LODs, our protocol provided comparable LOD values [53], or higher using MS detection [54]. In any case, this drawback does not reduce the good features of our protocol such as the easy and cheap preparation of the sorbent combined with the accessible equipment required, make this protocol a feasible method to monitor benzomercaptans and their derivatives in environmental samples.

4. Conclusions

In this research, n-butylamine modulated ZIF-8 nanocrystals were successfully synthesized, characterized and applied as SPE sorbent to the extraction of
benzomercaptans in environmental matrices. Prior to the SPE optimization, the addition of BA (at several molar ratios) as modulator to the reaction mixture was done to evaluate its impact on the crystal growth and adsorption capacity of the resulting MOFs. As a result of this study, NH$_2$-ZIF-8 containing 10 mmol of BA as modulator was the most appropriate sorbent. Then, several experimental parameters of the SPE protocol (such as sample pH, desorption solvent composition, among others) were investigated in detail using this MOF as SPE phase. The highly selective and efficient retention of benzomercaptans on this MOF take advantages of multiple interactions between these targets and the framework.

Moreover, the fruitful combination of NH$_2$-ZIF-8-BA 1 followed by HPLC-UV analysis led to high extraction recovery values, low LODs, excellent enrichment factors and satisfactory reusability. All these figures of merits prove that the present protocol constitutes a simple, cost-effective, and appropriate methodology for extraction and preconcentration of these pollutants in complex environmental samples. As far as we know, this study reports the first study of influence of modulator content on the morphology and extraction performance of organic pollutants and the subsequent application of the best modulated ZIF-8 for the extraction of benzomercaptans and demonstrate that it could be a promising SPE sorbent for further applications.

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Declaration of competing interest: None.
References


<table>
<thead>
<tr>
<th>Reference</th>
<th>Title</th>
<th>Journal</th>
<th>Year</th>
</tr>
</thead>
</table>


Development of a hydrophilic magnetic amino-functionalized metal-organic framework for the highly efficient enrichment of trace bisphenols in river water samples. Talanta, 211 (2020) 120713.


Table 1. Influence on different BA content modulator of textural parameters of synthesized MOFs and their extraction performances.

<table>
<thead>
<tr>
<th>MOF</th>
<th>Modulator</th>
<th>Particle size (nm)</th>
<th>Surface area (m² g⁻¹)</th>
<th>N amount (atomic, %)</th>
<th>Recoveriesa (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZIF-8</td>
<td>None</td>
<td>89 ± 6</td>
<td>1456</td>
<td>45.3</td>
<td>60-63</td>
</tr>
<tr>
<td>NH₂-ZIF-8-BA 1</td>
<td>BA, 10 mmol</td>
<td>61 ± 2</td>
<td>1365</td>
<td>52.6</td>
<td>94-102</td>
</tr>
<tr>
<td>NH₂-ZIF-8-BA 2</td>
<td>BA, 5 mmol</td>
<td>47 ± 5</td>
<td>856</td>
<td>47.7</td>
<td>64-77</td>
</tr>
<tr>
<td>NH₂-ZIF-8-BA 3</td>
<td>BA, 20 mmol</td>
<td>72 ± 7</td>
<td>1213</td>
<td>56.3</td>
<td>99-102</td>
</tr>
</tbody>
</table>

aExtraction conditions: sample concentration, 500 µg L⁻¹; volume, 5 mL; eluting solvent, 0.5 mL of 50:50 MeOH:H₂O (v/v) with 0.5 M NaOH.

Table 2. Figures of merit of the developed material used as SPE sorbent in the extraction and analysis of benzomercaptans.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Calibration range (µg L⁻¹)</th>
<th>Within-device recovery (%) ± RSD (n=3)</th>
<th>Between-device recovery (%) ± RSD (n=3)</th>
<th>LODa (µg L⁻¹)</th>
<th>LOQa (µg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBO</td>
<td>5 – 2500</td>
<td>109 ± 2</td>
<td>107 ± 2</td>
<td>1.6</td>
<td>5</td>
</tr>
<tr>
<td>MBT</td>
<td>7 – 5000</td>
<td>95 ± 7</td>
<td>101 ± 1</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>NMBT</td>
<td>7 – 5000</td>
<td>100 ± 7</td>
<td>95 ± 1</td>
<td>2</td>
<td>7</td>
</tr>
</tbody>
</table>

aInstrumental values (without applying the extraction protocol)
Table 3. Recovery study of benzomercaptans in spiked environmental samples analyzed following the recommended SPE protocol. Recovery (%) ± SD (n=3)\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Gandia sewage water (influent)</th>
<th>Paterna sewage water (effluent)</th>
<th>Tap water</th>
<th>Soil 1 (Sueca)</th>
<th>Soil 2 (Burjassot)</th>
<th>Soil 3 (Valencia)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spiked level (µg L\textsuperscript{-1})</td>
<td>Spiked level (µg kg\textsuperscript{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- 5</td>
<td>- 5</td>
<td>- 5</td>
<td>- 5</td>
<td>- 500</td>
<td>- 500</td>
</tr>
<tr>
<td>MBO</td>
<td>&lt; LOD</td>
<td>76 ± 2</td>
<td>&lt; LOD</td>
<td>94 ± 2</td>
<td>&lt; LOD</td>
<td>117 ± 9</td>
</tr>
<tr>
<td>MBT</td>
<td>&lt; LOD</td>
<td>76 ± 5</td>
<td>&lt; LOD</td>
<td>82 ± 2</td>
<td>&lt; LOD</td>
<td>81 ± 4</td>
</tr>
<tr>
<td>NMBT</td>
<td>&lt; LOD</td>
<td>74 ± 5</td>
<td>&lt; LOD</td>
<td>75 ± 6</td>
<td>&lt; LOD</td>
<td>74 ± 8</td>
</tr>
</tbody>
</table>

\textsuperscript{a}The recovery values were calculated by dividing the concentration found by the concentration added (calibration curves).
### Table 4. Comparison with other reported methods for benzomercaptans extraction and analysis.

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Material (sorbent amount)</th>
<th>Method</th>
<th>Sample matrix</th>
<th>Recoveries (%)</th>
<th>LOD (ng L$^{-1}$/ng kg$^{-1}$)</th>
<th>Reusability</th>
<th>EF$^a$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBT, MBO</td>
<td>Copper oxide nanoparticles (14 mg)</td>
<td>SPE / HPLC-DAD</td>
<td>Industrial water samples</td>
<td>56-93</td>
<td>-</td>
<td>3</td>
<td></td>
<td>[23]</td>
</tr>
<tr>
<td>MBT, MBO, MBI</td>
<td>Copper oxide nanoparticles (14 mg)</td>
<td>SPE / HPLC-DAD</td>
<td>Environmental water sample</td>
<td>96-104</td>
<td>1900-2700</td>
<td>3</td>
<td>200</td>
<td>[24]</td>
</tr>
<tr>
<td>MBT</td>
<td>Oasis HLB cartridge (200 mg)</td>
<td>SPE / HPLC-MS</td>
<td>Municipal wastewater samples</td>
<td>60-70</td>
<td>16.7</td>
<td>-</td>
<td>7-14</td>
<td>[28]</td>
</tr>
<tr>
<td>MBT</td>
<td>Oasis MAX (150 mg)</td>
<td>SPE / HPLC-MS</td>
<td>Environmental water samples</td>
<td>80-118</td>
<td>1-4</td>
<td>-</td>
<td>20-100</td>
<td>[30]</td>
</tr>
<tr>
<td>MBT</td>
<td>Oasis HLB (500 mg)</td>
<td>SPE / HPLC-MS</td>
<td>Water samples</td>
<td>70-85</td>
<td>7</td>
<td>-</td>
<td>21-83</td>
<td>[31]</td>
</tr>
<tr>
<td>BT, HBT, MTBT, ABT</td>
<td>Strata™-X cartridges (200 mg)</td>
<td>SPE / HPLC-MS/MS</td>
<td>Dewatered sewage sludge</td>
<td>50-116</td>
<td>40-13000</td>
<td>-</td>
<td>-</td>
<td>[51]</td>
</tr>
<tr>
<td>MBO, MBT, NMBT</td>
<td>Poly-Sery HLB cartridge (60 mg)</td>
<td>SPE / UPLC-MS/MS</td>
<td>Road dust / Roadside soil</td>
<td>56-105</td>
<td>20-120</td>
<td>-</td>
<td>-</td>
<td>[52]</td>
</tr>
<tr>
<td>MBO, MBT, NMBT</td>
<td>NH$_2$-ZIF-8-BA 1 (20 mg)</td>
<td>SPE / HPLC-UV</td>
<td>Environmental samples</td>
<td>74-117</td>
<td>16-21 / 400-500</td>
<td>10</td>
<td>4$^b$; 100$^c$</td>
<td>This Work</td>
</tr>
</tbody>
</table>

$^a$The EF from the overall SPE protocol was calculated using the ratio between the final (eluent) extract and the maximum sample volume load (for aqueous samples) or the amount of solid weighed (for soil samples); $^b$soil samples; $^c$aqueous samples

Figure 1. SEM (A) and TEM (B) micrographs of ZIF-8 (left part) and NH$_2$-ZIF-8-BA 1 (right part) materials.

Figure 2. Effect of elution solvent composition on the recovery values of analytes using NH$_2$-ZIF-8 as SPE sorbent (left part), eluent compositions: A) MeOH, B) 50:50 MeOH:H$_2$O (v/v), C) 50:50 MeOH:H$_2$O (v/v) with 0.1 M NaOH, D) 50:50 MeOH:H$_2$O (v/v) with 0.2 M NaOH and E) 50:50 MeOH:H$_2$O (v/v) with 0.5 M NaOH; effect of loading sample volume on the extraction efficiency of benzomercaptans (right part). Error bar = SD (n = 3).
Figure 3. HPLC-UV chromatograms of sewage water (Gandía’s plant) (left part) and extracts of soil samples (right part) spiked with 5 µg L$^{-1}$ of each benzomercaptan, respectively, without applying (A) and applying the proposed SPE protocol (B). The right part shows an extract of soil sample without (A) and with spiking of analytes at 500 µg kg$^{-1}$ (B) subjected to the SPE treatment. Chromatographic conditions are given in Experimental Section and Supplementary Information. Peak identification: 1, MBO; 2, MBT; 3, NMBT.