Magnetic material based on mesostructured silica functionalized with β-cyclodextrin to extract opium alkaloids in poppy seed infusions

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ABSTRACT

Sample preparation methods tend to be fast, simple, with the use of new adsorbents and with lower amounts of organic solvents to make them more environmentally friendly. In this work, a magnetic solid-phase extraction (MSPE) protocol for the quantification of six opium alkaloids (OAs) in poppy seeds infusions has been optimised followed by analysis with liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS). For this purpose, a mesostructured silica magnetic composite with β-CD ($Fe_3O_4@SiO_2@mSiO_2@β$-CD) was synthesised and characterized. To obtain the highest level of functionalisation, three proportions of β-CD monomer/material (12.5, 25 and 100%) were determined, demonstrating that 25% showed the highest level. Adsorption studies were carried out in water at different pH (1, 2, 7, 9 and 11) and times (1, 5, 10 and 20 min), confirming the high adsorption capacity at pH 9 for 1 min. Then, the elution conditions of MSPE procedure were optimised (50 mg material, 2 mL of water/EtOH at 50% with 1% formic acid for 1 min). The methodology was successful validated with low detection and quantification limits, negligible matrix effect and good recovery values (89–94%). The methodology was applied to analysis of home-made infusions with four different poppy seeds, observing in one of them a worrying amount of OAs which imply a content in the seeds of at least four times that of the legislation.

1. Introduction

Sample preparation prior to instrumental analysis plays an important role in analytical methodologies. In fact, to reduce interferences of complex matrices, efficient sample treatment methods are needed for selective isolation and concentration of target analytes at suitable levels before the analysis. In addition, the purification step can considerably increase the lifetime of the equipment by obtaining cleaner extracts. However, sample preparation is still the most labour-intensive analytical step and can affect both accuracy and precision of the results [1].

In general, sample preparation is based on extraction techniques and one of the most used is solid-phase extraction (SPE). However, SPE have some limitations and drawbacks, including the wide use of organic solvents and waste generated, but also because it requires too much labour, is time-consuming and relatively expensive. For this reason, different extraction techniques have emerged as greener and sustainable alternatives to classical sample preparation procedures, with the aim of improving the selectivity and sensitivity of analytical methods, while simultaneously reducing the deleterious side effects of traditional techniques for both the operator and the environment [2–5]. In this regard, some methodologies have been developed in the last years for the analysis of

different natural toxins, based on new sample preparation techniques trying to use fewer organic solvents and sorbents, to be more environmentally friendly, and to synthesize new effective sorbent materials, to be able to avoid matrix effects and preconcentrate the extracts to fewer instrumental limits [6]. For example, protocols based on miniaturized SPE (μ-SPE) with functionalized mesostructured silicas have been proposed recently for the analysis of toxic alkaloids [7,8] and good results have been obtained thanks to the advantages of this type of materials, such as large surface area, porosity, facile functionalization, good mechanical and chemical stability [9].

A powerful SPE alternative is magnetic solid phase extraction (MSPE), based on the use of magnetic sorbents. It has several advantages of high adsorption efficiency, simple operation, low cost, and time saving. MSPE involves dispersing the magnetic material in a solution with the sample/extract for a few minutes. Once it is at equilibrium, it is recovered with the help of a magnetic field and finally the analytes are desorbed, which can avoid tedious filtration, centrifugation or sedimentation steps [10]. Magnetic sorbents are mainly based on magnetite (Fe_3O_4) nanoparticles (MNPs), that show excellent features such as high magnetic moment, simple synthesis and low production cost, besides small size and high surface area that increases their sorption capacity [11]. However, to stabilize bare MNPs and to achieve a

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selective extraction towards the target analytes, it becomes necessary to modify their surface with some functional groups. For this reason, MNPs are usually coated with an adequate organic or inorganic material, and different MNP composites have been prepared with carbon, polymers, silica, metal-organic frameworks, etc. The coating layer prevents oxidation and increases the durability of the MNPs, but also provides an adequate colloidal dispersion [11,12]. In that respect, core coated MNPs with different mesostructured silicas (SBA-15, MCM-41, KIT-6, etc.) have been successfully applied as sorbents in MSPE for extraction of different analytes [9]. Compared with analogous amorphous silica coated particles (i.e., $Fe_3O_4@SiO_2$), these materials displayed higher binding capacity faster mass transfer and higher extraction efficiencies owing to the mesostructured silica coating [13]. For example, recently MNPs coated with amorphous and mesostructured silica, both functionalized with C18 or C8, were evaluated for MSPE to determine opium alkaloids (morphine, codeine, thebaine, papaverine, noscapine and oripavine) in edible poppy seeds samples. The best results were obtained with the non-functionalised mesostructured silica coated MNPs ($Fe_3O_4@SiO_2@mSiO_2$) and the sample extracts were purified in just 4 min using 50 mg of sorbent. However, for morphine and oripavine the recoveries obtained were around 50%, which may be since they were the smallest analytes and showed higher intra-particle diffusion and were, therefore, more difficult to desorb, resulting in lower recoveries [13].

On the other hand, in recent years, there has been an increasing interest of the scientific community the use of cyclodextrin (CD) with different purposes, which are natural products obtained from the enzymatic degradation of starch and therefore have a low price, negligible environmental impact and non-toxicity [14–16]. CDs are a family of natural torus-shaped cyclic oligosaccharides composed of 6 (α-CD), 7 (β-CD), and 8 (γ-CD) D-glucopyranose units linked by $\alpha-1,4$-glycosidic bonds, possessing hydrophobic inner cavities and hydrophilic outer faces. The unique structures of CD make possible the formation of inclusion complexes with aliphatic and aromatic non-polar compounds of suitable size by a variety of forces such as hydrogen bonding, hydrophobic and van der Waals interaction, especially when they are in aqueous media [14,15]. The use of MNPs composites as support is a versatile strategy to enhance the active surface where the CDs must be located. In this regard, some works have been explored these materials for the analysis of PAHs [17], pesticides [18], etc., but to the best of our knowledge, they have not yet been used for opium alkaloids (OAs).

The seeds of the *Papaver somniferum* L. plant, commonly known as opium poppy, are increasingly being used to elaborate infusions [19–21]. The problem with this practice is that seeds can be contaminated with OAs [13,22]. The consumption of infusions with poppy seed could lead to cases of severe intoxications [23–27] because the concentration of OAs in poppy seeds can be dangerously high [13,22]. However, the OAs content in the seeds is not the same as what can appear in the infusion, because in this process some of these compounds can be thermally degraded in different percentages depending on the analyte and heating conditions [28]. Therefore, it is necessary to study the levels of OAs that can be found in poppy seed infusions to estimate the real risk that the ingestion of this product represents for the population.

Accordingly, the potential use of a mesostructured silica magnetic composite functionalized with β-CD ($Fe_3O_4@SiO_2@mSiO_2@\beta$-CD) as MSPE sorbent in a new proposal for the extraction and quantification of six OAs in infusions is assessed. After an initial comparison with magnetite and amorphous silica-coated magnetite containing β-CD ($Fe_3O_4@\beta$-CD and $Fe_3O_4@SiO_2@\beta$-CD) to evaluate its performance, the analytical features of the developed method have been established to obtain a selective and more environmentally friendly protocol and to improve the recoveries obtained with previous methodologies. Finally, home-made infusions prepared with different commercially available poppy seeds have been analysed by using the validated method.

2. Materials and methods

2.1. Reagents and materials

Standards of morphine, codeine, thebaine and oripavine were received from Alcaliber S.A.U. (Madrid, Spain). Noscapine, papaverine, morphine-d3 and codeine-d3 (internal standard, IS) were obtained from Sigma-Aldrich (Zwijndrecht, The Netherlands). Individual stock standard solutions were prepared at 1000 mg/L in methanol, and working standard solutions were prepared at 1 mg/L in water/ethanol 75/25 (v/v) with 10% formic acid. All of these were stored in darkness at −20 °C.

Ferric chloride 6-hydrate ($FeCl_3 \cdot 6H_2O$) 99% and ferrous chloride 4-hydrate ($FeCl_2 \cdot 4H_2O$) 99% were purchased from Labkem (Barcelona, Spain) and Acros Organics (Geel, Belgium), respectively. Tetraethylorthosilicate (TEOS) 98%, hexadecyltrimethylammonium bromide (CTAB), 3-isocyanatopropyltriethoxysilane 98% and β-cyclodextrin $\geq$ 97% were purchased from Sigma-Aldrich. Ethanol absolute, formic acid (98%) and ammonia 32% (w/w), isopropanol, toluene, pyridine, and diethyl ether were of synthesis grade and purchased from Scharlab (Barcelona, Spain). N, N-dimethylformamide (DMF) were purchased from Merk (Darmstadt, Germany). Acetonitrile, methanol, and ethanol used were HPLC-MS quality and were purchased from Scharlab (Barcelona, Spain). Ultrapure water (resistivity 18.2 MΩ cm) was obtained from a Milli-Q water purification system (Millipore, Billerica, MA, USA). The Nd-Fe-B magnet ($5 \times 5 \times 2$ cm) with force 200 kg used in the MSPE procedure was obtained from Superimanes S.L. (Sevilla, Spain).

2.2. Infusions with poppy seed samples

Four samples of edible seeds (PS-01, PS-02, PS-03 and PS-04) were purchased in Spain in early to mid-2022 from supermarkets and herbalists. More information detailed in Table S1. To prepare the infusions (PS-I-01, PS-I-02, PS-I-03 and PS-I-04) with these poppy seeds, International Standard ISO 3103 protocol was used [29]. For it, 2.00 ± 0.01 g of poppy seeds were weighted, and 100 mL of boiling water was added. The infusion was covered for 5 min and then, it was removed and cooled to room temperature. Finally, the infusion was filtered through a nylon syringe filter and the MSPE procedure was applied with $Fe_3O_4@SiO_2@mSiO_2@\beta$-CD followed by HPLC-MS/MS analysis. The studies were done in triplicate, taking again 2 g of seeds in each infusion to determine the variation that the same seed batch could give, as observed in previous studies [13,30].

2.3. Preparation of the magnetic materials with β-CD

The schematic preparation process of the magnetic materials is shown in Fig. 1. First, the β-CD monomer was synthesised to functionalise the MNPs. For this purpose, 2.5 g of previously dried β-CD were added to 50 mL of dry pyridine. Once dissolved, 1.56 g of 3-isocyanatopropyltriethoxysilane was added (with a 1:3, β-CD:3- isocyanatopropyltriethoxysilane, stoichiometry). The reaction was maintained at 80 °C for 24 h under magnetic stirring at 500 rpm in an inert N_2 atmosphere. Then, the solvent was evaporated, and the resulting light-yellow solid was dried at room temperature under vacuum for 24 h. Secondly, such as shown in Fig. 1, MNPs were prepared using co-precipitation according to the work of Zhang and Shi [31]. Then, the first layer of amorphous silica ($Fe_3O_4@SiO_2$) was added to the magnetite, followed by a second layer of mesostructured silica ($Fe_3O_4@SiO_2@mSiO_2$) (Fig. 1), according to the protocol used in our previous work [13]. Thirdly, the final material ($Fe_3O_4@SiO_2@mSiO_2$) was functionalised with the β-CD (Fig. 1). For this purpose, the amount of β-CD monomer (weight percentage related to mass of material) added to the magnetic material were optimised (12.5, 25 and 100%). Finally, 25% by weight of the formed β-CD monomer (0.75 g) was added to the
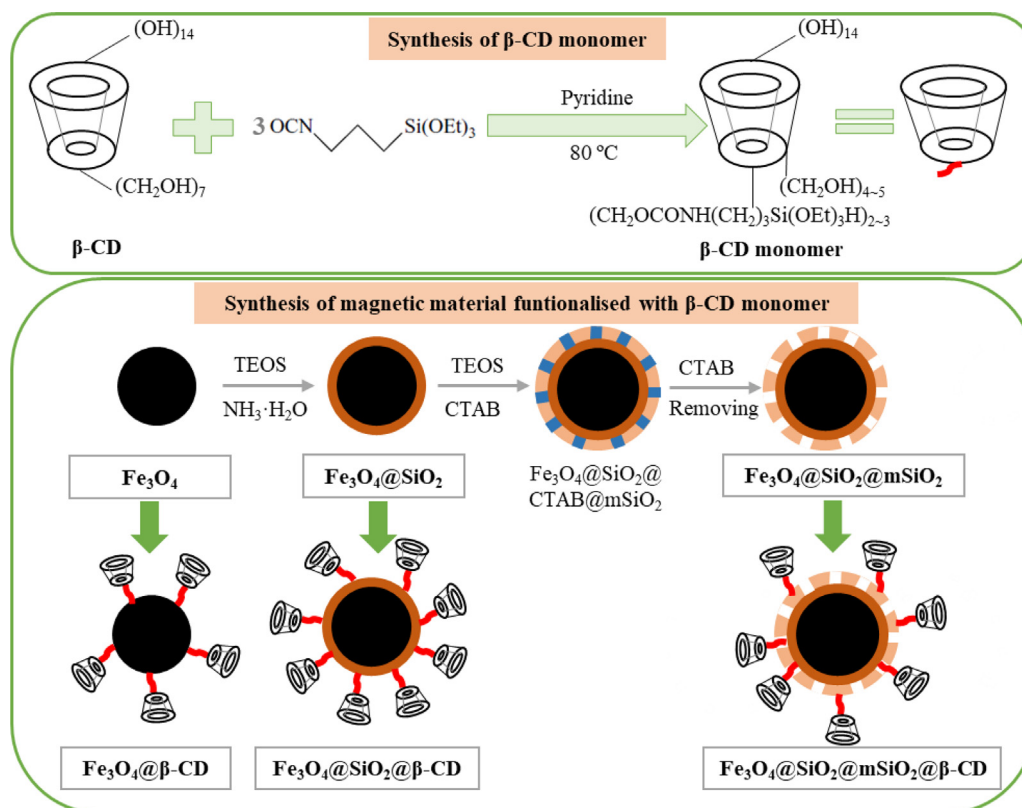
Fig. 1. Scheme of synthesis of β-CD based monomer and synthesis of each of magnetic composite functionalized with β-CD.

magnetic material (3 g) with approximately 50 mL of DMF. The reaction took place at 70 °C for 24 h under magnetic stirring at 500 rpm and in N_2 atmosphere. After the reaction time, the material was collected with the help of an external magnet, washed with approximately 30 mL of each solvent: DMF, ethanol and diethyl ether and dried under vacuum for 24 h.

2.4. Characterization of the magnetic materials with β-CD

The magnetic materials with β-CD were characterized by Attenuated Total Reflection Fourier-Transform Infrared (ATR-FT-IR), elemental analysis (% N), nitrogen gas adsorption–desorption isotherms and X-ray diffraction (XRD). Details of the equipment and conditions can be found in Supporting Information S1.

2.5. Discontinuous adsorption studies of $Fe_3O_4@SiO_2@mSiO_2@\beta$-CD as MSPE sorbent and comparison with $Fe_3O_4@\beta$-CD and $Fe_3O_4@SiO_2@\beta$-CD

To evidence that the mesostructured silica magnetic composite with β-CD is an efficient magnetic material to do the MSPE procedure a discontinuous adsorption study was performed. In addition, $Fe_3O_4@SiO_2@mSiO_2@\beta$-CD was compared with the $Fe_3O_4@\beta$-CD and $Fe_3O_4@SiO_2@\beta$-CD materials to confirm their higher adsorption capacity. For this purpose, 50 mg of each of the three synthesised materials was added to 2 mL of water with different pH values (1, 3, 7, 9 y 11) containing 1 mg/L of each of the six analytes. Finally, the supernatants of each of the different solvents were analysed by HPLC-MS/MS after a fixed adsorption time (1, 5, 10 and 20 min). All the studies were done in duplicate, and the adsorption percentages of each of the materials on the different solvents were calculated and compared to determine the optimal adsorption conditions. The adsorption (%) was calculated by the following formula: 100 – (value of the supernatant divided by value of the simulated sample, sample subjected to the same procedure but spiked at the concentration level prior to HPLC-MS/MS analysis).

2.6. Adsorption kinetic and isotherm experiments with $Fe_3O_4@SiO_2@mSiO_2@\beta$-CD material

For adsorption kinetics, 50 mg of the material was added to a water solution at pH 9 containing each of the six analytes under study (2 mL, 1 mg/L). The mixtures were subjected to ultrasound at different times (1 – 20 min), and the supernatants in equilibrium were analysed by HPLC-MS/MS. The adsorption capacity was calculated by Equation (S1) in Table S2. The adsorption kinetics were determined by Lagergren's pseudo-first-order [32], pseudo-second-order [33] and intra-particle diffusion kinetic models [34] (Table S2). For adsorption isotherm, a series of 2.0 mL solutions of different concentrations of the six analytes (0.1 – 40 mg/L) was added to 50 mg of the material under optimum time. The isotherms of the six opium alkaloids adsorption on the magnetic particles were analysed using the commonly used Langmuir [35] and Freundlich [36] models (Table S2).

2.7. Optimization of MSPE conditions with $Fe_3O_4@SiO_2@mSiO_2@\beta$-CD material

For this purpose, 2 mL of 1 mg/L of each of the six analytes was used. The parameters of the MSPE procedure that were optimised were the following: the amount of adsorbent (between 5 and 50 mg); the adsorption time (from 1 to 20 min); the pH of the initial solution (ranging from 1 to 11); the desorption eluent (water with ethanol, acetonitrile, or methanol at 50%) with 1% of formic acid. All studies were carried out in triplicate. In addition, different batches of the $Fe_3O_4@SiO_2@mSiO_2@\beta$-CD material were evaluated to ensure that the results obtained with all of them were reproducible. For this purpose, RSD (relative standard deviation,%) of the recoveries obtained with 0.4 mg/L (medium validation level) standard solutions at the optimum conditions used for the MSPE procedure was calculated.

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2.8. Optimised MSPE methodology and HPLC-MS/MS analysis for quantification of opium alkaloids in poppy seeds infusion

In the optimised analysis methodology, 50 mg of $Fe_3O_4@SiO_2@mSiO_2$ (conditioned with 1 mL of water at pH 9 for 1 min in ultrasound were added with 2 mL of the poppy seed infusion (previously filtered and adjusted at pH 9) and then, the mixture was treated with 1 min ultrasound until the analytes arrived at an adsorption equilibrium with the adsorbent material. Later, the material with analytes was separated by an external magnet from the solution, and the analytes were eluted from the magnetic particles with 2 mL water/ethanol at 50% with 1% formic acid for another minute. Subsequently, 950 µL was recovered and 50 µL of a 1 mg/L solution of morphine-d3 and codeine-d3 was added as an internal standard and analysed by HPLC-MS/MS.

Analysis of OAs was carried out with a Varian 1200/1200 LC (Varian Ibérica, Madrid, Spain) equipped with a ProStar 410 autosampler (100 µL loop) coupled with a TQ tandem mass spectrometer detector (1200 L TQ) with an electrospray ionization (ESI) ion source. For data acquisition, the system was MS Workstation Varian with a version 6.8. Chromatographic separation was performed as mentioned in our previous works [37,38] using a C18 KromaPhase 100 column (150 × 2.0 mm, 3.5 µm particle size, Scharlab, Barcelona, Spain) at 30 °C. The injection volume was 10 µL (partial injection), and the flow rate was set at 0.25 mL/min. The gradient elution was used with a mobile phase of water (A) and acetonitrile (B), both with 0.1% of formic acid as follows: 90−30% A (0 − 6 min), 30−90% A (6 − 9 min), and 90% A (9 − 11 min) for column reequilibration. Positive mode electrospray ionization (ESI +) with MRM mode was used for mass spectrometry acquisition. The drying and nebulizer gas was N_2. The drying gas was set at 350 °C and 22 psi, and the nebulizer gas was set at 58 psi. The collision gas used was argon at 1.90 mTorr and the detector voltage of 1480 V. The capillary voltage was held at 5000 V and shielded at 600 V and the cone voltage 72 V. In Table S3 was showed the optimal mass spectrum parameters.

2.9. Method validation

Currently, there is no official regulation on analytical performance requirements for OAs in food or feed. For this reason, the method validation was performed following the criteria described in the SANTE/11,312/2021 document [39], in regulation EC No 401/2006 [40], and in the Q2(R1) ICH guidelines (Internation Council for Harmonisation, 2005) [41]. The method validation was done in terms of linearity, method detection and quantification limits (MDL, MQL), matrix effect, accuracy, precision, and selectivity (more details in Supporting Information S2). To carry out the validation, washed and dried poppy seeds were used to avoid variation of OAs content in the same batches as confirmed in previous work [13]. After infusion following International Standard ISO 3103 protocol [29] (2.00 ± 0.01 g with 100 mL of boiling water for 5 min) the infusion was cooled to room temperature, filtered through a nylon syringe filter and spiked at three concentration levels: a low (0.2 mg/L), medium (0.4 mg/L) and high (0.8 mg/L). The spiked levels were selected according to the legislation (20 mg/kg in seeds) which calculated at 2 g/100 mL of infusion was 0.4 mg/L.

3. Results and discussion

3.1. Preparation and characterization of β-CD monomer

To attach the β-CD to the mesoporous silica structure, two or three triethoxysilyl groups were covalently attached to the β-CD via carbamate bonds (Fig. 1). For this purpose, the β-CD is reacted with 3-isocyanatopropyltriethoxysilane with a 1:3 stoichiometry [42]. However, the reaction medium used in different works to prepare del monomer is different, in DMF [42], in pyridine [43,44] and in toluene [45]. Therefore, a previous study was carried out to synthetise to

monomer with each of the three solvents to determine the most efficient one.

First the ATR-FT-IR spectrum of the β-CD is shown in Fig. S1a where the characteristic bands around 100 cm^{-1} can be seen. Subsequently, the spectra of the β-CD monomer were made with the three solvents (Fig. S1b) in which, in addition to the bands characteristic of β-CD, the bands of the 3-isocyanatopropyltriethoxysilane ligand were also seen. In toluene, the characteristic IR bands of the 3-isocyanatopropyltriethoxysilane ligand are less intense. The stretching bands νC–H(-CH_3 groups) (2850 cm^{-1}) and νC–H(-CH_2 groups) (2900 cm^{-1}) of the ethoxy groups (–OCH_2_CH_3) of the 3-isocyanatopropyltriethoxysilane ligand anchored to the β-CD moieties appear much less pronounced than in DMF this also happens with the band at 1083 cm^{-1} due to C–O-C bonds. In addition, a higher yield of monomer was obtained in DMF. These facts suggest a lower yield in the reaction between de CDs groups and the 3-isocyanatopropyltriethoxysilane ligands, using toluene as solvent medium, that can be explained due to a lower solubility of the β-CD in toluene. The β-CD monomer was isolated from the reaction medium through DMF evaporation. Nevertheless, the use of DMF presents some drawbacks, like its high boiling point and hydrogen bonding formation with the hydroxyl groups of the monomer, which means that for total removal of the DMF from the monomer, high vacuum, high temperature and long drying time are required, and even with that extreme conditions some rests of DMF can still be found in some situations, as the presence of carbonyl bands of the DMF in the ATR-FT-IR spectra of the β-CD monomer (1650 cm^{-1}) revealed. To avoid these drawbacks, pyridine was tested, as the reaction solvent, which due to its lower evaporation point and weaker interaction with the monomer, allowed better solvent removal, and produced the highest yield (90%). The ATR-FT-IR spectra of the monomers (Fig. S1b) show all the characteristic adsorption bands of the 3-isocyanatopropyltriethoxysilane ligand anchored to the β-CD moieties. In addition, the main bands due to β-CD groups can be detected in the range of 900–1400 cm^{-1} and at 3000 cm^{-1} that it belongs to the valence vibrations of the C–H bonds in the CH and CH_2 groups of the β-CD. Finally, the band at 3400 cm^{-1} is characteristic of the stretching band of the O–H groups. All these IR bands show the appropriate modification of the β-CD monomer with the 3-isocyanatopropyltriethoxysilane ligand. For all the above-mentioned reasons, pyridine was selected as the solvent to obtain the β-CD monomer.

3.2. Characterization of $Fe_3O_4@SiO_2@mSiO2@β$-CD material compared with $Fe_3O_4@β$-CD and $Fe_3O_4@SiO_2@β$-CD

The mesostructured silica magnetic composite was characterised before and after functionalisation. In addition, the Fe_3O_4 and $Fe_3O_4@SiO_2$ materials, before and after functionalisation, were also characterised for comparative purposes.

First, ATR-FT-IR spectra was performed of $Fe_3O_4@SiO_2@mSiO_2$ material before and after functionalisation with 100% of β-CD monomer to verify the formation of the silica layer and the functionalisation with the β-CD monomer. As shown in Fig. 2a, the infrared bands at 575 cm^{-1} were observed, which were associated with the stretching and twisting vibrational modes of the Fe-O bonds of magnetite at tetrahedral sites. Besides, the band at 3400 cm^{-1} is due to the protonation states by extensive hydrolysis of the interfacial water molecules with Fe_3O_4. Furthermore, the lack of this band in the functionalised materials due to the deprotonation of the hydroxyl groups, suggests a bonding between magnetite and β-CD monomer in the formed complex. In addition, the material both before and after functionalisation showed the characteristic band of Si-O bands at 1000 cm^{-1}. Therefore, it was determined that the material was functionalised with β-CD monomer and that the silica layer has formed around the particles. Besides, ATR-FT-IR spectrum of $Fe_3O_4@SiO_2$ (Fig. 2b) and Fe_3O_4 (Fig. 2c) before and after of functionalisation are also performed and the signals observed in the spectra also confirm the presence of silica and β-CD.
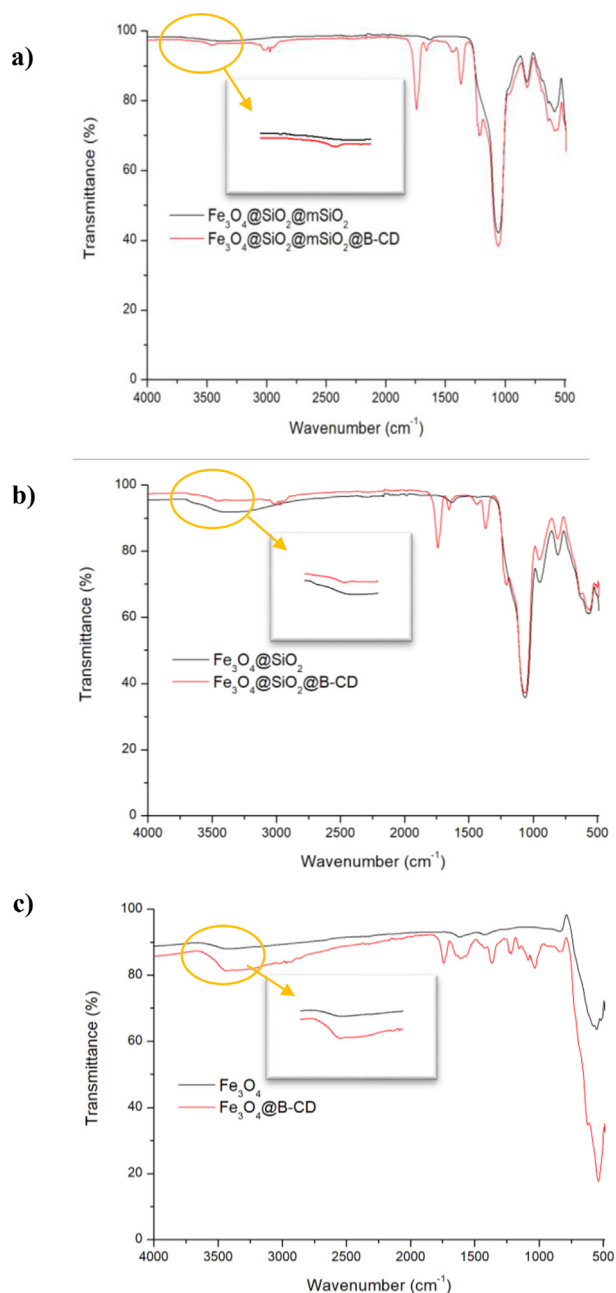
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Fig. 2. ATR-FT-IR spectra of mesostructured silica magnetic composite with β-CD (Fe₃O₄@SiO₂@mSiO₂) before and after of functionalization with β-CD (a) and spectra of Fe₃O₄@SiO₂ (b) and Fe₃O₄ (c) before and after of functionalization with β-CD for comparative purposes. Functionalization were carried out with 100% of β-CD.

Then, an elemental analysis was done to optimise the amount of β-CD monomer added to the magnetic material in the synthesis. For this purpose, three different proportions of β-CD monomer (12.5, 25 and 100%) added to Fe₃O₄@SiO₂@mSiO₂ material were evaluated and the functionalisation (mmol β-CD/g material) was estimated with the N content (%). As shown in Fig. 3, the Fe₃O₄@SiO₂@mSiO₂ material synthesised with 100% of β-CD monomer, (i.e., 1 g of β-CD monomer + 1 g of material), resulted in less functionalisation than with 25%. This fact can be explained because of the pore entrance blocking that can happens when a large amount of ligand is used in the modification process, avoiding the functionalization of free -OH groups inside de pores. Adding 12.5% decreases the degree of functionalisation considerably, with a similar

value that in the case of 100%. Therefore, it was decided to perform the functionalisation with 25% β-CD monomers in all materials, because a similar trend was observed in the other two materials (Fe₃O₄ and Fe₃O₄@SiO₂) such as shown in Fig. 3. However, the one with the highest degree of functionalisation was the one with a single silica layer (Fe₃O₄@SiO₂), with 0.147 mmol ligand/g as a maximum with 25% of β-CD monomer. This could be because, although the mesostructured silica bilayer gives a higher number of -OH groups and a higher surface area (402 vs. 220 m²/g), its pore size is smaller than the non-ordered silica (34.9 vs. 127.4 Å). Bulky ligand, like the β-CD monomer, could provoke steric impediments in the pores entrance, and the functionalisation taking place mainly the surface, resulting in a lower functionalisation. Nevertheless, with the addition of 25%, a similar functionalization degree was obtained with Fe₃O₄@SiO₂@mSiO₂@β-CD material (0.134 mmol ligand/g). For this reason, 25% was the optimum to allow surface and in-pore functionalisation in Fe₃O₄@SiO₂@mSiO₂@β-CD material.

In addition, the N₂ adsorption-desorption isotherms of mesostructured silica magnetic composite Fe₃O₄@SiO₂@mSiO₂@β-CD before and after of functionalization were performed (Fig. 4a). The adsorption isotherms can be assigned as Type IV, according to the IUPAC classification [46], which is characteristic of mesoporous materials. The isotherms show an initial part characteristic of monolayer adsorption and a significant increase of the adsorbed amount at intermediate relative pressures, characteristic of a multilayer filling mechanism. An H4 hysteresis loop is shown in material both before and after of functionalization (Fig. 4a). The textural properties of the Fe₃O₄@SiO₂@mSiO₂ material are shown in Table 1. As it can be seen, the material experiments a decrease in specific surface area BET (S_BET) and pore volume after functionalization due to anchoring of the β-CD monomers to the surface and inside the pores. In addition, the Fe₃O₄@SiO₂ material was also characterised before and after functionalisation (Fig. 4b) and as it was expected, lower surface area and pore volume was found in these materials (Table 1).

Finally, XRD was done to mesostructured silica magnetic composite before and after of functionalisation with β-CD to know its long-range order. The low-angle XRD pattern (Fig. S2a) reveals the Miller index (100) characteristic of materials with mesoscopic order, and in the wide-angle XRD patterns (Fig. S2b) the material before and after functionalisation showed the theoretical pattern of Fe₃O₄ with six discernible diffraction peaks in the 2θ region from 20° to 70° (220, 311, 400, 422, 511 and 440) that correspond with the Miller index diffraction peaks of the magnetic core (JCPDS card: 19–0629), showing that it is still present after modification.

3.3. Discontinuous adsorption studies with Fe₃O₄@SiO₂@mSiO₂@β-CD as MSPE sorbent compared with Fe₃O₄@β-CD and Fe₃O₄@SiO₂@β-CD

To evidence that the mesostructured silica magnetic composite with β-CD is an efficient magnetic material to do the MSPE procedure a discontinuous adsorption study was performed. To do this, 2 mL of water at different pH values (1, 2, 7, 9 and 11) with 1 mg/L of each of the six analytes and 50 mg of material. For comparative purposes the same studies were carried out with the Fe₃O₄@β-CD and Fe₃O₄@SiO₂@β-CD materials. The addition of the silica layers improved the adsorption capacity of the materials since, as shown in Fig. 5, the lowest adsorption (%) was in general with Fe₃O₄@β-CD material, then with Fe₃O₄@SiO₂@β-CD and the highest adsorption was obtained with Fe₃O₄@SiO₂@mSiO₂@β-CD. On the other hand, the optimum adsorption time was 1 min since increasing the adsorption time does not increase the adsorption. Moreover, the more acidified the medium, the lower the adsorption and at pH 11 it is like that obtained at pH 7, being pH 9 the pH at which the best adsorption results were obtained. Consequently, the highest adsorptions obtained were with Fe₃O₄@SiO₂@mSiO₂@β-CD with water at pH 9 and for 1 min, obtaining adsorptions of 95% for morphine, 94% for codeine, 97% for thebaine, 99% for papaverine, 95% for noscapine and 93% for oripavine. This confirms that the use of a β-CD functionalised mesostruc-
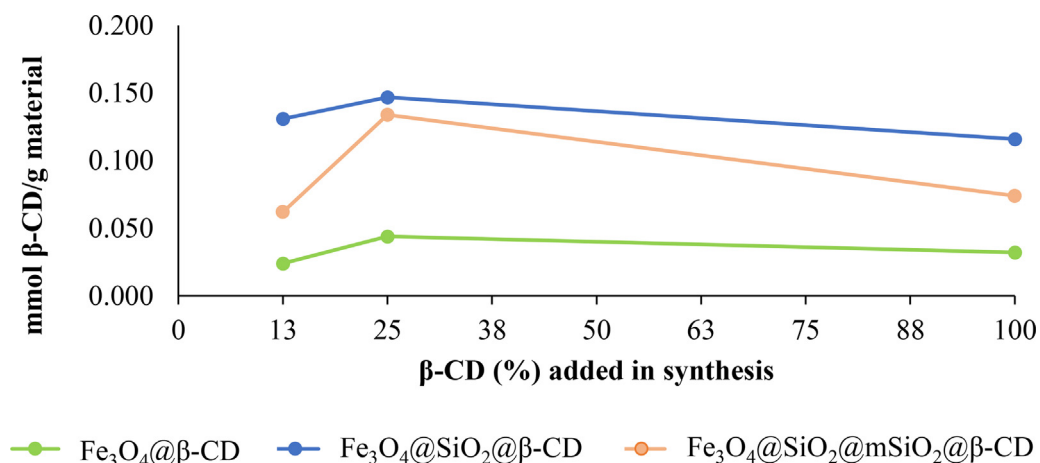
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Fig. 3. Functionalization degree of the three synthetized materials (mmol *β*-CD/g material) at three *β*-CD monomer proportions (weight percentage related to mass of material) added to the magnetic material (12.5, 50 and 100%) obtained by elemental analysis of N.
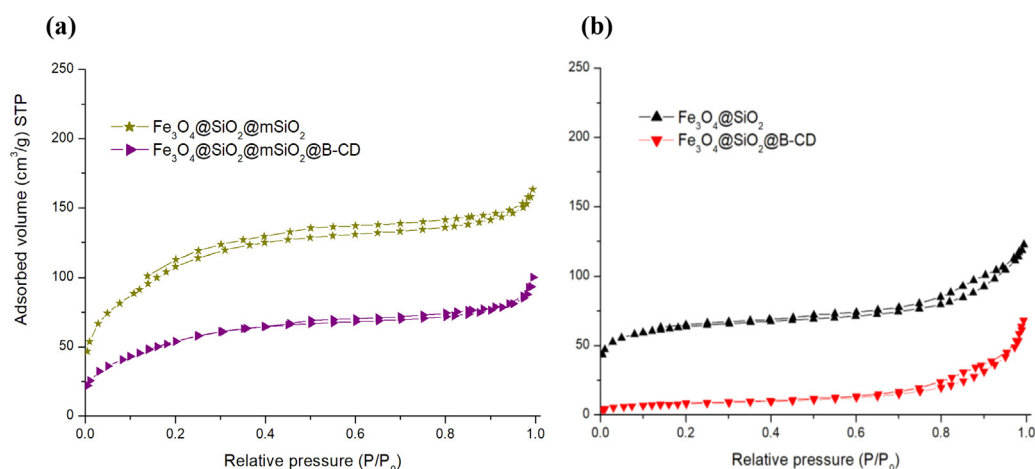

Fig. 4. N$_2$ adsorption-desorption isotherms of mesostructured silica magnetic composite before and after of functionalization (a) and for comparative purposes those of Fe$_3$O$_4$@SiO$_2$ (b).

Table 1
Textural properties of the magnetic materials synthesized.

Material	S$_{BET}$ (m^2/g)[a]	Pore volume (cm^3/g)[b]	Pore diameter (Å)[c]
Fe$_3$O$_4$@SiO$_2$	220	0.17	38.9, 91.9, 127.4
Fe$_3$O$_4$@SiO$_2$@*β*-CD	28	0.08	38.6, 91.4, 128.9
Fe$_3$O$_4$@SiO$_2$@mSiO$_2$	402	0.23	20.4, 34.9
Fe$_3$O$_4$@SiO$_2$@mSiO$_2$@*β*-CD	203	0.13	20.5, 39.0

[a] S$_{BET}$: Specific surface area calculated by Brunauer-Emmett-Teller (BET) method.
[b] Total pore volume was measured at relative pressure (P/P$_0$) = 0.97.
[c] Pore diameter estimated by using the BJH (Barrett, Joyner and Halenda) model applied on the desorption Branch.

tured silica magnetic composite, Fe$_3$O$_4$@SiO$_2$@mSiO$_2$@*β*-CD, is more efficient than the material with magnetite alone or amorphous silica, due to its higher adsorption capacity.

An additional test was performed with the non-functionalised material (Fe$_3$O$_4$@SiO$_2$@mSiO$_2$) which was used in a previous work [13] to quantify OAs in poppy seeds. In that work, adequate recovery values were obtained but those of morphine and oripavine only achieved up to 50% at all validation levels. Therefore, to improve this material, it was decided to add a ligand that interacts with these analytes to achieve better recovery values. To verify that the good adsorption values, obtained in the previous assay, are not only due to the interac-

tions between the free -OH groups of the silica, but also to the formation of inclusion complexes with the *β*-CD, the optimal conditions selected for the material with *β*-CD and the material without *β*-CD were compared. For this purpose, the adsorption was determined with a 1 mg/L standard solution in water at pH 9 at different times. The adsorptions obtained with Fe$_3$O$_4$@SiO$_2$@mSiO$_2$ were significantly lower than with Fe$_3$O$_4$@SiO$_2$@mSiO$_2$@*β*-CD, around 70% for thebaine, papaverine and noscapine, 60% for codeine and only 40% for morphine and oripavine. These results show an improvement of this type of material with mesostructured silica and, in addition, the high efficiency of *β*-CD in the extraction of OAs.
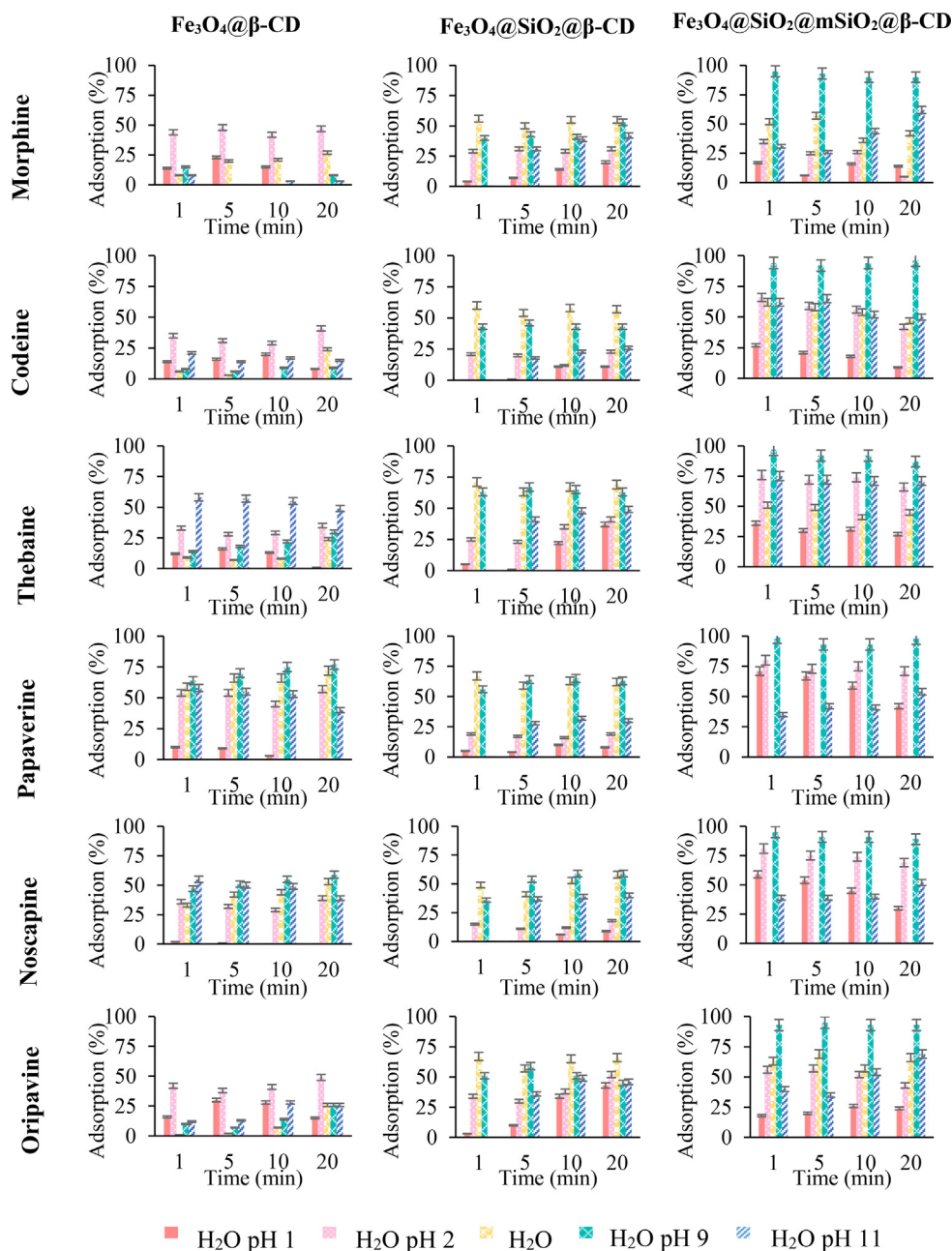
Fig. 5. Adsorption of morphine, codeine, thebaine, papaverine, noscapine and oripavine obtained at different times with the three magnetic materials. The adsorption (%) was calculated: 100 − (value of the supernatant divided by value of the simulated sample).

3.4. Adsorption kinetic and isotherm experiments with mesostructured silica magnetic composite $Fe_3O_4@SiO_2@mSiO_2@\beta$-CD

In Fig. S3a is shown the adsorption isotherms of the six OAs. This shows how the amount of OAs adsorbed increases over the equilibrium time until it reaches a maximum and remains constant. In addition, Langmuir [35] and Freundlich [36] models were applied (equations 5 and 6 in Table S2). As shown in Fig. S4b, the R^2 obtained by Langmuir model was more closed to 1 than by Freundlich model. So, adsorption occurred monolayer on the uniform surface of material [47].

As shown in Fig. S3b the adsorption kinetics is fast for all OAs. It is due because in the first minute all adsorption is obtained and kept it constant. The adsorption kinetics were determined by Lagergren's pseudo-first-order [32], pseudo-second-order [33] and intraparticle diffusion kinetic models (equations 2–4 in Table S3). The results were compiled in Fig. S4a and the important data of the three-kinetics model were shown in Table S4. The kinetics does not comply with pseudo-first order. The

linear regression coefficients (R^2) were closed to 1 in the pseudo-second-order kinetics, indicating a chemical adsorption mechanism [47]. In addition, all the analytes showed a low intraparticular diffusion rate with Kp values of −0.008 and −0.011 mg/g min².

3.5. Optimization of MSPE conditions with mesostructured silica magnetic composite $Fe_3O_4@SiO_2@mSiO_2@\beta$-CD

Once the good adsorption capacity of the material has been verified, with the adsorption solvent (water at pH 9) and the equilibrium time (1 min), the desorption and the amount of material required to obtain good recovery values were optimized. For this purpose, 2 mL of 1 mg/L of each of the six analytes in water with pH 9 was used. The desorption eluent was evaluated with 1 min of elution time by ultrasound. The desorption eluent evaluated were water with ethanol, acetonitrile, or methanol at 50% all of them with 1% of formic acid. As shown in Fig. S5, the mixture of 50% water/ethanol with 1% formic acid showed the

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Table 2
Validation parameters of the proposed methodology for the quantification of six OAs in poppy seed infusions.

Analytes	Linear range (mg/L)	Matrix-matched calibration (R^2) [a]	MDL (µg/L) [b]	MQL (µg/L) [c]	ME [d]	Accuracy [e]		Precision	
						Recovery (% ± SD)	Mean recovery (% ± SD)	Intra-day Precision (RSD%)	Inter-day Precision (RSD%)
Morphine	0.005–1	$y = 7.3 \times 10^7 x + 1.2 \times 10^6$ (0.999)	0.3	1.3	5.8	91 ± 4^L 95 ± 3^M 90 ± 3^H	92 ± 3	4^L 3^M 3^H	8^L 4^M 9^H
Codeine	0.005–1	$y = 1.4 \times 10^7 x + 1.1 \times 10^6$ (0.999)	0.3	1.3	−2.1	96 ± 9^L 97 ± 2^M 88 ± 4^H	94 ± 5	10^L 2^M 4^H	11^L 5^M 11^H
Thebaine	0.001–1	$y = 1.3 \times 10^9 x + 1.1 \times 10^7$ (0.996)	0.1	0.3	2.5	93 ± 7^L 99 ± 6^M 90 ± 4^H	94 ± 6	7^L 6^M 4^H	8^L 7^M 10^H
Papaverine	0.001–1	$y = 2.7 \times 10^9 x - 2.6 \times 10^6$ (0.996)	0.1	0.3	1.9	86 ± 3^L 88 ± 2^M 94 ± 5^H	89 ± 3	3^L 2^M 5^H	10^L 9^M 6^H
Noscapine	0.001–1	$y = 2.9 \times 10^9 x + 4.9 \times 10^7$ (0.997)	0.1	0.3	4.7	83 ± 4^L 91 ± 2^M 93 ± 1^H	89 ± 2	4^L 2^M 1^H	11^L 8^M 9^H
Oripavine	0.005–1	$y = 1.1 \times 10^8 x + 1.8 \times 10^5$ (0.999)	1.5	5	1.3	83 ± 6^L 90 ± 3^M 93 ± 2^H	89 ± 4	6^L 3^M 2^H	9^L 8^M 7^H

[a] The calibration line is in the units: mg/L;.

[b] MDL: method detection limit;.

[c] MQL: method quantification limit;.

[d] ME: matrix effect (purified matrix slope / solvent slope −1) × 100;.

[e] Accuracy and precision were obtained by spiking infusion with poppy seeds at three concentration levels: low (L, 0.2 mg/L), medium (M, 0.4 mg/L) and high (H, 0.8 mg/L); Accuracy: $n = 6$; Intra-day precision: $n = 6$, 1 day; Inter-day precision: $n = 9$, 3 days.

highest recovery percentages. It was 94% for morphine, 98% codeine, 97% thebaine, 85% papaverine, 90% noscapine and 91% oripavine.

In addition, different amounts of magnetic composite (5, 10, 25 and 50 mg) were evaluated to determine if lower amounts of adsorbent material could be used. As shown in Fig. S6, as the amount of material decreased the recovery values decreased, so the optimum amount of material to obtain good recovery values was 50 mg.

3.6. Method validation

The analytical methodology proposed for the quantification of six OAs in poppy seed infusions was carried out in terms of linearity, limits of detection and quantification of the method, matrix effect, accuracy, precision inter and intra-day and selectivity. The validation results are shown in Table 2. Calibration lines between 0.005 or 0.001 and 1 mg/L with adequate R^2 between 0.996 and 0.999 were obtained and the deviation of the back-calculated concentrations of the calibration standards from the true concentrations in the matrix calibration lines were between −0.3 and 10.6%. Therefore, these results demonstrated the good linearity of the method, which claims good linearity when the deviation of back calculated concentrations is $\leq \pm 20\%$ [39]. In addition, the matrix effect was calculated by comparing the slopes of the matrix calibration curves after the proposed method with the solvent calibration curves. No matrix effect was observed for poppy seed infusions after performing the proposed method with a purification step by MSPE with $Fe_3O_4@SiO_2@mSiO_2$, as the matrix effect values were between −2.1 and 5.8% (Table 2), so for all analytes the matrix effect was < +/- 20%. This means according to the validation guideline [39] that the developed purification procedure was able to eliminate all possible matrix effects for the six target analytes in the poppy seed infusion. The MDL and MQL values were sufficiently low to be able to detect and quantify the concentration of OAs in infusions originating from seeds with the quantity established in the legislation on poppy seeds (20 mg/kg) prepared with the ISO protocol [29], being 1.5 and 5 µg/L for oripavine, 0.3 and 1.3 µg/L for morphine and codeine, 0.1 and 0.3 µg/L for thebaine, papaverine and noscapine, respectively (Table 2). The limits obtained were lower than those achieved by other researchers such as Li et al.

which were between 10 and 50 µg/L using gas chromatography coupled to mass spectrometry (GC–MS) [21].

Accuracy and precision were evaluated at three different concentration levels, low (0.2 mg/L), medium (0.4 mg/L) and high (0.8 mg/L). Good recovery values were shown at all validation levels for all analytes, showing values between 83 and 99% (Table 2), which complies with the appropriate values established in the validation guidelines of between 70 and 120% (Table 2). In addition, satisfactory results were obtained for intra-day and inter-day precision at the three concentration levels, since the RSD values were lower than 10 and 11% respectively (Table 2), which complies with what is established in the validation guides, since they are lower than 20%. In addition, a good selectivity of the method was obtained, since when comparing the chromatograms of the extracted ions obtained for each of the OAs in a standard solution with those obtained in the sample, it was obtained that the variation of the t_R was ≤ 0.1 min and the ion ratios of the sample extracts were within $\pm 30\%$ (relative abundance) of the mean of the standards for each analyte, as established by the validation guidelines [39]. Regarding the results of the validation parameters for poppy seed infusion samples, in the present work, besides having successful recovery values and inter- and intra-day precision values, good quantification limits were achieved (between 0.3 and 5 µg/L),

3.7. Comparison of the proposed methodology with previously methods for OAs

One of the most important advantages of the methodology proposed in this work is that the adsorbent material used is a magnetic material, which allows the developed methodology to be faster and simpler than the classical solid phase extraction (SPE) used in other works to quantify OAs [38]. Even the developed methodology is faster and simpler compared to other previously published MSPE methodologies. For example, the work of Li et al. (2021) although they only used 5 mg of material, required 20 min of adsorption, a centrifugation to accelerate the subsequent separation with the magnet and 10 min of desorption, after which they had to centrifuge again to remove the material with the magnet [21]. In contrast, the methodology proposed in the present

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Table 3

Occurrence of OAs in poppy seed infusions analysed (n = 3).

Code sample[a]	OAs in poppy seed infusions (mean, mg/L ± standard deviation)					
	Morphine	Codeine	Thebaine	Papaverine	Noscapine	Oripavine
PS-I-01	1.7 ± 0.5	0.6 ± 0.2	1.1 ± 0.1	<MDL	<MDL	1.4 ± 0.2
PS-I-02	0.11±0.04	0.003±0.02	0.4 ± 0.1	0.01±0.01	0.06±0.02	<MDL
PS-I-03	0.10±0.05	0.008±0.02	<MDL	<MQL	0.03±0.05	<MDL
PS-I-04	0.01±0.01	<MQL	<MQL	<MQL	<MQL	<MDL

[a] PS-I: poppy seed infusion; <MDL: lower than method detection limit; <MQL: lower than method quantification limit but higher than method detection limit. PS-I-01 was diluted 1:3 to quantify it within the calibration line and then recalculated to obtain the final concentration.

work required only 1 min adsorption and 1 min desorption without any centrifugation step, which meant that it was a much faster and simpler methodology. In addition, the most remarkable aspect of the method proposed in this work is the use of β-CD as a ligand, which is natural and more environmentally friendly than others [48]. In the validation results, it has been possible to confirm its high effectiveness, showing very satisfactory recovery values. This is a great advantage with respect to the previous work in which the same material was used without this ligand [13]. This improvement is observed especially in the case of morphine and oripavine, rising from 46 to 52% to 92 and 89%, respectively.

All in all, the present methodology presented several advantages that make it very effective for the analysis of this family of natural toxins. However, a future challenge is the reuse of the material for several cycles [49]. In this way, the methodology could be more environmentally friendly by reducing the amount of waste and extending the lifetime of the material.

3.8. Reproducibility of mesostructured silica magnetic composite $Fe_3O_4@SiO_2@mSiO_2@\beta$-CD

An important parameter for adsorbent evaluation is the reproducibility of the material synthesis. That is, to ensure reproducible results during the MSPE procedure. For this purpose, the recoveries obtained with different batches of mesostructured silica magnetic composite $Fe_3O_4@SiO_2@mSiO_2@\beta$-CD were calculated. For this purpose, five batches were synthesized and evaluated at the medium validation level (0.4 mg/L). Table S5 shows that the RSDs were less than 9%, which indicated that the mesostructured silica magnetic composite with β-CD have acceptable reproducibility.

3.9. Application of the developed method to real samples of infusions with poppy seeds

The presence of OAs in poppy seed infusion from different poppy seed samples was determined. The areas obtained could have been interpolated on the solvent calibration line directly since the matrix effect was negligible [39]. However, to confirm the reliability of the proposed methodology, internal standards were used to correct the signals of matrix-matched calibration. Therefore, the obtained areas were corrected with the signals obtained from the internal standards, morphine-d3 was used to correct the signals of all except codeine which was corrected by codeine-d3. The corrected areas were interpolated to the respective calibration lines of the internal standard matrix-matched to calculate the OAs concentrations obtained in each of the infusions.

As shown in Table 3, all OAs were quantified in some of the poppy seed infusion samples analysed. Moreover, as can be seen, the calculated standard deviations of the three replicates were in some cases high. This is due to the external heterogeneous contamination suffered by the seeds that depends on multiple factors, such as plant variety, climate, harvesting conditions, amongst others [13,30,50]. Of the four poppy seed infusions analysed, two of them (PS-I-03 and PS-I-04) showed lower contents of all OAs, with morphine being the analyte with the highest concentration in both infusions 0.1 μg/L and 0.01 μg/L, respectively (Table 3). It should be noted that in sample PS-I-02 all analytes were quantified, except for oripavine which was below the MDL. In this PS-I-02 sample the OAs concentration was in general low, but for thebaine a significant amount (0.4 mg/L) was found (Table 3). This opium alkaloid is not legislated and therefore this poppy seed sample complies with current legislation. However, the importance of studying all opium alkaloids and not only morphine and codeine should be emphasized, since, as health authorities state, they can become even more toxic [22]. This has been observed in recent cases of intoxication due to consumption of poppy seed infusions in Australia, where seeds consumed were contaminated with high concentrations of thebaine [27]. On the other hand, sample PS-I-01 showed a higher OAs content, especially morphine (1.7 mg/L), codeine (0.6 mg/L) and thebaine (1.1 mg/L) (Table 3). This sample was diluted 1:3 to quantify it within the calibration line and then recalculated to obtain the final concentration. Therefore, the estimated amount of OAs in PS-01 poppy seeds will be 91 mg/kg, as morphine equivalents (morphine + 0.2 codeine), assuming a transfer rate of 100% of both analytes. It is well above the legislated limit established by the European Commission on December 2021 (Regulation (EU) 2021/2142) for poppy seeds of 20 mg/kg morphine equivalents [51]. It should be noted that the legislation states that if the seeds were commercialised before 1 July 2022, they can remain on the market until the best-before date is reached, so even if the maximum content is exceeded, they can still be marketed according is best-before date (see Table S1) [51].

In addition, the acute dose of morphine equivalents set by EFSA in 2018 is 10 μg/kg body weight. So, for a 20 kg child the acute dose would be 200 μg and for a 60 kg adult it would be 600 μg morphine equivalents. In the infusion with the highest amounts of OAs (PS-I-01), 182 μg of morphine equivalent were found, so for a child this would be close to the acute dose and an adult could drink three cups of this infusion. It is also a main problem because many packages do not give recommendations for use, and it is up to the consumer to take the amount of seeds they want in each infusion. In addition, it can be found in numerous internet forums that the preparation of infusions with very high quantities of seeds is recommended, as in the reported case of a man who consumes poppy seed tea with dependence, consuming up to 2 L of poppy seed tea per day, which requires about 4 kg of poppy seeds per day [21,52]. Therefore, the consumption of infusion from these seeds poses a health risk for both adults and children. This highlights the need to continue controlling this public health problem and the importance of developing analytical methods to analyse them to establish a legislation accordingly. In addition, an important aspect to note is that all four seed samples are labelled as poppy (*Papaver rhoeas* L.), which does not contain OAs in its latex and therefore its seeds cannot be contaminated. In this regard, the determination of OAs in their infusions confirms the mislabelling of the product as observed in previous studies [13]. Therefore, the need to correctly label the product and specify that they are opium poppy seeds (*P. somniferum* L.) and, therefore, must comply with the legislated maximum limit is claimed [51].

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4. Conclusions

A fast, efficient, and environmentally friendly method for the control of OAs poppy seed infusions has been developed. For this purpose, the synthesis of a mesostructured silica magnetic composite with β-CD ($Fe_3O_4@SiO_2@mSiO_2@\beta$-CD) and the magnetic solid-phase extraction (MSPE) procedure have been optimised for the quantification of six OAs in infusions with poppy seeds followed by liquid chromatography coupled to tandem mass detector (HPLC-MS/MS). This material achieved a fast (1 min adsorption and 1 min desorption) extraction of the analytes in a very efficient way (with successful recovery values between 89 and 94%). It was confirmed that the use of mesostructured silica layer confers higher adsorption capacities when compared by discontinuous adsorption studies with $Fe_3O_4@\beta$-CD and $Fe_3O_4@SiO_2@\beta$-CD. Furthermore, the efficacy of β-CD as an OAs-interacting ligand was confirmed when compared to the unfunctionalized material, being a more environmentally friendly alternative to other ligands used in the literature. Finally, the method was applied to the analysis of infusions with four different poppy seeds, showing in one of the seed samples using to make infusion a worrying concentration of OAs (91 mg/kg morphine equivalents) which imply a content in the seeds of at least four times that established in the current legislation (20 mg/kg).

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Data Availability

Data will be made available on request.

Acknowledgement

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sampre.2023.100056.

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