Modified ZSM-5 zeolite/Fe₂O₃ composite as sorbent for magnetic dispersive solid-phase microextraction of cadmium, mercury and lead from urine samples prior to inductively coupled plasma optical emission spectrometry†

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Abstract

The purpose of this work is to present a simple, fast, cost effective and environmental friendly magnetic dispersive solid-phase microextraction (MDSPME) method, employing a composite based on ZSM-5 zeolite decorated with iron oxide magnetic nanoparticles (i.e., ZSM-5/Fe₂O₃) as a valuable sorbent, for the simultaneous separation and preconcentration of cadmium (Cd), mercury (Hg) and lead (Pb) from urine samples for subsequent measurement by inductively coupled plasma optical emission spectrometry. The composite was first saturated with zinc and then modified with hexadecyltrimethylammonium bromide surfactant and sodium diethyldithiocarbamate trihydrate chelating agent. A two-step multivariate strategy, using Plackett-Burman and circumscribed central composite designs,

has been employed to optimize experimental parameters affecting MDSPME. The method has been evaluated under optimized extraction conditions (i.e., amount of sorbent, 50 mg; sample pH, 4.0; extraction time, 3 min; eluent solvent, nitric acid; eluent solvent volume, 432 μL ; eluent solvent concentration, 11.8 M and elution time, 2 min) using standard addition calibration. Calibration curves using standards between 0 to 100 $\mu\text{g L}^{-1}$ gave a good linearity with correlation coefficient values ranging from 0.998 to 0.9997 for Cd, from 0.998 to 0.9998 for Hg and from 0.9996 to 0.9999 for Pb (N=8). The repeatability of the method has been evaluated at 3 and 50 $\mu\text{g L}^{-1}$ obtaining coefficients of variation between 2 and 5% (n=6). Limits of detection values ranged from 0.15 to 0.20 $\mu\text{g L}^{-1}$ for Cd, from 0.42 to 0.73 $\mu\text{g L}^{-1}$ for Hg and from 0.23 to 0.39 $\mu\text{g L}^{-1}$ for Pb. These values satisfy the threshold limit (3.4 $\mu\text{g L}^{-1}$ for Cd, 10.0 $\mu\text{g L}^{-1}$ for Hg and 85.0 $\mu\text{g L}^{-1}$ for Pb) established by the Ministry of Labour and Social Affairs (Spain) for normal content of these metals in human urine and by the World Health Organization for normal mercury content in urine (i.e., 10 $\mu\text{g L}^{-1}$). Finally, three urine samples were used to assess the applicability of the developed procedure. Recovery values ranged between 87-107% showing negligible matrix effects.

Keywords: ZSM-5 zeolite, magnetic dispersive solid-phase microextraction, inductively coupled plasma optical emission spectrometry, cadmium, mercury, lead, urine samples.

1. Introduction

According to the World Health Organization (WHO), among 13 elements

of highest concern, Cd, Hg and Pb are considered as toxic and extremely dangerous even at trace levels, since they are not metabolized by the body and accumulate in soft tissues [1]. Heavy metals may enter the human body via food, water, air or absorption through the skin in agriculture, manufacturing, pharmaceutical, industrial or residential settings [2]. Exposure to these metals is known to cause a range of diseases that detrimental to human health. For example, Hg and Pb accumulation can cause higher rates of Parkinsonism, cognitive decline, depression, nausea, fatigue, developmental delays, neurological and movement disorders; and Cd toxicity is associated with high risk for osteoporosis, hepatic injury, hypertension, renal dysfunction and lung damage [3–5]. For this reason, the determination of these heavy metals in biological samples (e.g., urine) is extremely important in clinical medicine to assess the influence of the exposure to these toxic metals and prevent health effects. Nevertheless, due to the complexity of these matrices and the trace concentration of heavy metals it is necessary to employ sample pretreatment techniques.

Among the developed sample pretreatment techniques, solid-phase extraction (SPE) is the most often used technique and has been applied worldwide for trace enrichment (preconcentration), matrix simplification (sample clean-up) and medium exchange (transfer from the sample matrix to a different solvent) [6]. Briefly, SPE consists of columns packed with sorbent where analytes are retained on it. Then, the analytes are recovered by elution using a solvent prior to determination [6]. Different sorbents such as inorganic oxides (e.g., silica gel, zeolites) [7,8], polymeric materials [9], biosorbents (e.g., algae, bacteria) [10], among others, have been employed for preconcentration of toxic

metals in SPE. It should be noted that zeolites have been scarcely employed as sorbents in SPE for the preconcentration of heavy metals [8,11].

Zeolites are microporous crystalline aluminosilicates, which have a framework molecular structure composed of TO_4 tetrahedra ($\text{T} = \text{Si}, \text{Al}$) with O atoms connecting neighboring tetrahedral. The presence of Al atoms into the structure makes that the framework is negatively charged due to difference between the $(\text{AlO}_4)^{5-}$ and $(\text{SiO}_4)^{4-}$ tetrahedral. This negative charge is compensated by extraframework cations (e.g., alkaline and alkaline earth) [12–14]. Most of zeolites are natural products, but there are also synthetic zeolites and the number of synthetic zeolites is constantly increasing every year. Today, more than 200 different structural types of zeolites are known, and most of them are synthetic [12]. The most important properties are high surface area, excellent stability in chemical and thermal processes, catalytic action, high adsorption capacity and molecular selectivity and ion-exchange capacity, among others [14]. Both composition of zeolites, especially the Si/Al ratio [12], and structure of zeolites, which is described in terms of the size, geometry and connectivity/dimensionality of the pore space [15], determine their properties. Furthermore, zeolites possess advantages as their low cost of extraction, their availability in great volumes and they can be modified to get physical and chemical properties desired [14]. Regarding to this, in order to increase the heavy metal preconcentration capacity, the modification of the zeolites by different materials such as surfactants and chelating agents was reported [16–19]. The referred works employed conventional in-column SPE. However, it presents disadvantages such as the packing of columns and the time-consuming process of loading large volume of samples. For this reason,

dispersive solid-phase microextraction (DSPME) [20] was presented as an alternative to overcome most of the disadvantages. However, one of the main problems in DSPE is the separation of the sorbent from the sample since it becomes exhausting and time consuming, mostly due to the dispersion of the sorbent in the sample during the extraction process. Moreover, in some cases even centrifugation of the solution fails to complete the separation of the solid dispersed particles of the sorbent [21].

Recently, some publications have reported the use of magnetic materials as sorbents [22–24] due to their fast and easy separation. This SPE mode is named magnetic dispersive solid-phase microextraction (MDSPME). In MDSPME, magnetic sorbent is directly dispersed in the sample solutions and after extraction, sorbent is separated from sample by an external magnetic field [24]. Finally, analytes can be eluted using a proper solvent for further determination. In addition, magnetic sorbents unlike non-magnetic sorbents avoid time-consuming filtration or centrifugation steps for phase separation due to their easy manipulation. Different magnetic sorbents (e.g., ferrites, magnetic material coated with silica, alumina or polymer and materials decorated with magnetic nanoparticles) have been employed for preconcentration of toxic metals in samples such as water, food and urine, among others [22]. To our knowledge, only a publication has reported the use of zeolites modified with magnetic nanoparticles as sorbent for preconcentration of heavy metals in MDSPME [25]. In this study, a magnetic NaY zeolite modified with 2-(3,4-dihydroxyphenyl)-1,3-dithiane was employed to determine cadmium(II) and copper(II) in water and soil samples [25]. However, there is still much to investigate in the field of magnetic zeolites.

Therefore, the purpose of this work is to present a simple MDSPME method, employing a composite based on ZSM-5 zeolite decorated with iron oxide magnetic nanoparticles (i.e., ZSM-5/Fe₂O₃) as a valuable sorbent, for the simultaneous separation and preconcentration of Cd, Hg and Pb in urine samples for subsequent measurement by inductively coupled plasma optical emission spectrometry (ICP OES). To our knowledge, this is the first report of an analytical method in which a zeolite-based MDSPME is employed to determine Cd, Hg and Pb in urine samples. Determination of the optimum conditions for MDSPME was carried out using an experimental design strategy. Finally, method was validated and applied to real urine samples.

2. Experimental

2.1. Reagents

Deionized water was used throughout the experimental work (resistivity higher than 18 MΩ cm). Stock standard solutions of Cd, Hg and Pb (1000 mg L⁻¹ in 2% nitric acid (HNO₃)) and zinc (Zn) (10000 mg L⁻¹ in 2% HNO₃) were obtained from High-Purity Standards (Charleston, SC, USA). Cd, Hg and Pb working solutions (5-100 µg L⁻¹) were prepared by proper dilution of each stock standard solution with deionized water. In addition, a working solution of Zn (200 mg L⁻¹) was prepared by diluting stock standard solution. All working solutions were acidified by adding high-purity HNO₃ (65% (w/w)) (Merck, Darmstadt, Germany) up to 1% (w/v).

ZSM-5 zeolite (CBV 3024E, SiO₂/Al₂O₃ mole ratio=30) in the ammonium nominal cation form was obtained from Zeolist International (Conshohocken, PA, USA). FeCl₃·6H₂O and FeSO₄·7H₂O reactive grade were obtained from

Sigma-Aldrich (St. Louis, MO, USA) and NaOH (97% purity, pellets) from Scharlau Chemie (Sentmenat, Spain). Hexadecyltrimethylammonium bromide (HDTMABr, $\geq 99\%$ purity) from Sigma-Aldrich was employed for preparing a 0.5% (w/v) solution in deionized water/ethanol mixture (50/50, v/v), being HPLC-grade ethanol absolute from Scharlau Chemie. Sodium diethyldithiocarbamate trihydrate (DDTC, $\geq 99\%$ purity) from Sigma-Aldrich was employed for preparing a 0.5% (w/v) solution in deionized water.

H_3PO_4 (85% purity) from Scharlau Chemie and KH_2PO_4 and K_2HPO_4 pro-analysis from Merck were employed for preparing buffer solutions. Sodium acetate anhydrous from Panreac (Barcelona, Spain) and HPLC-grade acetic acid glacial from Scharlau Chemie were employed for buffering urine samples before being analyzed. Sodium chloride (99% purity) from Scharlau Chemie was employed to adjust the ionic strength of urine samples before being analyzed.

2.2. Instrumentation

An Agilent 720-ES inductively coupled plasma optical emission spectrometer (Melbourne, Australia) was employed for all measurements. The operational parameters of the ICP OES used in this work are shown in Table S1. The instrumental conditions were optimized achieving the maximum signal intensity for each analyte using a standard solution containing $100 \mu\text{g L}^{-1}$ of Cd, Hg and Pb.

It is important to note that for Cd, the MDSPME optimization was performed with analytical emission line Cd II 226.502 nm, while the validation of the method and the analysis of the real urine sample was performed with Cd II

228.802 nm. This is due to the fact that an iron spectral interference (i.e., Fe I 226.505 nm) was observed in the determination of Cd II 226.502 nm at the lowest standard concentration (i.e., 5 $\mu\text{g L}^{-1}$ of Cd). In the MDSPME optimization a high standard concentration (i.e., 100 $\mu\text{g L}^{-1}$ of Cd) was used and the spectral interference was negligible. Although emission line Cd II 226.502 nm is more sensitive than emission line Cd II 228.802 nm, emission line Cd II 228.802 nm is used because it eliminates Fe spectral interference. The presence of Fe in the MDSPME procedure is due to the Fe of the magnetic component of the composite (ZSM-5/ Fe_2O_3).

X-ray photoelectron spectroscopy (XPS) was used for the chemical characterization of iron oxide in the ZSM-5/ Fe_2O_3 composite. XPS measurements were performed with an automatic K-Alpha spectrometer from Thermo-Scientific (Waltham, MA, USA). The spectra were recorded with pass energy of 50 eV, X-ray spot size of 400 m, and step size of 0.1 eV [26].

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy technique was used for the chemical characterization of the different modified ZSM-5/ Fe_2O_3 composites. ATR-FTIR measurements were performed with a FT/IR-4700 fourier transform infrared spectrometer from JASCO (Tokyo, Japan) equipped with light source of mid infrared, a diamond crystal, a beam splitter of KBr encapsulated in germanium and a DLaTGS detector. The spectra of the samples were recorded in the range of 500-4000 cm^{-1} with a resolution of 4 cm^{-1} . It should be mentioned that the region between 1900 and 2400 cm^{-1} was deleted since the diamond crystal absorbs in this region. Each spectrum was collected from 128 scans in the transmittance mode.

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) techniques were used, respectively, to study the surface morphology and particles size of the ZSM-5 and modified ZSM-5 composites. SEM images were performed with a S3000N Scanning electron microscope from Hitachi (Japan) and TEM images were performed with JEM-2010 transmission electron microscope from JEOL (Japan).

2.3. Synthesis of ZSM-5/Fe₂O₃ composite

Synthesis of ZSM-5/Fe₂O₃ composite, described in detail in our previous work [26], was performed in a ZSM-5/Fe₂O₃ weight ratio of 3:1. This weight ratio was chosen to avoid a decrease of available surface area and to obtain an easy manipulation of the composite under a magnetic field [26,27]. The composite was prepared from a suspension of 3 g of ZSM-5 zeolite in 250 mL solution with FeCl₃·6H₂O (2.335 g) and FeSO₄·7H₂O (1.201 g). Then, 15 mL of NaOH 5 M was added drop wise to precipitate the iron oxide. For 2 h the mixture was stirred at room temperature. The separation of the composite from the solution was carried out using a Ni-coated neodymium magnet (S-45-30-N), N45 grade, dimensions 45 x 30 mm from Supermagnete (Gottmadingen, Germany) as an external magnetic field. The obtained composite was washed with deionized water until getting clear washing waters at neutral pH. Lastly, the composite was dried at 110 °C overnight.

2.4. Modification of ZSM-5/Fe₂O₃ composite

The modified ZSM-5/Fe₂O₃ composite was prepared by the following procedure: firstly, NH₄⁺-form of ZSM-5/Fe₂O₃ composite was calcinated at 380

°C for 2 h to prepare H⁺-form of ZSM-5/Fe₂O₃ composite [8,17,28], since this form of zeolite had much higher ion-exchange capacity than NH₄⁺-form [28]. Then, pores of H⁺-form of ZSM-5/Fe₂O₃ composite was saturated with Zn by stirring 10 g of ZSM-5/Fe₂O₃ composite with 400 mL of Zn solution (200 mg L⁻¹) for 2 h to obtain a uniform substrate and prevent the entrance of analytes into pores of ZSM-5/Fe₂O₃ composite so that in MDSPME, adsorption of metal takes place at surface [17]. This was followed by 3 rinses with HNO₃ 4 M and rinses with deionized water until neutral pH washing waters were obtained, and finally the composite was dried at 120 °C for 3 h. The resulting solid was zinc-saturated ZSM-5/Fe₂O₃ composite (Zn-ZSM-5/Fe₂O₃). Secondly, surfactant modification was performed by stirring 10 g of Zn-ZSM-5/Fe₂O₃ composite with 150 mL of the HDTMABr solution (0.5%, w/v) for 24 h at room temperature. Then, HDTMA-modified zinc-saturated ZSM-5/Fe₂O₃ composite (HDTMA-Zn-ZSM-5/Fe₂O₃) was filtered in a Büchner funnel connected to a vacuum pump, washed with deionized water and dried at 120 °C for 3 h. Finally, 10 g of HDTMA-Zn-ZSM-5/Fe₂O₃ composite was added to 300 mL of the DDTC solution (0.5%, w/v), stirred for 24 h at room temperature, filtrated by vacuum, cleaned with deionized water and dried at 120 °C for 3 h. The resulting solid was DDTC-HDTMA-modified zinc-saturated ZSM-5/Fe₂O₃ composite (DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃).

The modification of Zn-ZSM-5/Fe₂O₃ composite with surfactant and chelating agent is schematically shown in Fig. S1(a) [29]. This modification was carried out by the following procedure: first, surfactant is sorbed on zeolite surface by cation exchange [30], but if the surfactant concentration in solution exceeds the critical micellar concentration (CMC), as in this case, then the

hydrophobic tails of the surfactant molecules associate to form a bilayer, which has a counterion associated in solution [31]. Then chelating agent is sorbed by anion exchange since the surfactant has a counterion associated in solution.

2.5. Magnetic dispersive solid-phase microextraction

Firstly, 50 mg of DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃ composite was added to a 22 mL glass vial. Then, 20 mL of sample solution adjusted to pH 4.0 was transferred and the mixture was handily shaken vigorously for 3 min. After extraction, to remove easily the aqueous phase using a glass pipette, the separation of the composite from the solution was carried out using a Ni-coated neodymium magnet as an external magnetic field, avoiding time-consuming filtration or centrifugation steps for phases separation. Then, the adsorbed analytes on the composite were eluted with 432 μ L of HNO₃ 11.8 M using an ultrasonic bath for 2 min. Finally, eluate was separated from the composite using the neodymium magnet, withdrawn with a syringe, filtered with 0.45 μ m pore size polyvinylidene fluoride (PVDF) filters (Millipore, Madrid, Spain) and transferred to a vial for further determination by ICP OES.

2.6. MDSPME Optimization

Determination of the optimum conditions for MDSPME was carried out using a multivariate approach. Firstly, a Plackett-Burman design was used as a screening study to identify the significant factors. Then, a circumscribed central composite design (CCCD) was employed for optimizing the significant ones. The statistical software NEMRODW[®] ("New Efficient Methodology for Research using Optimal Design") from LPRAI (Marseille, France) was used to build the

matrices of experiments and evaluate the experimental results. A 20 mL of an aqueous standard with $100 \mu\text{g L}^{-1}$ of Cd, Hg and Pb was used for all MDSPME experiments. Analytical signal of Cd (i.e., Cd II 226.502 nm), Hg (i.e., Hg II 194.164 nm) and Pb (i.e., Pb II 220.253 nm) obtained with ICP OES were individually used as response functions for optimization.

2.7. Evaluation of the method

Under optimum MDSPME conditions, the analytical figures of merit of the proposed MDSPME-ICP OES methodology were evaluated. Preliminary experiments showed that there were negative matrix effects, so standard addition calibration was employed to compensate matrix effects in the urine sample analysis. The calibration curves were constructed at eight concentration levels of Cd, Hg and Pb from 0 to $100 \mu\text{g L}^{-1}$. The standard addition calibration was carried out by the following procedure: firstly, eight aliquots of 24.5 mL of urine samples were spiked with 0, 25, 50, 100, 175, 250, 375 and 500 μL of a 5 mg L^{-1} of Cd, Hg and Pb aqueous standard solution and secondly, a proper volume of deionized water (HNO_3 1% (w/v)) was added to each aliquot, reaching a final volume of 25 mL. Finally, 20 mL of each aliquot was transferred to vial glass to perform the MDSPME procedure.

2.8. Urine samples

Three urine samples were provided by healthy human volunteers, who were unexposed to Cd, Hg and Pb. These three samples were collected in sterilized containers and stored at 4°C . Before using the urine samples, these were adjusted to pH 4.0 with a buffer solution of acetic acid/sodium acetate and

only few droplets were added in urine samples, therefore the pH adjustment does not produce a significant dilution. Samples were filtered with 0.45 μm pore size nylon filters in order to remove suspended particles.

Urine samples were initially analyzed and its Cd, Hg and Pb content was detected with the proposed method using standard addition calibration (Section 2.7). Consequently, urine samples were spiked at 5, 15 and 30 $\mu\text{g L}^{-1}$ levels of Cd, Hg and Pb and analyzed to assess matrix effects (i.e., trueness).

3. Results and discussion

3.1. Characterization studies of modified ZSM-5/ Fe_2O_3 composite

3.1.1. XPS study

A high-resolution XPS spectra in the Fe 2p region, which are shown in Fig. S2, were used to investigate the oxidation state of Fe in ZSM-5/ Fe_2O_3 composite and calcined ZSM-5/ Fe_2O_3 composite since visually the calcined composite had a brown color more obscure than the non-calcined composite. Spectra of ZSM-5/ Fe_2O_3 composite (blue spectrum) and calcined ZSM-5/ Fe_2O_3 composite (red spectrum) show a main Fe $2p_{3/2}$ peak detected at 711 eV approximately, which confirms the presence of Fe_2O_3 . The Fe $2p_{3/2}$ peak has satellite peaks associated. The satellite peak of Fe $2p_{3/2}$ for Fe_2O_3 is located approximately 8 eV higher than the main Fe $2p_{3/2}$ peak, being located at 719 eV. This is also characteristic of Fe^{3+} [32,33]. Therefore, after the comparison of spectra obtained with database spectra [32], it can be concluded that Fe is in its highest oxidation state (Fe^{3+}) forming Fe_2O_3 .

3.1.2. ATR-FTIR study

ATR-FTIR spectra, which are shown in Fig. 1, were used to investigate the loading of HDTMABr surfactant and DDTC chelating agent onto surface of ZSM-5/Fe₂O₃ composite. In spectrum of Zn-ZSM-5/Fe₂O₃ composite (Fig. 1(a)), the peak located at 1629 cm⁻¹ is due to the bending vibration mode of surface hydroxyl groups, while the peak located at 3411 cm⁻¹ belongs to stretching vibration mode [29].

Comparison of spectra of Zn-ZSM-5/Fe₂O₃ composite (Fig. 1(a)) with HDTMA-Zn-ZSM-5/Fe₂O₃ composite (Fig. 1(b)) shows that in the latter spectrum appears new characteristic lines of HDTMABr surfactant. These peaks are located at 1470, 2853 and 2922 cm⁻¹, which are related to N-C, C-C, and C-H vibrations of HDTMABr, respectively [29]. However, comparison of the spectrum of HDTMA-Zn-ZSM-5/Fe₂O₃ composite (Fig. 1(b)) with the spectrum of DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃ composite (Fig. 1(c)) does not show characteristic peaks of DDTC chelating agent. This fact could be due to characteristic vibration modes of DDTC are weak in the studied range. Based on ATR-FTIR results, it can be concluded that HDTMABr was successfully immobilized onto the surface of ZSM-5/Fe₂O₃ composite.

3.1.3. SEM and TEM study

SEM and TEM images of composites are shown in Fig. S3. Images 'a' and 'b' present SEM pictures of ZSM-5 and DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃, respectively. The surface of DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃ presents aggregates larger than ZSM-5 composite. Images 'c' and 'd' present TEM pictures of ZSM-5 and DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃, respectively. TEM images show there were Fe₂O₃ particles and HDTMA and DDTC molecules on

the surface of ZSM-5.

3.2. Preliminary experiments

3.2.1. Elution solvent nature

Based on previous experiences [17,34], a volume of 500 μL of HCl 1 M and of HNO_3 2 M were tested for analyte elution from the sorbent. HNO_3 was selected as eluent because it performed better elution than the other solvent evaluated (data not shown).

3.2.2. Extraction efficiency of modified composites

The effect of the different modified ZSM-5/ Fe_2O_3 composites for the extraction of Cd, Hg and Pb was studied in preliminary experiments both in water and in urine (data showed in Fig. 2(a) and Fig 2(b), respectively). ZSM-5/ Fe_2O_3 composite, Zn-ZSM-5/ Fe_2O_3 composite and DDTC-HDTMA-Zn-ZSM-5/ Fe_2O_3 composite were used as sorbents. Firstly, extraction experiments were carried out by shaking 50 mg of ZSM-5/ Fe_2O_3 , Zn-ZSM-5/ Fe_2O_3 or DDTC-HDTMA-Zn-ZSM-5/ Fe_2O_3 in 20 mL of an aqueous standard with 50 $\mu\text{g L}^{-1}$ of Cd, Hg and Pb for 3 min. After extraction, the aqueous standard was removed and metals were eluted with 500 μL of HNO_3 10 M using an ultrasonic bath for 2 min. For ZSM-5/ Fe_2O_3 and Zn-ZSM-5/ Fe_2O_3 composites, metals were retained by the composite through cationic exchange, while for DDTC-HDTMA-Zn-ZSM-5/ Fe_2O_3 metals were mainly retained by the complex formation mechanism graphically shown in Fig. S1(b). For all analytes, there were significant differences between ZSM-5/ Fe_2O_3 and Zn-ZSM-5/ Fe_2O_3 composites, obtaining better results for ZSM-5/ Fe_2O_3 in the case of Cd and Hg and for Zn-ZSM-

5/Fe₂O₃ in the case of Pb. A possible explanation was that in Zn-ZSM-5/Fe₂O₃ composite, Zn could block active sites where Cd and Hg could be retained. However, this did not happen for Pb. This hypothesis was contrasted by quantifying the analyte content in the supernatant (remaining in the sample after extraction) (see Fig. S4(a)). In the case the Zn-ZSM-5/Fe₂O₃ composite, the content of Cd and Hg in the supernatant was higher than in the case of ZSM-5/Fe₂O₃ composite (i.e., for Cd, 77% and 31% of initial concentration, respectively, and for Hg, 62% and 26% of initial concentration, respectively). However, the content of Pb in the supernatant was similar for both composites. This was because all the lead content present in the solution was retained in both sorbents, concluding that there were no differences in the sorption step. However, in Fig. 2(a), the results showed that there were significant differences between ZSM-5/Fe₂O₃ and Zn-ZSM-5/Fe₂O₃ composites. In order to explain this, it should be borne in mind that extraction process is the combination of two steps: (i) sorption and (ii) elution. A possible explanation was that in Zn-ZSM-5/Fe₂O₃ composite, Zn could block internal sites where Pb could be retained, and therefore if Pb was retained in more external sites, elution step is more efficient. Secondly, extraction experiments were carried out by shaking 50 mg of ZSM-5/Fe₂O₃, Zn-ZSM-5/Fe₂O₃ or DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃ in 20 mL of a urine sample spiked with 50 µg L⁻¹ of Cd, Hg and Pb for 3 min. After the extraction, the urine sample was removed and metals were eluted with 500 µL of HNO₃ 10 M using an ultrasonic bath for 2 min. DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃ composite demonstrated the best capacity for the three analytes investigated. For Pb, there were not significant differences between the composites (Fig. 2(b)). However, for Hg there were significant differences

among composites. For ZSM-5/Fe₂O₃ and Zn-ZSM-5/Fe₂O₃ composites, the extraction of Hg in urine samples was not performed. A possible explanation was that urine contains certain compounds (i.e., proteins and large biomolecules) that act as chelating agents and could, therefore, form complexes with the studied analytes [35], as is the case of Hg. As in the previous experiment with aqueous standards, this hypothesis was contrasted by quantifying the analyte content in the supernatant (see Fig. S4(b)). For ZSM-5/Fe₂O₃ and Zn-ZSM-5/Fe₂O₃ composites the Hg content in supernatant was higher than for DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃ composite (i.e., for ZSM-5/Fe₂O₃ and Zn-ZSM-5/Fe₂O₃, it was 75% of Hg initial concentration of the spiked urine sample and for DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃, it was 10% of initial concentration). Therefore, a quantity of chelating agent was necessary on the surface of the zeolite to compete with the natural complexing agents present in the urine, forming complexes with the Hg. Consequently, DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃ composite was chosen as sorbent and it was evaluated at three different DDTC concentrations (0.05, 0.1 and 0.5 % (w/v)) during the modification of HDTMA-Zn-ZSM-5/Fe₂O₃. Results (see Fig. S5) of the analyte content in the supernatant showed that the adsorbed metal amount by the DDTC depended significantly on the DDTC percentage immobilized on the surface of ZSM-5/Fe₂O₃ composite, so we could conclude that DDTC was successfully immobilized onto the surface of ZSM-5/Fe₂O₃ composite. However, for Hg and Pb, in the case of DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃ composite with 0.5 % (w/v) of DDTC, analyte content in the supernatant was negligible (i.e., similar to blank). In the case of Cd, results of DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃ composite with 0.5 % (w/v) showed a high content of Cd in the supernatant (i.e.,

72% to initial content). It should be noted that the content of Cd in supernatant was higher for ZSM-5/Fe₂O₃ and Zn-ZSM-5/Fe₂O₃ composites than for DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃ (see Fig. S4(b)) (i.e., 80% and 98% of initial concentration, respectively, against 72%). The first hypothesis is that the Cd was also retained by natural complexing agents, however this was rejected because the signal obtained in urine for ZSM-5/Fe₂O₃ and DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃ was similar (i.e., 12199 cps and 11270 cps, respectively in Fig. 2(b)), therefore there was no improvement with a sorbent with DDTC. The second hypothesis is that in the urine there are cations (i.e., calcium (Ca²⁺) or magnesium (Mg²⁺)) that could block active sites on the surface of the composite, forming complexes with the DDTC. To verify that in our case the metal interference was not the cause of the matrix effect, DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃ composite with 0.75 % (w/v) of DDTC was tested and non-significant differences were found with respect DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃ composite with 0.5 % (w/v) of DDTC (data not shown). The third hypothesis was that ionic strength (% NaCl, w/v) affected the extraction process. The results (Fig. S6) show that the ionic strength had a negative influence on extraction for Cd (i.e., the signal of Cd decreased 90% with respect to the signal of an aqueous standard, which confirmed that the matrix effect was mainly given by the ionic strength and not by the cations present in the sample. Taking into account all that discussed above, DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃ composite with 0.5 % (w/v) of DDTC was chosen as sorbent. Finally, to study matrix effects, data obtained for the DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃ composite (Fig. 2(a) and Fig. 2(b)) were compared in Fig. S7. Data show that on the one hand, a noticeable negative matrix effect was found for Cd (i.e., the signal of Cd

decreased 44% with respect to the signal of an aqueous standard) whereas on the other hand, matrix effects were not significant for Hg and Pb.

3.3. MDSPME optimization

3.3.1. Screening study

Screening experiments are usually performed in the early stages of development of a method when many of the factors initially considered likely have little or no effect on the response. Therefore, screening designs are experiments in which many factors are considered and the objective is to identify those factors (if any) that have large effects. Plackett-Burman is a two-level fractional factorial design for studying $k=N-1$ factors in N runs, where N is a multiple of 4 [36]. This design assumes that the interactions between factors can be ignored, therefore it calculates main effects with a reduced number of experiments.

In present work, a saturated Plackett-Burman design was used to evaluate different factors. Based on previous experience the real factors investigated were: amount of sorbent, sample pH, extraction time, eluent solvent volume, eluent concentration and elution time. The considered experimental factors and levels are showed in Table S2. A matrix of experiments (Table S3) was constructed, including six real factors (F1-F6) and five fictitious factors or dummies (F7-F11) studied in twelve experiments. The five dummies included in the design were used to assess the variability of the procedure throughout experiments [37]. The twelve experiments were carried out in random order generated by the software program.

The data obtained were evaluated by ANOVA and the results are showed with the Pareto charts (Fig. S8). These Pareto charts contain a bar for each factor and the length of each bar is proportional to the relative influence of that factor in the MDSPME procedure. In addition, those bars to the right indicate a positive effect in the response when increasing the value of the factor from the lower to the upper level. In contrast, those bars to the left indicate a negative effect. Moreover, those bars that exceed reference vertical lines (dashed lines) can be considered significant with 95% probability. As Plackett-Burman design was saturated, dashed lines were calculated using the Eq. (1) [36,37]:

$$t_{(0.05; 5)} \cdot (SE) = t_{(0.05; 5)} \cdot \sqrt{\frac{\sum E_{dummy}^2}{n_{dummy}}} \quad (1)$$

where t is the tabulated t -value with 5 degree of freedom at a significance level of 95% probability, E_{dummy} is the coefficient of a dummy factor and n_{dummy} is the number of dummies to evaluate the standard error (SE).

According to Fig. S8, the significant factors were: for Cd (Fig. S8(a)), eluent solvent volume; for Hg (Fig. S8(b)), amount of sorbent, sample pH, extraction time and eluent solvent concentration; and for Pb (Fig. S8(c)), sample pH and eluent solvent volume.

The amount of sorbent presents a positive effect for all analytes since probably in the lowest level the amount of sorbent is not enough to extract the metals. In respect of sample pH, it is observe that the sign is not the same for all analytes (Cd presents a positive effect while Hg and Pb present a negative effect). It could be related with the species formation. At pH 8, the species $Pb(HPO_4)$ and $Hg(HPO_4)$ can be formed. In addition, the product constants (K_{ps})

for $\text{Cd}(\text{OH})_2$, $\text{Hg}(\text{OH})_2$ and $\text{Pb}(\text{OH})_2$ are $7.2 \cdot 10^{-15}$, $3.6 \cdot 10^{-26}$ and $1.4 \cdot 10^{-20}$, respectively, so, at pH 8, $\text{Hg}(\text{OH})_2$ and $\text{Pb}(\text{OH})_2$ are less soluble than $\text{Cd}(\text{OH})_2$. Therefore, for these reasons sample pH for Hg and Pb present a negative effect. Extraction time presents a negative effect for Hg and Pb and positive effect for Cd. The negative effect could be because if the interaction between the modifier agents with the zeolite is weak, the higher time of agitation, the greater the loss of the functional groups of the sorbent and therefore, the concentration of analyte that remains in the supernatant is high. And the positive effect for Cd is probably due to complex of Cd needs longer time to form. The eluent solvent volume presents a negative effect for all analytes since when the smaller the volume of eluent solvent the higher analyte concentration in the eluate. The eluent solvent concentration presents a positive effect for Cd and Hg and negative effect for Pb, but this factor is only significant for Hg. It could be due to the complex formation between chelating agent and Hg presents a high formation constant, which means a high affinity, and for this reason a high concentration of HNO_3 is necessary to break the complex. Finally, the elution time presents a negative effect for all metals although it is not significant. It can be explained using a similar reason used for the factor of extraction time, in this case, the eluate matrix is more complex at longer elution times and probably the signal decreases.

The use of a CCCD approach is hardly practicable for more than three factors, since a five factor quadratic model has 48 runs to be measured. For this reason, sample pH, eluent solvent volume and eluent solvent concentration were chosen as main significant factors because they had the highest influence (i.e., -13917.93 for eluent solvent volume in Fig. S8(a), 341.52 for eluent solvent

concentration in Fig. S8(b) and -923.20 for sample pH in Fig. S8(c)). Thus, next optimization step, sample pH, eluent solvent volume and eluent solvent concentration were optimized using a CCCD.

The other factors (i.e., amount of sorbent, extraction time and elution time) were fixed at the most convenient level. The amount of sorbent was fixed at high level with 50 mg and the elution time was fixed at low level with 2 min elution time. Finally, the extraction time was fixed at low level (i.e., 3 min) because it presented a significant negative effect for Hg (Fig. S8(b)).

3.3.2. Optimization study

CCCD was used to optimize significant factors in previous screening step. This type of design was employed to assess the main effects, interactions effects and quadratic effects of sample pH, eluent solvent volume and eluent solvent concentration. CCCD consists in two-level factorial design (2^k), with a central point which is repeated n times and $2k$ star points, where k is the number of factors to optimize. The value of star points depends on desired properties of design and number of factors. Star points fixed at $\alpha = \sqrt[4]{2^k} = 1.68$ in order to ensure the rotatability of the model and central point was repeated six times to ensure its orthogonality [36]. Low, central and high levels, and the star points of the studied factors are showed in Table S4. The matrix of experiments of CCCD is showed in Table S5.

The data obtained were evaluated by ANOVA and the response surfaces of the CCCD are shown in Fig. S9-S11. For the three emission lines, the quadratic regression model was significant with 95% probability. The repeatability of the central point ($n=6$) was assessed, obtaining coefficients of

variation between 5 and 16%. Table S6 shows the optimum MDSPME conditions obtained from the response surface and it can be observed that the MDSPME conditions are analyte-dependent and hence a compromise value was chosen in order to select the most favorable conditions for simultaneous MDSPME procedure of the three metals investigated. According to Table S6, firstly, the optimum eluent solvent volume was 432 μL of HNO_3 in all cases. This result can be easily explained since when the lower the volume of eluent solvent the higher analyte concentration in the extract. Secondly, optimum values for the eluent solvent concentration were 7.3, 11.8 and 8.4 M for Cd, Hg and Pb, respectively. The high eluent solvent concentration for Hg is according the Plackett-Burman design and this has already been explained before. Eluent solvent concentration was not a significant factor for Cd and Pb both in the screening design and in the CCCD. For this reason, a value of 11.8 M was selected as optimum value. Finally, optimum values for the sample pH were 6.2, 4.0 and 3.1 for Cd, Hg and Pb, respectively. The sample pH is an important factor because it may affect in the metal chelates formation. The optimum value obtained for each metal was different. However, there is not a well-defined maximum in the response surfaces (Fig. S9-S10) but there is a range of maximum values for the three metals. In this case, a compromise value of 4.0 was selected because the Cd emission intensity severely reduced when the sample pH was less than 4.0. Sample pH was adjusted to 4.0 with a buffer solution of acetic acid/sodium acetate.

As a conclusion, the MDSPME optimum conditions selected for simultaneous extraction of Cd, Hg and Pb were: amount of sorbent, 50 mg;

sample pH, 4.0; extraction time, 3 min; elution solvent volume, 432 μL ; eluent solvent concentration, HNO_3 11.8 M; and elution time, 2 min.

3.4. Sorbent reutilization study

The possibility of reusing DDTC-HDTMA-Zn-ZSM-5/ Fe_2O_3 composite as sorbent was studied. After each extraction, the composite was first subjected to additional elution steps (i.e., cleaning steps). The composite was eluted twice consecutively with 432 μL of nitric acid 11.8 M using an ultrasonic bath for 2 min. In addition, the two eluates of the cleaning step were analysed by ICP OES and the concentration of the three analytes was negligible in the last eluate (data not shown). Secondly, the composite was cleaned with deionized water three times and finally it was dried at 120 $^\circ\text{C}$ for 1 h. Three consecutive extractions were carried out in triplicate using the same composite. The extractions were performed using a urine sample spiked with 20 $\mu\text{g L}^{-1}$ of Cd, Hg and Pb. As observed in Fig. S12, signals remained practically constant for Pb, however for the other metals the extraction efficiency significantly decreased. This can be explained according to Fig. 1, where comparison of spectrum of reused composite (Fig. 1(d)) with spectrum of DDTC-HDTMA-Zn-ZSM-5/ Fe_2O_3 composite (Fig. 1(c)) shows that in spectrum (Fig. 1(d)) did not appear characteristic lines of HDTMABr surfactant. This could be related with removal of DDTC and HDTMABr in elution step. From the results obtained, it can be confirmed that Cd and Hg were adsorbed through complex formation while Pb was adsorbed either through complex formation (first extraction) or through cationic exchange (second and third extraction), as confirmed in section

3.2.2. As a conclusion, the composite cannot be reused for Cd and Hg extraction.

3.5. Validation of the method

. According to section 3.2.2. and data showed in Fig. S7, negative matrix effects for Cd were found when analyzing urine samples. Therefore, standard addition calibration was employed to evaluate quality analytical parameters under optimized extraction conditions for the three urine samples investigated. All analytical figures of merit are summarized in Table 1. The studied linear range was set from 0 to 100 $\mu\text{g L}^{-1}$ (number of calibration points, N=8) for Cd, Hg and Pb in urine samples. Standard addition calibration curves gave a high level of linearity with correlation coefficients (r) values ranging from 0.998 to 0.9997 for Cd, from 0.998 to 0.9998 for Hg and from 0.9996 to 0.9999 for Pb. The sensitivity of the instrumental measurements estimated by the slope of the calibration curves ranged from 161 ± 3 to 166 ± 1 cps μg^{-1} L for Cd, from 59 ± 1 to 82 ± 1 cps μg^{-1} L for Hg and from 88 ± 3 to 120 ± 1 cps μg^{-1} L for Pb. The repeatability of the method was assessed by six replicate analysis of urine samples spiked at 3 and at 50 $\mu\text{g L}^{-1}$ of Cd, Hg and Pb, obtaining coefficients of variation between 2 and 5%. Limit of detection (LOD) and limit of quantification (LOQ) values were calculated following the $3\sigma_{\text{blank}}$ and $10\sigma_{\text{blank}}$ criteria, respectively, being σ_{blank} the standard deviation of 10 measurements of urine matrix blank [39]. LOD values ranged from 0.15 to 0.20 $\mu\text{g L}^{-1}$ for Cd, from 0.42 to 0.73 $\mu\text{g L}^{-1}$ for Hg and from 0.23 to 0.39 $\mu\text{g L}^{-1}$ for Pb. These values satisfy the threshold limit established by the Ministry of Labour and Social Affairs (Spain) for normal content of these metals in human urine (i.e., 3.4 $\mu\text{g L}^{-1}$ for

Cd, $10 \mu\text{g L}^{-1}$ for Hg and $85 \mu\text{g L}^{-1}$ for Pb) [40] and by the World Health Organization for normal mercury content in urine (i.e., $10 \mu\text{g L}^{-1}$) [41]. LOQ values ranged from 0.5 to 0.7, from 1.4 to 2.4 and from 0.8 to $1.3 \mu\text{g L}^{-1}$ for Cd, Hg and Pb, respectively. Finally, the enrichment factors (EFs) were evaluated in urine samples. For each urine sample, a set of standards for each analyte studied was prepared using the standard addition calibration methodology. In all cases, the analyte concentration ranged from 5 to $100 \mu\text{g L}^{-1}$. Subsequently, all calibration sets were measured directly by ICP OES (i.e., calibration without MDSPME). Next, MDSPME was applied to the same sets of calibration standards and the eluates were measured by ICP OES (i.e., calibration with MDSPME). Finally, the EFs values for each analyte were calculated as the ratio between sensitivities with MDSPME and without MDSPME. EFs values ranged between 3.68 ± 0.03 and 4.47 ± 0.09 for Cd, between 18.1 ± 0.4 and 19.6 ± 0.4 for Hg and between 22.3 ± 0.4 and 24.4 ± 0.5 for Pb. The low EF value obtained for Cd can be explained through optimized extraction conditions chosen. Optimum sample pH and eluent concentration for Cd were 6.2 and 7.3, respectively (Table S6). However, sample pH 4.0 and HNO_3 11.8 M were chosen as optimum extraction conditions for the proposed method since these were obtained for Hg, and sensitivity for Hg is much lower than sensitivity for Cd (Table 1).

3.6. Analysis of real urine samples

Three urine samples were initially analyzed and its Cd, Hg and Pb content was detected with the proposed method. However Hg content of urine sample 3 resulted under the LOQ of the present approach (Table 1).

Consequently, three urine samples were spiked at 5, 15 and 30 $\mu\text{g L}^{-1}$ levels of Cd, Hg and Pb and analyzed by the standard addition calibration (Table 2). According to these results, there were not significant differences between the concentrations added and those found in the urine samples, obtaining relative recoveries ranged between 87-107%. Therefore, matrix effects were corrected with the proposed methodology.

3.7. Comparison with other methods

A comparison between this work and the previously reported procedures combining different SPE techniques and spectrochemical detection methods for the determination of Cd, Hg and Pb in urine and water samples is shown in Table 3. The extraction time of the proposed method is much shorter (3 min) than other previous works (i.e., 7, 15 and 20 min) [21,42–44] and the amount of sorbent (50 mg) is comparable to these works (i.e., 32.5, 25 and 100 mg) [21,44,45]. It should be noted that DDTC-HDTMA-Zn-ZSM-5/ Fe_2O_3 is a sorbent significantly cheaper than sorbents based on graphene oxide [42,44], since zeolites are more economical products, abundant and low cost. The LODs in this work are similar or even lower than those LODs obtained in previous publications using ICP OES as detection method [45,46]. It should be point out that the LOD of Pb in this work is comparable to the one obtained using magnetic solid-phase extraction (MSPE) and ICP-MS [42] employing a more economical analytical instrumentation. In addition, the developed extraction technique presents advantages versus others such as in-tube- μ -SPE [46] and SPE [21,44], because it avoids the column preparation and centrifugation steps. It should be noted that in many analytical methods it is necessary a digestion

sample step before extraction procedure to determine heavy metals in urine [43,45,46], however in our work urine samples were used directly for MDSPME. Finally, even though these publications determinate heavy metals in urine samples, to our knowledge, a modified ZSM-5/Fe₂O₃ composite has not been employed to determine Cd, Hg and Pb in urine samples.

4. Conclusions

A new analytical method based on the synergetic association MDSPME-ICP OES has been developed in this work. ZSM-5/Fe₂O₃ composite saturated with Zn and modified with HDTMABr surfactant and DDTC chelating agent has been employed as a sorbent for first time for the simultaneous separation and preconcentration of Cd, Hg and Pb in urine samples. The developed sample preparation method is fast and economical since sorbent is low cost and easy handling by applying an external magnetic field. Additionally, the proposed sample preparation avoids acid digestion of urine samples resulting in a more environmentally friendly procedure and reducing risk of analyte losses (i.e., Hg) or contamination. It should be noted that the DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃ composite cannot be reused since modification is lost during the elution step, but ZSM-5/Fe₂O₃ composite could be modified again. High repeatability was obtained for the three metals and good extraction efficiencies for Hg and Pb were obtained. LODs values satisfy the threshold limit established by the Ministry of Labour and Social Affairs (Spain) for normal content of these metals in human urine (i.e., 3.4 µg L⁻¹ for Cd, 10 µg L⁻¹ for Hg and 85 µg L⁻¹ for Pb) [40] and by the World Health Organization for normal mercury content in urine (i.e., 10 µg L⁻¹) [41], and obtained LOQs values satisfy the Biological Threshold Limit

(BTL) (i.e., 17 $\mu\text{g L}^{-1}$ for Cd, 85 $\mu\text{g L}^{-1}$ for Hg and 255 $\mu\text{g L}^{-1}$ for Pb) [40]. Finally, the applicability of the proposed method has been successfully studied for determination of Cd, Hg and Pb in three real urine samples.

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FIGURES

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150 **Fig. 1.** ATR-FTIR spectra of Zn-ZSM-5/Fe₂O₃ composite (a), HDTMA-Zn-ZSM-
151 5/Fe₂O₃ composite (b), DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃ composite (c) and
152 reused DDTC-HDTMA-Zn-ZSM-5/Fe₂O₃ composite (d).

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(a)

(b)

Fig 2. Extraction efficiency of different modified ZSM-5/Fe₂O₃ composites in aqueous standard (a) and in a urine sample (b) spiked with 50 µg L⁻¹ of Cd, Hg and Pb, respectively. The error bars are the standard deviation of three replicates.

TABLES

Table 1. Analytical figures of merit of the proposed method (MDSPME-ICP OES) in urine samples by standard addition calibration method.

Emission Lines (nm)	Linear range ($\mu\text{g L}^{-1}$)	r^a	Sensitivity ^b (cps μg^{-1} L)	CV ^c (%)		LOD ^d ($\mu\text{g L}^{-1}$)	LOQ ^e ($\mu\text{g L}^{-1}$)	EF ^f
				3 $\mu\text{g L}^{-1}$	50 $\mu\text{g L}^{-1}$			
Cd II (228.802)	0-100	0.998-0.9997 (8)	161 $\pm$ 3 - 166 $\pm$ 1	2	4	0.15-0.20	0.5-0.7	3.68 $\pm$ 0.03 - 4.47 $\pm$ 0.09
Hg II (194.164)	0-100	0.998-0.9998 (8)	59 $\pm$ 1 - 82 $\pm$ 1	2	5	0.42-0.73	1.4-2.4	18.1 $\pm$ 0.4 - 19.6 $\pm$ 0.4
Pb II (220.253)	0-100	0.9996-0.9999 (8)	88 $\pm$ 3 - 120 $\pm$ 1	4	4	0.23-0.39	0.8-1.3	22.3 $\pm$ 0.4 - 24.4 $\pm$ 0.5

^a Correlation coefficient (r): number of calibration standards in parenthesis.

^b Slope $\pm$ standard deviation.

^c Coefficient of variation (CV): mean value for 6 replicate analyses of a 3 $\mu\text{g L}^{-1}$ and 50 $\mu\text{g L}^{-1}$ spiked solution.

^d Limit of detection (LOD): calculated following the $3\sigma_{\text{blank}}$ criteria, being σ_{blank} the standard deviation of 10 measurements of urine matrix blank.

^e Limit of quantification (LOQ): calculated following the $10\sigma_{\text{blank}}$ criteria, being σ_{blank} the standard deviation of 10 measurements of urine matrix blank.

^f Enrichment factor (EF) $\pm$ expanded uncertainty. EF: calculated as the ratio of the sensitivity obtained with and without MDSPME.

184 **Table 2.** Determination of Cd, Hg and Pb in real urine samples.

	Cd II (228.802 nm)			Hg II (194.164 nm)		Pb II (220.253 nm)		
	Spiked value ($\mu\text{g L}^{-1}$)	Found value ($\mu\text{g L}^{-1}$) $\pm$ SD ^a	Relative recoveries (%) (CV)	Found value ($\mu\text{g L}^{-1}$) $\pm$ SD ^a	Relative recoveries (%) (CV)	Found value ($\mu\text{g L}^{-1}$) $\pm$ SD ^a	Relative recoveries (%) (CV)	
Sample 1	0	1.9 $\pm$ 1.1	-	1.6 $\pm$ 1.0	-	3.1 $\pm$ 0.5	-	187
	5	6.9 $\pm$ 0.5	98 (10)	6.0 $\pm$ 0.4	87 (10)	7.8 $\pm$ 0.3	91 (7)	
	15	17.0 $\pm$ 1.0	100 (6)	16.0 $\pm$ 1.0	95 (7)	16.9 $\pm$ 1.1	92 (8)	188
	30	31.9 $\pm$ 1.5	100 (5)	30.3 $\pm$ 2.2	96 (8)	32.7 $\pm$ 0.8	98 (3)	
Sample 2	0	1.6 $\pm$ 0.6	-	1.8 $\pm$ 0.3	-	4.5 $\pm$ 0.3	-	189
	5	6.6 $\pm$ 0.4	98 (8)	7.0 $\pm$ 0.5	101 (9)	9.9 $\pm$ 0.3	105 (6)	
	15	16.0 $\pm$ 0.5	96 (3)	17.0 $\pm$ 0.6	101 (4)	18.2 $\pm$ 0.6	91 (4)	190
	30	31.3 $\pm$ 1.1	99 (4)	31.4 $\pm$ 1.0	99 (3)	33.7 $\pm$ 0.8	97 (3)	
Sample 3	0	1.2 $\pm$ 0.2	-	<LOQ	-	3.8 $\pm$ 0.2	-	191
	5	6.2 $\pm$ 0.6	99 (12)	5.0 $\pm$ 0.7	100 (14)	9.1 $\pm$ 0.8	104 (15)	
	15	15.1 $\pm$ 0.8	93 (6)	14.3 $\pm$ 1.1	95 (8)	17.3 $\pm$ 0.9	90 (7)	192
	30	29.0 $\pm$ 1.0	91 (3)	32.1 $\pm$ 1.1	107 (3)	34.6 $\pm$ 0.8	103 (2)	

193 ^a Standard deviation values were calculated using the s_{XE} (i.e., standard deviation of x-value estimated using the regression line)
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199 **Table 3.** Comparison of different SPE techniques for Cd, Hg and Pb determination in urine and water samples

Sorbent	Sample	Sample digestion	Extraction technique	Amount of sorbent (mg)	Extraction time (min)	Detection method	LOD ($\mu\text{g L}^{-1}$)	Ref.
SDS-coated Fe_3O_4 NPs	water	Yes	MSPE	32.5	1	ICP OES	0.04 (Hg)	[45]
$\text{Fe}_3\text{O}_4/\text{GO}$	urine plasma	No	MSPE	6	7	ICP MS	0.16 (Pb)	[42]
Fe_3O_4 NPs	water urine	Yes	MSPE	10	15	ETAAS	0.01 (Hg)	[43]
Black stone	urine	Yes	in-tube- μ -SPE	20	-	ICP OES	0.3 (Cd) 1.1 (Pb)	[46]
MoS_2 -rGO	urine saliva water	No	SPE	25	15	AAS	0.7 (Pb)	[44]
Synthetic zeolite A-4	water	No	SPE	100	20	GFAAS	0.002 (Cd)	[21]
Modified ZSM-5/ Fe_2O_3	urine	No	MDSPME	50	3	ICP OES	0.15 - 0.20 (Cd) 0.42 - 0.73 (Hg) 0.23 - 0.39 (Pb)	This work

200 SDS-coated Fe_3O_4 NPs, sodium dodecyle sulphate-coated magnetite nanoparticles; MSPE, magnetic solid-phase extraction; ICP
201 OES, inductively coupled plasma optical emission spectrometry; GO, graphene oxide; ICP-MS, inductively coupled plasma mass
202 spectrometry; ETAAS, electrothermal atomic absorption spectrometry; μ -SPE, solid-phase microextraction; MoS_2 , molybdenum
203 disulfide; rGO, reduced graphene oxide; SPE, solid-phase extraction; AAS, atomic absorption spectrometry; GFAAS, graphite
204 furnace atomic absorption spectrometry; MDSPME, magnetic dispersive solid-phase microextraction.