Magnetic In-Tube Solid Phase Microextraction

Y. Moliner-Martínez,† Helena Prima-García,‡ Antonio Ribera,‡ Eugenio Coronado,‡ and P. Campíns-Falcó*†

†Departamento de Química Analítica, Facultad de Química, Universidad de Valencia, Dr. Moliner 50, E46100 — Burjassot, Valencia, España
‡Instituto de Ciencia Molecular (ICMol), Universidad de Valencia, Catedrático José Beltrán 2, E46980 — Paterna, Valencia, España

ABSTRACT: We report a new in-tube solid phase microextraction approach named magnetic in-tube solid phase microextraction, magnetic-IT-SPME. Magnetic-IT-SPME has been developed, taking advantage of magnetic microfluidic principles with the aim to improve extraction efficiency of IT-SPME systems. First, a magnetic hybrid material formed by Fe₃O₄ nanoparticles supported on SiO₂ was synthesized and immobilized in the surface of a bare fused silica capillary column to obtain a magnetic adsorbent extraction phase. The capillary column was placed inside a magnetic coil that allowed the application of a variable magnetic field. Acetalsalicylic acid, acetaminophen, atenolol, diclofenac, and ibuprofen were tested as target analytes. The application of a controlled magnetic field resulted in quantitative extraction efficiencies of the target analytes between 70 and 100%. These results demonstrated that magnetic forces solve the low extraction efficiency (10−30%) of IT-SPME systems, which is one of their main drawbacks.

Magnetism is nowadays one of the most exciting trends in analytical chemistry. Thus, analytical methodologies have started to take advantage of recent advances in the development of magnetic nanoparticles, hybrid magnetic (nano)materials, or magnetic composites, to improve the performance of existing methodologies. Magnetic forces offer great advantages in analytical applications as magnetic interactions are not influenced by chemical variables such as pH, concentration, or surface charges. In addition, they allow controlling fluid motion in microsystems, an important advance for chromatographic separations in microfluidic systems. These applications are especially focused on the development of sensor and biosensors, purification/remediation processes, separation techniques, sample pretreatment, and microfluidic technology to develop miniaturized total analysis systems. Reviews related with the application of magnetic solids have recently been published.

In 1997, Eisert and Pawliszyn coupled solid phase microextraction to HPLC to develop in-tube solid phase microextraction (IT-SPME). In this technique extraction, desorption and injection can be performed automatically, allowing a shortening time and better precision and accuracy. The main disadvantage of IT-SPME is the low extraction efficiency due to large breakthrough volume and a small amount of adsorbent phases. Several efforts have been devoted to developing new adsorbent phases to improve the extraction efficiency. Polypyrrole (PPY) and molecular imprinted polymers have been described as capillary coatings that improve these parameters. In addition, monolithic capillary columns have also been proposed as an alternative to IT-SPME.

Recent studies point out to the use of a magnetic field as an alternative to preconcentrate analytes in microfluidic devices by exploiting the phenomenon of diamagnetic repulsion in these systems. This theory is based on differences in the magnetic susceptibility between the analytes and the medium in which they are immersed. Under these conditions, diamagnetic forces can be used to focus or trap analytes as a function of their magnetic nature. Concretely, in the case of diamagnetic compounds immersed into paramagnetic media, analytes will concentrate in the areas in which the magnetic field is minimum. Choosing the adequate dimensions, IT-SPME could be considered a microfluidic device and so advantages of magnetic forces could be exploited to improve the features of these preconcentration systems.

This work is aimed at the development of a new IT-SPME approach based on the use of magnetism, namely, magnetic-IT-SPME. Thus, we have immobilized a magnetic adsorbent phase formed by SiO₂ supported Fe₃O₄ nanoparticles in a fused silica capillary column. Then, we have investigated the influence of an external magnetic field in the adsorption–desorption processes in the IT-SPME extraction system. As a result, we have demonstrated that the implementation of magnetism as a new variable for IT-SPME systems leads to a significant improvement of the extraction efficiency. The paper is focused...
on environmental science, and acetylsalicylic acid, atenolol, acetaminophen, diclofenac, and ibuprofen have been selected as target analytes as they are pharmaceuticals of major consumption. Those compounds are considered emerging contaminants in waters as they are biological active substances and so they could act as endocrine disrupters. Other areas of application of the magnetic-IT-SPME could be biochemistry, toxicology, pharmacy, cosmetics, and food chemistry in accordance with the proposals of the IT-SPME technique.

■ EXPERIMENTAL SECTION

Materials and Methods. Iron(III) acetylacetonate (Fe(acac)$_3$), 1,2-hexadecanodiol, oleylamine, oleic acid, acetylsalicylic acid, atenolol, acetaminophen, diclofenac, ibuprofen, tetraethylorthosilicate (TEOS), hexadecyltrimethylammonium bromide (CTAB), polyethylene glycol (PEG), and phenyl ether were purchased from Sigma-Aldrich (Steinheim, Germany). Methanol and acetonitrile were purchased from Panreac (Barcelona, Spain). Stock standard solutions (100 mg/L) of acetylsalicylic acid, acetaminophen, diclofenac, and ibuprofen were prepared in water. Working standard solutions were made by appropriate dilution of the stock standard solutions in water.

Preparation of the Silica Supported Fe$_3$O$_4$ Nanoparticles (Fe$_3$O$_4$−SiO$_2$) Capillary Columns. Organic-phase synthesis of Fe$_3$O$_4$ nanoparticles was based on the Sun and Zeng procedure:24 Fe(acac)$_3$ (0.706 g) was mixed with 2.013 g of 1,2-hexadecanodiol, 1.695 g of oleic acid, 1.605 g of oleylamine, and 20 mL of phenyl ether under Ar stream (20 min). The mixture was refluxed at 263 °C during 30 min. After being cooled to room temperature, 80 mL of ethanol was added to the reaction mixture and centrifugation (10 000 rpm, 10 min) was used to separate the dark-brown material. This material was redissolved in hexane (20 mL) to give 5 nm nanoparticles (see Figure S-1A, Supporting Information).

Water-soluble Fe$_3$O$_4$ nanoparticles (Fe$_3$O$_4$−CTAB) were obtained with 0.1 M solution of CTAB. Fe$_3$O$_4$ nanoparticles hexane extracts (20 mL) were mixed with 20 mL of 0.1 M CTAB solutions, respectively. Water Fe$_3$O$_4$−CTAB colloidal solution was then rotaevaporated until complete elimination of hexane.25

The preparation conditions of the capillary were similar to those reported earlier:26 A solution of PEG (0.9 g) and urea (0.9 g) in 10 mM acetic acid (10 mL) was prepared. Then, 2.5 mL of this solution was mixed with 1 mL of Fe$_3$O$_4$−CTAB water dispersion, adjusted to pH 11 with 1 M NaOH (7 mL) and 1 mL of TEOS, which polymerizes to give rise to a silica matrix embedding the Fe$_3$O$_4$−CTAB micelles. This mixture was stirred until it became a homogeneous gel, which was injected into the fused silica capillary column (340 mm, i.d. 75 μm), previously treated with 1 M NaOH. The ends of the capillary were sealed and placed into an oven at 43 °C. Then, the temperature was raised to 120 at 0.5 °C/min and kept constant for 2 h, followed by water and methanol washes. After drying, the capillary was heated at 330 °C for 25 h.

Scanning electron microscopy (SEM) images were obtained with a HITACHI-S4100 equipment operated at 20 kV. High resolution scanning electron microscopy (HRTEM) images were obtained with Philips Tecnai F20 equipment operating at 200 kV. The samples were prepared by deposition of a drop of the synthesized material suspension onto a lacey carbon/Formvar-coated copper grid. The digital analysis of the HRTEM micrographs was done using Digital Micrograph TM 1.80.70 for GMS1.8.0 Gatan. The magnetic measurements were carried out in a Quantum Design MPMS Squid Magnetometer with variable-temperature (T = 2 K) and field-dependent (applied field: 150 Oe) dc measurements. The same device was used in the ac susceptibility measurements at different frequencies of the oscillating field of 17 G amplitude.

Instrument and Chromatographic Conditions. The capillary chromatographic system used consisted of a LC isocratic capillary pump (Jasco Corporation, Tokyo, Japan) and a UV−vis diode array detector (Agilent, 1200 series) equipped with a 80 nL flow cell. The analytical signal was recorded.
between 190 and 400 nm. For the separation of the analytes, a Zorbax SB C<sub>18</sub> (150 mm × 0.5 mm, i.d. 3.5 μm) analytical column (Agilent) was used. The mobile-phase was a mixture of methanol/water 70:30 (v/v) at mobile-phase flow rates 6 μL/min. All solvents were filtered through 0.45 mm nylon membranes (Teknokroma) before use.

The SiO<sub>2</sub>–Fe<sub>3</sub>O<sub>4</sub> capillary column was connected to a conventional six-port injection valve and used as the injection loop. The SiO<sub>2</sub>–Fe<sub>3</sub>O<sub>4</sub> capillary column was wrapped with a magnetic coil such that the external magnetic field intensity (B) could be adjusted by varying the current applied to the coil by a power supply. Magnetic coils with linear and circular configuration were studied. Part A of Figure 1 shows the schematic diagram of the linear configuration (5 and 15 cm) for adsorption (—) and desorption (--) steps. Part B of Figure 1 illustrates the diagram of the circular configuration (15 cm) for both steps involved in the extraction procedure, adsorption (—) and desorption (--). Capillary connections to the valve were facilitated by the use of a 2.5 cm sleeve of 1/32 in. polyether ether ketone (PEEK) tubing; 1/32 in PEEK nuts and ferrules were used to complete the connections. Aliquots of 200 μL of the samples were passed manually through the magnetic capillary column (17 cm length) at 10 μL/s using a syringe and at different magnetic fields from 25 to 250 G. Next, the valve was manually rotated, so the analytes were desorbed from the extractive phase of the magnetic capillary, with the mobile-phase combined with the change of the polarity at different magnetic fields (25–200 G), and transferred to the analytical column for separation and detection. After each injection, the capillary column was cleaned with 300 μL of methanol. All the experiments were carried out in triplicate and at room temperature.

**RESULTS AND DISCUSSION**

**Magnetic Capillary Columns.** Fe<sub>3</sub>O<sub>4</sub> nanoparticles supported in a silica matrix (Fe<sub>3</sub>O<sub>4</sub>–SiO<sub>2</sub>) were deposited on capillary columns. The material is formed by Fe<sub>3</sub>O<sub>4</sub>–CTAB micelles (with particle size of 5 nm) and CTAB surfactant micelles surrounded by the silica matrix. The HRTEM image shows that the Fe<sub>3</sub>O<sub>4</sub> nanoparticles are homogeneously distributed in the silica matrix (Figure S-1B, Supporting Information). Such network structure involves the interaction of the ammonium groups of the CTAB, acting as templates, with the oligosilic species provided by TEOS in order to form, of the ammonium groups of the CTAB, acting as templates, with the oligosilic species provided by TEOS in order to form, an interpenetrating network. Thus, it presents a favorable mass transfer for extraction purposes since the organic analytes can interact with the adsorbent phase.

The immobilization of the supported Fe<sub>3</sub>O<sub>4</sub> nanoparticles in SiO<sub>2</sub> at the inner surface of a 75 μm-fused silica capillary column results in a coating 10 μm thick of Fe<sub>3</sub>O<sub>4</sub>–SiO<sub>2</sub> as can be seen in the SEM photographs (Figure S-1C, Supporting Information). This coating is formed by a highly porous interpenetrating network. Thus, it presents a favorable mass transfer for extraction purposes since the organic analytes can interact with the adsorbent phase.

The magnetic behavior of these nanoparticles embedded in the silica matrix shows that at room temperature these nanoparticles are superparamagnetic and well-isolated. Thus, the magnetization vs field curve shows at room temperature a sigmoidal dependence with a sharp linear increase in the region of 0–150 G (Figure S-2, Supporting Information) and without hysteresis. At higher fields, this increase is lower as magnetization (M) tends to saturate at fields above 10 000 G. That means that the sample becomes easily magnetized with fields as small as 200 G (in fact at this field the magnetization has already reached 16% of the saturation value). The well magnetic isolation of the nanoparticles in the silica matrix can be demonstrated from the ac magnetic measurements performed at low temperatures. Thus, one observes that both the in-phase and the out-of-phase susceptibility signals show a maximum in the region of 50–60 K, which is frequency dependent. Such a behavior is accompanied by the presence of a hysteresis loop with a coercive field of ca. 500 G. It indicates that below these temperatures the spins of the superparamagnetic nanoparticles undergo a magnetic blocking. An analysis of these experimental data follows an Arrhenius law with an activation energy barrier for the spin reversal of 870 K and a τ<sub>f</sub> atomic spin flip time of 2.5 × 10<sup>−13</sup> seconds (Figure S-3, Supporting Information). This τ<sub>f</sub> value falls in the range typical for noninteracting superparamagnetic nanoparticles (from 10<sup>−9</sup> to 10<sup>−13</sup> seconds). This result is not unexpected as, in view of the size of the nanoparticles (5 nm) and the composition of the Fe<sub>3</sub>O<sub>4</sub>–SiO<sub>2</sub> nanocomposite (5 wt % of Fe<sub>3</sub>O<sub>4</sub>), one can estimate that the average separation between nanoparticles is of 10–25 nm (depending the values of the Fe<sub>3</sub>O<sub>4</sub> nanoparticles and silica density).

In summary, these magnetic capillary columns are formed by well-dispersed and isolated superparamagnetic nanoparticles of Fe<sub>3</sub>O<sub>4</sub>, which can be easily magnetized and demagnetized upon application of a small magnetic field. These features make the hybrid material suitable for its evaluation as a magnetic sorbent phase for developing the magnetic-IT-SPME based technique.

**Magnetic-in-Tube Solid Phase Microextraction (Magnetic-IT-SPME).** As we have pointed out before, in the present method, the target analytes interact with the adsorbent phase of the SiO<sub>2</sub> supported Fe<sub>3</sub>O<sub>4</sub> nanoparticles through hydrophobic interactions. Let us study now the influence of magnetic fields in adsorption and desorption processes of the target analytes in the IT-SPME pretreatment step. Two different configurations of the magnetic coil have been evaluated, namely, the lineal configuration and the circular configuration. The capillary column was placed inside the magnetic coil and connected to the six port valve (see Figure 1A,B). At load position (adsorption step), the analytes were passed through into the capillary column. Then, at the injection position (desorption step), the analytes were transferred to the analytical column for their detection. The length and the magnetization were constant for the lineal and circular configuration. As the former one leads to a more homogeneous magnetic fields, we will focus primarily on this kind of configuration. The results obtained have been evaluated in terms of extraction efficiency of the analytes estimated from the calibration equations obtained through the direct injection of 2 μL of standard solution at concentration from 0.5 to 10 μg/mL for each analyte. The target analytes selected for this aim are acetylsalicylic acid, atenolol, acetaminophen, diclofenac, and ibuprofen. All these molecules are diamagnetic, and thus, they must be repelled by a magnetic field, moving to the regions in which the magnetic field felt by the molecules is minimum. The application of an external magnetic field to the capillary column magnetizes the Fe<sub>3</sub>O<sub>4</sub> nanoparticles homogeneously distributed all along the...
The extraction efficiencies obtained for the second set of experiments showed that initially trapped analytes by in the IT-SPME, they will be subjected to magnetic forces that may influence the partitioning of the analytes between the liquid phase (water/methanol) and the sorbent phase of the capillary in such a way that these analytes will be trapped in the minima of the magnetic field forces generated by these nanomagnets. After that, the analytes had to be released from the sorbent phase to the analytical column in the desorption step. The resulting values were comprised of those between 36 and 57%. These values are similar to those obtained if the magnetic field is switched off in the desorption step. Hence, one can conclude that the effect of switching off the magnetic field is negligible.

In the last set of experiments in which the magnetic field direction was reversed, the extraction efficiencies significantly increase to values of 70 ± 4, 100 ± 7, 80 ± 5, 99 ± 5, and 89 ± 6% for acetylsalicylic acid, atenolol, acetaminophen, diclofenac, and ibuprofen, respectively. From these results, one can conclude that the reversal in the polarity of the magnetic field is crucial for the release of the molecular analytes, being necessary to get a quantitative desorption of the analytes.

Bearing in mind these results, a deeper study of the influence of the magnetic field on the extraction efficiencies were carried out. First, we investigated the influence of magnetic fields in the adsorption step of the IT-SPME system. This effect is studied by injecting 200 μL of a mixture of acetylsalicylic acid, atenolol, acetaminophen, diclofenac, and ibuprofen (50 μg/L) in the SiO2 supported Fe3O4 nanoparticle capillary column while increasing magnetic fields between 25 and 250 G. We observe that this effect is quite significant (Figure 2). Thus, all the analytes show a similar tendency with a maximum at 150 G, in which the extraction capacities are in the range of 70–100%. It should be noted that the typical extraction efficiencies achieved with IT-SPME do not exceed 30%. This is the main drawback of IT-SPME as the analytes are not completely recovered. The results obtained in this work showed that the application of a magnetic field in a capillary column previously functionalized with magnetic nanoparticles resulted in a quite significant improvement of the IT-SPME technique. In particular, a

inner surface of the capillary. Therefore, when the analytes are processed in the IT-SPME, they will be subjected to magnetic forces that may influence the partitioning of the analytes between the liquid phase (water/methanol) and the sorbent phase of the capillary in such a way that these analytes will be trapped in the minima of the magnetic field forces generated by these nanomagnets. After that, the analytes had to be released from the sorbent phase to the analytical column in the desorption step.

On the basis of the above hypothesis, four sets of experiments were carried out. In the first set of experiments, the magnetic field was not applied; in the second one, a magnetic field was applied to trap the analytes in the adsorption step and then switched off in the desorption step; in the third set of experiments, a constant magnetic field was applied in both the adsorption and desorption steps; in the last one, the analytes were adsorbed at a magnetic field, and then, the magnetic field direction was reversed (from positive to negative values) in the desorption step. Notice that this study was done using a B = 150 G and with a 15 cm magnetic capillary column. Table 1 shows the extraction efficiency values (%) obtained for the analytes under the previously described conditions.

The extraction efficiencies in absence of the magnetic field were similar to those obtained with existing sorbent phases for IT-SPME, typically between 10 and 30%. The extraction efficiencies obtained for the second set of experiments showed that initially trapped analytes by influence of the magnetic field are partially released giving rise to extraction efficiencies of 36 ± 3%, 45 ± 5%, 35 ± 5%, 56 ± 2%, and 40 ± 3% for acetylsalicylic acid, atenolol, acetaminophen, diclofenac, and ibuprofen, respectively. The same study was carried out keeping the magnetic field at positive polarity in the adsorption and desorption step (third set of experiments). The resulting values were comprised of those between 36 and 57%. These values are similar to those obtained if the magnetic field is switched off in the desorption step. Hence, one can conclude that the effect of switching off the magnetic field is negligible.

In the last set of experiments in which the magnetic field direction was reversed, the extraction efficiencies significantly increase to values of 70 ± 4, 100 ± 7, 80 ± 5, 99 ± 5, and 89 ± 6% for acetylsalicylic acid, atenolol, acetaminophen, diclofenac, and ibuprofen, respectively. From these results, one can conclude that the reversal in the polarity of the magnetic field is crucial for the release of the molecular analytes, being necessary to get a quantitative desorption of the analytes.

Table 1. Extraction Efficiencies Obtained under Different Experimental Conditions*

<table>
<thead>
<tr>
<th>magnetic field</th>
<th>adsorption step</th>
<th>desorption step</th>
<th>extraction efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>acetylsalicylic acid</td>
<td>atenolol</td>
<td>acetaminophen</td>
</tr>
<tr>
<td>H=0</td>
<td>4 ± 1</td>
<td>34 ± 5</td>
<td>34 ± 4</td>
</tr>
<tr>
<td>H=B</td>
<td>36 ± 3</td>
<td>45 ± 5</td>
<td>39 ± 4</td>
</tr>
<tr>
<td>H=B</td>
<td>34 ± 4</td>
<td>40 ± 2</td>
<td>35 ± 5</td>
</tr>
<tr>
<td>H=B</td>
<td>70 ± 4</td>
<td>100 ± 7</td>
<td>80 ± 5</td>
</tr>
</tbody>
</table>

*B = 150 G.

Figure 2. Extraction efficiency (%) obtained for (a) acetylsalicylic acid, (b) atenolol, (c) acetaminophen, (d) diclofenac, and (e) ibuprofen. Concentration of the analytes, 50 μg/L. Injection, 200 μL; mobile phase, methanol/water 70:30 (v/v), 6 μL min⁻¹; B_{adsorption} = 150 G and B_{desorption} = 150 G (reversed polarity).
quantitative recovery, with values of $99 \pm 5\%$ and $100 \pm 7\%$, has been achieved for atenolol and diclofenac, respectively.

Previously, we have demonstrated that the extraction efficiency of the target analytes in the magnetic IT-SPME system sharply increases in the presence of a magnetic field. Here, we study the effect of a magnetic field in the desorption process. In this study, retention of the analytes from the adsorbent phase, a methanol/water mobile phase has been used. After each injection, the magnetic capillary column is cleaned with 300 $\mu$L of methanol to remove remaining analytes in the sorbent phase. In Figure 3, we can see that these maxima are reached for an applied magnetic field of $-150$ G.

The processed volume of a sample and the amount of analytes extracted in traditionally IT-SPME systems depends on the internal diameter, coating thickness, and length. Therefore, we have also studied if the analytical response is improved in our magnetic IT-SPME system by increasing the processed volume. The internal volume of our capillary is 3 $\mu$L. Sample volumes between 50 and 500 $\mu$L have been assayed for a mixture of the target analytes. Figure 4 illustrates the variation of the analytical signal as a function of the processed volume.

As expected, the analytical signal increases with the increment on the sample volume. However, similar values are obtained with 200, 400, or 500 $\mu$L as partial autoelution of the analytes took place when volumes of samples higher than 200 $\mu$L were processed. Therefore, we have selected 200 $\mu$L to achieve the maximum analytical response.

In order to investigate the effect of the magnetized area on the extraction efficiency, we have tested two magnetic coil lengths, 5 and 15 cm. Figure 5 compares the extraction capacity values obtained for both coils at $B_{retention} = +150$ G and $B_{elution} = -150$ G (reversed polarity). As can be seen, the higher the length of the magnetic coil, the higher is the extraction efficiency.

In addition, we have evaluated the results obtained with a SiO$_2$ deposited capillary in the absence of magnetic nanoparticles with the 5 cm magnetic coil. Retention and elution were done under the same conditions than those mentioned above. The extraction capacities with this nonmagnetic capillary in the presence of a magnetic field are in between 12 and 34% for all the analytes; these values are identical to those obtained in the absence of a magnetic field. In this case, analytes are not inside a paramagnetic medium and so they are not submitted to any repulsion forces that help the adsorption of the analytes into the coating. Without Fe$_3$O$_4$ nanoparticles in the adsorbent phase, movement and adsorption of the analytes is governed by

Figure 3. Influence of the magnetic field in the desorption step, (a) acetylsalicylic acid, (b) atenolol, (c) acetaminophen, (d) diclofenac, and (e) ibuprofen. Target analytes at 50 $\mu$g/L. Injection, 200 $\mu$L; mobile phase, methanol/water 70:30 (v/v), 6 $\mu$L min$^{-1}$; $B_{retention} = 150$ G; elution with reversed polarity.

Figure 4. Analytical response (peak area) as a function of the analytical volume processed: (a) acetylsalicylic acid, (b) ibuprofen, (c) atenolol, (d) diclofenac, and (e) acetaminophen. Concentration, 50 $\mu$g/L.

Figure 5. Influence of the magnetic coil length in the extraction capacity of the target analytes (50 $\mu$g/L).
the hydrodynamic flow and the hydrophobic interactions with the coating of the capillary column, thus reaching the typical extraction capacities of IT-SPME.

Besides, the circular magnetic coil configuration was evaluated (Figure 1B). The effect of magnetic field in the adsorption and desorption processes, change of polarity, and demagnetization were systematically studied as previously described for the lineal configuration. Figure 6 shows the extraction efficiencies as a function of the applied magnetic field obtained for ibuprofen and diclofenac in the adsorption and desorption steps. As can be seen, quantitative extraction efficiencies were achieved with this configuration by adsorbing the analytes at 150 G and desorbing them at the same magnetic field changing the polarity. It should be noted that the response of the extraction of the analytes was not a function of the magnetic coil configuration. However, the relative standard deviations (RSD) achieved with the circular magnetic coil were higher than 12% for all the analytes. These RSD values are higher than those obtained with the lineal magnetic coil (lower than 8%). The cause of this difference can be the lower homogeneity of the applied magnetic field on the circular magnetic coil.

**Precision.** The reproducibility was evaluated at two concentration levels. Relative standard deviations (%RSD) at different concentration levels are shown in Table 2. These results indicated satisfactory %RSD values and were independent of the concentration level. In this sense, a capillary-to-capillary reproducibility study was performed. For this aim, we compared the extraction efficiency obtained for a mixture of the target analytes (50 μg/L) with two SiO2 supported Fe3O4 nanoparticle capillary columns prepared under identical conditions. Table 2 shows the results obtained. As can be seen, run-to-run %RSD values were lower than 8% and capillary-to-capillary %RSD values were lower than 13% for all the analytes. The low RSD values bear evidence to the fact that the application of an external magnetic field to the capillary coated with the synthesized magnetic hybrid material was a reliable and reproducible approach to achieved quantitative extraction capacity in IT-SPME extraction by means of magnetic-IT-SPME. On the other hand, Figure 7 shows the chromatograms obtained at different concentrations. No carry over is observed; any analytical signals from a previously injected standard or sample appear after the injection of blank solution. In addition, the magnetic capillary columns were stable as the sol–gel technology guarantees the stability of the Fe3O4 nanoparticles, because the stiffness of the silica matrix avoids the magnetite nanoparticle interactions, and therefore, no agglomerations are done, keeping the size of the nanoparticles constant. On the other hand, the silica matrix protects the nanoparticles against their incorporation to the flow into the column, because the nanoparticles are trapped in the silica matrix. In fact, the magnetic capillary columns were used 200 times without loss of the extraction efficiency (see Figure S-4, Supporting Information).

**Detection Limit.** Detection limits were obtained experimentally by injecting successive dilutions of samples containing mixtures of the analytes. The concentration that provided a signal/noise ratio of three corresponded to the LOD value. The LODs for acetylsalicylic acid, atenolol, acetaminophen, diclofenac, and ibuprofen were 5, 5, 2.5, 1.7, and 2 ppb, respectively. As expected, LODs were improved between 60 and 100 times compared to the LOD obtained by direct injection of the analytes in the chromatographic (2 μL) as

<table>
<thead>
<tr>
<th>Analyte</th>
<th>%RSD run-to-run</th>
<th>%RSD capillary-to-capillary</th>
</tr>
</thead>
<tbody>
<tr>
<td>acetylsalicylic acid</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>atenolol</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>acetaminophen</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>diclofenac</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>ibuprofen</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

*C* 15, 15, 10, 6, and 6 μg/L for acetylsalicylic acid, atenolol, acetaminophen, diclofenac, and ibuprofen, respectively. *b* 50 μg/L for each analyte.
quantitative extraction efficiencies were achieved with the newly magnetic-IT-SPME. However, these LODs can be lowered using more sensitive detection techniques such as capillary LC-MS or MS-MS.

CONCLUSION

The use of the IT-SPME approach coupled to magnetic fields has given rise to the development of a new analytical approach, namely, magnetic-IT-SPME. In this work, we have demonstrated that quantitative extraction efficiencies can be achieved with this new approach.

Thus, it has been demonstrated that the application of an external magnetic field in a SiO2 supported Fe3O4 nanoparticle deposition capillary significantly improves the extraction efficiencies of acetylsalicylic acid, atenolol, acetaminophen, diclofenac, and ibuprofen, yielding to extraction capacities between 70 and 100%, which are much higher than those reached using an IT-SPME approach (in the range of 10–30%).

The reason for this improvement is undoubtedly related with the magnetization of the isolated superparamagnetic Fe3O4 nanoparticles when a magnetic field is applied. Thus, in the case of the IT-SPME process, analytes are injected into the capillary column and follow a hydrodynamic flow in the x-direction and partial adsorption of the analytes occurs. If a magnetic field is applied, the nanoparticles are magnetized creating around them regions with different magnetic field gradients. The diamagnetic analytes tend to be trapped in the regions in which this field gradient is minimal, increasing thus the adsorption of the analytes inside the interconnecting network created by the adsorbent SiO2 phase. This trapping seems to be strong, as in the presence of an external magnetic field the analytes did not release from the adsorbent phase by exclusively using the elutropic capacity of the mobile phase. For the desorption process, the magnetization of the nanoparticles need to be inverted by changing the polarity of the applied magnetic field. During the application of this external stimulus, the magnetic forces in the vicinity of the nanoparticles undergo rapid changes (for example, a rapid demagnetization/magnetization process). These rapid changes in the magnetic strengths lead to a detrapping of the adsorbed analytes causing the desorption of the analytes to the mobile phase, and so to the analytical column, and finally to the detector. Therefore, magnetic-IT-SPME was shown to be a potential alternative to solve one of the main disadvantages of the IT-SPME, the low extraction efficiency, especially for polar compounds, and so it is a promising parameter to include in this extraction technique.

ASSOCIATED CONTENT

3 Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: pilar.camps@mav.es. Tel: 34-96-3543002. Fax: 34-96-3544436.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors are grateful to the European Union (ERC Advanced Grant SPINMOL) and Spanish Ministerio de Economía y Competitividad (projects CTQ 2011-26760, CTQ 2011-26507, MAT2007-51584, MAT2011-22785, and CSD2007-00010) and to the Generalidad Valenciana (Prometeo Program). Y.M.-M. expresses her gratefulness for a JdC research contract.

REFERENCES

(21) Zhang, M.; Wei, F.; Feng, Y. Q.; Nie, J.; Feng, Y. Q. J. Chromatogr., A 2006, 1102, 294–301.