Evaluation of bi-functionalized mesoporous silicas as reversed phase/cation-exchange mixed-mode sorbents for multi-residue solid phase extraction of veterinary drug residues in meat samples

Natalia Casado, Damián Pérez-Quintanilla, Sonia Morante-Zarcero, Isabel Sierra*

Departamento de Tecnología Química y Energética, Tecnología Química y Ambiental, Tecnología Mecánica y Química Analítica, E.S.C.E.T, Universidad Rey Juan Carlos, C/ Tulipán s/n, 28933 Móstoles, Madrid, Spain

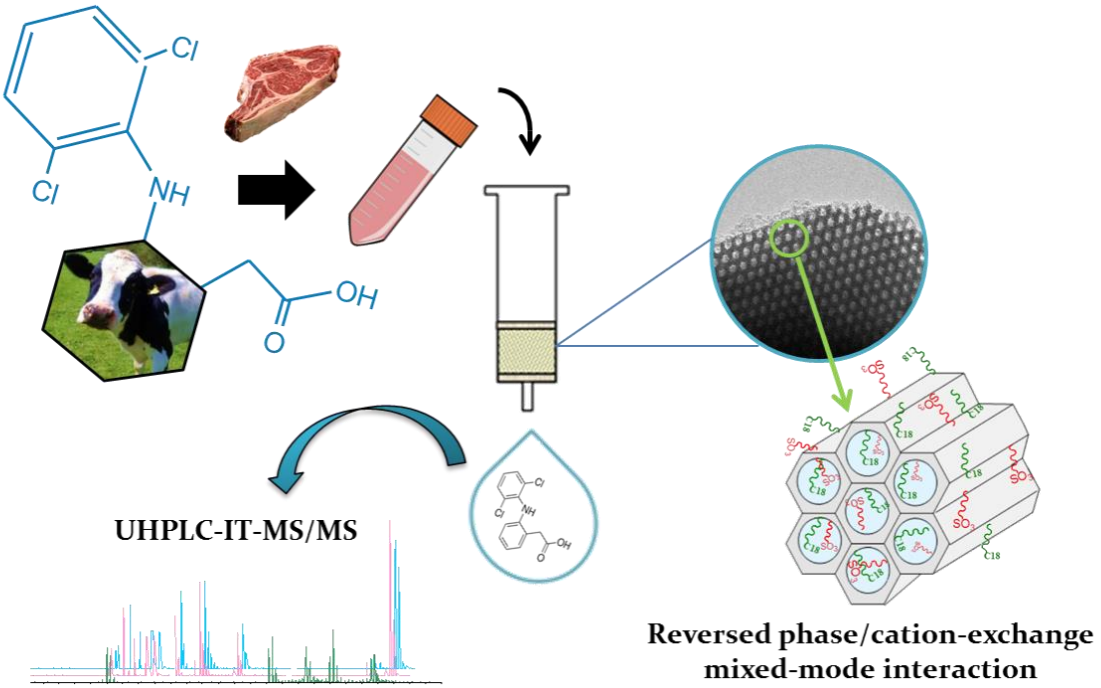
***** Corresponding author: Tel.: (+34) 914887018; fax: (+34) 914888143.

E-mail address: isabel.sierra@urjc.es

ABSTRACT

A SBA-15 type mesoporous silica was synthesized and bi-functionalized with octadecylsilane (C18) or octylsilane (C8), and sulfonic acid (SO_3^-) groups in order to obtain materials with reversed-phase/strong cation-exchange mixed-mode retention mechanism. The resulting hybrid materials (SBA-15-C18-SO_3^- and SBA-15-C8-SO_3^-) were comprehensively characterized. They showed high surface area, high pore volume and controlled porous size. Elemental analysis of the materials revealed differences in the amount of C18 and C8. SBA-15-C18-SO_3^- contained 0.19 mmol/g of C18, while SBA-15-C8-SO_3^- presented 0.54 mmol/g of C8. The SO_3^- groups anchored to the silica surface of the pore walls were 0.20 and 0.09 mmol/g, respectively. The bi-functionalized materials were evaluated as SPE sorbents for the multi-residue extraction of 26 veterinary drug residues in meat samples using ultra-high-performance liquid chromatography coupled to mass spectrometry detector (UHPLC-MS/MS). Different sorbent amounts (100 and 200 mg) and organic solvents were tested to optimize the extraction procedure. Both silicas showed big extraction potential and were successful in the extraction of the target analytes. The mixed-mode retention mechanism was confirmed by comparing both silicas with SBA-15 mesoporous silica mono-functionalized with C18 and C8. Best results were achieved with 200 mg of SBA-15-C18-SO_3^- obtaining recoveries higher than 70% for the majority of analytes.

Keywords: bi-functionalized mesoporous silicas, reversed phase/cation-exchange mixed-mode sorbents, veterinary drug residues, meat, ultra-high performance liquid chromatography


Cl
Cl
NH
OH
O
UHPLC-IT-MS/MS
Reversed phase/cation-exchange
mixed-mode interaction

1. Introduction

Veterinary drugs are considered a type of so-called “emerging contaminants”, thus their presence in food products has been one of the main concerns of food safety during the last years. Although they are generally administered in animal production with a therapeutic purpose, sometimes they can be used fraudulently due to their action as growth promoters [1-4]. Therefore, to ensure food safety the European Union (EU) has established maximum residue limits (MRLs) and recommended concentrations (RC) for these substances in products of animal origin [5-6].

Determination of veterinary drug residues in animal edible tissues constitutes a difficult task, since normally they occur at very low concentration levels (µg/kg to ng/kg) and are subjected to interferences or inhibited due to the complex nature of the sample matrix. Thus, a clean-up and pre-concentration step are often needed before analysis. Most of the veterinary drug multi-residue methods published only report the detection of compounds belonging to the same chemical drug family [2-3, 7-12] and there are only few true multiclass multi-residue procedures in meat samples [1, 4, 13-16]. Regarding sample preparation, solid phase extraction (SPE) has widely been used to extract and pre-concentrate veterinary drug residues from meat solvent extracts [1, 7-9, 13-15] due to its high recovery, low solvent consumption and easy operation. One of the challenges when developing a multi-residue method is to select a suitable sorbent that allows extracting a wide range of compounds of different nature. To solve this problem, mixed-mode reversed-phase/ion-exchange sorbents are specifically designed to interact with ionic species by combining effective reversed-phase chemistry with ion-exchange groups (acidic or basic groups). Therefore, many mixed-mode commercial polymeric or silica-based sorbents (such as Oasis MAX, Oasis MCX, Bond Elut Certify, etc.) have been used for the extraction of veterinary drug residues in food samples [1, 7-8, 16-18].

Recent advances in the development of new materials as SPE sorbents have had a direct and lasting impact on sample preparation. In this sense, some authors have developed new mixed-mode silica-based materials and have applied them as sorbents for the extraction of organic contaminants in different matrices [19-22]. In recent years, mesoporous silicas have gained increasing research interest in sample preparation due to their textural properties, since they present uniform and ordered arrangement, high pore volume and high surface area. In addition, their high flexibility in functionalization enables the introduction of hydrophilic, hydrophobic, polar a well as charged functional moieties on their surface, which allow an efficient and selective extraction of the target analytes [23-24]. For all these reasons, mesoporous silicas could be used as excellent SPE sorbents and may be a good alternative to classical sorbents, such as amorphous silica and polymeric materials, to prepare mixed-mode sorbents for this purpose.

Therefore, the aim of this work was to prepare novel mixed-mode sorbents for the multi-residue extraction of veterinary drug residues in meat samples. SBA-15 mesoporous silica was bi-functionalized in order to obtain hybrid silicas with reversed-phase/strong cation-exchange mixed-mode retention mechanism. Results were compared with mono-functionalized silicas with reversed-phase retention mechanism (C8 and C18 groups). Then, a SPE method was proposed for the simultaneous determination of 26 drugs in meat using UHPLC-MS/MS. To best of our knowledge this is the first time that bi-functionalized mesoporous silicas are prepared and applied as reversed-phase/strong cation-exchange mixed mode sorbents in the preparation of food samples.

2. Experimental

2.1. Chemicals, reagents and standard solutions

Tetraethylorthosilicate (TEOS) 98%, poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (EO20PO70EO20, Pluronic 123) Chloro(dimethyl)octadecylsilane (C18), chloro(dimethyl)octylsilane (C8), toluene, and diethylic ether were purchased from Sigma - Aldrich (St. Louis, MO, USA). Acetonitrile (ACN) and methanol (MeOH) LC-MS grade, hydrochloric acid 35% (HCl), hydrogen peroxide 30% (H_2O_2), ammonia solution 32%, and sodium hydroxide (NaOH) were obtained from Scharlab (Barcelona, Spain). (3-mercaptopropyl)triethoxysilane 94 % was from Alfa Aesar (Karlsruhe, Germanny). Trichloroacetic acid (TCA) was purchased from VWR Chemicals (Radnor, PA, USA). Formic acid and ammonium acetate LC-MS grade were from Fluka (Busch, Switzerland). Sodium chloride (NaCl), sodium acetate trihydrate and ethanol were from Panreac Química (Castellar del Vallès, Bacerlona, Spain). Water (resistance 18.2 MΩ cm) was obtained from a Millipore Milli-Q-System (Billerica, MA, USA).

All pharmaceutical standards used were of high-purity grade ≥ 98%. Cimaterol, terbutaline acetate salt hemihydrates, clenproperol, ractopamine hydrochloride, mabuterol hydrochloride, carazolol, naproxen, diclofenac sodium salt, flunixin, tolfenamic acid, carprofen, and vedaprofen were purchased from Fluka (Busch, Switzerland). Salbutamol, atenolol, acebutolol hydrochloride, betaxolol hydrochloride, metoprolol tartrate salt, clenbuterol hydrochloride, brombuterol hydrochloride, tulobuterol hydrochloride, labetalol hydrochloride, propranolol hydrochloride, α-zearalanol, ketoprofen, meloxicam, and ibuprofen were supplied by Sigma-Aldrich (St. Louis, MO, USA).

Stock standard solutions (1000 mg/L) were prepared by diluting in MeOH adequate amounts of each compound and stored at -20 ºC. Working solutions (40 μg/L – 20 mg/L)

were prepared by appropriate dilution of the stock solutions with MeOH and were stored at 2 -10 ºC.

2.2.Synthesis of mesoporous silicas

SBA-15 mesoporous silica was prepared according to Pérez-Fernández et al. [25]. For the synthesis of hybrid mesoporous silicas, the obtained SBA-15 was firstly functionalized with C8 or C18. For this purpose, 8 g of SBA-15 were heated at 150 ºC for 20 h and a 15% of C8 or C18 was added with respect to the mass of SBA-15. The mixture was heated at 80 ºC for 24 h at 500 rpm. Bi-functionalized mesoporous silicas, SBA-15-C8-SO_3^- and SBA-15-C18-SO_3^-, were prepared using 4 g of the obtained SBA-15-C8 and SBA-15-C18 respectively, and adding 4 mL of (3-mercaptopropyl)triethoxysilane stirring at 80 ºC for 24 h. The material was recovered by filtration and was washed with two fractions of 50 mL of toluene, ethanol, and ethylic ether. Then, the solid was oxidized adding 120 mL of H_2O_2 (30% v/v); then it was stirred for 48 h at room temperature. Finally, it was filtered and washed with water and ethanol to remove the excess of H_2O_2.

2.3.Characterization of mesoporous silicas

Mesoporous silicas were characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), nitrogen adsorption-desorption isotherms, ^{29}Si solid-state nuclear magnetic resonance spectroscopy (CP-MAS-NMR), elemental analysis and thermogravimetric analysis. To verify the presence of SO_3^- moieties in the bi-functionalized mesoporous silicas, an acid-base titration was performed according to Margolese et al. [26] (For details see SM1 in Appendix A).

2.4. Preparation of meat samples

Prepacked preparations of minced bovine meat with a 10-20 % fat (w/w) according to their labels were purchased from randomly chosen supermarkets in Madrid and stored at -20 ºC until analysis. The meat samples were analyzed with our previous validated method [27], in order to verify that the analyte concentration of these samples was under the MQLs (method quantification limits) of the method. Then, meat samples were spiked with the working standard solutions prior to the extraction procedure in order to obtain the target screening concentration as follows [28]: (a) For substances with an established MRL, the MRL was chosen as the spiked level, (b) For unauthorized substances, permitted substances, and unregulated substances with and without RC, the spiked level was defined as a "specific level of interest" based on RC levels or based on the drug characteristics and its detection in the method. Although a MRL has been established for carprofen, it was spiked at a concentration level below its MRL to prevent the amount of compound detected being outside the range for which the ion trap is linear and to prevent overloading of the analytical column (see the spiked level for each analyte in Table 1). The samples which were verified to be analyte-free were spiked with the standard solution and vortexed for 2 min for homogenization. A 30-min period was allowed for equilibration at room temperature according to Gentilli et al. [11] prior to the sample extraction procedure.

2.5. Sample extraction procedure

The sample treatment involved first a solvent extraction procedure followed by SPE. For the evaluation of bi-functionalized mesoporous silicas as SPE sorbents, the optimized extraction procedure of the target analytes was as follows: 2 g of spiked minced meat were mixed with 10 mL of 5% TCA, the mixture was vortexed for 1 min, then centrifuged at 3500 rpm for 10 min. The supernatant was collected, and the residue was extracted again with 5 mL

of 5% TCA and 5 mL of ACN, vortexed for 1 min and centrifuged at 5000 rpm for 10 min. The resultant supernatant was collected with the previous one, and then filtered under vacuum for subsequent purification by SPE. SPE cartridges (65 mm length, 11 mm diameter) were packed with 100 or 200 mg of SBA-15-C18-SO_3^- or SBA-15-C8-SO_3^- and plugged with polyethylene frits at both ends. To prevent the material lost during sample loading, a 0.45 µm pore size nylon filter membrane was also inserted at the bottom of the mesoporous silica bed. Cartridges were initially conditioned by passing 2 x 2 mL MeOH and 2 x 2 mL 5% TCA at a flow rate of 1 mL/min. Once the entire extract was loaded, cartridges were dried with a Supelco Visiprep™ DL solid phase extraction vacuum manifold 12 port model (Sigma Aldrich, St. Louis, MO, USA) connected to a vacuum pump at 7.6 psi, and then washed with 1 x 5 mL acetate buffer 0.2 M (pH 5.2) to remove interferences. Elution was performed by passing 2 mL ACN, 2 mL MeOH and 2 x 2 mL MeOH containing 3% ammonia (pH 9.6). Finally, the eluate was evaporated to dryness and re-dissolved with 500 µL of MeOH for subsequent analysis in the chromatographic system. For the evaluation of SBA-15-C8 and SBA-15-C18 as SPE sorbents, the extraction procedure was performed according to our previous work [27]. SPE cartridges were prepared as indicated above, but packing them with 100 or 200 mg of SBA-15-C8 or SBA-15-C18.

In each set of experiments, four different samples were extracted: three of them were meat samples spiked with the 26 analytes, and the other one was a simulated sample prepared in the same way but spiked with the analytes at the end of the sample treatment process. The recoveries obtained in each experiment were calculated by comparison of the areas of the meat samples with the areas of the simulated sample.

2.6.Chromatographic analysis

Chromatographic separation was performed according to our previous work [27] on an UHPLC system (Dionex UltiMate 3000, Thermo Scientific, MA, USA) connected to an ion trap mass spectrometer detector (Bruker). Separation was achieved using an ACE Excel 2 C18 column (100 mm x 2.1 mm, 2 µm particle size, ACE, UK) at 30 ºC. The flow rate was 0.3 mL/min and the injection volume was 10 µL. Separation conditions were as follows: ACN (A) and H_2O (B), both containing 0.1% formic acid and 4 mM ammonium acetate. The initial composition was 20% A and 80% B. For the gradient elution phase A increased linearly to 100% in first 6 min, then returned to initial conditions in 2 min, and was equilibrated for 2 min before the next injection. The total run-time of the method was 10 min. Mass spectrometry acquisition was carried out using electrospray ionization interface (ESI) operating in both positive and negative ion mode. The capillary voltage was held at -4500V, and the end plate offset at -500V. The nebulizer was set at 20 psi, the dry gas at 10 L/min, and the dry temperature at 200 ºC. Multiple reaction monitoring (MRM) mode was used for all analytes. Table 1 lists the retention time, the product ions (daughter ions and granddaughter ions) and the fragmentation amplitude selected for each compound during MRM acquisition.

Table 1. Mass spectrum parameters, retention time and spiked level for the target analytes using the developed UHPLC-IT-MS/MS method.

Analyte	Spiked level (µg/Kg)	Ionization mode	Retention time (min)	Precursor ion (*m/z*)	Fragmentation amplitude	MS^2. Daughter ions [1] (*m/z*)	Fragmentation amplitude	MS^3. Granddaughter ions (*m/z*)
Cimaterol	5[a]	ESI (+)	1.4	220	0.60	202*, 160		
Terbutaline	10[b]	ESI (+)	1.4	226	0.70	170, 152*		
Salbutamol	5[b]	ESI (+)	1.4	240	0.60	222*, 166		
Atenolol	5[a]	ESI (+)	1.4	267	0.50	225, 190*		
Ractopamine	10[c]	ESI (+)	1.6	302	0.50	284*, 164		
Clenproperol	5[a]	ESI (+)	1.8	263	0.70	245*, 203		
Acebutolol	5[a]	ESI (+)	1.8	337	0.60	319*, 260		
Metoprolol	5[a]	ESI (+)	2.1	268	0.60	191*, 116		
Tulobuterol	0.2[a]	ESI (+)	2.1	228	0.70	172, 154*		
Clenbuterol	0.2[c]	ESI (+)	2.2	277	0.60	259*, 203		
Brombuterol	5[a]	ESI (+)	2.6	367	0.70	349*, 293		
Carazolol	5[c]	ESI (+)	2.9	299	0.70	222*, 116		
Labetalol	5[a]	ESI (+)	2.9	329	0.50	311*, 207		
Mabuterol	5[a]	ESI (+)	2.9	311	0.70	293*, 237		
Propranolol	5[a]	ESI (+)	3.4	260	0.50	183*, 116		
Betaxolol	5[a]	ESI (+)	3.5	308	0.70	177, 116*		
α-Zearalanol	2[c]	ESI (-)	4.3	321	0.70	303, 277*		
Ketoprofen	5[a]	ESI (+)	4.9	255	0.50	209*	0.70	194,131, 105
Naproxen	10[b]	ESI (+)	4.9	231	0.80	185*	0.70	170
Meloxicam	20[c]	ESI (+)	4.9	352	0.60	141, 115*		
Flunixin	20[c]	ESI (+)	5.2	297	0.70	279*, 257		
Carprofen	50[a]	ESI (-)	5.6	272	0.60	228*	0.40	226
Diclofenac	5[c]	ESI (-)	5.8	294	0.70	250*	0.70	214, 178
Ibuprofen	100[a]	ESI (-)	6.1	205	1.00	161*, 159		
Tolfenamic acid	50[c]	ESI (-)	6.7	260	0.50	216*	0.60	180
Vedaprofen	50[c]	ESI (+)	7.3	300	1.00	201, 155*		

[1] Predominant product ions. * Ions used for quantitation. Isolation width (*m/z)* is 4. [a] Specific level of interest based on the drug characteristics and its detection in the ion trap mass spectrometer and in the analytical method.[b] Recommended concentration (EURL requirements). [c] Maximum Residue limit (MRL). Chromatographic conditions with the optimized gradient elution: t = 0 min, 20% A – 80% B, t = 6 min, 100% A, t = 8 min, 20% A – 80% B (2 min) (ACN as mobile phase A and water as mobile phase B, both containing 0.1% formic acid and 4 mM ammonium acetate). The flow rate was 0.3 mL/min.

3. Results and discussion

3.1. Characterization of mesoporous silicas

XRD pattern of the SBA-15 showed that this material display well-resolved pattern at low 2θ values, with a very sharp (100) diffraction peak around 0.92 Å and two well-resolved weak diffraction peaks (110) at 1.55 Å and (200) at 1.81 Å, that indicates a 2D ordered hexagonal mesostructure. The XRD pattern of the functionalized silicas also suggested the maintenance of the structural orderness of the synthesized material after functionalization. TEM micrographs of the prepared silicas confirmed a clear arrangement of hexagonal pores with uniform size. From SEM images, uniform particles with cylindrical shape (1 µm in one axis and 450 nm in the other) could be observed. These results indicated that the modification of the SBA-15 did not affect the morphology or pore distribution of the materials. Fig. 1 shows TEM and SEM images of SBA-15 and SBA-15-C18-SO_3^-.

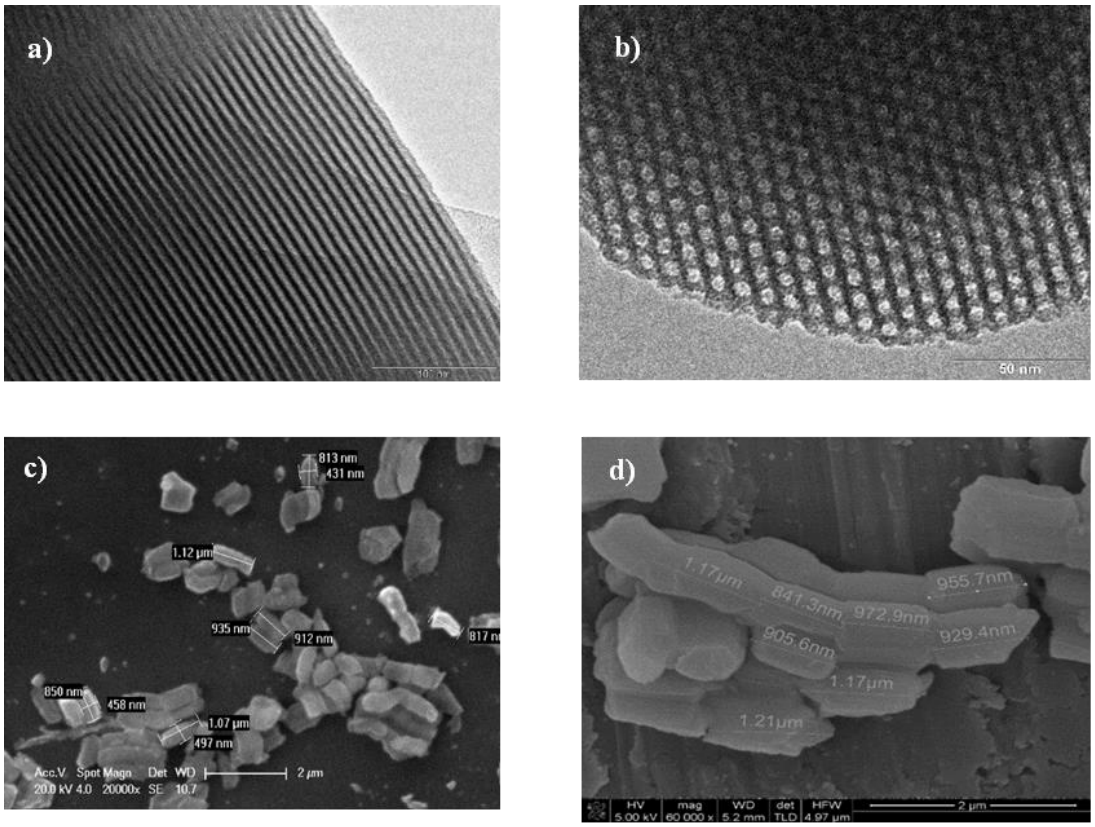


Fig. 1. TEM images of **a)** SBA-15 and **b)** SBA-15-C18-SO_3^-. SEM images of **c)** SBA-15 and **d)** SBA-15-C18-SO_3^- .

N_2 adsorption-desorption isotherms for all the synthesized silicas were of type IV, according to the I.U.P.A.C. classification, with an H1 hysteresis loop that is representative of materials with pores of constant cross-section (see SM2 in Appendix A). As it can be seen in Table 2, the surface area and pore volume of the mono and bi-functionalized materials decreased after the modification procedure from 849 m^2/g and 0.85 cm^3/g, respectively, in the SBA-15 to 542 m^2/g and 0.59 cm^3/g, respectively, in the SBA-15-C8. On the other hand, the pore diameter distribution of the materials, before and after modification procedure, was quite narrow centered in values around 50 Å in all the cases (see SM3 in Appendix A). This fact can be attributed to the presence of the C18, C8 and SO_3^- group moieties on the silica surface instead on the interior of the mesopores. Fig. 2 shows N_2 adsorption-desorption isotherms of SBA-15 and SBA-15-C18-SO_3^-.

Table 2. Textural properties and functionalization degrees of the synthesized materials.

Material	Surface Area $_{BET}$ (m^2/g)	Pore Volume (cm^3/g)	Pore Diameter (BJH)[a] (Å)
SBA-15	849	0.85	48.7
SBA-15-C18	654	0.71	48.7
SBA-15-C8	542	0.59	50.3
SBA-15-C18-SO_3^-	619	0.69	49.3
SBA-15-C8-SO_3^-	617	0.66	48.3

[a] Barret-Joyner-Halenda

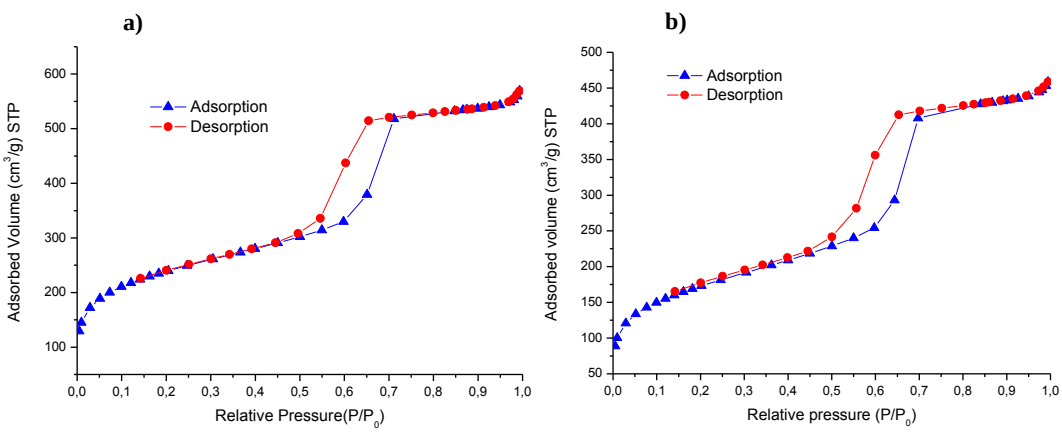


Fig. 2. N2 adsorption-desorption isotherms of a) SBA-15 and b) SBA-15-C18-SO3-.

The elemental analysis performed on the materials revealed different functionalization degree in the mesoporous silicas. Thus, regarding the % C in the mono-functionalized materials, it was estimated the presence of 0.73 mmol/g of C8 and 0.24 mmol/g or C18 attached on the SBA-15-C8 and SBA-15-C18, respectively. These results revealed that the functionalization with C8 group moieties allows a higher functionalization degree of the silica in comparison with the functionalization with C18, probably as a result of the shorter chains of C8. On the other hand, regarding de % C and % S in the bi-functionalized mesoporous silicas, it was estimated the presence of 0.19 mmol/g of C18 and 0.27 mmol/g of SO_3^- in the SBA-15-C18-SO_3^- and 0.54 mmol/g of C8 and 0.12 mmol/g of SO_3^- in the SBA-15-C8-SO_3^-. However, tritration analyses for the determination of SO_3^- groups in the modified silicas showed a conversion rate of 75 and 73% for SBA-15-C18-SO_3^- and SBA-15-C8-SO_3^- respectively, which are similar to the conversion rate (77%) reported by Margolese et al. [26]. Therefore, the concentration of SO_3^- in both silicas was recalculated and found to be 0.20 and 0.09 mmol/g for SBA-15-C18-SO_3^- and SBA-15-C8-SO_3^-, respectively. It is noteworthy to mention that in the SBA-15-C8-SO_3^- a reduction in the amount of C8 groups (besides an increase in the surface area and pore volume) was observed after the bi-functionalization procedure, in comparison with the SBA-15-C8 silica (Table 2). This fact could be explained as a consequence of the acid treatment carried out in the bi-functionalized material to obtain the SO_3^- groups that could cause partial leaching of the C8 organic chains.

The ^{29}Si MAS-NMR spectrum in the solid state for materials confirmed the covalent bond formed between the C8 or C18 ligand and the silanol groups dispersed on the SBA-15 surface (Fig. 3 and SM4 in Appendix A). Finally, TGA of the materials (see SM5 in Appendix A) showed that an exothermic degradation process occurred between 250-600ºC with a weight loss around 8-9% that demonstrated the good thermal stability of the material.

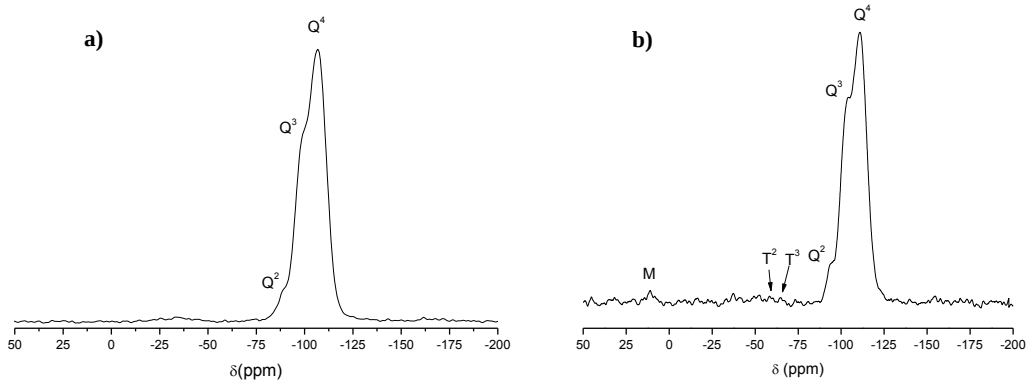


Fig. 3. ^{29}Si NMR spectra of a) SBA-15 and b) SBA-15-C18-SO_3^-.

3.2. Method performance with mono-functionalized mesoporous silica.

In our previous work [27] we developed and optimized a multi-residue method for the determination of 23 veterinary drug residues in meat samples using a SBA-15-C18 mesoporous silica as SPE sorbent. Therefore, the same methodology was followed for the evaluation of the SBA-15-C8 mesoporous silica. The extraction procedure involved a first extraction step with acetate buffer (pH 5.2) followed by a second extraction step using a combination of acetate buffer and MeOH (50:50 v/v). The extract was purified by SPE for subsequent analysis on the UHPLC-IT-MS/MS. The developed method was validated in accordance with the Commission Decision 2002/657/EC [28] for a quantitative screening method in terms of identification, selectivity, linearity, decision limit, detection capability, accuracy and precision.

Table 3 shows the recovery percentages for the 26 veterinary drug residues using SBA-15-C8 and SBA-15-C18 mesoporous silicas as SPE sorbents. As it can be observed, good recoveries values were found for the majority of the analytes employing 100 mg of SBA-15-C8, except for the most polar compounds, such as cimaterol, terbutaline, salbutamol, atenolol and ractopamine. Comparing these results with the ones previously reported for the SBA-15-

C18 mesoporous silica [27] it can be noticed that functionalization with C18 is more effective for the extraction of the most polar β-agonists and β-blockers, while C8 significantly improves the extraction of the less polar NSAIDs, such as carprofen, ibuprofen, tolfenamic acid and vedaprofen. This fact could be due to the differences in the amount of C18 and C8 groups attached on the surface of the mesoporous silicas. The L_0 of SBA-15-C8 (0.73 mmol/g) is approximately three times the L_0 of SBA-15-C18 (0.24 mmol/g), therefore there are more hydrophobic interactions in the SBA-15-C8 that enhance the retention of the less polar compounds. Nevertheless, the high L_0 of the SBA-15-C8 leads to a lesser amount of residual non-modified silanol groups in the silica surface in comparison with SBA-15-C18, thus there are less polar secondary interactions (by hydrogen bonding interactions) and consequently the retention of the most polar analytes is reduced. In this context, the amount of sorbent packed in the cartridges was increased and the SPE procedure was performed with 200 mg of SBA-15-C8 and 200 mg of SBA-15-C18 (Table 3). Recoveries values using 200 mg of SBA-15-C8 barely changed in comparison with the ones obtained with 100 mg, and the same trend in the recoveries of the target analytes was observed. Nevertheless, with 200 mg of SBA-15-C18 the recoveries of NSAIDs improved when compared to use 100 mg of this material, since there were more C18 groups available for hydrophobic interactions; however no significant changes were found in the recoveries achieved for β-agonists, β-blockers and α-zearalanol. These results underline that the amount of C8 or C18 is important to achieve the highest possible recoveries of the less-polar compounds, as well as the benefit of the presence of silanol groups on the surface of mesoporous materials that favors the retention of polar compounds.

Table 3. Recovery percentages obtained for the 26 veterinary drug residues in spiked meat samples using 100 mg and 200 mg of SBA-15-C18 and SBA-15-C8 mesoporous silicas as SPE sorbents (n = 6).

Analyte	SBA-15-C18		SBA-15-C8	
	% Recovery (RSD, %)		% Recovery (RSD, %)	
	100 mg*	200 mg	100 mg	200 mg
Cimaterol	96 (11)	90 (9)	46 (15)	49 (14)
Terbutaline	27 (27)	24 (12)	15 (19)	9 (14)
Salbutamol	67 (4)	64 (8)	25 (14)	27 (11)
Atenolol	75 (13)	77 (3)	67 (5)	66 (8)
Ractopamine	63 (23)	62 (7)	56 (17)	51 (9)
Clenproperol	94 (13)	91 (2)	91 (16)	95 (2)
Acebutolol	79 (7)	73 (9)	100 (4)	97 (5)
Metoprolol	89 (8)	89 (2)	96 (2)	95 (5)
Tulobuterol	94 (4)	92 (4)	85 (8)	89 (7)
Clenbuterol	92 (6)	97 (6)	97 (8)	96 (6)
Brombuterol	81 (9)	85 (3)	109 (7)	100 (3)
Carazolol	80 (8)	84 (2)	92 (4)	95 (5)
Labetalol	91 (4)	90 (2)	98 (3)	96 (3)
Mabuterol	89 (10)	88 (2)	104 (3)	98 (5)
Propranolol	92 (8)	90 (11)	89 (3)	89 (4)
Betaxolol	83 (1)	86 (12)	102 (2)	97 (6)
α-Zearalanol	77 (8)	73 (8)	105.0 (0.2)	95 (6)
Ketoprofen	92 (4)	93 (3)	60 (9)	89 (9)
Naproxen	56 (17)	65 (5)	54 (3)	55 (9)
Meloxicam	83 (8)	97 (4)	75 (15)	91 (7)
Flunixin	81 (16)	92 (7)	87 (15)	84 (5)
Carprofen	38 (10)	75 (12)	80 (5)	90 (7)
Diclofenac	86 (15)	92 (6)	96 (2)	97 (5)
Ibuprofen	57 (33)	70 (10)	81 (4)	83 (4)
Tolfenamic acid	50 (31)	80 (11)	83 (6)	83 (7)
Vedaprofen	27 (13)	54 (2)	55 (1)	68 (12)

*Recovery values reported in the work Casado et al. [27] except for acebutolol, betaxolol and α-zearalanol.

3.3. Method performance with bi-functionalized mesoporous silicas

Bi-functionalized mesoporous silicas were prepared with the aim of improving the results obtained with the mono-functionalized mesoporous silicas in order to achieve good recoveries values for all the target analytes, and therefore to synthesized sorbents that allow to develop accurate and precise multi-residue methods for the determination of veterinary drug residues of different nature. NSAIDs are acidic compounds with pKa values of 3-5, while β-blockers, β-agonists and α-zearalanol are basic compounds with pKa values in the range of 8-10, thus SBA-15 mesoporous silica was bi-functionalized with C18 or C8, and SO_3^- groups in order to obtain hybrid mesoporous silicas with a reversed-phase/strong cation-exchange mixed-mode retention mechanism. In this context, under acidic conditions, basic compounds may experience a cation-exchange interaction with the SO_3^- groups, while acidic compounds might have a reversed-phase interaction with the C18 or C8 groups of the silica. For the evaluation of these bi-functionalized mesoporous silicas, SBA-15-C18-SO_3^- and SBA-15-C8-SO_3^-, the extraction procedure and the SPE performance were optimized. First, the extraction procedure developed and optimized in our previous work was tested [27], using a combination of acetate buffer 0.2 M (pH 5.2) and MeOH in order to achieve extraction under acidic conditions. However, the recoveries values obtained were unsatisfactory (7-62 %). In some works ACN has been proposed as extraction solvent since it affords protein precipitation and it is more acidic than MeOH [4, 14-15, 29-30]. Hence, a combination of acetate buffer 0.2 M (pH 5.2) and ACN was also tested. Recoveries values improved with ACN, but in general they remained low for β-agonists and β-blockers (24-64 %). Therefore, in view of these results a more drastic acid pH was tested. For this purpose, TCA was the best choice, since it acts as extraction reagent due to its strong acidity and as denaturing agent providing protein precipitation in matrices of animal origin. Sai et al. [1] evaluated different concentrations of TCA for the extraction of β-blockers and β-agonists in meat samples for subsequent SPE with mixed-mode cation-exchange sorbents, and in the end the concentration 5% TCA was

selected as the most appropriate extraction solvent. Therefore, a first extraction step with 10 mL of 5% TCA was performed, followed by a second extraction step using a combination of 5% TCA and ACN (50:50 v/v). Acceptable recoveries values of β-agonists and β-blockers were found (Table 4), thus these combinations were selected for the extraction procedure.

The SPE procedure was designed according to previous works described in the literature which used mixed-mode strong-cation exchange SPE sorbents [1-2, 7-8, 18, 31-35]. All of them used acidic conditions for the extraction of analytes. In this sense, basic compounds such as β-blockers and β-agonists, under acidic conditions are positively charged and therefore, they are able to strongly interact with the SO_3^- moieties of the silica surface, while acidic compounds such as NSAIDs, could be retained at that pH by the reversed phase (C18 or C8 groups). For the elution step, acidic compounds can be easily eluted with MeOH or ACN, but basic compounds need to be desorbed with alkaline organic solvents in order to break the strong cation exchange interactions with the sorbent. For this purpose, authors usually used ammonium hydroxide in MeOH at different concentration levels (5%, 3% and 2%) [1-2, 7, 18, 32-34]. Accordingly, for the SPE elution step 2 mL of ACN followed by 2 mL of MeOH were passed through the cartridge for the elution of acidic compounds, and afterwards basic compounds were eluted with 4 mL of MeOH with 3% of ammonia solution (pH 7.4). However, poor recoveries were achieved for β-agonist and β-blockers (7-64 %), since the elution pH was not enough to desorb these analytes. Therefore, the pH was adjusted, and in the end MeOH containing 3% of ammonia solution (pH 9.6) was found to give the most satisfactory results.

First, the SPE procedure was performed with 100 mg of SBA-15-C18-SO_3^- and 100 mg of SBA-15-C8-SO_3^-. When comparing the results (Table 4), SBA-15-C18-SO_3^- was more effective in the extraction of the most polar compounds, such as cimaterol, terbutaline, salbutamol, atenolol, ractopamine and clenproperol, while SBA-15-C8-SO_3^- improved the

extraction of NSAIDs, which are less polar compounds. This is due to the differences observed in the amount of SO_3^- moieties and C8 and C18 groups attached on the surface of the two bi-functionalized mesoporous silicas. The amount of SO_3^- moieties on the SBA-15-C18-SO_3^- silica surface (0.20 mmol/g) is more than twice times the amount on the SBA-15-C8-SO_3^- (0.09 mmol/g), thus the higher presence of SO_3^- on the SBA-15-C18-SO_3^- enhances the retention of β-agonists and β-blockers, particularly of the most polar ones, whereas the amount of SO_3^- on the SBA-15-C8-SO_3^- surface is not enough to achieve good recoveries. On the other hand, the SBA-15-C8-SO_3^- was more effective in the reverse phase retention, since more C8 groups were attached on the silica surface (0.54 mmol/g) in comparison with the C18 groups attached on the SBA-15-C18-SO_3^- (0.19 mmol/g). The largest number of C8 groups on the mesoporous silica surface improved the retention of NSAIDs, mainly of the less polar ones such as vedaprofen, carprofen, tolfenamic acid and ibuprofen. Therefore, the same trend was observed in the reverse phase retention that the one previously described for the mono-functionalized mesoporous silicas, since the amount of C8 and C18 groups was similar.

Afterwards, in order to achieve the best recovery results, the SPE procedure was performed with 200 mg of SBA-15-C18-SO_3^- and 200 mg of SBA-15-C8-SO_3^-, thus more C18 groups will be available to improve the retention of NSAIDs in the SBA-15-C18-SO_3^-, while the presence of more SO_3^- groups in the SBA-15-C8-SO_3^- may increase the retention of the basic compounds which are positively charged under acidic conditions. By increasing the amount of sorbent, recoveries values improved in both cases (Table 4). With 200 mg of SBA-15-C18-SO_3^- the retention of NSAIDs significantly improved, while 200 mg of SBA-15-C8-SO_3^- enhanced the cation-exchange interaction of β-agonists and β-blockers with SO_3^- moieties, due to the increase in the silica amount used. Therefore, 200 mg were selected as the best amount of sorbent for the SPE since both silicas were successful in the multi-residue extraction and purification of the target analytes; however, when comparing both materials best results were achieved with 200 mg of SBA-15-C18-SO_3^-, since the recoveries values

obtained for the majority of the analytes were higher than 70% (Table 4). These results proved the reversed-phase/strong cation-exchange mixed mode retention mechanism in both bi-functionalized mesoporous silicas.

Table 4. Recovery percentages obtained for the 26 veterinary drug residues in spiked meat samples using 100 mg and 200 mg of SBA-15-C18-SO_3^- and SBA-15-C8- SO_3^- mesoporous silicas as SPE sorbents (n = 6).

Analyte	SBA-15-C18- SO_3^-		SBA-15-C8- SO_3^-	
	% Recovery (RSD, %)		% Recovery (RSD, %)	
	100 mg	200 mg	100 mg	200 mg
Cimaterol	96 (12)	92 (1)	50 (19)	96 (4)
Terbutaline	74 (8)	77 (18)	35 (6)	68 (6)
Salbutamol	84 (7)	103 (3)	36 (6)	83 (6)
Atenolol	72 (1)	103 (8)	45 (16)	88 (13)
Ractopamine	87 (17)	86 (4)	27 (9)	50 (4)
Clenproperol	105 (3)	101 (7)	39 (8)	53 (17)
Acebutolol	54 (16)	73 (17)	45 (9)	58 (12)
Metoprolol	83 (12)	103 (7)	70 (19)	76 (10)
Tulobuterol	73 (17)	82 (2)	70 (17)	85 (10)
Clenbuterol	96 (2)	95 (6)	88 (12)	93 (8)
Brombuterol	89 (5)	101 (4)	90 (6)	85 (1)
Carazolol	91 (14)	90 (17)	76 (8)	79 (11)
Labetalol	64 (13)	72 (11)	44 (10)	66 (5)
Mabuterol	92 (17)	96 (14)	78 (13)	82 (17)
Propranolol	105 (11)	104 (3)	93 (7)	98 (13)
Betaxolol	49 (14)	88 (3)	90 (8)	82 (4)
α-Zearalanol	66 (5)	96 (7)	83 (10)	89 (2)
Ketoprofen	97 (5)	97 (3)	71 (13)	95 (4)
Naproxen	87 (4)	96 (2)	96 (6)	99 (7)
Meloxicam	58 (10)	101 (13)	97 (6)	98 (5)
Flunixin	73 (10)	98 (10)	76 (3)	82 (3)
Carprofen	30 (14)	76 (18)	87 (3)	87 (2)
Diclofenac	98 (8)	98 (4)	99 (11)	101 (1)
Ibuprofen	56 (3)	72 (3)	80 (8)	78 (14)
Tolfenamic acid	32 (3)	89 (4)	76 (6)	78 (5)
Vedaprofen	24 (17)	54 (17)	61 (11)	64 (2)

3.4. Comparison of mono- and bi-functionalized mesoporous silicas

By comparing the results obtained with the mono-functionalized and bi-functionalized mesoporous silicas under the optimized extraction conditions described for each of them (Fig. 4) an improvement in the retention of β-agonists and β-blockers is evidenced as a result of the strong cation-exchange interaction provided by the SO_3^- moieties of the bi-functionalized materials. Best results were achieved when using 200 mg of SBA-15-C18-SO_3^- in comparison with 200 mg of SBA-15-C18 (Fig. 4a), since it clearly improved the retention of basic compounds, principally that of the most polar ones, such as terbutaline, salbutamol, atenolol, ractopamine and clenproperol. On the other hand, SBA-15-C8-SO_3^- also provided better recoveries values for the most polar analytes in comparison with SBA-15-C8 mesoporous silica (Fig. 4b), nevertheless the lesser amount of SO_3^- groups attached on the surface concerned the retention of some basic compounds such as clenproperol, acebutolol, metoprolol, brombuterol, carazolol, labetalol, betaxolol and α-zearalanol. Therefore, it was successfully confirmed the mixed-mode interaction in both bi-functionalized mesoporous silicas.

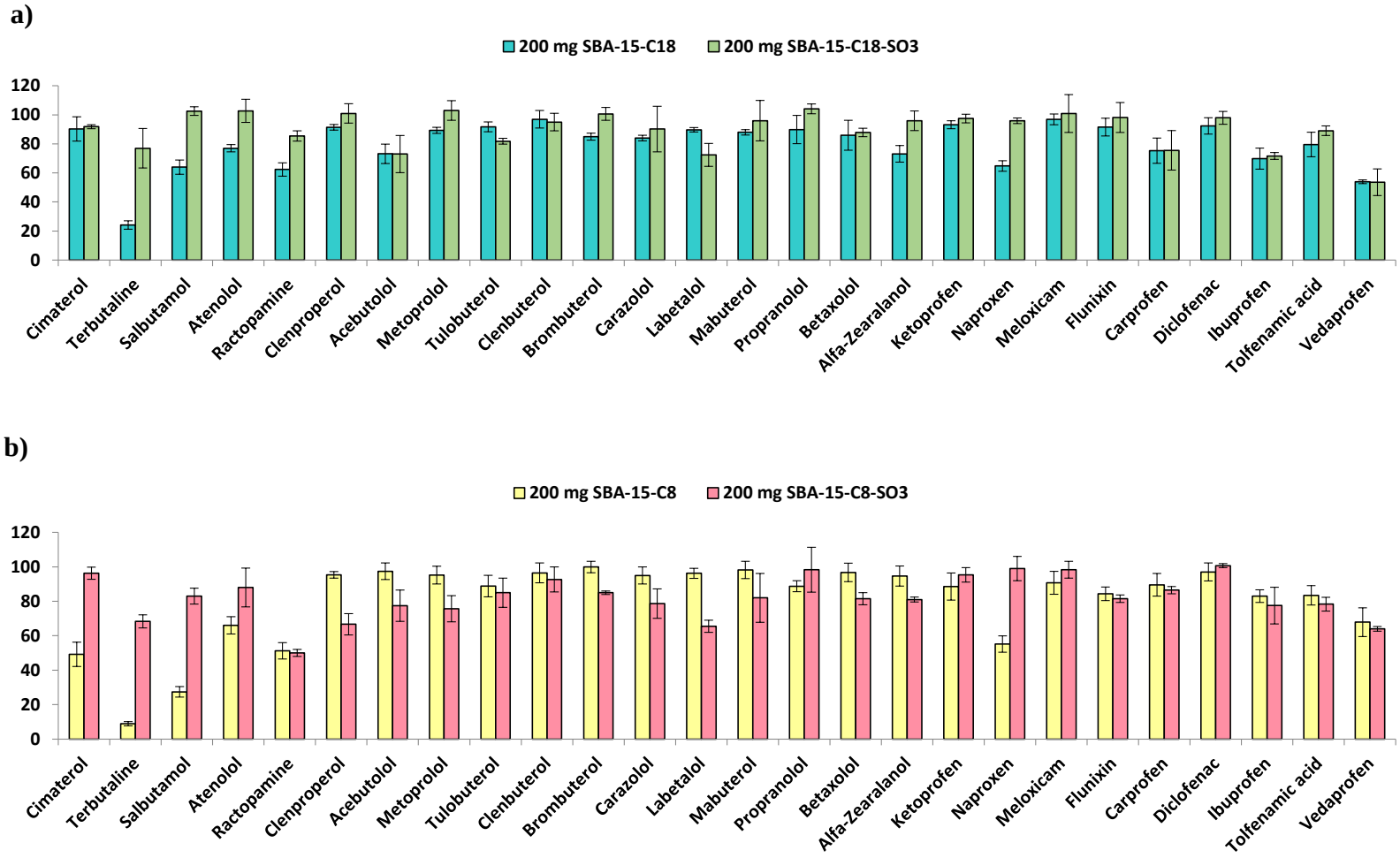


Fig. 4. a) Comparison of the recovery percentages obtained from the analysis of spiked meat samples extracted with SPE cartridges packed with 200 mg of SBA-15-C18 and 200 mg of SBA-15-C18-SO3. **b)** Comparison of the recovery percentages obtained from the analysis of spiked meat samples extracted with SPE cartridges packed with 200 mg of SBA-15-C8 and 200 mg of SBA-15-C8-SO3. Error bars represent the standard deviation of samples replicates (n=6).

4. Conclusion

In this work, a SBA-15 type mesoporous silica was synthesized and bi-functionalized with C18 or C8, and SO_3^- groups in order to obtained novel materials with reversed-phase/strong cation-exchange mixed-mode retention mechanism. The resulting hybrid materials were successfully applied and evaluated as SPE sorbents for the simultaneous extraction of 26 veterinary drug residues (7 β-blockers, 9 β-agonists, 9 NSAIDs and 1 mycotoxin) in meat samples at very low concentration using UHPLC-IT-MS/MS. Results showed that both silicas had a big extraction potential and the mixed-mode interaction was confirmed by comparing them with SBA-15 mesoporous silicas mono-functionalized with C18 and C8. Finally, best results were achieved with SBA-15 mesoporous silica bi-functionalized with C18 and SO_3^- groups, since high recoveries values were achieved for all the target analytes as a result of the hydrophobic and cation-exchange interactions provided by the material.

Acknowledgments

Authors thank financial support from the Comunidad of Madrid and European funding from FEDER program (Project S2013/ABI-3028, AVANSECAL).

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2016.12.057.

References

[1] F. Sai, M. Hong, Z. Yunfeng, C. Huijing, W. Yongning, Simultaneous detection of residues of 25 β_2-agonists and 23 β-blockers in animal foods by high-performance liquid chromatography coupled with linear ion trap mass spectrometry, J. Agric. Food Chem. 60 (2012) 1898-1905.

[2] W. Xiu-Juan, Z. Feng, D. Fei, L. Wei-Qing, C. Qing-Yu, C. Xiao-Gang, X. Cheng-Bao, Simultaneous determination of 12 β-agonists in feeds by ultra-high-performance liquid chromatography-quadrupole-time-of-flight mass spectrometry, J. Chromatogr. A 1278 (2013) 82-88.

[3] T. Peng, A.L. Zhu, Y.N. Zhou, T. Hu, Z.F. Yue, D.D. Chen, G.M. Wang, J. kang, C.L. Fan, Y. Chen, H.Y. Jiang, Development of a simple method for simultaneous determination of nine subclasses of non-steroidal anti-inflammatory drugs in milk and dairy products by ultra-performance liquid chromatography with tandem mass spectrometry, J. Chromatogr. B 933 (2013) 15-23.

[4] C. Robert, N. Gillard, P.Y. Brasseur, G. Pierret, N. Ralet, M. Dubois, P. Delahaut, Rapid multi-residue and multi-class qualitative screening for veterinary drugs in foods of animal origin by UHPLC-MS/MS, Food Addit. Contam. A 30 (2013) 443-457.

[5] Council Regulation 37/2010/EC of 22 December of 2009 on pharmacologically active substances and their classification regarding maximum residues limits in foodstuffs of animal origin, Brussels, Belgium, Official Journal of the European Union, L 15 (2010).

[6] CRL Guidance paper of 7th December 2007, CRLs view on state of the art analytical methods for national residue control plans, p. 1.

[7] B. Shao, X. Jia, J. Zhang, J. Meng, Y. Wu, H. Duan, X. Tu, Multi-residual analysis of 16 β-agonists in pig liver, kidney and muscle by ultra performance liquid chromatography tandem mass spectrometry, Food Chem. 114 (2009) 1115-1121.

[8] C. Liu, W. Ling, W. Xu, Y. Chai, Simultaneous determination of 20 βagonists in pig muscle and liver by high-performance liquid chromatography/tandem mass spectrometry, J. AOAC Int. 94 (2011) 420-427.

[9] T. Hu, T. Peng, X. J. Li, D.D. Chen, H.H. Dai, X.J. Deng, Z.F. Yue, G.M. Wang, J.Z. Shen, X. Xia, S.Y. Ding, Y.N. Zhou, A.L. Zhu, H.Y. Jiang, Simultaneous determination of thirty non-steroidal anti-inflammatory drug residues in swine muscle by ultra-high-performance liquid chromatography with tandem mass spectrometry, J. Chromatogr. A 1219 (2012) 104-113.

[10] N. Van Hoof, K. De Wasch, S. Poelmans, H. Noppe, H. De Brabander, Multi-residue liquid chromatography/tandem mass spectrometry method for the detection of non-steroidal anti-inflammatory drugs in bovine muscle:optimization of ion trap parameters, Rapid Commun. Mass Spectrom. 18 (2004) 2823-2829.

[11] A. Gentili, F. Caretti, S. Bellante, L. Mainero Rocca, R. Curini, A. Venditti, Development and validation of two multiresidue liquid chromatography tandem mass spectrometry methods based on a versatile extraction procedure for isolating non-steroidal anti-inflammatory drugs from bovine milk and muscle tissue, Anal. Bioanal. Chem. 404 (2012) 1375-1388.

[12] P. Gallo, S. Fabbrocino, F. Vinci, M. Fiori, V. Danese, L. Serpe, Confirmatory identification of sixteen non-steroidal anti-inflammatory drug residues in raw milk by liquid chromatography coupled with ion trap mass spectrometry, Rapid Commun. Mass Spectrom. 22 (2008) 841-854.

[13] A. Kaufmann, P. Butcher, K. Maden, M. Widmer, Quantitative multiresidue method for about 100 veterinary drugs in different meat matrices by sub-2μm particulate high-performance liquid chromatography coupled to time of flight mass spectrometry, J. Chromatogr. A 1194 (2008) 66-79.

[14] R.J.B. Peters, Y.J.C. Bolck, P. Rutgers, A.A.M. Stolker, M.W.F. Nielen, Multi-residue screening of veterinary drugs in egg, fish and meat using high-resolution liquid chromatography accurate mass time-of-flight mass spectrometry, J. Chromatogr. A 1216 (2009) 8206-8216.

[15] A. Azzouz, B. Souhail, E. Ballesteros, Determination of residual pharmaceuticals in edible animal tissues by continuous solid-phase extraction and gas chromatography-mass spectrometry, Talanta 84 (2011) 820-828.

[16] J. Chrusch, S. Lee, R. Fedeniuk, J.O. Boison, Determination of the performance characteristics of a new multi-residue method for non-steroidal anti-inflammatory drugs, corticosteroids and anabolic steroids in food animal tissues, Food Addit. Contam. 25 (2008) 1482-1496.

[17] L.D. Williams, M.I. Churchwell, D.R. Doerge, Multiresidue confirmation of β-agonists in bovine retina and liver using LC-ES/MS/MS, J. Chromatogr. B 813 (2004) 35-45.

[18] J. Pleadin, A. Vulic, N. Persi, N. Vahcic, Clenbuterol residues in pig muscle after repeat administration in a growth-promoting dose, Meat Sci. 86 (2010) 733-737.

[19] M.M. Zheng, G.D. Ruan, Y.Q. Feng, Hybrid organic-inorganic silica monolith with hydrophobic/strong cation exchange functional groups as a sorbent for micro-solid phase extraction, J. Chromatogr. A 1216 (2009) 7739-7746.

[20] X. Zhang, H. Niu, Y. Pan, Y. Shi, Y. Cai, Modifying the surface of Fe_3O_4/SiO_2 magnetic nanoparticles with C_{18}/NH_2 mixed group to get an efficient sorbent for anionic organic pollutants, J. Colloid Interface Sci. 362 (2011) 107-112.

[21] Y. Zhu, S. Yang, G. Chen, J. Xing, Single "click" synthesis of a mixed-mode silica sorbent and application in matrix solid-phase dispersion extraction of β-agonists from porcine liver, J. Chromatogr. A 1354 (2014) 101-108.

[22] Y. Li, J. Yang, C. Huang, L. Wang, J. Wang, J. Chen, Dendrimer-functionalized mesoporous silica as a reversed-phase/anion-exchange mixed-mode sorbent for solid phase extraction of acid drugs in humane urine, J. Chromatogr. A 1392 (2015) 28-36.

[23] L. Zhao, H. Qin, R. Wu, H. Zou, Recent advances of mesoporous materials in sample preparation, J. Chromatogr. A 1228 (2012) 193-204.

[24] I. Sierra, D. Pérez-Quintanilla, Heavy metal complexation on hybrid mesoporous silicas: an approach to analytical applications, Chem. Soc. Rev. 42 (2013) 3792-3807.

[25] V. Pérez-Fernández, S. Morante-Zarcero, D. Pérez-Quintanilla, M.A. García, M.L. Marina, I. Sierra Alonso, Evaluation of mesoporous silicas functionalized with C18 groups as stationary phases for the solid-phase extraction of steroid hormones in milk, Electrophoresis 35 (2014) 1666-1676.

[26] D. Margolese, J.A. Melero, S.C. Christiansen, B.F. Chmelka, G.D. Stucky, Direct syntheses of ordered SBA-15 mesoporous silica containing sulfonic acid groups, Chem. Mater 12 (2000) 2448-2459.

[27] N. Casado, S. Morante-Zarcero, D. Pérez-Quintanilla, I. Sierra, Application of a hybrid ordered mesoporous silica as sorbent for solid-phase multi-residue extraction of veterinary drugs in meat by ultra-high-performance liquid chromatography coupled to ion-trap tandem mass spectrometry, J. Chromatogr. A 1459 (2016) 24-37.

[28] Commission Decision 2002/657/EC of 12 August 2002 concerning the performance of analytical methods and the interpretation of results, Brussels, Belgium, Official Journal of the European Communities, L 221 (2002).

[29] H. G. J. Mol, P. Plaza-Bolaños, P. Zomer, T.C. de Rijk, A.A.M. Stolker, P.P.J Mulder, Toward a generic extraction method for simultaneous determination of pesticides, mycotoxins, plant toxins, and veterinary drugs in feed and food matrixes, Anal. Chem. 80 (2008) 9450-9459.

[30] M. L. Gómez-Pérez, R. Romero-González, P. Plaza-Bolaños, E. Génin, J.L. Martínez Vidal, A. Garrido Frenich, Wide-scope analysis of pesticide and veterinary drug residues in meat matrices by high resolution MS: detection and identification using Exactive-Orbitrap, J. Mass Spectrom. 49 (2014) 27-36.

[31] A.A. Salem, I.A. Wasfi, S.S. Al-Nassibi, Trace determination of β-blockers and β_2-agonists in distilled and waste-waters using liquid chromatography-tandem mass spectrometry and solid-phase extraction, J. Chromatogr. B 908 (2012) 27-38.

[32] N. León, M. Roca, C. Igualada, C.P.B. Martins, A. Pastor, V. Yusá, Wide-range screening of banned veterinary drugs in urine by ultra high liquid chromatography coupled to high-resolution mass spectrometry, J. Chromatogr. A 1258 (2012) 55-65.

[33] M. Gros, S. Rodríguez-Mozaz, D. Barceló, Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry, J. Chromatogr. A 1248 (2012) 104-121.

[34] M. Huerta-Fontela, M.T. Galceran, F. Ventura, Fast liquid chromatography-quadrupole-linear ion trap mass spectrometry for the analysis of pharmaceuticals and hormones in water resources, J. Chromatogr. A 1217 (2010) 4212-4222.

[35] M. Lavén, T. Alsberg, Y. Yu, M. Adolfsson-Erici, H. Sun, Serial mixed-mode cation- and anion-exchange solid-phase extraction for separation of basic, neutral and acidic pharmaceuticals in wastewater and analysis by high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry, J. Chromatogr. A 1216 (2009) 49-62.

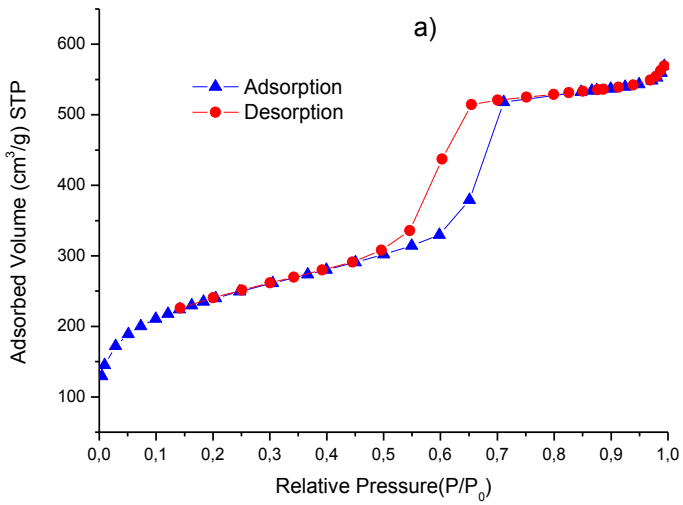
Appendix A. Supplementary material

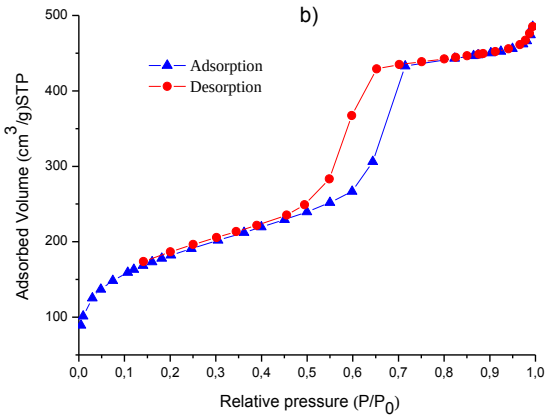
SM1. Characterization of mesoporous silicas.

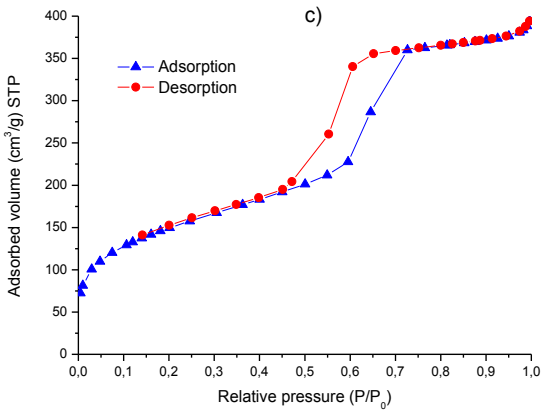
X-ray diffraction (XRD) patterns of the silicas were obtained on a Philips Difractometer model PW3040/00 X'Pert MPD/MRD at 45 KV and 40 mA, using a wavelength Cu Kα (λ = 1.5418 Å). Scanning electron micrographs and morphological analysis were carried out on a XL30 ESEM Philips with an energy-dispersive spectrometry system (EDS). The samples were treated with a sputtering method with the following parameters: sputter time 100 s, sputter current 30 mA, and film thickness 20 nm using sputter coater BAL-TEC SCD 005. Conventional transmission electron microscopy (TEM) was carried out on a TECNAI 20 Philips microscope operating at 200 kV, with a resolution of 0.27 nm and ± 70º of sample inclination, using a BeO sample holder. Nitrogen gas adsorption-desorption isotherms were performed using a Micromeritics ASAP 2020 analyzer. Cross-polarization magic-angle spinning (CP-MAS) ^{13}C and ^{29}Si solid-state nuclear magnetic resonance spectra (CP-MA-NMR) were recorded on a Varian-Infinity Plus Spectrometer at 400 MHz operating at 100.52 MHz proton frequency (4 ms 908 pulse, 4000 transients, spinning speed of 6 MHz, contact time 3 ms, pulse delay 1.5 s). Infrared spectra were recorded on a Thermo Nicolet 380 FT-IR spectrophotometer in the region 4000 to 400 cm^{-1} by using spectra quality KBr powder. Elemental analysis (% H, % C, % N and % S) were performed using a microanalyser model LECO CHNS-932. The thermal stability of the modified nanostructured silicas was studied using a Setsys 18 A (Setaram) thermogravimetric analyzer with a 100 mL platinum crucible. A synthetic air atmosphere was used and the temperature increased from 25 ºC to 800 ºC at a speed of 10 ºC per minute. To verify the presence of SO_3^- moieties in the bi-functionalized mesoporous silicas, an acid-base titration was performed according to Margolese et al. [26]. Briefly, 10.5 mL of NaCl 2 M were mixed with 0.05 g of material and stirred for 90 min at room temperature to equilibrate the resulting suspension. Afterwards it was titrated

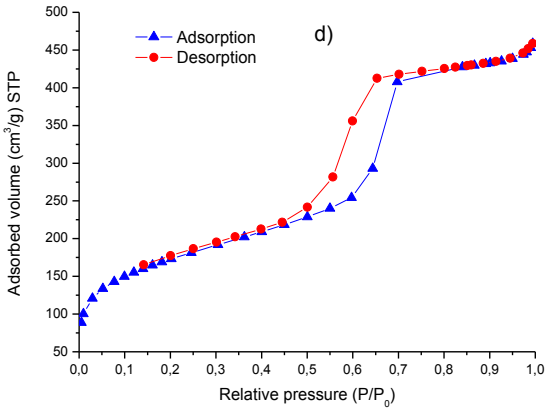
potentiometrically by dropwise addition of NaOH 0.01 M. The ion-exchange capacities of the bi-functionalized mesoporous silicas obtained were defined as mmol SO_3^-/g SiO_2, and the results were compared with the content of S, measured by elemental analysis, in order to determine the conversion rate (%) of thiol groups.

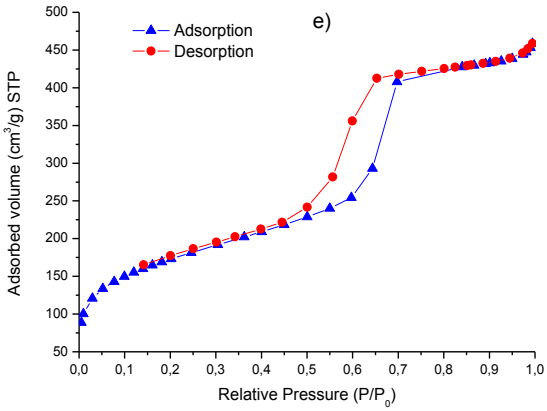
SM2. N_2 adsorption-desorption isotherms of: a) SBA-15, b) SBA-15-C18, c) SBA-15-C8, d) SBA-15-C18-SO_3^- and e) SBA-15-C8-SO_3^-.



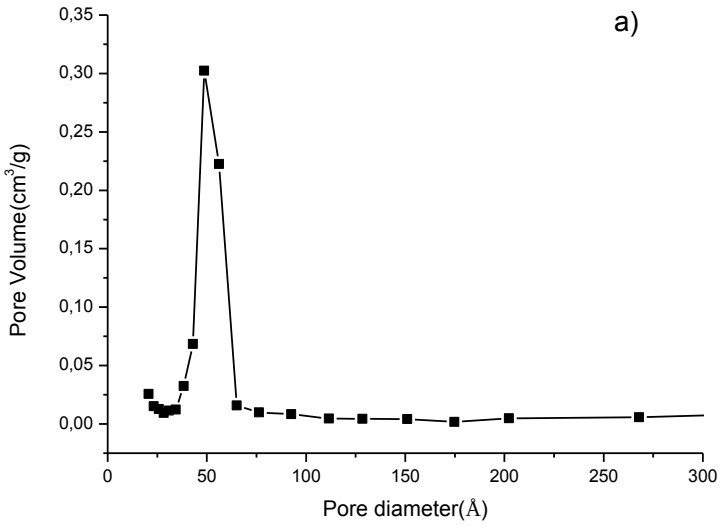


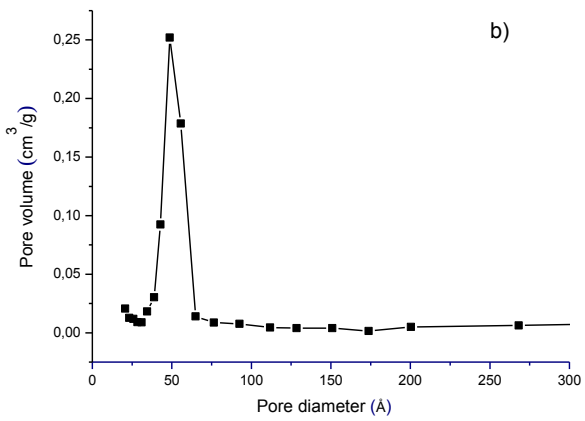


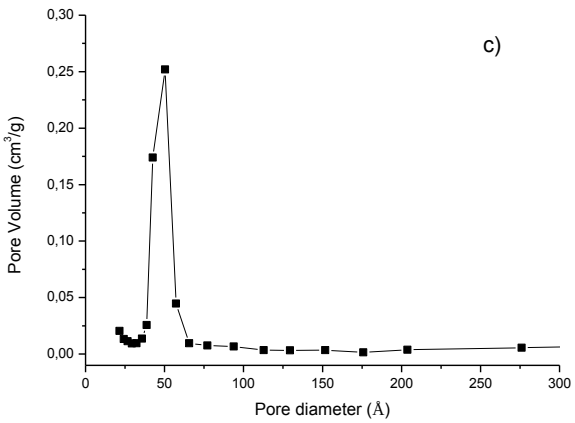


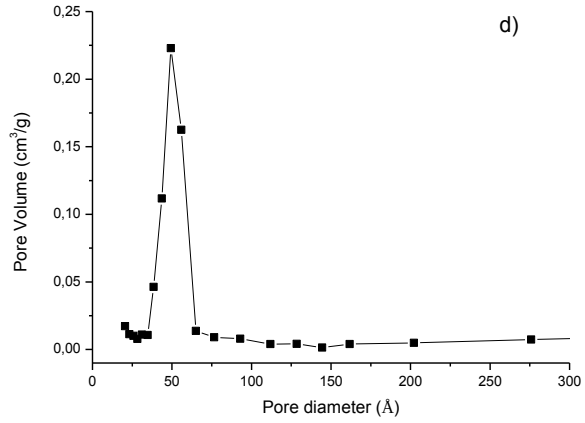


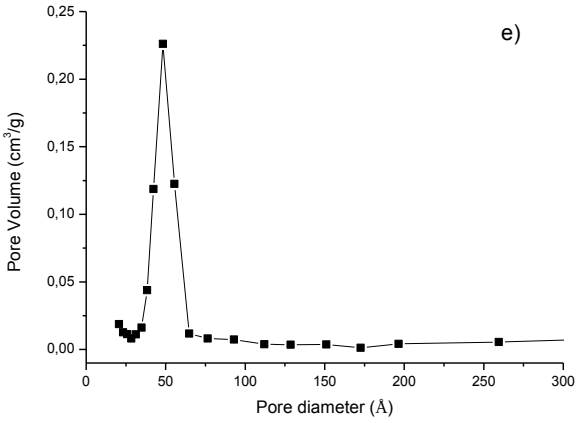
SM3. Pore size distribution of: a) SBA-15, b) SBA-15-C18, c) SBA-15-C8, d) SBA-15-C18-SO_3^- and e) SBA-15-C8-SO_3^-.



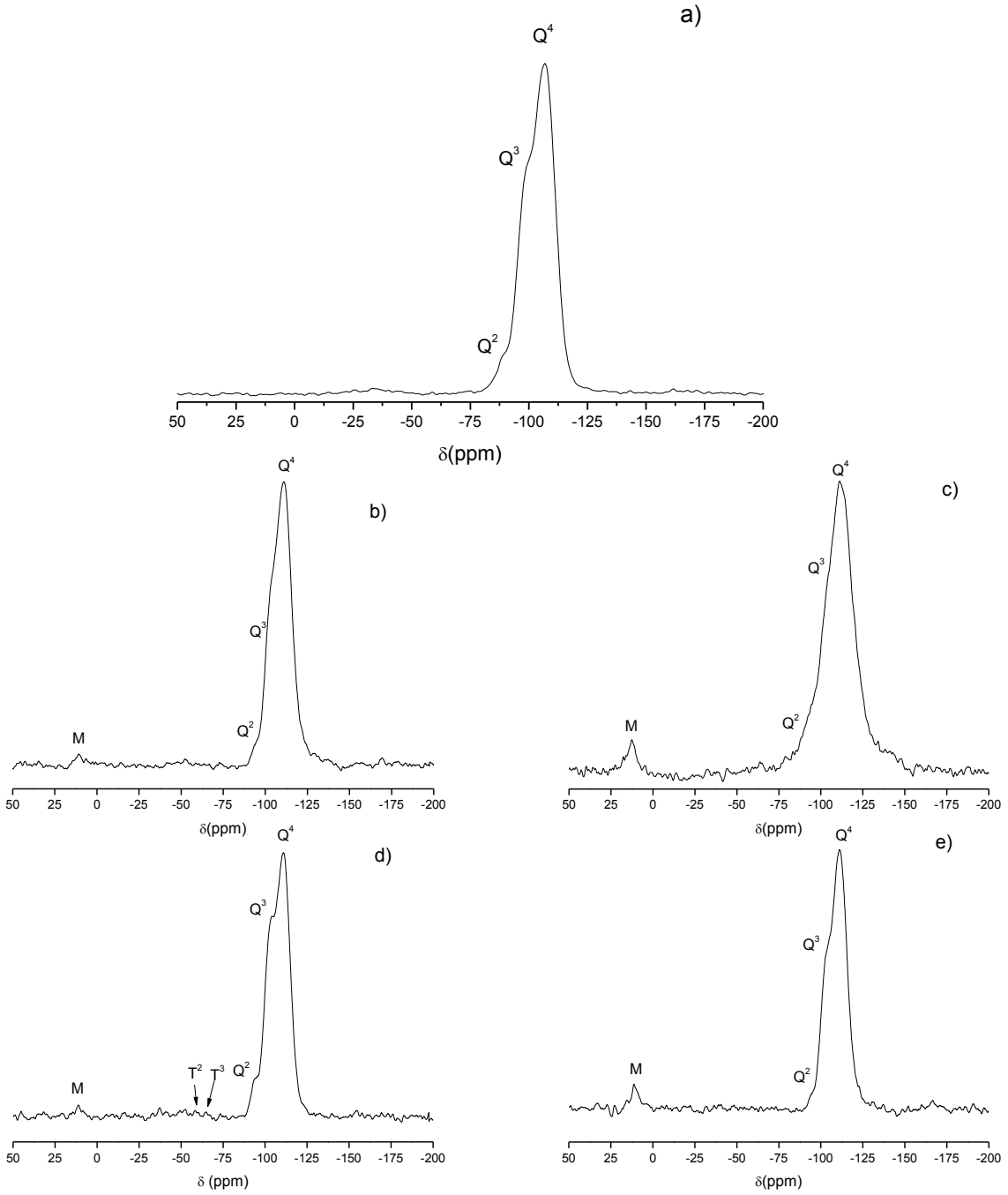








SM4. ^{29}Si NMR spectra of: a) SBA-15, b) SBA-15-C18, c) SBA-15-C8, d) SBA-15-C18-SO$_3^-$ and e) SBA-15-C8-SO$_3^-$.



SM5. Thermogravimetric curves of: a) SBA-15, b) SBA-15-C18, c) SBA-15-C8, d) SBA-15-C18-SO_3^- and e) SBA-15-C8-SO_3^-.