Poly(ethylene glycol) diacrylate-based solid-phase extraction for determination of sulfonamides in meat samples

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ABSTRACT

In this work, a sorbent based on poly(ethylene glycol) diacrylate (PEGDA) polymer has been synthesized for solid-phase extraction (SPE) of sulfonamides (sulfaguanidine, sulfadiazine, sulfathiazole, sulfapyridine, sulfamethazine, sulfamonomethoxine, sulfamethoxazole, sulfisoxazole) from different meat matrices, which were subsequently determined by HPLC-DAD. Several extraction parameters such as loading and elution solvents as well as other variables (loading capacity, breakthrough volume and reusability) influencing on the analytical performance of the sorbent were optimized. Under the optimal conditions, the developed method was successfully applied to determine the eight sulfonamides in meat samples including pork bacon, pork liver and chicken muscle. Satisfactory recovery values of sulfonamides comprised between 70% and 108% were obtained. The results demonstrated the suitability of the PEGDA-700 material to be used as sorbent in SPE for effective clean-up and extraction of sulfonamides in complex samples.

Keywords: poly(ethylene glycol) diacrylate; sulfonamides; solid-phase extraction, meat samples

1. Introduction

Antibiotics, such as sulfonamides, are widely used in livestock production with many purposes, such as disease treatment and/or prevention and to improve feeding efficiency. Nevertheless, the abuse of these drugs in food-producing animals could cause toxic effects, with direct impact on public health [1]. For this reason, governmental agencies have set maximum residue limits for certain veterinary drugs in foods, being the maximum allowable amount for sulfonamides of 100 μg kg^{-1} [2].

Sulfonamides extraction and determination has been carried out in different food matrices, such as meat [1,3,4] or milk [5,6]. However, the extraction of these analytes from these complex matrices remains a challenging task due to their low concentrations and the difficulty of removing proteins, fats and other matrix interferences.

Regarding the detection of these analytes, high-performance liquid chromatography coupled to mass spectrometry in tandem mode (HPLC-MS/MS) is one of the most extended techniques [1,5,7-16] however it requires the use of an expensive and complex instrumentation, which is not always available for routine monitoring. Other approaches also used to determine sulfonamides are HPLC with UV detection [4,17-21], capillary electrophoresis (CE) [22,23] and capillary electrochromatography (CEC) [24-26]. However, these techniques not always provide the desired sensitivity, being necessary an appropriate sample preparation to reduce matrix effects prior to the determination of these antibiotics and also to enhance the analytical properties of the detection step. In this sense, more selective sample treatments for the extraction of sulfonamides in food samples have been reported in the last years. These sample pretreatment methodologies include liquid-liquid extraction [5,6,10,21], solid-phase extraction (SPE) [15,20,23,27-31], the use of QuEChERS [12,14,17] and molecularly imprinted materials [4,18],

among others. Within these extraction techniques, SPE using commercial cartridges, such as Oasis HLB, were used to extract sulfonamides from complicated matrices [8,16,17]. However, the use of new sorbent materials, such as a hybrid monolith of polypyrrole-coated graphene oxide embedded in polyvinyl alcohol cryogel [32], metal-organic frameworks [33,34], a molybdenum disulfide-based core-shell magnetic nanocomposite [35], functionalized carbon nanotubes [36,37] and monoliths of graphene oxide [30] and phenyl–boronic acid [38], among others, have been also successfully proposed to extract sulfonamides. Indeed, the extraction performance of these sorbents was also demonstrated to extract other analytes from complex matrices such as polycyclic aromatic hydrocarbons [39-41] and anti-inflammatory drugs [42,43]. However, the synthesis of other new sorbents with good extraction performance is still a challenge in the analytical chemistry field.

Recently, our research group tested a poly(ethylene glycol) diacrylate (PEGDA)-based monolith as stationary phase for CEC for sulfonamides separation [26]. In that work, PEGDA monomers with different PEG chain length (PEGDA 250, 575, and 700) were synthesized, being the monolith prepared with PEGDA-700 the one that offered the best separation performance. There are also other works previously published in literature that used PEGDA-based monoliths as stationary phases in capillary (electro)separation techniques to separate proteins [44,45] and small molecules (such as phenols, hydroxy benzoic acids, and alkyl parabens) [46]. Recently, other acrylate-based monolith polymer was fabricated in a syringe to extract anticonvulsants drugs (carbamazepine and 10-hydroxy-carbamazepine) from human serum [47]. However, and to our knowledge, the use of PEGDA-based polymers as SPE sorbents for sulfonamides extraction in complex matrices has not been yet explored.

In this work, the use of a polymer based on PEGDA-700 as SPE sorbent to extract sulfonamides from meat samples is proposed. For this purpose, the polymer was synthesized and packed into an empty plastic SPE column, being different extraction parameters (such as loading and elution solvents) investigated. The optimized SPE procedure was applied to the analysis of eight sulfonamides from several meat samples.

2. Materials and methods

2.1. Chemicals

Poly(ethylene glycol) diacrylate (PEGDA, M_n 700), 2,2-dimethoxy-2-phenylacetophenone 99% (DMPA), disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), sodium acetate, boric acid, sodium tetraborate, and sulfonamides standards (sulfaguanidine (SGD, pK_{a1} = 2.75, pK_{a2} = 12.10); sulfadiazine (SDZ, pK_{a1} = 1.64, pK_{a2} = 6.50); sulfathiazole (STZ, pK_{a1} = 2.31, pK_{a2} = 7.24); sulfapyridine (SPY, pK_{a1} = 2.90, pK_{a2} = 8.54); sulfamethazine (SMZ, pK_{a1} = 2.28, pK_{a2} = 7.42); sulfamonomethoxine (SMM, pK_{a1} = 1.42, pK_{a2} = 6.67); sulfamethoxazole (SMX, pK_{a1}= 1.8; pK_{a2}= 5.57); sulfisoxazole (SXZ, pK_{a1} = 1.52, pK_{a2} = 4.83)) [24, 48] were purchased from Sigma-Aldrich (St. Louis, MO, USA). Glacial acetic acid (HAcO), acetonitrile (ACN), ethyl ether (Et_2O) and methanol (MeOH) were purchased from Scharlau (Barcelona, Spain). Anhydrous sodium sulfate, potassium hydroxide (KOH) and hydrochloric acid (37%) were supplied by Panreac (Barcelona, Spain). Unless otherwise stated, other chemicals used were of analytical grade. Deionized water was obtained with a B30 water purification system (Adrona, Rīga, Latvia). Sulfonamides standard solutions were prepared each one at 1 mg mL^{-1} in ACN, while

working standard solutions were obtained from the stock solution by proper dilution in the adequate solvent and kept at 4 ºC until use.

A total of 6 samples coming from edible animal tissues, which included 2 bacon and 2 liver samples from pork, and 2 muscle samples from chicken, were purchased from local markets in Paterna (Valencia, Spain).

2.2. Preparation of PEGDA 700-based polymer

Briefly, a polymerization mixture was prepared in a 50 mL glass vial by weighing 21.93 wt% PEGDA-700, 78.02 wt% Et_2O and 0.05 wt% DMPA. Photopolymerization was carried out by irradiation of the glass vial in a UV irradiation chamber (Model CL1000, UVP, Upland, CA, USA) equipped with five UV lamps (5 × 8 W, 254 nm) at 0.9 J cm^{-2} for 90 min. Polymerization was performed at 0ºC by placing the glass vial on a copper plate mounted on ice in advance. Then, the polymeric material was washed with MeOH to remove the porogenic solvent and possible unreacted monomer. Next, the monolithic bulk material was dried at 60ºC, ground with a mortar and sieved with a steel sieve with sizes comprised between 100 to 200 µm. The resulting polymeric material was characterized by scanning electron microscope (SEM) with a scanning electron microscope (S-4100, Hitachi, Ibaraki, Japan) provided by a field emission gun, an EMIP 3.0 image data acquisition system. Also, attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectrum of powdered material was recorded on a Tensor 27spectrometer from Bruker (Bremen, Germany). Measurements were recorded using a DLaTGS detector and a Dura Sample IR II ATR accessory from Smiths Detection Inc. (Warrington, UK) equipped with a nine-reflection diamond/ZnSe Dura Disk plate. Spectrum was collected in the region compressed between 4000 and 550 cm^{-}

^{1}, with a resolution of 4 cm^{-1} and 50 average scans. Background spectrum was acquired under the same measurement conditions. The surface area of the polymer was also accomplished through the Brunauer–Emmett–Teller (BET) method with a ASAP2020 (Micromeritics, USA) at 77 K. Prior to the measurement, the polymer was completely outgassed using an overnight vacuum of 10^{-8} bar at 393 K.

2.3. Sample preparation

Sample preparation protocol was adapted from Bele *et al.* [3]. Ten grams of each one of the 6 meat samples (bacon and liver from pork and muscle from chicken) was placed into polypropylene centrifuge tubes (50 mL), jointly with 20 mL ACN and 5 g anhydrous sodium sulfate. After vortexing for 1 min, the mixture was sonicated for 10 min and centrifuged at 4000 rpm for 10 min (centrifuge Girozen 416, Daejeon, Gimpo, Korea). These steps were repeated twice and both extracts were combined, transferred to another tube and evaporated to dryness in a vacuum rotary evaporator (RE300DB, Stuart, UK). Finally, the resulting residue was reconstituted with 2 mL of a 50 mM HAcO at pH 2 aqueous solution. In order to remove the interferences produced during the extraction (mainly due to the high fat content of the sample and turbidity), the extracts were saponified with 500 µL of 2 M ethanolic potassium hydroxide, being the pH adjusted to 2 after saponification. Additionally, 10 more grams of each one of the 6 meat samples were fortified with 100, 1000 and 4000 µg kg^{-1} of each one of the 8 sulfonamides, and subjected to the same extraction protocol. At least replicates were performed for each one of the 6 meat samples; therefore, a total of 48 extracts were collected.

164 *2.4. SPE protocol*

165

166 Fifty milligrams of the ground PEGDA 700-based polymer was packed between two
167 frits (1/16', 20 μm, Análisis Vínicos, Tomelloso, Spain) into 1 mL empty propylene
168 disposable SPE cartridge (Análisis Vínicos). SPE procedure was performed in an
169 Agilent Vac Elut 12 manifold (Waldbronn, Germany) connected to a vacuum pump
170 DINKO D-95 (Barcelona, Spain). Firstly, sorbent activation was done with ACN (200
171 μL) and then it was equilibrated with 50 mM HAcO at pH 2 (500 μL) at 0.7 mL min^{-1}.
172 Next, 500 μL of sulfonamide standard mixture (prepared in aqueous 50 mM HAcO at
173 pH 2) or meat sample extract (properly adjusted at pH 2) were loaded onto the SPE
174 cartridge at a flow rate of 0.1 mL min^{-1}. Then, the material was washed with the same
175 solution (500 μL) at 0.7 mL min^{-1}, and finally the retained analytes were eluted at 0.1
176 mL min^{-1} with 100 μL of 50 mM HAcO at apparent pH 2 in MeOH. All SPE fractions
177 were collected and analyzed by HPLC-DAD under the experimental conditions
178 mentioned below.

179

180 *2.5. Determination of sulfonamides by HPLC-DAD*

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182 Chromatographic separation and determination of sulfonamides were made with a
183 1100 series liquid chromatograph (Agilent Technologies) provided with a quaternary
184 pump, a degasser, a thermostated column compartment, an automatic sampler and a
185 UV-vis diode array detector. The chromatographic method was adapted from Karimi et
186 al. [4]. Concretely, a C18 Kromasil column (250 mm × 4 mm, 5 μm; Análisis Vínicos)
187 and a mobile phase composed by water containing 1% HAcO (pH=3.2) (eluent A) and
188 ACN with 1% HAcO (eluent B), was used. A gradient elution using both eluents was

189 performed by varying their proportion as follows: the initial phase (10% B) was
190 gradually changed to 23% B in 5 min and kept for 5 more min, followed by an increase
191 of eluent B up to 50% B at 25 min, which was maintained for 2 more min; finally, the
192 column was equilibrated with the initial composition for 2 min. UV detection was done
193 at 280 nm. The column temperature was set at 25 °C, and the flow rate was 1 mL min^{-1}.
194 The injection volume was 2 μL.
195
196 **3. Results and discussion**
197
198 *3.1 Characterization of PEGDA-700 polymer*
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200 Once the PEGDA-700 monolith was synthesized, it was grounded to particles of
201 100-200 μm size, and these particles were packed into the SPE cartridges. In order to
202 carry out the characterization of the material, SEM analysis was performed in first place
203 (Fig. S1, Supplementary material). As observed, the resulting material presented a
204 cauliflower-like structure with grains sizes comprised between 0.5 and 1 μm, which
205 were in agreement with the values described in literature [26]. In this article, it was
206 demonstrated that this polymeric sorbent provided a large permeability, which is key to
207 facilitate the sample solutions pass through the cartridges. Next, the ATR-FTIR
208 spectrum of the polymer was recorded (Fig. S2) to confirm its formation from the
209 starting monomer. It should be noted that the PEGDA monomer contains C=C bonds
210 (alkenes), which have typical absorption bands at a frequencies around 1660-1620 cm^{-1}.
211 During the crosslinking reaction of PEGDA monomers, the -C=C- bonds are consumed,
212 resulting in a completely disappearance of these bands. Indeed, the bands at 1732 cm^{-1}
213 and 1253 cm^{-1} are attributed to the stretching of -C=O and –C-O (carbonyl group) of the

214 acrylate moieties of PEGDA. Also, the band at 2873 is characteristic of the symmetric
215 stretching of C-H (CH_2). The specific surface area of the PEGDA polymer was also
216 measured by BET, giving a value close to 70.8 $m^2 g^{-1}$.
217
218 *3.2 Optimization of SPE conditions*
219
220 To evaluate the possibility to quantitatively extract sulfonamides using the PEGDA-
221 700 polymeric sorbent, different parameters such as loading and elution conditions were
222 considered. A factor by factor method was used for the optimization of the parameters
223 affecting the extraction efficiency, being at least three replicates performed for each
224 experiment.
225 First, the effect of sample pH on the loading SPE step was evaluated. The selection
226 of an optimal loading pH plays an important role in the retention of sulfonamides onto
227 the PEGDA polymeric surface. Thus, the first and second ionization constants of the
228 studied sulfonamides are in the respective ranges of 1.40-2.90 and 4.83-8.54 and
229 accordingly, their interaction with the sorbent is pH dependent. To evaluate the
230 influence of this variable on the sulfonamides retention, a 20 µg mL^{-1} standard mixture
231 was prepared in the following aqueous loading solutions (keeping constant the ionic
232 strength close to 50 mM): HAcO (pH 2), acetate buffer (pH 4.75), phosphate buffer (pH
233 7) and borate buffer (pH 9). For each pH assayed, 500 µL of the standard mixture were
234 passed through the cartridge, and the sulfonamides content found in the percolated
235 fraction was compared with the starting sample content. The best results were obtained
236 at pH 2, where the retention of all studied sulfonamides was *ca*. 96% (mean value). This
237 behavior could be explained taking into account that at acidic pH most of the
238 sulfonamides present a positive charge, which provided a retention mechanism due to

hydrogen bonding and dipole-dipole interactions with polar ethylene chains on the PEGDA sorbent. Thus, pH 2 was selected for further studies.

Next, the elution step was investigated by introducing into the cartridge 100 µL of the following elution solvents: MeOH, acidic MeOH (containing 50 mM HAcO) and basic MeOH (containing 50 mM KOH). In this case, recovery percentage was calculated from the sulfonamides content present in the starting sample and content eluted from the cartridge. The results obtained are shown in Fig. 1. As it can be seen, the highest recoveries for most of the studied sulfonamides were obtained when acidic MeOH was employed as elution solvent, reaching values comprised between 70.8% and 104.4% for SGD and SXZ, respectively. This result can be attributed to the fact that this protic solvent competes with the sorbent by weakening the interactions formed between the analytes and the polymer, thus favouring their elution. Therefore, the polymer used could be considered a pseudo cation exchanger.

3.3. Analytical features of the PEGDA-700 sorbent

Other important parameters that could affect the extraction efficiency in a SPE protocol are maximum loading capacity, breakthrough volume and sorbent reusability and stability, which will be next evaluated.

In order to establish the maximum loading capacity of the sorbent (using 50 mg), 500 µL of mixtures containing a total content of 0.2, 0.4, 0.8, 2.0 and 2.8 mg of the 8 sulfonamides were passed through the cartridge (see Fig. 2A). As observed in this figure, recovery values of *ca*. 80-96% were obtained for all sulfonamides at 40 mg g^{-1} sorbent. Therefore, this value could be adopted as the maximum loading capacity, although it should be advisable to work in a range comprised between 15-20 mg g^{-1} to

assure loading capacities higher than 90% for all sulfonamides. The maximum loading capacity value could be explained taking into account that not all glycol groups of the polymer are accessible to the analytes tested, so the material cannot retain concentrations higher than 20-40 mg g^{-1} (depending on the sulfonamide tested) when all the active points are occupied and therefore a recovery decrease was observed. It should be noted that even a loading capacity of 20 mg g^{-1} is higher than the values obtained in literature for different SPE sorbents [18,32,34]. In particular, it has been stated that typical values for loading capacity are comprised between 1 and 10% of the bed weight, meaning that 5 mg of analyte could be potentially retained (under the proper condition) per 50 mg of sorbent material [49,50], so, the loading capacity of the proposed sorbent is clearly above these values.

Next, the breakthrough volume was evaluated by percolating through the cartridge different sample volumes (comprised between 0.1 and 5 mL) of the 8 sulfonamides standard solution in which the amount of the analytes was kept constant (0.1 mg of each sulfonamide). Fig. 2B shows the breakthrough curve obtained using the developed sorbent, where recoveries comprised between 86% and 99% up to 2 mL were achieved. However, recoveries decreased when 5 mL of solution were passed through the cartridge. This result indicated a possible saturation of adsorption sites present in the sorbent. In any case, a volume of 500 µL was chosen for further SPE studies by considering operational aspects (such as the tube dimension (1 mL), reduction of extraction times) and green considerations thus favouring a high-throughput extraction.

The reusability of the sorbent by passing through the cartridge meat sample extracts was also tested. Sorbent could be reused after its regeneration with 5 mL of 50 mM HAcO at pH 2 in MeOH. Moreover, it was observed that the sorbent was stable up to 20 retention–elution cycles and could be repeatedly used without significant loss of their

289 uptake capacity (*ca*. 90%). A stability study of a PEGDA-based polymer batch, which
290 was synthesized near 1 year ago, was also made. No significant differences in extraction
291 performances were observed, achieving similar recoveries (71.5%-107.0%).

292 Concerning to the selectivity of the sorbent, this material could retain other organic
293 analytes with a certain polar character (for example, phenols, hydroxy benzoic acids,
294 parabens [46]) based on hydrogen bonding and dipole–dipole interactions with the
295 sorbent. However, the selected SPE conditions provide enough selectivity compared
296 with other commercial SPE phases (*e.g.* C18) to perform an accurate determination of
297 target compounds.

298

299 *3.4 Analytical parameters of the HPLC method and application to the determination of*
300 *sulfonamides in meat samples*

301

302 A chromatogram showing the separation of the 8 sulfonamides standards considered
303 in this study (20 µg mL^{-1} dissolved in acidic methanol) obtained using the
304 chromatographic conditions indicated in Section 2.4 is shown in Fig. 3 (continuous
305 black line). As it can be observed, all the considered sulfonamides were eluted in less
306 than 16 min.

307 In order to proceed with sulfonamides quantification, external calibration curves of
308 peak areas were constructed by injecting six standard solutions (prepared in acidic
309 MeOH) in the range 0.1-100 µg mL^{-1}. Additionally, standard addition calibration curves
310 were also obtained by adding to each sample eluate (pork bacon, pork liver and chicken
311 muscle) the same six standard solutions. As included in Table 1, all external and
312 standard addition calibration curves were linear with $R^2 > 0.992$; however, the slopes of
313 the standard addition calibration curves differ significantly from those obtained with the

external calibration method. This difference could be explained considering the high fat content present in the meat extracts that can interfere in the determination of sulfonamides. Therefore, it can be concluded that there is matrix effect in the determination of sulfonamides in the meat samples analyzed, and as a consequence standard addition calibration curves will be next employed to quantify sulfonamides.

Intra- and inter-day repeatabilities of peak areas were also evaluated by injecting a mixture composed of three sulfonamide at different concentration levels (2, 20 and 50 μg mL^{-1}) 10 times per day during 3 days (see Table 1). In all cases, the relative standard deviation (RSD) values were lower than 4.1%.

On the other hand, the limits of detection (LODs) and limits of quantification (LOQs) (by considering an enrichment factor of 5) were estimated for signal-to-noise ratios of 3 and 10, respectively. As observed in Table 1, LODs were comprised between 7.5 and 16.2 μg kg^{-1}, and LOQs between 25.0 and 51.6 μg kg^{-1}.

Next, the applicability of the PEGDA-700 sorbent to perform the quantitative extraction of sulfonamides was evaluated by percolating through the SPE-cartridge 500 μL of each one of the meat extracts included in the study (bacon and liver from pork and chicken muscle). However, none of the 8 sulfonamides was detected in the eluate of the non-fortified meat extracts (see Fig. 3 (dashed line) for a pork liver extract), even after removal of matrix interferents (see Fig. 3 (continuous grey line) for comparison), which demonstrated that the synthesized polymer provided a satisfactory clean-up of samples.

As a consequence, the meat samples were subsequently fortified at 100, 1000 and 4000 μg kg^{-1} concentration levels directly into the meat (as explained in Section 2.3) and then were subjected to the optimized SPE procedure. Fig. 4 shows the chromatograms of the pork bacon, pork liver and chicken muscle extracts at one of the

three fortified levels (4000 μg kg^{-1}) after passing them through the PEGDA-700 sorbent. The recovery values, which were calculated by dividing the concentration found by the concentration added (expressed in percentage) for the three concentration levels, are given in Table 2. As it can be seen, acceptable recoveries comprised between 70.0% (for SMZ) - 102.3% (for SDZ) for pork bacon, 74.1% (for SGD) - 108.1% (for SDZ) for pork liver, and 73.3% (for SMZ) - 104.9% (for SXZ) for chicken muscle, were obtained. On the other hand, when the recovery values of the different meat samples were compared; pork bacon and chicken muscle extracts provided slightly lower recovery values than those of the pork liver, which may be due to their more complex composition (higher fat content), which could be considered the main source of error. This statement is partially in accordance with other studies related to sulfonamides from meat samples [16].

A comparative study of our method with other protocols in which sulfonamides from meat samples were extracted was also done (see Table 3). As it can be observed, the proposed method provided similar LODs than those reported by other authors UV detection, and similar or even higher recoveries of the other methods. Additionally, the proposed SPE protocol provided several distinct advantages such as easy and cheap preparation of sorbent, facile manipulation and execution (traditional evaporation and centrifugation steps are avoided) and satisfactory sorbent reusability (until 20 times), which make this protocol economically feasible. Moreover, analytes determination was done using common accessible equipment (such as UV detector) in most analytical laboratories, which makes the protocol transferable to industry.

4. Conclusions

In this work, a SPE sorbent based on PEGDA polymer has been synthesized and used to extract several sulfonamides from complex food matrices, followed by their separation and quantification by HPLC-DAD. Several extraction parameters such as loading and elution solvents as well as other variables (loading capacity, breakthrough volume and reusability) that can affect the analytical performance were also established. The best results were obtained using 50 mM HAcO at pH 2 and 50 mM HAcO in MeOH as loading and elution solvents, respectively. In addition, under the optimal conditions, the extraction of the eight sulfonamides from different meat tissues (bacon and liver from pork and chicken muscle) was conducted, obtaining in all cases acceptable recovery values. In addition, the synthesis of this polymer is cheap and easier when compared to other commercial polymeric sorbents, since PEDGA-700 acts as monomer and cross-linker at the same time, allowing a higher reproducibility in the preparation. Moreover, its use resulted in an effective clean-up of sample, which allowed a successful determination of these analytes. To sum up, the present SPE procedure is simple and suitable for routine analysis and it should be noted that the sorbent can be reused until 20 times without significant loss of retention capability, which also means a reduction of analysis costs.

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Conflict of interest

The authors have declared no potential conflict of interest.

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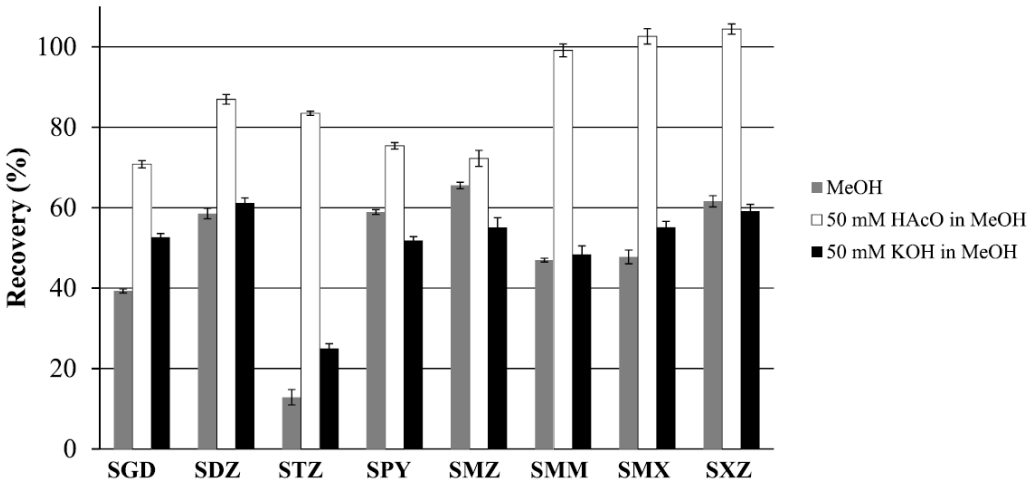
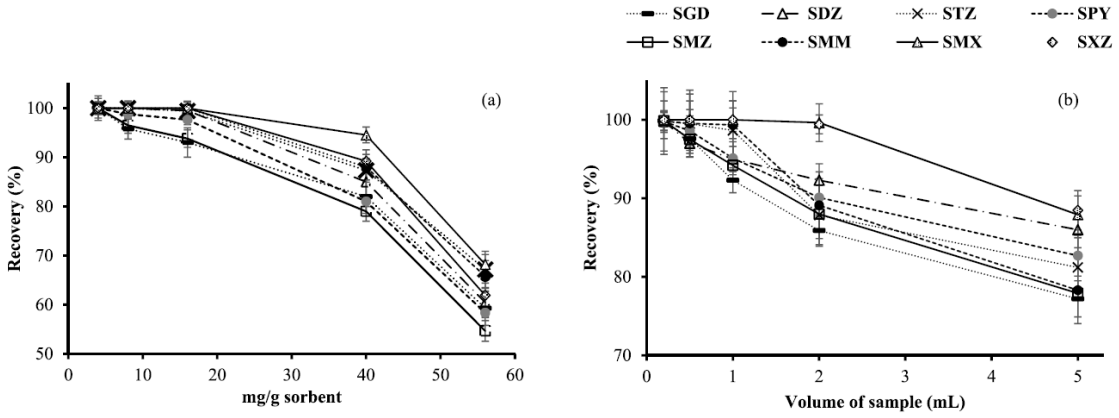


596 **Fig. 1**. Effect of the elution solvent on the recovery of sulfonamides from the PEGDA-
597 700 sorbent.

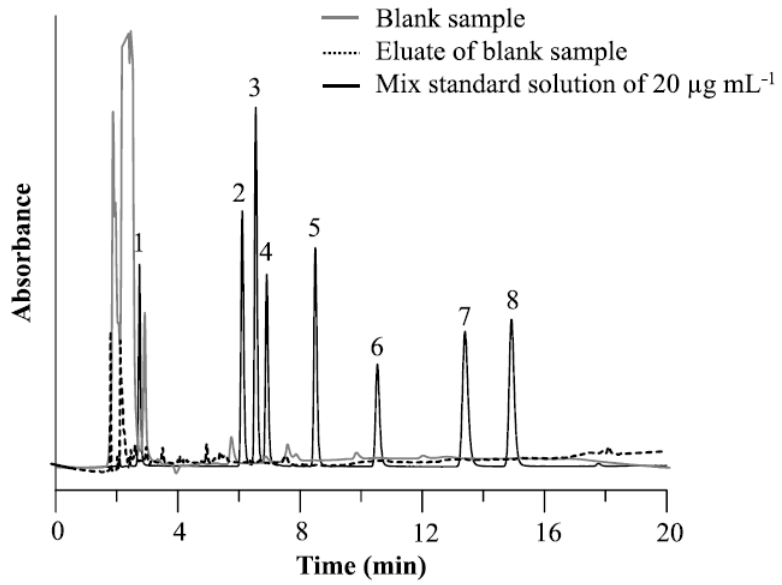
598



599

600 **Fig. 2**. Evaluation of the loading capacity of the PEGDA-700 sorbent at increasing
601 sulfonamides amounts (A) and influence of the breakthrough volume of sulfonamides
602 using the PEGDA-700 sorbent (B).

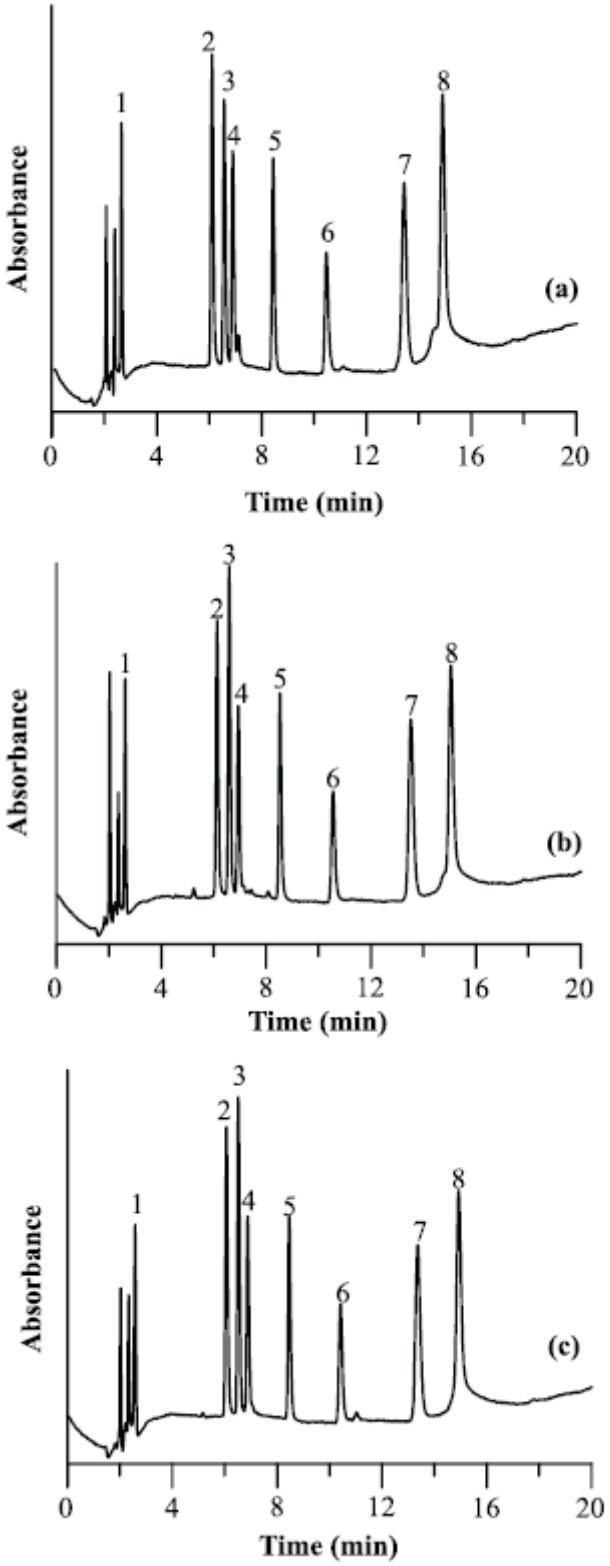
603



604

605 **Fig. 3**. HPLC-DAD chromatograms of a 20 µg mL^{-1} sulfonamide standard mixture
606 prepared in acidic methanol (continuous black line), of an eluate of a non-fortified pork
607 liver extract (dashed line) and of the same non-fortified pork liver extract directly
608 injected into the HPLC (continuous grey line). Peak identification: (1) sulfaguanidine,
609 SGD; (2) sulfadiazine, SDZ; (3) sulfathiazole, STZ; (4) sulfapyridine, SPY; (5)
610 sulfamethazine, SMZ; (6) sulfamonomethoxine, SMM; (7) sulfamethoxazole, SMX; (8)
611 sulfisoxazole, SXZ. Other experimental conditions as indicated in Section 2.5.

612



613

614 **Fig. 4**. HPLC-DAD chromatograms of the eluted fractions of pork bacon (A), pork liver

615 (B) and chicken muscle (C) fortified with 4000 µg kg^{-1}. Peak identification as in Fig. 3.

616 Other experimental conditions as indicated in Section 2.4.

Table 1. Intra- and inter-day peak area repeatabilities, sensitivities, R^2 and limits of detection (LOD) and quantitation (LOQ) for the determination of sulfonamides by HPLC-DAD.

Analyte	Peak area repeatability*, RSD (%)		Sensitivity ($mAU \cdot mL \cdot \mu g^{-1}$)	R^2	LOD ($\mu g\ kg^{-1}$)	LOQ ($\mu g\ kg^{-1}$)
	Intra-day (n=10)	Inter-day (n=3 days)				
SGD	1.0[a] 0.2[b] 0.3[c]	3.1[a] 2.5[b] 2.8[c]	3.42[d] 3.77[e] 4.16[f] 3.77[g]	0.9991[d] 0.9995[e] 0.9961[f] 0.9998[g]	15.0	50.0
SDZ	1.2[a] 0.3[b] 0.3[c]	3.2[a] 1.6[b] 1.8[c]	5.93[d] 6.77[e] 7.49[f] 6.78[g]	0.9983[d] 0.9997[e] 0.9941[f] 0.9997[g]	7.5	25.0
STZ	1.1[a] 0.2[b] 0.2[c]	2.3[a] 1.5[b] 1.8[c]	7.63[d] 8.88[e] 9.86[f] 8.96[g]	0.9985[d] 0.9996[e] 0.9933[f] 0.9995[g]	11.3	37.1
SPY	1.8[a] 0.6[b] 0.5[c]	3.0[a] 1.2[b] 1.1[c]	4.25[d] 5.94[e] 6.51[f] 5.97[g]	0.9971[d] 0.9995[e] 0.9920[f] 0.9991[g]	12.5	40.8
SMZ	1.2[a] 0.2[b] 0.2[c]	2.8[a] 1.8[b] 1.8[c]	5.27[d] 6.44[e] 7.21[f] 6.45[g]	0.9988[d] 0.9998[e] 0.9949[f] 0.9998[g]	13.8	48.3
SMM	1.3[a] 0.5[b] 0.4[c]	3.2[a] 3.4[b] 3.2[c]	6.67[d] 7.57[e] 8.13[f] 7.94[g]	0.9992[d] 0.9998[e] 0.9869[f] 0.9992[g]	13.7	46.5
SMX	1.1[a] 0.5[b] 0.5[c]	2.3[a] 1.4[b] 1.2[c]	6.24[d] 6.92[e] 7.89[f] 6.81[g]	0.9953[d] 0.9944[e] 0.9933[f] 0.9998[g]	11.3	37.1
SXZ	2.3[a] 1.3[b] 1.2[c]	4.1[a] 2.5[b] 2.8[c]	6.09[d] 7.04[e] 7.60[f] 7.04[g]	0.9957[d] 0.9991[e] 0.9955[f] 0.9997[g]	16.2	51.6

*Obtained from ten injections of a sulfonamide mixture at different concentration levels (2[a], 20[b] and 50[c] $\mu g\ mL^{-1}$) in 1 day and along three consecutive days, respectively.

[d]Values corresponding to the external calibration curve.

[e]Values corresponding to the pork bacon standard addition calibration curve.

[f]Values corresponding to the pork liver standard addition calibration curve.

[g]Values corresponding to the chicken muscle standard addition calibration curve.

Table 2. Recovery results of the determination of sulfonamides in pork bacon and liver and chicken muscle spiked at three different concentration levels.

Analyte[a]	Spiked level (μg kg^{-1})	Pork bacon recovery (%)	Pork liver recovery (%)	Chicken muscle recovery (%)
SGD	100	94.5	97.6	96.1
	1000	82.8	87.9	86.2
	4000	71.1	74.1	73.6
SDZ	100	100.9	108.1	102.9
	1000	89.4	97.9	96.5
	4000	83.1	87.7	86.1
STZ	100	98.1	102.5	101.6
	1000	92.5	98.6	99.2
	4000	83.1	86.7	85.2
SPY	100	95.1	99.8	98.6
	1000	87.8	93.6	89.7
	4000	72.1	76.0	75.5
SMZ	100	100.1	104.2	103.9
	1000	91.7	96.8	96.0
	4000	70.0	75.0	73.3
SMM	100	102.3	105.8	104.4
	1000	99.4	100.7	100.0
	4000	94.5	98.2	97.1
SMX	100	100.4	105.4	104.7
	1000	95.2	99.7	98.5
	4000	93.0	95.0	94.3
SXZ	100	102.3	105.6	104.9
	1000	96.7	99.4	98.8
	4000	93.1	97.6	96.3

[a] Analyte identification: sulfaguanidine, SGD; sulfadiazine, SDZ; sulfathiazole, STZ; sulfapyridine, SPY; sulfamethazine, SMZ; sulfamonomethoxine, SMM; sulfamethoxazole, SMX; sulfisoxazole, SXZ.

Table 3. Comparison of the proposed method with other published methods to determine sulfonamides in meats.

Pretreatment (Adsorbent)	Technique	Sample	Nº sulfonamides	LOD (μg kg^{-1})	Recovery (%)	Time	Reference
SPE (Discovery® DSC-SCX (Supelco))	HPLC-UV	Cattle, swine, chicken and sheep muscle tissues	10	3-14	66.3-71.5	SPE+29 min separation	[20]
Dispersive (magnetic molecularly imprinted polymer)	HPLC-UV	Chicken meat	4	0.5-150.0	95-99	Dispersive+25 min separation	[4]
SPE (InertSep K-solute (Diatomaceous Earth) (GL Science))	HPLC-MS	Chicken muscle	7	0.12-0.30	75-93	SPE+20 min separation	[31]
Liquid-liquid extraction, LLE (--)	HPLC-DAD	Chicken meat and eggs	7	--	85.8-108.1	LLE+30 min separation	[21]
Online-SPE (graphene oxide-based monolith)	HPLC-MS/MS	Chicken muscle and milk	16	0.3 (milk) and 0.6 (chicken muscle)	70.3-108.0	Total 23 min	[30]
SPE (PEGDA polymer)	HPLC-DAD	Pork bacon, pork liver, hicken muscle	8	7.5-16.2	70-108	SPE+16 min	This work