Research Article

Application of capillary zone electrophoresis with large-volume sample stacking to the sensitive determination of sulfonamides in meat and ground water

A CZE method with UV-Vis detection has been established and validated for the determination of nine sulfonamides: sulfapyridine, sulfamethazine, sulfamerazine, sulfameth, sulfadiazine, sulfadimethoxine, sulfamethoxazole, sulfachlorpyridazine, and sulfamethizole. Optimum separation was obtained on a 64.5 cm × 75 μm bubble cell capillary using a buffer containing 45 mM sodium phosphate and 10% methanol at pH 7.3, with temperature and voltage of 27°C and 25 kV, respectively. p-Aminobenzoic acid was used as an internal standard. Taking into account the lack of sensitivity of the UV-Vis detection, the application of an on-line preconcentration methodology, such as large-volume sample stacking with polarity switching has been proposed. This procedure combined with a solvent extraction/SPE method applied for off-line preconcentration and cleanup provides a significant improvement in the LODs, ranging from 2.59 to 22.95 μg/L for the studied compounds; the quantification of these residues being possible below the levels established by EU legislation in animal food products, such as meat. Satisfactory recoveries were also obtained in the analysis of these compounds in ground water.

Keywords: Capillary zone electrophoresis / Large-volume sample stacking / Meat / Solid phase extraction / Sulfonamides / Water DOI 10.1002/elps.200600166

1 Introduction

Many veterinary drugs are used for the treatment of food-producing animals. Among them, there is a class of antibiotics named sulfonamides which are antibacterial and anti-infective drugs, commonly used for the treatment of diseases in medicine and veterinary practice, such as gastrointestinal and respiratory infections, being administrated orally or mixed with animal feed with therapeutic and prophylactic purposes. Because of their broad spectrum of activity and their low costs [1], they are widely used nowadays, but the problem is that sometimes they are applied without respecting safety recommendations which results in undesirable residues in animal tissues, meat, or biofluids such as milk. As it has been observed that most of the sulfonamides are suspected to be carcinogenic, and produce thyroid tumors in rodents [2], affecting also human [3], the use of these compounds is of great public health concern and their residues have to be controlled. Due to the fact that these analytes may be present in minute concentrations, and may pose a health threat to consumers, the European Union (EU) has adopted a maximum sulfonamide residue level (MRL) of 100 μg/kg [4], and their determination in multiple animal tissues, meat and other animal by-products such as milk or eggs, have drawn great attention in the last years. As a consequence of nonadequate treatment of human and animal excretions, antibiotic residues can be present in the environment for a long period of time, leading to the appearance of antimicrobial resistance [5]. Sulfonamides can be used against human diseases; they spread to the surface water through urban waste water because the

Correspondence: Professor Ana M. García-Campaña, Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Avd. Fuente Nueva s/n, E-18071 Granada, Spain
E-mail: amgarcia@ugr.es
Fax: +34-958-249510

Abbreviations: HLB, hydrophilic–lipophilic balance; IS, internal standard; LVSS, large-volume sample stacking; MRL, maximum residue level; NSM, normal stacking mode; PABA, p-aminobenzoic acid; SCP, sulfachlorpyridazine; SDM, sulfadimethoxine; SDZ, sulfadiazine; SM, sulfameth; SP, sulfapyridine; SMR, sulfamerazine; SMT, sulfameth; SMX, sulfamethoxazole; SMZ, sulfamethazine
actual procedure for waste water treatment cannot completely remove these compounds [5]. Also, because of their use in veterinary care, they are found in soils, ground, and surface waters due to the use of animal excretions as manure.

There are several methods for the determination of sulfonamides, mainly using chromatographic techniques, especially HPLC with UV, fluorescence, electrochemical detector, and MS detectors [7–16]. Recently, chemiluminescence detection has been proposed as sensitive detection for the HPLC analysis of sulfonamides of veterinary use in milk [17]. Among all of these detection systems employed with HPLC, diode-array detection is the most widely used and it has been applied for the determination of sulfonamides in milk [18, 19] but, sometimes, it presents a lack of selectivity, being necessary the application of efficient matrix cleanup procedures [14] or a very selective detector. Also, HPLC-MS has been used for the establishment of multimethods for the analysis of pharmaceutical residues in environmental samples, including sulfonamides [20, 21]. GC coupled to MS (GC-MS) is relatively sensitive and selective, but routine residue analyses are not feasible because multiple purification steps are required prior to the analysis of thermally labile and nonvolatile sulfonamides. However, several methods involving GC-MS have been developed for detecting sulfonamides after derivatization [22–24].

CE is a popular and powerful separation technique nowadays, mainly because of many advantageous features of this technique, including high resolution, great efficiency, rapid analysis, and small consumption of both sample and solvent in comparison with HPLC. The development of CE methods to separate diverse analytical samples has been growing very rapidly over the past decade [25–29], and the technique has demonstrated its efficiency in many applications [30, 31], including the determination of antibiotics [32–34]. Despite these, CE is not very common for the determination of sulfonamides. Some papers have been published, using mainly UV [35–37] or amperometric detection [38], applying the CZE mode [39–43] or MEKC [41, 44–46] in different matrices such as pharmaceutical compounds, biological fluids, or food of animal origin. The coupling of CE to MS for the determination of sulfonamides is gaining importance in the last years. A first approach for their determination in food by CE-MS was carried out [47], and the characterization of isomeric sulfonamides using CE coupled with the MS/MS was proposed to identify residues in milk extracts. Recently, a new methodology for the screening and analytical confirmation of seven sulfonamide residues in milk using CE-MS has been published [48].

The first use of CE for the analysis of seven sulfonamides in meat was reported by Ackermans et al. [39], studying the corresponding migration times and the influence of the meat extract depending on the running buffer nature. A simple sample pretreatment consisting on extraction with ACN and centrifugation was applied, obtaining detection limits of 2–9 μg/mL. Due to the complex nature of meat, sample pretreatments are often required to remove proteins, fat, and potential interferences. Also, an important problem in the application of CE coupled to UV-Vis detection is the relatively high detection limits, which are an important drawback for its application in the control of drug residues in food, according to European recommendations. In this sense, solvent extraction and an SPE method were employed for sample cleanup and preconcentration, prior to the quantitative CE determination of eight sulfonamides in meat, with detection limits ranging 5–10 μg/kg [35].

Several on-column sample preconcentration methods can also be combined with CE to increase the amount of the analyte introduced into the capillary obtaining an improvement in sensitivity. Some reviews have been published about different sample stacking techniques [49–54]. Among the different modes, the simplest one is normal stacking mode (NSM), which is done by dissolving the sample in a low conductivity matrix and by injecting the resulting sample solution hydrodynamically. Focusing happens at the interface between the low conductivity matrix and the buffer, due to the abrupt change in electrokinetic velocity. A limitation of NSM is the short optimum sample plug length that can be injected into the capillary without loss of separation efficiency or resolution [50]. However, large-volume sample stacking (LVSS) [55] implies the introduction of a volume, greater than that found optimum in NSM. In this case, the sample matrix must be pumped out from the capillary in order to preserve separation efficiency. Pumping is carried out with external pressure or with EOF, with the direction of pumping always opposite to that of the electrophoretic movement of charged solutes, and its velocity lower than the electrophoretic velocity of the charged solutes. Using this strategy, only positive or negative solutes can be effectively concentrated at one time. Concentration factors of more than 100 are reported for LVSS, improving LOD from two orders of magnitude. In LVSS two modalities exist, with or without change of polarity. For the case of anions, LVSS with polarity switching is a mode that permits to control the EOF in CZE separations, involving high EOF conditions to carry the separate analytes to the detector. This is done by introducing hydrodynamically, a large plug of low conductivity sample into the capillary, and applying negative voltage at the injection end. The large solvent plug is then electroosmotically...
pushed out of the capillary while the negative species stack-up at the boundary between the sample zone and the BGE. Once the main part of the low-conductivity zone has been pushed out of the capillary, the positive voltage is applied to carry out the separation.

In this paper, we have developed a new method for the extraction and determination of nine sulfonamides of veterinary use (sulfapyridine (SPD), sulfamethazine (SMZ), sulfamerazine (SMR), sulfameter (SMT), sulfadiazine (SDZ), sulfadimethoxine (SDM), sulfamethoxazole (SMX), sulfachlorpyridazine (SCP), and sulfamethizole (SMI)), whose maximum residue levels (MRLs) are regulated by the EU [4]. Their chemical structures are shown in Fig. 1. For the first time, an on-line preconcentration step has been developed and applied for the analysis of these compounds by CE, improving significantly the detection limits. The combination of an adequate SPE procedure with the selection and optimization of the LVSS procedure permits the quantitative determination of these sulfonamides with satisfactory accuracy. The SPE-LVSS-CZE method has shown its usefulness in the simultaneous monitoring of these residues in meat and water, at the very low μg/L levels.

Figure 1. Chemical structures of the studied sulfonamides.
2 Materials and methods

2.1 Chemicals

All reagents were analytical reagent grade. Solvents were HPLC grade and sulfonamides were analytical standard grade. ACN, methanol, sodium dihydrogen phosphate, disodium hydrogen phosphate, and sodium hydroxide were obtained from Panreac-Química (Madrid, Spain). Ultrapure water (Milli-Q plus system, Millipore, Bedford, MA, USA) was used throughout the work. A stock standard solution of 100 mg/L of each sulfonamide (SPD, Riedel-de-Haën and SMZ, SMR, SMT, SDZ, SDM, SMX, SCP, and SMI, Vetranal, Sigma-Aldrich Química, Madrid, Spain) was prepared by dissolving 10 mg of the product in 100 mL of methanol (E. Merck, Darmstadt, Germany) in a calibrated flask. The solutions were stable for at least 2 months, stored in the dark at 4°C. Working standard solutions containing all the sulfonamides were freshly prepared by dilution of the stock solutions with imidazole solution (10 mM, pH 9.8) in the presence of methanol (10%) (Sigma-Aldrich Química). p-Amino-benzoic acid (PABA) (Fluka, Sigma-Aldrich Química) was used as internal standard (IS). A stock solution of 100 mg/L of PABA was prepared by dissolving 10 mg of the product in 100 mL of water. The solution was stable for at least 1 month.

Extraction cartridges containing an Oasis® hydrophilic–liphophilic balance (HLB; 60 mg, 3 cc; Waters, Milford, MA, USA) and Alumina N (500 mg, 3 cc; E. Merck) in homemade cartridges, were used.

2.2 Instrumentation

CE experiments were carried out with an HP3D CE instrument (Agilent Technologies, Waldbronn, Germany) equipped with a diode array detector (DAD). Data were collected using the software provided with the HP ChemStation version A.09.01. Separation was carried out in a bare fused-silica capillary (64.5 cm × 75 μm id, effective length 56 cm) with an optical path length of 200 μm (bubble cell capillary from Agilent Technologies). For pH measurements, a pH meter (Crisom model pH 2000, Barcelona, Spain) was employed with a resolution of ±0.01 pH unit. The extraction and preconcentration process was achieved with a vacuum manifold system from Supelco (Bellafonte, PA, USA) coupled with a vacuum pump (Büchi model B-169, Switzerland). Rotavapor (Büchi RE 121, Büchi Laboratories-Technik AG, Flawil, Switzerland), was used for sample preparation.

2.3 Electrophoretic procedure

Before the first use, the capillary was conditioned by flushing with 1 M NaOH for 10 min at 60°C, then with water for 5 min, and finally with the BGE solution for 20 min. A pressure of 1 bar was applied. At the beginning of each day, the capillary was prewashed with an N2 pressure of 7 bar for 1 min with 0.1 M NaOH, 1 min with water and 2 min with running buffer. After each run, the capillary was postwashed with 7 bar for 0.75 min 0.1 M NaOH, 0.5 min with deionized water, and 1 min with buffer to maintain an adequate reproducibility of run-to-run injections. The electrophoretic separation was achieved with a voltage of 25 kV (normal mode). The running buffer was an aqueous solution of sodium dihydrogen phosphate/disodium hydrogen phosphate 45 mM adjusted to pH 7.3 with orthophosphoric acid and containing 10% v/v methanol. All the sulfonamides were monitored at 265 nm with a bandwidth of 16 nm. The temperature of the capillary was kept constant at 27°C. Injection of the sample occurred using the following LVSS procedure.

2.4 LVSS procedure

Samples containing the analytes were dissolved in imidazole solution (pH 9.8, 10 mM, methanol 10%) and loaded with a pressure of 7 bar for 0.5 min. In this way, the whole capillary column was filled with the sample solution. After sample injection, a negative voltage (−28 kV) was applied. Sample matrix removal from the capillary was indicated by monitoring the electric current, which progressively increased to its normal value as the low-conductivity injected zone was eliminated from the capillary. At this stage the stacking process could be considered complete. The high voltage was then switched from negative to positive (25 kV).

2.5 Sample treatment

2.5.1 Meat samples

Pork meat samples purchased from a local market were used in this study. For extraction, preconcentration, and cleanup, we have developed a sample treatment with some modifications from one previously proposed [35], based on the use of ACN for sample extraction and SPE cartridges for sample cleanup. Meat (3 g) was spiked with different concentration levels of sulfonamides. After spiking and homogenizing, 20 mL of ACN was added and the samples were vortexed for 2 min. ACN extracted a lot of endogenous substance from meat, therefore, two different SPE cartridges were
needed in order to cleanup and concentrate the samples. The first one, Alumina N, is a polar sorbent SPE cartridge. The extract was percolated through the cartridge at a flow rate of approximately 1 mL/min. Then, a mixture of ACN/water (80:20 v/v) was used as a washing solution. The loading and washing solutions were collected, dried off with a rotavapor and redissolved with 10 mL of water by vortexing for 2 min. The water extract was passed through the second cartridge, an Oasis HLB, to remove potential interferences and to cleanup. The cartridge was previously activated with 2 mL of methanol and 2 mL of water. After sample percolation, final elution was carried out with methanol. This methanol solution was evaporated to dryness under a gentle nitrogen current at 50°C, and then reconstituted with the imidazole solution containing PABA (150 μg/L). Detailed sample preparation steps of the procedure are shown in Fig. 2.

![Figure 2](image)

**Figure 2.** Extraction procedure for meat samples.

### 2.5.2 Water samples

Different samples of ground water were collected near a cow farm in Santa Fe (Granada, Spain). In this case, an HLB-SPE cartridge was used in order to cleanup and to preconcentrate the samples. Water (10 mL) was spiked with different concentration levels of sulfonamides. The water extracts were loaded through the cartridge after conditioning with 3 mL of methanol and 3 mL of acetic acid (50%, pH 3). Methanol was used to elute the compounds. This methanol solution was evaporated to dryness under nitrogen gas at 50°C and then reconstituted with imidazole solution containing PABA (150 μg/L).

### 3 Results and discussion

#### 3.1 Optimization of CE experimental conditions

Preliminary studies were carried out in order to optimize the experimental parameters affecting the CE separation of the target compounds by UV-Vis detection. The UV-Vis spectra of the analytes were registered choosing 265 nm with a bandwidth of 16 nm for monitoring the select sulfonamides. To find a running buffer suitable for the separation of the analytes we checked different buffers in a pH range of 2–10. Finally, a pH of 7.3 was selected, because the best resolution was obtained at this value, using sodium dihydrogen phosphate/disodium hydrogen phosphate as a suitable buffer. A significant improvement in resolution was observed with the addition of methanol to the electrophoretic buffer, demanding a study using different percentages (5, 10, and 15%). An important interaction was observed between the pH and the methanol percentage, achieving the best results for a pH 7.3 and 10% methanol (adjusting the pH after the addition of methanol), with a resolution higher than 1.5 for all the peaks. A voltage of 25 kV was applied as optimum so as to achieve a good compromise between the running time and the electric current.

Different buffer concentrations were tested (40, 45, and 50 mM); 45 mM was selected as the optimum concentration, in order to obtain the best resolution with an adequate electric current. The effect of temperature on the separation was investigated in the range of 25–30°C, as lower or higher values did not provide an adequate resolution for all the analytes. A capillary temperature of 27°C provided the separation of all the sulfonamides with satisfactory resolutions, thus this temperature was selected as optimum. The summary of the optimized experimental conditions is shown in Table 1.

#### 3.2 Optimization of the LVSS with polarity switching procedure

Considering the poor sensitivity of CE using the UV-Vis detection, the direct use of this technique for the direct analysis of sulfonamides would not be appropriate for its monitoring in foodstuff of animal origin, obtaining LODs above their MRL values imposed by legislation. For this reason, in the present work we have combined the SPE with an on-line preconcentration protocol, providing a simple and inexpensive methodology for
Separation buffer Sodium phosphate, 45 mM containing increasing percentages of methanol (0, 5, 10, and 25% v/v) were tested as sample solvent. The best results were obtained in the presence of 10% methanol. Below a 10%, lower signals were obtained and above this percentage, peaks were significantly overlapped. For the optimization of the size of plug sample, different percentages (50, 70, 90, and 100%) of the capillary were filled in each experience. These percentages were estimated, based on the diameter and length of the capillary and the applied pressure. The results showed that it was possible to fill the whole volume of the capillary, being achieved by applying a pressure of 7 bar for 0.5 min. The negative voltage was studied in the interval of −25 to −30 kV. Finally, −28 kV was selected as optimum, as this value permits a rapid filling of the capillary in a stable system. Values higher than −28 kV frequently produce the capillary rupture. Once all these optimum values were selected, the whole LVSS process was completed in around 3.5 min, getting an improvement of the sensitivity of around 15 times.

Electropherograms of a meat sample spiked with 100 μg/kg, and of a water sample spiked with 50 μg/L of each sulfonamide, respectively, obtained after application of the corresponding SPE procedures, and using the optimized LVSS at the selected CE experimental conditions are shown in Fig. 3. No interferences from the matrix were observed in water samples. In meat sample one interfering peak appeared at the same migration time of SPD, so its detection was not possible in this matrix.

### 3.3 Validation of the electrophoretic procedure

#### 3.3.1 Linearity, LOD, and LOQ

The linearity of the response was established from eight calibration levels corresponding to 10, 25, 50, 75, 100, 150, 200, and 250 μg/L of each sulfonamide except for SPD. For this compound, calibration levels were 50, 75, 100, 125, 150, 200, 250, and 300 μg/L. In all cases, 150 μg/L of PABA was added as IS.

Each concentration level was injected by triplicate. Calibration curves were established by considering the relative corrected peak areas (as the ratio analyte peak per migration time to IS peak, per migration time) as a function of the analyte standard concentration. The statistic parameters calculated by least-square regression, and the performance characteristics are presented in Table 2. The satisfactory determination coefficients confirm that sulfonamide responses were linear over the studied range. LODs and LOQs have been calculated using the S/N ratio and have been calculated considering the analytical procedure without taking into account the previous

---

**Table 1. Summary of the optimized CE experimental conditions**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary</td>
<td>Fused-silica capillary 64.5 cm × 75 μm id, optical path length of 200 μm</td>
</tr>
<tr>
<td>Separation buffer</td>
<td>Sodium phosphate, 45 mM with 10% methanol</td>
</tr>
<tr>
<td>pH</td>
<td>7.3</td>
</tr>
<tr>
<td>Separation voltage</td>
<td>25 kV</td>
</tr>
<tr>
<td>Temperature</td>
<td>27°C</td>
</tr>
<tr>
<td>LVSS solution</td>
<td>Imidazole 10 mM with 10% methanol</td>
</tr>
<tr>
<td>LVSS voltage</td>
<td>−28 kV</td>
</tr>
</tbody>
</table>

Improving sensitivity. The buffer conditions and polarity allowed the use of LVSS with polarity switching for anions, considering that all the molecules are negatively charged. At the start, a large volume of sample prepared in a low conductivity matrix is injected and a voltage at negative polarity is applied for focusing of zones and removal of sample matrix. When the anions are completely focused and most of the sample matrix is removed, voltage is stopped, and polarity is reversed. This occurs when the current reached 95–99% of its value. Finally, a voltage at positive polarity is applied in order to separate and detect the focused zones.

In LVSS we have optimized three significant parameters: sample solvent, size of plug sample, and voltage. The most relevant variable in this type of preconcentration methodology is the sample solvent, because it requires a very low conductivity to obtain the best focusing of the analytes. Different sample solvents were studied: water, methanol, and an imidazole solution (10 mM, pH 9.8, 10% methanol), considering their low conductivity and a suitable pH in which all the sulfonamides are charged in the negative form [56]. Diluted BGE was not tested as sample solvent, as it does not provide a suitable pH for all the sulfonamides to be charged, and it provides higher conductivity than the other tested solvents, decreasing the effectiveness of the LVSS. Only imidazole medium allows us to apply the LVSS process, obtaining satisfactory results. Using methanol, it was not possible to carry out the stacking procedure because the capillary cannot be filled with the separation buffer. In the case of water, it was not possible to carry out the stacking for SPD, because this compound was not charged. In order to increase the efficiency of LVSS, we studied the effect of the presence of organic solvent in the imidazole solution. With this purpose, different 10 mM imidazole solutions, containing increasing percentages of methanol (0, 5, 10,
Figure 3. (A) Pork meat sample spiked with 100 μg/kg of each sulfonamide. (B) Ground water sample spiked with 50 μg/L of each sulfonamide. Separation conditions as indicated in Table 1.

Table 2. Statistics and performance characteristics of the proposed method (concentration vs. relative corrected peak areas)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Linearity range (μg/L)</th>
<th>Intercept (μg/L)</th>
<th>Slope</th>
<th>$R^2$</th>
<th>LOD (μg/L)</th>
<th>LOQ (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPD</td>
<td>23–300</td>
<td>−0.076</td>
<td>0.0035</td>
<td>0.9919</td>
<td>22.95</td>
<td>25.83</td>
</tr>
<tr>
<td>SMZ</td>
<td>10–250</td>
<td>−0.023</td>
<td>0.0036</td>
<td>0.9863</td>
<td>7.59</td>
<td>10.39</td>
</tr>
<tr>
<td>SMR</td>
<td>11–250</td>
<td>−0.021</td>
<td>0.0040</td>
<td>0.9949</td>
<td>8.33</td>
<td>10.85</td>
</tr>
<tr>
<td>SMT</td>
<td>11–250</td>
<td>−0.026</td>
<td>0.0039</td>
<td>0.9939</td>
<td>7.93</td>
<td>10.51</td>
</tr>
<tr>
<td>SDZ</td>
<td>6–250</td>
<td>−0.013</td>
<td>0.0046</td>
<td>0.9920</td>
<td>3.68</td>
<td>5.87</td>
</tr>
<tr>
<td>SDM</td>
<td>13–250</td>
<td>−0.045</td>
<td>0.0044</td>
<td>0.9906</td>
<td>11.19</td>
<td>13.48</td>
</tr>
<tr>
<td>SMX</td>
<td>5–250</td>
<td>−0.008</td>
<td>0.0046</td>
<td>0.9936</td>
<td>2.59</td>
<td>4.78</td>
</tr>
<tr>
<td>SCP</td>
<td>9–250</td>
<td>−0.019</td>
<td>0.0036</td>
<td>0.9926</td>
<td>6.50</td>
<td>9.30</td>
</tr>
<tr>
<td>SMI</td>
<td>12–250</td>
<td>−0.038</td>
<td>0.0042</td>
<td>0.9922</td>
<td>10.00</td>
<td>12.40</td>
</tr>
</tbody>
</table>

3.3.2 Recovery studies

In order to check the applicability of the proposed methodology, trueness of this method was examined using recovery studies by adding sulfonamides in sulfonamide-free pork meat and ground water samples. Pork meat samples were spiked with a mixture of the eight sulfonamides (SMZ, SMR, SMT, SDZ, SDM, SMX, SCP, and SMI) at different levels (50, 100, and 150 μg/kg for each one), selected according to the recommendations of the European Commission concerning the monitoring of drugs [57]. Each level was prepared by triplicate and it was injected three times. The identification of each sulfonamide was based on both their migration times and absorption spectra. The results are shown in Table 3 and as can be seen, the proposed method provides good results in terms of both trueness and precision.
Table 3. Recovery for each sulfonamide at different spiked levels in pork meat samples

<table>
<thead>
<tr>
<th>Added  (50 µg/L)</th>
<th>SMZ</th>
<th>SMR</th>
<th>SMT</th>
<th>SDZ</th>
<th>SDM</th>
<th>SMX</th>
<th>SCP</th>
<th>SMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>R (%)</td>
<td>83.9</td>
<td>94.8</td>
<td>83.8</td>
<td>98.7</td>
<td>90.2</td>
<td>93.3</td>
<td>96.2</td>
<td>87.1</td>
</tr>
<tr>
<td>RSD (º)</td>
<td>6.0</td>
<td>4.9</td>
<td>3.9</td>
<td>4.8</td>
<td>8.6</td>
<td>7.6</td>
<td>3.7</td>
<td>4.7</td>
</tr>
</tbody>
</table>

Table 4. Recovery for each sulfonamide at different spiked levels in ground water samples

<table>
<thead>
<tr>
<th>Added  (5 µg/L)</th>
<th>SPD</th>
<th>SMZ</th>
<th>SMR</th>
<th>SMT</th>
<th>SDZ</th>
<th>SDM</th>
<th>SMX</th>
<th>SCP</th>
<th>SMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>R (%)</td>
<td>89.2</td>
<td>91.9</td>
<td>92.8</td>
<td>97.4</td>
<td>94.7</td>
<td>90.7</td>
<td>93.6</td>
<td>98.2</td>
<td>75.6</td>
</tr>
<tr>
<td>RSD</td>
<td>8.8</td>
<td>7.7</td>
<td>7.6</td>
<td>3.4</td>
<td>8.6</td>
<td>7.2</td>
<td>4.7</td>
<td>4.5</td>
<td>6.8</td>
</tr>
</tbody>
</table>

Mean value (n = 9).

Ground water samples were spiked with a mixture of nine sulfonamides (SPD, SMZ, SMR, SMT, SDZ, SDM, SMX, SCP, and SMI) at different levels (5, 20, and 50 µg/L for each one) selected in relation to the low concentrations, possibly found in this kind of samples. Each level was prepared by triplicate and it was injected three times. The results are shown in Table 4. Accuracy was demonstrated considering the satisfactory results of this study.

4 Concluding remarks

A sensitive and rapid electrophoretic method has been developed and validated for the analysis of sulfonamides. For the first time, to the best of our knowledge, SPD has been quantified by CZE. Previously to the CE analysis, a solvent extraction/SPE procedure has been applied for extraction, off-line preconcentration and sample cleanup, and for the first time, on-line preconcentration using LVSS has been carried out, improving the sensitivity of the detection and making it suitable for the monitoring of these residues in foodstuffs of animal origin (pork meat) and in environmental samples (ground waters). The separation takes place in less than 13 min and in the case of meat samples, this new combined method provides good recoveries, ranging from 81.2 to 99.3%. The LODs obtained are low enough for quantifying these residues in meat below the legislated MRL established by the EU, being among the fastest and more sensitive methods for the analysis of sulfonamides. In the case of water samples, at this moment, no established levels exist for these emerging pollutants, but the method shows its usefulness for their satisfactory detection in environmental samples.

The National Institute of Agricultural and Food Research and Technology (INIA, Ministerio de Agricultura, Pesca y Alimentación, Project Ref. CAL03-087-C2-1) and EU funds (FEDER) supported this work. LGG is grateful to the Plan Propio of the University of Granada for a research contract. JJST is grateful to “Fundación La Caixa” for a predoctoral grant.

5 References
