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Rapid separation and highly sensitive detection methodology for sulfonamides in shrimp using a monolithic column coupled with BDD amperometric detection

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ABSTRACT

In this report, we aimed to extend our previous efforts toward the evaluation of sulfonamides (SAs) with a boron-doped diamond (BDD) electrode. We improved this method by reducing the analysis time using a monolithic column coupled with amperometric detection to determine seven sulfonamides (sulfaguanidine, sulfadiazine, sulfamethazine, sulfamonomethoxine, sulfamethoxazole, sulfadimethoxine and sulfaquinoxaline). Because of its rapid separation, low back-pressure and high separation efficiency compared to a particle-packed column, a monolithic column (100 mm × 4.6 mm) was used for sulfonamide separation. Chromatographic separation was performed in less than 8 min. The analysis was carried out using phosphate buffer (0.1 M, pH 3): acetonitrile: methanol in a ratio of 80:15:5 (v/v/v) as the mobile phase with a flow rate of 1.5 mL min^{-1}. The optimal detection potential using hydrodynamic voltammetry was found to be 1.2 V versus Ag/AgCl. The method was applied to determine seven sulfonamides in shrimp after sample preparation by solid-phase extraction. The recoveries of the sulfonamides in spiked shrimp samples at 1.5, 5 and 10 μg g^{-1} were in the range of 81.7 to 97.5% with a relative standard deviation (R.S.D.) between 1.0 and 4.6%. Our methodology produced results that were highly correlated with HPLC–MS data. Therefore, we propose a method that can be used for the rapid, selective and sensitive evaluation of sulfonamides in contaminated food.

1. Introduction

Currently, sulfonamides (Fig. 1) are widely used as broad-spectrum synthetic antibiotics due to their low cost. They are used for therapeutic, prophylactic and growth-promoting purposes in animals. The use of SAs can cause residual problems in meat because of excessive or uncontrolled dosages to animals before sales to consumers. The hazardous SAs can cause allergic reactions and antibiotic resistance, and be carcinogenic in humans [1,2]. To limit these and other impacts, the European Union (EU) set a maximum residue limit (MRL) of 100 ng g^{-1} for SA residues in original animal food [3]. In addition, each country has set different limits for antibiotic residues in food. In Thailand, shrimp is one of the top 10 exports sent to other countries. To overcome the limitations of trade based on SA residues, a rapid, accurate, selective and sensitive method for the quantification of SAs in shrimp is necessary. Several methods have been developed for the determination of residual SAs including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), high-performance liquid chromatography–mass spectrometry (HPLC/MS) [4–11], gas chromatography (GC), gas chromatography–mass spectrometry (GC/MS), capillary electrophoresis (CE) [12,13] and the enzyme-linked immunosorbent assay (ELISA) [14]. The common method used for the separation of sulfonamides is HPLC coupled with ultraviolet (UV) [15–22] and fluorescence detectors [1,23], which exhibits high sensitivity and selectivity. However, there is a high cost for the equipment and laboratories and a requirement for significant labor and analytical resources, which can potentially cause substantial delays in obtaining results. The electrochemical (EC) detector [24,25] is an alternative method for SA determination and has the benefits of simplicity, speed, sensitivity and low cost.

The conventional column employed for the separation of these analytes is particle-packed, which has disadvantages, such as high flow resistance, high back-pressure and particle splitting at elevated flow rates. These drawbacks can lead to non-reproducibility, low separation efficiency and reduced sample throughput [26]. Recently, the monolithic column was discovered and verified as an alternative material instead of using a conventional column. This column is classified according to its base, such as a polymer- or silica-based monolithic column [27–30]. The advantage of silica-based monolithic column over the polymer-based monolithic column is that it provides a high tolerance for organic

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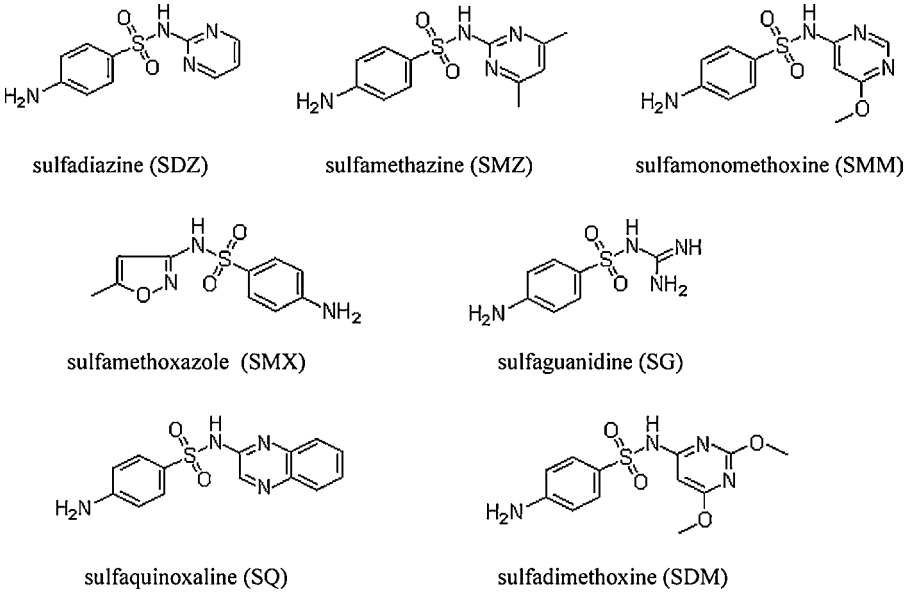

Fig. 1. Chemical structures of the studied sulfonamides.

solvent, which leads to a longer lifetime. Therefore, in this work, we selected a silica-based monolithic column for the separation of analytes. The monolithic columns have single piece, through-pore and cross-linked skeletons. The silica-based monolithic column has a small-sized skeleton and bimodal pores (both macropores and mesopores in one structure). The mesopores have an average diameter of 13 nm, and large macropores have an average diameter of 2 μm. Advantageously, the total porosity in a silica single piece is more than 80%. With a lower back-pressure than a particle-packed column, even at high flow rates, the separation can be performed in a silica-based monolithic column with a shorter analysis time and high sample throughput. Because of this strong performance, the monolithic column was applied for the separation of several analytes in food [31–34], cosmetics [35], pharmaceuticals [36,37] and environmental samples [38].

The important problem for the determination of residual sulfonamides in animal tissues and environmental is the matrix. Therefore, sample extraction techniques are required before analysis. The sample preparation techniques that have been reported for SAs consist of liquid–liquid extraction (LLE) [19,24], matrix solid-phase dispersion (MSPD) [39], solid-phase microextraction (SPME) [20] and solid-phase extraction (SPE) [1,4,6–10,21]. SPE was used for simultaneous extraction and cleanup analytes. The advantages of SPE are selective, simple and short time-consumption. Among the SPE materials reported, Oasis HLB is attractive. It is a hydrophilic–lipophilic balanced sorbent in SPE that is composed of two monomers (N-vinylpyrrolidone and divinylbenzene). This material has exhibited excellent retention capacity for a wider polarity of analytes [4,10].

We have reported on the determination of sulfonamides in egg samples by conventional HPLC, using a boron-doped diamond thin film electrode [24]. This work is an extension of our previous efforts on the determination of sulfonamides with a diamond electrode using chromatography. This is our first report on the use of a diamond electrode for sulfonamide quantification in 'real world' contaminated samples. We observed strong analytical figures with limits of detection in the low ppb range, good sensitivity, excellent response precision and stability. We developed a rapid, highly sensitive and accurate method by exploiting a monolithic column coupled with a diamond electrode and validated it through a comparison measurement using HPLC–MS. The methodology was applied to determine residual sulfonamides (sulfaguanidine (SG), sulfadiazine (SDZ), sulfamethazine (SMZ), sulfamonomethoxine (SMM), sulfamethoxazole (SMX), sulfadimethoxine (SDM) and sulfaquinoxaline (SQ)) in shrimp using Oasis HLB cartridges for sample extraction.

2. Experimental

2.1. Chemicals and reagents

HPLC-grade acetonitrile, ethanol and methanol were obtained from Merck (Darmstadt, Germany). Milli-Q water from Millipore ($R \geq 18.2\,M\Omega\,cm$) was used throughout this experiment. Potassium dihydrogen orthophosphate (KH_2PO_4) was purchased from BDH (VWR International Ltd., England). Disodium hydrogen phosphate dehydrate (Na_2HPO_4) and citric acid were purchased from Merck (Darmstadt, Germany). Ethylenediaminetetraacetic acid disodium salt dehydrate (Na_2EDTA) was obtained from Sigma–Aldrich (St. Louis, MO, USA). The extraction solution, Na_2EDTA–McIlvaine buffer, pH 4, was prepared by dissolving 13.52 g of Na_2HPO_4, 13.02 g of citric acid and 3.72 g of Na_2EDTA in one liter of Milli-Q water. All solutions and solvents were filtered with 0.45 μm Nylon membranes before use.

2.2. Standards solutions

Sulfadiazine, sulfamethazine, sulfamonomethoxine, sulfamethoxazole, sulfadimethoxine and sulfaquinoxaline were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sulfaguanidine was obtained from ICN Biomedicals Inc. (USA). A stock standard solution ($500\,\mu g\,mL^{-1}$) of each SAs was prepared by dissolving 5 mg of SA in 10 mL of an acetonitrile:Milli-Q water (50:50; v/v) solution in a volumetric flask and stored at 4 °C in the dark. The working solutions, containing a mixture of seven SAs, were prepared by suitable dilution of the stock standard solutions with the mobile phase.

2.3. HPLC experiment and apparatus

The HPLC-EC measurement, using a BDD electrode as an amperometric detector, was carried out in the mobile phase, which consisted of a phosphate buffer solution (0.1 M KH_2PO_4, pH 3), acetonitrile and ethanol in the ratio of 80:15:5 (v/v/v), with an

applied potential of 1.2 V versus Ag/AgCl. The flow rate was set at 1.5 mL min^{-1}. The HPLC system consisted of an HPLC compact pump model 2250 (Bischoff, Germany), a 20 μL-sample loop injection (Rheodyne No. 7125, USA), a thin-layer flow cell (GL Sciences, Inc.), an amperometric detector, and a data acquisition system (Eco-chemice Netherland). The chromatographic column was a Chromolith® Performance RP-18e silica-based monolithic column (100 mm × 4.6 mm i.d.) from Merck (Darmstadt, Germany). The experiment was performed at room temperature (25 °C).

2.4. Electrochemical measurement

The thin-layer flow cell consisted of three electrodes: a BDD working electrode, an Ag/AgCl reference electrode (Bioanalytical system Inc., USA) and a stainless steel tube counter electrode. The geometric area of the BDD electrode in the flow cell was estimated to be 0.37 cm^2 by using a 1 mm-thick silicon rubber gasket as a spacer. The measurement step was performed in a copper faradaic cage for reducing the electronic noise. An Autolab Potentiostat 100 (Eco-chemice Netherland) was used for amperometric controlling and signals processing.

2.5. Sample preparation and apparatus

The apparatuses for sample preparation consisted of a vortex mixer (Mixer Uzusio LMS. Co. Ltd., Japan), a centrifuge (MSE limited, London, UK), an ultrasonic bath (ESP chemicals, Inc., MA, USA), a vacuum manifold system (Phenomenex, Inc., CA, USA), a model 112 nitrogen evaporator (Organomation Associates Inc., MA, USA). Shrimp were purchased from a local supermarket. Two grams of a homogeneous shrimp sample was placed in a 20 mL-amber glass bottle, and 10 mL of Na_2EDTA–McIlvaine's buffer solution was added into the bottle. The mixture was well mixed on a vortex mixer for 5 min at high speed. Then, the mixture was placed in an ultrasonic water bath following centrifugation at 3500 rpm for 10 min. The collected supernatant was continually extracted and cleaned up with 200 mg Oasis HLB SPE cartridges (Water, Milford, MA, USA) connected to a 12-position vacuum manifold system.

For the solid-phase extraction, the SPE cartridges were conditioned with 5 mL of methanol and equilibrated with 5 mL of Milli-Q water, 5 mL of Na_2EDTA–McIlvaine buffer solution. The supernatant was loaded on Oasis HLB cartridges. During this step, the SA compounds are retained on the cartridges. The SA compounds were eluted with 7 mL of methanol at a flow rate of 1 mL min^{-1}. The eluted fraction was evaporated under a gentle stream of nitrogen and reconstituted with 10 mL of the mobile phase. The solution was filtered through a 0.45 μm Nylon membrane filter before injection into the HPLC-EC system.

3. Results and discussion

As mentioned, we previously reported the determination of SAs in an egg sample using liquid chromatography coupled with electrochemical techniques [24]. The Inertsil C4 particle-packed column was employed for the separation of four SAs, but required a long separation time (~18 min). In this work, we further developed this method for the separation of seven SAs by using a monolithic column in order to reduce the total analysis time. The BDD electrode was used as the working electrode to measure the oxidation reaction of SAs. Because of its low and stable background, the current led to high sensitivity and reproducibility for SA determination.

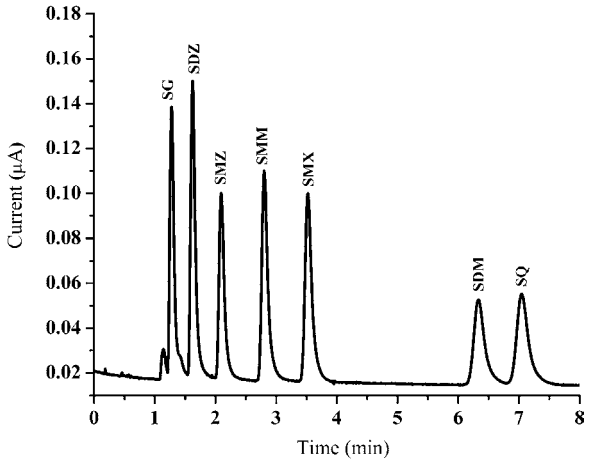

Fig. 2. HPLC-EC chromatogram of a 2.5 μg mL^{-1} mixture of seven standard SAs separated on a monolithic column (100 mm × 4.6 mm i.d.) using a mobile phase of phosphate buffer (0.1 M, pH 3), acetonitrile and ethanol in the ratio of 80:15:5 (v/v/v). The detection potential was 1.2 V vs. Ag/AgCl using a BDD electrode. The injection volume was 20 μL, and the flow rate was 1.5 mL min−^{1}.

3.1. Liquid chromatography with amperometric detection

3.1.1. HPLC separation

The seven SAs were separated by using a monolithic column and an isocratic system. The optimal mobile phase consisted of a phosphate buffer (pH 3), acetonitrile and ethanol in the ratio of 80:15:5 (v/v/v) at flow rate of 1.5 mL min^{-1}. A phosphate buffer was chosen as the most suitable solution for SA detection, because it provided the lowest background current. The chromatogram for the separation of a standard solution of seven SAs is presented in Fig. 2. The retention times were 1.15, 1.52, 2.01, 2.79, 3.56, 6.59 and 7.35 min for SG, SDZ, SMZ, SMM, SMX, SDM and SQ, respectively. From the chromatogram obtained, it can be seen that this method was more rapid (less than 8 min) than the previous methods (around 10 min) [24]. In addition, the present study reported not only four SAs, but also allowed for the separation of seven SAs in one injection. The monolithic column has a total porosity higher than 80%, and thus can use a higher flow rate, which leads to a faster mass transfer between the stationary and mobile phases. Table 1 shows a comparison of the total retention times for the determination of SAs by the HPLC methods, and clearly indicates that our developed method has the highest performance in terms of analytical speed.

3.1.2. Hydrodynamic voltammetry

Hydrodynamic voltammetry was employed to optimize the detection potential for SA detection. The detection potential ranging from 1.0 to 1.3 V versus Ag/AgCl was investigated. The obtained hydrodynamic voltammogram was an average of three 20 μL-injections of a 10 μg mL^{-1} SA standard mixture at the BDD electrode. Fig. 3(A) shows the hydrodynamic voltammetric *i*–*E* curve of seven SAs and the background current at each potential. The oxidation current of the SAs and the background current were significantly affected by the detection potentials. Therefore, the net current after background subtraction (S/B) was considered. Fig. 3(B) shows S/B ratios versus the potential curve. The signals increased when the potential increased up to 1.2 V versus Ag/AgCl for SMX, SDM and SQ. These three SAs had lower oxidation signals than the rest of the SAs. In order to compromise the highly sensitive detection of seven SAs in one injection, a detection potential at 1.2 V versus Ag/AgCl was selected as the optimal potential for the amperometric detection of SAs following their HPLC separation.

Table 1
Comparison of the total retention times of HPLC methods in the determination of sulfonamides.

Analytical column	Number of SAs	Flow rate (mL min^{-1})	Retention time	Reference
Alltech C18 (4.6 cm × 25 cm i.d. × 5 μm)	10	1	35	[2]
Mightysil RP 4 GP(150 mm × 3.9 mm i.d. × 4 μm)	3	1	7.2	[15]
NOVAPACK C18 (150 mm × 3.9 mm i.d. × 4 μm)	3	0.75	22	[16]
Alltech C18 (4.6 cm × 25 cm i.d. × 5 μm)	3	1	16	[18]
Metachem Inertsil Phenyl (150 mm × 4.6 mm i.d. × 5 μm)	6	1	12.5	[19]
Hypersil ODS (200 mm × 4.6 mm i.d. × 5 μm)	5	0.5	18	[20]
Inertsil C4 (150 mm × 4.6 mm i.d. × 5 μm)	4	1	18	[24]
Inertsil CN 3 (150 mm × 4.6 mm i.d. × 5 μm)	3	1	6	[25]
Lichrospher 100 RP18 (250 mm × 4.6 mm i.d. × 5 μm)	6	1	16	[39]
Chromolith® performance (100 mm × 4.6 mm i.d.)	7	1.5	8	This work

3.2. Sample extraction

3.2.1. pH of extracting solution

The pH of the extracting solution significantly affected the extraction efficiency of residual SAs in the sample. Na_2EDTA–McIlvaine buffer solution is a popular extracting solution employed for extraction of residual SAs in animal tissues due to its enhancement of the percentage recoveries [7,40,41]. EDTA, within the Na_2EDTA–McIlvaine buffer solution, acts as a chelating agent that binds with metals and cations in the matrix sample. This binding is useful for the prevention between metals/cations and the SPE cartridges, which also results in the improvement of the extraction efficiency [7,8]. The optimal pH of the Na_2EDTA–McIlvaine buffer solution for the extraction of seven SA residues in shrimp was studied over the range of pH 3–7. At pH values higher than pH 5, the mixture was a jelly-like solution, perhaps from the high protein and lipid concentrations in the shrimp samples. Moreover, the sample cannot be deprotonated or eluted through the SPE cartridges when using the Na_2EDTA–McIlvaine buffer solution in a high pH extraction. The highest percentage recoveries of the seven SAs were obtained at pH 4. At this pH, the seven SAs were kept in their neutral form because the pH of the extracting solution was lower than the pK_a values of the SAs (SG: 11.3, SDZ: 6.4, SMZ: 7.5, SMM: 6.5, SMX: 5.6, SDM: 6.0 and SQ: 5.5) [5,20,42], and had enhanced retention on the Oasis HLB cartridges. Therefore, the Na_2EDTA–McIlvaine buffer solution at pH 4 was selected as the optimal pH for the extraction of seven SAs contaminated in shrimp.

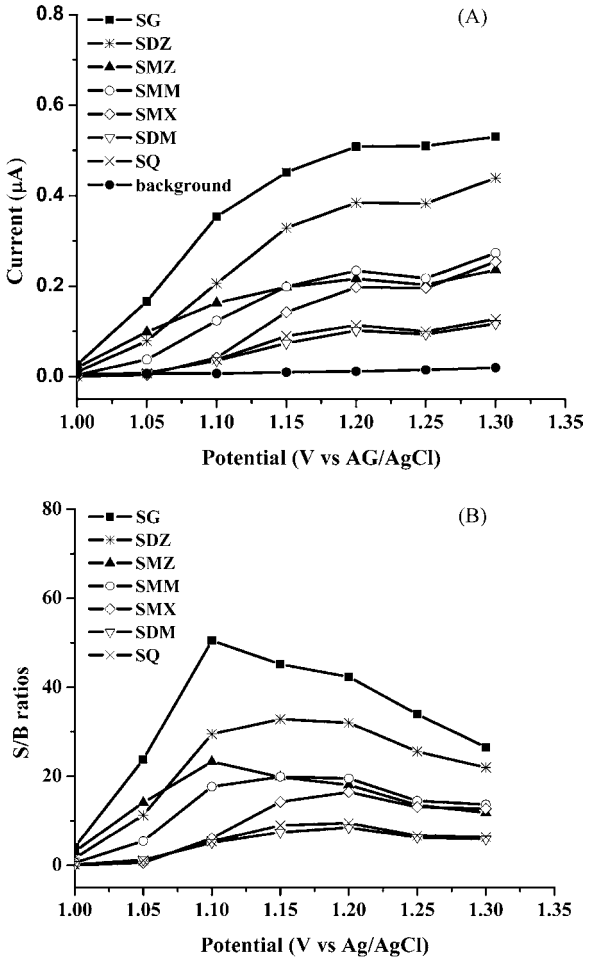

Fig. 3. Hydrodynamic voltammetric results for a 10 μg mL^{-1} mixture of seven SAs. (A) (■) SG, (✳) SDZ, (▲) SMZ, (○) SMM, (◊) SMX, (▽) SDM, (×) SQ, (●) background; (B) hydrodynamic voltammogram of signal-to-background ratios. The other conditions are the same as Fig. 2.

3.2.2. Selection of SPE materials

Typically, SPE materials significantly affected the recovery. Thus, we compared the C18 and Oasis HLB SPE cartridges. The conditions of the use of SPE procedure were 5 mL of methanol, equilibrated with 5 mL of Milli-Q water and 5 mL of Na_2EDTA–McIlvaine buffer solution, pH 4. Then, 10 mL of a 10 μg ml^{-1} SA standard mixture solution, prepared in a Na_2EDTA–McIlvaine buffer solution, was loaded and 7 mL of methanol was used to elute the SAs from the SPE cartridge. Fig. 4 demonstrates the percentage recoveries obtained from the C18 and Oasis HLB cartridges for seven SAs. The Oasis HLB and C18 cartridges presented high recoveries of SMZ, SMM, SMX, SDM and SQ. For SG and SDZ, the C18 cartridges provided lower percentage recoveries than Oasis HLB cartridges, which can be explained by the polarity differences of SG and SDZ. Because SG and SDZ are more polar than SMZ, SMM, SMX, SDM and SQ, the C18 cartridges were not suitable. In contrast, Oasis HLB can be used

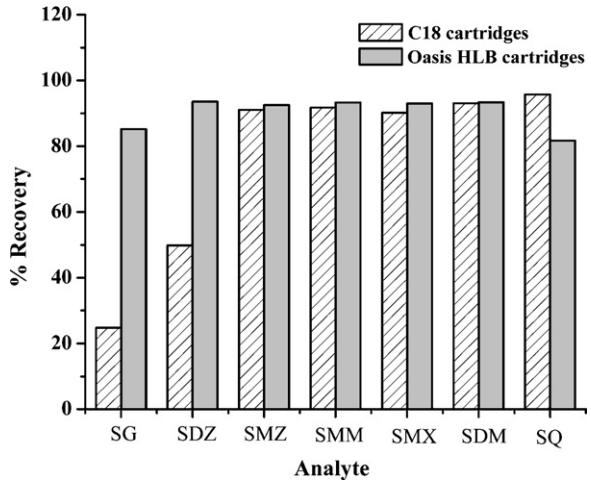

Fig. 4. Recoveries obtained of a 10 μg mL^{-1} standard mixture of seven SAs using C18 and Oasis HLB cartridges. The other conditions are the same as Fig. 2.

Table 2
Linearity, limit of detection and limit of quantitation of the method.

Analyte	Linear dynamic range (μg mL^{-1})	(peak areas units/ μg mL^{-1})	Intercept (μA)	R^2	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
Sulfaguanidine	0.01–50	0.1964	0.0638	0.9982	3.4	11.3
Sulfadiazine	0.01–50	0.2616	0.0659	0.9988	1.9	6.2
Sulfamethazine	0.01–100	0.1761	0.1348	0.9977	2.2	7.3
Sulfamonomethoxine	0.01–100	0.2239	0.2375	0.9961	1.4	4.6
Sulfamethoxazole	0.01–50	0.2285	0.0448	0.9995	1.2	4.1
Sulfadimethoxine	0.01–120	0.1582	0.1413	0.9985	2.0	6.8
Sulfaquinoxaline	0.01–120	0.1925	0.2244	0.9980	1.9	6.4

for a wider polarity range of analytes because it contains a mixture of hydrophilic N-vinylpyyolidone and lipophilic divinylbenzene in its structure. Therefore, Oasis HLB cartridges were used for sample preparation in order to maximize the sensitivity.

3.3. Linearity, limit of detection and limit of quantitation

The calibration of the peak areas against concentrations generated linear functions for all of the analytes within a range between 0.01 and 120 μg mL^{-1}, and the coefficients of determination (R^2) were higher than 0.99. The limits of detection (LOD) and limits of quantitation (LOQ) were calculated from 3 and 10S_{bl}/S, where S_{bl} is the standard deviation of the blank measurement (n = 10) and S is the sensitivity of the method or the slope of the linearity [43]. The data are summarized in Table 2. The detection limits obtained from our developed method are better than the detection limits obtained from previous reports [12,24,25,44,45].

3.4. Application to real sample

To assess the applicability of the proposed method, target compounds in food, shrimp samples from local supermarkets were investigated by standard addition. The typical chromatogram obtained from the analysis of a shrimp sample is illustrated in Fig. 5(B). The peaks were identified by comparison with the retention times of the reference compounds, which were determined by the injection of standard solutions (Fig. 5(A)). Sensitivities obtained from chromatograms in Figs. 2 and 5 are about 2 times different. It can be explained that protein and lipid contents in shrimp are very high although we have used Na_2EDTA–McIlvaine buffer solution and SPE for sample preparation.

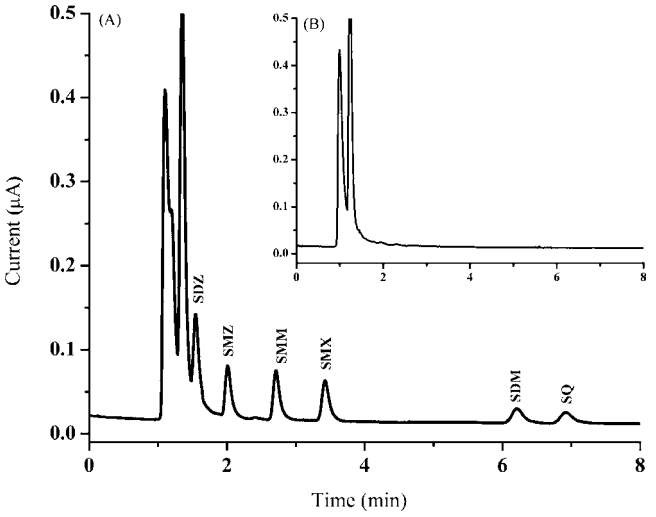

Fig. 5. HPLC-EC chromatogram of (A) a shrimp sample spiked with 2.5 μg mL^{-1} of standard mixture of seven SAs; (B) a blank shrimp sample separated on a monolithic column (100 mm × 4.6 mm i.d.). The other conditions are the same as Fig. 2.

The method determined SDZ, SMZ, SMM, SMX, SDM and SQ, but SG overlapped with interferences. The precision of the analytical process was calculated by determining the relative standard deviation for the repeated injection of solutions containing the complete set of standard compounds. To evaluate the repeatability of the analytical process, three concentrations (1.5, 5 and 10 μg ml^{-1}) were studied. These spiked concentration levels were chosen in order to compare the results to the ones from our previous work [24]. The

Table 3
Intra- and inter-day precisions and recoveries of the method.

Analyte	Spiked level (μg g^{-1})	Intra-day Mean of recovery (%) ± SD[a]	R.S.D (%)	Inter-day Mean of recovery (%) ± SD[a]	R.S.D (%)
Sulfadiazine	1.5	84.8 ± 1.0	1.1	86.7 ± 2.3	2.7
	5	82.0 ± 1.5	1.8	85.3 ± 3.0	3.5
	10	83.3 ± 1.0	1.2	83.3 ± 1.6	2.0
Sulfamethazine	1.5	94.6 ± 1.1	1.1	94.9 ± 0.9	1.0
	5	88.7 ± 1.5	1.7	88.9 ± 1.9	2.1
	10	82.3 ± 1.0	1.2	81.7 ± 1.2	1.5
Sulfamonomethoxine	1.5	93.6 ± 1.0	1.0	95.2 ± 1.5	1.6
	5	86.3 ± 2.3	2.6	88.3 ± 1.7	1.9
	10	84.3 ± 1.4	1.7	86.2 ± 1.6	1.9
Sulfamethoxazole	1.5	90.7 ± 1.3	1.3	89.1 ± 1.9	2.2
	5	87.9 ± 2.3	2.6	92.1 ± 4.3	4.6
	10	84.3 ± 1.3	1.5	85.6 ± 2.5	2.9
Sulfadimethoxine	1.5	97.5 ± 1.6	1.6	95.4 ± 1.8	1.9
	5	95.7 ± 1.0	1.0	97.3 ± 1.3	1.4
	10	94.1 ± 1.6	1.1	92.2 ± 1.8	1.9
Sulfaquinoxaline	1.5	95.5 ± 1.6	1.6	95.7 ± 1.5	1.5
	5	95.1 ± 2.2	2.3	97.3 ± 1.8	1.9
	10	95.0 ± 1.6	1.7	93.0 ± 2.1	2.2

[a] Mean of recovery (%) ± standard deviation of triplicate measurements.

percentage recoveries of both methods were found to be comparable. Table 3 presents the intra- and inter-day precision and recovery of the proposed method. For each concentration, three measurements were performed. The intra- and inter-day relative standard deviations (R.S.D) and recoveries of seven SAs were determined over the ranges of 1.0 to 4.6 and 81.7 to 97.5%, respectively.

For comparison, a paired *t*-test at the 95% confidence interval was performed on the results obtained from three spiked samples with different concentrations. The statistical *t*-value (4.303) was significantly higher than the experimental *t*-values between the two pairs of assays. The experimental *t*-values obtained by the proposed method were 0.710, 2.737, 0.306, 1.106, 2.722 and 0.702 for spiked concentrations at 5 $\mu g\,ml^{-1}$ and 0.550, 0.262, 3.051, 0.055, 0.577 and 0.169 for spiked concentrations at 10 $\mu g\,ml^{-1}$ of SDZ, SMZ, SMM, SMX, SDM and SQ, respectively. It is successfully found that there is no significant difference between the two sets of results obtained from the proposed methodology as compared with HPLC–MS, suggesting that these results are acceptable.

4. Conclusion

This work presents the extension of our previous efforts on the determination of sulfonamides with a diamond electrode using chromatography in 'real world' contaminated samples. Particular attention was focused on the development of a method for the rapid separation of sulfaguanidine, sulfadiazine, sulfamethazine, sulfamonomethoxine, sulfamethoxazole, sulfadimethoxine and sulfaquinoxaline with a monolithic column. The potential of 1.2 V versus Ag/AgCl was applied as the optimal value for SA determination. The limits of detection obtained from this method were lower than previous publications as well as a maximum residue limit of EU. The simultaneous determination of seven SAs in samples yielded satisfactory addition–recovery tests with an analysis time of 8 min; the obtained SA concentration values are similar to those obtained using a HPLC–MS method. Therefore, the proposed methodology was found to be effective for the separation and determination of SAs, while being very fast, inexpensive, and highly sensitive.

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References

[1] T.A. Gehring, B. Griffin, R. Williams, C. Geiseker, L.G. Rushing, P.H. Siitonen, J. Chromatogr. B 840 (2006) 132.
[2] G. Fang, J. He, S. Wang, J. Chromatogr. A 1127 (2006) 12.
[3] Commission of the European Community, The rules governing medical products in the European Community IV, Brussels, 1991.
[4] H. Chang, J. Hu, M. Asami, S. Kunikane, J. Chromatogr. A 1190 (2008) 390.
[5] E.L. McClure, C.S. Wong, J. Chromatogr. A 1169 (2007) 53.
[6] R. Sheridan, B. Policastro, S. Thomas, D. Rice, J. Agric. Food Chem. 56 (2008) 3509.
[7] U. Koesukwiwat, S. Jayanta, N. Leepipatpiboon, J. Chromatogr. A 1149 (2007) 102.
[8] M.S. Díaz-Cruz, M.Js. García-Galán, D. Barceló, J. Chromatogr. A 1193 (2008) 50.
[9] H. Li, P.J. Kijak, S.B. Turnipseed, W. Cui, J. Chromatogr. B 836 (2006) 22.
[10] Z. Ye, H.S. Weinberg, M.T. Meyer, Anal. Chem. 79 (2007) 1135.
[11] K. Choi, S. Kim, C. Kim, S. Kim, Chemosphere 66 (2007) 977.
[12] T. You, X. Yang, E. Wang, Analyst 123 (1998) 2357.
[13] M.S. Fuh, S. Chu, Anal. Chim. Acta 499 (2003) 215.
[14] X. Wang, K. Li, D. Shi, N. Xiong, X. Jin, J. Yi, D. Bi, J. Agric. Food Chem. 55 (2007) 2072.
[15] N. Furusawa, Anal. Chim. Acta 481 (2003) 255.
[16] R.H.M.M. Granja, A.M.M. Niño, F. Rabone, A.G. Salerno, Anal. Chim. Acta 613 (2008) 116.
[17] M. Gratacós-Cubarsí, M. Castellari, A. Valero, J.A. García-Regueiro, Anal. Bioanal. Chem. 385 (2006) 1218.
[18] J. He, S. Wang, G. Fang, H. Zhu, Y. Zhang, J. Agric. Food Chem. 56 (2008) 2919.
[19] J.E. Roybal, A.P. Pfenning, S.B. Turnipseed, S.A. Gonzales, Anal. Chim. Acta 483 (2003) 147.
[20] Y. Wen, M. Zhang, Q. Zhao, Y.Q. Feng, J. Agric. Food Chem. 53 (2005) 8468.
[21] H. Niu, Y. Cai, Y. Shi, F. Wei, J. Liu, S. Mou, G. Jiang, Anal. Chim. Acta 594 (2007) 81.
[22] B. Roudaut, M. Garnier, Food Addit. Contam. Part A 19 (2002) 373.
[23] K.E. Maudens, G. Zhang, W.E. Lambert, J. Chromatogr. A 1047 (2004) 85.
[24] A. Preechaworapun, S. Chuanuwatanakul, Y. Einaga, K. Grudpan, S. Motomizu, O. Chailapakul, Talanta 68 (2006) 1726.
[25] T.N. Rao, B.V. Sarada, D.A. Tryk, A. Fujishima, J. Electroanal. Chem. 491 (2000) 175.
[26] G. Guiochon, J. Chromatogr. A 1168 (2007) 101.
[27] K.K. Unger, R. Skudas, M.M. Schulte, J. Chromatogr. A 1184 (2008) 393.
[28] N. Tanaka, H. Kobayashi, Anal. Bioanal. Chem. 376 (2003) 298.
[29] K. Hosoya, N. Hira, K. Yamamoto, M. Nishimura, N. Tanaka, Anal. Chem. 78 (2006) 5729.
[30] O. Núñez, K. Nakanishi, N. Tanaka, J. Chromatogr. A 1191 (2008) 231.
[31] G.F. Barbero, A. Liazid, M. Palma, C.G. Barroso, Food Chem. 107 (2008) 1276.
[32] P.D. Tzanavaras, D.G. Themelis, Anal. Chim. Acta 581 (2007) 89.
[33] R. Muñiz -Valencia, R. Gonzalo-Lumbreras, A. Santos-Montes, R. Izquierdo-Hornillos, Anal. Chim. Acta 611 (2008) 103.
[34] P. Viñas, M.J. Soler-Romera, M. Hernández-Córdoba, Talanta 69 (2006) 1063.
[35] J.F. García-Jiménez, M.C. Valencia, L.F. Capitán-Vallvey, Anal. Chim. Acta 594 (2007) 226.
[36] V.F. Samanidou, A.S. Ioannou, I.N. Papadoyannis, J. Chromatogr. B 809 (2004) 175.
[37] S.E. Deeb, L. Preu, H. Wätzig, J. Pharm. Biomed. Anal. 44 (2007) 85.
[38] M. Seifrtová, A. Pena, C. Lino, P. Solich, Anal. Bioanal. Chem. 391 (2008) 799.
[39] K. Kishida, N. Furusawa, J. Chromatogr. A 937 (2001) 49.
[40] H. Berrada, F. Borrull, G. Font, J.C. Moltó, R.M. Maré, J. Chromatogr. A 1157 (2007) 281.
[41] M. Hernandez, F. Borrull, M. Calull, Chromatographia 54 (2001) 355.
[42] M.Y. Haller, S.R. Müller, C.S. McArdell, A.C. Alder, M.J. Suter, J. Chromatogr. A 952 (2002) 111.
[43] I. Taverniers, M.D. Loose, E.V. Bockstaele, TrAC Trends Anal. Chem. 23 (2004) 535.
[44] A. Momberg, V.M.E. Carrera, B.D. Von Baer, C. Bruhn, F. Anal. Chim. Acta 159 (1984) 119.
[45] C.D. Souza, O.C. Braga, I.C. Vieira, A. Spinelli, Sens. Actuators B: Chem. 135 (2008) 66.