Research Article
Automated Online Solid-Phase Extraction Coupled with Sequential Injection-HPLC-EC System for the Determination of Sulfonamides in Shrimp

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The use of fully automated online solid-phase extraction (SPE) coupled with sequential injection analysis, high-performance liquid chromatography (HPLC), and electrochemical detection (EC) for the separation and determination of sulfonamides has been developed. A homemade microcolumn SPE system coupled with sequential injection analysis (SIA) was used to automate the sample cleanup and extraction of sulfonamides. The optimal flow rate of sample loading and elution was found to be 10 µL/s, and optimal elution time of zone was 20–24 s. Under the optimal conditions, a linear relationship between peak area and sulfonamide concentrations was obtained in the range of 0.01–8.0 µg mL⁻¹. Detection limits for seven sulfonamides were between 1.2 ng mL⁻¹ and 11.2 ng mL⁻¹. The proposed method has been applied for the determination of sulfonamides in shrimp. Recoveries in the range of 84–107% and relative standard deviations (RSDs) below 6.5% for intraday and 13% for inter-day were received for three concentration levels of spiking. The results showed that the present method was simple, rapid, accurate and highly sensitive for the determination of sulfonamides.

1. Introduction

Sulfonamides (SAs) have been widely used as effective chemotherapeutics and growth promoters in animals’ feeding. They are a group of synthetic antibiotic agents that played important role for the treatment in both human and veterinary medicines. SAs represent one of the most commonly used families of antibiotics in veterinary medicine because of their low cost, low toxicity, and excellent activity against common bacterial diseases. Nowadays, they are much used for therapy, prophylaxis, and growth promotion in livestock in cattle farm [1, 2]. Residues of SAs may occur in animal tissues if the adequate withdrawal time has not been observed or if SAs have been improperly administered. Therefore, long-term use of sulfonamide agents can cause serious side effects, such as Stevens-Johnson syndrome and carcinogenicity because of human consumption [3, 4]. Additionally, antibacterial drug in food can cause anaphylaxis in sensitive patients and can foster the development of antibiotic resistance in pathogenic organisms. With considerable attention to the potential human health, the European Union (EU) has set the maximum residual level of sulfonamides at 100 ng g⁻¹ in edible tissues and in milk [5]. In Thailand, shrimp is one of the top ten of our exports sent to other countries. To safeguard human health and to overcome the limitations of trade based on SAs residues, a rapid, accurate, selective, sensitive,
and efficient method for the monitoring of SAs in shrimp is of great significance.

Several conventional methods have been successfully applied for the separation and determination of the sulfonamide content in different samples including gas chromatography (GC), gas chromatography-mass spectrometry (GC/MS), capillary electrophoresis (CE) [6, 7], enzyme-linked immunosorbent assay (ELISA) [8, 9], thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC-UV) [10–17], and high-performance liquid chromatography-mass spectrometry (HPLC/MS) [18–25]. Although the mentioned methods offer good resolution of organic compound, their sensitivities are often in sufficient for direct determination of trace sulfonamides residue in food. Therefore, sample preparation is required in order to obtain higher concentration and avoid interference by matrix constituents. Recently, offline sample preparation procedures such as liquid-liquid extraction (LLE) [14, 26], matrix solid-phase dispersion (MSPD) [27], solid-phase microextraction (SPME) [15], and solid-phase extraction (SPE) [1, 16, 18, 20–24, 28] have been reported for the extraction of sulfonamides. Unfortunately, the recovery of the analytes and analytical precision of these methods are reduced during the preparation of samples. Moreover, they also consume the large volumes of sample and solvent. To overcome these problems, online solid-phase extraction (SPE) coupled with HPLC has been developed [29–36]. Online SPE is an attractive sample preparation technique because it not only improves analytical precision but also reduces the solvent consumption.

In recent years, sequential injection analysis (SIA) has become apparent that the scope of SIA can be extended to encompass a variety of more complex, online, sample manipulation, and pretreatment procedure. Therefore, the use of SIA for a fully automated online SPE procedure prior to subsequently analyzing on HPLC to enhance productivity and reproducibility as well as reduce labor intensive is an attractive choice.

From our previous report, offline SPE using Oasis HLB material was successfully proposed and applied to extract the residual sulfonamides in shrimp [28]. This material is a hydrophilic-lipophilic balanced (HLB) sorbent that is composed of two monomers (N-vinylpyrrollidone and divinylbenzene). It exhibited excellent retention capacity for a wide polarity of analytes [18, 24]. To increase the performance and sensitivity in HPLC-EC by means of fully automation, in this work, we used online Oasis HLB SPE procedure to extend our previous efforts on the determination of sulfonamides instead of using offline SPE before their HPLC separation. The common detectors for chromatography with online SPE are ultraviolet (UV) [10–17], fluorescence [1, 4, 37] and MS [18–25]. Even though these methods provide high sensitivity and selectivity, there are the expensive equipment and a requirement for significant labor and analytical resources. Nowadays, the alternative determination for the detection of sulfonamides is the electrochemical detection (EC) due to its high sensitivity, low cost, fastness, and simplicity [26, 28, 38].

In this paper, the ultimate goal was to develop a fully automatic online SPE technique coupled with SIA-HPLC-EC for the separation and determination of seven sulfonamides (sulfaguanidine (SG), sulfadiazine (SDZ), sulfamethazine (SMZ), sulfamonomethoxine (SMM), sulfamethoxazole (SMX), sulfadimethoxine (SDM), and sulfaquinoxaline (SQ)). A silica-based monolithic column was employed for sulfonamides separation because of its high tolerance for organic solvent, which led to a longer lifetime and lower back-pressure, compared to other traditional columns. A meticulous optimization process was carried out in order to get an optimum performance of the online system. Likewise, a detailed evaluation of the methodology was developed to demonstrate that this method can be satisfactorily applied to determine residual concentrations of the seven sulfonamides in shrimp using the Oasis HLB SPE material for sample extraction.

2. Experimental

2.1. Reagents and Standards. All solvents and reagents used were HPLC or analytical grade. Acetonitrile was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol was obtained from Carlo Erba (Val de Reuil, France). Methanol, disodium hydrogen phosphate dehydrate (Na2HPO4), and citric acid were obtained from Merck (Darmstadt, Germany). Potassium dihydrogen orthophosphate (KH2PO4) was obtained from BDH (VWR International Ltd., England). Ethylene diamine tetraacetic acid disodium salt dehydrate (Na2EDTA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure Water (R ≥ 18.2 MΩ cm−1) from a Milli-Q system (Millipore) was used throughout the experiment. The extraction solution (Na2EDTA-McIlvaine buffer, pH 4) was prepared by dissolving 13.52 g of Na2HPO4, 13.02 g of citric acid and 3.72 g of Na2EDTA in one liter of ultrapure water. Prior to use, all solution and solvents were filtered with 0.45 µm nylon membranes.

Sulfadiazine, sulfadimethoxine, sulfamethazine, sulfamethoxazole, sulfamonemethoxine, and sulfaquinoxaline were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sulfaguanidine was obtained from ICN Biomedicals Inc. (USA). A stock standard solution (100 µg mL−1) of each SA was prepared by dissolving 3 mg of SA in 30 mL of an acetonitrile: ultrapure water solution in (1:1 v/v) and stored at 4°C in the dark. The working solutions were prepared by diluting the stock standard solutions with the mobile phase.

2.2. Materials and Instrumentations. Oasis HLB with particle size of 30 µm was obtained from Water (Milford, MA, USA). A homemade micrometer SPE (4.0 cm × 1.59 mm i.d.) packed with 60 mg of Oasis HLB was used for the sample cleanup and extraction of SAs.

The HPLC-EC system consisted of an HPLC compact pump model 2250 (Bischoff, Germany) and a thin-layer flow cell (Bioanalytical system Inc., Japan) consisting of three electrodes: a BDD working electrode, an Ag/AgCl reference electrode (Bioanalytical system Inc., Japan), and a stainless
steel tube counter electrode. The chromatographic column was a Chromolith Performance RP-18 silica-based monolithic column (100 mm × 4.6 mm i.d.) from Merck (Darmstadt, Germany). The HPLC was carried out in the mobile phase, which consisted of a phosphate buffer solution (0.05 M KH₂PO₄, pH 3), acetonitrile, and ethanol in the ratio of 80:15:5 (v/v/v). The flow rate was set at 1.5 mL min⁻¹. The amperometric detection was used in this experiment, with an applied potential at +1.2 V versus Ag/AgCl. The sequential injection system, Auto-Pret EC 01P (M&G CHEMATECHS Japan Co. Ltd., Okayama Japan) with 2.5 mL syringe pump, 2.5 mL holding coil, 8-port selection valve (Hamilton, Nevada, USA) and 6-port switching valve (Rheodyne MXT 715-000, USA) was used for sample processing. The experiment was performed at room temperature (25°C). The CHI1232A (CH Instrument, USA) was used for amperometric controlling and signals processing.

2.3. Online SPE-HPLC Procedure. A schematic diagram for the online solid-phase extraction coupled to SIA-HPLC-EC for the determination of SAs in shrimp is depicted in Figure 1. The online SPE-HPLC-EC consists of three parts. Part I is online SPE consisting of a 3-way syringe pump (2.5 mL), 8-port selection valve, 6-port switching valve, holding coil (2.5 mL), loop (34 μL), and SPE microcolumn. Part II is HPLC system including an HPLC pump and analytical column. Part III is an EC detector consisting of a thin-layer flow cell and a data acquisition system (CHI1232A). The online SPE procedure consists of six steps including conditioning, loading, washing, elution, reconditioning, and injection summarized in Table 1. The first is the column condition step. The SPE microcolumn was conditioned with 1 mL of Na₂EDTA-McIlvaine buffer. In the second, sample loading, 1 mL of sulfonamides solution is introduced into the SPE microcolumn. In the third step, washing, 1 mL of ultrapure water is used to remove the interferences from SPE microcolumn, while the analyte was retained on the sorbent. For the fourth step, elution, 0.2 mL of methanol is used to elute sulfonamides from the sorbent, and then the eluate zone was kept in sample loop. In the fifth step, the SPE microcolumn is cleaned. 5 mL of methanol and 2.5 mL of ultrapure water are sequentially passed through the sorbent to solvate the functional groups of the sorbent. In the final step, eluate was transferred to the analytical column by changing the switching valve from loading to injection position. Next, the separation was performed on RP-18 silica-based monolithic analytical column.

2.4. Sample Preparation. The apparatuses for sample preparation consisted of a vortex mixer (Mixer Uzusio LMS. Co Ltd., Japan), an ultrasonic bath (ESP chemicals, Inc., MA, USA), and a centrifugate (Cole Parmer, Illinois, USA). Shrimps were obtained from a local supermarket (Bangkok, Thailand). One gram of a homogeneous shrimp sample was placed in a 15 mL amber glass bottle, and 5 mL of Na₂EDTA-McIlvaine's buffer solution was then 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 for 10 min following centrifugation at 20,000 rpm for 10 min. Prior to loading the supernatant into online system, it was filtered through a 0.20 μm nylon membrane filter.

3. Results and Discussion

3.1. Optimization of SPE Condition. In order to obtain the optimal conditions, the effects of various parameters were investigated. These included the effect of eluent component, sample loading flow rate, and elution time zone. During the optimization of these parameters, a 10 μg mL⁻¹ of SAs standard mixture solution was utilized, and the results were evaluated as the highest current signal.

3.1.1. Optimization of the Eluent Component. From our previous report [28], methanol was selected as the most suitable eluent for offline SPE to achieve the complete extraction of sulfonamides (SAs) in shrimp and the mixture of phosphate buffer : acetonitrile : ethanol (80:15:5 v/v/v) was used as a mobile phase to obtain the high separation efficiency. In this work, seven SAs were eluted from online SPE and directly flowed through the analytical column (RP-18 silica-based monolithic column) and then detected by electrochemical detection, respectively. The eluent was not only used to elute SAs from SPE but also used to carry analytes into a monolithic column and electrochemical cell. Therefore, the eluent component has directly affected on the efficiency of elution, performance of separation, and sensitivity of electrochemical signal. The ratio of methanol to mobile phase was studied across 50:50, 60:40, 70:30, 80:20, 90:10, and 100:0 (v/v) ratios. The results were illustrated in Figure 2. By the use of 50% methanol, there are only three peaks of SG, SDZ, and SMZ observed. Interestingly, increasing percentage methanol up to 90% and 100%, all seven peaks of SAs can be recorded. However, the retention time of all analytes decreased with increasing the methanol ratio. Hence, an eluent of 100% methanol was selected as the best eluent for the next experiment because this condition led to the complete elution of SAs from SPE column, good separation of seven SAs following their HPLC, and highest electrochemical signals.

3.1.2. Optimization of the Sample Loading Flow Rate. The sample loading flow rate will influence on the retention of SAs on the SPE column. The sample loading flow rate was therefore studied in the range of 0.48, 0.54, 0.60, and 0.66 mL min⁻¹. From the results as shown in Figure 3, it can be observed that the peak current decreased at sample loading flow rate higher than 0.60 mL min⁻¹. This effect is caused from the short interaction time between analyte and sorbent in SPE column. The higher retention efficiency can be got at the lower sample loading flow rate; however, the total analysis time is also increased. Therefore, the sample loading flow rate of 0.60 mL min⁻¹ was selected as an optimal value to compromise between the interaction time and the total analysis time. Moreover, the changing of sample loading flow rate did not affect on the resolution of the seven SAs.

3.1.3. Optimization of the Elution Time Zone. After SAs were eluted from the SPE column, the eluate would be kept in
the sample loop (34 µL) whereas the switching valve was set at loading position. Then, this solution was transferred to the analytical column via the left position of injection. According to the different distribution of SAs in eluted zone, the eluate zone with the highest SAs concentration is preferably injected in the HPLC system in order to obtain maximum sensitivity. To deliver the most concentrated zone of eluate into the sample loop, optimization was needed. From the manifold diagram (Figure 1), it was found that the eluent (100% methanol), at least 200 µL, was used to completely fill eluent in SPE column and SIA line between port 6 of the switching valve and port 8 of the selection valve. In this online SIA-HPLC system, the elution time zone was varied from 20–24 s, 25–29 s to 30–34 s at constant eluting flow rate of 0.60 mL min\(^{-1}\) (10 µL s\(^{-1}\)) using syringe pump. The movement of the eluate zone was then passed to the analytical column and monitored by EC system, respectively. Results showed that the peak current for the seven SAs decreased when elution time increased as shown in Figure 4. The maximum signal was obtained at approximately 20–24 s from the start of the elution step. This indicated that the concentration of SAs in eluate zone was related to the elution time zone from the start to the stop of elution step. Hence, the elution time zone of 20–24 s was regarded as optimum for the delivery of the eluate zone into sample loop and then analytical HPLC system.

### Table 1: The SIA operating sequence for sulfonamides analysis.

<table>
<thead>
<tr>
<th>Step</th>
<th>Syringe pump Action</th>
<th>Position SL</th>
<th>Position SW</th>
<th>Volume (mL)</th>
<th>Flow rate (mL min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Column condition</td>
<td>(a) Aspirate</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(b) Dispense</td>
<td>8</td>
<td>1</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>(2) Sample loading</td>
<td>(a) Aspirate</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(b) Dispense</td>
<td>8</td>
<td>1</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>(3) Washing</td>
<td>(a) Aspirate</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(b) Dispense</td>
<td>8</td>
<td>1</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>(4) Elution</td>
<td>(a) Aspirate</td>
<td>2</td>
<td>Loading</td>
<td>0.24</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>(b) Dispense</td>
<td>8</td>
<td></td>
<td>0.24</td>
<td>0.6</td>
</tr>
<tr>
<td>(5) Column cleaning</td>
<td>(c) Aspirate</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(b) Dispense</td>
<td>8</td>
<td>5</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(d) Dispense</td>
<td>8</td>
<td>2.5</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>(6) Injection</td>
<td>HPLC pump</td>
<td>—</td>
<td>Injection</td>
<td>—</td>
<td>1.5</td>
</tr>
</tbody>
</table>

3.2. Analytical Performances. The methodology was validated with respect to linearity, accuracy, precision, and sensitivity in order to evaluate the reliability of results provided by methodology. Under the optimal condition, the calibration curves were established by measuring the peak areas of seven compounds of various concentrations with three injections of each concentration of standard SAs. The studied
Figure 2: HPLC chromatograms of SAs (10 µg mL⁻¹) at different ratio of methanol and mobile phase (a) 100% methanol, (b) 90% methanol and 10% mobile phase, (c) 80% methanol and 20% mobile phase, (d) 70% methanol and 30% mobile phase, (e) 60% methanol and 40% mobile phase, and (f) 50% methanol and 50% mobile phase. HPLC pump flow rate was set at 1.0 mL min⁻¹.

Figure 3: HPLC chromatograms of seven SAs (10 µg mL⁻¹) at different sample loading flow rates (a) 0.66, (b) 0.60, (c) 0.54, (d) 0.48 mL min⁻¹. HPLC pump flow rate was set at 1.5 mL min⁻¹. (1) SG, (2) SDZ, (3) SMZ, (4) SMM, (5) SMX, (6) SDM, and (7) SQ. Other conditions are the same as in Figure 2.
concentrations were in the range of 0.05–10.0 μg mL⁻¹. The calibration curves were found to be linear in the concentration range 0.01–8.0 μg mL⁻¹ for SG, SDZ, SMZ, SMM, and SMX and 0.1–8.0 μg mL⁻¹ for SDM and SQ. The correlation coefficients generally exceeded 0.994. The limits of detection (LOD) and limits of quantitation (LOQ) were calculated from 3Sbl/S and 10Sbl/S, where Sbl is the standard deviation of the blank measurement (n = 10) and S is the sensitivity of the method or the slope of the linearity. The data are summarized in Table 2.

3.3. Application to Shrimp Samples. To demonstrate the utilization of the proposed method, online SPE-HPLC-EC was applied for the determination of SG, SDZ, SMZ, SMM, SMX, SDM, and SQ in shrimp samples that were sampled from the local supermarkets by standard addition. Typically, the important problem for shrimp analysis is large lipid content in shrimp. Prior to loading sample into online system, the Na₂EDTA-MacIlvaine’s buffer solution (pH 4) was used to extract SAs from shrimp. This method can be used to determine SMZ, SMM, SMX, SDM, and SQ, but SG and SDZ peaks overlapped with interfering compound. This may concern the very large protein and lipid content in shrimp that could not be precipitated by high-speed centrifugation and cleanup with SPE.

The accuracy of the present method was expressed as a parameter of percentage recovery. The recovery was calculated at three concentration levels of 2, 4, and 6 μg g⁻¹ by measurement of responses from spiked blank shrimp. These concentrations of SAs were chosen to demonstrate the accuracy at overall SAs level: the low, medium, and high levels. The average recoveries of five SAs (SMZ, SMM, SMX, SDM, and SQ) in shrimp were obtained in the range of 84–107% as shown in Table 3. The precision of the method was characterized by parameter of repeatability. The relative standard deviation was calculated for three consecutive measurements of the repeated injections of solution containing the complete set of standard compounds. Three spiked concentrations (2, 4, and 6 μg g⁻¹) of SAs were studied to evaluate the repeatability of the proposed method. Intraday and interday precision were determined by injection of the spiked blank shrimp in the same day (n = 3), and, similarly, these methods were analyzed three times at different day (n = 3); results are shown in Table 3. The relative standard deviations of all concentrations of SAs were less than 6.5% for intraday and 13% for interday, respectively. The results indicated that this method provided the acceptable accuracy and precision for the SAs determination in shrimp.

To validate the developed method, the results from the developed method were compared to those obtained by HPLC-MS from Laboratory Center for Food and Agricultural Products Company Limited (Table 4). It can be seen that no significant difference was found at the 95% confidence level of the paired t-test method. Thus, the analyzed values of SAs in shrimp can be acceptable and reliable.

4. Conclusion

Fully automated sample preparation, separation, and determination of SAs in shrimp were developed using an online SPE coupled with SIA prior to HPLC electrochemical detection. This work is 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 online sample preparation. Automated online coupling of SPE to SIA-HPLC electrochemical detection has not been previously proposed to determine sulfonamides; therefore, for the first time, we have employed the present system for the automatic sample preparation and determination of SAs in real samples. Method exhibits similar sensitivity as our previous work. Anyhow, the developed method can reduce sample...
Table 2: Linearity, limit of detection (LOD), and limit of quantitation (LOQ) of the online SPE-HPLC-EC method (n = 3).

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Linearity (µg mL⁻¹)</th>
<th>Slope (peak area units/µg mL⁻¹)</th>
<th>Intercept (µA)</th>
<th>R²</th>
<th>LOD (ng mL⁻¹)</th>
<th>LOQ (ng mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SG</td>
<td>0.01–8</td>
<td>0.5277</td>
<td>0.1085</td>
<td>0.9943</td>
<td>11.2</td>
<td>33.6</td>
</tr>
<tr>
<td>SDZ</td>
<td>0.01–8</td>
<td>7.6757</td>
<td>0.0403</td>
<td>0.9982</td>
<td>1.2</td>
<td>4.0</td>
</tr>
<tr>
<td>SMZ</td>
<td>0.01–8</td>
<td>6.5834</td>
<td>0.0769</td>
<td>0.9990</td>
<td>1.3</td>
<td>4.2</td>
</tr>
<tr>
<td>SMM</td>
<td>0.01–8</td>
<td>5.2178</td>
<td>0.3764</td>
<td>0.9695</td>
<td>1.5</td>
<td>5.0</td>
</tr>
<tr>
<td>SMZ</td>
<td>0.1–8</td>
<td>2.4536</td>
<td>0.0679</td>
<td>0.9994</td>
<td>2.9</td>
<td>9.8</td>
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<tr>
<td>SDM</td>
<td>0.1–8</td>
<td>2.3970</td>
<td>0.2668</td>
<td>0.9597</td>
<td>3.1</td>
<td>10.3</td>
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<tr>
<td>SQ</td>
<td>0.1–8</td>
<td>0.8786</td>
<td>0.0419</td>
<td>0.9997</td>
<td>7.4</td>
<td>24.6</td>
</tr>
</tbody>
</table>

Table 3: Intra- and Interday precision and recoveries of the proposed method.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Spiked level (µg g⁻¹)</th>
<th>Intraday Mean of recovery (%) ± SD* RSD (%)</th>
<th>Interday Mean of recovery (%) ± SD* RSD (%)</th>
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<tbody>
<tr>
<td>SMZ</td>
<td>2</td>
<td>87.8 ± 5.5 6.2 10.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>86.3 ± 2.7 3.1 10.3</td>
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<td></td>
<td>6</td>
<td>97.6 ± 2.2 2.3 1.4</td>
<td></td>
</tr>
<tr>
<td>SMM</td>
<td>2</td>
<td>100.3 ± 6.5 6.5 5.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>85.2 ± 3.7 4.3 6.9</td>
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<tr>
<td></td>
<td>6</td>
<td>104.6 ± 3.4 3.2 1.4</td>
<td></td>
</tr>
<tr>
<td>SMX</td>
<td>2</td>
<td>106.5 ± 5.3 4.9 5.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>94.0 ± 1.9 2.0 1.3</td>
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<td></td>
<td>6</td>
<td>95.6 ± 1.9 2.0 1.3</td>
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<tr>
<td>SDM</td>
<td>2</td>
<td>102.1 ± 3.0 3.0 4.9</td>
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<tr>
<td></td>
<td>4</td>
<td>93.0 ± 3.9 4.2 3.1</td>
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<td></td>
<td>6</td>
<td>101.0 ± 5.6 5.5 1.8</td>
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<td>SQ</td>
<td>2</td>
<td>105.6 ± 1.3 1.2 13.0</td>
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<td></td>
<td>4</td>
<td>91.2 ± 1.2 1.3 5.8</td>
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<td></td>
<td>6</td>
<td>102.5 ± 1.1 1.0 1.3</td>
<td></td>
</tr>
</tbody>
</table>

*SD: standard deviation (n = 3).

Table 4: Determination of SAs levels in shrimp samples by the traditional HPLC-MS method and the developed online SPE-HPLC-EC method.

<table>
<thead>
<tr>
<th>Spiked level (µg g⁻¹)</th>
<th>Analytes</th>
<th>Online SPE-HPLC-EC method (conc. ± SD*, µg g⁻¹)</th>
<th>HPLC-MS (conc. ± SD*, µg g⁻¹)</th>
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<tbody>
<tr>
<td>2</td>
<td>SMZ</td>
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<tr>
<td></td>
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<td>2.01 ± 0.13 1.82 ± 0.11</td>
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<tr>
<td></td>
<td>SDM</td>
<td>2.04 ± 0.06 1.70 ± 0.09</td>
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<tr>
<td></td>
<td>SQ</td>
<td>2.11 ± 0.03 1.49 ± 0.09</td>
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<td>6</td>
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<tr>
<td></td>
<td>SDM</td>
<td>6.06 ± 0.34 6.02 ± 0.17</td>
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<tr>
<td></td>
<td>SQ</td>
<td>6.15 ± 0.07 5.79 ± 0.15</td>
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*SD: standard deviation (n = 3).
preparation time, reagent consumption, and labor intensive while the enable effective cleanup of sample can be obtained. Additionally, method also permits the simultaneous determination of seven SAs with good recoveries, precision, and detection limits. Overall, the present method is promising for the automation of online sample preparation before HPLC analysis. Therefore, the proposed method could be recommended as alternative method for the routine analysis of residual SAs in shrimp.

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References


[22] M. S. Díaz-Cruz, M. J. García-Galán, and D. Barceló, "Highly sensitive simultaneous determination of sulfonamide antibiotics and one metabolite in environmental waters by liquid..."


