Research Article

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

Pimkwan Chantarateepa,¹ Weena Siangproh,² Shoji Motomizu,³
and Orawon Chailapakul^{4,5}

¹ Program in Biotechnology, Faculty of Science, Chulalongkorn University, Patumwan, Bangkok 10330, Thailand

² Department of Chemistry, Faculty of Science, Srinakharinwirot University, Sukhumvit 23, Wattana, Bangkok 10110, Thailand

³ Department of Chemistry, Faculty of Science, Okayama University, 3-1-1 Tsushimaoka, Okayama 700-8530, Japan

⁴ Department of Chemistry, Faculty of Science, Chulalongkorn University, Patumwan, Bangkok 10330, Thailand

⁵ Center for Excellence Petroleum, Petrochemicals and Advanced Materials, Chulalongkorn University, Patumwan, Bangkok 10330, Thailand

Correspondence should be addressed to Orawon Chailapakul, corawon@chula.ac.th

Received 26 April 2011; Accepted 20 June 2011

Academic Editor: Carlos Alberto Martinez-Huitle

Copyright © 2012 Pimkwan Chantarateepa et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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 $\mu\text{L/s}$, 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 $\mu\text{g mL}^{-1}$. 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 insufficient 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 those 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-vinylpyrrolidone 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 detection for the determination 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 (Na_2HPO_4), and citric acid were obtained from Merck (Darmstadt, Germany). Potassium dihydrogen orthophosphate (KH_2PO_4) was obtained from BDH (VWR International Ltd., England). Ethylene diamine tetraacetic acid disodium salt dehydrate (Na_2EDTA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure Water ($R \geq 18.2 \text{ M}\Omega \text{ cm}^{-1}$) from a Milli-Q system (Millipore) was used throughout the experiment. 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 ultrapure water. Prior to use, all solution and solvents were filtered with $0.45 \mu\text{m}$ nylon membranes.

Sulfadiazine, sulfadimethoxine, sulfamethazine, sulfamethoxazole, sulfamonomethoxine, and sulfaquinoxaline were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sulfaguanidine was obtained from ICN Biomedicals Inc. (USA). A stock standard solution ($100 \mu\text{g mL}^{-1}$) 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 $30 \mu\text{m}$ was obtained from Water (Milford, MA, USA). A homemade microcolumn SPE ($4.0 \text{ cm} \times 1.59 \text{ mm i.d.}$) 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 $\times$ 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 KH_2PO_4 , 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 ECD-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 Na_2EDTA -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 centrifuge (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 Na_2EDTA -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 $0.20 \mu\text{m}$ 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 $10 \mu\text{g mL}^{-1}$ 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

FIGURE 2: HPLC chromatograms of SAs ($10 \mu\text{g mL}^{-1}$) 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 ($10 \mu\text{g mL}^{-1}$) 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.

FIGURE 4: HPLC chromatograms of SAs ($10 \mu\text{g mL}^{-1}$) at different elution time zone (a) 20–24 s, (b) 25–29 s, (c) 30–34 s. Other conditions are the same as in Figure 2.

concentrations were in the range of $0.05\text{--}10.0 \mu\text{g mL}^{-1}$. The calibration curves were found to be linear in the concentration range $0.01\text{--}8.0 \mu\text{g mL}^{-1}$ for SG, SDZ, SMZ, SMM, and SMX and $0.1\text{--}8.0 \mu\text{g mL}^{-1}$ for SDM and SQ. The correlation coefficients generally exceeded 0.994. The limits of detection (LOD) and limits of quantitation (LOQ) were calculated from $3S_{\text{bl}}/S$ and $10S_{\text{bl}}/S$, 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. 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 Na_2EDTA -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 $6 \mu\text{g g}^{-1}$ 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 $6 \mu\text{g g}^{-1}$) 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 ($n = 3$).

Analytes	Linearity ($\mu\text{g mL}^{-1}$)	Slope (peak area units/ $\mu\text{g mL}^{-1}$)	Intercept (μA)	R^2	LOD (ng mL^{-1})	LOQ (ng mL^{-1})
SG	0.01–8	0.5277	0.1085	0.9943	11.2	33.6
SDZ	0.01–8	7.6757	0.0403	0.9982	1.2	4.0
SMZ	0.01–8	6.5834	0.0769	0.9990	1.3	4.2
SMM	0.01–8	5.2178	0.3764	0.9965	1.5	5.0
SMZ	0.01–8	2.4536	0.0679	0.9994	2.9	9.8
SDM	0.1–8	2.3970	0.2668	0.9957	3.1	10.3
SQ	0.1–8	0.8786	0.0419	0.9997	7.4	24.6

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

Analyte	Spiked level ($\mu\text{g g}^{-1}$)	Intraday		Interday	
		Mean of recovery (%) $\pm$ SD*	RSD (%)	Mean of recovery (%) $\pm$ SD*	RSD (%)
SMZ	2	87.8 $\pm$ 5.5	6.2	84.6 $\pm$ 8.7	10.3
	4	86.3 $\pm$ 2.7	3.1	96.5 $\pm$ 9.9	10.3
	6	97.6 $\pm$ 2.2	2.3	99.8 $\pm$ 2.3	2.3
SMM	2	100.3 $\pm$ 6.5	6.5	94.5 $\pm$ 5.2	5.5
	4	85.2 $\pm$ 3.7	4.3	92.5 $\pm$ 6.4	6.9
	6	104.6 $\pm$ 3.4	3.2	102.8 $\pm$ 1.5	1.4
SMX	2	106.5 $\pm$ 5.3	4.9	103.5 $\pm$ 5.6	5.4
	4	94.0 $\pm$ 1.9	2.0	95.0 $\pm$ 1.3	1.3
	6	95.6 $\pm$ 1.9	2.0	100.9 $\pm$ 4.9	4.9
SDM	2	102.1 $\pm$ 3.0	3.0	101.2 $\pm$ 9.7	9.6
	4	93.0 $\pm$ 3.9	4.2	90.6 $\pm$ 2.8	3.1
	6	101.0 $\pm$ 5.6	5.5	103.1 $\pm$ 1.8	1.8
SQ	2	105.6 $\pm$ 1.3	1.2	96.9 $\pm$ 12.6	13.0
	4	91.2 $\pm$ 1.2	1.3	96.2 $\pm$ 5.6	5.8
	6	102.5 $\pm$ 1.1	1.0	100.9 $\pm$ 1.3	1.3

* 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.

Spiked level ($\mu\text{g g}^{-1}$)	Analytes	Online SPE-HPLC-EC method (conc. $\pm$ SD*, $\mu\text{g g}^{-1}$)	HPLC-MS (conc. $\pm$ SD*, $\mu\text{g g}^{-1}$)
2	SMZ	1.76 $\pm$ 0.11	1.77 $\pm$ 0.10
	SMM	2.01 $\pm$ 0.13	1.82 $\pm$ 0.11
	SMX	2.13 $\pm$ 0.11	2.04 $\pm$ 0.11
	SDM	2.04 $\pm$ 0.06	1.70 $\pm$ 0.09
	SQ	2.11 $\pm$ 0.03	1.49 $\pm$ 0.09
4	SMZ	3.45 $\pm$ 0.11	4.39 $\pm$ 0.14
	SMM	3.41 $\pm$ 0.15	4.44 $\pm$ 0.14
	SMX	3.76 $\pm$ 0.08	4.57 $\pm$ 0.16
	SDM	3.72 $\pm$ 0.16	4.34 $\pm$ 0.14
	SQ	3.65 $\pm$ 0.05	4.34 $\pm$ 0.14
6	SMZ	5.85 $\pm$ 0.13	6.56 $\pm$ 0.18
	SMM	6.28 $\pm$ 0.21	6.30 $\pm$ 0.18
	SMX	5.74 $\pm$ 0.12	6.61 $\pm$ 0.18
	SDM	6.06 $\pm$ 0.34	6.02 $\pm$ 0.17
	SQ	6.15 $\pm$ 0.07	5.79 $\pm$ 0.15

* 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.

Acknowledgments

This work was supported by the 90th Anniversary of Chulalongkorn University Fund, Innovation for the Improvement of Food Safety and Food Quality for New World Economy Project. This work is also supported by the Thai Government Stimulus Package 2 (TKK2555), under the Project for Establishment of Comprehensive Center for Innovative Food, Health Products and Agriculture (PERFECTA), University Project of CHE and the Ratchadaphisaksomphot Endowment Fund (Project code AM1009I).

References

- [1] T. A. Gehring, B. Griffin, R. Williams, C. Geiseker, L. G. Rushing, and P. H. Siitonen, "Multiresidue determination of sulfonamides in edible catfish, shrimp and salmon tissues by high-performance liquid chromatography with postcolumn derivatization and fluorescence detection," *Journal of Chromatography B*, vol. 840, no. 2, pp. 132–138, 2006.
- [2] G. Z. Fang, J. X. He, and S. Wang, "Multiwalled carbon nanotubes as sorbent for on-line coupling of solid-phase extraction to high-performance liquid chromatography for simultaneous determination of 10 sulfonamides in eggs and pork," *Journal of Chromatography A*, vol. 1127, no. 1-2, pp. 12–17, 2006.
- [3] F. J. Lara, A. M. García-Campaña, C. Neussüss, and F. Alés-Barrero, "Determination of sulfonamide residues in water samples by in-line solid-phase extraction-capillary electrophoresis," *Journal of Chromatography A*, vol. 1216, no. 15, pp. 3372–3379, 2009.
- [4] K. E. Maudens, G. F. Zhang, and W. E. Lambert, "Quantitative analysis of twelve sulfonamides in honey after acidic hydrolysis by high-performance liquid chromatography with post-column derivatization and fluorescence detection," *Journal of Chromatography A*, vol. 1047, no. 1, pp. 85–92, 2004.
- [5] Commission of the European Community, "The rules governing medical products in the European Community IV," 1991, Brussels, Belgium.
- [6] T. You, X. Yang, and E. Wang, "Determination of sulfadiazine and sulfamethoxazole by capillary electrophoresis with end-column electrochemical detection," *Analyst*, vol. 123, no. 11, pp. 2357–2360, 1998.
- [7] M. R. S. Fuh and S. Y. Chu, "Quantitative determination of sulfonamide in meat by solid-phase extraction and capillary electrophoresis," *Analytica Chimica Acta*, vol. 499, no. 1-2, pp. 215–221, 2003.
- [8] X. Wang, K. Li, D. Shi et al., "Development of an immunochromatographic lateral-flow test strip for rapid detection of sulfonamides in eggs and chicken muscles," *Journal of Agricultural and Food Chemistry*, vol. 55, no. 6, pp. 2072–2078, 2007.
- [9] C. Cháfer-Pericás, Á. Maquieira, R. Puchades, J. Miralles, and A. Moreno, "Fast screening immunoassay of sulfonamides in commercial fish samples," *Analytical and Bioanalytical Chemistry*, vol. 396, no. 2, pp. 911–921, 2010.
- [10] N. Furusawa, "Rapid high-performance liquid chromatographic determining technique of sulfamonomethoxine, sulfadimethoxine, and sulfaquinoxaline in eggs without use of organic solvents," *Analytica Chimica Acta*, vol. 481, no. 2, pp. 255–259, 2003.
- [11] R. H. M. M. Granja, A. M. M. Niño, F. Rabone, and A. G. Salerno, "A reliable high-performance liquid chromatography with ultraviolet detection for the determination of sulfonamides in honey," *Analytica Chimica Acta*, vol. 613, no. 1, pp. 116–119, 2008.
- [12] M. Gratacós-Cubarsí, M. Castellari, A. Valero, and J. A. García-Regueiro, "A simplified LC-DAD method with an RP-C12 column for routine monitoring of three sulfonamides in edible calf and pig tissue," *Analytical and Bioanalytical Chemistry*, vol. 385, no. 7, pp. 1218–1224, 2006.
- [13] J. He, S. Wang, G. Fang, H. Zhu, and Y. Zhang, "Molecularly imprinted polymer online solid-phase extraction coupled with high-performance liquid chromatography-UV for the determination of three sulfonamides in pork and chicken," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 9, pp. 2919–2925, 2008.
- [14] J. E. Roybal, A. P. Pfenning, S. B. Turnipseed, and S. A. Gonzales, "Application of size-exclusion chromatography to the analysis of shrimp for sulfonamide residues," *Analytica Chimica Acta*, vol. 483, no. 1-2, pp. 147–152, 2003.
- [15] Y. Wen, M. Zhang, Q. Zhao, and Y. Q. Feng, "Monitoring of five sulfonamide antibacterial residues in milk by in-tube solid-phase microextraction coupled to high-performance liquid chromatography," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 22, pp. 8468–8473, 2005.
- [16] H. Niu, Y. Cai, Y. Shi et al., "Evaluation of carbon nanotubes as a solid-phase extraction adsorbent for the extraction of cephalosporins antibiotics, sulfonamides and phenolic compounds from aqueous solution," *Analytica Chimica Acta*, vol. 594, no. 1, pp. 81–92, 2007.
- [17] B. Roudaut and M. Garnier, "Sulphonamide residues in eggs following drug administration via the drinking water," *Food Additives and Contaminants*, vol. 19, no. 4, pp. 373–378, 2002.
- [18] H. Chang, J. Hu, M. Asami, and S. Kunikane, "Simultaneous analysis of 16 sulfonamide and trimethoprim antibiotics in environmental waters by liquid chromatography-electrospray tandem mass spectrometry," *Journal of Chromatography A*, vol. 1190, no. 1-2, pp. 390–393, 2008.
- [19] E. L. McClure and C. S. Wong, "Solid phase microextraction of macrolide, trimethoprim, and sulfonamide antibiotics in wastewaters," *Journal of Chromatography A*, vol. 1169, no. 1-2, pp. 53–62, 2007.
- [20] R. Sheridan, B. Policastro, S. Thomas, and D. Rice, "Analysis and occurrence of 14 sulfonamide antibacterials and chloramphenicol in honey by solid-phase extraction followed by LC/MS/MS analysis," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 10, pp. 3509–3516, 2008.
- [21] U. Koesukwiwat, S. Jayanta, and N. Leepipatpiboon, "Solid-phase extraction for multiresidue determination of sulfonamides, tetracyclines, and pyrimethamine in Bovine's milk," *Journal of Chromatography A*, vol. 1149, no. 1, pp. 102–111, 2007.
- [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

- chromatography-quadrupole linear ion trap-mass spectrometry," *Journal of Chromatography A*, vol. 1193, no. 1-2, pp. 50–59, 2008.
- [23] H. Li, P. J. Kijak, S. B. Turnipseed, and W. Cui, "Analysis of veterinary drug residues in shrimp: a multi-class method by liquid chromatography-quadrupole ion trap mass spectrometry," *Journal of Chromatography B*, vol. 836, no. 1-2, pp. 22–38, 2006.
- [24] Z. Ye, H. S. Weinberg, and M. T. Meyer, "Trace analysis of trimethoprim and sulfonamide, macrolide, quinolone, and tetracycline antibiotics in chlorinated drinking water using liquid chromatography electrospray tandem mass spectrometry," *Analytical Chemistry*, vol. 79, no. 3, pp. 1135–1144, 2007.
- [25] K. J. Choi, S. G. Kim, C. W. Kim, and S. H. Kim, "Determination of antibiotic compounds in water by on-line SPE-LC/MSD," *Chemosphere*, vol. 66, no. 6, pp. 977–984, 2007.
- [26] A. Preechaworapun, S. Chuanuwatanakul, Y. Einaga, K. Grudpan, S. Motomizu, and O. Chailapakul, "Electroanalysis of sulfonamides by flow injection system/high-performance liquid chromatography coupled with amperometric detection using boron-doped diamond electrode," *Talanta*, vol. 68, no. 5, pp. 1726–1731, 2006.
- [27] K. Kishida and N. Furusawa, "Matrix solid-phase dispersion extraction and high-performance liquid chromatographic determination of residual sulfonamides in chicken," *Journal of Chromatography A*, vol. 937, no. 1-2, pp. 49–55, 2001.
- [28] H. Sangjarusvichai, W. Dungchai, W. Siangproh, and O. Chailapakul, "Rapid separation and highly sensitive detection methodology for sulfonamides in shrimp using a monolithic column coupled with BDD amperometric detection," *Talanta*, vol. 79, no. 4, pp. 1036–1041, 2009.
- [29] M. J. García-Galán, M. S. Díaz-Cruz, and D. Barceló, "Determination of 19 sulfonamides in environmental water samples by automated on-line solid-phase extraction-liquid chromatography-tandem mass spectrometry (SPE-LC-MS/MS)," *Talanta*, vol. 81, no. 1-2, pp. 355–366, 2010.
- [30] A. V. Pereira and Q. B. Cass, "High-performance liquid chromatography method for the simultaneous determination of sulfamethoxazole and trimethoprim in bovine milk using an on-line clean-up column," *Journal of Chromatography B*, vol. 826, no. 1-2, pp. 139–146, 2005.
- [31] R. V. Oliveira, A. C. De Pietro, and Q. B. Cass, "Quantification of cephalexin as residue levels in bovine milk by high-performance liquid chromatography with on-line sample cleanup," *Talanta*, vol. 71, no. 3, pp. 1233–1238, 2007.
- [32] L. Oliferova, M. Statkus, G. Tsylin, and Y. Zolotov, "On-line solid-phase extraction and high performance liquid chromatography determination of polycyclic aromatic hydrocarbons in water using polytetrafluoroethylene capillary," *Talanta*, vol. 72, no. 4, pp. 1386–1391, 2007.
- [33] E. Yamamoto, S. Takakuwa, T. Kato, and N. Asakawa, "Sensitive determination of aspirin and its metabolites in plasma by LC-UV using on-line solid-phase extraction with methylcellulose-immobilized anion-exchange restricted access media," *Journal of Chromatography B*, vol. 846, no. 1-2, pp. 132–138, 2007.
- [34] L. Tasso and T. D. Costa, "High performance liquid chromatography for quantification of gatifloxacin in rat plasma following automated on-line solid phase extraction," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 44, no. 1, pp. 205–210, 2007.
- [35] F. Gosetti, U. Chiuminatto, D. Zampieri, E. Mazzucco, E. Marengo, and M. C. Gennaro, "A new on-line solid phase extraction high performance liquid chromatography tandem mass spectrometry method to study the sun light photodegradation of mono-chloroanilines in river water," *Journal of Chromatography A*, vol. 1217, no. 20, pp. 3427–3434, 2010.
- [36] G. Z. Fang, J. X. He, and S. Wang, "Multiwalled carbon nanotubes as sorbent for on-line coupling of solid-phase extraction to high-performance liquid chromatography for simultaneous determination of 10 sulfonamides in eggs and pork," *Journal of Chromatography A*, vol. 1127, no. 1-2, pp. 12–17, 2006.
- [37] R. Fernandez-Torres, M. O. Consentino, M. A. B. Lopez, and M. C. Mochon, "Simultaneous determination of 11 antibiotics and their main metabolites from four different groups by reversed-phase high-performance liquid chromatography-diode array-fluorescence (HPLC-DAD-FLD) in human urine samples," *Talanta*, vol. 81, no. 3, pp. 871–880, 2010.
- [38] T. N. Rao, B. V. Sarada, D. A. Tryk, and A. Fujishima, "Electroanalytical study of sulfa drugs at diamond electrodes and their determination by HPLC with amperometric detection," *Journal of Electroanalytical Chemistry*, vol. 491, no. 1-2, pp. 175–181, 2000.