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Synchronized separation, concentration and determination of trace sulfadiazine and sulfamethazine in food and environment by using polyoxyethylene lauryl ether-salt aqueous two-phase system coupled to high-performance liquid chromatography

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ABSTRACT

Polyoxyethylene lauryl ether (POELE10)-$Na_2C_4H_4O_6$ aqueous two-phase extraction system (ATPES) is a novel and green pretreatment technique to trace samples. ATPES coupled with high-performance liquid chromatography (HPLC) is used to analyze synchronously sulfadiazine (SDZ) and sulfamethazine (SMT) in animal by-products (i.e., egg and milk) and environmental water sample. It was found that the extraction efficiency (E%) and the enrichment factor (F) of SDZ and SMT were influenced by the types of salts, the concentration of salt, the concentration of POELE10 and the temperature. The orthogonal experimental design (OED) was adopted in the multi-factor experiment to determine the optimized conditions. The final optimal condition was as following: the concentration of POELE10 is 0.027 g mL^{-1}, the concentration of $Na_2C_4H_4O_6$ is 0.180 g mL^{-1} and the temperature is 35 °C. This POELE10-$Na_2C_4H_4O_6$ ATPS was applied to separate and enrich SDZ and SMT in real samples (i.e., water, egg and milk) under the optimal conditions, and it was found that the recovery of SDZ and SMT was 96.20–99.52% with RSD of 0.35–3.41%. The limit of detection (LOD) of this method for the SDZ and SMT in spiked samples was 2.52–3.64 pg mL^{-1}, and the limit of quantitation (LOQ) of this method for the SDZ and SMT in spiked samples was 8.41–12.15 pg mL^{-1}.

1. Introduction

Sulfonamides (De Zayas-Blanco et al., 2004; Msagati and Nindi, 2004; Wen et al., 2005) are a type of synthetic antibacterial agent which can inhibit the synthesis of folic acid in susceptible microorganisms. Sulfadiazine (SDZ) and sulfamethazine (SMT) belong to the medium efficiency sulfonamides that have stable curative effects on infectious disease. However, sulfonamides have toxic effects on the urinary system and hematopoietic function of human and animal, and it was reported (Weber and Smedley, 1989) that sulfonamides could cause thyroid cancer. Therefore, it is very important to establish a rapid stable separation and measurement technique for SDZ and SMT in the animal by-products (egg and milk) and environmental water samples.

Nowadays, the main technologies used in the separation, enrichment and measurement of SDZ and SMT are dispersive liquid-liquid microextraction combined with ultra-high performance liquid chromatography (DLLME-UHPLC) (Herrera-Herrera et al., 2013), magnetic solid-phase extraction combined with high performance liquid chromatography (MSPE-HPLC) (Ibarra et al., 2014), pipette tip graphene solid-phase extraction (PT-G-SPE) coupled with liquid chromatography fluorescence detection (LC-FD) (Sun et al., 2014), dispersive micro-solid phase extraction (DMSPE) combined with mode-mismatched thermal lens spectrometry (MTLS) (Kazemi et al., 2016) and micro-solid phase extraction (μ-SPE) coupled with high performance liquid chromatography (HPLC) (Zhou and Fang, 2015). Although these technologies can meet the requirement of detection limit that the sulfonamides residues don't exceed the 50 μg kg^{-1}, but they are not used widely due to their shortcomings, such as tedious operation, long operation time and lower enrichment factor.

Aqueous two-phase system (ATPS) was more and more widely applied in the separation field as a powerful green extraction technique. It has been used successfully in the separation and extraction of nucleic acids (Luechau et al., 2009), proteins (Ooi

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et al., 2011; Rawdkuen et al., 2011; Yücekan and Önal, 2011), viruses (Luechau et al., 2011), antibiotics (Bi et al., 2009; Li et al., 2009; Xie et al., 2011) and other biological molecules (Azevedo et al., 2009; Gomes et al., 2009; Silva et al., 2009). ATPS extraction is more effective than traditional organic solvent extraction because it has some advantages for being simple, efficient, time-saving, green and eco-friendly (de Alvarenga et al., 2015). Compared with the traditional organic solvent extraction and solid phase extraction, ATPS is considered to be environmentally friendly due to the fact that the main ingredient at the two phases is water and no volatile organic solvent is added through the whole operation procedure (Li et al., 2009). The ATPSs mainly divides into four kinds that are the polymer-polymer ATPSs (Li and Cao, 2010), polymer-salt ATPSs (Zafarani-Moattar and Hosseinpour-Hashemi, 2012), ion liquid-salt ATPSs (Han et al., 2012) and micromolecule alcohol-salt ATPSs (Lu et al., 2013b; Zafarani-Moattar et al., 2012). In our previous articles (Lu et al., 2012a, 2012b, 2013a), it was found that nonionic surfactant polyoxyethylene (10) lauryl ether (POELE10, $C_{32}H_{66}O_{11}$) was an appropriate choice to form polymer-salt ATPS because it was consisted of the hydrophobic alkyl domain and hydrophilic polyoxyethylene tail. The phase behaviors of the ATPSs composed of POELE10 and various salts were studied, and it was found that the critical concentration of POELE10 was quite low for the POELE10-salt ATPS forming. Therefore, the POELE10-salt ATPSs can work well in the isolation and concentration of materials. We have applied POELE10-NaH_2PO_4 ATPS in the separation and enrichment of trace chloramphenicol in shrimp (Lu et al., 2016), and good effects are obtained. Although the phase-forming abilities of organic salts are not greater in comparison with the inorganic salts at the same valence states, but the organic salts are easily degradable and environment-friendly. Therefore, in this paper, we will study the POELE10-organic salt ATPS and apply it to extract and determine SDZ and SMT coupled with high-performance liquid chromatography (HPLC).

2. Experimental

2.1. Materials

Nonionic surfactants POELE10 with a quoted purity of greater than 0.99 mass fraction was obtained from Aladdin reagent company (Shanghai, China). Organic salts ($Na_2C_4H_4O_6 \cdot 2H_2O$, $K_2C_4H_4O_6 \cdot 1/2H_2O$, $(NH_4)_2C_4H_4O_6$, $K_2C_2O_4 \cdot H_2O$, $Na_3C_6H_5O_7 \cdot {}_2H_2O$, $K_3C_6H_5O_7 \cdot H_2O$) and $C_2HCl_3O_2$ were analytical grade reagents (GR, min. 99% by mass fraction), which were purchased from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The sulfadiazine and sulfamethazine standard sample was purchased from Chinese National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All reagents were used without further purification and the water used in experiments was double distilled.

2.2. Preparation of stock solution

0.5 g SDZ (or SMT) was put into 100 mL beaker and 1 mol L^{-1} of HCL was added. Stir until the analyte was completely dissolved. The solution was transferred to 1 L volumetric flask and deionized water was added to the volume. The stock solution with concentration of 500 μg mL^{-1} was stored at 4 °C in a refrigerator, which was replaced every two months. The standard working solutions were obtained by diluting the stock solution with appropriately deionized water.

2.3. Preparation of real samples

Milk samples were purchased from the local supermarket. 50 mL of sample and 20 mL of trichloroacetic acid (10%) were placed into a 100 mL tube, and the SDZ and SMT working solution was added. Then water was added into the tube until the total volume reached 100 mL. The mixture solution was shaken by using homogenizer-disperser (SJB-S450, Shanghai Siehe Instrument Co., China) until it was thoroughly mixed. The sample was put into centrifugal tube and was centrifuged at 4000 rpm for 30 mins (Anke TDL-4, Shanghai Chemical Machinery Plant Co., Ltd., China). The supernatant was taken out and filtered through the microfilter (Fushun filter paper factory, China) with a pore size of 0.45 μm to remove the proteins. The homogenization and centrifugation is carried out at room temperature. Finally, the filter liquor was stored at 4 °C.

Egg samples were also purchased from the local supermarket. Firstly, break the eggs into a bowl and stir them well. Secondly, 50 mL of eggs and 20 mL of trichloroacetic acid (10%) were placed into a 100 mL tube, and the SDZ and SMT working solution was added. The next step was identical to the steps that we prepared for milk samples.

Water samples were from Yangtze River and from the well located in Zhenjiang (China). Water samples were collected in 2.5 × 103 mL amber glass bottles. All the samples were centrifuged at 2000 rpm for 10 mins, and then the supernatant was collected. The collected supernatant was filtered through 0.45 μm filter, and the SDZ and SMT working solution was added. Finally, the solution were stored at 4 °C in the refrigerator for the further use.

2.4. Apparatus and procedure

An analytical balance (BS124S, Beijing Sartorius Instrument Co., Ltd, China) with an uncertainty of $\pm 1.0 \times 10^{-7}$ kg was used to weigh. A digital pH meter (Shanghai LIDA Instrument Factory, China) was used to determine the pH of solution. An HPLC (Agilent 1200, Agilent, USA) equipped with ultraviolet-visible (UV) detector was employed for the simultaneous qualitative and quantitative analysis of the SDZ and SMT. The Agilent ChemStation software was used to control the machine and process data. The calibration curves showed in Table 1 were obtained by means of injections of six levels of concentration (100–9000 pg mL^{-1}). Correlation

Table 1
Standard calibration data and instrumental LODs and LOQs of SDZ and SMT in spiked samples.

Analyte	Matrix	Linear range (pg mL^{-1})	Regression equation	Correlation coefficient (R^2)	LOD (pg mL^{-1})	LOQ (pg mL^{-1})
SDZ	Egg	100–9000	y = 0.0563x – 2.4973	0.9999	25.3	87.4
	Milk	100–9000	y = 0.0570x – 0.4759	0.9998	20.1	67.0
	Yangtze river water	100–9000	y = 0.0523x – 3.2442	0.9999	18.2	60.7
	Well water	100–9000	y = 0.0504x – 2.2271	0.9998	17.2	87.4
SMT	Egg	100–9000	y = 0.0478x – 1.0841	0.9992	27.7	92.4
	Milk	100–9000	y = 0.0490x – 1.9502	0.9991	21.9	93.1
	Yangtze river water	100–9000	y = 0.0469x – 0.3790	0.9997	20.7	69.0
	Well water	100–9000	y = 0.0469x – 0.4221	0.9995	18.2	60.7