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Dispersive liquid–liquid microextraction method based on solidification of floating organic droplet for the determination of thiamphenicol and florfenicol in environmental water samples

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ABSTRACT

Dispersive liquid–liquid microextraction with solidification of a floating organic droplet (DLLME-SFO) followed by high performance liquid chromatography-ultraviolet (HPLC-UV) detection was applied for the determination of thiamphenicol (TAP), florfenicol (FF) in water samples. 1-Undecanol was used as the extraction solvent which has lower density than water, low toxicity, and low melting point (19 °C). A mixture of 800 mL acetone (disperser solvent) and 80 μL of 1-undecanol (extraction solvent) was injected into 20 mL of aqueous solution. After 5 min, 0.6 g of NaCl was added and the sample vial was shaken. After 5 min, the sample was centrifuged at 3500 rpm for 3 min, and then placed in an ice bath. When the extraction solvent floating on the aqueous solution had solidified, it was transferred into another conical vial where it was melted quickly at room temperature, and was diluted with methanol to 1 mL, and analyzed by HPLC-UV detection. Parameters influencing the extraction efficiency were thoroughly examined and optimized. The extraction recoveries (ER) and the enrichment factors (EF) ranged from 67% to 72% and 223 to 241, respectively. The limits of detection (LODs) ($S/N=3$) were 0.33 and 0.56 μg L^{-1} for TAP and FF, respectively. Linear dynamic range (LDR) was in the range of 1.0–550 μg L^{-1} for TAP and 1.5–700 μg L^{-1} for FF, the relative standard deviations (RSDs) were in the range of 2.6–3.5% and the recoveries of spiked samples ranged from 94% to 106%.

1. Introduction

Thiamphenicol (TAP) and florfenicol (FF) are synthetic antibiotics with similar broad activity spectra; they are usually reserved for treating serious infections in animals and humans, but in humans they can show haematological toxicity (Dumont et al., 2006). TAP and FF are analogs of chloramphenicol, which have been suggested as potential substitutes. Large amounts of antibiotics are released into municipal waste water due to incomplete metabolism in humans or due to disposal of unused antibiotics (Nagulapally et al., 2009), which finally find their ways into different environmental water samples. TAP and FF have been found in various water samples, because of their wide spread availability and low cost. Therefore, it is necessary to establish simple, sensible and reliable analytical methods for the determination of TAP and FF in environmental water samples.

Sample pretreatment has probably been the main focus of research during many years in the environmental analytical chemistry area. Recently, much research has been directed toward efficient, economic, environment friendly and miniaturized extraction methods. Common extraction techniques used in environmental analysis are liquid–liquid extraction (LLE) (Puig and Barceló, 1996) and solid phase extraction (SPE) (Teng et al., 2009; Portet-Koltalo et al., 2007; Tran et al., 2013; Robert et al., 2010). These methods usually require large volume of organic solvents, as well as possessing the disadvantages of large quantities of environmentally unfriendly organic solvent and being laborious and time-consuming. First miniaturized techniques for aqueous samples were solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE), both derived from SPE (Luz et al., 2013). SPME is a powerful sample preparation technique that combines the microextraction and preconcentration of an analyte in a single step

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(Queiroz et al., 2003; Silva et al., 2008; Chaves et al., 2009). Although SPME is relatively new and an important sample preparation method with many advantages compared to other conventional methods, yet it is suffered from many weaknesses such as the fibre being fragile, expensive and has limited lifetime e.g. adsorption temperature, sample carry-over and reduced performance with time (Sanagi et al., 2012); moreover, coupling of SPME or SBSE to HPLC is not straightforward and requires either additional steps (desorption, evaporation, reconstitution) before analysis, or a special accessory for desorption in the injection system (Silva et al., 2008).

Recently, a new mode of liquid-phase microextraction based on solidification of floating organic droplet (LPME-SFO) was developed, which was initially introduced by Zanjani in 2007 (Zanjani et al., 2007). In this method, the extraction solvent was injected into a sample solution and then the sample solution was stirred at a very high speed. Furthermore, the extractant droplet can be collected easily by solidifying it in the lower temperature. However, the extraction was not rapid, so it cannot satisfy the demand of fast analysis. Later, the method was developed into a DLLME-SFO method (Leong and Huang, 2008), which was more rapid and simpler than LLME-SFO. In this method, the enormous contact area between extraction solvent and analytes in sample solution is beneficial for the fast mass transfer from the aqueous phase to the organic phase. Recently, it has been used for extraction of polycyclic aromatic hydrocarbons (PAHs) (Xu et al., 2009), organochlorine pesticides (OCPs) (Leong and Huang, 2009), organophosphorus pesticides (OPPs) (Pirsaheb et al., 2013), opium alkaloids (Toraj et al., 2013) and heavy metals (Mirzaei et al., 2011; Rezaee et al., 2010; Yamini et al., 2010) in water samples. In the extraction procedure, when a mixture of extraction and disperser solvents is injected into an aqueous sample, a cloudy solution is formed and the analytes are enriched in the extraction solvent. After centrifugation, the floated droplet is solidified in an ice bath and is easily collected for analysis.

In this paper, DLLME-SFO followed by HPLC-UV has been investigated for the simultaneous determination of TAP and FF in water samples. Furthermore, experimental variables, such as type of extraction and disperser solvents, volumes of extraction and disperser solvents, extraction time, pH, and salt addition were assessed and optimized.

2. Experimental

2.1. Reagents and samples

All chemicals were of reagent grade. Thiamphenicol (TAP) and Florfenicol (FF) were purchased from Sigma (USA). 1-undecanol, 1-dodecanol, n-hexadecane, methanol, acetone, acetonitrile and acetic acid were obtained from Chongqing Boyi Chemical Factory (Chongqing, China). NaCl was purchased from Merck (Darmstadt, Germany). Deionized water was prepared from a SZ-2 system (Shanghai Lu West Analytical Instruments, Shanghai, China).

The stock standard solution of TAP and FF was prepared in Methanol at a concentration of 500 mg L^{-1} and stored in a refrigerator at 4 °C. Real water samples were collected from Meixi, Yulin and Sujia River (Chongqing, China) and filtered through filter paper and stored in the refrigerator.

2.2. Instrumentation

The analyses were performed on a Shimadzu LC-20AT series HPLC System equipped with a solvent delivery pump, SPD-20A UV–Vis detector and a LC solution work-station (Shimadu, Japan). A high speed centrifuge was employed to centrifuge the sample solutions (Model 800, Shanghai, China).

The HPLC separation was performed on a Phenomenex C_{18} (150 × 4.6 mm i.d., 5 μm particle diameter) column. Mobile phase: methanol-0.6% acetic acid (35:65, v/v); flow rate: 0.8 mL/min; amount of injection: 20 μL; wavelength of detection: 225 nm; column temperature: 35 °C.

2.3. DLLME-SFO procedure

A mixed solution of 80 μL 1-undecanol (extraction solvent) and 800 μL acetone (dispersive solvent) was injected rapidly into 20 mL of aqueous solution containing 10.0 μg L^{-1} TAP and 20.0 μg L^{-1} FF in 25 mL vial, pH of the solutions were adjusted appropriately (pH=8.0), then the vial was placed in a water bath at 45 °C to reach a constant temperature of the sample and kept still for 5 min for equilibration. Afterwards, 0.6 g NaCl was added into the solution, and the vial was shaken by hand until the NaCl was completely dissolved. Followed by centrifugation at 3500 rpm for 3 min and on finally placing in an ice bath, the organic solvent droplet floated on the surface of the aqueous solution due to the low density below water. The sample vial was thereafter kept into an ice bath for 5 min, and at this time the floated solvent was solidified because of the low melting point. Then the solidified solvent was carefully transferred into ice water to wash away the NaCl, dispersers and other impurities on the surface. Then the solidified solvent (approximately 60 μL) was transferred to a conical vial where it melted quickly in the room temperature, and was diluted with methanol to a volume of exactly 1 mL. This final extract is then analyzed by HPLC-UV.

2.4. Calculation of the enrichment factor, extraction recovery, and relative recovery

The EF was defined as the ratio between the analyte concentration in the floated phase ($C_{floated}$) and the initial concentration of the analyte ($C_{initial}$) in the aqueous sample (Rezaee et al., 2006), as follows:

$$EF = \frac{C_{floated}}{C_{initial}} \quad (1)$$

The $C_{floated}$ was obtained by direct injection of the analyte standard solution in the extraction solvent. The extraction recovery (ER) was defined as the percentage of the total analyte (n_0) extracted into the floated phase ($n_{floated}$):

$$ER = \frac{n_{floated}}{n_o} \times 100 = \frac{C_{floated} \times V_{floated}}{C_{initial} \times V_{aq}} \times 100 \quad (2)$$

where $V_{floated}$ and V_{aq} are the volumes of the floated phase and sample solution, respectively. The relative recovery (RR) is defined by the following equation:

$$RR = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \quad (3)$$

where C_{found} represents the concentration of the analyte after adding a known amount of standard to the real sample, C_{real} is the concentration of the analyte in the real sample, and C_{added} refers to the concentration of a known amount of standard that was spiked in the real sample (Leong and Huang, 2009).