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Orthogonal array design for the optimization of hollow fiber protected liquid-phase microextraction of salicylates from environmental waters

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ABSTRACT

In the present study, a three phase-based hollow fiber protected liquid-phase microextraction (HF-LPME) method combined with high-performance liquid chromatography (HPLC) for the determination of salicylates in environmental waters was developed. The HF-LPME procedure was optimized by an $L_{16}(4^5)$ orthogonal array experimental design (OAD) with five factors at four levels. Under the optimal extraction condition (pHs of donor and receiving phases of 3.0 and 6.2, respectively, extraction time of 45 min, stirring speed of 1000 rpm, and salt addition of 20% (w/v)), salicylates could be determined in a linear range from 0.025 to 1.0 μg mL^{-1} with a good correlation ($r^2 > 0.9930$). The limits of detection (LODs) ranged between 0.6 ng mL^{-1} and 1.2 ng mL^{-1} for the target analytes. The relative standard deviations (RSDs) of intra-day and inter-day were in the range of 0.64–14.58% and 0.16–15.45%, respectively. This procedure afforded a convenient, sensitive, accurate and cost-saving operation with high extraction efficiency for the model analytes. The method was applied satisfactorily to the determination of salicylates in two environmental waters.

1. Introduction

With the growing activities of human beings and the advancement of the society, environmental pollution has become a serious issue. It is the duty of analystes to monitor the environment and give the public a prompt reminder. Generally, environmental analysis included several steps of sampling, sample preparation, instrumental analysis and data handling. Owing to low concentration of pollutants in varied and complex sample matrices, a suitable sample preparation is crucial to environmental analysis [1], which aims to clean up, isolate and concentrate the analytes of interest prior to instrumental analysis.

Liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are classical sample pretreatment methods and still remain popular choices in environmental analysis [2]. To develop the environmental-friendly and cost-effective sample pretreatment methodologies, miniaturization has been a key. Solid-phase microextraction (SPME) and liquid-phase microextraction (LPME) are popular miniaturized sample preparation methods. LPME was first introduced in middle-to-late 1990s by Jeannot and Cantwell [3–5]. The original LPME is based on a droplet organic solvent hanging at the end of a microsyringe needle, which is called single drop microextraction (SDME). Compared to LLE and SPME, SDME is simple, uses a small amount of organic solvent, avoids sample carry-over, and has a high enrichment factor due to the low volume ratio of the organic solvent to the sample solution [6–9]. The main problem of SDME is the instability of hanging drop, which is easily dislodged during the extraction [2,10].

In order to settle the problem of low stability, Pedersen-Bjergaard and Rasmussen proposed the use of a porous polypropylene hollow fiber membrane to hold the receiving phase [11], which was hollow fiber protected liquid-phase microextraction (HF-LPME). The porous hollow fiber membrane played dual roles, to stabilize the receiving phase and to prevent the entering of macromolecules or contaminations to the receiving phase. Besides, the hollow fiber was for single use to avoid sample carry-over [12,13]. Hence, compared to SDME, HF-LPME can afford cleaner and stabler extraction [14].

HF-LPME has two modes: two-phase and three-phase [15]. In two-phase HF-LPME, the receiving phase is filled in both the membrane pores and lumen. In three-phase HF-LPME, analytes are first extracted from aqueous donor phase (sample solution) to the organic solvent immobilized in the fiber pores, and then into another aqueous phase (the third receiving phase) inside the lumen. Compared to the two-phase HF-LPME, the three-phase extraction could afford much cleaner extractant, and is particularly suitable for the ionic compounds. HF-LPME, which has

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Table 1
Factors and level values in $L_{16}(4^5)$ matrix.

Levels	Factors				
	A pH of receiving phase	B pH of donor phase	C Extraction time (min))	D Stirring speed (rpm)	E Salt (%)
1	6.2	0	15	400	0
2	6.2	1.5	30	600	10
3	6.2	3.0	45	800	20
4	6.2	4.5	60	1000	30

demonstrated wide applications in environmental, pharmaceutical, forensic, biological analysis, etc. [2,16–20], is complementary to SPME.

Nowadays, the existence of pharmaceutical active compounds in aquatic environment is a serious pollution issue. Although drug residues are generally present in trace level, they may have chronic, long-term and cumulative adverse effects on human beings. Especially, due to their persistence and water solubility, some drugs are not even eliminated in sewage treatment plants and can reach surface and ground waters. Thus, ultimately, they are absorbed by the human beings. A sensitive analytical method is of great importance to monitor drugs and their metabolites [21–26]. The salicylates, salicylic acid (SA), para-aminosalicylic acid (PAS) and acetylsalicylic acid (ASA), are commonly used compounds. SA and PAS are important pharmaceutical intermediates. ASA (asprin) and its metabolite SA are widely used non-steroidal anti-inflammatory drugs. PAS is a traditional antituberculosis agent. These three salicylates have gastrointestinal toxicity, and high-level exposure to them may induce seizures, transient tinnitus and hearing lose [27,28]. In addition, PAS may induce skin rash, lymphadenopathy, joint pains and so on [29].

In this work, three-phase HF-LPME was used as the sample pretreatment method for the three salicylates, SA, ASA and PAS, prior to high-performance liquid chromatography (HPLC) analysis. To optimize the conditions of HF-LPME, an orthogonal array experimental design (OAD) based on five factors and four levels ($L_{16}(4^5)$) was employed. Analysis of variance (ANOVA) was used to assess the results of OAD. The significance of a factor and two-way interaction among different factors was presented by the significance value (F) [30–32].

2. Experimental

2.1. Chemicals and reagents

Three salicylates, SA, ASA and PAS were obtained from Alfa Aesar (Tianjin, China). HPLC-grade methanol, acetic acid, 1-octanol and sodium chloride were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Hydrochloric acid was from Kaifeng Dong Da Chemical Reagent Co., Ltd. (Henan, China). Ultrapure water (pH 6.2) was produced by Heal Fore NW system (Shanghai, China).

2.2. Apparatus

The determination of the three salicylates was performed on a Hitachi (Tokyo, Japan) HPLC system. It consisted of a Model L-2130 pump, a Rheodyne 7725i injector (Cotati, CA, USA) and an L-2400 UV–vis spectrophotometric detector. Data were collected and processed by a T3000P (Hangzhou Hui Pu Technology Co., Ltd., Hangzhou, China) software. Chromatographic separations were achieved on a Belta ODS (3.9 mm × 150 mm, 5 μm) column from Waters (Milford, MA, USA) at a temperature of 22 °C. A mixture of methanol and 1% acetic acid (35:65, v:v) were used as mobile phase. The flow rate was 1.0 mL min^{-1} and the injection volume was 5.0 μL. All the experiments were performed at least in triplicate. The pH values were measured with a Mettler Toledo Delta 320 pH meter (Shanghai, China). The UV wavelength was set at 280 nm. The hollow polypropylene fiber (Wuppertal, Germany) used in all the extractions was 600 μm I.D. with 200 μm wall thickness and 0.2 μm pore size.

2.3. Sample preparation

The stock solution containing the three salicylates (1 mg mL^{-1} of each analyte) was prepared in methanol. It was stored at 4 °C. Water samples were prepared by spiking ultrapure water with the analytes at a known concentration (0.5 μg mL^{-1}) to optimize extraction conditions.

2.4. Extraction procedure

The general procedure for HF-LPME was as follows: (1) prior to usage, the hollow fibers were cut into 4 cm each piece and ultrasonicated in acetone for 10 min to remove any possible contaminations. Then the fibers were taken out and exposed to the air till the acetone was evaporated completely; (2) one end of the hollow fiber was heat-sealed and the other end was connected to a 25 μL microsyringe needle; (3) 15 μL receiving phase contained in the microsyringe was injected to the lumen of hollow fiber completely and the fiber was immersed in 1-octanol for 30 s to impregnate the fiber pores; (4) the fiber was totally immersed into the sample solution (10 mL) through the small hole on the sample vial lid and the syringe was fixed at the same height for every extraction; (5) the sample solution with a stirring bar was stirred at a prescribed speed for a prescribed period of time for extraction; and (6) after the extraction, the syringe with the hollow fiber was taken out. The extractant was withdrawn into the syringe, and the hollow fiber was thrown away. The extractant (5.0 μL) was directly injected into HPLC system for analysis.

2.5. Optimization strategy

In order to optimize the HF-LPME conditions for the extraction of salicylates, an OAD based on the $L_{16}(4^5)$ matrix was employed in this work. Five factors at different levels were shown in Table 1.

After implementing the experiments based on the above OAD, ANOVA was used to assess the results. The significance value (F) was computed to assess the significance of a factor and two-way interaction among different factors.

2.6. Method validation

The intra-day repeatability was studied for three replicate experiments and the inter-day repeatability was investigated for consecutive three days at the optimized extraction condition for an aqueous sample containing 1.0, 0.1 and 0.025 μg mL^{-1} of the three salicylates, respectively. The linearity was investigated over a concentration range of 0.025–1.0 μg mL^{-1} and calculated by plotting corresponding HPLC peak areas (y) versus concentrations of