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Determination of monoamine neurotransmitters in human urine by carrier-mediated liquid-phase microextraction based on solidification of stripping phase

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ABSTRACT

A novel method was developed for the analysis of monoamine neurotransmitters (MNTs) in human urine by carrier-mediated liquid-phase microextraction based on solidification of stripping phase method (CM-LPME-SSP) coupled with high performance liquid chromatography-electrochemical detector (HPLC-ECD). By adding an appropriate carrier in organic phase, simultaneous extraction of hydrophilic analytes, MNTs, with high enrichment factors (22.6–36.1 folds) and excellent sample cleanup was achieved. A new strategy, solidifying the aqueous stripping phase in the back-extraction process, was developed to facilitate the collection of the stripping phase as small as a few microliters. Combined with HPLC-ECD analysis, the linear ranges of the established method were 0.015–2.0 μg/mL for NE, E, DA, and 0.020–2.0 μg/mL for 5-HT. The limits of detection and quantification were in the range of 5.5–10.8 ng/mL and 15–20 ng/mL, respectively. The relative recoveries were in the range of 87–108%, with intraday and interday relative standard deviations lower than 13%. This method was successfully applied to analysis of MNTs in real urine.

1. Introduction

Monoamine neurotransmitters (MNTs) including norepinephrine (NE), epinephrine (E), dopamine (DA), and 5-hydroxytryptamine (5-HT) (chemical structures shown in Fig. 1) play important roles in various biological, pharmacological and physical processes in brain tissue and body fluids [1,2]. It is significant for determining the content of MNTs in biological fluids for the clinical diagnosis of diseases, e.g. Depression and Alzheimer [3,4]. Among the reported analysis methods of MNTs, high performance liquid chromatography (HPLC) combining with different detectors, i.e. electrochemical detector (ECD) [5–7], fluorescence detector (FLD) [8,9], and mass spectrometry (MS) [10–12] was the most common technique. However, sample preconcentration and cleanup must be carried out before MNTs were determined using HPLC due to the low concentration of analytes and a high number of interferents existing in biological fluid.

Liquid–liquid extraction (LLE) [13] and solid phase extraction (SPE) [14] were mostly used in the separation and enrichment of analytes in biological samples. These traditional LLE and SPE methods are time-consuming, laborious and have high consumption of hazardous organic solvents and samples. Liquid phase microextraction (LPME), a solvent-saving and low consumption method, was developed in 1996 [15]. Thereafter, different LPME methods such as static liquid-phase microextraction (S-LPME) [16], single drop microextraction (SDME) [16], head space liquid-phase microextraetion (HS-LPME) [17], hollow fiber liquid-phase microextraction (HF-LPME) [18], and dispersive liquid–liquid microextraction (DLLME) [19] were proposed. Most studies focused on the extraction of hydrophobic compounds because it is difficult to obtain high extraction efficiency for hydrophilic compounds in LPME due to low distribution coefficient of hydrophilic compounds in the organic phase. To improve the extraction efficiency of hydrophilic compounds, some studies involved mixing various organic solvents or adding some additives for improving the distribution coefficient of the analytes in the organic phase were attempted [20–28]. Another challenge in LPME is the collecting process of the acceptor solution due to its very small volume. A novel LPME method based on solidification of floating organic drop (SFO) [29] solved the collection problem of the acceptor solution excellently by solidifying the organic acceptor droplet in two-phase LPME. However, the application of LPME-SFO was

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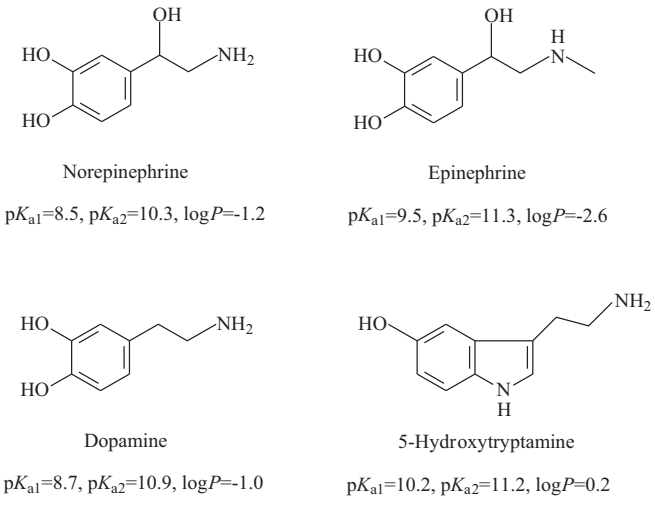

Fig. 1. Structures of the studied MNTs.

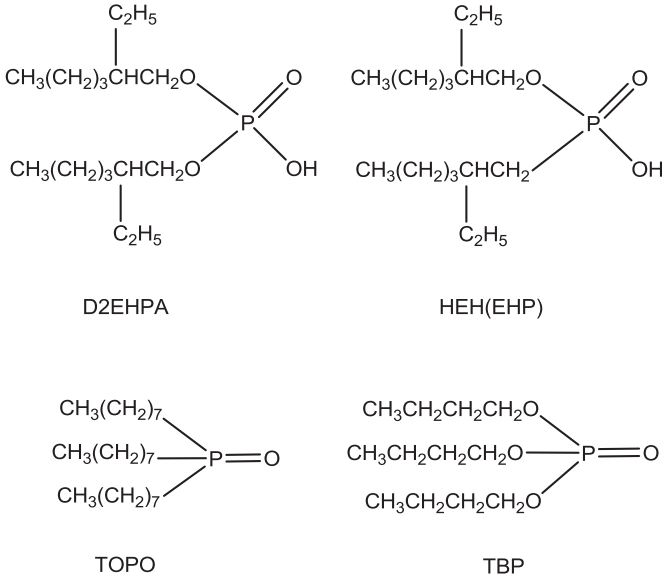

Fig. 2. Structures of the carriers.

limited because the cleanup effect is unsatisfactory due to the coextraction of the coexisting compounds in two-phase LPME and the incompatibility of organic acceptor with the mobile phase in HPLC.

In the present paper, a novel carrier-mediated liquid-phase microextraction based on solidification of stripping phase method (CM-LPME-SSP) was proposed for the separation and enrichment of MNTs in urine. Compared with the traditional LPME-SFO methods, this method solidified the aqueous stripping phase (aqueous acceptor phase) instead of the organic acceptor phase. This strategy makes the collection of the stripping phase as small as a few microliters become convenient. After separated from the organic phase, the solidified stripping phase was melted at the room temperature and directly analyzed by HPLC-ECD. By injecting the aqueous stripping phase instead of the organic acceptor phase in HPLC, not only was the pollution of chromatographic column by organic acceptor phase injection avoided, but also the compatibility of LPME injection solution with the HPLC mobile phase was enhanced. With the aid of the carriers, simultaneous extraction of hydrophilic analytes, MNTs, with high enrichment factors and excellent sample cleanup was achieved and the application of LPME was expanded to extract the hydrophilic substances from complex biological matrices. The proposed CM-LPME-SSP combined with HPLC-ECD analysis exhibited high selectivity, good precision and high sensitivity in analysis of MNTs in urine.

2. Experimental

2.1. Reagents and apparatus

The standard reference materials of E, DA, and 5-HT were obtained from National Institutes for the Control of Pharmaceutical and Biological Products (Beijing, China); NE was supplied by Aladdin Industrial Corporation (Shanghai, China). The carriers used, di(2-ethylhexyl) phosphoric acid (D2EHPA), 2-ethylhexyl 2-ethylhexyl phosphonic acid (HEH(EHP)), trioctylphosphineoxide (TOPO), and tributyl phosphate (TBP) (structures shown in Fig. 2) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China), Chemical Reagent Ltd. of Zhongkejian (Tianjin, China), Kefan Chemical Technology Co., Ltd. (Shanghai, China), and Hunan Normal University Reagent Factory (Changsha, China), respectively. All of the four carriers were of chemical grade and directly used without further purification. The organic solvent, *n*-hexane, toluene, *o*-xylene, isopropanol, and petroleum ether, were obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). HPLC-grade methanol, AR grade of HCl, H_3PO_4, CH_3COOH, and HCOOH were obtained from Damao Chemical Reagent Factory (Tianjin, China). Potassium dihydrogen phosphate (AR grade) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China) and 1-octanesulfonic acid sodium salt which used as ion-pairing agent was purchased from Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). The water used was purified with a Milli-Q system from Millipore (Bedford, MA, USA).

An HJ-2 magnetic stirrer, purchased from Hengfeng Instrument Factory (Jintan, Jiangsu, China) and a table-top low speed centrifuge, purchased from Anke Scientific Instrument Factory (Shanghai, China) were used in the LPME setup. The pH was measured with a PHS-3C digital pH meter manufactured by INESA Scientific Instrument Co., Ltd. (Shanghai, China).

2.2. Standard solution preparation, sample collection and preservation

Individual stock solution of NE, E, DA, and 5-HT at concentration of 500 μg/mL, containing 20 mmol/L antioxidant sodium metabisulfite to prevent the oxidation of analytes, was prepared in HCl solution (pH=3) and stored at 4 °C in darkness. Mixed standard solution containing 25 μg/mL of NE, E, DA, and 5-HT was daily prepared by pipetting 250 μL of each stock solution into 5 mL volumetric flask, diluting to the mark with HCl solution (pH=3) and stored at 4 °C in darkness. Fresh feed solution containing 5 μg/mL of NE, E, DA, and 5-HT was prepared by diluting the mixed standard solution with phosphate buffer (2.5 mmol/L, pH 8.0) unless other specified and used in the optimization of experimental conditions.

Twenty-four hours of urine samples were collected from five healthy volunteers (three males, 23 year old, 23 year old, 24 year old, and two females, 22 year old and 23 year old, respectively) and sodium metabisulfite was added to avoid the oxidation of analytes until the sodium metabisulfite concentration got to 0.8 mmol/L. All urine samples were stored at 4 °C and tested within 48 h except for the inter-day reproducibility test, for which the spiked urine samples were stored at −18 °C before test.