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Ionic liquid-based electromembrane extraction and its comparison with traditional organic solvent based electromembrane extraction for the determination of strychnine and brucine in human urine

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ABSTRACT

An ionic liquid-based electromembrane extraction (IL-EME) method was presented, and its performance was compared with 2-ethylnitrobenzene (ENB) based EME for the determination of strychnine and brucine in human urine. For the two methods, the fundamental extraction parameters such as supported liquid membrane, voltage, extraction time, pH values of sample solution and acceptor solution, temperature and salting-out effect were separately optimized. IL-EME provided 96- and 122-fold enrichment factors for strychnine and brucine, respectively, which were better than those obtained in EME (83- and 86-fold, respectively). The calibration curves were linear over the ranges of 20–720 $\mu g\,L^{-1}$ for strychnine and 20–640 $\mu g\,L^{-1}$ for brucine with the correlation coefficients higher than 0.9950. The repeatability of EME and IL-EME were evaluated by five parallel experiments giving the relative standard deviations of 5.12–6.98%. As the results indicated, compared with ENB based EME, the proposed IL-EME is more reliable and could provide better extraction performance for the determination of strychnine and brucine in human urine.

1. Introduction

For a long time, Semen Strychni has been part of traditional Chinese medicine used to improve blood circulation, alleviate rheumatic pain and treat cancer [1]. Strychnine and brucine, the main bioactive components of Semen Strychni [2], have been proved to have pharmacological effects and toxic effects [3]. At low doses, they exhibit excellent pharmacological activities [4] but are considered poisonous at high doses [5]. Researchers are most troubled by the narrow safety margin between the therapeutic and the toxic dose that makes evaluating the clinical dose difficult. Therefore, establishing an easy, fast and sensitive method is important to determine strychnine and brucine in biofluids for the areas of toxicological research, clinical study, forensic toxicology and medicine safety. During the evaluation of forensic toxicology, the concentration of target analytes in urine is usually determined to assess organism exposure. However, the concentration of strychnine and brucine in urine is very low. Therefore, developing an effective extraction method for analyzing such samples is necessary to overcome the complexity of biological matrices and the low level of target analytes.

In recent years, hollow fiber liquid phase microextraction (HF-LPME) has been given considerable attention in biomedical analysis [6]. HF-LPME has received merits for simplicity, low cost, low solvent consumption, excellent clean-up ability and high enrichment efficiency. However, HF-LPME has some main drawbacks, such as long extraction time, limited analyte variety, and easy loss of organic solvent. Aiming at solving these problems, some strategies have been successively introduced to HF-LPME. Direct current (DC) electric field was first introduced to the extraction mode in 2006 by Pedersen-Bjergaard [7]. This newly proposed mode was called electromembrane extraction (EME) in the following studies. In this mode, electrokinetic migration instead of passive diffusion serves as the main driving force, which dramatically shortens the extraction time. The experimental setup is the same as that in HF-LPME except for two additional platinum wires. When DC power is applied, the charged analytes directly migrate through the supported liquid membrane (SLM) composed of the

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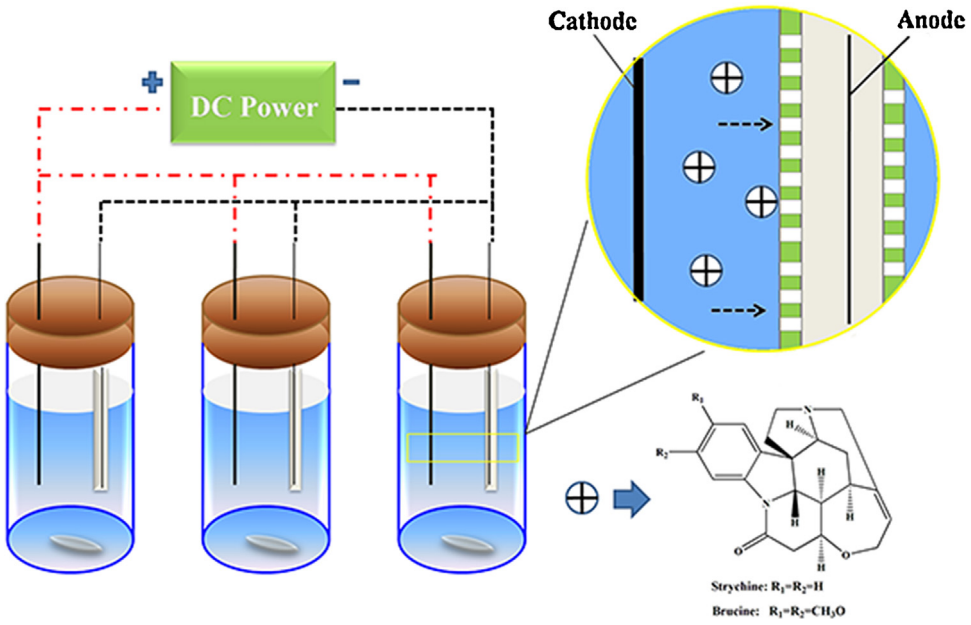

Fig. 1. The setup of EME and IL-EME and the structure of strychnine and brucine.

organic solvent, and then gather in the acceptor phase. Unlike in the conventional HF-LPME, satisfactory extraction efficiency is obtained in EME within a short period of extraction time (usually 5–15 min) [8–10]. Note that the extraction efficiency is not limited by the partition equilibrium. If the electric field is removed, the migration of the charged analytes will cease. Until now, the EME method has been successfully applied for the preconcentration of drugs [11,12], herbicides [13,14], and peptides [15] in complex biological matrices. Nevertheless, a relatively high voltage of power is required in EME that will cause unstable SLM and potential solvent loss. Recently, room temperature ionic liquids (ILs) have been proposed as an alternative to traditional organic solvents in HF-LPME [16]. ILs have distinct physical and chemical properties, such as low volatility and toxicity, high viscosity, adjustable polarity, and high extractability for both organic and inorganic compounds. However, the application of ILs in HF-LPME is limited because of its long extraction time (30 min to 8 h) [17–20]. This prolonged extraction time may be due to the high viscosity of ILs that slows down the mass transfer rate. Based on the above discussion, the respective advantages of EME, ILs, and HF-LPME were integrated to develop a novel microextraction method called ionic liquid-electromembrane extraction (IL-EME). In this mode, ILs are immobilized in the hollow fiber pores and used as SLM. The electric field is then introduced to serve as the main driving force of mass transfer. In this research, the proposed IL-EME combined with high performance liquid chromatography (HPLC) was applied to determine strychnine and brucine. The performance of IL-EME was compared with that of EME using the same analytes in the same human urine samples. By comparing the extraction procedure and efficiency with those of EME, the advantages and disadvantages of IL-EME were determined, and the applicability of IL-EME method was also confirmed.

2. Experimental

2.1. Chemical and reagents

Analytical standards, strychnine and brucine (Fig. 1) were obtained from the National Institute for the Control of Pharmaceutical Products (Beijing, China). Chromatographic-grade methanol and acetonitrile were purchased from Merck Co. (Darmstadt, Germany). 2-ethylnitrobenzene (ENB) was purchased from TCI Co. (Shanghai, China). The ILs were obtained from Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences (Lanzhou, China). Other chemicals are of analytical grade and obtained from Tianjin Chemical Reagent Co. (Tianjin, China). Ultrapure water supplied by an OKP ultrapure water system (Shanghai Laikie Instrument Co. Ltd., Shanghai, China) was used for the preparation of mobile phase and sample solution.

The D.C. power was supplied with a DC–AC converter with programmable voltage in the range of 1.5–12 V. Platinum wires (Tianjin Aidahengsheng Technology Co. Ltd., Tianjing, China) with diameter of 0.2 mm and 0.5 mm, respectively, were used as negative electrode and positive electrode. The PP Q2/1 polypropylene hollow fibers (Membrana, Wuppertal, Germany) with an internal diameter of 600 μm, a wall thickness of 200 μm and a pore size of 0.2 μm were used to immobilize the artificial liquid membrane and hold the acceptor solution.

2.2. Chromatographic apparatus and condition

Chromatographic analysis was performed on an Agilent 1260 HPLC system comprising a G1311C quarternary pump, a G1315D diode-array detector, a G1316A thermo stated column compartment, and a G1328C manual injector. Chromatographic separation of the analytes was performed on an Agilent Zorbax SB-C_{18} column (5 μm, 4.6 mm × 150 mm i.d.). Agilent ChemStation was employed to acquire and process chromatographic data. The mobile phase consisted of 0.1% aqueous phosphoric acid (A) and methanol (B) at a ratio of 78:22 (v/v). The flow rate was 1.0 mL min^{-1}. The column temperature was maintained at 25 °C and the detection wavelength was 260 nm.

2.3. Sample preparation

Standard stock solutions of strychnine (0.21 mg mL^{-1}) and brucine (0.19 mg mL^{-1}) were separately prepared in methanol and stored at 4 °C before use. The working solutions were freshly prepared by appropriate dilution of stock solution with ultrapure water, and used for optimization experiments. Then the pH value