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Determination of sildenafil, vardenafil and aildenafil in human plasma by dispersive liquid–liquid microextraction-back extraction based on ionic liquid and high performance liquid chromatography-ultraviolet detection

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ABSTRACT

A novel method which involved dispersive liquid–liquid microextraction (DLLME)-back extraction based on ionic liquid (IL) was developed for the determination of three phosphodiesterase-5 (PDE-5) inhibitors, sildenafil (SD), vardenafil (VD) and aildenafil (AD), in human plasma. DLLME based on IL as the extractant solvent and methanol as the dispersive solvent was the first step to extract PDE-5 inhibitors from sample solution; the other step of back extraction was followed by transferring target analytes from the IL to acidified aqueous solution. This two-step extraction ensured the compatibility of the final extractant phase, acidified aqueous solution herein, with the reversed phase high performance liquid chromatography-UV detection, and afforded clean extractant phase. The optimal extraction condition was obtained after systematical optimization. The sample solution (960 μL) was extracted by 20 μL of 1-octyl-3-methylimidazolium hexafluorophosphate in the presence of 20 μL methanol and 300 mg mL^{-1} NaCl with the assistance of vortex; IL phase enriched with the target analytes was then extracted by 10% acetic acid aqueous solution. Good linearity ranges (SD 1–500 ng mL^{-1}, VD 2–2000 ng mL^{-1} and AD 2–2000 ng mL^{-1}) with suitable r^2 (=0.9999) were achieved. Limits of detection (LODs) in pure water were 0.15 ng mL^{-1}, 0.30 ng mL^{-1} and 0.43 ng mL^{-1} for VD, SD and AD, respectively. Intra-day and inter-day relative standard deviations were below 6.38%. Finally, this method was applied for the determination of PDE-5 inhibitors in human plasma with satisfactory LODs of 0.92 ng mL^{-1}, 1.19 ng mL^{-1} and 2.69 ng mL^{-1} for VD, SD and AD, respectively. Acceptable absolute recoveries were obtained from 100.4% to 103.9%. The developed method afforded a convenient, fast and cost-saving operation with high extraction efficiency for the test analytes. It has potential to be applicable to biological samples.

1. Introduction

Sildenafil (SD), vardenafil (VD) and aildenafil (AD) are three typical phosphodiesterase-5 (PDE-5) inhibitors, which take action through inhibiting PDE-5 in the corpus cavernosum and are widely used to treat male erectile dysfunction [1–7]. These drugs were also deliberately added in dietary supplement [8–10], herbal products [11,12] and health care products [13]. If people take these drugs unreasonably, adverse effects, such as headache, vertigo, the reduction of blood pressure and aggravation of cardiovascular disease [14–16], may emerge. It is thus important to monitor the concentration level in biological samples for clinical or forensic purposes [17–19]. Since biological samples, e.g. human plasma, are complex, sample pretreatment is normally required prior to the instrumental analysis. Liquid–liquid extraction (LLE) [18], solid-phase extraction [19] and liquid–liquid–liquid microextraction [17] have been adopted to handle the biological samples related to the determination of PDE-5 inhibitors.

Dispersive liquid–liquid microextraction (DLLME) is an effective sample pretreatment technology introduced by Rezaee in 2006 [20]. It is based on a ternary component solvent system, in which a dispersive solvent, miscible with the aqueous solution and extractant solvent, is added; a water/dispersive solvent/extractant solvent emulsion system is thus formed [21–23]. In this way, target analytes could be transferred very fast from the aqueous phase to extractant solvent with the assistance of dispersive solvent. In the traditional LLE, large volume of organic solvent was required, while this technology consumes less solvent, less time and thus results in low cost with high enrichment efficiency. As well, compared to the miniaturized LLE procedure, e.g. liquid phase microextraction, DLLME has the merits of convenient operation and relative high recovery.

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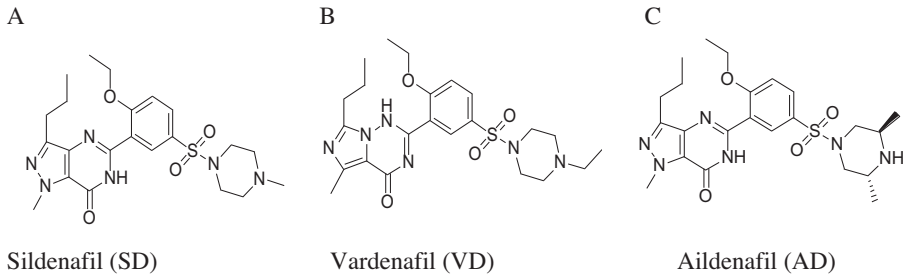

Fig. 1. Structures of the target analytes.

Ionic liquid (IL), which is composed of organic cations and organic or inorganic anions and presents a state of liquid molten salt at room temperature [24], possesses characteristics of low vapor pressure, good thermal stability and oxidation stability, non-volatility, low toxicity, etc. [25,26]. ILs have been explored to apply in analytical chemistry [27], especially as alternative solvents in sample preparation procedure [28,29]. In 2008, Ghareh-baghi [30] proposed one IL as the alternative extractant solvent to the traditional highly toxic chlorinated solvents in DLLME. This approach was environmental friendly and highly efficient, and it has demonstrated applications in environmental and biological samples [28,31,32].

In the present experiment, a two-step sample pretreatment method was proposed to extract SD, VD and AD from human plasma. DLLME based on IL as the extractant solvent was used to extract target analytes from human plasma; back extraction was followed to retrieve the target analytes from the IL to the other aqueous phase. Through optimizing a series of experimental conditions, good determination results were obtained, which provided an alternative approach for the determination of PDE-5 inhibitors in human plasma.

2. Experimental

2.1. Chemicals and reagents

Three PDE-5 inhibitors, SD, VD and AD, as shown in Fig. 1, were obtained from the laboratory of medicinal chemistry (Tongji School of Pharmacy, Huazhong University of Science and Technology), whose purities were all above 99.5%. Two ILs, 1-butyl-3-methylimidazolium hexafluorophosphate ($[C_4MIM][PF_6]$) and 1-octyl-3-methylimidazolium hexafluorophosphate ($[C_8MIM][PF_6]$), were purchased from Shanghai Cheng Jie Chemical Co. Ltd. (Shanghai, China). High performance liquid chromatography (HPLC)-grade methanol, acetonitrile, acetic acid (HAc), sodium hydroxide (NaOH) and sodium chloride (NaCl) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Acetone was from Tianjin Guangcheng Chemical Reagent Co., Ltd. (Tianjin, China). Hydrochloric acid (HCl) was from Kaifeng Dong Da Chemical Reagent Co., Ltd. (Henan, China). Ultrapure water was produced by a Heal Fore NW system (Shanghai, China).

2.2. Apparatus

Separation of the three PDE-5 inhibitors 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 T3000P (Hangzhou Hui Pu Technology, Hangzhou, China) software. The pH values were measured with a Mettler Toledo Delta 320 pH meter (Shanghai, China) with a combined electrode.

A Shimadzu (Kyoto, Japan) ODS RP-C_{18} column (250 mm × 4.6 mm, 5 μm) was used for chromatographic separation. The mobile phase consisted of 40% methanol- 60% water containing 1% HAc, and was applied in an isocratic mode at a flow rate of 1.2 mL min^{-1}. The UV wavelength was set at 254 nm. Injection volume was 20 μL for every analysis. All the experiments were performed at least in triplicate.

2.3. Sample preparation

The stock solutions containing the three PDE-5 inhibitors (1.000 mg mL^{-1} of each analyte) were prepared separately in methanol and stored at 4 °C. Water samples were prepared by spiking ultrapure water with the analytes at a known concentration (0.100 μg mL^{-1} SD, 0.200 μg mL^{-1} AD and 0.200 μg mL^{-1} VD) daily to optimize extraction conditions.

Human plasma was obtained from clinical laboratory of Tongji Hospital (Wuhan, China), and was directly handled with the proposed method.

2.4. Extraction procedure

The extraction procedure is shown in Fig. 2. 960 μL sample solution was placed in a 1.5 mL polyethylene tube and a mixture of certain volume of IL and dispersive solvent was injected rapidly into the sample solution. The mixture was gently shaken for several seconds and a cloudy solution was formed, which consisted of fine droplets of IL dispersed in the aqueous sample. The cloudy mixed solution was vortexed for the prescribed time. Phase separation was obtained by centrifugation at 10^4 rpm for 5 min. A pipet was used to remove the upper layer, and certain volume of the bottom layer was retrieved into another 1.5 mL tube, to which 40 μL aqueous HAc solution was added. The mixed solution was vortexed for 5 min and the phase separation was obtained by centrifugation at 10^4 rpm for 5 min. Twenty microliter of the upper layer was sucked by a HPLC syringe and injected into the HPLC system for analysis.

The best extraction conditions after a series of optimization were as follows. The sample solution was extracted by 20 μL of $[C_8MIM][PF_6]$ in the presence of 20 μL methanol and 300 mg mL^{-1} NaCl with the assistance of vortex; IL phase enriched with the target analytes of the first step was extracted by 10% HAc aqueous solution.

3. Results and discussion

3.1. HPLC separation of three PDE-5 inhibitors

The HPLC separation of SD, VD and AD was optimized as described in Section 2.2. The three analytes were well separated, as shown in Fig. 4A, with symmetrical peaks. As demonstrated in the following experiment, as shown in Fig. 4B, there was no