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A new approach for microextraction of non-steroidal anti-inflammatory drugs from human urine samples based on in-situ deep eutectic mixture formation

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ABSTRACT

A new strategy for the simple, environmental-friendly and highly-available microextraction of non-steroidal anti-inflammatory drugs (NSAIDs) from human urine samples has been proposed. The procedure assumes NSAIDs separation from aqueous sample phase via in-situ forming deep eutectic mixtures based on the formation of hydrogen bond between —OH group of menthol and the oxygen atom of —COOH group of NSAIDs. The developed strategy was used for the HPLC-UV determination of ketoprofen and diclofenac in human urine samples. Under optimal experimental conditions limits of detection, calculated from a blank test, based on 3σ were found to be 15 μg L^{-1} and 44 μg L^{-1} for ketoprofen and diclofenac, respectively. The microextraction process can reach equilibrium within 1 min with extraction recovery values from 93 to 97% for both analytes.

1. Introdaction

Liquid-liquid extraction (LLE) is one of the widely-used separation and preconcentration methods in analytical chemistry. Usually LLE is applied in analyses of complex samples to eliminate the negative effect of matrix components and improve sensitivity.

Despite advantages of conventional LLE such as simplicity and availability this method has some limitations in terms of time-consumption and frequent requirement of large amounts of hazardous organic solvents. To improve conventional LLE, various liquid-liquid microextraction (LLME) methods have been proposed. The principles, advantages and limitations of LLME methods are presented in numerous reviews [1,2].

Recently a new class of extractants for the LLME – deep eutectic solvents (DESs) has been proposed [3] and used in analytical chemistry [4]. DESs are generally composed of two or three cheap and safe components that are capable of self-association, often through hydrogen bond interactions, to form a eutectic mixture with a melting point lower than that of each individual component [5]. DESs have been studied for the extraction of various substances from organic [6] and aqueous [7] liquids as well from solid samples [8]. Usually, the LLME process based on DES assumes preliminary DES synthesis followed by DES mixing with sample resulting target analytes extraction. Recently, new approach for extraction of phenolic compounds from organic phase based on in-situ deep eutectic mixtures (DEM) formation has been proposed [9]. In this case, halogen-free zwitterions including quaternary ammonium cations (1-(propyl-3-sulfonate)-triethylaminium, etc.) and SO_3^- anion were used for phenols separation from oils via forming deep eutectic solvents based on the formation of hydrogen bond between —OH group on phenol and the oxygen atom on —SO_3^- group of the zwitterions.

The LLME of target analytes from aquoes sample phase based on in-situ DEM formation has not been presented in the literature. Recently, menthol has been reported as a green extractant for the LLME from aquoes phase [10,11]. Moreover, menthol has grown increasingly popular as a cheap and green component used for hydrophobic DES synthesis [12].

In [13] was shown, that menthol can form with non-steroidal anti-inflammatory drugs (NSAIDs) the so-called "therapeutic deep eutectic preparations" by mixing menthol and NSAIDs followed by heating the solid mixtures. In our study, phenomenon of in-situ DEM formation by mixing molten menthol and aquoes NSAIDs solution was established for the first time. As a result, a new approach for the simple, environmental-friendly and highly-available microextraction of NSAIDs from human urine samples has been proposed. The microextraction procedure assumes NSAIDs separation from aquoes sample phase via in-situ forming deep eutectic mixtures based on the formation of

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hydrogen bonding between —OH group of menthol and the oxygen atom on —COOH group of NSAIDs. The developed procedure was used for the HPLC-UV determination of ketoprofen and diclofenac in human urine samples without additional sample pretreatment. The performance of the suggested approach was demonstrated by the determination of ketoprofen and diclofenac (ESM. Fig. 1) in human urine samples using high-performance liquid chromatography with UV-VIS detection (HPLC-UV). NSAIDs are one of the most commonly used pharmaceuticals for human treatment. Their capability in reducing pain and preventing inflammation has resulted in their widespread use. However, their overdose can cause side-effects such as gastrointestinal bleeding, intestinal ulceration, renal failure and aplastic anemia [14]. Thus, an important task of personalized medicine is the NSAIDs determination in biological fluids for adjusting of dosages and regimens of drugs for treatment.

2. Experimental

2.1. Reagents and solutions

Menthol, hydrochloric acid, sodium hydroxide, sodium acetate, carbon tetrachloride, formic acid and acetic acid were purchased from Vecton (Russia). Ketoprofen and diclofenac sodium salts and methanol were purchased from Sigma-Aldrich (Germany). Stock solution of 1 g L^{-1} of each NSAIDs was prepared by dissolving an appropriate amount of substances in 0.01 mol L^{-1} NaOH solution and stored in the dark at +4 °C. Working solutions were prepared immediately before the experiments by dilution of the stock solution with ultra pure water. Ultra pure water from Millipore Milli-Q RG (Millipore, California, USA) was used for all experiments. All chemicals were of analytical reagent grade.

2.2. Samples

Urine samples were collected in 2 h after taking the drugs containing ketoprofen or diclofenac. Approval to conduct this study was granted by the ethics committee of Institute of Medicine, St. Petersburg State University, Russia. Before analysis, the urine sample (8 mL) was mixed with 1.5 mL of 1 mol L^{-1} NaOH and kept for 30 min at ambient conditions for the hydrolysis of acyl glucuronic acid conjugates [15]. Then, 1.5 mL of 1 mol L^{-1} HCl was added and sample was stored in a refrigerator.

2.3. Apparatus

A HPLC-UV system LC-20 Prominence liquid chromatograph (Shimadzu, Japan) was used for the NSAIDs determination. The pH values of the solutions were measured using an ORION 720A^{+} pH meter (Thermo Scientific, MA, USA) with a glass electrode. A synchronous thermal analysis device Netzsch STA 449 F3 Jupiter (Netzsch, Germany) was used for the DEMs study by the differential scanning calorimetry (DSC). Fourier transform-infrared IR Prestige-21 spectrometer (Shimadzu, Japan) was used to characterize DES, using KBr pellet technique.

2.4. Extraction procedure

The extraction procedure involved several steps (Fig. 1). At the first step 2 mL of acetate buffer solution (pH = 3.8) and 50 mg of solid menthol were added to the urine sample and heated at menthol melting point (40 °C) for 1 min. The obtained mixture of sample and liquid menthol was mixed and as result the formation of DEMs between the analytes and menthol directly into aqueous sample phase was observed. In this step, DEMs obtained were dissolved in menthol phase. In the second step, the obtained mixture of sample and liquid menthol phase containing DESs was placed in a refrigerator (5 °C) to solidify extract for 5 min. After solidification of menthol phase the aqueous sample phase was aspirated into a syringe and wasted. For the HPLC-UV determination of NSAIDs the solid extract was dissolved in 100 μL of methanol. 50 μL of obtained solution was introduced into a HPLC-UV system.

2.5. Chromatographic conditions

HPLC-UV analysis was performed in isocratic mode, using a Luna C18 column (150 × 4.6 mm, 5 μm particles size) with mobile phase consisting of methanol and 0.1% aqueous solution of formic acid at a ratio of 3:1 (v/v) at a mobile phase flow rate of 1 mL min^{-1}. The chromatograph was operated at 45 °C with detection at 280 nm for diclofenac and 257 nm for ketoprofen.

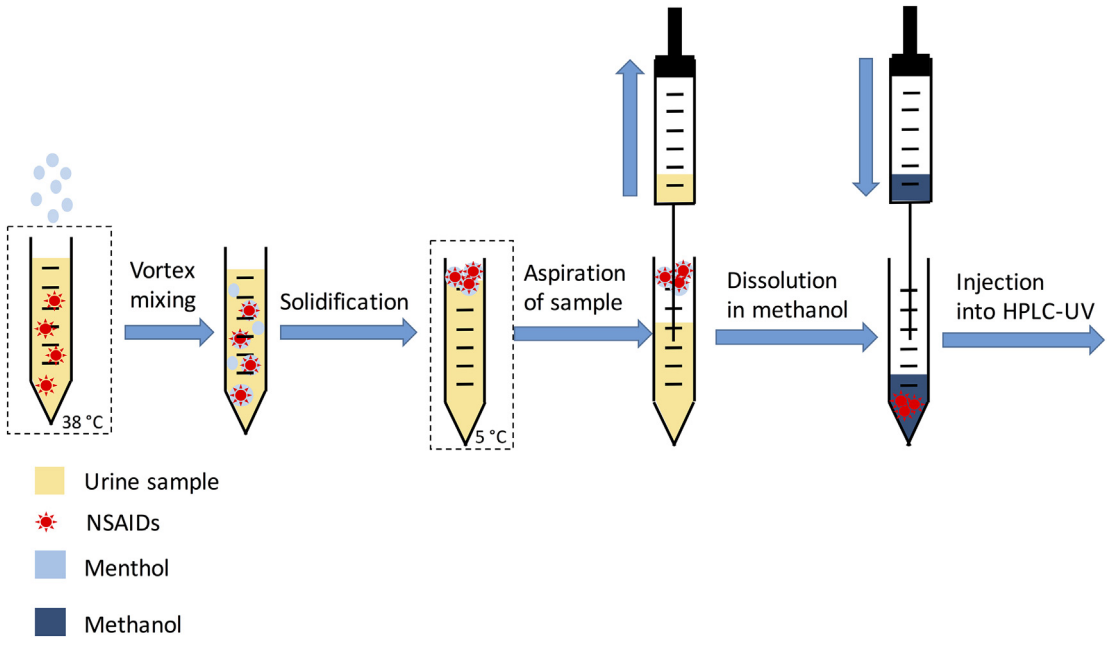

Fig. 1. The diagram of the proposed procedure.