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Ultrasound-assisted emulsification microextraction combined with ultra-high performance liquid chromatography–tandem mass spectrometry for the analysis of ibuprofen and its metabolites in human urine

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ABSTRACT

In this study, a fast, simple and efficient method based on ultrasound-assisted emulsification-microextraction (USAEME) coupled with ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) was successfully developed for the determination of ibuprofen (IBU) and its four metabolites (1-hydroxyibuprofen (1-HIBU), 2-hydroxyibuprofen (2-HIBU), 3-hydroxyibuprofen (3-HIBU), carboxyibuprofen (CIBU)) in human urine. For this purpose, the influence of the different parameters affecting the USAEME procedure was evaluated in order to optimize the efficiency of the process. The optimum conditions were found to be: 100 μL of 1-octanol as extraction solvent, 2 mL of urine sample, 15% (w/v) NaCl to control the ionic strength, ultrasonication for 10 min; and centrifugation for 5 min at 6500 rpm. After sample preparation, chromatographic separation was achieved on a Zorbax Rapid Resolution High Definition (RRHD) SB-C18 column using the mobile phase consisting of 0.1% formic acid in water and acetonitrile in an elution gradient. Detection was performed in a triple quadrupole tandem mass spectrometer using the multiple reaction monitoring (MRM) mode and negative ionization.

The proposed method showed satisfactory linearity over a wide concentration range (correlation coefficients over 0.9994). The lower limit of quantification (LLOQ) was 0.0005 ng/mL for IBU and its metabolites. The intra- and inter-day precisions were in the range of 2.19–10.8% and the accuracies were between −5.93% and 6.29%. The mean recovery of analytes ranged from 90.7 to 104%. As a result, this method has been successfully applied for the sensitive determination of IBU and its metabolites in human urine samples.

1. Introduction

Ibuprofen (IBU) is a non-steroidal anti-inflammatory drug (NSAID) that has been widely used in the treatment of pain and inflammation in rheumatic disease and other musculoskeletal disorders [1]. Pharmacokinetic studies have shown that about 66% of the drug is excreted in the urine whereas about 34% is excreted in the feces (biliary excretion). Recovery studies revealed that 60% of the given dose was excreted within the first 24 h [2]. Oxidative metabolism is the major route for biotransformation of IBU and four oxidative metabolites (1-hydroxyibuprofen (1-HIBU), 2-hydroxyibuprofen (2-HIBU), 3-hydroxy-ibuprofen (3-HIBU), carboxyibuprofen (CIBU)) have been identified in urine and plasma samples obtained from humans after the oral intake of ibuprofen. In humans, the parent drug, as well as the metabolites, is found to be conjugated with glucuronic acid [3]. The two major metabolites, 2-HIBU and CIBU, and their glucuronic acid conjugates were found to account for approximately 58% of the given dose of ibuprofen, whereas the two minor metabolites, 1-HIBU and 3-HIBU, and their glucuronic acid conjugates were found to be present in the urine in only very small concentrations [4].

For the diagnosis or, more importantly, the differential diagnostic exclusion of cases of acute overdose or chronic abuse, an analytical procedure is necessary for the determination of the drug and its metabolites in biological fluids. The chromatographic methods that are currently available to measure IBU include gas chromatography (GC) [5,6], capillary electrophoresis (CE) [7–10], high-performance thin-layer chromatography (HPTLC)

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[11] and high-performance liquid chromatography (HPLC) [12–26]. IBU was isolated from biological fluids by deproteination [10,23], liquid–liquid extraction [8,11,14,21,25,26], solid-phase extraction (SPE) [8,12,18,22] solid-phase microextraction (SPME) [13,17], hollow fiber-based liquid phase microextraction (HF-LPME) [15,16] and hollow-fiber liquid membrane-protected solid-phase microextraction (HFLM-SPME) [6]. Three papers have described methods for the simultaneous determination of IBU and its metabolites (only selected two metabolites) [12,14,17].

Recently, much attention is being paid to the development of miniaturized, more efficient and environmentally friendly extraction techniques which could greatly reduce the consumption of organic solvents. The ultrasound-assisted emulsification-microextraction method (USAEME) is an effective technique among these microextraction methods. This approach is based on the emulsification of a microvolume of organic extractant in an aqueous sample by ultrasound radiation and further separation of both liquid phases by centrifugation. The application of ultrasonic radiation accelerates the mass-transfer process between two immiscible phases, which, together with the large surface of contact between both phases, leads to an increment in the extraction efficiency in a minimal amount of time. In this way, USAEME can be employed as a simple, fast and efficient extraction and preconcentration procedure for organic compounds in aqueous samples [27].

The aim of the present work is to investigate and develop a rapid and efficient USAEME method coupled with ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) for the analysis of IBU and its metabolites in human urine samples. It is to be noted that this is the first report on the simultaneous determination of IBU and its four metabolites in human urine using USAEME, thus paving the way as a good alternative for routine analysis with the advantages of simplicity, reliability, cost effectiveness and minimized matrix interferences. The effect of various experimental conditions on the extraction of analyzed compounds is investigated and discussed. The optimized procedure was successfully applied to determination of the target analyte in human urine samples.

2. Experimental

2.1. Chemicals and reagents

Ibuprofen (IBU; purity >98.1%), 1-hydroxyibuprofen (1-HIBU; purity >98.5%), 2-hydroxyibuprofen (2-HIBU; purity >98.5%), 3-hydroxyibuporfen (3-HIBU; purity >98.5%), carboxyibuprofen (CIBU; purity >98.5%) and naproxen (NAP, used as an internal standard (IS); purity >98.3%) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC–MS grade), methanol (HPLC–MS grade) and water (HPLC grade) were purchased from Merck (Darmstadt, Germany). Formic acid, 1-decanol, 1-octanol, n-decane, n-undecane and n-hexadecane were obtained from Sigma–Aldrich (St. Louis, MO, USA). Sodium hydroxide (NaOH) was purchased from Stanlab (Lublin, Poland). Hydrochloric acid (HCl) was obtained from Chempur (Piekary Slaskie, Poland). Sodium chloride (NaCl) was purchased from Merck (Darmstadt, Germany).

2.2. Preparation of standard solutions, calibration standards and quality control (QC) solutions

The standard stock solutions of IBU and its metabolites from independent weighting were prepared in methanol at a concentration of 1.0 mg/mL. Working standard solutions of IBU and its metabolites ranging from 0.5 ng/mL to 5000 ng/mL were prepared by diluting the stock solutions with methanol. Finally, urine calibration standards (CS) were made at concentrations in the range of 0.0005–250 ng/mL for IBU, 1-HIBU, 2-HIBU, 3-HIBU and CIBU by spiking working standard solution to free-drug urine samples (blank sample). Also, quality control (QC) samples were prepared at four concentrations: lower limit of quantitation (LLOQ): 0.0005 ng/mL for IBU and its metabolites, low concentration quality control (LQC): 0.005 ng/mL for IBU and its metabolites, middle concentration quality control (MQC): 75 ng/mL for IBU and its metabolites, and high concentration quality control (HQC): 200 ng/mL for IBU and its metabolites.

Similarly, an IS stock solution (20 μg/mL) was also prepared in methanol and diluted with methanol to give an IS working solution (200 ng/mL). All solutions were stored in a refrigerator (4–8 °C) until used.

2.3. Sample preparation

Drug-free human urine samples used for the preparation of calibration and validation standards were collected from six different healthy subjects who were drug free. Additionally, urine samples were collected from ten patients being treated with IBU. All urine samples were stored in a freezer at −20 °C.

Human urine samples were prepared using the new USAEME method. The USAEME procedure was performed using five different extraction solvents: 1-decanol, 1-octanol, n-decane, n-undecane and n-hexadecane. In the optimization step, 1-octanol was selected, as the best solvent to extract the target analytes and this was used for all experiments.

Before the USAEME extraction, the hydrolysis reactions were performed according to Tan et al. [20] during 30 min. Urine samples (2 mL) containing IS (20.0 ng/mL) were alkalinized with 400 μL of 1 M NaOH for the hydrolysis of acyl glucuronic acid conjugates. The hydrolysis reaction was left to proceed for 30 min at room temperature and the hydrolyzed urine samples were then neutralized with 400 μL of 1 M HCl; the extraction was carried out as described in the following section.

After hydrolysis, urine samples were placed in glass centrifuge tubes and the ionic strength and pH of the solutions were adjusted to an appropriate level (sodium chloride, 15% (w/v); pH 2.0). Then, 100 μL of 1-octanol (extraction solvent) was quickly injected into the sample solution, and the tube was shaken manually for 10 s and so that small droplets of the extraction solvent formed an emulsion with the sample solution. The tube was immersed in an ultrasonic water bath, sonicated for 10 min and shaken manually for 10 s. The formation of tiny droplets greatly enlarges the contact area between the extraction solvent and aqueous phase, which enhances the extraction efficiency. Then, the emulsion was centrifuged at 6500 rpm (without 4912.5 × *g*) for 5 min in order to disrupt the emulsions and separate both phases (the organic phase remained at the bottom of the tube). After centrifugation, 50 μL of the extraction solvent floating on the surface was collected with a Hamilton microsyringe, from which 10 μL was dissolved in 90 μL of methanol. Finally, 5 μL of the obtained mixture was injected into the UHPLC–MS/MS system for subsequent analysis. Fig. 1 shows a scheme of the USAEME procedure.

2.4. Instrumentation and LC–MS/MS analytical conditions

Liquid chromatography was performed on a Dionex UPLC system (Dionex Corporation, Sunnyvale, CA, USA) consisting of an UltiMate 3000 RS (Rapid Separation) pump with on-line vacuum degasser, an UltiMate 3000 autosampler, an UltiMate 3000 column compartment with a thermostable column area and an UltiMate 3000 variable wavelength detector, all of which were operated using Dionex Chromeleon™ 6.8 software.

The chromatographic separation was performed on a Zorbax Rapid Resolution High Definition (RRHD) SB-C18 column