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Comparison of ultrasound-enhanced air-assisted liquid–liquid microextraction and low-density solvent-based dispersive liquid–liquid microextraction methods for determination of nonsteroidal anti-inflammatory drugs in human urine samples

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ABSTRACT

Two dispersive-based liquid–liquid microextraction methods including ultrasound-enhanced air-assisted liquid–liquid microextraction (USE-AALLME) and low-density solvent-based dispersive liquid–liquid microextraction (LDS-DLLME) were compared for the extraction of salicylic acid (the hydrolysis product of acetylsalicylic acid), diclofenac and ibuprofen, as instances of the most commonly used nonsteroidal anti-inflammatory drugs (NSAIDs), in human urine prior to their determination by gas chromatography with flame ionization detection (GC-FID). The influence of different parameters affecting the USE-AALLME (including type and volume of the extraction solvent, sample pH, ionic strength, and simultaneous sonication and number of extraction cycles) and the LDS-DLLME (including type and volume of the extraction and disperser solvents, sample pH, and ionic strength) were investigated to optimize their extraction efficiencies. Both methods are fast, simple and convenient with organic solvent consumption at μL level. However, the best results were obtained using the USE-AALLME method, applying 30 μL of 1-octanol as extraction solvent, 5.0 mL of sample at pH 3.0, without salt addition, and 5 extraction cycles during 20 s of sonication. This method was validated based on linearities ($r^2 > 0.971$), limits of detection (0.1–1.0 μg L^{-1}), linear dynamic ranges (0.4–1000.0 μg L^{-1}), enrichment factors (115 ± 3–135 ± 3), consumptive indices (0.043–0.037), inter- and intra-day precisions (4.3–4.8 and 5.6–6.1, respectively), and relative recoveries (94–103%). The USE-AALLME in combination with GC-FID, and with no need to derivatization step, was demonstrated to be a simple, inexpensive, sensitive and efficient method to determine NSAIDs in human urine samples.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) form a group of analgesic, antipyretic and anti-inflammatory agents that are frequently used in both humans and animals since they do not induce sedation, respiratory depression or addiction [1]. Because of their effectiveness in suppressing or preventing inflammation, NSAIDs are becoming the most commonly used medicines around the world. The pharmacological actions of NSAIDs are related to the inhibition of cyclooxygenase (COX), a key enzyme of prostaglandin biosynthesis, at the site of inflammation. Although NSAIDs are perceived to be safe drugs, they may lead to severe toxic effects in cases of acute over-dosage or chronic abuse. Therefore, they have been detected in clinical and forensic toxicological analyses [2]. For the diagnosis or, more importantly, the differential diagnostic exclusion of cases of acute over-dosage or chronic abuse, a fast and sensitive analytical procedure is necessary for the detection of these drugs in bio-fluid samples [3]. Determination of drugs in urine is useful to monitor drugs concentration and provide basic information about their bioavailability.

When the analytes of interest are present in a complex matrix, such as samples originating in the environment and human body, sample preparation is a crucial step in the analysis. In the last years, there has been an increasing interest in developing new sample pretreatment approaches to determine all type of analytes in several matrices; this is of special importance in the analysis

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of biological samples. Liquid–liquid extraction (LLE) is a classical and common technique used for the preconcentration and clean-up prior to chromatographic or electrophoretic analysis that requires large organic solvent consumption. It is also tedious and analyte-loss is frequent, due to multi-stage operations that cannot be neglected. However, extraction of pharmaceuticals from aqueous samples has usually been performed by off-line solid-phase extraction (SPE) [4,5]. Although SPE uses much less solvent than LLE, the sorbent needs pretreatment and can be relatively expensive.

Recently, much attention is being paid to the development of miniaturized, more efficient and environmentally friendly extraction methods which could greatly reduce the consumption of organic solvents. An interesting alternative for LLE and SPE methods is liquid-phase microextraction (LPME), because of its simplicity, effectiveness, low cost, minimum use of solvents and satisfactory sample clean-up ability. So far, different configurations of LPME have been developed which could be classified in three main categories including single-drop based LPME (SD-LPME) [6], hollow fiber-based LPME (HF-LPME) [7] and dispersive-based LPME (D-LPME) methods [8–10].

In 2006, Rezaee et al. introduced a new D-LPME method, termed as *normal dispersive liquid–liquid microextraction* (N-DLLME) [11]. It is generally based on a ternary component solvent system, in which extraction and disperser solvents are rapidly introduced into the aqueous sample to form a cloudy solution. Extraction equilibrium is quickly achieved, due to the extensive surface contact between the droplets of the extraction solvent and the sample. After centrifugation, extraction solvent is normally sedimented at the bottom of the tube (if the density is above that of water) and taken with a microsyringe for its later chromatographic analysis. The advantages of N-DLLME method are simplicity of operation, rapidity, low cost, high-recovery, high enrichment factor and decreasing waste generation [12–14]. The main disadvantage of the N-DLLME method is the use of chlorinated solvents as extraction solvent which are potentially toxic to humans and the environment. In addition, because the extraction solvent is incompatible with liquid chromatography (LC), the extract cannot be injected directly to LC system for analysis. Therefore, evaporation of the organic extraction solvent to dryness and reconstitution of analytes in a suitable solvent prior to LC is required. This is an effective but laborious approach and prone to loss of analytes during evaporation. Furthermore, in the determination of some important compounds using N-DLLME/GC with electron capture detector, chlorinated extraction solvents have a very high solvent peak which can interfere with some analytes peaks. In this way, low-density solvent-based dispersive liquid–liquid microextraction (LDS-DLLME) mode was developed using less toxic non-chlorinated solvents to replace chlorinated extraction solvents. However, the necessity of using a disperser solvent in both N-DLLME and LDS-DLLME methods – which can decrease the partition coefficients of analytes into the extraction solvent, and increase the cost and environmental pollution – is another drawback of this method [15].

Air-assisted liquid–liquid microextraction (*AALLME*) is one of the most recently used disperser solvent-free LPME methods, which has been reported by Farajzadeh in 2012 [16]. In this method, a few microliters of the extraction solvent (denser or lighter than water) is transferred into the aqueous sample solution in a conical centrifuge tube, and the mixture is then repeatedly withdrawn into a glass syringe and pushed out into the tube. By this action, fine organic droplets are formed, and the extraction solvent is entirely dispersed in the sample solution. After centrifugation of the cloudy solution formed, the extractant is settled down at the bottom of the centrifuge tube and used for further analysis [17–20]. Simultaneous application of ultrasound irradiations and common *AALLME* can lead to the rapid formation of sub-micron droplet size of the extractant in the aqueous solution, and the contact surface between both immiscible liquids is significantly enlarged. Smaller fine droplets of the extractant and enlarged interfaces lead to a significant increase in the analyte mass transfer into the extractant. Consequently, high extraction efficiency is achieved in a short period of time. In this way, ultrasound-enhanced AALLME (USE-AALLME) can be employed as a simple, fast and efficient extraction and preconcentration method for organic compounds in aqueous samples.

The aim of the present work was to investigate the ability and convenience of USE-AALLME method coupled to gas chromatography-flame ionization detection (GC-FID), for the simple and efficient determination of three widely used NSAIDs (salicylic acid (2-hydroxy-benzoic acid), *the hydrolysis product of the well-known acetylsalicylic acid (2-(acetyloxy)-benzoic acid)*, diclofenac (*2-[(2,6-dichlorophenyl)amino]-benzeneacetic acid*) and ibuprofen (*(R,S)-2-(4-isobutylphenyl)-propionic acid*), as model analytes, in human urine samples. Furthermore, in order to demonstrate if disperser solvent decreases the extraction efficiency or not, a LDS-DLLME method was also examined. In this way, the effect of various experimental conditions on the extraction of analyzed compounds was investigated. Two methods were compared and the obtained results showed that USE-AALLME, which excludes any disperser solvent and uses lower volume of organic solvent, could be applied as a more efficient and environmental friendly method for the determination of corresponding analytes in human urine samples. Also, comparison of the methods proved that the disperser solvent could increase the solubility of the target analytes and/or extraction solvent in the aqueous sample and decrease the extraction efficiency.

2. Experimental

2.1. Apparatus

Separation and detection of the analytes were performed by a gas chromatograph (GC-17A, Shimadzu, Japan) equipped with a splitless/split injector and a flame ionization detector. Helium (purity 99.999%) was used as the carrier gas at the constant flow rate of 4 mL min^{-1}. The temperatures of injector and detector were set at 280 and 290 °C, respectively. The injection port was operated at splitless mode and with sampling time 1 min. For FID, hydrogen gas was generated with a hydrogen generator (OPGU-2200S, Shimadzu, Japan). A 30 m BP-20 SGE fused-silica capillary column (0.32 mm i.d. and 0.25 μm film thickness) was applied for separation of target analytes. Oven temperature program was: started from 100 °C, held for 0 min, increased to 230 °C at 30 °C min^{-1}, held for 10 min, increased to 260 °C at 30 °C min^{-1} and then held for 7 min. The Hettich centrifuge, model EBA20 (Tuttlingen, Germany) was used for accelerating phase separation and a 10.0 μL ITO (Fuji, Japan) microsyringe applied for the collection of sedimented organic solvent and injection into the GC.

2.2. Chemicals and reagents

Standards of ibuprofen (IBP; purity >98%), sodium diclofenac (DIC; purity >98%), salicylic acid (SCA; purity >98%), and benzoic acid (as internal standard) were purchased from Sigma–Aldrich (St. Louis, MO, USA, www.sigmaaldrich.com). 1-octanol, 1-hexanol, toluene, *n*-heptane, *n*-hexane, acetone, methanol, sodium chloride, and ultra-pure water were all from Merck (Darmstadt, Germany, www.merck.de). Sodium hydroxide and concentrated hydrochloric acid, were bought from Merck used to adjust the pH of the samples. Other reagents were of analytical grade and obtained from Merck.

Stock standard solutions of each analyte were prepared separately by dissolving proper amounts of each drug in methanol at