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Low-toxic air-agitated liquid-liquid microextraction using a solidifiable organic solvent followed by gas chromatography for analysis of amitriptyline and imipramine in human plasma and wastewater samples

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ABSTRACT

A rapid and low-toxic microextraction method, namely air-agitated liquid-liquid microextraction (AALLME), was applied for the extraction of amitriptyline and imipramine from the human plasma and wastewater samples by solidification of the floating organic solvent droplets (SFO). Using very simple tools, the analytes contained in 10.0 mL of an aqueous sample solution were simply extracted into the solidifiable organic solvent 1-dodecanol. For this purpose, in the absence of an organic disperser solvent, a mixture of the aqueous sample solution and the extraction solvent was repeatedly aspirated and dispensed using a syringe, and by enlarging the surface area between the donor and acceptor phases, a fast and efficient extraction was achieved. The response surface methodology (RSM) was used to optimize the experimental parameters involved. 14.0 μL of the organic solvent used, a pH value of 12.0, 7.52% (w/v) salt addition, and 13 air-agitation cycles for the extraction number were found to be the optimal extraction conditions. Under the optimized experimental conditions, AALLME-SFO-GC-FID provided a good linearity in the range of 15–2000 ng mL^{-1}, low limits of detection (5.0–7.0 ng mL^{-1}), good extraction repeatabilities (relative standard deviations below 8.4%, n = 5), and enrichment factors (EFs) of 682–731. In order to verify the performance of the method, the extraction efficiencies of the method based on the solidifiable organic solvent and the conventional AALLME method based on halogenated solvents were compared.

1. Introduction

Nowadays, certain pharmaceuticals are attracting attention as a potentially-new class of water pollutants. These compounds that are synthetically-manufactured produce highly-toxic chemicals that not only affect the health of human beings but also potentially compromise the health of aquatic organisms [1,2]. Tricyclic antidepressants (TCAs) are one of the groups of these drugs that are used as the reference for the treatment of psychiatric disorders, mainly, major depressions [3]. The measurement of these compounds in water, especially, wastewater of pharmaceutical factories, can be important for a variety of purposes such as prevention of the entrance of these drugs into the environment [4]. Imipramine and amitriptyline are two most common TCAs. The therapeutic concentration range for most TCAs is approximately 100–300 ng mL^{-1}, while toxic effects can occur when the plasma concentration exceeds 500 ng mL^{-1}. As shown in Table 1, the therapeutic indices of these drugs were in the ranges of 125–250 and 80–200 ng mL^{-1} for imipramine and amitriptyline, respectively [5,6]. Hence, a simple, rapid, and robust analytical methodology is required to determine the concentration of these drugs in the human fluids for the diagnosis and effective treatment of intoxication. Despite the considerable advances in the analytical approaches and instruments, sample pre-treatment is required prior to the analysis of most samples. Liquid-liquid extraction (LLE) and solid-phase extraction (SPE) are the two most common sample preparation methods. However, these methods are time-consuming, and require moderate-to-large amounts of highly-expensive and potentially-hazardous organic solvents. Hence, these methods are overshadowed by the efficient, miniaturized, and environmentally-friendly extraction methods such as solid-phase microextraction (SPME) [7] and liquid-phase microextraction (LPME) [8]. SPME is a solvent-free extraction technique, which unifies extraction and preconcentration in a single step. However, the SPME fiber used is expensive and fragile, and it has a limited lifetime; sample carryover can also be a great problem in this method [9]. LPME has a simple experimental setup and a short preparation time. It is also environmentally-friendly due to a low consumption of organic solvents. Hollow-fiber-protected two-phase liquid-liquid microextraction (HF-LLME), hollow-fiber-protected three-phase liquid-liquid-liquid microextraction (HF-LLLME) [10–15], single-drop microextraction (SDME) [8], and dispersive liquid-liquid microextraction (DLLME) are some of the most popular LPME methods [16]. DLLME, which is a rapid, simple, and low-cost method with a high enrichment factor, has gained

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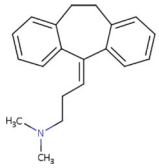
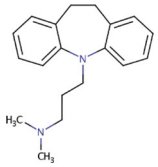
Table 1
Characteristics of TCA drugs.

Analytes	Molecular structure	Molar mass (g mol^{-1})	pK_a	Therapeutic concentration range (ng mL^{-1})
Amitriptyline		277.41	9.40	80–200
Imipramine		280.41	9.50	125–250

immediate and considerable attention by the researchers [17–20]. However, it is based upon a ternary component solvent system, in which utilization of the co-solvents (disperser solvents) leads to some disadvantages such as decrease in the partition coefficients for extraction of the analytes into the extracting solvent and increase in the cost and environmental pollution [21–24]. Since the invention of this method, continually-growing studies have been focused on it due to its remarkable virtues, and some disperser-free methods such as the ultrasound-assisted emulsification microextraction (USAEME) [25] and air-agitation liquid-liquid microextraction (AALLME) have been introduced [26]. AALLME requires very simple equipment that can be easily found in laboratories. However, the conventional AALLME suffers from the consumption of very toxic and even carcinogenic solvents such as the halogenated solvents [27]. Hence, solvents with fewer toxic elements as well as lower density ones such as long-chain alcohols and hydrocarbons have been considered. However, the main disadvantage of this approach is the difficulty to collect the small microdrops floating on the sample solution. DLLME based on solidification of floating organic droplets (DLLME-SFO) is an interesting idea that provides the utilization of lower-density solvents with a proper melting point, which would solidify at a low temperature and would, therefore, be easy to collect [28]. However using the disperser solvent is one of the disadvantages of this approach.

In this work, a disperser-free, very simple, and low-toxic microextraction method, namely air-agitated liquid-liquid microextraction using a solidifiable organic solvent (AALLME-SFO) was applied for the extraction of amitriptyline and imipramine from the human plasma and wastewater samples [29]. This method was introduced by X. You and coworkers, and using very simple tools, the analytes contained in an aqueous sample solution were simply extracted into an acceptor phase and analyzed by GC-FID. In order to verify the performance of the method, the extraction efficiencies of the method based on the solidifiable organic solvent and the conventional AALLME method were compared. Finally, the method was successfully used for the determination of the drugs under study in the wastewater and human plasma samples.

2. Experimental

2.1. Chemicals and materials

Amitriptyline (≥98.0%) and imipramine (≥99.0%) were obtained from Razi Drug Company (Tehran, Iran). Each analyte was dissolved in HPLC-grade methanol (Ameretat Shimi, Tehran, Iran) to obtain a stock solution (1 mg mL^{-1}). All the standard solutions were stored at 4 °C and re-prepared every 3 weeks. HPLC-grade water and acetonitrile were obtained from Ameretat Shimi (Tehran, Iran). H_3PO_4 (85%), NaCl (≥99.0%), HCl (37%), and NaOH (≥99.0%) were purchased from Merck (Darmstadt, Germany). Ammonia solution (25%) was purchased from Merck-Schuchardt (Munich, Germany). 1,2-dichloroethane (99.8%) and chloroform (≥99.0%) and carbon tetrachloride (≥99.9%) were obtained from Aldrich (Milwaukee, WI). 1-dodecanol (≥98.0%), 1-undecanol (≥97.5%), and n-tetradecane (≥99.0%) were all prepared from Merck (Darmstadt, Germany). All the other chemicals used were of reagent-grade or of the highest purity available.

2.2. Preparation of samples

The wastewater samples were collected from a pharmaceutical factory, and centrifuged to sediment their constituent solid compounds. The sample solution pH was adjusted at 12. The drug-free human plasma samples were obtained from Blood Transfusion Organization (Semnan, Iran). These samples were stored at −20 °C, thawed, and shaken before extraction. The spiked plasma samples were prepared as follows: 0.5 mL of the plasma was spiked by mixed standard solutions, and in order to decrease the protein bonding and protein precipitation, 2.0 mL of acetonitrile were added, followed by hand-shaking. The mixed solution was centrifuged for 7.0 min at 1398 × *g*. Then the supernatant solution was transferred into another vial and diluted to 10 mL using HPLC-grade water, and sample solution pH was adjusted at 12.

2.3. Apparatus

A Hettich centrifuge, model EBA20 (Tuttlingen, Germany) was used to accelerate phase separation, and sonication was carried out using a 50/60 KHz ultrasonic water bath (SW3, Switzerland). Separation and detection of TCAs were performed by a gas chromatograph (GC-17 A, Shimadzu, Japan) equipped with a splitless/split injector and a flame ionization detector. Helium (of 99.999% purity) was used as the carrier gas at a constant flow rate of 4 mL min^{-1}. The temperatures of the injector and detector were set at 280 °C. The injection port was operated at the splitless mode. For FID, hydrogen gas was generated by a hydrogen generator (OPGU-2200S, Shimadzu, Japan). A 30 m BP-5 SGE fused-silica capillary column (0.32 mm i.d. and 0.25 μm film thickness) was applied for the separation of TCAs. The oven temperature program was 100 °C for 3 min, which was increased to 280 °C at 20 °C min^{-1} and held for 2 min. A 10.0-μL ITO (Fuji, Japan) micro-syringe was applied to collect the acceptor phase and injection into the GC.

2.4. Procedure

At first, 10.0 mL of the sample solution (pH 12.0) was put into a 15.0-mL screw cap glass test tube with conic bottom. Then the extracting solvent, 1-dodecanol (14.0 μL), was added, and the mixture was repeatedly sucked from the tube and dispensed into it using a 10-mL glass syringe (the temperature of the sample solution was kept at ~50 °C). In both the sucking and dispensing steps, the solution became more and more turbid. After performing a pre-determined number of suction-dispersion cycles (thirteen times) and centrifugation for 4 min at 1132 × *g*, the mixture was cooled in ice bath for 2 min. The solidified 1-dodecanol was transferred into a conical vial, and 2 μL of it were injected into a gas chromatograph for analysis.

2.5. Calculation of enrichment factor and extraction recovery

The enrichment factor (EF) for the target analytes was calculated according to the following equation:

$$EF = \frac{C_{a,final}}{C_{s,initial}} \quad (1)$$

where $C_{a,final}$ is the final concentration of the analyte in the acceptor solution, and $C_{s,initial}$ is the initial analyte concentration in the sample (donor) solution.