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Optimization of ultrasound assisted dispersive liquid-liquid microextraction of six antidepressants in human plasma using experimental design

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ABSTRACT

A simple Ultrasounds Assisted-Dispersive Liquid Liquid Microextraction (UA-DLLME) method is presented for the simultaneous determination of six second-generation antidepressants in plasma by Ultra Performance Liquid Chromatography with Photodiode Array Detector (UPLC-PDA). The main factors that potentially affect to DLLME were optimized by a screening design followed by a response surface design and desirability functions. The optimal conditions were 2.5 mL of acetonitrile as dispersant solvent, 0.2 mL of chloroform as extractant solvent, 3 min of ultrasounds stirring and extraction pH 9.8.Under optimized conditions, the UPLC-PDA method showed good separation of antidepressants in 2.5 min and good linearity in the range of 0.02–4 $\mu g\,mL^{-1}$, with determination coefficients higher than 0.998. The limits of detection were in the range 4–5 $ng\,mL^{-1}$. The method precision (n = 5) was evaluated showing relative standard deviations (RSD) lower than 8.1% for all compounds. The average recoveries ranged from 92.5% for fluoxetine to 110% for mirtazapine. The applicability of DLLME/UPLC-PDA was successfully tested in twenty nine plasma samples from antidepressant consumers. Real samples were analyzed by the proposed method and the results were successfully submitted to comparison with those obtained by a Liquid Liquid Extraction-Gas Chromatography – Mass Spectrometry (LLE-GC–MS) method. The results confirmed the presence of venlafaxine in most cases (19 cases), followed by sertraline (3 cases) and fluoxetine (3 cases) at concentrations below toxic levels.

1. Introduction

The use of antidepressants (ATDs) has increased significantly in most countries belong to the Organization for Economic Co-operation and Development (OECD) since 2000 and Spain is the seventh country in the European Union with the highest consumption [1]. The statistical studies have estimated that 20% of patients from primary care in spanish health centers suffer well-defined mental disorders and this population increases to 40% when minor mental disorders were included. The association between severe psychopathology and suicide is real because there is a previous depressive episode in 70% of suicides and 58% of suicide attempts. Tricyclic antidepressants (TCAs) were the first-line treatment choice for depression, but they are not usually recommended because they cause unpleasant side effects and can be more dangerous if an overdose is taken. Most second-generation ATD relieve depression by affecting neurotransmitters serotonin or norepinephrine such as selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs) or noradrenergic and specific serotoninergic antidepressant (NaSSA) [2].

The importance of this study is justified because the use of ATD in Spain has gone from 26.5 DHD (the defined daily doses (DDD) per thousand inhabitants per day) in the year 2000 to 79.5 DHD in 2013, representing an increase of 200%. The use of tricyclic antidepressants has decreased by 14.7% in the period from 2000 to 2013 in Spain. Meanwhile, SSRIs have shown a marked increase (159.3%). Venlafaxine (SNRIs) consumption has increased significantly (8 times) as the NaSSA, mirtazapine (4 times) in the same period [3,].

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The wide use of these drugs needs the development of simple, accurate, sensitive, applicable and no expensive methods for their determination in human samples. Moreover, toxicological analyzes are based on sample preparation and this is a critical stage in the analytical process, since it has a direct effect on the quality of the results. There are many studies that report data obtained after applying traditional procedures, as LLE or solid phase extraction (SPE), to the determination of antidepressants in different biological fluids [4,5]. Other proposed methods for extraction of these drugs were stir bar sorptive extraction (SBSE) [6] and dispersive micro-solid-phase extraction (DMSPE) [7]. A recent technique such as DLLME, developed by Rezaee et al. [8,9], allows to simplify operations and reduce consumption of organic solvents and sample. The DLLME uses a ternary component solvent system and has two stages: First, injection of smaller volumes of solvent extractant and a dispersant in an aqueous sample containing the analytes of interest. Second, formation of a cloudy solution and subsequently the mixture is centrifuged. The main advantage is that equilibrium is reached quickly due to the large surface area between the extractant and the aqueous sample. The analytes are found in the sedimented phase and can be determined chromatographically. DLLME efficiency depends on the dispersing solvent and extractant and their respective volumes, as well as the salt effect, pH, time and type of agitation The most commonly used organic solvents as extractants are chlorobenzene, chloroform, carbon tetrachloride and tetrachlorethylene, and they are well separated from the aqueous sample because they have a higher density than water [9]. This technique has been successfully applied in the analysis of benzodiazepines in plasma [10] and urine [11], drugs of abuse in plasma [12] and blood [13] and other tricyclic antidepressants in urine, plasma and water samples [14,15].

Most of the published methods for determination of antidepressants involve separation by chromatographic techniques. GC methods with different detectors require a derivatization step to increase the volatility of these compounds on account of their thermolability [15,16]. This step is not necessary in liquid chromatography, which is becoming the technique of choice for the analysis of these drugs using different detectors like UV, PDA and MS [17–19].

In this work, UA-DLLME followed by UPLC-PDA was used for the determination of four SSRIs, which include escitalopram, fluoxetine, fluvoxamine and sertraline; a SNRI, venlafaxine, and a NaSSA, mirtazapine, in plasma samples from antidepressant users. The objectives of this study were the optimization of the parameters affecting the extraction, such as pH, ultrasonic time, and extraction and dispersion solvents, by experimental designs (a screening design, followed by a response surface design and desirability functions). The validation and application of UA-DLLME combined with UPLC-PDA for the screening analysis of six ATDs, demonstrate the suitability of the proposed method to analyse the target compounds in plasma samples which is compared with results obtained by LLE-GC–MS method [16].

2. Materials and methods

2.1. Chemicals and reagents

Standards of antidepressants were obtained from Cerilliant® (Round Rock, TX, USA). Acetonitrile (ACN, HPLC grade), methanol (MeOH, HPLC grade), acetone, chloroform, carbon tetrachloride, ethyl tetrachloride, chlorobenceno, dichloromethane, dichloroethane, potassium hydroxide, phosphoric acid, sodium hydroxide and potassium dihydrogen phosphate were purchased from Merck® (Darmstadt, Germany). Purified water was obtained from a Milli-Q water system from Millipore® (Le Mont-sur-Lausane, Switzerland). Phosphate buffer (0.05 M) was prepared dissolving potassium dihydrogen phosphate (0.68 g) in one liter of Milli Q water (pH adjusted to 6.5) with potassium hydroxide or (pH adjusted to 3.0) with phosphoric acid. Individual stock solutions of each drug were prepared in MeOH at 1 mg mL^{-1}. Working solutions at 0.02, 0.04, 0.1, 0.2, 0.4, 1.0, 2.0 and 4.0 μg mL^{-1} in mobile phase or plasma were prepared by successive dilutions of the stock solutions. All solutions were stored at −20 °C.

2.2. Apparatus

The determination of antidepressants was performed using a Waters®Acquity UPLC H-Class connected to a Waters® Acquity Photodiode Array Detector. Five microliters of sample extracts were injected onto an Acquity UPLC®BEH Shield RP18 (100 mm × 2.1 mm ID, 1.7 μm particle size) stainless steel column (Waters, Milford, MA, USA). The mobile phase was a mixture of ACN (A) and 0.05 M phosphate buffer pH 3 (B), at a flow of 0.4 mL min^{-1}. The gradient mode was selected as follows: 20% A (0 min), 40% A (0–0.7 min), 45% A (0.7–1.8 min) 30% A (1.8–2.0 min) 25% A (2.0–2.2 min) and 20% A (2.2–3 min) in order to get better resolution in a shorter analysis time. Based on the absorption spectra of the six antidepressants, the working wavelengths were: 250.9 nm for mirtazapine, 225.8 nm for venlafaxine, 239.3 nm for escitalopram, 252.1 nm for fluvoxamine, 227.0 nm for fluoxetine and 273.6 nm for sertraline. The identification of the drugs is based on their retention times using UPLC system (viz. 0.95 min for mirtazapine, 1.39 min for venlafaxine, 1.73 min for escitalopram, 2.00 min for fluvoxamine, 2.22 min for fluoxetine and 2.27 min for sertraline) and their UV spectral data.

2.3. Plasma samples

Drug-free plasma, supplied by Center of Transfusion of Galicia (Santiago de Compostela, Spain), was used for the preparation of calibration standards. Real plasma samples were obtained from patients poisoned with antidepressants and stored at 4 °C, unless the analysis was delayed, in which case the samples were frozen. All studies were conducted in accordance with the World Medical Association's "Ethical Principles for Medical Research Involving Human Subjects" [20].

2.4. UA-DLLME procedure

Five hundred microliters of human plasma sample containing a mixed standard solution were placed in a 10 mL glass test tube, and 2.5 mL of ACN were added firstly to plasma protein precipitation and secondly as disperser solvent. The mixture was centrifuged at 4000 rpm for 5 min. The supernatant was placed in a glass test tube and the extraction solvent (chloroform, 200 μL) was added. This mixture was rapidly injected using a glass syringe, into a conical test tube which contains 4.5 mL of Milli-Q water (pH adjusted to 9). The tube was immersed immediately in an ultrasonic water bath for 3 min (Selecta®, Barcelona, Spain). After centrifugation at 4000 rpm for 5 min the fine droplets of organic solvent were sedimented in the bottom of the conical tube and this phase was transferred to a glass vial with a glass syringe and evaporated to dryness under a stream of nitrogen at 40 °C. The extract was dissolved in 100 μL of the mobile phase, filtered through a 0.2 μm filter and 5 μL injected into the UPLC system.

2.5. Experimental design

Chemometric approach based on experimental designs allows the simultaneous variation of the experimental factors, reducing time and materials. This strategy is better than the traditional