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An improved analytical strategy combining microextraction by packed sorbent combined with ultra high pressure liquid chromatography for the determination of fluoxetine, clomipramine and their active metabolites in human urine

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ABSTRACT

A powerful and sensitive method, by microextraction packed sorbent (MEPS), and ultra-high performance liquid chromatography (UHPLC) with a photodiode array (PDA) detection, is described for the determination of fluoxetine, clomipramine and their active metabolites in human urine samples. The MEPS variables, such as sample volume, pH, number of extraction cycles (draw–eject), and desorption conditions (solvent and solvent volume of elution) were optimized. The analysis were carried out using small sample volumes (500 μL) and in a short time period (5 min for the entire sample preparation step). Good linearity was obtained for all antidepressants with the correlation coefficients (R^2) above 0.9965. The limits of detection (LOD) ranged from 0.068 to 0.087 μg mL^{-1}. The recoveries were from 93% to 98%, with relative standard deviations less than 6%. The inter-day precision, expressed as the relative standard deviation, varied between 3.8% and 8.5% while the intra-day precision between 3.0% and 7.1%. In order to evaluate the proposed method for clinical use, the MEPS/UHPLC–PDA method was applied to analysis of urine samples from depressed patients.

1. Introduction

Depression or major depressive disorder is one of the most common and widespread mental disorders in our society that affects millions of people around the world [1]. Treatment of depression includes various forms of psychotherapy as well as pharmacotherapy with antidepressant drugs [2]. This drug class is widely used for the treatment of this psychiatric disorder, and are frequently encountered in emergency toxicology screening, drug-abuse testing and forensic medical examinations [3].

Among the classical pharmacological treatments for depression currently available, four main classes can be cited: selective serotonin reuptake inhibitors (SSRI), selective noradrenaline reuptake inhibitors (SNRI), tricyclic antidepressants (TCA) and monoamine oxidase inhibitors (MAOI) [2,4].

Fluoxetine (FLX) [*N*-methyl-3-phenyl-3-[4-(trifluoromethyl)-phenoxy]propan-1-amine], also known by the trade name Prozac®, belongs to the SSRI class and is one of the most prescribed antidepressant [5]. FLX binds to serotonin reuptake transporter and is extensively metabolized by the cytochrome P450 in the liver to norfluoxetine (NORFLX) (Fig. 1).

As FLX, NORFLX also blocks the 5-HT transporter and is reported to be slightly more active than FLX and contributes to the long duration of the action of FLX in organism [7,8].

Clomipramine (CLP) [3-chloro-10,11-dihydro-*N*,*N*-dimethyl-5*H*-dibenz[*b*,*f*]azepine-5-propanamine], is a typical TCA reported as a potent inhibitor of 5-HT reuptake, with a wide clinical spectrum being used in major depressive, panic and obsessive–compulsive disorders [9,10]. Despite new atypical drugs such as FLX, CLP is still the reference compound in the treatment of this psychiatric disorder [9].

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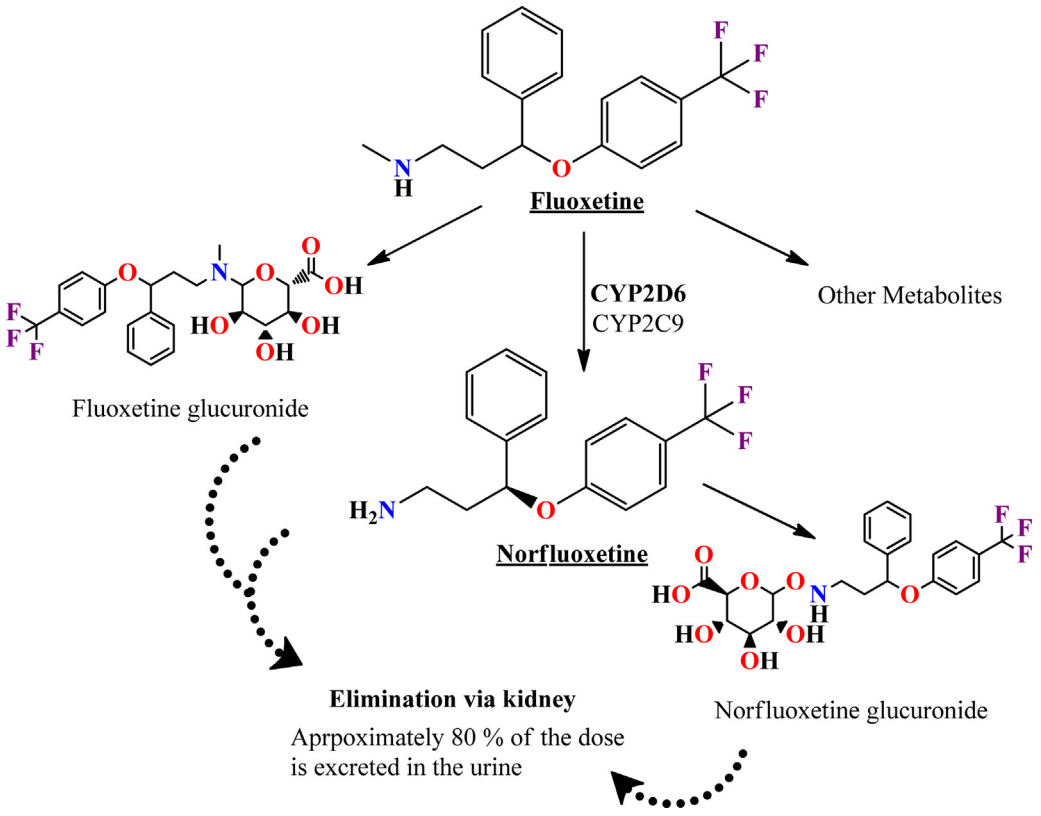

Fig. 1. Schematic representation of fluoxetine metabolism in human liver (*adapted from*M. Whirl-Carrillo et al. (2012) [6]).

As with other tricyclics, CLP undergoes considerable biotransformation before excretion. A major route of its hepatic first pass metabolism is the demethylation into desmethylclomipramine (DESCLP) by several cytochrome P450 enzymes [11]. DESCLP is the major active metabolite and strongly inhibits NA reuptake, but when compared to the parent drug CLP is a weaker inhibitor of 5-HT reuptake [12,13] (Fig. 2).

Due to the wide variety of antidepressants currently available, this drug class has rapidly gained importance in both clinical and forensic fields. These two classes of antidepressants can have a toxic effect in interaction with other drugs and paucity of information concerning drug intake prior to death is a common problem in forensic toxicology, as well as the potential abuse of these drugs can lead to a risk of suicidal attempts with the prescribed drug [14–16].

Consequently, the concentration of these therapeutic drugs and their metabolites in human specimens is very low, which complicate their detection. Therefore, there is a need to develop simple, reliable, fast and sensitive analytical methods based on recent technology that ensure a more rigorous control of these substances in biological matrices.

Several analytical methods have been reported for the analysis of FLX, CLP and in some cases their metabolites in biological fluids for TDM, bioavailability studies and toxicological purposes. The determination of these drugs in biological samples requires an initial sample pretreatment step for target analyte isolation. Most procedures use liquid–liquid extraction (LLE) [9,17–19] and solid-phase extraction (SPE) [16,20] techniques prior to high performance liquid chromatography (HPLC) [18–22] or gas chromatography [23,24].

The recent trends in sample preparation include miniaturization automation, high-throughput performance and on-line coupling with analytical instruments which leads to a reduction in solvent volume and time. This indicates that more advanced extraction techniques need to be developed. In this context, Abdel-Rehim [25] developed in 2004 the microextraction by packed sorbent (MEPS) a powerful technique based on the miniaturization of conventional SPE technique [26]. When compared to other extraction techniques, MEPS is very versatile, since its cartridge can be packed with any solid-phase material such as silica-based (C2, C8, C18), restricted access material (RAM) or molecularly imprinted polymers (MIPs) and can reduce the volume of solvent and sample needed [27]. Furthermore, it is fast and easy to use that includes the sample-processing, extraction and wash steps in the same device and can be connected on-line to a liquid chromatography or gas chromatography equipment [27,28].

This technique has been successfully applied to the qualitative and quantitative determination of a wide variety of drugs and metabolites in biological matrices, such as urine, plasma, saliva and blood [29–32]. MEPS could be of interest in different areas including clinical, pharmaceutical, forensic, toxicological, food and flavour, and environmental research [25,31,33–36].

According to literature, there are some studies that published the determination of antidepressants in biological samples using MEPS. In Chaves et al. [28] work, is described a method based on MEPS with liquid chromatography and UV detection for the determination of new generation antidepressants (sertraline, mirtazapine, fluoxetine, citalopram and paroxetine) in human plasma samples. More recently, Saracino et al. [37] reported a combined HPLC-fluorimetric detection and MEPS method for multi-matrix (saliva, plasma and dried blood spots) analysis of agomelatine. In the same year, Woźniakiewicz et al. [38] developed and validated a MEPS/UHPLC–MS method for the determination of TCA in human oral fluid.

In the present work, a method based on MEPS/UHPLC–PDA, for the simultaneous determination of two antidepressants from different classes, FLX, CLP and their active metabolites, NORFLX and DESCLP, in human urine samples, was developed and validated.