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Determination of selective serotonin reuptake inhibitors in plasma and urine by micellar liquid chromatography coupled to fluorescence detection

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ABSTRACT

Citalopram, paroxetine and fluoxetine are selective serotonin reuptake inhibitor (SSRIs) currently used in the treatment of psychiatric disorders. We present an analytical method using micellar liquid chromatography to quantify these three drugs in pharmaceutical formulations, plasma and urine. The resolution was performed using a mobile phase of 0.075 M SDS – 6% (v/v) butanol buffered at pH 7 running through a C18 column under isocratic mode at 1 mL/min at 25 °C. The analytes were eluted in less than 20 min. The fluorescence detection was programmed at the maximum excitation (236, 295 and 230 nm) and emission (310, 350 and 305 nm) wavelengths for citalopram, paroxetine and fluoxetine, respectively. The experimental procedure was expedited to 1/5 dilution of the sample in the micellar mobile phase and filtration, thus avoiding clean-up and extraction steps. An aliquot of 20 μL was injected after 80 min of preparation, to obtain maximum sensitivity. The method was validated according to the guidelines of the Food and Drug Administration (FDA) in terms of calibration range (20–500 ng/mL; $r^2 > 0.999$), sensitivity, accuracy (91.3–103.2%), precision (<9.3%), and robustness (<6.1%). The suitability of the method was successfully evaluated by analyzing plasma and urine samples from patients treated with SSRIs and checking the content of the active principle in tablets. Thus, the method can be applied to pharmacokinetics studies and in forensic cases, as well as in quality control of commercial pharmaceutical formulations.

1. Introduction

Citalopram (MW = 324.3 g/mol), paroxetine (MW = 32,937 g/mol) and fluoxetine (MW = 309.3 g/mol) [1] (Fig. 1) are antidepressants belonging to the family of selective serotonin (5-hydroxy-tryptamine, 5-HT) reuptake inhibitors (SSRIs). These drugs are used for psychiatric disorders, as depression, anxiety, panic and obsessive compulsive, post-traumatic, pre-menstrual and dysphoric disorder disorders [2], as well as for other diseases involving serotonin (5-HT) reuptake [3,4]. The treatment of these patients is of the utmost importance, as depression can lead to suicide attempts. The antidepressant effect of SSRIs is due to their property to block the 5-HT transporter, resulting in an increasing to 5-HT concentration in the presynaptic neurons of the central nervous system [2]. The SSRIs have a generally better tolerated, adverse side-effects than tricyclic antidepressants (TCAs) with approximately equivalent antidepressant efficacy [5]. Therefore, SSRIs are currently widely prescribed drugs to treat mental disorders. However, SSRIs are not effective in all cases. Many treated depressed patients did not respond to their treatment [6]. Although the toxicity of SSRIs is low, some cases of intoxication have been detected [7,8], and even several case reports of death suspecting these drugs as causative agents have been reported [9]. For these reasons, the development of a rapid and specific analytical method allowing the screening and the determination of these new antidepressant drugs in biological samples and pharmaceuticals could be of great interest either in therapeutic drug monitoring use or in toxicological screening in the case of the patients do not respond as expected due to drug interaction, non-compliance or other causes, as wells as to perform pharmacokinetics [10,11].

Several methods have been developed for the analysis of fluoxetine, paroxetine and citalopram in various biological matrices.

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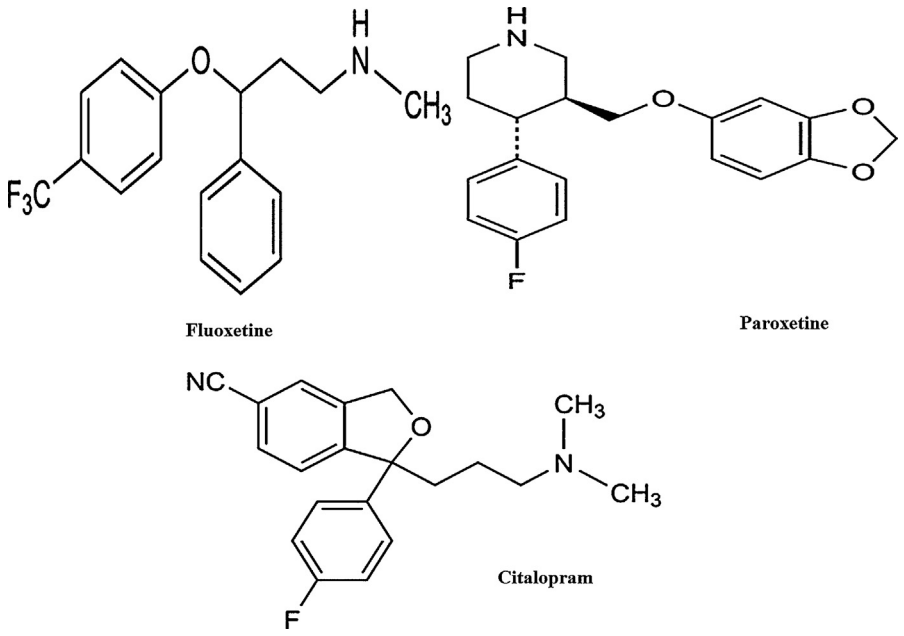

Fig. 1. Structures of the studied SSRIs.

Methods based on hydro-organic reverse phase-high performance liquid chromatography (RP-HPLC) coupled with UV–visible absorption (DAD) [12–14], electrochemical detection (ED) [15] and fluorescence detection (FLD) [9,14,16], have been applied in the quantification of these compounds in plasma and serum samples. Other reported methods analyze these SSRIs together with other antidepressant drugs in biological matrices such as plasma, serum, blood, urine or hair using HPLC coupled to mass spectrometry (MS) [14,17–21]. Gas chromatography has also been applied in SSRI quantification for plasma and urine samples [14,22–24]. However, these methods require time-consuming and tedious extraction and clean-up procedure to separate SSRIs to the endogenous compounds of plasma and urine, risking the loss of analyte and enlarging the use of toxic solvents.

Micellar liquid chromatography (MLC) is an RP-HPLC technique that uses a surfactant, as the main component in the mobile phase at a concentration over the critical micellar concentration [25]. The most used one is the anionic surfactant sodium dodecyl sulfate (SDS), because of its solubility in water, low critic micellar concentration (7–10 mM) [26], low cost, low toxicity and reduced viscosity, allowing an easy removing from the chromatographic system [27]. The addition of a modifier, such as propanol, butanol or pentanol, is also used to increase the elution power of the mobile phase and improve the efficiencies of the chromatographic peaks. MLC provides facility for the direct injection of physiological samples, because the proteins are denaturalized and dissolved by the surfactants and are scarcely retained by the chromatographic column [28]. Hence, this minimal sample handling reduces the cost, total time and complexity of analysis and decreases the sources of error in the sample preparation step, thereby improving the reproducibility of the method. In addition, the stable and reproducible behavior of micellar mobile phases allows accurate prediction of solute retention with a model that can also be used to optimize the separation of mixtures. MLC technique has been successfully used in the determination of a large number of drugs in biological fluids (plasma and urine), pharmaceutical preparations and foods, which can be injected directly into the chromatographic system [29–32].

The purpose of this study was to develop and validate a new MLC method for the simple, rapid and specific determination and quantification of three SRRIs: citaprolam, paroxetine and fluoxetine at clinical levels in biological fluids (plasma and urine) and in pharmaceutical formulations. The method was validated according to the Food and Drug Administration (FDA) guidelines [34]. The method would be applied to routine analyses in the quality control process of pharmaceutical samples and in pharmacokinetics studies, which require to detect SSRIs in physiological fluids at ng/mL levels.

2. Materials and methods

2.1. Chemicals and reagents

Sodium dodecyl sulfate (SDS, purity >99%) was purchased from Merck (Darmstadt, Germany). Sodium dihydrogen phosphate and HCl were supplied by Panreac (Barcelona, Spain). Methanol, 1-propanol, 1-butanol, 1-pentanol and NaOH, reagent grade for all cases, were purchased from Scharlab (Barcelona, Spain). All solutions and mobile phases were prepared using ultrapure water generated from distilled water using a Millipore device (S.A.S., Molsheim, France).

Citaprolam, patoxetine and fluoxetine, (purity >99.9%) were purchased to Zydus Health care (Sikkim, India). Plasma and urine samples (from patients and blanks) were provided for Hospital General (Castelló, Spain).

2.2. Apparatus

The HPLC analysis was carried out in an Agilent Technologies series 1100 apparatus (Palo Alto, CA, USA) equipped with a quaternary pump, a thermostated autosampler tray and column compartments, and a fluorescence detector. Instrumental control and chromatographic data acquisition were done with the Agilent ChemStation (Rev. B.03.01) software. The signal was acquired by a PC computer connected to the chromatograph, through an HP Chemstation.

A Crison GLP 22 (Crison, Barcelona) equipped with a combined Ag/AgCl/glass electrode was used to measure the pH of the solutions. The analytical balance employed was a Mettler-Toledo AX105 Delta-Range (Greifensee, Switzerland). The vortex shaker and sonication unit were obtained from Selecta (Barcelona, Spain).