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A fast and feasible microextraction by packed sorbent (MEPS) procedure for HPLC analysis of the atypical antipsychotic ziprasidone in human plasma

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ABSTRACT

An original high-performance liquid chromatographic method coupled to microextraction by packed sorbent (MEPS) was developed for the therapeutic drug monitoring (TDM) of psychiatric patients treated with the recent atypical antipsychotic ziprasidone.

The chromatographic separation was achieved on a RP C18 column, using an isocratic mobile phase and setting the wavelength at 320 nm. The analyte was extracted from human plasma by means of a fast and feasible innovative MEPS procedure, optimised on C2 sorbent and requiring only 100 μL of biological sample. A second pre-treatment procedure, based on solid phase extraction (SPE), has been also developed for comparison. The availability of different pre-treatment procedures allows the choice of the one best suiting the specific clinical, economic and scientific needs. The extraction yield values were always higher than 90% and sensitivity was also good, with a limit of quantitation (LOQ) of 1 ng/mL.

The method was successfully applied to plasma samples from ten subjects undergoing therapy with ziprasidone, thus confirming its suitability for the TDM of psychiatric patients, in order to personalise their pharmacological treatments.

1. Introduction

Ziprasidone (5-[2-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl]ethyl]-6-chloro-1,3-dihydro-2H-indol-2-one, ZPR, Fig. 1a) is one of the most recent atypical antipsychotics introduced into the market. It is used for the treatment of schizophrenia, acute mania and mixed state episodes associated with bipolar disorder [1]. ZPR is also used off-label for depression, bipolar disorder maintenance therapy, anxiety, aggression, dementia, attention deficit hyperactivity, autism and stress disorders [2]. ZPR efficacy in treating the positive symptoms of schizophrenia is primarily mediated by antagonism of the dopamine D_2 receptors. Blockade of 5-HT_{2A} and 5-HT_{2C} receptors and activation of 5-HT_{1A} receptors, as well as serotonin and norepinephrine reuptake inhibition, may all contribute to its ability to alleviate also negative symptoms. Unlike many other antipsychotics, ZPR has no significant affinity for muscarinic cholinergic receptors (mAChRs), for this reason it does not show any anticholinergic side effects [3]. Moreover ZPR is not generally associated with weight gain or hyperlipidemia [4]. Clinically effective doses are 80–160 mg/day [5], corresponding to plasma levels ranging from 20 to 160 ng/mL [6]. Orally administered ZPR is easily absorbed and its absorption increases two-fold in the presence of food. ZPR is extensively metabolised by phase I (primarily cytochrome P450 3A4) and phase II metabolic pathways, resulting in several metabolites which do not seem to contribute to the overall antipsychotic effect [7]. ZPR most frequent side-effects are mild or moderate headache, first-dose postural hypotension and mild histaminergic sedative effect [8]. The most important severe toxic effect of the drug is the QT syndrome: ZPR has been shown to prolong the cardiac corrected QT (QTc) interval, which is linked to fatal ventricular tachyarrhythmias and torsade de pointes [9]. Thus, ZPR should be avoided in patients with some types of cardiac diseases, uncontrolled electrolyte disturbance or in polypharmacy with other drugs that prolong the QT interval.

The importance of having at disposal feasible but reliable analytical methods for the therapeutic drug monitoring (TDM) of patients

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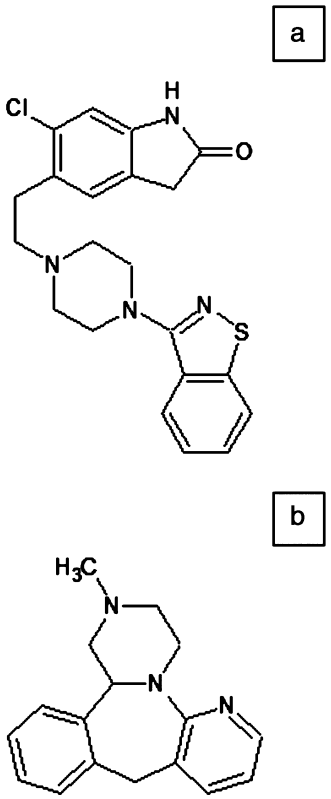

Fig. 1. Chemical structures of (a) ziprasidone (ZPR) and (b) mirtazapine, used as the internal standard (IS).

treated with ZPR is thus evident, in order to personalise and optimise pharmacological therapies, minimising side and toxic effects.

The method presented herein allows ZPR plasma determination by means of a fast and feasible microextraction by packed sorbent (MEPS) pre-treatment and HPLC-UV analysis. The methodology does not require expensive instrumentations and exploits a sample pre-treatment procedure, based on a miniaturised extraction, that reduces sample volumes and analysis time, while granting sound results.

To the best of our knowledge, only a few papers can be found in the literature, which specifically deal with the analysis of ZPR in biological fluids: those take advantage of chromatographic separations (HPLC) coupled to spectrophotometric (UV) [10,11], spectrofluorometric (F) [12], mass spectrometry (MS) [13–19] detection and describe pre-treatment techniques based on liquid–liquid extractions with organic solvents [11–13,15–18] or on protein precipitation [14,19].

This original MEPS-HPLC method is therefore proposed as a promising analytical improvement compared to already published papers.

2. Materials and methods

2.1. Chemicals

ZPR and mirtazapine ((±)-2-methyl-1,2,3,4,10,14b-hexahydropyrazino[2,1-a]pyrido[2,3-c][2]benzazepine, Fig. 1b), used as the Internal Standard (IS), reference pure compounds, were purchased from Sigma–Aldrich (St. Louis, USA). HPLC-grade acetonitrile and methanol, monobasic sodium phosphate, sodium hydroxide, 85% (w/w) phosphoric acid and diethylamine, all pure for analysis, were bought from Sigma–Aldrich. Ultrapure water (18.2 MΩ cm) was obtained by means of a MilliQ apparatus by Millipore (Milford, USA).

2.2. Instrumentation and chromatographic conditions

The chromatographic system was composed of a Jasco (Tokyo, Japan) PU-980 isocratic pump, equipped with a Jasco UV-975 spectrophotometric detector, set at 320 nm. Separations were obtained on a Phenomenex (Torrance, CA, USA) Gemini® reversed-phase column (50 mm × 3.0 mm I.D., 5 μm), kept at room temperature. The mobile phase was a mixture of acetonitrile (30%, v/v) and a pH 2.5, 50 mM phosphate buffer containing 0.2% (v/v) diethylamine (70%, v/v), flowing at 0.5 mL/min. The injections were carried out through a 10-μL loop. Data processing was handled by means of a Jasco ChromNAV 1.16 software.

2.3. Stock and standard solution preparation

Stock solutions of ZPR and IS (1 mg/mL) were prepared by dissolving suitable amounts of each pure substance in methanol. Standard solutions were obtained by diluting stock solutions with the mobile phase and injected into the HPLC-UV system. Stock solutions were stable for at least one month when stored at −20 °C (as assessed by HPLC assays), while standard solutions were prepared fresh every day.

2.4. Plasma collection and pre-treatment

Blood samples were centrifuged (within 1 h from collection) at 1400 × *g* for 10 min, the supernatant (plasma) was transferred into glass vials and stored at −20 °C until analysis.

MEPS procedure was performed on a SGE Analytical Science (Ringwood, Australia) apparatus, consisting of a 100-μL HPLC syringe with a removable needle, fitted with a BIN (Barrel Insert and Needle) containing a C2 sorbent. The sorbent was conditioned with 200 μL of methanol and equilibrated with 200 μL of ultrapure water. The loading solution was a mixture of 100 μL of plasma, 100 μL of ultrapure water and 5 μL of IS solution prepared in mobile phase; the loading mixture was drawn into the syringe and discharged back 10 times. The cartridge was then washed with 100 μL of water, 100 μL of a water/methanol mixture (90/10, v/v) and eluted by drawing and discharging 500 μL of methanol. The eluate was dried under vacuum, re-dissolved with 100 μL of mobile phase and injected into the HPLC-UV system.

The SPE procedure, developed as a comparison, was carried out on a Macherey-Nagel (Düren, Germany) Chromabond® vacuum apparatus, using Biotage (Uppsala, Sweden) Isolute C2 cartridges (50 mg, 1 mL) that were activated by passing 2 mL of methanol and then conditioned by passing 2 mL of ultrapure water. To 250 μL of plasma, 500 μL of ultrapure water and 10 μL of IS solution prepared in mobile phase were added and the resulting mixture was loaded onto a previously conditioned cartridge. The cartridge was then washed with 1 mL of ultrapure water and 1 mL of a water/methanol mixture (90/10, v/v), finally eluted with 1 mL of methanol. The eluate was dried under vacuum, re-dissolved with 125 μL of mobile phase and injected into the HPLC-UV system.

2.5. Method validation on blank plasma samples

Method validation was carried out on blank plasma samples from ten healthy volunteers, not treated with ZPR, according to USP [20] and Crystal City [21] guidelines.

2.5.1. Linearity

Aliquots of 10 μL of ZPR standard solutions at seven different concentrations, containing IS at a constant concentration, were