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Effective phospholipids removing microelution-solid phase extraction LC-MS/MS method for simultaneous plasma quantification of aripiprazole and dehydro-aripiprazole: Application to human pharmacokinetic studies

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ABSTRACT

A simple liquid chromatography-tandem mass spectrometry (LC-MS/MS) method has been developed and validated for simultaneous quantification of aripiprazole and its active metabolite, dehydro-aripiprazole, in human plasma. Stable isotopically labeled aripiprazole, aripiprazole-D8, has been used as the internal standard (IS) for both analytes. Only 200 μl of human plasma was needed for analyte extraction, using effective phospholipids-eliminating three-step microelution-solid-phase extraction (SPE, Oasis PRiME HLB 96-well μElution Plate). An ACE C18-PFP column was applied for chromatographic separation at 25 °C, protected by a 0.2-μm on-line filter. A combination of ammonium formate (5 mM)-acetonitrile (pH 4.0; 65:35, v/v) was used as mobile phase and the chromatogram was run under gradient conditions at a flow rate of 0.6 ml/min. Run time lasted 5 min, followed by a re-equilibration time of 3 min, to give a total run time of 8 min. Five μl of the sample was injected into the chromatographic system. Aripiprazole, dehydro-aripiprazole and IS were detected using the mode multiple reaction monitoring in the positive ionization mode. The method was linear in the concentration range of 0.18–110 ng/ml and 0.35–100 ng/ml for aripiprazole and dehydro-aripiprazole, respectively. Our method has been validated according to the recommendations of regulatory agencies through tests of precision, accuracy, recovery, matrix effect, stability, sensitivity, selectivity and carry-over. Our microelution-SPE method removes more than 99% of main plasma phospholipids compared to protein precipitation and was successfully applied to several bioequivalence studies.

Abbreviations: ARI, aripiprazole; AUC, area under curve; CAL, calibration standard; CID, collision-induced dissociation; C_{max}, maximum plasma concentration; CV, coefficient of variation; CYP, cytochrome P450; DAD, diode array detection; DARI, dehydro-aripiprazole; EDTA, ethylenediaminetetraacetic acid; EMA, European Medicines Agency; ESI, electrospray ionization; FDA, US Food and Drug Administration; HLB, hydrophilic-lipophilic balance; HPLC, high-performance liquid chromatography; IS, internal standard; LC-MS, liquid chromatography-mass spectrometry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LLE, liquid-liquid extraction; LLOQ, lower limit of quantification; MRM, multiple-reaction monitoring; PFP, pentafluorophenyl; PPT, protein precipitation; QC, quality control; R^2, correlation coefficient; tR, retention time; SD, standard deviation; SEM, standard error of mean; SIL-IS, stable isotopically labeled internal standards; SLE, supported liquid extraction; SPE, solid phase extraction; SRM, selected ion monitoring; T_{max}, time of occurrence of C_{max}; $T_{1/2}$, half-life; TIC, total ion chromatogram; UHPLC-MS/M, sultra-high performance liquid chromatography-tandem mass spectrometry; UV, ultraviolet; XIC, extraction ion chromatogram.

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1. Introduction

Aripiprazole (ARI), a quinolinone derivative, is an atypical antipsychotic drug indicated in schizophrenia and schizoaffective disorder [1]. It displays a partial agonist/antagonist activity at dopamine D2 and D3 receptors and serotonine 5-HT1A receptors, whereas it acts as an antagonist at serotonine 5-HT2A receptors [2].

ARI is metabolized to dehydro-aripiprazole (DARI), its main active metabolite, mainly by the cytochrome P450 (CYP) CYP3A4 and CYP2D6 isoenzymes. Both enzyme systems are subjected to drug interactions and genetic polymorphism, dose-adjustment is therefore necessary, especially when it is co-administered with CYP3A4 and CYP2D6 inhibitors or CYP3A4 inductors [3]. Additionally, allelic variants in *ABCB1* and *ABCG2* genes were found to influence the plasma concentrations of antipsychotics, including ARI. The *ABCB1* 2677TT/3435TT genotype has been linked to a significantly lower ARI plasma concentration/dose ratio if compared to patients carrying other *ABCB1* genotypes [4]. Therefore, ARI plasma monitoring is suggested to improve efficacy, avoid drug-drug interactions or decrease adverse effects.

To date, numerous analytical methods have been used for ARI and DARI human plasma determination by high performance liquid chromatography (HPLC, also called 'LC') with ultraviolet detection (UV) and diode array detection (DAD) [5–7]. However, the lower limit of quantification (LLOQ) is lower or the run time is shorter in case of using LC-MS/MS methods with electrospray ionization (ESI). Despite improved sensitivity and selectivity of the LC-MS/MS-based methods, one of the main problems of ESI sources is the ion suppression or enhancement caused by the sample matrix (also known as a 'matrix effect') and interferences from metabolites [8]. Endogenous phospholipids cause ion suppression in both positive and negative ESI modes leading to matrix effect [9] and consequently increased variability and irreproducibility in bioanalytical LC-MS/MS methods. Lipids are the most variable components in human plasma. They can vary significantly among individuals according to diet and metabolic rate. However, glycerophosphatidylcholines, such as phosphatidylcholine, the main phospholipids circulating in the human plasma, constitute up to 70% of total plasma phospholipids [10]. Lysophospholipids, including lysophosphatidylcholine, compose up to 10% of total phospholipids [11]. Therefore, an optimization of an appropriated extraction method, which is able to eliminate phospholipids, combined with the use of stable isotopically labeled internal standards (SIL-IS) is extremely important in order to achieve reliable results and maintain the LC/MS-MS system clean [8,12].

Nonetheless, the majority of LC-MS/MS and ultra-LC-MS/MS (UHPLC-MS/MS) methods with ESI have used protein precipitation (PPT) or liquid liquid extraction (LLE) in addition to non SIL-IS instead of solid phase extraction (SPE) and stable SIL-IS [13–18]. PPT is the fastest, but the least effective sample preparation technique, often resulting in significant matrix effects mainly due to the presence of endogenous phospholipids [11]. As follows, some HPLC-UV or LC-MS/MS methods choose SPE, including supported liquid extraction (SLE) [19], a type of SPE, as an extraction method. Reversed-phase SPE methods are known to provide cleaner extracts and reduce matrix effects compared to PPT [11,12,20]. In spite of the fact that SPE has been applied in many published methods, it is characterized by longer performing times, need of evaporation, reconstitution steps and no usage of SIL-IS [20,21]. Among all these methods, only studies employing SIL-IS based SPE [22] and SLE [19] were able to achieve the best results. Patel et al. also applied UHPLC, which offers shorter analyte separation time due to a higher pressure in the UHPLC system. However, none of the cited methods investigated phospholipids' elimination efficacy of the extraction method applied.

Thus, the aim of the present study was to develop a simple LC-MS/MS method based on effective phospholipids removing three-step microelution-SPE method compared to PPT for simultaneous plasma quantification of ARI and DARI as well as its further application to pharmacokinetic studies and clinical practice.

2. Materials and methods

2.1. Chemicals and reagents

ARI, DARI and SIL-IS [2H8]-ARI (ARI-D8) were provided by Toronto Research Chemicals (North York, Canada). Acetonitrile, methanol, ammonia solution (at 25%) and ammonia hydroxide 5N (HPLC Grade) were purchased from SYMTA (Madrid, Spain). Formic acid was supplied by Sigma-Aldrich (Madrid, Spain). All chemicals were analytical or LC-MS grade. The water for HPLC was obtained using a Milli-Q system (Millipore-Ibérica, Madrid, Spain). Blank human plasma samples from different individual human donors were supplied by the Transfusion Center of the Autonomous Community of Madrid (Madrid, Spain).

2.2. Stock solutions, calibration standards (CALs), and quality controls (QCs)

Stock solutions of CALs and QCs of ARI, DARI and ARI-D8 were prepared by dissolving an accurately weighed quantity in 0.5% formic acid solution in methanol to obtain a concentration of 1 mg/ml for all compounds. CALs were made from independent dilutions of each stock solution and spiked in the blank plasma to obtain 8 CALs with the concentrations of 0.18, 0.5, 1.0, 5.0, 25, 50, 100 and 120 ng/ml for ARI and 0.35, 0.5, 1.0, 2.5, 10, 50, 90 and 110 ng/ml for DARI. QC samples were prepared in the same fashion to obtain the four corresponding QC levels (LLOQ, low, medium, and high), as follows: 0.18, 0.5, 55, 110 ng/ml for ARI and 0.35, 0.7, 45, and 90 ng/ml for DARI. Secondary IS solution of 1000 ng/ml ARI-D8 was diluted 20 times to give a working solution of 50 ng/ml.

A drug-free blank plasma sample and a drug-free zero plasma sample (processed with IS) were included according to the recommendations for bioanalytical method validation of the US Food and Drug Administration (FDA) [23], the European Medicines Agency (EMA) [24] and the International Conference on Harmonisation (ICH) [25]. All CALs, QC, and IS solutions were stored at −80 °C until use or analysis.

2.3. Chromatographic conditions

The HPLC system consisted of a 1200 Series separation module (Agilent Technologies, Madrid, Spain) controlled by Agilent MassHunter Workstation Data Acquisition software for programming samples and chromatographic conditions. Separations were carried out at 25 °C in an ACE C18-PFP column (3-μm, 4.6 × 100 mm; SYMTA, Madrid, Spain) at 0.6 ml/min. The mobile phase consists of ammonium formate (5 mM, solvent A)-acetonitrile (solvent B) (pH 4.0; 65:35, v/v). The chromatogram was run under gradient condition as follows: initial conditions: 65% (A) and 35% (B); 0–0.1 min, gradually increasing eluent B to 75% (B); 0.1–0.5 min, gradually increasing eluent B to 90%; 0.5–1.5 min, gradually increasing eluent B to 99% and maintain from 1.5 to 3.0 min; 3.0–3.2 min returning to the initial conditions (65% A and 35% B) and maintain from 3.2 to 5.0 min. The chromatogram was followed by a re-equilibration time of 3.0 min. The volume injected into the HPLC was 5 μl.