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Development and validation of an LC–MS/MS method for the quantitative determination of aripiprazole and its main metabolite, OPC-14857, in human plasma

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Abstract

An accurate, sensitive, reproducible, and selective liquid chromatography/tandem mass spectrometry (LC–MS/MS) method for determination of aripiprazole and its main metabolite, OPC-14857, in human plasma was developed and validated. Chromatographic separation was achieved isocratically on a C18 reversed-phase column within 7.5 min. The calibration curve, ranging from 0.1 to 100 ng/ml, was fitted to a $1/y^2$-weighted linear regression model. The assay showed no significant interference. Lower limit of quantitation (LLOQ) for both analytes was 0.1 ng/ml using 0.4 ml of plasma. Intra- and inter-assay precision and accuracy values for aripiprazole and OPC-14857 were within regulatory limits.

Keywords: Aripiprazole; OPC-14857; LC–MS/MS

1. Introduction

Aripiprazole, 7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy]-3,4-dihydro-2(1H)-quinolinone (chemical structure shown in Fig. 1), is a novel antipsychotic developed by Otsuka Pharmaceutical Co., Ltd. [1]. Results of in vitro studies have indicated that aripiprazole is metabolised by two isozymes of human cytochrome P450, CYP 2D6 and CYP 3A4 [2]. OPC-14857 (dehydro-aripiprazole, chemical structure shown in Fig. 1) is the main metabolite of aripiprazole in humans. At steady state, OPC-14857 represents about 40% of aripiprazole AUC in plasma. Pharmacological studies have indicated that OPC-14857 has pharmacological activity equivalent to that of aripiprazole [2].

In the present study, we investigated and validated a selective and sensitive method for simultaneous determination of aripiprazole and its main metabolite, OPC-14857, in human plasma using liquid chromatography/tandem mass spectrometry (LC–MS/MS). The validation was conducted by determining specificity, linearity, accuracy, precision, recovery, and stability.

2. Experimental

2.1. Chemicals and reagents

Aripiprazole, OPC-14857, and OPC-14714, which was used as an internal standard (I.S.), were supplied by Otsuka

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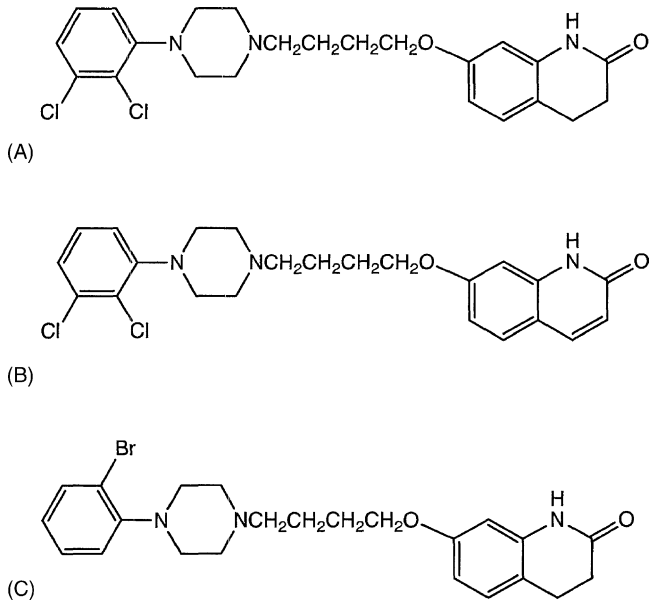

Fig. 1. Chemical structures of aripiprazole (A), its metabolite OPC-14857 (B), and the internal standard OPC-14714 (C).

Pharmaceutical Co. (Tokushima, Japan). Chemical structures are shown in Fig. 1. HPLC-grade acetonitrile, methanol, acetic acid, dimethyl sulfoxide, diethyl ether, and sodium hydrogen carbonate were purchased from Wako Pure Chemical Industries (Osaka, Japan). Heparinised blank human plasma was purchased from Nippon Bio-Supply Center (Tokyo, Japan). Deionised water was prepared in-house using a Milli-Q water system (Millipore, Milford, MA, USA).

2.2. Instrumentation

The HPLC system consisted of an HP1100 binary pump, autosampler, column oven, and online degasser (Agilent Technologies, Palo Alto, CA, USA). The analytical column used was a reversed-phase Chemcobond ODS-W (150 mm × 2.1 mm i.d., 5 μm; Chemco, Osaka, Japan). A mobile phase of 0.1% acetic acid in water/acetonitrile (65:35, v/v) was pumped at a flow rate of 0.2 ml/min. The column temperature was 30 °C. The LC eluate was introduced directly into a TSQ700 triple quadrupole (Thermo Electron, San Jose, CA, USA; OS: UNIX 4.0E, software: ICIS 8.3.0 SP2 and ICL 7.5) equipped with an electrospray ionisation source. The triple quadrupole mass spectrometer was operated in the positive ion mode and the selected reaction monitoring (SRM) chromatograms obtained were used for quantification. SRM transitions of *m/z* 448 → 285, *m/z* 446 → 285, and *m/z* 458 → 295 were respectively optimised for aripiprazole, OPC-14587, and OPC-14714. The detailed mass spectrometer conditions were as follows: electrospray voltage, 4500 V; heated capillary temperature, 240 °C; sheath gas (nitrogen) pressure, 482.633 kPa; auxiliary gas (nitrogen) flow rate, 2978 ml/min; collision gas (argon) thickness, 2 mTorr (1 Torr = 133.3 Pa); collision energy, 30 eV for aripiprazole, 25 eV for OPC-14857, 35 eV for OPC-14714.

2.3. Preparation of calibration standards and quality control

Aripiprazole was dissolved in dimethyl sulfoxide and then diluted in methanol to obtain a stock solution at 200 μg/ml. OPC-14587 was dissolved and then diluted in methanol to obtain a stock solution at 200 μg/ml. Aliquots of the two stock solutions were mixed and further diluted with methanol to obtain working standard solutions with concentrations ranging from 4 to 4000 ng/ml. The I.S. was dissolved and then diluted in methanol to obtain a stock solution at 200 μg/ml, and this solution was further diluted with methanol to obtain a working I.S. solution at 2000 ng/ml. Calibration standards and quality control (QC) samples were prepared in blank human plasma by spiking with the working standard solutions. The calibration standards comprised eight concentrations (0.1, 0.2, 0.5, 2, 5, 20, 50, and 100 ng/ml) for each analyte. The QC samples comprised three concentrations (0.2 ng/ml, low QC; 5 ng/ml, middle QC; and 80 ng/ml, high QC) for each analyte. QC samples at the lower limit of quantitation (LLOQ, 0.1 ng/ml) and upper limit of quantitation (ULOQ, 100 ng/ml) were also prepared. Calibration standards were freshly prepared prior to use. QC samples were prepared and divided into individual tubes and stored frozen until use for the analysis.

2.4. Extraction procedure

The plasma sample (0.4 ml) was placed in a glass centrifuge tube and 10 μl of working I.S. solution was added. The mixed sample was made weakly basic by adding 0.5 ml of saturated solution of sodium hydrogen carbonate followed by 4 ml of diethyl ether. The tube was shaken reciprocally for 5 min. After centrifugation at 1500 × *g* for 5 min at 4 °C, the organic phase was decanted into a clean glass test tube. The organic solvent was evaporated under a gentle stream of nitrogen gas at 40 °C. The residue was reconstituted with 200 μl of mobile phase and vortex-mixed for 10 s. A 50 μl aliquot of the reconstituted solution was injected into the LC–MS/MS system.

2.5. Linearity

Eight calibration standards were pretreated and analysed. Calibration curves were calculated by least-squares linear regression using $1/y^2$ weighting. Concentrations were evaluated on the basis of the corresponding calibration curve, and deviations from the theoretical concentrations were required to be within ±20% for the LLOQ and within ±15% for other concentrations. Correlation coefficients (*r*) were required to be 0.99 or higher.

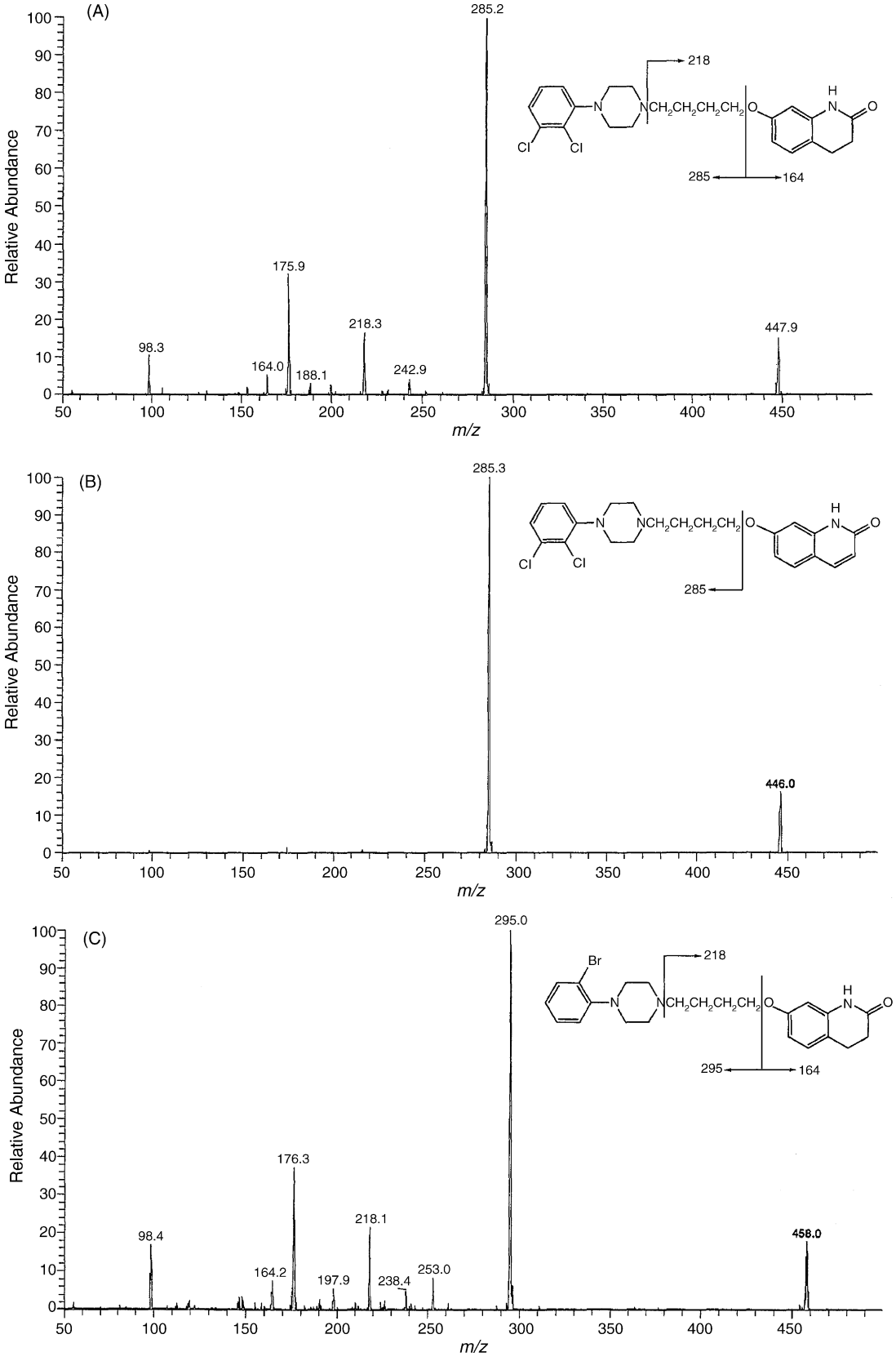

Fig. 2. Product ion mass spectra of aripiprazole (A), OPC-14857 (B), and OPC-14714 (C). The protonated molecules were used as precursor ions for MS/MS.

2.6. Intra-assay accuracy and precision

Three QC samples, LLOQ, ULOQ, and middle QC (5 ng/ml), were prepared and analysed in replicate ($n = 5$) together with calibration standards prepared independently from the QC samples. Accuracy was determined as the percent difference between the mean of observed concentrations and the theoretical concentration, and was required to be within ±20% for the LLOQ and within ±15% for other concentrations. The coefficient of variation (CV) was used to express precision, and the %CV was required to not exceed 20% for the LLOQ and 15% for other concentrations.

2.7. Inter-assay accuracy and precision

Three QC samples, low QC (0.2 ng/ml), middle QC (5 ng/ml), and high QC (80 ng/ml), were prepared and analysed together with calibration standards prepared independently from the QC samples. This procedure was repeated for 5 days. Accuracy was required to be within ±15%. The %CV was required to not exceed 15%.

2.8. Recovery

The recovery rates (%RE) for aripiprazole, OPC-14857, and OPC-14714 were calculated from the following equation by comparing the peak responses of aripiprazole, OPC-14857, and OPC-14714 in extracted samples and post-extracted spiked samples prepared from three human plasma samples.

$$\%\mathrm{RE} = \frac{[\text{Response of extracted sample}]}{[\text{Response of post} - \text{extracted spiked sample}]} \times 100$$

2.9. Specificity

Six individual human plasma samples containing neither of the analytes nor the I.S. were prepared for comparison with LLOQ samples. These samples were pretreated according to the described procedures and analysed. It was required that no significant interference be seen in the chromatograms at the retention times of aripiprazole, OPC-14587, and the I.S.

2.10. Stability

The stability of aripiprazole and OPC-14857 in human plasma after three freeze-thaw cycles and under a storage condition of −20 °C was evaluated on the basis of accuracy. The stability of processed samples in the autosampler (10 °C) was also determined. Aripiprazole and OPC-14857 were considered to be stable in human plasma or extracts when accuracy was within ±15%. The stability experiments were performed in triplicate and at two concentrations in plasma (0.2 and 80 ng/ml). The stability of aripiprazole, OPC-14857, and OPC-14714 in the stock and working solutions was evaluated under a storage condition of 4 °C. Aripiprazole, OPC-14597, and OPC-14714 were considered stable in the stock and working solutions when the percent change was within ±15%.

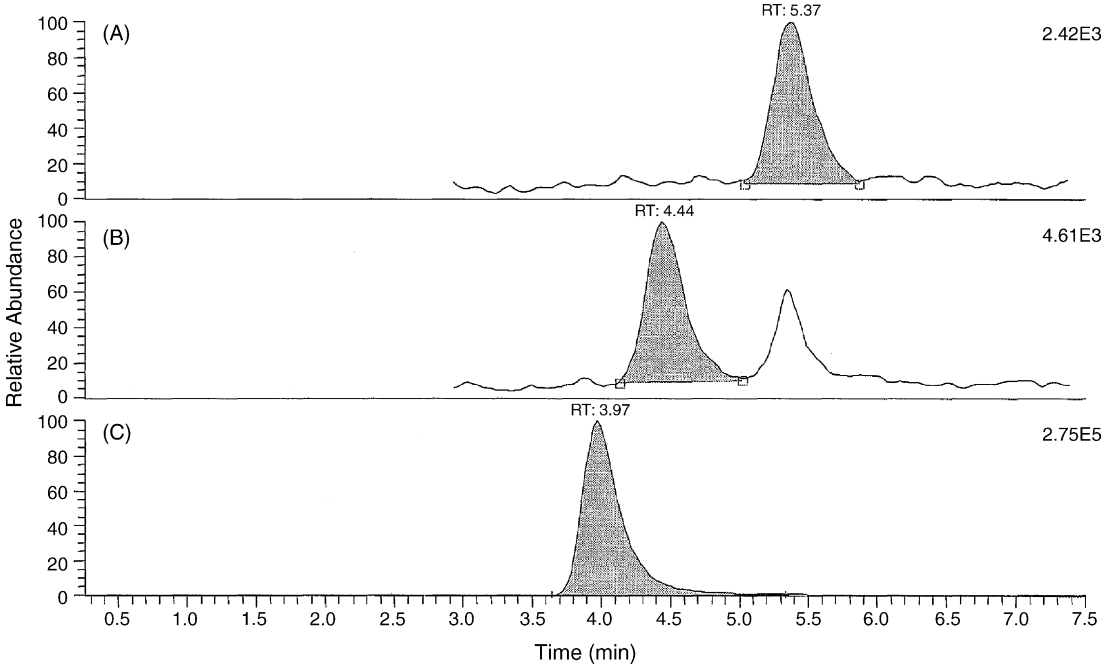

Fig. 3. Typical SRM chromatograms for LLOQ samples of aripiprazole (A) (0.1 ng/ml), OPC-14857 (B) (0.1 ng/ml), and internal standard (C) (50 ng/ml) in human plasma.

3. Results

3.1. LC–MS/MS conditions

ESI mass spectra of aripiprazole, OPC-14587, and OPC-14714 revealed predominant protonated molecules $[M + H]^+$ at *m/z* 448, 446, and 458, respectively.

Product ion spectra of the three compounds were recorded by allowing the protonated molecules to fragment in the collision cell. The resulting product ion spectra are presented in Fig. 2.

In the product ion mass spectrum of aripiprazole (Fig. 2A), it was estimated that the main product ion observed at *m/z* 285 is produced by onium cleavage of the oxygen–carbon bond, as indicated in the figure, resulting in protonated 4-[4-(2,3-dichlorophenyl)-1-piperazinyl] butane. The fragment ion at *m/z* 164 is also produced by the same cleavage. The fragment ion at *m/z* 218 is produced by onium cleavage of the nitrogen-carbon bond between the piperazine and butane moieties. In the product ion mass spectrum of OPC-14857 (Fig. 2B), the main product ion observed at *m/z* 285 was the same as for aripiprazole. In the product ion mass spectrum of OPC-14714 (Fig. 2C), the main product ion observed at *m/z* 295 is produced by onium cleavage of the oxygen–carbon bond. The product ion at *m/z* 285 was used for quantitation of aripiprazole and OPC-14857, and the ion at *m/z* 295 was used for quantitation of OPC-14714, in combination with their $[M + H]^+$ precursors.

Since the difference between the monitoring precursor ions of aripiprazole and OPC-14857 is 2 mass, in order to prevent interference it was necessary to separate the peaks of the two analytes on the chromatograms. Under these LC conditions, peak separation of aripiprazole and OPC-14857 was excellent (Fig. 3).

3.2. Linearity

Typical calibration curves and linear regression parameters are presented in Fig. 4. Calibration standards were analysed in a dynamic range of 0.1–100 ng/ml for aripiprazole and OPC-14857. Correlation coefficients of the calibration curves for aripiprazole and OPC-14857 were 0.9995 and 0.9999, respectively. The calibration concentrations were back-calculated from the peak response. Accuracy for aripiprazole was −3.6% to 5.5% at all concentrations. Accuracy for OPC-14857 was −0.6% to 1.6% at all concentrations.

3.3. Accuracy and precision

Accuracy and precision data for aripiprazole and OPC-14857 are summarised in Table 1. In the assay of aripiprazole, precision and accuracy data for the intra-day assay met the requirements at the LLOQ and at other concentrations, as did precision and accuracy data for the inter-day assay. Similarly, for OPC-14857, precision and accuracy data for intra-day and inter-day assays were within the required limits [3].

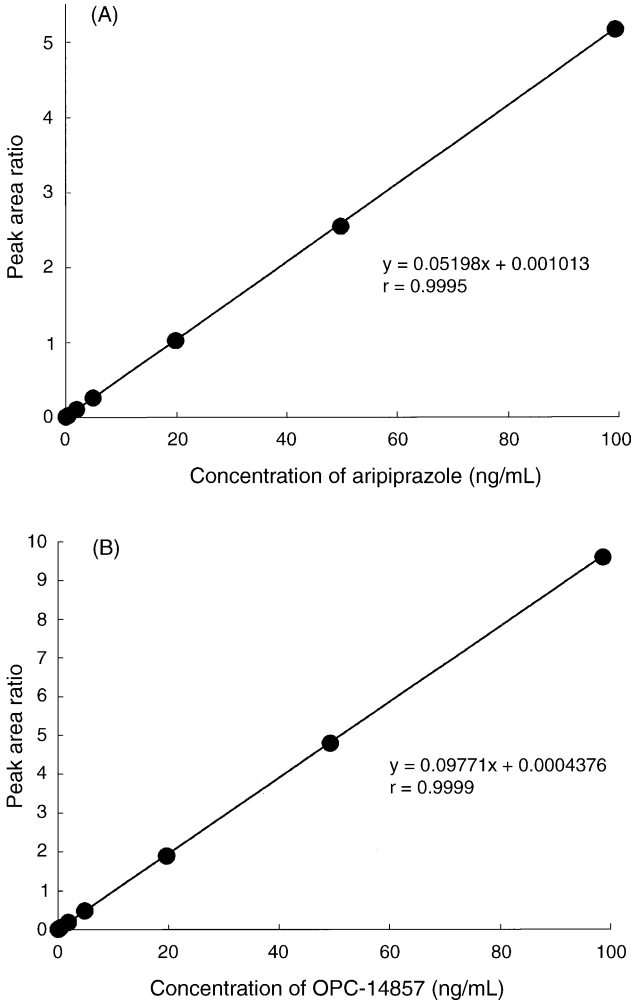

Fig. 4. Typical calibration curves of aripiprazole (A) and OPC-14857 (B).

Table 1
Intra-day and inter-day assay precision and accuracy of QC samples for aripiprazole and OPC-14857

	Theoretical concentration (ng/ml)				
	0.1 (LLOQ)	0.2	5	80	100 (ULOQ)
Aripiprazole					
Intra-day ($n = 5$)					
Precision (%)	5.1	–	1.7	–	1.4
Accuracy (%)	−12.0	–	4.8	–	2.8
Inter-day ($n = 5$)					
Precision (%)	–	6.2	3.7	1.5	–
Accuracy (%)	–	−8.0	1.8	5.4	–
OPC-14857					
Intra-day ($n = 5$)					
Precision (%)	15.9	–	1.6	–	1.3
Accuracy (%)	14.0	–	2.6	–	2.1
Inter-day ($n = 5$)					
Precision (%)	–	5.3	7.8	2.9	–
Accuracy (%)	–	−5.0	1.8	5.5	–

–, not examined.

Table 2
Extraction recovery for aripiprazole, OPC-14857, and the internal standard

	Theoretical concentration (ng/ml)		
	0.2	50	80
Aripiprazole			
Recovery (%)	91.5	–	93.2
OPC-14857			
Recovery (%)	83.1	–	93.6
OPC-14714			
Recovery (%)	–	84.4	–

All data generated in triplicate; –, not examined.

Table 3
Stability of aripiprazole and OPC-14857 in plasma and processed samples

	Theoretical concentration (ng/ml)	
	0.2	80
Aripiprazole		
−20 °C[a]	1.7	0.1
Freeze-thaw[b]	5.0	8.6
Processed samples[c]	−8.3	−7.0
OPC-14857		
−20 °C[a]	3.3	9.5
Freeze-thaw[b]	−3.3	6.7
Processed samples[c]	−8.3	−2.9

Figures represent the average ($n = 3$) % difference from the theoretical concentration.
[a] Stability in plasma for 3 months at −20 °C.
[b] Stability in plasma after three freeze-thaw cycles.
[c] Stability in final extracted solution for 72 h at 10 °C.

3.4. Recovery

Extraction recovery for aripiprazole and OPC-14587 from plasma was respectively 91.5% to 93.2% and 83.1% to 93.6%, and extraction recovery for the I.S. (OPC-14714) was 84.4% (Table 2).

3.5. Specificity

Typical chromatograms of LLOQ samples are shown in Fig. 3. There were no interfering peaks at the retention times of aripiprazole, OPC-14857, and the I.S. in the SRM chromatograms of six individual human plasma samples. Each peak had good shape and was clearly separable from the other peaks in the SRM chromatograms of LLOQ samples.

3.6. Stability

Stability results are shown in Table 3. Aripiprazole and OPC-14857 were shown to remain stable in human plasma after three freeze-thaw cycles. The two analytes were also found to be stable in human plasma for at least 3 months at −20 °C, and for 72 h in processed samples at 10 °C.

Aripiprazole, OPC-14857, and OPC-14714 (I.S.) were stable in stock solutions for at least 1 month at 4 °C.

4. Conclusion

An LC–MS/MS method for the determination of aripiprazole and its main active metabolite, OPC-14857, in human plasma was developed and validated.

This method met regulatory requirements for selectivity, sensitivity, precision, accuracy, and stability. Since validation, the method has been successfully applied to the analysis of clinical samples in pharmacokinetics, bioequivalence, and clinical studies of aripiprazole.

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