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Research Article

A sensitive column-switching HPLC method for aripiprazole and dehydroaripiprazole and its application to human pharmacokinetic studies

A simple and sensitive column-switching HPLC-UV method was developed for the simultaneous determination of aripiprazole, a novel atypical antipsychotic drug, and its active metabolite, dehydroaripiprazole in human plasma. Aripiprazole, its active metabolite and 7-[5-[4-(3-chloro-2-methylphenyl)-1-piperazinyl]pentyloxy]-3,4-dihydro-2(1H)-quinolinone (OPC-14558) as an internal standard were extracted from 1 mL of plasma using a mixture of chloroform/*n*-heptane (3:7, v/v), and the extract was injected into a column I (TSK BSA-ODS/S precolumn, 5 μm) for cleanup and column II (C_{18} STR ODS-II analytical column, 5 μm) for separation. Peaks were detected with an UV detector set at a wavelength of 254 nm, and the total time for chromatographic separation was ~20 min. Mean absolute recoveries were 74.0 and 74.7% for aripiprazole and dehydroaripiprazole, respectively. Intra- and inter-day CVs were less than 7.5 and 7.1% for aripiprazole concentrations ranging from 2 to 600 ng/mL, and 9.2 and 4.5% for dehydroaripiprazole concentrations ranging from 2 to 160 ng/mL. The validated concentration ranges for this method were 1–500 ng/mL and the limits of detection were 0.5 ng/mL for both aripiprazole and dehydroaripiprazole. This method was applied to pharmacokinetic study in human volunteers and patients taking aripiprazole.

Keywords: Aripiprazole / Column switching / Dehydroaripiprazole / HPLC
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1 Introduction

Aripiprazole (7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl] butoxy]-3,4-dihydro-2(1*H*)-quinolinone, Fig. 1) is a recent atypical antipsychotic drug that is effective for the treatment of patients with psychiatric disorders, especially schizophrenia [1, 2]. It has a unique pharmacological profile that consists of partial agonism at both the dopamine 2 (D_2) and the serotonin 1A ($5HT_{1A}$) receptors, and antagonism at the serotonin 2A ($5HT_{2A}$) receptor [3]. Similar to olanzapine and risperidone, aripiprazole is classified as a second-generation antipsychotic and compared with other second-generation antipsychotics has a lower side effect profile for weight gain, hyperlipidemia and hyperprolactinemia [4].

Aripiprazole is rapidly absorbed after oral administration, and its maximum plasma concentration is reached 3–5 h after dosing [5]. In addition, a steady-state concentration is achieved 14 days after initiation of aripiprazole treatment [6] (http://www.abilify.com). Aripiprazole is mainly metabolized by the cytochrome P450 (CYP) 3A4 and CYP2D6 in the liver and the active metabolite, dehydroaripiprazole has a similar pharmacological profile to the parent drug [2, 6, 7]. Therefore, it is clinically important to determine the detailed pharmacokinetics of dehydroaripiprazole. Hence, the simultaneous determination of aripiprazole and its active metabolite may improve the clinical effects of patients taking aripiprazole and may explain possible mechanisms of potential aripiprazole–drug interactions.

Several HPLC methods for the determination of aripiprazole concentrations have been reported previously [8–12]. There have been a few articles of chromatographic methods for the simultaneous determinations of aripiprazole and its metabolite that use MS [13–15], as well as a single report of an analytical HPLC method that uses diode array detection [16]. However, these methods are expensive for the routine analysis of antipsychotics in clinical situation and are not standard practice in most of hospitals. Therefore, new methods for the determination of aripiprazole and dehydroaripiprazole are required in the clinical setting, which are operationally simple, sensitive and cost effective. We describe a simple and sensitive HPLC method for the simultaneous determination of aripiprazole and dehydroaripiprazole in human plasma. In addition, the assay was validated using Food and Drug Administration guidance [17] (http://www.fda.gov/cder/guidance/4252fnl.pdf) and

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Abbreviation: IS, internal standard

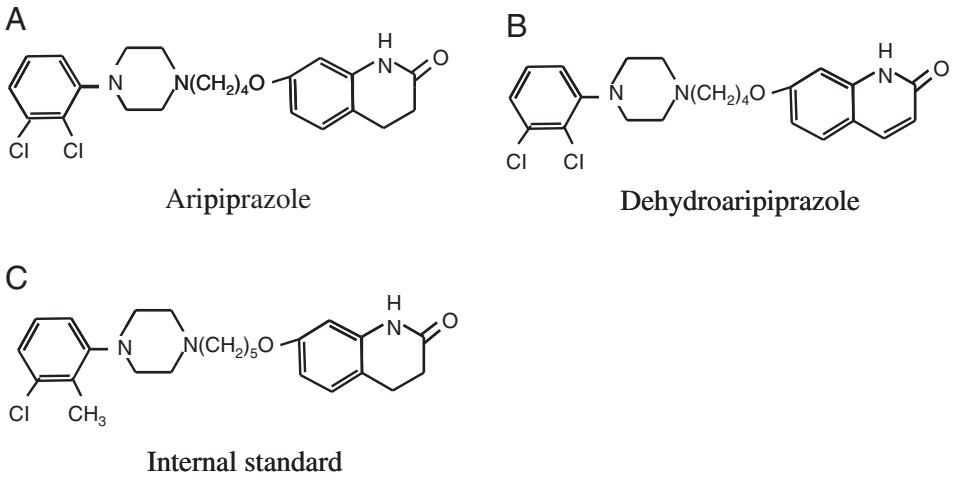

Figure 1. Chemical structures of aripiprazole (A), dehydroaripiprazole (B) and the IS (C).

was applied to pharmacokinetic studies of healthy volunteers and patients taking aripiprazole.

2 Materials and methods

2.1 Chemicals and reagents

Aripiprazole (purity 99.96%), its metabolite, dehydroaripiprazole (purity 99.85%) and OPC-14558 (purity 99.95%), as an internal standard (IS) (Fig. 1) were kindly provided by Otsuka Pharmaceutical (Tokyo, Japan). Potassium dihydrogen phosphate, acetonitrile, methanol, *n*-heptane, chloroform and perchloric acid were purchased from Wako Pure Chemical Industries (Osaka, Japan). Water was deionized and purified using a Milli-Q system (Millipore, Bedford, MA, USA). All chemicals used in this study were the high-purity ''HPLC grade''.

2.2 Preparation of stock and working solutions

Stock solutions of aripiprazole, dehydroaripiprazole and IS to generate standard curves were prepared by dissolving an appropriate amount of each compound in methanol to yield a concentration of 1.0 mg/mL. Working standard solutions of aripiprazole and dehydroaripiprazole (100, 10, 1 and 0.1 μg/mL) were prepared by serial dilution with methanol. The working standard solution of IS (10 μg/mL) was prepared by a 100 times dilution of the stock solution (1.0 mg/mL) with methanol. Stock solutions for analysis were stable at −30°C for at least 6 months.

2.3 Preparation of calibration standards and quality control samples

Drug-free plasma from healthy donors was used for validation studies. Calibration curves were prepared by spiking 10–50 μL of working solution in 1 mL of blank plasma to yield the final concentrations of aripiprazole (1, 5, 10, 50, 200 and 500 ng/mL plasma) and dehydroaripiprazole (1, 5, 10, 50, 200 and 500 ng/mL plasma). Standard curves were prepared daily and were constructed by linear regression analysis of the compounds/IS peak-area ratio *versus* the respective concentrations of aripiprazole and dehydroaripiprazole. Stock solutions of aripiprazole, dehydroaripiprazole and IS were prepared for quality controls in the same manner as for the standard curves. Quality control samples were obtained by spiking 16–80 μL of working plasma solution in 1 mL of blank plasma to yield the final concentrations of 2, 80, 250 and 600 ng/mL plasma for aripiprazole, and 2, 40, 80 and 160 ng/mL plasma for dehydroaripiprazole, and were stored at −30°C until analysis. All standard curves were checked using these quality control samples.

2.4 Sample preparation

The working standard solution of IS, 10 μL of 10 μg/mL and 0.5 mL NaOH (0.5 M) were added to 1 mL of plasma and 0.5 mL of water. The tubes were vortex mixed for 10 s and then 5 mL of chloroform/*n*-heptane (3:7, v/v) was added as an extraction solvent. After 10 min of vortex mixing, the mixture was centrifuged at 3000 × *g* for 10 min at 4°C (himac CF16RX, Hitachi, Tokyo, Japan), and the organic phase was evaporated *in vacuo* at 50°C to dryness (EYELA MG-2200, Tokyo Rikakikai, Tokyo, Japan). The residue was dissolved with 800 μL of phosphate buffer (pH 4.5, 0.02 M)/acetonitrile/perchloric acid (eluent A) (82.25:17.5:0.25, v/v/v) and a 500 μL aliquot was injected onto the column.

2.5 Instrumentation

The column-switching HPLC system consisted of two Shimadzu LC-10ADVP high-pressure pumps (for eluents

A and B), a Shimadzu CTO-10AVP column oven, and a Shimadzu Workstation LC solution chromatography integrator (Kyoto, Japan), a Shimadzu SPD-10AV (Kyoto, Japan) and a Shimadzu SIL-10ADVP (500 μL injection volume) (Tokyo, Japan). A TSK BSA-ODS/S precolumn was used for sample cleanup (column I: 10 mm × 4.6 mm id, particle size 5 μm; Tosoh, Tokyo, Japan) and a C_{18} STR ODS-II column was used as an analytical column (column II: 150 mm × 4.6 mm id, particle size 5 μm; Shinwa Chemical Industry, Kyoto, Japan).

2.6 Chromatographic conditions

The column-switching chromatographic conditions were set based on our previous report [18]. A 500 μL portion of the extract was automatically injected into the HPLC system. From 0 to 8.0 min after sample injection, the assay agents were separated from interfering substances present in the extract on column I with a mobile phase (eluent A) of phosphate buffer (pH 4.5, 0.02 M), acetonitrile and perchloric acid (60%) (82.25:17.5:0.25, v/v/v). Between 8.0 and 9.0 min after the injection, aripiprazole, dehydroaripiprazole and IS retained on column I were eluted with a mobile phase (eluent B) consisting of phosphate buffer (pH 4.5, 0.02 M), acetonitrile and perchloric acid (60%) (57.25:42.5:0.25, v/v/v), and the effluent from column I was switched to column II. Aripiprazole, dehydroaripiprazole and IS were separated on column II by eluting with eluent B (between 9.0 and 20 min). The flow rates of eluents A and B were 1.2 and 0.8 mL/min, respectively. The temperatures for columns I and II were 40°C, respectively. Peaks were detected by an UV detector set at a wavelength of 254 nm. The retention times of dehydroaripiprazole, aripiprazole and IS were 13.8, 15.3 and 17.6 min, respectively. The peak areas were used for the quantification of aripiprazole and dehydroaripiprazole.

2.7 Method validation

Intra- and inter-day precision and accuracy were evaluated by assaying quality control samples with different concentrations of aripiprazole and dehydroaripiprazole. Intra- and inter-day precision were assessed by analyzing six quality control samples at each concentration on the same day and mean values of a quality control sample over 6 days, respectively. The precision determined at each concentration level should not exceed 15% of the CV except for the lower LOQ, where it should not exceed 20% of the CV [17]. The limit of detection was defined as an analyte response that was at least five times greater than a blank response (signal-to-noise ratio = 5). Accuracy was calculated as percent error (relative error) ((measured concentration−spiked concentration)/spiked concentration) × 100 (%), whereas precisions were quantified by calculating intra- and inter-CV values. In addition, recovery from plasma was calculated by comparing the peak areas of pure standards prepared in working solution and injected directly onto the analytical column with those of extracted plasma samples containing the same amounts of the test compounds ($n = 6$ each).

2.8 Pharmacokinetic study design

Four healthy Japanese volunteers (two males and two females) and nine patients (two males and seven females) were enrolled in these studies after giving written informed consent.

For the healthy volunteers, the mean (±SD) age and body weight were 25.0 ± 0.8 years (range, 24–26 years) and 52.5 ± 5.8 kg (range, 47–60 kg), respectively. Each subject was deemed physically healthy by clinical examination and routine laboratory testing and had no history of significant medical illness or hypersensitivity to any drugs. The purpose of this pharmacokinetic study was to precisely determine how pharmacokinetic parameters of aripiprazole and dehydroaripiprazole could be defined in healthy subjects with this analytical method.

One tablet containing 6 mg of aripiprazole (Abilify®, Otsuka Pharmaceutical) was orally administered to each of the four healthy volunteers. Blood samples were obtained before and at 1, 2, 3, 4, 6, 8, 12, 24, 48, 72 and 168 h after the dosing. Blood samples of 10 mL were collected in heparinized tubes and were immediately centrifuged at 3000 × *g* for 10 min. Plasma samples were frozen and kept at −30°C until analyzed.

Data from the nine patients taking aripiprazole are summarized in Table 5. The purpose of this patient' study was to examine whether and to what extent the concentrations of aripiprazole and dehydroaripiprazole could be determined in patients taking aripiprazole.

Study protocols were approved by the Ethics Committee of Hirosaki University School of Medicine, and written informed consent was obtained from each participant prior to the study.

2.9 Pharmacokinetic analysis and statistical analyses

The maximum plasma concentration (C_{max}) of aripiprazole and its metabolite and the time to reach C_{max} (t_{max}) were determined directly from the individual concentration–time data. Pharmacokinetic parameters were calculated by noncompartmental methods. The terminal elimination rate constant (ke) was obtained by linear regression analysis using at least three sampling points of the terminal log–linear declining phase to the last measurable concentration. The apparent elimination half life ($t_{1/2}$) was calculated as 0.693 divided by ke. The area under the plasma concentration–time curve from time zero to the last sampling time (AUC_{0-t}) was calculated by the trapezoidal rule.

3 Results and discussion

3.1 Chromatographic optimization

This article describes a new method for the simultaneous determination of aripiprazole and dehydroaripiprazole in human plasma by column-switching HPLC-UV. Our goal was to develop a more sensitive HPLC method than had previously been reported for measuring patient samples in a clinical setting. In this study, there were no interfering peaks from endogenous substances with retention times similar to the peaks of aripiprazole and dehydroaripiprazole in blank plasma samples from healthy subjects (Fig. 2A). Lancelin *et al.* [16] reported an excellent HPLC method with diode array detection that used a simple liquid–liquid extraction procedure for aripiprazole and its metabolite, and achieved a low LOQ (2 ng/mL) for aripiprazole and dehydroaripiprazole. However, we achieved a 1 ng/mL LOQ for aripiprazole and dehydroaripiprazole by a liquid–liquid extraction with a different organic solvent mixture (chloroform/*n*-heptane) and an automated column-switching system for plasma sample cleanup.

Additionally, there has been only one assay reported that combines an online column-switching purification and UV detection [10], but it was not used to analyze dehydroaripiprazole and its sensitivity was greater than 50 ng/mL for aripiprazole. To our knowledge, this report is the first that describes a column-switching method with UV detection that can simultaneously determine the amount of both aripiprazole and its metabolite in a human pharmacokinetic study and was validated in-line using Food and Drug Administration criteria. This study demonstrates a more sensitive method for monitoring plasma concentrations of aripiprazole and its metabolite up to 168 h after a 6 mg dose in human volunteers as well as for determining both drug concentrations from patients at steady state.

3.2 Linearity

Calibration curves were linear over the concentration range from 1 to 500 ng/mL ($r^2 = 0.9997$, and $F = 39\,504.5$, $p < 0.001$, $n = 6$) for aripiprazole, and from 1 to 500 ng/mL ($r^2 = 0.9998$, and $F = 94\,626.5$, $p < 0.001$, $n = 6$) for dehydroaripiprazole (Table 1). An *F*-test on lack-of-fit at the 99% confidence level was performed, and it exhibited nonsignificance (Table 1).

3.3 Specificity and sensitivity

The chromatogram of an extracted blank plasma sample is shown in Fig. 2A. A typical chromatogram of the working solution is shown in Fig. 2B; the retention times for dehydroaripiprazole, aripiprazole and IS were 13.8, 15.3, and 17.6 min, respectively. The chromatogram of an extracted sample spiked with 1 ng/mL each of aripiprazole and dehydroaripiprazole and 100 ng/mL of IS is shown in Fig. 2C. The chromatograms of extracted plasma samples obtained from one patient receiving 6 mg of aripiprazole did not have any interfering peaks (Fig. 2D). This patient repeated oral doses (6 mg) of aripiprazole for at least 4 wk, and the plasma concentrations of aripiprazole and dehydroaripiprazole were determined to be 146.4 and 41.0 ng/mL, respectively.

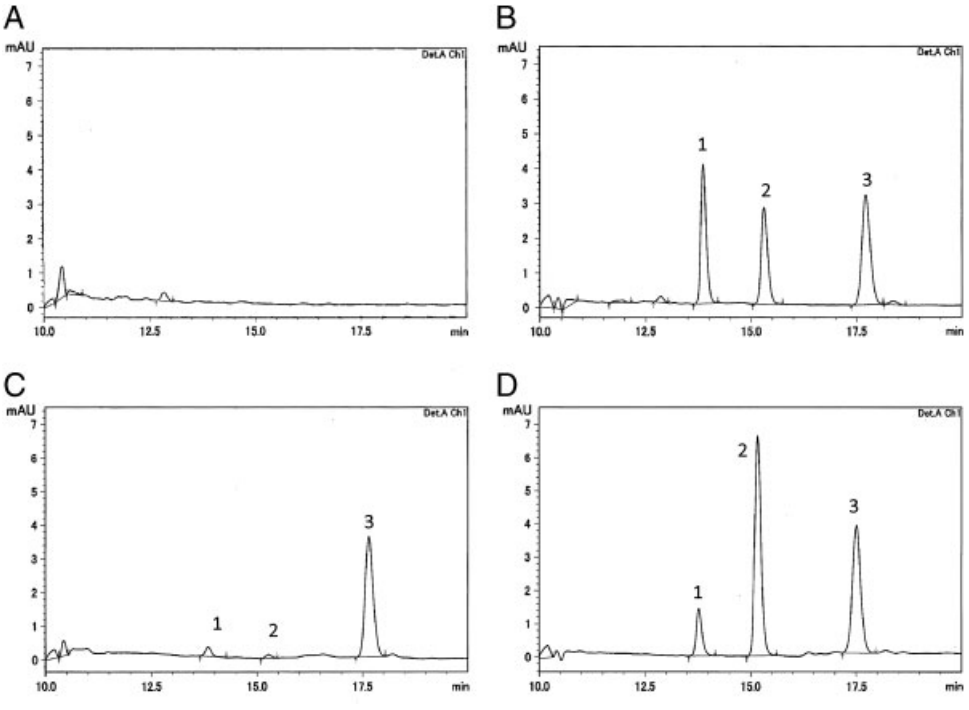

Figure 2. Typical chromatograms of an extracted blank plasma sample (A), working solution (B), an extracted sample spiked with 1 ng/mL each of aripiprazole and dehydroaripiprazole and 100 ng/mL of IS (C), and extracted plasma samples obtained from one patient receiving 6 mg of aripiprazole; the plasma concentrations of aripiprazole and dehydroaripiprazole were 146.4 and 41.0 ng/mL (D). Peaks: 1, dehydroaripiprazole; 2, aripiprazole and 3, IS.

Table 1. Individual and mean value for slope, intercepts, correlation coefficients and *F*-values of six calibration curves for analytes

Analyte	Curve	Slope	Intercepts	r^2	*F*-test for lack of linearity		Concentration added (ng/mL)	Found (mean ± SD)	Accuracy (%)	CV (%)	*n*
					F	*p*-value					
Aripiprazole	1	0.0100	0.0112	0.9998	38 752.5	<0.001	1	0.99 ± 0.12	98.82	12.14	6
	2	0.0092	0.0124	0.9999	104 177.9	<0.001	5	5.13 ± 0.25	102.51	5.06	6
	3	0.0096	0.0268	0.9998	28 386.9	<0.001	10	9.93 ± 0.38	99.30	3.87	6
	4	0.0099	0.0339	0.9995	25 994.7	<0.001	50	49.78 ± 0.49	99.57	0.99	6
	5	0.0098	0.0285	0.9995	6250.7	<0.001	200	202.98 ± 6.85	101.49	3.37	6
	6	0.0096	0.0284	0.9997	33 464.5	<0.001	500	499.93 ± 11.56	99.99	2.31	6
	Mean	0.0097	0.0235	0.9997	39 504.5						
	SD	0.0003	0.0094								
	SE	0.0001	0.0038								
Dehydroaripiprazole	1	0.0096	0.0376	0.9996	18 477.5	<0.001	1	0.91 ± 0.11	90.92	12.09	6
	2	0.0088	0.0312	0.9997	7606.2	<0.001	5	5.10 ± 0.13	101.95	2.63	6
	3	0.0088	0.0480	0.9999	491 618.9	<0.001	10	10.28 ± 0.19	102.80	1.85	6
	4	0.0084	0.0220	0.9999	26 740.6	<0.001	50	49.20 ± 3.26	98.40	6.63	6
	5	0.0083	0.0492	0.9998	11 087.6	<0.001	200	201.47 ± 5.72	100.74	2.84	6
	6	0.0080	0.0522	0.9998	12 228.1	<0.001	500	501.67 ± 13.37	100.33	2.67	6
	Mean	0.0087	0.0400	0.9998	94 626.5						
	SD	0.0006	0.0119								
	SE	0.0002	0.0048								

Table 2. Coadministrated drugs which do not interfere with aripiprazole or dehydroaripiprazole chromatography

Alprazolam	Diazepam	Nifedipine	Triazolam
Amitriptyline	Donepezil	Nitrazepam	Trihexyphenidyl
Amlodipine	Estazolam	Olanzapine	Troxipide
Amoxapine	Etizolam	Omeprazole	Valproate sodium
Atorvastatin	Famotidine	Paroxetine	Valsartan
Bezafibrate	Fexofenadine	Perospirone	Zolpidem
Biperiden	Flunitrazepam	Phenytoin	Zonisamide
Bisoprolol	Fluvoxamine	Pitavastatin	Zopiclone
Bromazepam	Furosemide	Propranolol	Zotepine
Brotizolam	Imipramine	Quetiapine	
Candesartan	Lamotrigine	Rebamipide	
Carbamazepine	Lithium carbonate	Risperidone	
Carvedilol	Lorazepam	Sertraline	
Cetirizine	Lormetazepam	Sulpiride	
Clobazam	Mianserin	Tandospirone	
Clonazepam	Milnacipran	Teprenone	
Clotiazepam	Mosapride	Tiapride	
Cloxazolam	Nicergoline	Topiramate	

The limit of detection, defined as an analyte' response that was at least five times greater than a blank response (signal-to-noise ratio = 5) was 0.5 ng/mL for both aripiprazole and dehydroaripiprazole. The lowest standard on the calibration curve was defined as the LOQ in which the analyte peaks for both compounds were identifiable, discrete and reproducible with a precision of 20% and an accuracy of 80–120%. The LOQs were 1 ng/mL for both aripiprazole and dehydroaripiprazole.

Potential interference from coadministered drugs in patients with psychiatric disorders was assessed by processing real patient plasma samples according to the method described and was found to be insignificant. The spectrum of drugs tested is listed in Table 2. There was no chromatographic interference from 63 drugs with respect to retention times similar to those of aripiprazole, dehydroaripiprazole and IS. These results show that the present method is more specific than a previous HPLC method [16] and the total time (within 20 min) for chromatographic separation may be clinically suitable.

3.4 Recovery (extraction efficiency) from matrix

Mean absolute recoveries were 71.6–75.6% for aripiprazole at 2, 250 and 600 ng/mL, and 71.8–76.5% for dehydroaripiprazole at 2, 80 and 160 ng/mL, respectively; their mean CV values were 2.9 and 3.3%, respectively. The mean recovery for the IS was 71.3%.

3.5 Accuracy and precision

Accuracy and precision are summarized in Tables 1 and 3. These extracts underwent the same column-switching procedure. Intra- and inter-day relative SDs were less than 7.5 and 7.1% for aripiprazole, and 9.2 and 4.5% for dehydroaripiprazole, respectively.

3.6 Stability

Stock solutions (methanol solution) of aripiprazole, dehydroaripiprazole and IS, and spiked aripiprazole and dehy-

Table 3. Precision and accuracy for the determination of analytes in spiked plasma ($n = 6$)

Analyte	Concentration added (ng/mL)	Found (mean ± SD) (ng/mL)	Accuracy (%)	Intra-day		Found (mean ± SD) (ng/mL)	Accuracy (%)	Inter-day	
				CV (%)	Relative error (%)			CV (%)	Relative error (%)
Aripiprazole	2	2.01 ± 0.15	100.53	7.46	0.53	2.03 ± 0.14	101.56	7.13	1.56
	80	82.12 ± 1.44	102.65	1.75	2.65	80.91 ± 2.23	101.14	2.76	1.14
	250	249.07 ± 7.77	99.63	3.12	−3.37	253.47 ± 10.43	101.39	4.11	1.39
	600	604.65 ± 20.44	100.78	3.38	0.78	596.71 ± 20.18	99.45	3.39	−0.55
Dehydroaripiprazole	2	1.99 ± 0.18	99.56	9.23	−0.44	2.01 ± 0.07	100.34	3.41	0.34
	40	38.74 ± 2.09	96.85	5.39	−3.15	41.02 ± 1.86	102.55	4.54	2.55
	80	77.88 ± 1.42	97.34	1.83	−2.66	79.05 ± 2.89	98.81	3.65	−1.19
	160	161.50 ± 6.03	100.94	3.74	0.94	158.43 ± 4.50	99.02	2.84	−0.98

droaripiprazole in blank plasma were stable at −30°C for at least 6 months. Plasma samples from the pharmacokinetic study were stored at −30°C and were analyzed within a month after sampling. Aripiprazole, dehydroaripiprazole and IS in extracts from plasma samples reconstituted in phosphate buffer/acetonitrile/perchloric acid (82.25:17.5:0.25, v/v/v) were stable at room temperature for 72 h in an autosampler. Three freeze–thaw cycles at −30°C did not affect the stability of aripiprazole and dehydroaripiprazole in plasma, as shown by the little deviation from nominal concentrations, which were within the acceptable limits of ±10% at all concentration levels. The stability experiments were performed in triplicate for two plasma concentrations (2 and 80 ng/mL).

3.7 Pharmacokinetic study

Figure 3 shows concentration *versus* time curves obtained after oral administration of aripiprazole (6 mg). The mean kinetic parameters of aripiprazole and dehydroaripiprazole are summarized in Table 4. The plasma concentrations at all sampling points were measurable for both drugs. There were inter-individual variabilities in not only plasma concentrations but also pharmacokinetic parameters for aripiprazole and dehydroaripiprazole.

The pharmacokinetic data of nine patients taking aripiprazole are summarized in Table 5. Plasma concentrations of aripiprazole were higher than those of dehydroaripiprazole in all patients. Accordingly, the concentration of dehydroaripiprazole in patients was 43.5% of the aripiprazole concentration at steady state. Therefore, these results combined with the healthy volunteer study results show that the dehydroaripiprazole concentration significantly increased in patients at steady state and the sum of both drugs concentrations may control the clinical effects. Although one patient (patient 6 in Table 5) showed very low aripiprazole and dehydroaripiprazole plasma concentrations, this patient did not dose aripiprazole for a long period of time. No interfering peaks were observed, despite the fact that 14 other drugs including alprazolam, biperiden and brotizolam were coadministered in patients.

Table 4. Pharmacokinetic parameters of aripiprazole and dehydroaripiprazole receiving aripiprazole 6 mg dose in four healthy subjects

	Parameters	Mean ± S.D.
Aripiprazole	C_{max} (ng/mL)	37.51 ± 1.41
	T_{max} (h)	2.50 ± 0.58
	$T_{1/2}$ (h)	61.13 ± 31.14
	AUC_{0-168} (ng h/mL)	1381.40 ± 464.80
Dehydroaripiprazole	C_{max} (ng/mL)	2.73 ± 0.41
	T_{max} (h)	78.00 ± 63.12
	$T_{1/2}$ (h)	270.14 ± 44.08
	AUC_{0-168} (ng h/mL)	243.31 ± 32.69

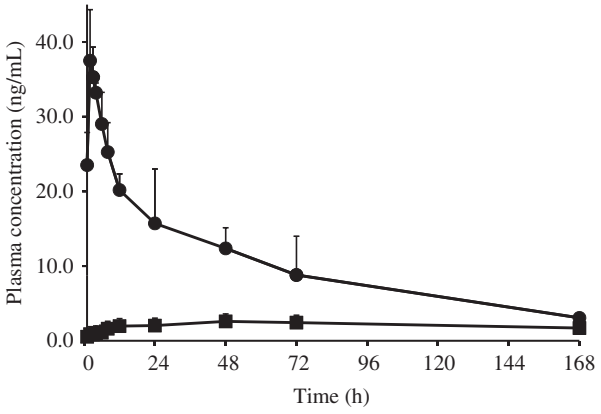

Figure 3. Plasma concentration–time curves (mean+SD) of aripiprazole (close circles) and dehydroaripiprazole (close squares) from 0 to 168 h in four healthy volunteers after a single 6 mg oral dose of aripiprazole.

4 Concluding remarks

The HPLC procedure described for the determination of aripiprazole and dehydroaripiprazole is suitable for human pharmacokinetic studies although it is somewhat time consuming. Satisfactory validation data were achieved for

Table 5. Clinical profile of nine patients taking aripiprazole

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Gender	F	F	F	M	F	M	F	F	F
Age (years)	21	29	36	17	45	32	19	33	23
Body weight (kg)	78	46	62	56	56	75	50	46	45
Aripiprazole dose administered (mg)	12	12	6	9	12	12	6	18	6
Aripiprazole concentration (ng/mL)	213.93	78.03	35.9	135.32	435.52	2.63	146.44	482.43	152.2
Dehydroaripiprazole concentration (ng/mL)	45.92	58.37	14.47	93.03	192.69	1.04	41.04	150.7	68.76

linearity, precision and recovery. The observed LOQ allows the measurement of therapeutic concentrations of aripiprazole and its metabolite.

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The authors have declared no conflict of interest.

5 References

[1] Raggi, M. A., Mandrioli, R., Pucci, V., Sabbioni, C., *Med. Chem. Rev.* 2004, *1*, 299–316.

[2] Harrison, T. S., Perry, C. M., *Drugs* 2004, *64*, 1715–1736.

[3] Shapiro, D. A., Renock, S., Arrington, E., Chiodo, L. A., Liu, L. X., Sibley, D. R., Roth, B. L., Mailman, R., *Neuropsychopharmacology* 2003, *28*, 1400–1411.

[4] Kerwin, R., Millet, B., Herman, E., Banki, C. M., Lublin, H., Pans, M., Hanssens, L., L'Italien, G., McQuade, R. D., Beuzen, J. N., *Eur. Psychiatry* 2007, *22*, 433–443.

[5] Mallikaarjun, S., Salazar, D. E., Bramer, S. L., *J. Clin. Pharmacol.* 2004, *44*, 179–187.

[6] Bristol-Myers Squibb Company and Otsuka America Pharmaceutical Inc. Abilify®, aripiprazole, tablets prescribing information, 2007.

[7] Kubo, M., Koue, T., Inaba, A., Takeda, H., Maune, H., Fukuda, T., Azuma, J., *Drug Metab. Pharmacokinet.* 2005, *20*, 55–64.

[8] Shimokawa, Y., Akiyama, H., Kashiyama, E., Koga, T., Miyamoto, G., *J. Chromatogr. B* 2005, *821*, 8–14.

[9] Musenga, A., Saracino, M. A., Spinelli, D., Rizzato, E., Boncompagni, G., Kenndler, E., Raggi, M. A., *Anal. Chim. Acta* 2008, *612*, 204–211.

[10] Kirschbaum, K. M., Mueller, M. J., Zernig, G., Saria, A., Mobascher, A., Malevani, J., Hiemke, C., *Clin. Chem.* 2005, *51*, 1718–1721.

[11] Zuo, X. C., Wang, F., Xu, P., Zhu, R. H., Li, H. D., *Chromatographia* 2006, *64*, 387–391.

[12] Kirchherr, H., Kuehn-Velten, W. N., *J. Chromatogr. B* 2006, *843*, 100–113.

[13] Choong, E., Rudaz, S., Kottelat, A., Guillarme, D., Veuthey, J. L., Eap, C. B., *J. Pharm. Biomed. Anal.* 2009, *50*, 1000–1008.

[14] Song, M., Xu, X., Hang, T., Xu, X., Hang, T., Wen, A., Yang, L., *Anal. Biochem.* 2009, *385*, 270–277.

[15] Kubo, M., Mizooku, Y., Hirao, Y., Osumi, T., *J. Chromatogr. B* 2005, *822*, 294–299.

[16] Lancelin, F., Djebrani, K., Tabaouti, K., Kraoul, L., Brovedani, S., Paubel, P., Piketty, M. L., *J. Chromatogr. B* 2008, *867*, 15–19.

[17] US Department of Health and Human Services, Food and Drug Administration, Guidance for Industry Bioanalytical Method Validation, 2001.

[18] Yasui-Furukori, N., Inoue, Y., Tateishi, T., *J. Chromatogr. B* 2003, *789*, 239–245.

