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High performance liquid chromatographic methods for the determination of aripiprazole with ultraviolet detection in rat plasma and brain: Application to the pharmacokinetic study

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Abstract

High performance liquid chromatographic (HPLC) methods were validated for the determination of aripiprazole (OPC-14597, Abilify™) in rat plasma and brain. Separation was by Nova-pak phenyl column; flow rate, 1.0 ml/min; mobile phase, acetonitrile–methanol–20 mM sodium sulfate–acetic acid (27:25:48:1, v/v/v/v); UV detection at 254 nm. Reproducibility in plasma and brain showed excellent precision (within 7.8 and 10.6%) and accuracy (96.0–102.4% and 99.0–108.7%) with calibration curve ranges 10.0–2000 ng/ml and 30.0–6000 ng/g, respectively. Validated HPLC methods were successfully applied to pharmacokinetic study of aripiprazole in rats, demonstrating brain concentrations after oral administration five times higher than plasma concentrations.

Keywords: Aripiprazole; OPC-14597; Abilify™

1. Introduction

Aripiprazole (OPC-14597, Abilify™), 7-(4-[4-(2,3-dichlorophenyl)-1-piperazinyl]butoxy)-3,4-dihydro-2(1*H*)-quinolinone, was synthesized and screened [1] by Otsuka Pharmaceutical Co. Ltd. (Tokyo, Japan) as a novel compound for the purpose of improving both negative and positive symptoms of schizophrenia without inducing extrapyramidal side effects. Aripiprazole is a novel atypical antipsychotic drug [2], and has already been on the market in the USA, European countries and several other countries. It is distinguished from all other antipsychotics by its unique pharmacologic profile – i.e. partial agonist activity at dopamine D_2 receptors [3], partial agonist activity at serotonin 5-HT_{1A} receptors [4], and antagonist activity at serotonin 5-HT_{2A} receptors [5]. In clinical studies, aripiprazole has been shown to improve both positive and negative symptoms in patients with schizophrenia and schizoaffective disorder [6–8]. This novel agent has also demonstrated a favorable and excellent safety profile and tolerability with a low liability for extrapyramidal symptoms and sedation, and no evidence for an increased risk of weight gain, prolactin elevation and QT_c prolongation [9,10].

It was thus considered that the measurement of concentrations of aripiprazole in the animal brain was important and worthwhile to understand the profile of aripiprazole in the human. The purpose of this work is to develop and validate simple, sensitive and accurate High performance liquid chromatographic (HPLC) methods for measuring aripiprazole in rat plasma and brain that can be used in the study of the distribution of aripiprazole in the rat. This is the first published report of analytical methods to determine aripiprazole in rat plasma and brain. In order to confirm whether the assay methods are accurate, the method validation was carried out in compliance with the guidance of the US Food and Drug Administration [11].

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2. Experimental

2.1. Materials

Aripiprazole (OPC-14597, Abilify^{TM}) and the I.S. (OPC-14558) were supplied by Otsuka Pharmaceutical Co. Ltd. (Tokyo, Japan). Their chemical structures are shown in Fig. 1. Heparinized blank plasma and brain of Sprague–Dawley rat were purchased from Kitayama Labes Co. Ltd. (Nagano, Japan) and Japan SLC Inc. (Shizuoka, Japan), respectively. All other chemicals were of the high purity available commercially.

2.2. Chromatographic system

The HPLC systems consisted of a LC-10A system (Shimadzu, Kyoto, Japan) equipped with a LC-10AD pump, a SIL-10AXL auto-injector, a SPD-10A UV detector for plasma, and a Waters HPLC system (Waters Associates, Milford, MA, USA) equipped with model 510 high-pressure pump, a model 717 automatic sample processor, a model 486 UV detector and a model C-R7A plus chromatopac (Shimadzu) for brain. The HPLC separation was achieved using a Nova-pak phenyl (150 mm × 3.9 mm i.d., 4 μm particle size, Waters) at 25 °C. The mobile phase was a mixture of acetonitrile–methanol–20 mM sodium sulfate–acetic acid (27:25:48:1, v/v/v/v). The effluent was monitored with UV detection at 254 nm at a flow rate of 1.0 ml/min. The quantification was accomplished based on the peak height ratio of aripiprazole to the I.S. Data calculations were processed with a pharmacokinetic data processing system (Otsuka Pharmaceutical Co. Ltd.) and a personal computer using Excel version 97 from Microsoft Co. (Redmond, WA, USA).

2.3. Preparation of standard solutions and standards

2.3.1. Rat plasma

Aripiprazole was dissolved in methanol to prepare working solutions of 100, 80, 25, 5, 1.25, 1 and 0.5 μg/ml. The I.S. was dissolved in methanol to prepare a working solution of 20 μg/ml. Working solutions of aripiprazole and the I.S. were stored at 4 °C and protected from light, and were stable for at least 33 and 39 days, respectively.

The calibration curves at the concentration range of 10.0–2000 ng/ml were prepared by spiking 10 μl of working solutions of aripiprazole to 0.5 ml of blank plasma. The quality control (QC) samples at eight concentration levels (10.0, 20.0, 25.0, 100, 200, 500, 1600 and 2000 ng/ml) were also prepared.

2.3.2. Rat brain

Aripiprazole was dissolved in methanol to prepare working solutions of 200, 160, 100, 20, 5, 2 and 1 μg/ml. The I.S. was dissolved in methanol to prepare a working solution of 20 μg/ml. These working solutions were stored at 4 °C and protected from light.

Brain was homogenized with two volumes of physiological saline. The calibration curves at the concentration range of 30.0–6000 ng/g were prepared by spiking 10 μl of working solutions of aripiprazole to 1 ml of blank brain homogenate. The QC samples at seven concentration levels (30.0, 60.0, 150, 600, 3000, 4800 and 6000 ng/g) were also prepared.

2.4. Extraction procedure

2.4.1. Rat plasma

To 0.5 ml of plasma sample, 10 μl of the I.S. working solution and 100 μl of 2 M hydrochloric acid were added. The mixture was shaken for 10 min with 5 ml of diethyl ether, and centrifuged at 1800 × *g* for 10 min at room temperature. After the organic layer was removed, 200 μl of 2 M sodium hydroxide and 5 ml of diethyl ether were added to the aqueous layer. The mixture was shaken for 10 min again, and then centrifuged at 1800 × *g* for 10 min at room temperature. Four millilitres of the organic layer was transferred to the other clean tube, and evaporated to dryness at 40 °C under a nitrogen gas stream. The extraction residue was reconstituted in 100 μl of acetonitrile–methanol–water–acetic acid (27:25:48:1, v/v/v/v), and a 30 μl aliquot was injected into HPLC system.

2.4.2. Rat brain

To 1 ml of brain homogenate sample, 10 μl of the I.S. working solution was added. The homogenate was deproteinized with 3 ml of acetonitrile, and centrifuged at 1800 × *g* for 10 min at room temperature. The supernatant (3.5 ml) was transferred to the other clean tube, and concentrated to remove acetonitrile at 40 °C under a nitrogen gas stream, and then 100 μl of 2 M hydrochloric acid was added. Aripiprazole and the I.S. were extracted in the same manner with the plasma.

2.5. Method validation parameters

2.5.1. Selectivity

Six different blank plasma and brain homogenate samples were extracted and analyzed to investigate whether intrinsic substances interfere with the analytical system.

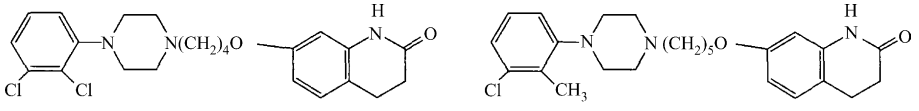

Fig. 1. Chemical structures of aripiprazole and the internal standard.

2.5.2. Calibration curve

The calibration curve samples were prepared in plasma at five concentration levels of 10.0, 25.0, 100, 500 and 2000 ng/ml for aripiprazole on 7 working days. In brain, the calibration curve samples were prepared at 5 concentration levels of 30.0, 150, 600, 3000 and 6000 ng/g for aripiprazole on 3 working days. The calibration curves were assessed by the coefficients of correlation (r) of the logarithmic equation using $\log Y = a \log X + b$ (a: slope, b: intercept, Y: peak height ratio, X: concentration) and the accuracy of the back-calculated concentrations for each concentration level.

2.5.3. Lower limit of quantification and intra-day assay precision and accuracy

The QC samples ($n = 5$) at five concentration levels (10.0, 25.0, 100, 500 and 2000 ng/ml in plasma, and 30.0, 150, 600, 3000 and 6000 ng/g in brain) were extracted and analyzed by 1 working day. The precision and accuracy were calculated for each concentration level to assess the lower limit of quantification and the intra-day assay variation.

2.5.4. Inter-day assay precision and accuracy

The QC samples ($n = 15$) at three concentration levels (10.0, 100 and 2000 ng/ml in plasma, and 30.0, 600 and 6000 ng/g in brain) were extracted and analyzed by 3 working days. The precision and accuracy were calculated for each concentration level to assess the inter-day assay variation.

2.5.5. Extraction recovery

Extraction recovery was evaluated with QC samples ($n = 3$) at two concentration levels (25.0 and 1600 ng/ml in plasma, and 60.0 and 4800 ng/g in brain) for aripiprazole, and with QC samples ($n = 6$) at one concentration level (400 ng/ml in plasma, and 600 ng/g in brain) for the I.S. Recovery was calculated by the comparison with their peak area and the corresponding control ones.

2.5.6. Stability of short-term storage

The stability of aripiprazole in plasma and brain homogenate following 24 h at room temperature and three freeze (−20 °C)/thaw cycles was tested with QC samples ($n = 3$) at two concentration levels (25.0 and 1600 ng/ml in plasma, and 60.0 and 4800 ng/g in brain). The QC samples kept 24 h at room temperature and after three freeze–thaw cycles were analyzed with the control QC ones. The stability was evaluated by the difference from the mean of control concentrations, and expressed as the percentage remaining (%).

2.5.7. Stability of long-term storage

The stability of aripiprazole in plasma and brain homogenate stored at −20 °C was tested with QC samples ($n = 3$) at three concentration levels (20.0, 200 and 1600 ng/ml in plasma, and 60.0, 600 and 4800 ng/g in brain). The samples were stored at −20 °C, and analyzed after 0, 3 and 6 months in plasma, and 0, 4 and 8 weeks in brain. The stability was evaluated by the difference from the mean of initial concentrations, and expressed as the percentage remaining (%).

2.6. Application for pharmacokinetic study

Plasma and brain samples were collected at 0.5, 1, 2, 3, 4, 6, 8 and 12 h after single oral administration of aripiprazole at 10 and 30 mg/kg to male Sprague–Dawley rats under fasted conditions. These samples were stored at −20 °C until the quantification. The pharmacokinetic parameters, t_{max} (time to maximum concentration), C_{max} (maximum concentration), AUC_{th} (area under concentration-time curve from 0 h to the last observable concentration at time t), $t_{1/2}$ (apparent elimination half-life) and brain concentration to plasma concentration ratio were calculated based on the concentrations of aripiprazole in the plasma and brain samples. The pharmacokinetic parameters were calculated by non-compartment analysis using WinNonlin Standard (Version 3.1, Pharsight Co., CA, USA).

3. Results and discussion

3.1. Development of assay methods

Aripiprazole (OPC-14597, Abilify™) was synthesized and screened [1] by Otsuka Pharmaceutical Co. Ltd. as a novel compound for the purpose of improving both negative and positive symptoms of schizophrenia without inducing extrapyramidal side effects. As its pharmacological profile, the agent was expected to be effective at low doses. Therefore, it was necessary to quantify nanogram ranges in plasma and brain. In general, HPLC is often used as highly simple, sensitive and accurate assay methods for the analysis of drugs. We therefore developed HPLC methods for the determination of aripiprazole with UV detection in rat plasma and brain.

Though the UV absorption spectrum of aripiprazole possessed peaks at 217, 254 and 284 nm, 254 nm was selected for the assay depending on the absorbance and the interference from biological substances. Aripiprazole and the I.S. could be extracted by using diethyl ether under neutral and basic conditions, but could not be extracted under acidic conditions. In order to decrease the interference from biological substances in plasma, a washing process by diethyl ether under acidic conditions was added before the extraction process under basic conditions. In brain, the homogenate diluted to 3 times by saline was used. Acetonitrile was selected as the solvent for deproteinization, before the extraction process by diethyl ether. There was no interference peak detected in the elution positions of aripiprazole and the I.S. for the extracted plasma and brain homogenate. The calibration curve was constructed in 10.0–2000 ng/ml for plasma, and it was constructed in 30.0–6000 ng/g for brain. The HPLC methods

developed in rat plasma and brain suggested that validation would be achieved.

3.2. Method validation

3.2.1. Selectivity

Six different lots of plasma and brain homogenate were extracted and analyzed as blank samples. The typical chromatograms for the plasma samples are shown in Fig. 2. No interference peak was detected in the eluting positions of aripiprazole and the I.S. in six blank chromatograms.

3.2.2. Calibration curve

The parameters for the calibration curve and back-calculated concentrations of the calibration curves in plasma and brain are shown in Table 1. The calibration curves were constructed over 10.0–2000 ng/ml in plasma, and 30.0–6000 ng/g in brain. The coefficients of correlation (r) in plasma and brain were 0.9998 and 0.9994, respectively. The mean accuracy in the back-calculated concentrations was 98.5–101.8% in plasma and 96.3–103.2% in brain.

3.2.3. Lower limit of quantification and intra-day assay precision and accuracy

The precision and accuracy at the lower limit of quantification (LLOQ) and the intra-day assay variation in plasma and brain are shown in Table 2. The precision at LLOQ was 2.0% in plasma and 5.2% in brain. The accuracy at LLOQ was 102.0% in plasma and 108.7% in brain. The intra-day assay precision was within 2.0% in plasma and within 10.6% in brain, and the intra-day assay accuracy was 96.0–102.4% in plasma and 99.0–108.7% in brain. The methods in plasma and brain showed excellent reproducibility and accuracy.

3.2.4. Inter-day assay precision and accuracy

The precision and accuracy of the inter-day assay variation in plasma and brain are shown in Table 3. The inter-day assay precision was within 7.8% in plasma and within 9.6% in brain. The inter-day assay accuracy was 96.0–102.0% in plasma and 99.8–104.0% in brain. The methods showed excellent reproducibility and accuracy.

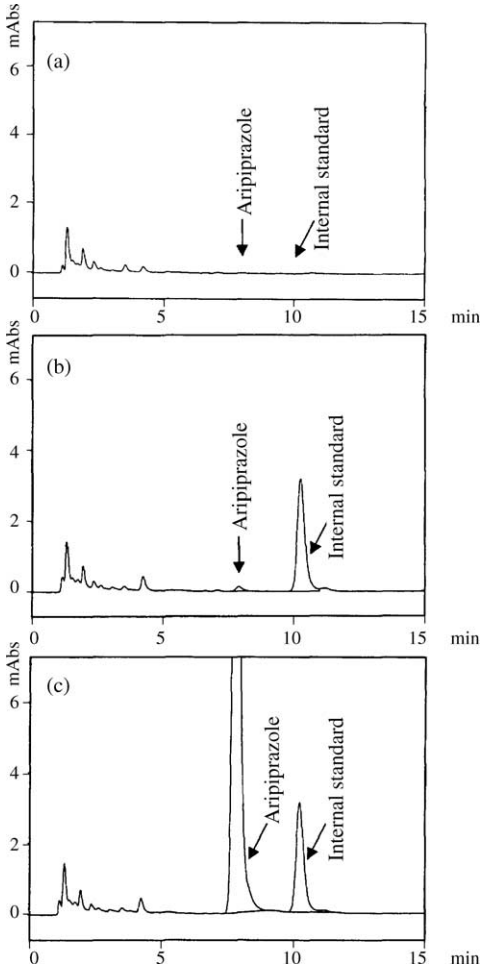

Fig. 2. HPLC chromatograms of extracted rat plasma. (a) Blank plasma, (b) plasma spiked with 10.0 ng/ml of aripiprazole (LLOQ) and the I.S., and (c) plasma spiked with 2000 ng/ml of aripiprazole and the I.S. The retention times of aripiprazole and the I.S. are 7.8 and 10.2 min, respectively.

3.2.5. Extraction recovery

The extraction recovery of aripiprazole and the I.S. from rat plasma and brain homogenate is shown in Table 4. The mean recovery of aripiprazole was 87.2–91.1% in plasma and 41.3–43.5% in brain. The mean recovery of the I.S. was 87.7% in plasma and 36.9% in brain. The reason for the lower recovery of both compounds from brain as com-

Table 1
Calibration curve parameters and back-calculated concentrations of the calibration curves for aripiprazole in rat plasma and brain

Matrix	Calibration curves	Back-calculated concentrations (ng/ml)					Curve parameters		
		10.0	25.0	100	500	2000	Slope	Intercept	r
Plasma	Mean (n = 7)	9.97	25.0	100	509	1970	0.9710	0.5657	0.9998
	Accuracy (%)	99.7	100.0	100.0	101.8	98.5	–	–	–
Matrix	Calibration curves	Back-calculated concentrations (ng/g)					Curve parameters		
		30.0	150	600	3000	6000	Slope	Intercept	r
Brain	Mean (n = 3)	29.4	154	619	2890	6070	1.0135	−2.5984	0.9994
	Accuracy (%)	98.0	102.7	103.2	96.3	101.2	–	–	–

The data result from the analysis on 7 and 3 working days in plasma and brain samples, respectively.

Table 2
Lower limit of quantification and intra-day assay precision and accuracy for aripiprazole in rat plasma and brain

Matrix	Concentration added (ng/ml)	Concentration found (ng/ml)	Precision (%)	Accuracy (%)	*n*
Plasma	10.0	10.2	2.0	102.0	5
	25.0	24.2	1.7	96.8	5
	100	100	1.0	100.0	5
	500	512	0.2	102.4	5
	2000	1920	1.0	96.0	5
Matrix	Concentration added (ng/g)	Concentration found (ng/g)	Precision (%)	Accuracy (%)	*n*
Brain	30.0	32.6	5.2	108.7	5
	150	162	5.6	108.0	5
	600	599	3.3	99.8	5
	3000	2970	10.6	99.0	5
	6000	6010	3.7	100.2	5

Concentration found and accuracy represent the mean values.

Table 3
Inter-day assay precision and accuracy for aripiprazole in rat plasma and brain

Matrix	Concentration added (ng/ml)	Concentration found (ng/ml)	Precision (%)	Accuracy (%)	*n*
Plasma	10.0	10.2	5.9	102.0	15
	100	97.8	3.3	97.8	15
	2000	1920	7.8	96.0	15
Matrix	Concentration added (ng/g)	Concentration found (ng/g)	Precision (%)	Accuracy (%)	*n*
Brain	30.0	31.2	9.6	104.0	15
	600	603	4.3	100.5	15
	6000	5990	5.0	99.8	15

Concentration found and accuracy represent the mean values.

pared to plasma is unclear, but it is thought that this may be because some brain components are being adsorbed to test substances.

3.2.6. Stability of short-term storage

The results of short-term stability of aripiprazole in plasma and brain homogenate kept for 24 h at room temperature and after three freeze–thaw cycles are shown in Table 5. The mean values of the percentage remaining kept for 24 h at room temperature were 98.8–107.4% in plasma, and 94.0–100.6% in brain. The mean values of the percentage remaining after 3 freeze–thaw cycles were 95.2–106.1% in plasma, and 97.9–99.2% in brain. These results showed that aripiprazole was stable in rat plasma and brain homogenate kept for 24 h at room temperature and after three freeze–thaw cycles.

3.2.7. Stability of long-term storage

The results of long-term stability of aripiprazole in plasma and brain homogenate stored at −20 °C are shown in Table 6. The mean values of the percentage remaining in plasma stored for 6 months were 100.9–111.1%, and the percentage remaining in brain stored for 8 weeks were 93.9–99.1%. These data showed that aripiprazole was stable for at least 6 months in plasma and for 8 weeks in brain after long-term storage at −20 °C.

Table 4
Extraction recovery of aripiprazole and the I.S. from rat plasma and brain

Matrix	Analyte	Concentration added (ng/ml)	Recovery (%)	Precision (%)	*n*
Plasma	Aripiprazole	25.0	87.2	2.4	3
		1600	91.1	3.7	3
	I.S.	400	87.7	2.5	6
Matrix	Analyte	Concentration added (ng/g)	Recovery (%)	Precision (%)	*n*
Brain	Aripiprazole	60.0	41.3	7.3	3
		4800	43.5	3.0	3
	I.S.	600	36.9	5.7	6

Recovery represents the mean values.

Table 5
Short-term stability of aripiprazole in rat plasma and brain homogenate kept for 24 h at room temperature and during three freeze–thaw cycles

Matrix	Concentration added (ng/ml)	Mean of percentage remaining (%)		*n*
		24 h at room temperature	Three freeze–thaw cycles	
Plasma	25.0	107.4	106.1	3
	1600	98.8	95.2	3
Matrix	Concentration added (ng/g)	Mean of percentage remaining (%)		*n*
		24 h at room temperature	Three freeze–thaw cycles	
Brain	60.0	100.6	97.9	3
	4800	94.0	99.2	3

Table 6
Long-term stability of aripiprazole in rat plasma and brain homogenate stored at −20 °C

Matrix	Concentration added (ng/ml)	Mean of percentage remaining (%)		*n*
		3 months	6 months	
Plasma	20.0	105.1	100.9	3
	200	96.9	105.3	3
	1600	98.9	111.1	3
Matrix	Concentration added (ng/g)	Mean of percentage remaining (%)		*n*
		4 weeks	8 weeks	
Brain	60.0	99.7	93.9	3
	600	98.6	98.2	3
	4800	101.8	99.1	3

Table 7
Pharmacokinetic parameters of aripiprazole in plasma and brain after single oral administration of aripiprazole at 10 and 30 mg/kg to male rats

Matrix	Dose (mg/kg)	t_{max} (h)	C_{max} (ng/ml)	AUC_{th} (ng h/ml)	$t_{1/2}$	$t_{1/2}$ calculated range (h)
Plasma	10	2	86	249_{6h}	2.2	2–6
	30	4	442	3044_{12h}	1.9	4–12
Matrix	Dose (mg/kg)	t_{max} (h)	C_{max} (ng/g)	AUC_{th} (ng h/g)	$t_{1/2}$	$t_{1/2}$ calculated range (h)
Brain	10	2	390	$1{,}074_{6h}$	1.8	2–6
	30	3	2232	$14{,}817_{12h}$	2.0	3–12

All pharmacokinetic parameters are calculated from the mean plasma and brain concentrations.

3.3. Application for pharmacokinetic study

The validated HPLC methods were successfully applied to the pharmacokinetic study of aripiprazole in rats. Mean concentrations of aripiprazole in plasma and brain after single oral administration of aripiprazole at 10 and 30 mg/kg to male rats are shown in Fig. 3. The pharmacokinetic parameters of aripiprazole are shown in Table 7. The maximum plasma concentration (C_{max}) of aripiprazole at 10 and 30 mg/kg doses reached 86 and 442 ng/ml, respectively, at 2 and 4 h after the dosing, and declined with $t_{1/2}$ of 2.2 and 1.9 h. AUC_{th} for 10 and 30 mg/kg were 249 and 3044 ng h/ml, respectively. On the other hand, the maximum brain concentration (C_{max}) of aripiprazole at 10 and 30 mg/kg doses reached 390 and 2232 ng/g, respectively, at 2 and 3 h after the dosing, and declined with $t_{1/2}$ of 1.8 and 2.0 h. AUC_{th} for 10 and 30 mg/kg were 1074 and 14817 ng h/g, respectively. Brain concentrations were about five times higher than plasma concentra-

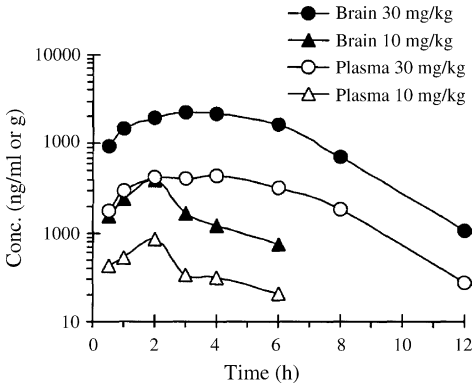

Fig. 3. Mean concentrations of aripiprazole in plasma and brain after single oral administration of aripiprazole at 10 and 30 mg/kg to male rats. Each data point represents the mean of four animals.

tions in both doses. These results suggest that aripiprazole is rapidly absorbed and penetrated into the brain.

4. Conclusion

HPLC methods for the determination of aripiprazole (OPC-14597, Abilify^{TM}) with UV detection in rat plasma and brain were developed and validated. The methods in plasma and brain showed excellent reproducibility and accuracy with the calibration curve ranges of 10.0–2000 ng/ml and 30.0–6000 ng/g, respectively. The validated HPLC methods were successfully applied to the pharmacokinetic study of aripiprazole in rats. The pharmacokinetic study demonstrates that aripiprazole is extensively distributed in rat brain.

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