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Detection and quantification of aripiprazole and its metabolite, dehydroaripiprazole, by gas chromatography–mass spectrometry in blood samples of psychiatric patients

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Abstract

Aripiprazole is a novel antipsychotic drug for the treatment of schizophrenia and schizoaffective disorders. In this study, a new method using gas chromatography–mass spectrometry (GC–MS) was developed and validated for the detection of aripiprazole and its main metabolite, dehydroaripiprazole, in plasma. Blood samples from seven psychiatric patients treated with aripiprazole (10–20 mg/day) underwent a solid-phase extraction (SPE) and *N*-methyl-*N*-trimethylsilytrifluoroacetamide (MSTFA) derivatization. The characteristic ions of mass spectra for aripiprazole and dehydroaripiprazole were *m*/*z* 306, 292, 218 and 304, 290, 218, respectively. Extraction recoveries from this method were 75.4% ($n = 5$) for aripiprazole and 102.3% ($n = 5$) for dehydroaripiprazole. The calibration curves of aripiprazole and dehydroaripiprazole were linear from 16 to 500 ng/ml ($r^2 = 0.999$) and 8 to 250 ng/ml ($r^2 = 0.999$), respectively. The respective limits of quantification (LOQs) for aripiprazole and dehydroaripiprazole evaluated in 0.5 ml of serum were 14.4 ng/ml and 6.9 ng/ml. Intra-assay and interassay precision and accuracy were within acceptable ranges. In this study, we also found that the mean trough concentrations in plasma at steady-state were 128.9 μg/l for aripiprazole and 30.1 μg/l for dehydroaripiprazole.

Keywords: Aripiprazole; Dehydroaripiprazole; Detection; GC–MS; Quantification

1. Introduction

Aripiprazole (Abilify™; 7-[4-[4-(2,3-dichlorophenyl)-1-piperazinyl] butoxy]-3,4-dihydro-2(1H)-quinolinone; $C_{23}H_{27}Cl_2N_3O_2$; MW 448.38) is an atypical antipsychotic agent. This drug has been approved recently by the US Food and Drug Administration (FDA) as the sixth second-generation antipsychotic for the treatment of schizophrenia and schizoaffective disorders [1,2]. Dehydroaripiprazole is the main metabolite with antipsychotic activity equivalent to that of aripiprazole [3,4]. Aripiprazole is a quinolinone derivative and the chemical structures of aripiprazole and dehydroaripiprazole are shown in Fig. 1.

Aripiprazole differs from other atypical antipsychotics and is considered to act as a partial dopamine D2 receptor agonist, partial serotonin1A (5-HT1A) receptor agonist and 5-HT2A receptor antagonist [1–3,5]. Because its pharmacological mechanism is different from other second-generation antipsychotic, aripiprazole has been referred to as a third-generation antipsychotic and a dopamine–serotonin system stabilizer [3].

One *in vitro* study has shown that aripiprazole is metabolized in the liver by P450, i.e., CYP 3A4 and CYP 2D6, isoenzymes

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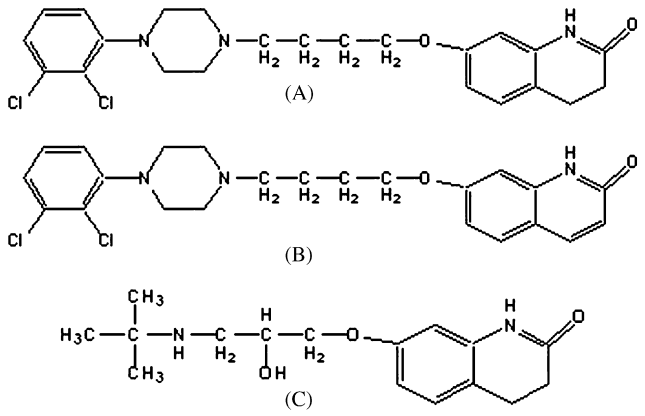

Fig. 1. Chemical structures of aripiprazole (A), dehydroaripiprazole (B) and carteolol as an internal standard (C).

[6]. Extensive pharmacokinetic variability can occur in psychiatric patients following aripiprazole administration, irrespective of dose or sex and may potentially be due to individual differences in CYP2D6 and CYP3A4 isoenzyme genotypes [7]. Early studies have indicated that aripiprazole is highly tolerated and relatively safe [1,8,9]. The most common side effects associated with aripiprazole administration are nausea, postural dizziness and somnolence, and the frequency and severity of these side effects are independent of dose [8].

Monitoring drug concentrations in blood plasma may not only ensure effectiveness and safety, but may also preclude side effects, especially for psychiatric patients with poor communication skills and impaired self-care. The commonly recommended therapeutic doses range from 10 to 30 mg/day with a starting dose of 10 or 15 mg/day [1,3,4]. One study has indicated that dose and concentration are proportional [7]; however, the relationship between aripiprazole concentration in blood plasma and drug effectiveness has not yet been established.

Establishing a reliable and accurate quantification method not only assists determining the relationship between drug concentration in plasma and its effectiveness, but also aids in pharmacokinetic studies. Previous studies on the detection and quantification of aripiprazole were limited to high performance liquid chromatography (HPLC) and liquid chromatography–tandem mass spectrometry (LC/MS/MS) [10,11]. To our knowledge, gas chromatography–mass spectrometry (GC/MS) has not yet been used in the analytical study of aripiprazole. This study demonstrates that GC/MS is a simple and reliable method for detection and quantification of aripiprazole and dehydroaripiprazole in small volumes of blood.

2. Experimental

2.1. Chemicals and standards

All reagents were analytical grade. Ethyl acetate, potassium carbonate and sodium hydrogen carbonate, dichloromethane, sodium dihydrogen phosphate, diethyl ether, hexane, methanol, triethylamine, *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA), trimethyliodosilane (TMIS) and trimethylchlorosilane (TMCS) were purchased from Sigma (St. Louis, MO, USA). Aripiprazole (OPC-14597, Abilify^{TM}) and dehydroaripiprazole (OPC-14857) reference standards were gifts from Otsuka Pharmaceutical Co. Ltd. (Tokyo, Japan). Ethanethiol was obtained from Fluka (Milwaukee, WI, USA). Carteolol (an internal standard; I.S.) was obtained from the Canadian Centre for Ethics in Sport (CCES). Purified water was generated from a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Derivatization mixture

A one-step derivatization process used to convert analytes to trimethylsilyl (TMS) enol–TMS ether derivatives was described previously [12,13]. Briefly, a trimethyliodosilane solution (TMIS; 0.1 M) was first prepared by mixing trimethyliodosilane, triethylamine and dichloromethane (70:1:430); the prepared TMS solution (60 μl), along with MSTFA (3 ml) and ethanethiol (60 μl) were mixed and stored at −20 °C.

2.3. Blood samples

Blood samples were obtained from seven psychiatric patients (ages 30–59; body weights 48.5–94.0 kg) who had been hospitalized for at least 2 years in the Yu-Li Hospital. The patients orally ingested aripiprazole 10 mg ($n = 5$) or 20 mg ($n = 2$) daily for various time periods that ranged from 2 to 8 months. Blood samples (10 ml) were collected at approximately 6 o'clock in the morning and stored at −20 °C until analysis. This study was conducted under the supervision of physicians.

2.4. Instrumentation and conditions

A Hewlett-Packard (HP, Palo Alto, CA USA) model 6890 GC equipped with a G1512A autosampler and interfaced to a 5973 mass selective detector (MSD) was used for sample analysis. The GC was equipped with a BPX5 5% phenyl polysilphenylene–siloxane capillary column (25 m × I.D. 0.22 mm; film thickness 0.25 μm). Helium, at a flow rate of 0.6 ml/min, was the carrier gas. Temperatures for the GC injection port and interface were maintained at 250 °C and 300 °C, respectively. The GC temperature started at 90 °C, increased 15 °C/min to 240 °C, 10 °C/min to 300 °C and was then held for 15 min. The mass spectrum was obtained by scanning from *m/z* 50 to 550. The mass spectrometer was operated in electron impact mode at an electron ionization energy of 70 eV. Splitless injection mode was used with an injection volume of 1 μl. Hewlett-Packard G1701AA version 0.300 Chemstation Software in the drug analysis mode was used for data acquisition and analysis.

2.5. Sample preparation and analysis

Stock solutions of aripiprazole and dehydroaripiprazole at 1 mg/ml in methanol were prepared as reference standards. 0.5 μg/ml of aripiprazole and dehydroaripiprazole in plasma were used as controls. Extraction of aripiprazole and dehydroaripiprazole from plasma was performed as follows. A

sample mixture 0.5 ml plasma, 1.5 ml phosphate buffer (0.1 M; pH 6.0) and 10 μl I.S. (carteolol; 0.2 μg/ml) were prepared in a 5 ml glass tube. All the samples were loaded onto the previously conditioned, (i.e., washed with 1.0 ml methanol and 1.0 ml phosphate buffer), SPE columns (Varian Bond Elut-Certify™ 130 mg 3 ml) that were connected to a vacuum manifold system. After washing with deionized water, the columns were acidified with 1.0 ml acetic acid (0.01 M; pH 3.3) and dried for 2 min under vacuum. Methanol (1.0 ml) was added and the columns were dried under vacuum. Elution was carried out with 1.5 ml (×2) of a freshly prepared mixture of ethyl acetate:28–30% ammonium hydroxide (98:2). The eluates were collected and evaporated to dryness under a nitrogen stream. Each sample was derivatized with 50 μl of previously prepared derivatization mixture (70 °C; 30 min) and injected for GC–MS analysis.

2.6. Calibration

Calibration solutions were prepared in triplicate by spiking drug-free human plasma samples with known amounts of aripiprazole and dehydroaripiprazole reference standards. Standard concentrations, 15.6, 31.3, 125.0, 250.0 and 500.0 ng/ml for aripiprazole and 7.8, 15.6, 62.5, 125.0 and 250.0 ng/ml for dehydroaripiprazole, were prepared for constructing a calibration curve for each compound. The concentration of the internal standard (I.S.; carteolol) was 200 ng/ml. Quantification was carried out by selected ion monitoring (SIM) mode with a selected ion *m/z* 306 for aripiprazole and an ion *m/z* 290 for dehydroaripiprazole and *m/z* 235 for I.S. The calibration curve for linear regression analysis was constructed by plotting the peak-area ratios of the analyte reference standards and the I.S. versus the standard concentrations.

2.7. Method validation

2.7.1. Limit of detection and limit of quantification

The limit of detection (LOD) was defined as a signal-to-noise (S/N) ratio ≥3. A single diagnostic ion *m/z* 306 and 290 was used for determining the LODs of aripiprazole and dehydroaripiprazole, respectively, for TMS-derivatized samples. The limit of quantification (LOQ) of the assay was determined mathematically when the concentration of the analyte required to produce a S/N ratio of greater or equal to 10 using a single diagnostic ion.

2.7.2. Recovery

To determine the recovery efficiency of the extraction procedure, a single target concentration, 125.0 ng/ml for aripiprazole and 62.5 ng/ml for dehydroaripiprazole, was prepared in the drug-free plasma and three replicates for each concentration were analyzed. In addition to this set (Set A), another set (Set B) of tubes containing drug-free plasma alone was prepared and all samples were extracted according to the protocol described above and eluted with the ethyl acetate:ammonium hydroxide mixture. Subsequently, the drug-free plasma samples in Set B were spiked with the target concentration of each analyte and 200 ng/ml of I.S. was added to all the samples in Sets A and B, which were then evaporated to dryness under a slow stream of nitrogen gas. Recovery percentages were determined by dividing the mean peak-area ratio in Set A by the corresponding mean peak-area ratio in Set B and then multiplying by 100%.

2.7.3. Accuracy and precision

The accuracy of the assays was determined by analyzing control samples in triplicate at 15.6, 125.0 and 500.0 ng/ml for aripiprazole and 7.8, 62.5 and 250.0 ng/ml for dehydroaripiprazole. The precision of each level in these two analytes was determined as the percent coefficient of variance (%CV).

3. Results

3.1. Analysis of aripiprazole and dehydroaripiprazole reference standards

To detect and characterize the chromatographic nature in the GC–MS analysis, a drug-free plasma sample spiked with aripiprazole and dehydroaripiprazole reference standards (200 ng/ml) was pretreated according to the procedure described in Section 2.5. The total ion chromatogram showed that the retention times (RTs) of aripiprazole and dehydroaripiprazole were 9.69 min (relative retention time or RRT 0.645) and 9.80 min (RRT 0.652), respectively (Fig. 2). The respective mass spectra of aripiprazole and dehydroaripiprazole were characterized by ions of *m/z* 218, 292, 306 and *m/z* 218, 290 and 304 (Fig. 3).

3.2. Method validation

The LODs of aripiprazole and dehydroaripiprazole were determined to be 4.8 and 2.3 ng/ml, respectively, when analyzed with a single diagnostic ion for TMS-derivatized samples by GC–MS. The respective LOQs for aripiprazole and dehydroaripiprazole were 14.4 and 6.9 ng/ml. The assay was linear over a range of concentrations from 15.6 to 500.0 ng/ml with a correlation coefficient of $r^2 = 0.9993$ and $y = 0.0004x - 0.0024$ for aripiprazole and over a range of

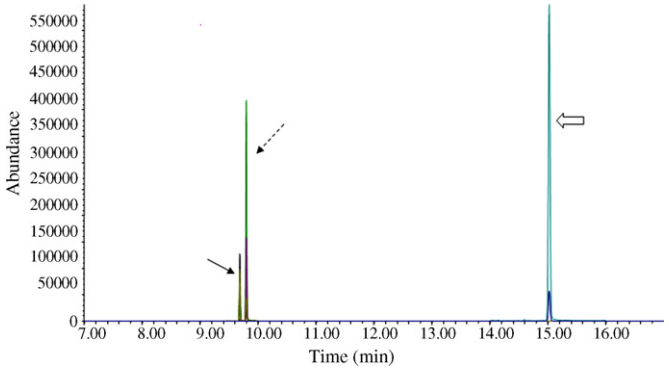

Fig. 2. Total ion chromatogram with selected ions of reference standards (250 ng/ml) spiked in plasma. Aripiprazole (arrow; RT 9.69 min), dehydroaripiprazole (dashed arrow; RT 9.80 min) and carteolol (open arrow; I.S., RT 15.02 min).

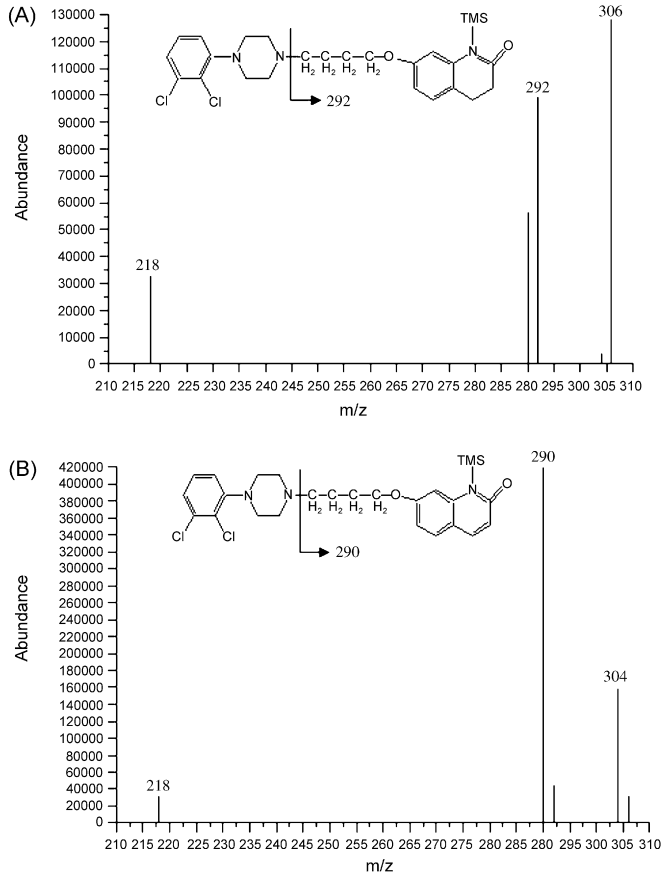

Fig. 3. Mass spectra of aripiprazole (A) and dehydroaripiprazole (B) spiked in drug-free plasma and derivatized with MSTFA.

7.8–250.0 ng/ml with a correlation coefficient of $r^2 = 0.9993$ and $y = 0.0019x - 0.0082$ for dehydroaripiprazole. The recovery of aripiprazole at 125.0 ng/ml was 75.4% ± 0.07 (mean ± SD), whereas 102.3% ± 0.13 of dehydroaripiprazole was recovered at 62.5 ng/ml. Intra-assay and interassay accuracy for the analysis of aripiprazole were measured to be 15.7, 124.0, 518.0 ng/ml and 15.7, 122.6, 512.9 ng/ml, respectively; for dehydroaripiprazole, the respective concentrations measured were 7.7, 66.6, 253.1 ng/ml and 7.8, 64.0, 246.1 ng/ml (Table 1). The precision values (%CV) for the intra- and interassay ranged from 1.3 to 11.2% (Table 1).

Table 2
Concentrations of unchanged aripiprazole and dehydroaripiprazole found in the plasma of patients treated with aripiprazole

Patient	Aripiprazole (μg/l) ± %CV	Dehydroaripiprazole (μg/l) ± %CV	A/DA ratio[a]
1	220.8 ± 3.3	36.5 ± 1.1	6.0
2	157.5 ± 1.1	47.2 ± 4.1	3.3
Mean	189.2	41.9	4.7
1	99.5 ± 3.8	20.0 ± 3.8	5.0
2	119.0 ± 2.2	36.0 ± 8.1	3.3
3	121.5 ± 7.5	33.5 ± 6.2	3.6
4	102.3 ± 7.1	19.3 ± 4.2	5.3
5	81.6 ± 8.1	18.3 ± 6.3	4.5
Mean	104.8 ± 15.5	25.4 ± 33.8	5.2
Total mean	128.9 ± 36.4	30.1 ± 36.8	4.3

[a] A denotes aripiprazole; DA denotes dehydroaripiprazole metabolite.

3.3. Metabolic products in the plasma of patients following aripiprazole treatment

Plasma samples from seven psychiatric patients were obtained during long-term treatment with aripiprazole. The unchanged parent compound and its major metabolite, dehydroaripiprazole, were detected in all the samples. As shown in Table 2, in the patients ($n = 5$) who received a dose of 10 mg/day, the mean concentrations of aripiprazole and dehydroaripiprazole were 104.8 ng/ml (range 81.6–121.5 ng/ml) and 25.4 ng/ml (range 18.3–36.0 ng/ml), respectively; and in the patients ($n = 2$) who received 20 mg/day, the mean concentrations were 189.2 ng/ml (220.8 and 157.5 ng/ml) and 41.9 ng/ml (36.5 and 47.2 ng/ml), respectively.

4. Discussion

Previous analytical studies of aripiprazole in human plasma were performed using HPLC and LC/MS/MS [7,10,11]. In this study, we developed and validated a simple, reproducible and reliable procedure using GC–MS to detect and quantify aripiprazole and its major metabolite, dehydroaripiprazole, in human plasma. To prepare a sample, a small volume of plasma (0.5 ml) was first extracted by a solid phase extraction (SPE) column and then derivatized by a mixture of

Table 1
Accuracy and precision of aripiprazole and its metabolite spiked in plasma[a]

Compound	Amount (ng/ml)	Intra-assay ($n = 9$)		Interassay ($n = 9$)	
		Concentration measured (mean ± SD)	%CV	Concentration measured (mean ± SD)	%CV
Aripiprazole	15.6	15.7 ± 1.8	11.2	15.7 ± 1.3	8.4
	125.0	124 ± 4.7	3.8	122.6 ± 1.9	1.6
	500.0	518.0 ± 6.7	1.3	512.9 ± 17.4	3.4
Dehydroaripiprazole	7.8	7.7 ± 0.5	6.6	7.8 ± 0.7	9.2
	62.5	66.6 ± 3.6	5.5	64.0 ± 3.0	4.6
	250.0	253.1 ± 7.6	3.0	246.1 ± 7.3	3.0

[a] Intra-assay and interassay values are means ($n = 9$) from three separated batches.

MSTFA and TMSI, which formed aripiprazole–TMS and dehydroaripiprazole–TMS molecules. The validation of this method was acceptable. However, despite their similar chemical structures, dehydroaripiprazole (102.3%) was recovered more efficiently than aripiprazole (75.4%). In the GC chromatogram, two peaks were identified (Fig. 2): the aripiprazole peak at RT 9.69 min (RRT 0.645) and the dehydroaripiprazole peak at RT 9.80 min (RRT 0.652). The mass spectra of aripiprazole and dehydroaripiprazole were characterized by fragmentation ions of *m/z* 218, 292, 306 (a base ion) and 218, 290 and 304, respectively; however, molecular ions of both compounds were not observed (Fig. 3). The major metabolite of aripiprazole found in the plasma is likely due to the loss of two hydrogens and thus was named dehydroaripiprazole in other studies [7,10].

In this study, plasma samples obtained from seven adult male psychiatric patients who had been receiving daily aripiprazole treatments for various periods of time, ranging from 2 to 8 months, were analyzed. Because all the patients had been treated with the drug once per day for more than 2 months during their hospitalization, we assumed that the concentrations of aripiprazole and its metabolite were at steady-state by the time when the blood samples were drawn from these patients [3,8]. Moreover, because the samples were drawn early in the morning, concentrations of aripiprazole and dehydroaripiprazole were mostly likely at the trough levels of the daily cycle. We observed a large variation in the steady-state concentrations of aripiprazole and dehydroaripiprazole among the seven patients. Such differences were observed in a previous study and were shown to be independent of dose and sex. The authors suggested that this variation was due to differences in both the formation and elimination of the metabolite and was found to be independent of dose and sex [7]. Previous studies suggested that aripiprazole has an elimination half-life of approximately 75 h and can reach steady-state after continuous oral administration for 14 days [6,8].

We found that the concentration of aripiprazole in plasma was approximately 4-fold (3.3- and 6.0-fold or 17% and 30% of dehydroaripiprazole with respect to aripiprazole) greater than dehydroaripiprazole in both the 10 mg and 20 mg doses used by the patients (Table 2). These results agree with previous studies that found that dehydroaripiprazole represents approximately 40% of aripiprazole's AUC in the plasma [14]. The concentrations of the compounds observed in plasma correlated with the doses administered; patients who were treated with 20 mg/day of drug had plasma concentrations approximately 2-fold higher than patients treated with 10 mg/day, even though the sample sizes in this study were relatively small for both groups.

5. Conclusions

The validated GC–MS method developed in this study provides a simple, accurate and reliable assay for the analysis of aripiprazole and dehydroaripiprazole in blood samples of psychiatric patients. The application of the SPE column, together with MSTFA-TMSI derivatization, generally produced better recovery and detection ability of the analytes than the liquid–liquid extraction (LLE) procedure. The detection limits of the analytes were far below the trough concentrations at steady-state indicating that this method is more than sufficient to detect aripiprazole and hydroxyaripiprazole in blood sample of the patients on commonly recommended doses. We suggest that GC–MS is the analytical instrument of choice for pharmacokinetic studies of aripiprazole in human blood sample.

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