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Original Article

The quantitative detection of aripiprazole and its main metabolite by using capillary-electrophoresis

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Abstract

Background: We aimed to establish a feasible and reliable method to measure the level of aripiprazole and its main metabolite, dehydroaripiprazole, using a capillary-electrophoresis (CE) machine.
Methods: Two blood samples were obtained from psychiatric patients hospitalized in Yu-Li Hospital who had been treated with aripiprazole for more than 4 weeks, at least 10 mg/d. Conditions for voltage, temperature and buffer concentration was optimized on a CE machine.
Results: The most optimal conditions for CE were 80 mM 2−3% DMSO-phosphate as a buffer under pH = 3.0, 15 KV, 20°C and a detection wavelength of 214 nm. The linear ranges of aripiprazole and dehydroaripiprazole concentration were from 0.5 to 50 ng/mL.
Conclusion: CE method is a feasible method to measure aripiprazole level with relatively low price compared with other analytical techniques for clinical use.

Keywords: Aripiprazole; Capillary-electrophoresis; Dehydroaripiprazole; Detection

1. Introduction

Aripiprazole (Abilify™) is an atypical antipsychotic agent, recently approved by the US Food and Drug Administration as the sixth second-generation antipsychotic for the treatment of schizophrenia, schizoaffective disorders, bipolar disorder and adjuvant therapy for major depression.[1] Dehydroaripiprazole, its main active metabolite, has an affinity for dopamine D2 receptors and thus has some pharmacological activities similar to that of its parent compound.[2,3]

Aripiprazole is considered a partial dopamine D_2 and D_3 receptor agonist, partial 5-HT_{1A} receptor agonist and 5-HT_{2A} receptor antagonist.[4–6] The partial agonist activity at the D_2 receptor may explain its efficacy in the treatment of both positive and negative symptoms of schizophrenia and its low probability for extra-pyramidal symptoms.[7] Its side-effects including weight gain, QTc prolongation and hyperprolactinemia. Nevertheless, it is not devoid of side-effects such as nausea, vomiting, lightheadedness, somnolence, constipation and postural dizziness.[8] Monitoring drug concentrations in plasma may not only ensure effectiveness and safety, but also preclude side-effects, especially for psychiatric patients with poor communication skills and impaired self-care. The commonly recommended therapeutic dosage prescribed for aripiprazole ranges from 10 mg/d to 30 mg/d, with a starting dose of 10 mg/d or 15 mg/d.[7] However, the relationship between aripiprazole concentration in plasma and drug effectiveness has not been well established yet.[2]

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Previous studies on detection and quantification of aripiprazole were mostly confined to high-performance liquid chromatography (HPLC),[9,10] for example, HPLC with photo diode array detection,[10] HPLC-tandem mass spectrometry,[11,12] LC-tandem mass spectrometry[2,13,14] and ultra-performance LC-electrospray tandem mass spectrometry.[15] The validated gas chromatography-mass spectrometry (GC-MS) method was developed in previous study and provides an accurate and reliable assay for analyzing of aripiprazole and dehydroaripiprazole in blood samples of psychiatric patients.[2]

Capillary-electrophoresis (CE) is one of the preferred techniques which has been frequently used in pharmaceutical quality control as well as clinical chemistry. The device has relatively high sensitivity, shorter analyzing time, and lower costs compared with HPLC. Furthermore, the precision of CE is as good as that of LC, and less effort for sample pre-treatments is needed in CE. Urine and even plasma can be directly injected without further pre-treatments.[16]

CE is used in analyzing and separating several antipsychotics, including zotepine, ziprasidone, clothiapine, clozapine, olanzapine and quetiapine.[17–19] There were limited reports about applying CE in detecting antipsychotics concentration in human blood.[8] CE was also used in detecting zotepine with solid-phase extraction and binary system of head-column field-amplified sample stacking method.[17] Raggi used a rapid CE method for the determination of clozapine and desmethylclozapine in human plasma and it proved to have good precision.[20]

Studies on using CE method for analysis of aripiprazole and its metabolites were limited. Musenga et al.[8] reported that a CE method with dual wavelengths was able to detect aripiprazole at 214 nm within 5 minutes (uncoated fused silica capillaries and a background electrolyte composed of 50 mM phosphate buffer at pH 2.5, 20 kV). The authors used loxapine as the internal standard, and the plasma sample was pre-treated by using solid-phase extraction on cyano cartridges, with extraction yield rate higher than 91.3%.[8] However, there are different detection modules available for CE. The CE machine used in the study of Musenga et al.[8] were equipped with dual wavelengths of 214 nm and 590 nm. The study used solid-phase extraction, a complicated and expensive method, for sample condensation. In this report, we describe the use of a liquid extraction method for sample condensation and the P/ACE MDQ System to detect aripiprazole and its metabolites. The broader wavelength range of The P/ACE MDQ System, between 190 nm and 600 nm, provides a more sensitive and alternative platform for such purpose.

2. Methods

2.1. Chemicals and standards

All reagents were analytical grade chemicals from Merck, including NaOH, HCl, phosphate, sodium dihydrogenphosphate, disodium hydrogenphosphate, methanol (MeOH), $NaHCO_3$, dimethyl sulfoxide (DMSO). Aripiprazole (OPC-14597, Abilify™), dehydroaripiprazole (OPC-14857) and the internal standard (OPC-14714) were obtained from the Otsuka Pharmaceutical Co. Ltd. (Tokyo, Japan) as a gift.

2.2. Standard mixture

Aripiprazole, OPC-14587 and OPC-14714 were dissolved in DMSO to make 1000 ng/mL solutions. Methanol was added to the solutions and adjusted to concentration of 200 ng/mL.

2.3. Running buffer

Four kinds of running buffer were prepared. Sodium dihydrophosphate (NaH_2PO_4) was used as a base, and phosphate (H_3PO_4) was added to adjust pH value to be around pH 2.0–4.0 as a phosphate buffer. MeOH and DMSO were then added to the above phosphate buffer, respectively and proportionally, to make 2.5–20% MeOH-phosphate buffer, 1–10% DMSO-phosphate buffer and MeOH-DMSO-phosphate buffer.

2.4. Blood samples and plasma extraction

Blood samples (No.030-205, No.038-205) were obtained from 2 schizophrenic patients who had been hospitalized in a psychiatric hospital with aripiprazole administered for more than 4 weeks, 10 mg/d. Participants who met DSM-IV criteria for schizophrenia or schizoaffective disorder were diagnosed by at least two board-certified psychiatrists. For the purpose of having stabilized plasma level of aripiprazole, patients with the first episode of illness were excluded and consent forms were obtained before the experiment began. A 200-μL aliquot of each patient's plasma was added to 5 μL of OPC-14714 (IS) (200 ng/mL). The mixture was then combined with 1 mL $NaHCO_3$ and extracted with 4 mL diethyl ether and then vortexed for 20 minutes. After mixing, the sample was centrifuged with 1500 g for 5 minutes. The sample was stored in an −80°C freezer for 90 minutes. The supernatant was moved into another tube, and dried by −40°C nitrogen gas. The residue was resolved by 25 μL of methanol and 25 μL running buffer (v/v, 50:50) with vortex (1 minute). Then, the sample was transferred to a 0.2-mL mini-vial which was placed into the sample tray of a Beckman P/ACE MDQ system for CE analyses.

2.5. Instrumentation and capillary conditioning

CE machine P/ACE MDQ System (Beckman, CA, USA) equipped with a photo diode array (PDA) detector which detected signals over 190–600 nm wavelength was used, and pH was detected by pH parameter 6,130 (Jenco, San Diego, CA, USA). The capillary had total length of 60 cm by 75 μm internal diameter; the capillary activation process was operated under 25°C and pressure of 20 psi. At the beginning of each day, the capillary was pre-treated by a sequence of methanol for 10 minutes, 1M HCl for 10 minutes, deionized water for 5 minutes, 1M NaOH for 10 minutes, deinoized water for 5 minutes, then running buffer for 10 minutes under pressure of 20 psi.

2.6. Optimizing conditions for testing

To discover the optimal conditions of detecting aripiprazole by means of our CE machine, PDA detector was used for finding the optimal detection wavelength, the optimal voltage (10 kv, 15 kv, 20 kv, 25 kv, 30 kv), the suitable operating temperature (18°C, 20°C, 25°C) and the suitable running buffer setting under different solutions: (1) pH 2.0–4.0 phosphate buffer; (2) 2.5–20% MeOH-phosphate buffer; (3)1–10% DMSO-phosphate buffer; (4) MeOH-DMSO-phosphate, respectively.

2.7. Method validation

To evaluate the quantitative application of the method, a repetition test was performed. The within-day variability test was repeated 3 times a day with 5 different concentrations of aripiprazole, OPC-14857 over the range of 10–50 ng/mL and fixed dose of OPC-14714 (IS) 20 ng/mL were analyzed. The between-day variability was tested in 8 consecutive days using 50 ng/mL of aripiprazole and OPC-14857.

2.8. Statistical method

We set up a linear regression equation which used the peak area of internal standard OPC-14714 as its X axis and its corresponding concentration (ppm) as Y axis, and quantified the concentration of aripiprazol and OPC-14857 by linear interpolation method.

The lowest detection range of concentration was based on the regression curve and its intersection at the Y axis. The analysis was done by P/ACE MDQ 32 Karat 7.0 software.

3. Results

3.1. Determine the optimal conditions for quantifying aripiprazole

3.1.1. General settings

Using the PDA detector, the strongest signal was found over wavelength of 214 nm during detection of aripiprazole and its metabolite OPC-14857. Thus, we thereafter used a 214-nm filter in ultraviolet/visible spectrophotometry for detection in the experiment. The greater the voltage, the shorter the migration time of the standard, and the shorter the peak distance between standards as well. Standards could be separated efficiently at a voltage of 15 kV and got better waveform. With higher temperature and shorter migration time of standards, however, the electric current will be increased. There was no significant difference between 18°C and 20°C. We chose 20°C as the temperature setting in this experiment for reducing the cost of Freon.

3.1.2. Optimization of running buffer

Pure phosphate buffer failed to separate aripiprazole from OPC-14857 under any pH value. We then tried to find out the influence of percentage of MeOH and DMSO used in the running buffer. The higher the concentration of MeOH, the better the waveform of separation. Nevertheless, because of the high volatility of MeOH, it was not suitable to use more MeOH in experimental procedures. Ten percent of MeOH-phosphate buffer was found to have better stability and separation of waveform.

Aripiprazole and its active metabolites are weak basic because of the tertiary amine group. The effect of pH was tested in 10% MeOH-phosphate buffer, and the separation curve showed better under pH = 3.0.

We could successfully separate aripiprazole from OPC-14857 under the concentration between 2.5% and 10% DMSO-phosphate buffer, but the higher concentration of DMSO in the buffer, the lower the peak height and area under the wave of the aripiprazole, OPC-14714 and OPC-14857 were demonstrated. Furthermore the migration time of analyses was increased along with increasing concentration of DMSO (Fig. 1). The migration time difference between 2% and 3% DMSO-phosphate buffer was not huge, but the integration area was much more stable under the 3% DMSO-phosphate buffer. Furthermore, aripiprazole could be separated from OPC-14857 under any concentration proportion of MeOH-DMSO-phosphate buffer. Thus, we chose 2–3% DMSO-phosphate buffer under pH 3.0 as the optimal running buffer.

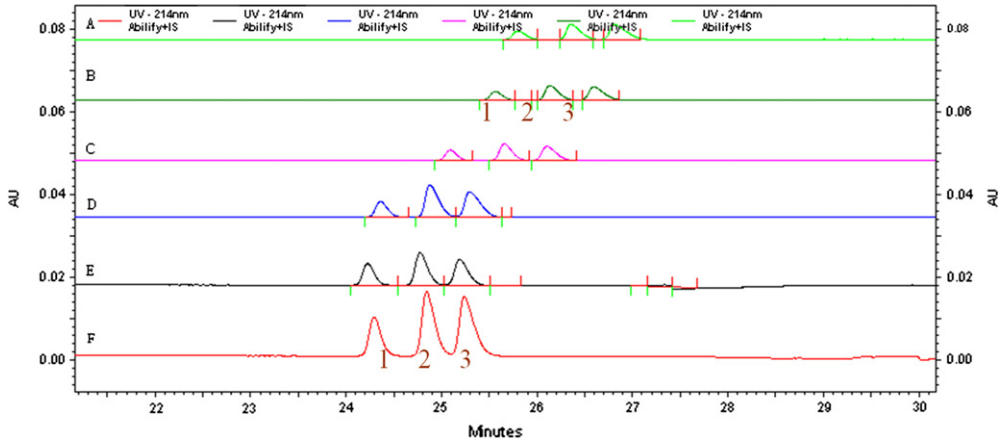

Fig. 1. The separations of standard in pH = 3, different concentration of DMSO-phosphate buffer: (A) 6% (B) 5% (C) 4% (D) 3% (E) 2% (F) 1% (1) OPC-14714: 50 ng/mL, (2) aripiprazole: 50 ng/mL, (3) OPC-14857: 50 ng/mL.

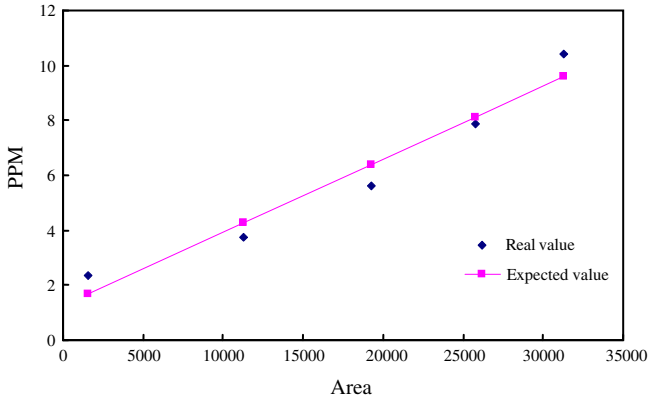

Fig. 2. The calibration line of 2～10 ng/mL aripiprazole by using the standard OPC-14714 concentrations of 2 ng/mL, 4 ng/mL, 6 ng/mL, 8 ng/mL, 10 ng/mL, respectively; detection range of aripiprazole: 1.5–10 ng/mL.

3.1.3. The result of calibration curve of aripiprazole and its active metabolite

The calibration curve was used to quantify the concentration of aripiprzaole and its metabolite OPC-14857. The standard mixture was prepared by adding aripiprazole, OPC-14587 and OPC-14714 (internal standard) into DMSO; then MeOH was added to adjust final concentration to 200 ng/mL. The concentration of standard mixture was adjusted by adding running buffers of 0.5 ng/mL, 1 ng/mL, 2 ng/mL, 4 ng/mL, 6 ng/mL, 8 ng/mL, 10 ng/mL, 20 ng/mL, 30 ng/mL, 40 ng/mL, and 50 ng/mL, respectively.

The calibration curve was set up by using the peak area of the internal standard as its X axis and the concentration of the internal standards as its Y axis, and then quantifying the concentration of aripiprazole and OPC-14857 using the linear interpolation method.

Using the method described above, three calibration curves was drawn (in concentrations of 10–50 ng/mL, 2–10 ng/mL (Fig. 2) and 0.5–4 ng/mL) of the aripiprazole and its metabolite OPC-14857, respectively. The respective limits of detection for aripiprazole and OPC-14857 were 1.5 ng/mL and 0.6 ng/mL (Table 1), respectively.

3.1.4. Validation of the method

To evaluate the quantitative applicability of the methods, five different concentrations of aripiprazole and dehydroaripiprazole were analyzed using OPC-14714 as an IS. Except for one obvious deviation in the third test of 10 ng/mL aripiprazole showing 21.93 ng/mL, which increased the mean concentration and RSD (relative standard deviation). Other results in Tables 2 and 3 show that the RSD of within-day and between-day variabilities in different concentrations, from high, medium–low, were all below 5%. The accuracy of aripiprazole and OPC-14857, obtained from the RE values at five different concentrations, were all below 5% for between-day assays. The results showed the accuracy of aripiprazole and OPC-14857 concentrations were also stable over 8 consecutive days.

Table 1
The respective limits of detection for aripiprazole and OPC-14857

Lowest detection concentration (ng/mL)	Aripiprazole (ng/mL)	OPC-14857 (ng/mL)
Curve 1 (10–50)	4	4
Curve 2 (2–10)	1.5	1.5
Curve 3 (0.5–4)	1.5	0.6

Table 2
The within-day variabilities ($n = 3$), 5 different concentrations of aripiprazole, OPC-14857 and fixed dose of OPC-14714 (IS) 20 ng/μL

Concentration known (ng/μL)	Mean concentration found (ng/μL)	RSD (%)	RE (%)
Aripiprazole within-day ($n = 3$)			
10	16.030 ± 3.021	18.84	60.3
20	20.087 ± 0.468	2.33	0.435
30	29.247 ± 0.219	0.75	2.51
40	38.180 ± 0.709	1.86	4.55
50	48.957 ± 0.609	1.24	2.09
OPC-14857 within-day ($n = 3$)			
10	10.963 ± 0.346	3.16	9.63
20	19.843 ± 0.951	4.79	0.79
30	29.650 ± 0.140	0.47	1.17
40	38.310 ± 0.699	1.82	4.23
50	48.940 ± 0.440	0.90	2.12

RE = relative error; RSD = relative standard deviation.

3.1.5. Application

Plasma samples of two schizophrenic patients were condensed into 4 fold by using liquid extraction method for sample purification and concentration because of the limitation of detection. The recovery rate after liquid extraction was 0.6. It was determined by estimating the concentrations of internal standard before and after the extraction.

The result of our samples checked from GC-MS method were A = 523.3 ng/L and B = 655 ng/L. The concentrations were to A = 1.255 ng/mL and B = 1.572 ng/mL in CE method, and the real concentrations from CE method were 1.772 ng/mL and 1.432 ng/mL, respectively. Sample A's concentration from CE method was higher than expected, but sample B was lower. The concentrations of the two samples were both located on the calibration curve.

4. Discussion

CE is a convenient and reliable approach to detect chemical reagents in different liquid phases such as serum or urine. From this study, we found the optimal analytic conditions for quantifying the concentrations of aripiprazole and its metabolites. Futhermore, the method is stable, and its results were repeatable without obvious error as well. The lowest concentration we could detect was about 1 ng/mL, the same as the limitation of our CE machine P/ACE by using PDA detector (Beckman). The lowest detectable range of CE method in our study was extended

Table 3
The between-day ($n = 8$) variability, Aripiprazole and OPC-14857 (50 ng/μL)

	Mean concentration found (ng/μL)	SD	RSD (%)
Aripiprazole	45.85	4.10	8.95
OPC-14857	50.46	3.31	6.56

RSD = relative standard deviation; SD = standard deviation.

Table 4
Comparison of different methods for detecting Aripiprazole

	Cost	Sensitivity	Speed	Pollution
GC-MS, LC-MS	High	High	Slow	High
HPLC	Moderate	High	Slow	High
CE	Low	Moderate	Fast	Low

CE = capillary-electrophoresis; GC-MS = gas chromatography-mass spectrometry; HPLC = high-performance liquid chromatography; LC-MS = liquid chromatography-tandem mass spectrometry.

by sample concentration. Furthermore, the commonly recommended therapeutic dose ranges from 10 mg/d to 30 mg/d, with a starting dose of 5 mg/d to 10 mg/d.[21] In our study, both of the patients were taking aripiprazole 10 mg/d. When applied clinically, plasma concentration might not be necessary because most patients take more than 10 mg of aripiprazole.

The sensitivity of GC-MS is 1000-fold higher than that of the CE method. However, the cost of HPLC, LC-tandem mass spectrometry and GC-MS are much higher than that of CE. By using CE method, we could save nearly 90% of cost spent in GC-MS, if the amount of study survey were large. We could also provide sensitivity enhancement by various approach, for example, head-column field-amplified sample stacking in binary system.[17] Furthermore, CE will not produce as much waste and pollutants as HPLC does during the experimental procedure (Table 4). Both Musenga et al. and our study found that CE method may be used to detect aripiprazole. Our study further indicates the lowest limitation of the method, which is relatively cheaper in concentration the plasma samples.

Monitoring drug plasma concentration in schizophrenia patients is helpful in the monitoring of existing drug level and compliance. The validated CE developed in this study provides a simple, rapid, reproducible, accurate and reliable assay for quantifying of aripiprazole and dehydroaripiprazole in blood samples of psychiatric patients and showed better stability in detection over higher concentrations. Applying the technique obtained from this study to clinical use could be used as a reference for monitoring aripiprazole's therapeutic effect and minimizing side-effects in clinical response. The patients' compliance for treatment responses and quality of life would increase.

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