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Research Article

Simultaneous determination of aripiprazole and its active metabolite, dehydro-aripiprazole, in plasma by capillary electrophoresis combining on-column field-amplified sample injection and application in schizophrenia

A sensitive high-performance CZE combining on-column field-amplified sample injection (FASI) has been developed for simultaneous determination of aripiprazole and its active metabolite, dehydroaripiprazole, in human plasma. A sample pretreatment by means of liquid–liquid extraction (LLE) (diethyl ether) with subsequent quantitation by FASI-CZE was used. The separation of aripiprazole and dehydroaripiprazole was performed using a BGE containing 150 mM phosphate buffer (pH 3.5) with 40% methanol and 0.02% PVA as a dynamic coating to reduce interaction of analytes with the capillary wall. Before sample loading, a methanol plug (0.3 psi, 6 s) was injected to permit FASI for stacking. The samples were injected electrokinetically (10 kV, 30 s) to introduce sample cations and the applied voltage was 20 kV with on-column detection at 214 nm. Several parameters affecting the separation and sensitivity of the drug and its active metabolite were studied, including reconstitution solvent, organic modifier, pH and concentration of phosphate buffer. The linear ranges of the method for test drug and its active metabolite, in plasma using amlodipine as an internal standard, were over the range 5.0–100.0 ng/mL. One female volunteer (25 years old) was orally administered a single dose of 10 mg aripiprazole (Abilify®, Otsuka) and blood samples were drawn over a 60 h period for pharmacokinetic study. The method was also applied to monitor the concentration of aripiprazole and dehydroaripiprazole in plasma collected after oral administration of 20 or 30 mg aripiprazole (Abilify®, Otsuka) daily at steady state in one schizophrenic patient.

Keywords:
Aripiprazole and dehydroaripiprazole in plasma / Field-amplified sample injection / Liquid–liquid extraction / Schizophrenia DOI 10.1002/elps.201000237

1 Introduction

Aripiprazole, an atypical antipsychotic agent, was approved for the treatment of schizophrenia, acute manic, mixed episodes associated with bipolar disorder at doses of up to 30 mg once daily. Aripiprazole will act as a dopamine antagonist in hyperdopaminergic condition and as an agonist in hypodopaminergic condition. It is commonly believed that aripiprazole appears to mediate its antipsychotic effects because the primary mechanism of action is functional selectivity at the D_2 receptor. Aripiprazole is also a partial agonist at the 5-HT_{1A} receptor, and like other atypical antipsychotics displays an antagonist profile at the 5-HT_{2A} receptor [1–3].

Aripiprazole displays linear kinetics (target concentrations of 150–300 ng/mL) [4] and has an elimination half-life of approximately 75 h. Steady-state plasma concentrations are achieved in about 14 days. The drug, aripiprazole, is metabolized to dehydroaripiprazole *via* the cytochrome P450 enzymes 3A4 and 2D6, and it was known the metabolite, dehydroaripiprazole, is also active [1]. Antipsychotics are widely used in the initial treatment either as monotherapy or in combination with other medications such as mood

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Abbreviations: AUC, area under the curve; **FASI,** field-amplified sample injection; **LLE,** liquid–liquid extraction; **IS,** internal standard

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stabilizers. Aripiprazole has recently been approved in USA for adjunctive use with antidepressants in major depressive disorder, based on the efficacy and safety findings of clinical trials [5]. Accordingly, coadministration of aripiprazole with medications that may inhibit or induce these metabolic enzymes may increase or decrease the concentration of aripiprazole, respectively. Drug plasma concentration monitoring in patients treated with psychotropic drugs is helpful to know the patients' compliance or detect an over dosage, thus reducing the risk of drug accumulation and optimizing the therapy. Thus, the analytical methods must be highly sensitive and selective for accurate and precise quantification because, generally, patients are frequently co-medicated with other drugs and may be affected by the concentrations of psychotropic drugs in plasma.

Literature studies of the concentration of aripiprazole and/or its active metabolite dehydroaripiprazole has been done by using gas chromatography with mass spectrometry [6], HPLC with mass spectrometry [7–12], UV [13, 14] or DAD [15] in human biological samples. CE is a powerful separation technique for the determination of ionic and neutral components. Compared with HPLC, only one CE method using SPE as sample pretreatment has been reported for the determination of aripiprazole in biological fluids [15]. To date, no CE with UV detection method for determining the highly similar structures of aripiprazole and its active metabolite, dehydroaripiprazole, in plasma or in schizophrenic patient's plasma has been developed. Further, CE produces less environmental pollution, gives high efficiency and causes with a low cost and small sample requirement. Owing to the short optical path length within the detection cell, on-column field-amplified sample injection (FASI) with electrokinetic injection has been shown that it can provide sensitivity enhancement. FASI is a simple and efficient technique for sensitivity enhancement, which was first described by Chien and Burgi [16]. Sample is prepared in a low-conductivity matrix and injected by voltage. The sample stacking is based on the presence of a short zone of low conductivity (water is usually used and the zone is referred as a water plug) at the capillary inlet end, across which an electric field up to several hundred times higher than that employ in normal CE is established which permits charged analytes to be injected at high velocity. During this electrokinetic injection process, the high velocity of sample ions reached high viscosity and high-conductivity running buffer; they slowed down and were condensed into a narrow zone.

The aim of this study was not only to develop a FASI-CE method for trace simultaneous determination of aripiprazole and dehydroaripiprazole in plasma, and to evaluate the time–concentration courses of aripiprazole and dehydroaripiprazole for healthy volunteer plasma after oral administration of 10 mg aripiprazole (Abilify®, Otsuka) in a single dose, but also to evaluate the concentration of aripiprazole and dehydroaripiprazole in one patient with schizophrenia at steady state after oral administration of 20 or 30 mg aripiprazole (Abilify®, Otsuka) once daily. In this study, we used liquid–liquid extraction (LLE) with diethyl ether for plasma pretreatment and then applied the head-column FASI technique to develop a sensitive and accurate CZE method for trace determination of aripiprazole and dehydroaripiprazole in plasma.

2 Materials and methods

2.1 Instrumentation

The Beckman P/ACE MDQ system (Fullerton, CA, USA) equipped with an UV detector and a liquid-cooling device was used. FASI-CZE was performed in an uncoated fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 40.2 cm (effective length 30 cm) × 50 μm id. The temperature of the separation was controlled at 25°C by immersion of the capillary in a cooling liquid circulating in the cartridge. The sample tray was at room temperature. Detection was carried out by the on-column measurement of UV absorption at 214 nm (cathode at the detection side). The Beckman P/ACE MDQ Microsoft system was used for data processing.

2.2 Chemicals and reagents

Aripiprazole and dehydroaripiprazole (Fig. 1) were kindly supplied by Otsuka Pharmaceuticals (Japan), whereas amlodipine used as the internal standard (IS) was supplied by Pfizer (USA). Sodium dihydrogen phosphate monohydrate, sodium hydroxide, diethyl ether, phosphoric acid (H_3PO_4), methanol and other reagents were of analytical grade from Merck (Darmstadt, Germany). PVA (M_W = 30 000–70 000), PVP (M_W = 55 000) and polyethylene oxide (PEO) (M_W = 8 000 000) were from Sigma (St. Louis, MO, USA).

2.3 Capillary conditioning

The new capillary was conditioned with methanol for 10 min, 1 M HCl for 10 min, deionized water for 2 min, 1 M NaOH for 10 min and deionized water for 2 min. The routine conditioning between runs every day was carried out using pressure with 1 M HCl (3 min), deionized water (2 min), 1 M NaOH (2 min), deionized water (2 min) and BGE (5 min) under positive pressure applied at the injection end. The processes of FASI in this study were as follows: the capillary was introduced to BGE (5 min) under positive pressure applied at the injection end as reinjection plug. After the capillary filled with BGE, 0.02% PVA in phosphate buffer (150 mM, pH 3.5) with 40% methanol, the inlet of capillary was dipped for 3 s in water for cleaning. A methanol plug (0.3 psi, 6 s) was introduced into the capillary followed by the sample injection. Sample loading was achieved by electrokinetic injection at a positive voltage of

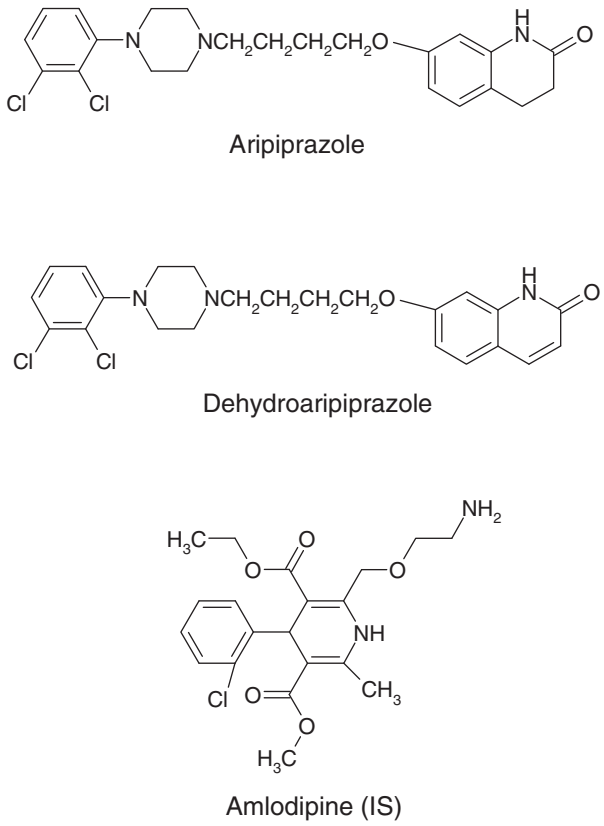

Figure 1. Chemical structures of aripiprazole, dehydroaripiprazole and amlodipine (IS).

10 kV for 30 s. A constant voltage of 20 kV was applied throughout the run under 150 mM phosphate buffer (pH 3.5) with 40% methanol and 0.02% PVA as BGE and the average current was approximately 60 μA. The hydrophilic polymeric PVA can be used as dynamic coatings to prevent adsorption of basic proteins to capillary surfaces. Otherwise, the polymer can also increase the viscosity of BGE and may enhance the effect of sample stacking in the proposed FASI-CE [17]. Temperature of separation was maintained at 25°C. A Beckman P/ACE MDQ Microsoft Software system was used for data processing.

2.4 Plasma extraction procedure

Drug-free human plasma samples were obtained from normal volunteers (two females; two males) used as controls. To prevent high protein concentrations in samples affecting the CE separation, LLE was employed for cleanup and preconcentration of the real samples. A simple pretreatment method using diethyl ether for extraction described in this article was based on the previously reported LLE procedures [7]. A 100 μL aliquot of patient's plasma or plasma spiked with aripiprazole and dehydroaripiprazole was pipetted into a 1.5 mL Eppendorf vial, and then added 50 μL of amlodipine (IS) (100 ng/mL). The mixture was extracted with 400 μL diethyl ether and then vortexed for 3 min. After mixing, the sample was centrifuged with $10\,000 \times g$ for 10 min. A 290 μL aliquot of supernatant was evaporated in centrifugal vaporizer (EYELA CVE-200 D, Japan) and the residue was reconstituted with 60 μL of methanol/0.15 mM H_3PO_4 (v/v, 50:50) with vortex (1 min). Then, the sample was transferred to a 0.2-mL minivial which was placed into the sample tray of a Beckman P/ACE MDQ system for CE analyses.

2.5 Application

The study protocol was approved by the Ethics Committee of the Kaohsiung Medical University Hospital. A 33-year-old Taiwanese with a diagnosis of schizophrenia received 20 mg aripiprazole (Abilify®, Otsuka) once daily for several months from his psychiatric prescription. However, after 1 month, his symptoms became aggravated. Aripiprazole was administrated with an increase of 30 mg/day by his psychiatric specialist for controlling his psychotic symptoms. Therefore, the patient's plasma after receiving 20 and 30 mg aripiprazole tablets (Abilify®, Otsuka) once daily from oral administration at steady state was measured. The concentration measured can be studied by the patient's compliance and the relationship of concentration with therapeutic outcome and further diagnosis. Venous blood sample was withdrawn and plasma fraction was separated immediately at 12 h after dosing. The plasma samples were stored frozen at −70°C until analyses.

3 Results and discussion

3.1 FASI investigation

Successful application of this FASI for sample stacking in binary system CE is shown to require an initially introduced low-conductivity zone (water plug) of >1 mm length. The literature stated that a methanol plug in processes of FASI is necessary for repeatability and sensitivity enhancement for the determination of heterocyclic aromatic amines [18]. Therefore, experiments were performed to discuss several parameters that would affect the assay method, including the length of water or methanol plug, and BGE system (concentration and pH of phosphate buffer, amount of PVA and organic modifier).

Compared with methanol and water as plug before sample injection, higher sample stacking was obtained using methanol as plug. To examine the effect of the methanol plug length, different injection periods (0, 3, 6 and 9 s) created by hydrodynamic injection of methanol (0.3 psi; 20.7 mbar) were investigated for the highest detection signal of the analytes. Comparing the difference between the presence and absence of methanol plug, higher sensitivity and repeatability were achieved when using the methanol plug before samples were injected for sample stacking. On

the other hand, there is no significant difference of sensitivity at the duration 6 or 9 s of methanol plug. A methanol plug (0.3 psi, 6 s) was selected for sample stacking.

3.2 Influence of nature of sample solvent

After LLE (diethyl ether) and evaporation of plasma samples, we tried to enhance the detection sensitivity by changing the nature of reconstructed sample solvents. Song *et al.* previously reported that the addition of alcohol to the sample solvent drastically influences stacking efficiency due to modification of conductivity [19]. Therefore, we studied the influence of two additional alcohols (methanol and isopropanol) on the reconstructed sample solvent in FASI mode by injecting the analytes dissolved in two different hydro-organic mixtures: water–methanol (50:50, v/v) and water–isopropanol (50:50, v/v). The heights of aripiprazole and dehydroaripiprazole peaks give a better response in the presence of methanol than isopropanol and the sensitivity ratios are 1.0 and 0.81 for water–methanol and water–-isopropanol, respectively. Thus, the water–methanol mixture was selected as the reconstructed sample solvent for further study.

3.2.1 Influence of the percentage of methanol within the sample solvent

The influence of volume fraction of methanol in sample solvent on sensitivity was studied. The peak response was improved when the methanol content in sample solvent increased from 10 to 90% v/v. The peak heights of aripiprazole and dehydroaripiprazole as the sensitivity ratios were 0.5, 0.6, 0.75, 0.8 and 1.0 at 10, 30, 50, 70 and 90%, respectively. However, higher repeatability of peak heights and migration times of analytes were obtained when using the 50% water–methanol v/v mixture as sample solvent.

3.2.2 Influence of the addition of phosphoric acid to the sample solvent

The stacking efficiency of on-column FASI was affected by sample matrix. With electrokinetic sample introduction, the amount of solute injected is proportional to the effective electrophoretic mobility. Although pure solvent has the lowest conductivity, neither water nor methanol has the ability to act as proton donator for aripiprazole ($pK_a = 7.6$). It has already been demonstrated that the addition of acid to the sample solvent may enhance the protonation of the cationic drugs and improve the sensitivity detection during FASI injection because a larger amount of higher mobility ions is introduced [20]. Thus, to charge the analytes, phosphoric acid was added to the sample for studying effective electrophoretic mobility of analytes. For the maximum stacking efficiency, the impacts of the phosphoric acid concentrations (50–180 μM) in the water–methanol mixture (50/50, v/v) for dissolving the sample residue were studied. The results indicated the effect of the concentration of phosphoric acid added to the sample solvent upon the sensitivity of aripiprazole and dehydroaripiprazole. Peak height increased as the concentration of phosphoric acid increased due to an enhanced protonation of positively charged aripiprazole and dehydroaripiprazole. The optimal H_3PO_4 concentration was found to be 150 μM. Thus, for robust operation with high sensitivity, the aripiprazole and dehydroaripiprazole need to be extracted from plasma samples by LLE and reconstructed in methanol/150 μM H_3PO_4 (50:50, v/v). The reconstructed sample solution was electrokinetically injected at a positive voltage of 10 kV for 30 s and then the inlet of capillary was dipped in water for 3 s.

3.3 Optimization of separation buffer

Due to the positive charge of aripiprazole and dehydroaripiprazole under acidic BGE, it is important to consider the interaction of the analytes with the wall of fused silica capillary. The migration time of analyte gradually became longer and less repeatability, when the BGE was phosphate 150 mM (pH 3.5) and 40% methanol without PVA. In order to minimize the adsorption of analytes, 0.02% of polymers, PVP, PEO and PVA, were added in BGE with the hope of dynamically modifying the capillary wall and the results as shown in Fig. 2. Broader peaks of aripiprazole and dehydroaripiprazole were observed when PEO was used as capillary modifier, probably due to its higher viscous property compared with the other solvents. On the other hand, an unstable baseline appeared when PVP or PEO was used as additives in BGE. The results show that PVA can provide better reproducibility of migration. In other words, the results showed that PVA can also confer better sensitivity and higher theoretical plate number than PEO and PVP. The sensitivity ratios are 0.55, 0.9 and 1.0 for PEO, PVP and PVA, respectively. The hydrophilic polymeric PVA interacts with the capillary surface and provides a barrier between the fused silica surface and the basic proteins. The effect of PVA concentration (0.005–0.025%) on the repeatability of migration time and sensitivity was also studied. With the concentration of PVA $\geq$0.015% in BGE buffer, reproducible migrations and peak areas of the test drugs were observed. PVA has been shown to rely on hydrophobic interaction or hydrogen bonding to dynamically bind or adsorb to wall of capillary and the repeatability of migration time of the analyte was obtained. Finally, 0.02% PVA was selected as dynamic coating on the surface wall of capillary to reduce analyte adsorption in this study. Without PVA, lesser repeatability of migration times of aripiprazole and dehydroaripiprazole was observed. Experiments were performed to determine the optimum conditions including phosphate buffer system (concentration and pH) and organic modify, methanol.

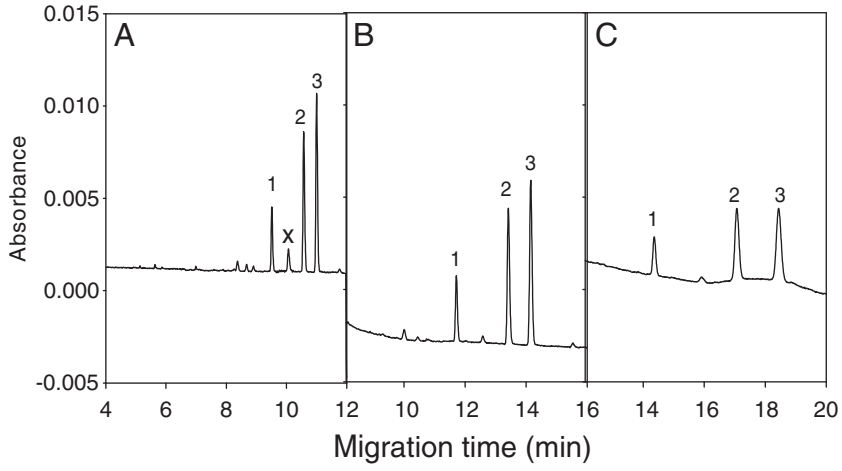

Figure 2. Effect of 0.02% of three polymers added to the BGE on the peak height of aripiprazole, dehydroaripiprazole and amlodipine. (A) PVA, (B) PVP and (C) PEO. Peaks: 1, amlodipine (IS); 2, aripiprazole; 3, dehydroaripiprazole; *x*, unknown peak. Separation CE conditions: uncoated fused silica capillary, 30 cm (effective length) × 50 μm id; wavelength, 214 nm; separation voltage, 20 kV (detector at cathode side); BGE: 150 mM phosphate (pH 3.5) with 40% methanol and 0.02% polymers.

3.4 Concentration and pH of phosphate buffer and methanol in BGE

The effects of the concentration of phosphate buffer 105–165 mM (pH 3.5) containing 40% methanol and 0.02% PVA were investigated spiked each 40 ng/mL of aripiprazole and dehydroaripiprazole in plasma. The results can give similar resolutions (Fig. 3) between aripiprazole and dehydroaripiprazole at different concentrations, but significant increase of migration times was obtained at high concentration. The migration time of aripiprazole increased from 9.85 (105 mM), 10.10 (120 mM), 10.55 (135 mM) and 10.69 (150 mM) up to 10.95 min (165 mM), respectively; migration time of dehydroaripiprazole increased from 10.12 (105 mM), 10.45 (120 mM), 10.92 (135 mM) and 11.11 (150 mM) up to 11.52 min (165 mM), respectively. In comparison of phosphate concentrations (from 105 to 165 mM) on the effect of the sensitivity for aripiprazole and dehydroaripiprazole, the produced sensitivity ratios varied in the range from 0.7 to 1.0 and the best result was found to be 150 mM. On the other hand, unknown peak, *x*, was observed that interfered with IS at the concentration of phosphate ≦120 mM in BGE buffer. Therefore, 150 mM of phosphate buffer was chosen for the separation.

The degree of protonation of species presented in the BGE system depends on the pH of the solution. Differences in the degree of ionization give rise to differences in electrophoretic mobilities. The analytes, aripiprazole and its active metabolite are weak basic (pK_a around 7.6), due to having tertiary amine group. The effect of pH (2.5, 3.0, 3.5, 4.0 and 4.5) of 150 mM phosphate buffer with 40% methanol and 0.02% PVA on the separation and sensitivity of analytes was studied. The pK_a of amlodipine (IS) was around 8.6. The analytes and IS dominate as the cationic species in electrolyte solution at the tested pH. Migration time decreased with increasing pH and a shorter migration time of the drug was obtained at high pH. However, an unknown peak *x* in endogenous plasma was observed that slightly interfered with IS or aripiprazole at pH 3.0 (Fig. 4B) or 4.0 (Fig. 4D), respectively. The better sensitivity and no interference for analytes' determination were found to be pH 3.5. Therefore, pH 3.5 of phosphate buffer was chosen for the determination of aripiprazole and its active metabolite, dehydroaripiprazole, in plasma.

The selectivity in CZE is altered by the addition of an organic additive such as methanol. The organic modifier alters the migration mechanism by changing the polarity and the viscosity of the BGE. Therefore, both the EOF and the electrophoretic mobility of the analytes are affected. The EOF mobility declines almost linearly by increasing the methanol concentration v/v [21], and hence expands the migration time window. The phosphate buffer at 150 mM (pH 3.5) with 0.02% PVA without organic modifier was studied to separate aripiprazole and dehydroaripiprazole in plasma from endogenous interference and poor resolution was found under this condition (Fig. 5). Therefore, four concentrations of methanol of additive were added in BGE. A better baseline resolution appeared when methanol was used as additive in BGE. On the other hand, results showed that methanol can also confer better sensitivity and higher theoretical plate number. The effect of concentrations of 0, 20, 40 and 60% methanol as organic additive added in phosphate buffer (150 mM; pH 3.5) with 0.02% PVA on separation of aripiprazole and dehydroaripiprazole in plasma was studied as shown in Figs. 5A–D, respectively. When the concentration of methanol was below 40%, lower resolution and broader peaks were observed, and this resulted in difficulty to assay. Baseline well resolution for the tested drugs was obtained at methanol concentration ≥40%. The 150 mM phosphate buffer (pH 3.5) with 40% methanol and 0.02% PVA was chosen as optimal separation buffer. The typical electropherogram of the FASI-CE separation of aripiprazole, its active metabolite, dehydroaripiprazole and amlodipine (IS) in plasma are shown in Fig. 6B. Repeatability of migration velocity of aripiprazole, dehydroaripiprazole and amlodipine in plasma was investigated ($n = 20$), and the observed migration times were 10.69 ± 0.09, 11.11 ± 0.10 and 9.61 ± 0.07 min for aripiprazole, dehydroaripiprazole and amlodipine, respectively.

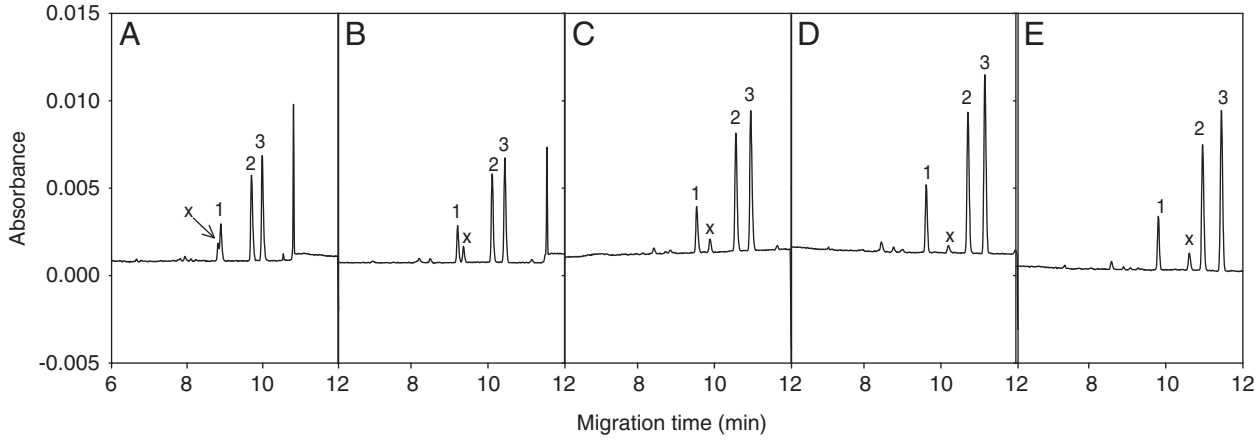

Figure 3. Effect of concentrations of phosphate buffer on the resolution of aripiprazole, dehydroaripiprazole and amlodipine. (A) 105 mM, (B) 120 mM, (C) 135 mM, (D) 150 mM and (E) 165 mM. CE Conditions: phosphate buffer (pH 3.5) with 40% methanol and 0.02% PVA. Peaks: 1, amlodipine (IS); 2, aripiprazole; 3, dehydroaripiprazole; *x*, unknown peak. The other CE conditions are the same as in Fig. 2.

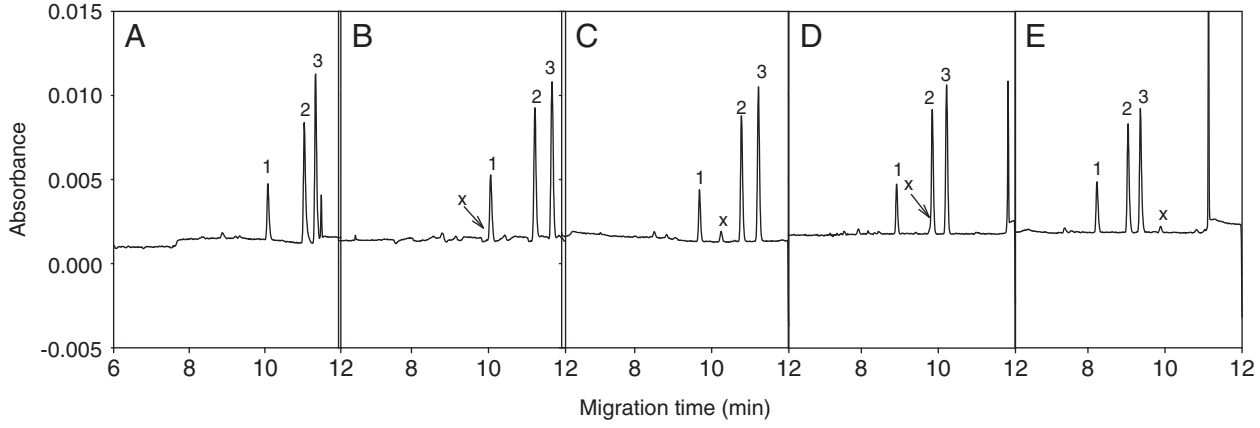

Figure 4. Effect of pH values (2.5–4.5) in phosphate buffer (150 mM) with 0.02% PVA on the resolution of aripiprazole, dehydroaripiprazole and amlodipine. Peaks: 1, amlodipine (IS); 2, aripiprazole; 3, dehydroaripiprazole; *x*, unknown peak. The other CE conditions are the same as in Fig. 2.

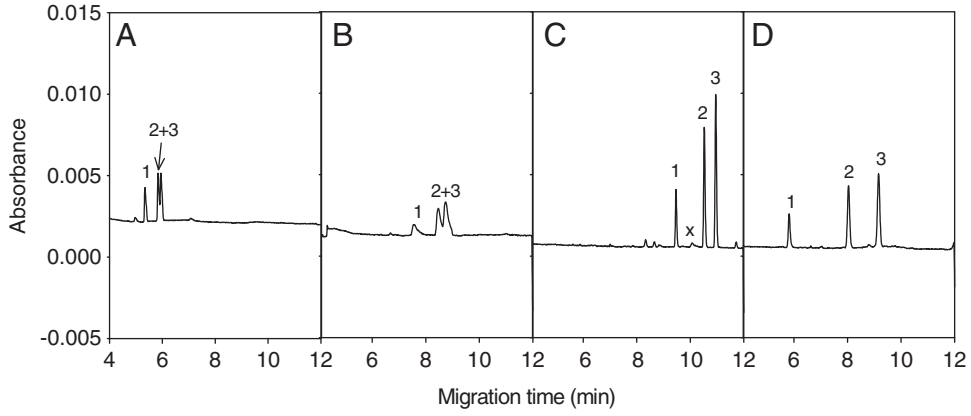

Figure 5. Effect of methanol concentration in BGE on the resolution of aripiprazole, dehydroaripiprazole and amlodipine. (A) 0%, (B) 20%, (C) 40%, (D) 60%. CE conditions: 150 mM phosphate (pH 3.5) with methanol and 0.02% PVA. Peaks: 1, amlodipine (IS); 2, aripiprazole; 3, dehydroaripiprazole; *x*, unknown peak. The other CE conditions are the same as in Fig. 2.

3.5 Method validation in human plasma

To evaluate the quantitative application of the method, five different concentrations over the range 5.0–100.0 ng/mL of aripiprazole and dehydroaripiprazole and fixed concentration of amlodipine (IS) spiked in plasma were analyzed. The linearity between the peak area ratios (y) of the tested drugs to IS and the concentration of the tested drugs (x, ng/mL) was

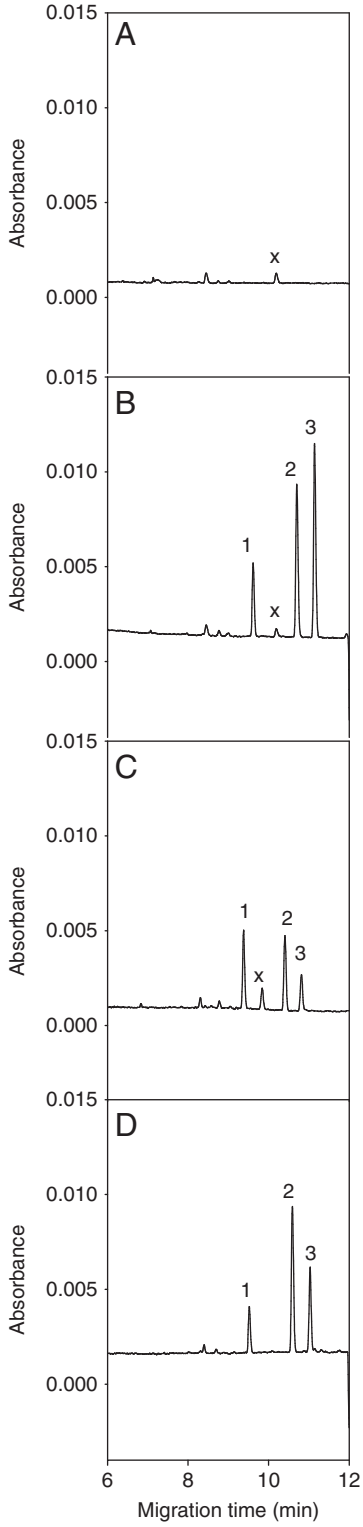

Figure 6. Electropherograms of aripiprazole, dehydroaripiprazole and amlodipine in biological sample determination. (A) Plasma blank, (B) plasma-spiked aripiprazole and dehydroaripiprazole each at 40 ng/mL, (C) oral administration of 10 mg aripiprazole tablet (Abilify®) single dosing in healthy volunteer plasma and (D) plasma from a schizophrenia patient receiving oral 30 mg of aripiprazole tablet (Abilify®). Peaks: 1, amlodipine (IS); 2, aripiprazole and 3, dehydroaripiprazole.

investigated. The linear regression equations were obtained as follows. For aripiprazole: $Y = (0.046 \pm 0.006)X + (0.080 \pm 0.070)$ and $Y = (0.047 \pm 0.001)X + (0.039 \pm 0.026)$ for intra-day ($n = 3$) and inter-day ($n = 5$), respectively; for dehydroaripiprazole: $Y = (0.055 \pm 0.007)X + (0.120 \pm 0.095)$ and $Y = (0.058 \pm 0.004)X + (0.052 \pm 0.074)$ for intra-day ($n = 3$) and inter-day ($n = 5$), respectively. The correlation coefficients of intra- and inter-day regression equations were all 0.999. These data demonstrate the high linearity of this method for intra- and inter-day assays. The precision of the proposed method was evaluated on spiked plasma sample and estimated by RSD value. In Table 1, the results show that the intra- and inter-day RSDs of different concentrations from high, medium to low were all below 9.0%. The accuracy of aripiprazole and dehydroaripiprazole obtained from the RE values at three concentrations which were all below 3.9% for intra- and inter-day assays. Compared with peak area ratio of the standard aripiprazole and dehydroaripiprazole, the absolute recoveries at 10.0 and 50.0 ng/mL are all above 70%. The LOQ of aripiprazole and dehydroaripiprazole was 5.0 ng/mL and the LODs were about 2.0 ng/mL for aripiprazole and 2.5 ng/mL for dehydroaripiprazole. Comparison with published CE method for aripiprazole determination [13] (LOD and LOQ are 35 and 70 ng/mL, respectively), the FASI-CE method has higher sensitivity. On the other hand, the method can also determine its active metabolite. The selectivity of the proposed method was briefly tested on the separation of aripiprazole, dehydroaripiprazole and amlodipine (IS) with other pharmacologically similar drugs including olanzapine, amisulpride, quetiapine and risperidone. Under present FASI-CE conditions, a complete separation of aripiprazole,

Table 1. Precision and accuracy for the analysis of aripiprazole and dehydroaripiprazole spiked in plasma

Concentration known (ng/mL)	Concentration found (ng/mL)	RSD (%)	RE (%)
Aripiprazole			
Intra-day[a] ($n = 3$)			
5.0	4.82 ± 0.44	9.1	3.6
25.0	24.37 ± 1.81	7.4	2.5
50.0	48.63 ± 2.84	5.8	2.7
Inter-day[a] ($n = 5$)			
5.0	5.20 ± 0.26	5.0	3.9
25.0	25.59 ± 1.73	6.8	2.4
50.0	49.99 ± 2.31	4.6	0.0
Dehydroaripiprazole			
Intra-day ($n = 3$)			
5.0	4.84 ± 0.22	4.5	3.2
25.0	25.16 ± 1.18	4.7	0.6
50.0	48.40 ± 2.17	4.5	3.2
Inter-day ($n = 5$)			
5.0	4.92 ± 0.25	5.1	1.6
25.0	25.1 ± 1.72	6.9	0.4
50.0	49.25 ± 1.28	2.6	1.5

a) Intra-day data were based on three replicate analyses and inter-day data were from three replications on five consecutive days.

dehydroaripiprazole and amlodipine from other antipsychotic agents was obtained and the observed migration times were 10.69, 11.11, 9.61, 7.66, 8.72, 9.25 and 9.82 min for aripiprazole, dehydroaripiprazole, amlodipine (IS), olanzapine, amisulpride, quetiapine and risperidone, respectively. In specific condition, clinicians may use aripiprazole in combination with other antipsychotic agents for control psychotic symptoms.

3.6 Application

One female volunteer (24 years old) was orally administered single dose of 10 mg aripiprazole (Abilify®, Otsuka) tablet. To study the concentration–time profiles of aripiprazole and active metabolite, dehydroaripiprazole, the plasma samples from the volunteer were measured at different time intervals. The concentration–time curves of aripiprazole and active metabolite, dehydroaripiprazole, in plasma after single dose oral administration are shown in Fig. 7 and the electropherogram is shown in Fig. 6C. The pharmacokinetic parameters were summarized in Table 2. From the results, the time (t_{max}) to reach the peak plasma concentration (C_{max}; 66.5 ng/mL) and area under the curve ($AUC_{0–60}$) were 3 h and 1764.1 h*ng/mL, respectively. The $AUC_{0–60}$ of active metabolite, dehydroaripiprazole, was 307.43 h*ng/mL. The elimination of aripiprazole from the blood was slower and had a long half life. Therefore, we measured the patient's plasma after oral administration 20 and 30 mg aripiprazole tablets (Abilify®, Otsuka) once daily at steady state. The electropherogram of an extracted plasma sample obtained from the schizophrenia patient receiving 30 mg/day of aripiprazole tablet (Abilify®, Otsuka) is shown in Fig. 6D and interference peaks did not appear. The concentrations of aripiprazole and dehydroaripiprazole after dosing at 12 h later were 304.1 and 189.3 ng/mL, respectively, at receiving 20 mg/day and 660.6 and 319.8 ng/mL, respectively, at receiving 30 mg/day, respectively. From the drug concentrations, the case has good patient's compliance. According to the definition and criteria by Chouinard and Jones [22], supersensitivity psychosis is suggested for the case. Early identification of this phenomenon would allow the psychiatrist to improve treatment strategies.

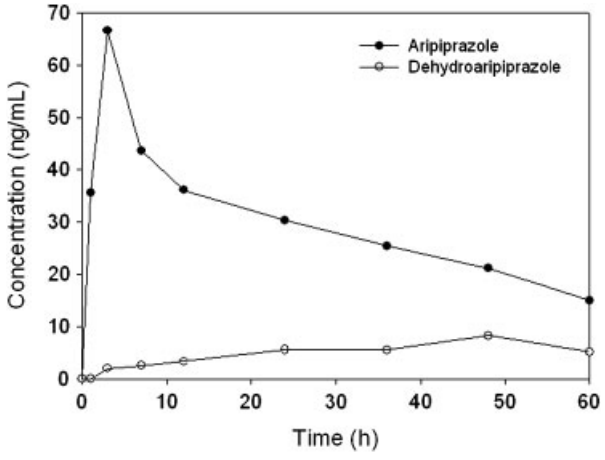

Figure 7. Concentration–time curves of aripiprazole and active metabolite, dehydroaripiprazole, in plasma after oral administration of single dose 10 mg of aripiprazole tablet (Abilify®). Data are expressed as mean ± SD, $n = 3$.

Table 2. Pharmacokinetic parameters after single oral administration of 10 mg aripiprazole in one healthy volunteer

Parameters	Volunteer
C_{max} (ng/mL)	66.5
t_{max} (h)	3.0
$t_{1/2}$ (h)	37.7
$AUC_{0–60}$ (h*ng/mL)	1764.1
$AUC_{0-\infty}$ (h*ng/mL)	2580.6
K_{el} (1/h)	0.018
Cl_{app} (mL/h)	3875.1
MRT_{app} (h)	52.8

K_{el}, elimination rate constant; Cl_{app}, appearance clearance and MRT, mean residence time.

4 Concluding remarks

A LLE coupling CZE with head-column FASI method for simultaneous determination of aripiprazole and dehydroaripiprazole in plasma described here represented a sensitive and efficient analytical method. The method was sensitive to ng/mL level measurement. Therefore, the method was suitable for the simultaneous analysis of aripiprazole and dehydroaripiprazole in plasma collected during pharmacokinetic investigations in humans and successfully applied to plasma samples from patients with schizophrenia. This analytical method might be applicable to therapeutic drug monitoring of aripiprazole and dehydroaripiprazole and investigate the relationship of concentrations of aripiprazole and dehydroaripiprazole in plasma and therapeutic effectiveness in schizophrenia patients.

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