Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1087 (2005) 189–196
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Trace analysis of zotepine and its active metabolite in plasma by capillary electrophoresis with solid phase extraction and head-column field-amplified sample stacking

Yu-Wei Chou[a], Wei-Shan Huang[b], Cheng-Chung Chen[c], Shun-Jin Lin[a], Hsin-Lung Wu[a], Su-Hwei Chen[a,b,*]

[a] *Graduate Institute of Pharmaceutical Sciences, Kaohsiung Medical University, Kaohsiung 807, Taiwan*
[b] *Department of Pharmacy, Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung 807, Taiwan*
[c] *Department of Psychiatry, Kaohsiung Medical University Chung-Ho Memorial Hospital, Kaohsiung 807, Taiwan*

Available online 8 December 2004

Abstract

A sensitive high-performance capillary zone electrophoresis (CZE) with head-column field-amplified sample stacking (FASS) in binary system has been developed for the simultaneous determination of zotepine and its active metabolite, norzotepine, in human plasma. The separation of zotepine and norzotepine was performed using a background electrolyte consisting of 50% ethylene glycol–borate buffer (20 mM, pH 8.0) solution with 20% methanol as the running buffer and on-column detection at 200 nm. Under the optimal FASS–CZE condition, good separation with high efficiency and short analysis time is achieved. Several parameters affecting the separation and sensitivity of the drug were studied, including sample matrix, pH and concentrations of the borate buffer, ethylene glycol and methanol. Using clozapine as an internal standard, the linear ranges of the method for the determination of zotepine and norzotepine in human plasma were over 3–100 ng/mL; the detection limits of zotepine and norzotepine in plasma were 2 and 1 ng/mL, respectively. A sample pretreatment by means of solid-phase extraction (SPE) with subsequent quantitation by FASS–CZE was used. The application of the proposed method for determination of zotepine and norzotepine in plasma collected after oral administration of 125 mg zotepine in one schizophrenic patient was demonstrated.

Keywords: Field-amplified sample stacking; Zotepine and norzotepine; Solid-phase extraction; Plasma

1. Introduction

Zotepine is an atypical antipsychotic with a high affinity for serotonin and dopamine receptors and it is effective in the management of patients with positive and negative schizophrenic symptoms [1–4] and it demonstrates fewer extrapyramidal adverse effects than conventional antipsychotics, such as haloperidol. N-demethylation of zotepine, the major metabolite of zotepine in humans, is pharmacologically active norzotepine and shows an affinity similar to that of zotepine. Zotepine is well absorbed from gastrointestinal tract after oral dosing for schizophrenia and the binding ratio of zotepine to human serum albumin is approximately 97%; the serum half-life is about 8 h. In administration of the drug for schizophrenia, serum concentrations vary among individuals; maximum plasma levels C_{max} = 30–240 ng/mL have been reported after a single dose of 100 mg of zotepine [1]. It is suggested that there is wide inter-patient variability in drug metabolism. There is little information on isoenzymes responsible for the metabolism of zotepine. At present, no definite relationship between clinical therapeutic efficiency and serum levels has been established. However, the side effect of the drug, akathisia and prolactin level increase, revealed a positive relationship with the zotepine serum concentration [1,5]. For these reasons, a reliable determination of the plasma concentrations of zotepine and norzotepine

* Corresponding author. Tel.: +886 7 3121101x2253; fax: +886 7 3210683.
E-mail address: suhwch@kmu.edu.tw (S.-H. Chen).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.11.061

is very important for evaluating therapeutic efficacy, monitoring drug safety and studying the mechanism of drug metabolism.

Several studies have been reported in the literatures including using gas chromatography-mass spectrometry (MS), high-performance liquid chromatography (HPLC) with MS–MS [6–9] or UV detection [10–12] on the study of zotepine concentration and/or its metabolite in human biological samples. To date, no capillary electrophoresis (CE) method for determining the structure highly similar zotepine and its active metabolite, norzotepine, has been developed. CE is a powerful separation technique for the determination of drugs in body fluids because of its high resolution and short analysis time. Owing to the short optical path length within the detection cell, a large volume of injection fluid is required for samples at low concentrations. However, a broadening of migration peak affecting the separation efficiency has been observed when the injection volume is excessive. Various approaches have been shown to provide sensitivity enhancement, including head-column FASS [13–25]. FASS is a simple and efficient technique for sensitivity enhancement, first introduced by Mikkers et al. [13]. This sample stacking is based upon the presence of a short zone of low conductivity (water is usually used and the zone is referred to as a water plug) at the capillary inlet end, across which an electric field up to several hundred times higher than that employed in normal CE is established which permits charged analytes to be injected at high velocity. During this electrokinetic injection process, the sample ions reach the high viscosity and high conductivity running buffer, they slow down and are condensed as a narrow zone. The head-column FASS may provide a sensitivity enhancement of up to 1000-fold [17,23–25]. For example, based on this stacking for determination of H_2-receptor blockers in plasma and phenoxy acid herbicides, sensitivity enhancements of up to 1000-fold have been reported by Wu et al. [23] and Zhu and Lee [24], respectively. In this paper, we used SPE with C_{18} cartridges for plasma pretreatment and then applied the head-column FASS technique to develop a sensitive and accurate CZE method for trace determination of zotepine and norzotepine in plasma. Application of the proposed method to analyze the analytes in one schizophrenia patient was evaluated and it proved to be satisfactory.

2. Experimental

2.1. Instrumentation

The Beckman P/ACE MDQ system (Fullerton, CA, USA) equipped with a photodiode-array detector (DAD) and a liquid-cooling device was used. FASS–CZE was performed in an uncoated fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) of 40.2 cm (effective length 30 cm) × 75 μm I.D. 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 200 nm (cathode at the detection side). The Beckman P/ACE MDQ Microsoft system was used for data processing.

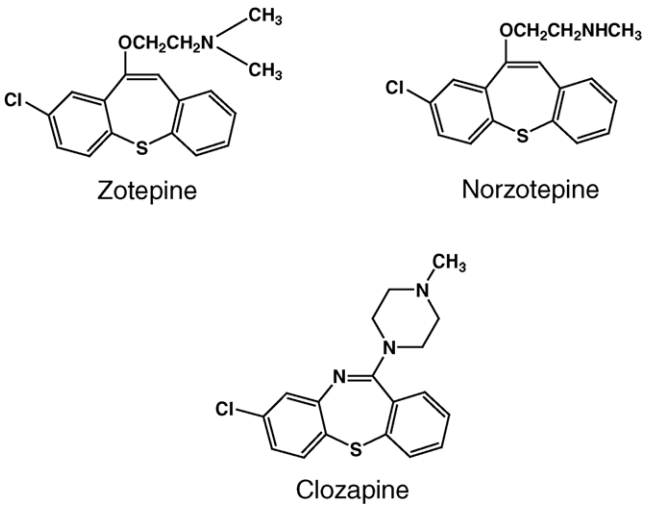

Fig. 1. Chemical structures of zotepine, norzotepine and clozapine (I.S.).

2.2. Chemicals and reagents

Zotepine and norzotepine were kindly supplied by Fujisawa Pharmaceuticals (Osaka, Japan), while clozapine used as the internal standard (I.S.) was from Sigma (St. Louis, MO, USA) (as Fig. 1). Disodium tetraborate, sodium hydroxide, ethylene glycol, phosphoric acid, methanol and other reagents were of analytical grade from Merck (Darmstadt, Germany).

2.3. Capillary conditioning

Capillary conditioning before startup was undertaken with methanol for 10 min, 1 M HCl solution for 10 min, deionized water for 2 min, 1 M NaOH for 10 min, deionized water for 2 min and electrolyte solution for 10 min. The conditioning between run was affected by rinsing with 1 M NaOH (5 min), deionized water (3 min), and electrolyte solution (3 min) under positive pressure applied at the injection end. The capillary tip was dipped for 3 s into a vial containing water for cleaning and a short water plug was inserted into the capillary. Then a water plug (application of 34.5 mbar for 0.1 min) was introduced into the capillary. Samples were electrokinetically injected at a positive voltage of 10 kV for 20 s (anode at the injection end) and the separation voltage was 20 kV. Due to the low conductivity of the sample zone, the current gradually increased to 10 μA within the first 10 s of power application and then slowly reached a level of about 16 μA at 30 s. The background electrolyte was composed of 50% ethylene glycol–borate buffer (20 mM, pH 8.0) solution with 20% methanol.

2.4. Sample pretreatment

Waters Oasis HLB 3 ml (60 mg) C_{18} cartridges (Milford, MA, USA) were used in a Varian (CA, USA) vacuum manifold apparatus for the SPE procedure. The cartridges were activated and conditioned with 1 mL of methanol and then 1 mL of water. An amount of 250 μL of blank plasma spiked with the various concentrations of zotepine and norzotepine and 50 μL of 120 ng/mL of clozapine (I.S.) or 250 μL of patient plasma and 50 μL of 120 ng/mL of clozapine (I.S.) were loaded onto the cartridge. After loading, the cartridge was washed with 1 mL of carbonate buffer (0.1 M, pH 10) and 1 mL of water and dried under vacuum aspiration for 10 s. The analytes were eluted with 1 mL of ethyl acetate and the cartridge was dried under vacuum again for 10 s. The 0.8 mL of ethyl acetate layer was then brought to dryness under freeze evaporator (EYELA UT-80 and EYELA CVE-2000) and redissolved with 30 μL of mixed solvent composed of 80% ethylene glycol and 120 μM H_3PO_4 by vortex mixing and then it was transferred into a 0.5 mL PCR sample vial that could be placed into the sampler of the CE apparatus.

2.5. Method validation

The calibration graphs of zotepine and norzotepine in biological matrix with five different concentrations of zotepine and norzotepine ranging from 3–100 ng/mL were established with the peak area ratio of zotepine or norzotepine to clozapine (I.S.) as ordinate (y) versus the concentration of zotepine or norzotepine in ng/mL as abscissa (x). The precision and accuracy of the method were estimated at 3, 20 and 100 ng/mL and from back-calculated standard concentration. The intra-day of mean precision was defined by relative standard deviation (RSD) and relative error (RE) from analyses on the same days. The inter-day precision and accuracy were calculated from repeated analyses of identical samples on five consecutive days for these concentrations of zotepine and norzotepine and expressed also as RSD and RE. The limit of quantitation (LOQ) is the minimum injected amount that gives precise measurements. The LOQ in plasma defined as the sample concentrations generating a peak height 10 times the level of the baseline noise. The limits of detection (LOD) were calculated on the basis of the baseline noise, which was defined as the sample concentration generating a peak of height three times the level of the baseline noise (signal-to-noise ratio of 3).

2.6. Application

A 42-year-old male psychotic patient was treated with a 125 mg zotepine tablet (Lodopin®, Fujisava). The patient was in a steady state (he had been taking zotepine for more than one month). Venous blood sample was withdrawn and plasma fraction was separated immediately at 8 h after dosing. The plasma sample was stored frozen at −40 °C until analyses.

3. Results and discussion

On column FASS is a simple and efficient technique for sensitivity enhancement. This approach can improve the sensitivity of the detection of trace components in biological samples without modification of the instrument. In this work, we describe the application of head-column FASS to develop a CZE approach to the antipsychotic drug, zotepine and its active metabolite, in microliter volumes of body fluids. Optimization and validation of injection process and separation mode were investigated.

3.1. Effect of water plug and sample matrix

This head-column FASS preceded as follows: The capillary was dipped into water for 3 s and then a 34.5 mbar 0.1 min water plug from a different vial was inserted. Dipping the capillary inlet end and electrode in a vial containing water was found to be necessary to prevent contamination of the sample solution with the high conductivity running buffer. Without this procedure, smaller and irreproducible peak height was observed. In principle, application of higher voltage and a longer injection time period should result in more solute injected resulting in more sensitivity. In practice, however, higher injection voltage and longer injection time such as 99 s were found to yield only a broader peak but not higher peak in our study. The standard solutions or extracted plasma samples from SPE were injected using a voltage of 10 kV for 20 s. The injection time at 34.5 mbar of the water plug was optimized for the highest detection signal of the analytes. The results show the 34.5 mbar 0.1 min water plug injection time provided the highest detection signal. Comparing the difference between presence and absence of water plug, we can gain higher reproducibility and higher sensitivity when using the water plug before samples are injected.

The stacking efficiency was affected by the sample matrix. The effect of the ethylene glycol concentration added to the sample matrix on the sensitivity was studied. The data presented in Fig. 2 show the impact of the volume fraction (0.2–0.8) of ethylene glycol in the sample on the sensitivity. The peak height signal was increased when the ethyl glycol content in sample matrix increased from 20 to 80% and decreased in 100%. As previously reported, the addition of ethylene glycol to the sample matrix drastically influences stacking efficiency, due to some conductivity modification [19]. The optimal volume fraction of ethylene glycol was found to be 0.8. 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 ethylene glycol has the ability to act as proton donator for zotepine (pK_a 7.0) [26], norzotepine and clozapine (pK_{a1}, 3.7; pK_{a2}, 7.6) [27]. Thus, to charge the analytes, phosphoric acid was added to the sample. It has already been demonstrated that the addition of phosphoric acid to the sample matrix may enhance the protonation of the cationic drugs and improve the sensitivity

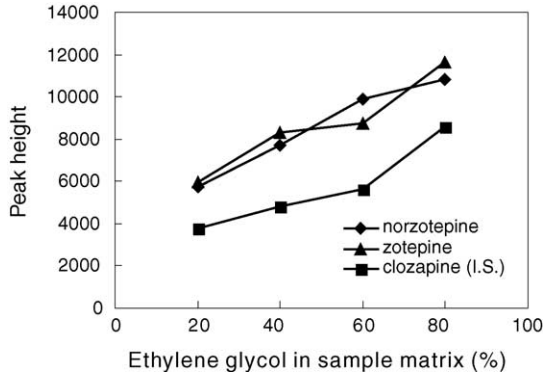

Fig. 2. Effect of the ethylene glycol concentration added to the sample matrix on the peak height of zotepine, norzotepine and clozapine each at 100 ng/mL. Separation CE conditions: uncoated fused-silica capillary, 30 cm (effective length) × 75 μm I.D.; wavelength, 200 nm; separation voltage, 20 kV (detector at cathode side); background electrolyte: 20 mM borate (pH 8.0)–50% ethylene glycol solution with 20% methanol.

detection during FASS injection because a larger amount of higher-mobility ions is introduced [19,20]. For the maximum stacking efficiency, the impact of the acid in sample solution was studied. With the sample solutions containing 30–90 μM of H_3PO_4, poor reproducibility was observed. The optimal H_3PO_4 concentration was found to be about 120 μM for analysis of positively charged analytes. Binary sample solutions of low conductivity and containing small amounts of weak acid are demonstrated to be most effective for stacking of positively charged analytes. Thus, for robust operation with high sensitivity, 80% ethylene glycol with 120 μM H_3PO_4 in sample matrix was used to prepare the sample. In order to optimize the separation of zotepine and its main active metabolite (norzotepine) by CZE with head-column FASS, we studied the effect of several experimental parameters such as background electrolyte concentration, pH and additives.

3.2. Optimization of the separation buffer

For binary CZE, the running buffer was composed of ethylene glycol, borate buffer and methanol. CZE with head-column FASS with 50% ethylene glycol–borate buffer (pH 8.0) at different concentrations (5–25 mM) with 20% methanol was studied in this experiment. The results in Fig. 3 show that the higher the concentration of buffer, the higher the sensitivity that was obtained. It is indicated that sample

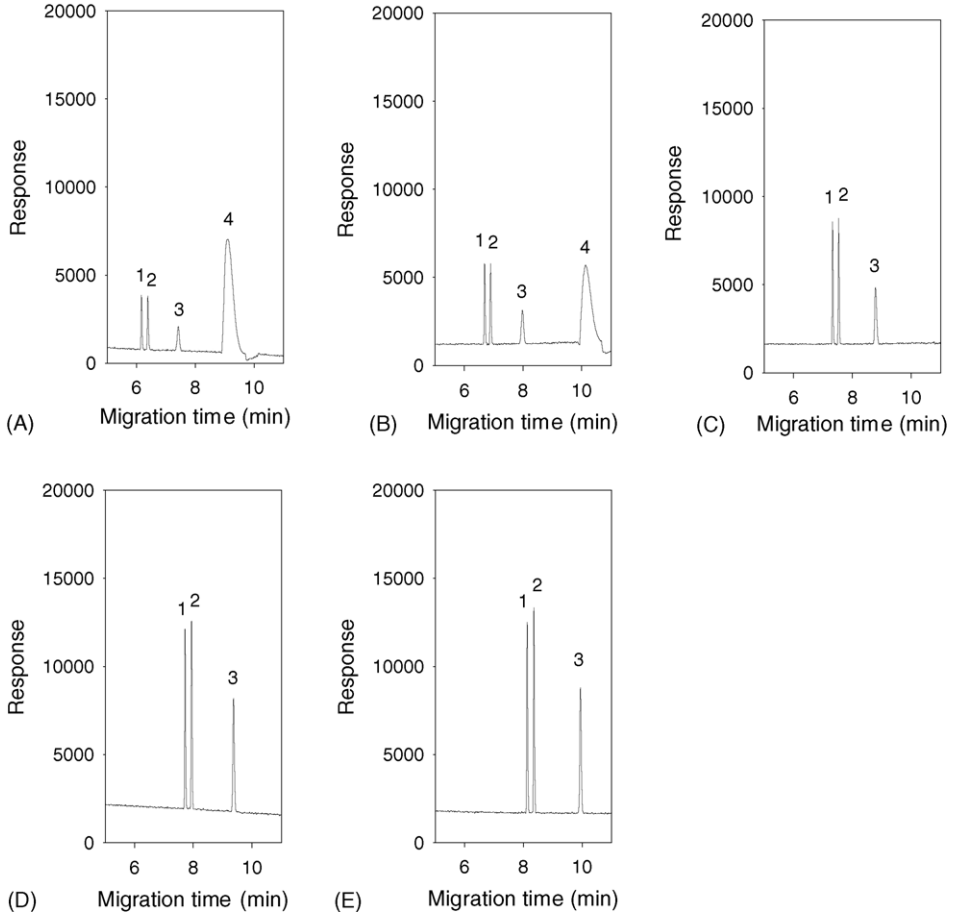

Fig. 3. Effect of borate buffer concentration on the separation and sensitivity of analytes each at 100 ng/mL. Electropherograms: (A) 5 mM, (B) 10 mM, (C) 15 mM, (D) 20 mM, (E) 25 mM. Peaks: 1, 2 and 3 are norzotepine, zotepine and clozapine (I.S.), respectively. Background electrolyte: 5–25 mM borate (pH 8.0)–50% ethylene glycol solution with 20% methanol. For other CE conditions as in Fig. 2.

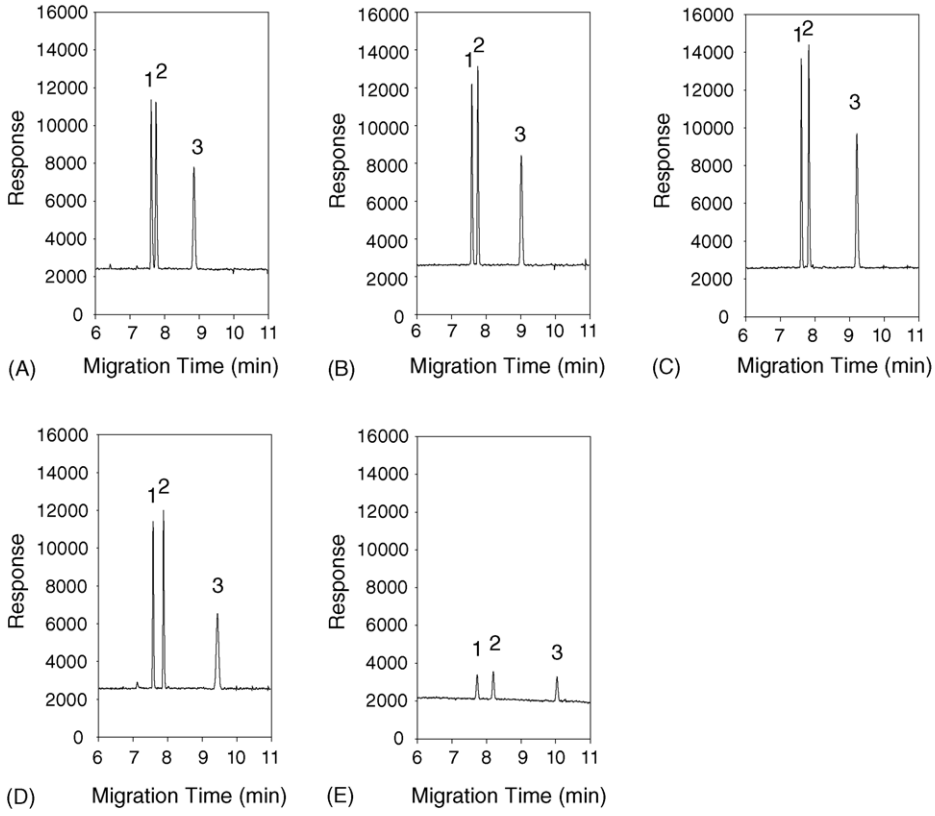

Fig. 4. Effect of borate buffer pH on the separation and sensitivity of analytes each at 100 ng/mL. Electropherograms: (A) 7.0; (B) 7.5; (C) 8.0; (D) 8.5, (E) 9.0. Peaks: 1, 2 and 3 are norzotepine, zotepine and clozapine (I.S.), respectively. Background electrolyte: 20 mM borate (pH 7–9)–50% ethylene glycol with 20% methanol. For other CE conditions as in Fig. 2.

condensation increased by introducing a high conductivity of running buffer to act as a temporary trap for solutes. The same stacking effect was obtained at 20 mM and 25 mM borate buffer. Peaks 1, 2, 3 and 4 represent norzotepine, zotepine, clozapine and electroosmotic flow (EOF). During FASS injection, the positively charged analytes moved at higher velocity than the neutral water. Therefore, EOF moved to detector is behind the analytes. However, a higher concentration of buffer caused a reduction in EOF, leading to longer analysis time. The optimal borate buffer is set at 20 mM.

The effect of pH (7.0–9.0) of 20 mM borate buffer-50% ethylene glycol solution with 20% methanol on the separation and sensitivity of analytes was also studied. That the migration velocity of zotepine, norzotepine, clozapine and EOF is not obviously influenced by pH can be seen in Fig. 4; however, pH obviously influenced the peak heights. It was found when background electrolyte was set at pH 8, the highest sensitivity and good resolution (R_S = 3.1) between norzotepine and zotepine were achieved as shown in Fig. 5.

The effects of ethylene glycol at 40, 50, or 60% (v/v) in 20 mM borate buffer (pH 8.0) with 20% methanol on the separation and sensitivity enhancement are discussed. The results show the resolution and sensitivity effect of norzotepine and zotepine in ethylene glycol concentration as shown as Fig. 6. More viscous buffer needs a longer separation time. The optimal ethylene glycol concentration is set at 50%, which can effectively retain and separate the drugs. In this condition, a slightly higher sensitivity than at 60% and higher resolution than at 40% (R_S = 1.9) were obtained.

Electrophoresis of the drugs in the absence of methanol as an additive results in partial resolution of zotepine and norzotepine (Fig. 7A). Organic solvents miscible with water are widely used as mobile phase modifier to adjust the capacity factor. The use of organic solvents can contribute to the

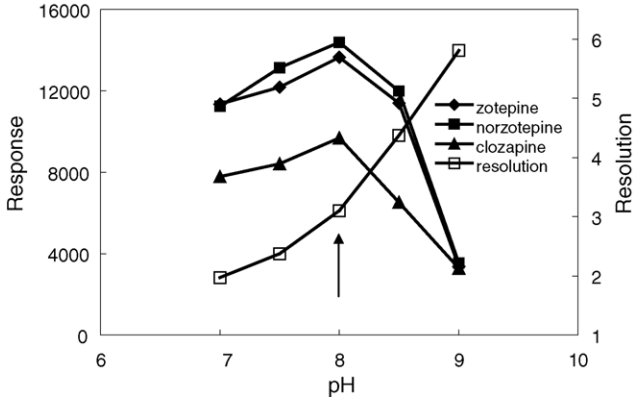

Fig. 5. Effect of pH (7.0–9.0) on the sensitivity of the analytes and resolution (□) between norzotepine and zotepine.

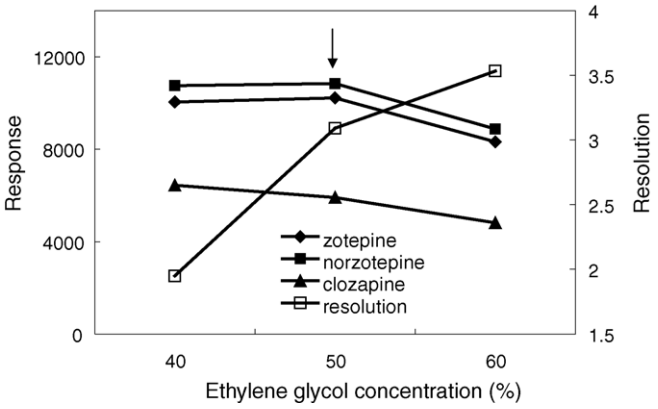

Fig. 6. Effect of ethylene glycol (40–60%) on sensitivity of analytes and resolution (□) between norzotepine and zotepine.

improvement of resolution or the alteration of selectivity. In general, the addition of organic solvents reduces the EOF and expands the migration time window. Therefore, the effects of concentration of 10, 20, 30 and 40% methanol added in 50% ethylene glycol–borate buffer (20 mM; pH 8.0) as organic modifier on separation of zotepine and norzotepine were studied as shown in Fig. 7B–E, respectively. Baseline resolution for zotepine and norzotepine was obtainable at a methanol concentration ≥10%. The 50% ethylene glycol–20 mM borate buffer solution (pH 8) with 20% methanol was the choice for optimal buffer concentration, pH and additives for determination of zotepine and norzotepine. Fig. 8A and B represent the typical electropherograms for human plasma blank and zotepine, norzotepine and clozapine spiked in plasma using CZE with head-column FASS. Peaks 1, 2 and 3 in electropherogram represent norzotepine, zotepine and clozapine (I.S.), respectively. The results indicate that simple separation mode of CZE based mainly on the differences of charge to mass ratios of the analytes in the tested conditions is able to resolve the zotepine and its active metabolite, norzotepine. The charge to mass ratio of norzotepine, N-demethylation of zotepine, is higher than the parent drug, zotepine. Therefore, electrophoretic velocity of norzotepine has faster migration in this CZE running buffer. The reproducibility of the migration rate of analytes was studied. With twenty repeated injections of the samples, observed migration times were 7.61 ± 0.03 min, 7.81 ± 0.03 min, 9.16 ± 0.05 min and

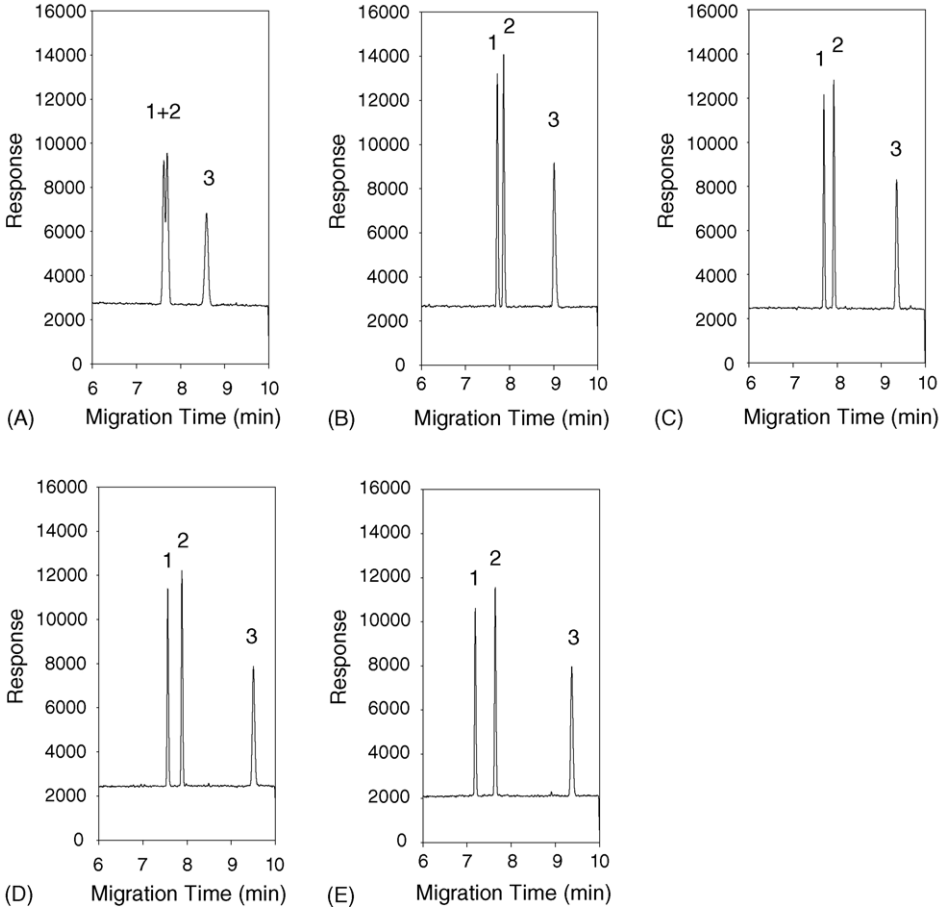

Fig. 7. Effect of concentration of methanol added in background electrolyte on the separation and sensitivity of analytes each at 100 ng/mL. Electropherograms: (A) without methanol; (B) 10%; (C) 20%; (D) 30%, (E) 40%. Peaks: 1, 2 and 3 are norzotepine, zotepine and clozapine (I.S.), respectively. Background electrolyte: 20 mM borate (pH 8)–50% ethylene glycol with (0–40%) methanol. Other CE conditions as in Fig. 2.

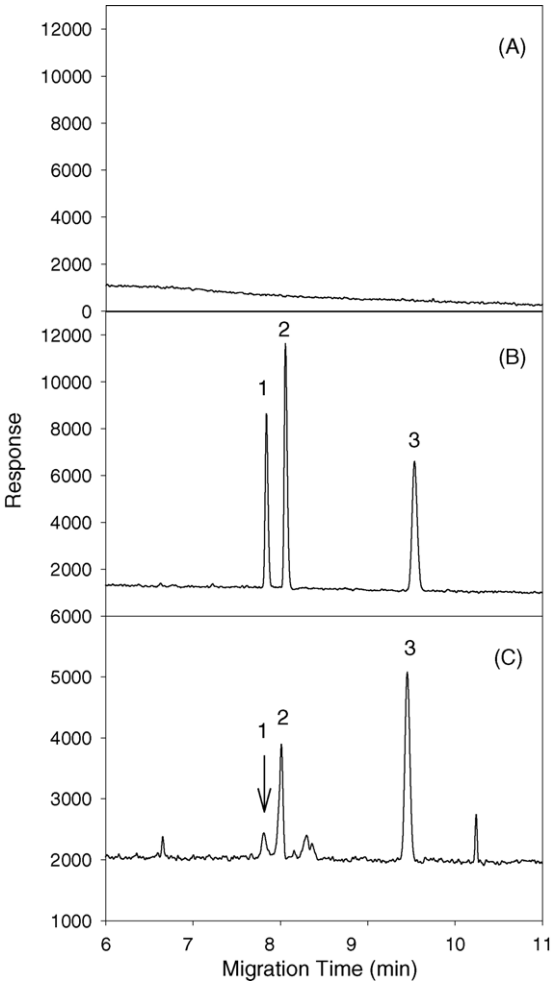

Fig. 8. Electropherograms of zotepine, norzotepine and clozapine in biological samples determination. (A) plasma blank; (B) plasma spiked zotepine, norzotepine and clozapine each at 100 ng/mL; (C) plasma from a schizophrenia patient receiving oral 125 mg of zotepine tablet. Peaks: 1, 2 and 3 are norzotepine, zotepine and clozapine (I.S.), respectively. Background electrolyte: 20 mM borate (pH 8)–50% ethylene glycol with 20% methanol. Other CE conditions as in Fig. 2.

11.67 ± 0.08 min for norzotepine, zotepine, clozapine and EOF, respectively. A high accuracy on retention times in intra- and inter-day was found with relative standard deviations (RSD) being generally less than 0.43%. The apparent mobility (μA) was calculated according to the equation: $\mu A = \mu E + \mu EOF = (lL/tV)$ where l = length along the capillary (cm) to detector, V = voltage, t = migration time (s) and L = total length (cm) of the capillary [28]. In optimized CE conditions, the apparent mobility values of norzotepine, zotepine, clozapine and EOF are 1.32×10^{-4} cm^2 V^{-1} s^{-1}, 1.29×10^{-4} cm^2 V^{-1} s^{-1}, 1.10×10^{-4} cm^2 V^{-1} s^{-1} and 8.61×10^{-5} cm^2 V^{-1} s^{-1}, respectively. According to the equation, $\mu A = \mu E + \mu EOF$, the electrophoretic mobility values (μE) of norzotepine, zotepine and clozapine in human plasma are 4.59×10^{-5} cm^2 V^{-1} s^{-1}, 4.29×10^{-5} cm^2 V^{-1} s^{-1} and 2.39×10^{-5} cm^2 V^{-1} s^{-1}, respectively.

Table 1
Regression analyses for determination of spiked zotepine and norzotepine in plasma

Concentration range (ng/mL)	Regression equation	Correlation coefficient (r)
Zotepine		
Intra-day[a]		
3.0–100.0	$y = (0.153 \pm 0.010)x - (0.223 \pm 0.095)$	0.999
Inter-day[a]		
3.0–100.0	$y = (0.156 \pm 0.012)x - (0.216 \pm 0.091)$	0.999
Norzotepine		
Intra-day[a]		
3.0–100.0	$y = (0.113 \pm 0.008)x - (0.256 \pm 0.038)$	0.999
Inter-day[a]		
3.0–100.0	$y = (0.116 \pm 0.009)x - (0.238 \pm 0.078)$	0.999

[a] Intra-day data were based on four replicate analyses and inter-day were from five consecutive days.

3.3. Validation of zotepine and norzotepine spiked in plasma

To evaluate the quantitative applicability of the methods, five different concentrations of zotepine and norzotepine were analyzed using clozapine as an I.S. The linear regression equations in plasma samples are listed in Table 1. The straight lines obtained from four or five separate experiments had correlation coefficients of 0.999. The data indicate high linearity of this method for the intra- and inter-day assays. Repeatability was determined by RSD of the slope of the linear regression equations. For plasma, the RSDs of intra- and inter-day average slope of the equations of analytes were below 7.0 and 7.8%, respectively. The precision of the proposed method for spiked samples was studied. The results in Table 2 show that the intra- and inter-day variances at the concentrations were all below 9.7%. The relative recoveries of zotepine and norzeptine were obtained from the calibration graph constructed from plasma spiked with different amounts of zotepine and norzotepine at low, medium and high concentration levels. Table 2 shows all the recoveries were >91%. The LOQs in plasma were 3 and 3 ng/mL for zotepine and norzotepine, respectively. The LODs of the proposed method for zotepine and norzotepine (electrokinetic injection 10 kV, 20 s) were found to be 2 and 1 ng/mL, respectively. Stock solution of zotepine and norzotepine in aqueous solution did not reveal any appreciable degradation after 20 days at +4 °C.

3.4. Application

After receiving 125 mg zotepine tablet (Lodopin®, Fujisava) from oral administration, we measured the patient's plasma 8 h later. The electropherogram of plasma is shown in Fig. 8C. The concentrations of zotepine and norzotepine after dosing at 8 h later were 53.2 and 13.6 ng/mL, respectively.

Table 2
Precision and accuracy for the recovery of spiked zotepine and norzotepine in plasma

Concentration known (ng/mL)	Concentration found (ng/mL)	RSD (%)	RE[a] (%)
Zotepine			
Intra-day[b] ($n = 4$)			
3.0	2.87 ± 0.16	5.6	−4.3
20.0	19.13 ± 1.63	8.5	−4.3
100.0	101.04 ± 5.69	5.6	1.0
Inter-day[b] ($n = 5$)			
3.0	2.88 ± 0.23	8.0	−4.0
20.0	18.64 ± 1.21	6.5	−6.8
100.0	100.57 ± 2.56	2.5	0.6
Norzotepine			
Intra-day[b] ($n = 4$)			
3.0	2.83 ± 0.11	3.9	−5.7
20.0	19.01 ± 1.17	6.2	−5.0
100.0	96.54 ± 2.21	2.3	−3.5
Inter-day[b] ($n = 5$)			
3.0	2.86 ± 0.28	9.7	−4.6
20.0	18.35 ± 1.67	9.1	−8.3
100.0	99.51 ± 3.28	3.3	−0.5

[a] RE is used to check the accuracy of the measurements by the propose method.
[b] Intra-day data were based on four replicate analyses and inter-day were from five consecutive days.

4. Conclusions

A SPE coupling CZE with head-column FASS method for determination of zotepine and norzotepine in plasma described here represented a sensitive and efficient analytical method. Validation of the methods for quantitation of zotepine and norzotepine in plasma showed that the methods have high sensitivity and accuracy. The zotepine and norzotepine in biological matrix was stable for at least 30 days when frozen at −40 °C. Therefore, the methods were suitable for the analysis of zotepine and norzotepine in plasma collected during pharmacokinetic investigations in humans and clinical use.

Acknowledgements

The authors are grateful to the National Science Council, ROC, and Fujisawa Pharmaceuticals for partial financial support of the study.

References

[1] A. Prakash, H.M. Lamb, Drug 2 (1998) 153.
[2] W.W. Fleischhacker, C. Barnas, C.H. Stuppäck, B. Unterweger, C. Miller, H. Hinterhuber, Psychopharmacol. Bull. 25 (1989) 97.
[3] C. Barnas, C.H. Stuppäck, C. Miller, C. Haring, B. Sperner-Unterweger, W.W. Fleischhacker, Int. Clin. Psychopharmacol. 7 (1992) 23.
[4] K. Otani, T. Kondo, S. Kaneko, T. Hirano, K. Mihara, Y. Fukushima, Hum. Psychopharmacol. 7 (1993) 331.
[5] T. Kondo, K. Otani, M. Ishida, O. Tanaka, S. Kaneko, Y. Fukushima, Ther. Drug Monti. 16 (1994) 120.
[6] O. Tanaka, T. Kondo, S. Kaneko, K. Mihara, K. Otani, N. Tokinaga, Ther. Drug Monit. 18 (1996) 294.
[7] J. Bickeboeller-Friedrich, H.H. Maurer, Ther. Drug Monit. 23 (2001) 61.
[8] C. Kratzsch, F.T. Peter, T. Kraemer, A.A. Weber, H.H. Maurer, J. Mass. Spectrom. 38 (2003) 283.
[9] M. Kollroser, G. Henning, R. Gatternig, C. Schober, Forensic Sci. Int. 123 (2001) 243.
[10] M. Hayashida, M. Nihira, T. Watanabe, J. Chromatogr. 506 (1990) 133.
[11] J. Joseph-Charles, M. Bertucat, Anal. Chim. Acta 284 (1993) 45.
[12] J. Joseph-Charles, M. Bertucat, J. Liq. Chromatogr. Rel. Technol. 21 (1998) 3047.
[13] F.E.P. Mikkers, F.M. Everaerts, T.P.E.M. Verheggen, J. Chromatogr. 169 (1979) 11.
[14] G. Hempel, Electrophoresis 21 (2000) 691.
[15] W. Thormann, A. Minger, S. Molteni, J. Caslavaka, P. Gebauer, J. Chromatogr. 593 (1992) 275.
[16] M. Albin, P.D. Grossman, S.E. Moring, Anal. Chem. 65 (1993) 489A.
[17] S. Pálmarsdóttir, L.E. Edholm, J. Chromatogr. A 693 (1995) 131.
[18] C.X. Zhang, W. Thormann, Anal. Chem. 68 (1996) 2523.
[19] J.Z. Song, J. Chen, S.J. Tian, Z.P. Sun, J. Pharm. Biomed. Anal. 21 (1999) 569.
[20] C.X. Zhang, W. Thormann, Anal. Chem. 70 (1998) 540.
[21] B. Wey, C.X. Zhang, W. Thormann, J. Chromatogr. A 853 (1999) 95.
[22] D.M. Osbourn, D.J. Weiss, C.E. Lunte, Electrophoresis 21 (2000) 2768.
[23] S.M. Wu, Y.H. Ho, H.L. Wu, S.H. Chen, H.S. Ko, Electrophoresis 22 (2001) 2717.
[24] L. Zhu, H.K. Lee, Anal. Chem. 73 (2001) 3065.
[25] S. Grard, P. Morin, J.P. Ribet, Electrophoresis 23 (2002) 2399.
[26] http://medicine.cug.net/index.htm.
[27] Merck Index, 13th ed., Merck & Co. Inc., New Jersey, 2001, p. 2451.
[28] K.D. Altria (Ed.), Capillary Electrophoresis Guidebook: Principle, Operation, and Application, Humana Press Inc., New Jersey, 1996, p. 7.