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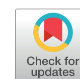
Original Article

Enantioseparation of phenothiazines through capillary electrophoresis with solid phase extraction and polymer based stacking



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ARTICLE INFO

Article history:
Received 18 September 2017
Received in revised form
15 November 2017
Accepted 7 December 2017
Available online 12 January 2018

Keywords:
Capillary electrophoresis
Chiral separation
On-line concentration
Phenothiazine
Solid phase extraction

ABSTRACT

This study developed a sensitive method involving capillary electrophoresis (CE) coupled with ultraviolet absorption for the simultaneous separation of chiral phenothiazine drugs at nanomolar concentration levels. The method consists of hydroxypropyl-γ-cyclodextrin (Hp-γ-CD) as a chiral selector and poly (diallyldimethylammonium chloride) (PDDAC)-based CE. Five pairs of D,L-phenothiazines were baseline separated using a background electrolyte containing 0.9% PDDAC, 5 mM Hp-γ-CD, and 100 mM tris(hydroxymethyl)aminomethane (Tris)-formate (pH 3.0). The five pairs were successfully stacked on the basis of the difference in viscosity between the PDDAC-containing background electrolyte and the sample solution, with almost no loss of resolution. The combination of a solid-phase extraction and PDDAC-mediated CE can efficiently improve the sensitivity of the phenothiazine enantiomers. Under optimal conditions, calibration graphs displayed the linear range between 6 and 1500 nM, with relative standard deviation values lower than 3.5% (n = 5). Detection limit ranged from 2.1 to 6.3 nM for target analytes, and 607- to 1555-fold enhancement was achieved. The practicality of using the proposed method to determine five pairs of D,L-phenothiazines in urine is also validated, in which recoveries between recoveries of all phenothiazines from urine ranged from 89% to 101%.

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Abbreviations: CD, cyclodextrin; CE, capillary electrophoresis; D,L-Eth, D,L-ethopropazine; D,L-Met, D,L-methotrimeprazine; D,L-Tri, D,L-trimeprazine; D,L-Pro, D,L-promethazine; D,L-Thi, D,L-thioridazine; Hp-γ-CD, hydroxypropyl-γ-cyclodextrin; PDDAC, poly(diallyldimethylammonium chloride); SPE, solid phase extraction; Tris, tris(hydroxymethyl)aminomethane.

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https://doi.org/10.1016/j.jfda.2017.12.002

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1. Introduction

Phenothiazines, such as D,L-thioridazine (D,L-Thi), D,L-methotrimeprazine (D,L-Met), D,L-ethopropazine (D,L-Eth), D,L-trimeprazine (D,L-Tri), and D,L-promethazine (D,L-Pro), are enantiomeric drugs [1,2]. They are widely used as antipsychotic, antihistaminic, antiemetic, anesthetic, and neuroleptic drugs, and their concentration in blood and urine is extremely low; approximately 2%–10% of a dose is excreted in urine [1,3–5]. An overdose of phenothiazines could cause coma, miosis, respiratory depression, and life-threatening conditions. When a patient consumes several phenothiazine drugs, fatal intoxication typically occurs [6]. For example, doses of 15 mg of chlorpromazine, 50 mg of thioridazine, and 75 mg of promethazine could be fatal to adults [1]. Therefore, it is urgent to differentiate rapidly the phenothiazines overdose from others. Furthermore, researchers have reported that the use of a single phenothiazine enantiomer as a drug might lead to a substantial therapeutic improvement [7]. Therefore, it is imperative to develop a rapid, low-cost, and reliable method for simultaneous quantifying trace amounts of phenothiazine enantiomers in human fluid samples.

Currently, separation methods, including high-performance liquid chromatography (HPLC) [8–10] and capillary electrophoresis (CE) [5,11], have been routinely used for determining a number of phenothiazine drugs in human fluids. CE has been shown to be a more powerful method than HPLC for separating phenothiazine enantiomers because it is characterized by low solvent consumption, short analysis time, high resolution, and high efficiency; moreover, it just requires a small volume of the sample to be injected [12,13], The introduction of a chiral selector into the background electrolyte enables researchers to separate D- and L-phenothiazines in CE [14,15]. For example, Martinez-Gomez et al. demonstrated that D,L-Tri and D,L-Pro could be completely enantioseparated by using affinity electrokinetic chromatography and human serum albumin as a chiral selector [16]. Lin et al. used sulfate-substituted cyclodextrin (CD) and neutral CD as dual chiral selectors for the CE enantioseparation of four pairs of D,L-phenothiazines within 18 min [17,18], However, D,L-Thi, D,L-Met, D,L-Eth, D,L-Tri, and D,L-Pro were not resolved simultaneously in a single run. Additionally, in the aforementioned CE methods, the chiral separation of D,L-phenothiazines is time-consuming and characterized by poor separation efficiency and sensitivity. Moreover, the methods are yet to be used for detecting D,L-phenothiazines in human fluids.

This study proposes the use of 2-hydroxypropyl-γ-cyclodextrin (Hp-γ-CD) as a chiral selector for simultaneously separating five pairs of phenothiazine enantiomers D,L-Thi, D,L-Met, D,L-Eth, D,L-Tri, and D,L-Pro through CE in the presence of poly (diallyldimethylammonium chloride) (PDDAC). PDDAC consists of quaternary amines and has been shown to be useful as a buffer additive for the on-line concentration and separation of positively charged analytes, including aminothiols, amine neurotransmitters, and biogenic and high pI proteins, under low pH conditions [19–22]. It has been suggested that the stacking mechanism is based on the difference in viscosity between a PDDAC-containing background electrolyte and a sample solution [21,23,24]. Our

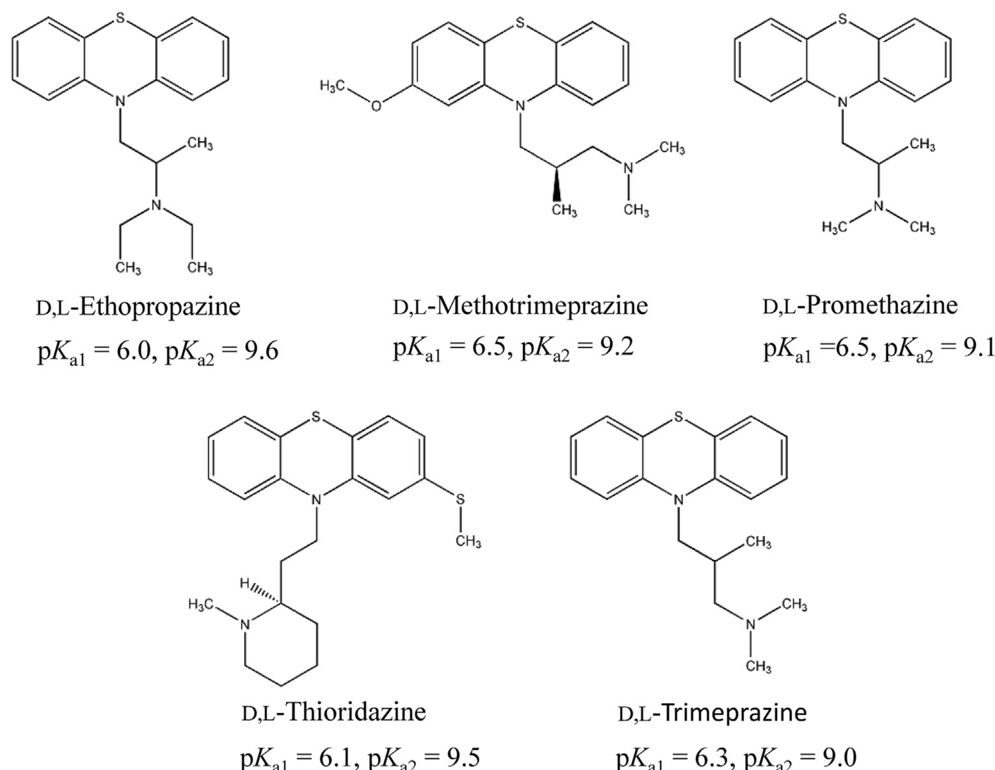

Fig. 1 – Chemical structures and pK_a values of the analytes.

previous study mainly goal to improve separation time and efficiency [25], but it had slightly less enough sensitivity to measure phenothiazine enantiomers in real samples. Thus, this study also showed that PDDAC is well suited for on-line stacking five pairs of phenothiazine enantiomers in CE without loss of resolution. Furthermore, to determine a number of phenothiazine enantiomers in real samples by using the proposed method, a C_{18} solid-phase extraction (SPE) column was chosen for sample preparation because of its ability to extract and purify analytes from complex matrices [26,27]. The combination of Hp-γ-CD uses as a chiral selector and PDDAC-based CE, and SPE was used for simultaneously determining the amounts of the phenothiazine enantiomers in urine samples.

2. Materials and methods

2.1. *Chemicals*

PDDAC (20 wt% in water; M.W. 400,000–500,000), mesityl oxide (MO), sodium hydroxide, formic acid, tris(hydroxymethyl)aminomethane (Tris), D,L-Thi, D,L-Tri, D,L-Eth, D,L-Pro, D,L-Met, α-CD, β-CD, γ-CD, hydroxypropyl-α-CD (Hp-α-CD), Hp-β-CD, and Hp-γ-CD were obtained from Sigma–Aldrich Corporation (St. Louis, MO). The pH of formic acid was adjusted to 2.0–4.0 by adding 100 mM Tris. Water used in all experiments was doubly distilled and purified using a Milli-Q system (Millipore, Milford, MA).

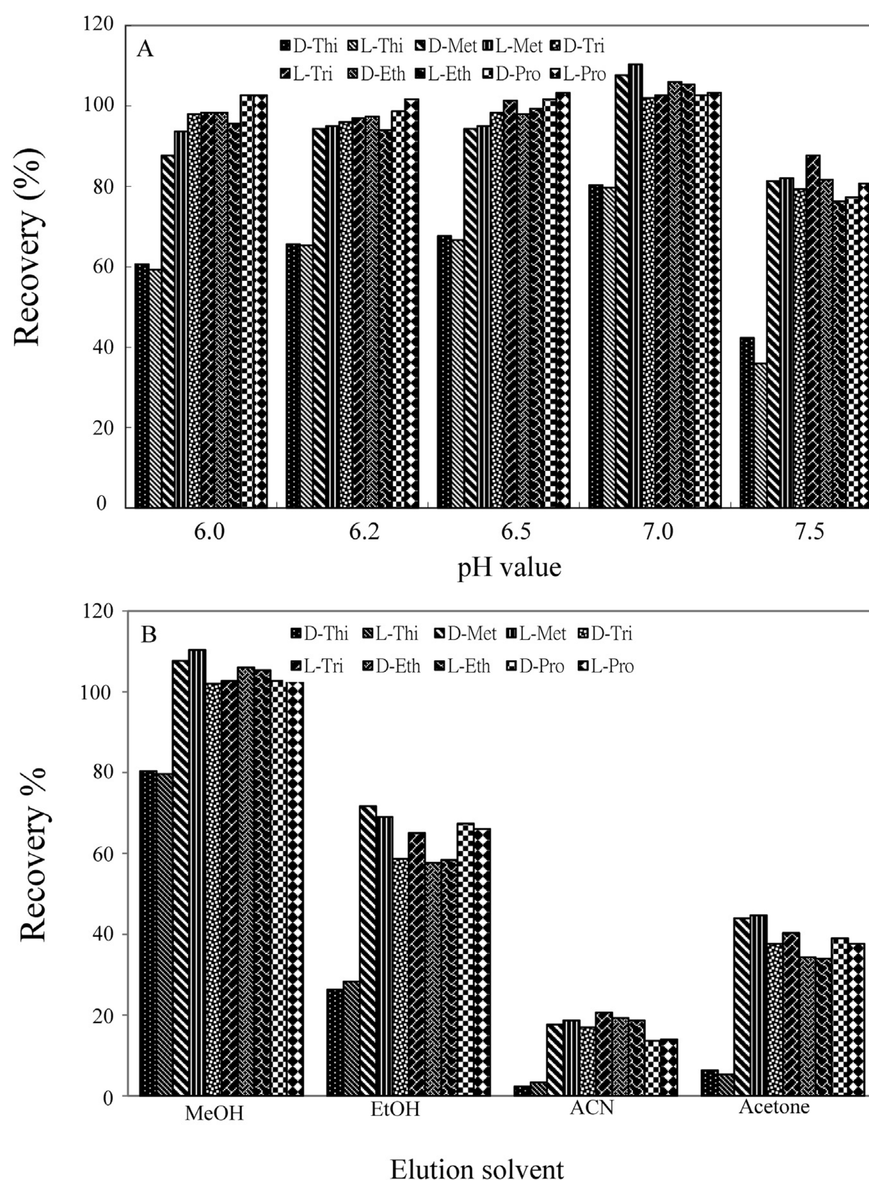

Fig. 2 – Influence of (A) the sample pH (6.0–7.5) and (B) elution solvent type on extraction efficiency of SPE. The cartridges were conditioned with 3 mL methanol and 3 mL phosphate (5 mM, pH 7.0). Sample loaded 1 mL containing ten phenothiazines (1 mM). After sample percolation, the cartridge was washed off the interfering compounds with 1 mL methanol: water (50:50 v/v) and water 2 mL. Then using 3 mL methanol eluted analytes, it was evaporated under centrifugal vaporizer. The residue was reconstituted with 3 mL aqueous solution.

2.2. Capillary electrophoresis

The detection wavelength of a commercial ultraviolet absorbance detector (ECOM, Germany) was set to 254 nm for phenothiazines. A high-voltage power supply (Bertan, Hicksville, NY) was used to drive electrophoresis. A computer connected to the Peak-ABC Chromatography Data-Handling System (Great Tide Instrument Company, Taipei, Taiwan) was used to record electropherograms and perform quantitative measurements of the peak area. Fused-silica capillaries (Polymicro Technologies, Phoenix, AZ) with a 75 μm I.D. and 365 μm O.D. were used for separating analytes. Prior to analysis, the capillaries were flushed with 0.5 M NaOH for 10 min by using a syringe pump (KD Scientific, New Hope, PA) at a flow rate of 5 μL min^{-1}, and were then water-flushed for 1 min. Subsequently, the capillary was treated with a solution containing 2% PDDAC and 5 mM tris-formate (pH 2.0–4.0) for 10 min, resulting in reversed EOF. Before performing separation, the capillary was filled with a solution containing 0–1.2% PDDAC and 0–15 mM CD derivative through syringe pumping at a flow rate of 5 μL min^{-1}. The viscosity of the solution containing 0.9% PDDAC is 5.4 mPa s. Phenothiazines were injected through hydrodynamic injection at a 20 cm height for 10 s. The sample volume was estimated by using MO as a UV absorbance marker. The time of the baseline shift indicated that the MO molecules migrated to the detection point, which allows calculation of the sample injection rate (nL s^{-1}). By using the injection rate and injection time, the sample injection volumes were estimated. The applied voltage in all separations was performed between −10 kV and −12 kV (250–300 V/cm). After each run, the capillary was flushed with 0.9% PDDAC for 10 min through syringe-pumping at a flow rate of 5 μL min^{-1}.

2.3. Quantification of the five pairs of D,L-phenothiazines

The calibration curve was drawn by plotting the peak area against the analyte concentration over the range of 0.006–1.5 μM. The estimated limits of detection (LODs) of the D,L-phenothiazines were considered to be the analyte concentration at an estimated signal-to-noise ratio of 3. The separation of the five pairs of D,L-phenothiazines was repeated three times on the same day to obtain the repeatability (intraday precision), and three times over five consecutive days to obtain the intermediate precision (interday precision); the repeatability and intermediate precision were expressed as relative standard deviation (RSD) values. Samples of human urine were collected from six healthy volunteers and were stored at −4 °C until analysis. Subsequently, the sample solution was adjusted to pH 7 with NaOH, thus centrifuged at 3000 rpm for 10 min. The urine supernatant (940 μL) of each sample was spiked without and with a solution of D,L-phenothiazines standard (50 μL, 0–3.0 mM). The calibration curves for standard solution and urine sample were both obtained by plotting the peak area against the spiked concentration of the analytes.

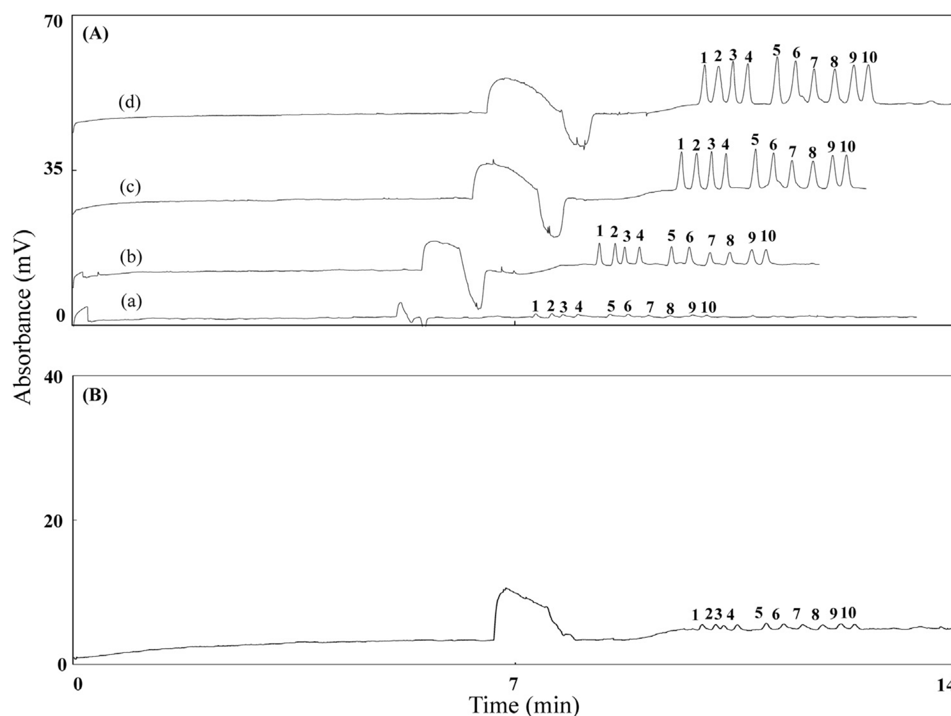

Fig. 3 – (A) Stacking and enantioseparation of (a) 9, (b) 108, (c) 216, and (d) 270 nL five pairs of phenothiazines by SPE-polymer based stacking-CE. Electrophoresis conditions: 50-cm capillary (40-cm to detector). Peak identities: 1. D-Thi (0.38 μM), 2. L-Thi (0.38 μM), 3. D-Met (0.13 μM), 4. L-Met (0.13 μM), 5. D-Tri (0.15 μM), 6. L-Tri (0.15 μM), 7. D-Eth (0.20 μM), 8. L-Eth (0.20 μM), 9. D-Pro (0.10 μM), and 10. L-Pro (0.10 μM). (b) Stacking and separation of 270 nL five pairs of phenothiazines by SPE-polymer based stacking-CE. Peak identities: 1. D-Thi (20 nM), 2. L-Thi (8 nM), 3. D-Met (9 nM), 4. L-Met (9 nM), 5. D-Tri (9 nM), 6. L-Tri (9 nM), 7. D-Eth (10 nM), 8. L-Eth (10 nM), 9. D-Pro (6 nM), and 10. L-Pro (6 nM).

Table 1 – Calibration line, coefficients of determination, LODs, RSDs of migration time and area for 10 phenothiazines of the proposed method.

Compound	LDR[a](nM)	Regression equation[b]	R^2	LOD (nM)[c]	RSD (%)[d]	
					Migration time (min)	Area
D-Thi	20–1500	y = 66.124x + 0.7546	0.9969	5.6	2.9	3.2
L-Thi	20–1500	y = 65.361x + 0.3562	0.9995	6.3	3.2	3.5
D-Met	9–600	y = 159.69x + 2.2137	0.9991	2.6	2.7	3.1
L-Met	9–600	y = 163.88x + 2.4783	0.9981	2.5	2.6	2.9
D-Tri	9–600	y = 169.83x + 2.2867	0.9993	2.6	2.5	3.0
L-Tri	9–600	y = 161.61x + 1.7387	0.9998	2.5	2.3	3.3
D-Eth	10–800	y = 145.22x + 1.2511	0.9992	2.9	2.8	3.1
L-Eth	10–800	y = 152.85x + 2.4520	0.9979	2.8	2.9	3.2
D-Pro	6–400	y = 295.56x + 3.2011	0.9997	2.3	2.5	2.8
L-Pro	6–400	y = 301.50x + 3.8776	0.9998	2.1	2.7	3.0

[a] linear dynamic range.
[b] y (peak area) = slope × [compound concentration (nM)] + intercept.
[c] Limit of detection.
[d] % Relative standard deviation ($n = 3$).

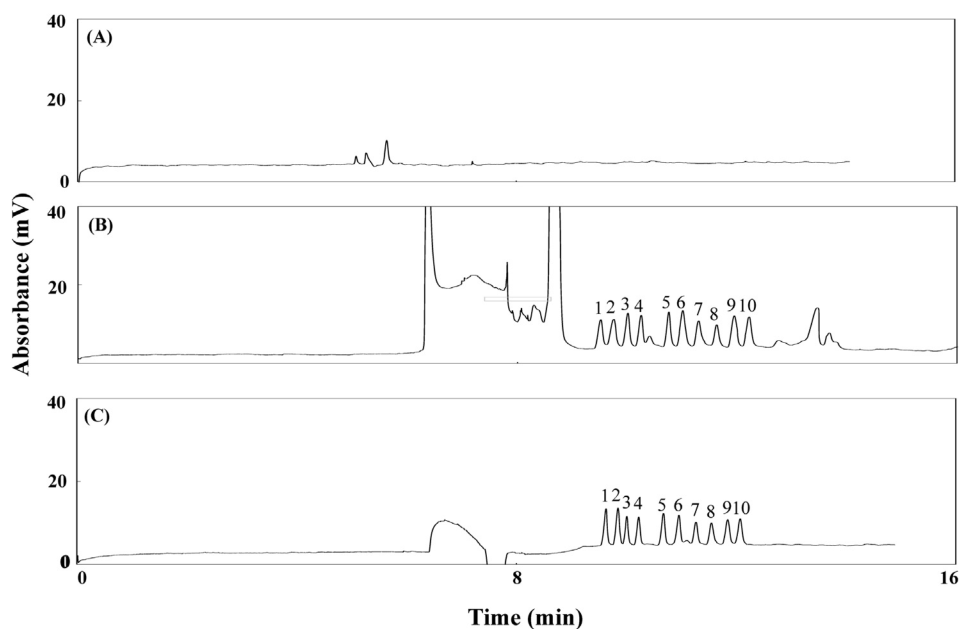

Fig. 4 – The electropherograms of the spiked urine samples ((A) and (B)) and standards prepared in aqueous solution (C) using CZE (A), and SPE-polymer based stacking-CE ((B) and (C)). The sample was spiked (A), (B) and (C) with five pairs of phenothiazines. The injected volume of sample was (A) 9 nL and (B), (C) 270 nL. Peak identities: 1. D-Thi (0.28 μM), 2. L-Thi (0.28 μM), 3. D-Met (0.13 μM), 4. L-Met (0.13 μM), 5. D-Tri (0.15 μM), 6. L-Tri (0.15 μM), 7. D-Eth (0.20 μM), 8. L-Eth (0.20 μM), 9. D-Pro (0.10 μM), and 10. L-Pro (0.10 μM).

2.4. *SPE cartridge of D,L-phenothiazines*

Samples of urine were spiked with different concentrations of five pairs of standard D,L-phenothiazines. The Sep-Pak Vac 3 cc (200 mg sorbent, 55–105 μm particle size) C_{18}-SPE cartridge (Waters, Milford, MA) was first conditioned with methanol (3 mL), followed by sodium phosphate buffer (3 mL, 5 mM; pH 7.0). The spiked samples were loaded onto a SPE cartridge. After that, the cartridge was washed with 1 mL method:water (50:50 v/v) and 2 mL deionized water. Final eluted was carried out with 3 mL methanol (100%). The eluted samples were evaporated in a centrifugal vaporizer (EYELA, CVE-200D). The dried samples were dispersed by adding a solution containing 15 μL methanol and 15 μL deionized water. Finally, the purified sample was analyzed by CE.

3. Results and discussion

3.1. *Effect of poly(diallyldimethylammonium chloride) concentration*

Our previous study indicated that a background electrolyte containing 75 mM phosphate buffer (pH 3.0) and 0.9% PDDAC is well suited for separating a mixture of three phenothiazines [6,21]. On the basis of this result, the separation of five pairs of D,L-phenothiazines (Fig. 1) was also performed in the presence of PDDAC. Fig. S1A shows that the separation of the five phenothiazines was unsuccessful in the absence of PDDAC. The efficiency of the separation of the five phenothiazines increased with the PDDAC concentration and was saturated for concentrations above 0.6% PDDAC (Fig. S1A–E). Obviously, the addition of PDDAC to the background electrolyte can improve the efficiency of CE separation of the five phenothiazines, mainly because of the high PDDAC concentration efficiently suppressed the adsorption of D,L-phenothiazines on the capillary surface. A similar improvement in separation efficiency was observed in a previous study when polyethylene oxide and dextran sulfate were used as the background electrolyte [28]. A possible reason for the improvement is that PDDAC reduces the adsorption of the analyte on the capillary surface and modifies the magnitude of the reversed EOF [21,25]. Therefore, the use of 0.9% PDDAC as a buffer additive is sufficient for the separation of the five phenothiazines.

3.2. *Effect of the type of cyclodextrin derivative*

Typical CD derivatives, including α-CD, β-CD, γ-CD, Hp-α-CD, Hp-β-CD, and Hp-γ-CD, were tested for the separation of the five pairs of D,L-phenothiazine enantiomers at pH 3.0 in the presence of 0.9% PDDAC (Fig. S2 and S3, supplementary material). Among them, Hp-γ-CD provided the most favorable chiral separation of the five pairs because of the interactions between Hp-γ-CD and phenothiazine enantiomers included hydrogen bonding, dipole and pi–pi interactions [29,30], which provide excellent selectivity for resolving D,L-phenothiazines. The effect of the Hp-γ-CD concentration was investigated by varying its concentration in the range of 0–15 mM. Fig. S4 shows that the best separation of the five pairs of D,L-phenothiazine enantiomers was accomplished

Table 2 – Accuracy of five pairs of phenothiazines from spiked urine sample using proposed method.

Compound	Spiked level (nM, ±SD)	Urine								
		Sample 1			Sample 2			Sample 3		
		A (%)[a]	RSD%[b]		A (%)[a]	RSD%[b]		A (%)[a]	RSD%[b]	
			Intra-day	Inter-day		Intra-day	Inter-day		Intra-day	Inter-day
D-Thi	25 (±0.1)	89	3.1	3.9	91	3.9	4.5	91	2.7	3.5
	400 (±0.2)	101	1.7	2.6	98	1.5	2.6	101	1.7	2.6
	700 (±1.9)	99	2.0	3.0	101	1.7	2.5	98	2.3	3.1
L-Thi	25 (±0.1)	93	3.0	3.8	93	4.6	5.1	92	3.3	4.0
	350 (±0.6)	101	2.2	2.9	101	2.3	3.2	99	1.8	2.8
	750 (±2.2)	97	1.9	2.7	100	2.0	2.9	101	2.2	3.1
D-Met	10 (±0.1)	91	2.7	3.6	90	4.7	5.1	90	2.3	2.9
	100 (±0.5)	100	2.1	3.2	100	2.6	3.2	101	2.5	3.2
	250 (±4.1)	99	2.3	3.3	99	2.7	3.3	100	2.5	3.4
L-Met	10 (±0.1)	90	2.6	3.5	89	4.7	5.0	90	2.8	3.6
	100 (±0.9)	99	1.9	2.6	101	3.4	3.9	101	2.3	3.1
	250 (±5.6)	99	2.4	3.1	99	2.9	3.4	96	3.3	4.1
D-Tri	10 (±0.1)	90	3.6	4.3	89	3.1	3.9	91	2.2	3.2
	150 (±0.2)	101	2.3	3.1	100	1.8	2.6	101	1.8	2.8
	300 (±2.2)	100	1.8	2.8	99	2.7	3.6	99	3.0	3.7
L-Tri	10 (±0.2)	89	3.5	4.0	94	3.1	3.9	89	3.2	3.9
	150 (±1.1)	100	2.2	2.9	101	1.6	3.0	101	2.0	3.0
	300 (±7.6)	100	2.1	3.1	99	2.4	3.2	98	2.2	3.1
D-Eth	10 (±0.3)	90	3.1	3.8	93	4.9	5.4	90	2.8	3.6
	200 (±1.9)	101	3.2	4.1	101	2.5	3.4	100	2.3	2.9
	400 (±8.6)	99	2.7	3.6	100	2.9	3.6	100	3.4	4.0
L-Eth	10 (±0.2)	90	3.6	4.3	89	4.1	4.7	90	2.6	3.2
	200 (±1.6)	101	2.8	3.3	101	2.3	3.1	98	1.9	3.0
	400 (±10.8)	99	2.3	3.1	100	2.4	3.0	99	2.8	3.5
D-Pro	5 (±0.05)	89	3.2	3.8	90	3.5	4.2	89	3.8	4.3
	100 (±1.5)	101	2.1	2.9	100	2.4	3.2	102	1.8	2.9
	200 (±14.4)	99	2.4	3.4	96	2.5	3.2	99	2.6	3.7
L-Pro	5 (±0.1)	91	3.5	4.2	90	4.5	4.9	91	3.1	3.8
	100 (±1.2)	100	1.8	2.8	101	2.0	3.1	101	2.1	2.9
	200 (±7.3)	99	2.7	3.7	99	2.0	2.9	99	2.8	3.3

[a] % Accuracy, percentage value obtained considering extraction yields from urine calibrations.
[b] % Relative standard deviation ($n = 3$).

within 7 min upon the addition of 5 mM Hp-γ-CD to the PDDAC-containing background electrolyte. Moreover, the separation time decreased as the Hp-γ-CD concentration increased. This result demonstrates that high Hp-γ-CD concentration promotes the formation of an inclusion complex between the D,L-phenothiazines and Hp-γ-CD, thereby reducing their effective electrophoretic mobility. To summarize, the use of Hp-γ-CD as a chiral selector not only helps achieve the baseline separation of the five pairs of D,L-phenothiazine enantiomers but also reduces the separation time.

3.3. *Optimization of solid-phase extraction conditions*

The determination of trace amounts of chiral drugs in biological fluids often requires the sample be cleaned before a separation procedure in order to suppress the matrix effect [31,32]. Because the SPE method has been demonstrated to be successful for extracting different types of chiral drugs from biological fluids and purifying the extracted drugs, this study selected a C_{18} SPE column coupled to the proposed separation system [1,26,33]. Because the sample volume was reduced from 3 mL to 30 μL, the detection sensitivity of the phenothiazine enantiomers could be efficiently improved. Prior to the separation of the extracted D,L-phenothiazine enantiomers, the factors affecting SPE efficiency were optimized by monitoring the sample recovery. When the solution pH varied between 6.0 and 7.0, the recoveries of all analytes gradually increased (Fig. 2A). At pH $\geq$ 7.5, D,L-phenothiazines were insoluble and caused the decrease of recoveries. Another important parameter that may affect the extraction efficiency is the type of elution solvent used. Fig. 2B reveals that methanol as an eluted solvent provides relatively high recoveries compared with other organic solvents. Therefore, for the extraction of the D,L-phenothiazines through SPE, the optimized pH and eluted solvent were chosen to be 7.0 and methanol, respectively.

3.4. *Solid-phase extraction coupled with the proposed separation system*

For the optimized separation (0.9% PDDAC, 5 mM Hp-γ-CD, and 100 mM tris-formate at pH 3.0) under the SPE conditions (pH 7.0 and methanol as the elution solvent), the extracted D,L-phenothiazine enantiomers were stacked through CE in the presence of PDDAC. The electropherograms (a–d) in Fig. 3A show that the peak intensities of 10 peaks corresponding to the five pairs of D,L-phenothiazine enantiomers considerably increased as the sample volume increased from 9 to 270 nL. This study showed that the CE stacking method exhibits high linearity (R^2 > 0.9922) between the peak area and injection time (5–150 s), confirming that each analyte was stacked well by PDDAC. When the sample volume exceeded 270 nL, the stacking efficiency became very low. Thus, the optimized sample volume was set to 270 nL in this study. Table 1 shows that the calibration curves obtained by plotting the peak areas of the five pairs of D,L-phenothiazine enantiomers against the concentrations of the enantiomers are linear (R^2 was greater than 0.99 for each.). The RSDs of the migration times and peak areas for the five pairs of D,L-phenothiazine enantiomers were

Table 3 – Comparison of the proposed method with other reported methods for preparation and determination of phenothiazines.

Analyte	Matrix	Sample preparation	Method	LOD (μM)	Chiral selector	Reference
D,L-Pro, D,L-Thi, D,L-Tri	urine	SPE	CZE-FASI-UV	0.018–0.024	–	[1]
D,L-pro, D,L-Tri	plasma	ACN	AEKC-DAD	45–79	HSA	[16]
D,L-Pro, D,L-Thi, D,L-Tri, D,L-Eth	–	–	CE-DAD	N.P.	γ-CD, Hp-β-CD, heptakis (2,3-dihydroxy-6-O-sulfo)-β-CD	[11]
D,L-Pro, D,L-Thi, D,L-Tri, D,L-Eth, D,L-Met	urine	–	CE-UV	2–8	Hp-γ-CD	[25]
D,L-Pro, D,L-Thi, D,L-Tri, D,L-Eth, D,L-Met	urine	SPE	CE-PDDAC based-UV	0.002–0.006	Hp-γ-CD	This work

Abbreviations: ACN, acetonitrile; AEKC, affinity electrokinetic chromatography; CD, cyclodextrin; CZE, capillary zone electrophoresis; DAD, diode array detector; FASI, field amplified sample injection; HSA, human serum albumin; Hp, hydroxypropyl; LLE, liquid–liquid extraction; N.P., not provided; PDDAC, poly(diallyldimethylammonium chloride); SPE, solid-phase extraction; UV, ultraviolet detector.

less than 3.2% and 3.5%, respectively. The LODs and limit of quantification for the five pairs of D,L-phenothiazine enantiomers ranged from 2.1 to 6.3 nM and 6.9–20.1 nM, respectively (estimated from Fig. 3B), and were considerably lower than the therapeutic and toxic levels of D,L-phenothiazine enantiomers in patient urine. In addition, the proposed method provided an improvement in the sensitivity by a factor in the range of 607–1555 compared with the results of capillary zone electrophoresis with a sample injection time of 10 s (Table S1, Supplementary Material).

3.5. *Analysis of urine samples*

The proposed method for the detection of D,L-phenothiazine enantiomers was applied to urine samples. Three individual urine samples from healthy volunteers (denoted as 1, 2, and 3) were spiked with standard solutions containing different concentrations of D,L-phenothiazine enantiomers. Without using a C_{18} SPE column and PDDAC-mediated CE stacking, the direct analysis of the spiked urine samples showed no peak corresponding to the five pairs of D,L-phenothiazine enantiomers (Fig. 4A). By contrast, a distinct peak pattern was observed when the spiked samples were extracted through SPE and PDDAC-mediated CE stacking was performed (Fig. 4B). We identified 10 peaks corresponding to the five pairs of D,L-phenothiazine enantiomers by comparing the migration time obtained from the SPE extraction and CE stacking of D,L-phenothiazine enantiomers in a urine sample (Fig. 4B) with that obtained from an aqueous solution sample (Fig. 4C). The peak areas of the D,L-phenothiazine enantiomers obtained from the spiked urine sample were similar to the peak area obtained for the aqueous solution, suggesting that the SPE method can substantially reduce the matrix effect of the urine samples. To confirm this suggestion, we recovered the five pairs of D,L-phenothiazine enantiomers in samples 1, 2, and 3; three replicate extractions at different concentrations were performed for each sample in Table 2. The table shows that the mean recoveries of the five pairs of D,L-phenothiazine enantiomers in samples 1, 2, and 3 varied from 87% to 107% and that the RSD values for the intraday and interday precisions were less than 7.1%. Furthermore, Table 3 shows that the sensitivity of the proposed method is greater than that of previously reported methods [1,9,11,16,25]. Compared to our previous study [25], this work combined SPE and PDDAC-mediated CE technique, provided high sensitivity and the LODs were down to nM. To summarize, the proposed method offers the advantages of simplicity, high enantioseparation efficiency, and high sensitivity.

4. Conclusions

A simple, low cost, the reproducible, reliable, and sensitive method was developed for the simultaneous separation of five pairs of D,L-phenothiazine enantiomers in urine samples; the method involved the use of Hp-γ-CD as a chiral selector, PDDAC as a stacking medium, and SPE for sample preparation. The detection sensitivity of the five pairs of D,L-phenothiazine enantiomers was efficiently improved by using the SPE method, which was followed by PDDAC-mediated CE. Consequently, the LODs of the five pairs of D,L-phenothiazine enantiomers were as small as 2.1–6.3 nM. This sensitivity enabled us to determine trace amounts of the five pairs of D,L–phenothiazine enantiomers in urine samples. Compared with previously reported separation methods [21–24,34], the proposed method provides the distinct advantages of high sensitivity, high separation efficiency, and short analysis time. To the best of our knowledge, this is the first paper to report the use of an on-line concentration and separation system and a C_{18} SPE column for the simultaneous detection of five pairs of D,L-phenothiazine enantiomers. The proposed technique was successfully extended to analyze 10 central nervous system drugs in the complex matrices of urine, and the method was found to be suitable for the detection of isomers of phenothiazines in real samples. We believe that, if combined with an appropriate chiral selector, the proposed method can be further applied for analyzing other chiral drugs.

Conflict of interest

The authors have declared no conflict of interest.

Acknowledgments

This work was financially supported by the Ministry of Science and Technology of Taiwan under contract numbers MOST 105-2113-M-017-003 and MOST 105-2113-M-143-002.

Appendix A. Supplementary data

Supplementary data related to this article can be found at: https://doi.org/10.1016/j.jfda.2017.12.002.

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