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Separation of Enantiomeric Ephedrine and Pseudoephedrine—High Pressure Liquid Chromatography and Capillary Electrophoresis*

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ABSTRACT: Ephedrine and pseudoephedrine (ψ-ephedrine), frequently found in packaged drugs of abuse, are common over-the-counter pharmaceuticals. Present in high concentrations, these compounds have reportedly caused false identification of methamphetamine in urine specimens. Furthermore, (−)-ephedrine and (+)-ψ-ephedrine are used for manufacturing (+)-methamphetamine. Thus, knowledge on the enantiomeric compositions of these compounds may help identify their sources, providing valuable information to the investigation process. High pressure liquid chromatography (HPLC) and capillary electrophoresis (CE) methods were evaluated and compared for their application in analyzing the enantiomeric compositions of these two compounds. A chiral column (Supelcosil LC-(S) Naphthyl Urea) was found effective in resolving the resulting four components when derivatized with both of the following two chiral derivatization reagents: *N*-trifluoroacetyl-*l*-prolyl chloride (*l*-TPC) and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC). A C18 column, although less effective, can also adequately resolve these four components for identification purposes. With CE, inclusion of 30 mM hydroxypropyl-β-cyclodextrin in 50 mM phosphate buffer (pH 2.5) was very effective in resolving all four components using an uncoated fused silica capillary without prior derivatization.

KEYWORDS: forensic science, ephedrine, pseudoephedrine, enantiomer, HPLC, capillary electrophoresis, chiral column, GITC, *l*-TPC, hydroxypropyl-β-cyclodextrin

Ephedrine and pseudoephedrine (ψ-ephedrine) (Fig. 1A-1D) are common over-the-counter pharmaceuticals. They are frequently used as adulterants in packaging drugs of abuse (1). (−)-Ephedrine has been a popular precursor for illicit manufacturing of (+)-methamphetamine (2,3). Recent investigation (4,5) of clandestine laboratory activities reported the use of ephedra plant (Ma Huang) material for methamphetamine manufacturing; (−)-ephedrine and (+)-ψ-ephedrine in this plant are extracted for conversion to methamphetamine in these illicit manufacturing processes. Thus, the identification of ephedrine and ψ-ephedrine and their enantiomeric composition in methamphetamine samples may help identify the drug's precursor material and provide valuable information to the investigation process.

Also of significant analytical concern is the reported false methamphetamine identification in urine specimens due to excessive consumption of ephedrine and ψ-ephedrine (6). The method hereby described can be used to analyze extracts resulting from biological samples. Thus, the analysis of ephedrine and ψ-ephedrine and their enantiomeric composition constitutes an important aspect of the overall analytical scheme in the forensic science laboratory.

In the first part of this study, the effectiveness of high pressure liquid chromatographic (HPLC) separation of these compounds using chiral and non-chiral derivatization reagents and stationary phases is evaluated. Effective chiral derivatizing reagents evaluated are *N*-trifluoroacetyl-*l*-prolyl chloride (*l*-TPC, Fig. 1E) and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC, Fig. 1F), while a C18 and a naphthyl urea column (chiral) are used as the stationary phases. Various tetrahydrofuran (THF)/water compositions are investigated for achieving optimal separation of the above-mentioned derivatization products and stationary phases.

In the most recent years, capillary electrophoresis (CE) has proven to be an effective new tool for drug analysis. Inclusion of cyclodextrins for in situ complex formation, provides great resolution power for enantiomers, and has recently been applied to the separation of various phenethylamines by one of the authors contributing to this study (7). The study hereby reported is much narrower in scope, focusing on the resolution of enantiomeric components of ephedrine and ψ-ephedrine, which possess much greater chemical similarity than the various amines previously studied.

In a second part of the present paper, the effectiveness of this emerging technique in chiral separation is demonstrated and compared with HPLC findings.

Materials and Methods

(+)- / (−)-Ephedrine, (+)- / (−)-ψ-ephedrine, and GITC were obtained from Sigma Chemical Co. (St. Louis, MO). *l*-TPC and inhibitor-free THF (purity > 99.9%) were obtained from Regis

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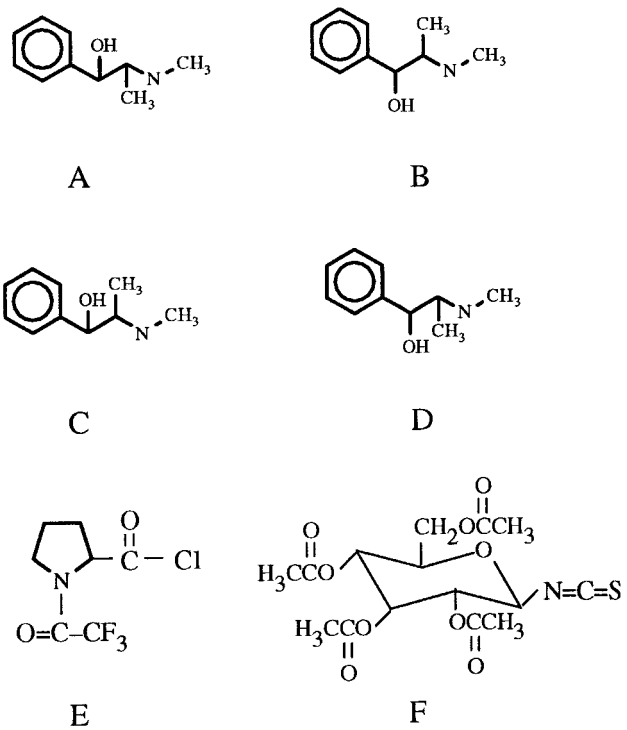

FIG. 1—*Structures of (+)-ephedrine (A), (−)-ephedrine (B), (+)-ψ-ephedrine (C), (−)-ψ-ephedrine (D), l-TPC (E), and GITC (F).*

Chemical Co. (Chicago, IL) and Aldrich Chemical Co. (Milwaukee, WI), respectively.

High Pressure Liquid Chromatographic System, Derivatizations, and Procedure

A Gilson chromatographic system (Gilson: Middleton, WI), consisting of a Gilson model 302 pump with Model 802B manometric module and a Gilson model 112 UV/Vis detector fixed at 254 nm, was used for this study. Injections were made using a 25-μL Hamilton #702-SNR (Hamilton: Reno, NE) blunt-end syringe. The sample was injected into a Rheodyne (Cotati, CA) model #7125 injection port equipped with a 20-μL injection loop. Chromatograms were registered with a Kipp and Zonen model BD 111 recorder (Kipp & Zonen: Delft, Holland).

The columns used were a Gilson Synchropak RP-P (25 cm × 4.6 mm; particle size 6.5 μm) C18 non-chiral column and a Supelco Supelcosil LC-(S) Naphthyl Urea (25 cm × 4.6 mm; particle size 5 μm) chiral column (Supelco: Belletcute, PA). A precolumn filter was used for both columns. Various compositions of THF and distilled water were used as the isocratic mobile phases.

The *l*-TPC derivatization procedure was similar to that adopted by Hays et al. (8). Briefly, the analyte (2.0 mg) was dissolved in 0.1 mL chloroform. 0.2 mL *l*-TPC derivatizing reagent was added to the solution and allowed to stand for 5 min. 20 μL triethylamine was added, followed by continuous shaking for 15 min. The mixtures were washed with 0.2 mL 6 N hydrochloric acid, followed by washing with 0.2 mL distilled water. The chloroform layer was removed and dried over magnesium sulfate ($MgSO_4$). The solution was then reconstituted with 1 mL chloroform and redried with $MgSO_4$. The liquid was then separated from the $MgSO_4$ and evaporated under a stream of air to dryness. The residue was then redissolved into THF/water mixture (30:70 v/v) to desired working concentrations prior to injection.

The GITC derivatization procedure was that adopted by Noggle and Clark (9). Briefly, a 10% molar excess of GITC, dissolved in 0.1 mL chloroform, was added to the analyte (2.0 mg in chloroform). The mixture was allowed to stand at room temperature for 10 min with intermediate shaking and then evaporated to dryness under a stream of air. The residue was in turn dissolved in 1 mL THF. The samples were diluted with THF/water mixture (30:70 v/v) to desired working concentrations prior to injection.

Capillary Electrophoresis System and Procedure

An automated MDQ/Pace electropherograph (Beckman Instruments: Fullerton, CA), fitted with a filter UV detector, was used. The capillary, made of fused silica and uncoated at the inner surface, had an internal diameter of 50 μm and a total length of 50 cm (40 cm to the detector). The optimized separation conditions were as follows: buffer: 50 mM phosphate (pH 2.5) with 30 mM hydroxypropyl-β-cyclodextrin (Beckman Instruments); 25 kV applied potential; injection by positive pressure (0.5 psi for 10 s); detection at 200 nm.

Individual enantiomers of ephedrine and ψ-ephedrine were diluted in water (1 mg/mL) to obtain a stock solution and kept at + 4°C. The stock solution was daily further diluted in water to obtain the desired working concentrations. All buffers and samples were filtered through 0.45-μm pore size nylon membranes.

New capillaries were treated before use with 1.0 M NaOH for 3 min, with 0.1 M NaOH for 10 min, with water 10 min, and with the selected buffer for 20 min. Between injections, the capillary was simply washed with water for 1 min and reconditioned with the selected buffer for 3 min.

Results and Discussion

We have previously studied GC methods for enantiomeric analysis of amphetamines and ephedrines (10–12) and LC approaches for amphetamine and methamphetamine (8). Since LC-based methods allow for the use of larger derivatizing groups which may facilitate enantiomeric resolution, the first part of this study will compare the effectiveness of GITC and *l*-TPC derivatization procedures for the analysis of ephedrine and ψ-ephedrine compositions by LC methods.

The second part of the study will determine optimum separation parameters for enantiomeric ephedrine and ψ-ephedrine by CE and contrast the results of LC and CE to determine which is more suitable for the separation of these enantiomeric drugs.

HPLC Optimization and Results

The retention characteristics of all analytes (as their respective *l*-TPC and GITC derivatives) under various mobile phase compositions and flow rates were first established by chromatographing each analyte with the stationary phase studied. The resulting retention time information was then used for the identification of individual components in a mixture.

Retention data of ephedrine and ψ-ephedrine enantiomers (as *l*-TPC and GITC derivatives) using the non-chiral and chiral columns and various mobile phase compositions are summarized in Tables 1 and 2. The optimal mobile phase was evaluated by observing the effect of changing the composition of the THF/H_2O mobile phase in 5% increments, e.g., from 20:80 (THF/H_2O) to 25:75 (THF/H_2O). The optimal mobile phase composition chosen represented a compromise between low retention time, minimum peak width, and maximum separation. The optimal mobile phase parameters for the

TABLE 1—*Retention data of l-TPC-derivatized ephedrines and ψ-ephedrines.*

THF:H_2O	Flow Rate (mL/min)	T_0	T_R (min) (−)-Ephedrine	(+)-ψ-Ephedrine	(+)-Ephedrine	(−)-ψ-Ephedrine
			C18 Column			
30:70	1.5	2.0	3.8	4.4	5.1	5.0
25:75	1.5	2.0	4.2	5.5	5.7	6.3
20:80	1.5	2.0	5.1	7.3	7.4	9.1
15:85	2.0	1.6	5.1	7.8	8.5	11.4
10:90*	2.0	1.6	8.0	16.0	13.5	24.4
10:90	2.5	1.4	6.8	13.4	11.4	19.2
			Chiral Column			
30:70	1.5	2.8	6.0	7.8	7.7	8.3
25:75	1.5	2.8	7.3	10.7	10.7	11.8
20:80†	1.5	3.0	9.9	15.8	14.2	18.4
15:85	2.0	2.2	10.1	17.0	15.2	20.2
10:90	2.5	1.9	12.2	20.7	17.7	26.2

* Optimal mobile phase conditions with C18 column.
† Optimal mobile phase conditions with chiral column. Chromatogram shown as Fig. 2.

TABLE 2—*Retention data of GITC-derivatized ephedrines and ψ-ephedrines.*

THF:H_2O	Flow Rate (mL/min)	T_0	T_R (min) (−)-Ephedrine	(+)-ψ-Ephedrine	(+)-Ephedrine	(−)-ψ-Ephedrine
			C18 Column			
30:70*	1.5	2.5	10.2	9.3	11.7	8.6
25:75	1.5	2.5	21.9	20.0	26.8	18.0
20:80	1.5	2.5	50.4	49.0	67.2	44.2
			Chiral Column			
35:65	1.5	2.5	13.7	12.9	15.1	11.9
30:70†	1.5	2.6	23.9	22.4	27.0	20.5
25:75	1.5	2.8	44.2	64.6	52.5	43.1

* Optimal mobile phase conditions with C18 column.
† Optimal mobile phase conditions with chiral column. Chromatogram shown as Fig. 3.

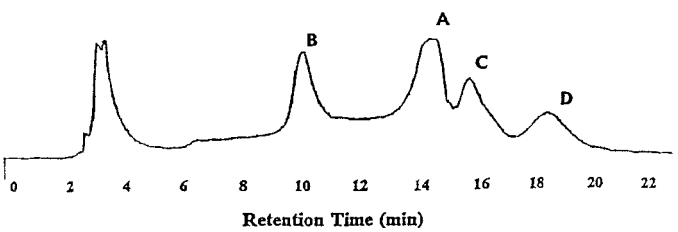

FIG. 2—*Chromatogram of l-TPC-derivatized ephedrines and ψ-ephedrines resulting from the liquid chromatographic system of chiral column; mobile phase composition, THF/H_2O (20:80, polarity index of mobile phase, 8.96); flow rate, 1.5 mL/min: (−)-ephedrine (B); (+)-ephedrine (A); (+)-ψ-ephedrine (C); (−)-ψ-ephedrine (D).*

various combination of the derivatizing reagents and stationary phases are footnoted in these two tables. Chromatograms showing optimal retention characteristics with both derivatizing reagents were obtained using the chiral column (Figs. 2 and 3).

Data in Tables 1 and 2 and Figs. 2 and 3 indicate differences in the elution orders of the resulting *l*-TPC and GITC derivatives. The order for the resulting *l*-TPC derivatives is: (−)-ephedrine, (+)-ephedrine, (+)-ψ-ephedrine, and (−)-ψ-ephedrine, while the order for the GITC derivatives is: (−)-ψ-ephedrine, (+)-ψ-ephedrine, (−)-ephedrine, and (+)-ephedrine.

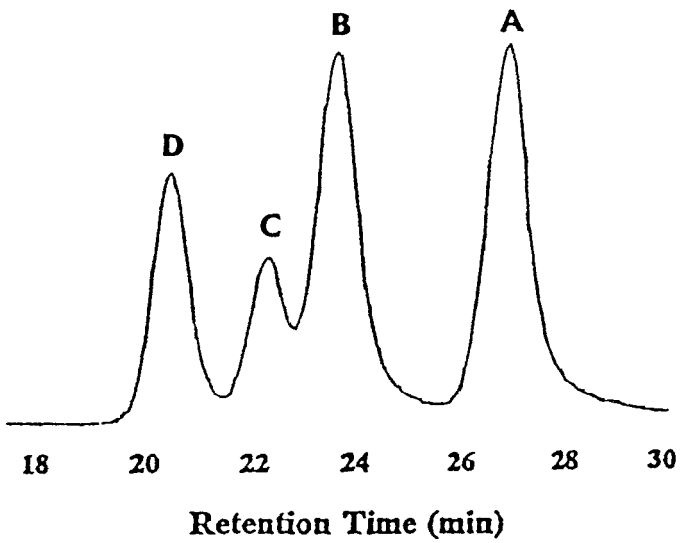

FIG. 3—*Chromatogram of GITC-derivatized ephedrines and ψ-ephedrines resulting from the liquid chromatograph system of chiral column; mobile phase composition, THF/H_2O (30:70, polarity index of mobile phase, 8.34); flow rate, 1.5 mL/min: (−)-ψ-ephedrine (D); (+)-ψ-ephedrine (C); (−)-ephedrine (B); (+)-ephedrine (A).*

TABLE 3—*Optimal resolution of ephedrine and ψ-ephedrine enantiomers as* l*-TPC and GITC (in parentheses) derivatives.*

	(−)-Ephedrine		(+)-ψ-Ephedrine		(−)-ψ-Ephedrine	
(+)-Ephedrine	1.64	(2.88)	2.24	(0.67)	3.35	(1.88)
(−)-Ephedrine	NA	NA	0.67	(3.46)	1.67	(4.19)
(+)-ψ-Ephedrine	2.24	(0.67)	NA	NA	1.0	(1.29)

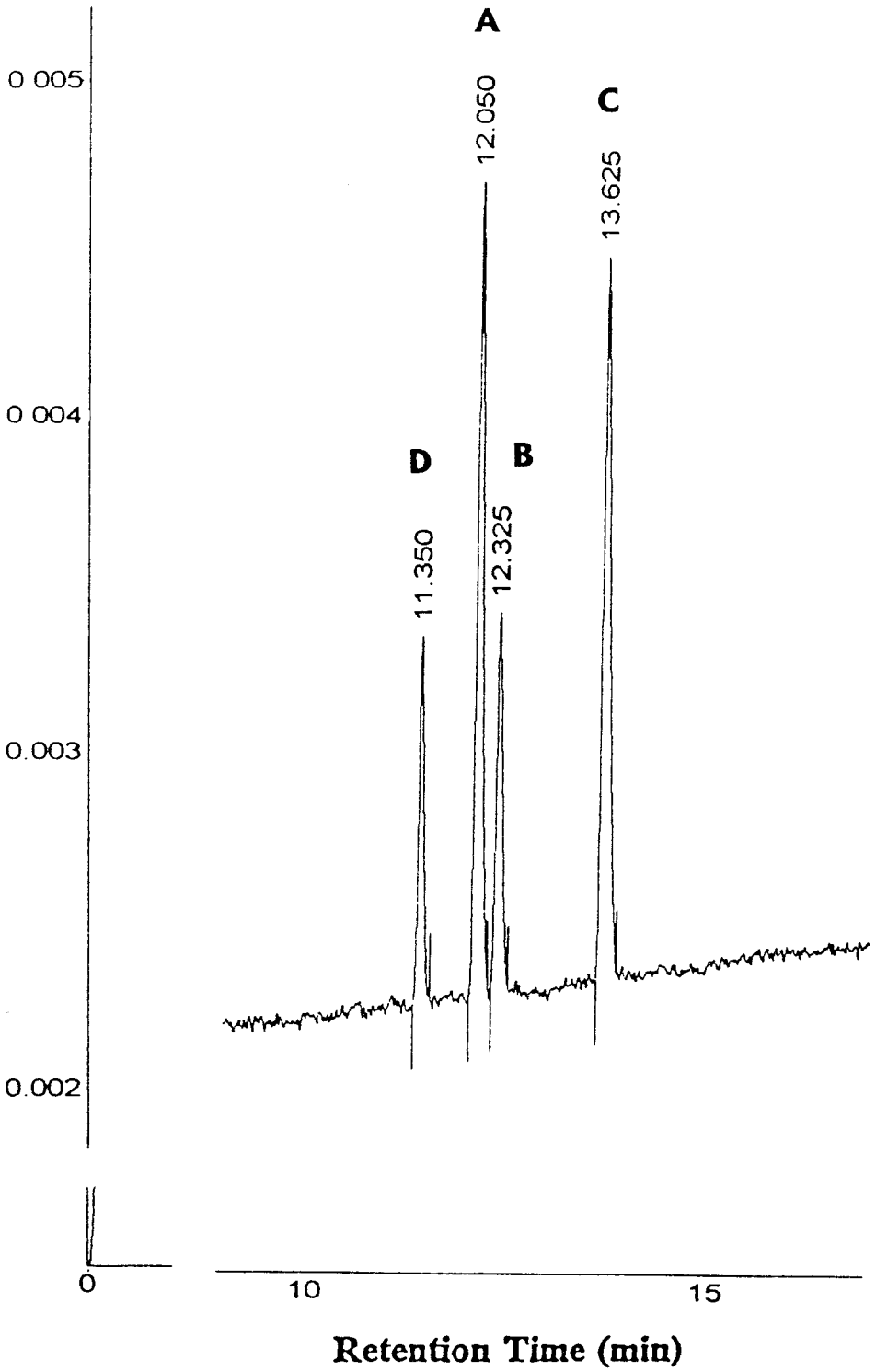

FIG. 4—*CE electropherogram of underivatized ephedrine and ψ-ephedrine obtained with 50 mM phosphate buffer (pH 2.5) including 30 mM hydroxypropyl-β-cyclodextrin under 25 KV: (−)-ψ-ephedrine (D); (+)-ephedrine (A); (−)-ephedrine (B); (+)-ψ-ephedrine (C).*

TABLE 4—*Precision (intra-day) of CE analysis of ephedrine and pseudoephedrine: absolute migration times and peak areas (average and RSD from four repeated injections).*

Compound	Migration Time (min)	Migration Time (RSD %)	Absolute Area	Absolute Area (RSD %)
(−)-ψ-Ephedrine	11.395	0.155	5187	4.29
(+)-Ephedrine	12.060	0.160	13506	3.17
(−)-Ephedrine	12.337	0.164	6122	3.07
(+)-ψ-Ephedrine	13.641	0.171	14363	3.67

With the HPLC parameters investigated, the C18 column does not provide adequate base-line resolution for the four components of ephedrine and ψ-ephedrine. With optimal HPLC parameters, the chiral column achieved base-line separation for *l*-TPC derivatives with the exception of (+)-ephedrine and (+)-ψ-ephedrine (Fig. 2) and similar base-line separation for GITC derivatives with the exception of (−)-ephedrine and (+)-ψ-ephedrine (Fig. 3). Resolutions of various pairs of drugs on the chiral column are shown in Table 3. Sequential analysis of *l*-TPC and GITC derivatives, with the naphthyl urea chiral column using THF/water compositions of 20:80 and 30:70, respectively, will allow for base-line separation and quantification of four enantiomeric compositions—(+)- / (−)-ephedrine and (+)- / (−)-ψ-ephedrine.

While evaluating separation parameters, it was noted that the detector's (254 nm) responses toward the GITC derivatives were approximately 60 times greater than that for the corresponding *l*-TPC derivatives. Approximate limits of detection for these four compounds range from 0.005 to 0.1 mg/mL for *l*-TPC and 0.003 to 0.01 mg/mL for GITC derivatizations.

Capillary Electrophoresis Optimization and Results

The choice of the separation buffer was made to keep as low as possible the electroendoosmotic flow by suppressing the ionization of the silica capillary silanols and to assure an adequate conductivity and optical transparency to the separation medium, as well as a full ionization of the cationic analytes. The adopted 50 mM phosphate buffer met all these requirements, allowing the achievement of a flat and stable baseline at the wavelength of 200 nm.

Preliminary tests using underivatized 15 mM β-cyclodextrin were partially unsuccessful because, notwithstanding a complete resolution of ephedrine and ψ-ephedrine enantiomers, there was a partial overlapping between two isomers of different compounds.

A more soluble hydroxypropyl-β-cyclodextrin at the concentration of 30 mM was found to achieve base-line separation for all four components in the order of: (−)-ψ-ephedrine, (+)-ephedrine, (−)-ephedrine, and (+)-ψ-ephedrine (Fig. 4). Chiral resolution for (+)- / (−)-ψ-ephedrine was excellent, with R = 14, while that for (+)- / (−)-ephedrine was lower (R = 1.54) but still sufficient for base-line separation. Enantiomeric components of ephedrine are also sufficiently resolved from that of ψ-ephedrine. Efficiency, calculated on (+)-ephedrine, the second peak in the electropherogram, was about 189,000 plates per column.

Under the adopted injection conditions (0.5 psi for 10 s) the concentration sensitivity for the individual enantiomers, with a signal-to-noise ratio of 3, was about 0.6 μg/mL. Assay linearity tested in the range of 0.6 and 40 μg/mL was satisfactory for all analytes, with coefficients of correlation between 0.9995 and 0.9998, while maintaining a good resolution of all peaks over the entire concentration range tested. At concentrations above 40–50 μg/mL, the resolution between (+)- and (−)-ephedrine became incomplete and not suitable for quantitative determination.

The intra-day reproducibility of absolute migration times (n = 4) was excellent, with relative standard deviation (RSD) values between 0.15 and 0.17%, while that of absolute peak areas was characterized by RSDs between 3.07 and 7.31% (Table 4).

Conclusion

This study has demonstrated that enantiomeric components of ephedrine and ψ-ephedrine can be adequately resolved using both HPLC and CE methods. It furthermore shows that capillary electrophoresis can provide efficient separation of these drugs without prior derivatization.

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