

P-ST016

INVESTIGATION OF CHIRAL ANALYSIS OF PSEUDOEPHEDRINE IN DECONGESTANT BY SIMPLE CAPILLARY ELECTROPHORESIS SYSTEM

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Abstract

Pseudoephedrine (PE) is an over-the-counter drug that is used to relieve nasal congestion caused by colds, allergies, and hay fever. It has been reported that PE is a precursor to the illegal synthesis of methamphetamine (MA). MA addiction is a serious problem in Thailand and throughout the world. Therefore PE has been banned from sale since 2012. PE and MA are chiral compounds that consist of *d*- and *l*- forms. In this research, we investigated PE in tablet and syrup form from different manufacturers using an in-house capillary electrophoresis (CE) system to separate *d*- and *l*-PE. Chiral separation of PE was successfully achieved using the following conditions: fused-silica capillary (length 59.00 cm and effective length 38.30 cm), phosphate buffer (20.0 mM, pH 2.70) with carboxymethyl- β -cyclodextrin (4.0 mM), applied electrical field strength (420 V/cm), electrokinetic injection (15 kV, 1 s) and UV detection (195 nm). The calibration curves for *d*- and *l*-PE showed a good linearity with correlation coefficients greater than 0.99. The percentage recoveries were in range of 84 ± 8 to $100 \pm 2\%$. It was found that only *d*-PE was observed in decongestant samples. The amount of *d*-PE ranges from 27 ± 2 to 61 ± 5 mg/tablet.

Keywords: chiral separation, pseudoephedrine, methamphetamine, capillary electrophoresis

Introduction

Pseudoephedrine (PE) is a sympathomimetic drug of the phenethylamine and amphetamine chemical class. It is in the class of medication called nasal/sinus decongestant, as a stimulant [1]. It is used to relieve nasal congestion caused by colds, allergies, and hay fever [2]. PE is commonly used as a decongestant and is an over-the-counter drug. In 2012, there were reported in the media that millions of PE tablets had disappeared from many hospitals in Thailand [3]. There is concern about the misuse of PE to produce illicit MA. Therefore, the Public Health Ministry had kept the use of PE under control and PE has been classified as a second type of psychotropic substance [4]. It has now been banned from sale in Thailand. MA is a highly addictive drug that affects the brain and central nervous system [5].

PE and MA are chiral compounds that have *d*, *l*-forms. Chemical structure of PE is similar to MA. *d*-MA can be synthesized from *d*-PE and also *l*-MA can be synthesized from *l*-PE (see Figure 1) [6]. In general, *d*- and *l*- forms are different in pharmacokinetics and pharmacodynamics properties [7]. *d*-MA has more potent physiologic and behavioral effects and higher abuse liability [8]. Thus the chiral form of PE in decongestant is of interest.

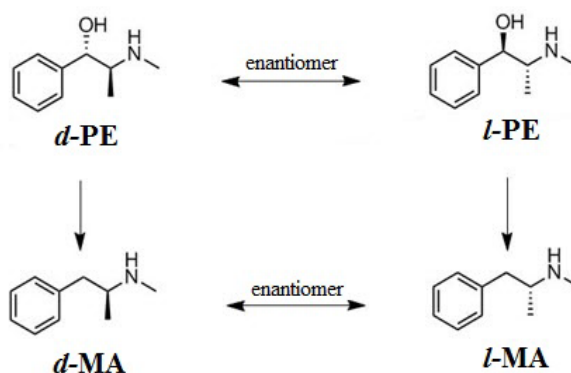


Figure 1. Synthesis of *d, l*-MA from precursors of *d, l*-PE.

Capillary electrophoresis (CE) is very useful technique in forensic research. It is a high efficiency separation technique. By adding a chiral selector, β -cyclodextrin into running buffer, enantiomeric separation can be achieved. In this research, an in-house capillary electrophoresis system was used for the investigation of chirality of pseudoephedrine (*d, l*-forms) in decongestant tablets and syrup from various manufacturers.

Methodology

Standard racemic methamphetamine, racemic pseudoephedrine, *d*-PE, *l*-PE, *d*-MA and *l*-MA have been kindly provided by Assoc.Prof.Prapin Wilairat from National Doping Control Centre, Mahidol University (Bangkok, Thailand). Pharmaceutical tablets containing PE were provided by Forensic Science Graduate Programme, Mahidol University (Bangkok, Thailand) and Anghong Medical 2 Hospital (Anghong Province, Thailand). The running buffer was phosphate buffer (20.0 mM, pH 2.70). All solutions were prepared with deionized ultra pure water (18.0 M Ω) prior to filtration through 0.45 μ m cellulose acetate membrane. Chiral selector; carboxymethyl- β -cyclodextrin, CMBCD (Cyclolab, Budapest, Hungary) was added into the running buffer. All solutions were degassed for 10 min before analysis.

Fused-silica capillary (50 μ m ID, 365 μ m OD) of 59.00 cm total length and 38.30 cm effective length was used for electrophoretic separation. Before the experiment, the fused-silica capillary was rinsed by hydrodynamic injection with 0.1 M sodium hydroxide (5 min), deionized-distilled water (5 min) and running buffer (5 min), respectively. In the experiment, all standard and sample solutions were introduced into a capillary column by electrokinetic injection at 15 kV for 1 s and a positive electrical field strength of 420 V/cm (25 kV) was applied for chiral separation with UV detection at 195 nm.

PE tablet samples were ground with mortar and pestle. The ground samples were accurately weighed (10 mg) and dissolved in 10.0 mL of water:methanol (8:2, v:v), sonicated for 2 minutes and filtered before CE analysis. Syrup sample was diluted with distilled water.

Results

The separation of standard *d, l*-PE was performed using phosphate buffer as running buffer (20.0 mM phosphate buffer, pH 2.70) with addition of CMBCD. Figure 2 shows electropherograms of standard *l*-PE and *d*-PE. The migration time orders of the enantiomers were *l*-PE (4.86 min) and *d*-PE (6.00 min), respectively.

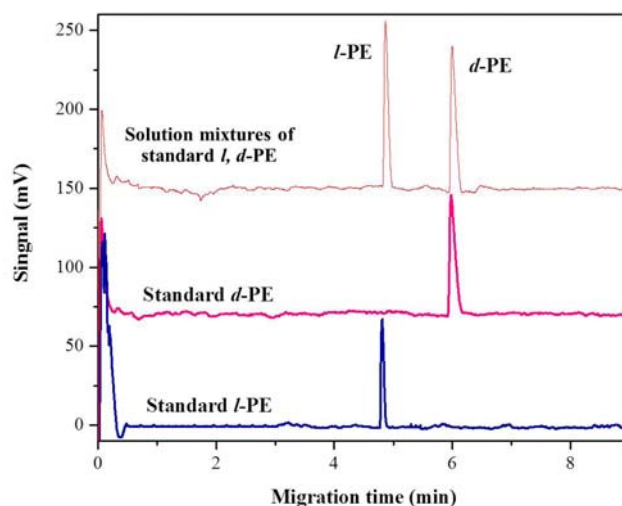


Figure 2. Electropherograms; standard *l*-PE, standard *d*-PE and solution mixtures of standard *l*, *d*-PE. CE conditions: capillary (59.00 cm long, 38.30 cm effective length), phosphate buffer (20.0 mM, pH 2.70) with CMBCD (4.0 mM), applied electrical field strength of 420 V/cm, electrokinetic injection (15 kV, 1 s) and UV detection (195 nm).

Calibration curves of standard solution mixtures of the *d*-PE and *l*-PE. *d*-PE and *l*-PE showed a good linearity over the concentration ranges of 5 μ M to 75 μ M. The linear regression equations were: $y=11.5475x+216.3000$ ($r^2=0.9903$) and $y=11.7018x+189.5724$ ($r^2=0.9905$) for the *l*- and *d*- enantiomers, respectively (Figure 3). The experiments were carried out in triplicates.

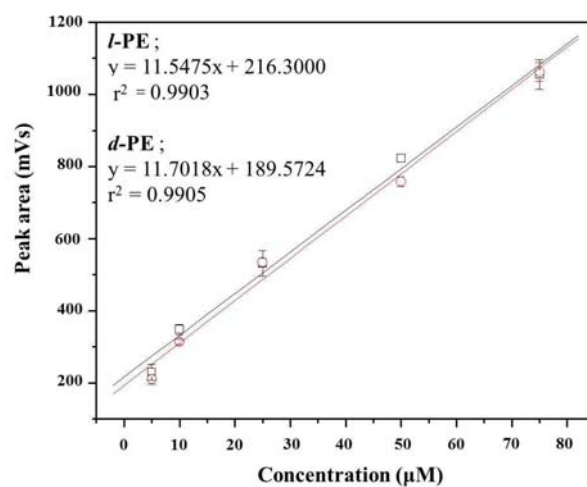


Figure 3. Calibration curves of standard *l*-PE and standard *d*-PE.

Validation of the CE developed method was done by measurement of percentage recovery. The percentage recoveries of spiked standard *d*-PE (10 μ M) in solution containing sample (Actifed, Colidine, Sulidine, Consint and Colidine syrup) were $100 \pm 2\%$, $90 \pm 7\%$, $90 \pm 11\%$, $90 \pm 2\%$ and $84 \pm 8\%$, respectively. PE tablet samples from different manufacturers were analyzed by chiral CE.

All PE drug samples were found to have only *d*-PE. To quantify *d*-PE in PE tablets and syrup, we used peak area. The amount of *d*-PE ranged from 27 ± 2 to 61 ± 5 mg/tablet. Concentration of *d*-PE contents in each sample obtained from CE analysis were compared with concentration of PE stated on the labels (Figure 4).

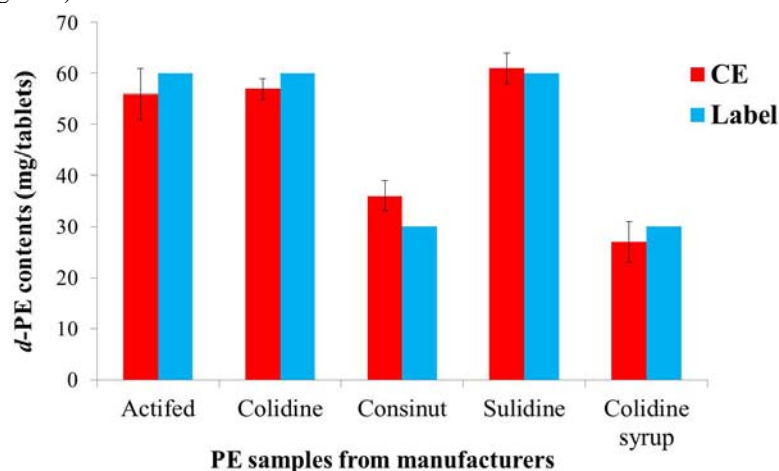


Figure 4. Comparison of *d*-PE concentrations in samples obtained from CE analysis with concentration of PE stated on the label.

The study of chiral analysis of MA in MA sample is shown in Figure 5. It was found that only *d*-MA compound was contained in an MA sample. Thus this *d*-MA in MA sample could possibly be synthesized from *d*-PE in decongestant.

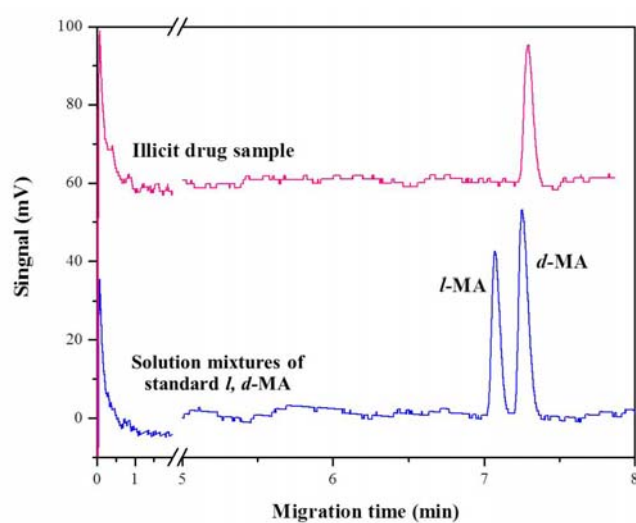


Figure 5. Electropherograms; solution mixtures of standard *l*, *d*-MA and *d*-MA in an MA sample. CE conditions: capillary (59.00 cm long, 38.30 cm effective length), phosphate buffer (20.0 mM, pH 2.70) with CMBCD (4.0 mM), applied electrical field strength of 420 V/cm, electrokinetic injection (15 kV, 1 s) and UV detection (195 nm).

Discussion and Conclusion

CE system for chiral analysis is very useful for forensic laboratory. The illicit drug and its precursor (such as MA and PE) can be separated in their chiral forms using CE system. The calibration curve of *d*, *l*-PE showed a good linearity with correlation coefficients more than 0.99. The percentage recovery was in range of 84 ± 8 to $100 \pm 2\%$. The investigation of PE drug samples from different manufacturers found that there was only *d*-PE in all drug samples. The amount of *d*-PE ranged from 27 ± 2 to 61 ± 5 mg/tablet. An MA sample has also been found to contain only *d*-MA (Figure 5). Thus, the stolen decongestant containing only *d*-PE may be used as a precursor to the synthesis of illicit *d*-MA.

Acknowledgements

This research project was supported by Forensic Science Graduate Programme and Department of Chemistry, Faculty of Science, Mahidol University. RC and PT acknowledge Center of Excellence for Innovation in Chemistry (PERCH-CIC) and Mahidol University for financial support. PT thanks The King Prajadhipok and Queen Rambhai Barni Memorial Foundation for the scholarship. BK would like to thank Anghthong Medical 2 Hospital for providing PE drug samples.

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