Technical Note

Rapid Chiral Separation of Dextro- and Levo- Methorphan using Capillary Electrophoresis with Dynamically Coated Capillaries

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ABSTRACT: The chiral differentiation of the Dextro- and Levo- Methorphan is obtained in under 4 minutes with excellent peak shapes using capillary electrophoresis with dynamically coated capillaries. Dynamic coating of the capillary surface is accomplished by rapid flushes of 0.1 N sodium hydroxide, water, a buffer containing a polycation coating reagent, and a reagent containing methanol and a polyanionic coating reagent containing hydroxypropyl-*beta*-cyclodextrin.

KEYWORDS: Dextromethorphan, Levomethorphan, Chiral Analysis, Capillary Electrophoresis, Dynamically Coated Capillaries, Forensic Chemistry

Introduction

Dextromethorphan is an antitussive agent commonly found in Over-the-Counter (OTC) cough and cold pharmaceuticals (and more recently in Ecstasy (MDMA) mimic or combination tablets). Levomethorphan is a narcotic analgesic that is not commercially available, and therefore is not commonly submitted to forensic laboratories. Nonetheless, the differentiation and identification of these enantiomers is important in the United States, since Dextromethorphan is not controlled while Levomethorphan is a Schedule II controlled substance.

However, the differentiation of Dextro- and Levo- Methorphan is challenging. Methorphan is a tertiary amine which is not amenable to derivatization; therefore, the use of chiral derivatizing reagents (to form diastereomers for analysis on an achiral gas chromatography (GC) or high performance liquid chromatography (HPLC) column) is not a viable approach. Instead, relatively expensive chiral columns are required to resolve the two isomers using either GC or HPLC [1].

Capillary electrophoresis (CE) allows for the separation of enantiomers on conventional capillaries by utilizing run buffers containing chiral additives. Micellar electrokinetic chromatography (MEKC) [2], and electrokinetic chromatography (ECC) [3], both with single wavelength UV detection, and free zone capillary electrophoresis (CZE) with secondary equilibria and PDA-UV detection [4], have all been previously used to resolve the enantiomers of methorphan. However, run times in excess of 15 minutes were required.

For the separation of basic drugs at low pH, faster, more precise migration times and higher plate counts are obtained using dynamically coated versus uncoated capillaries. The use of a chiral additive (such as a cyclodextrin) imparts the additional selectivity needed for the analysis of enantiomers [5]. Using the procedure developed by Chevigne and Janssens [6], the capillary, after base hydrolysis, is sequentially coated with a polycation and then with a polyanion. The run buffer (with or without an added cyclodextrin) is the final coating

reagent. This process produces coated capillaries with a higher and more robust electroosmotic flow (EOF) at lower pH values versus uncoated capillaries. In addition, the coated capillary surface has more favorable kinetics. In the present study, the rapid chiral analysis of methorphan using a dynamically coated capillary approach is reported.

Experimental

Chemicals

Standards of Dextro- and Levo- Methorphan were obtained from the reference collection of this laboratory. Sodium hydroxide 0.1 N, CElixir A (pH 2.5), CElixir B (pH 2.5), and CElixir B (pH 2.5) with 0.95 % (w/v) hydroxypropyl-ß-cyclodextrin, were all acquired from MicroSolv Technology (Long Branch, NJ). Hydroxypropyl-ß-cyclodextrin (HP-ß-CD) was obtained from Sigma (St. Louis, MO). HPLC-grade methanol was obtained from Burdick and Jackson (Muskegon, MI). Deionized and high purity water (that is, HPLC-grade water) was obtained from a Millipore Synergy 185 water system (Bedford, MA).

Instrumentation and Procedures

An Agilent Model HP^{3D} CE Capillary Electrophoresis System fitted with a diode array detector (Waldbronn, Germany) was used for CE separations. New, bare silica capillaries were conditioned following the same procedure used for regular analyses. That is, the capillaries were first flushed with 0.1N sodium hydroxide for 1 minute, followed by water for 1 minute, then CElixir Reagent A for 1 minute, and finally the run buffer for 2 minutes. Either 2.0 mL CE glass vials or 1.0 mL polypropylene vials are used as reservoirs. For glass vials, waste vials were filled with 500 µL of water. Flush, run buffer, standard and sample vials were filled with 1000 µL of liquid (for the sodium hydroxide 0.1 N vial add 500 µL to a polypropylene vial). When polypropylene vials were used, waste vials were filled with 250 µL of water, while all others were filled with 500 µL of liquid.

Standard and Sample Preparation

The injection solvent consisted of 75 mM phosphate monobasic, adjusted to pH 2.6 with phosphoric acid, and diluted 1:20 with HPLC-grade water. Alternatively, injection solvent concentrate (MicroSolv) was diluted 1:20 with HPLC-grade water.

For standard solutions, an appropriate amount of standard Dextro- and Levo- Methorphan was weighed into an appropriate volumetric flask and diluted to volume with injection solvent, in order to obtain a final concentration of approximately 0.05 mg/mL of each component. These were sonicated for 15 minutes, then filtered. For sample solutions, an appropriate amount of powder was weighed into a volumetric flask and diluted to volume with injection solvent, in order to obtain a final concentration approximately equal to that of standard. These were also sonicated for 15 minutes, then filtered. All standard and sample solutions were filtered with 0.45 µm Nylon syringe filters (MicroSolv).

Capillary Electrophoresis Conditions

For the chiral separation either a 50 µm ID 32 cm (23.5 cm to the detector) fused silica capillary obtained from Polymicro Technologies (Phoenix, AZ) or a 50 µm ID 33 cm (24.5 cm to the detector) pre-made capillary (Agilent) were used, at 15 ^{O}C. The run buffer consisted of 15 % methanol and 85 % (CElixir Reagent B (pH 2.5) + 0.95 % HP-ß-CD). For all CE runs a 50 mbar pressure injection of 2 second duration was used, followed by a 35 mbar pressure injection of water for 1 second. For electrophoresis, an initial 0.5 minute linear voltage ramp from 0 V to the final voltage of 20 kV was used.

Results and Discussion

Dahlen and Lenz used CZE with an uncoated capillary and with added HP-β-CD to resolve Dextro- and Levo-Methorphan in under 16 minutes [4]. In the present study, using the same run buffer but on a dynamically coated

capillary, identical results were obtained in under 4 minutes (see Figure 1). Highly precise separations were obtained, as demonstrated by excellent run-to-run migration time precision (% RSD = 0.1, n = 7). Because the peaks are so narrow, identification can be difficult based on migration time alone; however, co-injection of sample and either standard eliminates any ambiguities. Relative migration time data (relative to Dextromethorphan) of solutes commonly found with Dextromethorphan is given in Table 1. The non-controlled substances are included because they and Dextromethorphan are commonly combined in various OTC pharmaceuticals. The controlled substances are included since they and Dextromethorphan are occasionally identified in Ecstasy (MDMA) mimic and combination tablets.

If needed, *n*-butylamphetamine can be used as an internal standard in this method for the quantitation of Methorphan.

The present procedure is compatible with previously reported methodology for the CE analysis of a wide variety of seized drugs using the same capillary with dynamic coatings [5]. Classes of compounds that can be analyzed using this methodology (using higher CD concentrations than used in this study) include the phenethylamines and the methylenedioxyphenethylamines. Specific compounds that can be analyzed using this methodology include propoxyphene, cocaine, oxycodone, heroin, lysergic acid diethylamide (LSD), opium, psilocybe mushrooms, *gamma*-hydroxybutyrate (GHB), and *gamma*-butyrolactone (GBL).

References

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5. Lurie IS, Hays PA, Parker K. Capillary electrophoresis analysis of a wide variety of seized drugs using the same capillary with dynamic coatings. Electrophoresis 2004;25:1580-1591. [Note: Substrates analyzed in the study include the enantiomers of norpseudoephedrine, pseudoephedrine, ephedrine, amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxy-methamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDEA), and propoxyphene].

6. Chevigne R, Janssens J. US Patent #5,611,903, 3/18/97.

[Table 1 and Figure 1 Follow.]

Table 1. Relative Migration Times of Solutes Commonly Found with Dextromethorphan.

Solute	Relative Migration Time *
d,l-Methamphetamine HCl	0.798
Phenylpropanolamine HCl	0.836
d,l-Pseudoephedrine HCl	0.841
MDMA HCl	0.850
d,l-Ephedrine HCl	0.851
Phenylephrine	0.874
n-Butylamphetamine HCl (internal standard)	0.919
Diphenhydramine HCl	0.973
Dextromethorphan	**1.000**
Levomethorphan	1.010
Acetaminophen	2.371
Guaifenesin	2.388

* Relative to Dextromethorphan. Note that the concentration of the cyclodextrin used in this study was insufficient to resolve the enantiomeric pairs of the listed phenethylamines and methylenedioxyphenethylamines; therefore, only one (the average) RMT is reported.

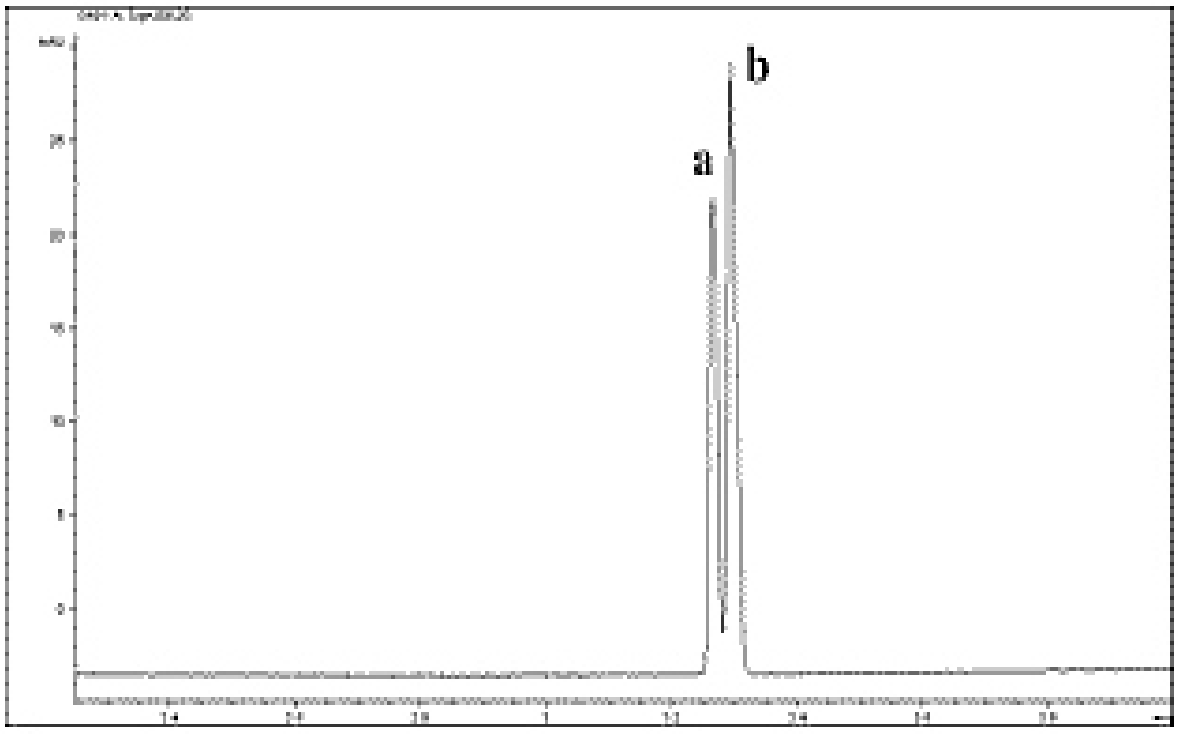

Figure 1. Electropherogram of a standard mixture of a) Dextromethorphan and b) Levomethorphan. A 33 cm (24.5 cm to the detector window) x 50 µm ID fused-silica dynamically coated capillary was used. Solute concentration of each enantiomer was approximately 0.05 mg/mL (CE conditions are described in the Experimental section).