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Separation of Levodopa, Carbidopa and Their Related Impurities by Capillary Electrophoresis

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Separation of Levodopa, Carbidopa and Their Related Impurities by Capillary Electrophoresis

Summary : *In this study a simple and rapid capillary electrophoresis (CE) method was developed for the simultaneous separation of levodopa, carbidopa and their related impurities. The influence of the pH of the electrolyte on the resolution was also investigated. A baseline separation was obtained at +20 kV voltage using 40/51 cm (effective/total length) uncoated fused-silica capillary (50 μm I.D.) at 20°C in 100 mM phosphate buffer (pH: 8.5) as the background electrolyte. Tryptophan was used as the internal standard. In these conditions, it is possible to separate levodopa, carbidopa and their related impurities in short analysis times (less than 8 min).*

Keywords *: Levodopa and impurities, carbidopa and impurities, capillary electrophoresis, method development*

Levodopa, Karbidopa ve Safsızlıklarının Kapiller Elektroforez ile Ayırımı

Özet *: Bu çalışmada levodopa, karbidopa ve ilgili safsızlıklarının bir arada ayırımı için basit ve hızlı kapiller elektroforez (CE) yöntemi geliştirilmiş; elektrolit pH'sının rezolüsyon üzerindeki etkisi incelenmiştir. Baseline ayırım +20 kV uygulanarak, 40/51 cm (efektif/total uzunlukta) kaplanmamış silika kapiller (50 μm I.D.) ile 20°C sıcaklıkta 100 mM fosfat tamponu (pH: 8.5) kullanılarak elde edilmiştir. İnternal standart olarak triptofan kullanılmıştır. Bu şartlar uygulanarak levodopa, karbidopa ve ilgili safsızlıklarını 8 dakikadan kısa analiz süresinde ayırmak mümkündür.*

Anahtar kelimeler : *Levodopa ve safsızlıkları, karbidopa ve safsızlıkları, kapiller elektroforez, metot geliştirme*

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INTRODUCTION

Parkinson's disease is a degenerative nervous system disorder, characterized by progressive tremor, bradykinesia and muscular rigidity. Levodopa or L-dopa [dihydroxyphenylalanine; (-)-3,4-dihydroxyphenyl-L-alanine] is used orally in the treatment of the prominent symptoms of Parkinson's disease[1]. In order to prevent *in vivo* destruction of levodopa in extracerebral tissues and to prolong its anti parkinsonial effect, it is formulated with carbidopa [L-2-hydrazino-2-methyl-3-(3,4-dihydroxy-phenyl) propanoic acid monohydrate], an inhibitor of dopa-decarboxylase. Several combinations of levodopa and carbidopa are commercially available in different formulations[2,3].

The major impurities reported in The United State Pharmacopeia National Formulary (XXIII) are 6-hydroxydopa and 3-methoxytyrosine for levodopa and methyldopa and 3-O-methylcarbidopa for carbidopa, respectively[4] (Fig. 1). The USP XXIII monograph given for levodopa-carbidopa tablet formulations requires that both the compounds should be assayed by high performance liquid chromatography (HPLC). The USP HPLC method was not developed for concurrent testing of impurities. Several HPLC methods have been reported for the simultaneous

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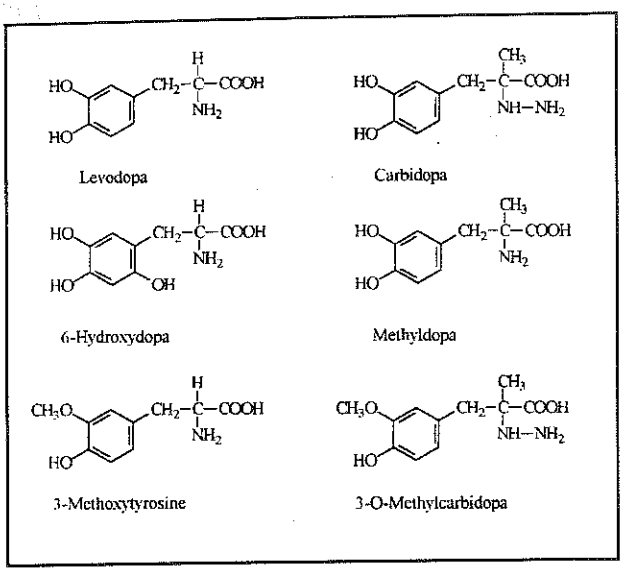

Figure 1. Chemical structures of levodopa, carbidopa and their related impurities.

separation of levodopa-carbidopa formulations[5-7]. Kafil and Dhingra[8] have developed a HPLC method for combined formulations which separates these drugs from their potential impurities.

The determination and identification of impurities are important aspects of drug analysis in order to fulfil official requirements and to develop a potential pharmaceutical product[9]. Various liquid chromatography (LC) methods can be used for the quantitation of the drug substance and its impurities. Capillary electrophoresis (CE) has been proved to possess several advantages for the analysis of pharmaceutical samples in comparison to LC, e.g. high efficiency, short time of analysis, fast method development and low cost[10-15].

A search of the literature showed that a CE method was not available for the simultaneous analysis of levodopa, carbidopa and their impurities. In this paper a simple and rapid CE method has been developed for the simultaneous separation and determination of levodopa, carbidopa and their impurities.

EXPERIMENTAL

Apparatus

All experiments were performed on a Beckman P/ACE MDQ capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA) equipped with an ultraviolet detector.

Chemicals and reagents

Levodopa, (-)-carbidopa, 3-methoxy-DL-tyrosine, 6-hydroxydopa and L-α-methyldopa were purchased from Sigma (Deisenhofen, Germany) and L-tryptophan from Degussa (Frankfurt-Mainz, Germany). Sodium dihydrogenphosphate, disodium monohydrogenphosphate, sodium phosphate and phosphoric acid were purchased from Merck (Darmstadt, Germany).

Electrophoretic technique

Electrophoretic separations were carried out in 51 cm (effective length 40 cm) x 50 μm I.D., uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) using 100 mM phosphate buffer, pH: 8.5. Between each run the capillary was rinsed with 0.1 M sodium hydroxide for 1 min followed by a rinse with the run buffer for 2 min. The applied voltage was +20 kV. The detection was processed at 214 nm. Sample solutions were injected into the capillary at the anodic end by hydrodynamic injection at a pressure of 0.5 psi for 3 s. The temperature of the cooling system of the capillary was kept at 20°C. Tryptophan was used as internal standard.

Sample solutions

The standard solutions were prepared by dissolving the compounds (1 mg) in 0.01 M HCl (1 ml) and filtered before injection.

RESULTS AND DISCUSSION

Due to zwitter ionic characters of the compounds, method development was begun by varying the pH value of the phosphate buffer solutions in the range of 2.5-10.5. Separation of carbidopa, levodopa and their impurities with an internal standard was not realised in acidic buffers. Depending on their pK_a values, the compounds will carry a negative charge at higher pH values. The change from acidic to basic electrolytes enabled the baseline separation of the compounds. In the pH range of 7.8-8.5 resolution was better; changing the pH from 8.5 to 10 increased the migration time of the compounds and peak shapes deteriorated

(Fig. 2). Thus, it was decided that 100 mM phosphate buffer at pH 8.5 was the best for the analysis of these compounds in a short time (ca. 8 min). Reproducibility of the migration times of all the compounds were studied and satisfactory results were obtained (Table 1). Borate buffer was also tested at the chosen pH but the use of this buffer had no beneficial effect on the separation of the analytes.

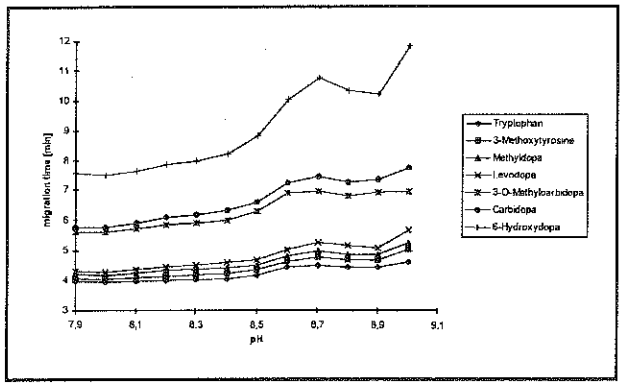

Figure 2. Effect of the pH of 100 mM phosphate buffer on migration time. Separation conditions as described in Table 1.

and levodopa with the CE conditions and the background electrolyte showed no interfering peaks at the detector wavelength. A representative electropherogram is shown in Fig. 3. This implied that, using the CE method, any of two compounds

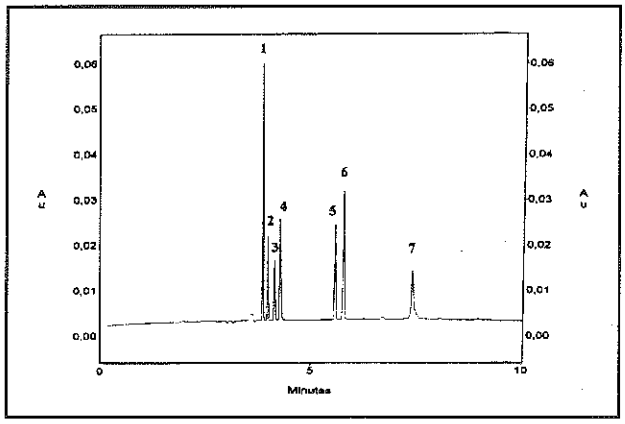

Figure 3: Electropherogram of a standard mixture of levodopa, carbidopa and their related impurities. Separation conditions as described in Table 1. Peak identification: 1= tryptophan (internal standard), 2= 3-methoxytyrosine, 3= methyldopa, 4= levodopa, 5= 3-O-methylcarbidopa, 6= carbidopa, 7= 6-hydroxydopa.

Table 1: Reproducibility of the migration times of levodopa, carbidopa and their related impurities in CE (n=5)*

Compound	Average migration time (min)	Relative standard deviation (RSD %)
Tryptophan	3.88	2.05
3-Methoxytyrosine	4.00	2.45
Methyldopa	4.16	1.58
Levodopa	4.29	2.06
3-O-Methylcarbidopa	5.59	1.39
Carbidopa	5.80	1.43
6-Hydroxydopa	7.40	1.23

*Buffer: 100 mM phosphate buffer (pH: 8.5); injection mode: hydrodynamic, 0.5 psi, 3s; capillary: uncoated fused-silica, 51 cm (effective length 40 cm) x 50 μm I.D.; applied voltage: +20 kV; λ: 214 nm.

In a second trial, a 20/31.2 cm (effective/total length) capillary was used in order to obtain a shorter analysis time. From some preliminary experiments, it was observed that the use of a short capillary had no reasonable advantage over the long capillary.

Several chemicals, such as tyrosine, phenylalanine, ibuprofen and tryptophan were tested as an potential internal standard. In order to optimize migration times of the compounds and to obtain good resolution, tryptophan was finally chosen as the internal standard.

The impurities were well resolved from carbidopa (carbidopa and levodopa) could be determined without any interference from their impurities.

CONCLUSION

CE is a complementary and alternative technique to HPLC in the area of drug analysis. Using the advantages of CE such as high separation efficiency, short analysis time, ease of use of instrumentation and preconditioning, it is possible to employ this method for the separation of levodopa and carbidopa from their potential impurities. The proposed CE method is simple and rapid. It is capable of validation and quantitation of levodopa and carbidopa in the

combined formulation and can be considered as a cost effective alternative to liquid chromatography for the analysis of these two drugs.

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