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Short communication

Enantiomeric separation and determination of antiparkinsonian drugs by reversed-phase ligand-exchange high-performance liquid chromatography☆

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Abstract

A simple and rapid high-performance liquid chromatographic method for the separation and determination of enantiomers of levodopa and carbidopa using a LiChrosper C_{18} column with aqueous copper–L-phenylalanine as mobile phase was developed. The separation between D- and L-enantiomers of levodopa and carbidopa was fairly good with separation factors of 1.63 and 2.38, respectively. The method was validated using synthetic mixtures and used for quality assurance of commercial formulations.

1. Introduction

Parkinson's disease, or paralysis agitans, is a degenerative nervous system disorder, characterized by progressive tremor, bradykinesia and muscular rigidity [1,2]. The causes of this disease are not known and its pathophysiology is poorly understood. However, it is known that the neuronal degeneration is difficult to arrest and hence leads to a significant depletion of dopamine. Therefore, any therapeutic attempt should involve correcting the dopamine depletion effectively. Dopamine is not administered directly because it does not cross the blood–brain barrier readily [3]. Therefore, its precursor levodopa (L-Dopa) is given orally for the treatment of Parkinson's disease. Levodopa is converted into dopamine by the enzyme decarboxylase and hence the concentration of dopamine is increased. This process of conversion of levodopa into dopamine is beneficial within the limits of the striatum but deleterious outside the blood–brain barrier because of the elevated levels of dopamine cause adverse reactions such as nausea, vomiting and cardiac arrhythmias (4). These side-effects are generally reduced by administering levodopa combined with a peripheral decarboxylase inhibitor, viz., carbidopa. Several combinations of levodopa and carbidopa are commercially available as different formulations. Although L-carbidopa is only used as a decarboxylase inhibitor, it may contain small amounts of the D-isomer owing to the procedures involved in its manufacture [5]. It has been reported that L-carbidopa is pharmacologically active whereas the D-form is not [6–9]. It may be noted that the use of racemic mixtures containing L-Dopa, D-Dopa, L-carbidopa and D-carbidopa may lead to serious side-effects such as dyskinesia and psy-

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chosis owing to differences in the metabolism of the active and non-active components of the racemates [10]. Therefore, development of methods for the separation and determination of optical isomers of the antiparkinsonian drugs is of great importance.

Several gas and liquid chromatographic methods for the separation and determination of levodopa and carbidopa have been reported [11–13], but these methods are not specific for the separation of enantiomers of levodopa and carbidopa. Hence they are not suitable for monitoring the levels of D-Dopa and D-carbidopa which are generally present in low concentrations, i.e., 0.1–2.0%, in commercial formulations. Recently, high-performance liquid chromatography (HPLC) has opened up new opportunities in the resolution of optical isomers of drugs, pharmaceuticals and agrochemicals [14–16]. Chiral columns, chiral ligand-exchange stationary phases, chiral mobile additives and diastereomerization techniques have been used extensively. Gilon et al. [17] studied three-point interactions involved in the resolution of amino acids using chiral eluents. Olerich et al. [18] demonstrated the usefulness of aqueous copper(II)–L-phenylalanine complex as mobile phases with reverse-phased C_{18} columns for the separation of enantiomers of methyldopa, tryptophan and hydroxytryptophan. Gelber and Neumeyer [19] adopted the same conditions for the determination of enantiomers of L-Dopa and its analogues in individual dosage forms by HPLC. Several others have reported the enantiomeric separation of L-Dopa using a variety of columns such as cellulose, ion-exchange resins and modified silica with chiral eluents as mobile phases by HPLC [20–22]. However, the simultaneous determination of enantiomers of L-Dopa and carbidopa, as used in the treatment of Parkinson's disease, has not been reported so far. In this paper, we describe the simultaneous separation and determination of enantiomers of levodopa and carbidopa in combined formulations using a LiChrospher C_{18} column and an eluent containing 0.003 *M* aqueous $CuSO_4$ and 0.006 *M* L-phenylalanine at ambient temperature.

2. Experimental

2.1. Materials and reagents

All reagents were of analytical-grade unless stated otherwise. Glass-distilled water was deionized using Nanopure II D 3700 cartridge (Barnstead). L-Phenylalanine (Loba Chemie, Bombay, India), Copper sulphate (BDH, Poole, UK), L-Dopa (Loba Chemie), D-Dopa (Fluka, Buchs, Switzerland) and L-carbidopa (Sun Pharmaceuticals, Bombay, India) were used. D-Carbidopa was prepared and purified in the laboratory following published methods [23,24]. Commercial formulations of levodopa and carbidopa were obtained from local firms.

2.2. Apparatus

A high-performance liquid chromatograph (Shimadzu, Kyoto, Japan) with a 20-μl loop injector having a high-pressure six-way valve was used. A Shimadzu SPD-6AU variable-wavelength UV–Vis spectrophotometric detector was connected after the column. A LiChrospher C_{18} (Merck, Darmstadt, Germany) column (125 mm × 4.0 mm I.D., particle size 5 μm) was used for separation. The chromatograms and the integrated data were recorded with a Chromatopac C-R3A processing system.

2.3. Mobile phase

A 0.9912-g amount of L-phenylalanine and 0.7488 g of $CuSO_4 \cdot 5H_2O$ were dissolved in 100 ml of doubly distilled, deionized water to give 0.003 *M* copper(II)–0.006 *M* L-phenylalanine complex, which was used as the mobile phase.

2.4. Chromatographic conditions

The mobile phase was 0.003 *M* aqueous copper(II) sulphate–0.006 *M* L-phenylalanine. The analysis was carried out under isocratic conditions at a flow-rate of 1 ml/min and a chart speed of 5 mm/min at room temperature (27°C). Chromatograms were recorded; at 280 nm.

2.5. Analytical procedure

Samples (10 mg) were dissolved in the mobile phase (10 ml) and a 5-μl volume of each sample was injected and chromatographed under the above conditions. Synthetic mixtures and commercial formulations were analysed under identical conditions. The amounts of enantiomers of levodopa and carbidopa were calculated from the corresponding areas of the peaks.

3. Results and discussion

The HPLC separation of enantiomers of levodopa and carbidopa is shown in Fig. 1. The peaks were identified by injecting individual authentic compounds. It can be seen from Fig. 1 that the compounds are well resolved under the conditions used. The separation factors were 1.63 and 2.38 for enantiomers of levodopa and carbidopa, respectively. The peaks were resolved with excellent symmetry and reproducibility. The resolution factors were determined and found to be fairly high, viz., 4.46 and 11.28 for enantiomers of levodopa and carbidopa, respectively. The LiChrospher C_{18} column with 0.003 *M* $CuSO_4$–0.006 *M* L-phenylalanine (50:50, v/v) was found to be an ideal system for separation. The retention data, viz., retention times, capacity factors (k') and separation factors (α) for the compounds under investigation are given in Table 1.

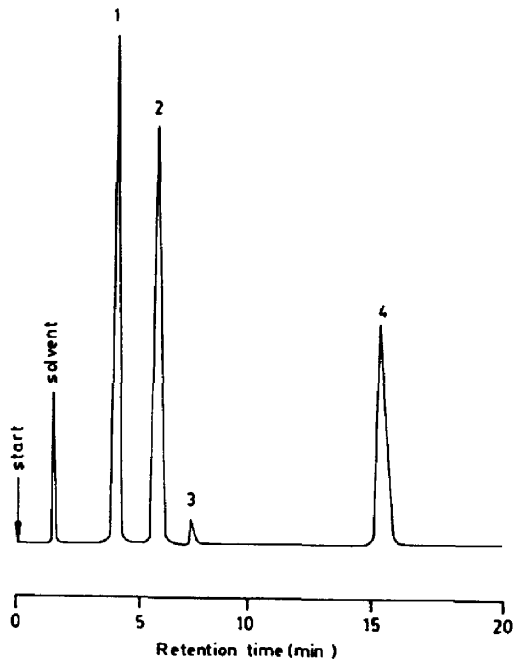

Fig. 1. Chromatogram of a typical mixture containing (1) D-Dopa (12 μg), (2) L-Dopa (10 μg), (3) D-carbidopa (0.1 μg) and (4) L-carbidopa (0.9 μg).

Table 1
Retention data

Compound	Retention time (min)	k'	α
D-Dopa	4.28	1.67	1.63
L-Dopa	5.96	2.73	
D-Carbidopa	7.55	3.72	2.38
L-Carbidopa	15.75	8.84	

The detector responses for D-Dopa and D-carbidopa were determined and the results are given in Table 2. The peak areas for $26.5 \cdot 10^{-9}$ g of D-Dopa and $10.4 \cdot 10^{-9}$ g of D-carbidopa were determined in triplicate and the average values for each were calculated. The relative standard deviations of these determinations were found to be 1.48% and 1.72% for D-Dopa and D-carbidopa, respectively. It is clear from Table 2 that the detector response for D-carbidopa was fairly high and it was found to be 4.5 times that of D-Dopa.

Standard mixtures containing different

Table 2
Detector response for D-Dopa and D-carbidopa

Compound	Amount (10^{-7} g)	Area	Relative standard deviation (%) ($n = 3$)
D-Dopa	0.2650	27114	1.48
D-Carbidopa	0.1040	47843	1.72

Table 3
Analytical data for standard mixtures

Sample No.	Compound	Taken (μg)	Found[a] (μg)	Error (%)	Compound	Taken (μg)	Found[a] (μg)	Error (%)
1	L-Dopa	98.04	97.18	−0.88	D-Dopa	1.96	2.01	+2.55
2	L-Dopa	94.59	95.92	+1.41	D-Dopa	5.41	5.33	−1.48
3	L-Dopa	90.43	89.26	−1.29	D-Dopa	9.57	9.82	+2.61
4	L-Carbidopa	97.25	98.79	+1.58	D-Carbidopa	2.75	2.67	−2.91
5	L-Carbidopa	95.38	96.25	+0.91	D-Carbidopa	4.62	4.73	+2.38
6	L-Carbidopa	91.24	90.17	−1.17	D-Carbidopa	8.76	8.58	+2.05

[a] Average of three determinations.

amounts of D-Dopa, D-carbidopa, L-Dopa and L-carbidopa were prepared and analysed by HPLC. The results are given in Table 3. It can be seen that the measured amounts of D-Dopa and D-carbidopa agreed well with the actual values to within 1.58% and 1.87%, respectively. The accuracy of the method was determined by the standard addition technique. Subsequent additions of D-Dopa and D-carbidopa were accurately reflected in their peak areas. Linear regression analysis data and the correlation coefficients are given in Table 4.

Fig. 2 shows the HPLC trace for a typical formulation of levodopa and carbidopa obtained commercially. It can be seen that very small amounts of D-Dopa and D-carbidopa which are known to be inactive are present in the formulations. The results are given in Table 5. These results show that the method is suitable for the determination of the enantiomeric excess of levodopa and carbidopa simultaneously using a reversed-phase C_{18} column with aqueous copper–L-phenylalanine as the mobile phase.

4. Conclusions

A simple and rapid HPLC method using a reversed-phase C_{18} column with aqueous copper–L-phenylalanine as the mobile phase has been developed for the simultaneous separation and determination of enantiomers of levodopa and carbidopa in mixtures. It is suitable for quality assurance of commercial formulations of levodopa and carbidopa used in the treatment of Parkinson's disease.

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Table 4
Linearity data

Compound	Concentration range (μg)	Correlation equation[a]	Correlation coefficient
L-Dopa	90.43–99.56	$y = 1.015x - 0.072$	0.989
D-Dopa	1.96–9.97	$y = 0.997x + 0.014$	0.985
L-Carbidopa	91.24–99.85	$y = 1.012x - 0.108$	0.998
D-Carbidopa	0.51–8.76	$y = 0.989x + 0.025$	0.978

[a] y = Amount found (μg); x = amount taken (μg).

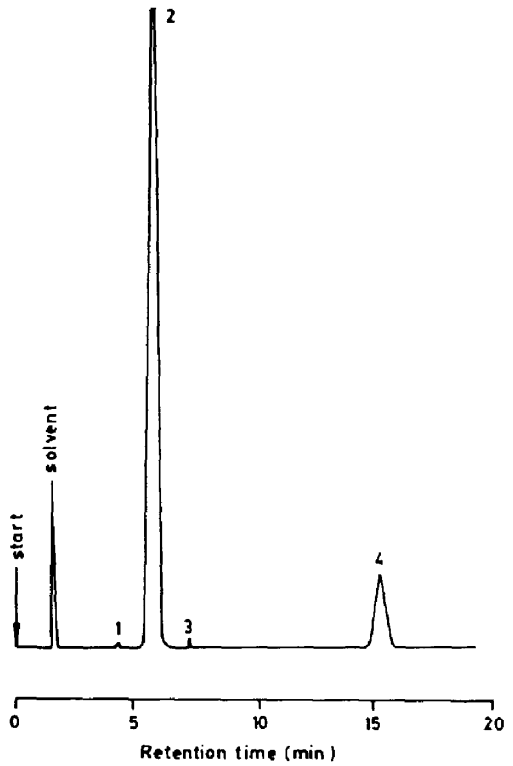

Fig. 2. Chromatogram of a commercial formulation containing L-Dopa (25 μg), L-carbidopa (2.5 μg), D-Dopa (0.15 μg) and D-carbidopa (0.02 μg). For identification of peaks, see Fig. 1.

Table 5
Levels of D-Dopa and D-carbidopa determined in samples of commercial formulations

Sample No.	Compound	Concentration (%)[a]	R.S.D. (%)
1	D-Dopa	0.89	1.6
	D-Carbidopa	–	–
2	D-Dopa	0.54	2.0
	D-Carbidopa	0.08	2.6
3	D-Dopa	0.27	2.4
	D-Carbidopa	0.15	2.2

[a] Average of three determinations.

References

[1] J.H. Stein (Editor), *Internal Medicine*, Little, Brown and Long, Boston, 1983, pp. 878–879.
[2] D.B. Clane, *N. Engl. J. Med.*, 329 (1993) 1021.
[3] L.S. Goodman and A. Gilman (Editors), *The Pharmacological Basis of Therapeutics* Macmillan, New York, 5th ed., 1975, pp. 227–229.
[4] E.F. Reynolds (Editor), *Martindale, The Extra Pharmacopoeia*, Pharmaceutical Press, London, 28th ed., 1982, pp. 883–890.
[5] B. Lausecker and F.M. Albert, *J. Chromatogr.*, 545 (1991) 115.
[6] L. Porter, *J. Pharmacol. Exper. Ther.*, 172 (1970) 406.
[7] S. Andreae, E. Schmitz, S. Schramm, F.M. Albert and D. Lohman, *GDR Pat.*, 230865 (1986); *C.A.*, 105 (1986) 227301j.
[8] S. Budavari (Editor), *The Merck Index*, Merck, Rahway, NJ, 11th ed., 1989, p. 1802.
[9] A.E. Harper, N.J. Benevenga and R.M. Wohlhueter, *Physiol. Rev.*, 50 (1970) 428.
[10] M. Goodall, *Adv. Neurol.*, 1 (1973) 517.
[11] S.T. Hamid and J. Walker, *Anal. Chim. Acta*, 105 (1979) 403.
[12] S. Ting, *J. Assoc. Off. Anal. Chem.*, 69 (1989) 169.
[13] L. Rihbany and M.F. Delaney, *J. Chromatogr.*, 248 (1982) 125.
[14] S.C. Chang, G.L. Reid, C.D. Chang and D.W. Armstrong, *Trends Anal. Chem.*, 12 (1993) 145.
[15] W.H. Pirkle, J.M. Finn, J.L. Schriner and B.C.H. Hamper, *J. Am. Chem. Soc.*, 103 (1981) 3964.
[16] S. Li and W.C. Purdy, *J. Chromatogr.*, 625 (1992) 109.
[17] C. Gilon, R. Leshem and E. Grushka, *J. Chromatogr.*, 203 (1981) 365.
[18] E. Olerich, H. Preusch and E. Wilhelm, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 269.
[19] L.R. Gelber and J.L. Neumeyer, *J. Chromatogr.*, 257 (1983) 917.
[20] G. Gilbitz, W. Jellenz and D. Schonleber, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 31.
[21] P.E. Hare and E. GilAv, *Science*, 204 (1979) 1226.
[22] H.G. Kicinski and A. Kettrup, *Fresenius' Z. Anal. Chem.*, 3200 (1985) 51.
[23] S. Karady, M.G. Ly Seemon, H. Pines and M. Sletzinger, *J. Org. Chem.*, 36 (1971) 1946.
[24] M. Sletzinger, J.M. Chemerda and F.W. Bollinger, *J. Med. Chem.*, 6 (1963) 101.