ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
19 (1999) 555–567

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Direct high-performance liquid chromatographic determination of the enantiomeric purity of levodopa and methyldopa: comparison with pharmacopoeial polarimetric methods

Milada Doležalová *, Magda Tkaczyková

State Institute for Drug Control, Šrobárova 48, 100 41 Prague 10, Czech Republic

Received 18 November 1997; received in revised form 28 April 1998; accepted 21 May 1998

Abstract

Chiral high-performance liquid chromatography was employed for determination of the enantiomeric purity of levodopa and methyldopa. The determination of D-DOPA in levodopa was accomplished using a chiral ligand-exchange chromatograpy with an ordinary C_{18} column and a chiral mobile phase containing *N*,*N*-dimethyl-L-phenylalanine and Cu(II) acetate or by means of LC on a teicoplanin column in conjunction with ethanol–water (65:35, v/v). Both methods gave good performance, however, the latter was faster and more convenient and suitable for routine analyses. For the determination of D-methyldopa a LC method based on the use of a teicoplanin column in polar organic mode with methanol-acetic acid-triethylamine (1000:0.05:0.05, v/v/v) mobile phase was developed. The precision, accuracy, linearity and selectivity were satisfactory. In comparison with pharmacopoeial polarimetric methods (according to the European Pharmacopoeia and the Pharmacopoea Bohemoslovaca), the LC methods proved to be much more sensitive giving detection limits 0.04% of D-DOPA and 0.3% of D-methyldopa.

Keywords: Levodopa; Methyldopa; Enantiomeric purity; Chiral liquid chromatography; Teicoplanin stationary phase; Chiral ligand exchange phase; Optical rotation

1. Introduction

About 25% of all therapeutics are marketed and administered as stereoisomeric mixtures, mostly racemates. Usually only one of the isomers fully contributes to the therapeutic activity and the other are classified as isomeric balast. However, until the recent great progress in enantioselective syntheses and analytical methodology has been made, such mixtures were regarded as single active substances.

If only one enantiomer of a chiral drug is used as an active substance all other stereoisomer(s) should be treated as impurities. However, in contrast with ongoing refinements of tests for other

* Corresponding author.

0731-7085/99/$ - see front matter
PII: S0731-7085(98)00257-X

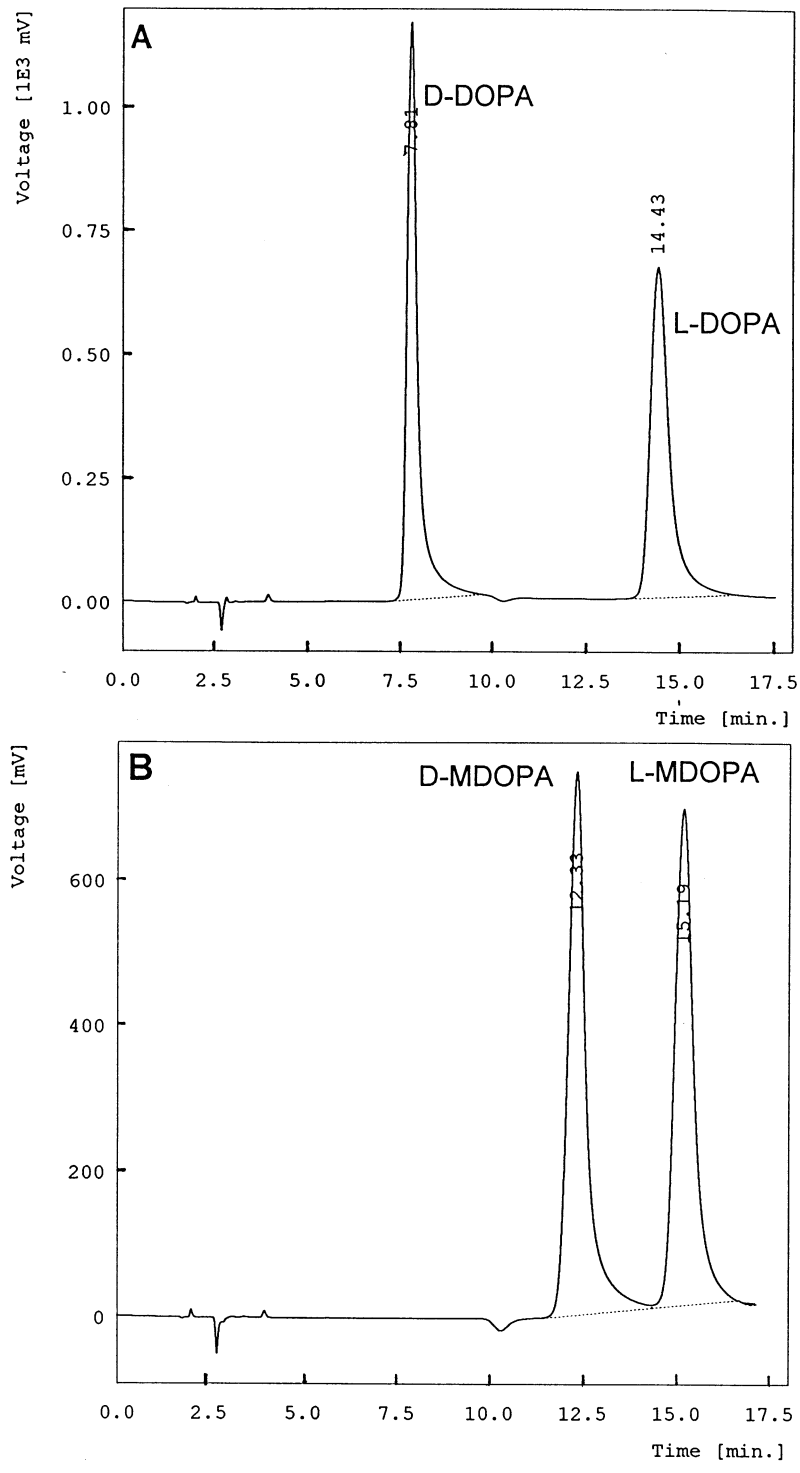

Fig. 1. Chiral LEC enantioseparation of: (A) DOPA; (B) MDOPA. Column: Lichrospher 100 RP – 18 (5 μm), 250 × 4.6 mm. Mobile phase: methanol-0.002 M *N*,*N*-dimethyl-L-phenylalanine and 0.001 M copper(II) acetate in water (pH 4.5) (1:9, v/v). Flow rate: 0.8 ml min^{-1}. Detection 228 nm.

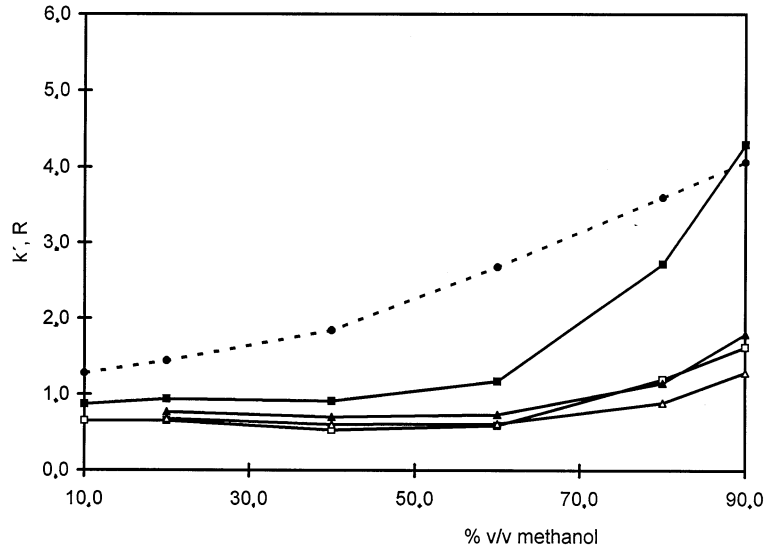

Fig. 2. Influence of percentage methanol on capacity factors k' of L-DOPA (□); D-DOPA (■); L-MDOPA (△); D-MDOPA (▲′) and enantioresolution of DOPA (● and dashed line). Column: Chirobiotic T (5 μm), 150 × 4.6 mm with a precolumn 10 × 3.2 mm. Mobile phase: methanol–water. Flow rate: 0.7 ml min^{-1}.

organic impurities in drugs, the enantiomeric impurities are still in most cases assessed using traditional, non-selective and often unsufficiently sensitive polarimetric methods. Until quite recently these methods were the only pharmacopoeial tests of enantiomeric purity.

Levodopa, (L-DOPA) (3-(3,4-dihydroxyphenyl)-L-alanine), which is extensively used for the treatment of Parkinson's disease and methyldopa, (L-MDOPA) (2-methyl-3-(3,4-dihydroxyphenyl)-L-alanine), which is a widely used antihypertensive, are examples of drugs that have been marketed as single enantiomers for many years and even now are tested for the enantiomeric purity by measurement of optical rotation.

The US Pharmacopeia (USP) [1] specifies tests for the following impurities in L-DOPA and L-MDOPA: 3-methoxytyrosine, 3-(3,4,6-trihydroxyphenyl)alanine and 3-methoxymethyldopa, respectively. The enantiomeric purity is controlled by measurement of optical rotation as well as in European Pharmacopoeia (Ph. Eur.) [2] and Pharmacopoea Bohemoslovaca (PhBs) [3].

The objective of the present work was to investigate applicability of chiral HPLC for determination of the enantiomeric purity of L-DOPA and L-MDOPA and to compare such methods with the pharmacopoeial tests of optical rotation measurements.

There are several approaches to HPLC enantioselective separations, either indirect separations using derivatization with chiral reagents to diastereoisomers prior to separation, or direct separations that can be performed on chiral stationary phases or with the use of chiral mobile phases in conjunction with achiral stationary phases. Today the direct separations are preferred as they avoid derivatization with its possible difficulties.

Direct separations of aromatic amino acids can be achieved with the use of a chiral ligand-exchange chromatography (LEC) [4], a chiral crown ether selector applied as a bonded chiral stationary phase or a mobile phase additive [5,6], an α-cyclodextrin stationary phase in reversed-phase mode [7] or a recently introduced stationary phase with covalently bonded glycopeptide antibiotic teicoplanin in reversed phase mode [8,9]. Three of the above-mentioned types of chiral HPLC have been successfully used for separation of enantiomers of DOPA—a chromatographic system composed of a crown ether stationary phase and a

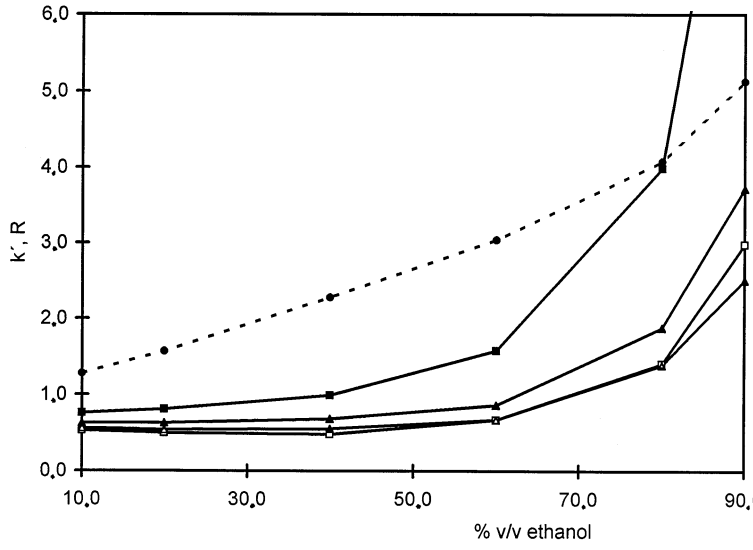

Fig. 3. Influence of percentage ethanol on capacity factors k' and enantioresolution R. Mobile phase: ethanol–water. Symbols and other conditions as in Fig. 2.

mobile phase with perchloric acid [10], a chiral LEC with L-phenylalanine as the mobile phase additive [11] and a teicoplanin stationary phase in combination with an ethanol–water mixture as the mobile phase [8]. The only type of direct HPLC enantioseparations of MDOPA reported in literature is LEC with chiral mobile phases containing either L-phenylalanine or *N*,*N*-dimethyl-L-phenylalanine [11,12].

In order to elaborate chiral HPLC methods suitable for quality control purposes we tested two different types of direct enantioseparations of DOPA and MDOPA: A. ligand-exchange chromatography based on the use of a chiral mobile phase containing *N*,*N*-dimethyl-L-phenylalanine and a conventional C18 stationary phase, B. chromatography on a chiral stationary phase with bonded teicoplanin.

The suggested HPLC methods were compared to optical rotation measurements carried out according to Ph. Eur. [2] and PhBs [3].

2. Experimental

2.1. Reagents and chemicals

L- and D-DOPA, L- and DL-MDOPA, 3,4,6-trihydroxyphenyl-DL-alanine and 3-methoxy-DL-tyrosine were products of Sigma (St. Louis, MO), reference standard 3-methoxymethyldopa was obtained from European Department for the Quality of Medicines-European Pharmacopoeia (Strasbourg, France). L-DOPA and L-MDOPA were dried at 105 and 130°C, respectively, for 4 h prior to use. Methanol (gradient grade), acetonitrile (gradient grade), ethanol 96% (extra pure), *N*,*N*-dimethyl-L-phenylalanine (Lichropur) and copper(II) acetate monohydrate (extra pure) were obtained from Merck (Darmstadt, Germany). Water was passed through an Elgastat UHQ water purification unit (Elga, Lane End High Wycombe Bucks, UK). Triethylamine (super purity solvent) was purchased from Romil (Loughborough, UK). All other chemicals used were of analytical-reagent grade.

2.2. Chromatography

HPLC measurements were performed with two chromatographic systems: A. an HPLC apparatus consisting of a Constametric 3500 pump, a Rheodyne 7725i injector, a Spectromonitor 4100 variable-wavelength detector (Thermo Separation Products, Riviera Beach, FL), a datastation CSW DataApex (Prague, Czech Republic) and a column

thermostat Model K-5 Techlab, (Erkerode, Germany), B. a Shimadzu Model LC-6A liquid chromatograph (Kyoto, Japan) equipped with a variable-wavelength detector and a Rheodyne 7125 injector. The HPLC columns used were Lichrospher 100 RP-18 (5 μm), 250 × 4.6 mm I.D., (Merck, Darmstadt, Germany) and Chirobiotic T (5 μm), 150 × 4.6 mm with a guard column Chirobiotic T (5 μm), 10 × 3.2 mm I.D., (Astec, Whippany, NJ).

The mobile phase for chiral LEC was prepared by mixing methanol and water containing 0.002 M *N*,*N*-dimethyl-L-phenylalanine and 0.001 M copper(II) acetate (pH 4.5) in ratio 1:9, v/v and passing the mixture through a 0.45 μm Millipore filter before use. The flow rate was kept at 0.8 ml/min and the detection wavelength was 228 nm. The mobile phases used in conjunction with a column Chirobiotic T were a mixture of ethanol–water (65:35, v/v) for quantitative determination of D-DOPA and a mixture of methanol-acetic acid-triethylamine (1000:0.05:0.05, v/v/v) for quantitative determination of D-MDOPA. The flow rates were kept at 0.7 ml min^{-1} and 0.9 ml min^{-1}, respectively. The detection wavelength was 210 nm (TSP detector) or 280 nm (Shimadzu detector). The column temperature was ambient, only separations on the teicoplanin column in polar organic mode were performed at controlled temperature 25°C.

The hold-up time (t_0) was determined by injecting water or methanol and measuring the elution time of the disturbance peak.

For RP LC on the teicoplanin column 0.5 mg ml^{-1} solutions of DOPA or MDOPA were prepared in 0.04 M acetic acid and a 5-μl aliquot was injected into the chromatograph, for the other LC methods the samples were dissolved in the mobile phases and the amount injected was 10 μl.

2.3. Polarimetry

Measurements of optical rotation were performed on a Perkin-Elmer Model 241 polarimetr (Norwalk, CT) with a jacketed microcell and a thermostat kept at 20°C. Optical rotation of solutions of DOPA and MDOPA in 1 M hydrochloric acid (40.0 mg ml^{-1}) and in water (20.0 mg ml^{-1}), respectively, was measured and specific optical rotation calculated following the method of PhBs [3]. According to Ph. Eur. [2] optical rotation of DOPA solutions in 1 M hydrochloric acid (8 mg ml^{-1}) containing hexamethylenetetramine (200 mg ml^{-1}) was measured.

3. Results and discussion

3.1. Chiral LEC separation

Chiral LEC is based on formation of a ternary complex in the presence of a metal ion (Cu II) and an α-amino acid. The chromatographic system proposed for enantioseparation of MDOPA [12] was chosen to be examined in our work as it

Table 1
Capacity factors (k') and enantioresolution (R) of DOPA, MDOPA and related compounds

Compound	Chiral LEC			Teicoplanin column					
				RP mode			PO mode		
	k'_L	k'_D	R	k_L'	k'_D	R	k'_L	k'_D	R
DOPA	6.2	2.9	10.0	0.9	2.4	3.6	2.9	9.3	6.2
MDOPA	6.5	5.1	3.4	0.8	1.1	1.0	2.1	3.4	2.9
Carbidopa	>16	>16		1.5	2.2	1.7	5.6	8.5	2.6
3-Methoxytyrosine	15.8	7.8	11.5	0.9	1.6	2.4	2.5	4.1	3.1
3-(3,4,6-Trihydroxy-phenyl)alanine	5.6	12.8	4.6	0.1	0.1				
3-Methoxymethyldopa				0.8			1.7		
L-Tyrosin	8.4			0.9					

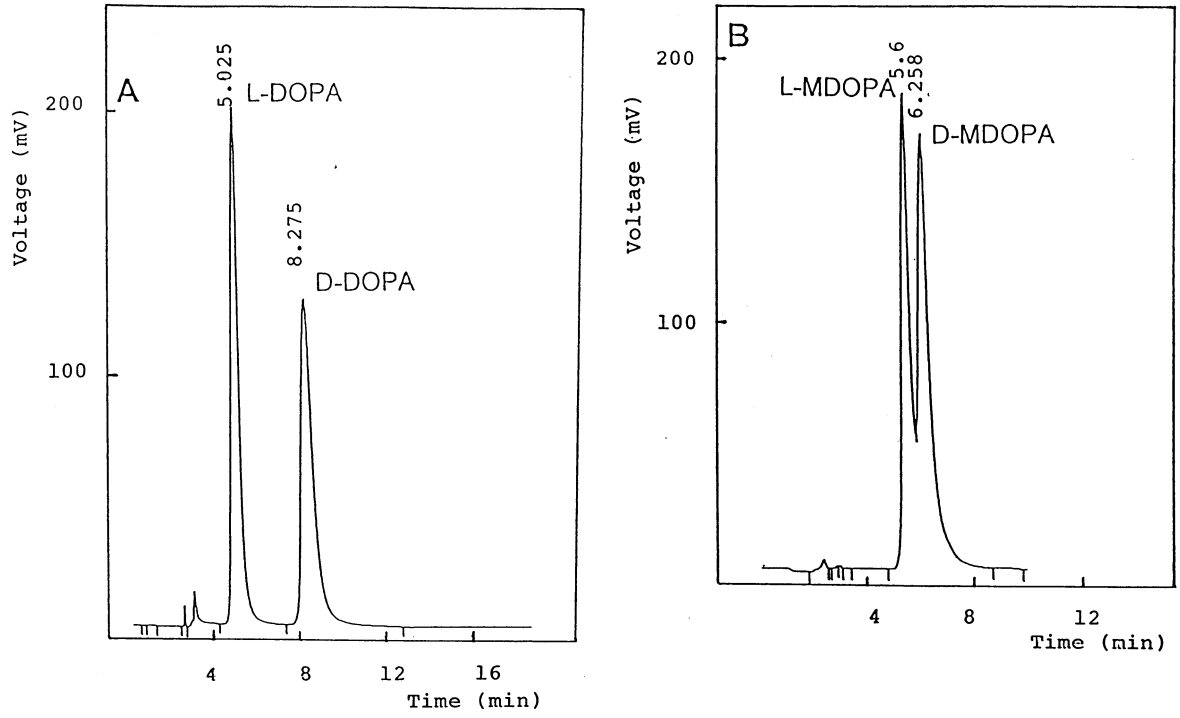

Fig. 4. Reversed-phase enantioseparations of: (A) DOPA; (B) MDOPA. Column: Chirobiotic T (5 μm), 150 x 4.6 mm with a precolumn 10 × 3.2 mm. Mobile phase: ethanol–water (65:35, v/v). Flow rate: 0.7 ml min^{-1}. Detection: 280 nm.

circumvents the need of a special column packed with a chiral stationary phase and would be thus easily accessible to control laboratories equipped with conventional HPLC columns.

Before analyses a chiral mobile phase composed of *N*,*N*-dimethyl-L-phenylalanine and Cu(II) acetate was passed through an ordinary C18 stationary phase for 5 to 6 h until the stable baseline was detected. After the equilibrium had been reached it was convenient to recycle the mobile phase.

Both MDOPA and DOPA were resolved into the enantiomers under the chromatographic conditions published for MDOPA [12], as shown in Fig. 1. The capacity factors and resolution are given in Table 1.

Retention and resolution of the enantiomers were slightly influenced by changes of pH in the range of 3.5 to 4.6. Increasing the concentration of the chiral ligand and Cu(II) in mobile phase resulted in a higher background absorbance, whereas at lower concentrations a peak distortion was observed. As expected, an increasing the content of methanol in mobile phase produced a decrease of retention of all enantiomers.

Elution order of both compounds was favourable for determination of small amounts of D-enantiomer in the presence of an excess of L-enantiomer and the enantioresolution was in both cases sufficiently large. However, quantitation of D-MDOPA traces in L-MDOPA was difficult due to a large negative peak observed closely to D-MDOPA. Consequently, limit of quantitation of D-MDOPA was about 1% (related to D + L-MDOPA).

Hence the LEC method was used only for determining the enantiomeric purity of L-DOPA. The pharmacopoeial impurities and related substances did not interfere with the determination as the chromatographic conditions enabled their separation (Table 1).

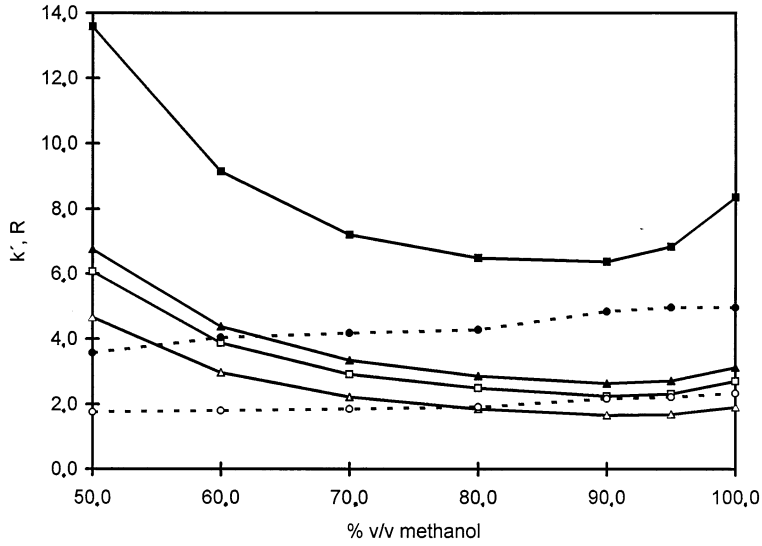

Fig. 5. Influence of percentage methanol on capacity factors k′ and enantioresolution R; o and dashed line = enantioresolution of MDOPA, other symbols as in Fig. 2. Column: Chirobiotic T (5 μm), 150 × 4.6 mm with a precolumn 10 × 3.2 mm. Mobile phase acetonitrile-methanol-acetic acid-triethylamine (100-x: x: 0.01: 0.01, v/v/v). Flow rate 0.9 ml min^{-1}.

3.2. Separation on teicoplanin stationary phase in reversed-phase mode

This mode was proposed for the class of amino acids. In accordance with the published data [8], DOPA was resolved on the teicoplanin column using mobile phases composed of methanol or ethanol and water. As can be seen in Figs. 2 and 3, capacity factors and resolution of the enantiomers increased significantly with the increasing content of ethanol or methanol in mobile phase, ethanol giving better enantioselectivity than methanol. Similar results were obtained with mobile phases containing triethylamine acetate buffer of pH 4.1 instead of water.

The data presented in Figs. 2 and 3 also indicated that substituting methyl group at the assymetric carbon of DOPA dramatically decreases the enantioresolution. While the capacity factors of L-MDOPA and L-DOPA were very close, D-MDOPA was much less retained than D-DOPA. Hence the complete enantioseparation of MDOPA could not be achieved on the teicoplanin column used in RP mode.

On the basis of the experimental results plotted in Fig. 3, the optimum mobile phase ethanol–water (65:35, v/v) was found for separation of DOPA enantiomers. The enantioresolution was high enough for sensitive determination of the enantiomeric purity of L-DOPA, despite the fact that the elution order was unfavourable as the major enantiomer L-DOPA was eluted before traces of D-DOPA. Fig. 4 shows the separations of DOPA and MDOPA racemates, obtained under the optimal conditions.

Table 1 demonstrates that related substances did not interfere with the determination of traces of D-DOPA. However, L-enantiomers of DOPA, MDOPA, tyrosin and 3-methoxytyrosin were not separated under this conditions.

3.3. Separation on teicoplanin stationary phase in polar organic mode

The above chiral chromatographic system proved to have low enantioselectivity for MDOPA. However, the curves in Fig. 2 indicated that polar organic (PO) mode of separation, which was originally devised for cyclodextrine stationary phases, could be feasible. A teicoplanin stationary phase appears to have a great success in this mode, in which potential for all interactions is enhanced [13].

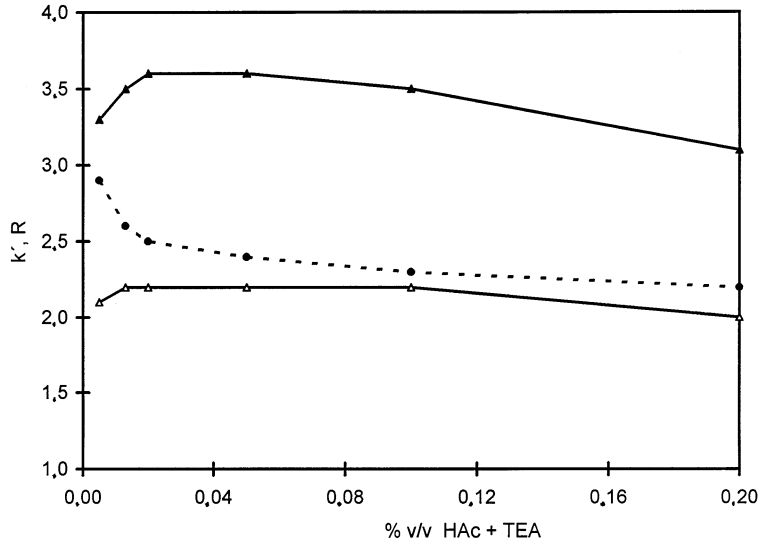

Fig. 6. Dependence of capacity factors k' of L-MDOPA (△)and D-MDOPA (▲′) and enantioresolution R (● and dashed line) on total amount of acetic acid (HAc) and triethylamine (TEA) added to neat methanol, Relative amounts of HAc and TEA = 1:1 v/v. Temperature: 25°C. Other conditions as in Fig. 5.

PO mobile phases consist of four components-acetonitrile, methanol, glacial acetic acid and triethylamine. In Fig. 5 the function of methanol as a retention modifier is shown for both amino acids studied. As expected, increasing the concentration of methanol from 50 to 90% (v/v) caused the retention to decrease. Despite this retention decrease, the enantioresolution of both DOPA and MDOPA increased. In neat methanol the enantioresolution of MDOPA further slightly increased, the retention of the enantiomers being slightly enhanced this time.

Unlike RP mode, the capacity factors of both L-enantiomers were different (Fig. 5). Enantioresolution of MDOPA was again lower than that of DOPA, however, complete separation could be achieved in this mode.

In all these experiments mobile phases with the same amount of acetic acid and triethylamine (0.05% v/v) were used. In the next step influence of total amount of the polar components added to neat methanol was followed. The data plotted in Fig. 6 show that increasing the total amount of acetic acid and triethylamine added to methanol depressed slightly enantioresolution of MDOPA. The greatest values were obtained for zero or very low total amount (0.01% v/v maximumly) of the polar components. The retention was affected only slightly—after a small initial increase it decreased with increasing the total concentration of the polar additives.

At the total amount of acetic acid and triethylamine as low as 0.01% v/v the enantioselectivity was not very sensitive to their relative ratio. Very similar values of resolution and retention were received with the mobile phases composed of methanol and 0.005% of acetic acid or methanol, 0.005% of acetic acid and 0.005% of triethylamine or methanol without any additive.

The experiments, which were done in an attempt to reach as high enantioresolution of MDOPA as possible, lead us to a simple mobile phase composed of methanol- acetic acid-triethylamine (1000:0.05:0.05, v/v/v). Enantioseparations of DOPA and MDOPA racemates under these conditions are depicted in Fig. 7, the capacity factors and resolution are given in Table 1.

The enantioresolution of MDOPA was not influenced by column temperature in the range of 16–35°C.

Table 1 also shows that 3-methoxymethyldopa, levodopa and carbidopa did not interfere with determination of D-MDOPA.

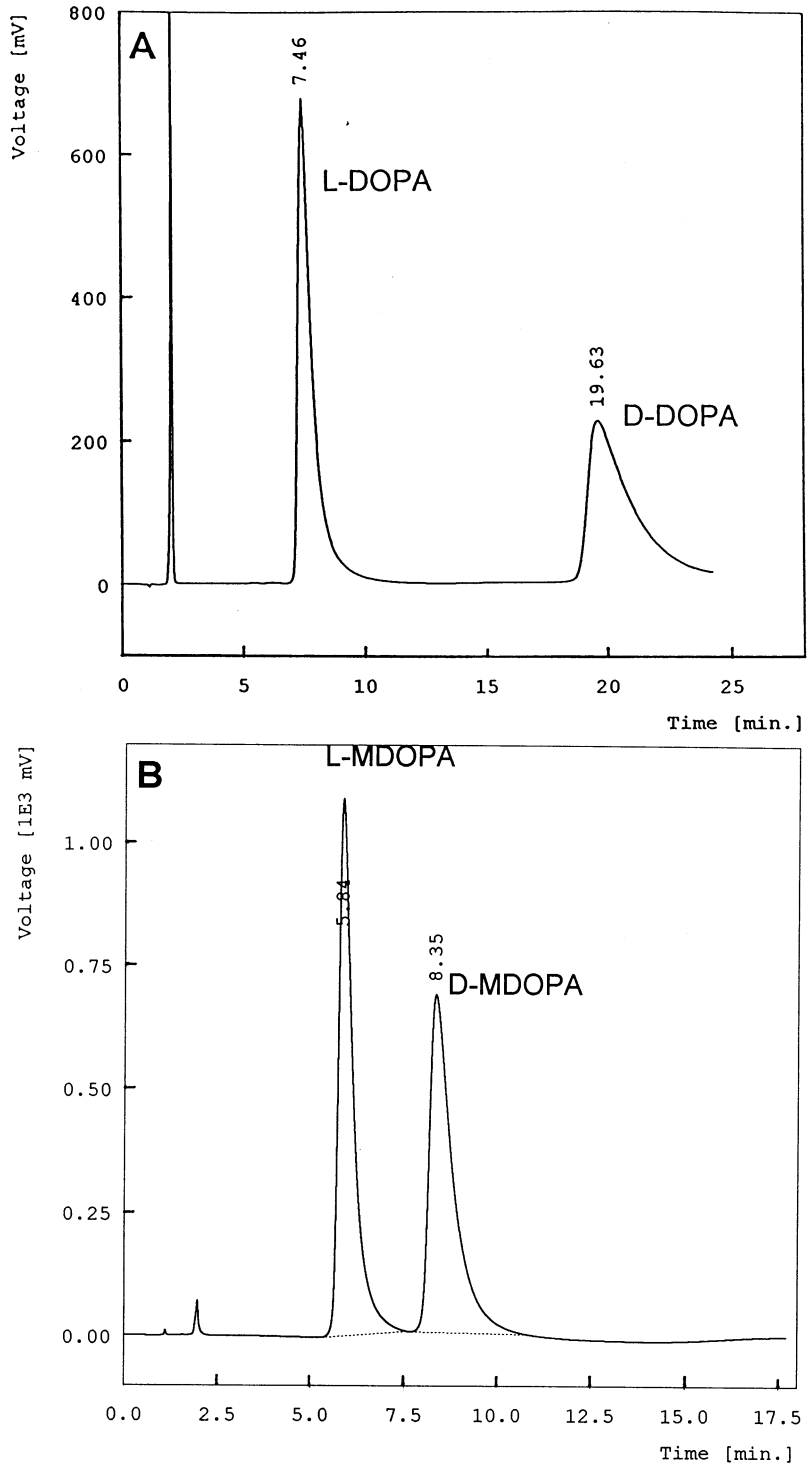

Fig. 7. Enantioseparation of (A) DOPA; (B) MDOPA in PO mode. Column: Chirobiotic T (5 μm), 150 × 4.6 mm. Mobile phase: methanol-acetic acid-triethylamine (1000:0.05:0.05, v/v/v). Flow rate: 0.9 ml min^{-1}. Temperature: 25°C. Detection: 210 nm.

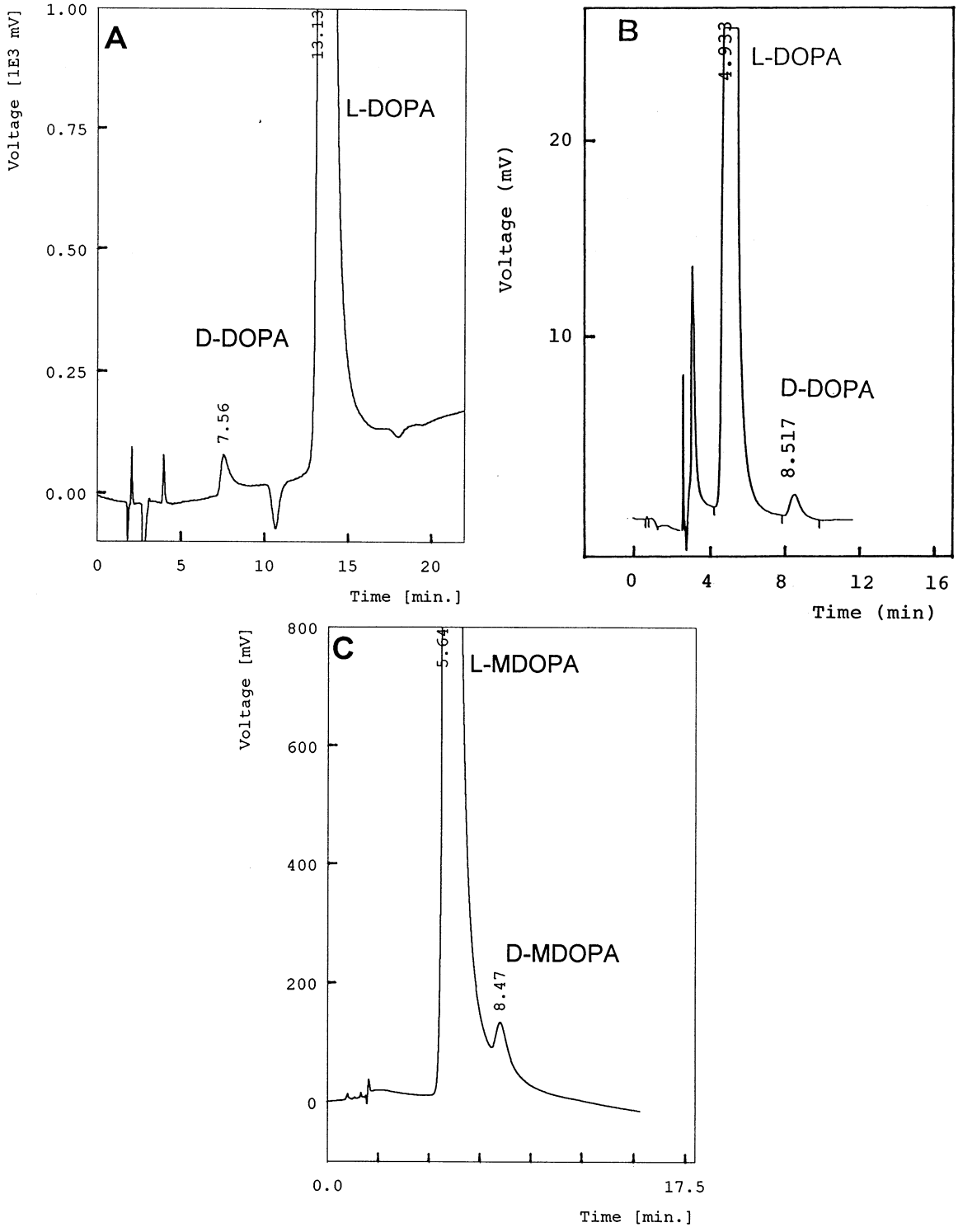

Fig. 8. Typical chromatograms of: (A, B) L-DOPA containing 0.5% of D-DOPA; (C) L-MDOPA containing 1.0% of D-MDOPA. Chromatographic conditions: (A) as in Fig. 1; (B) as in Fig. 4; (C) as in Fig. 7.

Table 2
Precision, accuracy and linearity of the HPLC methods for determining the enantiomeric purity of DOPA and MDOPA

Compound/ HPLC method	D enantiomer added[a] (% m m^{-1})	RSD (%) ($n=4$) intraday	Interday	Recovery (%)	Range (μg ml^{-1})	Correlation coefficient
DOPA/LEC	1.19	1.4	3.5	102.6	1–25	0.9991
	2.10	3.8	3.3	102.7		
DOPA/Teicoplanin-RP	0.50	1.0	1.4	104.3	2–27	0.9999
	0.99	1.2	-	99.5		
	3.60	1.7	2.9	97.7		
MDOPA/Teicoplanin-PO	1.47	1.8	1.5	83.2	5–27	0.9815
	2.97	0.4	2.2	96.2		

[a] Relative content of D enantiomer in known mixtures of L+D.

3.4. Validation

Validation studies of the chromatographic methods for determining the enantiomeric purity of L-DOPA (the chiral LEC and the RP LC on a teicoplanin column) and MDOPA (the LC on a teicoplanin column in PO mode) were done in order to evaluate suitability of the methods for the intended purpose.

Data calculated from results of analyses of known D + L mixtures are summarized in Table 2. Detection limits were evaluated from chromatograms of samples of L-enantiomers containing very low amounts of D-enantiomers. 0.04% and 0.05% of D-DOPA were considered to be detection limits for the LEC method and the RP LC on a teicoplanin column, respectively, and 0.3% of D-MDOPA for the LC on a teicoplanin column in PO mode.

Excellent results were obtained for both methods of determination of the enantiomeric purity of L-DOPA. The methods are accurate, precise, selective and very sensitive. Small changes in the mobile phases did not influence determination. However, using the chiral LEC method a long equilibration of the column was necessary for reproducible and stable chromatographic conditions.

Somewhat lower accuracy and sensitivity were assessed using the method of determination of the enantiomeric purity of L-MDOPA. The enantioresolution found under the optimized chromatographic conditions was high ($R_S = 2.9$ for the racemic mixture), however, it should be noted at this point that the requirements for determining small amounts of distomer in the presence of excess of eutomer are very rigorous. Generally, resolution greater than 2 for a racemic mixture is needed [14] for trace analyses. If a trace component is eluted after a dominant enantiomer the situation is more complicated because of tailing from the major peak, as is illustrated in Fig. 8 showing typical chromatograms obtained with the use of the three suggested methods. Tailing of the major peak of L-MDOPA (Fig. 8C) resulted in more difficult quantitation of D-MDOPA peak and hence in somewhat lower accuracy and sensitivity, as compared with the methods for determining D-DOPA.

Nevertheless, the developed method proved to be more acceptable for routine analyses than the published chiral LEC method [11]. The sensitivities of both methods are comparable (0.42% of D-MDOPA is the published detection limit), however, the present method did not suffer from experimental problems as reported in [11]. It is more convenient, simple and faster.

3.5. Polarimetric methods

The optical purity of samples prepared as mixtures of L- and D-enantiomers of DOPA and MDOPA was assessed using pharmacopoeial methods [2,3]. The Ph. Eur. method for MDOPA was not accomplished as various problems with it were reported [11].

Table 3
Optical rotation[a]

A. D enantiomer added (% m m^{-1})	Specific optical rotation $[\alpha]_D^{20}$	B. D-DOPA added (% m m^{-1})	Optical rotation α_D^{20}
DOPA			
0	$-11.84° \pm 0.07°$	0	$-1.310° \pm 0.003°$
0.50	$-11.41°$	0.50	$-1.313°$
1.00	$-11.30°$	1.00	$-1.299°$
2.00	$-11.04°$	1.98	$-1.278°$
4.99	$-10.54°$	5.02	$-1.260°$
MDOPA			
0	$-15.42° \pm 0.03°$		
0.50	$-15.09°$		
1.00	$-14.84°$		
1.97	$-14.73°$		
4.79	$-13.91°$		

[a] A. Measurements according to PhBs [3] (the limits are -11.0 to $-13.0°$ for DOPA and -12.5 to $-15.5°$ for MDOPA). B. Measurements according to Ph. Eur. [2] (the limits are -1.27 to $-1.34°$).

The results summarized in Table 3 show that the pharmacopoeial methods tested are not sufficiently sensitive. The limits set in the pharmacopoeias were not exceeded untill more than 2% of D-DOPA or 5% of D-MDOPA was present in the tested samples. Indeed, in contrast with the chiral chromatographic methods the pharmacopoeial tests of optical rotation proved to be unable to detect traces of D-enantiomers.

4. Conclusion

A new approach to determination of the enantiomeric purity of L-DOPA and L-MDOPA was suggested. With the use of the described chromatographic systems the enantioseparation of DOPA and MDOPA can be achieved and the enantiomeric purity of the drugs determined.

The present chiral HPLC methods give high sensitivity and precision for the determination of traces of D-enantiomers in the presence of excess of L-enantiomers. The impurities specified in the USP did not interfere with the determination.

The HPLC methods using a teicoplanin stationary phase are superior to the chiral LEC, as they did not suffer from problems such as slow equilibration of a column, small stability of baseline during measurements at high detector sensitivities, occurence of negative peaks.

The results obtained show the potential of using chiral HPLC methods for the determination of the enantiomeric purity, especially in comparison with the pharmacopoeial polarimetric methods that proved to be insensitive to trace levels of D-enantiomers in levodopa and methyldopa.

Acknowledgements

Support of this work by Ministry of Health of Czech Republic (No 3607-3) is gratefully acknowledged.

References

[1] The United States Pharmacopeia 23rd Rev., U.S. Pharmacopeial Convention, Rockville, MD, 1995.
[2] European Pharmacopoeia 3rd Ed., Council of Europe, Strasbourg, 1996.
[3] Pharmacopoea Bohemoslovaca IV, Prague, 1987.
[4] V.A. Davankov, in: J.C. Giddings, J. Cazes, P.R. Brown (Eds.), Advances in Chromatography, vol. 22, Marcel Dekker, New York, 1983, p. 71.
[5] M. Hilton, D.W. Armstrong, J. Liq. Chromatogr. 14 (1991) 9–28.
[6] T. Shinbo, T. Yamaguchi, K. Nishimura, M. Sugiura, J. Chromatogr. 405 (1987) 145–153.

[7] D.W. Armstrong, X. Yang, S.M. Han, R.A. Menges, Anal. Chem. 59 (1987) 2594–2596.
[8] A. Berthod, Y. Liu, Ch. Bagwill, D. Armstrong, J. Chromatogr. 731 (1996) 123–137.
[9] D. W. Armstrong, Y. Liu, K.H. Ekborgott, Chirality 7 (1995) 474–497.
[10] Application Guide for Chiral Column Selection, 2. edition, Daicel Chemical Industries, Tokyo, Japan.
[11] L.R. Gelber, J.L. Neumeyer, J. Chromatogr. 257 (1983) 317–326.
[12] E. Merck, in: Chiral HPLC Application Guide, Merck, Darmstadt, Germany.
[13] Chirobiotic Handbook, Advanced Separation Technologies, Whippany, USA, 1996.
[14] T.D. Doyle, in: S. Ahuja (Ed.), Chiral Separations by LC (ACS Symposium Series 471), American Chemical Society, Washington, DC, 1991, p. 27.