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Simultaneous Determination of Levodopa and Benserazide Using Derivative Spectrophotometry

Levodopa ve Benserazid'in Türev Spektrofotometrisi ile Birarada Tayini

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SUMMARY

Direct spectrophotometric methods for the simultaneous determination of levodopa and benserazide in combined formulations are presented. The methods are based on the use of **UV** derivative spectrophotometric measurements -the first derivative at 271.6 nm **(I)** and the second derivative at 239.4 nm (II) for levodopa (in 0.1 N hydrochloric acid) and the second derivative at 219.4 nm for benserazide (in 0.1 N hydrochloric acid). The mean percentage recoveries of the drugs by standard addition method were 100.6 % (I) and 99.2 % (II) for levodopa and 100.2 % for benserazide, respectively. In replicate analyses of a sample of Madopar capsules, satisfactory precision was demonstrated by the relative standard deviations of 1.41 % (I) and 1.16% (II) for levodopa and 1.49 % for benserazide.

Keywords: Drug analysis, levodopa, benserazide, simultaneous determination, derivative spectrophotometry.

ÖZET

Bu çalışmada, levodopa ve benserazidin kombine preparatlarından birarada tayini için spektrofotometrik yöntemler geliştirilmiştir. Yöntemler, levodopanın 0.1 N HCI'deki çözeltisinin birinci türev spektrumunda 271.6 nm de (I), ikinci türev spektrumunda 239.4 nm de (II) ve benserazidin 0.1 N HC1 deki çözeltisinin ikinci türev spektrumunda 219.4 nm de ölçümüne dayanmaktadır. Sözkonusu bileşiklerin standart ilavesi yöntemiyle % geriye kazanımları levodopa için % 100.6 (I) ve % 99.2 *(11),* benserazid için ise % 100.2 dir. Bu

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etken maddeleri (4:1) oranında içeren Madopar kapsüllerinin analizinde bağıl standart sapma levodopa için % 1.41 (I) ve % 1.16 (II), benserazid içinse % 1.49 bulunmuştur.

Anahtar Kelimeler: İlaç analizi, levodopa, benserazid, birarada tayin, türev spektrofotometrisi.

INTRODUCTION

Madopar[R], used in the treatment of Parkinsonism, is a combined formulation of levodopa and benserazide (as the hydrochloride) in the ratio of 4:1 (m / m). Assay of the drugs in tablets or capsules by conventional spectrophotometric procedures is difficult owing to the weak absorption of the minor component in the presence of the strongly absorbing major component and to interference from irrelevant absorption of the excipients. In preceding works, difference spectrophotometry (1,2) was used to overcome this difficulty. This paper presents derivative spectrophotometric methods for the simultaneous determination of levodopa and benserazide, which are simple, rapid and accurate.

Derivative spectrophotometry (3-6) is a relatively modern technique, which has proved to be very advantageous in eliminating spectral interferences caused by co-formulated drugs (7,8), excipients (9,10) and degradation products (11, 12).

EXPERIMENTAL

Instrument

Derivative spectra of solutions were recorded in matched 10-mm quartz cells using a Shimadzu UV-160 UV-Visible recording spectrophotometer. Settings were as follows: Spectral slit width, 3 nm; scan speed, 8 nm s^{-1}. Derivative spectra in the region of interest were recorded with an ordinate scale of ± 0.5 absorbance units. For the first- and second derivative spectra N was 5 and 7, respectively.

Reagents and Materials

Levodopa, benserazide hydrochloride and Madopar samples were provided from ROCHE A.Ş., Istanbul, Türkiye. Hydrochloric acid (BDH), ferrous sulfate. 7 H_2O (Panreac), sulfuric acid (Merck), sodium acetate. 3 H_2O (Panreac), dimethyl formamide (Merck) were

analytical reagent grade. Amber-coloured or aluminum foil-wrapped calibrated flasks were used for solutions containing levodopa, benserazide hydrochloride and both of them.

Stock Solutions of Benserazide Hydrochloride and Levodopa

100 mg of benserazide hydrochloride powder was accurately weighed and transferred into a 250-ml volumetric flask. It was dissolved in and diluted to volume with 0.1 N hydrochloric acid (B).

Levodopa stock solution (L) was prepared in the same manner and concentration. But it needs to be stirred for 10 min for complete dissolution (magnetic stirrer or ultrasonicator).

Stock solutions were prepared freshly everyday.

Calibration Graphs of Benserazide Hydrochloride and Levodopa

For calibration, 0.15-1.5 ml aliquots of (B) were pipetted into 50-ml volumetric flasks and diluted to volume with 0.1 N hydrochloric acid. The second derivative spectra of each solution was recorded and the absorbance at 219.4 nm was read against 0.1 N hydrochloric acid as blank (Table 1).

For levodopa, 0.5-6.0 ml aliquots of (L) was pipetted into 50-ml volumetric flasks and diluted to volume with 0.1 N hydrochloric acid. The first- and second derivative spectra of each solution was recorded and the absorbance of the first- at 271.6 nm and of the second derivative spectra at 239.4 nm were read against 0.1 N hydrochloric acid as blank (Table 1).

Investigation of interference by Simultaneous Determination

Series of solutions containing a fixed concentration of the one drug corresponding to the actual quantity of it in Madopar with varying concentrations of the second drug were prepared (Table 2). The measurements were made as mentioned for the standard solutions.

Sample Treatment

The content of 20 "Madopar 125" capsules were weighed and the average mass of a capsule content was determined. Then an amount of powder equivalent to 100 mg of levodopa (and 25 mg of benserazide) was accurately weighed and transferred to a 250-ml volumetric flask. It was diluted to volume with 0.1 N hydrochloric acid and dissolved

by stirring for 10 minutes (magnetic stirrer or ultrasonicator). The insoluble excipients were removed by filtration through Whatman No. 42 filter paper. 2.6 ml of the filtrate was transferred to a 50-ml volumetric flask and diluted to volume with 0.1 N hydrochloric acid. The measurements were made as mentioned for the standard solutions.

Methods of Analysis Used by the Manufacturer

Solutions of Benserazide, HC1 **Assay**

— Ferrous sulfate reagent was prepared by dissolving 500 mg $FeSO_4.7H_2O$ in 50 ml of 1 N H_2SO_4 and making up to 250 ml with distilled water.

— Sodium acetate solution was prepared by dissolving 37.5 g Na-acetate. $3H_2O$ in distilled water and making up to 250 ml with distilled water.

— For the standard solution of benserazide. HCI, approximately 110 mg of benserazide. HCI was weighed accurately into a 250-ml volumetric flask, 37.5 ml of dimetyl formamide was added and the solution was brought to volume with 0.1 N sulfuric acid.

— For the sample solutions, total amount of 10 capsules were weighed into a 100-ml volumetric flask. Approximately 80 ml of dimethyl formamide was added and the solution was shaken for 10 min. Then the solution was brought up to volume with the same solvent. The solution was filtered -The forerun was discarded- and 15.0 ml of the filtrate was diluted to 100 ml with 0.1 N sulfuric acid.

Benserazide. HC1 **Assay**

Following solutions were pipetted into eight 100-ml volumetric flasks:

	Blank(l)	Standard(2,3)	Sample(4-8)
— Ferrous sulfate reagent	20.0 ml	20.0 ml	20.0 ml
— 0.1 N H_2SO_4	10.0	—	—
— Standard solution of (B)	—	10.0	—
— Sample solution	—	—	10.0
— Distilled water	40.0	40.0	40.0
— Sodium acetate solution	20.0	20.0	20.0

They were mixed immediately and brought to volume with distilled water. The absorbance of the solutions were measured 15 minutes after the addition of sodium acetate solution. And the measurements were made at 575 nm against the blank solution. Calculations were made using the equation below:

$$\text{mg benserazide.HCI (per capsule)} = \frac{E_s \,.\, A \,.\, 1000 \,.\, B}{E_{st} \,.\, 375 \,.\, C}$$

E_s = Extinction of the sample solution

Est = Extinction of the standard solution

A = Amount of benserazide.HCI for the standard solution taken in mg

B = Average capsule fill-weight in mg

C = Amount of capsule powder taken in mg

Levodopa Assay

Total amount of 10 capsules were weighed into a 250-ml volumetric flask. They were shaken after adding approximately 200 ml of 0.1 N sulfuric acid and brought to volume with 0.1 N sulfuric acid.

A part of this solution was filtered -the forerun was discarded- and 5 ml of the filtrate was diluted to 100 ml with 0.1 N sulfuric acid. 15 ml of the diluted soution was diluted again to 100 ml. The absorbance of this solution was measured at 280 nm against 0.1 N sulfuric acid as blank. Calculations were made using the equation below:

$$\text{mg levodopa (per capsule)} = \frac{E \,.\, 500.000 \,.\, A}{142 \,.\, 1.5 \,.\, B} - \frac{26.4 \,.\, C}{142}$$

E = Extinction of the measuring solution at 280 nm

A = Average capsule fill-weight in mg

B = Amount of capsule powder taken in mg

C = Content of benserazide HCI (mg per capsule)

142 = E (% 1,1 cm) - value for levodopa in 0.1 N sulfuric acid at 280 nm

26.4 = E (% 1,1 cm) - value for benserazide HCI in 0.1 N sulfuric acid at 280 nm.

RESULTS AND DISCUSSION

To prevent oxidation, levodopa and benserazide solutions were prepared in. 0.1 N hydrochloric acid (13). During a period of 90 min. no significant change in the concentration of the solutions were observed.

In Fig. 1, the zeroth-, first- and second order-spectra of levodopa and benserazide hydrochloride were drawn onto each other, separately. It can be seen that levodopa can be determined from the zeroth-, first- and second order spectra by reading the absorbances at 288.0, 271.6 and 239.4 nm, respectively. On the other hand, benserazide can. be determined only from the second derivative spectra by reading the absorbance at 219.4 nm. In. this study, just the derivative spectrophotometric determination, of the drugs were investigated, since it can eliminate possible matrix interference.

Calibration data of standard solutions of levodopa and benserazide prove that measurements made on spectra of any derivative-order mentioned above allow to obtain reliable and reproducible results (Table 1).

The accuracy of the procedure for the simultaneous determination of levodopa and benserazide was investigated by assaying a number of standard mixtures containing different concentrations of the drugs. For levodopa, good recoveries even by 100 % excess of benserazide and for benserazide. by 50 % excess of levodopa (compared with the formulation ratio in Madopar) were obtained indicating the applicability of the method (Table 2).

Samples of Madopar 125 capsules were assayed with the proposed first- and second derivative methods. The concentrations found show good agreement with the stated contents of the dosage units. And according to statistical data, no significant difference was observed between the results obtained by the proposed derivative methods and the spectrophotometric method currently used by the manufacturer (Table 3 and 4). However, the second derivative spectrophotometric method should be preferred for sample analysis. Because it supplies data for both drugs.

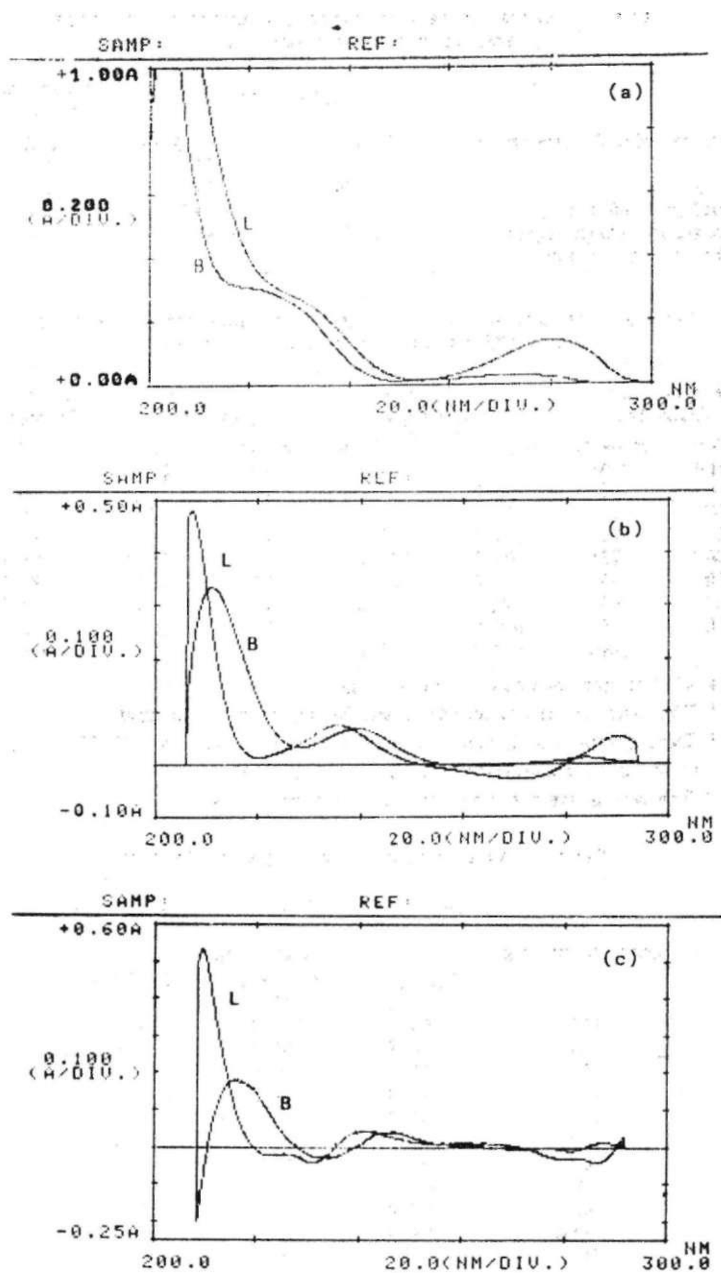

Figure 1. a) Zero-order spectra, b) first derivative spectra, c) second derivative spectra of levodopa (10.4 μg.ml^{-1}) and benserazide.HCl (10.4 ***μg.ml^{-1}).*** Solvent 0.1 N HC1.

Table 1. Calibration data of standard solutions of Levodopa and Benserazide hydrochloride.

	LEVODOPA		BENSERAZIDE.HC1
Derivative-order (walevength,mn)	D_1(271.6)	D_2(239.4)	D_2(219.4)
Slope	1.73	2.05	7.56
Intercept	-0.309	0 990	0.173
Correlation coefficient (r)	0.9999	0.9999	0.9997
Concentration range (μ g.ml^{-1})	4-48	4-48	1.2-12
Number of data points	7	7	7

Table 2. The accuracy of simultaneous determination of Levodopa and **Benserazide in standard solutions.

The ratio (m / m) (Mixture)		% Recovery (Levodopa)		The ratio (m / m) (Mixture)		% Recovery (Benserazide)
Levo-dopa	Bensera-zide	D_1(271. 6)	D_2(239.4)	Bensera-zide	Levo-dopa	D_2(219.4)
100	0	100.0	100.0	25	0	100.0
100	10	100.0	100.0	25	50	98.1
100	20	100.0	100.0	25	75	98.1
100*	25*	100.0	101.2	25*	100*	98.8
100	30	100.0	101.2	25	125	98.1
100	40	100.0	102.4	25	150	100.0
100	50	100.0	101.2	25	175	95.1a
	Mean:	100.0	100.9			98.9

a 95.1 is not included in the average

* The ratio (m / m) of levodopa and benserazide in Madopar

** Data obtained with benserazide hydrochloride (mol wt. 293.75) and presented in terms of benserazide base (mol wt. 257.25).

*** The results are the mean of three determinations.

Table 3. Assay results of Levodopa in Madopar

LEVODOPA

Label claim (mg)	Found (mg)		
	D_1(271.6)	*D_0(280.0)	D_2(239.4)
100	100.6	100.7	98.6
100	100.6	100.7	98.6
100	100.6	100.2	98.6
100	100.6	100.0	101.0
100	97.9	99.8	101.0
100	97.9	—	101.0
100	97.9	—	101.0
100	97.9	—	101.0
100	97.9	—	101.0
100	97.9	—	101.0
Mean:	99.0	100.3	100.3
SD :	1.39	0.41	1.16
% RSD:	1. 41	0.41	1.16

* Method supplied by the manufacturer.

** t_{calc} = 2.00 for D_1 and D_0 (t_{teo} = 3.01 for $p < 0.01$)

*** t_{calc} = 0 for D_2 and D_0 t_{teo} = 3.01 for $p < 0.01$).

Table 4. Assay results of Benserazide in Madopar.

B E N S E R A Z I D E *		
Label claim (mg)	Found (mg)	
	D_2(219.4)	**D_0(575.0)
25	24.7	24.9
25	24.7	24.8
- 25	24.7.	24.5
25	24.7	25.1
25	24.0	24.6
25	24.0	—
25	24.0	—
25	24.0	—
25	24.0	—
25	24.0	—
Mean :	24.3	24.8
SD :	0.36	0.24
%RSD :	1.49	0.97

* Data obtained with benserazide hydrochloride (mol wt 293.75) and presented in terms of benserazide base (mol wt. 257.25).

** Method supplied by the manufacturer.

*** t_{cale} = **2.78** for D_2 and D_0 (t_{teo} = 3.01 for $p < 0.01$)

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