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Difference spectrophotometric assay of 1,2-diphenolic drugs in pharmaceutical formulations — III. The simultaneous assay of levodopa and benserazide

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Abstract: A simultaneous assay of levodopa and benserazide in combined formulations is described that utilizes the different spectral properties of the drugs in the presence of germanium dioxide. Difference absorbances are measured at 238 and 292.5 nm between pH 6 solutions of the drugs complexed with germanium dioxide relative to equimolar solutions of the uncomplexed drugs. The concentrations are calculated using two simultaneous equations derived from the difference absorptivities of the individual drugs at 238 and 292.5 nm. The choice of wavelengths for maximum accuracy and precision is discussed. Good recoveries of the drugs in standard mixtures were obtained and satisfactory precision was demonstrated by the relative standard deviations of 0.46% and 1.07% for levodopa and benserazide, respectively, obtained in the replicate analyses of a sample of Madopar capsules. The accuracy of the procedure and the absence of interference from excipients were confirmed by the good agreement in the results for the assay of several batches of Madopar capsules and one of Madopar tablets with those obtained by the manufacturer by an HPLC procedure.

Keywords: *Levodopa; benserazide; difference spectrophotometry; Madopar.*

Introduction

Madopar®, used in the treatment of Parkinsonism, is a combined formulation of levodopa and benserazide (as the hydrochloride) in the ratio 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.

Recently, two new reagents, boric acid [1] and germanium dioxide [2], have been developed for the assay of 1,2-diphenolic drugs, based upon the measurement of difference absorbance at about 292 nm. The procedures were shown to be selective for 1,2-diphenolic drugs in the presence of absorbing substances, such as monophenols and excipients, that interfere in a direct spectrophotometric assay, provided that the absorbance of the interferents at the wavelength of measurement is unchanged by the reagents.

During the development of these procedures it was noted that all catechol (1,2-diphenolic) derivatives, such as adrenaline and levodopa, exhibit two bands, of approximately equal intensity, in their difference absorption spectra near 240 nm and 290 nm, whereas pyrogallol (1, 2, 3-triphenolic) derivatives are less similar in their wavelengths and intensity of maximum difference absorbance. For example, the difference absorption spectrum of benserazide induced by germanium dioxide comprises an intense band at 238 nm and a weak band at 283 nm. These different spectral properties of levodopa and benserazide in the presence of germanium dioxide have been used in the present work as the basis of their simultaneous assay in Madopar formulations by measurement of difference absorbance at two wavelengths, rather than by direct absorbance measurement, thereby eliminating interference from the excipients.

Experimental

Spectrophotometer

Absorption and difference absorption spectra of solutions in 1-cm silica quartz cells were recorded using a Perkin–Elmer 552 UV-visible spectrophotometer. The slitwidth was 1 nm, the scan rate 1 nm s^{-1} and the response 0.5 s. The difference absorbance values of the standard, sample and blank solutions at 238 and 292.5 nm were read from the digital display after entering the appropriate wavelength via the keyboard.

Reagents

Stock buffer pH 6 was prepared by dissolving 9.67g of citric acid (A.R.; BDH Chemicals, Poole, UK) and 22.40g of anhydrous disodium hydrogen orthophosphate (A.R.; BDH Chemicals) in water and diluting to 500 ml. The working buffer pH 6 was prepared by diluting 100 ml of the stock buffer to 500 ml with water; the germanium dioxide reagent was prepared by dissolving 500 mg of germanium dioxide (BDH Chemicals) in 100 ml of stock buffer, with the aid of gentle warming, and diluting to 500 ml with water.

Levodopa and benserazide hydrochloride were gifts from the manufacturer (Roche Products, Welwyn Garden City, UK) and were used without further purification.

Procedure

Standard solutions. Standard solutions of levodopa and benserazide hydrochloride containing 200 $\mu g\ ml^{-1}$ were prepared by dissolving 50 mg of each compound, accurately weighed, in water containing 2.5 ml of 0.1 M hydrochloric acid and diluting to 250 ml in volumetric flasks. A 5-ml aliquot of each standard solution was transferred to two 25-ml flasks containing 10 ml of buffer pH 6 and 10 ml of germanium dioxide reagent, respectively, and diluted to volume with water. Five minutes after preparation of the solutions, the difference absorbances of each standard solution, containing germanium dioxide relative to the solution of the uncomplexed drug and of the blank buffer solutions diluted (2:3, v/v) with water, were measured at 238 nm (ΔA_{238}) and 292.5 nm ($\Delta A_{292.5}$).

Samples. Twenty tablets or the contents of twenty capsules were weighed and ground to powder. An accurately weighed quantity of the powder, containing 50 mg of levodopa and 12.5 mg of benserazide (as the hydrochloride), was shaken with 2.5 ml of 0.1 M hydrochloric acid and about 200 ml of water and the extract was diluted to 250 ml.

Alternatively, for unit dose assays, a powdered tablet or the contents of a capsule was shaken with a volume (in ml) of 0.001 M hydrochloric acid equal to four times the total weight of the two drugs (in mg) in the dosage unit. The extract was filtered through Whatman No. 1 filter paper and the first 10 ml of filtrate was discarded; a 5-ml aliquot of the filtrate was transferred to two 25-ml volumetric flasks containing 10 ml of pH 6 buffer and 10 ml of germanium dioxide reagent, respectively, diluted to volume, and the ΔA_{238} and $\Delta A_{292.5}$ were measured as described for the standard solutions.

Treatment of the results. The difference absorptivities ($\Delta A_{1\,cm}^{1\%}$) of benserazide hydrochloride at 238 nm (b_1) and at 292.5 nm (b_2) and of levodopa at 238 nm (l_1) and at 292.5 nm (l_2) were calculated from the ΔA values of the standard solutions ($\Delta A_{1\,cm}^{1\%} = \Delta A/C$ where C is the concentration in g/100 ml) corrected for any small difference absorbance of the blank buffer solutions. The net ΔA_{238} (m_1) and $\Delta A_{292.5}$ (m_2) of the sample solutions were calculated. The concentrations of levodopa (C_l) and of benserazide hydrochloride (C_b) are given by equations (1) and (2) respectively:

$$C_l = \frac{b_1m_2 - b_2m_1}{b_1l_2 - b_2l_1} \tag{1}$$

$$C_b = \frac{l_2m_1 - l_1m_2}{b_1l_2 - b_2l_1} . \tag{2}$$

Results and Discussion

Figure 1a, which shows the absorption spectra of levodopa and benserazide, demonstrates the impracticability of assaying a mixture of the drugs by a direct spectrophotometric technique. The weak absorption of benserazide, its low concentration and the absence of two maxima preclude the application of the two wavelength (simultaneous equations) technique using normal absorbance values. The interference from the excipients would further reduce the accuracy of such a procedure.

The difference absorption spectra of the drugs induced by germanium dioxide (Fig. 1b) each shows two bands near 240 and 290 nm that are known to be free of interference from the excipients [2]. The normal criterion for the selection of the two wavelengths at which measurements are made in the simultaneous equations procedure is that they should maximize the difference between the ratio of absorptivities of one component at the two wavelengths and the corresponding ratio for the other component [3]. Thus, 238 nm, the lower wavelength maximum in the difference absorption spectrum of benserazide, and 292.5 nm, the longer wavelength maximum in the difference absorption spectrum of levodopa, which give the maximum difference in the $\Delta A_{1\,cm}^{1\%}$ ratios b_2/b_1 and l_2/l_1, were selected.

The proportionality of the ΔA values at 238 and 292.5 nm to the concentrations of levodopa and benserazide was checked using a five-point calibration series of each drug in the concentration range 0–75 $\mu g\ ml^{-1}$. The statistical data in Table 1 confirm that each drug gives ΔA values at 238 and 292.5 nm that are in proportion to their concentrations.

Table 1 also shows the precision data obtained in ten replicate assays of a sample of Madopar capsules (125 mg). The relative standard deviations of the concentrations of

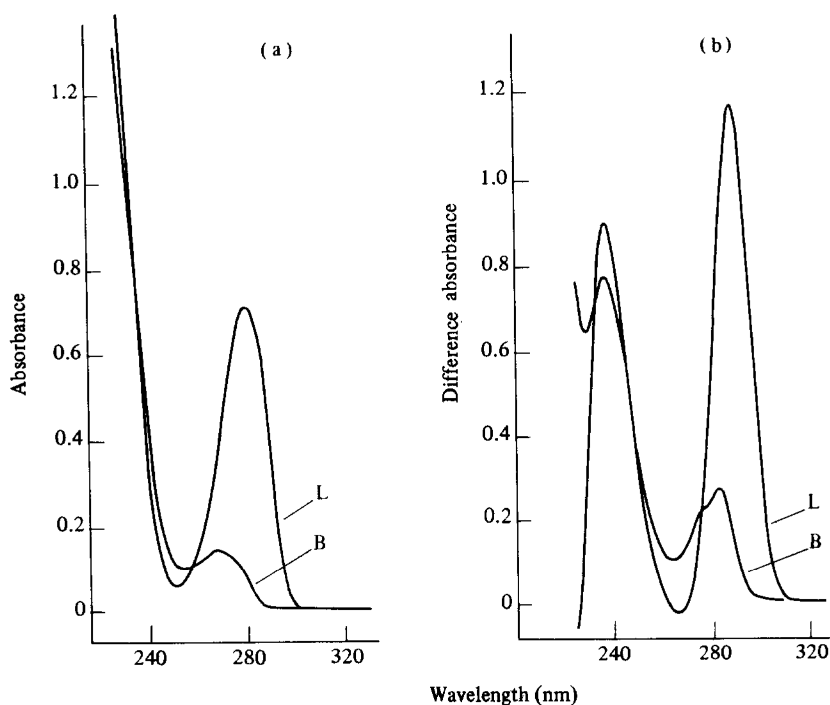

Figure 1
(a) The absorption spectra of uncomplexed levodopa (L) and benserazide hydrochloride (B) in solutions (50 μg ml^{-1}) buffered at pH 6.
(b) The difference absorption spectra of levodopa (L) and benserazide hydrochloride (B) (50 μg ml^{-1}) complexed with germanium dioxide relative to equimolar solutions of the uncomplexed drugs.

Table 1
Calibration and precision data

		Levodopa		Benserazide*	
Calibration		ΔA_{238}	$\Delta A_{292.5}$	ΔA_{238}	$\Delta A_{292.5}$
	Slope†	159.5	226.1	154.1	11.3
	Intercept	0.0013	0.0007	−0.0004	−0.0004
	Correlation coefficient	0.9996	0.9995	0.9997	0.994
	Concentration range (μg ml^{-1})	0–75		0–65.7	
	Number of data points	5		5	
Precision					
	Sample	Madopar capsules 125 mg			
	Number of replicate results	10			
	Weight found (mg)‡	98.4		24.3	
	Standard deviation	0.45		0.26	
	Relative standard deviation (%)	0.46		1.07	

*Data obtained with benserazide hydrochloride (mol. wt 293.75) and presented in terms of benserazide base (mol. wt 257.25).
† ΔA per g/100 ml, that is $\Delta A^{1\%}_{1\,cm}$.
‡ Calculated for a capsule of average weight of contents.

levodopa and benserazide were 0.46% and 1.07%, respectively. Criteria for obtaining maximum precision, based upon absorbance ratios, have been suggested that place limits on the relative concentrations of the components of mixtures [3]. The requirements are that the ratios $(m_2/m_1)/(b_2/b_1)$ and $(l_2/l_1)/(m_2/m_1)$ should lie outside the range 0.1–2.0 for the precise determination of levodopa and benserazide, respectively. At the relative concentrations of levodopa and benserazide of 4:1, the ratios are 15.71 and 1.235, respectively. The compliance of the former ratio explains the excellent precision of the levodopa assay. The similarity of the values m_2/m_1 and l_2/l_1, giving the latter ratio of 1.235, results in a larger but still satisfactory relative standard deviation of the benserazide concentrations. The reproducibility of the procedure is due to the high precision with which the measurements of the difference absorbances were made. The wavelength repeatability and absorbance accuracy of modern microprocessor-controlled spectrophotometers, such as that used in the present work, yield more precise absorbance values and concentrations than the older single-beam instruments of the type used to establish the exclusion range of 0.1–2.0 [3, 4]. It would appear, therefore, that careful analytical technique and the use of a high-performance spectrophotometer will permit acceptable precision with absorbance ratios closer to 1.0 than was previously thought. The $(m_2/m_1)/(b_2/b_1)$ and $(l_2/l_1)/(m_2/m_1)$ ratios obtained by the use of boric acid as the complexing reagent were slightly lower (14.95 and 1.17 respectively) than those given by germanium dioxide. Consequently, the latter reagent was used to induce difference absorbance.

The accuracy of the procedure for the simultaneous determination of the concentrations of levodopa and benserazide was investigated by assaying a number of standard mixtures containing different concentrations of the drugs. Good recoveries were obtained (Table 2) indicating the general applicability of the method to mixtures of levodopa and benserazide other than the 4:1 combination of the drugs in Madopar formulations.

Samples from several batches of capsules and one batch of tablets were assayed for levodopa and benserazide. The concentrations found (Table 3) show good agreement

Table 2
Assay results of standard mixtures

	Concentration of levodopa		Concentration of benserazide†	
Mixture*	Added (μg ml^{-1})	Found (as % of concentration added)	Added (μg ml^{-1})	Found (as % of concentration added)
1	250	99.7	50	97.3
2	200	100.7	50	98.8
3	150	100.1	100	99.0
4	100	101.4	150	100.8
5	50	101.4	200	100.5
6	50	98.3	250	99.1
	Mean	100.3	Mean	99.3

* Assayed by diluting 5 ml aliquots to 25 ml as described in the Experimental section.

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

Table 3
Assay results of Madopar samples

Sample	Content of levodopa			Content of benserazide†		
	The present method (mg)	HPLC* method (mg)	% Difference	The present method (mg)	HPLC* method (mg)	% Difference
Capsules 250 mg	194.9	198.0	−1.6	49.3	48.0	+2.7
Capsules 125 mg	102.1	99.5	+2.6	25.7	24.8	+3.5
Capsules 125 mg	98.4	100.0	−1.6	24.3	24.6	−1.1
Capsules 62.5 mg	50.1	50.8	−1.4	12.8	12.4	+2.8
Capsules 62.5 mg	50.8	51.4	−1.2	12.1	12.3	−1.4
Tablets 250 mg	197.7	199.6	−1.0	47.5	48.9	−2.9
			Mean −0.7			Mean +0.6

*Data supplied by the manufacturer.
†Data calculated as benserazide hydrochloride and presented in terms of benserazide base for comparison with the stated amount.

with the stated contents of the dosage units and with the manufacturer's results obtained by an unpublished high-pressure liquid chromatographic procedure. The results confirm the accuracy of the spectrophotometric procedure and the absence of interference from the excipients.

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