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Spectrophotometric Method for the Determination of Carbidopa Using Neotetrazolium Chloride

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Abstract—A new high-sensitive spectrophotometric method for the determination of microquantities of carbidopa is described. The method is based on the reduction of neotetrazolium chloride by carbidopa in an alkaline ethanolic medium. The formazan formed exhibits an absorption maximum at $\lambda = 507$ nm, with an apparent molar absorptivity of $\varepsilon = 6{\cdot}63 \times 10^4$ M^{-1} cm^{-1} and a corresponding Sandell's sensitivity of 3·68 ng cm^{-2}. Beer's law is obeyed over the range of 0·10–6·0 μg mL^{-1} while the optimum concentration range is 0·125–5·0 μg mL^{-1}. The regression line equation was calculated as: $A = 0{\cdot}271C + 0{\cdot}0023$ with a correlation coefficient of 1·0000 (n = 28). The accuracy and the precision of the method were considered as very satisfactory. The results obtained from the determination of carbidopa using both the described procedure and the corresponding USP. XXII and BP 1988 official methods were statistically compared by means of Student's *t*-test as well as by the variance ratio F-test, and no significant difference was observed.

For the analytical estimation of carbidopa, besides the official compendia which specify HPLC (US Pharmacopeia XXII, 1990) and potentiometry (British Pharmacopoeia, 1988), various methods have been reported (Steup et al 1986; Ting 1986, 1987; Foerster et al 1988), including three spectrophotometric procedures from our laboratories (Issopoulos 1989a, b, 1990). This paper describes the use of neotetrazolium chloride (NTZC) to determine carbidopa.

Materials and Methods

Apparatus

All the absorbance measurements were recorded in a 10·0 mm quartz cell in a Hitachi, Model 100–80, ratio recording spectrophotometer. A Varian, HPLC complete system (Variable Detector 2550; HPLC Pump 2510; Integrator 4290) and a WTW 522 pH-meter, with a glass-modified calomel electrode in which the usual aqueous solution was replaced with a 1·0% w/v solution of lithium perchlorate in glacial acetic acid, were used to perform the US Pharmacopeia XXII and British Pharmacopoeia 1988 official methods. A thermostated constant temperature waterbath, accurate to ±0·5°C, was employed throughout.

Reagents and solutions

Carbidopa standard solution. A standard stock solution (500 μg mL^{-1}) of carbidopa (Merck, Sharp & Dohme, UK) calculated as the hydrous substance was prepared by dissolving carbidopa in ethyl alcohol and a working solution (10·0 μg mL^{-1}) was prepared by suitable dilution of the appropriate volume of the standard stock solution with the same solvent. Both solutions were stored in a dark, cool place.

NTZC standard solution. Five hundred milligrams of NTZC (Fluka, Art. No 72150) were dissolved in ethyl alcohol and this solution was diluted to 250 mL with the same solvent. The final NTZC solution (2 mg mL^{-1}) should be freshly prepared although it may be kept for longer periods (8–10 days) if it is stored in a dark, cool place (0–4°C).

Sodium hydroxide solution. A 0·1% w/v NaOH solution was prepared in ethyl alcohol. Precautions were taken to minimize the absorption of CO_2 by the prepared solution.

Ethyl alcohol (96°, puriss. p.a., Ph. Helv. (Fluka)) was used throughout.

General procedure and preparation of calibration graph

Into a 20 mL calibrated flask, the following solutions were pipetted in order: 0·2–12·0 mL of the carbidopa working solution, 2·0 mL of NTZC standard solution and 2·0 mL of 0·10% w/v NaOH ethanolic solution. The mixture was diluted to volume with ethyl alcohol and homogenized by shaking. After mixing, the solution was transferred immediately into a 50 mL test tube, kept in the water-bath at 50 ± 0·5°C for 30 min and then cooled to room temperature (20–25°C).

The absorbance of the formazan formed solution was measured at 507 nm, against a carbidopa blank solution which was prepared and treated similarly.

Results and Discussion

The most common analytical application of the tetrazolium salts is the spectrocolorimetric assay of those pharmacopoeial steroids which possess the reducing functional group of the α-ketol side chain (—$COCH_2OH$) attached to the steroid D ring. Thus, the tetrazolium salts oxidize this α-ketol moiety of the C_{17} side chain, in strongly alkaline ethanolic solution, and so are reduced quantitatively to highly coloured formazans, which consequently are measured colorimetrically.

In the present investigation, where the reducing agent is carbidopa analysed, the redox reaction occurs as shown in Scheme 1.

The reduction of NTZC could take place in two steps, since this reagent contains two reducing tetrazolium rings.

The reddish colour of the solution resulting from the redox reaction corresponding to a λ_{max} of 507 nm, suggests the

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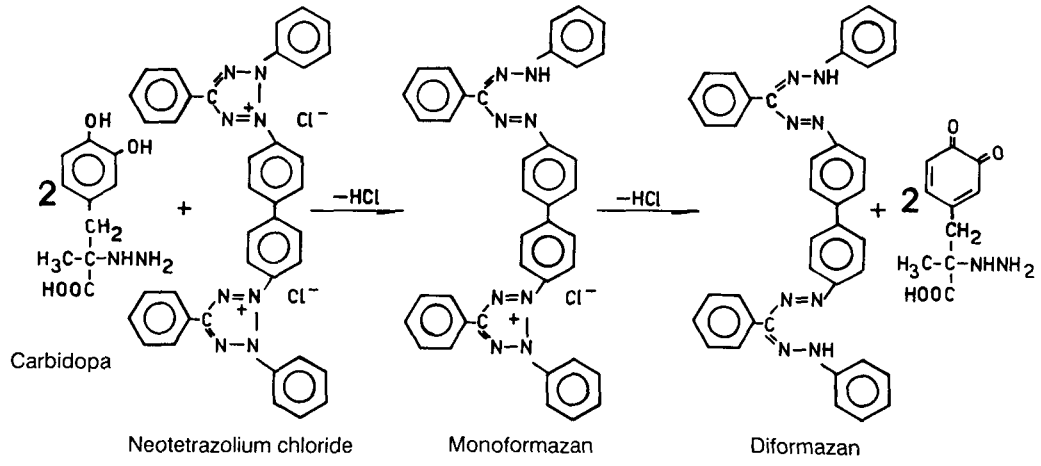

SCHEME 1.

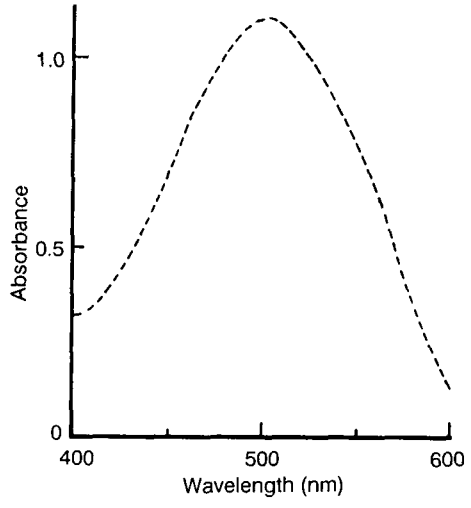

FIG. 1. Absorption spectrum of formazan formed by the redox reaction between carbidopa (4·0 μg mL^{-1}) and NTZC.

Table 1. Spectral characteristics and other analytical factors.

Characteristic	Numerical data
λ_{max} (nm)	507
ε (M^{-1}cm^{-1})[a]	$6{\cdot}63 \times 10^4$
Sandell's sensitivity (ng cm^{-2})[a]	3·68
Regression line equation: A = mC + z	
m (slope, μg mL^{-1})	0·271
z (intercept)	$2{\cdot}30 \times 10^{-3}$
Correlation coefficient (r) (n = 28)[b]	1·0000
Adherence to Beer's law (μg mL^{-1})	0·10–6·0
Optimum concentration range (μg mL^{-1})	0·125–5·0

[a]Average of 28 determinations.
[b]Four replicates for seven different concentrations.

formation of a monoformazan (Görög & Horváth 1978) as the diformazans of the tetrazolium salts usually have a blue colour and their formation is subject to several variables, such as the temperature, the nature of the solvent, the pH of

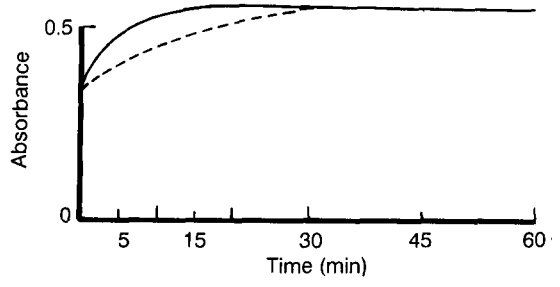

FIG. 2. Effect of temperature and heating time on absorbance of the formazan formed. - - - - 25°C, —— 50°C, carbidopa concn = 2·0 μg mL^{-1}.

the medium and the ratio of the concentrations of the reacted substances (Graham et al 1976, 1977).

Although the simultaneous presence, in equilibrium, of both forms of formazans cannot be excluded, the experimental data (colour of the solution formed, λ_{max}) indicate that the monoformazan is formed almost quantitatively.

Absorption spectrum

The absorption spectrum of the above cited formazan was scanned in the double-beam mode against a carbidopa blank solution over the range 400–600 nm. Automatic baseline correction was employed, while the same baseline is determined and checked with both sample and blank cells filled with the carbidopa blank solution used. Fig. 1 shows these absorption spectrum. The apparent molar absorptivity and other spectral characteristics are presented in Table 1.

Table 2. Evaluation of the accuracy and the precision of the proposed analytical method.

Carbidopa (μg mL^{-1}) Added	Found ± s.d.*	rsd%	s.a.e. (s.d./$\sqrt{n}$)	Confidence limits (P = 0·05; ((n − 1) = 4)
0·5	0·4998 ± 0·0078	1·53	0·00343	0·4998 ± 0·0095
1·5	1·5014 ± 0·0133	0·89	0·00595	1·5014 ± 0·0165
3·0	3·0058 ± 0·0231	0·77	0·01033	3·0058 ± 0·0287
5·0	5·0110 ± 0·0297	0·59	0·01331	5·0110 ± 0·0369

*Average of five determinations.

Table 3. Determination of carbidopa by the proposed procedure and two official methods.

Sample	Proposed method	Official method I[a]	Official method II[b]		
	Recovery% ± s.d.[e]	Recovery% ± s.d.[e]	Recovery% ± s.d.[e]	*t*-value[c]	F-value
Carbidopa, pure form	100·09 ± 0·76	99·74 ± 0·37		0·937	4·25
			99·94 ± 0·69	0·327	1·22

[a] US Pharmacopeia, XXII (1990). [b] British Pharmacopoeia (1988). [c] Tabulated *t*-value for $P = 0{\cdot}05$ and eight degrees of freedom, is equal to 2·306. [d] Tabulated F-value for $P = 0{\cdot}05$ and $f_1 = f_2 = 4$, is equal to 6·39. [e] Average of five determinations.

Calibration graph

The calibration graph was established using known concentrations of carbidopa processed as above. The linearity of this graph (four replicates for seven different concentrations) was checked by a linear least-squares treatment using a Macintosh SE computer. Beer's law limits, the regression line equation, the correlation coefficient and the Sandell sensitivity (for log Io/I = 10^{-3} absorption units) are summarized in Table 1.

Effect of temperature and heating time on formazan formation

In order to examine the effect of temperature and the contribution of the heating time to formazan formation, the reduction of NTZC by carbidopa was studied as a function of time at two different temperatures (25 ± 0·5 and 50 ± 0·5°C) using a thermostated waterbath (Fig. 2).

Sensitivity, accuracy and precision

The mean Sandell's sensitivity for the method is summarized in Table 1. To determine and confirm experimentally the accuracy and the precision of the method, solutions containing four different concentrations of carbidopa (0·5, 1·5, 3·0 and 5·0 $\mu g\ mL^{-1}$) were prepared and analysed in quintuplicate as recommended above. The results of this examination are demonstrated in Table 2.

Statistical comparison with two official methods

The results of the proposed new analytical procedure have been successfully compared by means of both the Student's *t*-test and variance ratio F-test, with those obtained from the application of the US Pharmacopeia XXII (1990) and of the British Pharmacopoeia (1988) official methods (HPLC and potentiometry, respectively), on samples of carbidopa from the same batch (Table 3).

The mean *t*-test value which results for a 95% confidence level and eight degrees of freedom, did not exceed the theoretical tabulated value of $t = 2{\cdot}306$, indicating no significant difference between the methods compared.

Likewise, the mean F-value, which was calculated for $P = 0{\cdot}05$ and $f_1 = f_2 = 4$, did not exceed the theoretical tabulated value of $F = 6{\cdot}39$. This confirms that there is no significant difference between the precision of the described procedure and those of the official methods.

The present paper describes the first application of NTZC for the spectrophotometric determination of micro amounts of carbidopa in pure form.

Besides its acceptable sensitivity (125 ppb, CV < 2·0%), the method is simple, rapid, accurate and uses inexpensive reagents and instruments.

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