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Spectrophotometric determination of carvedilol in pharmaceutical formulations through charge-transfer and ion-pair complexation reactions

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Received April 26, 2006, accepted May 11, 2006

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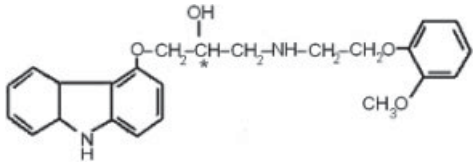
Pharmazie 62: 34–37 (2007) *doi: 10.1691/ph.2007.1.6075*

Simple extraction-free spectrophotometric methods have been developed for the determination of carvedilol (CAR). The methods were based either on charge-transfer reaction of the drug with the σ-acceptor iodine, in acetonitrile, or on ion-pair formation with the acidic sulphophthalein dyes bromothymol blue (BTB) and bromocresol green (BCG), in chloroform. The obtained complexes showed absorbance maxima at 363, 411 and 414 nm, respectively for iodine, BTB and BCG. Beer's law validation, accuracy, precision, and other aspects of analytical merit are presented in the text. The proposed methods were applied for the determination of CAR in tablets and compounded capsules. The results were in good agreement with those obtained by an established UV spectrophotometric method.

1. Introduction

Carvedilol (CAR) is an anti-hypertensive agent with non-selective β and α_1 blocking activities, and also has an anti-oxidant effect (Bristow et al. 2003; Stroe and Gheorghiade 2004). CAR is a racemic compound chemically described as 2(*RS*)-1-(9*H*-carbazol-4-yloxy)-3-[[2-(2-methoxiphenoxy)ethyl]amino]propan-2-ol (European Pharmacopoeia 2003). The non-selective β blocking activity is essentially limited to (*S*)-carvedilol whereas both enantiomers exhibit the same α_1 adrenergic antagonism. It is available in tablets and compounded capsules and has been used in treatment of hypertension, ischemic heart disease and congestive heart failure (Tenero 2000). The official method for the assay of CAR in bulk form is a non-aqueous titration (European Pharmacopoeia 2003). No official methods are available for CAR pharmaceutical formulations. Few methods have been reported for the CAR determination including: UV spectrophotometry and non-aqueous titration (Cardoso et al. 2005), fluorimetry (Xiao et al. 2005; Xu et al. 2005), differential pulse voltammetry (Radi and Elmogy 2005), chemiluminometric (Pires et al. 2005) and liquid chromatography (Stojanovic et al. 2005). Some β-adrenergic blocking drugs have been determined spectrophotometrically after formation of complexes with the σ acceptor iodine (Salem 2002) or with an ion-pair complex (Al-Ghannam 2006). However, colorimetric methods were not applied for CAR determination yet. Thus, this paper describes the application of iodine and acidic dyes to the spectrophotometric determination of CAR in tablets and compounded capsules. The charge transfer or ion pair complexes require no extraction step and CAR can be measured directly in the solvents used. The proposed methods are simple and can be used in laboratories where expensive or specifics equipments, such as a liquid chromatography system, spectrofluorimeter or voltammetry, are not available.



2. Investigations, results and discussion

2.1. Charge-transfer method

Some N-donor drugs react with the σ electron acceptor iodine forming charge-transfer complexes followed by triiode ion pair formation (Bebawy et al. 1999). It is known that β-adrenergic blockers drugs act as good N-electron donors (Salem 2002). Hence iodine was used in the proposed method for the determination of CAR in pharmaceutical formulations. The immediate change of the violet color of iodine in acetonitrile to a lemon yellow colour upon reaction with CAR was taken as suggestive of charge transfer complex formation. The formed complex showed two maxima of absorption at 285 nm and 363 nm (Fig. A). Measurements were carried out at 363 nm due to the interference from the native UV absorption of CAR at 285 nm. It is described in the literature that iodine reacts with basic centers of some solvent donors of electrons, forming the triiodide ion, as acetonitrile, in which iodine reacts with the nitrogen present in its molecule (Moustafa 2000). However, in spite of this interaction, acetonitrile was shown to be a good solvent for the for-

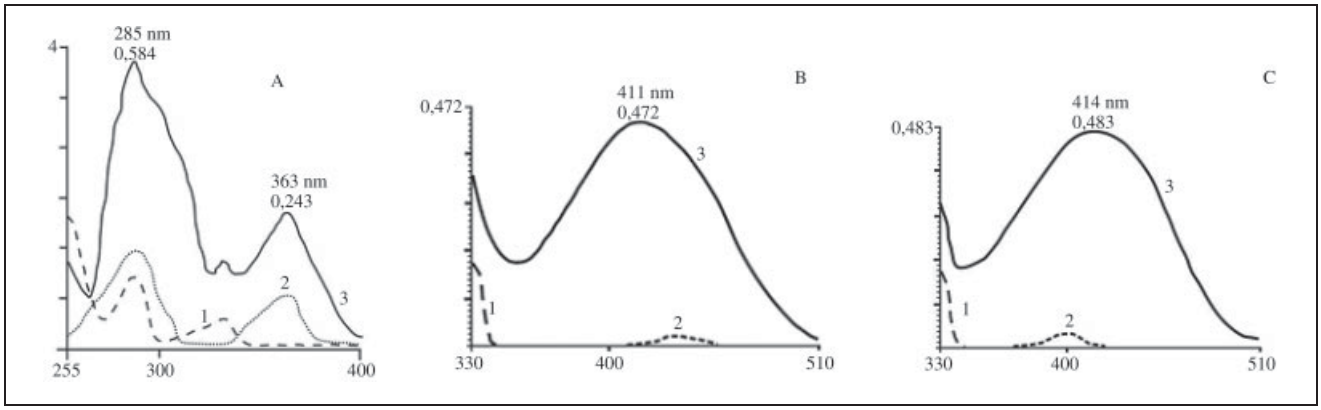


Fig.: Absorption spectrum of carvedilol: **(A)** Iodine method: 1) CAR in acetonitrile (4 μg · mL^{-1}), 2) Iodine 0.001 M in acetonitrile, 3) Complex between carvedilol and iodine in acetonitrile (4 μg · mL^{-1}); **(B)** BTB method: 1) CAR in chloroform (10 μg · mL^{-1}); 2) BTB 0.0005 M in chloroform. 3) Complex between carvedilol and BTB in chloroform (10 μg · mL^{-1}); and **(C)** BCG method: 1) CAR in chloroform (10 μg · mL^{-1}), 2) BCG 0.00025 M in chloroform, 3) Complex between carvedilol and BCG in chloroform (10 μg · mL^{-1})

mation of the complex between CAR and iodine, that did not interfere in the validation. Chloroform was also tested as solvent, but the formation of the complex between CAR and iodine could not be demonstrated. The experimental conditions were studied and it was found that an amount of 4 mL of iodine (1×10^{-3} M) was sufficient to produce a maximum and reproducible complex, stable for at least 2 h.

2.2. Ion-pair methods

The ion-pair spectrophotometry has received considerable attention for quantitative analysis of many pharmaceutical compounds (Rahman et al. 2004; Ashour and Al-Kalil 2005; El-Dien et al. 2006; Saffajat et al. 2006). In most of the reported methods the concentrations of the resulting ion-pair complexes are determined after extraction procedures. Only few papers have been reported for the spectrophotometric determination of drugs through ion-pair formation without an extraction step. Extraction-free methods have been proposed by Al-Ghannam (2006) for the quantitative analysis of some β-blockers in pure form and in their dosage forms. These methods were based on the interaction of atenolol, timolol and nadolol, in chloroform, with 0.1% chloroformic solutions of bromophenol blue (BPB), bromothymol blue (BTB) and bromocresol purple (BCP) to form stable, yellow-colored ion-pair complexes that could be measured at 415 nm. We have used the acid dyes bromothymol blue (BTB) and bromocresol green (BCG) to form the ion-pair complexes with CAR in chloroform. In this solvent, CAR is not an absorbing species in the visible region, and the dyes used have negligible absorbances. In contrast, when a solution of BTB or BCG in chloroform was added in large excess to the drug solution, an intense yellow color was immediately produced. In our method the CAR measurements could be done directly in chloroform solutions without an extraction procedure, as proposed by Al-Ghannam (2006). Complexes formed showed absorptions maximum at 411 nm and 414 nm, respectively for BTB and BCG (Fig. B and C). It was found that 4 mL of BTB or BCG was sufficient to produce maximum and reproducible colors, at room temperature, that were stable for at least 2 h.

2.3. Validation of the methods

The optimal characteristics of the methods, such as Beer's law limit, apparent molar absorptivity, correlation coefficient, slope, intercept, and confidence limits are presented in Table 1. The correlation coefficients were > 0.999. The linearity data were validated by the analysis of variance (ANOVA), which demonstrated significant linear regression and no significant linearity deviation ($P < 0.05$). The results obtained for the determination of CAR in tablets

Table 1: Analytical parameters for the determination of carvedilol with iodine, bromothymol blue (BTB) and bromocresol green (BCG) methods

Parameters	Iodine[a]	BTB[a]	BCG[a]
Volume of the dye (mL)	4	4	4
λ_{max} (nm)	363	411	414
Beer's law limit (μg · mL^{-1})	2–7	2,5–15	2,5–15
Apparent molar absorptivity (L mol^{-1} · cm^{-1})	24.733	19161	19323
Regression equation			
Slope ± standard error	0.062× ± 0.001	0.048× ± 0.001	0.050× ± 0.001
Confidence limit of slope[b]	0.003	0.001	0.002
Intercept ± standard error	−0.005 ± 0.006	−0.006 ± 0.005	−0.018 ± 0.007
Confidence limit of intercept[b]	0.016	0.013	0.018
Correlation coefficient (r^2)	0.9989	0.9999	0.9998
Variance			
Linear regression[c]	9.788 (4.96)	20.697 (4.96)	54.054 (4.96)
Linearity deviation[c]	2.69 (3.71)	0.76 (3.71)	3.42 (3.71)

[a] Data obtained from three calibration curves;
[b] 95% confidence limit,
[c] Figures in parentheses corresponding critical values for F at P = 0.05

Table 2: Intra and inter-day assay variations of carvedilol by the proposed and reported methods

Method	Tablets A				Tablets B				Compounded capsules			
	Intra-day[a]		Inter-day		Intra-day[a]		Inter-day[a]		Intra-day[a]		Inter-day[a]	
	% ± s.e.m.	%RSD	% ± s.e.m	%RSD	% ± s.e.m	%RSD	% ± s.e.m	%RSD	% ± s.e.m	%RSD	% ± s.e.m	%RSD
Iodine	96.1 ± 0.1	0.4	96.3 ± 0.1	0.3	102.1 ± 0.4	0.9	102.3 ± 0.2	0.4	99.5 ± 0.6	1.4	99.5 ± 0.2	0.4
BTB	97.1 ± 0.4	1.0	97.1 ± 0.2	0.4	101.5 ± 0.2	0.6	101.9 ± 0.2	0.4	98.7 ± 0.5	1.2	98.9 ± 0.3	0.6
BCG	96.7 ± 0.2	0.5	96.9 ± 0.1	0.2	102.4 ± 0.3	0.6	102.3 ± 0.1	0.2	99.3 ± 0.4	0.9	99.4 ± 0.1	0.1
UV	96.5 ± 0.1	0.2	96.4 ± 0.1	0.1	102.2 ± 0.2	0.5	102.4 ± 0.1	0.2	99.6 ± 0.6	1.5	100.0 ± 0.2	0.4

[a] mean of six determinations
[b] mean of three determinations
sem = standard error of the mean

Table 3: Determination of carvedilol in formulations by the standard addition method

Formulations	Iodine			BTB			BCG		
	Spiked ($\mu g \cdot mL^{-1}$)	Found ($\mu g \cdot mL^{-1}$)	Recovery (%)	Spiked ($\mu g \cdot mL^{-1}$)	Found ($\mu g \cdot mL^{-1}$)	Recovery (%)	Spiked ($\mu g \cdot mL^{-1}$)	Found ($\mu g \cdot mL^{-1}$)	Recovery (%)
Tablets A	1.00	1.00	100.0	2.50	2.48	99.2	2.50	2.47	98.8
	2.00	1.97	98.5	5.00	5.08	101.6	5.00	4.89	97.8
	3.00	2.90	96.7	7.50	7.56	100.8	7.50	7.48	99.8
Tablets B	1.00	1.00	100.0	2.50	2.50	100.0	2.50	2.49	99.4
	2.00	2.04	102.0	5.00	5.06	101.2	5.00	4.99	99.7
	3.00	3.01	100.3	7.50	7.57	100.9	7.50	7.31	97.5
Compounded capsules	1.00	0.97	97.0	2.50	2.47	98.8	2.50	2.47	98.8
	2.00	1.93	96.5	5.00	5.02	100.4	5.00	5.03	100.6
	3.00	2.98	99.3	7.50	7.45	99.3	7.50	7.43	98.9

A, B and compounded capsules are shown in Table 2. No interferences from the excipients were observed with the proposed methods. The obtained % RSD values for the intra-day and inter-day were less than 2%, indicating a satisfactory precision. No significant recovery differences were observed ($P < 0.05$ Table 3). The results obtained by the proposed methods were compared with a reported UV method. There was no significant difference between the UV-spectrophotometric (Cardoso et al. 2005) and the proposed methods (Table 4).

3. Experimental

3.1. Chemicals

Carvedilol reference substance (assigned purity, 99.63%) was obtained from DEG (Brazil). Tablets and compounded capsules were purchased at the local market and were claimed to contain 25 mg CAR each. Two commercially available tablets (A and B) and one batch of compounded capsules were evaluated by the proposed methods. Iodine resublimed: 25.5 mg was dissolved in 50 mL of acetonitrile (10^{-3} M). Acidic dyes: 15.6 mg of BTB was dissolved in 50 mL ($5 \cdot 10^{-4}$ M) and 17.5 mg of BCG was dissolved in 100 mL ($2.5 \cdot 10^{-4}$ M), both in chloroform. The iodine and acid dyes solutions were found to be stable for at least a week at 5 °C.

3.2. Equipment

A Spectronic Genesis 2 UV-VIS spectrophotometer (Milton Roy G., USA) with a fixed slit width (2nm) and a 10 mm quartz cell was used to obtain spectrum and absorbance measurements.

3.3. Recommended procedure and calibration curve

Different aliquots of a diluted solution of CAR reference standard containing 20 $\mu g \cdot mL^{-1}$ in acetonitrile (for iodine) and 25 $\mu g \cdot mL^{-1}$ in chloroform (for BTB and BCG) were transferred to 20 mL volumetric flasks. In each flask 4 mL of each specified reagent (iodine, BTB or BCG) was added and brought to volume with either acetonitrile (iodine) or chloroform (BTB and BCG). The final concentrations are in the range stated in Table 1. Absorbances of the resulting colored solutions were measured at the specified wavelength (Table 1) against a reagent blank similarly prepared.

3.4. Sample preparation

Twenty tablets were weighed and the average tablet weight was determined. The tablets were ground to a homogeneous powder. An amount of powder equivalent to 12.5 mg of CAR was placed in a 50 mL volumetric flask and about 25 mL of solvent (acetonitrile or chloroform) was added. After shaking for 15 min the volume was made up with the same solvent. The solution was filtered through a quantitative paper filter (Schleicher & Schuell). Further dilution of the filtrate was made with the same solvent in order to give a final concentration of 4.0 $\mu g \cdot mL^{-1}$ (for iodine) and 10 $\mu g \cdot mL^{-1}$ (for BTB and BCG), adding to each flask 4 ml of specified reagent (iodine, BTB or BCG). The compounded capsules were submitted to the same conditions of the tablets. The absorbances were measured at a specified wavelength (Table 1) against a reagent blank similarly prepared.

3.5. Method validation

The methods were validated by the determination of the following characteristics: linearity, specificity, precision and accuracy (USP 28 2004, ICH Q2R1 2005). Calibration curves (three different days) were obtained with six concentrations of the standard solution in the ranges given in Table 1. The results obtained were used to calculate the equation of the line using linear regression by the least square regression method, and data were evaluated by the analysis of variance (ANOVA). The influence of commonly used tablets and capsule excipients (lactose, sucrose, povidone, crospovidone, colloidal anhydrous silica, magnesium stearate, iron oxide yellow, iron oxide red; microcrystalline cellulose, starch, sodium lauryl sulfate and talc) was investigated before the determination of the drug in dosage forms. The precision of the procedures was determined through repeatability (intra-day precision). Six samples of tablets and compounded capsules at the same concentration (4.0 $\mu g \cdot mL^{-1}$ for iodine and 10.0 $\mu g \cdot mL^{-1}$ for BTB and BCG methods) were assayed during the same day and under the same experimental conditions. The analyses were repeated on different days ($n = 3$) in order to evaluate the intermediate precision (inter-day). The recovery (accuracy) was determined by adding known amounts of CAR reference substance (1.0, 2.0 and 3.0 $\mu g \cdot mL^{-1}$ for iodine and 2.5, 5.0 and 7.5 $\mu g \cdot mL^{-1}$ for BTB and BCG) to the samples and submitting the samples to some procedure described above. The absorbances were measured at specified wavelength (Table 1) against a reagent blank similarly prepared and the percentage recovery was calculated using the formula proposed by A.O.A.C. (1990).

Acknowledgement: This work was supported by FAPERGS-Brazil.

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