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DETERMINATION OF CARVEDILOL BY ITS QUENCHING EFFECT ON THE LUMINESCENCE OF TERBIUM COMPLEX IN DOSAGE FORM

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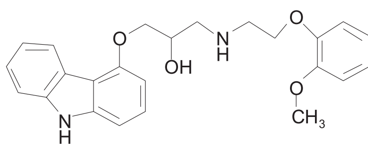
Abstract: A new, simple, sensitive luminescence methods for the determination of carvedilol have been developed and validated. Carvedilol was remarkably quenching the luminescence intensity of the Tb(III) ion in the new terbium complex with 1-butyl-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid-(4-methyl-pyridin-2-yl)-amide (R) in aqueous solutions containing urotropine buffer (pH 7.5) at λ_{ex} = 317 nm and λ_{em} = 545 nm. Under optimal conditions, the quenching of luminescence intensity was found to be proportional to the concentration of carvedilol in the range of 0.5–400 µg/mL. The detection limit was 0.16 µg/mL. This method was applied for the determination of carvedilol in tablets "Coryol".

Keywords: carvedilol, luminescence, quenching effect, terbium(III)

Carvedilol (CAR) is a (±)-[3-(9*H*-carbazol-4-yloxy)-2-hydroxypropyl][2-(2-methoxyphenoxy)ethyl]amine (IUPAC name), which is used for the treatment of hypertension, ischemic heart disease and congestive heart failure (1). It is a non-selective, β-adrenergic receptor antagonist and an α_1-adrenoceptor blocker.



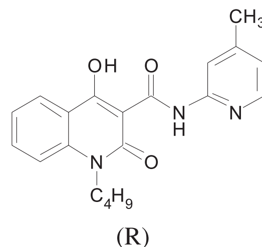
Carvedilol

Many various methods for determination of CAR in pharmaceutical preparations and biological samples have been described. The most popular method is high-performance liquid chromatography (HPLC) for determination of CAR in biological fluids (2–7).

Other reported methods for determination of CAR in different samples are voltammetry (8, 9), spectrophotometry (10–12), first-order derivative fluorometry (13), spectrofluorimetry (14, 15), and chemiluminescence (16). The suggested method has the advantage of being simple, sensitive and suitable for routine analysis in a quality control laboratory.

The analytical application of lanthanide-sensitized luminescence is of great interest. The main advantages of lanthanide chelates in fluorescence spectrometry include large Stokes shifts, narrow emission bands and long fluorescence lifetimes (17). The strong ion emission of these complexes is a result of the intramolecular energy transfer process from the ligand (drug) to the lanthanide ion. In the last few years, an opportunity of analytical use of the lanthanide ions luminescence sensitization effect as well as their decrease/enhancement effects by some inorganic and organic anions was applied for the determination of drugs, which are not Ln luminescence sensitizers (18–20). The lanthanide complexes are often used as a fluorescence probes for indirect determination of some drugs: zidovudin (21); catecholamines (22); enoxacin (23), omeprazole (24), ramipril (25).

The aim of this study was to develop a new fluorimetric method for determination of CAR in pharmaceutical preparation (tablets) based on luminescence quenching of Tb(III) complex with 1-butyl-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid-(4-methyl-pyridin-2-yl)-amide (R):



(R)

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EXPERIMENTAL

Apparatus

All luminescence measurements (luminescence spectra, excitation spectra and lifetimes) were performed using a Cary Eclipse luminescence spectrometer (Varian, Australia) equipped with a 150-W xenon lamp, using 1.0 cm quartz cells. The excitation and emission monochromator band widths were 5 nm. The excitation wavelength was set at 317 nm and the fluorescence was measured using the peak height at 545 nm. All measurements were performed at room temperature (21–23°C). The pH values of solutions were measured using Lab 850 pH meter (Schott Instruments GmbH, Germany) with a glass electrode. Absorption spectra were recorded with a UV-2401 PC (Shimadzu) spectrophotometer.

Table 1. Summary of validation parameters.

Parameters	Results for carvedilol
Linearity range	0.5–400.0 μg/mL
The limit of detection (LOD)	0.16 μg/mL
Correlation coefficient (r)	0.9971
Accuracy (n = 6) Inter-day (n = 10) Intra-day (n = 10)	100.5% Precision 2.5% 3.6%
Specificity	specific

Materials and methods

The standard solution of terbium(III) chloride (1×10^{-1} mol/L) was prepared from a high purity oxide. The concentration of the metal was determined by complexometric titration with Arsenazo I as the indicator.

The ligand R was synthesized as described elsewhere (26). The standard solutions of reagent (1×10^{-3} mol/L) were prepared by dissolving accurate weights of the solid compound in dimethylformamide (DMF).

Pharmaceutical preparation, "Coryol" – tablets containing 3.125 mg of carvedilol, were provided by KRKA pharmaceutical company (Slovenia).

An accurately weighted 50 mg sample of carvedilol (Sigma, C 3993) was placed into a 50 mL volumetric flask, mixed with 30 mL of ethanol, stirred and diluted with the same solvent up to 50

Table 2. Recovery of carvedilol in model solutions (n = 6, p = 95%).

Label claim (mg/tablet)	Amount added (%)	Amount added (mg)	Amount found (mg)	Recovery (%)	RSD %
Carvedilol 3.125	80	2.5	2.45 ± 0.10	98.0	3,9
	100	3.1	3.16 ± 0.10	101.9	3.1
	120	3.7	3.76 ± 0.11	101.6	2.7
				Average recovery: 100.5	

Table 3. Tolerance limits of various interferents in the determination of 100 μg/mL of CAR.

Interferents	Interferent-to-analyte ratio	ΔI (%)
Lactose anhydrous (Granulac 200)	50:1	–4.0
Cellulose microcrystalline	10:1	2.5
Calcium stearate	0.7:1	–5.5
Talk	1.3:1	2.1
Maize starch	7:1	–5.1
Macrogol (PEG 6000)	3:1	7.6
Sodium carboxymethylcellulose	2:1	6.5
Gelatin	1:1	–0.9
Povidone (K-17)	13:1	–2.2
Disodium hydrogen phosphate dihydrate	30:1	–8.3
Mannitol	7:1	7.9

mL. A standard solution of the concentration 1.0 mg/mL was obtained. The working solution of carvedilol was obtained by dilution with the same solvent to final concentration of 100 μg/mL.

An urotropine buffer was prepared by dissolving 40.0 g of urotropine in 100 mL volumetric flask with water. The pH of solutions was maintained at 6.5 with 40% urotropine solution.

All of the used chemicals were of analytical grade or chemically pure; doubly-distilled water was used.

Construction of calibration curve

The volumes of 0.05, 0.3, 0.5 and 1.0 mL of carvedilol working solution (100 mg/mL) and 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 2.0, 3.0 and 4.0 mL of carvedilol standard solution (1 mg/mL) were placed in 10 mL volumetric flasks. The solutions were diluted with ethanol up to 4 mL. One milliliter of a working terbium(III) chloride solution (1×10^{-4} mol/L), 0.1 mL of R working solution (1×10^{-3} mol/L) and 0.4 mL of urotropine buffer (40%) were added to each volumetric flask. Simultaneously, a blank solution, which contained all the components with the exception of carvedilol, was prepared. The solutions were diluted with water up to 10 mL and stirred. After 5 min, the intensity of luminescence was measured at λ_{em} = 545 nm (λ_{ex} = 317 nm).

Procedure for commercial tablets "Coryol" (3.125 mg)

Twenty tablets of an analyzed drug were weighed to calculate the average tablet weight, and were then powdered and mixed. The powder equiv-

Table 4. Determination of carvedilol in tablets "Coriol" – 3.125 mg (n = 5, p = 95%).

Batch No.	Found, mg	RSD, %
N62929	3.09 ± 0.13	3.4
N62919	3.13 ± 0.14	3.5
N62909	3.10 ± 0.10	2.7

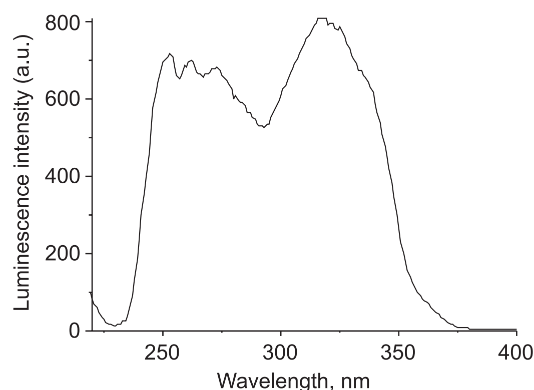

Figure 1. Excitation spectra of Tb(III)–R complex (C_{Tb} = C_R = 1×10^{-5} mol/L; λ_{em} = 545 nm)

Table 5. Overview on selected assays for determination of carvedilol.

Method	Linear range [μg/mL]	Limit of detection [μg/mL]	Sample	Ref.
Liquid chromatography (HPLC)	0.005–0.500	-	Biological fluids	6
Liquid chromatography (HPLC)	1–40	-	Biological fluids	7
Voltammetry	2.5–22.5	1.7	Pharmaceutical preparation and biological fluids	8
Voltammetry	0.08–8.0	-	Pharmaceutical preparation and biological fluids	9
Spectrophotometry	5.0–25.0	1.5	Pharmaceutical preparation and biological fluids	10
Spectrophotometry	2.0–7.0	0.7	Pharmaceutical preparation	11
Spectrophotometry	0.6–2.0	0.21	Pharmaceutical preparation and biological fluids	12
First-order derivative fluorometry	0.002–0.02	0.001	Pharmaceutical preparation and biological fluids	13
Spectrofluorimetry	0.04–0.40	-	Pharmaceutical preparation	15
Chemiluminescence	0.05–1.22	-	Pharmaceutical preparation	16
Luminescence	0.5–400	0.16	Pharmaceutical preparation	this work

alent to 10.0 mg of the active ingredient was placed into a 10 mL volumetric flask, mixed with 7 mL of ethanol, stirred, diluted with the same solvent up to 10 mL and filtered to remove insoluble materials. One milliliter of the filtrate solution was placed into the 10 mL volumetric flask. Further, 1.0 mL of a working terbium(III) chloride solution (1×10^{-4} mol/L), 0.1 mL of R working solution (1×10^{-3} mol/L) and 0.4 mL of urotropine buffer were added to each of these volumetric flasks, then water was added up to the volume of 10 mL and luminescence intensity was measured at λ_{em} = 545 nm (λ_{ex} = 317 nm). I_{lum} of standard solution – 1.0 mL of carvedilol standard solution (1 mg/mL), was placed into the volumetric flask, then all components (with the exception of 1 mL of the filtrate solution) were added as described above – was recorded at the same time.

The content of carvedilol (X) in one tablet in milligrams was calculated using the formula:

$$X_1 = \frac{I_1 \cdot m_0 \cdot b \cdot 1 \cdot 10 \cdot 10}{I_1 \cdot m_1 \cdot 50 \cdot 10 \cdot 1} = \frac{I_1 \cdot m_0 \cdot b \cdot 0.2}{I_1 \cdot m_1}$$

where I_1 – the luminescence intensity of the assay, I_0 – the luminescence intensity of standard, m_0 – the weight of standard in mg, m_1 – the weight of powdered tablets in mg, b – the average tablet weight in mg.

RESULTS AND DISCUSSION

Spectral characteristics

The absorption spectra of R in water solution are characterized by the presence of band with high molar absorption coefficient (ε) in the UV region of the spectra at λ_{max} = 313 nm (ε = 2.46×10^4 $L \cdot mol^{-1} \cdot cm^{-1}$). This coefficient give the possibility for effective absorption of light energy. The energy of triplet level (T) of R (22150 cm^{-1}) was calculated from phosphorescence spectra of its Gd(III) complex at 77 K. This energy is higher than the energy of level of the first excited Tb(III) ion state ($^5D_4$; 20500 cm^{-1}), resulting in the possiblity of energy transfer from ligand to lanthanide ions.

The excitation spectra of the complex monitored at 545 nm show an excitation maximum at 317 nm (Fig. 1). Especially the 545 nm-band is hypersensitive to changes of the coordination environment of the respective complex. Therefore, the changes of the luminescence intensity of this band are most often used for analytical applications with Tb(III) complexes. The lifetime (τ) of the test complex is rather long.

Effect of pH, stoichiometry

The complexation of Tb(III) with the ligand occurs in a wide range of pH values from 3 to 10. The maximal luminescence intensity of the complexes Tb(III)–R is observed at pH 7.0–9.5. The pH of solutions was maintained at 7.5 with urotropine buffer.

Applying the restricted-logarithm method to the luminescence data, it was found that in case of reagents' shortage or at equimolar ratio Tb forms complex compounds with R at the component ratio Tb(III) : R = 1:1 (τ = 850 µs) and if reagent is in excess, terbium forms complex compounds with R at the component ratio Tb(III) : R = 1:3 (τ = 1130 µs).

The influence of ligand concentration on the luminescence intensity was investigated at constant Tb(III) concentration of 10.0 mM. The optimal conditions were: equal concentrations (10.0 mM) of Tb(III) and R, which were chosen for further experiments.

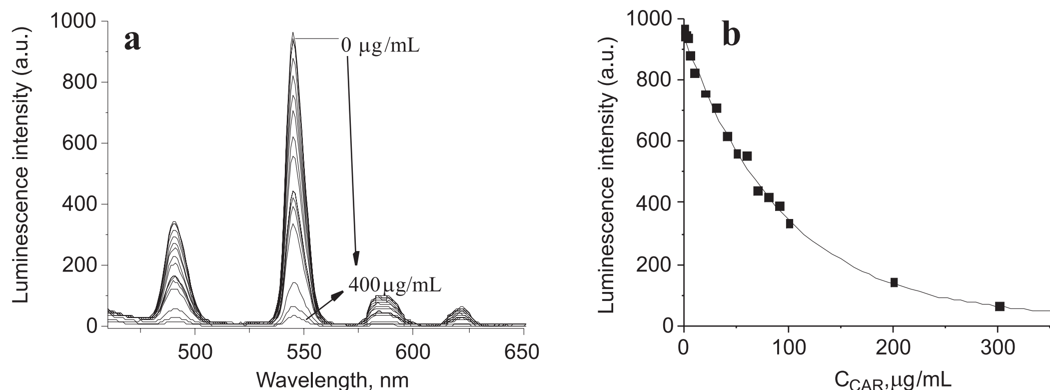

Figure 2. Luminescence spectra (a) and dependence of the luminescence intensity of Tb(III)–R in the presence of different concentration of CAR (b) ($C_{Tb} = C_R = 1\times10^{-5}$ mol/L; λ_{em} = 545 nm; λ_{exc} = 317 nm)

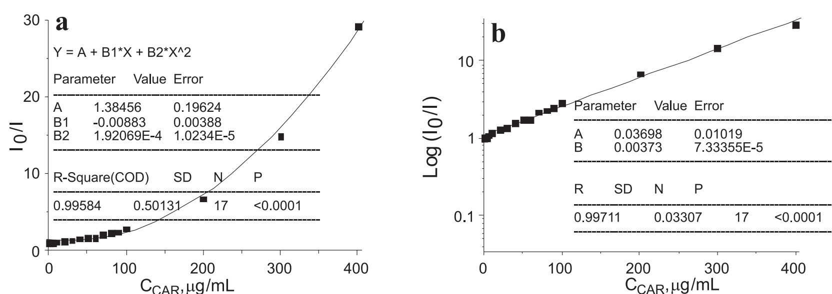

Figure 3. Non-linear Stern-Volmer plot (a) and modified linear Stern-Volmer plot (b) for carvedilol determination ($C_{Tb} = C_R = 1\times10^{-5}$ mol/L; λ_{em} = 545 nm; λ_{exc} = 317 nm)

Analytical performance

The proposed method was validated in terms of linearity, accuracy, inter and intra-day precision and specificity (Table 1).

The method for the luminescent determination of CAR concentration was developed using Tb(III)–R complex. Luminescence quenching experiments were carried out. The carvedilol was used as a quencher in this experiment (Fig. 2). Different concentrations of CAR were added to Tb(III)–R = 1:1 complex. I_0 and I were measured at λ_{exc} = 317 nm and λ_{em} = 545 nm. The Stern-Volmer plot yielded $I_0/I = 1.38 - 0.009C_{CAR} + 0.0002C_{CAR}^2$; correlation coefficient is 0.996; where I_0 and I are the relative luminescence intensities of the system without and with CAR, respectively, C_{CAR} is the concentration of carvedilol in µg/mL (Fig. 3 a). As can be seen from Fig. 3 a, the Stern-Volmer plot had been found to be non-linear with an upward curvature and obeyed the polynominal equation. The result suggests that both static and dynamic quenching processes are responsible for the observed positive deviation in the Stern-Volmer plot.

The linear relationship between the luminescence intensity of Tb(III)–R complex and the CAR concetration can be obtained when I_0/I was given in logarithmic form. The linear relationship was obtained between the log (I_0/I) and CAR concetration: $Log(I_0/I) = 0.04 + 0.004C_{CAR}$; correlation coefficient is 0.997 (Fig. 3b). The calibration curve is linear in the 0.5–400.0 µg/mL range of CAR. The signal-to-noise ratio of 3 was considered as the limit of detection (LOD). The LOD for carvedilol was found to be 0.16 µg/mL.

Accuracy of the analysis was evaluated by carrying out a recovery study at three different levels (80, 100 and 120%). The results of recovery study indicate that the proposed method is accurate for estimation of drug in tablet dosage form (Table 2).

The precision of the method was established by testing the analytical signal corresponding to a carvedilol concentration of 100 µg/mL. For a series of 10 measurements, the relative standard deviation was 2.5% for the intra-days and 3.6% for the inter-days analysis (p = 95% confidence level) for carvedilol.

The specificity of the proposed method was investigated and no interferences were observed between CAR and some common excipients for tablets formulations. The interference of typical excipients was studied by addition of these compounds at different concentrations to the solution of 100 µg/mL of CAR. As shown in Table 3, most excipients either had no effect or had little effect on the determination of CAR. Hence, specificity achieved by the proposed method is good and it is possible to determine CAR in the presence of the excipients.

This method was used to assay the active ingredient – carvedilol, in dosage form – tablets "Coryol" («KRKA», Slovenia) – 3.125 mg. The content of carvedilol in dosage form in milligrams was calculated by the standard sample method using the above formula. Three batches of carvedilol tablets were analyzed. The results are shown in Table 4**.**

In comparison with the spectrophotometry and voltammetry methods reported in Table 5, the method proposed in this paper offers higher sensitivity and a wider linear range. In addition, this method is more quick and simpler than HPLC method. The proposed luminescence method for the determination of CAR is simple, reliable, sensitive with the advantage of a wide determination range that does not require extraction.

CONCLUSIONS

The new terbium complex with 1-butyl-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid-(4-methyl-pyridin-2-yl)-amide has high sensitivity, selectivity and characteristic peaks. It was found that carvedilol can remarkably quench the luminescence intensity of this complex. On this basis, as a result of this study, a new spectrofluorimetric method was developed for determination of CAR. The proposed method is simple, accurate and easy to perform and can be used for the routine determination of CAR in dosage forms.

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