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Determination of drotaverine hydrochloride in dosage forms by its quenching effect on the luminescence of terbium complex

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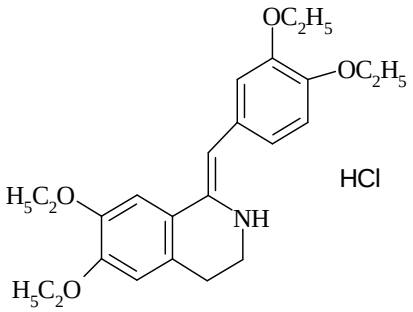
ABSTRACT

A new, simple, sensitive luminescence method for the determination of drotaverine hydrochloride is developed and validated. The Drotaverine hydrochloride can remarkably quench the luminescence intensity of the Tb^{3+} ion in terbium complex with 1-bythyl-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 is directly proportion to the concentration of Drotaverine hydrochloride in the range of 0.5-300 μg/mL and detection limit is 0.16μg/mL. This method was applied for the determination of Drotaverine hydrochloride in tablets "No-spa".

INTRODUCTION

Drotaverine hydrochloride (DH), 1-(3,4-diethoxy benzylidene)-6,7-diethoxy-1,2,3,4-tetrahydroisoquinoline is an antispasmodic drug widely used to relieve cramps or spasms of the stomach, intestines and bladder (Sweetman, 2011). Drotaverine hydrochloride is an analogue of papaverine with smooth muscle relaxant properties. It is a non-anticholinergic antispasmodic, which selectively inhibits phosphodiesterase IV and is accompanied by a mild calcium channel-blocking effect. Few methods have been reported for the determination of drotaverine hydrochloride in biological fluids and in pharmaceutical formulation including voltammetric (Ziyatdinova *et al.*, 2007, Zayed *et al.*, 2009), high performance liquid chromatography (HPLC) (Mezei *et al.*, 2006, Dahivelkar *et al.*, 2009, Topagi *et al.*, 2010), spectrophotometric (Metwally *et al.*, 2006, Amin *et al.*, 2007, Dahivelkar *et al.*, 2007, Abdellatef *et al.*, 2007, Rajmane *et al.*, 2009, Daabees *et al.*, 2000, Metwally *et al.*, 2007, Metwally *et al.*, 2008), membrane selective electrodes (El-Saharty *et al.*, 2006, Ibrahim *et al.*, 2005) and spectrofluorometric (El-Wasseef *et al.*, 2008) methods. The analytical application of lanthanide-sensitized luminescence has a great interest.

The main advantages of lanthanide chelates in luminescence spectrometry include large Stokes shifts, narrow emission bands and long luminescence lifetimes (Georges *et al.*, 1993).



Drotaverine hydrochloride (DH)

The strong ion emission of these complexes as 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 (Duerkop *et al.*, 2008; Aleksandrova *et al.*, 2008; Aleksandrova *et al.*, 2009).

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The lanthanide complexes are often used as a luminescence probes for inderect determination of some drugs: zidovudin (Araujo *et al.*, 2002); catecholamines (Takahashi *et al.*, 2002); enoxacin (Karim *et al.*, 2007), omeprazole (Shaghaghi *et al.*, 2008), ramipril (Attia *et al.*, 2010). This work designed a novel platform for effective fluorimetric determination of DH in pharmaceutical preparation based on luminescence quenching of terbium complex. The suggested method is rapid, simple, sensitive and can be used for the determination of DH in tablets.

GENERAL EXPERIMENTAL

Apparatus

All luminescence measurements (luminescence spectra, excitation spectra and lifetimes) are carried out on Cary Eclipse (Varian, Australia) luminescence spectrophotometer in the range (220-700 nm) equipped with a 150-W xenon lamp, 1.0 cm quarts cell. The excitation and emission monochromator band widths were 5 nm. The excitation wavelength was set at 317 nm and the luminescence was measured using the peak height at 545 nm. All measurements were performed at room temperature (21–23 °C). A pH meter (Lab 850, Schott Instruments GmbH, Germany) was used for pH adjustment. Absorption spectra are recorded with a UV-2401 PC (Shimadzu, Japan) spectrophotometer.

Material and reagents

All of the used chemicals were of analytical grade or chemically pure; doubly-distilled water was used. Pharmaceutical preparation, “No-spa”, tablets containing 40 mg of drotaverine hydrochloride, produsedby “Chinoin Chemical Pharmaceutical” (“Sanofi-Aventis”, Hungary) are purchased from local market. The standard solution of terbium (III) chloride (1 10^{-1} mol L^{-1}) 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 1-bythyl-4-hydroxy-2-oxo-1,2-dihydro quinoline-3-carboxylic acid-(4-methyl-pyridin-2-yl)-amide (R) was synthesized as described elsewhere (Ukrainets et al., 2004). The standard solutions of reagent (1 10^{-3} mol L^{-1}) was prepared by dissolving accurate weights of the solid compounds in dymethylformamide (DMFA). An accurately weighted 50 mg standard sample of drotaverine hydrochloride (Akums Drugs & Pharmaceuticals Ltd., Haridwar, India) was dissolved in water, placed into a 50 mL volumetric flask, stirred and diluted to the mark with water and mixed well. A standard solution of the concentration 1.0 mg mL^{-1} was obtained. The stock standard solution of DH was diluted to 100 μg mL^{-1} before being used.

An urothropine buffer was prepared by dissolving 40.0 g of urothropine in 100 mL volumetric flask with water.

General procedure

Preparation of lanthanide complex

The complex of R with the Tb^{3+} ion was prepared by mixing the R at concentration of 1 10^{-4} mol L^{-1} and 1 10^{-4} mol L^{-1} in a molar ratio of 1:1 in water at room temperature.

Measurement procedures of the luminescence spectrum of the Tb(III)-R complex in presence of different concentration of DH

0.05; 0.1; 0.5; 1.0 mL of DH working solution (100 μg mL^{-1}) and 0.2; 0.3; 0.5; 0.6; 0.7; 0.8; 0.9; 1.0; 1.5; 2.0; 3.0; 4.0 mL of DH standard solution (1 mg mL^{-1}) were placed into volumetric flasks. 1.0 mL of a working terbium chloride solution (1 10^{-4} mol L^{-1}), 0.1 mL of R working solution (1 10^{-3} mol L^{-1}) and 0.4 mL of urothropine buffer (40 %) were added to each of these volumetric flasks. The solutions were diluted with water up to 10 mL and stirred. In 5 minutes the luminescence intensity is measured at $\lambda_{ex}/\lambda_{em}$ = 317 / 545 nm.

The determination of DH in pharmaceutical preparation (“No-spa”-40 mg)

Twenty tablets of an analyzed drug are weighed to calculate the average tablet weight, and are then powdered and mixed.

The powder equivalent to 40.0 mg of the active ingredient are placed into a 200 mL volumetric flask, mixed with 50 mL of water, stirred, diluted with the same solvent up to 200 mL and filtered to remove insoluble materials. 5 mL of the filtrate solution are placed into the 10 ml volumetric flask. Further 1.0 mL of a working terbium chloride solution (1 10^{-4} mol L^{-1}), 0.1 mL of R working solution (1 10^{-3} mol L^{-1}) and 0.4 mL of urothropine buffer were added to each of these volumetric flasks, then water was added up to the volume of 10 mL and luminescence intensity (I_{as}) are measured at $\lambda_{ex}/\lambda_{em}$ = 317 / 545 nm. I_{lum} of standard solution (I_{st}) - 1.0 mL of DH standard solution (1 mg mL^{-1}) was placed into the volumetric flask, then all components (with the exception of 5 mL of the filtrate solution) were added as described above - was recorded at the same time. I_{lum} of control solution (I_0) which contents all components with the exception of DH was recorded at the same time. I_0 was considered at calculation of I_{lum} of an investigated solution and standard solution.

The content of DH (X) in one tablet in milligrams is calculated using the formula:

$$X = \frac{I_1 \cdot m_0 \cdot 1 \cdot 200 \cdot 10 \cdot \mathrm{b}}{I_2 \cdot m_1 \cdot 50 \cdot 10 \cdot 5} = \frac{I_1 \cdot m_0 \cdot 0.8 \cdot b}{I_2 \cdot m_1}$$

I_1 - the luminescence intensity of the assay (I_0/I_{as});
I_2 - the luminescence intensity of standard (I_0/I_{st});
I_0 - the luminescence intensity of control solution;
m_0 - the weight of standard, mg;
m_1 - the weight of powdered tablets, mg;
b – the average tablet weight, mg

RESULTS AND DISCUSSIONS

Spectral characteristics

The absorption spectrum of R in DMFA-water solution is 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 $L \cdot mol^{-1} \cdot cm^{-1}$) is shown in Fig. 1.

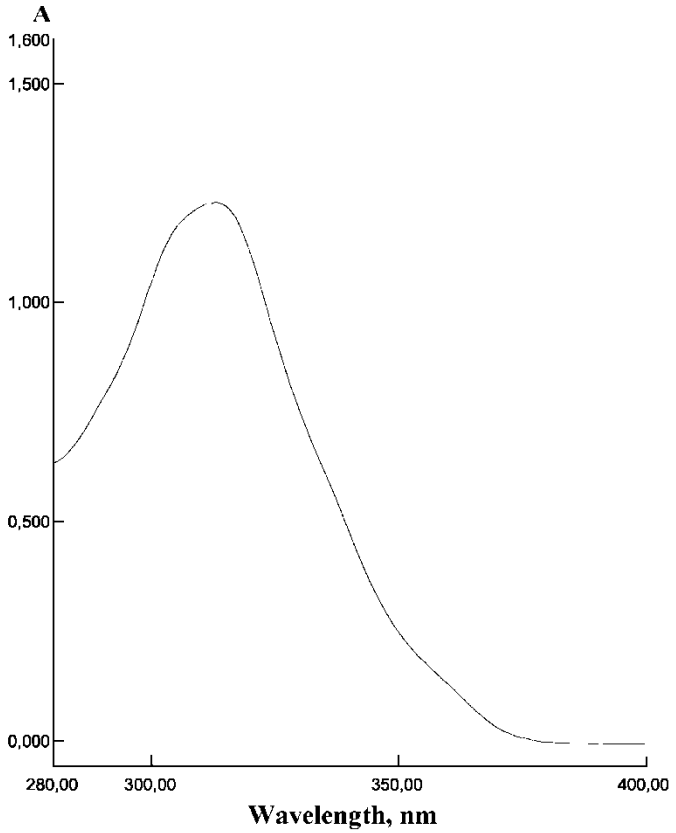
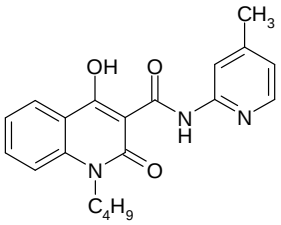


Fig. 1: The absorption spectrum of R. Conditions: [R], 5 10^{-5} mol L^{-1} in 50 % v/v DMFA.

This coefficient gives the possibility for effective absorption of light energy. The energy of triplet level (T) of R (22150 cm^{-1}) is calculated from phosphorescence spectra of its Gd complex at 77 K. This energy is higher than the energy of level of the first excited Tb^{3+} ion state ($^5D_4$; 20500 cm^{-1}), resulting in the possibility of energy transfer from ligand R to lanthanide ion.



(R)

The excitation spectra of the complex monitored at 545 nm shows an excitation maximum at 317 nm (Fig. 2, a). It was found that with the excitation wavelength at 317 nm, the Tb(III)-R complex emitted the characteristic luminescence of Tb^{3+} ion with the emission peaks of 490 nm, 545 nm, 590 nm and 620 nm, which corresponded to $^5D_4 \rightarrow {}^7F_6$, $^5D_4 \rightarrow {}^7F_5$,$^5D_4 \rightarrow {}^7F_4$ and $^5D_4 \rightarrow {}^7F_3$ transitions of Tb^{3+}, respectively. 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.

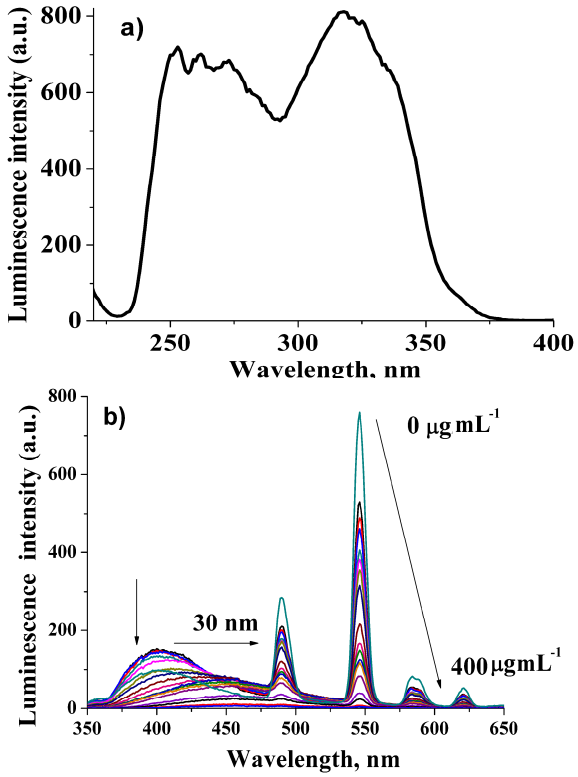


Fig. 2: Excitation spectra of Tb(III)-R complex (a) and luminescence spectra of Tb(III)-R in the presence of different concentration of DH (b). Conditions: [Tb=R], 1 10^{-5} mol L^{-1} ($\lambda_{ex}/\lambda_{em}$ = 317 / 545 nm).

The effect of different experimental conditions

Effect of pH

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 7.0-8.5. The pH of solutions was maintained at 7.5 with urothropine buffer.

Effect of stoichiometry

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 lifetime (τ) of the test complex is rather long. The influence of ligand concentration on the luminescence intensity was investigated at constant Tb^{3+}concentration of 10.0 μ mol. The optimal conditions were equal concentrations (10.0 μ mol) of Tb^{3+} and R which were chosen for further experiments.

The effect of the amount of DH

Fig. 2 displays the spectral changes that occur when DH is added to a solution of Tb(III)-R. The luminescence intensity of Tb(III)-R complex was quenched and luminescence intensity of R was decreased in presence of various concentrations of DH. The interaction of the Tb-R with DH causes a bathochromic shift of 30 nm in the luminescence maximum (from 400 nm to 430 nm).

Analytical performance

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

Table. 1: Summary of validation parameters.

Parameter	Results of DH
Linear range (µg mL^{-1})	0.5-300.0
LOD (µg mL^{-1})	0.16
Correlation Coefficient (r)	0.9947
Accuracy (n=6) (%)	99.86
Precision	
Inter-day (n=10) (%)	2.4
Intra-day (n=10) (%)	3.1
Specificity	specific

LOD : Limit of detection (µg mL^{-1})

The different concentrations of DH were added to Tb(III)-R=1:1 complex. The plot of Stern-Volmer was obtained (Fig. 3, a). I_0 and I were measured at λ_{exc} = 317 nm and λ_{em} = 545 nm. The plot of Stern-Volmer was obtained $I_0/I = 2.42 - 0.086C_{DH} + 0.0014C_{DH}^2$; correlation coefficient is 0.9962; where I_0 and I are the relative luminescence intensities of the system without and with DH, respectively, C_{DH} is concentration of drotaverine hydrochloride, µg mL^{-1}. 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.

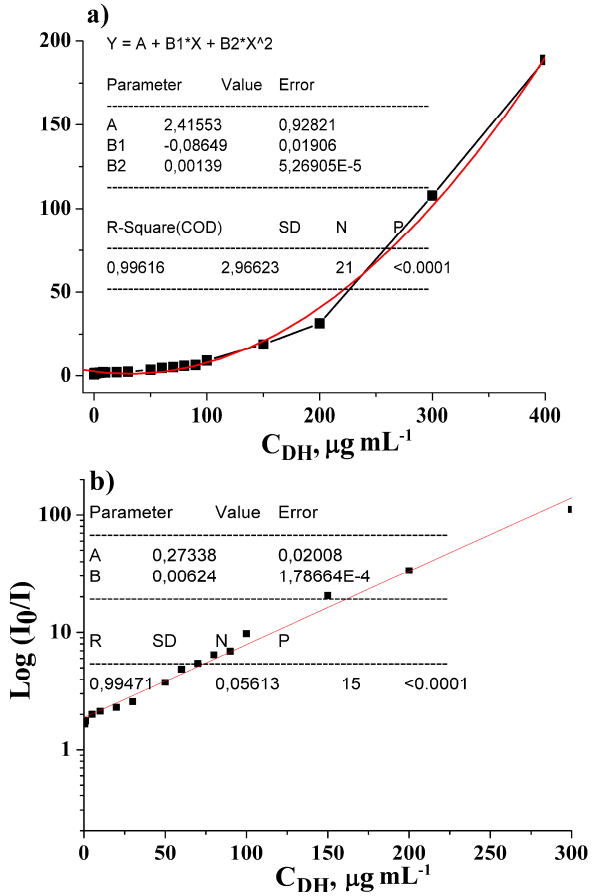


Fig. 3: Non-linear Stern-Volmer plot (a) and modified linear Stern-Volmer plot (b) for DH determination. Conditions:[Tb=R],110^{-5} mol L^{-1}($\lambda_{ex}/\lambda_{em}$=317/545 nm).

When I_0/I was modified by logarithm, a linear relationship between the Log (I_0/I) and concentration of DH was obtained: $Log(I_0/I) = 0.27 + 0.006\ C_{DH}$; correlation coefficient is 0.9947 (Fig. 3, b). The calibration curve is linear in the 0.5-300.0 µg mL^{-1} range of DH. The signal-to-noise ratio of 3 was considered as the limit of detection (LOD). The LOD for drotaverine hydrohloride was found to be 0.16 µg mL^{-1}.

Accuracy of the analysis was evaluated by carrying out a recovery study at three different levels namely 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).

Table. 2: Recovery of DH in model solutions (n = 6, P = 95 %).

Label claim (mg/tablet)	Amount added (%)	Amount added (mg)	Amount found (mg)	Recovery (%)	RSD (%)
No-spa 40	80	32	31.80 ± 1.23	99.38	3.7
	100	40	40.20 ± 1.35	100.50	3.2
				99.69	
	120	48	47.85 ± 1.41	Average recovery: 99.86	2.8

RSD : Relative standard deviation

The precision of the method was established by testing the analytical signal corresponding to a Drotaverine hydrohloride concentration of 100 µg mL^{-1}. For a series of 10 measurements, the relative standard deviation was 2.4 % for the intra-days and 3.1 % for the inter-days analysis (P = 95 % (confidence level)) for DH. The specificity of the proposed method was investigated and no interferences were observed between DH and some common excipients for tablets formulations. The interference of typical excipients was studied by addition of the concentration of these compounds to solution of 100 µg mL^{-1} of DH. As shown in the Table 3, all excipients either had no effect or had little effect on the determination of DH. Hence specificity achieved by the proposed method is good and it is possible to determine DH in the presence of the excipients.

Table. 3: Tolerance limits of various interferents in the determination of 100 µg mL^{-1} of DH.

Interferents	Interferent-to-analyte ratio	ΔI (%)
Lactose anhydrous (Granulac 200)	50:1	-3.0
Magnum stearate	0.7:1	-3.5
Talk	1.3:1	2.1
Maize starch	7:1	-3.1
Povidone (K-17)	13:1	2.2

This method was used to assay the active ingredient - Drotaverine hydrochloride in dosage form - tablets "No-spa" – 40 mg. The content of DH in dosage form in milligrams was calculated by the standard sample method using the above formula. Three batches of DH tablets were analyzed. The results are shown in Table 4**.** In comparison with the spectrophotometry, membrane selective electrodes and voltammetry methods reported, as shown in Table 5, the proposed method in this paper offers higher sensitivity and a

wider linear range. In addition, this method is more quick and simple than HPLC method. The proposed luminescence method for the determination of DH is simple, reliable, sensitive with the advantage of a wide determination range that does not require extraction.

Table. 4: Determination of DH in tablets "No-spa" – 40 mg (n = 5, P = 95 %).

Batch №	Found (mg)	RSD (%)
0V097	39.85 ± 1.24	2.5
0V073	40.14 ± 1.35	2.7
0V092	39.97 ± 1.49	3.0

RSD : Relative standard deviation

Luminescence quenching mechanism

Luminescence quenching experiments were carried out. The drotaverine hydrochloride was used as a quencher in this experiment. Quenching mechanism DH on luminescence of complex is combined static and dynamic. The life time of the terbium ion in the Tb(III)-R complex (τ = 880 μs) decreases (Fig. 4) in the presence of a various concentrations of DH (τ = 787-700 μs), that confirms the contribution of dynamic quenching.

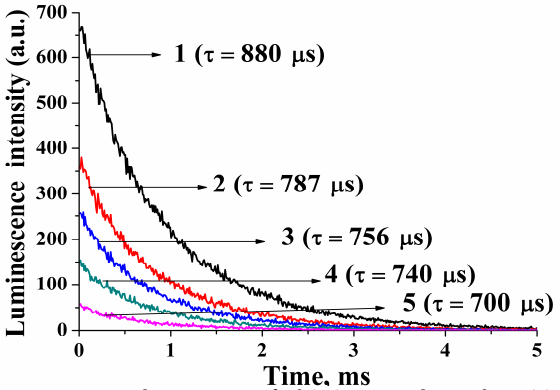


Fig. 4: Luminescence decay curves of Tb(III)-R complex without (1) and in presence (2 - 5) of DH. Conditions: [DH], 2 – 2 μg mL^{-1}; 3 – 20 μg mL^{-1}; 4 – 50 μg mL^{-1}; 5 – 100 μg mL^{-1}; [Tb=R], 1 10^{-5} mol L^{-1} ($\lambda_{ex}/\lambda_{em}$ = 317 / 545 nm).

Luminescence spectra of R, Tb(III)-R, R-DH, Tb(III)-R-DH, DH, Tb(III)-DH (Fig. 5) were studied for confirmation of interaction (static mechanism) in the system of Tb(III)-R-DH. As can be seen from figure 5, there is overlapping of excitation spectrum of DH (curve 1) with the luminescence spectrum of R (curve 2), that testifies to the possible energy transfer between these components of the system. I_{lum} quenching of R confirmes the intramolecular energy transfer from the reagent R (curve 2) to the terbium ion in the complex Tb(III)-R (curve 3).

Luminescence quenching of reagent (curve 2) and luminescence increase of DH (curve 4) in comparison to I_{lum} of free DH (curve 6) are observed because the energy transfer from the R to DH. As follows from curves 5 and 6 drotaverine does not form a complex with the terbium ions. There is quenching of intensity of luminescence of complex Tb(III)-R (curve 3) in the presence of DH can be explained by energy transfer from the reagent to DH. The results suggest that both static and dynamic quenching processes are responsible for the observed positive deviation in the Stern-Volmer plot.

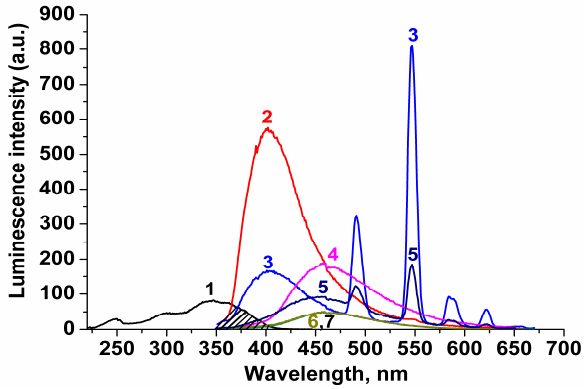


Fig. 5: Excitation spectra of DH (1) and luminescence spectra: 2 – R; 3 – Tb(III)-R; 4 – R-DH; 5 – Tb(III)-R-DH; 6 – DH; 7 – Tb(III)-DH. Conditions: [Tb=R], 1 10^{-5} mol L^{-1}; [DH], 50 μg mL^{-1} (λ_{ex} = 317 nm).

CONCLUSION

The new terbium complex with 1-bythyl-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylic acid-(4-methyl-pyridin-2-yl)-amide has high sensitivity and selectivity characteristic peaks. The intensities of these peaks are quenched by increasing the concentration of drotaverine hydrohloride. On this basis a new spectrofluorimetric method was developed for determination of DH. The proposed method is simple, accurate and easy to perform and can be used for the routine determination of DH in dosage forms.

Table. 5: Overview on selected assays for determination of Drotaverine hydrochloride.

Method	Linear range [μg mL^{-1}]	Limit of detection [μg mL^{-1}]	Sample	Reference
voltammetry	9.67–126.58	9.36	Pharmaceutical preparations	Ziyatdinova *et al.*, 2007
liquid chromatography	0.2–100	0.05	Biological fluids	Mezei *et al.*, 2006
liquid chromatography	0.032–0.96	0.011	Biological fluids	Dahivelkar *et al.*, 2009
liquid chromatography	10–50	1.13	Pharmaceutical formulations	Topagi *et al.*, 2010
spectrophotometry	2–10	-	Pharmaceutical preparations	Metwally *et al.*, 2006
spectrophotometry	4.34–60.76	-	Pharmaceutical preparations	Amin *et al.*, 2007,
spectrophotometry	4–32	-	Pharmaceutical preparations	Dahivelkar *et al.*, 2007
spectrophotometry	2–40	0.4	Pharmaceutical preparations	Abdellatef *et al.*, 2007
spectrophotometry	10–50	-	Pharmaceutical preparations	Rajmane *et al.*, 2009
spectrophotometry	2–40	0.4	Pharmaceutical preparations	Metwally *et al.*, 2007
spectrophotometry	2–8	-	Pharmaceutical preparations	Metwally *et al.*, 2008
membrane selective electrodes	4.34–4335.0	2.94	Tablets and biological fluids	El-Saharty *et al.*, 2006
membrane selective electrodes	0.867–4335.0	0.87	Pharmaceutical preparations	Ibrahim *et al.*, 2005
spectrofluorimetry	0.16–4	0.032	Pharmaceutical preparation	El-Wasseef *et al.*, 2008
luminescence	0.5–300.0	0.16	Pharmaceutical preparation	this work

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