LUMINESCENCE METHOD FOR THE DETERMINATION OF LORAZEPAM IN TABLETS

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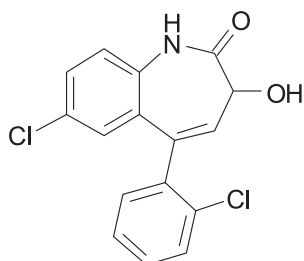
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Abstract: New terbium complex of 7-chloro-5-(2-chlorophenyl)-3-hydroxy-1,2-dihydro-3H-1,4-benzdiazepin-2-one (lorazepam, L), which is highly luminescent and do not require luminescence enhancers, is reported. The luminescence intensity of the Tb – L complex was enhanced by the addition of Tergitol 7 in water solution. The Tb – L – Tergitol 7 complex with a components ratio 1:1:2 was proposed to be used as the analytical form for the luminescence determination of drug – lorazepam. The calibration curve is linear in the 0.05–20.0 µg/mL range of lorazepam (LOD is 0.016 µg/mL). This method was applied for the determination of lorazepam in dosage form – tablets "Apo-lorazepam" – 2.5 mg.

Key words: lorazepam; luminescence, sensitization, terbium (III)

In medicine lorazepam: 7-chloro-5-(2-chlorophenyl)-3-hydroxy-1,2-dihydro-3H,4-benzdiazepin-2-one (L) is used as an anxiolytic and sedative agent.

Lorazepam (L)

The HPLC method is the official Pharmacopeia method for the determination of lorazepam in tablets (1). The extensive application of this preparation in medical practice necessitates its determination in various pharmaceutical preparations and biological fluids (serum, urine, plasma).

Most of the proposed methods for L analysis are spectrophotometric (2, 3), voltammetric (4) and luminescence (5, 6) methods.

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 (7).

The strong ion emission of these complexes is a result of the intramolecular energy transfer process from the ligand to the lanthanide ion. Previously, the determinations of fluoroquinolones (8, 9), non-steroidal anti-inflammatory drugs (10), antibiotics (11), steroids (12) by lanthanide-sensitized luminescence in the dosage forms and in various biological fluids were also described.

In case of anxiolytics based on 1,4-benzodiazepine core, the 4f-luminescence of terbium (III) and europium (III) ions is proposed for 3-hydroxy derivative (oxazepam) (13) and bromazepam (14), respectively, due to their ability to form complexes with Ln (III) ions.

The aim of this research was a detailed study on the sensitization of terbium ion luminescence by lorazepam in the presence of sodium heptadecyl sulfate (Tergitol 7) and the development of a simple, rapid, precise and sensitive method for its determination in tablets without the need of extraction.

EXPERIMENTAL

Apparatus

Luminescence and excitation spectra and lifetimes were measured using a Cary Eclipse luminescence spectrometer (Varian, Australia) with a 150-

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W xenon lamp. All the measurements were performed at room temperature (21–23°C). The pH values of solutions were measured on Lab 850 pH meter (Schott Instruments GmbH, Germany) with a glass electrode. Absorption spectra were recorded on a UV-2401 PC (Shimadzu) spectrophotometer.

Materials and methods

The standard solution of terbium (III) chloride (0.1 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.

An accurately weighted 50 mg of lorazepam (Sigma, L1764) was placed into a 50 mL volumetric flask, mixed with 30 mL of ethanol, stirred and diluted with the same solvent up to 50 mL. Standard solution of the concentration 1.0 mg/mL was obtained. Working solution of lorazepam was obtained by dilution with the same solvent to final concentration of 100 µg/mL.

The solution of sodium heptadecyl sulfate (Tergitol 7) at a concentracion of1×10^{-2} mol/L was prepared by dissolution of an accurately weighed substance in water. The pH of solutions was maintained at 6.5 with 40% urothropine solution.

An urothropine buffer was prepared by dissolving 40.0 g of urothropine in 100 mL volumetric flask with water and adjusting the pH to 6.5 with HCl.

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

Construction of calibration curve

Working solutions of 0.05; 0.1; 0.2; 0.3; 0.4; 0.5; 0.6; 0.7; 0.8; 0.9 mL of lorazepam (100 mg/mL) and 0.1; 0.2 mL of lorazepam standard solution (1 mg/mL) were placed in volumetric flasks. The solutions were diluted with ethanol up to 1 mL, 1.0 mL of a working terbium chloride solution (1×10^{-2} mol/L), 1.5 mL of solution of Tergitol 7 (1×10^{-3} mol/L) and 1.0 mL of urothropine buffer were added to each of these volumetric flasks. Simultaneously, a blank solution, which contained all the components with the exception of lorazepam, 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_{exc} = 270 nm).

Procedure for commercial tablets "Apo-lorazepam" – 2.5 mg

Twenty tablets of the analyzed drug were weighed to calculate the average tablet weight, and were then powdered. The powder equivalent to 5.0 mg of the active ingredient was placed into a 50 mL volumetric flask, mixed with 30 mL of ethanol, stirred, diluted with the same solvent up to 50 mL and filtered to remove insoluble materials. One mL of the filtrate solution was placed into the 10 mL volumetric flask. Further, 1.0 mL of a working terbium chloride solution (1×10^{-2} mol/L), 1.5 mL of Tergitol 7 solution (1×10^{-3} mol/L) and 1.0 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 was measured at λ_{em} = 545 nm (λ_{ex} = 270 nm). I_{lum} of standard solution – 1.0 mL of lorazepam stock solution (100 µg/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 lorazepam (X_1) in one tablet in milligrams was calculated using the formula:

$$X_1 = \frac{I_1 \cdot m_0 \cdot b \cdot 5 \cdot 50 \cdot 10}{I_0 \cdot m_1 \cdot 50 \cdot 50 \cdot 10} = \frac{I_1 m_0 \cdot b \cdot 0.1}{I_0 \cdot m_1}$$

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

RESULTS AND DISCUSSION

Spectral characteristics

The absorption spectrum of lorazepam in ethanol solution is characterized by the presence of two bands with high molar absorption coefficients (ε) in the UV region of the spectrum at λ_{1max} = 221 nm (ε = 2.5 10^4 L·mol^{-1}·cm^{-1}) and λ_{2max} = 339 nm (ε = 3.7 10^2 L·mol^{-1}·cm^{-1}). These coefficients give the possibility for effective absorption of light energy. The energy of triplet level (T) of lorazepam (21550 cm^{-1}) was calculated from the 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 (20500 cm^{-1}), resulting in the possibility of energy transfer from the ligand to the lanthanide ion. The lifetimes (τ) of the test complexes are rather long.

Applying the restricted-logarithm method to the luminescence data, it was found that in the case of reagents' shortage or at equimolar ratio Tb forms complex compounds with L at the component ratio Tb:L_1 = 1:1 and if the reagent is in excess, terbium forms complex compounds with L at the component ratio Tb:L = 1:3.

Table 1. Summary of validation parameters.

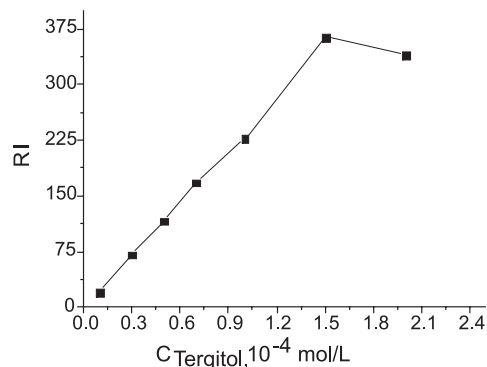| Parameters	Results for lorazepam
Linearity range	0.05-20.0 µg/mL
Limit of detection (LOD)	0.016 µg/mL
Correlation coefficient (r)	0.9985
Accuracy (n = 6)	99.1%
Precision Inter-day (n = 10) Intra-day (n = 10)	 2.8% 3.9%
Specificity	specific

Figure 1. Dependence of the luminescence intensity of the Tb(III)–L complex on concentration of Tergitol 7 ($C_{Tb} = 1 \times 10^{-3}$ mol/L, $C_L = 20$ µg/mL)

Influence of Tergitol 7 and terbium concentracion

The presence of sodium heptadecyl sulfate (Tergitol 7) significantly increased the $I_{lum.}$ Tb(III) in complex with lorazepam. The dependence of I_{lum} on the Tergitol 7 concentration is shown in Figure1. The maximum luminescence was observed at $1,5 \cdot 10^{-4}$ mol/L of Tergitol 7. The increase of luminecsence in the presence of Tergitol 7 could be explained by the structure microregulation and rigidity of the compound formed and by the displacement of water molecules from the inner sphere.

From the luminescence intensity data for the complex, the ratio of component Lorazepam: Tb^{3+}:Tergitol 7 was established as equal to 1:1:2 by the restricted-logarithm method.

The effect of terbium concentration on the analytical signal for lorazepam – Tb^{3+} – Tergitol 7 ternary complex was studied. A terbium concentration of $1 \cdot 10^{-3}$ mol/L was selected for the measurements.

It was established that luminescence intensity of the complex Tb(III)–L–Tergitol 7 reached the maximum in 5 min after preparation of solutions and remained constant for 2 h that proved its photostability. The interaction in Tb(III)–L–Tergitol 7 system was proved by the increase in lifetime of the excited state of $^5D_4$ Tb(III) ions in ternary system (Fig. 2a). The excitation spectra (b) of Tb(III)–L indicated strong interaction between Tb–L and Tergitol 7 (Fig. 2b).
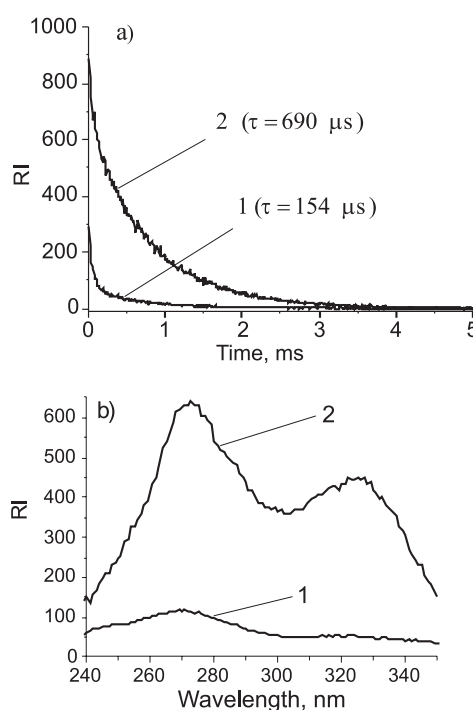

Figure 2. Fluorescence decay curves (a) and excitation spectra (b) of Tb(III)–L complex without (1) and in the presence (2) of Tergitol 7 ($C_{Tb} = 1 \times 10^{-3}$ mol/L; $C_L = 20$ µg/mL; $C_{Tergitol\ 7} = 1,5 \times 10^{-4}$ mol/L ;pH 6.5; $\lambda_{em} = 545$ nm)

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

Label claim (mg/tablet)	Amount added (%)	Amount added (mg)	Amount found (mg)	Recovery (%)	RSD %
Lorazepam 2.5	80	2.0	1.95 ± 0.086	97.5	4.2
	100	2.5	2.57 ± 0.092	102.8	3.4
	120	3.0	2.91 ± 0.089	97.0	2.9
				Average recovery: 99.1	

Table 3. Determination of lorazepam in tablets "Apo-lorazepam"
– 2.5 mg (n = 5, p = 95%).

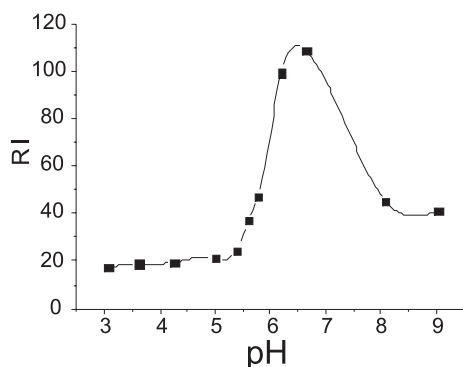| Batch No.	Found, mg	RSD %
871009	2.47 ± 0.09	2.9
881109	2.54 ± 0.10	3.1
891209	2.52 ± 0.08	2.7

Figure 3. Dependence of the luminescence intensity of the Tb(III)–L complex on pH (C_{Tb} = 1×10^{-3} mol/L, C_L = 20 µg/mL)

Figure 4. Luminescence spectra (a) and calibration curve (b) for lorazepam determination (C_{Tb} =1×10^{-3} mol/L, $C_{Tergitol\ 7}$ = 1.5×10^{-4} mol/L; pH 6.5)

Influence of pH and ligand amount of the components

The complexation of Tb(III) with the ligand occurs in a wide range of pH values from 3 to 9 (Fig. 3). The maximal luminescence intensity of the complexes Tb(III)–L and Tb(III)–L–Tergitol 7 is observed at pH 6.2–6.7. Upon the lower pH values (in acid solutions, pH < 2) the complex, evidently, does not form or the degree of its formation is very low. In alkaline solutions (pH > 8) the decomposition of complexes with formation of the terbium hydroxide is observed. The pH of solutions was maintained at 6.5 with urothropine buffer.
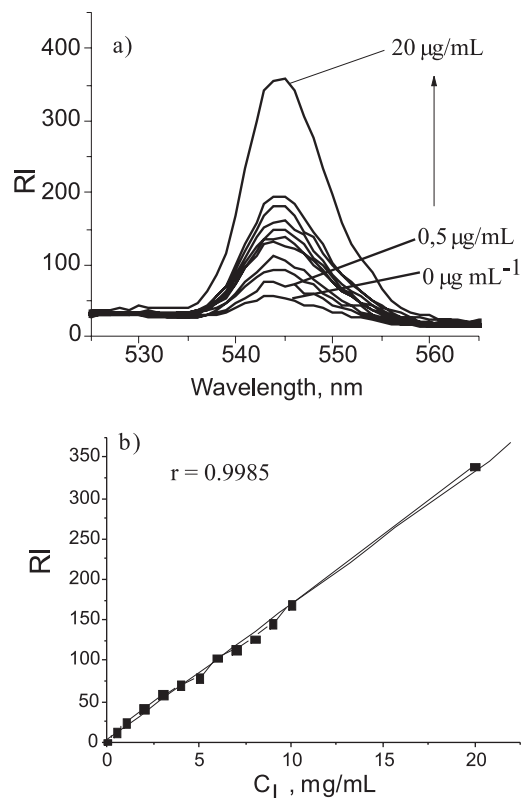
Analytical performance

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 lorazepam concentration was developed using Tb(III)–L–Tergitol 7 complex. The proposed method was evaluated by statistical analy-

Table 4. Overview on selected assays for determination of lorazepam.

Method	Linear range	Limit of detection [µg/mL]	Sample [µg/mL]	Ref.
spectrophotometry	0.5 – 8.75	–	Pharmaceutical dosage form	2
spectrophotometry	1.0 – 20.0	0.33	Pharmaceutical dosage form	3
voltammetry	0.05 – 1.15	0.019	Pharmaceutical formulations and biological fluids	4
luminescence	1.0 – 8.0	0.004	Pharmaceutical preparation	5
luminescence	0.05 – 5.0	0.016	Pharmaceutical preparation (drug in tablets)	6
luminescence	0.05 – 20.0	0.016	Pharmaceutical preparation (drug in tablets)	this work

sis of the experimental data by fitting the overall least squares line according to RI = a + bc (RI = 2.99 + 16.57 c; correlation coefficient is 0.9985), where RI is the luminescence intensity of Tb(III)-L-Tergitol 7 complex that was calculated as RI = (I-I_0) /I_0, where I_0 and I are the relative luminescence intensities of the system without and with lorazepam, respectively; c is the concentration of lorazepam (µg/mL). The calibration curve is linear in the 0.05–20.0 µg/mL range of lorazepam (Fig. 4). The signal-to-noise ratio of 3 was considered as the limit of detection (LOD). The LOD for lorazepam was 0.016 µg/mL.

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).

The precision of the method was established by testing the analytical signal corresponding to lorazepam concentration of 10 µg/mL. For a series of 10 measurements, the relative standard deviation was 2.8% for the intra-days and 3.9% for the inter-day analysis (p = 95% (confidence level)) for lorazepam.

In order to confirm the specificity of the proposed method the effect of some common excipients used in pharmaceutical preparations (sucrose, lactose, glucose, sorbitol, starch, magnesium stearate, talc, microcrystalline cellulose) was studied by analyzing sample solutions containing a fixed amount of lorazepam (10 µg/mL) with various amounts of each excipient. No interference could be observed with the proposed method.

This method was used to assay the active ingredient – lorazepam in dosage form – tablets "*Apo-lorazepam*" («*Apotex*», Canada) – 2.5 mg. The content of lorazepam in dosage form in milligrams was calculated by the standard sample method using the above formula. Three batches of lorazepam tablets were analyzed. The results are shown in Table 3**.**

In comparison with the spectrophotometric methods reported, as shown in Table 4, the proposed method in this paper offers higher sensitivity and a wider linear range. In addition, this method is faster and simpler than HPLC method. Tb(III)–L–Tergitol 7 complex shows a large Stokes' shift of 275 nm which facilitates the separation of excitation and emission spectra. This can be very useful if simple luminescence instrumentation with filters or microplate readers are employed for detection.

CONCLUSIONS

In this work, it was found that lorazepam can sensibilize the intrinsic luminescence of the lanthanide ion. We determined the spectroscopic and luminescence characteristics of Tb(III)–L complex . When Tergitol 7 was added to the above systems, the luminescence was significantly increased. The complex Tb(III)–L–Tergitol 7 was used for the determination of lorazepam. The proposed luminescence method for the determination of lorazepam is simple, reliable, sensitive with the advantage of a wide determination range that does not require extraction. This method can be successfully applied in the micro determination of lorazepam in pharmaceutical formulations.

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