J Fluoresc (2011) 21:2229–2235
DOI 10.1007/s10895-011-0927-y

ORIGINAL PAPER

Cilostazol Determination by the Enhancement of the Green Emission of Tb^{3+} Optical Sensor

M. S. Attia · W. H. Mahmoud · A. O. Youssef · M. S. Mostafa

Received: 19 May 2011 / Accepted: 28 July 2011 / Published online: 5 August 2011

Abstract The efficiency of excited-state interaction between Tb^{3+} and the industrial product Cilostazol (CIL) has been studied in different solvents. High luminescence intensity peak at 545 nm of terbium complex in acetonitrile was obtained. The photophysical properties of the green emissive Tb^{3+} complex have been elucidated, the terbium was used as optical sensor for the assessment of CIL in the pharmaceutical tablets and body fluids at pH 3.1 and λ_{ex}= 320 nm with a concentration range 1.0×10^{-9}–1.0×10^{-6} mol L^{-1} of CIL, correlation coefficient of 0.998 and detection limit of 7.5×10^{-10} mol L^{-1}.

Keywords Cilostazol · Terbium(III) · Enhancing · Luminescence · Optical sensor

Introduction

Cilastazol (CIL), 6-[4-(1-cyclohexyl-1*H*-tetrazol-5-yl) butoxy]-3, 4-dihydro-2(1*H*)-quinolinone [Fig. 1] is a potent phosphodiesterase inhibitor; its major effects are prevention of platelet aggregation and dilation of blood vessels via an increase in tissue CAMP levels [1–4]. CIL is approved for the treatment of intermittent claudication. Only few methods were performed for the determination of CIL. It was determined in the presence of some of its metabolites in liver microsomal solutions [5], and in human plasma and urine using HPLC with gradient elution and by either UV [6, 7] or MS detection [8]. Also, HPLC methods were reported for its determination in human plasma according to the ICH guidelines [9, 10]. The HPLC/tandem mass spectrometry assay for the determination of CIL in Wistar rat plasma has been reported [11]. In this work, CIL concentration was determined by the complexation between CIL as a ligand and the Tb^{3+} ion and the possibility of the enhancement of the Tb^{3+} luminescence sensitized by CIL was established and investigated. The absorption and emission spectra of CIL and (CIL-Tb^{3+}) complex were measured in acetonitrile at pH 3.1. This method is simple, accurate and can successfully be applied to the determination of CIL in pharmaceutical preparation and in serum samples with remarkably satisfactory results.

M. S. Attia (✉) · A. O. Youssef · M. S. Mostafa
Department of Chemistry, Faculty of Science,
Ain Shams University,
Cairo, Egypt
e-mail: Mohamed_sam@yahoo.com

W. H. Mahmoud
Chemistry Department, Faculty of Applied Science,
Taibah University,
Al-Madinah Al-Munawarah, Kingdom of Saudi Arabia

Experimental

Materials

Pure standard CIL supplied by the National organization for Drug control and Research (Giza, Egypt). Pharmaceutical preparation of Sedotazole tablets containing 50 mg of CIL produced by SEDIO Pharmaceutical Co., 6 October City, Egypt is purchased from local market.

Reagents

All chemicals used are of analytical grade and pure solvents were purchased from (Aldrich). A stock solution of CIL

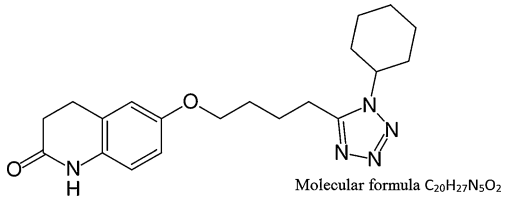

Fig. 1 Chemical structure of Cilostazol

(10^{-2} mol L^{-1}) was freshly prepared by dissolving 0.093 g in 25 ml pure methanol. More diluted solution (1.0×10^{-4} mol L^{-1}) was prepared by appropriate dilution with acetonitrile. Stock and working solutions are stored at 20 °C when are not in use.

A Tb^{3+} ion stock solution (10^{-2} mol L^{-1}) was prepared by dissolving 0.0109 g$Tb(NO_3)_3.5H_2O$ (delivered from Aldrich-99.99%) in small amount of ethanol in 25 ml measuring flask, then dilute to the mark with ethanol. The working solution of Tb^{3+} ion of 3×10^{-4} mol L^{-1} was obtained by appropriate dilution with acetonitrile. The pH=3.1 was adjusted by using 0.1 mol L^{-1} of NH_4OH/HCl solutions.

Apparatus

All luminescence measurements were carried out on Shimadzu RF5301 (PC) spectrofluorophotometer in the range 290–750 nm. The absorption spectra were recorded with a Unicam UV-Visible double beam spectrophotometer from Helios. The spectrophotometer is equipped with a temperature-controller cell holder. All pH measurements were made with a pHs-JAN-WAY 3040 ion analyzer.

General Procedure

To 10 ml measuring flasks, solutions were added in the following order: 0.1 ml of 1×10^{-2} mol L^{-1} CIL solution and 0.3 ml of 1×10^{-2} mol L^{-1} Tb^{3+} solution to give 1×10^{-4} mol L^{-1} of CIL and 3×10^{-4} mol L^{-1} of Tb^{3+}. The mixture was diluted to the mark with acetonitrile and pH was adjusted at 3.1 by using 0.1 mol L^{-1} of NH_4OH/HCl solutions. The above procedure was used for the subsequent measurements of absorption, emission spectra and effect of pH and solvents. The luminescence intensity was measured at $\lambda_{ex}/\lambda_{em}$=320/545 nm.

Determination of CIL in Pharmaceutical Preparations

Five tablets of Sedotazole were carefully weighed and ground to finely divided powders. Accurate weights equivalent to 1.5 mg sedotazole were dissolved in 50 ml acetonitrile and mixed well and filtered up using 12 mm filter papers. The concentration of the drug was determined by using different concentrations from the corresponding calibration graph.

Determination of CIL in Serum Solution

3 mL of trichloroacetic acid was added to 1.0 mL serum of a real health volunteer and the solution was centrifuged for 15 min at 4,000 r/min to remove proteins, then 100 μL of the serum was added to 0.3 mL of Tb^{3+} stock solution (1×10^{-2} mol L^{-1}) in 10 mL measuring flask and complete to the mark with acetonitrile and the pH was adjusted to 3.1. The luminescence intensity of the test solution was measured before and after addition of Tb^{3+} optical sensor. The change in the luminescence intensity was used for determination of CIL in serum sample.

Determination of CIL in Urine Solution

Urine sample of healthy people was collected from volunteer who received a single oral dose of 50 mg of Sedotazole tablet. The treatment procedure of used urine sample was carried out according to the method described by N.A. Al-Arfaj [12]. 1 mL urine sample was pipetted into clean 10 mL centrifugation vial. 0.1 mL of 0.1 mol/L NaOH solution was added, shaken for few seconds, followed by the addition of 5 mL dichloromethane. The mixture was vortex mixed at high speed for 2 min and then centrifuged at 4,000 rpm for 10 min. The resulting supernatant was transferred to a small conical flask. The extract was evaporated to dryness at 60 °C and the residue was dissolved in 0.5 mL water and then analyzed according to the proposed procedure

Result and Discussion

Absorption and Emission Spectra

The absorption spectra of CIL and Tb^{3+}- CIL complex are shown in Fig. 2. Comparing the spectrum of the drug with its spectra after the addition of different concentrations of Tb^{3+} ion into CIL in acetonitrile, a red shift was observed and the absorbance is also enhanced which indicates that CIL can form a complex with Tb^{3+} ion.

The emission spectra of Tb^{3+}– CIL complex in different concentrations of CIL are shown in Fig. 3. After the addition of different concentrations of CIL into the Tb^{3+} ion in acetonitrile, the intensity of the characteristic peak at 545 nm of Tb^{3+} was enhanced indicating that CIL can form a complex with Tb^{3+} ion. The characteristic peaks of Tb^{3+} ion appear at ($^5D_4 \rightarrow {}^7F_6$=490 nm, $^5D_4 \rightarrow {}^7F_5$=545 nm, $^5D_4 \rightarrow {}^7F_4$=590 nm, $^5D_4 \rightarrow {}^7F_3$=620 nm and $^5D_4 \rightarrow {}^7F_2$= 650 nm).

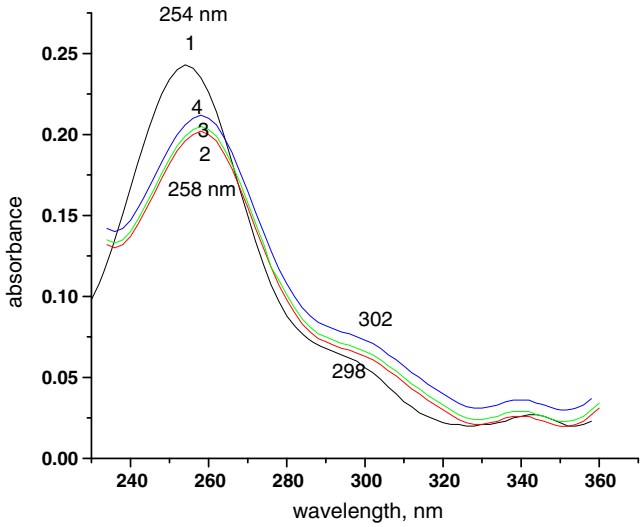

Fig. 2 Absorption spectra of CIL in the presence of different concentrations of Tb^{3+}

Effect of Experimental Variables

Effect of the Amount of CIL and Tb^{3+}

The ion titration revealed that the complex formed M : L (3 : 1) for Tb^{3+} and CIL, which indicates that the metal may coordinate to the ligand from different coordination sites and not only through oxygen of the ketone ring, but also the more preferred coordination sites are the O of the ketone group and the nitrogen of the amide ring because they have the highest negative charges as indicated by DFT calculations [12].

The unusual stability of lanthanide ion with tetrazine ring that have one or two binding sites was dominated by two primary factors: (1) ion-dipole interaction between metal ion and keto-amide ring donating oxygens, and (2) long-range interaction between metal ion and nitrogen atoms of the tetrazine ring [12].

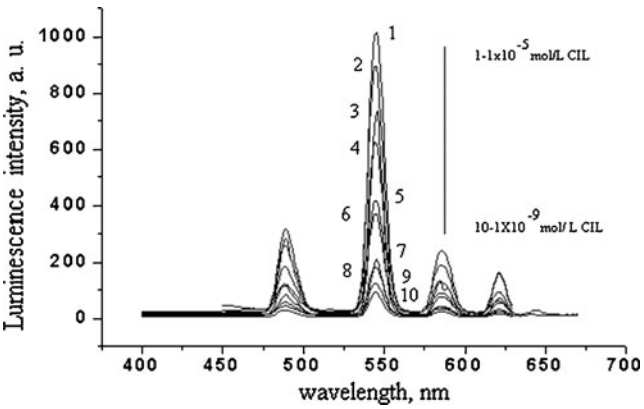

Fig. 3 Luminescence spectra of Tb^{3+} in presence of different concentrations of CIL in acetonitrile at λ_{ex}=320 nm

Effect of pH

The pH of the medium has a great effect on the luminescence intensity of the Tb-CIL. The pH has been adjusted using NH_4OH and HCl solutions. The optimum pH value where the peak at 545 nm has the highest intensity was obtained at pH=3.1, Fig. 4.

Effect of Solvent

The influence of the solvent on the luminescence intensities of the solutions containing 1.0×10^{-4} mol L^{-1} of CIL and 3.0×10^{-4} mol L^{-1} Tb^{3+} was studied under the conditions established above. The results show the enhanced emission of Tb^{3+}-CIL in CH_3CN. This can be attributed to the formation of anhydrous solvates of Tb^{3+}-CIL complex introducing solvent molecules in the first coordination sphere of Tb^{3+}-CIL leads to the enhancement of the intensity of all transitions ($^5D_4 \rightarrow {}^7F_6$=490 nm, $^5D_4 \rightarrow {}^7F_5$=545 nm, $^5D_4 \rightarrow {}^7F_4$=590 nm, $^5D_4 \rightarrow {}^7F_3$=620 nm and $^5D_4 \rightarrow {}^7F_2$= 650 nm). especially $^5D_4 \rightarrow {}^7F_5$ transition in Tb^{3+} Fig. 5.

By increasing the radiative rate, Tb^{3+} excited states will become less sensitive to deactivation processes, ultimately resulting in a more efficiently emissive Tb^{3+} complex. Also, the luminescence intensities for the complexes in CH_3CN solutions are stronger than in ethanol as hydroxy solvent. This may be due to vibrational energy transfer to the solvent molecules. It is well know that the excited state of the lanthanide ions is efficiently quenched by interactions with high-energy vibrations like O-H groups thereby the luminescence of this complex in –OH containing solvents can be quenched easily because of the O-H oscillators [13–15].

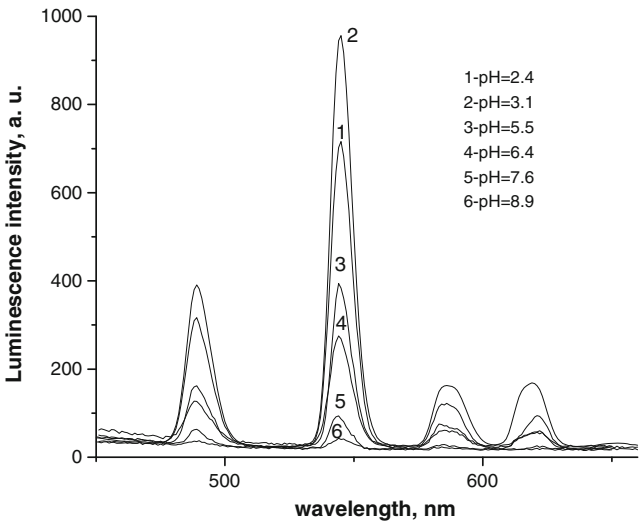

Fig. 4 Luminescence spectra of Tb $^{3+}$- CIL in different pH at λ_{ex}= 320 nm

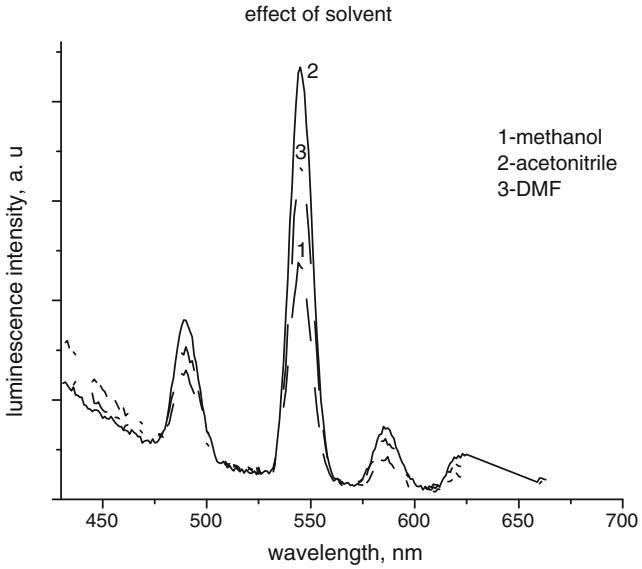

Fig. 5 Luminescence spectra of Tb^{3+}- CIL in different solvents

Analytical Performance

Method Validation

Analytical Parameters of Optical Sensor Method A linear correlation was found between luminescence intensity of CIL– Tb^{3+} complex at λ_{em}=545 nm and concentration of CIL in the ranges given in Table 1 and Fig. 6. The six-points (1,000, 500, 200, 150, 20, and 1 n mol L^{-1}) calibration curve was obtained by plotting the peak intensity of Tb^{3+} at λ_{em}=545 nm versus the concentration of CIL and the graph was described by the regression equation:

$$Y = a + bX$$

(Where Y = luminescence intensity of the optical sensor at λ_{em}=545 nm; a = intercept; b = slope and X = concentration in ng mL^{-1}). Regression analysis of luminescence intensity data using the method of least squares was made to evaluate the slope (b), intercept (a) and correlation coefficient (r) and the values were presented in Table 1. The limit of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines [16] using the formulae:

$LOD = 3.3S/b$ and $LOQ = 10S/b$, (where S is the standard deviation of blank luminescence intensity values, and b is the slope of the calibration plot) are also presented in Table 1. The low value of LOD indicates the high sensitivity of the proposed method when compared by other methods (Table 2).

Accuracy and Precision of the Method To compute the accuracy and precision, the assays described under "general procedures" were repeated three times within the day to determine the repeatability (intra-day precision) and three times on different days to determine the intermediate precision (inter-day precision) of the method.

These assays were performed for three levels of analyte. The results of this study are summarized in Table 3. The percentage relative standard deviation (% RSD) values were 1.29–1.19% (intra-day) and 1.13–1.11% (inter-day) for sedotazole tables, (%RSD) values were 1.26–1.15% (intra-day) and 1.11–1.02% (inter-day) for serum sample and (%RSD) values were 2.35–1.86% (intra-day) and 2.35–1.51% (inter-day) for urine sample indicating high precision of the method. Accuracy was evaluated as percentage relative error (RE) between the measured mean concentrations and the taken concentrations of CIL. Bias{bias% = [(Concentration found–known concentration1) × 100/known concentration]} was calculated at each concentration and these results are also

Table 1 Sensitivity and regression parameters for chemosensor

Parameter	Method
λ_{em}, nm	545
Linear range, mol L^{-1}	1.0×10^{-6}–1.0×10^{-9} (0.369–369 μg L^{-1})
Limit of detection (LOD), mol L^{-1}	7.5×10^{-10} (0.28 μg L^{-1})
Intercept (a)	96.5
Slope (b)	0.6×10^{9}
Standard deviation	14.9
Regression coefficient (r)	0.998

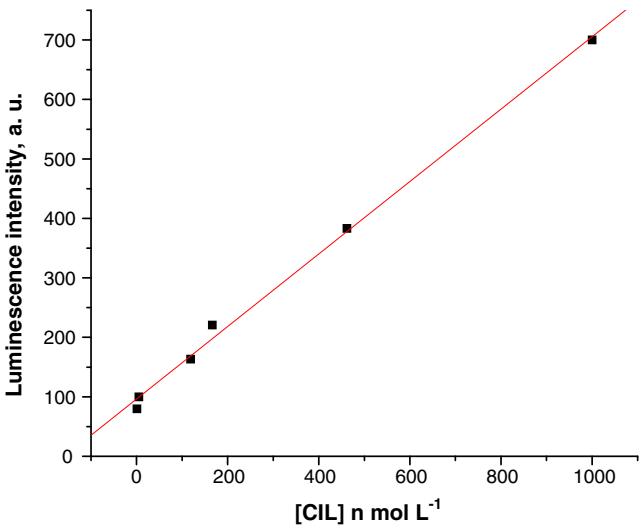

Fig. 6 Linear relationship between luminescence intensity of Tb^{3+}-CIL and different concentrations of CIL

Table 2 Comparison of spectrofluorimetric technique with some existing methods for the determination of cilostazol

Method	Linear range	Detection limit	Reference
HPLC-UV in human liver microsomal solution	1,000–20,000 μg L^{-1}	1,000 μg L^{-1}	[5]
HPLC in human plasma with gradient elution and by UV detection	20–1,200 μg L^{-1}	20 μg L^{-1}	[6]
HPLC in human urine with gradient elution and by UV detection	100–3,000 μg L^{-1}	100 μg L^{-1}	[7]
HPLC in human plasma with gradient elution and by mass detection	5.0–1200.0 μg L^{-1}	5.0 μg L^{-1}	[8]
HPLC in human plasma	25–2,000 μg L^{-1}	25 μg L^{-1}	[9]
According to ICH guidelines	1–31×10^3 μg L^{-1}	24×10^3 μg L^{-1} 72×10^3 μg L^{-1}	[10]
HPLC in Wistar rat plasma	20–2,000 μg L^{-1}	20 μg L^{-1}	[11]
CIP-Tb^{3+} optical sensor (Chemosensor method)	0.369–369 μg L^{-1}	0.28 μg L^{-1}	Present work

presented in Table 3. Percent relative error (%RE) values of 0.23–0.75% (intra-day) and 0.83–2.5% (inter-day) for sedotazole tablets;%RE values were 0.33–2.75% (intra-day) and 0.25–1.62% (inter-day) for serum sample and% RE values were 0.83–4.0% (intra-day) and 2.33–4.75% (inter-day) for urine sample demonstrates the high accuracy of the proposed method.

Selectivity The proposed method was tested for selectivity by placebo blank and synthetic mixture analysis. A placebo blank containing talc (250 mg), starch (300 mg), lactose (30 mg), calcium carbonate (50 mg), calcium dihydrogen orthophosphate (20 mg), methyl cellulose (40 mg), sodium alginate (70 mg) and magnesium stearate (100 mg) was extracted with water and solution made as described under "analysis of dosage forms". A convenient aliquot of solution was subjected to analysis according to the recommended procedures. In the method of analysis, there was no interference by the inactive ingredients.

A separate test was performed by applying the proposed method to the determination of CIL in a synthetic mixture. To the placebo blank of similar composition, different amount of CIL of sedotazole product was added, homogenized and the solution of the synthetic mixture was prepared as done under "analysis of dosage forms". The filtrate was collected in a 100-mL flask. 1.5, 4 and 6.5 mL of the resulting solution was assayed (n=3) by proposed method which yielded a% average recovery of 100.2±1.43, 99.6±0.7 and 103.1±1.70 for tablet, serum and urine samples, respectively (Table 4). The results demonstrated the accuracy as well as the precision of the proposed

Table 3 Evaluation of intra-day and inter-day accuracy and precision

Sample	CIL taken[a]	Intra-day accuracy and precision (n=3)			Inter-day accuracy and precision (n=3)		
		CIL Average Found ± CL[b]	%RE[c]	%RSD[d]	CIL average found ± CL	%RE	%RSD
Sedotazol	4.0	4.03±0.13	0.75	1.29	4.06±0.11	1.50	1.13
	6.0	5.95±0.18	0.83	1.23	6.05±0.17	0.83	1.12
	8.0	8.19±0.24	2.37	1.19	8.20±0.23	2.50	1.11
Serum sample	4.0	4.11±0.13	2.75	1.26	3.99±0.11	0.25	1.11
	6.0	6.02±0.18	0.33	1.21	6.04±0.16	0.66	1.06
	8.0	7.89±0.26	1.37	1.15	8.13±0.21	1.62	1.02
Urine sample	4.0	4.16±0.23	4.00	2.22	4.19±0.21	4.75	2.01
	6.0	6.05±0.28	0.83	1.86	6.14±0.36	2.33	2.35
	8.0	8.19±0.48	2.37	2.35	8.23±0.31	2.87	1.51

[a] The values are multiplied by 10^{-7} mol L^{-1} for method

[b] CL. Confidence limits were calculated from: . (The tabulated value of t is 4.303, at the 95% confidence level; S = standard deviation and n = number of measurements.

[c] % RE. Percent relative error

[d] % RSD. relative standard deviation

Table 4 Determination of (CIL) in biological fluids and pharmaceutical preparations using Tb^{3+} - (CIL)—optical sensor

Drug	Added ($\times 10^{-8}$ mol L^{-1})	Found ($\times 10^{-8}$ M)	Average[a]	Average recovery ± R.S.D. (%)	B.P. (LC)
Sedotazol	1.5	1.52, 1.48, 1.57	1.52	100.2±1.43	99.5±1.0
	4.0	3.97, 4.05, 4.03	4.01		
	6.5	6.55, 6.46, 6.46	6.49		
Serum sample	1.5	1.49, 1.53, 1.52	1.51	99.6±0.70	99.8±0.4
	4.0	3.95, 3.99, 3.98	3.97		
	6.5	6.51, 6.45, 6.48	6.48		
Urine sample	1.5	1.39, 1.43, 1.62	1.48	103.1±1.70	98.8±0.9
	4.0	3.85, 3.89, 3.88	3.87		
	6.5	6.41, 6.55, 6.58	6.51		

[a] Average of nine measurements

methods. These results complement the findings of the placebo blank analysis with respect to selectivity.

Application to Formulations The proposed method was applied to the determination of CIL in one representative tablet of sedotazol was purchased from local market and containing other inactive ingredients and in serum sample of the health state human. The results in Table 4 show that the method is successful for the determination of CIL and that the excipients in the dosage forms did not interfere. The results obtained (Table 4) were statistically compared with the official British Pharmacopoeia [B.P] method [17]. The average recovery and R.S.D for the tablet, serum and urine sample in proposed method were (100.2%, 99.6 and 103.1%) respectively. Data obtained by B. P method average recovery 99.5%, 99.8 and 98.8 for the tablet, serum and urine samples respectively; and R.S.D was also presented for comparison and shows a good correlation with those obtained by the proposed method. The results obtained by the proposed method agreed well with those of reference method and with the label claim (Table 4).

Stability No significant loss of CIL (5.22, 5.63 and 8.22% R. S.D. for tablet, serum and urine samples, respectively) was observed after storage of pharmaceutical tablet and serum samples at room temperature for at least 24 h (Table 5). Pharmaceutical tablet, serum and urine samples were stable over at least three freeze–thaw cycles (Table 5), indicating that the pharmaceutical tablet, serum and urine samples can be frozen and thawed at least three times prior to analysis.

Conclusion

The Tb^{3+} ion in acetonitrile has high sensitive and characteristic peaks in the presence of CIL. The proposed method for the determination of CIL offers simple, rapid and sensitive method for the analysis of CIL in acetonitrile and pH 3.2 with a linear range of 1×10^{-9}–1×10^{-6} mol L^{-1} and detection limit of 7.5×10^{-10} mol L^{-1}. The developed optical sensor is selective, accurate and attractive for routine control analysis of the drug.

Table 5 Freeze–thaw stability of CIL in pharmaceutical tablets and human biological fluids (n=3)

Drug	Normal concentration $\times 10^{-8}$ mol L^{-1})	Found average recovery $\times 10^{-8}$ mol L^{-1}			R.S.D. (%)
		0 day	15 days	30 days	
Sedotazol	10	10.2	10.6	09.6	5.22
	40	40.0	40.1	40.3	
	80	80.2	79.7	79.5	
Serum sample	10	10.2	10.4	10.7	5.63
	40	40.2	40.4	40.6	
	80	79.9	79.7	79.5	
Urine sample	10	10.4	10.4	11.1	8.22
	40	40.7	40.9	41.0	
	80	79.5	79.5	79.2	

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