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Spectrofluorimetric assessment of Ramipril using optical sensor Samarium ion–doxycycline complex doped in sol–gel matrix

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ABSTRACT

A new, simple, sensitive and selective spectrofluorimetric method for the determination of Ramipril is developed. The Ramipril can remarkably quench the luminescence intensity of the Sm^{3+} ion in Sm^{3+}–doxycycline complex at λ_{ex} = 375 nm in sol–gel matrix. In the same time the intensity of the emission band of the Ramipril in DMSO at 454 nm is increased due to the energy transfer from the Sm^{3+}–doxycycline complex to Ramipril in the excited stated. The quenching of luminescence intensity of Sm^{3+}–doxycycline complex doped in the sol–gel matrix and the enhancement of the emission band of Ramipril at 454 nm are directly proportion to the concentration of Ramipril with a dynamic ranges of 3.4×10^{-9}–1.0×10^{-7} mol l^{-1} and 2.4×10^{-9}–1.0×10^{-7} mol l^{-1} and detection limits of 6.0×10^{-10} and 5.2×10^{-10} mol l^{-1}, respectively.

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1. Introduction

Ramipril, 2-[*N*-[(*S*)-1-ethoxy carbonyl-3-phenyl propyl]-lalanyl]-(1*S*, 3*S*, 5*S*)-2-azabicyclo [3,3,0]-octane-3-carboxylic acid is a prodrug [1] which is rapidly hydrolyzed with the cleavage of an ester group through hepatic metabolism in human body forming an active metabolite, i.e., Ramiprilat.

The spectrophotometric methods have been reported for the assessment of drug content in commercial dosage forms, which are based on the formation of ternary complex of the drug with Cu (II)–eosin [2] and Fe (III)–ammonium thiocyanate [3]. The drug content in pharmaceutical formulations has been determined spectrophotometrically in visible region based on the charge transfer reaction of Ramipril with π-acceptors such as 7,7,8,8-tetracyanoquinodimethane and *p*-chloranilic acid then subsequently measuring the absorbance at 840 and 520 nm, respectively [4]. The quantitation of Ramipril has been done by spectrophotometric and fluorimetric techniques using the reaction of the drug with 7-fluoro-4-nitrobenzo-2-oxo-1,3-diazole which exhibits maximum absorbance at 460 nm, and maximum fluorescence intensity at 530 nm after excitation at 465 nm [5].

More recently, a spectrophotometric kinetic method based on the reaction of the carboxylic acid group of the drug with a mixture of potassium iodate (KIO_3) and potassium iodide (KI) in aqueous medium has been reported [6]. A spectrophotometric and spectrofluorimetric method for determination of Ramipril based on the oxidation of the drug with 1-chlorobenzotriazole reagent (CBT) in strong alkaline medium is followed by measuring the absorbance at 350 nm has been reported [7].

The concentration of Ramipril in human plasma and pharmaceutical formulations were measured by gas chromatography–mass spectrometric (GC–MS) [8,9], high-performance liquid chromatography (HPLC) [10,11], voltammetric [12], radioimmunoassay [13], potentiometry [14,15] and flow-injection analysis [16].

However, these published methods suffered from either requiring time-consuming derivatization technique or had relatively high detection limits (i.e., in microgram level). In addition, Ramipril per se is characterized by its low ability to absorb light in the UV region.

In this work, the Ramipril concentration was determined by the optical sensor Sm^{3+}–doxycycline complex doped in the sol–gel matrix. The absorption and emission spectra of Ramipril, doxycycline and Sm^{3+}–doxycycline complex were measured in sol–gel matrix. In comparison with other spectrofluorimetric techniques, this method is simple, relatively interference free from coexisting substances and can successfully be applied to the determination of Ramipril in pharmaceutical preparations and in serum samples with remarkably satisfactory results.

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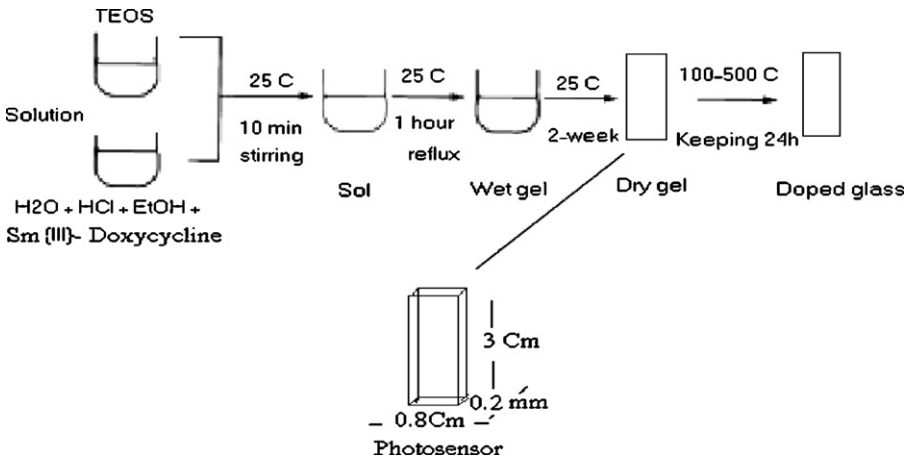

Fig. 1. Preparation of optical sensor (lanthanide complex doped in sol–gel matrix).

2. Experimental

2.1. Chemicals and reagents

All chemicals used are analytical-reagent of higher grade. Pure standard of Ramipril is either purchased from Sigma or supplied by the National Organization for Drug Control and Research (Cairo, Egypt). Pharmaceutical preparations, Corpril, Ramipril and Tritace tablets containing 5 mg for each tablet of Ramipril, produced by Aventis Co., Egypt are purchased from local market.

Distilled water and pure grade solvents from (Aldrich) are used for the preparation of all solutions and during the all determinations. A stock solution of Ramipril (5×10^{-4} mol l^{-1}) is freshly prepared and dissolved in ethanol and stored at 4 °C when not in use. The working standard solution of (5×10^{-5} mol l^{-1}) is freshly prepared by appropriate dilution with dimethylsulphoxide (DMSO). A stock solution of doxycycline hydrochloride (DC) (5×10^{-3} mol l^{-1}) is directly prepared and dissolved in ethanol. The stock solution given above is stored at 0–4 °C when not in use.

A Sm^{3+} ion stock solution (5×10^{-3} mol l^{-1}) is prepared by dissolving $SmCl_3$ (delivered from Aldrich, 99.99%) with a small amount of ethanol in 100 ml measuring flask, then diluting to the mark with ethanol.

2.2. Apparatus

All luminescence measurements are carried out on Shimadzu RF5301 spectrofluorophotometer in the range (290–750 nm). The absorption spectra are recorded with a Unicam UV–visible double-beam spectrophotometer from Helios Company. It employs a Tungsten filament light source and a deuterium lamp, which has a continuous spectrum in the ultraviolet region. The spectrophotometer is equipped with a temperature-controller cell holder. (All measurements are measured at Photoenergy Center, Faculty of Science, Ain Shams Univ.)

2.3. General procedure

2.3.1. Preparation of lanthanide complex doped in sol–gel matrix

The complex of (DC) with the Sm^{3+} ion was prepared by mixing the doxycycline at concentration of 3×10^{-4} mol l^{-1} and 1×10^{-3} mol l^{-1} of $SmCl_3 \cdot 6H_2O$ in a molar ratio of 1:3 (or above) in spectral grade dry ethanol at room temperature. A white precipitate was obtained and was separated from the solution by filtration. A hydrous complex was obtained from the solid by washing with ether and drying. Mixture consisting of TEOS (tetraethoxysilane), C_2H_5OH and H_2O in a molar ratio of 1:5:1 was refluxed for 1 h to give precursor sol solutions, using a few drops of diluted HCl solution as a catalyst. Subsequently, appropriate amount of the complex and the precursor solution were mixed and stirred together for 15 min until the mixture become homogeneous. The obtained complex-dispersed sol solution was casted into polystyrene cup with diameters (3 cm, 0.2 mm, 0.8 cm) and kept at 25 °C in air for 2 weeks then heating at 100–500 °C for 24 h to give solidified and transparent composite sample (Fig. 1).

2.3.2. Preparation of Ramipril solutions

To 10 ml clean and sterilized measuring flasks, the standard solutions of Ramipril are prepared by different additions of (5×10^{-5} mol l^{-1}) Ramipril solution to give different concentrations of Ramipril. The solutions are diluted to the mark with DMSO at room temperature. The above method is used for the subsequent measurements of absorption, emission spectra and effect solvents. The luminescence intensity is measured at $\lambda_{ex}/\lambda_{em}$ = 375/645 nm.

2.3.3. Measurement procedures of the luminescence spectrum of the optical sensor Sm–DC doped in sol–gel matrix in different standard solutions of Ramipril in DMSO

After the preparation of the different standard solutions of Ramipril in DMSO according to Section 2.3.2 the optical sensor Sm–DC doped in sol–gel matrix will immersed in each standard solution of Ramipril in the cell of the spectrofluorimetric device then the luminescence spectrum will be measured at the excitation wavelength. The optical sensor must be rinsed after each measurement by DMSO. Then draw the peak intensity at λ = 645 nm on *y* axis against 1/concentration of Ramipril on *x* axis and the peak intensity at λ = 454 nm of Ramipril against concentration of Ramipril (1×10^4, 8×10^3, 4×10^3, 800, 400, 80, 40, 8, 4, 0.8 nmol l^{-1}) on *x* axis.

2.4. Validation

2.4.1. Selectivity

The selectivity was performed on the three different products of pharmaceutical tablets and human serum from 3 individual healthy donors receiving no medication for the assessment of potential interferences with endogenous substances at the linear range of the determination of Ramipril.

2.4.2. Linearity

The pharmaceutical tablet samples and serum samples spiked with Ramipril were processed according to the procedure described above for the construction of calibration curves. The six-point

Table 1
Determination of (Ramipril) in serum and pharmaceutical preparations using Sm^{3+}–(DC)–Ramipril optical sensor.

Drug	Added ($\times10^{-8}$ M)	Found ($\times10^{-8}$ M)	Average[a]	Average recovery ± R.S.D. (%)	B.P. (LC)
Corpril (5 mg), Aventis Co., Egypt	2.5 3.0 3.5	2.52, 2.49, 2.51 2.97, 3.05, 3.03 3.51, 3.48, 3.49	1.001	100.1 ± 0.43	97.5 ± 1.0
Ramipril (5 mg), Aventis Co., Egypt	2.5 3.0 3.5	2.54, 2.49, 2.51 3.02, 3.03, 3.05 3.52, 3.46, 3.51	1.004	100.4 ± 0.61	98.5 ± 0.4
Tritace (5 mg), Aventis, Co., Egypt	2.5 3.0 3.5	2.44, 2.48, 2.52 2.96, 2.98, 3.03 3.49, 3.53, 3.49	0.997	99.7 ± 0.31	98.0 ± 0.4
Serum sample	2.5 3.0 3.5	2.49, 2.53, 2.52 2.95, 2.99, 2.98 3.51, 3.45, 3.48	0.996	99.6 ± 0.30	96.3 ± 0.4

[a] Average of nine measurements.

(3.4, 5, 10, 25, 50, 100 nmol l^{-1}) calibration curve was obtained by plotting the peak intensity at $\lambda = 645$ nm of Sm–DC on y axis against 1/concentration (x) of Ramipril. The six-point (2.4, 5, 10, 25, 70, 100 nmol l^{-1}) calibration curve was obtained by plotting the peak intensity at $\lambda = 454$ nm of Ramipril against concentration of Ramipril. The concentrations of calibration standards were analyzed and the linearity was evaluated by comparing the correlation coefficient (r) between theoretical and back-calculated concentrations of calibration standard samples.

2.4.3. Precision

The intraday precision of optical sensor was evaluated by replicate ($n = 3$) analysis of the pharmaceutical tablet samples and serum samples containing Ramipril at three different concentrations (Table 1) of (25, 30, and 35 nmol l^{-1}). The interday precision was evaluated at the above concentration levels for 3 days. The precision was estimated by the relative standard deviation (R.S.D.%).

2.4.4. Recovery

The average recoveries of Ramipril were evaluated at three concentration levels of (25, 30, and 35 nmol l^{-1}) each one was repeated three times and from peak intensity of assayed samples comparison to the one of reference standards prepared in DMSO, then recoveries were calculated using the formula:

$$\%\text{Recovery} = \frac{\text{peak intensity serum}}{\text{peak intensity DMSO}} \times 100$$

2.4.5. Stability

The processed pharmaceutical tablet samples and serum samples (25, 30, and 35 nmol l^{-1}) treated as sample preparation were kept at room temperature for 24 h and then the stability was determined. The freeze–thaw stability was determined after three repeated freezing and thawing cycles on day 0, 15 and 30.

2.5. The determination of Ramipril in pharmaceutical preparations

Ten tablets each of Corpril or Ramipril or Tritace are carefully weighed and ground to finely divided powders. Accurate weights equivalent to 5.5 mg Corpril or Ramipril or Tritace are accurately transferred to 50 ml beaker and dissolved in DMSO and solutions are stand for about 10–15 min and filtered up using 12 mm filter papers then transferred to 100 ml volumetric flask and completed to the mark with DMSO to give the test solution. The concentration of the drug is determined by using 9 concentrations for each sample from the corresponding calibration graph.

2.6. The determination of Ramipril in serum solution

A 1.0 ml of samples of serum collected from various real patients is centrifuged for 15 min at 4000 rpm to remove proteins. The unknown amount of Ramipril in human serum samples is determined using the standard addition (spiking) techniques as follow; a known volume of the treated serum of the real patient is transferred into a calibrated 10 ml measuring flask and diluted by DMSO. The luminescence intensity of the test solution is measured before and after addition of 1.0 ml of previously prepared serum solution. The change in the luminescence intensity is used for determination of Ramipril in serum sample.

3. Results and discussions

3.1. Spectral characteristics of Sm^{3+}–(DC) complex in the presence of Ramipril

3.1.1. Absorption spectra

The absorption spectra of (1) (DC), (2) Ramipril, (3) (DC) + Sm^{3+}, (4) Ramipril + Sm^{3+}, (5) (DC) + Ramipril + Sm^{3+} in sol–gel matrix are shown in Fig. 2. Comparing curve 1 with curve 3 in Fig. 2, after the addition of Sm^{3+} ion into the (DC) in sol–gel matrix, a red shift is observed in the two bands by 3, and 2 nm respectively, and the absorbance is also enhanced, which indicates that (DC) can form a binary complex with Sm^{3+} ion. Comparing curve 3 with curve 5 in Fig. 2, the absorption peak at 360 nm of (DC)–Sm^{3+} system red shift a little, but absorption peak at 273 nm red shift about 9 nm and the absorbance of (DC)–Sm^{3+} is decreased, which

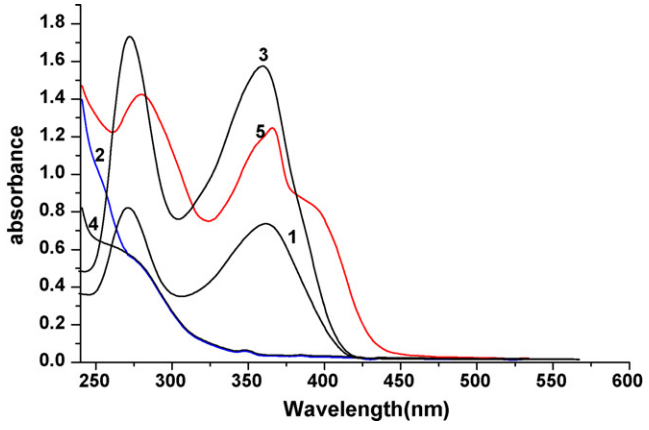

Fig. 2. The absorption spectra of (1) (DC), (2) Ramipril, (3) (DC) + Sm^{3+}, (4) Ramipril + Sm^{3+}, (5) (DC) + Ramipril + Sm^{3+} in sol–gel matrix.

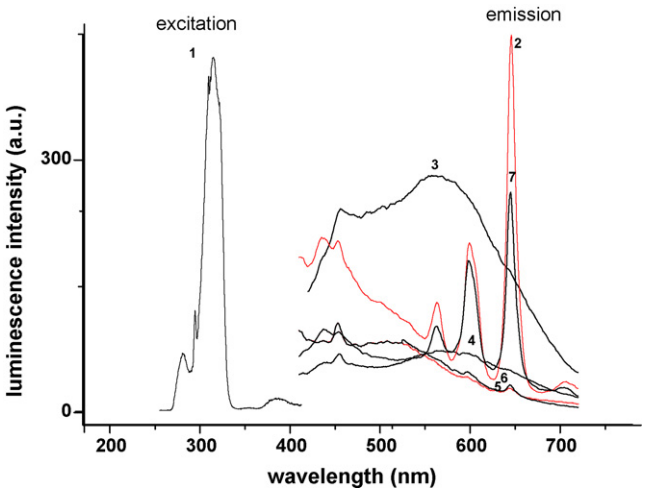

Fig. 3. The fluorescence excitation spectrum (1) Sm + (DC) and emission spectra of (2) (DC), (3) Ramipril, (4) Sm^{3+}, (5) Ramipril + Sm^{3+}, (6) (DC) + Sm^{3+}, (7) (DC) + Ramipril + Sm^{3+} in sol–gel matrix at $\lambda_{ex}/\lambda_{em}$ = 375/645 nm.

indicates that a Ramipril quenches the energy of the (DC)–Sm^{3+} complex.

3.1.2. Emission and excitation spectra

The fluorescence excitation spectrum (1) Sm + (DC) and emission spectra of (2) (DC), (3) Ramipril, (4) Sm^{3+}, (5) Ramipril + Sm^{3+}, (6) (DC) + Sm^{3+} and (7) (DC) + Ramipril + Sm^{3+} in sol–gel matrix are shown in Fig. 3. From curve 4 in Fig. 3, it can be seen that single Sm^{3+} ion in sol–gel matrix has nearly no peak. Comparing curve 2 with curve 6 in Fig. 4, after the addition of Sm^{3+} ion into the (DC) in sol–gel matrix, (DC) can form a binary complex with Sm^{3+} ion. So it appears the characteristic peaks of Sm^{3+} ion ($^4G_{5/2} \rightarrow {}^6H_{5/2}$ = 564 nm, $^6H_{7/2}$ = 599 nm, $^6H_{9/2}$ = 643 nm, $^6H_{11/2}$ = 707 nm), respectively.

Comparing curve 6 with curve 7 in Fig. 3. It can be seen that the characteristic peak of Sm^{3+} at 643 nm remarkably has been quenched after the addition of Ramipril, which indicates that Ramipril effectively quenches the energy of (DC)–Sm^{3+} complex.

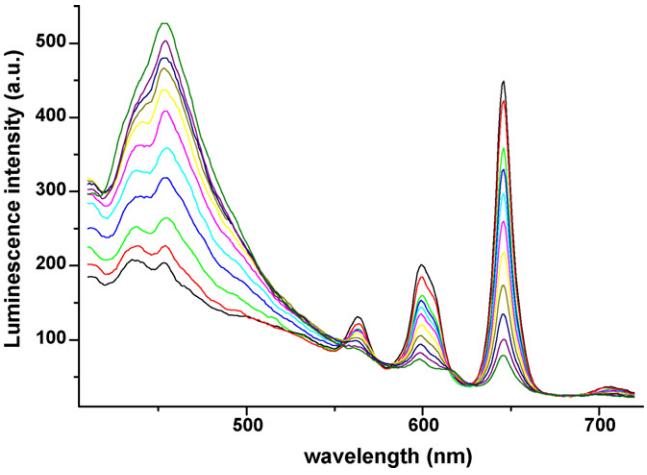

Fig. 4. Luminescence spectra of 3×10^{-3} mol l^{-1} of Sm^{3+} in the presence of 3×10^{-4} mol l^{-1} (DC) in sol–gel matrix in the presence of different molar concentration of (Ramipril) in DMSO at λ_{ex} = 375 nm.

3.2. The effect of different experimental conditions

3.2.1. The effect of the amount of doxycycline (DC)

The influence of the amount of (DC) on the luminescence intensities of the complex in the sol–gel matrix is studied. The luminescence intensity of Sm–DC complex was increased upon increasing the concentration of DC till 3×10^{-4} mol l^{-1} then becomes constant The experimental results showed that the luminescence intensity reached maximum and remained constant when (DC) solution is (3.0×10^{-4} mol l^{-1}) (DC) in the sol–gel preparations.

3.2.2. The effect of the amount of Sm^{3+}

The influence of the amount of Sm^{3+} ion on the luminescence intensities of 3.0×10^{-4} mol l^{-1} of (DC) in sol–gel matrix is studied under the conditions established above. The luminescence intensity of Sm–DC complex at 645 nm was increased upon increasing the concentration of Sm up to 1×10^{-3} mol l^{-1} then becomes constant. When the concentration of Sm^{3+} ion is 1.0×10^{-3} mol l^{-1}, the composition ratio for the Sm^{3+} to (DC) in the (DC)–Sm^{3+} system is 1:3. Thus, 1.0×10^{-3} mol l^{-1} Sm^{3+} ion concentration is used for further study in the sol–gel matrix.

3.2.3. The effect of solvent

The influence of the solvent on the luminescence intensity of the Sm^{3+} in the complex of 3.0×10^{-4} M of (DC) with 3.0×10^{-3} M of $SmCl_3 \cdot 6H_2O$ in sol–gel matrix was studied under the conditions established above. The results show that there is no quenching in the emission intensity of Sm^{3+}–(DC) in sol–gel matrix in the presence of DMSO.

3.2.4. The effect of the amount of Ramipril

The influence of the amount of Ramipril in DMSO on the luminescence intensities of the sol–gel matrix containing 3×10^{-4} mol l^{-1} of (DC) and 1×10^{-3} mol l^{-1} of $SmCl_3 \cdot 6H_2O$ was studied under the conditions established above. The luminescence intensity of Sm^{3+}–(DC) in sol–gel matrix was quenched by the increasing of the concentration of Ramipril up to 5×10^{-5} mol l^{-1} (Fig. 4).

4. Analytical application

4.1. Linear range and limit of detection

Under the chosen experimental conditions, there is an established linear relationship between luminescence intensity of Sm^{3+}–(DC) complex and 1/concentration of Ramipril within the range of 3.4×10^{-9}–1.0×10^{-7} mol l^{-1} with a correlation coefficient of 0.9996. The regression equation is luminescence intensity = 2,450,275,870 × 1/concentration (mol l^{-1}) + 94. Limits of detection (LOD) and -quantitation (LOQ) are defined as 3 sb and 10 sb, respectively [17] where sb is its standard deviation. LOD and LOQ are calculated to be 6.0×10^{-10} and 2.0×10^{-9} mol l^{-}, respectively.

Also, there is a linear relationship between luminescence intensity of Ramipril at 454 nm and (Ramipril) concentration in the range of 2.4×10^{-9}–1.0×10^{-7} mol l^{-1} with a correlation coefficient of 0.9999. The regression equation is luminescence intensity = 22,623,620 × concentration (mol l^{-1}) + 211. LOD and LOQ are calculated to be 5.2×10^{-10} and 1.7×10^{-9} mol l^{-1}, respectively.

4.2. The determination of (Ramipril) in pharmaceutical preparations and in serum

The developed method is applied to the determination of (Ramipril) in pharmaceutical preparations as shown in Table 1.

Table 2
Freeze–thaw stability of Ramipril in pharmaceutical tablets and human serum ($n = 3$).

Drug	Normal concentration (nmol l^{-1})	Found average recovery ± S.D. (nmol l^{-1})			R.S.D. (%)
		0 day	15 days	30 days	
Corpril (5 mg), Aventis Co., Egypt	25	25.2	25.6	24.6	2.00
	30	30.0	30.1	30.3	
	35	35.2	34.9	34.5	
Ramipril (5 mg), Aventis Co. Egypt	25	25.1	25.3	25.5	1.88
	30	30.3	30.6	30.8	
	35	35.0	35.2	34.7	
Tritace (5 mg), Aventis, Co., Egypt	25	24.9	24.8	24.5	1.89
	30	30.3	30.1	29.2	
	35	35.1	35.3	34.3	
Serum sample	25	25.0	25.1	25.7	1.90
	30	30.2	30.4	30.6	
	35	34.9	34.7	34.5	

For the assay of (Ramipril), the samples must be diluted appropriately within the linear range of determination of (Ramipril) and the sample solution is analyzed by the method developed above, using the standard calibration method. The average recovery and relative standard deviation (R.S.D.) are (100.1% and 0.45%) respectively. Data obtained by liquid chromatography method of British Pharmacopoeia [B.P. 2000] (average recovery 98.0% and S.D. 0.6%) are also presented for comparison and show a good correlations with those obtained by the proposed method. The developed method can be easily performed and offers good precision and accuracy when applied for the determination of (Ramipril) in pharmaceutical preparations.

The developed method is also, applied to the determination of (Ramipril) in human serum sample. Proteins in human serum interfere seriously for the system. So, 1.0 ml serum is centrifuged for 15 min at 4000 rpm to remove proteins. Then 100 μm of the serum of real patients is added to 9.8 ml of DMSO then analyzed by a standard addition method as mentioned above. The experimental results in Table 1 show that an average recovery of 99.6% with relative standard deviation of 0.30, which indicates that the developed method can be easily performed and offers good precision and accuracy when applied to human serum sample.

By comparison with some existing methods, the present methods have the advantages in terms of high sensitivity, good stability and wide a linear range of applications. It avoids potential background fluorescent emission interferences from the biological background. So this method may provide a new kind of luminescent sensor for the determination of biomolecular systems.

4.3. Stability

No significant loss of Ramipril (0.4%, R.S.D.) was observed after storage of pharmaceutical tablet samples and serum samples at room temperature for at least 24 h (Table 1). Pharmaceutical tablet samples and serum samples were stable over at least three freeze–thaw cycles (Table 2), indicating that the pharmaceutical tablet samples and serum samples can be frozen and thawed at least three times prior to analysis (1.86%, R.S.D.).

5. Conclusion

The Sm^{3+}–(DC) complex doped in sol–gel matrix has high sensitivity and selectivity characteristic peaks. The intensities of these peaks are quenched by increasing the concentration of Ramipril, in the same time, the intensity of the emission band at 454 nm characterizes to Ramipril is enhanced by increasing the Ramipril concentration, due to energy transfer from Sm–DC complex to the Ramipril in the excited state by collision. Therefore, the quenching of the peaks of the Sm^{3+} ion in Sm^{3+}–(DC) complex and enhancement of the emission band at 454 nm of Ramipril can be used for determination of Ramipril in pharmaceutical preparations and in serum samples.

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