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Gold nanoparticles-based fluorescence enhancement of the terbium–levofloxacin system and its application in pharmaceutical preparations

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ABSTRACT: A sensitive fluorescence (FL) technique is proposed for the determination of levofloxacin (LVX). The method is based on the fact that the weak FL signal of the Tb(III)–LVX system is strongly enhanced in the presence of gold nanoparticles. Gold nanoparticles were prepared by the citrate reduction of $HAuCl_4$ and characterized by transmission electron microscopy (TEM). Levofloxacin and Tb(III) ion form a fluorescence complex in aqueous solution, and its maximum emission wavelength was found at 545 nm. Optimal conditions for the formation of the levofloxacin–Tb(III) complexes were studied. Levofloxacin was detected by measuring the FL intensity, which increases linearly with the concentration of LVX in the range 6.2×10^{-10}–2.6×10^{-8} mol/L. Recovery of the target analytes was >96% with good quality parameters: linearity ($r^2 > 0.996$), limit of detection (LOD) and limit of quantification (LOQ) values 2.1×10^{-10} mol/L and 7.2×10^{-10} mol/L, and run-to-run and day-to-day precisions with relative standard deviations (RSDs) around 3%. Thus, the proposed method can be successfully applied to the routine determination of levofloxacin in pharmaceutical preparations. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: fluorescence; levofloxacin; terbium; gold nanoparticles; tablets

Introduction

Levofloxacin [LVX; (–)-(*S*)-9-fluoro-2.3-dihy-*p*-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7 *H*-pyrido (1,2,3-de)-1,4-benzoxazine-6-carboxylic acid hemihydrates] is a synthetic fluorinated quinolone derivative. Chemically, it is a chiral fluorinated carboxy quinolone, the pure (–)-(*S*)-enantiomer of the racemic drug substance ofloxacin and approximately two-fold more potent than the racemic mixture (1). LVX exhibits broad-spectrum *in vitro* bactericidal activities against Gram-positive and Gram-negative aerobes. It is also found to be active against intracellular pathogens responsible for atypical pneumonia (2). It exerts antibacterial activity via antagonism of the interaction between bacterial DNA gyrase and cell DNA (3). The mechanism of action of LVX involves the inhibition of bacterial topoisomerase IV and DNA gyrase (both of which are type II topoisomerases), enzymes required for DNA replication, transcription, repair and recombination (4). LVX exhibits high potency, a low incidence of resistance, high oral bioavailability, extensive tissue penetration, low protein binding and long elimination half-lives (5). LVX has been used in the treatment of community-acquired pneumonia, acute maxillary sinusitis and acute exacerbation of chronic bronchitis and is also suitable in treating bone diseases (1).

The few reported methods for the determination of LVX are spectrophotometry (6,7), CE with ECL detection (8), cyclic and square-wave voltammetry (4) and HPLC with UV detection (9). Ocana *et al.* (10) reported a flow-injection chemiluminescence assay for the determination of LVX. The method is based on the luminescent properties of the Ce(IV)–sulphite–LVX system and the addition of Eu(III) as the lanthanide cation for the emission sensitizer. However, most of the reported methods for the determination of LVX have shown poor selectivity and sensitivity. Although the liquid chromatographic methods have high sensitivities, they are expensive, involving the use of complex procedures with several sample manipulations and take a long time for analysis.

The fluorescence (FL) method has frequently been used for the analysis of pharmaceutical compounds because of its advantages of low detection limit, large linear dynamic range and relatively simple and inexpensive instrumentation. The sensitivity of the FL method has largely justified the use of FL for many analytical determinations. A large number of spectrofluorimetry methods have been used for the assay of drugs and metals and for the determination of low concentrations of other

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analytes (11–13). The terbium-sensitized fluorescence method has been used in the determination of fluoroquinolones (14–16).

In this work, gold nanoparticles (AuNPs) have been utilized in order to investigate their effect on the sensitivity of the FL method for the quantitative estimation of LVX. A substantial enhancement of terbium-sensitized FL by LVX was observed when the AuNPs were added to the system and the relative FL intensity was proportional to the amount of LVX added. The proposed analytical method is simple, sensitive and at the same time offers good reproducibility, and has been applied to the direct determination of LVX in commercial pharmaceutical tablets. The results of recovery of LVX in spiked tablets were satisfactory. The possible mechanism involved in fluorescence enhancement by gold nanoparticles is also described.

Materials and methods

Apparatus

All the spectrofluorimetric measurements were conducted with a SPEX Fluorolog-2 spectrofluorometer (Model F111, SPEX Industries, Edison, NJ, USA). The spectrometer used a 450 W xenon lamp (Model XBO 450 W/1, Osram, Germany) as the excitation light source and a photomultiplier tube (Model R928, Hamamatsu, Japan) powered at 950 V as the detector. Excitation and emission monochromator slit, increment and integration times were set at 1 mm, 1 nm and 1 s, respectively. All spectral data were obtained using a SPEX DM 3000F spectroscopy computer. A pH meter (Model 520A, Orion, USA) was used for pH measurements.

Reagents

Analytical reagent-grade chemicals were used throughout the experiment without further purification. Distilled deionized water (MilliQ Water System, Millipore, USA) was used throughout. LVX was purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions (1.0×10^{-3} mol/L) of LVX were prepared in deionized water. A stock standard solution of terbium (0.1 mol/L) was prepared by dissolving the required amount of terbium(III) chloride hexahydrate ($TbCl_3 \cdot 6H_2O$, 99.9%; Aldrich, Milwaukee, USA) in water. The working solution were prepared daily with appropriate dilution with water. Sodium citrate and $HAuCl_4$ were purchased from Sigma.

Sample preparation

Sample solutions for analysis were prepared as follows. The average tablet weights were calculated from the weight of each of 10 tablets, which were selected from the same group randomly. An accurately weighed portion of each homogenized sample containing 250 mg LVX (Levaquin and Tavanic) were transferred separately into a 1000 mL calibrated dark flask containing 500 mL deionized water and dissolved using an ultrasonic bath for 20 min, then diluted with deionized water up to the mark. The dissolved sample was filtered through Millipore membrane filter paper and diluted with deionized water to volume to obtain the appropriate concentration for analysis.

Preparation of AuNPs. All glassware used in the following procedure was cleaned in a bath of freshly prepared 3:1 HNO_3–HCl, rinsed thoroughly in water and dried in air. AuNPs were prepared according to the published protocol (17). In brief; 10 ml 38.8 mmol/L sodium citrate solution was quickly injected into 100 mL 1 mmol/L boiling $HAuCl_4$ in a 250 mL round-bottomed flask under vigorous stirring. The solution quickly changed colour from pale yellow to dark red. The solution was continuously heated at ~100°C for 10 min and was then kept stirring for another 15 min while cooling.

Analytical procedures

Apparent FL excitation and emission spectra were measured at room temperature and optimum excitation and emission wavelengths were determined from these spectra. To a 10 mL volumetric flask were added in the following order of: 2 mL of phosphate buffer solution, Tb(III) ion solution, LVX and AuNPs. The mixture was diluted to 10 mL with double-distilled water, mixed thoroughly and allowed to stand for 30 min. The solution was then put into the 1 cm quartz cell for measuring FL spectra and intensity. The FL intensity was measured with an excitation wavelength of 373 nm and an emission wavelength of 545 nm.

Results and discussion

TEM images of AuNPs

The size and shape of the synthesized AuNPs were characterized by transmission electron microscopy (TEM). The results are shown in Fig. 1. AuNPs were found to have an average diameter of 15 nm and the dispersion was found to be good.

Spectral characteristics

The phenomenon of intramolecular energy transfer from the ligand to Ln(III) is well known (14,15). The fluoroquinolones (FQs) are a family of antibiotics which have similar structures. All the FQs possess carboxylate and *keto*-oxygen, which make them able to form complexes with Tb(III). As can be seen from Fig. 2, the emission spectrum of Tb(III) ion in aqueous solution is very weak, but when LVX is added to the system the emission of Tb (III) is intensified several-fold. This phenomenon indicates that a complex of Tb(III) with LVX has formed and intramolecular energy transfer has occurred. The characteristic narrow emission bands of the terbium ion appear at 490, 545, 583 and 620 nm,

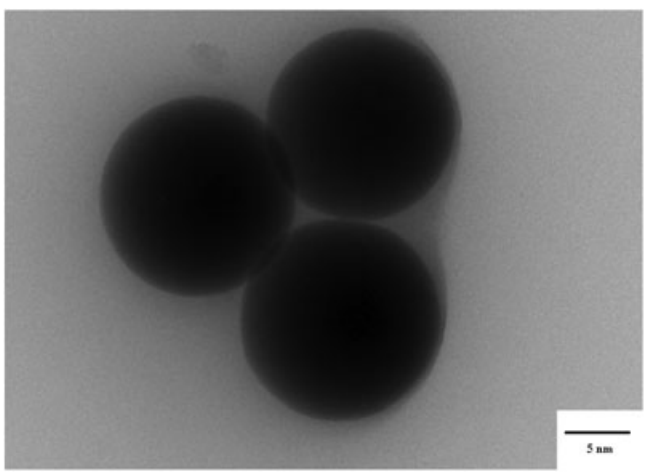

Figure 1. TEM image of AuNPs.

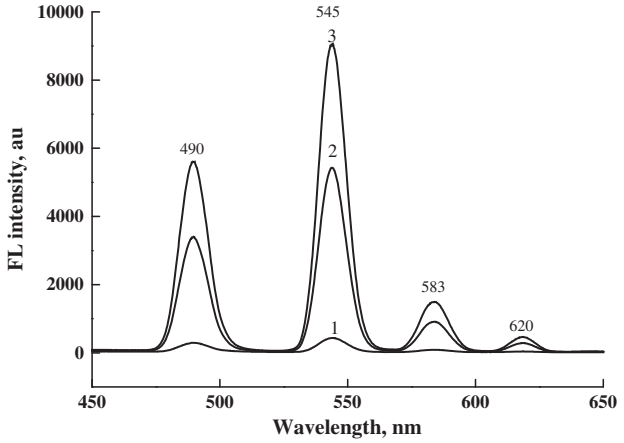

Figure 2. FL spectra. $\lambda_{ex} = 330$ nm: 1, Tb(III); 2, Tb(III)–LVX; 3, Tb(III)–LVX–AuNPs. Conditions: LVX, 1.0×10^{-5} mol/L; Tb(III) ion, 5.0×10^{-4} mol/L; AuNPs, 1.0×10^{-6} mol/L, pH 6.8.

and the maximum intense FL peak locates at 545 nm. These peaks correspond to the transitions of Tb(III), $^5D_4 \rightarrow {}^7F_6$, $^5D_4 \rightarrow {}^7F_5$, $^5D_4 \rightarrow {}^5F_4$ and $^5D_4 \rightarrow {}^7F_3$, respectively. From the point of view of the Förster theory, in order to promote energy transfer efficiently, there should be some overlaps between the FL spectrum of the donor and the absorption spectrum of the acceptor (18). As can be seen from Fig. 3, it can be elucidated that energy transfer occurred easily between LVX and Tb(III) because of the strong spectral overlap between the FL spectra of the donor, LVX, and the excitation spectra of the acceptor, Tb (III). It is well reported that the coordination number of Tb(III) is generally eight (19). Therefore, it can be suggested that there are unfilled sites on Tb(III). Since LVX exists as an anionic state in solution, it can easily combine with the Tb(III) ion via electrostatic interaction. Because of the effect of packing and cooperation in the ternary complex, the energy transfer can more easily occur and the non-radioactive energy loss can be greatly decreased, so the FL intensity of Tb(III) can be enhanced several times.

Additionally, it can be observed from Fig. 2 that the FL intensity was further increased when AuNPs were added to the Tb(III)–LVX system, indicating that AuNPs were beneficial for the excitation of LVX. The increments may be due to the fact that the AuNPs interact with the complex of Tb(III)–LVX, so the aggregation of AuNPs with the complex enlarged the size of particles (20) or may be by accelerating the energy transfer process between LVX and Tb^{3+} ion in aqueous solution. In the presence of AuNPs, the FL peak of LVX increased, indicating that more energy had been transferred to the Tb^{3+} ion.

The mechanism of FL enhancement through gold nanoparticles can be explained as follows. Nanomaterials have shown a narrow size distribution and particularly these spherical nanoparticles (Fig. 1) in the size range 5–15 nm are easy to mix with water or complex solutions, which could be responsible for the fluorescence enhancement of the reported FL system. At the same time, the presence of Au nanoparticles may decrease the interaction among Tb^{3+} complex molecules. In solution containing Au nanoparticles the electronic dipole transition rate of $^5D_4 \rightarrow {}^7F_5$ might be increased due to the enhanced local field surrounding the Tb^{3+} ions. On the other hand, the non-radiative contribution decreases with the decrease in particle size of the materials. So the non-radiative transition rate from $^5D_4$ also could be decreased in the presence of AuNPs owing to more decreased resonant energy transfer among terbium complex molecules. All these factors could lead to fluorescence enhancement of the reported FL system.

Effect of terbium (III) ion concentration

The effect of terbium ion concentration on the analytical signal for the LVX–Tb(III)–AuNPs complex was studied in the range 1.5×10^{-5}–1.5×10^{-2} mol/L. As can be seen from Fig. 4, the maximum FL is observed at a terbium concentration of 5.0×10^{-4} mol/L. Hence the terbium concentration of 5.0×10^{-4} mol/L was selected for the measurements. The enhanced intensity with increasing Tb(III) concentration can be attributed to the fact that excess Tb(III) makes the coordination equilibrium shift to the formation of the complex, and the efficiency of the energy transfer is enhanced when the LVX molecules are involved in the coordination. Further increase in the concentration of terbium ion does not much alter the signal.

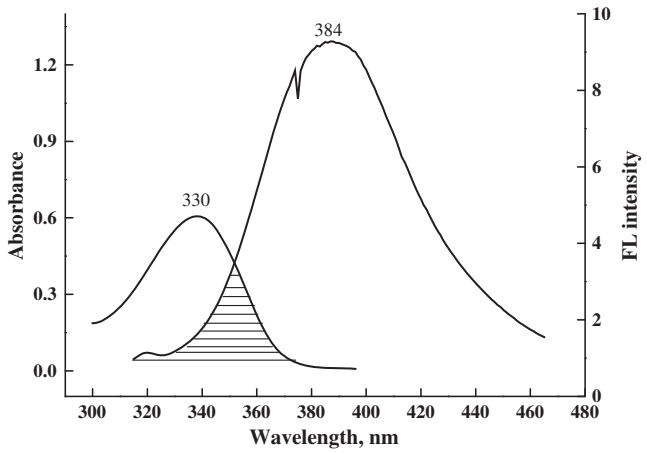

Figure 3. The overlap of the absorption spectrum of Tb(III) and emission spectrum of LVX. Conditions: LVX, 1.0×10^{-5} mol/L; Tb(III) ion, 1.0×10^{-4} mol/L.

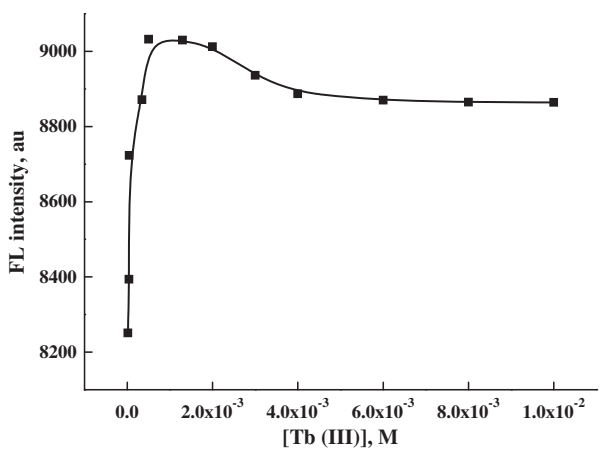

Figure 4. Investigation of the concentration of Tb(III) ion on FL intensity. Conditions: LVX, 1.0×10^{-5} mol/L; AuNPs, 1.0×10^{-6} mol/L; phosphate buffer, pH 6.0.

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Effect of pH

To find the optimum pH of the sample solution for the determination of LVX, the effect of pH on the FL intensity obtained from the Tb(III)-AuNPs–LVX complex system was investigated. The concentration used for LVX was 1.0×10^{-5} mol/L. The concentration of Tb(III) was selected as 5×10^{-4} mol/L. The FL intensity was examined over the pH range 5.8–8.2; the pH of the buffer solution was adjusted by 0.2 mol/L Na_2HPO_4 and 0.2 mol/L NaH_2PO_4 solutions. The volume of added buffer was fixed at 2 mL. The experimental results are shown in Fig. 5, from which it can be seen that the FL intensity remains almost constant over the pH range 6.2–7.0. The increased intensity of the Tb(III)–LVX–AuNPs system at higher pH may be because the increase of $-COO^-$ groups of LVX leads to the formation of Tb(III)–LVX complex, which further facilitates the dissociation of the carboxylic proton. The complex is theoretically more favourable at higher pH, but the intensity decreases owing to the precipitation of terbium hydroxide (21). pH 6.8 was chosen for subsequent experiments.

Effect of AuNPs concentration

The effect of the AuNPs was studied in the range 0.1×10^{-6}–1.0×10^{-5} mol/L. The FL emission increased with the concentration of AuNPs and reached a maximum at 1.0×10^{-6} mol/L; higher concentrations of AuNPs led to a decrease in the FL signal. Thus, 1.0×10^{-6} mol/L concentration was selected for further study. In this study, the maximum FL intensity was observed at the following concentrations: LVX, 1.0×10^{-5} mol/L; Tb(III) ion, 5.0×10^{-4} mol/L; AuNPs, 1.0×10^{-6} mol/L with pH 6.8. These parameters were selected for the further investigation.

Calibration curve for LVX

To evaluate analytical characteristics of this method, a calibration curve of LVX by plotting concentrations versus FL intensities was constructed. Eight standard solutions were prepared for the calibration curve, using the optimum conditions, which were Tb(III), 5×10^{-4} mol/L and pH 6.8. The results obtained (Fig. 6) show that the FL intensity of LVX is linearly proportional to the concentration of LVX in the range 6.8×10^{-10}–2.7×10^{-8} mol/L. The correlation coefficient for the calibration curve was 0.9999. The regression equation for LVX is $Y = 6650.4 + 5.7\ E10X$. The limit of detection as defined by IUPAC, $C_{LOD} = 3S_b/m$, where S_b is the standard deviation (SD) of the blank signals and m is the slope of the calibration graph, was found to be 2.1×10^{-10} mol/L. The relative standard deviation (RSD) for six repeated measurements of 1.0×10^{-5} mol/L LVX was 2.35%.

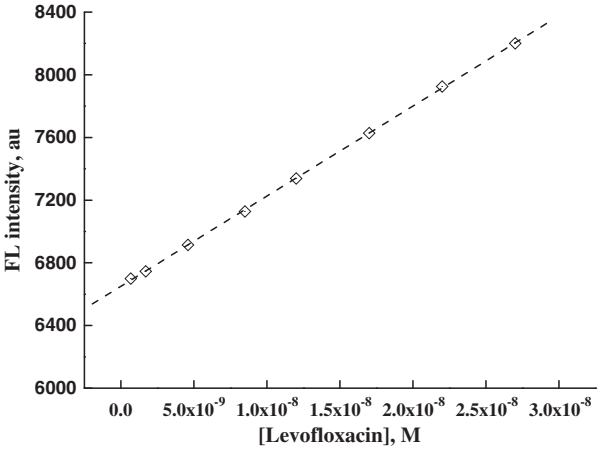

Figure 6. Calibration curve for the quantitative analysis of LVX.

Interference studies

In a real sample, the analyte under investigation will be in the presence of interferents. They may suppress or enhance the FL signal. Therefore, a study of some potential interfering substances, such as metal ions and some organic compounds commonly used in pharmaceutical tablets, was tested. The interference study was carried out by using samples containing a fixed amount of LVX (1×10^{-9} mol/L) and variable concentrations of foreign substances. Starch, glucose and fructose do not cause interference at molar ratios of foreign substance/levofloxacin < 300. The fluorescence measurements of LVX (1×10^{-9} mol/L) were also carried out in presence of some metal ions typically present in the human body; Na^+, K^+, Zn^{2+}, Mg^{2+}, Cu^{2+}, Al^{3+} and Fe^{3+} do not cause interference at molar ratios of cation/levofloxacin < 3000. The tolerance limit was estimated to be < ±4% of the error for the determination of LVX (Table 1). So no interference from the presence of these foreign substances is expected in this FL method. From Table 1 it

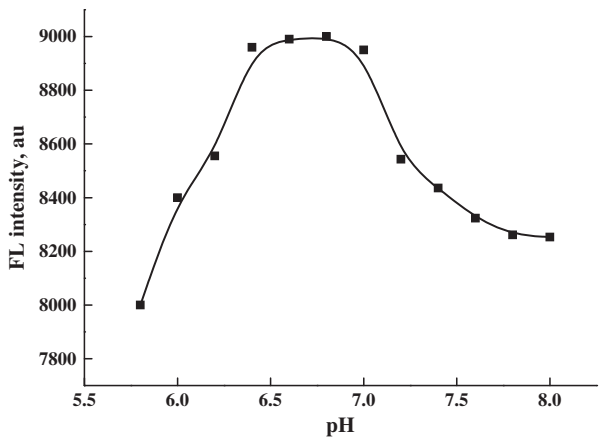

Figure 5. Dependence of pH on the FL spectrum of Tb(III)–LVX–AuNPs.

Table 1. Effect of interfering substances for quantitative determination of 1×10^{-9} mol/L LVX (< 4% error)

Foreign substance	Foreign substances/LVX (molar ratios)	Change of relative intensity (%)
Na^+	2500	−2.5
K^+	2200	−3.6
Zn^{2+}	2000	+3.5
Mg^{2+}	3000	−1.40
Cu^{2+}	100	−0.25
Al^{3+}	10	+2.25
Fe^{3+}	45	−1.39
Fructose	144	+1.25
Glucose	100	+0.54
Starch	300	−1.23

Table 2. Assays of LVX in pharmaceutical formulations by the proposed method

Sample	Active ingredient labelled	Found ± RSD[a]	Added ($\times 10^{-9}$ mol/L)	Found ($\times 10^{-9}$ mol/L)	Recovery mean ± RSD[a] (%)
Levaquin	250 mg LVX	246.3 ± 0.31	2.0	1.98	99.0 ± 1.7
			4.0	3.93	98.2 ± 0.3
Tavanic	500 mg LVX	488.6 ± 0.27	2.0	1.92	96.0 ± 0.4
			4.0	3.89	97.2 ± 0.6

[a]Mean of three measurements.

Table 3. Quality parameters of the proposed method

Analyte	LOD[a] (mol/L)	LOQ[b] (mol/L)	Run–to–run precision (RSD%)[c]	Day–to–day precision (RSD%)[c]
Levofloxacin	2.1×10^{-10}	7.2×10^{-10}	2.7	3.1

[a]Limit of detection was estimated at a signal:noise ratio of 3.
[b]Limit of quantification was estimated at a signal:noise ratio of 10.
[c]Relative standard deviation ($n = 3$).

can be seen that for all the foreign substances, the tolerance ratio was much higher than the usual concentrations found in pharmaceutical tablets.

Analytical applications

In order to check the performance of the method, two commercial pharmaceutical formulations were assayed, such as Levaquin (containing 250 mg LVX) and Tavanic (containing 500 mg LVX). Assay of the active ingredients in the tablets was performed by weighing 10 tablets (calculating the average weight of one tablet), grinding the tablet mass, using the average weight of one tablet and dissolving it in deionized water with shaking for 20 min in an ultrasonic bath. The solution was filtered through Millipore membrane filter paper and diluted with water to volume to obtain the appropriate concentration for analysis. The results obtained and the percentages of recovery are summarized in Table 2. The results were in good agreement with the label content claimed by the manufacturer, which indicates that the proposed method can be applied successfully to the determination of LVX in pharmaceutical formulations.

The standard addition method was applied for the determination of LVX in the spiked tablets to check the accuracy of the developed method. Recovery studies were carried out after the addition of known amounts of pure LVX to Levaquin and Tavanic formulation tablets of LVX. The amounts of LVX added to the tablets and recovered from them are listed in Table 2. The overall recovery of the compounds was about 96–99%.

Validation parameters

Validation parameters were studied to assess the performance of the method. These were linearity, LOD, LOQ, repeatability (run-to-run precision) and reproducibility (day-to-day precision). Table 3 shows the validation parameters of the proposed analytical method. Satisfactory results were obtained for each compound and were found to be in agreement with label claims. The proposed method showed good reproducibility and repeatability when applied to real samples.

Conclusions

The determination of levofloxacin by a gold nanoparticles-enhanced terbium-sensitized fluorimetric method has been proposed. Gold nanoparticles were prepared by the citrate reduction of $HAuCl_4$ and were found to be good enhancers for the FL of the Tb(III)–LVX system. Levofloxacin and Tb(III) ion form a fluorescence complex in aqueous solution, and its maximum emission wavelength is located at 545 nm. Optimum conditions for the formation of the LVX–Tb(III) complexes have been studied. Under optimized parameters, the concentration of levofloxacin was found to be linear in the range 6.2×10^{-10}–2.6×10^{-8} mol/L and the detection limit was found to be 2.1×10^{-10} mol/L, with a limit of quantification (LOQ) value of 7.2×10^{-10} mol/L. This method was applied to the determination of levofloxacin in pharmaceutical preparations and expands the scope of analytical application of gold nanoparticles.

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