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Determination Of Lomefloxacin By Flow Injection Chemiluminescence

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ABSTRACT

A rapid and sensitive flow injection chemiluminescence (CL) method is described for the determination of lomefloxacin hydrochloride based on the CL generated during its reaction with either cerium(IV) and sodium sulphite in acid medium (system1) or Ce(IV) in acid condition sensitized by rhodamine 6G (system2) or luminol-KIO_4-calcein in alkali medium (system3). The effect of analytical and flow injection variabled on these CL systems and determination of lomefloxacin hydrochloride are discussed. The proposed procedures allow the determination of lomefloxacin hydrochloride over the following concentration range 0.01-15.0 mg L^{-1} with a detection limit of 0.005 mg L^{-1}, 0.10-15.0 mg L^{-1} with a detection limit of 0.06 mg L^{-1}, 1.0-20.0 mg L^{-1} with a detection limit of 0.50 mg L^{-1} respectively. The method is successfully applied to the determination of lomefloxacin hydrochloride in pharmaceutical preparations.

KEYWORDS

Chemiluminescence(CL);
Lomefloxacin hydrochloride;
Pharmaceutical analysis;
Flow injection analysis (FIA)

INTRODUCTION

Lomefloxacin(LFLX) [1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylix acid] (figure 1) is one of the synthetic antibacterial fluoroquinolone agents of the third generation, which exhibits high activity against abroad spectrum of gram-negative and gram-positive bacteria. This synthetic fluoroquinolone derivative is used for the control of the urinary tract and respiratory infections[1]. The widespread use of this compound and the need for clinical and pharmacological study require fast and sensitive analytical techniques to determine the presence of the drug in commercial formulations and biological fluids. Up to now the most common techniques for the determination

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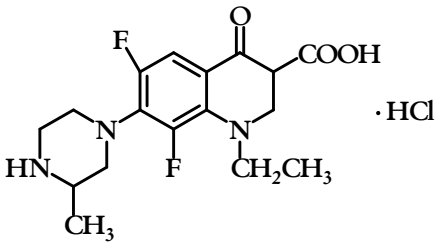

Figure 1: Structure of lomefloxacin hydrochloride

of the drug in commercial formulations and biological fluids have been based on HPLC with UV detection[2] or with fluorimetric detection[3] methods, but capillary zone electrophoresis[4], micellar electrokinetic capillary chromatographic[5], spectrophotometric[6], voltametric method[7-8] and photochemical-fluorimetric[9] methods, have also been used.

Analytical procedures applying (CL) measurements in flow-injection (FI) setups combine the advantages of instrument simplicity (no monochromator required), rapidity in signal detection (normally 0.1-10s), sensitivity and ease of use. Since many CL reactions are very fast, they give rise to imprecise measurements as a result of irreproducible mixing of sample and reagents, but the reproducibility and selectivity of the CL analysis can be improved by combination with an FI method. There had been no reports on CL methods for the determination of lomefloxacin.

In this paper, the method is based on the CL reactions of lomefloxacin, cerium(IV) and sulphite in acid medium; lomefloxacin hydrochloride with Ce(IV) in acid condition sensitized by rhodamine 6G; lomefloxacin and luminol-KIO_4-calcein in alkali condition, respectively. The simplicity sensitivity and rapidity of the proposed method are better than other previously reported methods[2-9]. The proposed method is applied successfully for the determination of lomefloxacin.

EXPERIMENTAL

Instruments and flow system

A schematic diagram of the flow injection chemiluminescence analyzer was shown in figure 2 ((Xi'an Ruimai Electronic Equipments Company). The 4-channel peristaltic pump was equipped with silicon rubber tubes (1 mm i.d.). The sample stream merged with oxidant solution stream in a spiral flow cell in front of a photomultiplier tube (PMT). The signals from the PMT were sent to a computing integrator and then to an compatible computer.

Reagents

Analytical reagent grade chemicals and double distilled water were used throughout.

Lomefloxacin was kindly provided by Henan Institute for Drug Control.

Stock solution of lomefloxacin(100 mg mL^{-1}) was prepared by dissolving 0.1000 g of lomefloxacin hydrochloride in water, and diluting to 1000 ml with water.

Stock solution of Cerium(IV)(2.0×10^{-3} mol·L^{-1}) was prepared by dissolving 0.8086 g $Ce(SO_4)_2$ in 0.5 mol L^{-1} sulphite and diluting to 1000 mL.

Stock solution of Rhodamine 6G (1.0×10^{-4} mol·L) was prepared in water

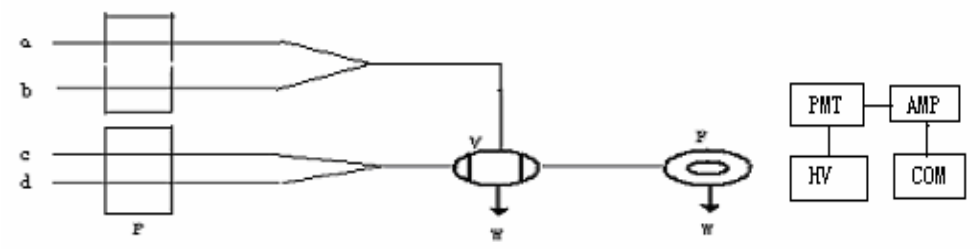

Figure 2: Schematic diagram of flow injection CL assembly
System1: (a) Cerium(IV)(b) Sulphite(c) Lomefloxacin (d) Sodium sulphite.
System2: (a) Cerium(IV)(c) Lomefloxacin (d) Rhodamine6G.
System3: (a) luminol(b)Calcein (c)KIO_4 (d) Lomefloxacin (P)Peristaltic pump,(V)eight-way valve, (F)Flow cell, (W)Waste, (PMT)photomultiplier tube, (AMP)amplifier, (COM)computer, (HV)high voltage

Stock solution of Sodium sulphite(2.0×10^{-3} mol·L)was prepared in water.

Stock solution of luminol(1.0×10^{-2} mol·L) was prepared by dissolving 0.1772 g luminol in sodium hydroxide and diluting in buffer solution of sodium carbonate and bicarbonate(PH = 10.08) to 100 mL.

Stock solution of KIO_4(1.0×10^{-2} mol·L) was prepared by dissolving 0.2299 g KIO_4 in water and diluting to 100 mL.

Stock solution of calcein(1.0×10^{-2} mol·L) was prepared by dissolving 0.6225 g calcein in water and diluting to 100 mL.

The minimum number of dilution steps possible was used for preparation of more dilute solutions. All other common laboratory chemicals were of the best grade available.

General procedure

In order to achieve good mechanical and thermal stability, the instrumental system was allowed to run for 10 min before the first measurement was made. The FIA configuration used was outlined in figure 2 The sample stream merged with the oxidant solution stream in the spiral flow cell in front of the PMT. The light emitted was detected by PMT with no wavelength discrimination and the CL signal was recorded. The concentration of lomefloxacin was quantified via the peak height of the relative CL emission intensity which was obtained by subtracting the blank CL intensity from that of the sample or standard hydrazine solution.

Procedure for dosage forms (capsules)

Weigh accurately a quantity of the mixed contents of 10 capsules (Ultracef 100 mg per capsule: China) or measure accurately. Transfer into a 100 ml volumetric flask and dilute to the mark with distilled water. Proceed as described above under procedure for calibration. Calculate the nominal content from the corresponding calibration graph or regression equation.

RESULTS AND DISCUSSION

The flow-injection chemiluminometric determination of lomefloxacin was studied using three different systems which they were lomefloxacin, cerium(IV) and sulphite in acid medium; lomefloxacin hydrochloride with Ce(IV) in acid condition sensitized by rhodamine 6G and lomefloxacin and luminol-KIO_4-Calcein in alkali condition. The CL of lomefloxacin was all obtained and very satisfactory.

Optimization of experimental variables

A series of experiments were conducted to establish the optimum analytical variables. The parameters optimized included reagent concentrations and the flow rate.

1. Effect of oxidant concentrations in different systems

Figure 3 shows the effect of Ce(IV) concentration on the CL intensity in system 2. The greatest CL response was obtained with 2.0×10^{-3}mol/L. Larger concentrations of Ce(IV) lowered the CL intensity. Therefore, 2.0×10^{-3} Ce(IV) was used. At the same time in system 1 and 3, the optimum concentrations of Ce(IV) and KIO_4 were 4.0×10^{-3} mol·L and 4.0×10^{-5} mol·L, respectively.

2. Effect of sensitizers in different systems

Based on the observation that some of the fluorescing compounds can be used for energytransfer in the CL reactions with an enhancement of the intensity[10-11], various fluorophores were investigated for obtaining maximum yields in CL intensity. In system 2, different concentrations of rhodamineB, fluorescein, rhodamine6G and calcein were investigated. It was found that only rhodamine6G enhanced the CL signal when dissolved in the carrier. Figure 4 shows that about 1.0×10^{-4} mol·L of rhodamine6G

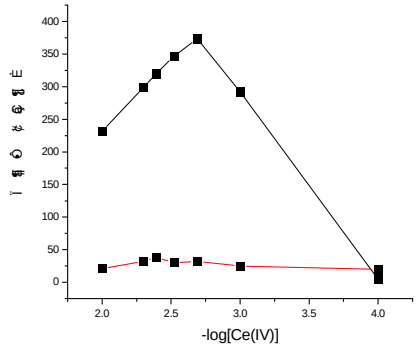

Figure 3: Effect of concentration of Cerium(IV) (mol·L) on the emission intensity. 1. LMX10.0 mg·L 2. blank

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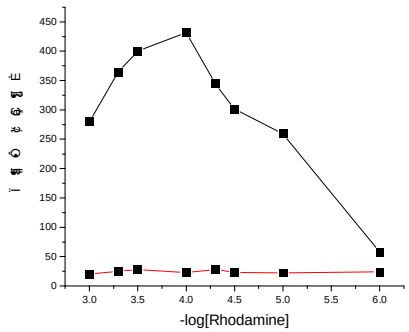

Figure 4: Effect of Rhodamine6G concentration (mol·L) on the emission intensity. 1. LMX10.0 mg·L 2. blank

gave rise to the most intense signal, so this concentration of rhodamine6G was used in all subsequent studies. The same procedure in system 3, only calcein enhanced the CL signal when dissolved in the carrier. About 5.0×10^{-5} mol·L of calcein gave rise to the most intense signal, so this concentration of calcein was used.

3. Effect of concentration of luminol and its buffers solution's pH in system 3

From the results of the experiments, the optimum concentration was 6.0×10^{-4} mol·L, and the pH of the buffer solution of sodium carbonate and bicarbonate was 10.08.

4. Effect of reagents flow-rate

In this paper, the reagents via pump (a) and (b) was looked as carrier stream, and their flow rate affected the CL emission seriously. The effect of flow rate on the emission intensity of three CL systems was studied over the range of 2.3-8.5 ml·min^{-1} in each stream. An increase in the emission intensity was obtained with increase in flow rate in the range of 2.3-6.0 ml·min^{-1} for three CL systems, but higher flow rates caused a decrease in emission intensity. Thus, a flow rate of 6.0 ml·.min^{-1} was chosen as the optimum flow rate. The reagents via pump (c) and (d) as sample stream, and their flow rates affect the CL emission hardly. In order to achieve good CL emission's stability, the flow rates of the sample stream were allowed 5.5 ml·min^{-1}.

5. Interference study

Interferences from various pharmic excipient were investigated by studying their effects on the determination of 1.0 mg/L lomefloxacin in three systems. TABLE 1 shows the effect of various pharmic excipient on CL determination of lomefloxacin.

Performance of the system for lomefloxacin measurements

Calibration graphs were obtained for each of the CL systems described above. Under the optimum conditions described in TABLE 2, calibration equations, concentration ranges of applications and detection limits are given in TABLE 3. Results show that lomefloxacin can readily be determined over a wide concentration range, down to ≤ 0.01mg·L^{-1} by CL measurements. Repeatability was measured with standard solutions. The relative standard deviations for ten measurements of 1.0mg.L^{-1}, 1.0 mg·L^{-1} and 5.0 mg·L^{-1} lomefloxacin were 1.1, 1.2 and 0.9% for three systems, respectively.

TABLE 1: Effect of various pharmic excipient on the determination of 1.0 mg·L lomefloxacin.

Species	Times system1	Times system2	Times system3
Dextrine	50	50	55
Starch	50	50	55
Glucose	50	50	55
Fructose	50	50	60
Galactose	20	20	20
Lactose	20	20	20
Gelatine	20	20	25
Polyethylene Glycol4000	10	15	15
Sucrose	10	10	15

Above the TABLE 3 RCI is the relative CL intensity; C is hydrazine concentration (mg·L^{-1}); Detection limit was theoretical detection limit (blank plus three times its standard deviation)[12].

Analysis of pharmaceutical formulations

The proposed methods were applied to the determination of lomefloxacin in capsules. Satisfactory results were obtained for three systems (TABLE 4). Moreover, to check the validity of the proposed methods; the standard addition method was applied by adding lomefloxacin to the previously analyzed capsules. The recovery of lomefloxacin was calcu-

TABLE 2: The optimum conditions in three systems

Series	Flow-rate ($mL \cdot min^{-1}$)	C.of Ce(IV) (mol·L)	C.of H_2SO_4 (mol·L)	C.of Na_2SO_3 (mol·L)	C.of R6G (mol·L)	C.of Luminol (mol·L)	pH in Luminol	C.of KIO_4 (mol·L)	C.of Calcein (mol·L)
System1	-	4.0×10^{-3}	0.05	2.0×10^{-3}	-	-	-	-	-
System2	-	2.0×10^{-3}	0.5		1.0×10^{-4}	-	-	-	-
System3	-	-	-	-	-	6.0×10^{-4}	10.08	4.0×10^{-5}	5.0×10^{-5}

TABLE 3: Analytical figures of merit for the lomefloxacin determination by three different CL systems

System	Range of application($mg \cdot L^{-1}$)	Calibration equation (RCIvs. C)	Detection limit($mg \cdot L^{-1}$)
1	0.01–15.0	RCI=1190+2822C(r=/0.9985 for n=10)	0.005
2	0.10-15.0	RCI=349.1+25.7C(r=/0.9906 for n=10)	0.06
3	1.0-20.0	RCI=9.00+836.6C(r=/0.9918 for n=10)	0.50

lated by comparing the concentration obtained from the mixtures with those of the pure lomefloxacin. TABLE 4 shows the results of analysis of the capsules.

4. Possible CL mechanism

The CL mechanism of three systems may be attributed to the deoxidation of lomefloxacin. In system 1, lomefloxacin and sodium sulphite deoxidized Ce(IV) together in acid medium, but the CL intensity weren't the addition of the lomefloxacin and sodium sulphite separately.

In system 2, the CL mechanism may be attributed to the following reactions:

Lomefloxacin + Ce(IV). Oxidized Lomefloxacin* + Ce(III)

In the presence of rhodamine 6G, the energy resulting from the redox reaction can be effectively transferred to rhodamine6G which in turn generates CL emission.

Oxidized Lomefloxacin* + Rhodamine6G— Oxidized Lomefloxacin* + Rhodamine6G*

Rhodamine6G*--- Rhodamine6G + light

The CL emission of oxidized Lomefloxacin (II) may be ascribed to the oxidation of the carboxyl group in the para position of the benzene ring. In system 3, the CL mechanism may be attributed to the following reactions:

Luminol + IO_4^---- Luminol* + light the CL reaction (1)

TABLE 4: Determination of lomefloxacin in capsules

System	Capsule batch number (100mg per grain)	Found (mg)	Added (mg)	Recovery (%)	Reference[13]method recovery(%)
1	(01)-0901	0.099	50	106.0	105.6
			100	98.6	97.5
	(01)-0701	0.098	50	96.5	98.0
			100	101.2	99.6
2	(01)-0901	0.099	50	102.8	106.0
			100	98.6	99.2
	(01)-0701	0.099	50	99.0	99.3
			100	100.7	100.2
3	(01)-0901	0.097	50	103.3	102.2
			100	98.6	99.0
	(01)-0701	0.098	50	95.4	96.0
			100	106.0	101.0

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lomefloxacin + IO_4--- lomefloxacin(II) the restrained reaction(2)

CONCLUSION

A simple, rapid and highly sensitive flow injection chemiluminometric method is described for the determination of lomefloxacin in dosage forms. The proposed methods are suitability for the routine quality control of the drug alone and in capsules without fear of interference caused by the excipients expected to be present in capsules. The method had been also applied successfully to the determination of the active constituent in a commercial pharmaceutical.

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