

*Original article***Micelle enhanced spectrofluorimetric method for the determination of ofloxacin and lomefloxacin in human urine and serum****Kuldeep Kaur, Baldev Singh and Ashok Kumar Malik\****Department of Chemistry, Punjabi University Patiala-147002, Punjab, India**\*Corresponding author: E-mail address: malik\_chem2002@yahoo.co.uk***Abstract:**

A spectrofluorimetric method for the determination of two fluoroquinolone drugs, ofloxacin and lomefloxacin in biological fluids has been developed. The method is based on native fluorescence of these drugs in micellar medium of sodium dodecyl sulfate (SDS). The wavelengths of excitation and maximum emission were 295 nm and 485 nm for ofloxacin, and 285 nm and 449 nm for lomefloxacin, respectively. The method allows the determination of 10-1,000 ng mL<sup>-1</sup> of ofloxacin and 20-2,000 ng mL<sup>-1</sup> of lomefloxacin in a 0.1 M acetic acid-sodium acetate buffer solution of pH 6. The SDS concentration of 0.01 M was selected for both the drugs. The method was successfully applied to determination of the two drugs in human urine and serum samples.

**Keywords:** Fluorescence; Lomefloxacin; Micelle; Ofloxacin; Sodium dodecyl sulfate

## Introduction

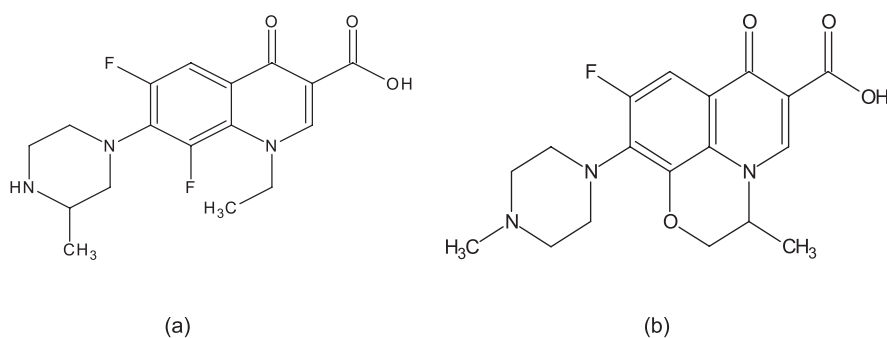
Fluoroquinolones (FQs) are a class of important synthetic antibiotics, which are active against both Gram (+) and Gram (-) bacteria through inhibition of their DNA gyrase [1]. Their excellent pharmacokinetic profile, good tissular penetration and wide scope of activity have made these drugs very useful in human and veterinary medicine [2]. Lomefloxacin which is ( $\pm$ ) 1-ethyl-6,8-difluoro-1,4-dihydro-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid (Fig. 1a) is one of the synthetic antibacterial fluoroquinolone agents of the third generation, and is often used for treatment of respiratory tract, urinary tract, skin and skin-structure infections. Lomefloxacin is generally administered to patients in 400 mg per day doses. At this dose level, lomefloxacin was well absorbed and achieved peak plasma concentrations approximately 1 h after administration and elimination half-life of 7-8 h were found for this dosage. A 400 mg oral dose provided a mean peak plasma concentration of  $3.43 \mu\text{g mL}^{-1}$  and urine concentration of greater than  $80 \mu\text{g mL}^{-1}$  during the 12-to 24-h period after the dose [3].

Ofloxacin is ( $\pm$ ) 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid (Fig. 1b). It is the second generation fluoroquinolone drug which is useful for the treatment of systematic infections including urinary tract, respiratory and gastro-intestinal infections [4]. Following oral administration, there is rapid and extensive oral absorption from gastrointestinal tract achieving peak

serum concentration within 1-3 h and levels in excess of  $100 \mu\text{g mL}^{-1}$  in urine. The maximum serum concentration after 400 mg oral dose was reported to vary from 3.5 to  $5.3 \mu\text{g mL}^{-1}$  after a time of 1.1-1.4 h. The elimination half-life is between 5 and 7 h and it is almost completely eliminated in the unchanged form by the kidneys and about 80% is excreted in 24 h [5].

The widespread use of these fluoroquinolone drugs as medicines and need for clinical and pharmacological study requires fast and sensitive analytical methods for determination of these drugs in biological fluids. The most common techniques used for determination of these drugs in biological fluids include high performance liquid chromatography (HPLC) [6-7], microbiological assay [8-9], capillary electrophoresis (CE) using solid phase extraction (SPE) [10], capillary electrophoresis-electrochemical detection [11], chemiluminescence methods [12-13], spectrophotometric [14] and fluorimetric methods [15]. However, there is always a need for development of new analytical procedures which are simple, inexpensive and sensitive and can be used alternative to HPLC methods.

In this paper, a micelle enhanced spectrofluorimetric method for determination of ofloxacin and lomefloxacin has been proposed. The method has been successfully applied for the determination of these drugs in plasma and urine samples. The proposed method is inexpensive and fast as compared to commonly used HPLC methods. The sample preparation is simple and do not require elaborate procedures. The method is also highly sensitive.



**Figure 1** Structures of lomefloxacin (a) and ofloxacin (b)

## Experimental

### Apparatus

Fluorescence spectra were recorded and intensity measurements were made on RF-5301 PC spectrofluorophotometer (Shimadzu, Japan). Excitation and emission spectra were recorded with monochromators set at a slit width of 10 nm. A UV-1700 Pharma Spec UV-Visible spectrophotometer (Shimadzu, Japan) was used to record absorbance spectra. An automatic temperature compensation (ATC) pH meter model 132-E (Electronics, India) was used for all pH measurements.

### Reagents

All chemicals used were of analytical reagent grade unless stated otherwise and purchased from Merck, Germany. Triple distilled water was used throughout the experiment. Stock standard solutions of  $0.1 \text{ mg mL}^{-1}$  for both the drugs were prepared by exact weighing of the standard drug and dissolution in deionised water. The solutions were stable for at least one week if stored in the dark at  $4^\circ\text{C}$ . Working solutions were obtained by appropriate dilution of the stock solution daily as needed. Solutions of sodium dodecyl sulphate (SDS), cetyl pyridinium bromide (CPB) and Triton-X (TX-100) were separately prepared at 0.1 M. Solutions of sodium acetate-acetic acid buffer solutions of required pH were prepared at 0.5 M from sodium acetate and acetic acid.

### Methods

To two separate 10 ml volumetric flasks, an aliquot of working standard solution of each drug was added and then following solutions were added in sequence: 2 ml of 0.5 M acetate buffer solution of pH 6 and 1 ml of 0.1 M SDS solution. The resultant mixture was diluted to the mark with distilled water, mixed thoroughly by

shaking and then allowed to stand for 10 minutes. A blank sample containing all the reagents except the drug was also prepared. The fluorescence intensities of the sample and blank were then measured in a 10 mm path-length quartz cell at an emission wavelength of 485 nm for ofloxacin and 449 nm for lomefloxacin. The fluorescence intensity was represented as  $\Delta F = F - F_0$ , where  $F$  and  $F_0$  were the fluorescence intensities of the system with and without the drug, respectively.

### Serum and urine sample preparation

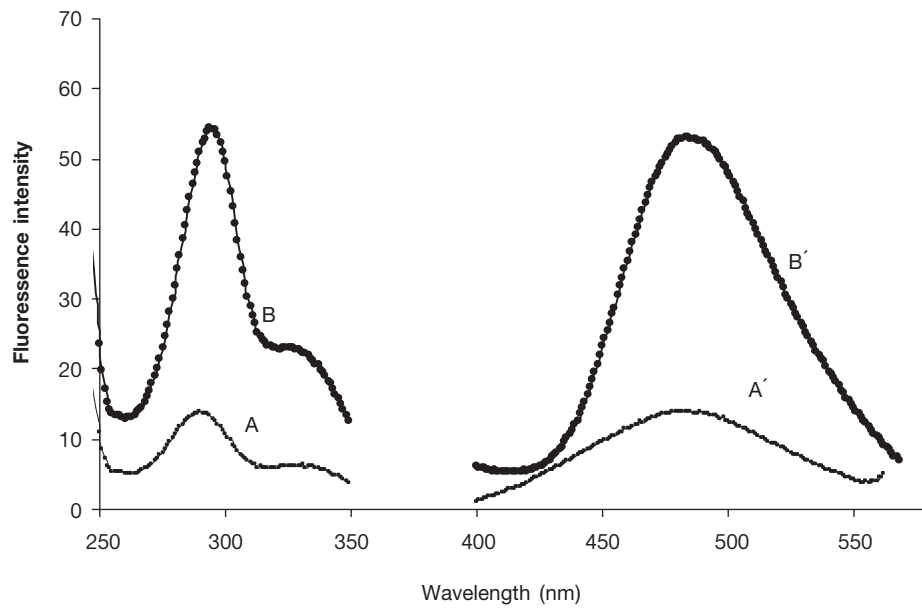
Urine samples were obtained from healthy volunteers and serum samples were obtained from a local hospital. Urine samples were centrifuged at 4,000 rpm for 10 min and the clear supernatant obtained was collected. Suitable aliquots of this supernatant were taken, spiked at 100, 200 and  $300 \mu\text{g mL}^{-1}$  levels for ofloxacin and at 60, 80 and  $100 \mu\text{g mL}^{-1}$  levels for lomefloxacin. The plasma samples were centrifuged at 4,000 rpm for 5 min. The clear supernatant obtained was collected. Suitable aliquots of this supernatant were taken and spiked at three different levels: 3, 4 and  $6 \mu\text{g mL}^{-1}$  for ofloxacin and at 3, 4 and  $5 \mu\text{g mL}^{-1}$  levels for lomefloxacin. These samples were then subjected to the analytical procedure described above.

## Results and Discussion

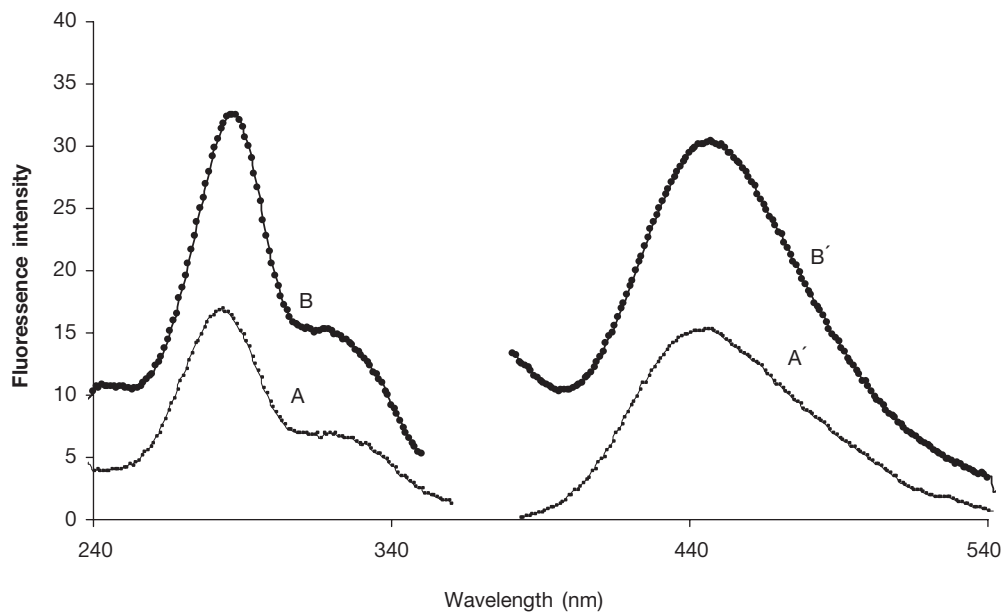
### Fluorescence spectra

Both ofloxacin and lomefloxacin show native fluorescence in an aqueous solution. The fluorescence spectra of both the drugs in an aqueous solution were recorded. For ofloxacin, an excitation wavelength was 292 nm and an emission maximum was obtained at 482 nm (Fig. 2a). For lomefloxacin, an excitation wavelength was 283 nm and an emission maximum was obtained at 445 nm (Fig. 2b).

(a)

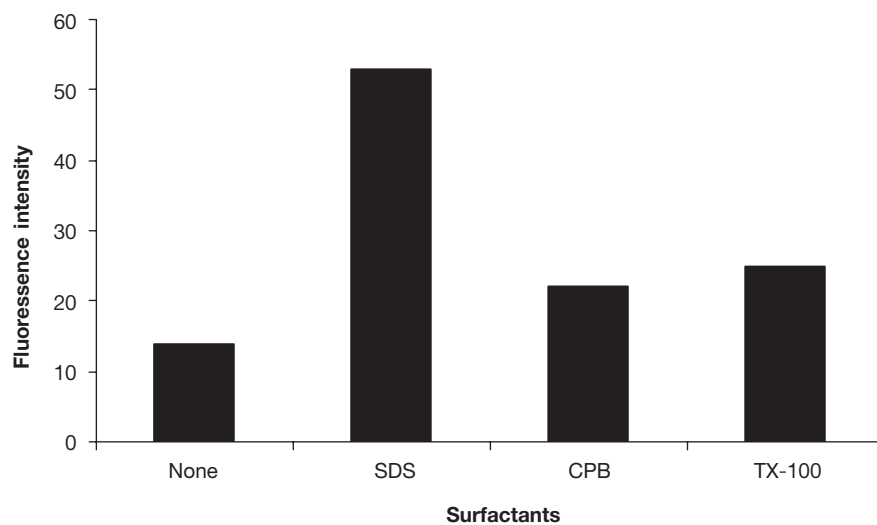


(b)

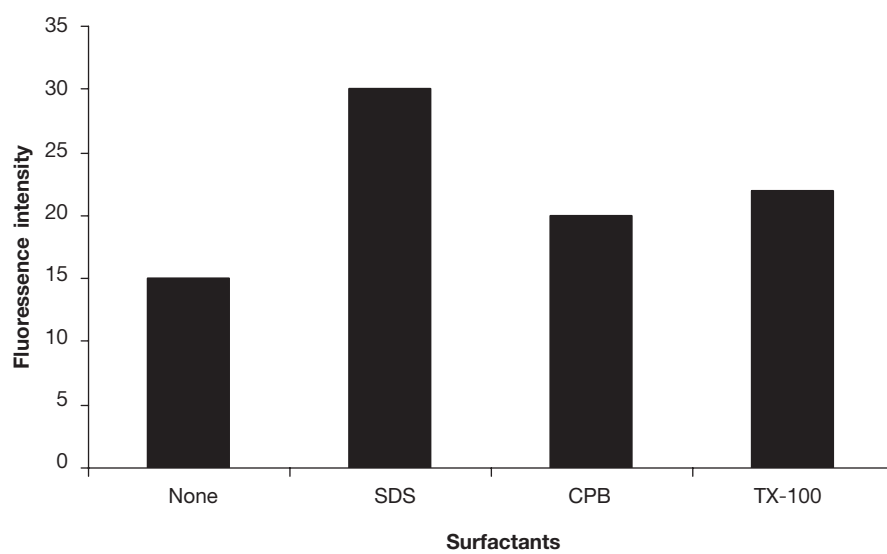


**Figure 2** Fluorescence spectra of ofloxacin ( $50 \text{ ng mL}^{-1}$ ) (a) and lomefloxacin ( $60 \text{ ng mL}^{-1}$ ) (b) in aqueous solution [excitation (A), emission (A')] and in micellar medium of 10 mM SDS [excitation (B), emission (B')]

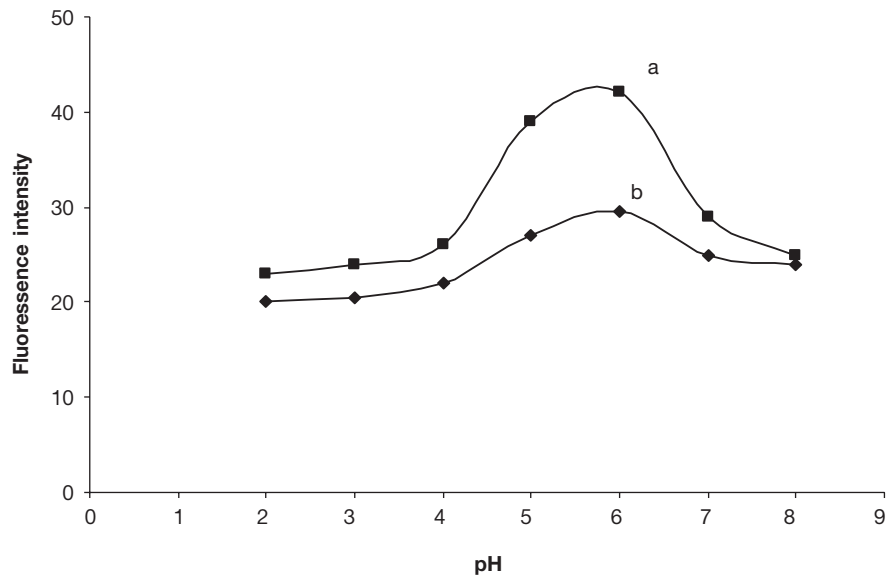
(a)



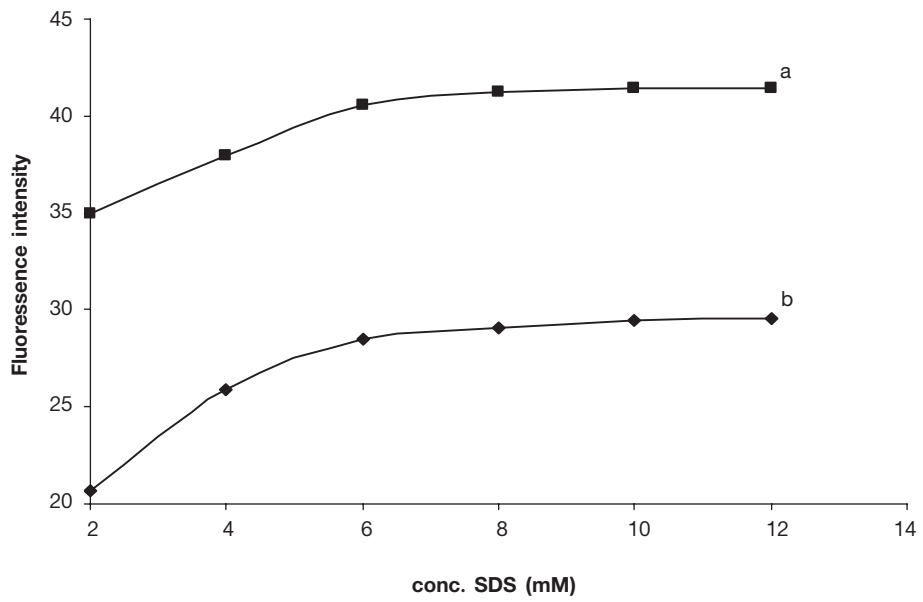
(b)



**Figure 3** Effect of surfactants on fluorescence intensity of 50 ng mL<sup>-1</sup> ofloxacin (a) and 60 ng mL<sup>-1</sup> lomefloxacin (b)



**Figure 4** Effect of pH on fluorescence intensity; (a) ofloxacin (40 ng mL<sup>-1</sup>) and (b) lomefloxacin (60 ng mL<sup>-1</sup>) in 10 mM SDS solution



**Figure 5** Effect of the SDS concentration on fluorescence intensity; (a) ofloxacin (40 ng mL<sup>-1</sup>) and (b) lomefloxacin (60 ng mL<sup>-1</sup>)

### **Effect of surfactants**

Addition of a surfactant above its critical micelle concentration (CMC) increases the fluorescence intensity of many fluorophores. This fact has been used to develop improved methods for spectrofluorimetric determination of many compounds [16-18]. In the present paper, the effect of three surfactants; an anionic (SDS), a cationic (CPB) and a neutral surfactant (TX-100) on fluorescence intensity of ofloxacin and lomefloxacin was investigated. Figure 3 shows that the enhancement of the fluorescence intensity of both drugs was maximum in the SDS solution and therefore SDS was chosen for further study. Figure 2a shows the fluorescence spectrum of ofloxacin in the SDS micellar solution having an excitation maximum at 295 nm and an emission maximum at 485 nm. The fluorescence spectra of lomefloxacin in SDS micellar solution had an excitation maximum at 285 nm and an emission maximum at 449 nm (Fig. 2b). The modification of the features of these fluorescence spectra occurs due to micellar solution of SDS which causes a change in the environment of the drug.

### **Effect of pH**

The effect of pH on the enhanced fluorescence of drugs in micellar medium was studied. The results showed the optimum pH at pH 6 for both ofloxacin and lomefloxacin (Fig. 4). Different buffer solutions (acetate, phosphate and Britton Robinson buffer) were tested, and the acetic acid-sodium acetate buffer solution was found to be most successful for both drugs. The effect of buffer strength was also investigated, and a 0.1 M buffer concentration gave an adequate buffer capacity without an excessive loss of sensitivity. Further changes in the buffer concentration produced little change in fluorescence intensity.

### **Effect of SDS concentration**

The effect of SDS concentration on fluorescence of both the drugs was studied. With increase in SDS concentration, the fluorescence intensity increased and reached a stable value at 8 mM concentration of SDS which is above the CMC value of 6 mM for SDS (Fig. 5). Further increase in concentration of SDS produced little

variation in fluorescence intensity. Therefore, a 10 mM concentration was selected as the optimum concentration of SDS for carrying out further investigations.

### **Effect of addition order of reagents**

A series of solutions were prepared by changing the sequence of addition of reagents while keeping the same concentration and fluorescence intensity was measured. It was observed that change in addition order of the reagents produced little variation in fluorescence intensity. The optimal order of addition of reagents selected was: drug, buffer and SDS.

### **Stability test**

It was observed that fluorescence intensity of the system reached a maximum value in about 10 minutes and remained stable for about 2 hours after the addition of the reagents. Therefore the fluorescence measurements were made only after completion of 10 minutes.

### **Analytical parameters**

The conditions were optimised and calibration graphs were constructed for determination of ofloxacin and lomefloxacin. The fluorescence intensity ( $\Delta F$ ) of the system showed a linear relationship with concentration of the drug in the concentration range of 10-1,000 ng mL<sup>-1</sup> and 20-2,000 ng mL<sup>-1</sup> for ofloxacin and lomefloxacin respectively. Six replicates were used for each of the five standards prepared to obtain the calibration curve. The regression equation of the calibration line for ofloxacin was  $\Delta F = 0.941C + 0.087$  with a correlation coefficient of 0.9999. For lomefloxacin, the regression equation was  $\Delta F = 0.379C + 0.039$  with a correlation coefficient of 0.9996 where C = concentration of drug in ng mL<sup>-1</sup>. The detection limit ( $3\sigma$ ) was found to be 2.1 ng mL<sup>-1</sup> for ofloxacin and 4.1 ng mL<sup>-1</sup> for lomefloxacin. The quantification limit ( $10\sigma$ ) was found to be 6.9 ng mL<sup>-1</sup> and 13.5 ng mL<sup>-1</sup> for ofloxacin and lomefloxacin respectively.

The precision of the proposed method was measured for ofloxacin concentrations of 50, 200, 500, 800 ng mL<sup>-1</sup> and the R.S.D. values obtained were 1.8, 1.5, 1.0, 1.9% respectively. For lomefloxacin

concentrations of 100, 500, 1,000, 1,500 ng mL<sup>-1</sup>, relative standard deviations (R.S.D.) obtained were 2.0, 1.1, 1.9, 2.1% respectively. The values were obtained by performing six independent determinations.

#### **Analysis of spiked serum and urine samples**

The proposed method was used to determine drug content of serum and urine samples. The samples were analyzed by standard addition method to avoid matrix effects. The results for analysis of lomefloxacin and ofloxacin in serum and urine samples are provided in Table 1. The accuracy of the proposed method was assessed by investigating the recovery of each of the studied drugs at three concentration levels covering the specified range (six replicates of each conc.). The tabulated recoveries indicate good accuracy.

#### **Luminescence mechanism**

Ofloxacin and lomefloxacin are hydrophobic drugs having very low polarity. In the presence of micellar medium of SDS the solubility of the drug in the micellar medium is greatly increased. Due to change in micro environment of the drug, there is decrease in non-radiative

energy loss through molecular collisions and quantum efficiency of fluorescence is greatly increased [18]. The optimum concentration of SDS is above the CMC value (6 mM) which indicates that formation of micelles greatly increases the fluorescence intensity of the system.

#### **Conclusion**

A simple and sensitive micelle enhanced spectrofluorimetric method for determination of ofloxacin and lomefloxacin is presented. The method was successfully applied to urine and plasma samples with good results. The method can detect these drugs at ng level and a quite low detection limit was obtained. The method is simple, not involving elaborate sample preparation steps, and less time consuming as compared to HPLC methods. The method offers high sensitivity and has a wide linear range. The sensitivity, accuracy and detection limit offered by this method is comparable to the HPLC methods used for the determination of these drugs in biological fluids. However the proposed method is much simpler, inexpensive, involves easy sample preparation and can be used alternative to HPLC methods.

**Table 1** Determination of lomefloxacin and ofloxacin in human samples

Sample	Ofloxacin			Lomefloxacin		
	Spiked ( $\mu\text{g mL}^{-1}$ )	Found <sup>a</sup> ( $\mu\text{g mL}^{-1}$ )	Recovery (%)	Spiked ( $\mu\text{g mL}^{-1}$ )	Found <sup>a</sup> ( $\mu\text{g mL}^{-1}$ )	Recovery (%)
Urine-1	100	100.30 $\pm$ 0.70	100.3	60	60.40 $\pm$ 0.80	100.6
Urine-2	200	198.00 $\pm$ 1.20	99.0	80	81.00 $\pm$ 0.90	101.2
Urine-3	300	303.00 $\pm$ 1.50	101.0	100	99.50 $\pm$ 1.10	99.5
Serum-1	3	3.05 $\pm$ 0.08	101.6	3	3.10 $\pm$ 0.05	103.0
Serum-2	4	3.96 $\pm$ 0.07	99.0	4	3.96 $\pm$ 0.10	99.0
Serum-3	6	6.15 $\pm$ 0.10	102.6	5	4.94 $\pm$ 0.09	98.8

<sup>a</sup>average value  $\pm$  S.D. of six determinations

## References

- [1] L. L. Shen. Quinoline DNA interaction. *Quinolone Antimicrobial Agents* (2<sup>nd</sup> ed.), American Society for Microbiology, Washington D.C., 1993, pp. 77-95.
- [2] D. C. Hooper, and J. S. Wolfson. Mechanisms of quinolone action and bacterial killings. *Quinolone Antimicrobial Agents* (2<sup>nd</sup> ed.), American Society for Microbiology, Washington D.C., 1993, pp. 53-57.
- [3] T. G. Mant. Multiple-dose pharmacokinetics of lomefloxacin: rationale for once-a-day dosing, *Am. J. Med.* 92: 26S-32S (1992).
- [4] R. G. Finch. The role of new quinolones in the treatment of respiratory tract infections, *Drugs* 49, 144-151(1995).
- [5] H. A. Okeri, and I. M. Arhewoh. Analytical profile of the fluoroquinolone antibacterials ofloxacin, *African J. Biotechnol.* 7: 670-680 (2008).
- [6] F. T. Robert, C. A. Robert, M. P. Frano, and J.A. Longstreth, Determination of certain fluoroquinolones in bulk drug and dosage forms by high-performance liquid chromatography, *J. Pharm. Biomed. Anal.* 13: 1243-1248 (1995).
- [7] A. E. Mansilla, A. M. Pena, D. G. Gomes, and F. Salinas. HPLC determination of enoxacin, ciprofloxacin, norfloxacin and ofloxacin with photoinduced fluorimetric detection and multiemission scanning: application to urine and serum, *J. Chromatogr. B* 822: 185-193 (2005).
- [8] A. M. Shibl, A. M. Shibl, A. F. Tawfik, S. Houfy, and F. J. Shammery. Determination of lomefloxacin in biological fluids by high-performance liquid chromatography and a microbiological method, *J. Clin. Pharm. Ther.* 16: 353-9 (1991).
- [9] P. Gurumurthy, G. Ramachandran, A. K. Hemanth Kumar, P. Venkatesan, V. Chandrasekharan, and P.R. Narayanan. Simple spectrofluorimetric and microbiological assay methods for the estimation of ofloxacin in biological fluids, *Indian J. Pharmacol.* 30: 263-266 (1998).
- [10] M. Hernández, F. Borrull, and M. Calull. Determination of quinolones in plasma samples by capillary electrophoresis using solid-phase extraction, *J. Chromatogr. B* 742: 255-265 (2000).
- [11] S. Hanwen, L. Liqing, and S. Ming. Simultaneous determination of lidocaine, proline and lomefloxacin in human urine by CE with electrochemiluminescence detection, *Chromatographia* 67: 399 (2008).
- [12] S. Hanwen, L. Liqing, and C. Xueyan. Flow-injection chemiluminescence determination of ofloxacin and levofloxacin in pharmaceutical preparations and biological fluids, *Anal. Sci.* 2: 1145-1149 (2006).
- [13] L. H. Nie, H. C. Zhao, X. Wang, L. Yi, Y. Lu, L. P. Jin, and H. M. Ma. Determination of lomefloxacin by terbium sensitized chemiluminescence method, *Anal. Bioanal. Chem.* 374: 1187-1190 (2002).
- [14] Y. M. Issa, F. M. Abdel-Gawad, M. A. A. Table, and H. M. Hussein. Spectrophotometric determination of ofloxacin and lomefloxacin hydrochloride with some sulphonphthalein dyes, *Anal. Lett.* 30: 2071-2084 (1997).
- [15] S. Ulu. Highly sensitive spectrofluorimetric determination of lomefloxacin in spiked human plasma, urine and pharmaceutical preparations, *Eur. J. Med. Chem.* 44: 3402-3405 (2009).
- [16] J. A. Ocana, F. J. Barragan, and M. Callejon. Fluorescence and terbium-sensitized luminescence determination of garenoxacin in human urine and serum, *Talanta* 63: 691-697 (2004).
- [17] A. M. Garcia-Campana, J. J. Aaron, and J. M. Bosque-Sendra. Micellar-enhanced photochemically induced fluorescence detection of chlorophenoxyacid herbicides: flow injection analysis of mecoprop and 2,4-dichlorophenoxyacetic acid, *Talanta* 55: 531-539 (2001).
- [18] C. L. Tong, Y. Zhu, and W. P. Liu. Study on the co-luminescence system of Dy-Gd-1,6-bis (1-phenyl-3-methyl-5-pyrazol-4-one) hexanedione-cetyltrimethyl ammonium bromide and its analytical application, *Analyst* 126: 1168-1171 (2001).