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Short communication

Simplified reversed-phase LC method with spectrophotometric detection for estimation of sparfloxacin in human plasma

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1. Introduction

Sparfloxacin is a long acting new quinolone antibacterial agent and has a broad antibacterial spectrum against gram-positive and gram-negative bacteria [1,2]. Sparfloxacin is well absorbed following oral administration and distributed extensively to the target tissues, and it has a long plasma elimination half-life of 16 h [3,4]. Consequently, when conducting pharmacokinetic studies with this compound, knowledge of drug concentrations in plasma/blood are required.

Few LC methods are reported to estimate sparfloxacin in human plasma [3,5,6]. These methods involve solid phase extraction and liquid–liquid extraction techniques for sample preparation. The proposed method avoids these tedious extraction procedures and introduces a simple, one step, rapid sample preparation technique, with a better level of detection. This method was found to be economic, sensitive, specific and reproducible.

2. Materials and methods

2.1. Reagents and materials

Sparfloxacin and lomefloxacin (internal standard) were obtained from Sun Pharma. Analytical grade sodium citrate, citric acid, O-phosphoric acid, perchloric acid (70%) and HPLC grade acetonitrile were obtained from Spectrochem (Mumbai, India).

The protein precipitating solution was a mixture of acetonitrile–perchloric acid in the ratio 1:1 (v/v).

2.2. Apparatus and conditions

The HPLC system (Alliance) consisted of a waters 2487 dual wavelength detector, a 2690 seperation module, the millennium version 2.15.01 software. The columns used were PRODIGY, 5 μ,

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ODS, Phenomenex, USA 30×4.6 mm (guard column) and 250×4.6 mm (analytical column), respectively.

The mobile phase used was acetonitrile–citrate buffer (pH 2.4, 0.02 M) (50:50 v/v). Citrate buffer was prepared by dissolving 5.88 g of sodium citrate and 4.2 g of citric acid in 900 ml of HPLC grade water. The pH of the above solution was adjusted to 2.4 with O-phosphoric acid and volume adjusted to 1000 ml. Flow rate was 1.0 ml min^{-1}, detection wavelength 290 nm, and the injection volume was 20 μl.

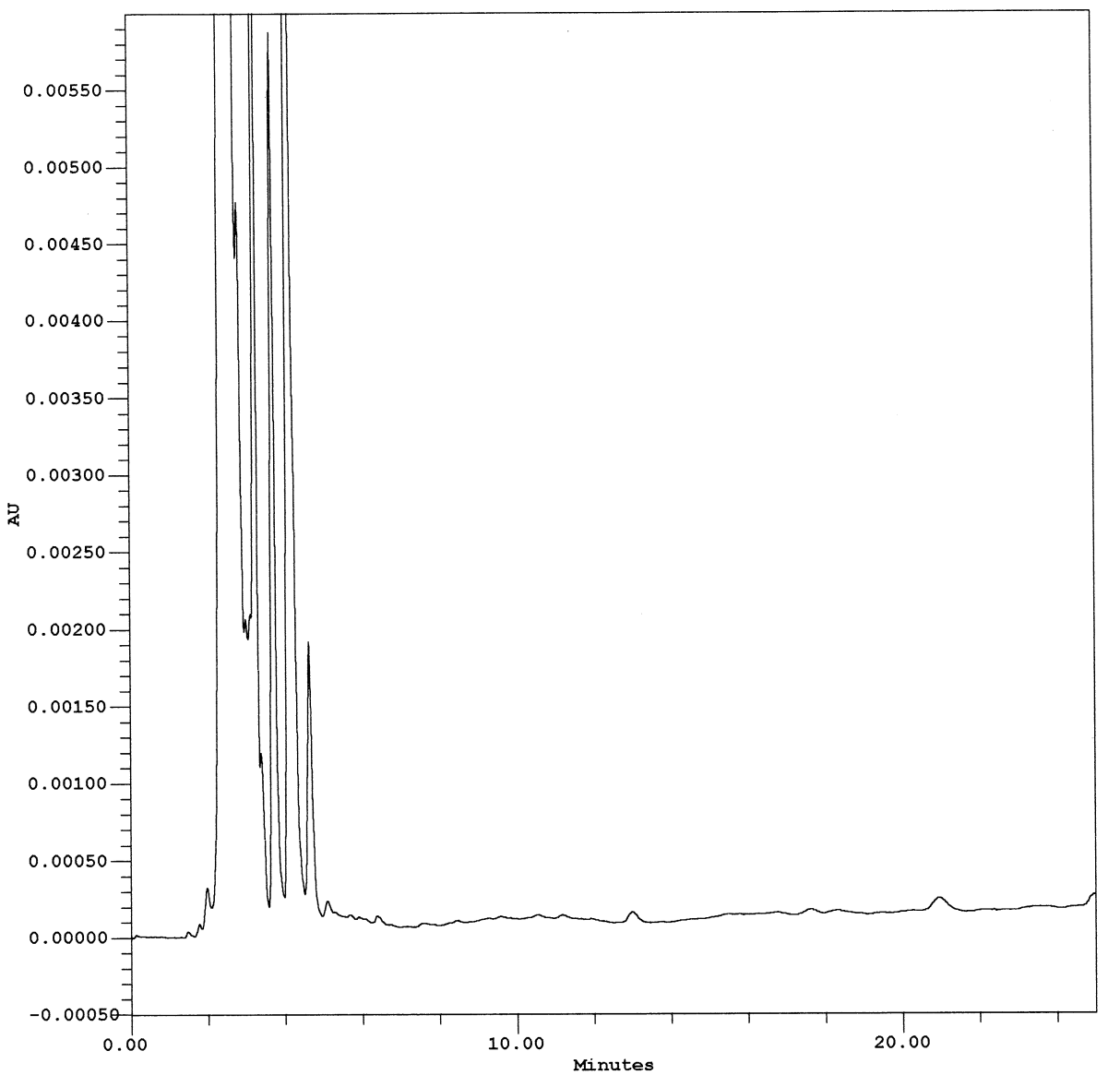

Fig. 1. Chromatogram of blank human plasma.

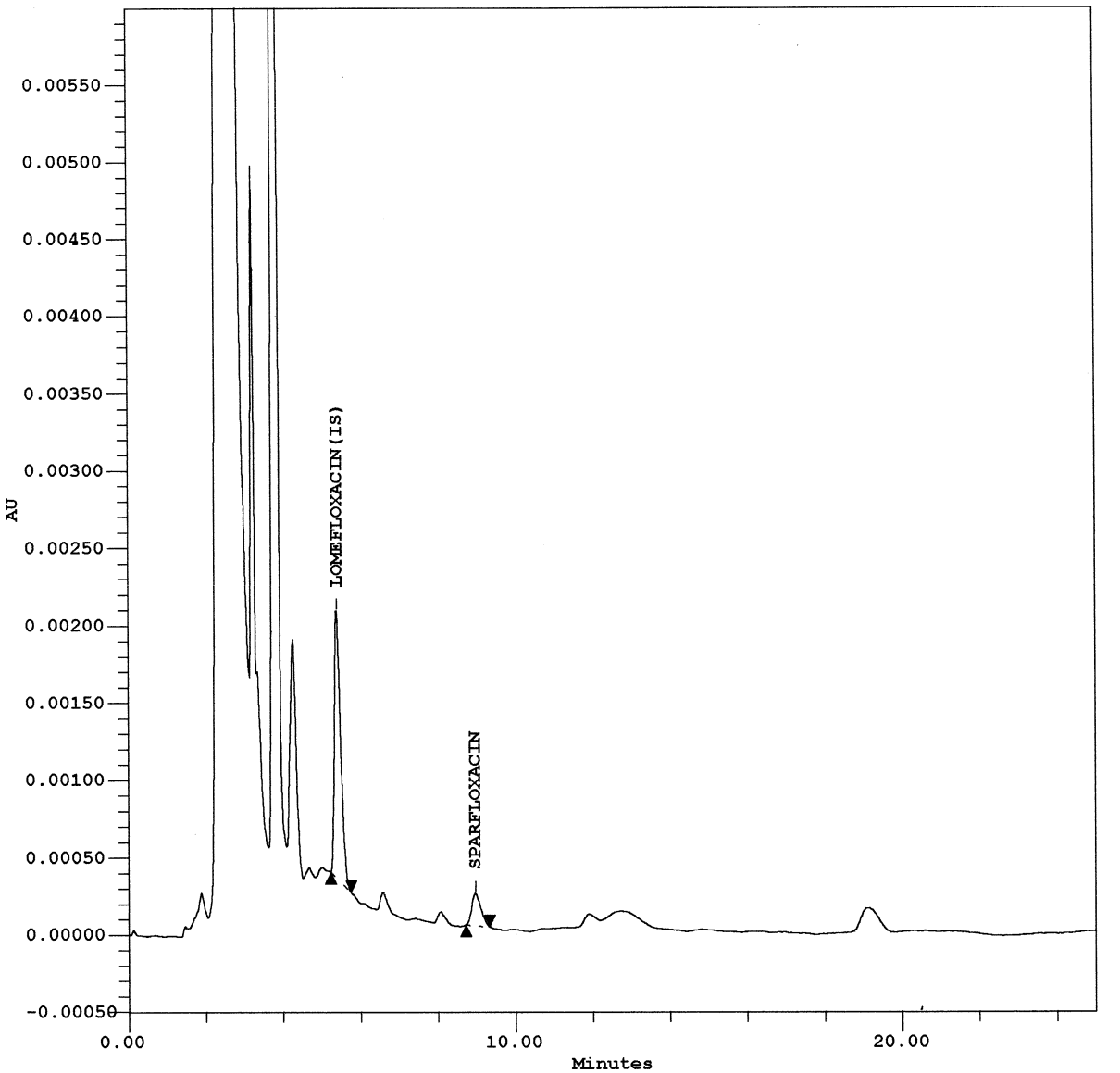

Fig. 2. Chromatogram of 100 ng ml^{-1} calibration standard.

2.3. Standard solutions and calibrations

A standard stock solution of sparfloxacin (0.5 mg ml^{-1}) was prepared in mobile phase. The solution was stable for three weeks at −20°C. Lomefloxacin (internal standard) was prepared (5 μg ml^{-1}) in mobile phase and was stable for 2 weeks at −20°C. Plasma standards were prepared containing 100, 200, 300, 400, 600, 800, 1200, and 1500 μg ml^{-1} of the analyte by diluting appropriate aliquot (100 μl) of stock solution with drug-free plasma. The calibration curve was obtained by linear regression of the peak-area ratio versus concentration. Peak area ratios were fit to a least squares linear regression algorithm with a $1/x^2$ weighting. The correlation coefficients were greater than 0.99 for all curves ($n = 5$).

2.4. Sample preparation

One ml of plasma was transferred to a clean 5 ml borosilicate glass tube, 50 μl of internal standard (IS) was added and vortex mixed for 10 sec. To this, 200 μl of protein precipitating solution was added and vortex mixed for 1 min. After centrifugation at 3000 rpm for 20 min, 20 μl of the clear supernatant was then injected into the HPLC.

3. Results and discussion

3.1. Chromatography

The precision of the retention times observed for sparfloxacin and internal standard ($n = 25$), expressed as RSD were less than 0.5%. The representative chromatograms of blank plasma and plasma spiked with sparfloxacin and IS shown in

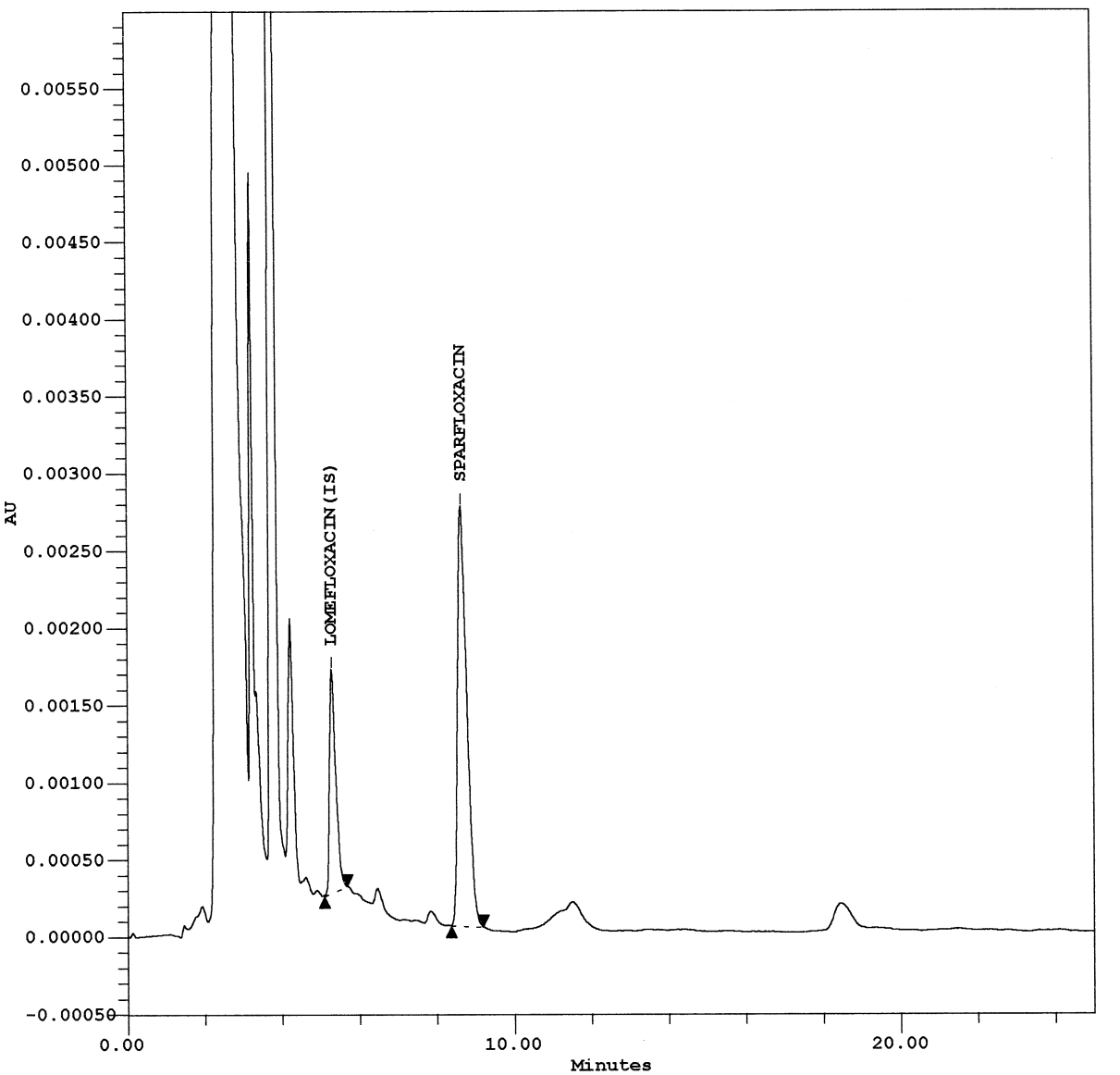

Fig. 3. Chromatogram of 1600 ng ml^{-1} calibration standard.

Table 1
Data from calibration curves of sparfloxacin

Day	Slope	Intercept	R^2
1	0.001664	−0.001976	0.9961
2	0.001639	−0.001432	0.9969
3	0.0018	−0.0015	0.9938
4	0.001686	−0.021779	0.9988
5	0.001566	−0.026397	0.9978
Mean	0.0017	−0.0105	0.9967
SD	9.35×10^{-5}	0.0124	0.0019
% RSD	5.5841	118.6708	0.1906

Figs. 1–3 indicate that there are no endogenous interfering components and co-eluting with sparfloxacin or the IS.

3.2. Linearity of calibration standard curves

Table 1 contains the linear regression results for calibration curves performed on five different days. The mean correlation coefficients (r^2), slopes and intercepts are included in the Table 1. Calibration curves were linear in the range 100–1500 ng ml^{-1} with r^2 values more than 0.99.

3.3. Accuracy and reproducibility

The limit of detection (LOD) for this assay was set at 5 ng ml^{-1} and the limit of quantitation (LOQ) was 50 ng ml^{-1}. The intra and inter-day accuracy and reproducibility was assessed by replicate analysis of three QC samples (200, 800, and 1600 ng ml^{-1}). The inter-day analysis precision was assessed over a period of five days. The mean RSD values for intra-day and inter-day assay reproducibility ($n = 5$) were 2.04 and 6.2%, respectively.

Table 2
Interday variability of the assay of quality control samples at three concentration levels

Added concentration (ng ml^{-1})	Mean analysed concentration ± SD (ng ml^{-1}) ($n = 30$)	% CV	% Bias
200	201.52 ± 12.25	6.08	0.76
800	745.95 ± 53.96	7.24	−6.82
1600	1522.50 ± 80.39	5.28	−4.84

Table 3
Intraday variability of the assay of quality control samples at three concentration levels

Added concentration (ng ml^{-1})	Mean analysed concentration ± SD (ng ml^{-1}) ($n = 30$)	% CV	% Bias
200	202.87 ± 4.70	2.32	−1.44
800	795.13 ± 18.13	2.28	−0.61
1600	1589.64 ± 25.9	1.63	−0.65

Table 4
Freeze-thaw effect on sparfloxacin recovery

Concentration (ng ml^{-1})	% Recovery of sparfloxacin in freeze-thaw cycle			Mean ± SD	CV%
	1	2	3		
200	88.8	86.2	82.1	85.7 ± 3.38	3.94
800	89.2	91.3	88.6	89.7 ± 1.42	1.58
1600	85.3	86.9	89.4	87.2 ± 2.07	2.37

Tables 2 and 3 summarize the assessment of both interday and intraday reproducibility of the method. Data presented in Tables 2 and 3 are the coefficients of variability (CV%) for each check sample processed.

3.4. Absolute recovery

Absolute recoveries were determined by comparing the ratio peak areas of sparfloxacin to internal standard for the standard preparations against those of the same preparations in supernantant obtained after treatment of drug free plasma with protein precipitating solution. Recoveries performed at three clinically relevant concentrations (200, 800, and 1600 ng ml^{-1}) were greater than 80% ($n = 6$). Three freeze thaw cycles did not alter the recovery of the analyte significantly (82–91%).

3.5. Stability

Evaluation of the effect of short term storage of extracted plasma samples on the standard curve characteristics and chromatographic behaviour of sparfloxacin and IS were also performed. Regression analysis of the standard curve data gave correlation coeffecients and values for the slope and Y intercept within the same order of magnitude following storage of samples at -20°C from 1–30 days. The chromatographic behaviour was also unaffected by storage of extracted plasma samples in auto sampler at 20°C for 48 h. Freeze-thaw analysis of three cycles did not show any effect on the recovery of sparfloxacin. The results are shown in Table 4.

4. Conclusions

The HPLC assay described here is simple, selective, precise and accurate for quantitation of sparfloxacin in human plasma. The sensitivity, rapidity and economy of the method was the main achievement which can be applied for routine therapeutic monitoring of the drug and will prove useful in supporting pharmacokinetics and drug interaction studies.

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