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HPTLC Method Development for Pharmacokinetic Study of Sparfloxacin in Plasma

R. R. SHAH*[1], B. N. SUHAGIA, I. S. RATHOD, S. A. SHAH AND D. M. PATEL[2]
L. M. College of Pharmacy, Navarangpura, Ahmedabad-380 009
[1]Shri Swaminarayan Sanskar Pharmacy College, Zundal-382 421
[2]Shri B. M. Shah College of Pharmaceutical Education and Research, Modasa-383 315.

A simple, sensitive, specific and precise method has been developed for the analysis of the sparfloxacin in plasma. Extraction of sparfloxacin from plasma was done with dichloromethane. Standard solutions and extracted samples were spotted on precoated silica gel G 60 F 254 plates and developed using chloroform:toluene:methanol:diethylamine (4:4:1.5:0.2 v/v) as mobile phase. Densitometric scanning was performed at 301 nm. The method had linearity range of 80-200 ng/spot and average recovery was found to be 89.17 %. This method has been successfully used for the estimation of sparfloxacin in plasma samples of volunteers. The pharmacokinetic parameters like C_{max}, T_{max}, and $AUC_{0-\alpha}$ were computed.

Sparfloxacin,[5-amino-1-cyclopropyl-6,8-difluoro-7-(cis-3,5-dimethyl-piperazinyl)-4 (1H)-oxoquinolone-3-carboxylic acid], is a fluoroquinolone, which has demonstrated potent, broad antibacterial spectrum of activity against gram-negative bacteria, mycoplasma pneumonia, and some intracellular organisms; including major respiratory pathogens[1-2]. Activity of sparfloxacin against gram-positive bacteria particularly *Streptococcus pneumonia* shows that it fulfills the gap seen with other fluoroquinolones. It is not official in any pharmacopoeia.

Several analytical methods for estimation of sparfloxacin have been reported which includes spectrophotometric methods[3-7] and high performance liquid chromatographic methods for estimation of sparfloxacin in dosage forms and in biological fluids[8-11]. The spectrophotometric methods are not suitable to determine low concentration of the drug present in plasma after oral administration of sparfloxacin tablet.

In present work simple, rapid, specific, precise and accurate HPTLC method was developed for the estimation of sparfloxacin in human plasma after extraction with dichloromethane. The developed method was applied to study the pharmacokinetic parameters of sparfloxacin in human volunteers.

*For correspondence
E-Mail: ragin_shah@rediffmail.com

MATERIALS AND METHODS

Instruments used for the estimation were Camag Linomet IV automatic sample spotter with Hamilton syringe, Camag TLC Scanner III and CATS 4 software for interpretation of data. Precoated silica gel G 60 F 254 plates having 200 µm thickness were used for spotting of the standard solutions and extracts. Standard sparfloxacin was obtained from Torrent Pharmaceuticals Limited, Ahmedabad. Sparfloxacin formulations (Torospar of Torrent Pharmaceuticals Ltd., Sparlox of Sun Pharmaceuticals Ltd., Rexpar of Ranbaxy Laboratories Ltd.) were procured from the local pharmacy. Methanol (AR grade), dichloromethane (AR grade), acetonitrile (AR grade), chloroform (S. D. Fine chemicals), diethylamine (S. D. Fine chemicals) and sodium sulphate (AR grade) were used.

Standard stock solution of sparfloxacin was prepared by dissolving accurately weighed sparfloxacin (5 mg) in methanol:dichloromethane (1:1) mixture and diluted to 10 ml with the same solvent. One millilitre of standard stock solution was diluted further to 10 ml with methanol: dichloromethane (1:1) mixture to obtain working standard solution.

Chromatographic parameters:

Standard solutions and extracts were spotted to precoated silica gel G 60 F 254 plate with the help of Camag Linomet IV automatic sample spotter with Hamilton syringe as 3 mm bands with a distance of 5 mm between the bands. Plate was developed using chloroform: toluene: methanol: diethylamine (4:4:1.5:0.2 v/v) as mobile phase in a twin trough glass chamber. Saturation time was 25 min and ascending migration distance for mobile phase was 55 mm. After removal from the chamber, the plate was dried in air for 15 min and was scanned and quantified at 301 nm using Camag TLC Scanner III and CATS 4 software. Data of the peak area of each band was recorded. Standard curve for sparfloxacin in the range of 80–200 ng/spot was generated by plotting the peak area against concentration of the standard solutions of sparfloxacin prepared in methanol:dichloromethane (1:1) mixture.

Spike study:

To 1 ml of plasma, varying volumes (8, 10, 12, 14, 16, 20 µl) of working standard sparfloxacin solutions having concentration of 50 µg/ml were added and test tubes were shaken in cyclomixer for 1 min. Acetonitrile (300 µl) was added to each test tube and shaken on cyclomixer and allowed to stand for 5 min. Free drug was extracted with 5 ml of dichloromethane by vortexing followed by centrifugation of the test tubes at 403.67 'g' for 15 min. Organic layer from each test tube was carefully separated and transferred. Sodium sulphate was added to dry the organic phase and anhydrous supernatant organic layer was then evaporated carefully on water bath. Residue was dissolved in 50 µl of methanol: dichloromethane (1:1) mixture. Ten microlitre of reconstituted solutions were spotted on precoated silica gel G 60 F 254 plates, developed and quantified with Camag TLC Scanner III and CATS 4 software at 301 nm. Calibration curve was obtained by plotting the peak area of spiked plasma concentrations of sparfloxacin against spiked concentrations. Unknown sparfloxacin concentrations in plasma samples were determined using this calibration curve.

Method validation:

The recovery of sparfloxacin from plasma was determined by comparing peak area, obtained from spiked plasma with different volumes (8, 10, 12, 14, 16, 20 µl) of working standard sparfloxacin solution (50 µg/ml), with the peak area obtained with standard sparfloxacin solutions. The intra-day and inter-day precisions were determined. Linearity of detector response was tested by analyzing standards 5 times for each concentration ranging between 80–200 ng/spot.

Clinical studies:

A single dose of 200 mg of sparfloxacin tablet (Torospar of Torrent Pharmaceuticals Ltd.) was orally administered to 3 healthy male volunteers in the age group of 21-24 y. The volunteers were kept on normal diet during the study and no other drugs were taken by volunteers, two weeks before and during study. Blood specimens were collected from antecubital vein into heparinised tube at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 h after the drug was given and were immediately centrifuged at 403.67 'g' for 15 min. Supernatant separated plasma samples were stored in refrigerator at -20° until analysis. Concentrations of sparfloxacin in plasma samples were calculated from the calibration curve. Standard sparfloxacin solution (10 µl) was spotted every time to keep check on the experimental conditions. Peak plasma concentration (C_{max}) and time to reach the peak plasma concentration (T_{max}) were obtained from plasma concentration – time data. The area under the curve ($AUC_{0-\alpha}$) was calculated by trapezoidal method. The elimination rate constant (K_{el}), elimination half-life ($T_{1/2el}$) and absorption half-life ($T_{1/2a}$) were also calculated.

RESULTS AND DISCUSSION

Several analytical methods have been reported for estimation of sparfloxacin in plasma, but HPTLC method was selected owing to its simplicity, specificity, sensitivity and reproducibility. It provides rapid analysis with minimum amount of sample. Extraction procedure always remains quite critical to obtain good recovery of analyte from plasma sample. In the proposed procedure, plasma sample was extracted with dichloromethane. Thus a single step simple extraction procedure was developed for effective extraction (with average recovery of 89.17 %) of drug from plasma. By using chloroform: toluene: methanol: diethylamine (4:4:1.5:0.2 v/v) as mobile phase, Camag TLC Scanner III and CATS 4 software, it was observed that sparfloxacin was separated well from the endogenous plasma components (fig.1). The least square linear regression analysis of peak area (Y) versus concentration of sparfloxacin in plasma gave equation of straight line Y=561.98X+1258.7 with a correlation coefficient of 0.9945 over a range of 80-200 ng/spot. Limit of detection and limit of quantification were found to be 50 ng/spot and 80 ng/spot, respectively. Overall average recovery of various concentrations of sparfloxacin from plasma was 89.17 % (Table 1). The method was validated by determining reproducibility and accuracy for a spiked plasma samples (Table 1). Intra-day and inter-day coefficients of variation for analysis of plasma samples were

TABLE 1: SUMMARY OF VALIDATED PARAMETERS FOR THE PROPOSED HPTLC METHOD FOR ESTIMATION OF SPARFLOXACIN IN PLASMA.

Parameters		Results
Precision (% CV)		092-4.97
Accuracy (% recovery)		84.21-97.11 (Avg 89.17)
Limit of detection (ng/spot)		50
Limit of quantification (ng/spot)		80
Specificity		Specific
Linearity (correlation coefficient)		0.9945
Range (ng/spot)		80-200
Intra-day Coefficient of	100 ng	2.08
Variation (%COV)	200 ng	1.14
Inter-day Coefficient of	100 ng	1.35
Variation (%COV)	200 ng	2.11

calculated which were varies from 1.14 % to 2.08 % and 1.35 to 2.11 %, respectively (Table 1). Proposed HPTLC assay method for sparfloxacin estimates the drug concentrations in volunteer plasma samples with sufficient sensitivities to allow clinical study. The overlay time versus plasma concentration curve for three volunteers showed no significant differences of T_{max} values of sparfloxacin in plasma (fig.2). Calculated pharmacokinetic parameters were summarized in Table 2.

TABLE 2: SUMMARY OF PHARMACOKINETIC PARAMETERS

Pharmacokinetic parameters	Values by proposed method*
Elimination rate constant (K_{e1}) h^{-1}	0.04145
Absorption rate constant (Ka) h^{-1}	1.4949
Elimination Half life ($T_{1/2el}$) h	16.719
Absorption Half life ($T_{1/2a}$) h	0.4636
Maximum Plasma Concentration (C_{max}) mg/l	0.502
Time for maximum concentration (T_{max}) h	2.47
Area Under Curve (AUC) mg/l h	12.644
Area Under Moment Curve (AUMC) mg/l	4665.67
Volume of Distribution (Vd/F) l/kg	6.35

*Average of three determinations

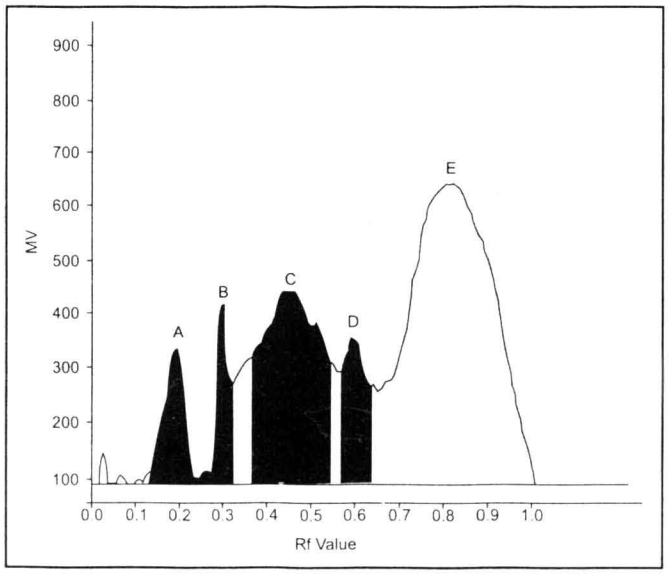

Fig. 1: HPTLC Chromatogram of sparfloxacin in plasma. A represents peak of sparfloxacin and B, C and D represent peaks of plasma constituents

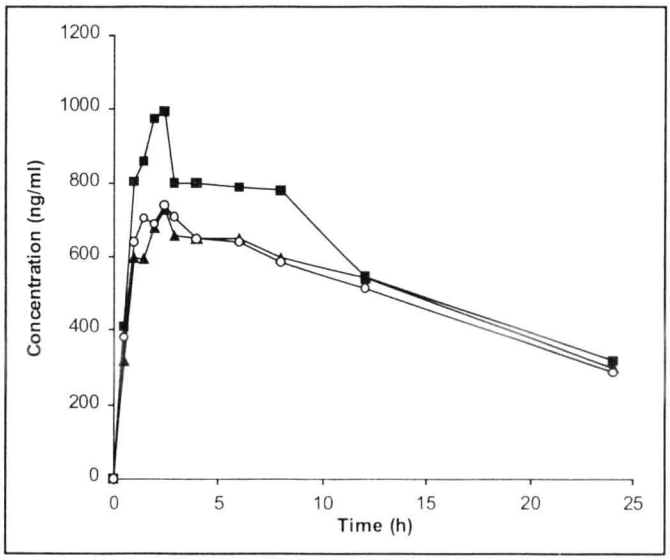

Fig. 2: Overlay of plasma concentration v/s time curve after sparfloxacin administration.

Volunteer 1 (–▲–), volunteer 2 (–■–) and volunteer 3 (–o–)

The proposed HPTLC method is rapid, specific, sensitive and accurate. This method was successfully used to analyze sparfloxacin in plasma and can also be used to evaluate pharmacokinetic parameters and for bioequivalence study.

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