Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences
ISSN- 0975-1491 Vol 6, Issue 9, 2014

Original Article

# DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF SITAFLOXACIN IN BULK AND PHARMACEUTICAL DOSAGE FORMS

**NAGA RAJU POTNURI*[1], DEVALA RAO G[2], RAJENDRA PRASAD Y[3]**

**[1]Department of Pharmaceutical Analysis, Joginpally B. R. Pharmacy College, Yenkapally, Moinabad, R. R. Dist, A. P, India, [2]Department of Pharmaceutical Analysis, K. V. S. R Siddhartha College of Pharmaceutical Sciences, Vijayawada, A. P, India, [3]Department of Pharmaceutical Chemistry, University Colleges of Pharmaceutical Sciences, Andhra University, Vishakhapatnam, A. P, India**
**Email: nagaraju_potnuri@yahoo.co.in**

*Received: 16 Jun 2014 Revised and Accepted: 04 Sep 2014*

**ABSTRACT**

**Objective:** A simple, specific, and precise reversed phase high performance liquid chromatography method was developed and validated as per the ICH guidelines for the determination of Sitafloxacin in bulk and tablet dosage forms.

**Methods:** The quantitation was carried out by using Symmetry $C_{18}$ ((5 µm, 100X4.6 mm) column at $30^0$c with Water: Acetonitrile in ratio of 70:30 % V/V as mobile phase. The flow rate is 0.9 mL/min and the estimation was carried out by using PDA detector at 300 nm.

**Results:** The retention time of Sitafloxacin was 2.198 minutes. The linearity was observed from 5-25 µg/mL with correlation coefficient 0.9999. The LOD and LOQ were found to be 0.429µg/mL and 1.415µg/mL respectively.

**Conclusion:** The Statistics data for the Sitafloxacin was concluded that the method was found to be simple, reliable, selective, reproducible and accurate. The method was successfully used for quality control analysis of Sitafloxacin in bulk and Pharmaceutical dosage forms.

**Keywords**: Sitafloxacin (SFX), RP-HPLC, Validation.

## INTRODUCTION

Sitafloxacin is an orally active of fourth generation new fluoroquinolone antibiotic. The chemical name of the Sitafloxacin is 7-[(4S)-4-Amino 6-azaspiro [2, 4] heptan-6 yl]-8-chloro-6-fluoro-1-[(2S)-2-fluorocyclopropyl]-4-oxoquinoline 3-carboxylic acid [1, 2]which includes amino pyrrolidine substituent at C-7 and a fluorocyclopropyl group at N-1. It contains a chlorine substituent at position C-8 of the quinolone nucleus and its molecular formula is $C_{19}H_{18}ClF_2N_3O_3$. It is very high activity against gram-positive, gram-negative, and anaerobic organisms [3]. It has more potentactivity against gram-positive and gram-negative bacteria compared to currently available quinolones such as Ofloxacin, ciprofloxacin,to sufloxacin, and sparfloxacin[4, 5, 6 ] and reduced toxicity compared to other fluoroquinolones. The structure of Sitafloxacin shown in Figure 1. SFX is active against methicillin-resistant staphylococci, Streptococcus pneumoniae and other type of streptococci with reduced susceptibility to levofloxacin and other fluoro quinolones. The doses are given once daily because the post-antibiotic effect lasts greater than 6 hours and the serum half-life is 7 hours [7, 8]. Some of the recently developed quinolones have shown excellent anti-chlamydial activity in vitro and in vivo, as well as inclinical studies [9]. Only one analytical method was reported in the literature, such as determination of sitafloxacin in human plasma by using LC-MS/MS [10]. However literature survey reveals that, there is no method for estimation of sitafloxacin in bulk and pharmaceutical dosage forms by RP-HPLC. So we have developed and validated this method used for routine quality control analysis of Sitafloxacin.

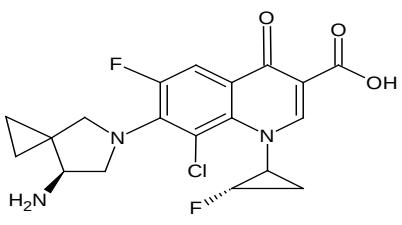



**Fig. 1: Chemical structure of SFX**

## MATERIALS AND METHODS

### Materials

Sitafloxacin pure drug was obtained as a gift sample from Daiichi Sankyo Co., Ltd, Japan. HPLC grade Acetonitrile, and water [filtered through 0.2µ filters] were purchased from Merck, Mumbai, India.

### Preparation of Solutions

### Stock solution

100 mg of Sitafloxacin pure drug was dissolved in 100 ml of HPLC grade water: Acetonitrile (70:30) to get a concentration of 1000 µg/mL.

### Standard solution

10 ml of stock solution was taken in 100 ml volumetric flask and diluted up to the mark with HPLC grade water to get a concentration of 100µg/mL.

### Sample solution

20 tablets of Sitafloxacin were powdered and an amount of the powder equivalent to 100 mg of the drug was accurately weighed and transferred to the 100 ml volumetric flask, made up to the volume with diluent. The solution was placed in an ultrasonicator for 20 minutes and filtered through a 25 mm, 0.45 µm nylon syringe filter. 10 ml of this solution was taken and diluted to 100 ml by using a HPLC grade water to get a final concentration of 100 µg/mL. Five replicate sample solutions were prepared in the similar manner.

### Hplc instrumentation and conditions

### Instrumentation

Waters HPLC system consisting of WATERS 2695 separation module, an inbuilt auto sampler, column oven and WATERS 2996 (PDA) detector was employed for throughout the analysis. Chromatography was performed on a Symmetry $C_{18}$ column. A sonerex sonicator was used for sonication. The data were acquired by using the EM Power[2] software and other details of the instrumentation are given in Table 1.

**Optimized chromatographic conditions**

Chromatography was performed on a Symmetry $C_{18}$ column using mobile phase containing mixture of Water: Acetonitrile 70:30% V/V. The mobile phase was filtered through membrane filter (0.45 µm), and vacuum degassed by sonication prior to use.

**Table 1: Instrumentation and Optimized chromatographic conditions for proposed method**

| S. No. | Instrumentation | Optimized Chromatographic Conditions |
|---|---|---|
| 1 | HPLC | Waters: 2695 Separation Module |
| 2 | Detector | Waters: 2996 PDA |
| 3 | Column | Symmetry $C_{18}$ (5 µm, 100X4.6 mm) |
| 4 | Column temperature | $30^0$C |
| 5 | Flow rate | 0.9 mL/min |
| 6 | Injection volume | 20 µL |
| 7 | Wavelength | 300 nm |
| 8 | Run time | 10 minutes |
| 9 | Diluent | Water (HPLC Grade) |
| 10 | Mobile phase composition | Water: ACN 70:30% V/V |

The pump pressure and run time was maintained at 1500-2000 psi and 10 minutes respectively. Chromatography was performed at ambient temperature with flow rate at 0.9 mL/min and detection was carried out at 300 nm. Instrumentation and optimized chromatographic conditions for proposed method details are shown in Table 1.

**RESULTS AND DISCUSSION**

**Validation study of Sitafloxacin**

The Method validation was performed as per ICH guidelines for the determination of Sitafloxacin in bulk, and dosage forms. The method was validated with respect to parameters including accuracy, precision, linearity, robustness, specificity, system suitability, LOD and LOQ [11].

**Assay of Sitafloxacin tablets**

The developed method was applied to the assay of SFX tablets. The drug content was estimated with an average of six determinations, and its results were given in Table 2. The results were similar to the labeled claim of market formulations. The standard and sample chromatograms of Sitafloxacin were shown in Figure 2 and 3 receptively.

**Table 2: Assay results of Sitafloxacin formulations**

| S. No. | Formulations | Standard Peak area | Sample Peak area | Labeled Amount(mg) | Amount Found(mg) | %Assay ±RSD* |
|---|---|---|---|---|---|---|
| 1 | M. F | 1318938 | 1318929 | 100 | 99.96 | 99.96±0.12 |

* Average of 6 determinations

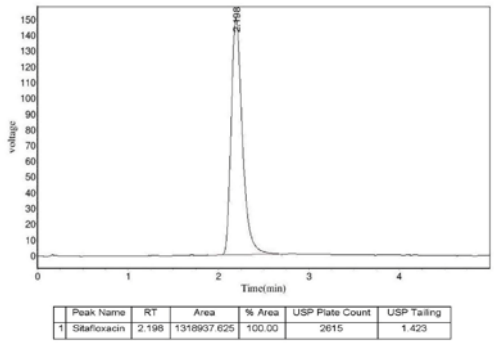



**Fig. 2: RP-HPLC Chromatogram of Sitafloxacin**

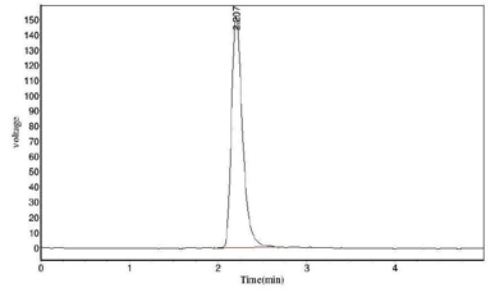



**Fig. 3: RP-HPLC chromatogram of Sitafloxacin formulation (Tablets)**

**Specificity**

The specificity of the proposed method was established by injecting the placebo and mobile phase solution in triplicate and the chromatograms were recorded. Comparison of chromatograms revealed that there were no interactions between the placebo and sample peaks. Finally, it was indicated that the method was specific.

**Accuracy**

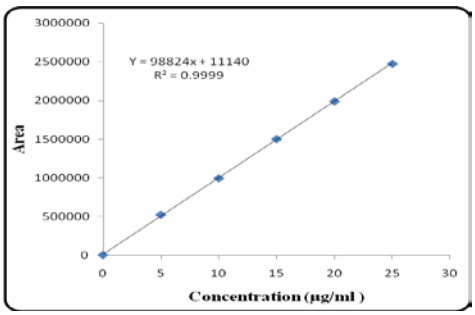
The accuracy was determined by calculating the recovery of Sitafloxacin at 50%, 100%, and 150% and they were added to pre quantified sample solution. Recovery studies were carried out in the tablet in triplicate each in the presence of placebo. The mean percentage recovery of SFX at each level was not less than 99%, and not more than 102%. The percentage recovery of Sitafloxacin was found to be in the range of 100 to 101%. The results are shown in the Table 3.

**Precision**

Precision should be investigated by using authentic, and homogeneous samples. The Precision of this method was expressed as S. D and %RSD of series of repeated measurements. Precision of SFX determination by proposed method was ascertained by repeated analysis of homogeneous samples of Sitafloxacin standard solutions in the intraday under the similar conditions. The system precision and method precision results were shown in Table 4.

**Linearity**

Linearity of the proposed method was established by using series of standard solutions of Sitafloxacin at concentration levels from 5 to 25 µg/mL, like 5, 10, 15, 20, and 25 µg/mL. These linearity studies are repeated in triplicate with different stock solutions.




**Graph 1: Linearity Graph of Sitafloxacin**

The curve obtained by concentration on x-axis and peak area on y-axis against showed linearity in the concentration range of 5-25 µg/mL of SFX and linearity graph is shown in Graph 1. The regression equation was found to be Y = 98824x + 11140 with correlation coefficient is $r^2$ = 0.9999. The Linearity and statistical analysis of data is shown in Table 5.

**Robustness**

The robustness was evaluated by the analysis of Sitafloxacin under different experimental conditions such as slight changes in chromatographic conditions like change of temperature (± 5$^0$C), flow rate (± 0.2 mL/min), and mobile phase composition (± 5%). It was distinguished that there were no changes in the chromatograms, and the parameters were within the limits, which indicates that the method was robust and suitable for routine use. The complete results are shown in Table 6 and the method is having good system suitability.

**System suitability**

This test was conducted on freshly prepared SFX standard solution and was used for the evaluation of the system suitability parameters such as retention time, area, USP tailing and theoretical plates, limit of detection and limit of quantification.

Five replicate injections for a system suitability test were injected into the chromatographic system and results were given in Table 7.

**Table 3: Recovery data for the proposed RP-HPLC method**

| S. No. | Concentration level | Amount added (µg/mL) | Amount found (µg/mL) | Area obtained | Mean %Recovery ± S. D* | %RSD* |
|---|---|---|---|---|---|---|
| | | | 5.00 | 1908799 | | |
| 1 | 50% | 5 | 4.97 | 1956765 | 100±0.57 | 0.627 |
| | | | 5.03 | 1999014 | | |
| | | | 9.98 | 2566768 | | |
| 2 | 100% | 10 | 9.97 | 2663052 | 100.13±0.67 | 0.664 |
| | | | 10.09 | 2513953 | | |
| | | | 15.01 | 3349282 | | |
| 3 | 150% | 15 | 14.97 | 3278576 | 100.06±0.27 | 0.266 |
| | | | 15.05 | 3331193 | | |

***S. D & %RSD** is standard deviation and percentage of relative standard deviation

**Table 4: System and Method Precision results of the proposed RP-HPLC method**

| | | System Precision | | Method Precision | |
|---|---|---|---|---|---|
| S. No. | Injections | Retention Time | Peak Area | Retention Time | Peak Area |
| 1 | 1 | 2.207 | 1283904 | 2.190 | 1281340 |
| 2 | 2 | 2.198 | 1262428 | 2.198 | 1333464 |
| 3 | 3 | 2.207 | 1287461 | 2.198 | 1298485 |
| 4 | 4 | 2.207 | 1294271 | 2.207 | 1287461 |
| 5 | 5 | 2.198 | 1283464 | 2.207 | 1283904 |
| 6 | 6 | 2.207 | 1283921 | 2.207 | 1283854 |
| 7 | MEAN | 2.204 | 1282574.83 | 2.2011 | 1294751.33 |
| 8 | SD | 0.004 | 10685.87 | 0.007 | 19909.99 |
| 9 | %RSD | 0.210 | 0.833 | 0.319 | 1.537 |

**Table 5: Linearity and Statistical analysis data for Sitafloxacin**

| S. No. | Concentration (µg/mL) | Area | Average Area | Statistical Analysis | | |
|---|---|---|---|---|---|---|
| | | | | Slope | Y-Intercept | Correlation Coefficient |
| 1 | 5 | 520374 | | | | |
| 2 | 10 | 992290 | 7478620 | 98824 | 11140 | **0.9999** |
| 3 | 15 | 1500786 | | | | |
| 4 | 20 | 1989536 | | | | |
| 5 | 25 | 2475634 | | | | |

**Table 6: Robustness results of the proposed RP-HPLC method**

| S. No. | Parameters | | | Peak Areas | Retention Time (Rt) | USP | |
|---|---|---|---|---|---|---|---|
| | Optimized | | Used | | | Plate Count | Tailing Factor |
| | | | 0.7 | 1495177 | 2.465 | 2639 | 1.396 |
| 1 | Flow rate (±0.2) | **0.9 mL/min** | 0.9 | 1318938 | 2.198 | 2615 | 1.423 |
| | | | 1.1 | 1166289 | 2.023 | 2491 | 1.442 |
| | | | 25 | 1468912 | 2.421 | 2594 | 1.394 |
| 2 | Temperature (±5$^0$c) | **30$^0$c** | 30 | 1318938 | 2.198 | 2615 | 1.423 |
| | | | 35 | 1167859 | 2.018 | 2569 | 1.685 |
| | Mobile phase composition (± 5%) | | 65:35 | 1412799 | 2.357 | 2608 | 1.393 |
| 3 | | **70:30** | 70:30 | 1318938 | 2.198 | 2615 | 1.423 |
| | | | 75:25 | 1179849 | 1.973 | 2457 | 1.648 |

**Limit of Detection:** The limit of detection (LOD) has established the minimum concentration at which the analyte can be reliably detected. LOD is determined by the signal to noise ratio and generally acceptable detection limit ratio is 3:1 and it was found to be 0.429 µg/mL.

**Limit of Quantification:** The limit of quantification (LOQ) has established the minimum concentration at which the analyte can be reliably quantified. LOQ is determined by the signal to noise ratio and a typical signal to noise ratio is 10:1 is acceptable for estimating the quantification limit and it was found to be 1.415 µg/mL. Finally

the proposed method is having good system suitability and its parameters were shown in Table 7.

**Table 7: System suitability parameters of proposed RP-HPLC method**

| S. No. | Parameters | Values |
|---|---|---|
| 1 | Wavelength (λ max) | 300 nm |
| 2 | Linearity range(µg/mL) | 5-25 |
| 3 | Regression equation | Y=98824x+11140 |
| 4 | Correlation coefficient($r^2$) | 0.9999 |
| 5 | Retention time (minutes) | 2.198 |
| 6 | Theoretical plates | 2615 |
| 7 | Tailing factor | 1.423 |
| 8 | Limit of Detection (µg/mL) | 0.429 |
| 9 | Limit of Quantification (µg/mL) | 1.415 |
| 10 | Asymmetry Factor | 1.672 |
| 11 | Capacity factor (k) | 0.147 |

## CONCLUSION

A RP-HPLC method for quantitative estimation of SFX in bulk and pharmaceutical dosage forms is established. The method is simple, accurate, linear, sensitive and reproducible as well as economical for the effective quantitative analysis of SFX in bulk and tablet dosage forms. The method was validated, and all the method validation parameters were tested and shown to yield satisfactory results. The method is free from interactions of the other ingredients and excipients used in the tablet formulations. Finally it is concluded that the method is suitable for use in the routine quality control analysis of Sitafloxacin in API and in pharmaceutical dosage forms.

## CONFLICT OF INTERESTS

Declared None

## ACKNOWLEDGEMENTS

The authors would like to thank the management of Daiichi Sankyo Co., Ltd, Japan for the gift sample of drug used in this investigation.

## REFERENCES

1. Merck & co. Inc. The Merck Index, an Encyclopedia of Chemicals, Drugs and Biologicals, 14th Edn. white house station: New Jersey;2006. p. 8551.
2. Sean C, Sweetman. Martindale: the complete drug reference, 37th Edn. Pharmaceutical Press: London;2011. p. 360.
3. Milatovic D, Schmitz FJ, Brisse S, Verhoef J, Fluit AC. *In vitro* activities of sitafloxacin (DU-6859a) and six other Fluoroquinolones against 8,796 clinical bacterial isolates. Antimicrob Agents Chemother 2000;44:1102-7.
4. Nakane T, S Iyobe, K Sato, S Mitsuhashi. *In vitro* antibacterial activity of DU-6859a; a new fluoroquinolone. Antimicrob Agents Chemother 1995;39:2822-6.
5. Sato K, K Hoshino, M Tanaka, I Hayakawa, Y Osada. Antimicrobial activity of DU-6859, a new potent fluoroquinolone, against clinical isolates. Antimicrob Agents Chemother 1992;36:1491-8.
6. Tanaka M, K Hoshino, M Hohmura, H Ishida, A Kitamura, K Sato, I Hayakawa, T Nishino. Effect of growth conditions on antimicrobial activity of DU-6859a and its bactericidal activity determined by the killing curve method. J Antimicrob Chemother 1996;37:1091-102.
7. Giamarellou-Bourboulis EJ, Sambatakou H, Grecka P, Chryssouli Z, Giamarellou H. Sitafloxacin (DU-6859a) and trovafloxacin: postantibiotic effect and *in vitro* interactions with rifampin on methicillin-resistant Staphylococcus aureus. Diagn Microbiol Infect Dis 1999;34:301-7.
8. Nakashima M, Uematsu T, Kosuge K, Umemura K, Hakusui H, Tanaka M. Pharmacokinetics and tolerance of sitafloxacin (DU-6859a), a new fluoroquinolone, after single and multiple oral doses in healthy volunteers. Antimicrob Agents Chemother 1995;39:170-4.
9. Hammerschlag MR, CL Hyman, PM Roblin. *In vitro* activities of five quinolones against Chlamydia pneumoniae. Antimicrob Agents Chemother 1992;36:682-3.
10. Huang K, Yang J, Zhang J, Ding Y, Chen L, Xu WY, *et al*. Determination of sitafloxacin in human plasma by liquid chromatography-tandem mass spectrometry method: application to a pharmacokinetic study. J Chromatogr B Analyt Technol Biomed Life Sci 2014;957:36-40.
11. ICH Q2 (R1), Validation of Analytical Procedures: Text and Methodology. 2005.