Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2014; 6(1): 63-67

ISSN- 0975 1556

Research Article

Analytical Method Development and Validation of S-Nadifloxacin in Pure Form by HPLC

*Devhadrao Nitin[1], Bansode Ashwini[2], Bansode Ajit[2], Yeole Ravindra[3]

[1] *Sharadchandra Pawar College of Pharmacy, Dumbarwadi, Otur. (Pune)-412409 Maharashtra, India.*
[2] *Vjsm's Vishal Institute of Pharmaceutical Education and Research Ale, Junnar (Pune) Maharashtra, India.*
[3] *New Drug Discovery Department Wockhardt Ltd, Aurangabad, Maharashtra, India.*

Available online: 1st January 2014

ABSTRACT

A simple, rapid, precise and cost effective HPLC method was developed for S-Nadifloxacin in pure drug and pharmaceutical dosage forms. The separation was carried out using Zorbax SB C18 (150 × 4.6 mm ID. 5 µm particle size) column, with mobile phase comprising of 0.05 %v/v tri fluoro acetic acid and acetonitrile in the ratio of 70 : 30 (v/v). The flow rate was 1.0ml/min and the detection was carried out using UV-visible detector at 237nm. The method was validated by evaluation of different parameters such as accuracy, precision, linearity, ruggedness, robustness, LOD and LOQ. The retention time were found to be 10.5. Calibration curves were linear with correlation coefficient (r^2) 0.999 and concentration range of 0.05 ppm to 5 ppm. The percentage recovery for S-Nadifloxacin was found to be in the range between 98.33-100.30. Method was found to be reproducible with relative standard deviation (RSD) for intra and inter day precision less than 2%.

Keywords: S-Nadifloxacin, HPLC, Trifluoro acetic acid, Validation.

INTRODUCTION

S-Nadifloxacin is a second generation Fluoroquinolones Antibiotic. It is used for the treatment of skin infections with susceptible bacteria, Acne vulgaris[1; 2].It has broad spectrum activity against Gram-negative and Gram-positive bacteria including *S. aureus, P. acnes and S. epidermidi.*[3, 4, 5]. The bactericidal action of S-Nadifloxacin is mediated by inhibiting the formation of supercoiled DNA by DNA gyrase (Topoisomerase II), an enzyme responsible for bacterial DNA replication[6, 7]. The Fluoroquinolones are quinolones with fluorine at position 6 of Naphthyridine ring[8]. It also inhibits the generation of O^{2-} and OH radicals by neutrophils and inhibits the production of inflammatory cytokines. According to literature review only HPTLC methods has been reported for the determination of S-Nadifloxacin in plasma, formulation and bulk drug. For routine analysis, a simple and cost effective analytical method is preferred [9, 10]. A simple and rapid assay procedure using the sample preparation as described in Ref.[11] and the mobile phase as given in Ref. [12] the objective of present study was to develop a simple, precise, accurate and economic HPLC analytical method with better detection range for the estimation of S-Nadifloxacin in bulk drugs. The developed method was validated as per ICH guidelines[13]. And suitable statistical tests were performed on validation data. (Figure 1)

Experimental

Chemical and Reagents: Pure S-Nadifloxacin was obtained as gift sample from Wockhardt R&D center. Aurangabad, India. Acetonitrile, HPLC grade was from Merck (India) and the tri-fluro acetic acid used were of analytical grade. Deionised water was processed through a Milli-Q water purification system. All other chemicals and the reagents used were of Analytical AR grade.

Analytical conditions

A double beam UV-Visible Spectrophotometer (Pharmaspec-UV1700, Shimadzu, Japan) was used for carrying all the data analysis. The instrument was provided with an automatic wavelength accuracy of 0.1 nm and matched quartz cells of 10 mm path length. A liquid chromatography Chemstation (Agilent 1100 with VWD or DAD) was use for estimation of S-Nadifloxacin and Liquid Chromatography (Shimadzu LC-2010 C HT) was use for Ruggedness study.

Chromatographic system: The Chromatographic separation were achieved on a Zorbax SB C-18 column with 150 mm x 4.6 mm ID with 5 µm particle size using

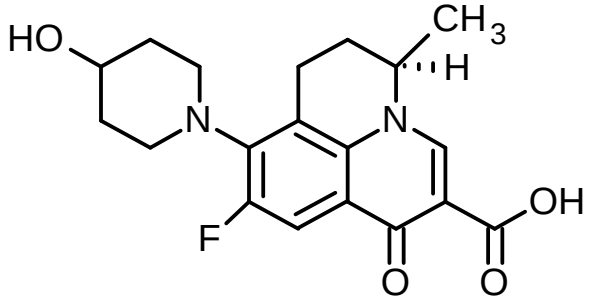

Figure 1: Structure of S-Nadifloxacin

*Author for correspondence: E-Mail: devhadraonitin9@gmail.com

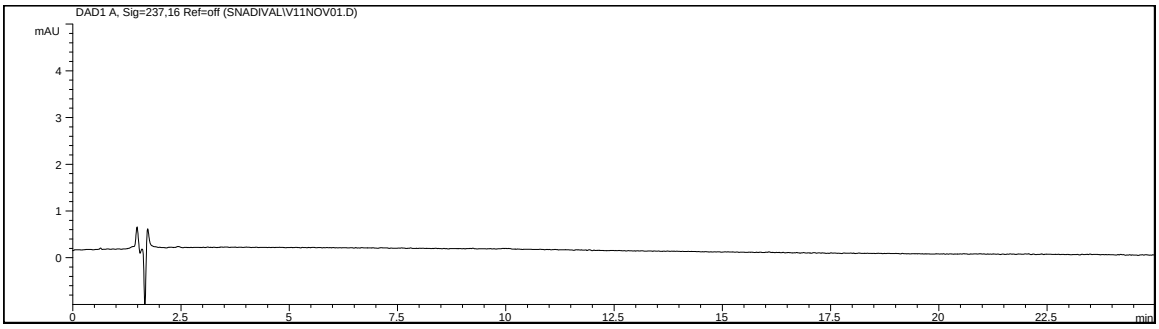

Figure-2 Blank

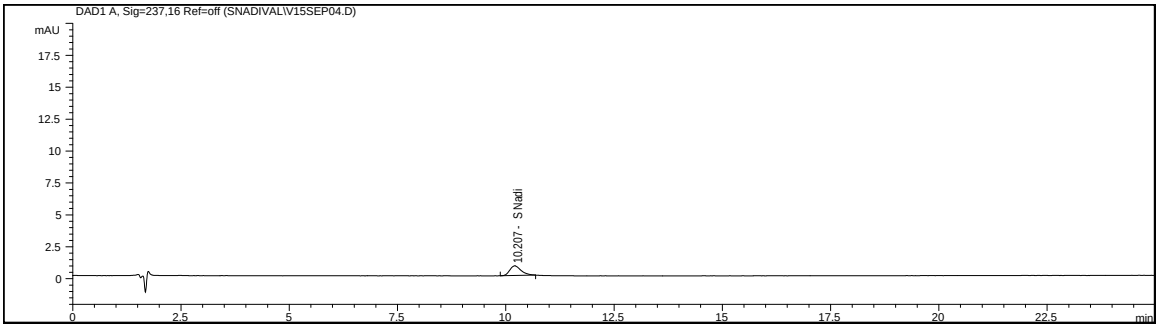

Figure-3 Standard solutions

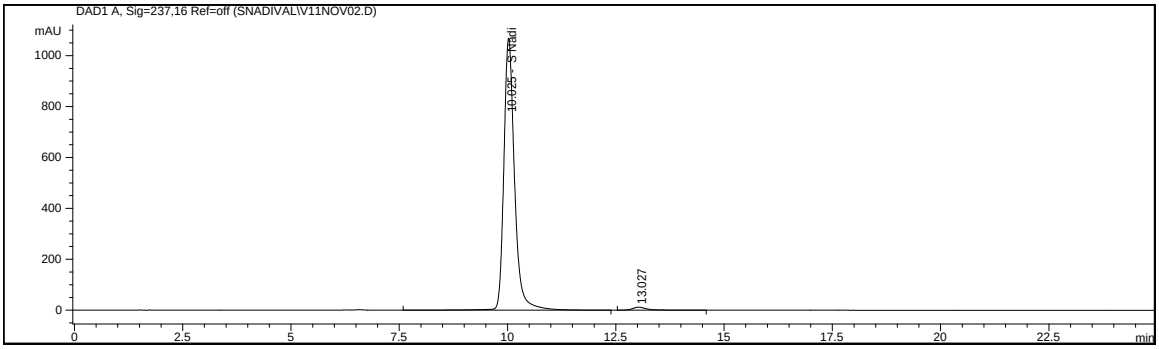

Figure- 4: A chromatogram of S-Nadifloxacin

Table no 1: System suitability test for S-Nadifloxacin

S-Nadifloxacin	Retention Time	Peak Area	TP	TF
Inj. 1	10.492	13.80004	63219	1.13
Inj. 2	10.501	14.14504	63250	1.13
Inj. 3	10.514	14.21628	63230	1.14
Inj. 4	10.512	14.59954	63215	1.12
Inj. 5	10.527	14.33659	63220	1.13
Inj. 6	10.518	14.29326	63218	1.12
Average	10.51067	14.23179	63225.333	1.12833
S.D	0.0125	0.2624	13.109	0.00752
%RSD	0.12	1.8	0.0207	0.66

Table no 2: Precision of S-Nadifloxacin

Precision Level	Time (t)	Found mean ± S.D (µg/ml)	(%) RSD
Intra-day	3hr	1.0 ± 0.005	0.58
	6hr	1.0 ± 0.01	0.99
	9hr	1.0 ± 0.005	0.57
Inter day	1st day	1.0 ± 0.01	0.98
	2nd day	1.0 ± 0.015	1.51
	3rd day	1.0 ± 0.02	1.98

Table no 3: Recovery results for S-Nadifloxacin

Standard concentration Level (%)	Found mean ± S.D (µg/ml)	Recovery (%)
0%	0.044	0
80%	0.132± 0.46	97.99
100%	0.157± 0.51	101.16
120%	0.173± 0.32	95.84

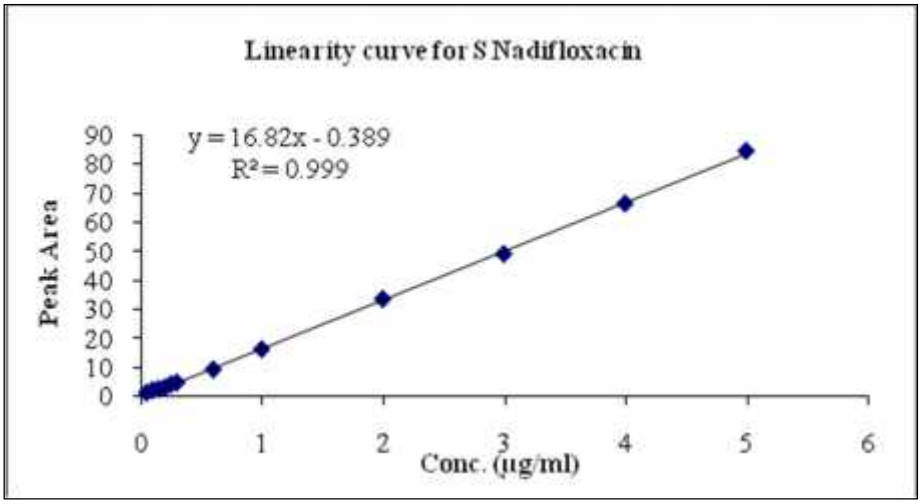

Figure 5: Calibration curve of S-Nadifloxacin at 237 nm

Table no 4: Linearity result for S-Nadifloxacin

Standard concentration (µg/ml)	Mean peak height ±SD (%RSD)
0.049	0.99± 0.0001 (0.01)
0.099	1.90± 0.0014 (0.07)
0.15	2.36± 0.0165 (0.69)

the solvent A as 1 ml of tri-fluro acetic acid (0.05%v/v) was added in 2000 ml water and sonicated (as a buffer) and solvent B as Acetonitrile with a isocreatic with a pre-mix run time of 10min at a flow rate of 1.0 ml/min and a column temperature was maintained at 40ºC and the detection was carried out at 237nm.the test concentration was about 1000ppm and the injection volume was 5µl. The effluent was monitored by PDA detector. A degassed mixture of water and acetonitrile in the ratio of 70:30(v/v) was used as diluent during the standard and test sample preparation.

Preparation of Blank (Diluent): Diluent was prepared by addition of Milli-Q water and acetonitrile in the ratio of 50:50 and pH was adjusted to 8.5 by using liquid ammonia. (Figure no-2)

Preparation Standard solutions: 10 mg S-Nadifloxacin was weighed accurately. Transferred into 10 ml volumetric flask and sufficient amount of diluent was added. Then it was sonicated to dissolve the drug and volume was made up with diluent (1000 ppm). 1 ml of this solution was transferred to 10 ml volumetric flask and made up the volume with diluent (100 ppm). 0.1 ml from 100 ppm solution was transferred to 10 ml volumetric flask and made up the volume with diluent (1 ppm). (Figure no-3)

Sample solution:10mg Test sample of S-S-Nadifloxacin was transferred in to 10 ml volumetric flask, sufficient amount of diluent was added and sonicated for 2 min. Then volume was made up with diluent. (Figure- 4)

Method Validation: Once the chromatographic method was developed and optimized, it must be validated. After optimization of the chromatographic conditions, the parameters of linearity, precision, accuracy and selectivity, limit of detection and limit of quantitation were evaluated to validate the process. The validation of an analytical method verifies that the characteristics of the method satisfy the requirements of the application domain. The proposed method was validated as per ICH Guidelines[14].

System Suitability Test: A system suitability test of the chromatographic system was performed. Six replicate injections for a system suitability test were injected into the chromatographic system. Relative standard deviation and column efficiency for the six suitable injections were determined. For all sample analyses, the efficiency and %RSD were found ≥ 2000 Theoretical plate and ≤ 2%. USP tailing factor and capacity factor was found to be ≤ 1.5.(Table no 1)

Precision: The precision of the method was demonstrated by inter-day and intra-day variation studies. In the intra-day studies, six injections of standard solution were injected into the chromatographic system in different time interval within a day. In the inter-day variation studies, six injections of standard solution were injected at different days. % RSD was calculated. the precision of the method was evaluated by analyzing containing three same concentration of S-Nadifloxacin. Within day and between day Variations were determined by assaying each sample in triplicate for 3 day. (Table no 2).

Accuracy /Recovery: The Accuracy of an analytical procedure expresses the closeness of agreement between

Table no 5: Robustness of S-Nadifloxacin

Robustness		Retention time	Peak Area± SD (%RSD)
Flow	1.1	9.45	14.48± 0.1021 (0.7)
	0.9	11.54	17.91± 0.1307 (0.7)
	+2%	8.32	18.35±0.1388 (0.8)
Organic composition	-2%	13.65	17.95±0.4421 (2.5)
	42ºc	10.23	16.96± 0.4354 (2.6)
Temperature	38ºc	10.61	17.93± 0.6488 (3.6)

the value, which is accepted either as a conventional true value or an accepted reference value and the value found[15]. Accuracy was performed in triplicate after spiking pure drug equivalent to 80, 100 and 120% in bulk drug sample. The percentage recoveries were calculated from the slope and y-intercept of the calibration curve. The results obtained indicated that recovery was excellent and not less than 100% ± 5.(Table no 3)

Linearity study: The linearity of an analytical procedure is its ability (within a given range) to obtained test result, which are directly proportional to the concentration of the analyte in the sample[16]. Calibration curve was constructed for S-Nadifloxacin pure drug by plotting the concentration of compound versus peak area response. Standard solutions containing 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.6, 1.0, 2.0, 3.0, 4.0 and 5.0 and ppm of S-Nadifloxacin were injected into the HPLC column. The linearity was calculated by the least square regression method. The regression equations were calculated from the calibration graphs. The correlation coefficient obtained was greater than 0.9998.(Figure 5 and Table no 4)

Robustness: The standard solution was prepared and injected in replicate for six times with (±0.1) flow rate i.e. 0.9 ml/min and 1.1 ml/min respectively (actual flow 1.0ml/min). Then the standard solution was prepared and injected in replicate for six times with (absolute ±2%) mobile phase composition i.e. (68:32) %v/v and (72:28) %v/v respectively. The sample solution was prepared and injected in replicate for three times with (absolute ±2%) mobile phase composition. The peak area of standard and sample mixture was compared. Then the standard solution was prepared and injected in replicate for six times with ($\pm 2^0$C) Column oven temperature i.e. 42^0C and 38^0C respectively. The sample solution was prepared and injected in replicate for three times with ($\pm 2^0$C) column oven temperature. The peak area of standard and sample mixture was compared. (Table no 5)

Ruggedness: Ruggedness of the method was studied by different analysts and on different make of instruments (LC-2010 C HT liquid chromatography SHIMADZU).

Limit of detection (LOD) and limit of Quantitation: The LOD and LOQ of sample were estimated at a signal to noise ratio of 3:7 and 9:1, respectively[17, 18]. By injecting a series of diluted solution with known concentration. Precision study was also carried at the LOQ level by injecting six individual preparations of sample and calculated the percentage RSD of the area.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions: The main target of the chromatography method is to get the separation of sample. the sample eluted by using different stationary phase like C8,cyno, XTerra and Zorbax and different mobile phases containing buffers like phosphate, sulphate and acetate with different PH (4-5) and using organic modifiers like methanol and ethanol in the mobile phase. Apart from the elution of sample, poor peak shape was noticed. Phosphate buffer and water at 1.0mL/min flow was chosen for initial trail with a 250mm length × 4.6mm ID column and 5µm particle size C18 stationary phase. When sample was injected the shape and analyte was poor. To get the good shape and resolution. The chromatographic conditions were adjusted in order to provide a good performance of the assay. The method involved a mobile phase consisting of solvent A as 0 .05 %v/v tri-fluro acetic acid: Solvent B as Acetonitrile with isocratic program (70: 30) with a post –run time of 25 min at detection wavelength 237 nm by using an Zorbax SB C-8 column (150 mm x 4.6 mm, 5 µm) at a flow-rate of 1.0 ml/min and the injection volume was 5 µl. Was successful in separation of drug. Under the above conditions, result were as follows : retention time of S-Nadifloxacin was 10.5 min, with a tailing factor of 1.1, number of theoretical plates (N) for the S-Nadifloxacin peak was 63225 and %RSD for six replicate injection was 1.8%. Peak purity of S-Nadifloxacin was checked by using a photodiode array detector of Agilent 1100 series.

Result of method validation

Linearity: Linearity calibration plot for the assay method was obtained over the calibration tested. i.e. 80-120% of assay analyte concentration and the correlation coefficient obtained was greater than 0.9998. Linearity was checked for the assay method over the same concentration range for two consecutive days (table 4).

Accuracy: The percentage (%) recovery of S-Nadifloxacin in bulk drug sample range from 95.84 to 101.16 (table 3).

Precision: Precision within –run relative standard deviation (RSD) ranged from 0.57 to 0.99% and the precision between-run RSD ranged from 0.98 to 1.98% (table 2).

limit of detection (LOD) and limit of quantitation (LOQ): The LOD of S-Nadifloxacin was 0.007µgmL^{-1} respectively (of analyte concentration, i.e 0.074 µgmL^{-1}). The LOQ of S-Nadifloxacin was 0.025 µgmL^{-1} respectively (of analyte concentration, i.e 0.246 µgmL^{-1}).

CONCLUSION

The optimized and validated HPLC method was shown to be simple, sensitive, reliable, and reproducible. Precise and accurate and hence can be used for the routine analysis of S-Nadifloxacin in bulk and pharmaceutical preparation. The sample recoveries from all formulations were in good agreement with their respective label claims, and sensitivity of this method is within the range.

ACKNOWLEDGEMENT

The authors wish to thank Dr. C. Bothiraja for his valuable suggestion in preparing the manuscript and Dr. A. Battase for his technical advice. The authors acknowledge the support provided by Dr. R. yeole, Director New drug discovery department of Wockhardt Pharmaceutical Limited (R&D), Aurangabad, India.

REFERENCES

1. Parfitt, K : *Martindale the Complete Drug Reference*, Pharmaceutical Press, London. 32nd ed.1999
2. O'Neil, M. J. *Merck Index*: John Merck research laboratories division of Merck and Co. INC.: Whitehouse station, NJ, USA.14th ed. 2006
3. J.M. Smith, Br. J. Pharm. Pract. 10 (1988) 18.
4. V. Lorian, Antibiotics in Laboratory Medicine, 4th ed., Williams and Wilkins. Baltimore, 1996,pp. 591-592.
5. V.F. Samanidou, C.E. Demetriou, I.N. Papadoyannis, Anal. Bioanal. Chem. 375. 2003; 623-629.
6. J. Gore, Z. Bryant, M.D. Stone, M. Nollmann, N.R. Cozzarelli, C. Bustamante, Nature 439 2006; 100-104.
7. K. Sato, Y. Matsura, M. Inoue, T. Ueno, Y. Osada, H.Ogawa, M. Mitsuhashi, Antimicrob. Agent Chemother. 22.1982; 548.
8. A.H. Arteseros, J. Barbosa, R. Compano, J. Chromatogr. A 45.2002; 1-24.
9. Kulkarni, A. A.; Nanda, R. K.; Ranjane, M. N.; Ranjane, P. N: Simultaneous estimation of S-Nadifloxacin and Mometasone Furoate in topical cream by HPTLC method. *Der Pharma Chemica*, 2010; 2(3): 25-30.
10. Kumar, A., Sinha, S.; Agrawali, S. P.; Ali, J.; Ahuja, A.; Baboota, S: Validated Stability-Indicating Thin Layer Chromatographic Determination of S-Nadifloxacin in Microemulsion and Bulk Drug Formulations. *Journal of Food and Drug Analysis* (2010); 18(5): 358-365.
11. D.J. Griggs, R. Wise, J. Antimicrob, Chemother. 24. 1989; 437.
12. K. Kosuge, T. Uematsu, S. Araki, H. Matsuno, K. Ohashi, M. Nakashima, Antimicrob. Agent Chemother. 42, 1998; 1298.
13. The European agency for the evaluation of medical/products. ICH Topic Q2B note for guidance on validation of analytical procedures: methodology GPMP/ICH/281/195.1996.
14. ICH, International Conference on Harmonization, IFPMA, Geneva, 2006.
15. Validation of Chromatographic method ,24th ed., Reviwer Guidance, Center for Drug Evaluation and Research (CDER), EDA, Rockville, 1994(CMC 3).
16. Validation of Compendial Method, 24th ed., the United State Pharmacopeia, 2000 (USP 24, Section <1225>).
17. G. A. shabir. J. chromatogr. A 987,2008; 57-66.
18. M. Ribani, C.B.G. Bottoli, C.H. Collins, I.S.F. Jardim, L.F.C. Melo, Quim. Novo 27, 2004. 771.