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Development and validation of a RP-HPLC method for the quantification of sparfloxacin in pharmaceutical dosage forms.

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ABSTRACT

A rapid and sensitive reversed phase high performance liquid chromatographic method has been developed for quantitative analysis of sparfloxacin in pharmaceutical preparations. The method was validated according to FDA, ICH and USP guidelines with respect to accuracy, precision, specificity and linearity. The method was developed using the mobile phase comprising 1% aqueous solution of acetic acid and acetonitrile in the ratio of 71: 29% (v/v) at a flow rate of 0.7 mL/min over C-8 bonded silica column at ambient temperature. The recovery was found to be more than 97% for each spiked samples of sparfloxacin demonstrative of accuracy of the protocol. Intraday and interday precision of the new method were less than the maximum allowable limit (RSD% 2.0) according to FDA. The method showed linear response with correlation coefficient value of 0.998. Therefore, the method was found to be accurate, reproducible, sensitive and less time consuming, and can be successfully applied for the assay of sparfloxacin formulations. .

KEY WORDS: Sparfloxacin; HPLC; method development; validation

RESUMEN

Un método rápido y sensible de cromatografía líquida en fase inversa de alta resolución ha sido desarrollado para el análisis cuantitativo de sparfloxacino en preparaciones farmacéuticas. El método fue validado de acuerdo a las normas de precisión, especificidad y linealidad aportadas por la FDA, ICH y USP. El método fue desarrollado utilizando la fase móvil compuesta por solución acuosa de ácido acético al 1% y acetonitrilo en la proporción 71:29% (v/v) bajo una velocidad de flujo de 0.7 ml/min sobre una columna de sílice c-8 a temperatura ambiente. La recuperación fue de más del 97% para cada muestra adicionada de sparfloxacino lo que demuestra la precisión del protocolo. La precisión intradía e interdía fue inferior al límite máximo permitido ($RSD \leq 2.0$), de acuerdo a la FDA. El método mostró una respuesta lineal con un coeficiente de correlación del 0.998. Por lo tanto, el método fue preciso, reproducible, sensible y rápido, por lo que puede ser utilizado para el análisis de formulaciones de sparfloxacino.

PALABRAS CLAVE: Sparfloxacino, HPLC, desarrollo de métodos, validación.

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INTRODUCTION

Sparfloxacin, a fluoroquinolone antibiotic, has been reported to be more active than other fluoroquinolones against some Gram-positive organisms such as *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Mycobacterium* and *Chlamydia* species. Sparfloxacin is a potential antibiotic for use in the treatment of upper and lower respiratory tract infections including sinusitis, acute exacerbation of chronic bronchitis, community and hospital acquired pneumonia; urinary tract infections such as gonococcal and non-gonococcal urethritis, chancroid and other sexually transmitted diseases; skin and soft tissue infections, and prophylactic use in different urological and ophthalmic surgical procedures¹⁻⁴.

Like other drugs sparfloxacin also requires some absolute quality parameters like quality, potency etc. to serve its best activities. It is obvious that a little change in the formulation or variations in the manufacturing process or use of low quality materials can affect the product stability and efficacy. It has been reported that due to inadequate therapeutic content in antibiotic formulations, susceptible organisms gain resistant tremendously and many pathogenic organisms have already shown resistance to a number of antimicrobial drugs⁵. Therefore, quality and efficacy assessment and maintenance of proper dosage schedule of the antimicrobial agents are strongly emphasized to ensure their efficacy and safety which can be ensured by analyzing the products during and after manufacturing and at various intervals during the shelf life of the product. Effective process validation contributes significantly to assuring drug quality. The basic principle of quality assurance is that a drug should be produced that is fit for its intended use and does not expose the consumers to risks⁶.

Although several methods have been reported previously in the literature for determination of sparfloxacin in the pharmaceutical formulations⁷⁻¹², among them many articles have described the use of buffers to develop reversed-phase high pressure liquid chromatographic (RP-HPLC) method. The use of buffer as a mobile phase in HPLC has many limitations as it requires filtration before use, crystallization may occur when the organics come in contact with leftover buffers in system which may damage the pump and column, and also produce corrosion of stainless steel lines. Besides these buffers require to prepare everyday because especially phosphate buffer is a good medium for bacterial and fungal growth. To overcome the limitations of buffers, the objective of this work was to develop a more simple, accurate and

rapid liquid chromatographic analytical method for the assay of sparfloxacin in pharmaceutical formulations and to validate the method in accordance with the guidelines of FDA (Food and Drug Administration, United States of America), USP (United States Pharmacopoeia) and ICH (International Conference on Harmonization) with respect to accuracy, reproducibility, linearity and specificity¹³⁻¹⁵.

We, herein, describe an isocratic HPLC method using 1% aqueous solution of acetic acid and acetonitrile (71:29) with UV detection instead of buffer. To the best of our knowledge, this is the first report of using non-buffer containing 1% aqueous solution of acetic acid with acetonitrile to assay sparfloxacin of pharmaceutical preparations.

MATERIALS AND REAGENTS

Working standard of sparfloxacin (potency 99.93%) was a kind gift of Drug International Ltd., Dhaka, Bangladesh. For the estimation of sparfloxacin formulated as tablets, samples were purchased from different manufacturers and from retail pharmacies on random basis and were coded as Sample-1, Sample-2 and Sample-3. HPLC grade acetic acid and acetonitrile were procured from local sources.

Apparatus

HPLC system

High Performance Liquid Chromatographic system (Shimadzu-UFLC Prominence), equipped with an auto sampler (Model- SIL 20AC HT) and UV-Visible detector (Model-SPD 20A) was used for the analysis. The data was recorded using LC-solutions software.

Column

Analytical reversed phase C-8 column [Luna C-8(2), 5 μ , 150 x 4.6 mm, Phenomenex, Inc] was used to analyze the samples.

Mobile phase

Aqueous solution of 1% acetic acid and acetonitrile were prepared and sonicated for 10 minutes and then it was filtered through a 0.22 μ m millipore filter. HPLC grade acetonitrile was also filtered and degassed before using.

Chromatographic conditions

All analyses were done at ambient temperature under isocratic condition. The mobile phase consisted of 1% aqueous solution of acetic acid and acetonitrile in the ratio of 71% and 29% (v/v) at a flow rate of 0.7 mL/min. The injection volume was 20 μ L for standard and samples. Before analysis, every standard and sample were filtered through 0.45 μ m filter tips. The column eluate was monitored at 290 nm.

Preparation of standard solutions

Solutions of the standard drug was prepared by dissolving 25.02 mg of sparfloxacin powder (equivalent to 25.0 mg sparfloxacin) in a 25 mL volumetric flask using 10 mL of the mobile phase. Then the volume was made up to the mark with the same solvent. The final concentration obtained was 1.0 mg/mL. Appropriate volume from this solution was further diluted to get standards of varying concentrations (25, 30, 35, 40, 45, 50, 60 µg/mL).

Preparation of test sample

Ten tablets in each of the samples (Sample-1, Sample-2 and Sample-3) were weighed, made into fine powder in a mortar with pestle and average weight was taken. Accurately weighed powder equivalent to average weight of each tablet (25 mg of sparfloxacin) was taken in a 25 mL volumetric flask and 10 mL of mobile phase was added and sonicated to mix uniformly. The final volume was adjusted with mobile phase to get the concentration of 1.0 mg/mL. The solution was further diluted 20 times to get the concentration of 50 µg/mL, filtered through 0.45 µm filter tips, and aliquots of 20 µL from this solution was injected into the HPLC by using an auto injector. The average content of the tablets was determined using the calibration curve.

Method validation*Specificity*

The specificity of the LC method was evaluated to ensure that there was no interference from the excipients present in the pharmaceutical product. The specificity was studied by injecting the excipients, standard solution and pharmaceutical preparation of sparfloxacin.

Accuracy/ Recovery

The accuracy of an analytical method expresses the nearness between the expected value and the value found. It is expressed by calculating the percent recovery (R%) of analyte recovered by assay of spiked samples. In this case, aliquot equivalent to 100 µg/mL of sparfloxacin tablet solution was taken in three 10 mL volumetric flasks and 20 µg/mL, 40 µg/mL and 60 µg/mL standard sparfloxacin solutions were added to get the final concentrations as 60 µg/mL, 70 µg/mL and 80 µg/mL, respectively. These three solutions were analyzed in successive analysis (n = 3) using the proposed method to evaluate the accuracy of the method. The data of the experiment were statistically analyzed to study the reproducibility and validity of the proposed method.

Precision/ Reproducibility

Precision of the assay was investigated with respect to

both repeatability and reproducibility. The precision of an analytical method is the degree of agreement among individuals test result where the method is applied repeatedly to multiple samplings. It was checked by intra- and inter-day repeatability of responses after replicate injections and expressed as RSD % amongst responses using the formula [RSD (%) = (Standard deviation/Mean) × 100 %]. In the current method development and validation protocol, precision was determined by three replicate analyses of each of the concentrations of 40 µg/mL, 50 µg/mL, 60 µg/mL of standard sparfloxacin solutions using the proposed method.

Linearity

Five different concentration levels (25 µg/mL, 30 µg/mL, 35 µg/mL, 40 µg/mL and 45 µg/mL) were prepared from standard solution by diluting with the 1% aqueous acetic acid solution. Then 20 µL from each solution was injected into the HPLC using auto-sampler and the analyses were monitored at 290 nm and repeated four times. The average peak areas were plotted against concentrations. The linearity of the proposed method was evaluated by using calibration curves to calculate coefficient of correlation, slope and intercept values.

RESULTS AND DISCUSSION

A reversed phase HPLC method has been developed and validated as per ICH, USP and FDA guidelines for determination of sparfloxacin in pharmaceutical formulations by using the mobile phase comprising aqueous solution of 1% acetic acid and acetonitrile in the ratio of 71% and 29% (v/v) at ambient temperature at flow rate of 0.7 mL/min with UV detection at 290 nm. The injection volume was kept at 20 µL for standard and all samples. The retention time of sparfloxacin was obtained at 3.1 ± 0.1 min (Figure 1).

The specificity of the method was monitored by analyzing the placebo (containing all the ingredients of the formulation except the analyte), standard solution and market preparation containing sparfloxacin. No peak was detected close to the retention time of sparfloxacin hence proving the high degree of specificity of the method.

The accuracy was evaluated at three different concentrations with spikes which were conducted in successive analysis (n = 4) using the proposed method and the value was expressed as percentage of recovery between the mean concentrations found and added concentration for sparfloxacin. The average percentage of recovery was found to be 97.65%, 97.41% and 98.11% for 60 µg/

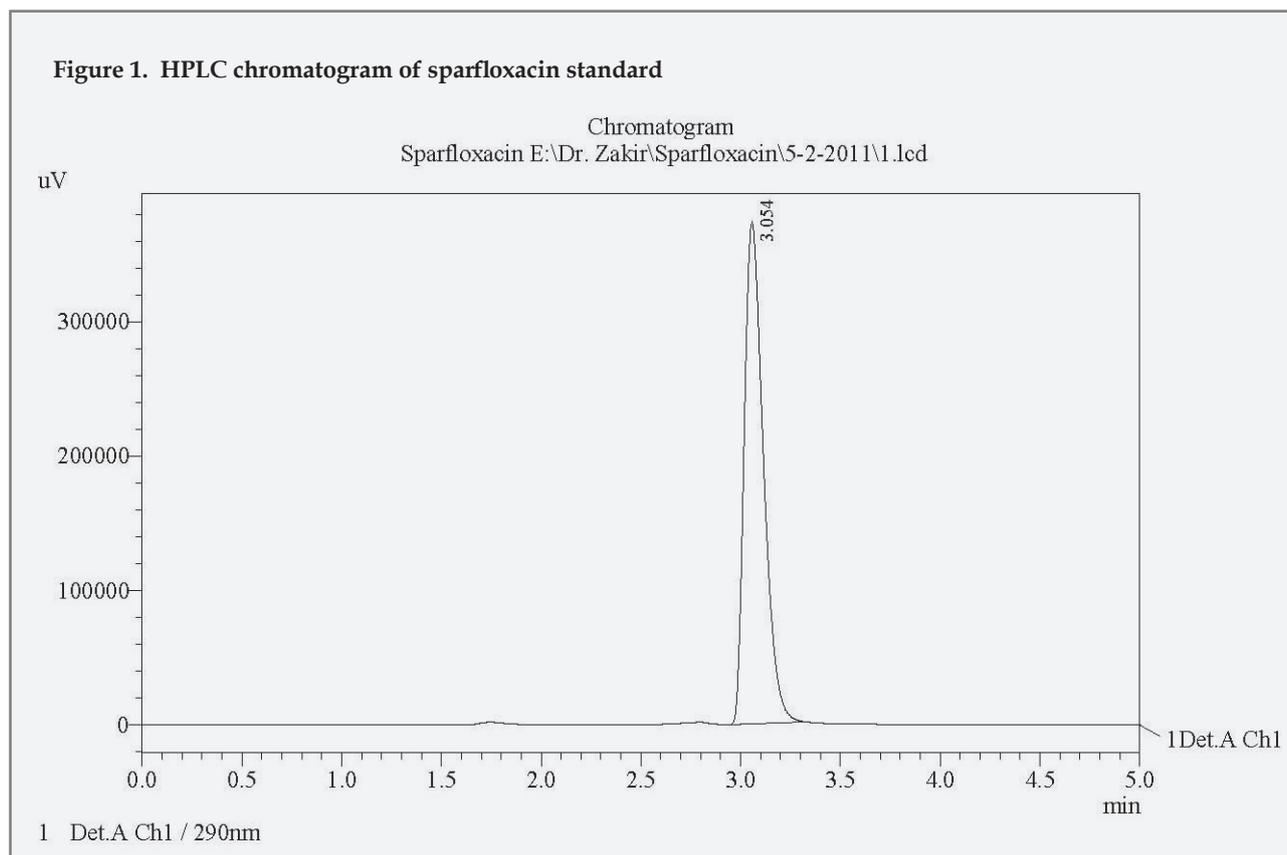


Table 1. Accuracy of the developed method

Standard sparfloxacin + Spike ($\mu\text{g/mL}$)	Total concentration for analysis ($\mu\text{g/mL}$)	Recovered Mean ($n = 3$) \pm SD	% Recovery
10 + 50	60	58.59 \pm 0.20	97.65
20 + 50	70	68.19 \pm 0.10	97.41
30 + 50	80	78.49 \pm 0.31	98.11

mL, 70 $\mu\text{g/mL}$ and 80 $\mu\text{g/mL}$, respectively (Table 1). All experimental results were in the range of the acceptability for precision and accuracy¹⁵, which indicated that the developed method is sensitive enough and accurate for determination of sparfloxacin.

The precision of the method was evaluated by repeatability of sample application and measurement of peak area using three replicates of each of the 40 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$, and 60 $\mu\text{g/mL}$ standards, where the mean concentrations were found as 40.49 $\mu\text{g/mL}$, 49.93 $\mu\text{g/mL}$ and 59.15 $\mu\text{g/mL}$ with associated %RSDs of 0.32%, 1.66%, and 0.08%, respectively. The %RSD values for intra- and inter-day assays of sparfloxacin performed in the same laboratory did not exceed more than 1.3% (Table 2).

When peak area (y) was plotted against concentration levels of 25 $\mu\text{g/mL}$, 30 $\mu\text{g/mL}$, 35 $\mu\text{g/mL}$, 40 $\mu\text{g/mL}$ and 45 $\mu\text{g/mL}$, a good correlation coefficient was obtained. For the equation of calibration curve, correlation coefficient (r^2)

was obtained as 0.998 which was within the accepted range of guidelines and showed good linear relationship of the newly developed method with the slope (m) and intercept (c) of the calibration curve as 106705.52 and -153223, respectively (Table 3, Figure 2).

By employing this method, the content of sparfloxacin was determined in marketed tablets. The sparfloxacin formulations available in Bangladesh were purchased from different manufacturers on random basis from retail pharmacies and coded as Sample-1, Sample-2, and Sample-3, and the content of drug was found as 100.07%, 101.52% and 98.12%, respectively (Table 4), which were also in the acceptable range¹³⁻¹⁶.

CONCLUSION

The activity of a drug depends on some absolute requirements such as quality, potency etc. It is obvious that a little change in the formulation or variations in the

Table 2. The precision of the developed method

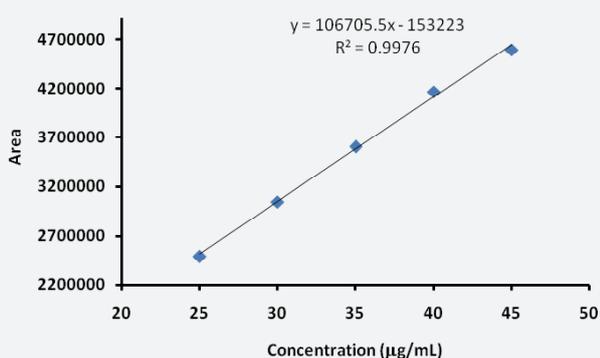
Injected conc. ($\mu\text{g/mL}$)	Intraday			Interday		
	Recovered Mean (n = 3) \pm SD	% Recovery	RSD %	Recovered Mean (n = 3) \pm SD	% Recovery	RSD %
40	60	40.49 \pm 0.13	0.32	40.54 \pm 0.07	101.35	0.18
50	70	49.93 \pm 0.83	1.66	49.49 \pm 0.63	98.98	1.26
60	80	59.15 \pm 0.05	0.08	59.02 \pm 0.19	98.36	0.32

Table 3. Linearity of the method

Concentration ($\mu\text{g/mL}$)	Mean Area (y) (n=4)	Slope (m)	Intercept (c)	Correlation coefficient (r^2)
25	2490542	106705.52	-153223	0.998
30	3045976			
35	3604752			
40	4169826			
45	4596255			

Table 4. Determination of drug content in the sparfloxacin tablets

Formulation code	Amount of sparfloxacin label claimed per tablet (mg)	Amount of sparfloxacin found by new method (mg)	Amount of sparfloxacin found in percentage
Sample-1	200	200.14	100.07%
Sample-2	200	203.04	101.52%
Sample-3	200	196.24	98.12%

Figure 2. Linearity of curve for standard sparfloxacin

manufacturing process or use of low quality materials including API (Active Pharmaceutical Ingredients) and fake or expired drugs can affect the efficacy of the drug as well as may produce harmful effect to the patient to a high risk level. Therefore, quality and efficacy assessment and maintenance of proper dose schedule are strongly emphasized to ensure the effectiveness of the drug. To ensure the requisite quality, drug manufacturers are required to test their products during and after

manufacturing processes and at various intervals during the shelf life of the product. Hence, we planned to study and determine the potency and efficacy of most commonly used antimicrobial preparations like sparfloxacin, a broad spectrum antibiotic which is widely prescribed in Bangladesh.

To attain this objective, a rapid and sensitive reversed phase high performance liquid chromatographic method was developed and validated according to the guidelines of FDA, ICH and USP with respect to accuracy, precision, specificity and linearity. The developed method was found to be simpler, accurate, reproducible, efficient and less time consuming, and was applied successfully for the study of sparfloxacin formulations.

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