Quantification of sparfloxacin in pharmaceutical dosages and biological samples

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Abstract: A simple and fast method for spectrophotometric determination of sparfloxacin using *p*-dimethyl-aminobenzaldehyde (DMAB) has been developed. A yellow coloured product formed from reaction between sparfloxacin and DMAB as a result of condensation reaction at room temperature. The maximum absorbance was found at 392 nm with molar absorptivity of 4.9×10^3 L mol^{-1} cm^{-1}. All parameters for the reaction, as concentration of DMBA reagent, molarity of sulphuric acid, and reaction temperature were studied. Under the conditions studied, a linear relationship between absorbance of the condensation product and concentration of sparfloxacin in the range of 2.0-80.0 µg mL^{-1} was found with good correlation coefficient (0.9997). The limits of detection (LOD) and quantification (LOQ) for the proposed method were found to be 0.22 and 0.75 µg mL^{-1} respectively. The repeatability and accuracy (model) of the method was studied at three different concentrations of sparfloxacin and found with value of relative standard deviation less than 2.0%. The method was found selective for determination of sparfloxacin in the presence of commonly used excipients in dosage forms. The developed method was validated statistically and applied successfully to the analysis of the drug in pure form, pharmaceutical preparations, and spiked blood plasma and urine samples with good accuracy (real) and precision. The percentage recovery was found from 99.0-100.0% with relative standard deviation less than 1%. The results of the proposed method were compared statistically with the results of literature HPLC method.

Keywords: Sparfloxacin, *p*-dimethylaminobenzaldehyde, spectrophotometry, formulations.

INTRODUCTION

Fluoroquinolones are one of the most important antibacterial agents used in human medicines, which are active through inhibition of their DNA gyrase against both Gram positive and Gram negative bacteria.

Sparfloxacin has a broad spectrum of activity and is more potent in vitro than the non-fluorinated quinolone and more favourable pharmacokinetics allowing its use in systemic infections. It has been used in the treatment of urinary tract infection, lung infection and cutaneous allergy (Martindale 2009).

Analytical methods so far reported for determination of sparfloxacin in pharmaceutical preparation are DC polarography (Jain *et al.,* 2003), HPLC (Marona *et al*., 1999, Kowalczuk *et al*., 2003, Cao *et al*., 2001) and capillary zone electrophoresis (Faria *et al*., 2006, Sun *et al*., 1999, Fierrens *et al*., 2000). Spectrophotometric methods have also been reported based on the oxidation of sparfloxacin (Jan *et al*., 2010), indirect determination of sparfloxacin with N-bromosuccinimide (Askal *et al*., 2007), ion-pair complex with bromothymol blue (Marona and Schapoval, 2001), coloured product with ammonium reineckate as a precipitate and redissolution in acetone (Al-Ghannam 2008), Pd (II) and eosin ternary complex (El-Didamony 2007) and UV-spectrophotometry (Kaur *et al*., 2008). Some spectrofluorimetric methods have also been reported based on intrinsic fluorescence property (El-Didamony 2011) or complexation reaction with aluminum chloride (Rizk *et al*., 2000), lutetium (III)-sparfloxacin (Wang 2004) and terbium (III)-sparfloxacin complex (Fangtian *et al*., 1999).

The present work was carried out with the aim to develop a selective, precise, accurate, and sensitive method for spectrophotometric determination of sparfloxacin in pure, different pharmaceutical preparations and biological samples. The validated method should also be according to the guidelines of ICH. The method is based on the spectrophotometric analysis of the studied fluoro-quinolones through its condensation reaction with *p*-dimethylaminobenzaldehyde (DMAB) in acidic medium at room temperature.

MATERIALS AND METHODS

Instruments

UV/Vis Spectrophotometer (Model SP-3000 plus, Optima, Japan) with matched 1 cm quartz cells for all spectrophotometric measurements and thermostatically controlled water bath (Yu Jia, China) were used. Centrifugation of plasma and urine samples was carried out on a clinical centrifuge (Model 800, Jiangsu, China).

Reagents and solutions

All reagents used were of high grade purity. *p*-

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dimethylaminobenzaldehyde, DMAB (Merck, Germany), sulphuric acid (95-98% Merck), ethanol (100% Riedel-deHaën, Germany), standard reference sparfloxacin (gifted by Libra Pharmaceutical Industry (Pvt.) Ltd., Pakistan), and commercial preparations of sparfloxacin (Quspar 100 mg/tablet manufactured by The Schazoo Laboratories (Pvt) Ltd, Lahore, Pakistan; Sparcin 100 mg/tablet manufactured by FOZAN Pharmaceutical Industries (Pvt) Ltd. Industrial Estate Hayatabad, Peshawar, Pakistan; Sparaxin 100 mg/tablet manufactured by Abbott Laboratories (Pakistan) Ltd Karachi, and Lowspar 100 mg/tablet manufactured by Lowitt Pharma (Pvt) Ltd, Industrial Estate Hayatabad, Peshawar, Pakistan) were purchased from local market.

p-dimethylaminobenzaldehyde (DMAB) solution (0.3%) was prepared by dissolving 0.15 g of DMAB in sufficient ethanol and made the volume up to mark in 50 mL volumetric flask. 1000 µg mL^{-1} solution of standard reference sparfloxacin was prepared in ethanol and sulfuric acid solution (3 mol L^{-1}) was used for acidic medium adjustment.

Calibration method

Aliquots of 1 mL of 0.3% DMAB solution were added to 10 mL volumetric flasks followed by the successive addition of 1 mL of 3 mol L^{-1} H_2SO_4 solution (0.3 mol L^{-1}concentration after dilution) and sparfloxacin solution from 100 µg mL^{-1} with concentration in the range of 2.0 – 80.0 µg mL^{-1}. The solutions were equilibrated for 20 minutes at room temperature. The mixture was diluted up to mark in the flask with ethanol. Blank was also prepared using the same procedure except the addition of sparfloxacin. Absorbance of the analyte against the blank was measured at 392 nm.

Linearity, intercept and slope were calculated with help of regression equation. Repeatability and recoveries of the propose method were studied according to the ICH guidelines (ICH 1996). Precision and accuracy were calculated at three concentration levels in triplicate and 95% confidence limits.

Analysis of dosage form

Tablets (five) of each dosage form were weighed, grounded and mixed. After powdering of the tablets, an accurate weight of the sample equivalent to 10 mg of sparfloxacin was dissolved in 30 mL of ethanol (100%) with constant shaking. Diluted to 100 mL with ethanol and filtered. The same method was adopted as described above and sparfloxacin concentration in dosage form was determined from the calibration plot.

Recovery experiment

To the pre-analyzed tablet powder of sparfloxacin commercial formulations, a known and fixed amount of standard sparfloxacin was added at three different concentration levels in triplicate. Total sparfloxacin was determined by the proposed method and the percent recovery of standard sparfloxacin added was calculated.

Analysis of spiked human urine and plasma

Control samples of urine and plasma were collected from three healthy volunteers for spiking after approval of the ethical committee of Khyber Teaching Hospital, University of Peshawar, Pakistan. 20 mL of urine sample in triplicate of three volunteers was spiked with the standard sparfloxacin in the concentration from 15 to 25 µg mL^{-1} and for 15 minutes the samples were centrifuged at 3000 rpm. The clear supernatant was used in further analysis and proceeded in the same way as described for standard.

Five milliliters of plasma sample in triplicate was spiked with standard sparfloxacin in the concentration of 10 to 20 µg mL^{-1} and deproteinated by the addition of 10 mL of acetonitrile and centrifuged at 3500 rpm for 30 minutes. The clear supernatant was used in further analysis. The same method was adopted as described above.

RESULTS

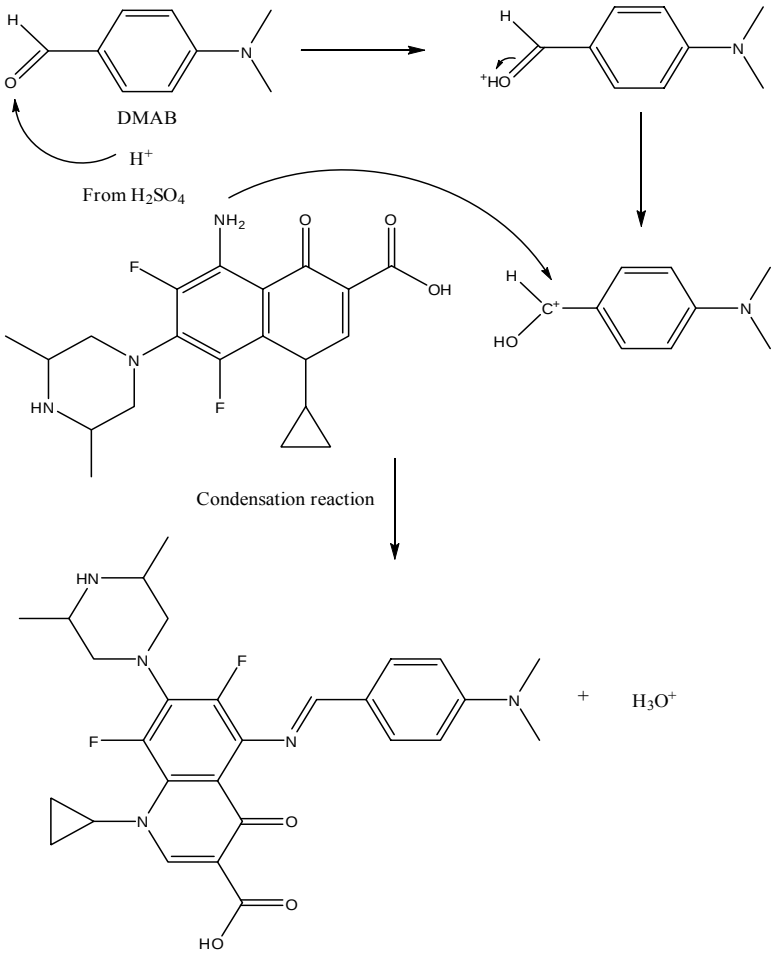
Method development

Sparfloxacin reacted with DMAB in acidic medium; condensation reaction took place and resulted in a yellow coloured product. The ethanolic solutions of both sparfloxacin and *p*-dimethylaminobenzaldehyde are colourless. The condensation reaction of DMAB occurs in the presence of an acid and condensation product form as a result of internal rearrangement. The condensation product shows maximum absorbance at 392 nm where neither DMAB nor sparfloxacin has any significant absorbance. The proposed reaction mechanism is shown in scheme 1.

Optimization of experimental conditions

The effects of temperature and reaction time on the condensation reaction were studied in the range of room temperature to 100˚C and 5-60 min, respectively. Maximum absorbance of the analyte against reagent blank was found at room temperature with 20 min reaction time. It appears that high temperature causes decomposition of the condensation product and leads to decrease in absorbance.

Effect of sulphuric acid concentration on the reaction was carried out in the range of 0.0-5.0 mol L^{-1} sulphuric acid. Maximum absorbance of the condensation product against reagent blank was obtained with 1 mL of 3 mol L^{-1} H_2SO_4 with final concentration of 0.3 mol L^{-1} after dilution. Concentrations of acid below and above 3 mol L^{-1} gave lower values of absorbance. It indicates that in lower concentration of the acid, reaction is incomplete while higher acid concentration catalyzes breakdown of the product.



Scheme 1: Proposed reaction mechanism for sparfloxacin with DMAB

Effect of concentration of DMAB was studied in the range of 0.1-2.0%. It was found that with increase in concentration of DMAB from 0.1-1.0%, absorbance remained almost constant and onward it decreases. Therefore, 0.3% DMAB concentration was selected as an optimum reagent concentration.

Quantification

A linear response between the absorbance and concentration of sparfloxacin was observed under the studied experimental conditions of the proposed method. Beer's law was obeyed in a concentration range of 2.0-80.0 µg mL^{-1} with molar absorptivity of 4.9 x 10^3 L mol^{-1} cm^{-1}. The linear regression equations, slopes, intercepts, correlation coefficients and relative standard deviation are given in table 1.

The limit of detection (LOD) and limit of quantification (LOQ) were determined using the formula 3.3 s/b and 10 s/b respectively, where s is standard deviation of blank and b is slope of the calibration curve. The LOD value was calculated to be 0.22 µg mL^{-1}, and LOQ value was found to be 0.75 µg mL^{-1}.

Table 1: Analytical parameters for the spectrophotometric determination of sparfloxacin

Parameter	Value
λ_{max} (nm)	392
Beer's law limits (µg mL^{-1})	2.0-80.0
Molar absorptivity (L mol^{-1} cm^{-1}.)	4.9×10^3
Limit of detection (µg mL^{-1})	0.22
Limit of quantification (µg mL^{-1})	0.75
Slope (b)	0.0124
Intercept (a)	0.0132
RSD (%)	2.0
Correlation coefficient (r)	0.9996

A review of the reported spectrophotometric methods is given in table 2 along with their figures of analytical merits. Most of the reported spectrophotometric methods

suffers from the disadvantages like the reaction products required either tedious extraction, heating at high temperature and long time for the completion of the reaction (Jan *et al*., 2010, Askal *et al*., 2007, Marona and Schapoval 2001, Al-Ghannam 2008) or lack the sensitivity (Marona and Schapoval 2001, Al-Ghannam 2008, Kaur *et al*., 2008) or prone to interferences by reducing substances (Jan *et al*., 2010, Askal *et al*., 2007) and most of them are even not applied to the interferences effect of excipients as well as to biological samples. The proposed method is sensitive, economical, simple, independent of temperature and matrix interferences, applicable to wide linear range of concentration and also applied to biological samples. As compared to HPLC literature method (Marona *et al*., 1999, Kowalczuk *et al*., 2003, Cao *et al*., 2001), the reagents required in the proposed method are easily available and the method does not require any critical reaction conditions like heating, solvent extraction or sample preparation.

Method validation

The selectivity of the developed method was evaluated by analysis of pharmaceutical preparations of sparfloxacin, the interferences effect of various pharmaceutical additives such as glucose, lactose, sucrose, sorbitol, magnesium stearate, talc and starch on the efficiency of the presented method were studied. Sparfloxacin solutions and each of the excipients taken separately in concentrations of five, ten and fifty times greater than that of the sparfloxacin were analyzed by the developed method. The results are shown in table 3. Recoveries of sparfloxacin in selectivity study were found to be 97.0- 102.0% with relative standard deviation of 0.4-0.9%. The results indicated that there were no significant interferences produced by these excipients substances on the developed method for the analysis of sparfloxacin.

The repeatability of the developed method was determined with three different concentrations (10-30 µg mL^{-1}) of sparfloxacin commercial formulations in triplicate. The results are presented in table 4. The relative standard deviations (RSD) were between 0.03% and 1.02% which are less than 2%. The data indicate good repeatability of the developed method.

Table 2: Review of spectrophotometric methods for determination of sparfloxacin

Reagent	$\lambda_{max\ (nm)}$	Range µg mL^{-1}	Detection Limit µg mL^{-1}	Remarks	Ref.
Ammonium meta venadate	530	0.8-28	0.15	Sensitive, indirect, temp. dependent, prone to interferences by reducing substances.	Jan *et al*., 2010
N-bromosuccinamide, p-phenylenediamine	530	3-25	0.3-1.29	Indirect method, interferences by reducing substances, no applications to biological samples	Askal *et al*., 2007
Bromothymol blue, dichloromethane	385	2-12	NA	Less sensitive, ion pair formation, solvent extraction method, narrow linear range, no applications to biological samples	Marona and Schapoval 2001
Ammonium reineckate, acetone	525	100-150	30-45	Less sensitive, tedious and time consuming, precipitation method require redissolution in acetone, narrow linear range, no applications to biological samples	Al-Ghannam 2008
Pd (II), eosin and Titron X-100	550	1.6-16	2.0×10^{-2}	Sensitive but time and temp. dependent, no applications to biological samples	El-Didamony 2007
No reagent and methanol as a solvent	295.2	2-12	NA	UV-range where the interferences from excipients is serious, less sensitive, narrow linear range, no applications to biological samples	Kaur *et al*., 2008
Ferric chloride	510	0.7-160	NA	Sensitive but lengthy, no applications to biological samples	Kaur *et al*., 2008
Cerric ammonium sulphate	484	10-80	NA	less sensitive, no applications to biological samples	Kaur *et al*., 2008
p-dimethylamino benzyldehyde	392	2-80	0.22	Sensitive, economical, simple, independent of temp. and matrix interferences, wide linear range, applied to biological samples	Proposed method

Table 3: Selectivity study of sparfloxacin (10 µg mL^{-1}) in the presence of excipients

Excipient	Excipient added (µg mL^{-1})	Recovery (%) ± % RSD
Glucose	50	100.0 ± 0.8
	100	101.0 ± 0.4
	500	102.0 ± 0.8
Sucrose	50	100.0 ± 0.4
	100	101.0 ± 0.6
	500	102.0 ± 0.5
Sorbitol	50	101.0 ± 0.4
	100	101.0 ± 0.8
	500	102.0 ± 0.5
Lactose	50	100.0 ± 0.4
	100	102.0 ± 0.8
	500	102.0 ± 0.5
Starch	50	99.0 ± 0.5
	100	98.0 ± 0.4
	500	97.0 ± 0.4
Talc	50	98.0 ± 0.9
	100	99.0 ± 0.6
	500	97.0 ± 0.4
Mg. stearate	50	99.0 ± 0.4
	100	99.0 ± 0.5
	500	99.0 ± 0.6

(Mean ± %RSD, n = 3)

Table 4: Precision of the proposed method

Formulation (Tablets)	Concentration taken (µg mL^{-1})	Recovery (%) ± % RSD
1	10.0	99.0 ± 1.0
	20.0	100.0 ± 0.50
	30.0	99.7 ± 0.33
2	10.0	98.7 ± 1.22
	20.0	99.4 ± 0.03
	30.0	99.4 ± 0.20
3	10.0	100.0 ± 0.70
	20.0	99.5 ± 0.35
	30.0	99.7 ± 0.23
4	10.0	97.3 ± 0.62
	20.0	98.5 ± 0.51
	30.0	99.0 ± 0.24

(Mean ± %RSD, n = 3)

Analytical applications

The accuracy of the proposed method in dosage form of four different brands of tablets was evaluated by standard addition method. For this, known quantities of sparfloxacin in the range of 10-30 µg mL^{-1} were added to definite amounts (10 µg mL^{-1}) of pre-analyzed sparfloxacin commercial formulations and the mixture was analyzed by the proposed method. The average percent recoveries obtained were in the range of 99.3-100.0% with relative standard deviation from 0.2-0.8 which is less than 1.0% and shows good accuracy of the proposed method (table 5).

Table 5: Recovery test of sparfloxacin in tablets by the standard addition method

Pharma-ceutical formulations	Concen-tration taken (µg mL^{-1})	Concen-tration added (µg mL^{-1})	Recovery (%) ± % RSD
1	10.0	10.0	100.0 ± 0.8
	10.0	20.0	100.0 ± 0.4
	10.0	30.0	100.0 ± 0.2
2	10.0	10.0	99.5 ± 0.5
	10.0	20.0	99.7± 0.5
	10.0	30.0	99.7 ± 0.2
3	10.0	10.0	99.5 ± 0.5
	10.0	20.0	99.7 ± 0.6
	10.0	30.0	100.0 ± 0.3
3	10.0	10.0	100.0 ± 0.4
	10.0	20.0	99.3 ± 0.2
	10.0	30.0	99.5 ± 0.4

(Mean ± %RSD, n = 3)

The developed method applied successfully for determination of sparfloxacin in commercial formulations (tablets). The results obtained for pharmaceutical dosage forms (table 6) were compared statistically with respect to accuracy by Student t-test at 95% confidence level and precision by the variance ratio F-test with those of the literature HPLC method (Cao *et al*., 2001). There was also no significant difference in precision between the developed and literature methods. The results show similar precision and accuracy in the analysis of pharmaceutical dosage forms.

Good sensitivity as shown by the limit of quantification (LOQ) value, high precision and accuracy, as revealed by the repeatability and recovery study obtained by the proposed method suggested the determination of sparfloxacin in urine and plasma samples. Sparfloxacin is recommended orally at doses of 100 mg two times daily, which results about 2-4 µg mL^{-1} concentration in urine. Table 7 shows the results of the recovery studies of sparfloxacin from spiked urine and plasma. The results are in the range of 99.0-101.0% with a relative standard deviation from 0.2-0.5% which is less than 1.0%. The data indicate the results are precise and accurate.

Table 6: Determination of sparfloxacin in commercial formulation by proposed and literature method

Commercial formulation		Mass (mg) determined	
		Proposed method	Literature method (8)
1 100 mg/tab	mean ± %RSD F (9.28) t (2.776)	99.91 ± 0.22 0.015 1.73	101.85 ± 1.78
2 100 mg/tab	mean ± %RSD F (9.28) t (2.776)	100 ± 0.33 0.083 0.912	99.81 ± 0.32
3 100 mg/tab	mean ± %RSD F (9.28) t (2.776)	99.5 ± 0.17 0.028 0.81	99.97 ± 1.04

(Three determinations were carried out for both the proposed and literature method)

Table 7: Determination of sparfloxacin in spiked urine and plasma by the proposed method

Samples	Amount added (µg mL $^{-1}$)	Recovery ± % RSD
urine 1	15.0	99.3 ± 0.5
urine 2	20.0	101.0 ± 0.4
urine 3	25.0	100.0 ± 0.7
plasma 1	10.0	100.0 ± 0.3
plasma 2	15.0	100.0 ± 0.6
plasma 3	20.0	99.0 ± 0.2

(Mean ± %RSD, n = 3)

DISCUSSION

A simple and fast method for spectrophotometric determination of sparfloxacin using *p*-dimethyl-aminobenzaldehyde (DMAB) has been developed. Sparfloxacin reacted with DMAB in acidic medium; condensation reaction took place and resulted in a yellow coloured product. The ethanolic solution of sparfloxacin and ethanolic solution of *p*-dimethylaminobenzaldehyde are colourless. The condensation reaction of DMAB occurs in the presence of an acid and the carbonyl carbon becomes positively charged. Amino group of the sparfloxacin then donates a lone pair of electrons to the positively charged carbon and a condensation product forms as a result of internal rearrangement. The condensation product shows maximum absorbance at 392 nm where neither DMAB nor sparfloxacin has any significant absorbance. All parameters for the reaction, as concentration of DMBA reagent, molarity of sulphuric acid, and reaction temperature were studied and optimized. Under the optimized conditions, a linear relationship between absorbance of the condensation product and concentration of sparfloxacin in the range of 2.0-80.0 µg mL^{-1} was found with good correlation coefficient (0.9997). The limits of detection (LOD) and quantification (LOQ) for the proposed method were found to be 0.22 and 0.75 µg mL^{-1} respectively. The molar absorptivity of the product was found to be 4.9×10^3 L mol^{-1} cm^{-1}.The repeatability and accuracy (model) of the method was studied at three different concentrations of sparfloxacin and found with value of relative standard deviation less than 2.0%. The method was found selective for determination of sparfloxacin in the presence of commonly used excipients in dosage forms. The developed method was validated statistically and applied successfully to the analysis of the drug in pure form, pharmaceutical preparations, and spiked blood plasma and urine samples with good accuracy (real) and precision. The percentage recovery was found from 99.0-100.0% with relative standard deviation less than 1%. The results of the proposed method were compared statistically with the results of literature HPLC method.

CONCLUSION

The developed method is accurate, simple, rapid, sensitive, precise, and suitable for the determination of sparfloxacin in pure form and can be satisfactorily applied to the quality control laboratory of pharmaceutical preparations and biological samples analysis.

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