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Spectrophotometric Determination of Gemifloxacin Mesylate in Pharmaceutical Formulations Through Ion-Pair Complex Formation

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Abstract: Four simple and sensitive ion-pairing spectrophotometric methods have been described for the assay of gemifloxacin mesylate (GFX) either in pure form or in pharmaceutical formulations. The developed methods involve formation of colored chloroform extractable ion-pair complexes of the drug with safranin O (SFN O) and methylene blue (MB) in basic medium; Naphthol blue 12BR (NB 12BR) and azocaramine G (AG) in acidic medium. The extracted complexes showed absorbance maxima at 525, 650, 620 and 540 nm for SFN O, MB, NB 12BR and AG, respectively. Beer's law is obeyed in the concentration ranges 3-15, 4-20, 2-10 and 2-10 $\mu\text{g/mL}$ with molar absorptivity of 2.81×10^4 , 2.20×10^4 , 4.02×10^4 and $4.15 \times 10^4 \text{ L mole}^{-1} \text{ cm}^{-1}$ and relative standard deviation of 0.077, 0.104, 0.080 and 0.103% for SFN O, MB, NB 12BR and AG, respectively. These methods have been successfully applied for the assay of drug in pharmaceutical formulations. No interference was observed from common pharmaceutical adjuvants. Results of analysis were validated statistically and through recovery studies.

Keywords: Gemifloxacin Mesylate, Ion-pair complex formation, Spectrophotometry.

Introduction

Gemifloxacin, (R,S)-7-(3-aminomethyl-4-syn-methoxyimino-1-pyrrolidinyl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid methanesulfonate, is a new fluoroquinolone antibacterial compound with enhanced affinity for bacterial topoisomerase IV and is being developed for the treatment of respiratory and urinary tract infections. The compound has a broad spectrum of activity against Gram-positive and Gram-negative bacteria¹⁻³. Literature survey revealed that few analytical methods have been reported for the estimation of GFX; they include high-performance liquid chromatography-tandem mass

spectrometry (LC-MS-MS)^{4,5}, microchip electrophoresis⁶, chiral high-performance liquid chromatography⁷ and chiral counter-current chromatography⁸. To the best of our knowledge, there is no work in the literature reported about the spectrophotometric method for the analysis of GFX in either biological fluids or pharmaceutical formulations. Hence the author has made an attempt to develop four simple and rapid spectrophotometric methods for the estimation of GFX in bulk drugs and in pharmaceutical formulations. The developed methods involve formation of colored chloroform extractable ion-pair complexes of the drug with safranin O and methylene blue in basic medium; naphthol blue 12 BR and azocaramine G in acidic medium. The extracted complexes showed absorbance maxima at 525, 650, 620 and 540 nm for SFN O, MB, NB 12BR and AG, respectively.

Experimental

Apparatus

All spectral and absorbance measurements were made on a Systronic Model 117 digital spectrophotometer with 10mm matched quartz cells.

Materials and reagents

All chemicals used were of analytical reagent grade and double distilled water was used for preparing the reagent solutions. SFN O (0.2%) solution was prepared by dissolving 200 mg of safranin O in 100 mL of distilled water. MB (0.2%) solution was prepared by dissolving 200 mg of methylene blue in 100 mL of distilled water. Ammonia-ammonium chloride buffer solution (pH 9.8) was prepared by mixing 7 g of ammonium chloride with 56.8 mL of liquor ammonia solution and diluted to 100 mL with distilled water and pH was adjusted to 9.8. NB 12 BR (0.2%) solution was prepared by dissolving 200 mg of naphthol blue 12BR in 100 mL of distilled water. AG (0.05%) solution was prepared by dissolving 50 mg of azocaramine G in 100 mL of distilled water containing traces of sodium hydroxide. 0.1 M HCl was prepared by diluting 8.6mL of concentrated hydrochloric acid to 1000 mL with distilled water and standardized. pH 1.5 buffer solution was prepared by mixing 289 mL of glycine solution (37.52 g of glycine and 29.24 g of NaCl were dissolved in 500 mL distilled water) with 711 mL of 0.1 M HCl and the pH of the solution was adjusted to 1.5.

Preparation of standard stock solutions

Stock solution of GFX was freshly prepared by dissolving 100mg of GFX in 100mL of distilled water and then this was further diluted with distilled water so as to obtain working standard solution of 60 µg/mL for SFN O, 80 µg/mL for MB, 40 µg/mL for NB 12BR and AG.

General procedures

For SFN O and MB

Different aliquots of standard solution (60 µg/mL for SFN O and 80 µg/mL for MB) from 0.5-2.5mL and 1.0mL of pH 9.8 buffer solution were placed separately in a series of 125mL separating funnels. A volume of 1.5mL of SFN O or 0.5mL of MB was added respectively. The total volume in each funnel was adjusted to 10mL with distilled water. Then 10mL of chloroform was added to each separating funnel and the contents were shaken for 5 minutes and allowed to separate. The organic layer was collected through cotton plug and the absorbance was immediately measured at 525 nm for SFN O and at 650 nm for MB against the reagent blank. Both the colored species were stable for 1 hour. The calibration curves were constructed by plotting the absorbance *versus* final concentration of

GFX. The amount of the drug was computed either from calibration curve or from regression equation.

For NB 12BR and AG

In to a series of 125 mL separating funnels containing aliquots of standard GFX solution (0.5-2.5 mL, 40 µg/mL), 6.0 mL of 0.1M HCl (for NB 12BR), or buffer solution pH 1.5 (AG) and 2.0 mL of dye solution were added. The total volume of aqueous phase in each separating funnel was adjusted to 15mL with distilled water and 10 mL of chloroform was added. The contents were shaken for 2 minutes and allowed to separate. The organic layer was collected through cotton plug and the absorbance was immediately measured at 620 nm for NB 12BR and at 540 nm for AG against the reagent blank. Both the colored species were stable for 1 hour. The calibration curves were constructed by plotting the absorbance versus final concentration of the GFX. The amount of the drug was computed either from calibration curve or from regression equation.

Procedure for the assay of the tablets

Twenty tablets were weighed accurately and ground in to a fine powder. An amount of powder equivalent to 50 mg of GFX was weighed into a 100 mL volumetric flask, about 20 mL of water containing 10 mL of the methanol were added and shaken thoroughly for about 20 min, then the volume was made up to the mark with the water, mixed well and filtered using a whatman filter paper #42 and the first few milliliters of the filtrate were discarded. The procedure was continued as mentioned under general procedures

Results and Discussion

GFX forms ion-pair complexes in basic buffer with dyestuffs such as SFN O and MB and these complexes are quantitatively extracted into chloroform. The ion-pair complexes with SFN O and MB absorbed maximally at 525 and 650 nm respectively. GFX also forms ion-pair complexes in acidic medium with dyestuffs such as NB 12BR and AG and these complexes are quantitatively extracted into chloroform. The ion-pair complexes with NB 12BR and AG absorbed maximally at 620 and 540 nm respectively. Optimum conditions established for these methods were presented in Table 1 and 2.

The optical characteristics such as Beer's law limits, Sandell's sensitivity, molar absorptivity, percent relative standard deviation (calculated from eight replicate samples containing 3/4th of the amount of the upper beer's law limits) were calculated for all the methods and the results are summarized in Table 3. Regression characteristics like standard deviation of slope (S_b), standard deviation of intercept (S_a), standard error of estimation (S_e), % range of error (0.05 and 0.01 confidence limits) and detection limit were calculated for all the methods and are shown in Table 3.

Commercial formulation of GFX was successfully analyzed by the proposed methods. The values obtained by the proposed methods are presented in Table 4. The reliability of the proposed method was checked by standard addition method. The results (Table 5) show that the mean recoveries were found in the range 100.0-100.05 with RSD \leq 0.55% for SFN O, 100.01-100.08 with RSD \leq 0.54 for MB, 100.05-100.12 with RSD \leq 0.61 for NB 12BR and 100.01-100.16 with RSD \leq 0.57 for AG. Interference studies revealed that the common excipients and other additives usually present in dosage form did not interfere in the proposed methods. The performance order of the proposed methods is AG > NB 12BR > SFN O > MB.

Table 1. Optimum conditions established for SFN O and MB

Parameter	Optimum range	Conditions in procedure	Remarks
Effect of buffer pH on color development	9.0-10.0	pH-9.8	Variations of the pH <6 and >11 resulted in low absorbance values
Volume of buffer required for maximum intensity of color	0.5-1.5 mL	1.0 mL	1 mL of buffer is preferable for maintenance of pH 9.8
Effect of volume of dye	1.0-2.0 mL for SFN O 0.3-0.8 mL for MB	1.5 mL of SFN O 0.5 mL of MB	1.5 mL of SFN O and 0.5 mL of MB was necessary for covering the broad range of Beer's law.
Choice of the organic solvent for the extraction of the colored species	Chloroform	Chloroform	The other water immiscible solvents tested for the extraction of the colored complex into organic phase include chlorobenzene, dichloromethane, carbon tetrachloride, <i>n</i> -butanol and benzene. Chloroform was preferred for its selectivity extraction of the complex from the aqueous phase.
Effect of the ratio of aqueous to organic phase on extraction	1:1	1:1	The extraction of the colored species into chloroform layer was incomplete, where the ratio of aqueous to chloroform phase was more than the specified ratio in each case.
Effect of shaking time	3-8min	5min	Constant absorbance values were obtained for the shaking period 3-8 min.
Effect of temperature on the colored species	Laboratory temperature (28 ± 2°C)	Laboratory temperature	At low temperature (<20 °C) the extraction of the colored species was found to be improper. At high temperature (>35 °C) the stability of the colored species was found to be less.
Stability of the colored species	1-60 min	5 min	The colored species after separation from organic layer was stable for 60 min, afterwards the absorbance gradually decreased.

Table 2. Optimum conditions established for NB 12BR and AG

Parameter	Optimum range	Conditions in procedure	Remarks
Effect of acid concentration or buffer pH on color development	0.08-0.12 N HCl for NB 12BR 1.0-1.8 (pH) for AG	0.1 N HCl for NB 12BR pH -1.5 for AG	Variations of concentrations of the acid or pH of the buffer beyond the upper and lower limits resulted in low absorbance values.

Contd...

Volume of acid or buffer required for maximum intensity of color	5.0-7.0mL acid	6mL of acid or buffer (for B12BR) or buffer (AG)	Variation of volume of buffer or acid beyond the lower limit resulted in low absorbance values.
Effect of volume of dye	1.0-2.5 mL	2.0 mL	2.0mL of NB 12BR and AG was necessary for covering the broad range of Beer's law.
Choice of the organic solvent for the extraction of the colored species	Chloroform	Chloroform	The other water immiscible solvents tested for the extraction of the colored complex into organic phase include chlorobenzene, dichloromethane, carbon tetrachloride, <i>n</i> -butanol and benzene. Chloroform was preferred for its selectivity extraction of the complex from the aqueous phase.
Effect of the ratio of aqueous to organic phase on extraction	3:2	3:2	The extraction of the colored species into chloroform layer was incomplete where the ratio of aqueous to chloroform phase was more than the specified ratio in each case.
Effect of shaking time	1-5 min	2 min	Constant absorbance values were obtained for the shaking period 3-8 min.
Effect of temperature on the colored species	Laboratory temperature (28+ 2 ⁰ C)	Laboratory temperature	At low temperature (<20 ⁰ C) the extraction of the colored species was found to be improper. At high temperature (> 35 ⁰ C) the stability of the colored species was found to be less.
Stability of the colored species	1-60 min	5 min	The colored species after separation from organic layer was stable for 60 min, afterwards the absorbance gradually decreased.

Table 3. Optical, regression characteristics of the proposed methods for GFX

Parameter	SFN O	MB	NB 12BR	AG
λ_{max} , nm	525	650	620	540
Beer's law limits, $\mu\text{g mL}^{-1}$	3.0 – 15.0	4.0 – 20.0	2.0 - 10.0	2.0 - 10.0
Detection limits, $\mu\text{g mL}^{-1}$	0.058	0.074	0.045	0.053
Molar absorptivity, $\text{Lmole}^{-1} \text{cm}^{-1}$	2.81×10^4	2.20×10^4	4.02×10^4	4.15×10^4
Sandell's sensitivity $\mu\text{g cm}^{-2} / 0.001$ absorbance unit)	0.017	0.022	0.012	0.0116
Regression equation ($Y = a + bC$)				
Slope (b)	5.7×10^{-2}	4.5×10^{-2}	8.2×10^{-2}	8.5×10^{-2}
Standard deviation of slope (S_b)	0.11×10^{-3}	0.09×10^{-3}	0.19×10^{-3}	0.23×10^{-3}
Intercept (a)	0.5×10^{-3}	0.50×10^{-3}	0.30×10^{-3}	1.20×10^{-3}
Standard deviation of intercept (S_a)	1.13×10^{-3}	1.13×10^{-3}	1.26×10^{-3}	1.53×10^{-3}

Contd...

Standard error of estimation (S_e)	1.08×10^{-3}	1.08×10^{-3}	1.20×10^{-3}	1.46×10^{-3}
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999
Relative standard deviation, % ^a	0.077	0.104	0.080	0.103
% Range of error (Confidence limits) ^a				
0.05 level	0.064	0.087	0.067	0.086
0.01 level	0.095	0.129	0.100	0.128
% Error in bulk samples ^b	0.072	0.138	0.075	-0.036

^a Average of eight determinations; ^b Average of three determinations
In $Y = a + bC$, Y is absorbance and C is concentration.

Table 4. Results of analysis of tablet formulations containing GFX

Formulation	Labeled amount mg	% Recovery*				% RSD			
		SFN O	MB	NB 12BR	AG	SFN O	MB	NB 12BR	AG
Tablets-1	320	100.03	100.01	99.99	100.08	0.49	0.38	0.44	0.56
Tablets-2	320	100.06	100.0	100.02	100.06	0.57	0.53	0.41	0.32

* Average of six independent analyses.

Table 5. Results of recovery study by standard addition method

Formulation	Amount $\mu\text{g mL}^{-1}$ Taken +Added	% Recovery*				% RSD			
		SFN O	MB	NB 12BR	AG	SFN O	MB	NB 12BR	AG
Tablets-1	4+5	100.05	100.01	100.05	100.01	0.55	0.29	0.58	0.42
	5+5	100.03	100.02	100.10	100.08	0.47	0.35	0.55	0.57
Tablets-2	4+4	100.0	100.08	100.12	100.04	0.36	0.54	0.61	0.50
	5+4	100.02	100.06	100.09	100.16	0.51	0.38	0.53	0.54

* Average of six independent analyses

In conclusion the proposed spectrophotometric methods for the estimation of GFX were simple, sensitive and accurate and can be used for the routine quality control of the drug in bulk as well as in pharmaceutical formulations.

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