IOSR Journal Of Pharmacy www.iosrphr.org
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 8, Issue 4 Version. III (April 2018), PP. 01-08

Determination of Gemifloxacin in pure and pharmaceutical forms : A spectrophotometric study

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Abstract: Four simple and sensitive extractive spectrophotometric methods for the determination of Gemifloxacin in pure form and in pharmaceutical formulations using triphenyl methane dyes have been developed. The developed methods involve formation of coloured chloroform extractable ion-pair complexes of the drug with triphenyl methane dyes viz., bromothymol blue (BTB), bromophenol blue (BPB), bromocresol green (BCG) and bromocresol purple (BCP) in acidic medium. The extracted complexes formed with BTB, BPB, BCG and BCP showed absorbance maxima at 415, 416, 412 and 414 nm respectively**.** The stoichiometry of the ion-pair complex of Gemifloxacin with dyes is found to be 1:1 in each case. Beer's law is obeyed in the concentration range 2.5-25µg/ml of drug with dyes. The effect of concentration of dye, pH, and interference of excipients has been studied for optimization. The limits of detection and quantification have been determined for all the four methods. These methods have been validated as per the guidelines of ICH. The results of analysis were validated statistically through recovery studies.

Keywords: Gemifloxacin, Bromothymol blue, Bromophenol blue, Bromocresol green, Bromocresol purple, Factive tablet, Ion-pair complex, Spectrophotometry, Validation

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Date of Submission: 12-04-2018 Date of acceptance: 30-04-2018

I. INTRODUCTION

The present study is aimed at the development of four sensitive and simple spectrophotometric methods for the determination of Gemifloxacin using acidic triphenyl methane dyes in pure and pharmaceutical forms.

The chemical name of Gemifloxacin is R,S-7-(3-amino methyl-4- syn methoxyimino-1-pyrrolidinyl)-1-cyclopropyl - 6-flouro - 1,4-dihydro – 4 - oxo-1,8-napthyridine-3-carboxylic acid (Fig. 1) and it belongs flouroquinolone antibacterial type of compounds. It is used for the treatment of urinary tract and respiratory infections [1,2].

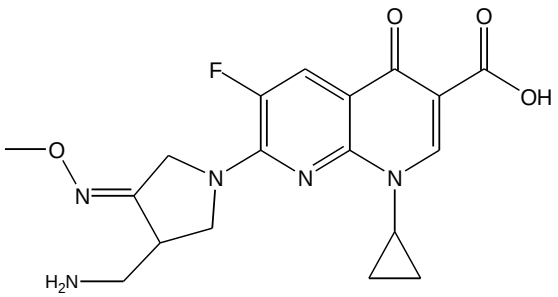

Fig 1: Structure of Gemifloxacin

Gemifloxacin is a broad-spectrum fluoroquinolone antibacterial agent. Its bactericidal activity depends on inhibition of DNA synthesis. This mode of action involves dual targeting of two bacterial enzymes: DNA gyrase and topoisomerase IV, which are essential for bacterial DNA replication and transcription [3,4]. Gemifloxacin is not official in any pharmacopoeia. The literature survey revealed several reported analytical approaches for its determination in pharmaceutical dosage forms and in biological fluids. Various Spectrophotometric[5-15] and Spectrofluorimetric [16-18] methods were described. Chromatographic methods were also reported and include capillary electrophoresis [19], HPTLC [20-22] and HPLC [23].

Development and validation of RP-HPLC method [24] for determination of gemifloxacin mesylate in bulk and tablet dosage form and simple spectrophotometric and conductometric methods [25] for its determination in pure and pharmaceutical dosage form and human urine were reported. Gemifloxacin in human

serum by online heart-cutting liquid chromatography [26] and spectrophotometric/chemometric assisted methods [27] for the simultaneous determination of imatinib, gemifloxacin, nalbuphine and naproxen in pharmaceutical formulations and human urine are available in the literature.

Although a considerable number of analytical methods have been applied by analytical chemists for the quantitative determination of Gemifloxacin, methods on spectrophotometric determination of the drug involving ion-pair complexes with common acidic dyes *viz.*, bromothymol blue (BTB), bromophenol blue (BPB), bromocresol green (BCG) and bromocresol purple (BCP) are not available in the literature. This encouraged the author to develop new extractive spectrophotometric methods for the determination of Gemifloxacin using acidic dyes mentioned above.

II. MATERIALS AND METHODS

Instruments

For recording UV-Vis spectra of the ion-pair complexes, SHIMADZU 140 double beam spectrophotometer and ELICO SL 210 UV-Visible double beam spectrophotometer with quartz cells of 10 mm path length have been used. For *p*H measurements, an Elico model Li-120 *p*H meter was employed.

Materials

The dyes *viz.*, Bromothymol blue, Bromophenol blue, Bromocresol green and Bromocresol purple of analytical grade supplied by SD Fine Chemicals Ltd. Mumbai, were used without any further purification. The solvent Chloroform (HPLC grade) and AR grade HCl and Sodium acetate supplied by SD Fine Chemicals, Mumbai were used in the study. The drug, Gemifloxacin analysed in the present study was procured as gift sample from Hetero Drugs Pvt. Ltd, Hydeabad, Telangana.

Methods

Method A

The basis for the method A is the interaction of the drug with dye, BTB to form chloroform extractable ion-pair complex which shows absorption around 415 nm. Increase in the absorbance with the concentration of drug formed a basis for the quantification of the drug. 0.025% solutions of dye stuff in doubly distilled water and CH_3COONa-HCl buffer of pH 2.8 were used and the pH of the reaction mixture in each case was adjusted to required value with the help of a pH meter.

Method B

The basis for the method B is the interaction of the drug with dye, BPB to form chloroform extractable ion-pair complex which shows absorption around 416 nm. Increase in the absorbance with the concentration of drug formed a basis for the quantification of the drug by this method. The *p*H of the solution was maintained at 2.5 using CH_3COONa-HCl buffer. All the other experimental details are similar as mentioned in method A.

Method C

The basis for the method C is the interaction of the drug with dye, BCG to form chloroform extractable ion-pair complex which shows absorption around 412 nm. The increase in the absorbance with the concentration of drug formed the basis for the quantification of the drug. The *p*H of the solution was maintained at 2.5 using CH_3COONa-HCl buffer. All the other experimental details are similar as mentioned in method A.

Method D

The basis for the method D is the interaction of the drug with dye, BCP to form chloroform extractable ion-pair complex which shows absorption around 414 nm. The increase in the absorbance with the concentration of drug formed the basis for the quantification of the drug. The *p*H of the solution was maintained at 3.5 using CH_3COONa-HCl buffer. All the other experimental details are similar as mentioned in method A.

III. RESULTS AND DISCUSSION

The developed methods are based on the formation of ion-pair complexes between drug and dyestuffs viz., BTB, BPB, BCG and BCP and these complexes were extracted into chloroform to measure the absorbance of colour complexes. Ion-pair complexes of Gemifloxacin with BTB, BPB, BCG and BCP absorbed maximally at 415, 416, 412 and 414 nm respectively (Fig. 2a, 2b, 2c and 2d). The reagent blank under similar conditions showed no absorption. The developed methods have the advantages of speed and simplicity besides being accurate and precise, and can be adopted by the pharmaceutical laboratories for industrial quantitative analysis.

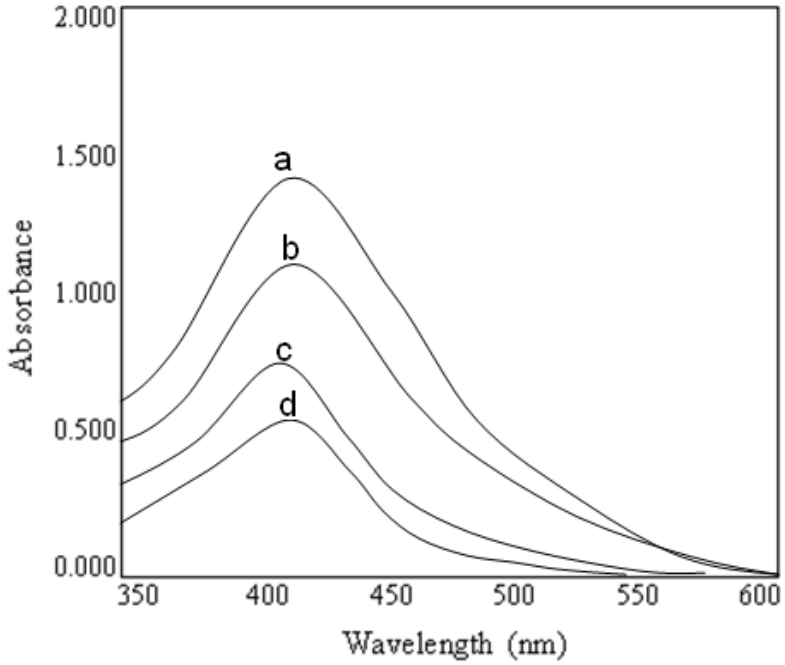

Fig 2: Absorption spectra of Gemifloxacin-dye complex extracted into 10 ml chloroform
a. drug = 25.0 μg ml^{-1} + 5 ml of 0.025% BTB + 5 ml of pH 2.8 buffer
b. drug = 22.5 μg ml^{-1} + 5 ml of 0.025% BPB + 5 ml of pH 2.5 buffer
c. drug = 20.0 μg ml^{-1} + 5 ml of 0.025% BCG + 5 ml of pH 2.5 buffer
d. drug = 17.5 μg ml^{-1} + 5 ml of 0.025% BCP + 5 ml of pH 3.5 buffer

Gemifloxacin contains a primary amine nitrogen which is protonated in acid medium, while sulphonic acid group present in BTB, BPB, BCG and BCP undergoes dissociation in the *p*H range 1-5. The colour is due to the opening of lactoid ring and subsequent formation of quinoid group which predominates in strong acidic medium. Finally the protonated Gemifloxacin forms ion-pairs with the dyestuffs which are quantitatively extracted into chloroform. The possible structure of ion pair complex formed between Gemifloxacin and dyes is given in Chart 1.

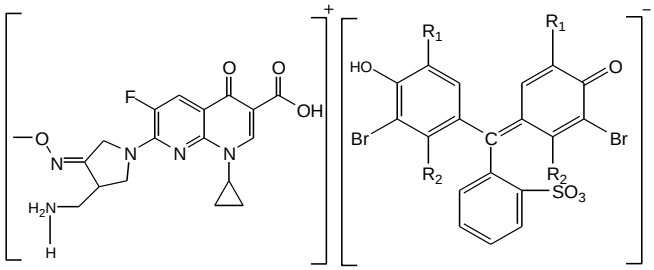

Chart 1: Gemifloxacin-dye ion pair complex
Bromothymol blue : R_1= isopropyl, R_2= -CH_3
Bromophenol blue ; R_1 = -Br, R_2 = -H
Bromocresol green : R_1 = -Br, R_2 = -CH_3
Bromocresol purple : R_1 = -CH_3, R_2 = -H

Calibration curve

Into a 125ml separating funnel, different aliquots of Gemofloxacin solution were taken and 5 ml of buffer of *p*H 2.8 for BTB, 2.5 for BPB, 2.5 for BCG and 3.5 for BCP and 5 ml of 0.025% dye solution were added and the total volume was set to 20ml with distilled water. Then, 10 ml of Chloroform was added and the contents in the funnel were shaken well for 5 min and kept for 5 min aside to allow the contents to separate into two layers. The stable coloured organic layer was separated and its absorbance was recorded around 419 nm against blank similarly prepared. It was observed that the calibration curves were linear for all the drugs analyzed by this method. The similar procedure was taken up to analyze the pure drug and its pharmaceutical forms. The calibration graphs (Fig. 3) are linear over the concentration ranges and are within the permissible range. The data in Table 1 represents the optical characteristics of ion-pair complexes and statistical data for the regression equation of the developed methods.

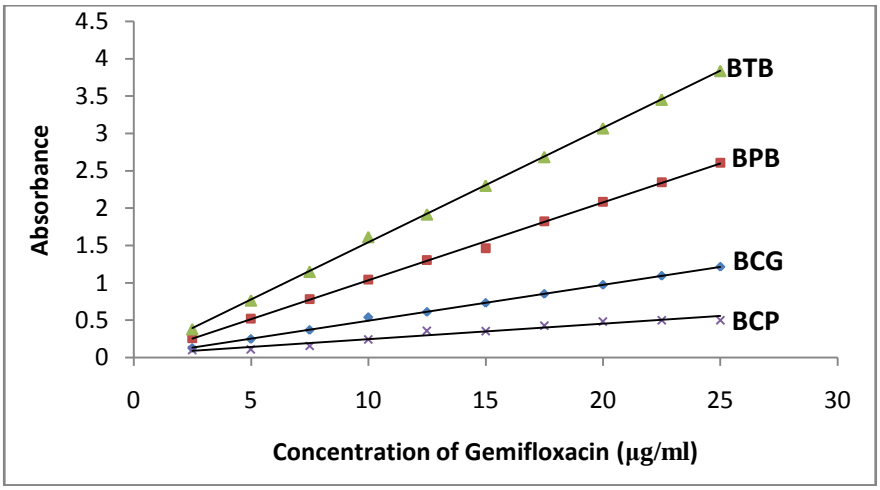

Fig 3: Calibration graph for Gemifloxacin-dye ion pair complexes

Table 1: Optical characteristics and statistical analysis for the regression equation of the proposed methods for the analysis of Gemifloxacin

Parameters		Extraction methods with[b]		
	BTB	**BPB**	**BCG**	**BCP**
λ_{max} (nm)	415	416	412	414
Beer's law limit (µg ml^{-1})	2.5-25	2.5-25	2.5-25	2.2-25
Molar absorptivity (L mol^{-1} cm^{-1})	22360	24093	23220	23421
Formation constant, K, M^{-1}	$5.15x10^6$	$4.23x10^6$	$1.97x10^6$	$8.12x10^5$
Sandell sensitivity (µg cm^{-2})	0.0217	0.0204	0.0213	0.0208
Slope (specific absorptivity), b	0.046	0.049	0.047	0.048
Intercept (a)	0.027	-0.011	0.005	-0.059
Correlation coefficient (r)	0.995	0.996	0.998	0.994
Standard deviation of intercepts (% n=6)	0.0094	0.0079	0.006	0.0102
Limit of detection, µgml^{-1}	0.678	0.534	0.421	0.706
Limit of quantification, µgml^{-1}	2.250	1.619	1.277	2.138
Regression equation[a]	Y=0.046C±0.027	Y=0.049C±0.011	Y=0.047C±0.005	Y=0.048C±0.059

[a]Withrespect to $Y=bc+a$, where C is the concentration (µg ml^{-1}) and Y is absorbance, [b]Six replicate samples

Procedure for the assay of pure drug

The recovery experiments were performed with five different solutions of pure Gemifloxacin in the range of calibration curve. The percent recoveries and their relative standard deviations are presented in Table 2.

Table 2: Application of proposed methods for the analysis of Gemfiloxacin in pure form

Taken (µg ml^{-1})	**Proposed method**								**Reference method Recovery (%)**
	Found (µg ml^{-1})				**Recovery (%)**				
	BTB	**BPB**	**BCG**	**BCP**	**BTB**	**BPB**	**BCG**	**BCP**	
4	4.032	3.97	4.03	4.02	100.8	99.25	100.75	100.5	98.75
8	8.03	7.96	8.02	8.06	100.38	99.50	100.31	100.75	101.02
12	12.07	11.95	12.08	12.08	100.58	99.58	100.65	100.66	101.88
16	16.06	15.97	16.12	16.09	100.38	99.81	100.75	100.56	101.06
									99.84
									101.58
									101.85
RSD (%)					0.20148	0.233	0.206	0.1101	1.10638
Mean±SD					100.53 ±0.203	99.53 ±0.232	100.62 ±0.208	100.62 ±0.1107	100.76 ±1.106

t-test					1.0542	0.2368	0.2218	0.2348	
F-test					0.3256	0.4265	0.4866	0.5489	

Procedure for the assay of dosage forms

Ten tablets of Factive 15mg each are powdered and dissolved in doubly distilled water and stirred thoroughly, filtered through a Whatman No. 42 filter paper. This solution was transferred into 100 ml standard volumetric flask and diluted with doubly distilled water as required. Different solutions of drug in the range of calibration curve were chosen and the assay was estimated using the calibration curve. The results of the recovery experiments are presented in Table 3.

Table 3: Application of proposed methods for the analysis of Gemfiloxacin in pharmaceutical form

Taken ($\mu g\ ml^{-1}$) Factive 15mg	**Proposed methods**								**Reference method Recovery (%)**
	Found ($\mu g\ ml^{-1}$)				**Recovery (%)**				
	BTB	**BPB**	**BCG**	**BCP**	**BTB**	**BPB**	**BCG**	**BCP**	
4	3.98	3.97	4.05	4.01	99.50	99.75	101.39	100.25	99.75
8	8.05	8.03	8.02	7.95	100.63	100.38	100.31	99.38	101.12
12	12.05	11.94	12.07	12.04	100.42	99.50	100.66	100.33	101.35
16	15.98	15.93	15.94	15.96	99.88	99.56	99.64	100.38	100.06
									99.85
									101.25
									100.81
RSD (%)					0.20148	0.233	0.206	0.1101	0.688
Mean±SD					100.53 ±0.203	99.53 ±0.232	100.62 ±0.208	100.62 ±0.1107	100.59 ±0.6923
t-test					1.0542	0.2368	0.2218	0.2348	
F-test					0.3256	0.4265	0.4866	0.5489	

Stoichiometry

In order to establish molar ratio between Gemifloxacin mesylate and dyestuffs used, the Job's method of continuous variation [28] has been applied. In this method, solutions of drug and dyestuff with identical molar concentrations ($8 \times 10^{-5} M$) were mixed in varying volume ratios in such a way that the total volume of each mixture was the same. The absorbance of each solution was measured and plotted against the mole fraction of the drug (Fig. 4). This measurement showed that 1:1 complex was formed with each dyestuff. The formation constants [29,30] were also estimated and found to be 5.15×10^6, 4.23×10^6 and 1.97×10^6, 8.12×10^5 K M^{-1} for complexes with BTB, BPB, BCG and BCP respectively.

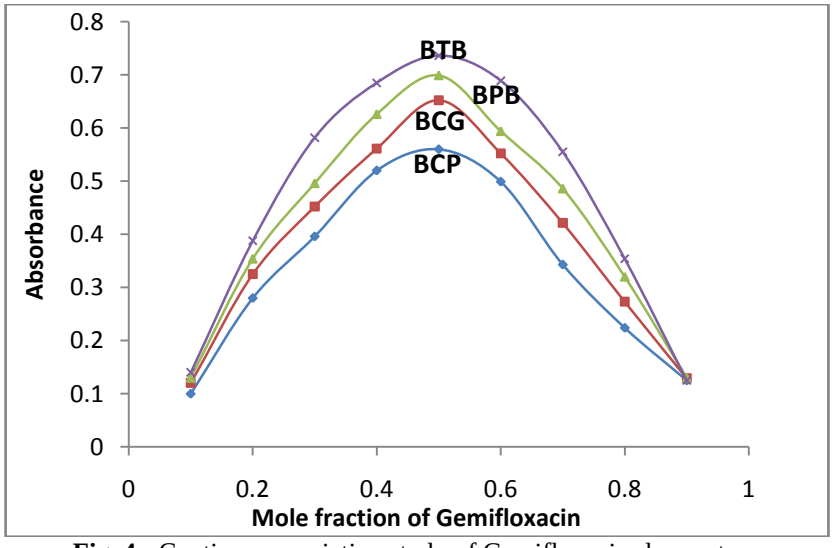

Fig 4: Continuous variation study of Gemifloxacin-dye systems
[Drug] = [Dye] = 8×10^{-5}M

Optimization of the factors effecting the absorbance

The influence of *p*H on the formation of ion-pair complexes of Gemifloxacin mesylate with various dyestuffs has been studied using sodium acetate-hydrochloric acid buffer. The results are shown in Fig. 5. It is evident that absorbance of complexes with BTB, BPB, BCG and BCP was found to be constant within the *p*H ranges 2.2-3.3, 2.0-3.0, 2.0-3.0 and 2.8-3.8 respectively.

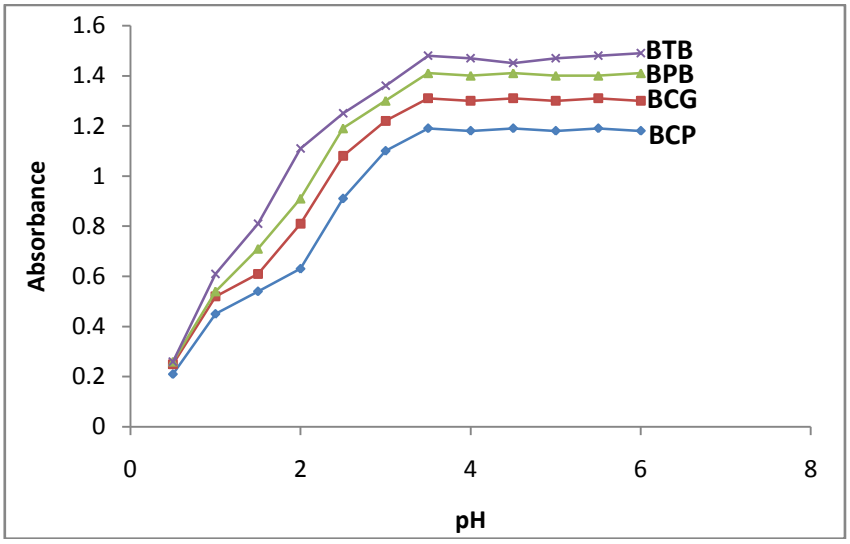

Fig 5: Effect of pH
[Gemifloxacin] = [8µg ml^{-1}], [Dye] = 5ml of 0.025%

The effect of dyestuff concentrations was also studied by adding different volumes of dyestuff to a constant amount of Gemifloxacin mesylate (8 µg ml^{-1}). It is apparent from Fig. 6 that the maximum absorbance, in each case, was found with 3.0 ml of dyestuff, beyond which absorbance was constant. Thus, 5 ml of each dyestuff was used for ion-pair formation throughout the experiment.

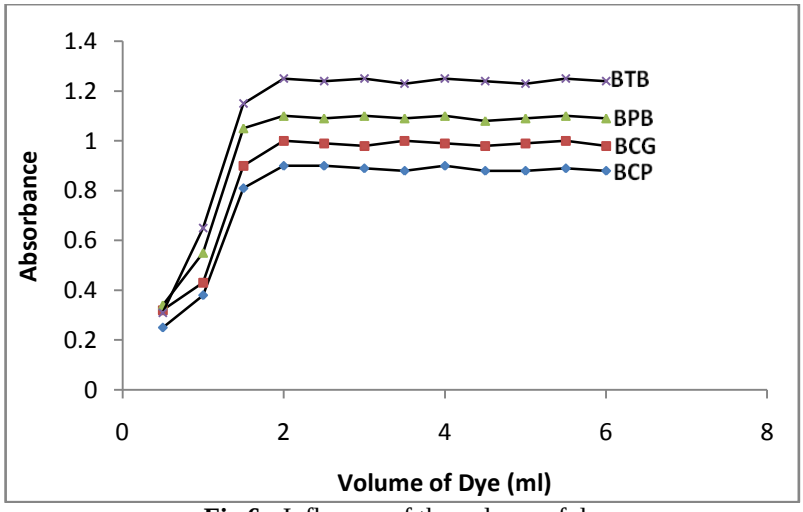

Fig 6: Influence of the volume of dye
[Gemifloxacin] = [8µg ml^{-1}]

A systematic study of the effect of foreign species present along with Gemifloxacin mesylate on its determination mesylate at 8 µg ml^{-1} levels was undertaken. This study was carried out by following the proposed procedures for a 10 ml sample system, by adding a known amount of foreign species to a Gemifloxacin mesylate solution of 8 µg ml^{-1}. Table 4 summarizes the results obtained. However, the drug content from the powdered capsules was extracted into chloroform, which completely removes any interference by the common excipients found in formulations.

Table 4: Interference study in the analysis of Gemifloxacin

Sl. No.	Excipients	Tolerance limit (µg ml^{-1})
1	Microcrystalline cellulose	98
2	Starch	148
3	Lactose	121
4	Povidone	55
5	Silicon dioxide	75
6	Titanium dioxide	45

Validation of the proposed methods

All the four proposed methods have been validated in terms of guidelines proposed by International Conference on Harmonization [31,32] *viz*. selectivity, specificity, accuracy, precision, limits of calibration curve, LOD, LOQ, robustness, ruggedness and regression equation. The student t-test and variance F-test have been performed in comparison with a reference method. Table 1 summarizes the values for Beer's law limits, molar absorptivity, regression equation, correlation coefficients and relative standard deviations. To test the reproducibility of the proposed methods, six replicate determinations of 10µg ml^{-1} of Gemifloxacin mesylate were made. The coefficient of variation was found to be less than 1.2% for all the procedures.

The proposed methods have been successfully applied to the determination of Gemifloxacin mesylate in pharmaceutical preparations. The performance order of the proposed methods is found to be BTB>BPB>BCG>BCP. The results obtained and shown in Table 2 and Table 3 were compared to those obtained by a reference method [32] by means of *t*-test at 95% confidence level. In all cases, the average results obtained by proposed methods and reference method were statistically identical. The recovery studies and comparative t- and F-tests grow confidence on the applicability of these methods for the determination of Gemifloxacin in pharmaceutical formulations.

IV. CONCLUSIONS

Gemifloxacin forms ion-pair complexes with acidic triphenylmethane dyes *viz.*, bromocresol green, bromophenol blue, bromothymol blue and bromocresol purple in 1:1 proportion. These complexes are extractable into chloroform and offer a basis for assay of the drug. The developed methods are simple, sensitive, and reproducible and can be used for routine analysis of Gemifloxacin in pure and formulation forms. The results obtained by the proposed methods proved that these methods can be considered as standard methods. Comparative t- and F-tests grow confidence on the applicability of the methods in pharmaceutical formulations. The results obtained are satisfactorily accurate and precise as indicated by the excellent percent recovery.

ACKNOWLEDGEMENTS

The authors are grateful to Prof. G. Venkateshwarlu, Department of Chemistry, Osmania University, Hyderabad for helpful discussion and to Sri M. Ravindra Reddy, Chairman, Managing Committee & Sri P. Shivaprakash, Principal of SAP College Vikarabad for providing facilities. The authors are thankful to the UGC for financial assistance under Major Research Project.

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