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Development and Validation of a Dissolution Test with Spectrophotometric Analysis Forgemifloxacin Mesylate and Ambroxol Hydrochloride in Tablet Dosage Form

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Abstract: The aim of this work was to develop and validate a dissolution test for Gemifloxacin mesylate and Ambroxol hydrochloride tablets using spectrophotometric method. The dissolution established conditions were: 900 mL of 0.01M HCl pH 2.0 as dissolution medium, using a paddle apparatus at a stirring rate of 50 rpm. The drug release was evaluated by UV spectrophotometric method at 271 nm for Gemifloxacin mesylate and 243.5 nm for Ambroxol hydrochloride. The method was validated to meet requirements for a global regulatory filing which includes linearity, specificity ,precision, accuracy robustness and ruggedness. In addition, filter suitability and drug stability in medium were demonstrated. The comparison of the obtained dissolution profiles of tablets, obtained from three different batches (A, B and C) of 320 mg Gemifloxacin mesylate and 75 mg Ambroxol hydrochloride of was performed and the results showed no significant difference among the products.
Keywords: In vitro release, Stability, Dissolution study of Gemifloxacin mesylate and Ambroxol hydrochloride, Spectrophotometry, Area under curve(AUC), Multicompoeant mode method , Validation.

INTRODUCTION

Gemifloxacin (GEM) (Fig. 1A) chemically R,S-7-(3 amino methyl 4- syn methoxyimino-1pyrrolidinyl)-1cyclopropyl-6-flouro1,4,dihydro 4- oxo-1,8 napthyridine-3-carboxylic acid methane- sulphonate[1-3] is a new flouroquinolone antibacterial compound with enhanced affinity for bacterial topoisomerase-IV and is being used for the treatment of respiratory and urinary tract infections, light brown powder, freely soluble in water and slightly soluble in Methanol. Ambroxol hydrochloride (AMB) (Fig. 1B) chemically, 4-[(2-amino-3,5-dibromophenyl)-methyl]-amino] cyclohexanol hydrochloride is a mucolytic expectorant and used to reduce the viscosity of mucous.[4-5]

FIG 1A STATURE OF GEM

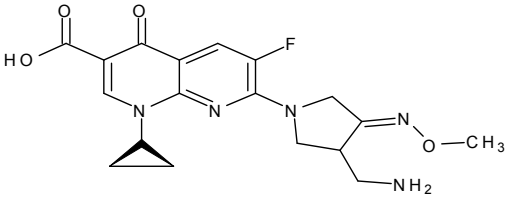
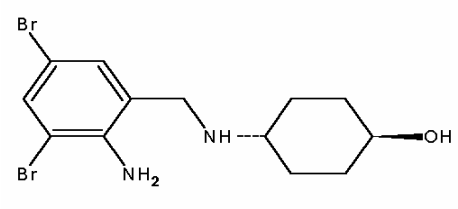


FIG 1AB STRUCTURE OF AMB



Literature survey revealed that few analytical method have been reported for the estimation of gemifloxacin, rapid and sensitive LC method for analysis of gemifloxacin in human plasma[6], spectrophotometric determination of gemifloxacin mesylate in pharmaceutical formulation trough ion-pair complexation [7]and validated stability indicating assay of gemifloxacin and lomefloxacin in tablet formulation by capillary electrophoresis[8]. Drug absorption from a dosage form after oral administration depends on the release of the drug from the pharmaceutical formulation, the dissolution and/or its solubilization under physiologicalconditions, and the permeability across the gastrointestinal tract. Because of the critical nature of the first two of these steps, *in vitro* dissolution may be relevant to the prediction of *in vivo* performance [9]. The dissolution test is a very important tool in drug development and quality control and the process of dissolution. At present time there are no official monograph for Gemifloxacin raw material and tablets and no dissolution test has been described in literature for these drugs. No single method is available for Gemifloxacin mesylate and Ambroxol hydrochloride in combination . The present paper describes the development and validation of dissolution test for quality control of Gemifloxacin in sustain release tablets. The best dissolution conditions were used to evaluate development and validation of a dissolution method with dissolutions profile of three different batches of tablets.

MATERIAL AND METHODS

Materials

Standard gift sample of Gemifloxacin mesylate and Ambroxol hydrochloride were provided by Hetero pharma Ltd., Himachal Pradesh . Tablet (G-CIN A) Gemifloxacin mesylate 320 mg and Ambroxol hydrochloride 75 mg manufactured by Hetero Pvt. Ltd were purchased from local market for analysis. All reagents and solvents used were analytical grade. 0.01 M HCl of pH2.0, pH 4.5 sodium acetate, pH 6.8 sodium phosphate and 2.1 simulated gastric fluid buffer solutions were prepared according to USP Pharmacopoeia [10].

Instrument [11]

Make: Electrolab
Model**:** TDT-06L
Specification**:** USP Standards tablet dissolution test apparatus multi-bath (n=6)
Dissolution test was performed in accordance to USP Pharmacopoeia [12] general method. The medium were vacuum degassed under in house vacuum and were maintained at 37.0 ± 0.5°C by using a thermostatic bath. A double-beam UV-Visible double beam spectrophotometer, make: SHIMADZU (model UV-1800) with a pair of 1 cm matched quartz cells. with spectral band width of 1 nm, was used for all absorbance measurements. Elico pH analyzer (Model: Elico 11610) was used to determine the pH of all solutions.

Solubility/stability determination and dissolution test optimization

Gemifloxacin and Ambroxol solubility was determined in 900 mL of Purified Water, 0.01M HCl, simulated gastric fluid (SGF) pH 2.1, sodium acetate buffer pH 4.5 and sodium phosphate buffer pH 6.8, using an amount of the drug equivalent a three times of dose in the pharmaceutical formulation [13]. Drug release tests were carried out according to conventional dissolution procedures recommended for singleentityproducts, usingpaddle(USPApparatus II) at 25 and 50 rpm. Sampling aliquots of 10.0 mL were withdrawn at 0, 5, 10, 30,60,90,120,150 and 180 minutes, and replaced with an equal volume of thefresh medium to maintain a constant total volume. At the end of each test time, samplesaliquots were filtered and diluted with dissolution medium, when necessary, andquantified. The assay of the Gemifloxacin and Ambroxol product was performed using previouslyvalidated spectrophotometric method [, and the content results were used to calculatethe percentage release on each time of dissolution profile. The cumulative percentage of drug released was plotted against time, in order to obtain the release profile and to
calculate the *in vitro* dissolution data (*n*=12). The filtration procedure of Gemifloxacin and Ambroxol and samples (tablets dissolved in dissolution medium, n=3) were evaluated using 0.1 µm,0.2 µm, 0.45 µm cellulose acetate membrane filter (Phenomenex), and quantitative filter.The absorbance of filtered and unfiltered (centrifuged) solutions in dissolution medium were

measured using concentration 35.6µg/mL and 8.4µg/mL for Gemifloxacin and Ambroxol respectively. To assess the stability of Gemifloxacin and Ambroxol in dissolution medium, samples were diluted in 0.01 M HCl, and tested after 24 h at room temperature and also kept at 37 ± 0.5 ºC for 2 h after dissolution. The stability of these solutions was studied by comparing values obtained with freshly prepared solutions.

METHODS

Dissolution Study Of Gemifloxacin and Ambroxol From Tablets Using Area under curve method:

The release kinetic of Gemifloxacin and Ambroxol from Tablets was studied by conducting dissolution tests. Dissolution tests performed using USP type II dissolution apparatus and 900 ml of 0.01M HCL at 37± 0.5 ^{0} C at 50 rpm. 10 ml sample were withdrawn at the intervals of 5, 10, 30, 60, 90, 120. 150,180 min. sampling was carried out and every time replaced with fresh 10 ml with 0.01M HCL. The absorbance of solution were recorded at 243.5nm and 271nm using 0.01M HCL as blank (fig 1). The dissolution studies were performed in triplicate (n=3).and result was calculated as % drug release of GEM and AMB, table 1 and (Fig 2).

Dissolution Study Of Gemifloxacin and Ambroxol From Tablets Using *Multicompoeant mode method*

Following the above procedure the absorbance of solutions were recorded at 271nm (GEM) and 249.5 nm (Isobastic Point) using 0.01M HCL as blank.The dissolution studies were performed in triplicate and result was calculated as % drug release of GEM and AMB, table 2 and (Fig 3).

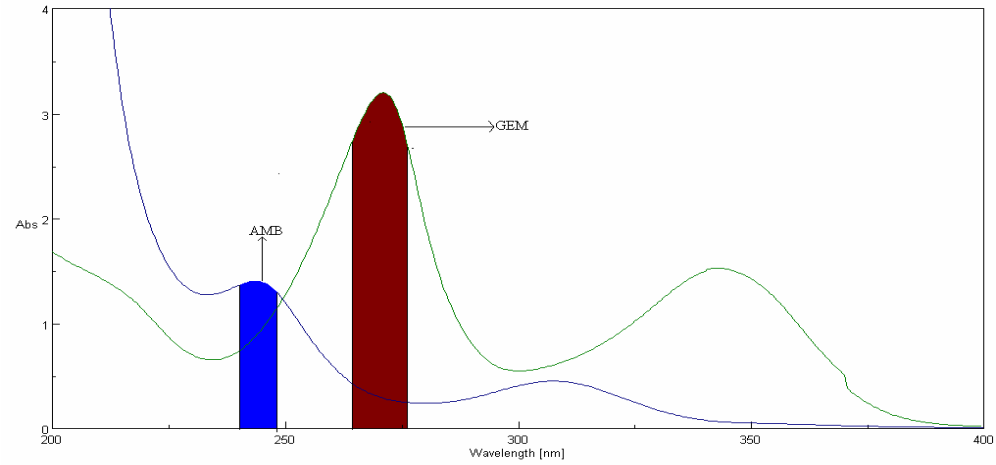


Figure 1 Overlain spectra of GEM and AMB in 0.01M HCL

Table 1 Calculation by AUC method

Sr. No	Sampling Time(Min)	Area		Percentage Released (%)	
		GEM (265-276nm)	AMB (236-249nm)	GEM	AMB
1	5	2.33	0.30	6.57	3.67
2	10	5.79	0.80	16.28	9.55
3	30	15.31	2.46	43.01	29.31
4	60	30.79	4.83	86.50	57.59
5	90	35.51	7.40	99.76	88.12
6	120	36.32	8.34	102.04	99.35
7	150	35.32	8.48	99.24	100.98
8	180	35.04	8.39	98.45	99.89

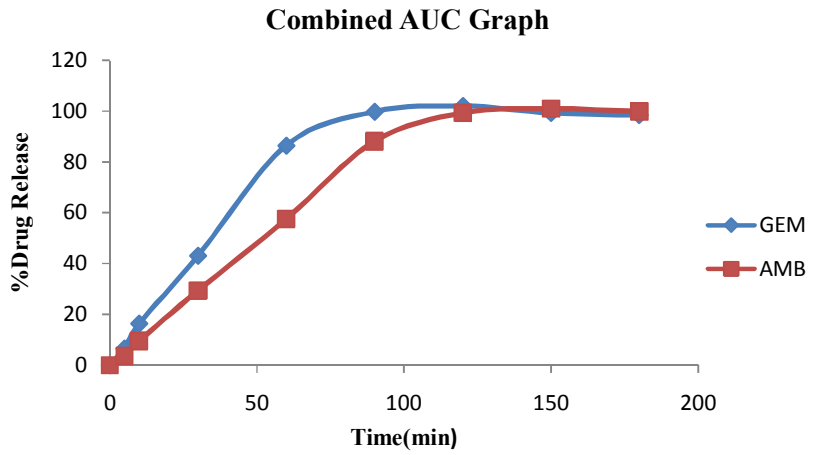


Figure 2 AUC method Graph

Table 2 Calculation by multicomponent mode method

Sr. No	Sampling Time(Min)	Absorbance		Percentage Released (%)	
		GEM (271nm)	AMB(243.5nm)	GEM	AMB
1	5	2.48	1.43	6.98	4.02
2	10	6.09	2.92	17.11	8.21
3	30	15.06	10.31	42.31	28.97
4	60	30.41	19.96	85.43	56.09
5	90	35.55	31.57	99.87	88.68
6	120	35.95	35.48	100.99	99.67
7	150	35.31	35.90	99.21	100.87
8	180	34.89	35.31	98.03	99.20

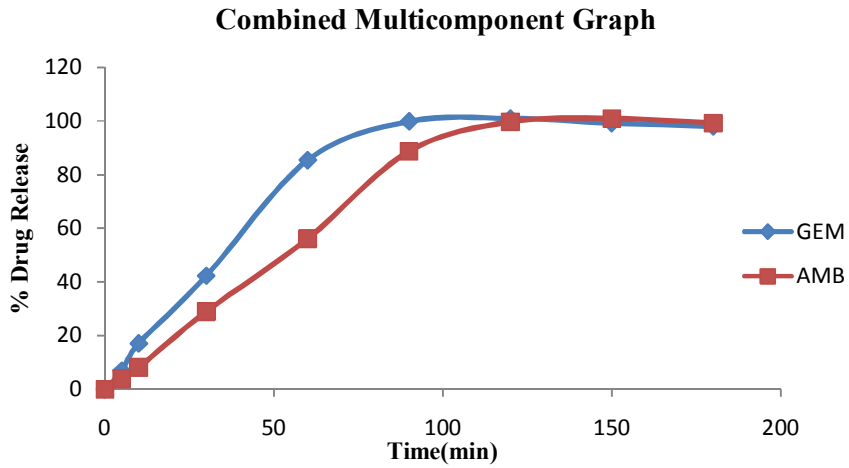


Figure 3 multicomponent mode method Graph

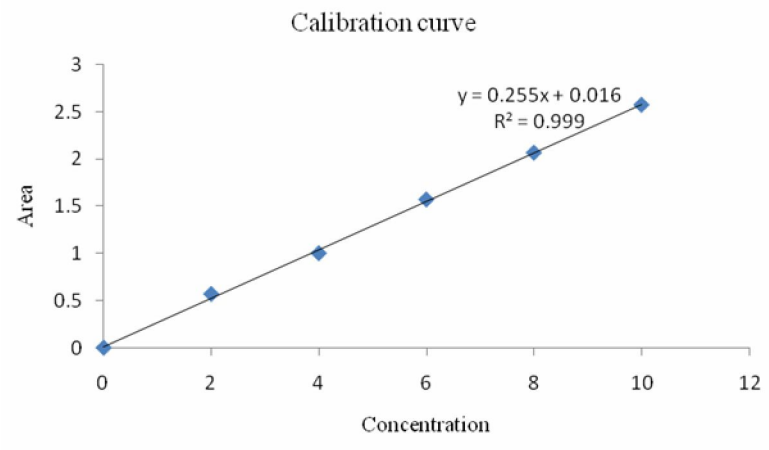


Figure 4 Calibration curve of AMB

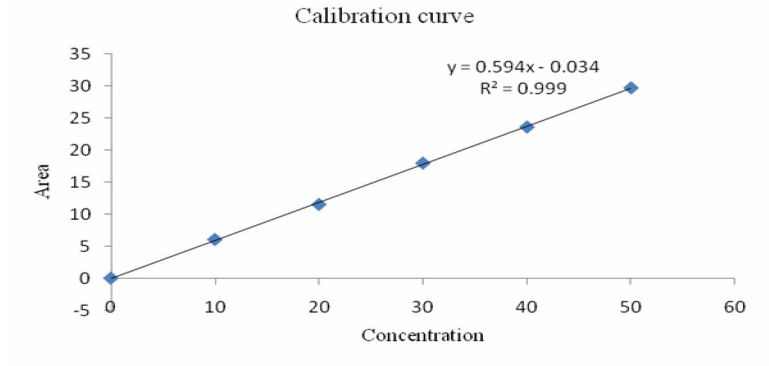


Figure 5 Calibration curve of GEM

Method validation

The UV spectrophotometric method used to analyze the Gemifloxacin and Ambroxol samples in 0.01 M HCl dissolution medium was validated for specificity, linearity, precision, ruggedness and robustness according to USP Pharmacopoeia [12] and ICH guideline [13]. All absorbance were measured at 271 nm and 243.5nm for Gemifloxacin and Ambroxol respectively

Linearity

The linearity of Gemifloxacin and Ambroxol response is evaluated from the range of 10–60 µg/mL and 2-12 µg/mL and showed a good correlation coefficient . To assess linearity, the standard curves of Gemifloxacin and Ambroxol ware constructed by plotting concentration (µg/mL) for GEM and AMB respectively.versus absorbance is shown in Figure 6. Linear regression is also calculated and the obtained equation is-

y = 0.255x + 0.016 for GEM and
y= 0.594x + 0.034 for AMB
where x is the concentration in µg/mL, y is amplitude for UV spectrophotometry. [Figure 4, 5.Linearity or calibration curve of GEM and AMB]

Precision

The precision of the method is evaluated by measuring the repeatability in two
different UV Vis spectrophotometers have shown %RSD value of 0.41 and 0.37 for GEM and AMB respectively for inter day .The%, RSD values obtained during intraday precision were 0.24and 0.22for GEM and AMB respectively. These results demonstrated the good precision of the proposed methods for dissolution test(**table 3).**

Table 3 Repeatability and intermediate precision of the dissolution method

Methods	Mean		Standard deviation		Coefficient of variation		Standard error	
Inter day								
	GEM	**AMB**	**GEM**	**AMB**	**GEM**	**AMB**	**GEM**	**AMB**
Area Under curve	99.96	100	0.016	0.0140	0.016	0.014	0.08	0.03
Multicomponent method	99.73	100	0.42	0.37	0.41	0.37	0.24	0.16
Intra day								
Area Under curve	99.96	99.98	0.24	0.22	0.24	0.22	0.109	0.10
Multicomponent method	99.73	99.93	0.31	0.082	0.31	0.082	0.12	0.03

Table 4 Results from accuracy as recovery studies

Method	Level of % Recovery	Amt.Present (mcg/tab)		Amt. of standard added (mcg/tab)		Total Amt. recovered (mcg)		% Recovery	
		GEM	**AMB**	**GEM**	**AMB**	**GEM**	**AMB**	**GEM**	**AMB**
Area Under curve	80	35.6	8.4	28.48	6.72	64.06	15.10	99.97	99.89
	100	35.6	8.4	35.6	8.4	71.21	16.81	100.02	100.08
	120	35.6	8.4	42.42	10.08	77.78	18.46	99.96	99.92
Multicomponent method	80	35.6	8.4	28.48	6.72	64.07	15.12	99.99	100.03
	100	35.6	8.4	35.6	8.4	71.22	16.79	100.04	99.98
	120	35.6	8.4	42.42	10.08	77.79	18.46	99.97	99.93

Table 5 Ruggedness results

Method	Analyst 1		Analyst 2	
	GEM	**AMB**	**GEM**	**AMB**
Area Under curve	98.98	99.69	99.73	100.32
Multicomponent method	99.97	100.08	99.86	100.08
Mean	99.58	99.58	99.92	99.82

Table 6 Robustness results

1. Revolution per minute

R.P.M	level	Time of Dissolution(Min)		% Drug Release	
		GEM	**AMB**	**GEM**	**AMB**
25	-25	150	165	99.12	99.35
50	0	90	120	99.90	99.53
75	+25	60	85	99.45	99.67

Mean ± S.D (n=3) GEM 99.49 ± 0.3915

Mean ± S.D (n=3) AMB 99.510.1604

2. Temperature changes:

Temperature	Level	Time of Dissolution(Min)		% Drug Release	
		GEM	AMB	GEM	AMB
32	-5	140	155	99.45	99.37
37	0	90	120	99.90	99.53
42	+5	100	130	99.61	99.39

Mean ± S.D (n=3) GEM 99.65 ± 0.2281
Mean ± S.D (n=3) AMB 99.43 0.0871

3. Molarity of HCL

HCL	level	Amt. of drug release (µg/ml)		%drug release	
		GEM	AMB	GEM	AMB
0.005	-5	34.12	8.09	95.84	96.30
0.01	0	35.56	8.36	99.90	99.53
0.05	+5	34.88	8.12	97.97	96.66

Mean ± S.D (n=3) GEM 97.9± 1.0308
Mean ± S.D (n=3) AMB 97.49± 1.7700

4. Filters

Filters	Amount of drug release		% Release	
	GEM	AMB	GEM	AMB
0.2 µm	35.27	8.31	99.09	99.02
0.45 µm	35.36	8.32	99.34	99.07
Whatmann	35.56	8.36	99.90	99.56

Mean ± S.D (n=3) GEM 99.44 ± 0.04147
Mean ± S.D (n=3) AMB 99.21± 0.02883

Accuracy

The accuracy is evaluated by applying proposed method to the analysis of mixture of the tablet and with known amount of the Gemifloxacin and Ambroxol working standard, corresponding to the concentrations of 80, 100 and 120%, which were subjected to dissolution test conditions described above. The accuracy was assessed from three replicate determinations of samples containing 35.6 µg/mL of GEM and 8.4 µg/mL of AMB respectively recoveries obtained with a mean value of 99.96-100.04% for GEM and 99.93-100.08% for AMB .demonstrated that the method is accurate for intended use. The percent recoveries obtained (Table 4) are considered acceptable

Ruggedness

Ruggedness of the method is determined by carrying out the analysis by two different analysts and the respective dissolution values are calculated**(** table 5).

Robustness

The evaluation of robustness should be considered during the development phase and depends upon the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in the method parameters. The parameters included pH, temperature, Revolution per minute (R.P.M.) Filters and molarity of HCL. The solution containing 35.6 mcg/ml of GEM and 8.4 mcg/ml were analyzed under different condition as above and results are represented in (table 6).

CONCLUSION

A simple dissolution test developed and validated for Gemifloxacin and Ambroxol tablets are considered satisfactory. The conditions that allowed the dissolution determination are 900 mL of 0.01 M HCl at 37.0 ± 0.5 ºC, paddle apparatus, 50 rpm stirring speed and filtration with 0.45 µ cellulose acetate membrane filters. In these conditions, the Gemifloxacin stability is good. The percent drug delivery is higher than 90% in 90 minutes for GEM and 120 minutes for AMB in evaluated products. Therefore, the proposed method is successfully applied and suggested for the quality control studies of Gemifloxacin and Ambroxol pharmaceutical dosage forms contributing to assure the therapeutic efficacy of the drug.

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