SIMULTANEOUS ESTIMATION OF GATIFLOXACIN AND AMBROXOL HYDROCHLORIDE BY UV-SPECTROPHOTOMETRY

S. Lakshmana Prabu*, S. Thiagarajan, M. Srinivasan, Queeni Marina
Dept. of Pharm. Technology, Anna University-Tiruchirappalli, Tiruchirappalli–620 024
***Email:** slaxmanvel@gmail.com

ABSTRACT

A novel, simple, sensitive, rapid spectrophotometric method has been developed for simultaneous estimation of gatifloxacin (GFC) and ambroxol hydrochloride (AMB). The method involved solving simultaneous equations based on measurement of absorbance at two wavelengths 286nm and 242 nm, λ max of GFC and AMB respectively. Beer's law was obeyed in the concentration range of 4-16 µg/ml and 10 – 50 µg/ml for GFC and AMB respectively. The method was validated for accuracy, precision and recovery studies. Statistical analysis proved the method was precise, reproducible, selective, specific, and accurate for analysis of GFC and AMB. The wide linearity range, sensitivity, accuracy, and simple procedure imply that the proposed technique demonstrated to be appropriate for routine analysis and quality control assays of tablets.

Keywords: Gatifloxacin, Ambroxol hydrochroride, simultaneous determination, spectrophotometry.

INTRODUCTION

Gatifloxacin (GFC) is chemically 1-cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-7- (3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxilic acid[1] has broader spectrum of antibacterial activity than the older fluoroquinolones and shows good activity against gram + ve and gram – ve microorganisms[2]. Ambroxol Hydrochloride (AMB) is chemically trans-4-(2-amino 3,5-dibromobenzyl)amino-cyclohexanol, as hydrochloride, is used to reduces bronchial hyper-reactivity, stimulates cellular surfactant production, increases the amount of antibiotic penetration and thus reduces daily dose of gatifloxacin and exhibits anti-inflammatory properties[3]. In dual drug therapy GFC and AMB are used for the treatment of upper respiratory tract infection for adults. Some methods can be found for the individual determination of GFC and AMB. High performance liquid chromatography[4] and LC/ESI-MS/MS[5] methods have been reported for the estimation of gatifloxacin in dosage forms and from human plasma. Methods available for the determination of ambroxol hydrochloride include capillary electrophoresis[6-8], spectrometry[9], gas chromatography[10-11] and LC with potentiometric detection[12], MS detection[13] and UV detection[14-18] methods have been reported for the estimation of ambroxol hydrochloride.

GFC and AMB are used in dual drug therapy for the treatment of upper respiratory tract infection for adults. In recent years pharmaceutical preparations containing both these drugs have been available commercially. UV detection is often preferred in ordinary laboratories because of its wide suitability and availability. The reported methods for the individual determination of the drugs cannot be easily applied for the simultaneous determination of both drugs in the formulation owing to their large differences in physicochemical properties. The present paper describes a simple and accurate UV spectroscopic method for the simultaneous determination of GFC and AMB in the tablet formulation.

MATERIALS AND METHODS

Instrument:

A Shimadzu UV/Vis double beam spectrophotometer model 1601 with spectral bandwidth of 0.1 nm, wavelength accuracy of ± 0.5 nm with automatic wavelength correction and with a pair of 3 mm quartz cells.

Chemicals:

GFC and AMB (Purity 99.89%w/w and 99.92% were procured as a gift sample from Aristo Pharma Ltd., India). Methanol AR grade (Merck India Ltd.,), distilled water were used in the present study.

METHOD VALIDATION

Linearity:

Standard stock solution of 500 µg/ml of GFC and AMB were prepared by dissolving separately in 50 ml of methanol in 100 ml volumetric flask, the volume was made up to mark with the same. Standard solutions were prepared by dilution of the stock solution with distilled water to give the final concentration range of 4-16 µg/ml and 10-50 µg/ml for GFC and AMB respectively.

Sample preparation:

Twenty tablets were weighed accurately. The average weight was determined and then ground to a fine powder. A quantity equivalent to 400 mg of GFC and 75 mg of AMB were transferred into a 100 ml volumetric flask. The contents were ultrasonicated for 15 min with 50 ml of methanol and made up to the mark with same. The resulting solution was allowed to settle for about an hour, and the supernatant was suitably diluted to give the desired concentration with distilled water.

Precision:

Intra-day and inter-day accuracy and precision of the assay samples containing (8, 10 and 12µg/ml) for GFC and (20, 30, 40µg/ml) for AMB were analyzed six times in the same day (intra-day) and for three consecutive days (inter-day).

Specificity:

The specificity of the method was assessed by analyzing standard drug, pharmaceutical product and placebo and comparing the λ max of the standard with that of the sample to determine whether the pharmaceutical product and placebo led to interfere.

Accuracy as Recovery studies:

Recovery studies were done at three different levels. The pre-analyzed sample was spiked with known concentration of the pure samples, and the mixtures were reanalyzed by the proposed method. Percentage recovery was calculated from the amount of drug found in the solution.

Robustness of the method:

Small deliberate changes in the wavelength (± 5 nm) were introduced and the effects on the results were examined.

RESULTS AND DISCUSSION

The development of simultaneous determination of drugs has received considerable attention in recent years because of their importance in the quality control of drugs and drug products. Since there are no literature reported methods of GFC and AMB simultaneously for routine analysis, our primary goal is to develop a simple UV spectroscopic method is to achieve simultaneous determination of GFC and AMB in the compound formulation under common conditions that are applicable for the routine quality control of this product in ordinary laboratories.

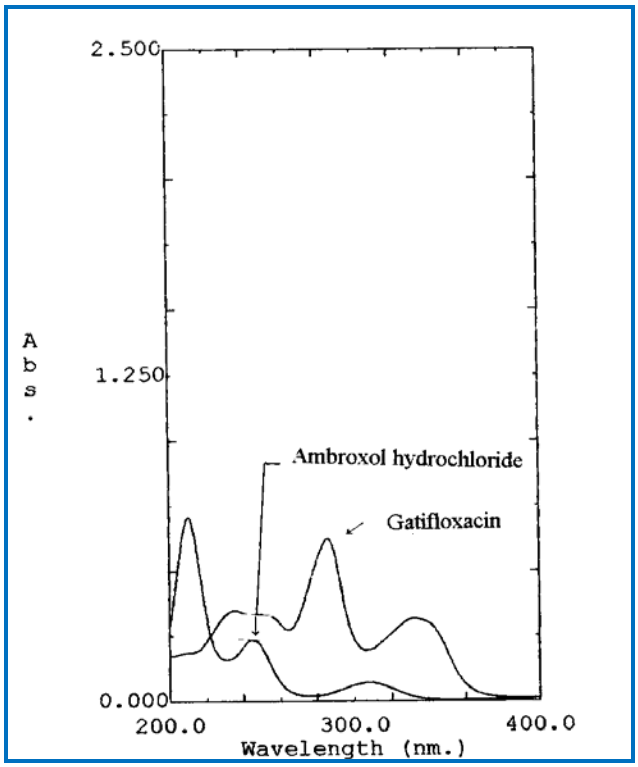
For simultaneous estimation of GFC and AMB, a series of standard solutions were prepared by diluting appropriate volumes of the standard stock solutions. The scanning of the solutions of GFC and AMB was carried out in the range of 200 to 400 nm against water as blank for obtaining the overlain spectra. The overlain UV spectra are shown in Figure 1. Absorbance and absorptivities of series of standard solutions were recorded at selected wavelengths λ_1 and λ_2. The method employed simultaneous equations using Cramer's rule and matrices ($C_1 = \lambda_2\varepsilon_2 . A\lambda_1 - \lambda_1\varepsilon_2 . A\lambda_2 / \lambda_1\varepsilon_1 . \lambda_2\varepsilon_2 - \lambda_1\varepsilon_2 . \lambda_2\varepsilon_1$ and $C_2 = \lambda_1\varepsilon_1 . A\lambda_2 - \lambda_2\varepsilon_1 . A\lambda_1 / \lambda_1\varepsilon_1 . \lambda_2\varepsilon_2 - \lambda_1\varepsilon_2 . \lambda_2\varepsilon_1$). A set of two simultaneous equations was framed using the mean of absorptivity values, as given below

$$A\lambda_1 = 236\ C_1 + 360C_2 \quad \text{--------1}$$

$$A\lambda_2 = 32\ C_1 + 670C_2 \quad \text{--------2}$$

Where, C_1 and C_2 are the concentrations of AMB and GFC respectively in simple solution (µg/ml). $A\lambda_1$ and $A\lambda_2$ are the absorbance of the sample solution measured at 242 and 286 nm, respectively.

Figure 1: Overlain spectra of Ambroxol Hydrochloride and Gatifloxacin



Linearity:

A set of six solutions of GFC and AMB at concentrations ranging from 4 to 16µg/ml and 10 to 50µg/ml were prepared. Each sample was analyzed in triplicate; calibration curve was constructed by plotting the absorbance against concentration using linear regression analysis. The correlation coefficient was found to be 0.9994 and 0.9952 for GFC and AMB respectively. The results show that an excellent correlation existed between absorbance and concentration of each drug within the concentration range tested.

Precision:

The intra-day precision of the developed method was determined by preparing the tablet samples of the same batch in six determinations with three concentrations. The RSD of the assay results, expressed as a percentage of the label claim, was used to evaluate the method precision. The obtained RSD values found to be 0.65 to 0.93% and 1.37 to 1.40 % for GFC and AMB respectively. The inter-day precision was also determined by assaying the tablets in triplicate per day for consecutive 3 days, which were found to be 0.82 to 0.98% and 0.86 to 1.44% for GFC and AMB respectively, the results are shown in Table 1. The results revealed that the good precision of the developed method.

Table 1: Intra and Inter day precision

Actual conc. (µg/ml)	Intraday Precision		Interday Precision	
	S.D.	% RSD	S.D.	% RSD
GFC				
8	0.06	0.72	0.07	0.82
10	0.08	0.93	0.1	0.98
12	0.08	0.65	0.1	0.87
AMB				
20	0.28	1.4	0.28	1.44
30	0.42	1.4	0.37	1.26
40	0.54	1.37	0.34	0.86

n=6

Specificity:

The specificity of the method was confirmed by comparing the λ max of standard with that of GFC and AMB in the marketed formulation. There is no interference from the excipients commonly present in the tablets. Hence the developed method is specific and selective.

Accuracy as Recovery studies:

The developed method was used to quantify GFC and AMB in tablet dosage; tablets of 400 mg of GFC and 75 mg of AMB label claim were analyzed and the average drug content was found to be 99.26 % and 99.32% for GFC and AMB respectively for labeled amount. It may therefore be inferred that degradation of GFC and AMB had not occurred in the formulation that were analyzed by this method. The low % RSD values indicated the suitability of this method for routine analysis of GFC and AMB in pharmaceutical dosage forms. The recovery results are shown in Table 2, the mean recoveries were found to be 98.98 ±0.23% and 99.15 ±0.11% and for GFC and AMB respectively. The obtained results suggested the accuracy of the developed method for the simultaneous determination of the two drugs in the formulation.

Robustness of the method:

The standard deviations of absorbances were calculated, the % RSD was found to be less than 2%. The low values of % RSD indicated robustness of the method.

Table 2: Recovery studies

Amount added (µg/ml)	Amount recovered (µg/ml)	Recovery (%) #
GFC		
2	1.985	99.25
4	3.955	98.88
6	5.928	98.81
AMB		
10	9.928	99.28
15	14.862	99.08
20	19.815	99.08
#n=6		

Table 3: Summary of validation parameters

Parameters	AMB	GFC
λ max	242 nm	286 nm
Beer's Law range	10-50 µg/ml	4 - 16 µg/ml
Molar Absorptivity (0.001 absorbance unit/mole. cm/dm3)	9.83×10^3	2.696×10^4
Sandell's Sensitivity (mg/cm2/0.001 absorbance unit)	0.04237	0.01529
Mean Recovery (%)	99.15± 0.11	98.98± 0.23
Precision (% RSD)		
Inter day	1.39	0.77
Intra day	1.19	0.89
Specificity	Specific	Specific
Robustness	Robust	Robust

CONCLUSION

The results of our study indicate that the proposed UV spectroscopic method is simple, rapid, precise and accurate. The developed UV spectroscopic method was found suitable for determination of GFC and AMB as bulk drug and in marketed solid dosage formulation without any interference from the excipients. Statistical analysis proves that, the method is repeatable and selective for the analysis of GFC and AMB. It can therefore be concluded that use of the method can save much time and money and it can be used in small laboratories with very high accuracy and a wide linear range.

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