



RESEARCH ARTICLE

**Spectrophotometric Method Development and Validation for Estimation of
Gatifloxacin and Prednisolone Acetate in Bulk and Eye Drop**

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ABSTRACT

UV spectrophotometric method has been developed and validated for the determination of Gatifloxacin and Prednisolone Acetate in bulk and eye drop. The λ_{max} of Gatifloxacin and Prednisolone Acetate were found to be 292 nm and 242nm, respectively, in methanol. Beer's law was obeyed in the conc. range of 0.6-3 μ g/ml and 2-10 μ g/ml with correlation coefficient of 0.999 and 0.999 for Gatifloxacin and Prednisolone Acetate, respectively. The result of analysis has been validated as per the ICH guidelines. The developed method is simple, selective and reproducible and can be used for routine analysis of formulations containing Gatifloxacin and Prednisolone Acetate.

KEYWORDS

UV Spectrophotometric method, Gatifloxacin, Prednisolone acetate

INTRODUCTION

The bactericidal action of Gatifloxacin results from inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV. DNA gyrase is an essential enzyme that is involved in the replication, transcription and repair of bacterial DNA. Topoisomerase IV is an enzyme known to play a key role in the partitioning of the chromosomal DNA during bacterial cell division. Prednisolone Acetate can inhibit leukocyte infiltration at the site of inflammation, interfere with mediators of inflammatory response, and suppress humoral immune responses. This combination of drugs will be used to treat optical infections.

The literature is enriched with several methods for determination of GATI and PRED in pharmaceutical dosage forms either as a single drug or in combination with some other drugs.

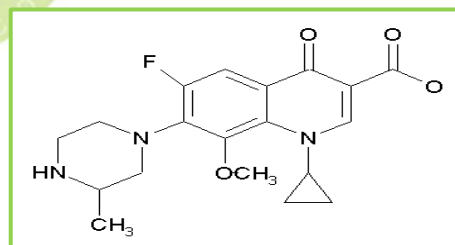


Figure 1. Chemical Structure of Gatifloxacin

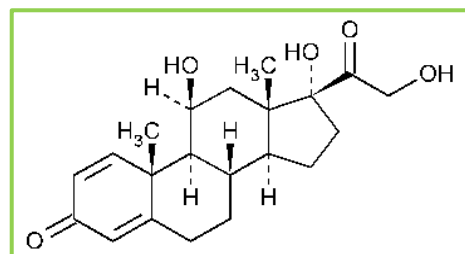


Figure 2. Chemical Structure of Prednisolone
Acetate

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The most extensively used technique for estimation of GATI and PRED are by UV, HPLC methods.

The aim of study is Development and Validation of Spectrophotometric method for Simultaneous Estimation of Gatifloxacin and Prednisolone Acetate in their combined Dosage form. The present study was designed to develop a simple, precise, and rapid analytical Second Derivative Spectrophotometric procedure, which can be used for the analysis of assay method for simultaneous estimation of Gatifloxacin and Prednisolone Acetate.

MATERIAL & METHODS

Instrument Used

A double beam UV spectrophotometer (Shimadzu, UV-VIS1800) was used with 10mm matched quartz cell.

Reagents and Materials Used

Standard samples of Gatifloxacin and Prednisolone acetate were generous gifts from Aditi Pharmaceuticals (Solapur, Maharashtra). Marketed formulation Gatiquin-P Eye Drops (CIPLA Ltd.), containing Gatifloxacin-0.3% and Prednisolone acetate IP-1% procured from local medical shop. Solvent methanol used was of pure analytical grade.

Solvent Selection

Methanol is used as a solvent because both drugs are soluble in methanol.

Spectrophotometric Conditions

The absorbance were measured at 292 nm for GATI and 242 nm for PRED. These absorbances vs concentration of standards were plotted to obtain the calibration curves from the concentration of the corresponding drugs were determined. Both the drugs obeyed Beer's law.

Preparation of Standard Stock Solution of GATI (30g/ml) and PRED (100 µg/ml):-

3mg of standard GATI and 10mg of standard PRED were weighed and transferred to a 10ml volumetric flask separately and then dissolved in methanol. The volume was made up to the

mark with same solvent to obtain conc. of 300µg/ml of GATI and 1000µg/ml. From the resulting solutions 1ml was diluted to 10ml with same solvent to obtain conc. of 30µg/ml of GATI and 100µg/ml of PRED. From the resulting solution 1ml was diluted to 10ml with same solvent to obtain conc. of 3µg/ml of GATI and 10µg/ml of PRED.

Preparation of Combined Working Standard Solution containing GATI and PRED in Ratio of 0.3:1

3mg of GATI and 10mg of PRED were weighed and transferred to a 10ml volumetric flask then dissolved in methanol. The volume was made up to the mark with same solvent to obtain conc. of 300µg/ml of GATI and 1000µg/ml. From the resulting solutions 1ml was diluted to 10ml with same solvent to obtain conc. of 30µg/ml of GATI and 100µg/ml of PRED. From the resulting solution 1ml was diluted to 10ml with same solvent to obtain conc. of 3µg/ml of GATI and 10µg/ml of PRED. This solution was diluted further to get the concentrations in range of 0.6, 1.2, 1.8, 2.4, 3 µg/ml Gatifloxacin and 2, 4, 6, 8, 10 µg/ml Prednisolone Acetate.

Method Validation

Linearity and Range

From the std. stock of GATI (3µg/ml) and PRED (10µg/ml) solution, solutions of 0.6, 1.2, 1.8, 2.4, 3µg/ml and 2, 4, 6, 8, 10µg/ml concentrations, respectively were prepared in a series of 10ml volumetric flasks. The correlation coefficient (R^2) of GATI and PRED was calculated from the calibration graph. The range of analytical method was decided from the interval between upper and lower level of calibration curve by plotting the curve.

Accuracy

Recovery study was carried out by standard addition method by adding the known amount of GATI and PRED (working standard) to the pre-analyzed sample at three different conc. levels that is 80%, 100%, 120% of assay conc. & percent recovery were calculated.

Precision

Repeatability

The precision of instrument was checked by repeated scanning and measurement of absorbance of solutions (n=6) for GATI and PRED (0.6µg/ml and 2µg/ml) without changing the parameter of the proposed spectrophotometry method.

Intraday and Interday Precision

Intraday and interday precision for method were measured in term of % RSD. The experiment was repeated three times in a day for intraday and on three different days for interday precision by taking 0.6µg/ml concentrations of GATI and 2µg/ml PRED.

Limit of Detection

Detection limit was determined based on calibration curve method. Solutions of GATI and PRED were prepared in the range of 0.6-3µg/ml and 2-10 µg/ml and LOD calculated by

$$\text{LOD} = 3(\text{SD}/\text{S})$$

where, SD- standard deviation; S-slope of curve

Limit of Quantification

Quantification limit was determined based on calibration curve method. Solutions of GATI and PRED were prepared in the range of 0.6-3µg/ml and 2-10 µg/ml and LOD calculated by

$$\text{LOQ} = 10(\text{SD}/\text{S})$$

where, SD=standard deviation; S=slope of curve

Analysis of Mixture

1 ml of GATIQUIN-P eye drops was taken in 10 ml volumetric flask and diluted up to 10 ml with methanol. From this stock solution, working standard solution of 3µg/ml GATI and 10µg/ml PRED was prepared by taking 1ml and diluted up to 10 ml with methanol. The responses of the sample solution were measured at 292 nm and 242 nm for quantification of GATI and PRED, respectively. The amounts of GATI and PRED present in sample solution were calculated by fitting the responses into

regression equation for GATI and PRED in proposed method.

RESULTS AND DISCUSSION

Validation

Linearity and Range

The linearity of measurement was evaluated by analyzing standard solutions of GATI and PRED in the range of 0.6-3µg/ml and 2-10µg/ml respectively for both drugs and calibration plot was constructed.

Table 1: Statistical Parameters for Gatifloxacin and Prednisolone Acetate

Statistical Parameters	Gatifloxacin	Prednisolone Acetate
Average Abs*	0.073	0.161
	0.129	0.324
	0.19	0.461
	0.246	0.611
	0.306	0.771
Concentration Range (µg/ml)	0.6-3	2-10
Straight Line Equation	$y = 0.097x + 0.013$	$y = 0.075x + 0.013$
Correlation Coefficient (R ²)	0.999	0.999
LOD (µg/ml)	0.01388	0.02782
LOQ (µg/ml)	0.04208	0.08432

Precision

The Relative Standard Deviation (%RSD) after six determinations was 0.53% at 0.6µg/ml for GATI and 0.43% at 2µg/ml for PRED (see Table 2).

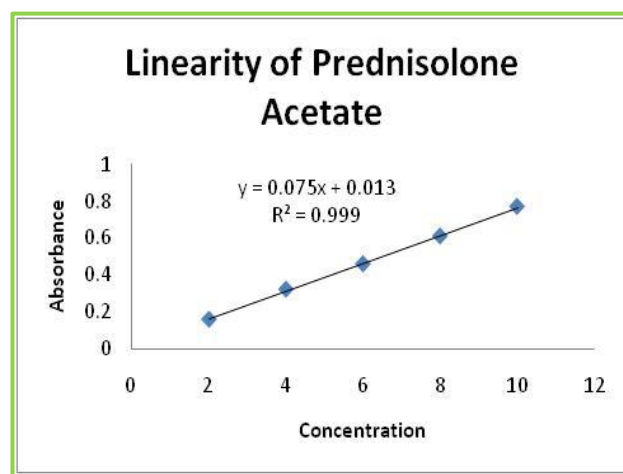
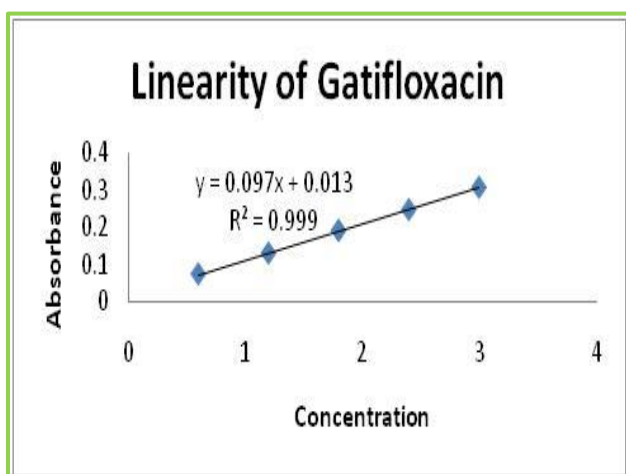
Table 2: Precision Data for Gatifloxacin and Prednisolone Acetate

Precision	Concentration* ($\mu\text{g/ml}$)		%RSD	
	Gatifloxacin	Prednisolone acetate	GATI	PRED
Repeatability	0.6	2	0.531	0.438
Intraday	0.6	2	0.532	0.439
Interday	0.6	2	0.531	0.439

*n=6

Table 3: Recovery Data for Gatifloxacin and Prednisolone Acetate

Drug	Level of % recovery	Conc. Taken ($\mu\text{g/ml}$)	Conc. Added ($\mu\text{g/ml}$)	Total Conc. Found ($\mu\text{g/ml}$)	Amount Recovered ($\mu\text{g/ml}$)	Mean Recovery (%)
Gatifloxacin	0	0.6	0	4.690	---	---
	80	0.6	0.48	5.169	0.479	99.79
	100	0.6	0.6	5.296	0.605	100.83
	120	0.6	0.72	5.417	0.726	100.97
Prednisolone acetate	0	2	0	13.52	---	---
	80	2	1.6	15.1	1.59	99.73
	100	2	2	15.52	2	100
	120	2	2.4	15.93	2.41	100.41



Precision

The Relative Standard Deviation (%RSD) after six determinations was 0.53% at 0.6µg/ml for GATI and 0.43% at 2µg/ml for PRED (see Table 2).

Accuracy

Accuracy of the method was calculated by recovery studies at three levels by standard addition method. The mean percentage recoveries obtained for GATI and PRED were 100.53% and 100.04%, respectively. (see Table 3)

Analysis of Market Formulation (see Table 4)

CONCLUSION

The proposed UV spectrophotometry method was used for the estimation of Gatifloxacin and Prednisolone Acetate in bulk and dosage form was found to be sensitive, accurate, precise, simple, and rapid. Hence the present method may be used for routine analysis of the raw materials as well as combined dosage formulations containing Gatifloxacin and Prednisolone Acetate.

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