AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Simultaneous Equation and Second Order Analytical Method Development and Validation of for the Simultaneous Estimation of Gatifloxacin and Loteprednol in Bulk and Dosage Form

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ABSTRACT

To develop and validate a simple, accurate & precise Spectrophotometry methods for simultaneous estimation of Gatifloxacin (Gati) and Loteprednol (Lote) in their combined pharmaceutical dosage form. Two simple, accurate, precise U.V Spectroscopy methods have been developed. First method was based on Simultaneous equation method. Here 286 nm was selected for the estimation of Gatifloxacin and 220 nm was selected for the estimation of Loteprednol. Second method was developed was second order derivative method, here Gatifloxacin was measured at 265.60nm ZCP of Loteprednol and 228.84 was selected for Loteprednol at ZCP of Gatifloxacin. Gatifloxacin and Loteprednol showed linearity in the range of 3-15µg/ml and 5- 25µg/ml in Simultaneous Equation method as well for second order derivative method. Correlation coefficient for Simultaneous estimation method is 0.9936 & 0.9968 whereas for Second order derivative it is 0.9986 & 0.9977 Both methods were validated by validation parameters and it show result where lie within its acceptance criteria as per ICH Q2 (R1) guideline. Hence, it can be successfully used for the routine analysis of Gatifloxacin and Loteprednol in their combined pharmaceutical dosage forms.

Keyword: Gatifloxacin, Loteprednol, 0.1N NaoH and Validation parameter.

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Received 09 November 2014, Accepted 17 November 2014

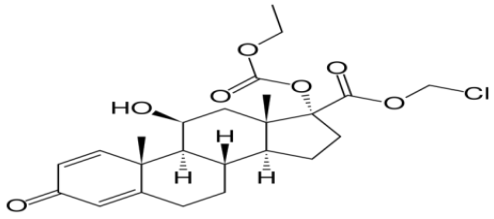
Please cite this article as: Raval K *et al.*, Simultaneous Equation and Second Order Analytical Method Development and Validation of for the Simultaneous Estimation of Gatifloxacin and Loteprednol in Bulk and Dosage Form. American Journal of PharmTech Research 2014.

INTRODUCTION

ZYLOPRED name which contain Loteprdnolang Gatifloxacin. It contains a sterile, topical anti-inflammatory corticosteroid for ophthalmic use. Loteprednol is a white to off-white powder.Loteprednol as the ester Loteprednoletabonate. Its chemical name ischloromethyl 17α-[(ethoxycarbonyl)oxy]-11β-hydroxy-3-oxoandrosta-1,4-diene-17β-carboxylate. Its Molecular formula &Molecular weight are $C_{21}H_{27}ClO_5$&394.889 respectively.

The structural formula is:

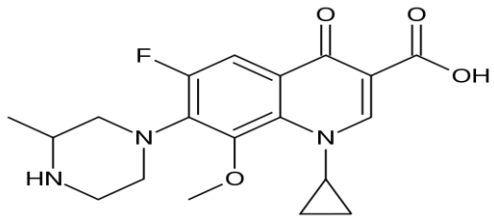
Structure of Loteprednol



Loteprednol etabonate is structurally similar to other glucocorticoids. However, the number 20 position ketone group is absent. It is highly lipid soluble which enhances its penetration into cells. Loteprednol etabonate is synthesized through structural modifications of prednisolone- related compounds so that it will undergo a predictable transformation to an inactive metabolite. Gatifloxacin is an antibiotic of the fourth-generation fluoroquinolone family that like other members of that family inhibits the bacterial enzymes DNA gyrase and topoisomerase IV. Bristol-Myers Squibb introduced Gatifloxacin in 1999 under the proprietary name Tequin® for the treatment of respiratory tract infections, having licensed the medication from Kyorin Pharmaceutical Company of Japan. Its Chemical name is (±)-1-cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid sesquihydrate. Its Molecular formula & Molecular weight are $C_{19}H_{22}FN_3O_4$& 402.42respectively

The structural formula is:

Structure of Gatifloxacin



Gatifloxacin is a synthetic broad-spectrum 8-methoxyfluoroquinolone antibacterial agent for oral or intravenous administration. is bactericidal and its mode of action depends on blocking of bacterial DNA replication by binding itself to an enzyme called DNA gyrase, which allows the

untwisting required to replicate one DNA double helix into two. Notably the drug has 100 times higher affinity for bacterial DNA gyrase than for mammalian. Gatifloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria.[1-3]. Up to now there are some methods developed on these two drugs but there is no method developed for this combination like Dual wavelength method etc.[4-18] after methods were developed, both methods were validated as per ICH guideline.

MARTIALS AND METHOD

Instrumentation, Reagents and Material

Jasco UV-1800 UV spectrophotometer, Gatifloxacin, Loteprednol, 0.1N NaoH

Preparation of standard solution

Preparation of standard stock solution of Gatifloxacin

Accurately weighed quantity of Gatifloxacin 100 mg was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with 0.1N NaoH. This will give a stock solution having strength of 1000 µg/ml.

Preparation of working standard solution of Gatifloxacin

100 µg/ml of Gatifloxacin solution was prepared by diluting 10 ml of stock solution to 100 ml with 0.1N NaoH

Preparation of standard stock solution of Loteprednol

Accurately weighed quantity of Loteprednol 100 mg was transferred into 100 ml volumetric flask, dissolved and diluted up to mark with 0.1N NaoH. This will give a stock solution having strength of 1000 µg/ml.

Preparation of working standard solution of Loteprednol

100 µg/ml of Loteprednol solution was prepared by diluting 10 ml of stock solution to 100 ml with 0.1N NaoH.

Simultaneous Equation Method

Determination of wavelength for measurement

0.3 ml of working standard solution of Gatifloxacin (100 µg/ml) and 0.5 ml of working standard of Loteprednol (100 µg/ml) was diluted to 10 ml with 0.1N NaoH to get 3 µg/ml of Gatifloxacin and 5 µg/ml of Loteprednol. Each solution was scanned between 200-400 nm. Here 286 nm was selected for the estimation of Gatifloxacin and 220 nm was selected for the estimation of Loteprednol. Which shown in figure 1.

Preparation of Calibration Curve

Calibration curve for Gatifloxacin(3-15µg/ml)

Calibration curve for Gatifloxacin consisted of different concentrations of standard Gatifloxacin solution ranging from 3-15µg/ml. The solutions were prepared by pipetting out 0.3, 0.6, 0.9, 1.2, and 1.5 ml of the working standard solution of Gatifloxacin (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with 0.1N NaoH then absorbance measured at 286nm and The straight-line equation was determined by plotting graph Concentration VS absorbance. Its data was recorded in table no. 1. And figure no. 2 & 3.

Calibration curve for Loteprednol(5-25 µg/ml)

Calibration curve for Loteprednol consisted of different concentrations of standard Loteprednol solution ranging from 5-25 µg/ml. The solutions were prepared by pipetting 0.5. 1, 1.5, 2 and 2.5 ml of the working standard solution of Loteprednol (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with 0.1N NaoH then absorbance measured at 220 nm and The straight-line equation was determined by plotting graph Concentration VS absorbance. Its data was recorded in table no. 1. And figure no. 2 & 4.

Validation of proposed method

Linearity

The linearity response was determined by analyzing independent levels of concentrations in the range of 3-15 and 5-25 µg/ml for Gatifloxacin and Loteprednol respectively six times. Absorbance of each solution was measured at 286 nm λmaxGatifloxacin& 220 nm λmaxLoteprednol. Linearity of 6 concentrations was measured six times.

Precision

Repeatability

6 replicates of 6 µg/ml concentrations of Gatifloxacin and 10 µg/ml of Loteprednol were prepared and absorbance was measured at 286 nm and 220 nm respectively. SD and RSD were calculated and recorded in table 1.

Intraday Precision

Standard solutions containing 3, 6 and 9 µg/ml Gatifloxacin and 5, 10 and 15 µg/ml Loteprednol were analyzed 3 times on the same day. The absorbance of solutions was measured at 286 nm and 220 nm respectively. SD and RSD were calculated and recorded in table 1.

Interday Precision

Standard solutions containing 3, 6 and 9 µg/ml Gatifloxacin and 5, 10 and 15 µg/ml µg/ml Loteprednol were analyzed 3 times on the three different days. The absorbance of solutions was measured at 286 nm and 220 nm respectively. SD and RSD were calculated and recorded in table 1

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. Recovery studies were carried out by addition of standard drug to the pre analysed sample at 3 different concentration levels (80, 100 and 120 %) taking into consideration percentage purity of added bulk drug samples. It was determined by calculating the recovery of Gatifloxacin and Loteprednol Sodium by standard addition method.

Preparation of sample solution for % recovery

An accurately weighed powder equivalent to about 100mg Gatifloxacin was transferred to 100 ml volumetric flask and the volume was made up to the mark using 0.1N NaoH as solvent and aliquate them to make final concentration 3 µg/ml Gatifloxacin and 5 µg/ml Loteprednol . The resulting solution was filtered through Whatman filter paper. Absorbance of sample solutions was measured at selected wavelength of Gatifloxacine and Loteprednol and concentration is calculated which is known as pre-analyzed sample. In pre-analyzed sample 80, 100 and 120 % of Gatifloxacin and Loteprednol was spiked. Absorbance of spiked samples was measured and total amount of drug was calculated and from which % recovery was calculated.

Limit of Detection (LOD) & Limit of Quantification (LOQ)

The LOD & LOQ are estimated from the set of 6 calibration curves used to determine method linearity.

LOD = 3.3 x (SD / Slope)

LOQ = 10 x (SD / Slope)

Where, SD = the standard deviation of Y- intercept of 6 calibration curves.

Slope = the mean slope of the 6 calibration curves.

Its data recorded in table no 1.

Analysis of marketed formulation

An accurately weighed eye drop equivalent to about 10 mg of Gatifloxacin was transferred to 100 ml volumetric flask and the volume was made up to the mark using 0.1N NaoH as solvent. The solution was sonicated for 20minutes. The solution was filtered through whatman Filter Paper No.42. First 0.3 ml of filtrate was discarded and was diluted to 10 ml with 0.1N NaoH. Resulting solution contain 3µg/mlGatifloxacin and 5 µg/ml Loteprednol. The absorbance of the resulting solution was measured at 286 nm for Gatifloxacin and 220 nm for Loteprednol. The concentration of each drug was calculated using simultaneous equation table no.1.

Development of Second Order Derivative Method

Determination of wavelength for measurement

1.5 ml of working standard solution of Gatifloxacin (100 µg/ml) and 2.5 ml of working standard of Loteprednol (100 µg/ml) was diluted to 10 ml with 0.1N NaoH to get 15µg/ml of Gatifloxacin and 25µg/ml of Loteprednol. Each solution was scanned between 200-400 nm. Then spectra were converted to 2nd order by keeping Delta Lemda 2 and Scaling factor 230. It will show that Gatifloxain is show ZCP at 228.84 and Loteprednol show ZCP at 265.60 nm so Gatifoxacin was measured at 265.60 (ZCP of Loteprednol) and Loteprednol was measured at 228.84nm (ZCP of Gatifloxacin) which is shown in figure no. 5

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Calibration curve for Loteprednol(5-25 µg/ml)

Calibration curve for Loteprednol consisted of different concentrations of standard Loteprednol solution ranging from 5-25 µg/ml. The solutions were prepared by pipetting 0.5. 1, 1.5, 2 and 2.5 ml of the working standard solution of Loteprednol (100 µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with 0.1N NaoH then absorbance was measured 265.60nm (ZCP of Gatifloxacin) The straight-line equation was determined by plotting graph Concentration VS absorbance. Its data was recorded in table no. 1. And figure no. 6 & 8.

Validation of proposed method

Linearity

The linearity response was determined by analyzing independent levels of concentrations in the range of 3-15 and 5-25 µg/ml for Gatifloxacin and Loteprednol respectively six times. Absorbance of each solution was measured at 228.84nm for Gatifloxacin& 265.60 for Loteprednol. Linearity of 6 concentrations was measured six times.

Precision

Repeatability

6 replicates of 3 µg/ml concentrations of Gatifloxacin and 5µg/ml of Loteprednol were prepared and absorbance was measured at 265.60 nm and 228.84 nm respectively. SD and RSD were calculated and recorded in table 1

Intraday Precision

Standard solutions containing 3, 6 and 9 µg/ml Gatifloxacin and 5, 10 and 15 µg/ml Loteprednol were analysed 3 times on the same day. The absorbance was measured at 265.60 nm and 228.84 nm respectively. SD and RSD were calculated and recorded in table 1

Interday Precision

Standard solutions containing 3, 6 and 9 µg/ml Gatifloxacin and 5, 10 and 15 µg/ml µg/ml Loteprednol were analyzed 3 times on the three different days. The absorbance was measured at 265.60 nm and 228.84 nm respectively. SD and RSD were calculated and recorded in table 1

Accuracy

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RESULTS AND DISCUSSION

Simultaneous Equation Method

3 µg/ml of Gatifloxacin and 5 µg/ml of Loteprednol. Each solution was scanned between 200-400 nm. Here 286 nm was selected for the estimation of Gatifloxacin and 220 nm was selected for the estimation of Loteprednol.

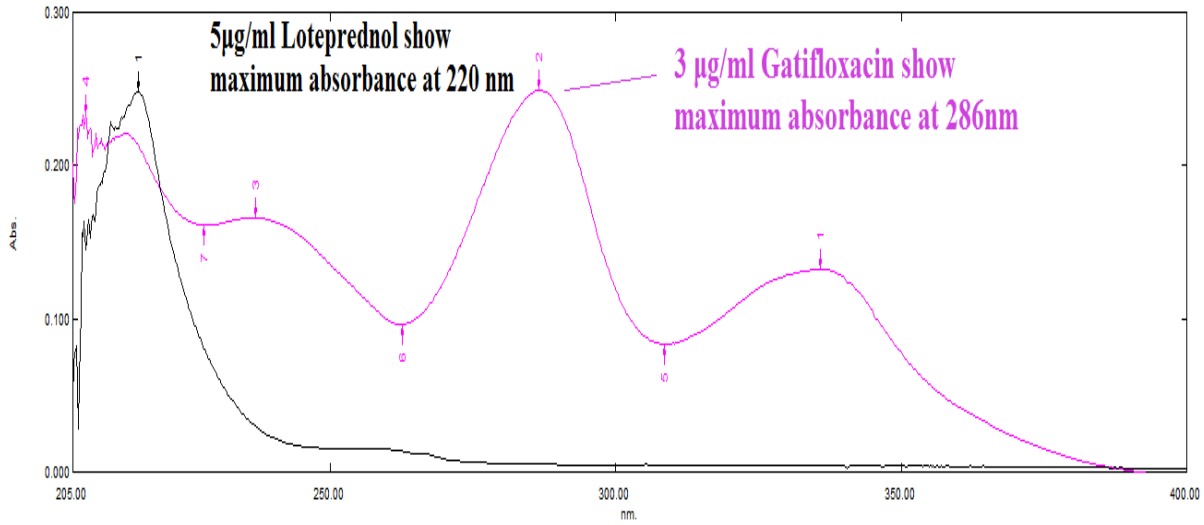

Figure 1: Determination of wavelength for measurement for Gatifloxacin&Loteprednol

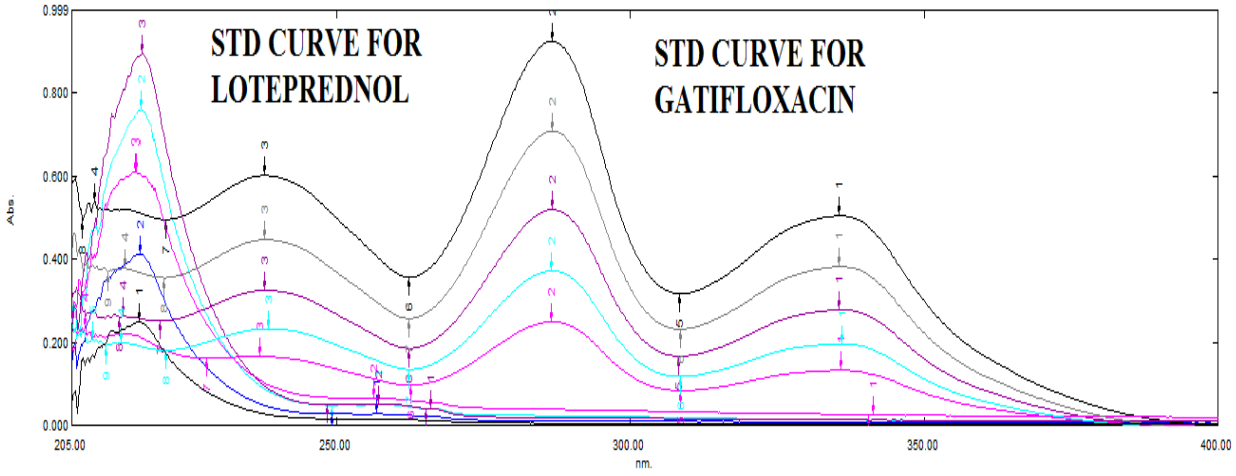

Figure 2: Standard curve Spectra of Gatifloxacin and Loteprednol

STD curve for both Loteprdnol and Gatifloxacin

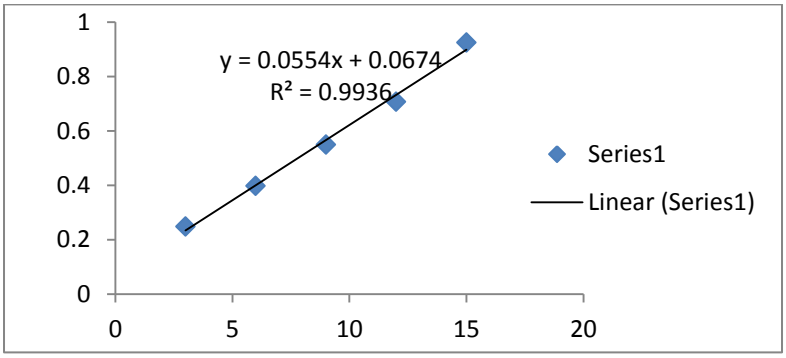

Figure 3: Standard curve Spectra of Gatifloxacin at 286nm

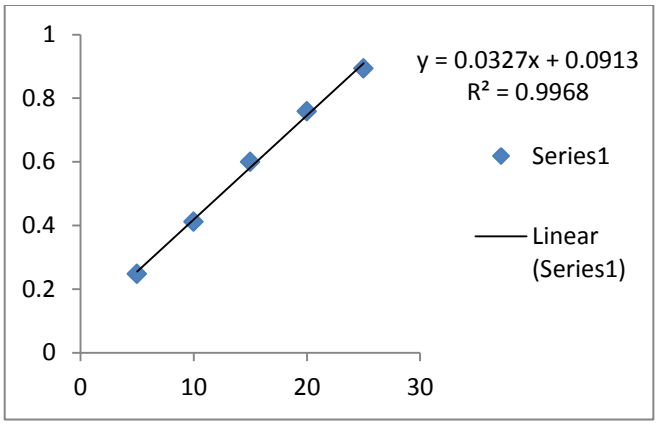

Figure 4: STD cure for Loteprdnol at 220 nm

Second Derivative Method

15µg/ml of Gatifloxacin and 25µg/ml of Loteprednol. Each solution was scanned between 200-400 nm. Then spectra were converted to 2nd order by keeping Delta Lemda 2 and Scaling factor 230. It will show that Gatifloxain is show ZCP at 228.84 and Loteprednol show ZCP at 265.60 nm so Gatifoxacin was measured at 265.60 (ZCP of Loteprednol) and Loteprednol was measured at 228.84nm (ZCP of Gatifloxacin).

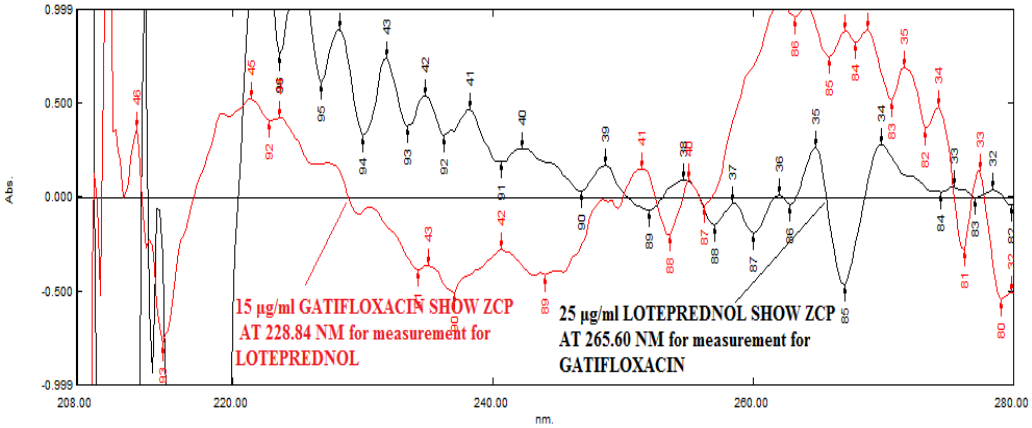

Figure 5: Second order UV spectra of Gatifloxacin and Loteprednolselection of wavelength

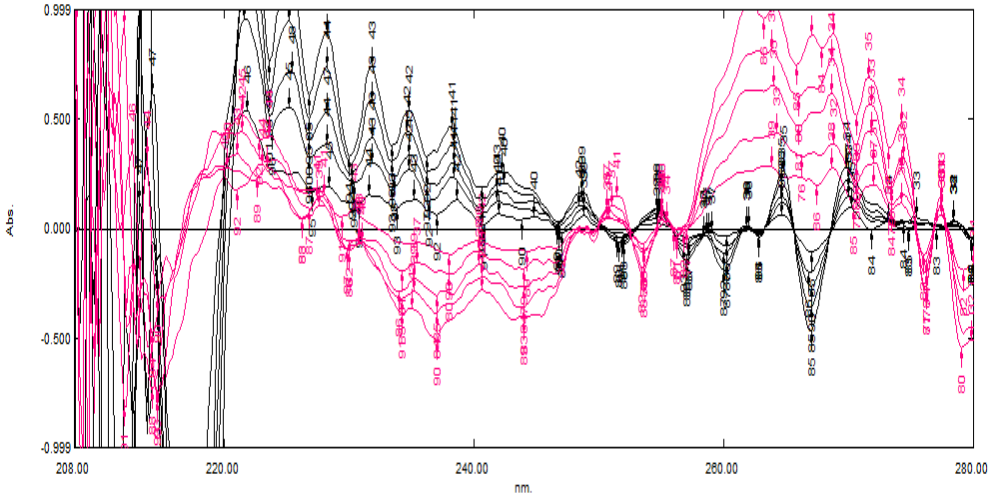

Figure 6: STD CURVE 2nd order for Gatifloxacin&Loteprednol

STD curve for both Loteprdnol and Gatifloxacin

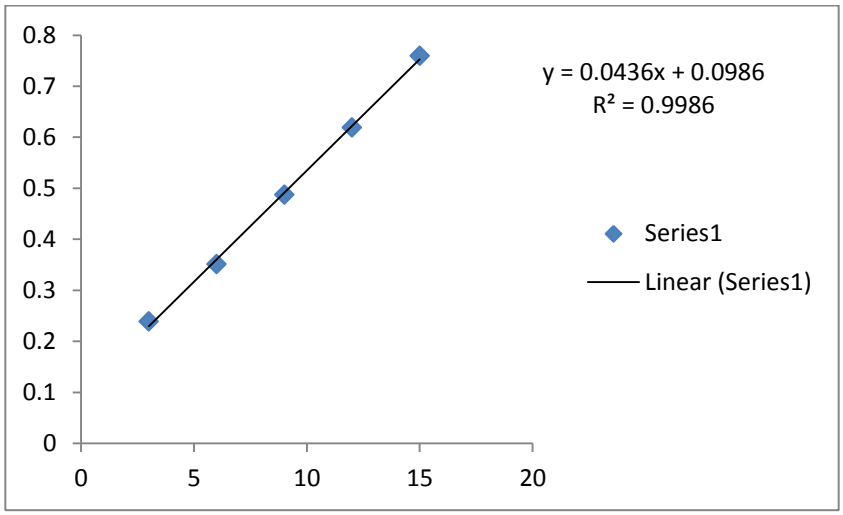

Figure 7: Calibration curve of Gatifloxacin at 265.60nm

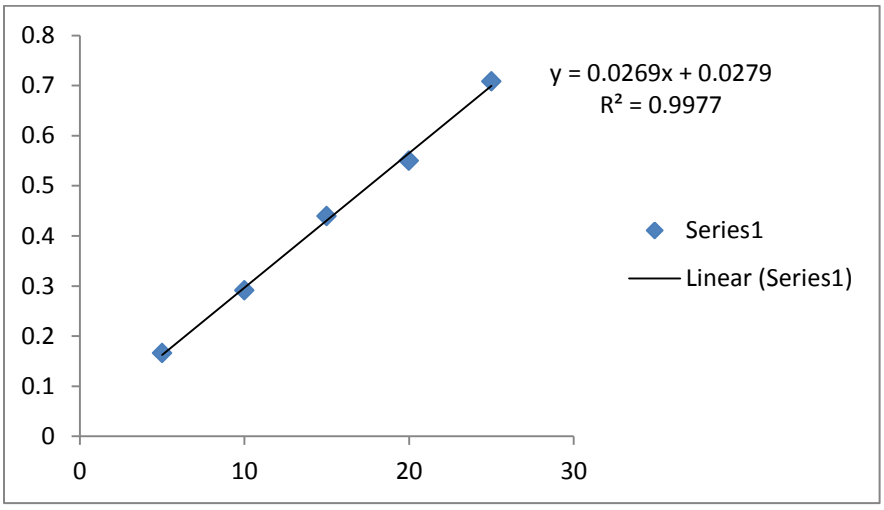

Figure 8: Calibration curve of Loteprednol at 228.84nm

Summary

All the data for Simultaneous equation method and Second order derivative methods are recorded in table 1

Table1: Summary for Simultaneous estimation and Second order methods

Methods	**Simultaneous Equation**		**Second order deravative**	
Parameter	**Gati**	**Lote**	**Gati**	**Lote**
Wavelength (nm)	286 nm	220 nm	265.60 nm (ZCP of Lote)	228.84 nm (ZCP of Gati)
Beer's law limit (µg/ml)	3-15 µg/ml	5-25 µg/ml	3-15 µg/ml	5-25 µg/ml
STD CURVE				
Regression equation	y = 0.0554x + 0.0674	y = 0.0327x + 0.0913	y = 0.0436x + 0.0986	y = 0.0269x + 0.0279
r^2	0.9936	0.9968	0.9986	0.9977
Slope (m)	0.05555	0.03425	0.0436	0.0268
Intercept (c)	0.001463	0.007402	0.002181	0.0018540
PRECISION	INTRADAY	INTRADAY	INTRADAY	INTRADAY
	0.248±0.0005	0.247±0.00115	0.238±0.00152	0.166±0.00124
	0.397±0.0010	0.409±0.00057	0.352±0.001732	0.290±0.00163
	0.551±0.0017	0.599±0.00057	0.488±0.002309	0.440±0.00124
	INTERDAY	INTERDAY	INTERDAY	INTERDAY
	0.245±0.0047	0.245±0.00458	0.235±0.003055	0.163±0.00294
	0.391±0.0065	0.406±0.00416	0.347±0.003512	0.286±0.00286
	0.542±0.0091	0.595±0.00557	0.484±0.004583	0.432±0.00571
Repeatability	6 µg/ml	10 µg/ml	3	5µg/ml
	0.396±0.00273	0.411±0.00325	µg/ml0.239±0.00194	0.167±0.00151
LOD	0.0867	0.7132	0.165	0.2282
LOQ	0.2628	2.16	0.5002	0.6917
Marketed formulation % Found	99.33%	101%	99%	102%

CONCLUSION

Two simple, accurate, precise, reproducible and economical UV Spectophotometric methods have been developed and validated for the estimation of Gatifloxacin and Loteprednolin pharmaceutical dosage form. All method validation parameters lie within its acceptance criteria as per ICH Q2 (R1) guideline so we can conclude that methods are specific, linear, accurate and precise. Hence, it can be successfully used for the routine analysis of Gatifloxacin and Loteprednolin pharmaceutical dosage forms.

ACKNOWLEDGEMENT

The author wishes to thanks mates who helped me lot for my work. And how can I forget

U. Srinivas, my guide who suggested me in all way.

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