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Estimation of gatifloxacin in pharmaceutical formulation by area under curve spectrophotometric method

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Abstract
Objective: The aim of present work was to develop an accurate, simple and cost effective UV spectrophotometric method for estimation of gatifloxacin.
Method: This method was based on area under curve of UV spectrum between 316 – 346 nm and validation as per ICH guideline Q2 (R1). The method has followed linearity in the range of 5-25 µg /ml. The value of correlation coefficient was 0.999.Satiafactory value of percent relative standard deviation for the intraday and inter-day precision indicates that method was precise.
Result: Result of the recovery studies (102%) shows accuracy of method. The method developed method can be used for routine estimation of gatifloxacin in bulk and dosage form.
Conclusion: The developed method can be concluded as accurate, sensitive and precise and can be easily applied to the pharmaceutical formulation.

Keywords: Gatifloxacin, AUC (area under curve), validation, ICH guideline Q2 (R1)

Introduction [1-4]

Gatifloxacin or 1-cyclopropyl-6-fluoro-1, 4-dihydro-8-methoxy-7-[3-methyl-1-piperazinyl]-4-oxo-3-quinolinecarboxylic acid (figure 1), is a synthetic broad-spectrum antimicrobial fluoroquinolone active against both Gramnegative and Gram-positive organisms and is used in the treatment of a wide range of infections. The distinct difference between gatifloxacin and other fluoroquinolones is the methoxy group at position. It is believed that this group mediates the binding of the DNA DNA gyrase complex to the DNA-topoisomerase complex and potentially decreases the likelihood of high-level resistance The most commonly reported adverse reactions include hypoglycemia, constipation, and feeling of enlarged abdomen, abdominal discomfort, nausea, abdominal pain, stomatitis, eczema, rash, pruritus, dermatitis and malaise. Literature survey reveals no AUC methods have been reported for the estimation of gatifloxacin in pure and tablet dosage forms.

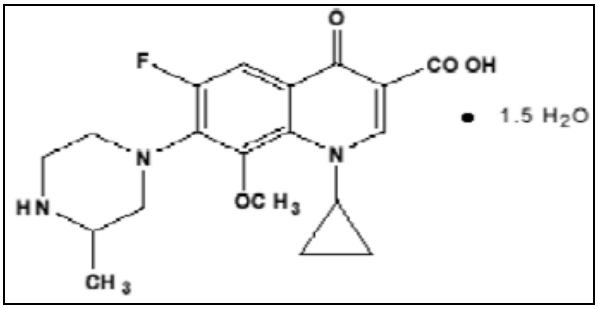

Fig 1: The chemical structure of gatifloxacin

Materials and Methods

Materials

Gatifloxacin (API) was obtained as gift sample from Barrett Hodgson Pakistan Ltd , India. A tablet formulation containing 200 mg of gatifloxacin (Glax) was purchased from local market, (shri ram medical, junner, pune). Manufacturing by Bosch Pharmaceutical Pvt Ltd Pakistan. methanol use as solvent purchase from S.D fine chemicals.

Instruments used

A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu) connected to computer loaded with spectra manager software UV Probe 2.21 with 10 mm quartz cells was used. The spectra were obtained with the instrumental parameters as follows: wavelength range: 200-400 nm; scan speed: medium; sampling interval: 1.0 nm; band width (Δλ):10.0 nm; spectral slit width: 1 nm. All weights were taken on electronic balance (Model Shimadzu AUX 120).

Preparation of standard solution
Accurately weighed 10mg of gatifloxacin was transferred into 100ml volumetric flask and dissolve in water and diluted up to the mark with water to get a stock solution containing of 100 µg /ml.

Selection of wavelength range
The standard solution of 10 µg /ml was scanned between 400nm to 200nm in UV spectrophotometer against water as a blank after baseline correction. The wavelength range was selected around wavelength maxima (332 nm). The wavelength range tried and final range selected between 316-346 nm was selected on the basis of linear relationship between area and corresponding concentration.

Area under curve [5]
The AUC (area under curve) method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ1 and λ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The above mentioned spectrums were used to calculate AUC. Thus, the calibration curve can be constructed by plotting concentration Versus AUC.

Preparation of calibration curve
Working solution were prepared from standard stock solution in methanol by further dilution with water to obtain the concentration of 5,10,15,20,25 µg /ml respectively. These solution were scanned from 400to 200nm and area under curve was integrated in the range of 316-346 nm. The calibration curve was plotted between areas under curve against concentration.

Assay of Tablet
Twenty tablets (gatifloxacin 200mg equivalent to 10mg) containing 10 mg of gatifloxacin weighed, average weight calculated and triturated to fine powder and then weight equivalent 10 mg of gatifloxacin transferred into 100ml of volumetric flask and dissolve in methanol and diluted up to the mark with water to get a solution containing of 100 µg /ml from the 2.5ml was transferred to 25ml volumetric flask and diluted up to the mark with water to get gatifloxacin solution containing 10 µg /ml of gatifloxacin.

Validation [6-9]
The developed method was validated as per ICH guidelines.

Linearity
The linearity was determine by using working standard solution between 5-25 µg/ml. The spectrum of these solution were recorded and area under curve was integrated in wavelength range 316-346 nm. Calibration curve of area under curve vs. concentration was plotted after suitable calculation and simple linear regression was performed. (see Table no.6 and Fig no.3)

Precision
The precision of the method was checked by repeatedly injecting (10 µg/ml). Area under curve of each of these solution was measured in the range of 316-346 nm. Percent relative standard deviation (RSD) was calculated. (see table no.7, 8, and 9).

Accuracy
The accuracy for the analytical procedure was determinedat 80% 100% and 120% level of standard solution. Area under curve was measured in the range 316-346 nmand result were expressed in term % recoveries. Three determination at each level were performed and %RSD was calculated. The result were tabulated in Table see 10, 11, 12.

Result & discussion
The calibration curve of gatifloxacin was performed and graph plotted concentration vs. area under curve. Show in fig.no.2

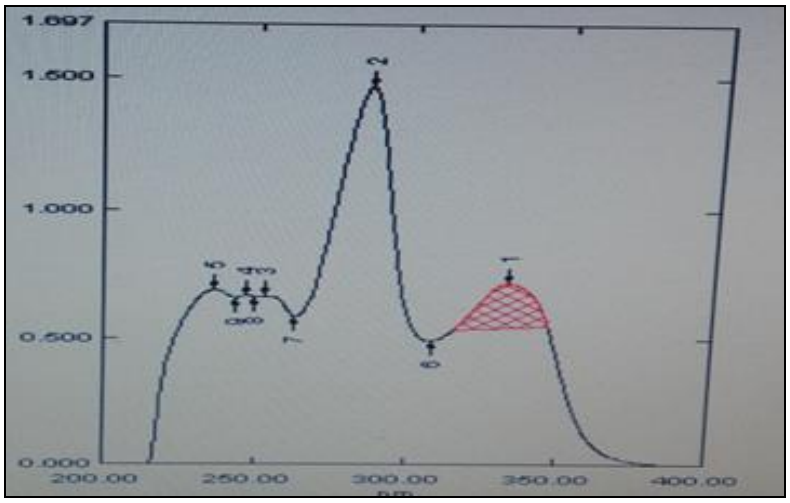

Fig 1: Spectrum of gatifloxacin for AUC (10 µg/ml)

Table 1: Calibration data of gatifloxacin for area under curve

Concentration (µg/ml)	AUC
5	1.420
10	3.023
15	4.34
20	5.78
25	7.34

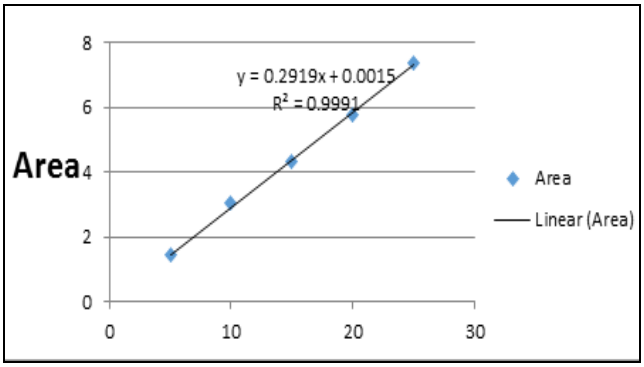

Fig 2: Calibration curve for gatifloxacin for AUC

Analysis of pure drug

The standard solution of 10 µg /ml was scanned between 400nm to 200nm in UV spectrophotometer against water as a blank after baseline correction. The area under curve of solution was measured as shown in table no.2.

Table 2: Analysis of pure drug.

Sr. No.	Amount taken (µg/ml)	AUC	Amount of drug found (µg/ml)	% amount Found
1	10	3.023	10.38	103.8
2	10	3.123	10.72	107.2
3	10	3.056	10.10	101.0
4	10	3.013	10.35	103.5
5	10	3.5120	10.08	100.8

Statistical evaluation of pure drug

Table 3: Statistical evaluation of pure drug

%Mean*	±S.D.*	%RSD*
102	0.0202	0.019214

*average of five determinations

Analysis of marketed formulation

Twenty tablets (gatifloxacin200mg equivalent to 10mg) containing 10 mg of gatifloxacin weighed, average weight calculated and triturated to fine powder and then weight equivalent 10 mg of gatifloxacin transferred into 100ml of volumetric flask and dissolve in water and diluted up to the mark with water to get a solution containing of 100 µg /ml from the 2.5ml was transferred to 25ml volumetric flask and diluted up to the mark with water to get gatifloxacin solution containing 10 µg /ml of gatifloxacin and result shown in table no.4.

Table 4: Analysis of marketed formulation.

Sr. No.	Amount taken (µg/ml)	AUC	Amount of drug found (µg/ml)	% amount Found
1	10	3.034	10.42	104.1
2	10	3.123	10.72	100.1
3	10	3.034	10.42	104.2
4	10	3.0132	10.35	103
5	10	3.001	10.30	103.0

Statistical evaluation of markrted formulation

Table 5: Statistical evaluation of marketed formulation.

%Mean*	±S.D.*	%RSD*
102.88	0.02038	0.01019

*average of five determinations

Linearity

Table 6: Calibration data of gatifloxacin for area under curve

Concentration (µg/ml)	AUC
5	1.420
10	3.023
15	4.34
20	5.78
25	7.34

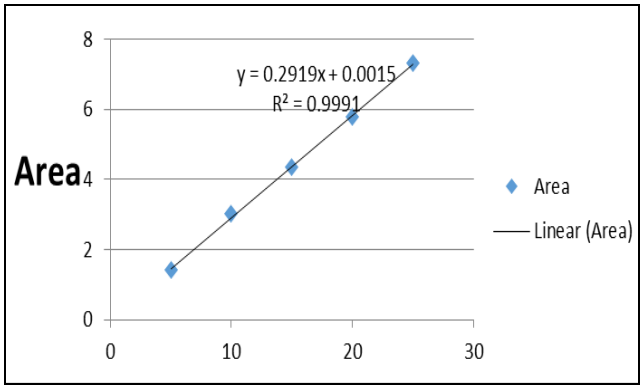

Fig 3: Calibration curve for gatifloxacin for AUC

Precision

Table 7: Analysis of inter-day precision

Sr.no	Amount taken (µg/ml)	Amount of drug found (µg/ml)	% Amount found
1	10	10.0700	100.70
2	10	9.93545	99.35
3	10	10.254	102.54
4	10	10.09	100.9
5	10	9.943	99.43
6	10	9.945	99.45

Table 8: Analysis of intra-day precision

Sr.no	Amount taken (µg/ml)	Amount of drug found (µg/ml)	% Amount found
1	10	10.07	100.70
2	10	9.890	98.90
3	10	10.09	100.9
4	10	9.934	99.34
5	10	10.020	100.20
6	10	10.04	100.4

Table 9: Statistical evaluation of inter-day and intra-day precision studies

Parameter	% Mean*	± S.D. *	%RSD *
Interday	100.30	1.0526	1.0504
Intraday	99.89	0.4980	0.5002

*Average of six determinations

Accuracy

Table 10: Results of recovery studies

Level of recovery (%)	Amount taken (µg/ml)	Amount of std. added (µg/ml)	AUC	Total amount recovered (µg/ml)	% Recovery
80	10	8	5.5509	17.93	100.89
80	10	8	5.5509	17.93	100.89
80	10	8	5.5418	17.71	98.64
100	10	10	5.2219	19.94	100.90
100	10	10	5.2311	19.95	100.97
100	10	10	6.2263	19.93	100.81
120	10	12	6.7477	21.63	98.12
120	10	12	6.7866	21.89	100.28
120	10	12	6.7349	21.78	99.36

Table 11: Statistical evaluation of recovery studies.

Level recovery	%mean	±S.D.*	%RSD*
80	100.14	1.0022	0.011
100	100.89	0.05	0.555
120	99.25	0.75	0.6352

Summary data of validation parameter

Table 12: summery data of validation parameter.

Sr.no.	Parameter	Auc method
1.	Linearity range	5-25ppm
2.	Regression equation	Y= 0.291x+0.001
3.	Correlation co-efficient	R2=0.999
4.	Precision	
	Intra day	1.0504%rsd
	Inter day	0.5002%rsd
5.	% recovery	0.6352-0.011%rsd

Discussion

An attempt was made to develop a simple and specific AUC spectrophotometric method for the determination of gatifloxacin in tablet dosage form. The generated regression equation was y=0.291x+0.001, (R^2=0.999). The area under curve between 316-346 nm, x is concentration and R is correlation coefficient. The R^2 value as 0.999 as indicates that developed method was linear. The proposed method was found to be precise %RSD value for inter day and intraday precision were satisfactory. The drug at each of the good recoveries (99.25%). Hence, it can be said that this method was accurate. The result of the analysis of pharmaceutical formulation by the developed method was consistent with the label claim, highly reproducible and reliable. The method can be used for routine analysis of the gatifloxacin. The validation parameter are summarized in tableno.12.

Conclusion

No any spectrophotometric methods have been described for AUC estimation of gatifloxacin. Therefore, simple, fast and reliable area under curve spectrophotometric method was developed for the routine analysis of gatifloxacin. The developed method can be concluded as accurate, sensitive and precise and can be easily applied to the pharmaceutical formulation.

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