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RESEARCH ARTICLE

Development and Validation of Difference Spectrometric Method for the Estimation of Garenoxacin mesylate in Bulk and Pharmaceutical Formulation

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ABSTRACT

A simple, specific and rapid difference spectroscopic method has been developed for the estimation of Garenoxacin mesylate in bulk and Pharmaceutical formulation. The proposed method was carried out by measuring the difference absorbance of Garenoxacin mesylate in two different conditions containing three different forms of drug generated by neutral (solvent), acidic (solvent) and basic (solvent) medium. The measurements of difference absorbance were carried out at 347nm and 348nm for two different conditions. The calibration curves were linear in the concentration range of 10-50µg/ml. The proposed method was validated as per ICH validation guideline Q_2 (R_1) for accuracy, robustness, LOD, LOQ etc. The method was found to be accurate, precise, robust and sensitive hence it can be applied in routine analysis of Garenoxacin mesylate in bulk and Pharmaceutical formulation in its quality control.

KEYWORDS

Difference Spectrometry, Garenoxacin Mesylate, 0.1N HCl, 0.1N NaOH, Distilled Water

INTRODUCTION

Garenoxacin mesylate is a synthetic, des-F(6)-quinolone antibacterial agent[1]. Chemically it is 1-Cyclopropyl-8-(difluoromethoxy)-7-[(1R)-1-methyl-2,3-dihydro-1H-isoindol-5-yl]-4-oxo1,4-dihydroquinoline-3-carboxylic acid mono methanesulfonate[1]. Garenoxacin (GAR) is an oxazolidinonespiro compound used as a drug in treatment of certain respiratory tract infections (RTIs), Urinary tract infection (UTI), otorhinolaryngological infections and Penicillin- and fluoroquinolone-resistant Streptococcus pneumonia[2,3]. It was approved by USFDA in Feb 2006[4]. The Extensive review revealed that few analytical methods like, RP-HPLC have been reported for estimation of garenoxacin mesylate in dosage form and biological fluid[5,6,7,8].

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But no any simple difference spectrophotometric method was available for GAR estimation in bulk and Pharmaceutical dosage forms. So it was thought of interest to develop and validate a rapid, cost effective and precise difference spectrophotometric method for the determination of GAR in bulk and Pharmaceutical formulation.

MATERIAL AND METHODS

Garenoxacin mesylate (GRA) was procured from Glenmark Pharma Pvt. Ltd., Bharuch, Gujarat. The Garenoxacin mesylate tablet (200mg) was procured from the market.

Preparation of Standard Stock Solution

Accurately weighed (25 mg) GRA was transferred to a 25 ml volumetric flask, dissolved in and diluted up to the mark with methanol to obtain a standard stock solution (1000µg/ml). An aliquot (1ml) was transferred to 10 ml volumetric

flask and diluted up to the mark with methanol to obtain the working standard solution (100µg/ml).

Preparation of Hydrochloric Acid (0.1N)

Accurately measured 0.85 ml concentrated hydrochloric acid (36%) was transferred to 100 ml volumetric flask and diluted up to the mark with distilled water.

Preparation of Sodium Hydroxide (0.1N)

Accurately weighed (0.4 gm) sodium hydroxide was transferred in to 100ml volumetric flask, dissolved in about 60 ml distilled water and diluted up to the mark with distilled water.

Preparation of Sample Solution

Twenty tablets (Zinox® Glenmark Pharma, India) were weighed accurately and powdered. Powder equivalent to 100 mg of Garenoxacin mesylate was transferred to 100 ml volumetric flask and then Methanol (50 ml) was added and sonicated for 15 minutes and diluted up to 100 ml with Methanol (1000µg/ml). The solution was filtered through Whatman filter paper No. 41. Aliquot (2.5 ml) was diluted to 25 ml with Methanol (100µg/ml). An aliquot (2.5 ml) was taken in triplicate 10 ml volumetric flasks, and volumes were made up to the mark with Water, 0.1N HCl and 0.1N NaOH respectively to prepare test solution containing (25µg/ml). The above solutions were scanned in the UV range of 200 nm to 400 nm to obtain difference spectra at the below mentioned conditions.

Selection of Wavelength for Determination

- Wavelength was determined for two conditions

Condition 1: HCl (Reference) - NaOH (Sample)

Different aliquots of appropriate volume were taken in a series of 10ml volumetric flasks from the working standard solution and diluted up to the mark with 0.1N NaOH and 0.1N HCl separately to prepare the concentration range of 10-50µg/ml. The solutions were scanned in the UV range of 200 nm to 400 nm to obtain difference spectra by keeping the acidic form (i.e. Garenoxacin mesylate in 0.1N HCl) in Reference cell and Basic form (i.e. Garenoxacin mesylate in 0.1N NaOH) in Sample cell using 0.1N HCl (in Reference cell) and 0.1N NaOH (in Sample cell) as blank. The maximum difference absorbance was observed at 347 nm which was selected for analysis.

Condition 2: HCl (Reference) - Water (Sample)

Different aliquots of appropriate volume were taken in a series of 10ml volumetric flasks from the working standard solution and diluted up to the mark with 0.1N HCl and Distilled water separately to prepare the concentration range of 10- 50µg/ml. The solutions were scanned in the UV range of 200 nm to 400 nm to obtain difference spectra by keeping the acidic form (i.e. Garenoxacin mesylate in 0.1N HCl) in Reference cell and neutral form (i.e. Garenoxacin mesylate in Distilled water) in reference cell using 0.1N HCl (in Reference cell) and water (in sample cell) as blank. The maximum difference absorbance was observed at 348 nm which was selected for analysis.

RESULTS AND DISCUSSION

The calibration standards of Garenoxacin mesylate were prepared in concentration range of 10-50µg/ml and analyzed spectrophotometrically to generate calibration curve of difference absorbance vs. concentration. A regression coefficient was obtained above 0.992 in both the condition.

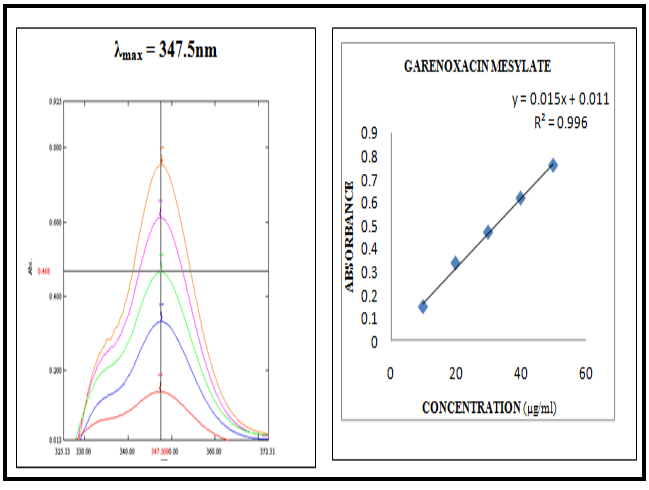

Figure 1: Overlaid UV- spectra of GRA in the Range of 10-50µg/ml in 0.1N HCl (Reference) and 0.1N NaOH (Sample)

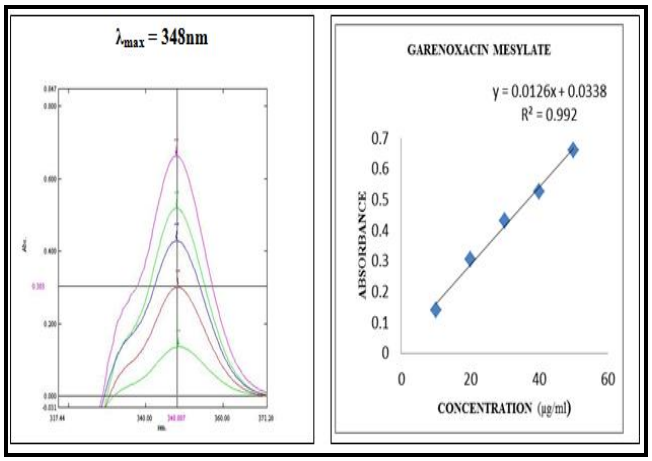

Figure: 2 Overlaid UV- spectra of GRA in the Range of 10-50µg/ml in 0.1N HCl (Reference) and Water (Sample)

Method Validation

Accuracy (% Recovery)

The accuracy study was carried out by spiking the standard solution of GRA to the pre-analysed test solution at three different concentration levels (50, 100,150) and % recovery was calculated. The percentage recoveries of GRA were found in the range of 96.24- 104.2 % and 97.68- 102.3% for above two mention conditions respectively (Table 1).

Precision

The % RSD for repeatability, intra-day and inter-day precision were found to be less than 2% (Table 2, 3 and 4).

Table 2: Results of Repeatability (n=6)

Sample No.	Difference Absorbance 0.1N HCl (Reference) and 0.1N NaOH(Sample) (20µg/ml)	Difference Absorbance 0.1N HCl (Reference) and Water(Sample) (20µg/ml)
1	0.333	0.305
2	0.332	0.307
3	0.330	0.301
4	0.332	0.303
5	0.331	0.307
6	0.330	0.305
Mean	0.331	0.304
SD	0.0012	0.0023
%RSD	0.365	0.767

Table 1: Results of Recovery Study (n=3)

Level of Recovery	Amount of Test Solution (µg/ml)	Amount of Standard Solution Added (µg/ml)	Total Concentration (µg/ml)	% Recovery 0.1N HCl (Reference) and 0.1N NaOH (Sample)	% Recovery 0.1N HCl (Reference) and Water (Sample)
0 %	15	0	15	103.30 ± 0.40	102.74 ± 1.96
50 %	15	7.5	22.5	96.24 ± 0.39	97.68 ± 1.95
100 %	15	15	30	104.28 ± 0.19	102.35± 0.98
150 %	15	30	45	99.28 ± 0.26	100.69 ± 0.99

Table 3: Results of Intra- day and Inter- day precision (n=3) for 0.1N HCl (Reference) and 0.1N NaOH (Sample)

GRA (µg/ml)	Intra- Day Precision (n=3)		Inter- Day Precision (n=3)	
	Mean ± SD	%RSD	Mean ± SD	%RSD
10	0.142 ± 0.0015	1.070	0.143 ± 0.002	1.768
30	0.463 ± 0.0020	0.448	0.462± 0.003	0.647
40	0.614 ± 0.0010	0.162	0.612 ± 0.003	0.617

Table 4: Results of Intra- day and Inter- day precision (n=3) for 0.1N HCl (Reference) and Water (Sample)

GRA (µg/ml)	Intra- Day Precision (n=3)		Inter- Day Precision (n=3)	
	Mean ± SD	%RSD	Mean ± SD	%RSD
10	0.141 ± 0.001	0.709	0.140 ± 0.0015	0.523
30	0.432 ± 0.001	0.231	0.431 ± 0.0026	0.613
40	0.525 ± 0.0015	0.290	0.523 ± 0.0035	0.671

LOD and LOQ

The values of LOD for condition-1 and condition-2 were found to be 0.32 and 0.46µg/ml respectively as well as LOQ was 1.09 and 1.55µg/ml respectively.

Robustness

The method was found to be robust as the results were not significantly affected by slight variation in wavelength (±2 nm) (Table 5).

Table: 5 Results of Robustness study (Wavelength change by ±2 nm)

Conditions	λmax (nm)	Concentration (µg/ml)	Difference Absorbance	%Recovered	Mean	S.D	%RSD
HCl reference / NaOH sample	345	25	0.406	104.6	104.8	0.23	0.220
	347		0.408	105.0			
	349		0.408	105.0			
HCl reference / water sample	346	25	0.362	104.1	104.2	0.23	0.221
	348		0.362	104.1			
	350		0.362	104.5			

Analysis of Tablet Dosage Form

The proposed UV spectrophotometric method was successfully applied for the determination of GRA in tablet dosage form. The percentage of GRA using condition-1 and condition-2 were 104.7% and 103.6% respectively (Table 6).

Table 6: Assay of GRA in Tablet Dosage Form (n=3)

Conditions	Concentration (µg/ml)	Difference Absorbance	%Recovered	Mean	S.D	%RSD
HCl reference /NaOH sample	25	0.408	105.0	104.7	0.3785	0.3615
		0.407	104.9			
		0.405	104.3			
HCl reference / water sample	25	0.360	103.5	103.6	0.4582	0.4422
		0.362	104.1			
		0.359	103.2			

Table 7: Summary of Validation Parameters

Sr. No.	Parameter	HCl (Ref.)- NaOH(Sample)	HCl (Ref.)- Water (Sample)
1	Linearity (µg/ml)	10- 50	10- 50
2	Regression coefficient (r^2)	0.996	0.992
3	Assay (%)	104.7	103.6
4	Accuracy (%)	96.24- 104.2	97.68- 102.3
5	Repeatability (%RSD)	1.55	1.34
6	Intra-day precision (%RSD)	0.16- 1.07	0.29- 0.70
7	Inter-day precision (%RSD)	0.61- 1.76	0.52- 0.67
8	Robustness (%RSD)	0.220	0.221
9	LOD (µg/ml)	0.32	0.46
10	LOQ (µg/ml)	1.09	1.55

CONCLUSION

A Difference spectrophotometric method has been developed and validated for the determination of GRA in tablet dosage form. The proposed method was found to be simple, accurate, precise, repeatable and robust. Hence, it can be used successfully for the routine analysis of GRA in bulk and Pharmaceutical formulation in its quality control.

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REFERENCES

1. http://ema.europa.eu/docs/en/document/wc500067888.pdf. Assessed on: Jul 26th 2014
2. http://nhs.uk/conditions/respiraory-tract-infections/pages/Introduction.aspx Assessed on: Nov 11th 2014
3. kidney.niddk.nih.gov/.../utiadult/Urinary_Tract_Infections_Adults_508APR14th2015.
4. http://taisho.co.jp/en/company/release/2007/2007100401-e.html Assessed on: Aug 01th2014
5. Cavazos-Rocha, N., Vera-Cabrera, L., Welsh-Lozano, O., Waksman-de-Torres, N., & de la Luz Salazar-Cavazos, M. (2007). Simultaneous determination and validation of antimicrobials in plasma and tissue of actinomycetoma by high-performance liquid chromatography with diode array and fluorescence detection. *Journal of pharmaceutical and biomedical analysis*, *43*(5), 1775-1781.
6. González, J. O., Mochón, M. C., & de la Rosa, F. B. (2005). Simultaneous determination of cefepime and the quinolones garenoxacin, moxifloxacin and levofloxacin in human urine by HPLC-UV. *Microchimica Acta*, *151*(1-2), 39-45.
7. Supriya G. N., Kandisa R. V., and RaviKumar, B. V. V. (2012). High performance liquid chromatographic method for determination of garenoxacin mesylate in pharmaceutical dosage form. *Journal of Biotechnol Biomater*, *2*(6), 194.
8. Unnisa, A., Ali, S. S., & Siva Chaithanya, K. (2014). Method development and validation for the assay of garnoxacin in pharmaceutical dosage forms by RP-HPLC. 3(10), 1767-1779.
9. *International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use*, Validation of analytical procedures: Methodology, ICH- Q_2 (R_1), Geneva, 1- 13.

