ISSN 2231–5667 (Print)
2231–5675 (Online)
DOI: 10.5958/2231-5675.2018.00015.7

Vol. 08| Issue-02|
April- June 2018

Available online at
www.anvpublication.org
www.asianpharmaonline.org

Asian Journal of Pharmaceutical Analysis
Home page *www. ajpaonline.com*

RESEARCH ARTICLE

RP-HPTLC Method for Determination of Garenoxacin mesylate in Bulk and in Tablet Formulation

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ABSTRACT:
Garenoxacin mesylate is used as an antifungal agent. A new, rapid, simple, economical and environmental friendly Reversed- Phase High-Performance Thin-Layer Chromatography (RP-HPTLC) has been developed and validated for quantitative determination of Garenoxacin mesylate in bulk and in tablet formulation. RP-HPTLC separation was performed on aluminum plates precoated with silica gel 60 RP-18 $F_{254}S$ as the stationary phase using *n*-butanol: methanol: triethylamine (60:20:20 % *v*/*v*/*v*) as mobile phase. Quantification was done by densitometric analysis at 257 nm over the concentration range of 100 - 600 ng/band. The method was found to give compact and well resolved band for Garenoxacin mesylate at retention factor (R*f*) 0.62 ± 0.02. The linear regression analysis data for calibration graph showed good linear relationship with $r^2 = 0.988$. The method was validated for precision, recovery, robustness, ruggedness and sensitivity as per International conference on Harmonization (ICH) guidelines. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were found to be 6.63 ng and 20.11 ng, respectively. The proposed developed RP-HPTLC method can be applied for identification and quantitative determination of Garenoxacin mesylate in bulk and in tablet formulation.

KEYWORDS: Garenoxacin mesylate, HPTLC, Validation.

1. INTRODUCTION:
Garenoxacin mesylate (GRN) is 1-Cyclopropyl-8-(difluoromethoxy)-7-(1-methylisoindolin-5-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid mono methane sulfonate [1]. The chemical structure of Garenoxacin mesylate is shown in **Fig. 1**.

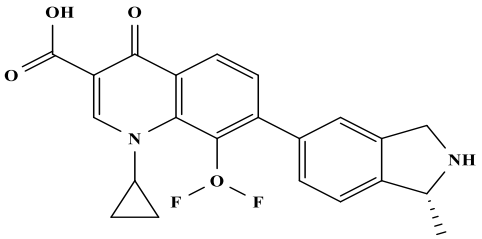

Fig. 1: Chemical Structure of Garenoxacin mesylate

Garenoxacin mesylate (GRN) is an oxazolidinone spiro 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]. A detail literature survey for Garenoxacin mesylate

Received on 28.02.2018 Accepted on 30.03.2018

Asian J. Pharm. Ana. 2018; 8(2):78-82.
DOI: 10.5958/2231-5675.2018.00015.7

revealed that few analytical methods like RP-HPLC have been reported for estimation of Garenoxacin mesylate in dosage form [3, 4] and biological fluid [5] and few spectroscopic methods [6] was reported.

So far no RP-HPTLC method for the analysis of Garenoxacin mesylate has been reported. Therefore, proposed research work illustrate a simple, accurate, sensitive and precise RP-HPTLC method has been developed for determination of Garenoxacin mesylate in the bulk and in tablet formulation.

2. EXPERIMENTAL:

2.1. Materials and Reagents:

Garenoxacin mesylate was obtained as gift sample from Alkem Pharmaceuticals Ltd, Mumbai, India. All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals, India.

2.2. Chromatographic Conditions:

The plates were prewashed with methanol and activated at 100°C for 5 min prior to chromatography. The drug standard and samples were spotted in the form of bands of 6 mm width with a Camag microlitre syringe on precoated silica gel aluminum plates 60 RP-18 $F_{254}S$ (10 x 10 cm, E. Merck), using a Camag Linomat 5 applicator. The slit dimension was kept at 6.00 x 0.45 mm (micro) and 20 mm/s scanning speed was employed. The mobile phase consisted of n-butanol: methanol: triethylamine (60:20:20 % *v*/*v*/*v*), and 10 mL of mobile phase was used. Linear ascending development was carried out in a 10 x 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 20 min at room temperature (25°C ± 2). The length of the chromatogram run was approximately 80 mm. After development; the HPTLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed on a Camag TLC scanner 3 and was operated by winCATS software (Version 1.3.0).

2.3. Preparation of Stock Standard Solution and Linearity Study:

Stock standard solution was prepared by dissolving 10 mg of Garenoxacin mesylate in 10 mL methanol to obtain concentration 1 mg/mL. Aliquots of standard solutions 0.2, 0.4, 0.6, 0.8,1.0 and 1.2 mL of Garenoxacin mesylate were transferred into six separate 10 mL volumetric flasks and volumes were made up to the mark using same solvent. An appropriate volume 5 µL was applied with the help of microlitre syringe, using Linomat 5 applicator on RP-HPTLC plate to obtain concentrations of 100, 200, 300, 400, 500 and 600 ng/band. The standard curves were assessed for within day and day-to-day reproducibility. Each experiment was repeated for six times.

3. Method Validation:

The proposed method was validated as per ICH guidelines [7].

3.1. Precision:

Precision can be performed at two different levels i.e. repeatability and intermediate precision. Repeatability of sample application and measurement of peak area were carried out using six replicates of the same band (300 ng/band of Garenoxacin mesylate). The intermediate precision results from the variations such as different days, analysts and equipment. The intra-day variation experiments were studied using three different concentrations over the linearity range within same day. The inter-day variations in the methods were assessed by studying three different concentrations for three different days over a period of week. The intra and inter-day variation for the determination of Garenoxacin mesylate was done at three different concentration levels of 200, 300, and 400 ng/band.

3.2. Limit of Detection (LOD) and Limit of Quantification (LOQ):

So as to determine limit of detection and limit of quantification, Garenoxacin mesylate concentrations in the lower part of the linear range of the calibration curve were used. Garenoxacin mesylate solutions of 100, 125, 150, 175 and 200 ng/band were prepared and applied on RP-HPTLC plate. The LOD and LOQ were calculated using equation LOD = 3.3 x N/B and LOQ = 10 x N/B, where, 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

3.3. Specificity:

The specificity of the method was determined by examining Garenoxacin mesylate standard and Garenoxacin mesylate extracted from the tablet formulation. The spot for Garenoxacin mesylate in sample was confirmed by comparing the R*f* values and spectra.

3.4. Ruggedness:

Ruggedness of the method was performed by spotting 300 ng/band of Garenoxacin mesylate by two different analysts keeping same experimental and environmental conditions.

3.5. Accuracy:

The pre- analyzed samples were spotted with extra 80, 100 and 120% of the Garenoxacin mesylate standard and the mixtures were re-analyzed by the proposed method. This was performed to check the recovery of the drug at different levels in the tablet formulation.

3.6. Robustness:

Robustness measures the capacity of an analytical method to remain unaffected by small but deliberate

various in method parameters. By introducing small changes in the mobile phase composition, the effects on the results were examined. Mobile phases having different compositions of *n*-butanol: methanol: triethylamine tried and chromatograms were run.

The amount of mobile phase, temperature and relative humidity varied in the range of ± 5%. Time from spotting to chromatography and from chromatography to scanning varied.

3.7. Application of Proposed Method to Tablet Formulation:

To determine the concentration of Garenoxacin mesylate in Zinox tablets (Label claim: 200 mg Garenoxacin mesylate), 10 tablets were accurately weighed and powdered. The powder equivalent to one tablet was transferred into 100 mL volumetric flask, to it 30 mL of methanol was added and sonicated for 15 min and volume was made up to the mark with methanol. The resulting solution was filtered using 0.41 lm filter (Millifilter, Milford, MA). From it, appropriate volume 0.3 mL was diluted to 10 mL with methanol. The appropriate volume 5 µL was applied on RP-HPTLC plate. The experiment was repeated six times.

4. RESULTS AND DISCUSSION:

4.1. Development of Optimum Mobile Phase:

Different proportion of the mobile phase for RP-HPTLC analysis were experimented with an objective to get high resolution and reproducible peaks. The required objective was achieved using *n*-butanol: methanol: triethylamine (60:20:20 % *v*/*v*/*v*) as the mobile phase. The wavelength of 274 nm was found to be optimal for the highest sensitivity. The peaks for the Garenoxacin mesylate were obtained at R*f* 0.62 ± 0.02 with the chamber saturation for 20 min at room temperature, **Fig. 2**.

4.2. Calibration Curve:

The acceptability of linearity data is often judged by examining the correlation coefficient and intercept of the linear regression line for the response versus concentration plot. The linear regression data for the calibration curves showed good linear relationship over the concentration range 100 - 600 ng/band. Linear regression equation was found to be Y = 6.62X +1065.8 (**Fig. 3**). The regression coefficient (r^2 = 0.988) is generally considered as evidence of acceptable fit.

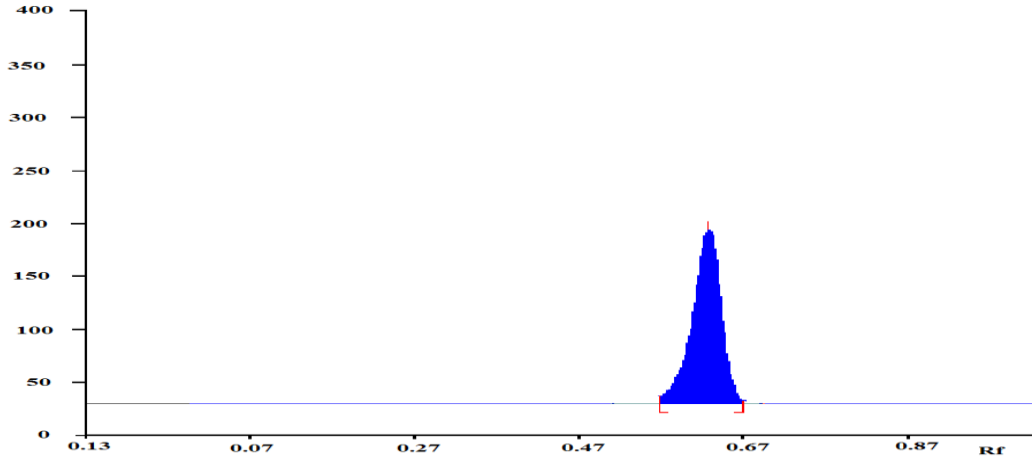

Fig. 2.The optimized chromatogram of Garenoxacin mesylate

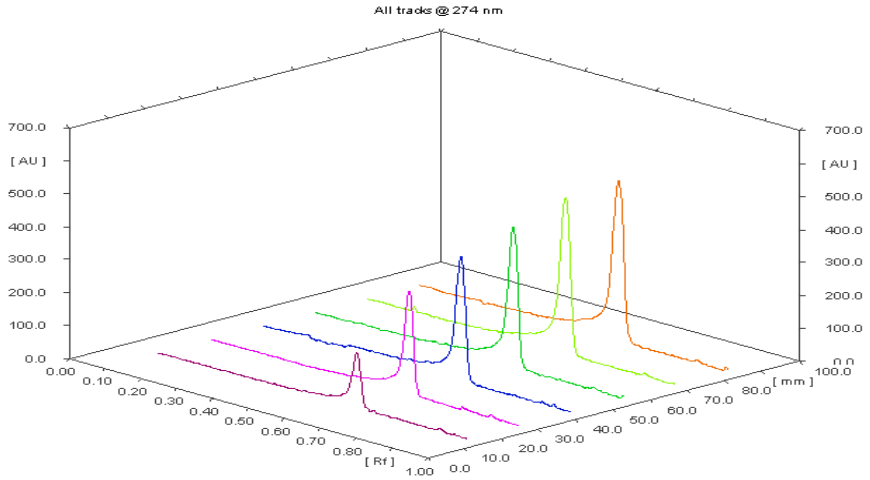

Fig. 3.The 3D view developed plate for calibration curve of Garenoxacin mesylate

4.3. Validation of Method:

4.3.1. Precision:

The precision of the developed RP-HPTLC method was expressed in terms of % relative standard deviation (% RSD).The % RSD value for repeatability of sample application and amount of Garenoxacin mesylate was estimated and was found to be less than 2. The results depicted revealed high precision of the method and are presented in **Table 1**.

Table 1: The Calibration curve for the proposed method

Parameters	Garenoxacin mesylate
Linearity range(ng/band)	100- 600
Slope	6.62
Intercept	+1065.8
Correlation coefficient	0.988

4.3.2. LOD and LOQ:

Detection limit and quantification limit were calculated by the method as described above. The LOD and LOQ were found to be 6.63 ng and 20.11 ng respectively. This indicates that the sensitivity of the method is adequate.

4.3.3. Specificity:

A typical absorption spectrum of Garenoxacin mesylate was shown in **Fig. 4.** Excellent correlation (r^2 = 0.99) was also obtained between standard and sample spectra of Garenoxacin mesylate. {correlation r (S, M) = 0.9995, r (M, E) = 0.9806}.

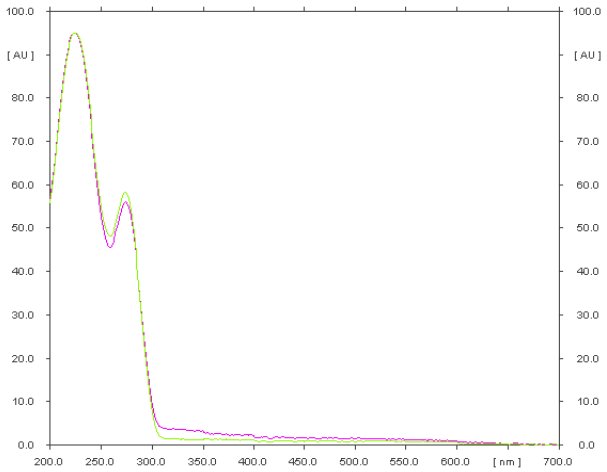

Fig. 4: peak - purity Spectra of Garenoxacin mesylate

3.4. Ruggedness:

The method was executed out by two different analysts under the same experimental and environmental conditions; the results were calculated in terms of % RSD of amount found. The % RSD was found to be less than 2 which indicate that the method is rugged.

4.3.5. Recovery Study:

The accuracy of the method is studied to measure that other components in the pharmaceutical formulation do not interfere with analytical method. The proposed method when used for extraction and subsequent quantification of Garenoxacin mesylate from the tablet formulation after over spotting with 80, 100 and 120% of additional drug, gives excellent recovery of Garenoxacin mesylate. The amounts of drug added were determined and the % recovery is shown in **Table 2**. The results obtained indicate that other components do not interfere with the analytical method.

Table 2: Precision studies

Drug	Concentration [ng/band]	Intra- day Amount found		Inter- day Amount Found	
		Mean ± SD	%RSD [n= 3]	Mean ± SD	%RSD [n= 3]
Garenoxacin mesylate	200	196.59 ± 2.31	1.17	196.65 ± 3.04	1.55
	300	298.64 ± 4.09	1.37	298.51 ± 4.53	1.52
	400	397.35 ± 5.76	1.45	397.46 ± 5.88	1.48

n- number of estimations

Table 3: Recovery studies

Drug	Initial amount (ng/band)	Amount added (ng/band)	% Recovery	% RSD [n = 3]
Garenoxacin mesylate	200	160 (80%)	98.34	1.86
	200	200 (100%)	98.87	1.11
	200	240 (120%)	98.59	1.63

n- number of estimations

4.3.6. Robustness of the Method:

The standard deviation of peak areas was calculated for each parameter and % RSD was found to be less than 2%. The low value of % RSD, **Table 3** indicates the reliability of analytical method during normal usage.

Table 4: Analysis of marketed formulation

Drug	Amount Taken (ng/band)	Drug content [%] ± SD	% RSD [n= 5]
Garenoxacin mesylate	300	98.30 ± 1.24	1.27

n- number of estimations

4.3.7. Assay of Zinox Tablets:

The chromatogram of Garenoxacin mesylate after extraction from tablet showed as single spot at R*f* 0.62 ± 0.02. It was further observed that there was no interference from the excipients commonly present in the tablet formulation. The % drug content ± S.D. was found to be 98.30 ± 1.24. The amount of drug estimated was found to be in close agreement with label claim which indicates the suitability of this method for routine analysis of Garenoxacin mesylate in pharmaceutical dosage forms.

5. CONCLUSION:

The present RP-HPTLC method is precise, specific, sensitive and accurate. Statistical analysis proved the method is reproducible and selective for analysis of Garenoxacin mesylate in the bulk drug and in tablets. The method can be used to determine the purity of the commercially available drug. The additives usually present in the tablet formulation of the assayed samples did not interfere with determination of Garenoxacin mesylate.

6. ACKNOWLEDGEMENTS:

Authors are thankful to the Alkem Pharmaceuticals Ltd, Mumbai, India for providing Garenoxacin mesylate as gift sample also thankful to Principal, Z. B. Patil Arts, Science and Commerce College, Dhule and R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur Dist: Dhule (MS) for providing necessary facilities to carry out this work.

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