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STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR GATIFLOXACIN AND PREDNISOLONE ACETATE

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ABSTRACT

A chromatographic RP-HPLC method was developed for the analysis of Gatifloxacin and Prednisolone acetate. It is concluded that the HPLC method developed for Gatifloxacin and Prednisolone acetate is capable of discriminating between the drug and degradation products. Linearity of this validated method was also proved by Fisher test, i.e., the F test. F_{cal} value for GAT and PRD was found to be 1.73 and 0.16, which is less than the F_{tab} value at a 95 % level. % recovery at 80, 100, and 120 % recovery level was found to be 100.80, 101.61, 100.53 for GAT and 100.32, 100.96, 101.60 for PRD. Assay was performed for two formulations, and assay percentage was found to be 100-102 % for GAT and PRD, respectively. Precision and robustness studies are also performed, and the method was found to be precise and robust for both GAT and PRD, respectively. The developed chromatographic RP-HPLC method was validated as per the guidelines of ICH for specificity, linearity, range, accuracy, precision, robustness, and the parameters were found to meet the predetermined acceptance criteria; hence, the method was simple, accurate, reproducible, and robust. Hence, this method can be introduced into routine use for the determination of Gatifloxacin and Prednisolone acetate.

Keywords: Gatifloxacin, Prednisolone, HPLC Method, ICH Guidelines, Stressed Condition

RASĀYAN*J. Chem.*, Vol. 18, No.4, 2025

INTRODUCTION

Gatifloxacin, as shown in Fig.-1, belongs to the carboxylic group of antimicrobial drugs. It is a white to off-white hygroscopic powder having a molecular weight of 375.394g/mol. Formula of C19H22FN3O4, melting point 182° to 185° C, and solubility in 1 in 5 parts methanol and ethanol.[1]

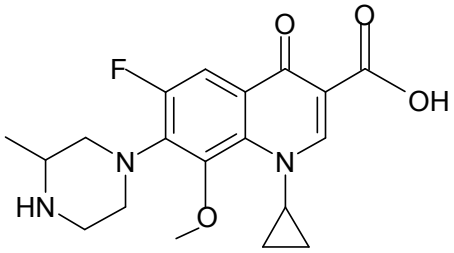

Fig.-1: Structure of Gatifloxacin

Prednisolone acetate is a corticosteroid drug, as shown in Fig.-2, with a white crystalline powder having a Molecular weight of 360.444 g/mol, Molecular formula C21H28O5, Soluble in methanol, Ethanol, acetonitrile, and M.P. 235° C.[2]

Acidic, alkaline, wet heat, oxidative, photolytic, and dry heat conditions were all used for the tests. Chromatographic separation of the stressed sample and the standard drug was performed, and the conditions necessary to resolve the drug peak from having any possible degradation products were optimized. [3, 4]

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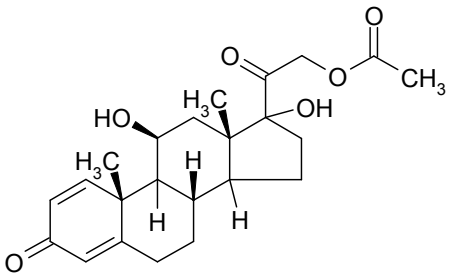

Fig.-2: Structure of Prednisolone

EXPERIMENTAL

Materials and Methods

Procurement of Drug Samples

Reference standards of Gatifloxacin (% purity 99.96) and Prednisolone acetate (%purity 99.82) were obtained from Sun Pharmaceuticals Ltd., Baroda, and Nulife Pharmaceuticals, Pune, respectively.

Instruments Used

HPLC System: The HPLC system (Waters Corp Ltd), equipped with a pump, an auto sampler, and a 2998 Photodiode Array (PDA) detector, operated at a wavelength range of 190 nm-800 nm. The column used.

Standard Stock Solution Preparation and Calibration

Standard solutions of GAT and PRD, 2000 µg/ml, were prepared separately with methanol. Mixed standard solutions containing 300 and 1000 µg/ml GAT and PRD was prepared from a standard stock solution in methanol. Various aliquots of mixed standard solutions was serially diluted to have concentrations in the ranges of 3-10.5µg/ml of GAT and 5-45µg/ml of PRD, and injected into the column and analyzed by utilizing chromatographic conditions that are optimized, and solutions filtered through a 0.45 µm filter.[5]

RESULTS AND DISCUSSION

Optimized Chromatographic Condition

Based on laboratory-generated information and information from a literature survey, various trials were conducted to get optimized conditions as shown in Table-1, and Fig.-3 shows the optimized chromatogram for standard GAT and PRD.[6]

Table-1: Optimized Chromatographic Conditions

Column	Cosmosil C_{18} (150 mm×4.6 mm,5.0µ)
Mobile Phase	Acetonitrile and Methanol (3:2): Phosphate buffer (3.9)
Rate of Flow/type	0.8 ml/min (Gradient Program)
Injection volume	10 µl/ml
Detection	272 nm
Column Temp.	40°c

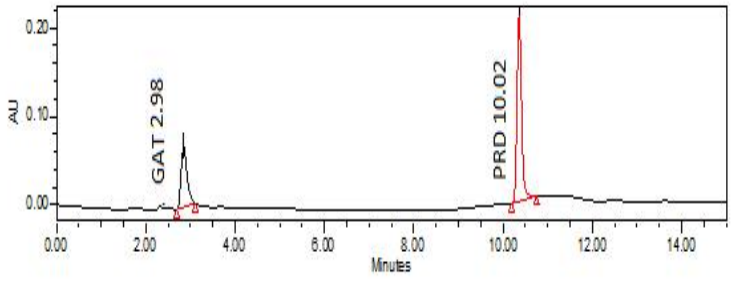

Fig.-3: Optimized Chromatogram for Standard GAT and PRD

System Suitability

To confirm that the equipment's repeatability is sufficient for analyzing as given in Table-2, system suitability tests were employed.

Table-2: System Suitability Parameters for Formulation

No.	Parameters	GAT	PRD
1	Retention time (t_R) ± SD	2.90 ± 0.07	9.98 ± 0.03
2	Tailing factor [a] (T_f) ± SD	1.45 ± 0.21	1.11 ± 0.17
3	No. of theoretical plates (N) ± SD	2959 ± 152.30	4890 ± 239.48
4	USP Resolution (R) ± SD	-	7.06
5	Selectivity Factor (K) ±SD	2.73	3.29

Method Validation Parameters

Linearity

Mixed standard stock solutions were diluted to create linearity solutions. For PRD, the concentration range was 10-35 µg/ml, and for GAT, it was 3-10.5 µg/ml.

Linear Function Analysis

The experimentally determined value of F is contrasted with the statistical tables' critical value of F.

F Test Calculation

Linear Function Analysis

The analytical method committee recommends the Fisher ratio test [F-test] as a dependable method to verify the linearity of the calibration function because a correlation coefficient near 1 is not a trustworthy indicator of linearity. The experimentally determined value of F is contrasted with the statistical tables' critical value of F.[7]

F Test Calculation

Error sum squares: The sum of three-square differences, or residual error sum of squares (SSr), experimental error sum of squares (SSe), and lack of fit sum of squares (SSlof), must be computed using the following formula after choosing several concentration levels (i) and their replicating concentrations (j) during the calibration process.

$$SS_r = \Sigma\Sigma(Y-Y^I)^2$$
$$SS_e = \Sigma\Sigma(Y-\bar{Y})^2$$
$$SS_{lof} = SS_r - SS_e,$$

Where Y is the response of each concentration.

$\bar{Y}$= average concentration.

$Y^{I=}$ predicted concentration i.e. concentration* slope + intercept

Degree of freedom (DF): Degrees of freedom associated with the above equation are calculated as follows,

$$DF_r = (ij - 2)$$
$$DFe\ (ij - 1)$$
$$DF_{lof} = (i - 2)$$

The pure experimental variance and the lack of fit variance are given by σ^2_e and σ^2_{lof} and are estimated by computing the quotients-

$$SS_e/ DF_e \text{ and } SS_{lof}/ DF_{lof}.$$

The calculated σ^2_e and σ^2_{lof} values are used to calculate the Fisher variance ratio or F test by the expression.

$$F\ (DF_{lof})/ DF_e = \sigma^2_{lof} / \sigma^2_e$$

For GAT

For degrees of freedom (DF),

$$DF_r = (ij - 2) = 16$$
$$DF_e = (ij - 1) = 17$$
$$DF_{lof} = (i - 2) = 4$$

σ^2_e is calculated using the formula SS_e/ DF_e, σ^2_e=27036037364
σ^2_{lof} is calculated by the formula SS_{lof}/ DF_{lof}, σ^2_{lof} =46789900669
The F value is calculated using the following expression and was found to be-

$$F\ (DF_{lof})/\ DF_e = \sigma^2_{lof} / \sigma^2_e = 1.73$$

Table-3: Observation Table for Experimental Error Sum of Square (SS_e)= $\Sigma\Sigma(\ Y-\bar{Y})^2$ for GAT.

Replicates. (i*3)	(Y-Ȳ)² at the concentrations (µg/ml)					
	3	4.5	6	7.5	9	10.5
I	50773.61	121	2601	27556	245025	65025
II	25814.85	6561	329476	299209	169744	160801
III	4182.209	8464	394384	145161	6889	31329
AVG	26923.56	5048.667	242153.6667	157308.7	140552.7	85718.33
% RSD	1.10	1.17	0.78	1.25	1.44	0.80
	$SS_e = \Sigma\Sigma(\ Y-\bar{Y})^2 = 4.325778 * 10^{11}$					

$$SS_e = \Sigma\Sigma(\ Y-\bar{Y})^2 = 4.325778 * 10^{11}$$

Table-4: Observation Table for Residual Error Sum of Squares $SS_r = \Sigma\Sigma(\ Y-Y^1)^2$ for GAT.

Replicates.	Peak areas at give concentration ((Y-Y¹)²					
	3	4.5	6	7.5	9	10.5
I	51337225	132365025	248062500	447830244	652189444	1037226436
II	45954841	133980625	264810529	418161601	606686161	995402500
III	47265625	130005604	227135041	456976129	623001600	1032208384
AVG	48185897	132117085	246669357	440989325	627292402	1021612440
	$SS_r = \Sigma\Sigma(\ Y-Y^1)^2 = 6.33462 * 10^{18}$					

For PRD

For degrees of freedom (DF),

$DF_r = (ij - 2) = 16$
$DF_e = (ij - 1) = 17$
$DF_{lof} = (i - 2) = 4$

σ^2_e is calculated using the formula SS_e/ DF_e, σ^2_e=5.33095*10¹²
σ^2_{lof} is calculated by the formula SS_{lof}/ DF_{lof}, σ^2_{lof} =8.89979*10¹¹

The F value is calculated using the following expression and was found to be-

$$F\ (DF_{lof})/\ DF_e, \text{ i.e., } F_{cal} = \sigma^2_{lof} / \sigma^2_e = 0.16$$

F value, i.e., F_{tab} at given degrees of freedom at 95% of confidence level as shown in Table 3,4 and Table 5,6 was found to be 3.01 for both GAT and PRD, respectively.

Table-5: Observation Table for Experimental Error Sum of Square(SS_e)= $\Sigma\Sigma(\ Y-\bar{Y})^2$ for PRD

Replicates. (i*3)	(Y-Ȳ)² at the concentrations (µg/ml)					
	10	15	20	25	30	35
I	25281	3569568	539725.3156	1649504	4752400	52592.25
II	270400	2339890	353240.0356	343009.3	96100	3256834
III	130321	129362.5	19695.3156	488139.8	3496900	2481665
AVG	142000.7	2012940	304220.2223	826884.2	2781800	1930364
% RSD	0.96	1.12	1.28	1.65	1.59	0.86
	$SS_e = \Sigma\Sigma(\ Y-\bar{Y})^2 = 6.39713 * 10^{13}$					

Table-6: Observation Table for Residual Error Sum of Squares $SS_r = \Sigma\Sigma(\ Y-Y^1)^2$

Replicates.	Peak areas at give concentration ((Y-Y¹)²					
	10	15	20	25	30	35
I	$3.76*10^9$	8298116836	$1.4976*10^{10}$	$2.2509*10^{10}$	$3.343*10^{10}$	$5.1042*10^{10}$
II	$3.85*10^9$	7686905625	$1.4652*10^{10}$	$2.1951*10^{10}$	$3.253*10^{10}$	$5.0127*10^{10}$
III	$3.74*10^9$	7893434025	$1.4762*10^{10}$	$2.1918*10^{10}$	$3.197*10^{10}$	$5.1652*10^{10}$
AVG	$3.78*10^9$	7959485495	$1.4797*10^{10}$	$2.2126*10^{10}$	$3.264*10^{10}$	$5.094*10^{10}$
	$SS_r = \Sigma\Sigma(\ Y-Y^1)^2 = 1.74895*10^{22}$					

As the F_{cal} values for GAT and PRD are less than the F_{tab} values at given degrees of freedom at 95 % confidence level, it indicates that there is an outstanding relationship between the peak area and the GAT and PRD analyte concentrations, respectively.

LOD and LOQ
The calibration curve was used to assess the LOD and LOQ. GAT and PRD were studied to have respective LODs of 0.11 and 0.33 g/ml. GAT and PRD were determined to have respective LOQs of 0.39 and 1.21g/ml.

Assay
Assays of two batches of eye formulations of label claim 0.3% and 1% w/v, respectively. Mean content of the drug and % RSD for F-I was observed as 101.60 %, 0.11 % and 101.06 %, 0.16 % for GAT, and for F-II, it is 100.64 %, 9.34% and 101.32%, 0.27 % for PRD, respectively.

Solution Stability
Over the course of eight hours, twenty-four hours, and a month, the analysis of each solution was conducted three times under various conditions.

Accuracy
According to the findings of recovery experiments conducted at different levels, the recovery ranges from 99.50% to 101.60% (maximum 98-102%). Using a calibration curve, the amount of drug present was calculated.

Precision
Precision of the method was studied for intraday and interday intervals. The %RSD of the precision study found below 0.6 % and 0.7 % for GAT and PRD, respectively.

Specificity
The purity angle was consistently lower than the analyte's purity threshold, which is 0.11,0.28 for GAT and 0.09,0.23 for PRD, respectively, in peak purity analysis using the light diode array detector.

Robustness
The effects were studied by injecting 7.5µg/ml for GAT and 25µg/ml for PRD; one factor was changed at a time to estimate the effect.[8]

Forced Degradation Study
Gatifloxacin and Prednisolone acetate were exposed to a variety of stress conditions to affect degradation up to about 5-20 %. The drugs was stressed under various stress conditions like acid, alkali, wet heat, affected by oxidation, light, and dry heat.[9]

Acid Degradation
2ml of 0.5M Hydrochloric acid was added to all solutions and kept aside for 4 hrs at 40°C. The degradation was found to be 28.35 % for GAT and 18.92% for PRD. In general, 5% to 20% degradation of the drug substance has been considered acceptable. Hence, the sample was degraded again with 0.1 M HCl for 4 hrs, and the degradation was found to be 11.34 % and 5.4 % for GAT and PRD, respectively. When stressed samples were analyzed, there was one additional peak at the retention time of 2.12 min for GAT, 11.15 for PRD, confirming the formation of a degradation product.

Base Degradation
Degradation was found when GAT and PRD were individually and mixed treated with NaOH 0.1N for 2 hrs. at 40 °C. When stressed samples were analyzed, an additional peak for GAT was found at RT 2.12 min and 11.12min for PRD, confirming the formation of a degradation product. Comparison of the average peak area of both GAT and PRD in stressed conditions with that of the average peak area of the standard drug sample gives 14.43 and 9.21 % degradation for GAT and PRD, respectively.

Oxidation Degradation

Oxidative degradation for individually and mixed GAT and PRD was studied using 2 % hydrogen peroxide (H_2O_2) for 2 hrs at 40 ^{0}C. When stressed samples were analyzed, there was an additional peak at retention time 2.56 min for GAT and 12.60 min for PRD, confirming the formation of a degraded product. Comparison of the average peak area of both GAT and PRD in stressed conditions with that of the average peak area of the standard drug sample gives 12.78% and 10.39 % degradation for GAT and PRD, respectively.

Wet Heat Degradation

Stability of GAT and PRD under wet heat for individual and mixed was studied by keeping it at 70 ^{0}C for 5 hrs. When stressed samples were analyzed, no degradation was found, and hence, the heating time was increased up to 12 hrs at the same temperature. The comparison between peak areas of stressed samples of GAT and PRD indicates there was no degradation.

Thermal Degradation

Stability of GAT and PRD under dry heat was studied by keeping both drugs in a hot oven for 12 hrs at 70^{0}C. The additional peaks at retention time 4.85 min for GAT and 11.89min for PRD confirm formation of degraded product. Comparison of the average peak area of both GAT and PRD in stressed conditions with that of the average peak area of the standard drug sample gives 5.03 and 6.09 % degradation for GAT and PRD, respectively.

Photo Degradation

GAT and PRD were exposed directly to UV light for 12 hours in the UV chamber. When stressed samples were analyzed, no degradation was found, and hence, exposure time was increased up to 18 hrs. There were no extra peaks found when stressed samples were examined. It was also determined that the medication was stable under photolytically stressed conditions because there was no change between the peak regions of the stressed sample and the standard, suggesting that there was no degradation. From the studies, it can be concluded that Gatifloxacin and Prednisolone acetate undergo acidic/alkaline/oxidative/dry heat degradation, giving primary degradation products. The retention times of Gatifloxacin and Prednisolone acetate were found to be 2.98 min and 10.01 min. Degradation were found to be 11.34%, 14.43 %, 12.78 %, 5.03 % for GAT and 5.4%, 9.21%, 10.39%, 6.09% for PRD, respectively.[10]

CONCLUSION

Gatifloxacin and Prednisolone acetate undergo acidic, alkaline, oxidative, and dry heat degradation, yielding principal degradation products, according to the forced degradation experiments. The retention times of Gatifloxacin and Prednisolone acetate were found to be 2.98 min and 10.01 min. Degradation in acidic conditions gave chromatograms at 2.12 and 2.97 minutes for GAT and 9.96 and 11.15 minutes for PRD. Degradation in alkaline, oxidation gave the chromatogram at 1.85, 2.12, 2.88/ 2.56/3.11 min for GAT and 9.97,11.15/ 10.11, 12.98 min for PRD. Degradation at dry heat conditions gave chromatograms at 2.85 and 4.12 minutes for GAT and 10.11 and 11.89 minutes for PRD. Degradation was found to be 11.34%, 14.43 %, 12.78 %, 5.03 % for GAT and 5.4%, 9.21%, 10.39%, 6.09% for PRD, respectively. The developed chromatographic RP HPLC method was found to be simple, precise, reproducible, and robust after it was validated in accordance with ICH guidelines for specificity, linearity, range, accuracy, precision, and robustness. Additionally, the parameters were found to meet the predetermined acceptance criteria. As a result, this technique can be used regularly to determine the levels of Gatifloxacin and Prednisolone acetate.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-9: Industry, Innovation, and Infrastructure*. All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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