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Research Article

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Method Development and its Validation for Quantitative Simultaneous Determination of Dexamethasone and Gatifloxacin in Ophthalmic Solution by RP-HPLC

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ABSTRACT

To develop a simple, rapid & accurate HPLC method for simultaneous quantitative determination of Dexamethasone and Gatifloxacin in ophthalmic solution. Chromatographic separation was achieved with PDA detector using Inertsil C18, 250 x 4.6mm, 5µ reverse phase analytical column. The mobile phase consist of buffer: acetonitrile (70: 30 v/v), was passed through the column at flow rate of 1.0 ml/min. The method was performed at wavelength 254. The experiment was carried out at 30°C. The calibration curves were linear in the concentration range of 25% to 150% of the working concentration (r2 >0.999). The lower limit of quantification was 1.6, 2.8 µg/ml for Dexamethasone and Gatifloxacin respectively. The accuracy of Dexamethasone and Gatifloxacin was also found to be in the limits (ie 98% - 102%). Developed procedure was used for simultaneous quantitative estimation of Dexamethasone and Gatifloxacin in ophthalmic solution and validated as per ICH and most useful for academic as well as industrial scale..

Key words: Dexamethasone, Gatifloxacin, RP-HPLC, Validation

INTRODUCTION

Dexamethasone is a glucocorticoid agonist. Unbound dexamethasone crosses cell membranes and binds with high affinity to specific cytoplasmic glucocorticoid receptors. This complex binds to DNA elements (glucocorticoid response elements) which results in a modification of transcription and, hence, protein synthesis in order to achieve inhibition of leukocyte infiltration at the site of inflammation, interference in the function of mediators of inflammatory response, suppression of humoral immune responses, and reduction in edema or scar tissue. The anti-inflammatory actions of dexamethasone are thought to involve phospholipase A2 inhibitory proteins, lipocortins, which control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes.[1-4] 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. [5-7] 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. It should be used only to treat or prevent infections that are proven or strongly suspected to be caused by bacteria [8-9]. The aim of this study was to develop a RP HPLC method for the quantitative simultaneous determination of Dexamethasone and Gatifloxacin. The method developed was validated as per ICH Q2 (R1).

MATERIALS & METHODS

Chemicals and reagents

HPLC grade acetonitrile, sodium dihydrogen, GAA and TEA were used to prepare the mobile phase and were purchased from Merck Specialties. The working standards of Dexamethasone and Gatifloxacin were purchased from LG Promochem. Deionized and purified water using a Mili-Q system (Millipore) was used for the mobile phase and the standard solutions preparation. All experiment was performed using 'A' class volumetric glassware. All other reagents were of analytical grade.

Instrument and Chromatographic Conditions [10-14]

Shimadzu LC 2010 C_{HT} HPLC was used for the chromatographic separation equipped with autosampler and Photo diode array (PDA) detector. The software used was LC Solution. The chromatographic separation of Dexamethasone and Gatifloxacin were carried out using Inertsil C18 250 x 4.6 mm, 5µ reverse phase analytical column. Mobile phase consisted of Acetonitrile: Buffer (0.1 M sodium di hydrogen phosphate and 1ml triethylamine in 1000 ml WFI and pH adjusted to 3.0 with GAA) in the ratio 30: 70. The mobile phase was filtered by passing it through 0.45 µm filter and the filtrate is degassed by using bath sonicator. Mobile phase was used as diluent. Injection volume was 10 µl. Oven temp was set at 30°C. The mobile phase was pumped at 1 ml/min at room temperature. Detection was carried by using wavelength 254.

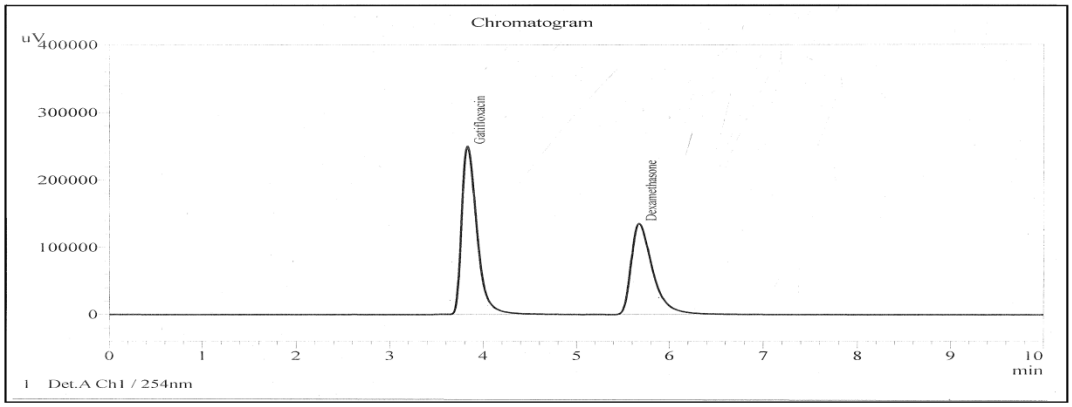

Figure 1: Chromatogram of Gatifloxacin and Dexamethasone in standard preparation

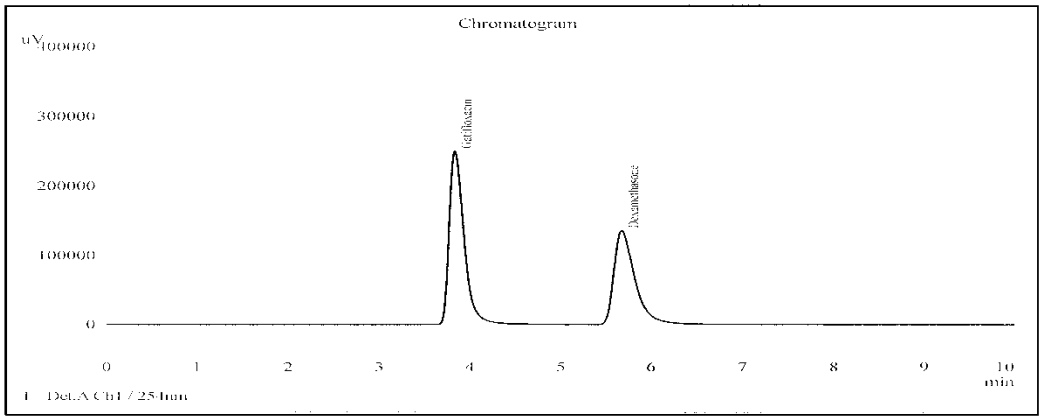

Figure 2: Chromatogram of Gatifloxacin and Dexamethasone in sample preparation

Preparation of standard and test solution

Prepare standard solution and test solution having concentration of Dexamethasone (0.04 mg/ml and Gatifloxacin (1.2 mg/ml) dilute to the mark by diluent (mobile phase).

RESULTS AND DISCUSSION

Method Validation
Specificity

The test was carried out by injecting 10 µl standard solutions of Dexamethasone (0.04 mg/ml), and Gatifloxacin (1.2 mg/ml) in five replicates. The RSD values for areas of Gatifloxacin and Dexamethasone standard were found 0.130 % and 0.573%, respectively. Resolution, Theoretical plates and Tailing factor were determined. Results are shown in table 1.

Table 1: System Suitability Parameters

	Resolution	Tailing factor	Theoretical plates
Gatifloxacin	-	1.23	2605.79
Dexamethasone	5.14	1.26	2707.47

Linearity

The linearity of an analytical procedure within a given range is its ability to obtain test results, which are directly proportional to the concentration of analyte in the standard. The range is derived from the linearity studies. A linearity standard solution was prepared at about 25%, 50%, 75%, 100%, 125% and 150% of the standard solution concentration and then linearity correlation coefficient of Gatifloxacin and Dexamethasone obtained from the graph obtained by plotting area count on Y axis and concentration on X axis. Correlation coefficient of Gatifloxacin and Dexamethasone are shown in table 2 and figure 3.

Table 2: Correlation Coefficient

Gatifloxacin	Dexamethasone
0.99996	0.99995

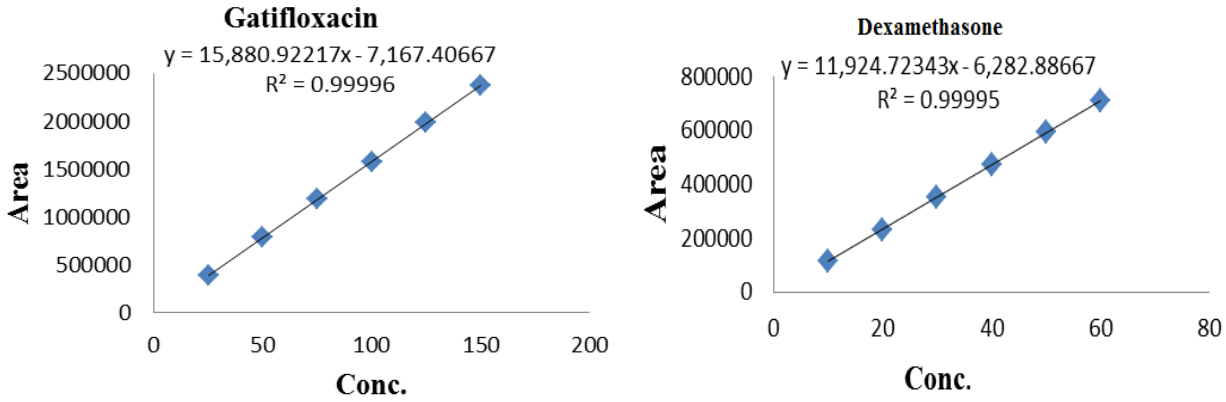

Figure 3: Linearity of Gatifloxacin and dexamethasone

Precision

System precision

The six injections of standard solutions were injected to the chromatographic system. The relative standard deviation for area and retention time of Gatifloxacin and Dexamethasone peak was determined and shown in table 3.

Table 3: System Precision and Method Precision

System Precision		
% RSD	**Gatifloxacin**	**Dexamethasone**
AREA	0.130%	0.573 %
RT	0.25%	0.46%
Method Precision		
% RSD of assay	0.49	0.53

Method Precision

Six sample of a single batch of Gatifloxacin and Dexamethasone peak were analyzed by proposed method and their assay was calculated and results are shown in table 4.

Accuracy (Recovery)

Table 4: Accuracy (Recovery)

Analyte	**Conc. Added (%)**	**Mean (%) Recovery**
Gatifloxacin	50%	99.59
	100%	99.51
	150%	99.70
Dexamethasone	50%	100.00
	100%	99.81
	150%	100.13

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the found value. Recovery samples were

prepared in triplicate and injected each sample in duplicate to the chromatography system. Dexamethasone and Gatifloxacin peak working standard was added with placebo and recovery solutions were prepared so that, the final concentration contains 50%, 100% and 150 % of the recovery levels of Gatifloxacin and Dexamethasone and results are shown in table 4.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.
The analysis was carried out used the method outlined in the method of analysis and by carried out the following alterations and results are shown in table 5 and 6.
a) By changing the flow rate of the HPLC System by ±0.2 mL/min.
b) By changing the column oven temperature by ± 5°.

Table 5: Robustness by changing flow rate

At flow rate 1.2 ml/ min		
	Gatifloxacin	**Dexamethasone**
% RSD	0.51	0.72
Tailing factor	1.13	1.09
Theoretical plates	2569.81	2682.19
At flow rate 0.8 ml/ min		
% RSD	0.63	0.69
Tailing factor	1.34	1.41
Theoretical plates	2726.34	2817.42

Table 6: Robustness by changing temperature

At Temp 25°C		
	Gatifloxacin	**Dexamethasone**
% RSD	0.47	0.59
Tailing factor	1.29	1.27
Theoretical plates	2559.29	2784.16
At Temp 35°C		
% RSD	0.61	0.54
Tailing factor	1.24	1.30
Theoretical plates	2668.14	2729.26

Limit of Detection and Quantification

The limit of detection and Quantification were calculated as per formulas given below

$$LOD = \frac{3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

Where σ is standard deviation and S is the slope of the calibration curve. The LOD and LOQ values of Gatifloxacin and Dexamethasone are shown in table 7.

Table 7: LOD and LOQ

	Gatifloxacin	**Dexamethasone**
LOD (ppm)	1.2	0.8
LOQ (ppm)	2.8	1.6

4. CONCLUSION

The present study shows that the method developed for the determination of Gatifloxacin and Dexamethasone were specific, linear, accurate, precise and robust. The method clearly shows that all the peaks had tailing factor less than 2. The RSD for areas and theoretical plates (> 2500) was also found to be satisfactory. Validation parameters were performed according to ICH Q2 (R1) guidelines. The recoveries achieved were highly significant in the developed method. Hence it can be concluded that the method developed can be effectively used in the industries as well as research purposes.

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