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Simultaneous Estimation of Gentamicin and Clobetasol in Bulk and Pharmaceutical Formulations by RP-HPLC Method

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ABSTRACT

A new simple, precise, accurate and reproducible RP-HPLC method for simultaneous estimation of gentamicin and clobetasol in bulk and pharmaceutical formulations. Separation of gentamicin and clobetasol was successfully achieved on a Zorbax C18 (150 mm x 4.6mm x 5µ) in an isocratic mode utilizing disodium hydrogen phosphate buffer and methanol (60:40 *v/v*) at a flow rate of 1.0 mL/min. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision, specificity and robustness. The response was found to be linear in the drug concentration range of 0.1-0.30 mg/mL for gentamicin and 0.05–0.15 mg/mL for clobetasol. The correlation coefficient was found to be 0.9997 for both the drugs. The LOD and LOQ for gentamicin were found to be 0.3525 µg/mL and 1.1751 µg/mL respectively. The LOD and LOQ for clobetasol were found to be 0.1938 µg/mL and 0.6460 µg/mL respectively. The proposed method was found to be good percentage recovery for gentamicin and clobetasol, which indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard solution with the sample solution. Therefore, the proposed method specifically determines the analyte in the sample without interference from excipients of pharmaceutical dosage forms.

Keywords: RP-HPLC, gentamicin, clobetasol, pharmaceutical formulation, analysis.

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INTRODUCTION

Gentamicin[1-3] is an aminoglycoside bactericidal antibiotic. Gentamicin composed of three closely related aminoglycoside sulfates, gentamicins C1, C2, and C1a. It is obtained from *Micromonospora purpurea* and related species. Gentamicin is active against a wide range of human bacterial infections, mostly gram-negative bacteria. Gentamicin exerts its activity by irreversibly binding to 30S subunit of the bacterial ribosome and interrupts the protein synthesis. The chemical structure of the gentamicin is shown in Figure 1. In literature chemiluminescent immunoassay[4,5], spectrophotometric assay[6,7], enzymatic radioassay[8], HPLC assay[9,10,11], and microbiological assay[10,11] methods are reported for the determination of gentamicin.

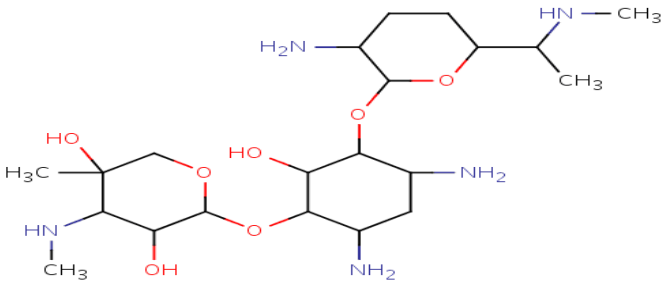

Figure 1: Chemical structure of gentamicin

Clobetasol[12,13,14] is a derivative of prednisolone with high glucocorticoid activity and low mineralocorticoid activity. Clobetasol is used in the treatment of itching, redness, dryness, crusting, scaling, inflammation and discomfort of various skin conditions. The precise mechanism of corticosteroids is uncertain. Corticosteroids are thought to act by the induction of phospholipase A_2 inhibitory proteins. These proteins control the biosynthesis of mediators of inflammation, prostaglandins and leukotrienes by inhibiting the release of arachidonic acid. Arachidonic acid is released from membrane phospholipids by phospholipase A_2. Clobetasol, like other corticosteroids, bind to the glucocorticoid receptor, which complexes, enteres the cell nucleus and modifies genetic transcription. The chemical structure of the clobetasol is shown in Figure 2.

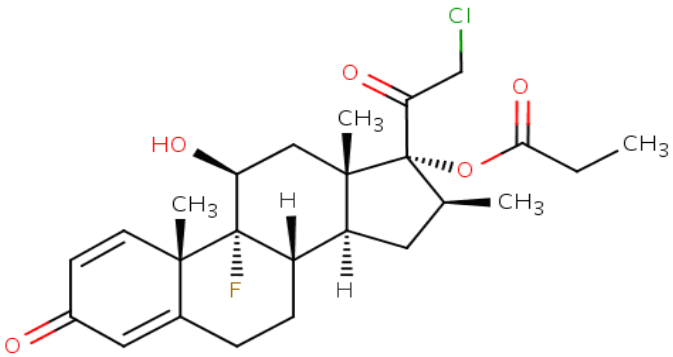

Figure 2: Chemical structure of clobetasol

In the literature, several analytical techniques were reported for the quantification of clobetasol. They include spectrophotometry[15,16], HPLC[16-22], HPTLC[23], LC-MS[24], and voltametry[25].

The detailed literature has indicted that there is no HPLC method for the simultaneous determination of gentamicin and clobetasol in pharmaceutical formulation. Hence, there is a need for developing a HPLC method for the simultaneous estimation of both drugs in pharmaceutical formulation. Therefore, the present study was focused on the development of simple and rapid RP-HPLC method for the routine simultaneous analysis of gentamicin and clobetasol in pharmaceutical formulations. The developed method was validated by following ICH guidelines.

MATERIALS AND METHOD

Chemicals and Reagents

Gentamicin and Clobetsol were obtained from Lara drugs pvt Ltd., Hyderabad as a gift sample. Disodium hydrogen phosphate of analytical reagent grade was purchased from Merck (India) Ltd., Mumbai. Methanol of HPLC grade was purchased from E. Merck (India) Ltd., Mumbai. Ortho phosphoric acid of analytical reagent grade was obtained from S.D. Fine Chemicals Ltd., Mumbai. Mille Q HPLC grade water was used throughout the process.

Instrumentation

Analysis was carried out using Waters 2695 alliance HPLC system with binary HPLC pump and Waters 2998 PDA detector. Waters Empower2 version software was used for the acquisition of the chromatographic data.

Chromatographic conditions

The HPLC separation and quantification of the gentamicin and clobetasol were made on the Zorbax C18 analytical column (150 mm length, 4.6 mm i.d and 5 µm particle size). An isocratic mobile phase consisting of disodium hydrogen phosphate and methanol in the proportion of 60:40% v/v at a temperature of 30 °C was the optimized mobile composition and column temperature. The eluate was monitored at 226 nm. The mobile was pumped into the column at a flow rate of 1.0 mL/min and the run time was 8 min. The volume of injection loop was 10µL. Prior to injection of the drug solution the column was equilibrated for at least 15 min with the mobile phase flowing through the system.

Preparation of standard solutions

0.05mg of gentamicin and 0.10mg of clobetsol was weighed accurately and transferred into 100 mL volumetric flask. It was dissolved in 10 mL of mobile phase, sonicated for 10 minutes and diluted upto the mark with mobile phase. The mobile phase was filtered through the 0.45µm filter

paper. 5 mL of the above prepared standard stock standard stock was transferred into 25 mL volumetric flask dilute to volume with mobile phase.

Preparation of sample solutions

Accurately weighed 100 mg of sample powder was transferred into 100 mL of volumetric flask containing 10 mL mobile phase and dissolved with the aid of sonication for 20 minutes. The volume was made upto the mark with mobile phase and filtered through the 0.45µm filter paper. Transfer 5 mL of above solution into 25 mL volumetric flask and made up the volume with mobile phase.

Method validation

The method was validated for system suitability, specificity, linearity and range, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and robustness as per International Conference on Harmonization (ICH) guidelines[27].

System Suitability Studies

System suitability for chromatographic separation was checked on each day of validation to evaluate the components of the analytical system in order to show that the performance of the system meet the standards required by the method. Mixed standard solution of gentamicin and clobetsol solution was injected in six replicates and system suitability parameters were determined System suitability parameters established for the developed method include number of theoretical plates, resolution and tailing factor.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. The specificity of the developed HPLC method was investigated by non-interference of excipients in pharmaceutical formulations.

Linearity and Range

Linearity was evaluated by analyzing five concentrations of gentamicin and clobetasol by the developed method. For linearity and range testing, stock solutions of gentamicin and clobetasol were prepared. Appropriate quantities of these stock solutions were mixed and diluted in a series of volumetric flasks to contain both the drugs in the concentration range of 0.05 – 0.15 mg/mL of clobetasol and 0.1 - 0.30 mg/mL of gentamicin.

Precision

The precision of the proposed method was performed by analyzing six sample solutions. The response factor of drug peaks and percentage RSD were calculated.

Accuracy

The accuracy of the method was determined through recovery experiments. The accuracy of the proposed method was demonstrated by preparing samples spiked with 50%, 100%, and 150% of the test concentration of gentamicin and clobetasol. Each concentration level was analyzed. Mean percent recovery and percent RSD were calculated for each concentration.

Robustness

The robustness test was performed by deliberately making the changes in chromatographic conditions. Retention time, tailing factor, resolution, and theoretical plates were measured to demonstrate the robustness of the method.

Limit of detection (LOD), limit of quantification (LOQ)

The Limit of quantification and detection determines the sensitivity of the method. The LOD and LOQ were calculated using the following formulas (a) and (b).

(a) $LOQ = 10\ \sigma / S$

(b) $LOD = 3.3\ \sigma / S$

Where

σ = residual standard deviation of response

S = slope of the calibration curve.

RESULTS AND DISCUSSION

System suitability studies

The column efficiency, resolution and tailing factor were calculated for the standard solutions (Table 1).The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within ± 2 % standard deviation range during routine performance of the method.

Table 1: System suitability parameters

Parameter	Gentamicin	Clobetsol
Retention time	6.267	4.405
Theoretical plates	7717	5688
Tailing factor	1.14	1.21
% RSD	0.2	0.4

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Specificity of the proposed method was demonstrated by comparing the chromatograms of standard solution of gentamicin and clobetasol with the chromatogram of formulation sample solution. The chromatograms are shown in Figures 3 and 4. There were not difference in the chromatograms of standard solution and formulation sample

solution indicating the specificity of the proposed method.

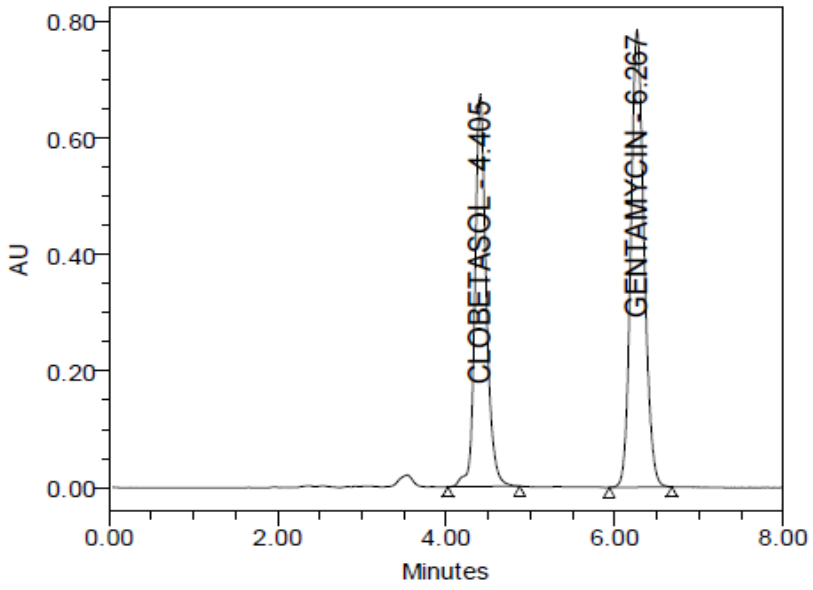

Figure 3: Chromatogram of standard gentamicin and clobetasol

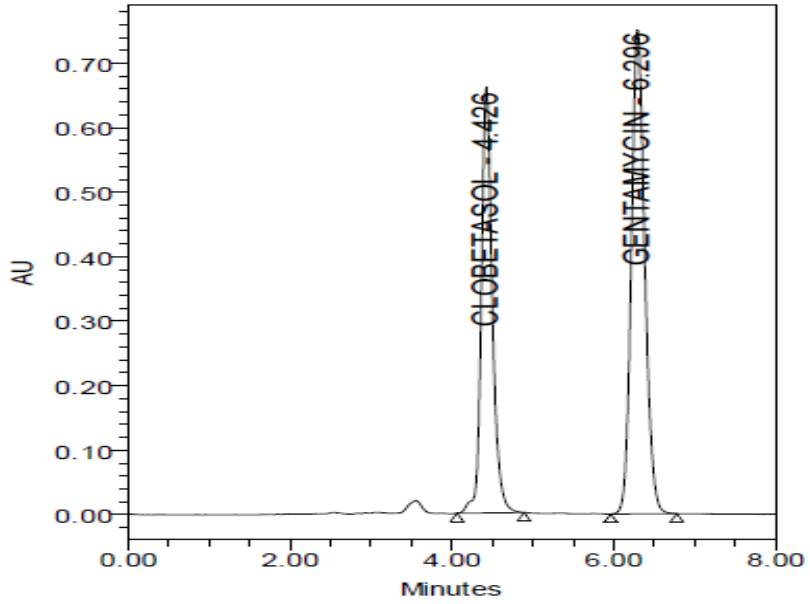

Figure 4: chromatogram of gentamicin and clobetasol in formulation.

Linearity and range

The linearity for the proposed HPLC method was established by least squares regression analysis of the calibration curve. Calibration curves were linear over the concentration range of 0.05 - 0.15 mg/mL for clobetasol and 0.1 - 0.30 mg/mL for gentamicin with a correlation coefficient (r^2) of 0.9997 and 0.9997, respectively. The results shows an excellent correlation exists between peak area and concentration of drugs within concentration range indicated above. The results for calibration data are shown in Table 2 and calibration curves are given in Figure 5 & 6.

Table 2: Linearity studies for clobetasol, gentamicin by proposed method

Linearity study for clobetasol			Linearity study for gentamicin		
% level	Area	Conc. mg/mL	% level	Area	Conc. mg/mL
50	3125948	0.05	50	4299189	0.1
75	4688488	0.08	75	6416057	0.15
100	6241531	0.10	100	8526982	0.2
125	7814044	0.125	125	10621750	0.25
150	9377287	0.15	150	12863706	0.30

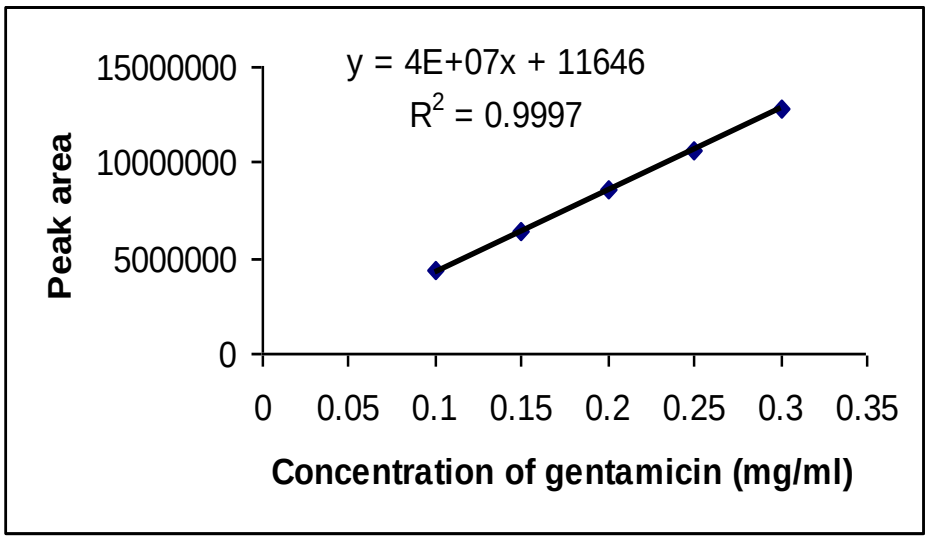

Figure 5: Linearity curve for gentamicin

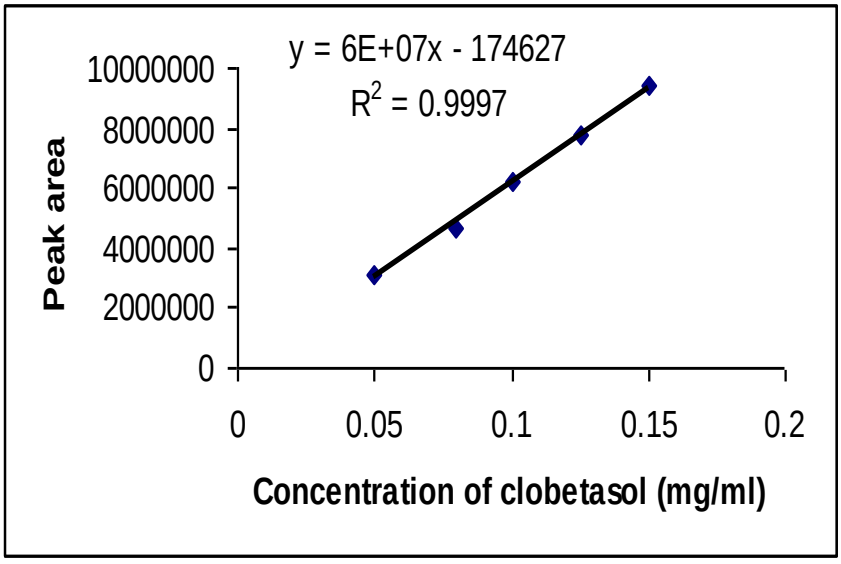

Figure 6: Linearity curve for clobetasol

Precision and accuracy

The results of method precision are presented in Table 3. The results were with in the acceptable limit and indicated that the method is precise.

Table 3: Precision studies

S.No	Sample Wt (mg)	Area (Clobetasol)	Area (Gentamicin)	%Assay (Clobetasol)	%Assay (Gentamicin)
1	100.00	6231916	8569118	99	100
2	100.00	6257184	8545461	99	100
3	100.00	6234180	8543818	99	100
4	100.00	6242835	8523243	99	99
5	100.00	6259317	8583093	99	100
6	100.00	6337110	8535472	100	100

Accuracy was checked by spiking the standard drugs gentamicin and clobetasol at three different concentration levels. Recovery of individual components was well within the acceptable limit. Results are presented in Tables 4 and 5. From the data obtained, added recoveries of standard drugs were found to be accurate. The chromatograms of three different levels are shown in Figures 7, 8 and 9.

Table 4: Accuracy for clobetasol

Accuracy level	Sample weight	mg/mL added	mg/mL found	% Recovery	% Mean
50%	50.00	0.050	0.05	100	100
	50.00	0.050	0.05	100	
	50.00	0.050	0.05	100	
100%	100.00	0.099	0.10	100	100
	100.00	0.099	0.10	100	
	100.00	0.099	0.10	100	
150%	150.00	0.149	0.15	100	100
	150.00	0.149	0.15	99	
	150.00	0.149	0.15	100	

Table 5: Accuracy for gentamicin

Accuracy level	Sample weight	µg/mL added	µg/mL found	% Recovery	% Mean
50%	50.00	0.100	0.10	102	100
	50.00	0.100	0.10	101	
	50.00	0.100	0.10	100	
100%	100.00	0.200	0.20	100	100
	100.00	0.200	0.20	100	
	100.00	0.200	0.20	100	
150%	150.00	0.300	0.30	100	100
	150.00	0.300	0.30	100	
	150.00	0.300	0.30	100	

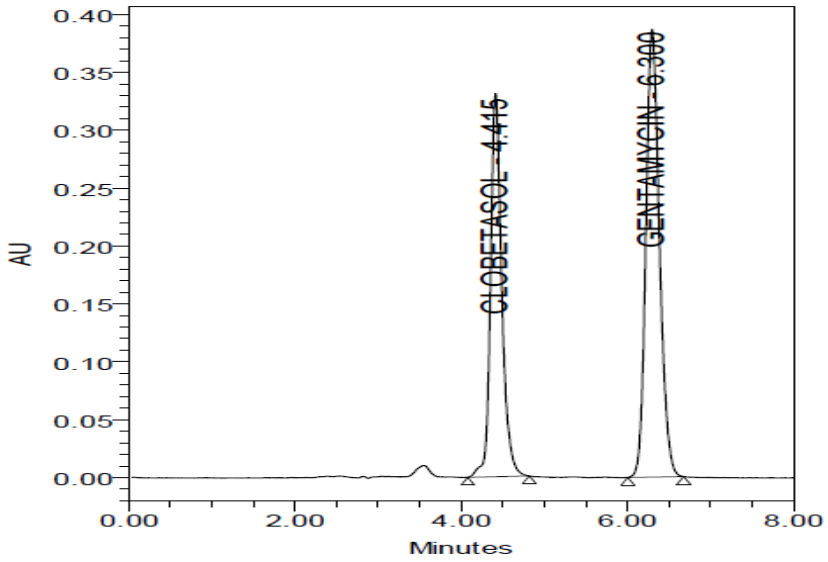

Figure 7: Chromatogram of gentamicin and clobetasol at 50% accuracy level

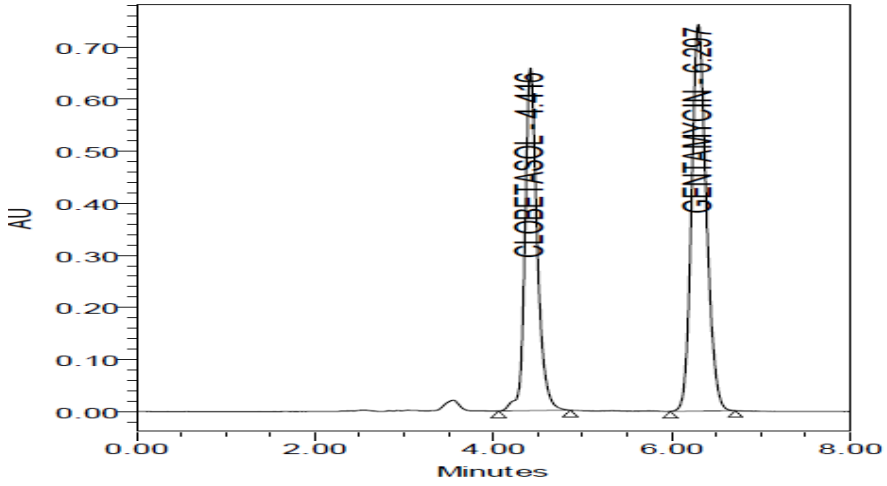

Figure 8: Chromatogram of gentamicin and clobetasol at 100% accuracy level

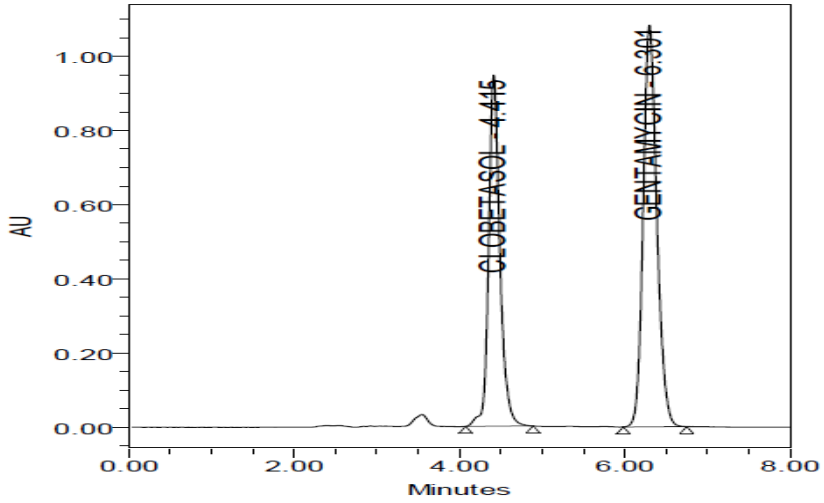

Figure 9: Chromatogram of gentamicin and clobetasol at 150% accuracy level.

Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions. In all the deliberate varied chromatographic conditions, the parameters like tailing factor, peak area and theoretical plates were not much affected, which shows that the method is robust. The results are shown in Table 6 and 7.

Table 6: Robustness for gentamicin

Sample No.	Sample Name	RT	Area	Theoretical plates	USP Tailing
1	Temp-1	8.598	13424277	6645	1.18
2	Temp-2	5.372	7081724	6872	1.19
3	Flow-1	7.857	10559955	7698	1.17
4	Flow-2	5.274	7123545	6356	1.21

Table 7: Robustness for clobetasol

Sample No.	Sample Name	RT	Area	Theoretical plates	USP Tailing
1	Temp-1	5.412	10257261	4809	1.25
2	Temp-2	3.771	5138485	5480	1.21
3	Flow-1	5.496	7721139	5963	1.27
4	Flow-2	3.682	5261439	4868	1.27

Limit of detection (LOD), limit of quantification (LOQ)

The LOD of gentamicin and clobetasol was found to be 0.3525 and 0.1938 µg/mL and the LOQ of gentamicin and clobetsol was 1.1751 and 0.6460 µg/mL, respectively. The chromatograms are shown in Figures 10 and 11. The results are summarized in Table 8. The results indicate that the method possess adequate sensitivity for the determination of gentamicin and clobetasol.

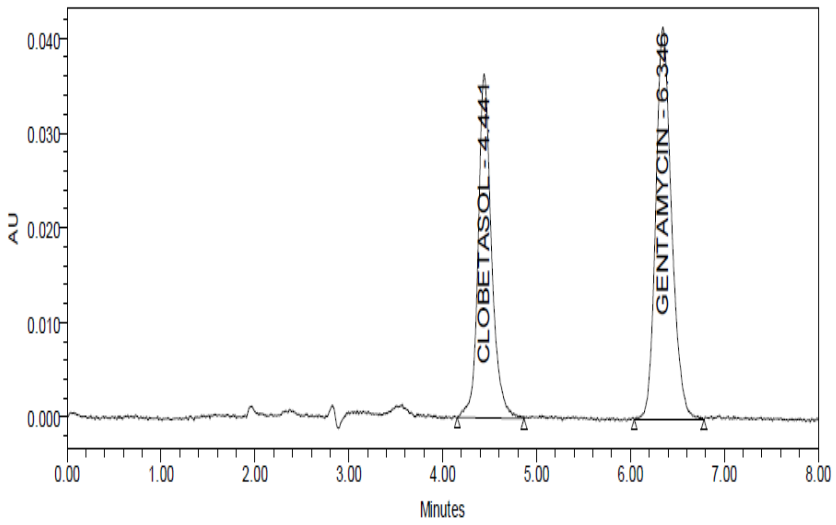

Figure 10: Chromatogram for LOD

Table 8: LOD and LOQ for gentamicin and clobetasol

Sample No.	Sample Type	Sample Name	RT	Area
1	LOD	Gentamicin	6.346	485478
2	LOQ	Gentamicin	6.315	955782
1	LOD	Clobetsol	4.441	358862
2	LOQ	Clobetsol	4.422	659488

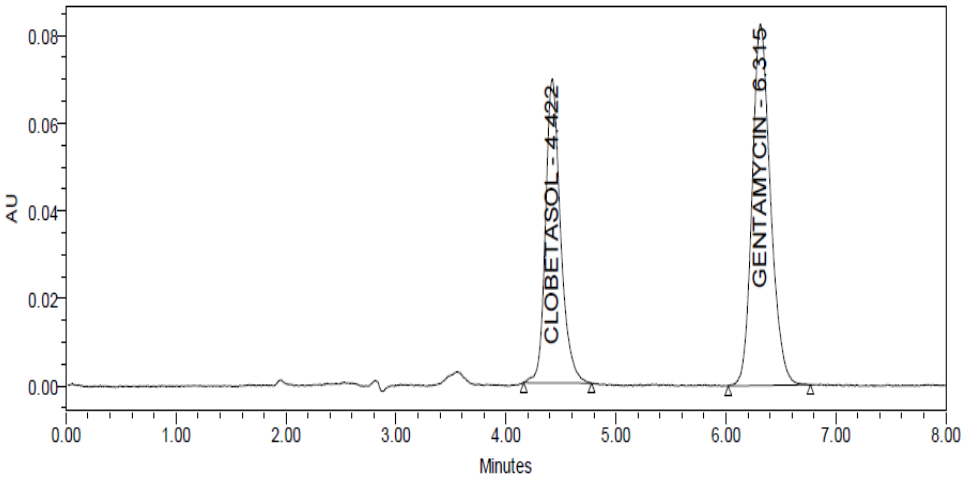

Figure 11: Chromatogram for LOQ

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of gentamicin and clobetasol pharmaceutical formulations. Hence, this method can easily and conveniently adopt for routine quality control analysis of gentamicin and clobetsol in pure and in its pharmaceutical formulations.

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