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Analytical Method Development and Validation by RP-HPLC for Simultaneous Estimation of Moxifloxacin and Prednisolone in Bulk Drug and Pharmaceutical Dosage

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A B S T R A C T

A simple, specific, and fast stability indicating reverse phase liquid chromatographic method was established for instantaneous determination of Moxifloxacin and prednisolone in bulk drugs and pharmaceutical formulations. For the mobile phase, the first variable to be decided is whether an organic or aqueous eluent should be used. With the RP-HPLC analysis, either an aqueous eluent or a very polar organic solvent such as methanol or acetonitrile should be fixed. If the K' values are too large with an aqueous solvent, organic solvent should be tried. If the K' value are too low with organic solvent the separation should be attempted using a mixture of two solvents with various properties. Optimum chromatographic separations among the Moxifloxacin, prednisolone and stress-induced degradation products were achieved within 10 minutes by use of BDS Hypersil C18 column (250 X 4.6 mm, 5 µm) as stationary phase with mobile phase Methanol HPLC Grade, Acetonitrile HPLC Grade. Detection was performed at 248 nm using diode array detector. The method was validated in accordance with ICH guidelines. Response was a linear function of concentrations over the range of 20–80 µg mL-1 for Moxifloxacin ($r2 \geq 0.998$) and 40–160 µg mL-1 for prednisolone ($r2 \geq 0.998$). The method was resulted in good separation of both the analytes and degradation products with acceptable tailing and resolution. The peak purity index for both the analytes after all types of stress conditions was ≥ 0.9999 indicated a complete separation of both the analyte peaks from degradation products. The method can therefore, be regarded as stability indicating.

Keywords: Reverse phase liquid chromatography, moxifloxacin, prednisoloneacetate, Degradation products, ICH guidelines

A R T I C L E I N F O

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1. Introduction

Pharmaceutical Analysis plays a very vital role in the quality assurance and quality control of bulk drugs and their formulations [1,2]. Pharmaceutical analysis is a specialized branch of analytical chemistry which involves separating, identifying and determining the relative amounts of components in a sample of matter [3,4]. It is concerned with the chemical characterization of matter both quantitative and qualitative. In recent years, several analytical techniques have been evolved of a monochromatic light by colorless compounds in the near ultraviolet path of spectrum (200-380nm [5,6].

Moxifloxacin is chemically known as 1-cyclopropyl-7-[(1S,6S)-2,8-diazabicyclo [4.3.0] nonan-8-yl]-6-fluoro-8-methoxy-4-oxoquinoline-3-carboxylic acid [7,8]. It is mainly used for treating eye infections caused by certain bacteria [9]. The bactericidal action of Moxifloxacin results from inhibition of the enzymes topoisomerase II (DNA Gyrase) and topoisomerase IV [10]. Prednisolone is chemically known as 11β)-11, 17, 21-trihydroxypregna-1, 4-diene-3,20-dione [11,12]. Glucocorticoids such as Prednisolone can inhibit leucocyte infiltration at the site of inflammation, interfere with mediators of inflammatory response, and suppress humoral immune responses [13,14,15].

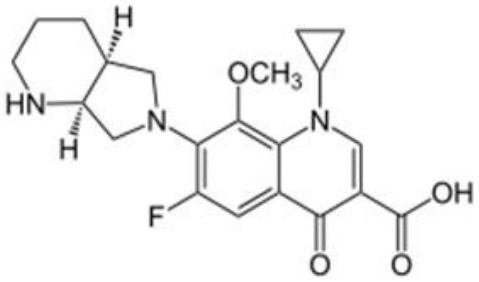

Figure 1: Structure of Moxifloxacin

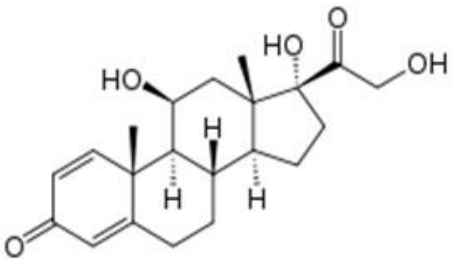

Figure 2: Structure of Prednisolone

2. Materials and Methods

Instruments:

HPLC –Waters Model NO.2690/5 series Compact System Consisting of

- Inertsil-C18 ODS column.
- Electronic balance (SARTORIOUS)
- Sonicator (FAST CLEAN)

Chemicals:

- Methanol HPLC Grade.
- Water HPLC Grade.

Raw Material:

Metformin and Vildagliptin Working Standards.

Method Development for HPLC:

The objective of this experiment was to optimize the assay method for simultaneous estimation of Metformin and Vildagliptin on the literature survey made. So here the trials mentioned describes how the optimization was done.

Trial: 1

Mobile Phase: Degassed Methanol and Water 90:10 V/V.

Preparation of Standard Solution:

Weigh down 10mg's of Moxifloxacin and Prednisolone Acetate drugs and dissolved in 10ml of Mobile phase taken in two 10ml of volumetric flasks separately and sonicated for 20 minutes to get 1000ppms and 1 ml was taken from each solution into a 10ml volumetric flask and diluted to 10 ml with mobile phase.

Chromatographic Conditions:

Flow rate : 1.0ml/min
Column : Inertsil - C18, BDS column
Detector wavelength: 248nm
Column temp : Ambient
Injection volume : 20µl
Run time : 10min
Retention time : 2.6min for Moxifloxacin and 3.06 for Prednisolone Acetate.

Observation: Two peaks are merged and not separated completely. The trial 1 chromatogram result was shown in Fig:3.

Trail: 2

Mobile Phase: Degassed Acetonitrile and Water in the ratio of 90:10 V/V.

Preparation of Standard Solution:

Weigh down 10mg's of Moxifloxacin and Prednisolone Acetate drugs and dissolved in 10ml of Mobile phase taken in two 10ml of volumetric flasks separately and sonicated for 20 minutes to get 1000ppms and 1 ml was taken from each solution and diluted to 10 ml with mobile phase.

Chromatographic Conditions:

Flow rate : 1ml/min
Column : Inertsil -C18, BDS column
Detector wavelength: 248nm
Column temp : Ambient
Injection volume : 20µl
Run time : 10min
Retention time : 2.6 min for Moxifloxacin and 4.1 min for Prednisolone Acetate.

Observation:

The two peaks are separated completely but peak shapes are not good. The trial 2 chromatogram result was shown in Fig: 4.

Trail: 3

Mobile Phase: Degassed Acetonitrile and Methanol in the ratio of 80:20 V/V.

Preparation of Standard Solution:

Weigh down 10mg's of Moxifloxacin and Prednisolone Acetate drugs and dissolved in 10ml of Mobile phase taken in two 10ml of volumetric flasks separately and sonicated for 20 minutes to get 1000ppms and 1 ml was taken from each solution and diluted to 10 ml with mobile phase.

Chromatographic Conditions:

Flow rate : 1.0ml/min
Column : Inertsil - C18, BDS column
Detector wavelength : 248 n.m
Column temp : Ambient
Injection volume : 20µl
Run time : 10min
Retention time : 2.7 min for Moxifloxacin and 3.1 min for Prednisolone Acetate.

Observation: Prednisolone Acetate and Moxifloxacin got peak fronting and base line between two peaks is not straight. The trial 3chromatogram result was shown in Fig:5.

Optimized Method

Mobile Phase: Degassed Methanol: Acetonitrile in the ratio of 80:20 V/V.

Preparation of stock solution:

Reference solution: The solution was prepared by dissolving 20.0 mg of accurately weighed Moxifloxacin and 20.0 mg Prednisolone Acetate in Mobile phase, in two 100.0 mL volumetric flasks separately and sonicate for 20min. From the above solutions take 10.0 mL from each solution into a 50.0 mL volumetric flask and then makeup with mobile phase and sonicate for 10min.

Preparation of working standard solution: The stock solutions equivalent to 20ppm to 80ppm with respect to both drugs were prepared in combination of Moxifloxacin and Prednisolone Acetate above, sonicated and filtered through 0.45µ membrane.

Observation:

Prednisolone Acetate and Moxifloxacin got peak fronting and base line between two peaks is straight. The optimization chromatogram result was shown in Fig:6.

Table 1: Optimized chromatographic conditions

Parameters	Method
Stationary phase (column)	Inertsil -ODS C18(250 x 4.6 mm, 5 µ)
Mobile Phase	Methanol : Acetonitrile (80:20)
Flow rate (ml/min)	1.0 ml/min
Run time (minutes)	10 min
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	248nm
Drug RT (min)	2.762min for Moxifloxacin and 3.161 for Prednisolone Acetate.

Method Validation

System Suitability:

A Standard solution was prepared by using Moxifloxacin and Prednisolone Acetate working standards as per test method and was injected Five times into the HPLC system. The system suitability parameters were evaluated from standard chromatograms by calculating the % RSD from five replicate injections for Moxifloxacin and Prednisolone Acetate, retention times and peak areas.

Acceptance Criteria:

a. The % RSD for the retention times of principal peak from 5 replicate injections of each Standard solution should be not more than 2.0 %
b. The % RSD for the peak area responses of principal peak from 5 replicate injections of each standard Solution should be not more than 2.0%.
c. The number of theoretical plates (N) for the Moxifloxacin and Prednisolone Acetate peaks is NLT 3000.
d. The Tailing factor (T) for the Moxifloxacin and Prednisolone Acetate peaks is NMT 2.0

Observation:

The %RSD for retention times and peak areas were found to be within the limit. As shown in fig:8,9,10& 11,12. And the table shown in fig:7&8.

Specificity:

Moxifloxacin and Prednisolone Acetate:

Solutions of standard and sample were prepared as per the test method are injected into chromatographic system.

Acceptance criteria:

Chromatograms of standard and sample should be identical with near Retention time.

Observation:

The chromatograms of Standard and Sample were same identical with same retention time.

Precision:

Repeatability:

System precision: Standard solution prepared as per test method and injected five times.

Method precision: Prepared six sample preparations individually using single as per test method and injected each solution.

Acceptance criteria:

The % relative standard deviation of individual Moxifloxacin and Prednisolone Acetate, from the six units should be not more than 2.0%.The individual assays of Moxifloxacin and Prednisolone Acetate should be not less than 98% and not more than 102.0%.

Observation:

Test results are showing that the test method is precise. Intermediate precision (analyst to analyst variability): A study was conducted by two analysts as per test method

Acceptance criteria: The individual assay of Moxifloxacin and Prednisolone Acetate should be not less than 98% and not more than 102% and %RSD of assays should be NMT2.0% by both analysts.

Observation:

Individual %assays and %RSD of Assay are within limit and passes the intermediate precision.

Accuracy (Recovery):

A study of Accuracy was conducted. Drug Assay was performed in triplicate as per test method with equivalent amount of Moxifloxacin and Prednisolone Acetate into each volumetric flask for each spike level to get the concentration of Moxifloxacin and Prednisolone Acetate equivalent to 50%, 100%, and 150% of the labeled amount as per the test method. The average % recovery of Moxifloxacin and Prednisolone Acetate were calculated.

Acceptance criteria: The mean % recovery of the Moxifloxacin and Prednisolone Acetate at each spike level should be not less than 98.0% and not more than 102.0% for both the drugs separately.

Observation

$$\% \text{ Recovery} = \frac{\text{Amount found}}{\text{Amount added}} \times 100$$

Linearity of Test Method:

A Series of solutions are prepared using Moxifloxacin and Prednisolone Acetate working standards at concentration levels from 20ppm to 80 ppm of target concentration .Measure the peak area response of solution at Level 1 and Level 6 six times and Level 2 to Level 5 two times.

Acceptance criteria:

Correlation Coefficient should be not less than 0.9990.

% of y- Intercept should be ±2.0.

% of RSD for level 1 and Level 6 should be not more than 2.0%.

Observation:

The linear fit of the system was illustrated graphically.

Ruggedness of Test Method:

a) System to system variability:

System to system variability study was conducted on different HPLC systems, under similar conditions at different times. Six samples were prepared and each was analyzed as per test method. Comparison of both the results obtained on two different HPLC systems, shows that the assay test method are rugged for System to system variability.

Acceptance criteria:

The % relative standard deviation of Moxifloxacin and Prednisolone Acetate from the six sample preparations should be not more than 2.0%.The % assay of Moxifloxacin and Prednisolone Acetate should be between 98.0%-102.0%.

Observation:

The % RSD was found within the limit.

Column to column variability:

Column to column variability study was conducted by using different columns. Six samples were prepared and each was analyzed as per test method

Acceptance criteria:

The %RSD of Moxifloxacin and Prednisolone Acetate tablets should be NMT2.0%. The %assay of Moxifloxacin and Prednisolone Acetate should be between 98.0% and 102.0% for individual drugs.

Observation: The results obtained by comparing with both two types were within limit.

Robustness:

a) Effect of variation of flow rate:

A study was conducted to determine the effect of variation in flow rate. Standard solution prepared as per the test method was injected into the HPLC system using flow rates, 1.0ml/min and1.2ml/min. The system suitability parameters were evaluated and found to be within the limits for 1.0ml/min and 1.2ml/min flow. Moxifloxacin and Prednisolone Acetate and was resolved from all other peaks and the retention times were comparable with those obtained for mobile phase having flow rates 1.0ml/min.

Acceptance criteria:

The Tailing Factor of Moxifloxacin and Prednisolone Acetate standards should be NMT 2.0 for Variation in flow.

Observation: The tailing factor for Moxifloxacin and Prednisolone Acetate was found to be within the limits.

b) Effect of variation of temperature:

A study was conducted to determine the effect of variation in temperature. Standard solution prepared as per the test method was injected into the HPLC system at 20ºC temperature. The system suitability parameters were evaluated and found to be within the limits for a temperature change of 20ºc. Similarly sample solution was chromatographed at 25ºC temperature. Moxifloxacin and Prednisolone Acetate were resolved from all other peaks and the retention times were comparable with those

Acceptance criteria:

The Tailing Factor of Moxifloxacin and Prednisolone Acetate standard and sample solutions should be NMT 2.0 for Variation in temperature.

Observation: The tailing factor for Moxifloxacin and Prednisolone Acetate. x is found to be within the limits.

$$\text{LOD} = \frac{3.3\,\sigma}{S}$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

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

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

3. Results and Discussion

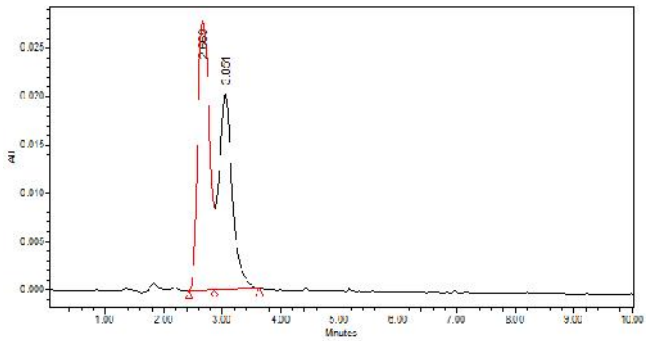

Figure 3: Chromatogram of Trial I

Table 2: Retention Time of Moxifloxacin and Prednisolone Acetate

S.NO	Name of the peak	Retention time (min)
1	Moxifloxacin	2.689
2	Prednisolone Acetate	3.061

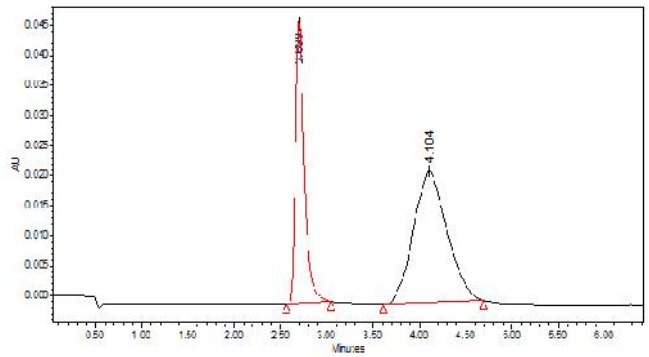

Figure 4: Chromatogram of Trial II

Table 3: Retention Time of Moxifloxacin and Prednisolone Acetate

S.NO	Name of the peak	Retention time (min)
1	Moxifloxacin	2.699
2	Prednisolone Acetate	4.104

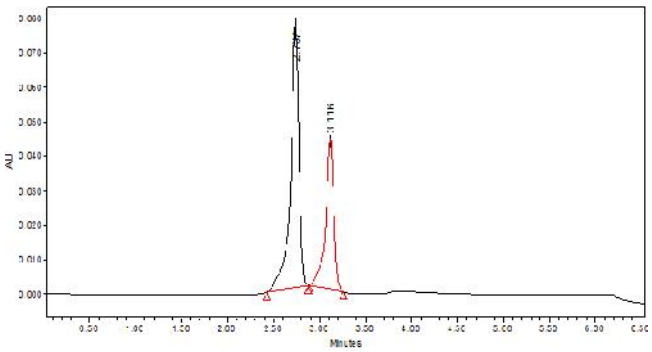

Figure 5: Chromatogram of Trial III

Table 4: Retention Time of Moxifloxacin and Prednisolone Acetate

S.NO	Name of the peak	Retention time (min)
1	Moxifloxacin	2.707
2	Prednisolone Acetate	3.116

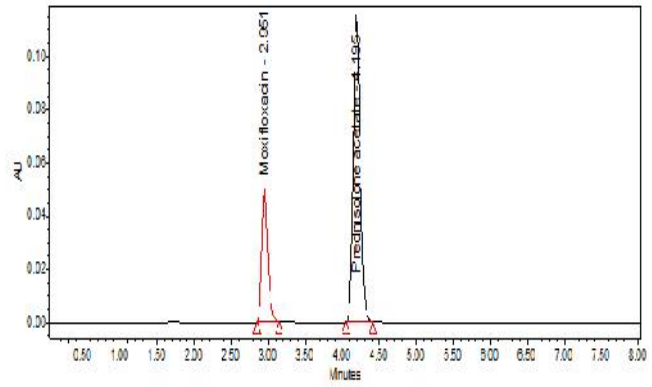

Figure 6: Chromatogram of standard

Table 5: Retention Time of Moxifloxacin and Prednisolone Acetate

S.NO	Name of the peak	Retention time (min)
1	Moxifloxacin	2.951
2	Prednisolone Acetate	4.193

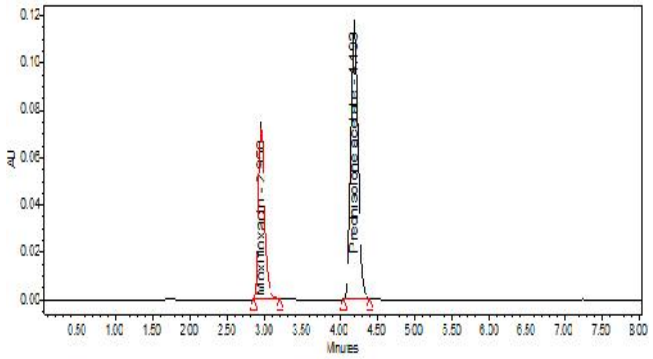

Figure 7: Chromatogram of sample

Table 6: Retention Time of Moxifloxacin and Prednisolone Acetate

S.NO	Name of the peak	Retention time (min)
1	Moxifloxacin	.950
2	Prednisolone Acetate	4.193

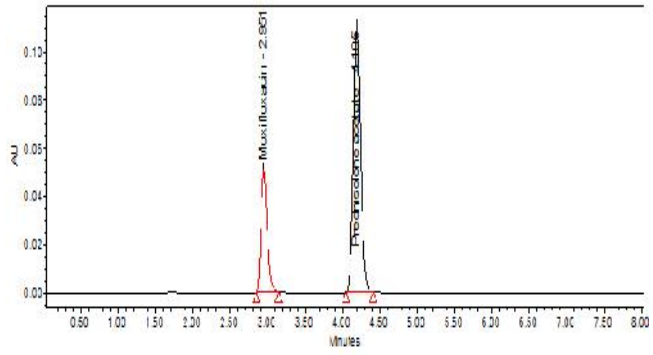

Figure 8: System suitability Chromatogram for std–1

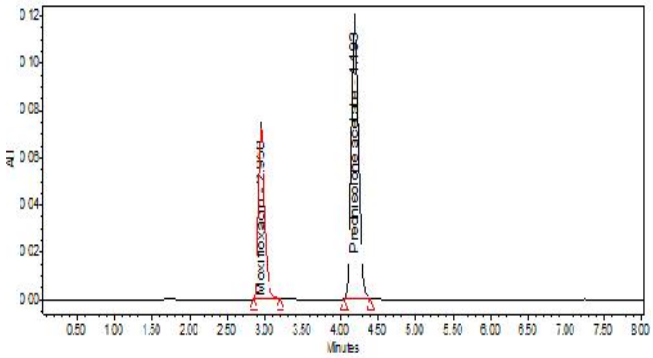

Figure 9: System suitability Chromatogram for std–2

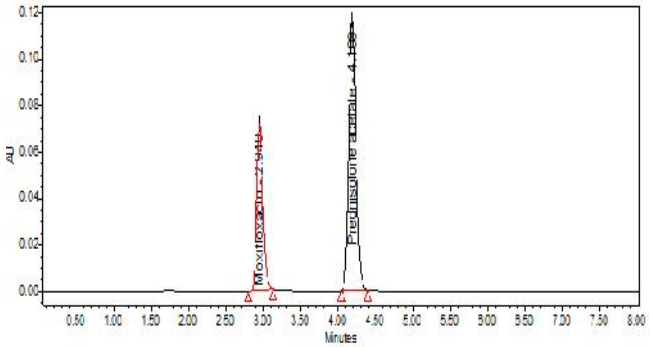

Figure 10: System suitability Chromatogram for std–3

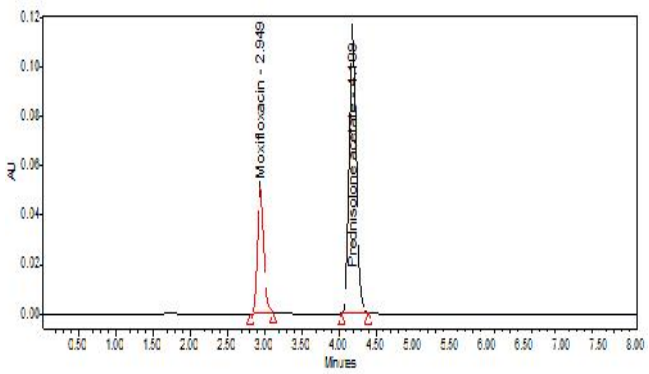

Figure 11: System suitability Chromatogram for std–4

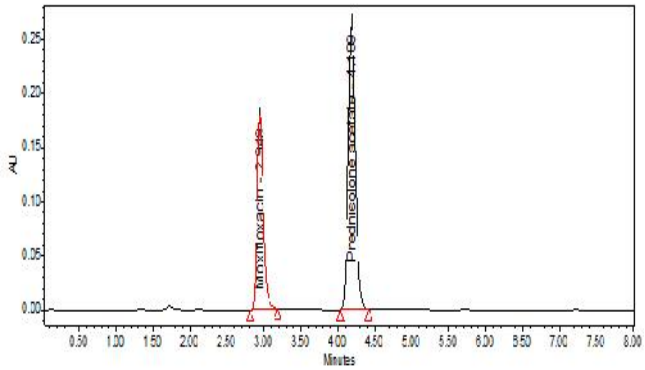

Figure 12: System suitability Chromatogram for std–5

Figure 13: 13 Linearity Plot (Concentration vs. Response) of Moxifloxacin

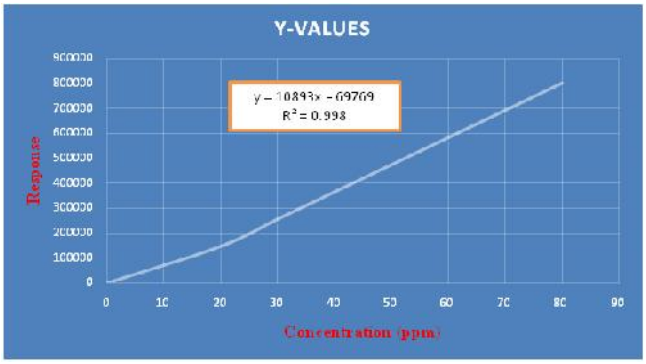

Figure 14: Linearity Plot (Concentration vs. Response) of Prednisolone Acetate

Table 7: Data of System Suitability for Moxifloxacin

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	3.161	2164732	6648.722084	1.119216
2	3.162	2161848	6673.911816	1.142210
3	3.163	2198427	6630.743655	1.167058
4	3.162	2231236	6778.292084	1.140170
5	3.161	2254490	6687.924039	1.132946
Mean	3.1618	2202147	6683.919	1.14032
SD	0.000837	40693.03	-------	-------
% RSD	0.026462	1.84788	-------	-------

Table 8: Data of System Suitability for Prednisolone Acetate

Injection	RT	Peak Area	USP Plate count	USP Tailing
1	2.951	4037216	5368.357111	1.496148
2	2.950	4063397	5441.422408	1.471228
3	2.948	4115511	5413.470928	1.484832
4	2.949	4126557	5357.317249	1.466473
5	2.949	4195611	5398.478881	1.506952
Mean	2.948546	4107658	5395.809	1.485127
SD	0.00114	61391.54	-------	-------
% RSD	0.041257	1.494563	-------	-------

Table 9: Data of Accuracy for Moxifloxacin

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50%Injection 1	20	20.04	100.22	**MEAN**	100.06
50%Injection 3	20	20.02	100.11	**%RSD**	0.18
100 % Injection 1	40	40.01	100.02	**MEAN**	100.04
100 %Injection 2	40	40.05	100.14		

100%Injection 3	40	39.98	99.96	**%RSD**	0.091
150%Injection 1	60	60.08	100.14	**MEAN**	100.02
150%Injection 2	60	59.97	99.96		
150%Injection 3	60	59.98	99.98	**%RSD**	0.09

Table 10: Data of Accuracy for Prednisolone Acetate

Concentration % of spiked level	Amount added (ppm)	Amount found (ppm)	% Recovery	Statistical Analysis of % Recovery	
50%Injection 1	20	20.15	100.75	**MEAN**	99.69333
50%-Injection3	20	19.80	99.02	**% RSD**	0.92
100 %Injection 1	40	39.88	99.70	**MEAN**	99.83333
100 %Injection 2	40	40.12	100.30		
100%Injection 3	40	39.80	99.50	**%RSD**	0.41
150%Injection 1	60	60.12	100.21	**MEAN**	99.97333
150%Injection 2	60	59.76	99.61		
150%Injection 3	60	60.06	100.10	**%RSD**	0.31

Table 11: Data of Linearity (Moxifloxacin)

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	10310
20	560960	y-Intercept	540.63
30	1602034	Correlation Coefficient	0.999

Table 12: Data of Linearity (Prednisolone Acetate)

Concentration (ppm)	Average Area	Statistical Analysis	
0	0	Slope	10893
20	2110652	y-Intercept	69769
30	3250149	Correlation Coefficient	0.998

4. Conclusion

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Moxifloxacin and Prednisolone acetate was done by RP-HPLC. Chromatographic conditions used are stationary phase Methanol, Mobile phase acetonitrile in the ratio of 80-20 and flow rate was maintained at 1ml/min, detection wave length was 288nm for Moxifloxacin and 247 for prednisolone acetate, Inertsil-C18 ODS column. Temperature was set to ambient and water is used as diluents. Linearity study was carried out between 50% to150 % levels. Retention time of moxifloxacin and prednisolone acetate were found to be 2.7 min and 3.1min. %RSD of the moxifloxacin and prednisolone acetate were and found to be not more than 2.0%. %Recover was Obtained as not less than 98.0% and not more than 102.0% for moxifloxacin and prednisolone acetate respectively. LOD, LOQ values are obtained from regression equations of moxifloxacin and prednisolone acetate were 20ppm, 40ppm and 60ppm, 80ppm respectively. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries. The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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