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ASSAY METHOD DEVELOPMENT AND VALIDATION OF MINOXIDIL FILM-COATED TABLETS.

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Abstract
A robust and precise reverse-phase high-performance liquid chromatographic (RP-HPLC) method has been developed and validated for the quantitative determination of Minoxidil in film-coated tablets. Chromatographic separation was achieved using an X-Bridge C18 column (150 mm x 4.6 mm ID, 5 µm). The mobile phase consisted of a buffer and acetonitrile mixture in a 55:45 (v/v) ratio. The diluent was prepared using water and acetonitrile in a 50:50 (v/v) ratio, while the needle wash solution was a 90:10 (v/v) acetonitrile-water mixture. The method employed a flow rate of 1.0 mL/min, a detection wavelength of 280 nm, an injection volume of 10 µL, and a column temperature of 40°C in isocratic pump mode. Minoxidil had a retention time of 6.6 minutes, with a total run time of 10 minutes. The assay exhibited a Minoxidil percentage range of 97%-102%, with an r^2 value of 1.000 for the concentration range tested. The %RSD values were less than 2%, indicating high accuracy and precision. The method demonstrated robustness within acceptable limits and was validated in accordance with ICH Q2 guidelines.

Keywords: Minoxidil, RP-HPLC, Chromatographic Method, Assay Development, Method Validation

Introduction

High Performance Liquid Chromatography (HPLC) is a pivotal analytical technique in the pharmaceutical industry, renowned for its capability to detect, separate, and quantify a wide range of compounds, including active pharmaceutical ingredients (APIs), impurities, and degradation products [1]. This technique is indispensable throughout the stages of drug development, from discovery through production, ensuring that products meet stringent quality standards. The development and validation of robust analytical methods are essential for the accurate and reliable determination of drug compounds in pharmaceutical formulations. A key objective in method development is to establish a procedure that is simple, economical, and feasible while delivering high sensitivity, precision, and accuracy. The choice of chromatographic parameters, such as mobile phase composition, column type, column temperature, detection wavelength, and pump mode, plays a critical role in achieving these objectives [2].

The focus of this research is on the development and validation of an HPLC method for quantifying Minoxidil in film-coated tablets. Minoxidil is widely used for treating hypertension and pattern hair loss, acting through multiple pathways including vasodilation and anti-inflammatory mechanisms. Given the clinical significance of Minoxidil, a reliable analytical method is crucial for ensuring the efficacy and safety of Minoxidil-containing formulations. Existing literature on HPLC methods for Minoxidil primarily addresses its estimation in various formulations, but there is a notable gap in the development of a method specifically for film-coated tablets [3, 4, 5]. This research aims to fill that gap by optimizing chromatographic conditions and validating the method according to International Conference on Harmonization (ICH) guidelines.

The aim of this study is to develop and validate a precise and robust reverse-phase high-performance liquid chromatographic (RP-HPLC) method for the quantitative determination of Minoxidil in film-coated tablets. The objectives include optimizing chromatographic conditions for Minoxidil using RP-HPLC, validating the developed method as per ICH guidelines, and determining the Minoxidil concentration in film-coated tablet formulations. The research plan involves a systematic approach beginning with the selection of Minoxidil as the target drug, followed by method optimization, development, and application to tablet assays, studying the drug release profile, and final method verification and validation. This structured approach ensures the development of a reliable, accurate, and efficient RP-HPLC method for the assay of Minoxidil in film-coated tablets, adhering to stringent validation criteria to meet regulatory standards.

Experimental Work

Solvents and Chemicals Used:
The solvents and chemicals employed in this study were of high purity and suitable for HPLC analysis. The chemicals used included methanol, glacial acetic acid, sodium lauryl sulfate, sulfo succinate sodium, potassium hydrogen phosphate, and sodium hydroxide. Specifically, methanol and glacial acetic acid were of HPLC grade, ensuring their suitability for highperformance liquid chromatography. Sodium lauryl sulfate was of MilliQ grade, while sulfo succinate sodium, potassium hydrogen phosphate, and sodium hydroxide were all of HPLC grade from Merck, a trusted supplier of highquality chemicals. These highpurity reagents ensured the accuracy and reliability of the experimental results.

Experiment No: 1 (Column and Chromatographic Optimization)
Background: Initial chromatographic conditions were adapted from the Minoxidil tablets USP monograph Assay by HPLC.
Objective: To optimize the chromatographic conditions for the Assay by HPLC method.
Chromatographic Conditions:
The chromatographic analysis was carried out using an XBridge C18 column (150 x 4.6 mm, 5 µm) or an equivalent column. The flow rate was set to 1.2 mL/min, with detection performed at a wavelength of 230 nm. A sample injection volume of 10 µL was used, and data acquisition was conducted over a period of 60 minutes. The column temperature was maintained at 40°C, while the sample temperature was kept at ambient conditions. The pump operated in isocratic mode throughout the analysis.
Buffer Preparation:
Mobile Phase: Mixed 1200 mL of methanol and 800 mL of water, stirred to mix, added 20 mL of glacial acetic acid and 4.0 grams of sodium lauryl sulfate, stirred to dissolve, and adjusted pH to 3.02 with glacial acetic acid.
Diluent: Mixed water and methanol in the ratio of 50:50 v/v.
Standard Preparation:
Weighed and transferred 25.7147 mg of Minoxidil WS into a 50 mL volumetric flask, added 30 mL of diluent, sonicated to dissolve, and made up to volume with diluent. Diluted 5 mL to 50 mL with diluent.
Sample Preparation:
Weighed 10 tablets into a 200 mL volumetric flask, added 130 mL of diluent, sonicated for 15 minutes on a rotary shaker, made up to volume with diluent, filtered through a 0.45 µm Whatman PVDF filter, discarded the first 5 mL of filtrate, and diluted 5 mL to 50 mL with diluent.
Observation and Conclusion:
The system suitability parameters were good with the XBridge C18 (150 x 4.6 mm, 5 µm) column, while poor peak shape was observed with Novapak C18 (150 x 4.6 mm, 5 µm) and Kinetic columns. Further trials are required to select better peak shapes.

Experiment No: 2 (Column and Chromatographic Optimization)
Objective: To optimize the chromatographic conditions for the Assay by HPLC method.
Chromatographic Conditions:
The chromatographic conditions utilized an XBridge C18 column (150 x 4.6 mm, 5 µm) with a flow rate of 1.2 mL/min. Detection was carried out at 230 nm, and the injection volume was 10 µL. Data acquisition time was set to 60 minutes. The column temperature was maintained at 40°C, with the sample temperature kept at ambient levels. The pump mode was isocratic throughout the analysis.
Buffer Preparation:
Mobile Phase: Mixed 2400 mL of methanol and 1600 mL of water, stirred to mix, added 40 mL of glacial acetic acid and 8.073 grams of sodium lauryl sulfate in 5000 mL MilliQ water, stirred to dissolve, and adjusted pH to 3.02 with glacial acetic acid.
Diluent: Mixed water and methanol in the ratio of 50:50 v/v.
Standard Preparation:
Weighed and transferred 25.02 mg of Minoxidil WS into a 50 mL volumetric flask, added 30 mL of diluent, sonicated to dissolve, and made up to volume with diluent. Diluted 5 mL to 50 mL with diluent.
Sample Preparation:
Weighed 10 tablets into a 200 mL volumetric flask, added 130 mL of diluent, sonicated for 15 minutes on a rotary shaker at 150 rpm, made up to volume with diluent, filtered through a 0.45 µm Whatman PVDF filter, discarded the first 5 mL of filtrate, and diluted 5 mL to 50 mL with diluent.

Experiment No: 3 (Mobile Phase Optimization)
Objective: To optimize the chromatographic conditions for the Assay by HPLC method.
Chromatographic Conditions:
The chromatographic conditions involved the use of both XBridge C18 columns (150 x 4.6 mm, 5 µm and 250 x 4.6 mm, 5 µm) with a flow rate of 0.8 mL/min. Detection was performed at 230 nm, with an injection volume of 10 µL.

The data acquisition time was set to 35 minutes. The column temperature was maintained at 40°C, while the sample temperature was kept at ambient levels. An isocratic pump mode was used throughout the analysis.
Buffer Preparation:
Mobile Phase: Mixed 2250 mL of methanol and 2750 mL of water, added 5 mL of trifluoroacetic acid and 10.04 grams of sodium heptane sulfonic acid, mixed well, filtered through a 0.22 µm membrane filter, and degassed.
Diluent: Mixed water and methanol in the ratio of 50:50 v/v.
Standard Preparation:
Weighed and transferred 27.17 mg of Minoxidil WS into a 50 mL volumetric flask, added 30 mL of diluent, sonicated to dissolve, and made up to volume with diluent. Diluted 5 mL to 50 mL with diluent.

Experiment No: 4 (Injection Volume Optimization)
Objective: To optimize the chromatographic conditions for the Assay by HPLC method.
Chromatographic Conditions:
The chromatographic conditions included the use of an XBridge C18 column (250 x 4.6 mm, 5 µm) with a flow rate of 1 mL/min. Detection was carried out at 280 nm, with injection volumes of 10 µL and 40 µL. The data acquisition time was set to 40 minutes, and the column temperature was maintained at 40°C. An isocratic pump mode was employed, with the sample temperature kept at ambient conditions.
Buffer Preparation:
Mobile Phase: Mixed 2400 mL of methanol and 1600 mL of water, 40 mL of glacial acetic acid, added 8.0 grams of sodium lauryl sulfate, stirred to dissolve, mixed well, adjusted pH to 3.03 with perchloric acid, filtered through a 0.22 µm membrane filter, and degassed.
Diluent: Mixed water and methanol in the ratio of 50:50 v/v.
Sample Preparation: (1 mg/mL solution)
Weighed 10 tablets into a 100 mL volumetric flask, added 70 mL of diluent, shaken for 20 minutes on a rotary shaker at 150 rpm, made up to volume with diluent, filtered through a 0.45 µm Whatman PVDF filter, discarded the first 5 mL of filtrate.
Injected 1 mg/mL sample solution, 0.002 mg/mL sample solution, individual impurities, and 1% and 5% spiked sample solutions with 10 µL and 40 µL injection volumes.

Experiment No: 5
Objective: To optimize the chromatographic conditions for the Assay by HPLC method for Minoxidil 10 mg tablets.
Chromatographic Conditions:
The chromatographic conditions utilized an XBridge C18 column (150 x 4.6 mm, 5 µm) with a flow rate of 1.0 mL/min. Detection was set at 280 nm, with an injection volume of 10 µL. Data acquisition was conducted over a 10-minute period, and the column temperature was maintained at 40°C. The pump operated in isocratic mode, with the sample temperature kept at ambient conditions, and the sampling rate was 5 points per second.
Buffer Preparation:
Mobile Phase: Transferred 5 grams of Sodium lauryl sulfate into 1000 mL of MilliQ water, added 10 mL of glacial acetic acid, and stirred well.
Mobile Phase: Mixed buffer and acetonitrile in the ratio of 550:450 v/v.
Diluent: Mixed water and acetonitrile in the ratio of 50:50 v/v.
Standard Preparation:
Weighed and transferred 25.31 mg of Minoxidil WS into a 50 mL volumetric flask, added 30 mL of diluent, sonicated to dissolve, and made up to volume with diluent. Diluted 5 mL to 50 mL with diluent.
To ensure the method was robust and reliable, it underwent thorough validation focusing on several key attributes. Specificity was checked to confirm that no impurities or excipients interfered with the detection of Minoxidil. Linearity was verified by plotting peak areas against concentrations and ensuring a correlation coefficient close to 1. Accuracy and precision were assessed by calculating the recovery and relative standard deviation (RSD) from multiple injections. Detection and quantification limits (LOD and LOQ) were determined using the signal-to-noise ratio. Robustness was examined by making slight changes to method parameters and observing their impact on results. Lastly, system suitability was monitored through parameters like resolution, tailing factor, and theoretical plate count.

Results and Discussion
UV Spectra
The typical UV spectra of Minoxidil reveal absorption maxima at approximately 230 nm and 280 nm (Figure 4). This characteristic helps in identifying and confirming the presence of Minoxidil in the sample.

UV SPECTRA:

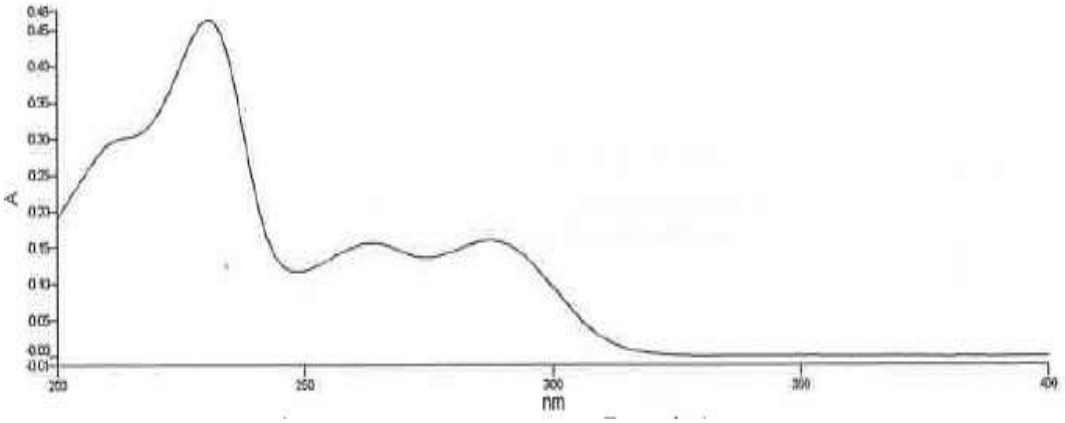

Figure 4. UV Spectra

Spiked Sample Chromatograms

Figures 5 and 6 depict the chromatograms of spiked samples at 1% and 5% levels, respectively. The analysis demonstrated the ability to detect Minoxidil and its impurities at these concentrations.

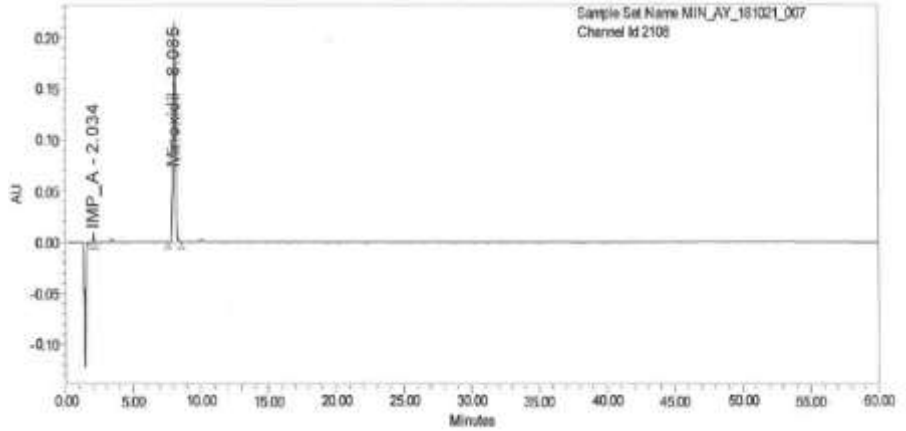

Figure 5. SPIKED SAMPLE CHROMATOGRAM (1% level)

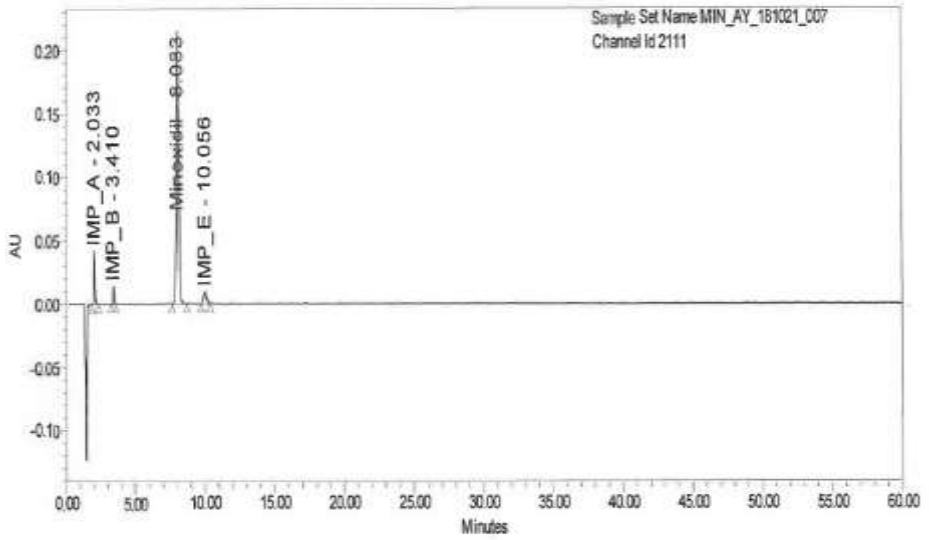

Figure 6. SPIKED SAMPLE CHROMATOGRAM (5% level)

Impurity Chromatograms

Chromatograms for Impurity-A, Impurity-B, and Impurity-E are shown in Figures 7, 8, and 9. The separation of these impurities is crucial for the method's specificity.

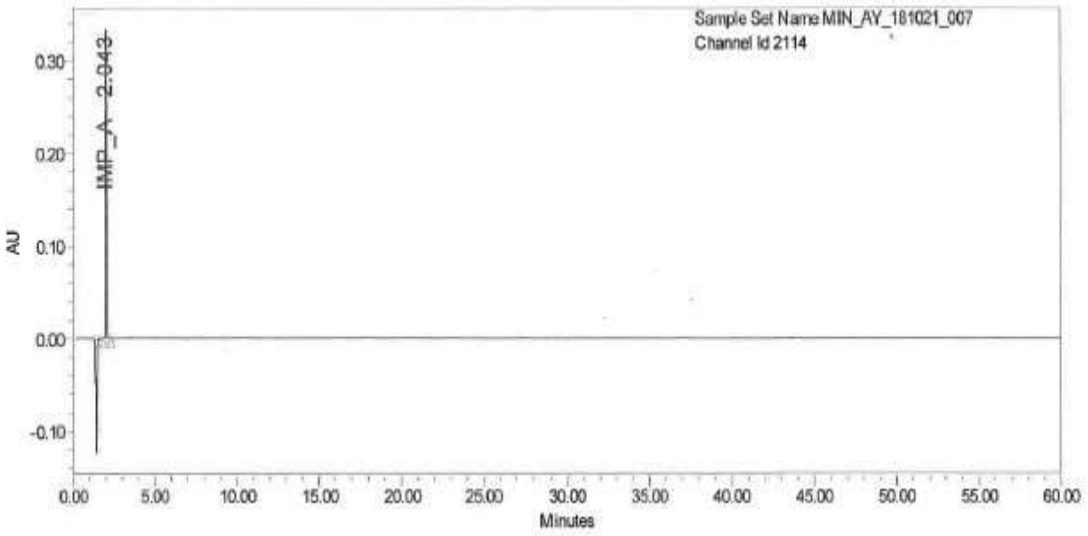

Figure 7. IMPURITY-A CHROMATOGRAM

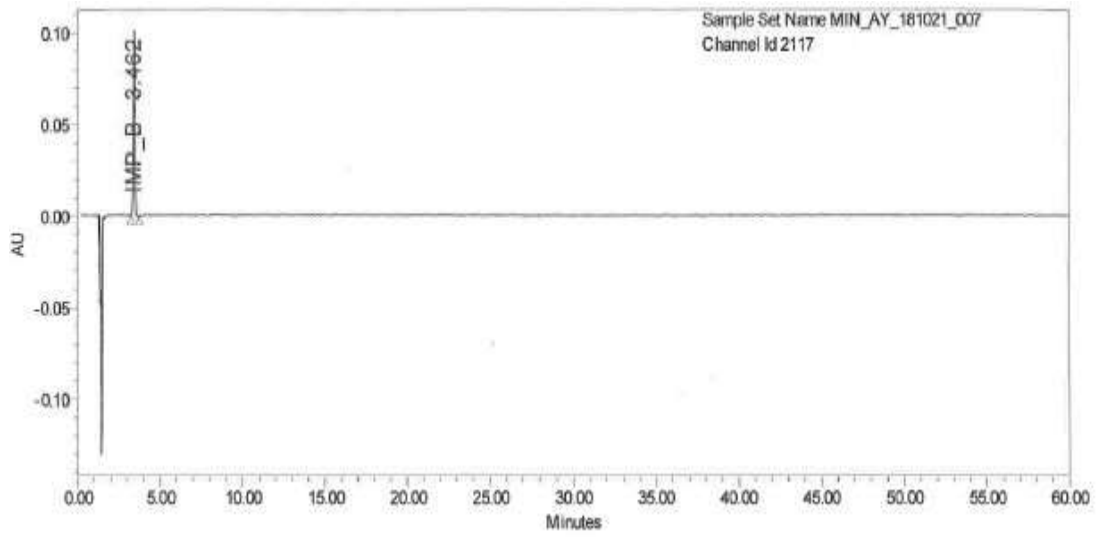

Figure 8. IMPURITY-B CHROMATOGRAM

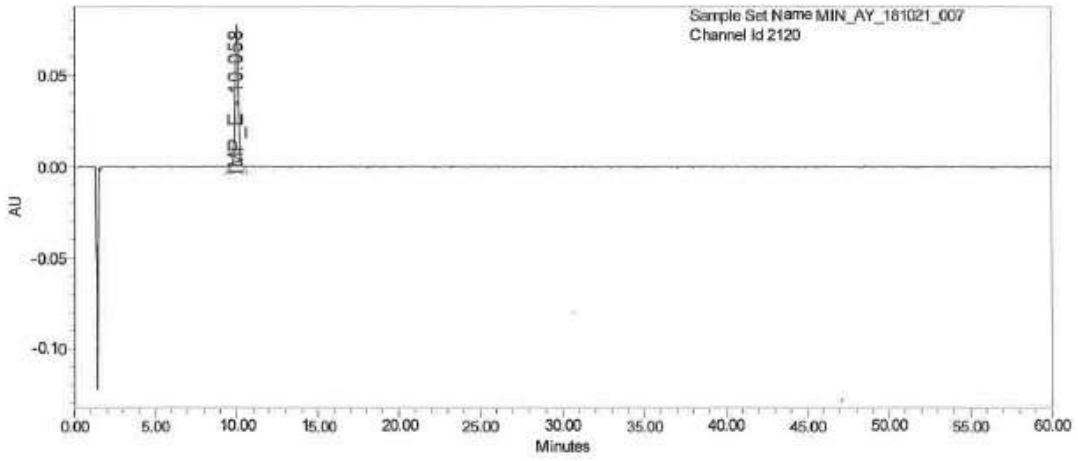

Figure 9. IMPURITY-E CHROMATOGRAM

Column Performance

The spiked sample preparations, as seen in Figures 10 and 11, were injected at 1% and 5% levels using an X-Bridge C18 (150 x 4.6 mm, 5 µm) column and an X-Bridge C18 (250 x 4.6 mm, 5 µm) column, respectively, both at a flow rate of 1.0 mL/min. Observations indicated a negative peak near Impurity-A, suggesting further optimization was needed for chromatographic conditions.

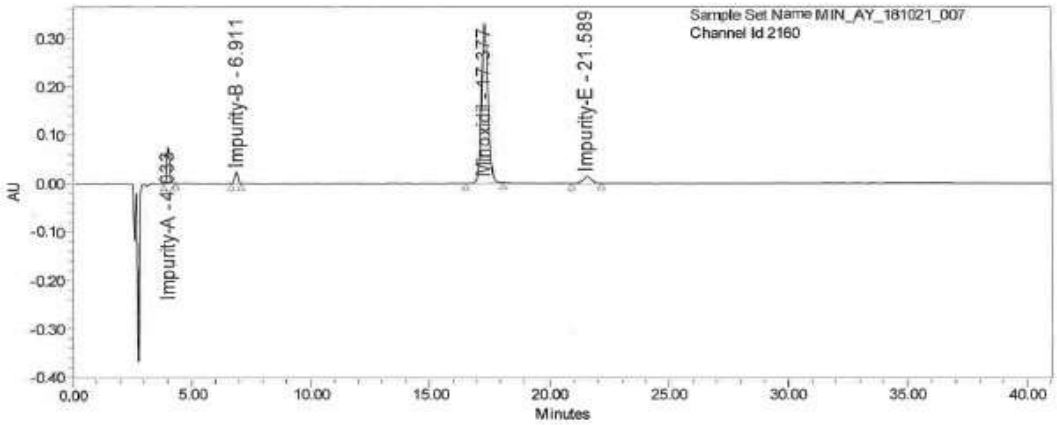

Figure 10. Spiked sample preparation

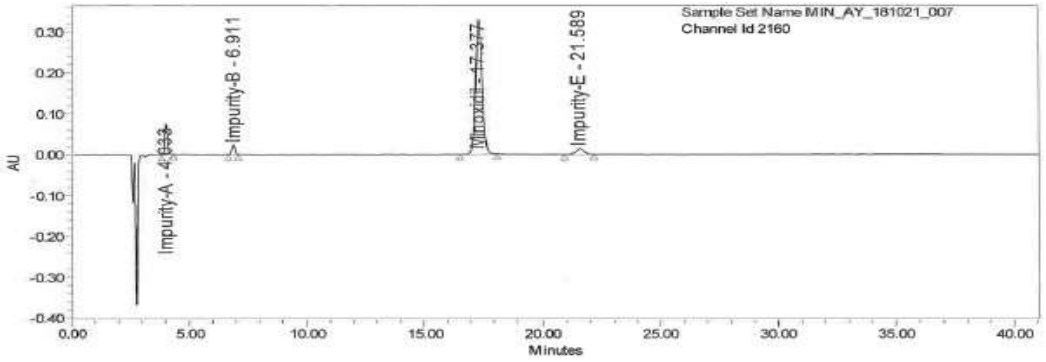

Figure 11. Spiked sample preparation

Figures 12 and 13 compare the performance of the X-Bridge C18 (150 x 4.6 mm, 5 µm) and X-Bridge C18 (250 x 4.6 mm, 5 µm) columns. Impurity-A and Impurity-B were closely eluted at retention times of 2.936 and 3.271 minutes, respectively. The X-Bridge C18 (250 x 4.6 mm, 5 µm) column provided better separation but showed broader peak shapes compared to a mobile phase with Sodium Lauryl Sulfate (SLS) composition.

Observation: A negative peak is eluting near to Impurity-A .Hence to separate the negative peak from Impurity-A further trails required to optimize the final chromatographic conditions.

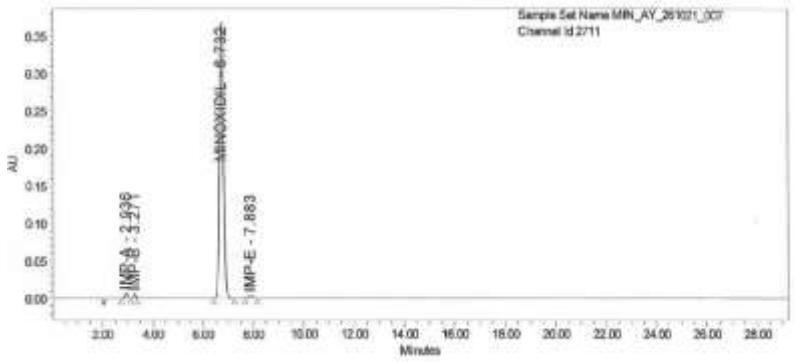

Figure: 12. X-Bridge C18 (150 x 4.6 mm, 5 µm) column

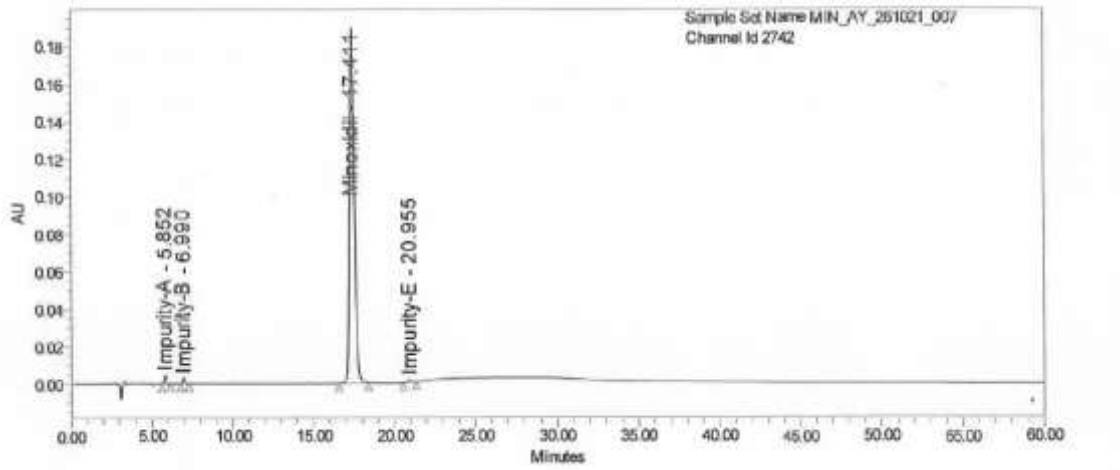

Figure 13. X-Bridge C18 (250 x 4.6 mm, 5 µm) column

Observation and conclusion:

1. In above mobile phase observed Impurity-A and Impurity-B eluted very closely at Rt'S2.936 and 3.271.
2. Peak shape brooding observed in X-Bridge C18 (150 x 4.6 mm, 5 µm) column
3. There is a well separation between Impurity-A and Impurity-B in X-Bridge C18 (250 x 4.6mm, 5 µm), but peak shapes are broaden compared to mobile phase with SLS composition.

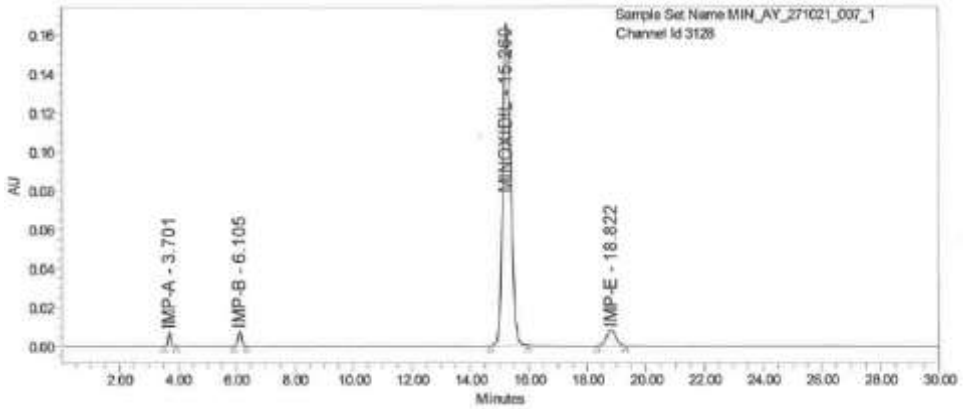

Figure 14. Spiked sample with 10µl injection volume

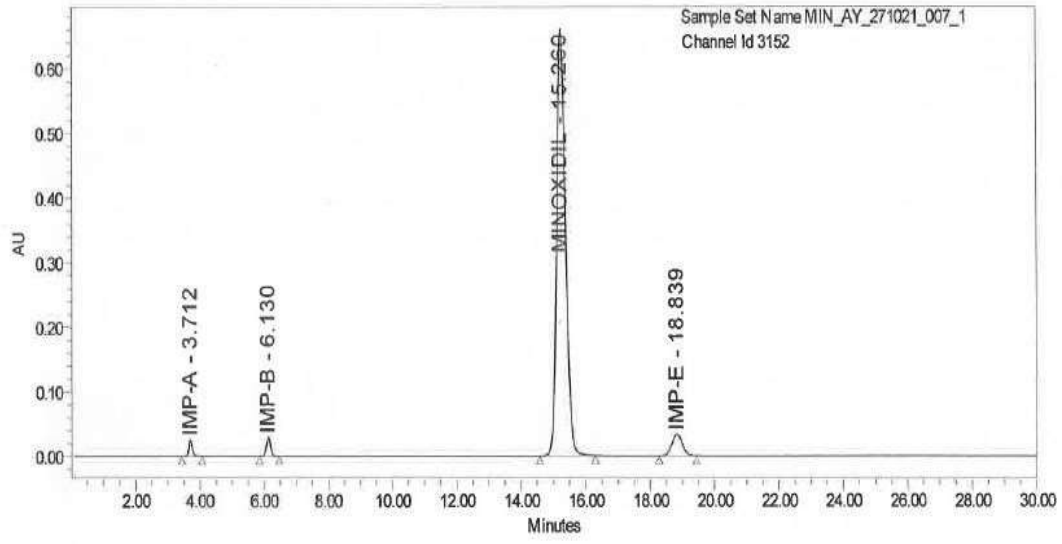

Figure 15. Spiked sample with 40 µl injection volume

Observations:
Better peak shapes observed with 10µl and 40µl injection volumes, hence selecting towards lower injection volumes, the final injection volumes shall be considered as **10µl** injection volume for chromatographic conditions.

Validation results
Validation of an analytical method is process to establish that the performance characteristicsof the developed method meet the requirements of the intended analytical application.
Typical Analytical Parameters used in Assay Validation are:

- Specificity
- Linearity and Range
- Accuracy
- Assay
- Precision
- Robustness

Table 7: Accuracy results: (ACCURACY WITH TABLETS)

Level	**Theoretical Concentration (mg/mL)**	**Experimental Concentration (mg/mL)**	**% Recovery**
50%-T1	0.0249979	0.0256162	102.5
50%-T2	0.0249979	0.0255605	102.3
100%-T1	0.0500083	0.0510698	102.1
100%-T2	0.0499979	0.0507514	101.5
150%-T1	0.0749855	0.0765746	102.1
150%-T2	0.0750104	0.0763457	101.8
300%-T1	0.1500062	0.1541164	102.7
Mean			**102.1**
Standard deviation			**0.41**
% RSD			**0.40**
Minimum			**101.5**
Maximum			**102.7**

Table 8: (ACCURACY WITH API+PLACEBO)

Level	**Theoretical Concentration (mg/mL)**	**Experimental Concentration (mg/mL)**	**% Recovery**
50%-T1	0.0125396	0.0127331	101.5
50%-T2	0.0126168	0.0128587	101.9
50%-T3	0.0125919	0.0128121	101.7
100%-T1	0.0250743	0.0255239	101.8
100%-T2	0.0250121	0.0253926	101.5
100%-T3	0.0249050	0.0253469	101.8
Mean			**101.7**
Standard deviation			**0.17**
% RSD			**0.16**
Minimum			**101.5**
Maximum			**101.9**

Table 9: (ACCURACY WITH API+PLACEBO) AT 600% LEVEL

Level	Theoretical Concentration (mg/mL)	Experimental Concentration (mg/mL)	% Recovery
600%-T1	0.1495643	0.1486414	99.4
600%-T2	0.1497611	0.1504655	100.5
600%-T3	0.1495046	0.1497892	100.2
Mean			**100.0**
Standard deviation			**0.57**
% RSD			**0.57**
Minimum			**99.4**
Maximum			**100.5**

Acceptance criteria: % Recovery of analyte should be between 97-103% with in specifiedrange.
Observation: Observed the % Recovery with in the acceptance criteria with in the specifiedrange for Tablets and API+Placebo method.

Table 10: ROBUSTNESS (SONICATION EFFECT)

Sample	Sonication Time	% Assay
600%-T1	15 minutes	**99.44**
600%-T2	30 minutes	**100.68**
600%-T3	60 minutes	**99.29**

Observation: No Effect of sonication time observed from 15 minutes to 60 minutes .Hence 15 minutes of sonication, time is sufficient for complete drug extraction.

Table 11: ROBUSTNESS (FILTER VARIABILITY STUDIES)

Sample ID	Saturation volume	% Assay
0.45µm Whatmann PVDF	10 ml	101.27
0.45µm Millipore PVDF	5 ml	100.10
0.45µm Millipore PVDF	10 ml	100.97
0.45µm Millipore Nylon PVDF	5 ml	100.80
0.45µm Millipore Nylon PVDF	10 ml	101.80
0.45µm Axiva PVDF	5 ml	101.16
0.45µm Axiva PVDF	10 ml	101.30

Observation: No Effect of Filtration by using different makes of filter observed.The results are with in the specification limit. Hence Whatmann PVDF, Millipore PVDF, Millipore Nylon, Axiva PVDF filters can be used for regular analysis for sample preparation.

Table 12: Linearity results

Linearity Solution Preparation		Dilution		Conc (in mg/mL)
Linearity Level-1	**20%**	1	100	0.0050
Linearity Level-2	**50%**	2.5	100	0.0124
Linearity Level-3	**80%**	4	100	0.0199
Linearity Level-4	**100%**	5	100	0.0249
Linearity Level-5	**600%**	7.5	25	0.1492

Results and observations:

Linearity graph:

Correlation Coefficient (R)=	1.0000
Regression Coefficient (R^2)=	1.0000
y-Intercept=	12144.6659
Slope of Regression line=	61182926.5404
Residual Sum of squares=	93159109.8300
Y-Bias	0.7874

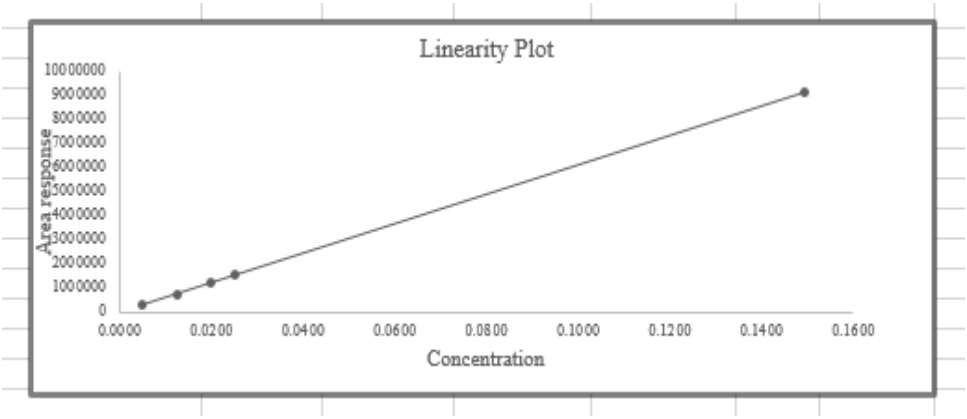

Figure 16. Linearity plot

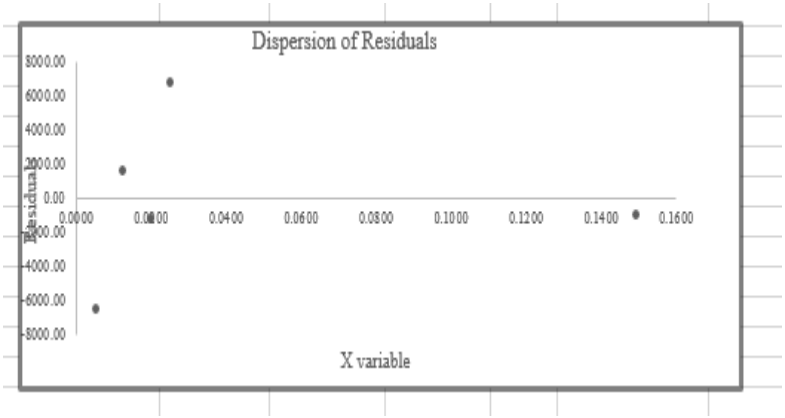

Figure 17. Dispersion of Residuals

Conclusion:

The correlation coefficient (R) is 1.0000. The Regression coefficient (R^2) is 1.0000. Regression analysis shows linear relationship between concentration and the response of Minoxidil is within specified range.

Table 13: SOLUTION STABILITY

S.No	Sample ID	WT OF STD	Std area	Similarity factor
01	Standard Initial	25.088	1544964	NA
02	Standard after 24 hours	25.088	1541758	1.00

(5mg Strength)

S.No	Sample ID	%Assay	%Difference
01	Initial sample	101.2	NA
02	Sample after 24 hours	100.0	**1.2**

(2.5 mg Strength)

S.No	Sample ID	%Assay	%Difference
01	Initial sample	102.1	NA
02	Sample after 24 hours	101.0	**1.1**

Acceptance criteria: Similarity factor for solution stability standard and fresh standardshould be between 0.98 to 1.02
Assay values of sample preparations should not differ from initial value by more than 2.

Conclusion: The results are meeting the acceptance criteria. Hence standards and samplesarestable up to 24 hours at room temperature

Table 14: ROBUSTNESS PARAMETER for Assay method of Minoxidil Tablets

Parameter	Actual (1.0 mL/minute)	Change-1 (0.8 mL/minute)	Change-2 (1.2 mL/minute)
% Labelled amount dissolved	100.98	100.60	100.01
Retention time of Minoxidil	5.9	7.3	5.0

Table 15: Chromatographic conditions: (Mobile phase composition change)

Parameter	Actual Buffer: Acetonitrile 55:45 v/v	Change-1 Buffer: Acetonitrile 50:50 v/v	Change-2 Buffer: Acetonitrile 57:43 v/v
% Labelled amount dissolved	100.98	100.60	99.42
Retention time of Minoxidil	5.9	7.3	7.1

Table 16: Chromatographic conditions: (Temperature variation) Mobile phase-1:
Mixed Buffer: Acetonitrile in the ratio of 50:50 v/v

Parameter	Actual 40°C	Actual 35°C	Actual 45°C
% Labelled amount dissolved	100.98	100.36	99.86
Retention time of Minoxidil	5.9	6.2	5.6

Acceptance criteria:
Results obtained by analytical method should not be affected by small variation in methodparameters.
System suitability should meet the acceptance criteria and results should meet specification.
Inference:
All the results met the acceptance criteria and from the above data, it was proposed run timeas 10 minutes

FINALISED ASSAY BY HPLC METHOD:

$$\text{Similarity factor} = \frac{\text{Average area of STD-I}}{\text{Average area of STD-II}} \times \frac{\text{Weight of STD-II}}{\text{Weight of STD-I}}$$

Minoxidil retention time is about 6.6 min. (For information only)

Table 17: Exemplary order of injections:

Name of the solution	Number of Injections
Blank	1
Standard solution-1	5
Standard solution-2	2
Sample preparation-1	1
Sample preparation-2	1
Bracketing standard	1

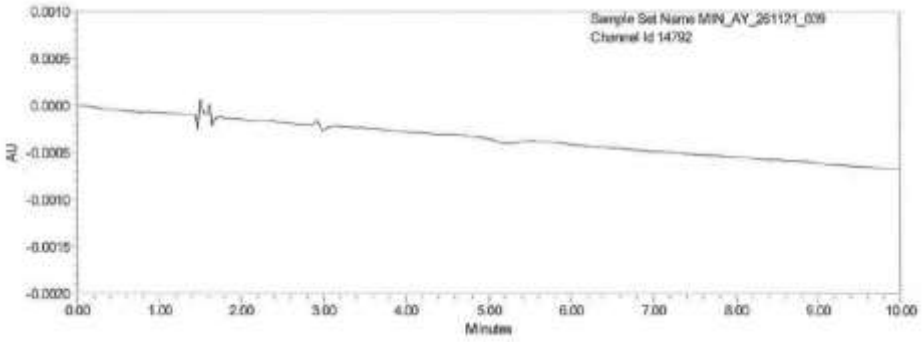

Figure 18. Time-dependent Absorbance Measurement for Sample MIN_AY_261121_039 (Channel ID 14792)"

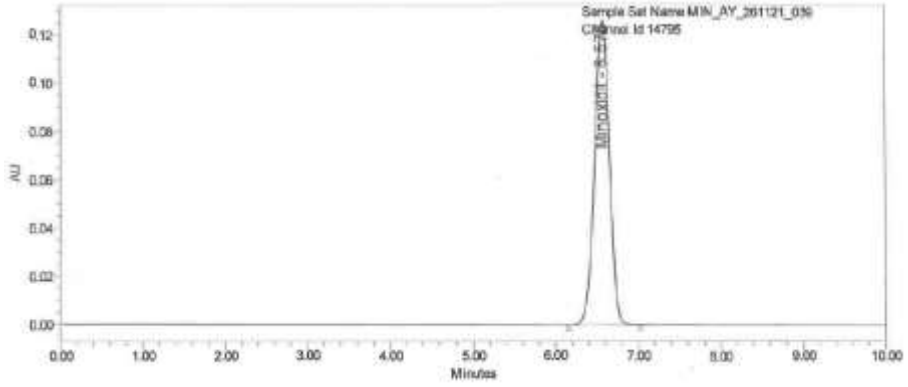

Figure 19. STANDARD CHROMATOGRAM

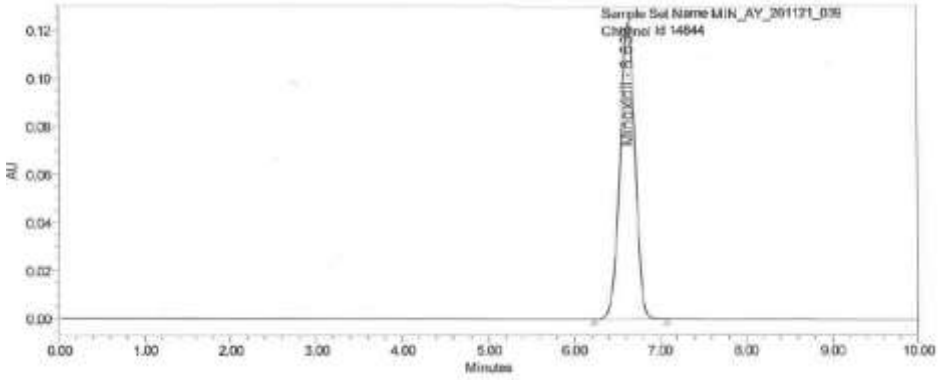

Figure 20. SAMPLE CHROMATOGRAM

Table 18: Summary of the present study (RP-HPLC)

S. No	Validation Parameter	Minoxidil
1	Mobile Phase	Buffer & acetonitrile in the ratio of 550:450 (%, v/v)
2	Flow Rate	1.0 mL/min
3	Detection Wavelength	280 nm
4	Retention Time	6.6 Min
5	Run Time	10 Min.
6	Specificity	Placebo, individual impurity and degradantinterference should not be observed
7	Linearity	(R^2) is 1.0000.
8	Accuracy	% RSD < 2
9	Recovery	97%-102%
8	Robustness	Should not be affected with small changes in method

Conclusion

The HPLC assay method for quantification of Minoxidil 10 mg tablets was found to be precise, accurate, and robust. This method is proposed for further method validation activities and can be considered for regular analysis support of Minoxidil drug products. The assay method for Minoxidil film-coated tablets was developed and validated using high-performance liquid chromatography (RP-HPLC). The chromatographic separation was achieved using an X-Bridge C18 column (150 mm x 4.6 mm ID, 5 µm) or an equivalent column. The mobile phase consisted of a mixture of buffer and acetonitrile in a 55:45 (v/v) ratio. The diluent was prepared with a mixture of water and acetonitrile in a 50:50 (v/v) ratio, and the needle wash solution was prepared with acetonitrile and water in a 90:10 (v/v) ratio. Key chromatographic parameters included a flow rate of 1.0 mL/min, detection wavelength of 280 nm, injection volume of 10 µL, data acquisition time of 10 minutes, and column temperature of 40°C. The pump operated in isocratic mode. The retention time for Minoxidil was 6.6 minutes with a total run time of 10 minutes. The assay results for Minoxidil ranged from 97% to 102%, with an R^2 value of 1.000 across the concentration range. The %RSD values were less than 2%, indicating the accuracy and precision of the method. Robustness testing showed that the method remained within acceptable limits under small variations in method parameters. The developed method was validated according to ICH Q2 guidelines.

Conflict of interest

None

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