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Research

ANALYTICAL METHOD VALIDATION FOR ASSAY OF ALPROSTADIL BY HPLC USING INTERNAL STANDARD

Bantu Pooja[1*], A. Shailaja[2], Mohammad Omar[3]

[1,2,3]*Arya college of Pharmacy, Kandi, Sangareddy, affiliated to Osmania University, Hyderabad, Telangana, India*

*Author for Correspondence: Bantu Pooja
Email: dollybantu07@gmail.com

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Abstract

An isocratic reversed-phase high performance liquid chromatographic (RP-HPLC) method has been developed and validated for the assay of Alprostadil using an Internal Standard. The successful separation of from its synthetic impurities and degradation products formed under stress conditions was achieved using 4.6mm x 25cm, Packing L1 or equivalent maintained at 25°C with a mobile phase of Methanol, acetonitrile and 0.1 M mono basic potassium phosphate (2:1:2) adjust with phosphoric acid to pH of 3.0. The mobile phase flow rate was 1.0 mL/min, and the detection wavelength was 200 nm. The developed HPLC method was validated with respect to linearity, accuracy, precision, specificity, and robustness. The developed HPLC method to determine the assay of alprostadil can be used to evaluate the quality of regular production samples. It can be also used to test the stability samples of Alprostadil.

Keywords: RP-HPLC, Alprostadil, Methanol, Acetonitrile, Validation, Assay

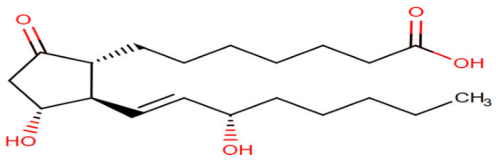
INTRODUCTION:

High-Performance Liquid Chromatography (HPLC): The HPLC is the method of choice in the field of analytical chemistry since this method is specific, robust, linear, precise, and accurate and the limit of detection analyses calculations are performed by the integrator itself.

Solvent and delivery system: In a continuous flow column, the mobile phase is continuously pumped under pressure from are savoir or reservoir. When it comes to the mobile phase, polarity, stationary phase, and sample characteristics all play a role in determining its ability to elute **Mobile phase:** When it comes to HPLC, most mobile phases are composed of organic solvents mixed with water or aqueous buffers. Organic solvents typically employed in HPLC.

DRUG PROFILE:

"Alprostadil": Molecular Formula: $C_{20}H_{34}O_5$. **Molecular weight:** 354.487 **Solubility**: Soluble in Water 0.0788mg/mL



MATERIALS & METHOD:

Details of Instruments, Column, Chemicals and Specification limits, Instruments**:** HPLC with UV-Visible or PDA detector Analytical Balance Glassware Class-A. Column4.6mm x 25cm, Packing L1 or equivalent Chemicals Mono basic potassium phosphate Acetonitrile, HPLC grade Water Methanol Ortho phosphoric acid Specification limits: Not less than 95.0 % and Not more than 105.0 %

Description of Analytical method (Methodology):

HPLC : Photo diode array detector or Equivalent capable of detecting UV Wavelength of 200 to 300 nm, HPLC Column: 4.6mm x 25-cm, Packing L1 or equivalent, Wavelength: 200 nm, Flow rate:1.0.mL/minute Injection Volume: 20 µL, Run time: 40 minutes Diluent: Methanol and water (90:10%v/v)

Preparation of Mobile Phase: Mix well Methanol, Acetonitrile and 0.1 M mono basic potassium phosphate (2:1:2) adjust with phosphoric acid to pH of 3.0.

Preparation of Internal Standard solution: Prepare 0.05mg/mL of ethyl Parabeen in diluent.

Preparation of Standard stock solution: 0.3 mg/mL of USP Alprostadil RS in diluent.

Preparation of Standard solution: 0.2 mg/mL of USP Alprostadil RS prepared by combining 2.0mL of standard stock solution with 1.0 mL of Internal standard solution.

Preparation of System suitability stock solution: 4.5µg/mL of USP Prostaglandin A1 RS in standard stock solution.

Preparation of System suitability solution: Combine 2.0mL of System suitability stock solution with 1.0 mL of internal standard solution.

Preparation of Sample stock solution: 0.3 mg/mL of Alprostadil in diluent.

Preparation of sample solution: 0.2 mg/mL of Alprostadil prepared by combining 2.0mL of Sample stock solution and 1.0 mL of Internal standard solution.

Procedure: Equilibrate the column using mobile phase to get a stable base line. Inject diluent as a blank (one injection), System suitability (One injection), and standard preparation (five injections) and check the system suitability parameters.

RESULTS AND DISCUSSION:

Validation Results: System Suitability: As per methodology, injected blank, System suitability and five replicate injections of standard solution intoHPLC system and calculated the % RSD for five replicate injections.

Results

Table 10: System suitability

Parameter	**System suitability**		**% RSD**
Result	19.7	14.2	0.4
Acceptance Criteria	The resolution between prostaglandin A1 and Alprostadil is NLT 7.5	resolution between Prostaglandin A1 and Ethyl paraben is NLT 2.0.	**NMT 2.0**

Acceptance criteria: The resolution between prostaglandin A1 and Alprostadil is NLT 7.5 and resolution between Prostaglandin A1 and Ethyl paraben is NLT 2.0. The % RSD of peak area ratio of

alprostadil to ethylparaben from six replicate injections of standard preparation should be not more than 2.0.

Conclusion: The above results reveal that the system meets the required system suitability criteria.

Results

Table 11: System suitability

Parameter	**System suitability**		**% RSD**
Result	19.7	14.2	0.4
Acceptance Criteria	The resolution between prostaglandin A1 and Alprostadil is NLT 7.5	resolution between Prostaglandin A1 and Ethyl paraben is NLT 2.0.	**NMT 2.0**

Table 12: Blank Interference Data

S. No.	**Name**	**Interference Due to blank (Yes/No)**
1	Alprostadil	No

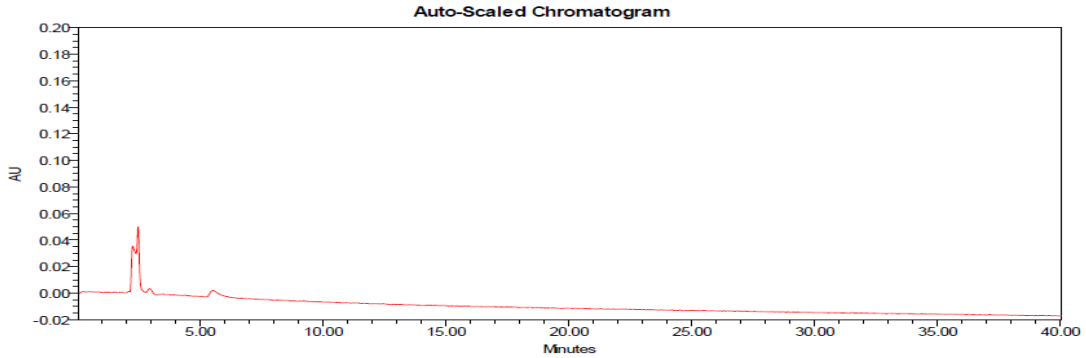


Figure 4: Typical chromatogram of Blank

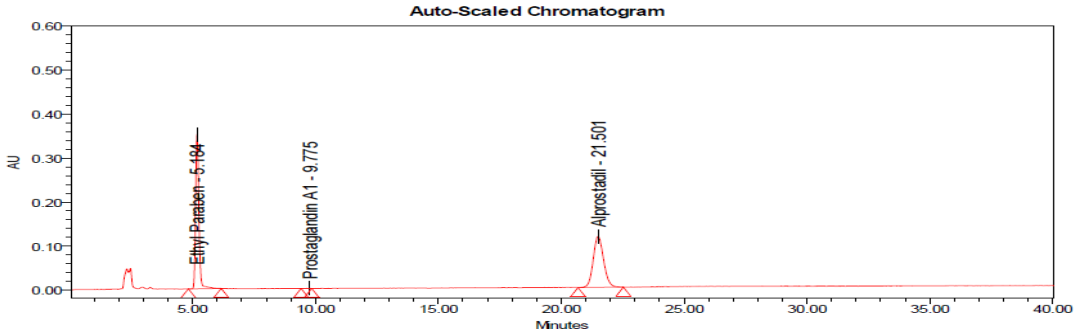


Figure 5: Typical chromatogram of System suitability solution

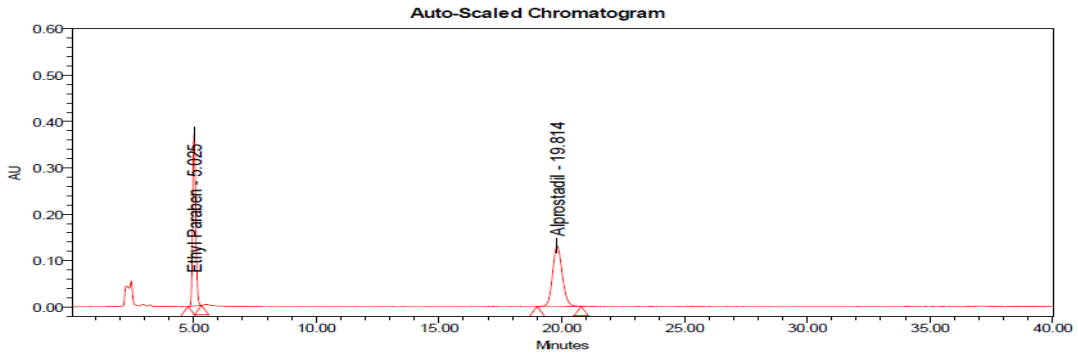


Figure 6: Typical chromatogram of standard solution

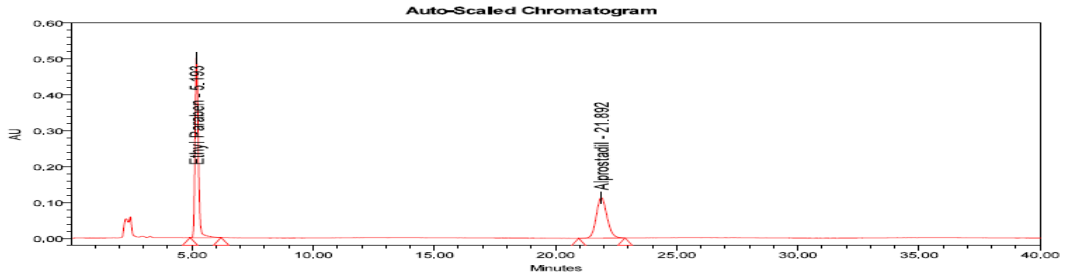


Figure 7: Typical chromatogram of sample solution

Acceptance criteria: The resolution between prostaglandin A1 and Alprostadil is NLT 7.5 and resolution between Prostaglandin A1 and Ethyl paraben is NLT 2.0.

- The % RSD of peak area ratio of alprostadil to ethylparaben from six replicate injections of standard preparation should be not more than 2.0.
- The blank should not show any interference at the retention time of Alprostadil peak in the standard and sample solution.

Conclusion: There is no interference observed from blank at the retention time of Alprostadil peak.

Precision:

System Precision: As per methodology, injected blank, standard solution for six times into the HPLC system.

Results

Table 13: System suitability

Parameter	**System suitability**		**% RSD**
Result	19.7	14.2	0.4
Acceptance Criteria	The resolution between prostaglandin A1 and Alprostadil is NLT 7.5	Resolution between Prostaglandin A1 and Ethyl paraben is NLT 2.0.	NMT 2.0

- The resolution between prostaglandin A1 and Alprostadil is NLT 7.5 and resolution between Prostaglandin A1 and Ethyl paraben is NLT 2.0.
-
- The % RSD of peak area ratio of alprostadil to ethylparaben from six replicate injections of standard preparation should be not more than 2.0.

Table 14: System Precision

Injection No.	**Alprostadil to Ethylparaben peak area ratio**
01	1.098
02	1.103
03	1.091
04	1.099
05	1.103
06	1.104
Average	1.1
STDEV	0.004885
% RSD	0.4

Conclusion: The above results reveal that the system is precise.

Method Precision:

Analysed six test preparations of Alprostadil as per the methodology and determined the % RSD of six sample preparations for Assay of Alprostadil.

Results

Table 15: System suitability for Assay

Parameter	**System suitability**		**% RSD**
Result	19.7	14.2	0.4
Acceptance Criteria	The resolution between prostaglandin A1 and Alprostadil is NLT 7.5	resolution between Prostaglandin A1 and Ethyl paraben is NLT 2.0.	NMT 2.0

Table 16: Method precision Result

Sample	**Alprostadil (%w/w)**
01	99.0
02	98.6
03	99.3
04	99.3
05	98.9
06	99.0
Average	99.0
S.D	0.270
%RSD	0.3

Acceptance criteria: The resolution between prostaglandin A1 and Alprostadil is NLT 7.5 and resolution between Prostaglandin A1 and Ethyl paraben is NLT 2.0. The % RSD of peak area ratio of alprostadil to ethylparaben from six replicate injections of standard preparation should be not more than 2.0. The % RSD for the assay of Alprostadil from the six preparations of the method precision solutions should be not more than 2.0.
Conclusion: The above results reveal that the method is precise.

Linearity: Linearity for Alprostadil was determined in the concentration range from 25 to 150 % levels of test concentration levels.

Results

Table 17: System suitability

Parameter	**System suitability**		**% RSD**
Result	19.7	14.2	0.4
Acceptance Criteria	The resolution between prostaglandin A1 and Alprostadil is NLT 7.5	resolution between Prostaglandin A1 and Ethyl paraben is NLT 2.0.	NMT 2.0

Table 18: Linearity Results of Alprostadil

Level (%w/w)	**Alprostadil Concentration (mg/mL)**	**Alprostadil to Ethylparaben peak area ratio**
25 (L1)	0.05	0.273
50 (L2)	0.1	0.546
100 (L3)	0.2	1.092
150 (L5)	0.3	1.649
Correlation Coefficient	0.9999	
Slope	5.50102	
Y-Intercept	-0.00392	
% Y-Intercept	-0.4	
Square of correlation	0.9999	

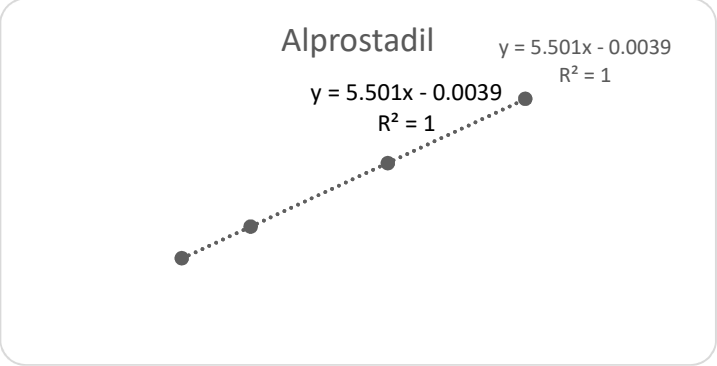


Figure 8: Linearity graph for Alprostadil

Acceptance criteria: The resolution between prostaglandin A1 and Alprostadil is NLT 7.5 and resolution between Prostaglandin A1 and Ethyl paraben is NLT 2.0. The % RSD of peak area ratio of alprostadil to ethylparaben from six replicate injections of standard preparation should be not more than 2.0. Report the slope &intercept, bias at 100 % level should be between ± 2.0 %. The Correlation coefficient should be not less than 0.99.

Conclusion: The above results reveal that the method is linear over the range of 15 % to 150% of working level concentration.

Accuracy: As per methodology, injected blank, 50%, 100, and 150% sample solutions and injected into the HPLC system and demonstrated the accuracy of the method. Calculated the system suitability parameters and % Inividual recovery and % mean recovery.

Table 19: System suitability

Parameter	**System suitability**		**% RSD**
Result	19.7	14.2	0.4
Acceptance Criteria	The resolution between prostaglandin A1 and Alprostadil is NT 7.5	resolution between Prostaglandin A1 and Ethyl paraben is NLT 2.0.	NMT 2.0

Table 20: Accuracy of Alprostadil

Sample No	**Spike level**	**Found (mg/mL)**	**Added (mg/mL)**	**'%' Recovery**	**'%' Mean recovery**	**%RSD**
1	50%	0.100	0.100	100.0	99.4	0.6
2	50%	0.099	0.100	99.0		
3	50%	0.099	0.100	99.0		
4	50%	0.099	0.100	99.0		
5	50%	0.099	0.100	99.0		
6	50%	0.098	0.100	98.0		

Table 21: Accuracy of Alprostadil

Sample No	**Spike level**	**Found (mg/mL)**	**Added (mg/mL)**	**'%' Recovery**	**'%' Mean recovery**	**%RSD**
1	100%	0.198	0.200	99.0	100.8	0.6
2	100%	0.197	0.200	98.5		
3	100%	0.199	0.200	99.5		
1	150%	0.302	0.300	100.7	100.4	0.8
2	150%	0.301	0.300	100.3		
3	150%	0.302	0.300	100.7		
4	150%	0.302	0.300	100.7		
5	150%	0.302	0.300	100.7		
6	150%	0.302	0.300	100.7		

Acceptance criteria : The resolution between prostaglandin A1 and Alprostadil is NLT 7.5 and resolution between Prostaglandin A1 and Ethyl paraben is NLT 2.0. The % RSD of peak area ratio of alprostadil to ethylparaben from six replicate injections of standard preparation should be not more than 2.0. Individual % recovery and mean % recovery value for Alprostadil at each level should be in between 97 to 103. % RSD for Individual % recovery at each level should not be more than 2.0

Conclusion: The above results reveal that the method is accurate.

CONCLUSION:

The current analytical method was validated according to the protocol, and it passes the acceptance criteria. Thus, it was determined that the analytical approach is particular, precise, linear, accurate, rugged, and robust. As a result, the current analytical approach is suitable for regular analysis and serves its intended function.

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