RP-HPLC METHOD DEVELOPMENTAND VALIDATION FOR THE DETECTION OF IMPURITIES IN BREXANOLONE PHARMACEUTICAL DOSAGE FORM

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Abstract

A simple, Accurate, Precise method was developed for the simultaneous estimation of the Erythromycin and Isotretinoin in dosage form. Chromatogram was run through Platisil ODS C18(4.6x250mm,5.0□m)MobilephasecontainingMethanolandOrthphosphoricacid taken with in the ratio70:30 was pumped through column on a flow rate at 1.0 ml/min. Buffer used in this method was Orthphosphoric acid. Ambient Temperature was maintained. Optimized wavelength selected was 245nm. Retention time of Brexanolone is 3.008 min respectively. %RSD of Brexanolone found to be 0.6 respectively. %Recovery was obtained as100.50 % for Brexanolone respectively. LOD, LOQ values obtained from regression equations of Brexanolone was found to be 2.97 and 9.97 respectively.

Key words: Brexanolone, Chromatogram, RP-HPLC, Method development, validation

Introduction

Although there are many other analytical procedures, such as dissolution testing for drug products or particle size determination for drug substance, these have not been addressed in the initial text on validation of analytical procedures. Validation of these additional analytical procedures are equally important to those listed herein and may be addressed in subsequent documents. Identification tests are intended to ensure the identity of an analyte in a sample. This is normally achieved by comparison of a property of the sample (e.g., spectrum, chromatographic behaviour,chemical reactivity etc) to that of a reference standard. Testing for impurities can be either a quantitative test or a limit test for the impurity in a sample. Either test is intended to accurately reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test.

Assay procedures are intended to measure the analyte present in a given sample. In the context of this document, the assay represents a quantitative measurement of the major component in the drug substance. For the drug product, similar validation characteristics also apply when assaying for the active or other selected component(s). [1]The same validation characteristics may also apply to assays associated with other analytical procedures (e.g., dissolution). Related substance by RP-HPLC method development and validation for the detection of impurities in brexanolone drug substance. Very few methods are reported for estimation of both drugs from formulation. We intend to develop RP-HPLC method by simultaneous determination with simple, rapid, greater sensitivity and faster elution.

Materials and methods

Method development:

Wavelength selection:
UV spectrum of 10 µg / ml Brexanolone in diluents (mobile phase composition) was recorded by scanning in the range of 200 nm to 400 nm. From the UV spectrum wavelength selected as 245.At this wavelength both the drugs show good absorbance.

Optimization of Column:
DIKMA Platisil ODSC18 (4.6x250mm,5µm) was found to be ideal as it gave good peak shape and resolution at 1.5 ml/min flow.

Preparation of buffer and mobile phase:

Preparation of 0.1% Ortho phosphoric acid buffer:
Pipetted 1ml of ortho phosphoric acid in1000 ml HPLC water

Preparation of mobile phase:
Mix a mixture of above buffer 300ml (30%), 700ml Methanol (70%) to1000 ml and degas in ultrasonic water bath for 5 minutes. Filter through0.45 µ filter under vacuum filtration.
Diluent Preparation: Use the Mobile phase as Diluents.

Preparation of Brexanolone solution:
Preparation of 0.04µg/ml solution:
Accurately weigh and transfer 10mg of Brexanolone working standard into a 10ml clean dryvolumetricflaskaddDiluentsandsonicatetodissolveitcompletelyandmakevolume up to the mark with the same solvent. (Stock solution). Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents. Further pipette 0.5ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with Diluent. Further pipette 0.4 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with Diluent.

Results and discussion

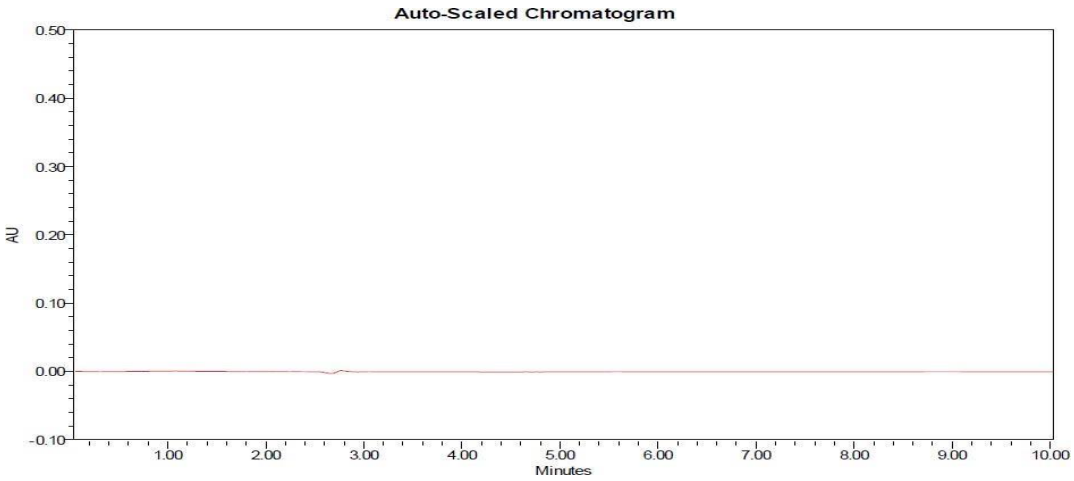

Figure-1: Chromatogram for system suitability

Resolution between two drugs must be not less than 2. Theoretical plates must be not less than 2000. Tailing factor must be not more than 2. It was found from above data that all the system suitability parameters for developed method were within the limit.

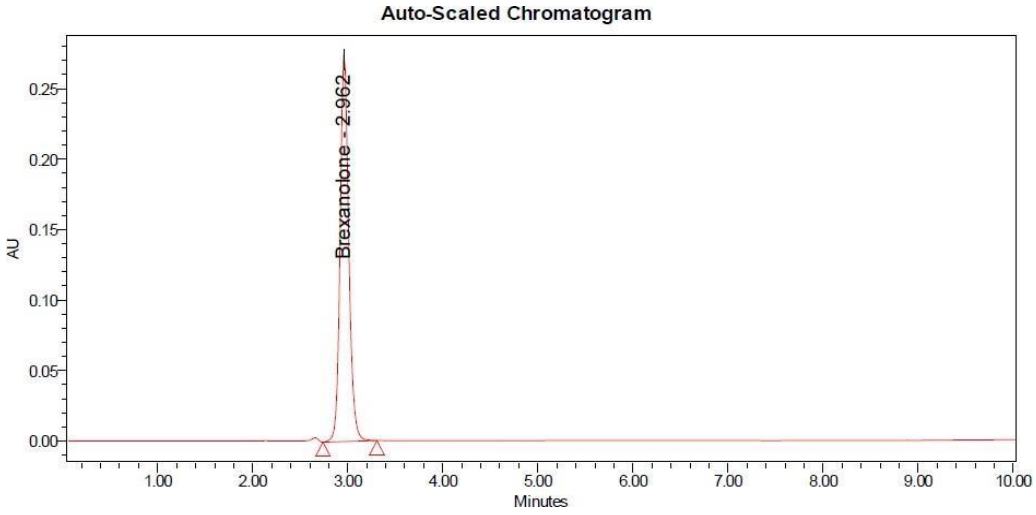

Figure-2: Chromatogram for standard

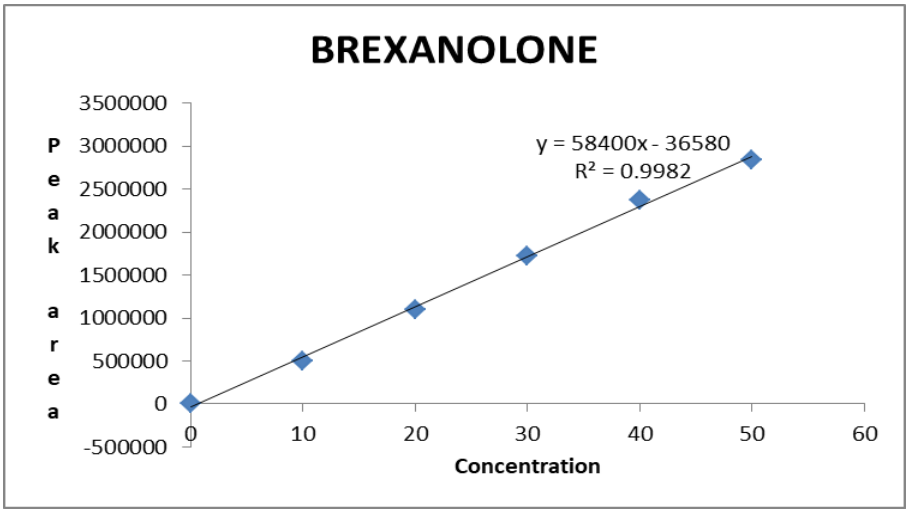

Figure-3: Chromatogram of linearity curve

Correlation coefficient (R^2) should not be less than 0.999. The correlation coefficient obtained was 0.999 which is in the acceptance limit.

Table-1: Results of Precision for Brexanolone

Injection	**Area**
Injection-1	1692267
Injection-2	1729767
Injection-3	1692267
Injection-4	1729767
Injection-5	1692267
Injection-6	1729767
Average	1711017.2
Standard Deviation	26516.4
%RSD	1.5

The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table-2: Results of intermediate Precision for Brexanolone

Injection	**Area**
Injection-1	1792267
Injection-2	1529767
Injection-3	1692267
Injection-4	1729767
Injection-5	1692267
Injection-6	1719767
Average	1601017
Standard Deviation	31516.4
%RSD	1.9

The % RSD obtained is within the limit, hence the method is rugged.

Table-3: Accuracy (recovery) data for Brexanolone

%Concentration(atspecification Level)	Area*	Amount Added(mg)	Amount Found(mg)	% Recovery	Mean Recovery
50%	864883	5	5.07	101.43	100.50
100%	1729767	10	9.92	99.21	
150%	2594650	15	15.13	100.88	

*Average of three determinations

The percentage recovery was found to be within the limit (97-103%). The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Degradation calculation:

Table-4: Degradation calculation

S.no	Types of forced degradation	Peak Area	% Degradation
1	**Standard**	1722323	-----
2	**Acid**	1642002	4.66
3	**Base**	1647791	4.33
4	**Peroxide**	1677084	2.63
5	**Thermal**	1688709	1.95
6	**Photo**	1646769	4.39

Summary of the results

Validation Parameters	Result	Limit
Assay(% meanassay)	99.76	90-110%
Linearity	0.999	NMT1
Precision	0.6%	2.0%
Intermediate precision	0.4	2.0%
acy(Percentage recovery)	100.50%	97.0%-103.0%.
LOD	2.97	3
LOQ	9.97	10

The estimation of Brexanolone was done by RP-HPLC. The assay of Brexanolone was performedwithtabletsandthe%assaywasfoundtobe99.76whichshowsthatthemethod is useful for routine analysis. The linearity of Brexanolone was found to be linear with a correlation coefficient of 0.999 which shows that the method is capable of producing good sensitivity. The acceptance criteria of precision are RSD should be not more than 2.0% and the method show precision 0.6 for Brexanolone which shows that the method is precise. The acceptance criteria of intermediate precision are RSD should be not more than 2.0% and the method show precision 0.4 for Brexanolone which shows that the method is repeatable when performed in different days also. The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.50% for Brexanolone. [2-4]

Conclusion

The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ is 3 and 10.The LOD and LOQforBrexanolonewasfoundtobe2.97and9.97. The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

References

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