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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF BELINOSTAT BY RP-HPLC METHOD IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

The Present work was to develop a simple, fast, accurate, precise, reproducible, reverse phase high performance liquid chromatographic method for estimation of Belinostat in pharmaceutical tablet dosage form marketed as doxinate. Chromatographic separation was done using Inertsil ODS RP C18 column having dimension of 4.6×250mm having particle size of 5µm, with mobile phase consisting of phosphate buffer pH 3 ±0.02 pH adjusted with ortho phosphoric acid and acetonitrile (50:50 %v/v), flow rate was adjusted to 1.0 ml/min and detection wavelength at 263nm. The retention times of Belinostat was found to be 2.35. The Proposed method has been validated for accuracy, precision, linearity; range and robustness were within the acceptance limit according to ICH guidelines. Linearity for Belinostat was found in range of 25µg-150µg and correlation coefficient was found to be 0.999. %RSD for method precision was found to be 0.76and for system precision was 0.80 respectively, % mean recovery for Belinostat succinate was found to be 99.18% The method was found to be robust even by change in the mobile phase ±5% and in less flow condition. The developed method can be successfully employed for the routine analysis of Belinostat in API and Pharmaceutical dosage forms.

INTRODUCTION

Belinostat is a novel investigational small molecule drug that inhibits the enzyme histone deacetylase (HDAC). PXD101 has been shown in preclinical studies to have the potential to treat a wide range of solid and hematologic malignancies either as a monotherapy or in combination with other active agents, and both an oral and intravenous formulation of the drug are being evaluated in clinical trial. Its IUPAC name is (2E)- N- hydroxy- 3- [3-Phenyl sulfamoyl) phenyl]prop-2-enamide.PXD101 is a small molecule HDAC inhibitor being investigated for its role in the treatment of a wide range of solid and hematologic malignancies either as a single-agent, or in combination with other active anti-cancer agents, and is currently being evaluated in a Phase II clinical trial for the treatment of multiple myeloma. UGT-1A is a uridine diphosphate glucuronosyl transferase (UDP-glucuronosyl transferase, UDPGT).

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Equipment and Apparatus used: HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Belinostat solutions.

Methods:

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of Standard stock solutions: Accurately weighed 50mg of Belinostatstat transferred10ml and volumetric flasks, 3/4 th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (5000µg/ml of Belinostat)

Preparation of Standard working solutions (100% solution): 1ml of Belinostatstat from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (500µg/ml of Belinostat)

Preparation of Sample stock solutions: 5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 10 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters.(5000 µg/ml of Belinostat)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (500µg/ml of Belinostat)

Preparation of buffer:

0.1%OPA Buffer: 1ml of Perchloric acid was diluted to 1000ml with HPLC grade water.

Precision:

Preparation of Standard stock solutions: Accurately weighed 50mg of Belinostat transferred to 10ml and volumetric flasks, 3/4 th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (5000µg/ml of Belinostat)

Preparation of Standard working solutions (100% solution): 1ml of Belinostat from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent.500 µg/ml of Belinostat)

System suitability parameters: The system suitability parameters were determined by preparing standard solutions of Belinostatstat (200ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

Linearity:

Preparation of Standard stock solutions: Accurately weighed 50mg of Belinostat transferred to two separately 10ml and volumetric flasks, 3/4 th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (5000µg/ml of Belinostat)

Accuracy:

Preparation of Standard stock solutions: Accurately weighed 50mg of Belinostat transferred to two separately 10ml and volumetric flasks ,3/4 th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (5000µg/ml of Belinostat)

Robustness: Small deliberatechanges in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

LOD sample Preparation:

0.25ml standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml Belinostat, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

LOQ sample Preparation: 0.25ml standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml Belinostat of,

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solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Degradation studies:

Oxidation: To 1 ml of stock solution of Belinostat, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 500µg/ml solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid degradation studies: To 1 ml of stock solution Belinostat, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c.The resultant solution was diluted to obtain 500µg/ml solution and10µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

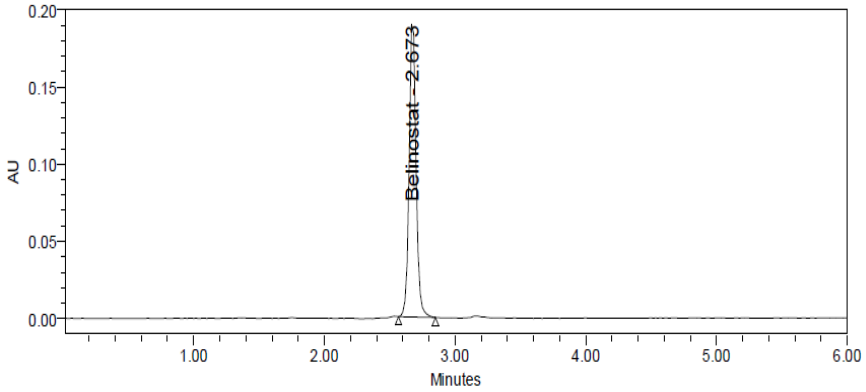

Fig. No. 1 Chromatogram showing Observation: Belinostat eluted with good peak shape and retention time and tailing was passed

S.No	Belinostat		
Inj	RT(min)	USP Plate Count	Tailing
1	2.662	12943	1.05
2	2.665	12940	1.04
3	2.667	13175	1.04
4	2.673	13507	1.12
5	2.673	13464	1.09
6	2.678	13088	1.05

Table No: 1 System suitability data

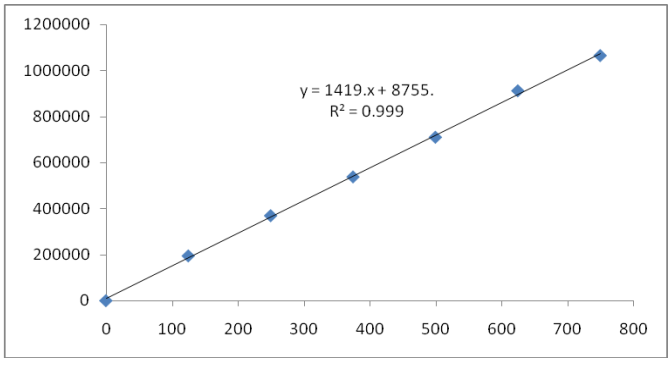

Fig. No. 2 Calibration graphs showing Belinostat.

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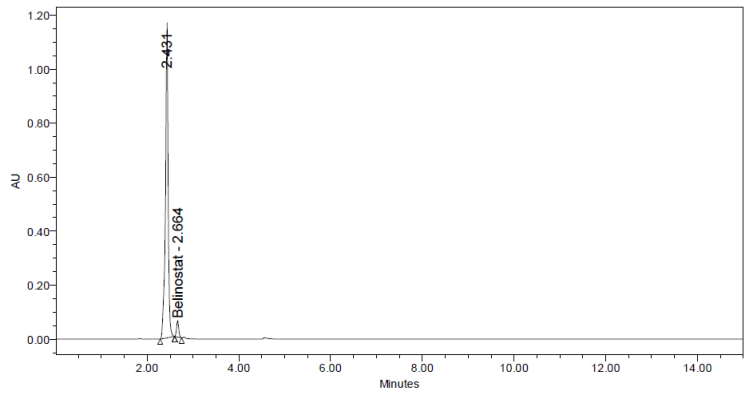

Fig. No.3 Chromatogram showing Observation:LOD &LOQ data of Belinostat.

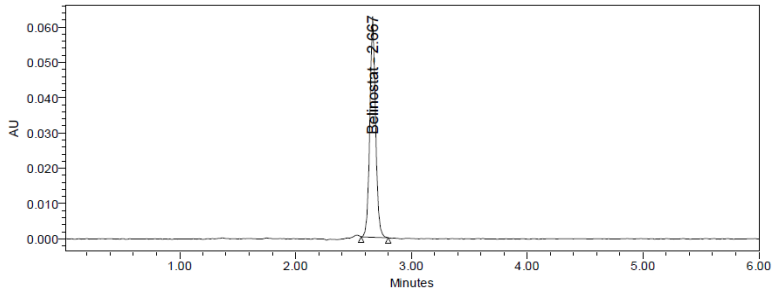

Fig. No.4 Chromatogram showing Observation:Accuracy data of Belinostat

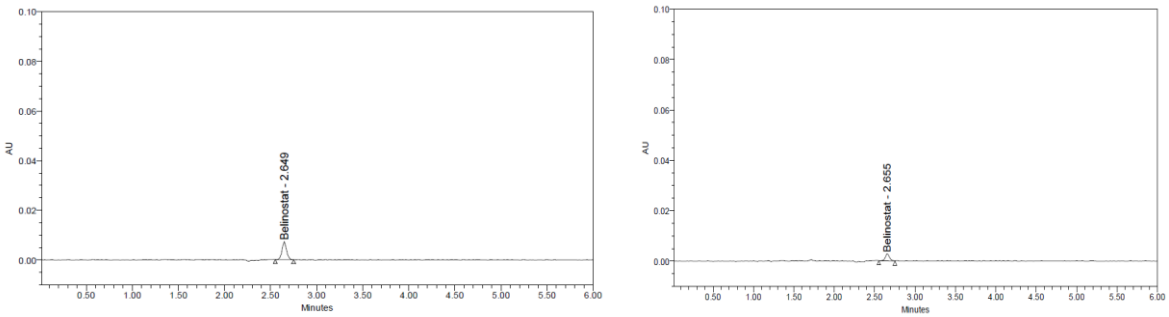

Fig. No.5 Chromatogram showing Observation: LOD & LOQ data of Belinostat.

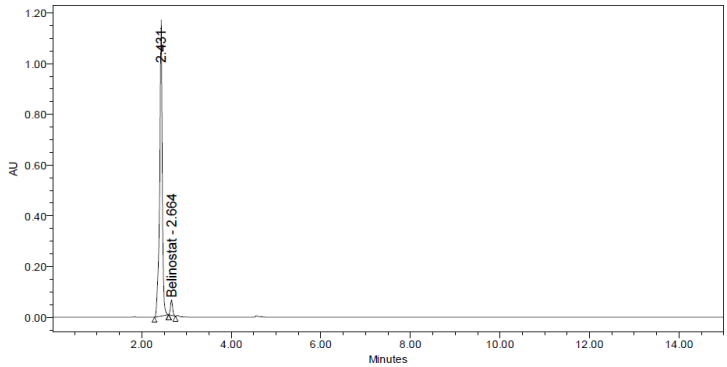

Fig no.6 Chromatogram showing Observation: Peroxide degradation Chromatogram of belinostat.

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Table no.2 Chromatogram showing Observation: Linearity125 µg/ml Chromatogram of belinostat

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	250	251.0655	100.43	**99.63%**
	250	250.6589	100.26	
	250	245.9225	98.37	
100%	500	495.771	99.15	
	500	491.358	98.27	
	500	500.9042	100.18	
150%	750	762.6575	101.69	
	750	738.7061	98.49	
	750	748.9866	99.86	

Table no.3 Chromatogram showing Observation: Degradation data of belinostat

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	4.01	0.295	0.345
2	Alkali	3.96	0.325	0.360
3	Oxidation	0.523	0.873	0.577
4	Thermal	0.51	0.193	0.328
5	UV	0.90	0.430	0.535
6	Water	0.07	0.264	0.331

CONCLUSION

The chromatographic technique for the assurance of test methods of Assay for Belinostat bulk drug, raw materials and tablets were simple, reliable, sensitive and less time consuming. The advantage of the current test techniques was that it doesn't need any complicated mobile phase and it is basic isocratic method. The current technique can be confidently be utilized for fast and exact quantitation of Belinostat, uncommonly this strategy can be a significant enthusiasm for analytical chemistry, since it offers a particular quality control in the test methodology of Assay of Belinostat. The current work shows a validated, highly sensitive and selective method for assurance of Terbinafine HCl in pharmaceutical dosage forms. These validated method parameters were applied to the oxidative degradation investigations of Belinostat, Which impact the stability of the active drug for example Belinostat.

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