Research Article

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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

A simple, Precise, Accurate method was developed for the estimation of Belinostat by RP-HPLC technique. Chromatographic conditions used are stationary phase Discovery c18 250 x 4.6 mm, 5µ. Mobile phase O-phosphoric acid buffer: Acetonitrile in the ratio of 50:50and flow rate was maintained at 1ml/min, detection wave length was 230nm, column temperature was set to 30^{o}C and diluent was Acetonitrile: Water (50:50), Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R^2 value was found to be as 0.999. Precision was found to be 0.2 for repeatability and 0.4 for intermediate precision. LOD and LOQ are 0.42µg/ml and 1.28µg/ml respectively. By using above method assay of marketed formulation was carried out 100.83% was present.

Keywords: HPLC Belinostat, Method development. ICH Guidelines

INTRODUCTION

Belinostat is a novel investigational small molecule drug that inhibits the enzyme histone deacetylase [1] (HDAC). PXD101 has been shown in preclinical studies [2] 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 [3,4]. Its IUPAC name is (2E)-N-hydroxy-3-[3-(phenylsulfamoyl)phenyl]prop-2-enamide. PXD101 is a small molecule HDAC inhibitor [5] 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 [6,7,8] for the treatment of multiple myeloma. UGT-1A is a uridine diphosphate glucuronosyltransferase (UDP-glucuronosyltransferase, UDPGT) [9, 10].

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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 [11-14]

- **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 Belinostat transferred10ml and volumetric flasks, 3/4th 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 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:** Belinostat equivalent to 50 mg was taken and 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 [15-17]

- **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 [18-20]

- **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 deliberate changes 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, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

DEGRADATION STUDIES [21-23]

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 600c. For HPLC study, the resultant solution was diluted to obtain 500µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies

To 1 ml of stock s solution Belinostat, 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 600c .The resultant solution was diluted to obtain 500µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

RESULTS

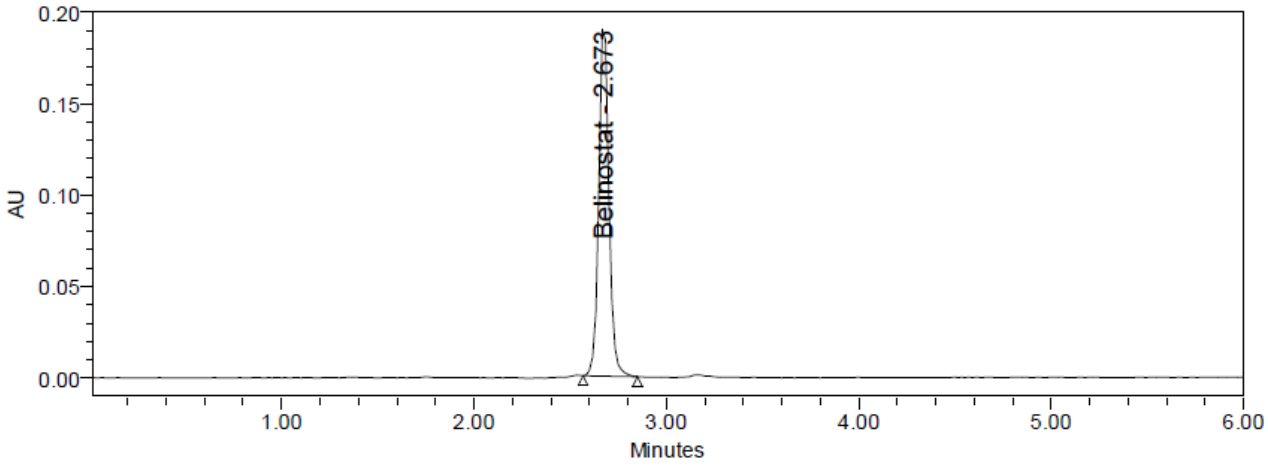

Fig. 1: Chromatogram of Belinostat eluted with good peak shape and retention time

Table 1: System suitability data

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

Linearity

To demonstrate the linearity of assay method, inject 6 standard solutions with concentrations of about 125ppm to 750ppm of Belinostat. Plot a graph to concentration versus peak area. Slope obtained was 1419, Intercept was 8755 and Correlation Co-efficient was found to be 0.999 and Linearity plot was in the figure 2.

Table 2: Linearity data

Concentration (ppm)	Peak Area
0	0
125	194577
250	369239
375	537632
500	709817
625	911590
750	1064489

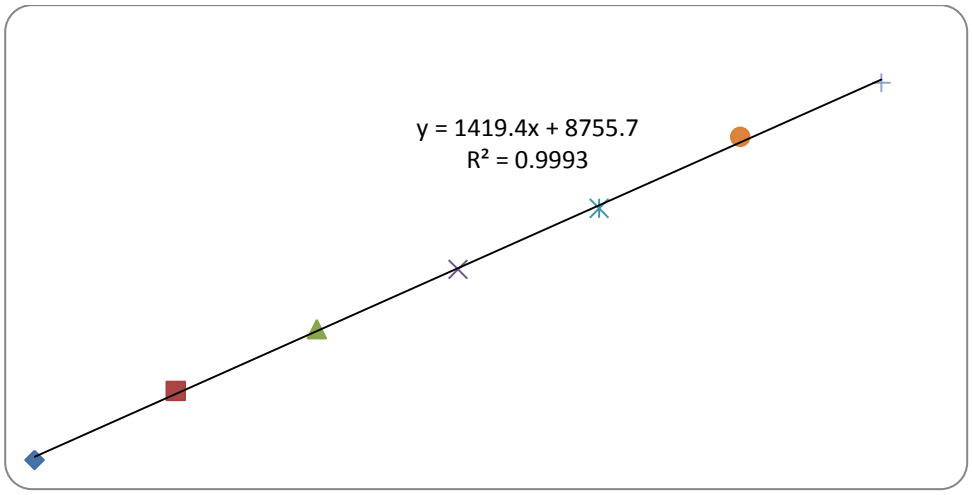

Fig. 2: Calibration curve of Belinostat

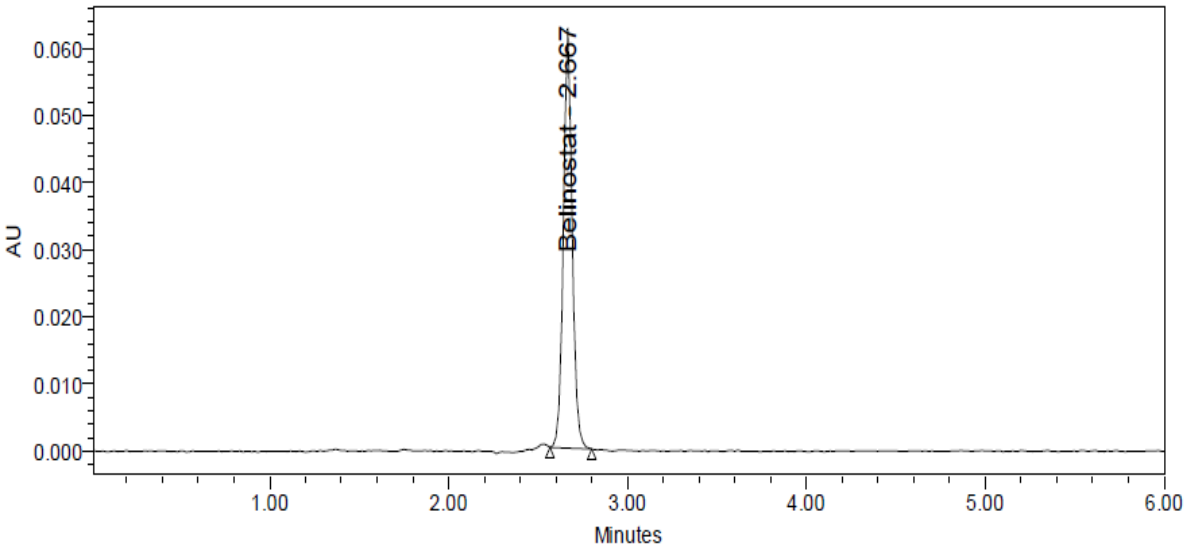

Fig.3: Chromatogram of belinostat at concentration of 125 µg/ml

Intermediate precision

Six working sample solutions of 500ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 0.4

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Table 3: Intermediate precision data

S.No	Peak Area
1	714158
2	714827
3	715051
4	714595
5	714749
6	707803
AVG	713531
STDEV	2821.7
%RSD	0.4

Table 4: Accuracy data

% 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	

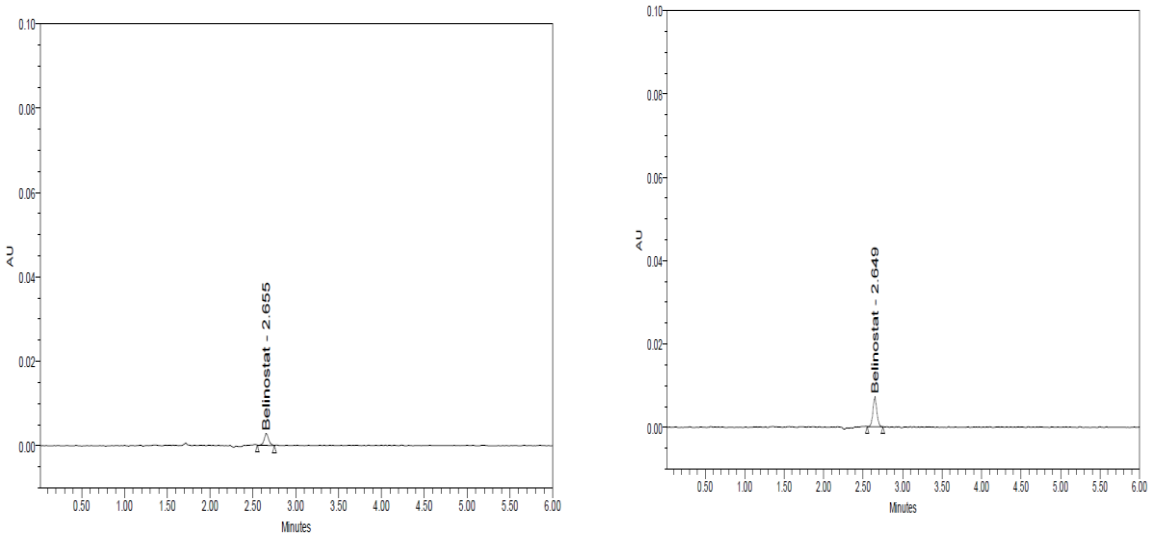

Fig. 4: Chromatogram showing Observation: LOD & LOQ data of Belinostat.

Table 5: 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

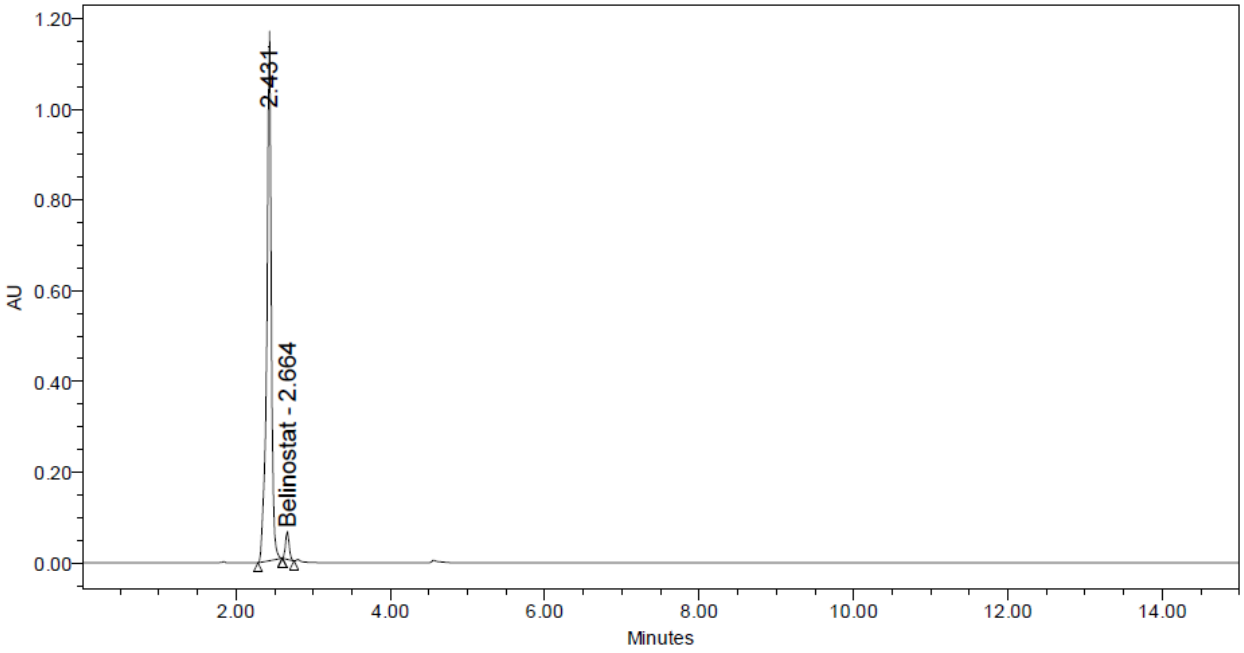

Fig 5: Chromatogram showing Peroxide degradation of belinostat

Table 6: Summary Table

Parameters	Belinostat
Calibration range (mcg / ml)	125-750 ppm
Optimized wavelength	230nm
Retention time	2.673min
Regression equation (Y)	y =1419x + 8755
Correlation coefficient(r^2)	0.999
Precision (% RSD*)	0.2
% Recovery	99.63
Limit of Detection (µg/ ml)	0.42
Limit of Quantitation (µg / ml)	1.28

CONCLUSION

Chromatographic conditions used are stationary phase Discovery c18 250 x 4.6 mm, 5µ. Mobile phase O- phosphoric acid buffer: Acetonitrile in the ratio of 50:50and flow rate was maintained at 1ml/min, detection wave length was 230nm, column temperature was set to 30^{o}C and diluent was Acetonitrile: Water (50:50), Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R^2 value was found to be as 0.999. Precision was found to be 0.2 for repeatability and 0.4 for intermediate precision. LOD and LOQ are 0.42µg/ml and 1.28µg/ml respectively. By using above method assay of marketed formulation was carried out 100.83% was present.

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