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Original Article

STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF PANOBINOSTAT LACTATEIN PHARMACEUTICAL DOSAGE FORMS BY UPLC

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ABSTRACT

Objective: The present study aimed to develop a stability indicating ultra-performance liquid chromatography (UPLC) method for the estimation of panobinostat lactate in pharmaceutical dosage form and validate the method in accordance with ICH guidelines.

Methods: The optimized conditions for the developed UPLC method are acquity UPLC hibar C18 (100 mm × 2.1 mm, 1.8µ) column maintained at 30°C with mobile phase consisting of 0.1% ortho phosphoric acid and acetonitrile in the ratio 50:50%v/v on isocratic mode at flow rate 0.3 ml/min. The sample was detected at 266 nm.

Results: The retention time for panobinostat was found to be 1.6 min. The developed method was validated for accuracy, precision, specificity, ruggedness, robustness and solution stability. The method obeyed Beer's law in the concentration range of 50µg/ml and 300µg/ml with correlation coefficient of 0.9998. Forced degradation studies were conducted by exposing the drug solution to various stress conditions such as acidic, basic, peroxide, neutral, photolytic and thermal conditions. The net degradation was found to be within the limits, indicating that drug is stable in stressed conditions.

Conclusion: The developed method for the estimation ofpanobinostat can be utilized for the routine analysis of pharmaceutical dosage form.

Keywords: Panobinostat lactate, Stability indicating, Method development, Validation, UPLC

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INTRODUCTION

Panobinostat [1–5] (fig. 1), chemically defined as 2-Hydroxypropanoic acid, compd. with 2-(E)-N-hydroxy-3-[4-[[[2-(2-methyl-1H-indol-3-yl)ethyl]amino]methyl]phenyl]-2-propenamide (1:1). It is white to slightly yellowish or brownish powder, slightly soluble in water. It has pKa values of 5.45 and 10.01. It belongs to anticancer category which acts by inhibiting multiple histone deacetylase enzymes, a mechanism leading to apoptosis of malignant cells via multiple pathways. It is used for the treatment of multiple myeloma [6, 7].

The literature survey reveals that there was only one method developed and validated for the estimation of Panobinostat using ultra-high performance liquid chromatography–mass spectrometry [8]. As there is no method reported using UPLC, the present study aimed to develop and validate an UPLC stability indicating method for the estimation of panobinostat in pharmaceutical dosage form.

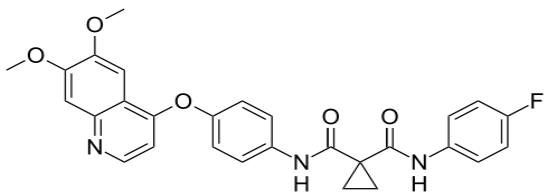

Fig. 1: Chemical structure of panobinostat

MATERIALS AND METHODS

Reagents and chemicals

Panobinostat working standard was procured from spectrum labs, Hyderabad as gift sample. The farydak tablets were purchased from local pharmacy. All the chemicals used were of AR grade purchased from Merck, Mumbai. All the solvents used were of HPLC grade purchased from Sigma-Aldrich, Mumbai.

Chromatographic conditions and instruments

The ACQUITY UPLC [9, 10] system equipped with binary solvent manager, sample manager, ultra violet (UV) detector and Hibar C18 (100 mm × 2.1 mm, 1.8µ) column was used for the determination of panobinostat. The optimized conditions included 0.1% ortho phosphoric acid (OPA) and acetonitrile (50:50%v/v) as mobile phase run on an isocratic mode at flow rate 0.3 ml/min. The column was maintained at 30°C and detection was done at 266 nm. Other equipment used in the method was pH meter, ultrasonic bath sonicator and weighing balance.

Preparation of diluent

Mixture of water and acetonitrile in the ratio 50:50%v/v was used as diluent.

Preparation of mobile phase

Mixture of 0.1% ortho phosphoric acid and acetonitrile in the ratio 50:50%v/v was used as mobile phase.

Preparation of Standard and Sample solutions

200 mg of panobinostat working standard was dissolved in 100 ml of diluent. 1 ml of the above stock solution was diluted to 10 ml using diluent in order to get a concentration of 200µg/ml.

20 Tablets (Farydak) were weighed accurately and average weight was calculated. An amount equivalent to 200 mg of drug was dissolved in 100 ml of diluent. Filtered the solution and diluted 1 ml of above solution to 10 ml with diluent.

Method validation

The developed method was validated in compliance with International Conference on Harmonization (ICH) guidelines [11, 12].

Specificity

The specificity of the method was determined by comparing the drug solution with the placebo solution and observed for the interference of placebo peak with drug peak.

Accuracy

Accuracy of the method was determined by % recovery. The drug solution along with sample was prepared in three concentration levels 50%, 100% and 150%. Then the % recovery was calculated.

Precision

Precision of the method was estimated by injecting the six solutions of standard into the UPLC system and % relative standard deviation (RSD) was calculated.

Linearity

Linearity of the method was determined by preparing series of dilutions ranging from 50µg/ml–300µg/ml and injecting them into UPLC system.

Ruggedness

Ruggedness was determined by injecting the six solutions of standard into UPLC for different days. The %RSD was calculated.

Robustness

Robustness of the method was determined by varying the optimized analytical conditions such as mobile phase composition by±5%, flow rate by±0.1 ml/min and column oven temperature by±5°C.

Solution stability

Solution stability was estimated by analyzing the standard drug solution after storage for 24hours under laboratory conditions.

Forced degradation studies

Forced degradation studies [13] were carried out for drug by exposing the drug solution to the various stress conditions such as acidic (2N hydrochloric acid for 30 min at 60 °C), basic (2N sodium hydroxide for 30 min at 60 °C), peroxide (20% hydrogen peroxide for 30 min at 60 °C), neutral (refluxing the drug in water for 6 hours at 60°C), photolytic (105 °C for 6h) and thermal (exposing the drug solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m² in photo stability chamber) conditions.

RESULTS

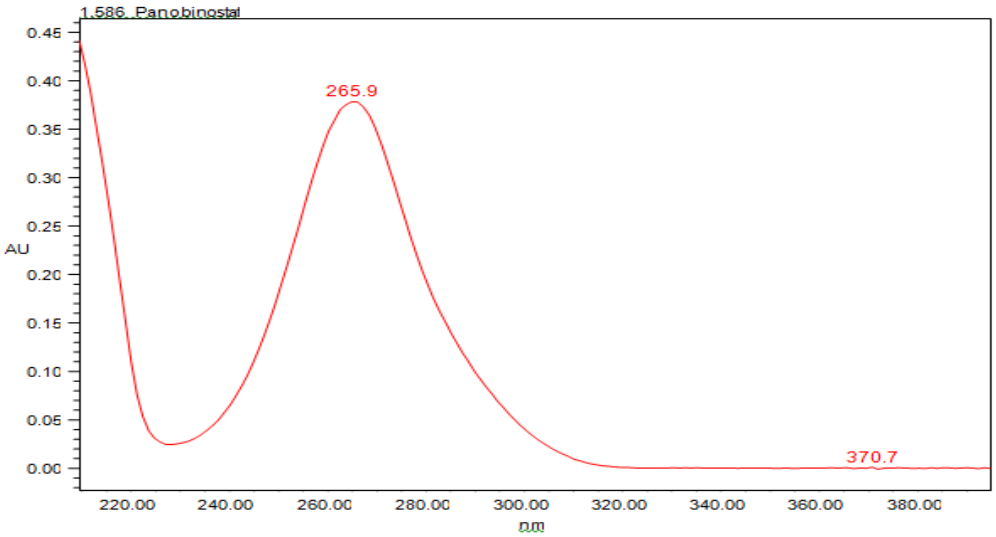

Fig. 2: UV Spectrum of panobinostat

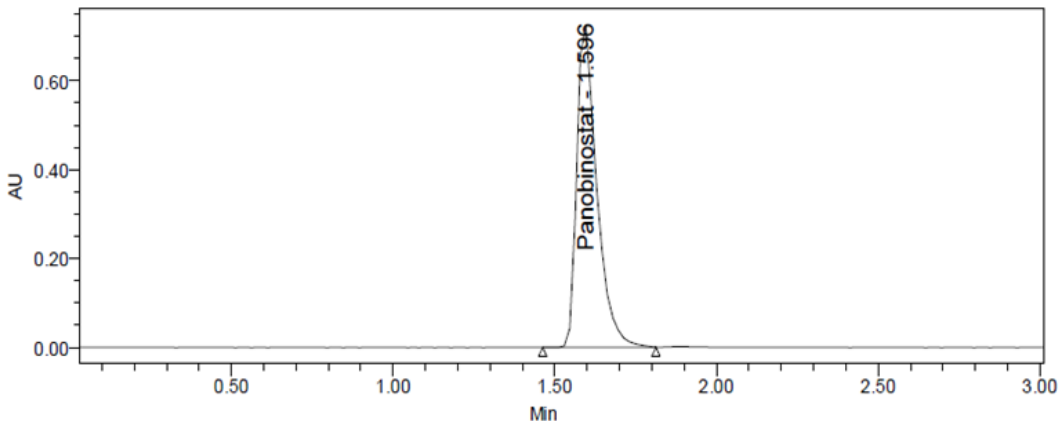

Fig. 3A: Standard chromatogram

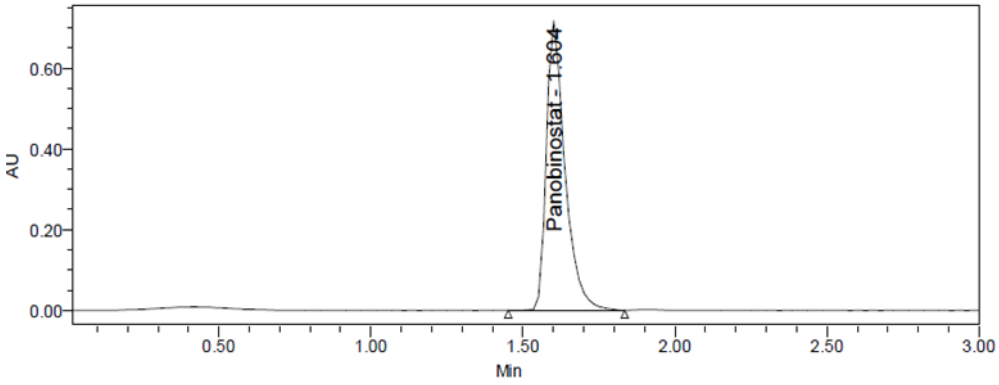

Fig. 3B: Sample chromatogram

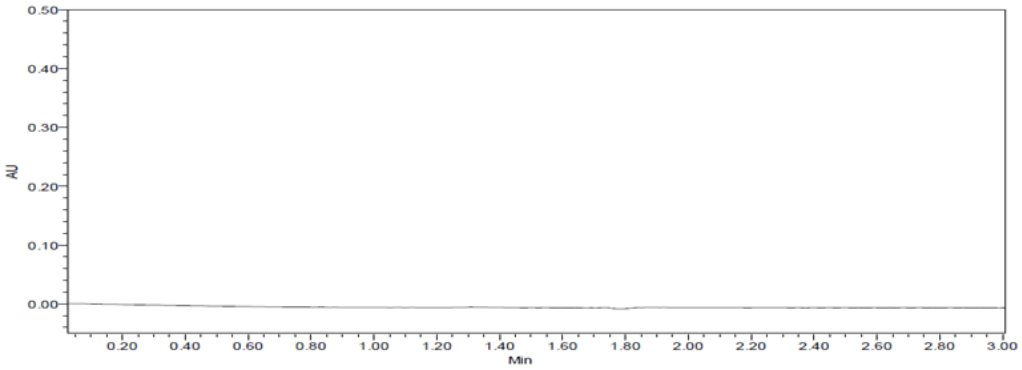

Fig. 3C: Blank chromatogram

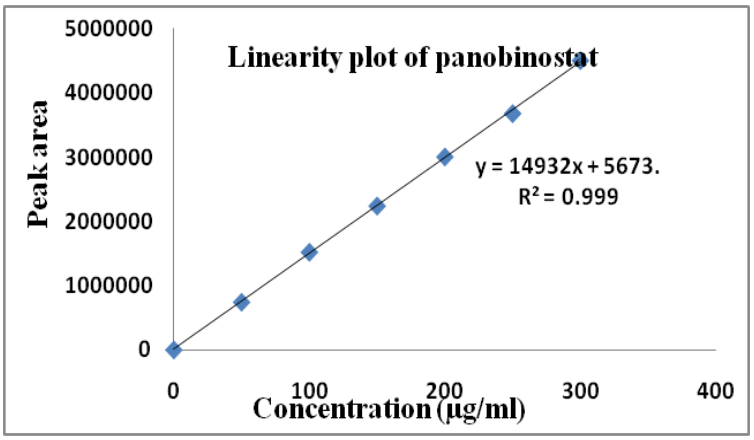

Fig. 4: Linearity plot of panobinostat

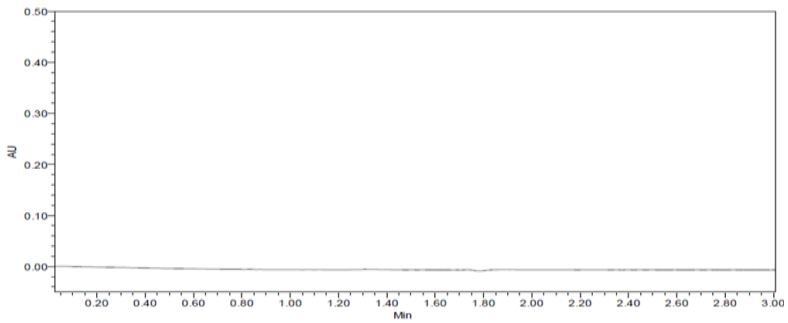

Fig. 5: Placebo chromatogram

Table 1: System suitability and validation parameter results

Parameter	Result(mean±SD)	
Precision (%RSD, n)	0.98(100.13±0.98)	
Accuracy (% Recovery, n mean±SD)	99.46% ±0.29- 99.98% ±0.16	
Specificity	Specific, No interference	
Linearity range (µg/ml)	50 – 300	
Correlation coefficient, r	0.9998	
Limit of Detection (µg/ml)	0.19	
Limit of Quantitation (µg/ml)	0.57	
Ruggedness (%RSD, n)	Day 1	Day 2
	1.7	1.7
Robustness (%RSD, n)	Less flow rate 1.4	More flow rate 0.3
(Organic)	Less mobile phase 1.3	More mobile phase (Organic) 1.0
	Less column temperature 0.8	More column temperature 1.1
Solution stability	Day 1 (0 h)	Day 2 (After 24 h)
(%RSD, n)	1.7	1.7
(% Assay, n)	100.13±0.98	99.80±1.67
USP Plate count	3434	
USP Tailing factor	1.46	

n–number of samples, i.e., six samples for estimation

Table 2: Forced degradation studies result

Stress condition	% Assay	% area of degradation peak	% Degradation
2N HCl for 30 min at 60 °C	95.90	3.65	4.10
2N NaOH for 30 min at 60 °C	94.37	-	5.63
20% H_2O_2 for 30 min at 60 °C	97.10	-	2.90
Water for 6h at60 °C	99.32	-	0.68
UV light 200wts/h or 7 d	98.68	-	1.32
105 °C for 6h	98.04	-	1.96

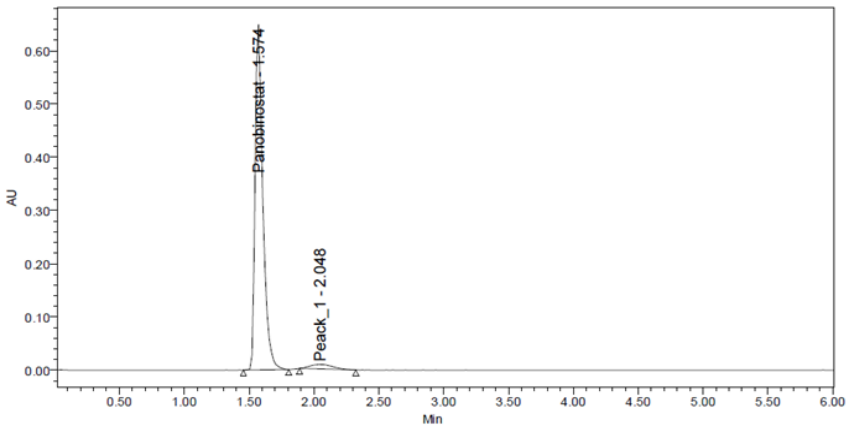

Fig. 6A: Acid degradation chromatogram

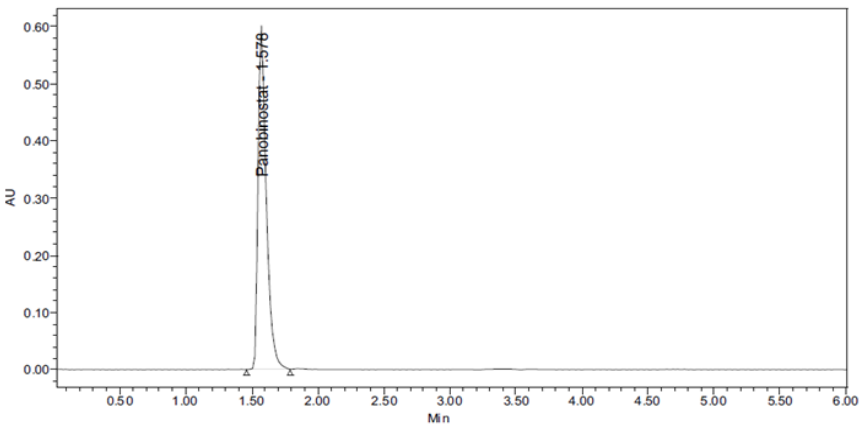

Fig. 6B: Base degradation chromatogram

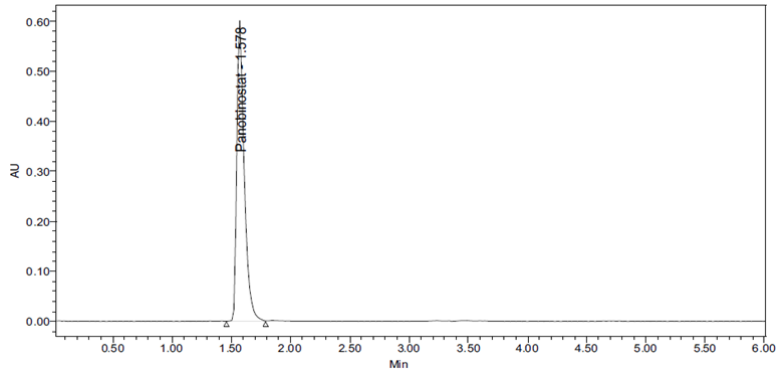

Fig. 6C: Peroxide degradation chromatogram

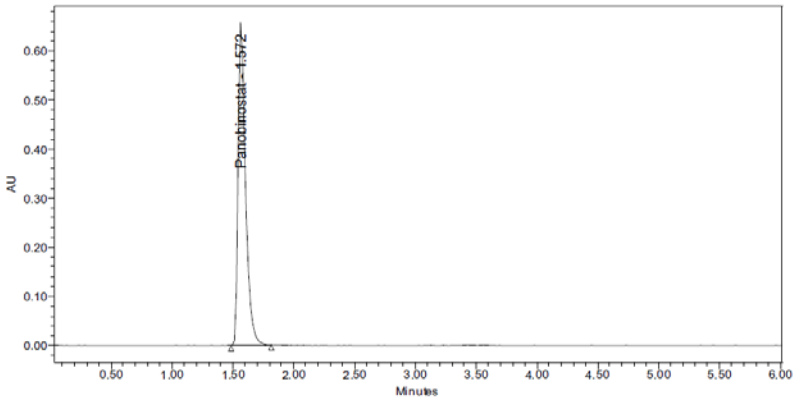

Fig. 6D: Water stress study chromatogram

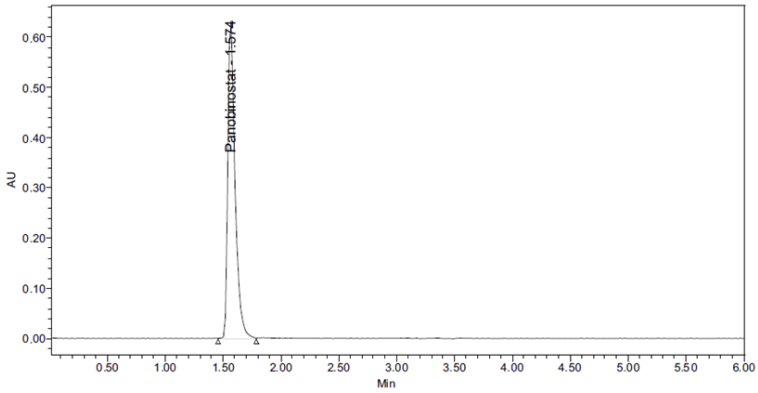

Fig. 6E: Photo stability degradation chromatogram

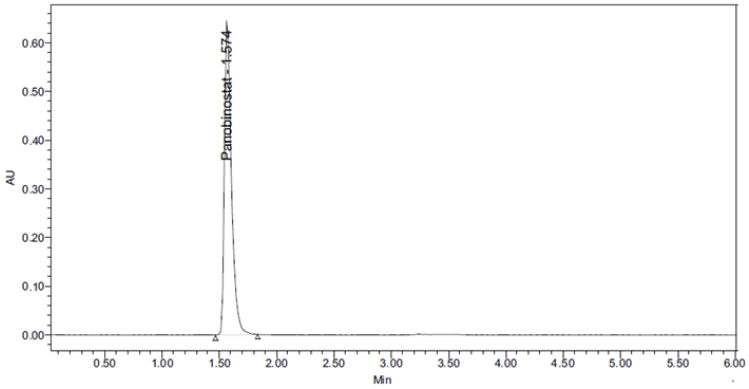

Fig. 6F: Dry heat study chromatogram

DISCUSSION

For the development of method for the estimation of panobinostat in pharmaceutical dosage form initially many mobile phases and many columns were tried to elute the drug peak with less tailing factor and more plate count.

Acquity UPLC Hibar C18 (100 mm × 2.1 mm, 1.8µ) column and 0.1% OPA: Acetonitrile (50:50%v/v) as mobile phase were selected based on peak parameters. The detection wavelength was found to be 266 nm as shown in fig. 2 of UV spectrum.

Prepared standard solution, sample solution and blank solution were injected into the UPLC system and system suitability parameters were noted as summarized in table 1 along with chromatograms as shown in fig. 3A, 3B and 3C respectively.

The developed method was found to obey Beer's law in the concentration range of 50µg/ml–300µg/ml with correlation coefficient of 0.9998. A linearity graph was plotted between concentration and peak area as shown in fig. 4 and results are summarized in table 1.

The method was found to be accurate as the % recovery was 99.46%-99.98% and was within the limits. The % RSD was found to be 0.98, indicates that the method was precise. The method was found to be specific, as there is no interference of retention time of placebo peak with that of drug peak. The placebo chromatogram was shown in fig. 5.

Forced degradation studies results indicate that the drug was found to be stable in various stress conditions as net degradation was found to be within the limits. The chromatograms were shown in fig. 6 and results were summarized in table 2.

CONCLUSION

A specific, accurate, precise stability indicating method was developed for the estimation of panobinostat in pharmaceutical dosage form using UPLC. The method was validated by using various validation parameters and the method was found to be linear, precise, accurate, specific and robust. From the degradation studies conducted it is concluded that panobinostat were more stable at more concentrations of acid, base, peroxide, thermal, UV and water stress study conditions. The run time was 3 min which enables rapid quantitation of many samples in routine and quality control analysis of tablet formulations.

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AUTHORS CONTRIBUTIONS

All authors contribute equally to this manuscript

CONFLICTS OF INTERESTS

The authors claim that they have no conflict of interest. It has not meant to publish elsewhere. And it has not meant simultaneously presented for publication elsewhere. All authors have decided to the submission to the journal.

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