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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR QUANTITATIVE ANALYSIS OF PANOBINOSTAT IN PURE AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, Precised, Accurate method was developed for the estimation of Panobinostat by RP-HPLC technique. Chromatographic conditions used are stationary phase Zodiac C18 150mm x 4.6 mm, 5µ, Mobile phase 0.01% KH2PO4:Acetonitrile in the ratio of 60:40 and flow rate was maintained at 1.0 ml/min, detection wave length was 230 nm, column temperature was set to 30^{o}C and diluent was mobile phase Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to 150% levels, R^2 value was found to be as 0.999. Precision was found to be 0.6 for repeatability and 0.2 for intermediate precision. LOD and LOQ are 0.050µg/ml and 0.151µg/ml respectively. By using above method assay of marketed formulation was carried out 99.98% was present. Degradation studies of Panobinostat were done, in all conditions purity threshold was more than purity angle and within the acceptable range.

KEYWORDS: HPLC, Panobinostat, Method development. ICH Guidelines.

1. INTRODUCTION

Panobinostat is an oral deacetylace (DAC) inhibitor approved by the FDA for the treatment of multiple myeloma.[1] The approval was accelerated based on progression-free survival, therefore confirmatory trials by the sponsor to demonstrate clinical efficacy in multiple myeloma treatment are in progress of being conducted.[2-4] Panobinostat is marketed by

Novartis under the brand name Farydak. Panobinostat acts as a non-selective histone deacetylase inhibitor (pan-HDAC inhibitor) and it is the most potent DAC inhibiting agent available on the market. In the present study, a new RP-HPLC method was developed which shown high reproducibility and sensitivity. The developed method was validated as per ICH guidelines.[5-11]

2. MATERIALS AND METHODS

2.1 Standards and Chemical Used

Panobinostat was gift sample for Noverties company, Hydrabad. All the chemicals Acetonitrile HPLC Grade, HPLC grade Water.

2.2 Instrumentation

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

2.3Preparation of Mobile phase: Into a 1000ml cleaned volumetric flask, HPLC grade, acetonitrile 400ml and Potassium dihyogen phosphate 600ml (0.01%w/v) which are filtered through 0.25mm membrane filters by vacuum filtration were slowly added, mixed well and sonicated upto 20min. Cool the above solution. This solution is again sonicated to 10min. Cool the solution to room temperature and use for chromatography method.

2.4 Preparation of Standard stock solutions: Accurately weighed 5mg of Panobinostat transferred 25ml of volumetric flask, and 3/4 Th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (200µg/ml of Panobinostat).

Preparation of Standard working solutions (100% solution): 1ml of Panobinostat from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (20µg/ml of Panobinostat).

2.5 Preparation of Sample stock solutions: 5 capsules were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 capsule was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further

the volume was made up with diluent and filtered by HPLC filters. (200µg/ml of Panobinostat).

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (20µg/ml of Panobinostat).

2.6 RP-HPLC Method Development

Based on nature and solubility characteristics of Panobinostat, reverse phase mode of HPLC was selected for chromatography. Among different RP-HPLC stationary phases tried, C_{18} column was found to be optimum.

In order to get sharp peak with base line separation from interfering peaks carried out a number of experiments by varying the composition of solvents and mobile phase flow rate. To have an ideal separation of the drug under isocratic conditions, mixtures of solvents like methanol, water and acetonitrile with or without different buffers in different combinations were tested as mobile phase. A mixture of 0.01% KH_2PO4 : Aceto nitrile (60:40) (v/v) was proved to be the most suitable of all the combinations, since the chromatographic peak obtained was better defined and resolved and almost free from tailing. The chromatographic conditions for the estimation of Panobinostat were discussed in table.

Table 1: Optimized chromatographic conditions for estimation of Panobinostat.

Parameter	Condition
Mobile phase	0.01% KH2PO4: Acetonitrile (60:40) (V/V)
Pump mode	Isocratic
Diluents	Mobile phase
Column	Zodiac C18 Column (150 x 4.6 mm, 5µ)
Column Temp	30^0C
Wavelength	230nm
Injection Volume	10µL
Flow rate	1.0 ml/min
Run time	10min

3. RESULT AND DISCUSSIONS

3.1 Analysis of Formulation

The sample solution was injected and a chromatogram was recorded. The injections were repeated six times and the peak areas were recorded. The amount of drug present in the pharmaceutical formulation was calculated using standard calibration curve (concentration in

µg/ml was taken on X –axis and average peak area on Y –axis). A representative chromatogram has been given in Fig. 1.

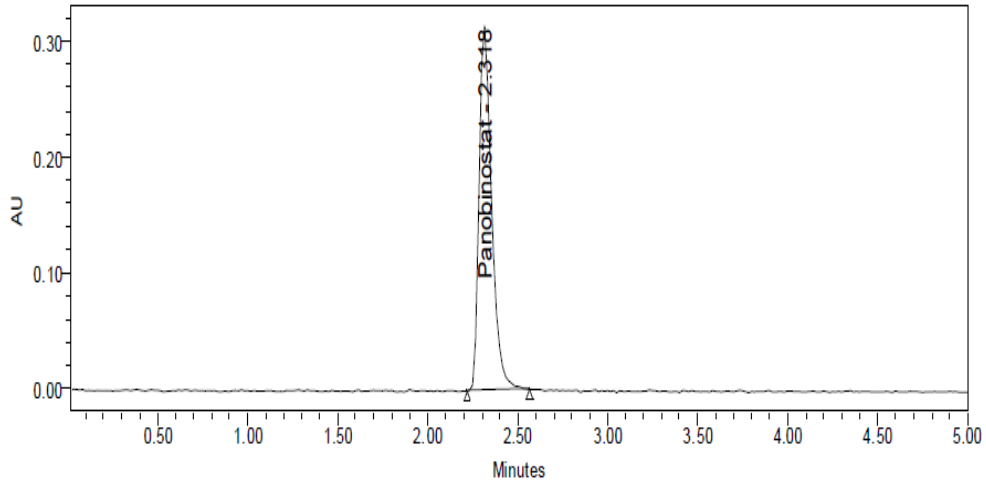

Fig. 1: Chromatogram of Panobinostat.

3.2 Validation of the Proposed Method

As an integral part of analytical method development is validation. The proposed method was validated as per ICH guidelines.

3.2.1 Linearity

To demonstrate the linearity of assay method, inject 5 standard solutions with concentrations of about 5 ppm to 30ppm of Panobinostat. Plot a graph to concentration versus peak area. Slope obtained was y = 80662x + 1849 and Correlation Co-efficient was found to be 0.999 and Linearity plot was shown in table.

Table 2: Linearity Results of Panobinostat.

Linearity Level (%)	Concentration (ppm)	Peak Area
0	0	0
25	5	394835
50	10	809969
75	15	1224311
100	20	1627235
125	25	2013229
150	30	2412872

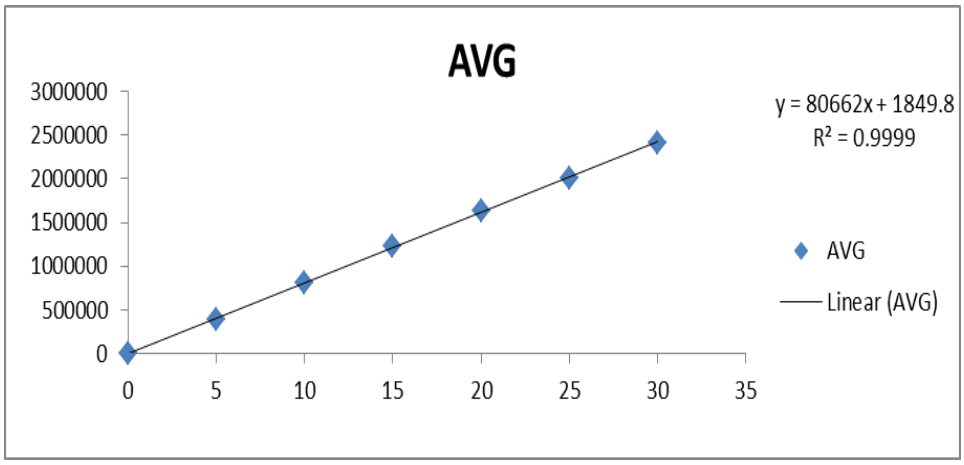

Fig. 2: Linearity Plot.

3.2.3 Precision

Repeatability: Six working sample solutions of 20ppm are injected and the % Amount found was calculated and %RSD was found to be 0.2.

Intermediate precision: Five working sample solutions of 20ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 0.3.

32.3. Ruggedness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC 2965 SYSTEM, Aglient HPLC By different operators using different columns of similar type like Hypersil C_{18} Hichron C_{18}. It was observed that there were no marked changes in the chromatograms, which demon started that the RP-HPLC method developed, is ruggedness.

3.2.4 Limit of Detection and Limit of Quantification

A Calibration curve was prepared using concentrations in the range of 5-30 µg/ml (expected detection limit range). The standard deviation of Y-intercepts of regression line was determined and kept in following equation for the determination of Detection limit and Quantitation limit. The results were reported in table 3.

$$\text{Limit of detection} = \frac{\sigma \times 3.3}{S}$$

$$\text{Limit of quantification} = \frac{\sigma \times 10}{S}$$

Where,

σ = the standard deviation of the response.

S = the slope of the calibration curve.

Table 3: Limit of Detection and Limit of Quantification for Panobinostat.

Parameter	Values
Limit of Quantification	0.151µg/ml
Limit of Detection	0.050 µg/ml

3.2.5 Accuracy: The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed sample solution Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner and %Recovery was calculated as 100.15%. And chromatograms were shown in fig 3 Recovery ranging from 99.12 to 101.04% were obtained by the proposed method.

Table 3: Accuracy data.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	10	10.104	101.04	100.15%
	10	10.023	100.23	
	10	10.040	100.40	
100%	20	20.181	100.90	
	20	20.057	100.28	
	20	20.005	100.02	
150%	30	30.039	100.13	
	30	29.771	99.24	
	30	29.737	99.12	

3.2.6 Robustness: Small Deliberate change in the method is made like Flow minus, flow plus, Mobile phase minus, Mobile phase plus, Temperature minus, Temperature Plus. %RSD of the above conditions are calculated.

Table 4: Robustness Data.

Parameter	% RSD
Flow Minus	1.6
Flow Plus	0.6
Mobile phase Minus	0.9
Mobile phase Plus	1.0
Temperature minus	0.8
Temperature plus	0.5

3.2.8 System Suitability

A Standard solution of Panobinostat working standard was prepared as per procedure and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention time, tailing factor, theoretical plates and peak areas from five replicate injections are within range and Results were shown in table 5.

Peak Name: Panobinostat

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Panobinostat	2.273	1612283	4834	1.40
2	Panobinostat	2.302	1638149	5157	1.36
3	Panobinostat	2.314	1627657	5195	1.38
4	Panobinostat	2.316	1632684	5238	1.37
5	Panobinostat	2.317	1611515	4596	1.40
6	Panobinostat	2.318	1632864	4823	1.36
Mean			1625859		
Std. Dev.			11313.6		
% RSD			0.7		

4. CONCLUSION

A convenient, rapid, accurate, precise RP-HPLC method has been developed for estimation of Panobinostat. The proposed method followed the ICH guidelines. The proposed method can be used for the routine analysis of Panobinostat in bulk preparations of the drug and in pharmaceutical dosage forms without interference of excipients.

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