Research Article

Development and Validation of Vorinostat in Bulk and Formulation by RP-HPLC

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ABSTRACT

A Rapid and Precise Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method was developed and validated for the estimation of Vorinostat, in its pure form, as well as in dosage form. The Chromatography was carried out on a Phenomenex Gemini C18 (4.6 x 250mm, 5µm) column using a mixture of Methanol and HPLC water (55:45 v/v) as the mobile phase at a flow rate of 0.9ml/min, the detection was carried out at 245nm. The retention time of the Vorinostat was found as 2.443 ±0.02 min. The method produce linear responses in the concentration range of 10-50µg/ml of Vorinostat. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations of Vorinostat.

Keywords: RP-HPLC, Vorinostat, Validation, Phenomenex Gemini, Precision.

INTRODUCTION

Vorinostat or Suberoyl Anilide Hydroxamic Acid (SAHA) is an Antineoplastic agent, which is currently under investigation for the treatment of Cutaneous T cell Lymphoma, a type of skin cancer. It is the first in a new class of agents known as Histone Deacetylase Inhibitors. Arecent study suggested that Vorinostat also possesses some activity against recurrent glioblastoma multiform, resulting in a median overall survival of 5.7 months (compared to 4-4.4 months in earlier studies). Chemically it is known as N-hydroxyl-N'-phenyl-octanediamide.[1] It has a molecular structure as shown in figure 1.
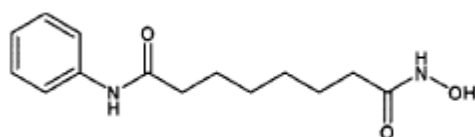

Figure 1: Structure of Vorinostat

Literature survey of the drug revealed that the drug was estimated by LC-MS methods in biological fluids [2-4], and the pharmacological action of the drug was also studied[5-7]. It was also estimated by RP-HPLC in rat plasma.[8]but, there were no methods for the estimation of Vorinostat in bulk and formulation by RP-HPLC. Hence the present research was aimed to develop and validate a new, simple, accurate and economical method for estimation of Vorinostat in bulk and formulation for the intended analytical application.

MATERIALS AND METHODS

Drugs, Chemicals and Solvents

Vorinostat API was kindly gifted by Sura Labs pvt Ltd, India. Zolinza tablets were purchased from local market.

All the chemicals, Solvents used were of analytical grade.

Instruments

The analysis was performed on Waters HPLC (with software Empower 2), fitted with a gradient pump, PDA Detector and Phenomenex Gemini C18 column (4.6×250mm, 5µm) which is maintained at an ambient temperature. The optimized mobile phase composition was Methanol: HPLC Water (55:45v/v). The mobile phase was run at a flow rate of 0.9ml/min. The injection volume was 10µl. The chromatographic run time was adjusted as 8min. The wavelength of the detector was set at 245nm for the analysis of the drug.

Preparation of Mobile phase

Accurately measured 450 ml of HPLC Water and 550 ml of Methanol were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45µ filter under vacuum filtration.

Method development

Preparation of standard solution

Accurately weighed 10mg of Vorinostat was taken in a 10ml standard volumetric flask, sonicated with few ml of methanol and then the volume was made up to the mark with methanol. Further, 0.3ml of this solution was diluted to 10ml with methanol.

The samples were injected by changing the chromatographic conditions. The chromatograms, their respective peak parameters were recorded. The Chromatogram of Standard Vorinostat was shown in Figure 2.

Preparation of Sample Solution

Accurately weighed 10mg of Vorinostat capsule powder was taken in a standard volumetric flask, sonicated with few ml of diluent and then the volume was made up to the mark with the same. Further, 0.3ml of this solution was diluted to 10ml with the diluent.

Then the sample was injected in replicate and the % purity was found to be 100%. The sample chromatogram was shown in Figure 3.

Mobile Phase Optimization

Initially the mobile phase tried was methanol: Water and Methanol: Phosphate buffer with varying proportions. Finally, the mobile phase was optimized to phosphate buffer (pH 3.5), Methanol in proportion 70:30 v/v respectively.

Optimization of Column

The method was performed with various C18 columns like X- bridge column, Xterra and Symmetry C18 column. Phenomenex Gemini C18 (4.6 x 150mm, 5μm) was found to be ideal as it gave good peak shape and resolution at 0.9ml/min flow rate.

Method Validation

The developed method was validated for Specificity, Linearity, Precision, Accuracy, Robustness, Limit of Detection (LOD) and Limit of Quantification (LOQ).

Specificity

Specificity is defined as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products and matrix components.

The analytical method was tested for specificity to measure accurately quantityVorinostat in drug product.

Linearity

From the Standard stock solution, 10, 20, 30, 40, 50 ppm solutions were made and their chromatograms were recorded. From the recorded chromatograms, their respective mean peak areas were calculated and the linearity plot was constructed using the mean peak areas at their respective concentrations. The correlation coefficient was found to be 0.999. The linearity data of Vorinostat was shown in Table 1 and the calibration plot was shown in Figure 4.

Precision

The precision of the method was evaluated by carrying Repeatability in the same day and Intermediate Precision. The percentage relative standard deviation (%RSD) of each study was calculated and was found to be less than 2% showing the method was precise. The results of Precision were shown in Table 2.

Accuracy

Accuracy of the method was determined by calculating the %recovery of Vorinostat by the standard addition method at 50%, 100% and 150% levels. The results were given in Table 3.

Robustness

Robustness was performed for the flow rate variations from 0.8ml/min to 1ml/min and mobile phase ratio variation from more organic phase to less organic phase for Vorinostat. The method was robust only in less flow condition and even by change in the mobile phase ±5%. There was no significant change in the parameters like Resolution, Tailing factor, Symmetric factor and Plate count. The results of Robustness study were shown in Table 4.

Limit of Detection (LOD)

LOD is the lowest level of concentration of analyte in the sample that can be detected, though not necessarily quantitated. It was calculated to be 1.181μg/ml using the formula,

$$LOD = 3.3\ \sigma/S$$

Where,
σ= Standard deviation of the response,
S= Slope of Calibration curve.

Limit of Quantitation (LOQ)

LOQ is the lowest concentration of analyte in a sample that may be determined with acceptable accuracy and precision when the required procedure is applied. It was calculated to be 5.502μg/ml using the formula,

$$LOQ = 10\ \sigma/S$$

Where,
σ= Standard deviation of the response,
S= Slope of Calibration curve.
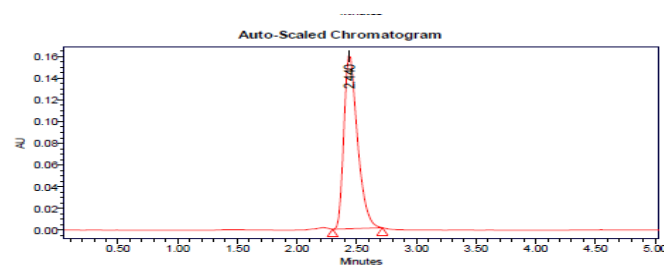
RESULTS AND DISCUSSION
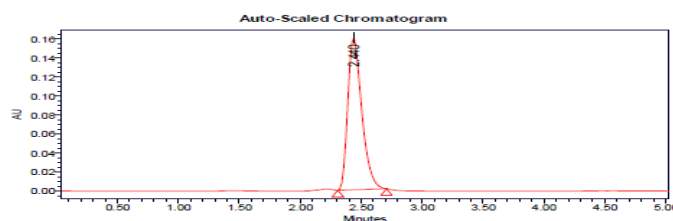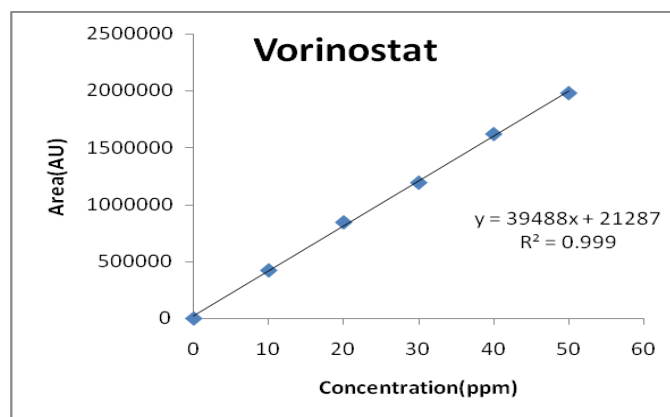

Figure 2: Standard chromatogram of Vorinostat

Figure 3: Sample chromatogram of Vorinostat

Table 1: Linearity data of Vorinostat

Concentration Level (%)	Concentration (μg/ml)	Average Peak Area
33	10	421796
66	20	842946
100	30	1191428
133	40	1618010
166	50	1976727

Figure 4: Calibration curve of Vorinostat

Table 2: Results of Precision

Parameter	Repeatability	Intermediate Precision
RT*	2.4376	2.4371
Std. dev*	0.000548	0.00132
%RSD*	0.022481	0.05

Table 3: Results of Accuracy

% Conc.	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	613351	15	14.9	99.3	
100%	12 28860	30	30.5	101	99%
150%	1793497	45	44.1	99	

Table 4: Results of Robustness

Actual Flow rate of 0.9 mL/min	1271072	2443	3422	1.39
Less Flow rate of 0.8 mL/min	839183	2865	2225	1.58
More Flow rate of 1.0 mL/min	837282	2.065	2492	1.58
More aqueous phase (about 5 % Increase in Methanol)	8 21092	2.156	2937	1.52
Less aqueous phase (about 5 % decrease in Methanol)	816281	2.921	2912	1.42

CONCLUSION

The proposed RP-HPLC method for estimation of Vorinostat in API and Formulation and its validation was carried out as per ICH guidelines. By studying various parameters, finally we conclude that the method is simple, precise, accurate, sensitive, robust, economic and specific and can be applied for the determination of Vorinostat in API and as well as pharmaceutical formulations. The method was found to be linear in the specified range. All the required validation parameters were estimated and were found to be within the limits.

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