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Research Article

RP-HPLC Method for Determination of Zinostatin in Bulk and Pharmaceutical Formulation

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ABSTRACT

A rapid and precise RP-HPLC method has been developed for the validated of Zinostatin in its pure form as well as in tablet dosage form. Chromatography was carried out on Apollo C18 (4.6×150mm, 5µ) column using a mixture of Methanol and water (80:20 v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 232nm. The retention time of the Zinostatin was 3.0 ±0.02min. The method produce linear responses in the concentration range of 25-115µg/ml of Zinostatin. 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.

1. Introduction

Zinostatin (Neocarzinostatin,vinostatin) is a cytotoxic enediyne that alkylates DNA and RNA. It is an antibiotic hybrid containing an aminoglycoside chromophore. Zinostatin is isolated from the bacterium Streptomyces carzinostaticus. It is chemically denoted as (1aS, 5R, 6R, 6aE)-6-{[(2R, 3R, 4R, 5R, 6R)-4, 5-Dihydroxy-6-methyl-3-(methyl-amino)-tetra-hydro-2H-pyran-2-yl]oxy}-1a-(2-oxo-1,3-dioxolan-4-yl)-2, 3, 8, 9-tetra-dehydro 1a, 5, 6, 9a-tetrahydro-cyclopenta-[5, 6]-cyclonona-[1, 2-b]-oxiren-5-yl-2-hydroxy-7-methoxy-5-methyl-1-naphthoate.

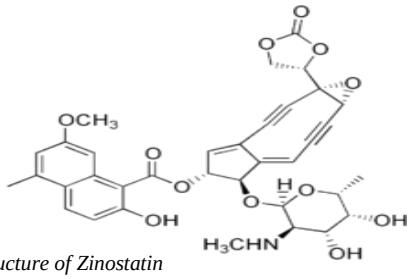

Fig 1: *Structure of Zinostatin*

The aminoglycoside component of Zinostatin intercalates into DNA and the benzene diradical intermediate of the enediyne core binds to the minor groove of DNA, resulting in single and double-strand breaks in DNA and apoptosis. It is used as antineoplastic and antibacterial agent. Literature review of Zinostatin shown that there were several analytical methods like Spectrophotometry.[1-2] Only few methods were reported for RP-HPLC for the estimation of this drug in bulk and in its formulation. Hence the present work targeted to develop a new precise, accurate and sensitive RP-HPLC [3-7] method for the determination of Zinostatin in API and formulation. The developed method validated as per ICH guidelines.[8-10]

2. Materials & Methods

Chemicals & Reagents: Zinostatin as pure standard reference drug was obtained from Sura labs, Hyderabad, India. Acetonitrile, Methanol used were of HPLC grade and purchased from Merck specialties Private Limited, Mumbai, India.

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Instrument: HPLC analysis was performed on chromatographic system of water 2695 separation module with empower software liquid chromatography comprising water 996 photo diode array detector, Column Apollo C18 (4.6×150mm) 5µ was used and an equipped with auto sampler. Derivative spectral and photometric absorbance measurements are done on UV spectrophotometer with software UV win, Lab India make 3092. 10mm path length quartz cells were used. Digital analytical balance Shimadzu make AUX 220 was used for weighing drug.

Experimental conditions: Chromatographic separation achieved using an analytical Column Apollo C18 (4.6×150mm) 5µ. Mobile phase consisted of Methanol: Water (80:20 v/v). The elution was achieved isocratically at a flow rate of 1.0ml/min with injection volume of 10µl, column temperature was maintained at 35ºC and chromatograph was recorded at wavelength 232nm.

Preparation of mobile phase: Accurately measured 800ml (80%) of HPLC Methanol and 200ml of HPLC Water (20%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

Preparation of standard solution: Accurately weigh and transfer 10 mg of Zinostatin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.75ml of the above Zinostatin stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Sample Solution: Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Zinostatin sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.75ml of the above Zinostatin stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Assay: Inject the three replicate injections of standard and sample solutions through autosampler into chromatographic system and peak areas were measured. The percentage of assays were calculated. The % purity of Zinostatin in pharmaceutical dosage form was found to be 99.8%.

Method development: Some important parameters like pH of the mobile phase, concentration of the acid, were tested for a good chromatographic separation. Trials showed that mobile phase with reverse phase C18 column gives symmetric and sharp peaks. After the optimization of chromatographic conditions, estimation of Zinostatin as carried out by the developed RP-HPLC method. Standard solution of drug was injected separately and chromatogram of Zinostatin was recorded in Figure 1. Now the sample solution was injected separately and chromatogram was recorded until the reproducibility of the peak areas were satisfactory (Figure 2).

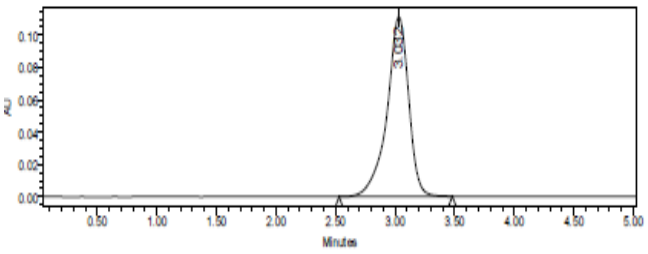

Fig 2: *Optimized Chromatogram (Standard)*

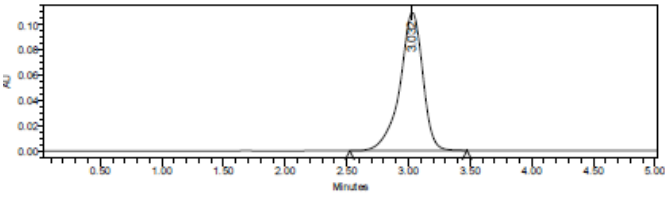

Fig 3: *Optimized Chromatogram (Sample)*

Analytical method validation: HPLC method was validated [11-13] according to the International Conference on Harmonization guidelines (ICH Q2B, validation of analytical procedures, methodology). The method was validated for parameters such as linearity, precision, accuracy, system suitability limit of detection, limit of quantification and robustness.

Linearity: Inject each level (25, 50, 75, 90 and 115µg/mL solutions (prepared from standard stock solution) into HPLC system and observed the linear relationship between concentration and peak area. in the concentration range of 25 – 115µg/mL.

Precision Repeatability: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was calculated.

Intermediate precision: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Accuracy: Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the amount found and amount added for Zinostatin and calculate the individual recovery and mean recovery values.

Robustness: Robustness was done by changing the actual chromatographic conditions like mobile phase ratio and flow rate. Results were determined by calculating the %RSD for six injections peak area values of each change in condition.

System suitability: This parameter used to know whether the HPLC system is suitable for actual chromatographic conditions or not. System suitability was estimated by injecting five standard solutions of Zinostatin and from the chromatograms %RSD, theoretical plates and peak symmetry were calculated.

Specificity: This parameter performed by injecting blank, standard and sample solutions and any interference with excipient and mobile phase were observed.

Limit of detection: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$LOD = 3.3 \times \sigma / s$

Quantitation limit: The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$LOQ = 10 \times \sigma / S$

3. Results & Discussion

Linearity and range: Linearity and range estimated by constructing the calibration curve by taking concentration on X-axis and peak area on Y-axis of 25, 50, 75, 90 and 115µg/mL solutions (prepared from standard

stock solution) and calculate the correlation coefficient. Correlation Coefficient (r) is 0.99, and the intercept is 10331. These values meet the validation criteria as shown in **Figure 4** and linearity values tabulated in **Table 1.**

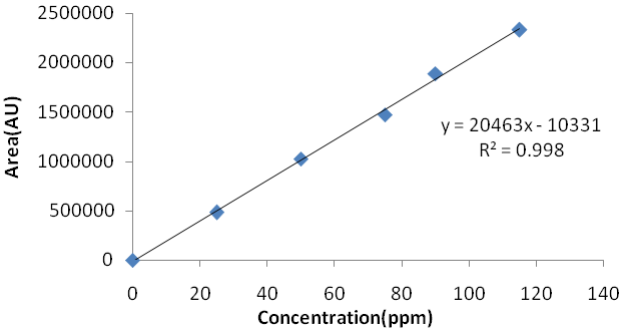

Fig 4: *Calibration curve of Zinostatin*

Table 1: Chromatographic data for linearity study

Concentration		Average
%	µg/ml	Peak Area
60	25	485219
80	50	1026232
100	75	1403734
120	90	1887785
140	115	2329488

Precision: Intermediate precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The results were shown in **Table2** for day 1 and 2. Calculated % RSD values were less than 2.

Accuracy: Inject the three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. The accuracy results for Zinostatin are recorded in **Table 3**.

Table 3: The accuracy results for Zinostatin

%Conc.	Area	Amount (ppm)		% Recovery	Mean Recovery
		Added	Found		
50	746164	37.5	37.46	99.8	99.8
100	1465242	75	74.8	99.7	
150	2194761	112.5	112.47	99.9	

Robustness: The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Zinostatin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Zinostatin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count. The results were recorded in **Table 4**.

Table 4: Results for Robustness

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Flow rate: 0.9mL/min	1409342	3.029	9404	1.1
Flow rate: 0.8mL/min	1472532	3.395	5967	1.2
Flow rate: 1.0mL/min	1499271	2.756	6645	1.5
About 5 % Less organic phase	1499261	3.592	8937	1.1
About 5 % More organic phase	1400285	2.715	9573	1.2

Specificity: The ICH documents define specificity 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. Analytical method was tested for specificity to measure accurately quantitated Zinostatin in drug product. The results for specificity of Zinostatin were cited in **Table 5**.

Repeatability: Multiple sampling from a sample solution was done and five working sample solutions of same concentrations were prepared, each injection from each working sample solution was given and obtained areas Standard Deviation and % Relative Standard Deviation are mentioned in **Table 6.**

System suitability: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits shown in **Table 6.**

Table 5: Peak results for Assay sample and standard of Zinostatin

Name	RT	Area	Height	USP Tailing	USP Plate Count	Inj
Sample	3.012	1397892	106006	1.2	9937	1
	3.014	1400977	106501	1.15	8755	2
	3.021	1412132	105495	1.15	5833	3
Standard	3.021	1412132	105495	1.12	6786	1
	3.021	1407910	105126	1.23	9957	2
	3.022	1406473	104887	1.1	4856	3

Limit of detection: Limit of detection is defined as lowest concentration of analyte that can be detected, but not necessarily quantified, by the analytical method. It is determined by the analysis of sample with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected, and it was found to be 7.3µg/ml of Zinostatin.

Table 2: Results of Intermediate precision Day 1 & 2 for Zinostatin

Peak Name	Day 1					Day 2				
	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
Zinostatin	3.007	1415046	106823	8953	1.1	3.029	1409342	107559	8937	1.33
	3.008	1411612	108281	9038	1.3	3.029	1410748	105215	9275	1.1
	3.008	1414509	107709	8849	1.13	3.030	1411727	105226	6674	1.45
	3.010	1412067	108063	7947	1.5	3.032	1409634	111714	9027	1.22
	3.012	1409788	108279	9937	1.2	3.032	1408759	109564	7547	1.1
	3.021	1412132	105495	9957	1.1	3.029	1464782	102847	8949	1.24
Mean		1412526					1419165			
Std. Dev.		1948.366					22372.74			
% RSD		0.137935					1.576471			

Table 6: Results of repeatability & system suitability for Zinostatin

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Zinostatin	3.029	1405533	107559	8758	1.1	3.029	1409342	107559	7837	1.1
2	Zinostatin	3.029	1416635	105215	9937	1.44	3.029	1410748	105215	9385	1.14
3	Zinostatin	3.030	1416874	105226	8827	1.13	3.030	1411727	105226	9937	1.33
4	Zinostatin	3.032	1402967	111714	9068	1.2	3.032	1409634	111714	8564	1.22
5	Zinostatin	3.032	1409927	109564	8846	1.1	3.032	1408759	109564	7746	1.1
Mean			1410387					1410042			
Std.dev			6323.527					1187.457			
%RSD			0.448354					0.084214			

Limit of quantification: Limit of quantification is the concentration that can be quantified reliably with a specified level of accuracy and precision. LOQ was found to be 22.2 µg/mL of Zinostatin.

4. Conclusion

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Zinostatin in bulk drug and pharmaceutical dosage forms. Zinostatin was freely soluble in ethanol, methanol and sparingly soluble in water. The %RSD values were within 2. The LOD and LOQ values were 7.3µg/ml and 22.2 µg/mL respectively. This method can be used for the routine determination of Zinostatin in bulk drug and in Pharmaceutical dosage forms.

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