

Development and Validation of a New RP- HPLC Method for the Zilucoplan in Pharmaceutical Dosage form as Per ICH Guidelines

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Abstract

The validation and reliability of a newly developed RP-HPLC method for quantifying zilucoplan in its pharmaceutical form, adhering to ICH guidelines. The method demonstrates specificity, accuracy, precision, and linearity within defined parameters. Robustness was confirmed through stress tests, ensuring suitability for routine quality control. Validation parameters, including system suitability, method precision, and stability testing, meet ICH criteria. Thus, this RP-HPLC method is a dependable tool for assessing zilucoplan quality, bolstering patient safety and drug efficacy assurance.

Keywords: zilucoplan, validation, reliability, RP-HPLC, ICH guidelines.

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1. Introduction

Zilucoplan is a prescription medication that belongs to a class of drugs called orexin receptor antagonists. It is used to treat insomnia, a condition that makes it hard to fall asleep or stay asleep. Zilucoplan works by blocking the activity of orexin, a chemical in the brain that promotes wakefulness[1-4]. By reducing orexin, Zilucoplan helps people with insomnia sleep better at night. Zilucoplan is taken by mouth, usually once a day before bedtime. The most common side effects of Zilucoplan are headache, drowsiness and following promising results in clinical trials. Pharmaceutical analysis plays a vital role in the Quality Assurance and Quality control of bulk drugs. Analytical chemistry involves separating, identifying, and determining the relative amounts of components in a sample matrix. Pharmaceutical analysis is a specialized branch of analytical Chemistry[5-8]. Pharmaceutical analysis derives its principles from various branches of sciences like physics, microbiology, nuclear science, and electronics etc. Qualitative analysis reveals the chemical

identity of the sample. Quantitative analysis establishes the relative amount of one or more of these species or analytes in numerical terms. Qualitative analysis is required before a quantitative analysis can be undertaken[9,10]. A separation step is usually a necessary part of both qualitative and quantitative analysis. The results of typical quantitative analysis can be computed from two measurements. One is the mass or volume of sample to be analyzed and second is the measurement of some quantity that is proportional to the amount of analyte in that sample and normally completes the analysis.

Chemicals & Reagents: Zilucoplan has been taken as a gift sample from Pharma Train, Ortho phosphoric acid has been purchased from Finar chemicals Pvt. Ltd. and all the other chemicals as well as reagents used for this study was of analytical grade.

HPLC Method Development[10-14]:

Wave length selection: UV spectrum of 10 µg / ml Zilucoplan in diluents (mobile phase composition) was

recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 290. At this wavelength both the drugs show good absorbance.

Optimization of Column: Spursil (4.6 x 150mm, 5 μ m) was found to be ideal as it gave good peak shape and resolution at 1 ml/min flow.

Optimized chromatographic conditions:

Instrument used : High performance liquid chromatography equipped with Auto Sampler and DAD or UV detector
Temperature : Ambient
Column : Spursil C 18 (4.6 x 150mm, 5.0 μ m)
Buffer : Ortho phosphoric acid buffer
Mobile phase : 30% Orthophosphoric Acid; 70% water.
Flow rate : 1 ml per min
Wavelength : 295 nm
Injection volume : 20 μ l
Run time : 10min.

2. Materials and Methods

Preparation of buffer and mobile phase:

Preparation of 0.1% Ortho phosphoric acid buffer:

Pipetted 1ml of ortho phosphoric acid in 1000 ml HPLC water.

Preparation of mobile phase:

Mix a mixture of above buffer 100ml (30%), OPA (70%) HPLC Water and degas in ultrasonic water bath for 10 minutes. Filter through 0.45 μ filter under vacuum filtration.

Diluent Preparation:

Use the Mobile phase as Diluents.

Validation parameters:

Assay:

Standard Solution Preparation:

- Accurately weigh and transfer 25mg of Zilucoplan working standard into a 25ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
- Further pipette 0.1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 25mg of Zilucoplan equivalent weight of the sample into a 25ml clean dry volumetric flask add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure: Inject 20 μ L of the standard, sample into the chromatographic system and measure the areas for the Zilucoplan peaks and calculate the % Assay by using the formulae.

Linearity:

Preparation of stock solution:

Accurately weigh and transfer 25mg of Zilucoplan working standard into a 25ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make

volume up to the mark with the same solvent. (Stock solution). Further pipette 0.1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Preparation of Level – I (10ppm of Zilucoplan):

0.1ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – II (20ppm of Zilucoplan):

0.2ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – III (30ppm of Zilucoplan):

0.3ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – IV (40ppm of Zilucoplan):

0.4ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Preparation of Level – V (50ppm of Zilucoplan):

0.5ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with Diluents.

Procedure:

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Precision:

Preparation of stock Solution:

Accurately weigh and transfer 10mg of Zilucoplan working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

- Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.
- Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure:

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.

Intermediate precision/ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day within the laboratory.

Preparation of stock solution:

Accurately weigh and transfer 10mg of Zilucoplan working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

- Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.
- Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.(30ppm)

Procedure: 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.

Accuracy:

For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

Preparation of Standard stock solution:

Accurately weigh and transfer 10mg of Zilucoplan working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

- Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.
- Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 5mg of Zilucoplan working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

- Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.
- Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 10mg of Zilucoplan working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

- Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.
- Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

For preparation of 150% solution (With respect to target Assay concentration): Accurately weigh and transfer 15mg of Zilucoplan working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

- Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.
- Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions.

Calculate the Amount found and Amount added for Zilucoplan and calculate the individual recovery and mean recovery values.

Limit of detection:

Preparation of Zilucoplan solution:

Preparation of 0.2µg/ml solution:

Accurately weigh and transfer 10mg of Zilucoplan working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

- Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.
- Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.
- Further pipette 0.2ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Limit of quantification:

Preparation of Zilucoplan solution:

Preparation of 0.9µg/ml solution:

Accurately weigh and transfer 10mg of Zilucoplan working standard into a 10ml clean dry volumetric flask add Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

- Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.
- Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.
- Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.
- Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluent.

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

- The flow rate was varied at 1.4 ml/min to 1.6ml/min.
- Standard solution 30 µg/ml of Zilucoplan prepared and analysed using the varied flow rates along with method flow rate.
- The Organic composition in the Mobile phase was varied from 63% to 77%

Standard solution 30 µg/ml of Zilucoplan was prepared and analyzed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

Forced Degradation Studies

Accurately weigh and transfer 10mg of Zilucoplan working standard into a 10ml clean dry volumetric flask add

Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with Diluents.

Acid degradation:

Pipette 3 ml of Zilucoplan of the above stock solution into a 10ml volumetric flask added about 3 ml of 0.1N Hcl and sonicated for 10minutes and kept it in darkness for 12 hours then refluxed under heat at 60 degrees in a heating mantle for 1 hours. Neutralized the sample solution using 0.1N NaOH and diluted up to the mark with diluents. The Final Sample was filtered through 0.44 micron Injection filters and injected into HPLC system.

Base Degradation:

Pipette 3 ml of Zilucoplan of the above stock solution into a 10ml volumetric flask added about 3 ml of 0.1N NAOH and sonicated for 10minutes and kept it in darkness for 12 hours then refluxed under heat at 60 degrees in a heating mantle for 1 hours. Neutralized the sample solution using 0.1N HCL and diluted up to the mark with diluents. The Final Sample was filtered through 0.44 micron Injection filters and injected into HPLC system.

Thermal Degradation:

Pipette 3 ml of Zilucoplan of the above stock solution into a 10ml volumetric flask and kept in oven under heat at 105 degrees for 12 hours and diluted up to the mark with diluents. The Final Sample was filtered through 0.44 micron Injection filters and injected into HPLC system.

Peroxide Degradation:

Pipette 3 ml of Zilucoplan of the above stock solution into a 10ml volumetric flask added about 3ml of 3% Hydrogen Peroxide (H2O2) and sonicated for 10 minutes and kept in darkness for 12 hours and refluxed under heat at 60 degrees in a heating mantle for 1 hours. The Final Sample was filtered through 0.44 micron Injection filters and injected into HPLC system.

Photo Degradation:

Pipette 3 ml of Zilucoplan of the above stock solution into a 10ml volumetric flask added about and kept in darkness for 12 hours. The Final Sample was filtered through 0.44 micron Injection filters and injected into HPLC system.

3. Results and Discussion

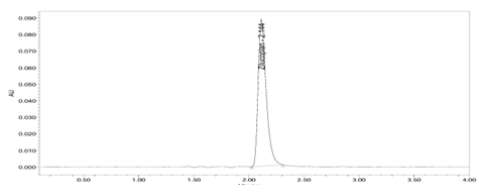


Figure 1: Chromatogram for Standard (30ppm)

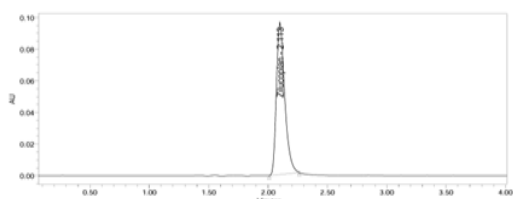


Figure 2: Chromatogram for Sample (30ppm)

Table 1: Results of Assay for Zilucoplan

	Label Claim (mg)	% Assay
Zilucoplan	384	101.85

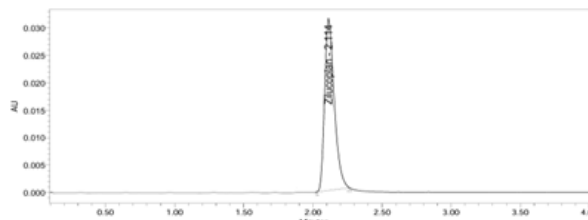


Figure 3: Chromatogram for linearity-1(10ppm)

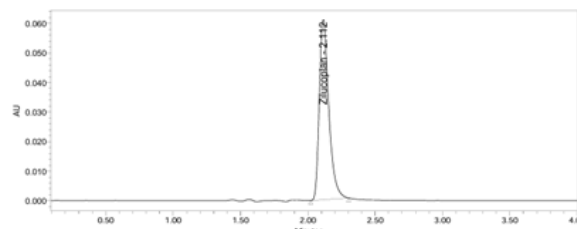


Figure 4: Chromatogram for linearity-2(20ppm)

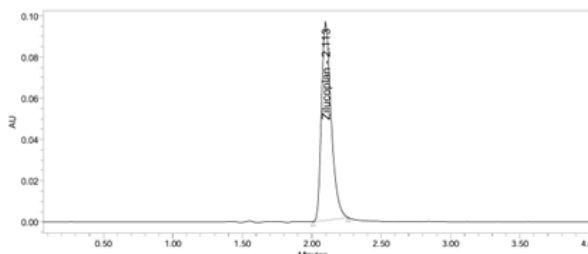


Figure 5: Chromatogram for linearity-3(30ppm)

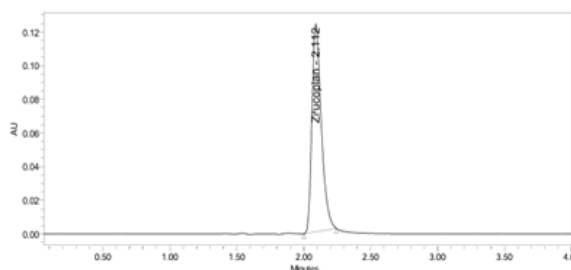


Figure 6: Chromatogram for linearity-4(40ppm)

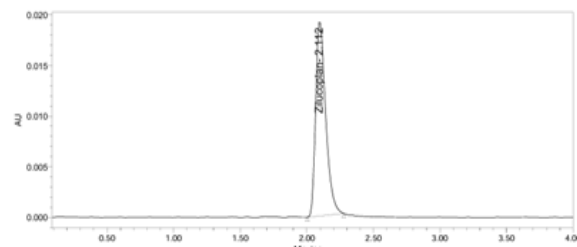


Figure 7: Chromatogram for linearity-5(50ppm)

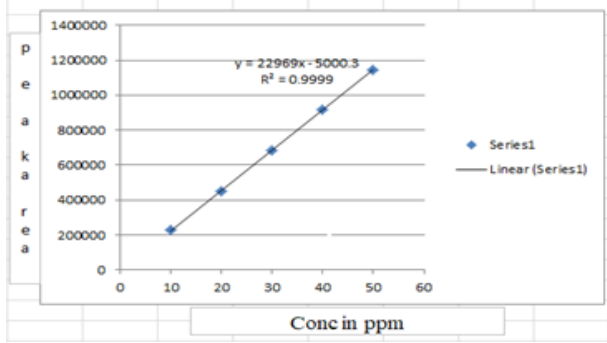


Figure 8: Calibration graph for Zilucoplan

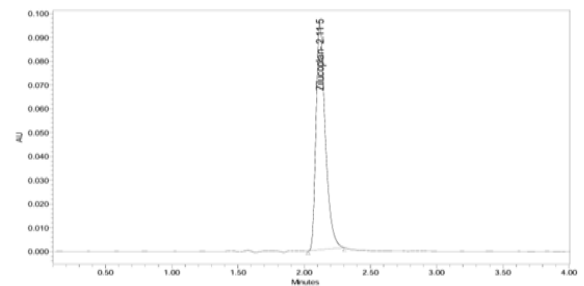


Figure 13: Chromatogram for Precision -5

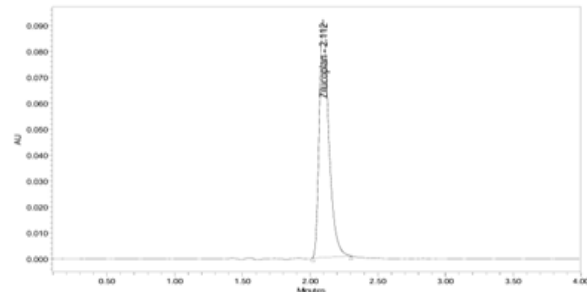


Figure 9: Chromatogram for Precision -1

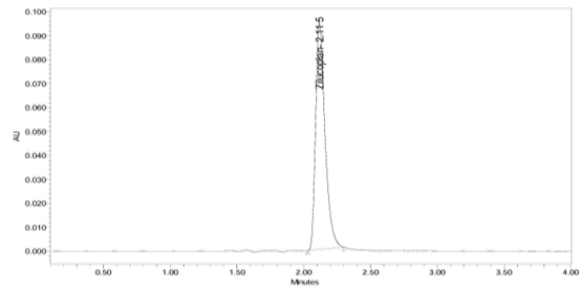


Figure 14: Chromatogram for Precision -6

Table 4: Results of Precision for Zilucoplan

Injection	Area
Injection-1	697409
Injection-2	690348
Injection-3	683414
Injection-4	682149
Injection-5	689131
Injection-6	679771
Average	687037
Standard Deviation	6522.873
%RSD	0.94

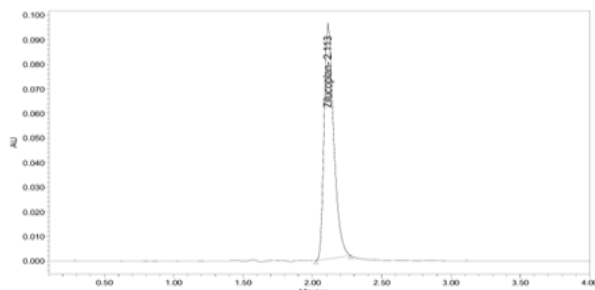


Figure 10: Chromatogram for Precision -2

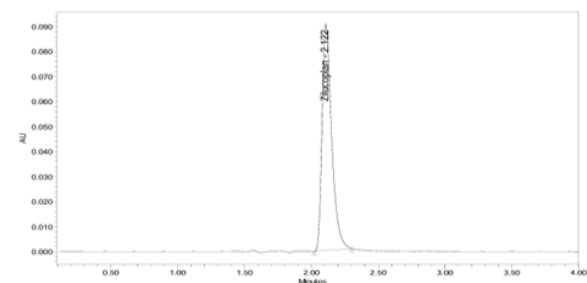


Figure 11: Chromatogram for Precision -3

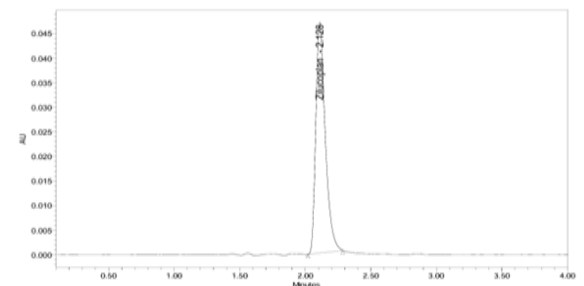


Figure 15: Chromatogram for Accuracy 50%-1

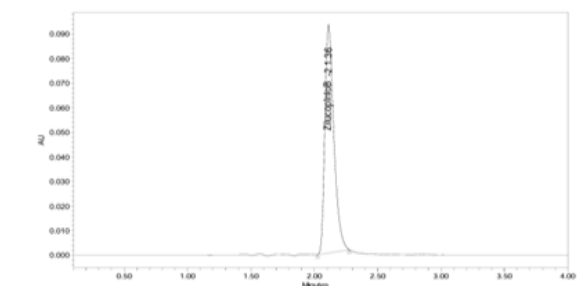


Figure 12: Chromatogram for Precision -4

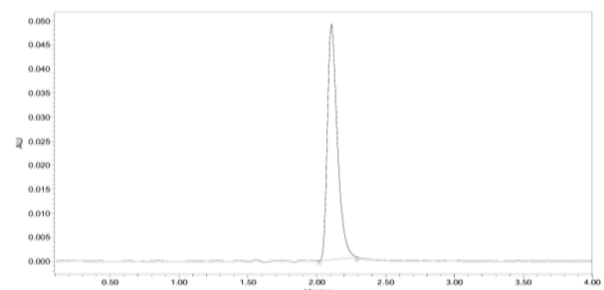


Figure 16: Chromatogram for Accuracy 50%-2

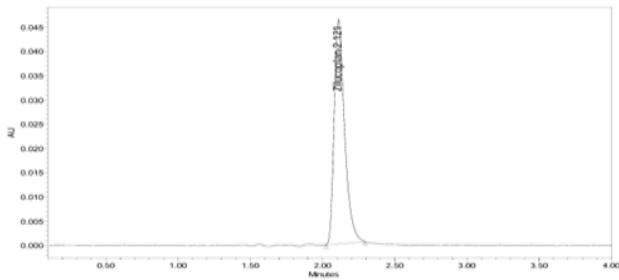


Figure 17: Chromatogram for Accuracy 50%-3

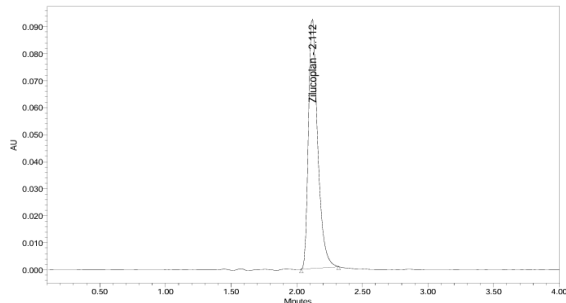


Figure 18: Chromatogram for Accuracy 100%-1

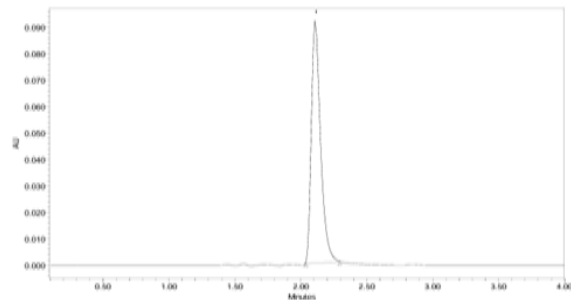


Figure 19: Chromatogram for Accuracy 100%-2

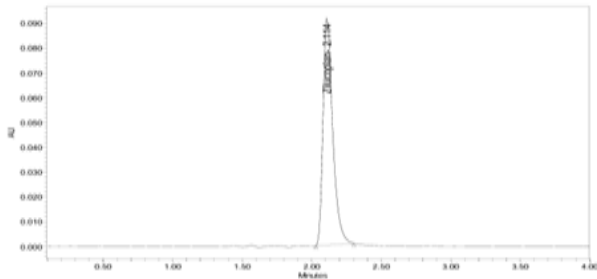


Figure 20: Chromatogram for Accuracy 100%-3

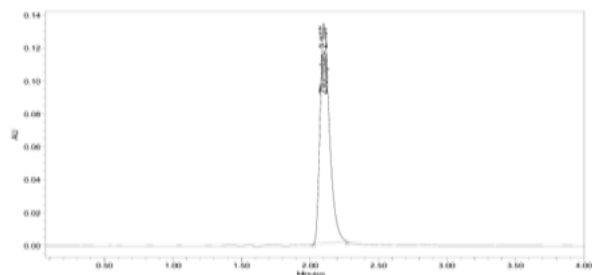


Figure 21: Chromatogram for Accuracy 150%-1

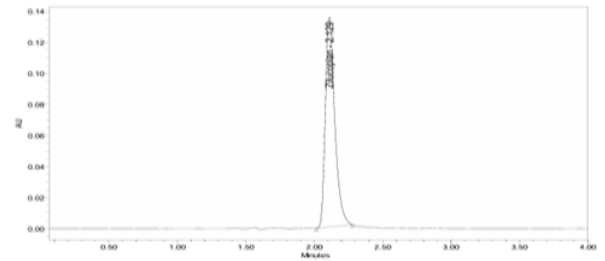


Figure 22: Chromatogram for Accuracy 150%-3

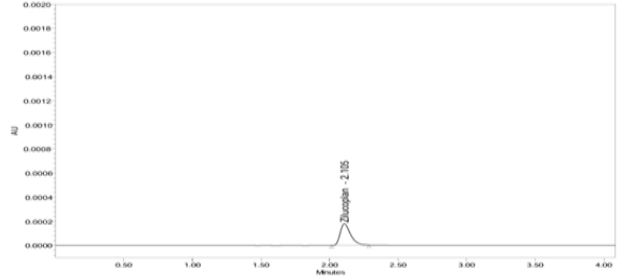


Figure 23: Chromatogram of Zilucoplan showing LOD

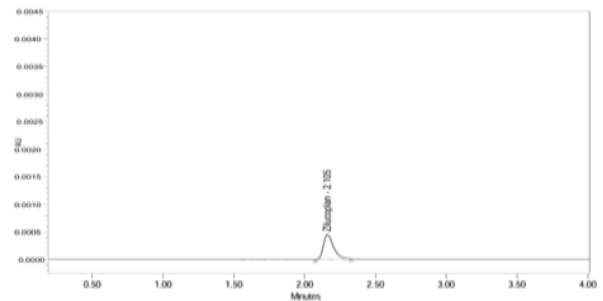


Figure 24: Chromatogram of Zilucoplan showing LOQ

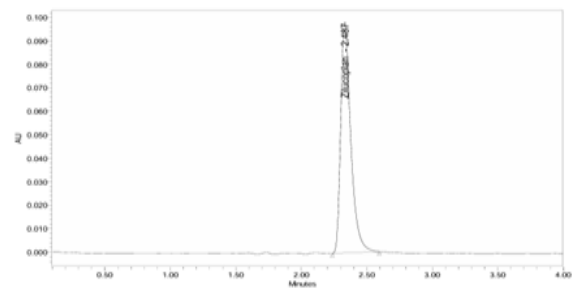


Figure 25: Chromatogram showing less flow

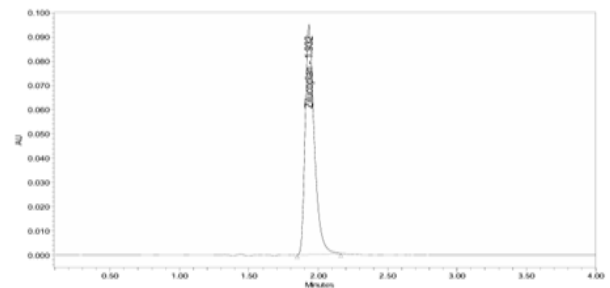


Figure 26: Chromatogram showing more flow

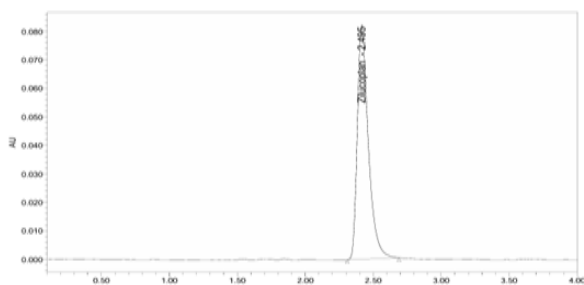


Figure 27: Chromatogram showing less organic

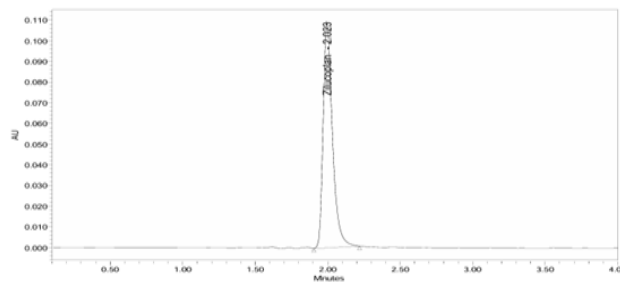


Figure 28: Chromatogram showing more organic

Table 5: Accuracy (recovery) data for Zilucoplan

% Concentration (at specification Level)	Area*	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	349165.3	12.5	12.4	99.2	99.6
100%	682988.7	25	24.98	99.9	
150%	1034600	37.5	37.4	99.7	

Table 6: Results of LOD

Drug name	Baseline noise(μV)	Signal obtained (μV)	S/N ratio
Zilucoplan	64	190	2.96

Table 7: Results of LOQ

Drug name	Baseline noise(μV)	Signal obtained (μV)	S/N ratio
Zilucoplan	64	638	9.96

Table 8: Results for variation in flow for Zilucoplan

S. No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	6908.71	1.11
2	1.0	3889.66	1.19
3	1.2	2374.78	1.32

Table 9: Results for variation in mobile phase composition for Zilucoplan

S. No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6908.71	1.11
2	*Actual	3889.66	1.13
3	10% more	2374.78	1.30

4. Conclusion

The newly developed RP-HPLC method for the quantitative analysis of zilucoplan in its pharmaceutical dosage form has been rigorously validated as per ICH guidelines. The method exhibits a high degree of specificity, accuracy, precision, and linearity within the specified range. The robustness of the method was confirmed through various stress tests, ensuring its reliability for routine quality control of zilucoplan. The validation parameters, including system suitability, method precision, and stability-indicating capability, meet all the criteria set forth by the ICH for analytical method validation. Therefore, this RP-HPLC method can be considered a reliable tool for the effective quality assessment of zilucoplan in its dosage form, contributing to the assurance of patient safety and efficacy of the drug.

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