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ORIGINAL ARTICLE

Development and validation of stability indicating RP-HPLC method for quantitative estimation of Plazomicin in Plazomicin parenteral dosage form

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ABSTRACT
For the quantitative determination of plazomicin in plazomicin parenteral dose form, a precise, accurate, and selective stability-indicating reverse phase high performance liquid chromatographic test method has been developed. Primesil C18 (250 x 4.6 mm, 5) was used as the stationary phase, and acetonitrile and pH 8.0 triethyl amine buffer were used as the mobile phase in a 50:50 volume/volume ratio. The run speed was 1 mL per minute. At a wavelength of 210 nm, a UV detector was used to identify plazomicin. Temperatures for the column oven and sample chiller were 40°C and 5°C, respectively. The run time was 60 minutes. A number of factors, including accuracy, precision, linearity, specificity, system appropriateness, solution stability, and robustness, were tested for the developed method in accordance with ICH guidelines. The results attained fit the criteria for approval. The urbanised technology can therefore be used successfully for the regular analysis of plazomicin in bulk and pharmaceutical dose forms because it is easy to use, precise, economical, safe, and non-toxic.
Keywords: *Plazomicin, Liquid chromatography, Forced degradation, Validation.*

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INTRODUCTION

Semi-synthetic aminoglycoside antimicrobial plazomicin sulphate, produced from sisomicin. Plazomicin sulphate is also known by its chemical name, (2"R,3"R,4"R,5"R).-2"-[(1S,2S,3R,4S,6R)-4-amino-6-[(2'"S)-4'"-amino-2'"-hydroxy butanamido)amino]-3-[(2'S,3'R)-3'-amino-6'-((2-hydroxy-ethylamino) methyl)-3',4'-dihydro-2H-pyran-2'-yloxy]-2-hydroxycyclohexyloxy]-5''-methyl-4''-(methylamino) sulphate of tetrahydro-2H-pyran-3'',5''-diol.

Based on full protonation, plazomicin sulphate [1–7] theoretically contains 2.5 molar equivalents of sulphate in relation to the freebase. Based on a 1:2.5 stoichiometric calculation, plazomicin sulfate's molecular weight is determined. Plazomicin sulphate, with a molecular weight of 837.89 g/mol and a freebase molecular weight of 592.69 g/mol, has the empirical formula $C_{25}H_{48}N_6O_{10}$ 2.5 H2SO4 (Plazomicin sulphate). Figure 1 depicts the Plazomicin's chemical composition.

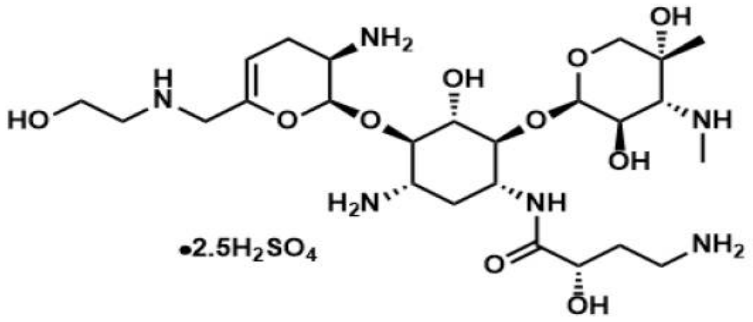

Fig. 1. Chemical structure of Plazomicin

A sterile, clear, colourless to yellow liquid for intravenous administration, plazomicin injection 500 mg/10 mL comes in 10-mL single-dose Type 1 glass bottles. Each vial contains 50 mg/mL of plazomicin sulphate, which is equivalent to 500 mg of plazomicin freebase at a pH 6.5 concentration. Additionally, each vial includes sodium hydroxide for pH correction and water for injection. Preservatives were not used in the formulation of this sterile, nonpyrogenic fluid.

The fictitious review reveals that major pharmacopoeias including USP, EP, JP, and BP do not list any HPLC procedures. For the determination of plazomicin using high performance liquid chromatography, only one method has so far been reported [8]. As a result, we worked to produce stability data for plazomicin in parenteral dose form using the HPLC method. According to ICH guidelines [9-10], the current study describes a straightforward HPLC method for the determination of plazomicin in plazomicin in parenteral dose form.

MATERIAL AND METHODS

Materials

Analytical-grade Triethylamine, orthophosphoric acid, Acetonitrile, Hydrochloric acid, Sodium hydroxide, Hydrogen peroxide and water, reagents and chemicals were procured from Merck Chemicals. Mumbai, India.

Instruments and Equipment

Waters HPLC model: e2695 with DAD, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model) and Analytical Balance (Metller Toledo Model) were used in the present study.

Method of Analysis

Preparation of Triethylamine buffer solution added 1.0 mL of triethylamine to 1000 mL of irrigate and thoroughly mixed it. using a diluted orthophosphoric acid solution, the pH was adjusted to 8.0.

Preparation of mobile phase 500 mL of triethylamine buffer and 500 mL of acetonitrile were combined in a 50:50 (%volume/volume) ratio. Filter the mixture through a 0.45 m membrane sieve and sonicate it to remove any gas.

Preparation of diluent Water is used as a diluent.

Preparation of standard solution Into a 50 mL volumetric flask, weighed precisely 25.18 mg of the plazomicin working standard. Added 25 mL of diluent, sonicate for two minutes to dissolve, then dilute to the desired amount with diluent and mixed well. Plazomicin is present in concentrations of the standard at about 500 g/mL.

Preparation of test solution 2 vials of sample were reconstituted with 10 mL of diluent and then transferred using an appropriate hypodermic needle and syringe into a 100 mL volumetric flask, each vial was rinsed twice with 10 mL of diluent before having its full contents transferred using an appropriate hypodermic needle and syringe to the same 100 mL volumetric flask. combined thoroughly and diluted with diluent to volume. This supernatant solution was further diluted by adding 5.0 mL to a 100 mL volumetric flask, making it up to volume with the diluent, and thoroughly mixing it.

Preparation of placebo solution 2 placebo vials were reconstituted with 10 mL of diluent and transferred using an appropriate hypodermic needle and syringe into a 100 mL volumetric flask. Each vial was rinsed twice with 10 mL of diluent before having its full contents transferred using an appropriate hypodermic needle and syringe to the same 100 mL volumetric flask. combined thoroughly and diluted with diluent to volume. This supernatant solution was further diluted by adding 5.0 mL to a 100 mL volumetric flask, making it up to volume with the diluent, and thoroughly mixing it.

Instrumentation Primesil C18 (250 x 4.6 mm, 5µm) mobile phase with a pH 8.0 triethylamine buffer and acetonitrile in a 50:50 volume/volume ratio was subjected to chromatographic analysis. The injection

volume was 20 µL, the column oven temperature was 40°C, and the sampler cooler temperature was 5°C. A photodiode array detector (PDA) was used for detection at 210 nm.

Method expansion and Optimization of Chromatographic Conditions The maximum UV absorbance (max) of the Plazomicin medicinal material was observed at 210 nm, in accordance with UV-spectroscopic examination. Dissimilar mobile phases were used to create a desirable peak shape in order to produce a suitable and reliable HPLC approach for the strength of plazomicin in plazomicin in parenteral dose form. The Zorbax SB C18 (250 x 4.6mm, 5 µm) was used as the base material for the technique expansion, along with various mobile phase compositions that included acetonitrile in an 85:15 volume/volume ratio and pH 8.0 triethylamine buffer in a volume-to-volume ratio. Higher retention times and peak tailing were found to be unsatisfactory when plazomicin was infused. The component was not suitable for the column stationary phase. Change the column for the subsequent experiment from Zorbax SB C18 to Hypersil BDS. Plazomicin was eluted at the tailing point, and the peak shape was poor. Change the column in the following trial from Hypersil BDS to Primesil C18 (250 x 4.6mm, 5µm). The peak fronting of the normal Plazomicin injection were unsatisfactory. For the following trial, the mobile phase's volume/volume ratio was modified to 50:50, the flow rate to 1.0 mL/min, the column temperature to 40°C, and the sampler cooler temperature to 5°C. At 210 nm, UV detection was carried out. At 20.81 minutes, the compound Plazomicin was eluted, and the zenith shape was determined to be favourable. Table 1 displays the method's results for system appropriateness.

Table 1. System suitability results

Name of the Component	Retention Time	Theoretical plates	Tailing factor
Plazomicin	20.81	9895	1.1

RESULTS AND DISCUSSION

The urbanized RP-HPLC technique was extensively authenticated for assay of Plazomicin in Plazomicin parenteral formulation using the following parameters.

Specificity and System suitability A swot to establish the meddling of blank and placebo were conducted. Diluent and placebo were infused keen on the chromatograph in the distinct above chromatographic circumstances and the blank and placebo chromatograms were recorded. Chromatogram of blank solution Figure 2. showed no zenith at the retention time of Plazomicin zenith. This indicates that the diluent solution used in sample preparation does not interfere with the inference of Plazomicin in Plazomicin parenteral dosage form. Similarly, chromatogram of the placebo solution Figure 3. showed no peaks at the retention time of Plazomicin zenith. This indicates that the placebo used in sample preparation does not interfere with the inference of Plazomicin in Plazomicin parenteral formulation. Similarly, chromatogram of the standard and sample solution Figure 4 and 5.

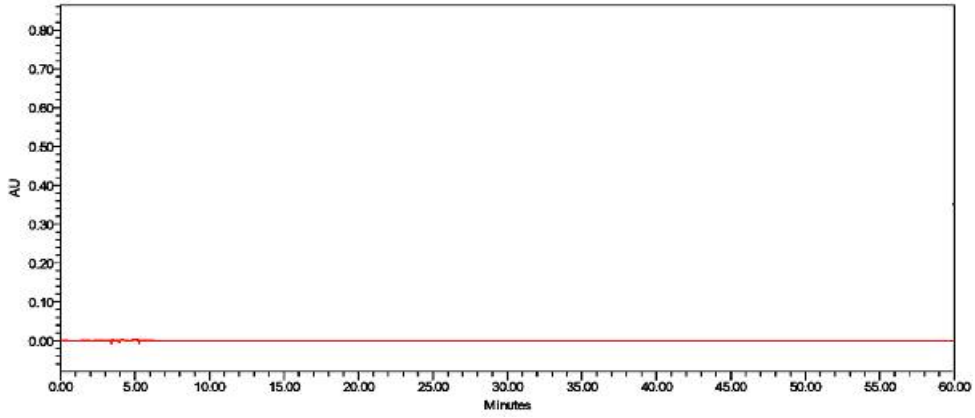

Fig. 2. Typical chromatogram blank

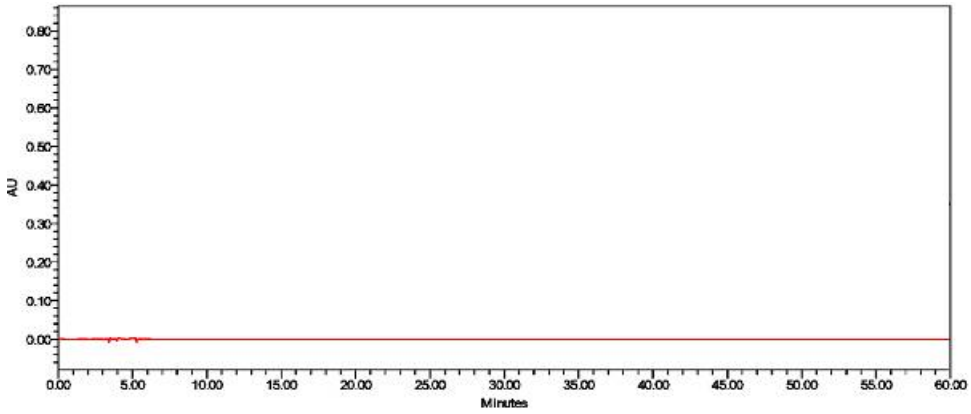

Fig. 3. Typical chromatogram placebo

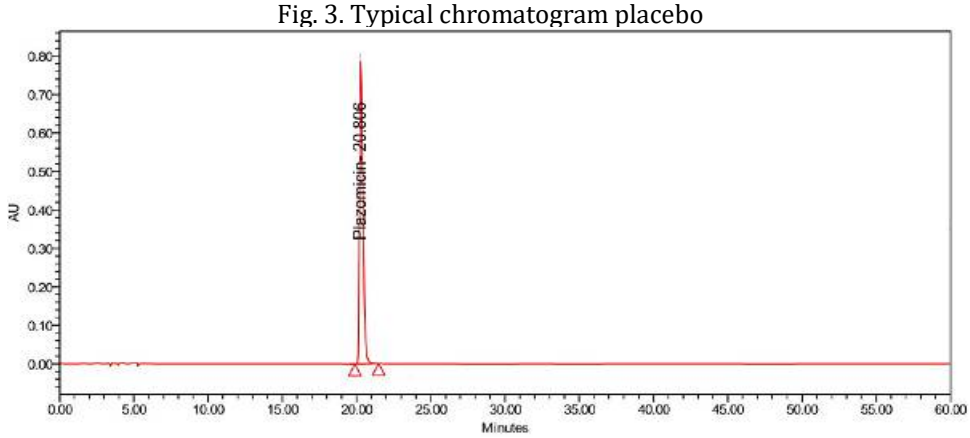

Fig. 4. Typical chromatogram standard

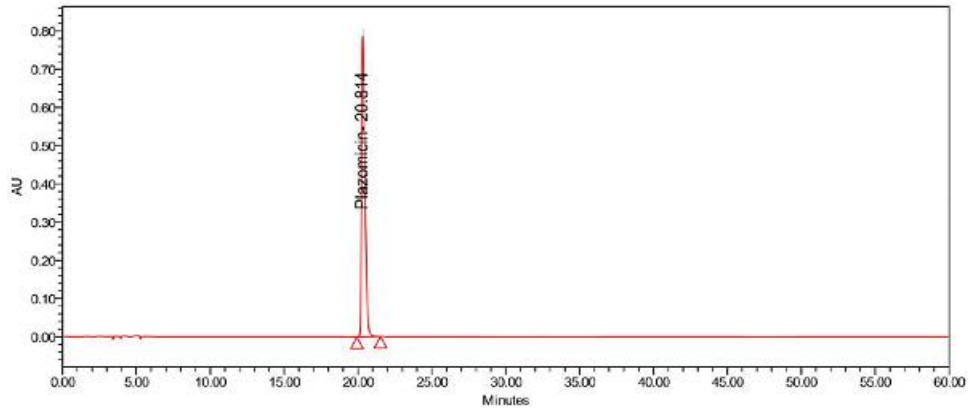

Fig. 5. Typical chromatogram sample

Table 2. Specificity results

S.No	Name	Retention Time (min)	Blank	Placebo
1	Blank	ND	NA	NA
2	Placebo solution	ND	NA	NA
3	Standard solution	20.806	No	No
4	Sample solution	20.814	No	No

Force Degradation studies A swot was conducted to show the Plazomicin's successful separation of degradants and contaminants. The following stress conditions were applied to separate parts of the sample and placebo solutions in order to cause deterioration. Samples were fed into the HPLC system using a PDA detector, both stressed and unstressed. The findings of the degradation investigation were displayed in Table 3.

Table 3. Forced degradation results

Stress condition	Degradation condition	% Assay	% Degradation
As such	Control sample	100.2	NA
Acid	1.0 N HCl/60°C/12 Hrs	99.1	0.36
Alkali	1.0N NaOH/60°C/12 Hrs	99.4	0.28
Oxidative	30% H_2O_2/BT/12 Hrs	84.9	15.5
Photolytic	1.2 million Lux hours or 200 watt hours/m^2 for 7 days	99.1	1.7
Humidity	90%RH Exposed for 2 days	100.2	0.21
Thermal	105°C/1 day	99.8	0.32

Significant degradation was pragmatic in the (oxidative) peroxide stress conditions. Hence it can be finished that Plazomicin is responsive to oxidation.

System precision

The criterion solution was set up in accordance with the test procedure, infused carefully on the HPLC system six times, and the percent RSD for the local responses was calculated. In Table 4, the statistics were shown.

Table 4. System precision results

S.No.	No. of injections	Peak area
1	Injection-1	2440054
2	Injection-2	2432545
3	Injection-3	2415684
4	Injection-4	2444381
5	Injection-5	2431122
6	Injection-6	2422394
Average		2431030
STDEV		10696.4076
% RSD		0.44

The relative standard deviation of six replicates criterion solution consequences were establish to be within the specification limit i.e.0.44%.

Method precision

By performing an assay for six samples of plazomicin parenteral (50 mg/mL), as per the test procedure, the exactitude of the test method was determined. Calculations were made to determine the label claim for Plazomicin's content in mg and percent for each test preparation. The six arrangements' median content and the percent RSD for the six observations were calculated. In Table 5, the statistics were shown.

Table 5. Method precision results

S. No	No. of Preparations	% Assay
1	Preparation 1	100.2
2	Preparation 2	100.7
3	Preparation 3	100.9
4	Preparation 4	100.1
5	Preparation 5	100.8
6	Preparation 6	100.3
Average		100.5
SD		0.3406
%RSD		0.34

Overall and individual % of Assay are complying as per test technique specification. The relative standard deviation of six assay preparations is 0.34%.

Linearity of detector response

The ability of an analytical procedure to produce test results that have a clear mathematical relationship to the attentiveness of the analyte is known as linearity. Plazomicin's linearity of response was determined to be between 25% and 150% (125.55-753.3 g/mL for Plazomicin). The calibration arc of the analytical method was graphically portrayed as a plot of attention against the acme area. It was determined that the correlation coefficient [r^2] was 0.9998. In order to show the linearity of the predicted technology, the HPLC method was established to be a linear criterion arc, which was estimated and shown in Figure 6. The approach was determined to be linear within the anticipated assortment based on the data collected, which is presented in Table 6.

Table 6. Linearity studies for Plazomicin

S.No	Linearity Level	Concentration (ppm)	Area response
1	Linearity at 25%	125.55	613575
2	Linearity at 50%	251.1	1235336
3	Linearity at 75%	376.65	1849218
4	Linearity at 100%	502.2	2452575
5	Linearity at 120%	627.75	3099527
6	Linearity at 150%	753.3	3669085
Correlation coefficient (r^2)			0.9998
Intercept			5871.3333
Slope			4886.7224
% Y-intercept			0.24

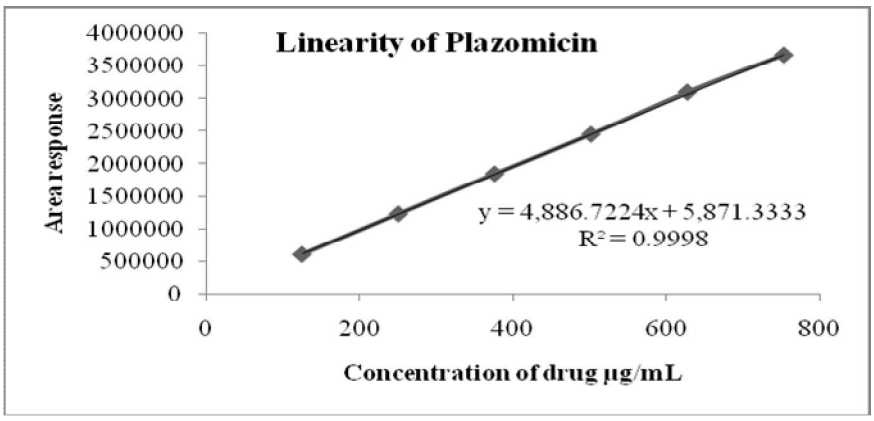

Fig. 6. Calibration curve for Plazomicin

Accuracy

By creating resurrection samples of Plazomicin at 50% to 150% of the target attentiveness level, the test method's accuracy was determined. With the exception of 50% and 150%, the resurrection samples were prepared in triplicate preparations on Plazomicin API spiked to placebo and analysed in accordance with the suggested procedure. The chromatography of the aforementioned samples allowed for the estimation of each sample's percentage resurrection for the amount added. calculated the relative standard deviation of six preparations for the implications of the 50% and 150% level recovery samples to assess the accuracy of the recovery at each level. The technique was proven to be accurate based on the statistics obtained, which are presented in Table 7.

Table 7. Recovery studies for Plazomicin

% Level	(mg) Recovered	(mg) Added	% Recovery	Mean % Recovery
Accuracy at 50 %-1	12.51	12.57	99.5	99.3
Accuracy at 50 %-2	12.37	12.49	99.0	
Accuracy at 50 %-3	12.46	12.54	99.4	
Accuracy at 100 %-1	25.26	25.21	100.2	100.0
Accuracy at 100 %-2	25.15	25.18	99.9	
Accuracy at 100 %-3	25.15	25.15	100.0	
Accuracy at 150 %-1	37.44	37.59	99.6	99.5
Accuracy at 150 %-2	37.39	37.65	99.3	
Accuracy at 150 %-3	37.49	37.60	99.7	

Solution stability of analytical solutions

Solution constancy standards and sample solutions were established at an assortment of circumstances for instance bench top at room temperature and in refrigerator 2-8°C. The constancy of standard and sample solutions was established by assessment of initially prepared criterion and sample solutions with freshly prepared criterion solutions. The statistics were revealed in Table 8-10.

Table 8. Results for solution stability of standard

Time Interval	Similarity factor	
	Room temperature	Refrigerator
Initial	NA	NA
12hr	1.01	1.00
24hr	1.02	1.01

Table 9. Results for solution stability of sample at room temperature

Time Interval	%Assay	%Assay difference
Initial	100.2	NA
12hr	100.4	0.2
24hr	100.8	0.6

Table 10. Results for solution stability of sample in Refrigerator

Time Interval	%Assay	%Assay difference
Initial	100.2	NA
12hr	100.3	0.1
24hr	100.5	0.2

Standard and sample solutions are steady for 24 hr when stored at room temperature and 2-8°C.

Robustness studies

The chromatographic performance in distorted conditions was assessed in comparison to the technique's nominal conditions in order to confirm the robustness of the method. At each of the following distorted situations, the criterion answer was infused. In Table 11, the statistics were shown.

Table 11. Robustness studies Results

Parameter		Theoretical plates	Tailing factor	%RSD of peak area
Flow variation ± 10%	0.9 mL	8534	1.2	0.58
	1.1 mL	10537	1.0	0.28
PH variation ± 0.2 units	7.8	9176	1.1	0.47
	8.2	9924	1.1	0.39
Temperature variation ± 5°C	35°C	8992	1.2	0.61
	45°C	10973	1.0	0.19
Organic phase variation ± 10%	505:495	8155	1.2	0.59
	495.505	10657	1.0	0.44

The technique is robust for modify like flow rate, column oven temperature, and the organic phase of the mobile phase.

CONCLUSION

According to ICH criteria, the RP-HPLC method for inferring plazomicin in plazomicin parenteral dosage form was urbanised and authenticated. For the determination of plazomicin in plazomicin parenteral dose form, a straightforward, accurate, and reproducible reverse phase HPLC technique was developed. The developed method underwent authentication for a number of factors, including accuracy, precision, linearity, specificity, system applicability, solution stability, and robustness, as specified by ICH regulations. The results attained fit the criteria for approval. The urbanised technology can therefore be used successfully for the regular analysis of plazomicin in bulk and pharmaceutical dose forms because it is easy to use, precise, economical, safe, and non-toxic.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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