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DEVELOPMENT AND VALIDATION OF SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF PRISTINAMYCIN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, specific, accurate and precise Spectroscopy method was developed and validated for the estimation of Pristinamycin in pharmaceutical dosage forms. The Standard solution was prepared by weighing 100 mg of Pristinamycin in 100 ml volumetric flask with Water. The final Standard solution was made to produce 1000 µg / ml with Water. Further dilutions were prepared as per procedure and were scanned at 230 nm. The linearity was found in the concentration range of 1-6 µg / ml. The Correlation coefficient was 0.999. The regression equation was found to be Y = 0.102 X + 0.009. The method was validated for linearity, accuracy, precision, limit of detection, limit of quantitation and ruggedness robustness. The limit of detection and limit of quantitation for estimation of Pristinamycin was found to be 0.04 (µg / ml) and 0.14 (µg / ml), respectively. The percentage recovery of Pristinamycin was found to be in the range of 98.2 ± 0.002 - 99.27 ± 0.002. Proposed method can be successfully applied for the quantitative determination of Pristinamycin in pharmaceutical dosage forms.

KEY WORDS: Pristinamycin; Method validation; UV Spectroscopy; ICH guidelines.

INTRODUCTION

Pristinamycin also spelled pristinamycine is an antibiotic used primarily in the treatment of staphylococcal infections[1], and to a lesser extent streptococcal infection. Pristinamycin is a mixture of two components that have a synergistic antibacterial action. Pristinamycin -IA is a macrolide, and results in pristine-espiralis[2] having a

similar spectrum of action of erythromycin. Pristinamycin -IIA (streptogrmin) is a depsipeptide. P-I and P-II are coproduced by s.pristinaespiralis in a ratio of 30:70. Each compound binds to the bacterial 50 s ribosomal subunit and inhibit the elongation process of the protein synthesis, thereby exhibiting only a moderate bacteriostatic activity. However, the combination of both substances acts synergistically and leads to a potent bactericidal activity that can reach up to 100 times that of the separate compounds Pristinamycin –IA is a chemically is N-[(6R,9S,10R,13S,15aS,18R,22S,24aS)-22-[p-(dimethylamino)benzyl]-6 ethyldocosahydro 10,23-dimethyl-5,8,12,15,17,21,24-heptaoxo-13phenyl-18-[[(3S)-3-quinuclidinylthio] methyl]12pyrido[2,1flpyrrolo[2,11] [1,4,7,10,13,16]oxapentaazacyclononadecin-9-yl]-3-hydroxypicolinamide. (fig.1) Pristinamycin –IIA is a chemically is 8,9,14,15,24,25-hexahydro-14-hydroxy-4,12-dimethyl-3-(1-methylethyl) (3R,4R,5E,10E,12E,14S) -3H-21, 18-nitrolo-1H,22Hpyrrolo\[2,1-c\]\[1,8,4,19\]-dioxadiazacyclotetracosine-1,7,16,22 (4H,17H) -tetrone. (fig.2).It is enhancement of pristinamycin production in the high-yielding[3] recombinants of Streptomyces pristinaespiralis obtained by genome shuffling were investigated by quantitative real-time PCR (Q-PCR) and amplified fragment length polymorphism (AFLP) techniques. In the biosynthesis of pristinamycins II and I component had more extended high expression in the recombinant than that in the ancestor during fermentation process, indicating their expression changes might be key factors during the biosynthesis of the antibiotic. In addition, the antecedent establishment of the high self-resistance to pristinamycin, because resistance gene started high-level expression ahead of the onset of the antibiotic production in the recombinant, might also lead to the increase of the antibiotics yield. AFLP analysis of these recombinants revealed genome variation of two novel genes, the homologs of AfsR regulatory gene and transposes gene, indicating these two gene variations were probably responsible for yield improvement of pristinamycin. This study provided several potential molecular clues for pristinamycin yield enhancement. Based on the literature survey it shows that very few analytical methods have been reported for the estimation of Pristinamycin which includes LC-MS, [4] HPLC-MS, [5,6] HPLC.[7]

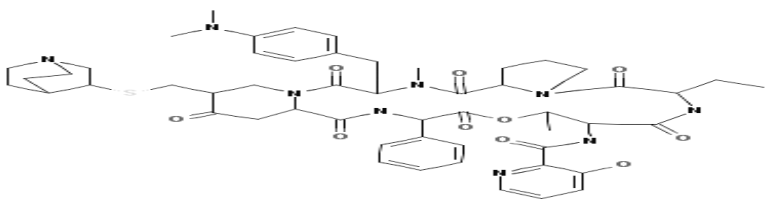

Fig.1: Structure of Pristinamycin -IA

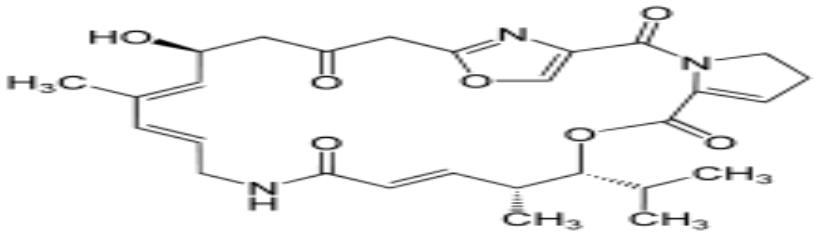

Fig.2: Structure of Pristinamycin -IIA

MATERIALS AND METHODS

1. Materials

Table No: 1. Materials used in present research work

S. No.	Materials	Source
1	Pristinamycin	Glad Care formulations Pvt.Ltd.
2	Water	Qualigens, Mumbai.

2. Equipments

Table No: 2. Equipments used in present research work

S. No.	Equipment	Source
1	UV Spectrophotometer	Elicosl SL 210, Mumbai
2	Sonicator	Wensar

Method

UV Spectrophotometry

Experimental: ELICO SL 210 UV / Vis double beam Spectrophotometer with 1 cm matched quartz cells was used for all spectral measurements. All chemicals used were of A.R. grade. Authentic drug sample of Pristinamycin was given as a gift sample by Glad Care formulations Pvt.Ltd., Hyderabad. Tablets of Pristinamycin were procured from local market.

Method Development

Solvent selection: In order to select suitable solvent for determination of pristinamycin various solvent like acetate buffer, phosphate buffer, Water, acetonitrile, acetate buffer tried for the solubility studies and it was found that pristinamycin was freely soluble in water. In the present investigation distilled water was selected as a solvent.

Using Water: UV Spectrophotometric method involves in the determination of Pristinamycin in bulk drug and pharmaceutical formulations and has an absorption maximum at 230 nm in water. It obeys Beer's law in the concentration range of 1-6 µg / ml.

Standard Solution: 100 mg of pristinamycin was dissolved in water in a 100 ml of volumetric flask and the solution was made up to volume with water.

Procedure: The standard solution of pristinamycin was subsequently diluted with water to obtain a series of dilutions containing 1, 2, 3, 4, 5, 6 µg/ml of pristinamycin. The absorbance of these solutions was measured in Elico SL 210 UV-Vis Spectrophotometer at 230 nm using water as blank. The concentration of pristinamycin and the corresponding absorbence are given in Table: 3.The absorbence was plotted against concentration of pristinamycin as shown in Fig: 3.

Sample preparation of pristinamycin: 20 tablets of pristinamycin were weighed and powdered in glass mortar and the powder equivalent to 10 mg of Pristinamycin was weighed accurately and transfer into a 100 ml standard volumetric flask. The contents were dissolved in Water and sonicated for few minutes. This solution was filtered through (0.45microns) membrane filter. 1 ml of the filtrate was diluted with Water to get the concentration of 10 µg / ml.

Validation of Spectrophotometric method

1. Linearity: To evaluate the linearity, serial dilution of analyte were prepared from the stock solution was diluted with solvent to get a series of concentration ranging from 1, 2,3,4,5 and 6 µg/ml. The prepared solutions were filtered through whatmann filter paper (No.41). Calibration curve was constructed by plotting the absorbance on Y- axis against the concentration on X- axis (Table: 3.)

2. Accuracy: Recovery studies by the standard addition method performed with a view to justify the accuracy of the proposed method. Previously analysed samples of pristinamycin (5µg/ml) were spiked with 80,100,120% extra pristinamycin standard and the mixture were analysed by the proposed method. The experiment was performed in triplicate and recovery of the pure drug. %RSD was calculated and reported in Table: 5.

3. Precision: The precision of the analysed method was studied by analysis of multiple sampling of homogeneous ample. The precision is expressed as standard deviation (or) relative standard deviation. The precision of the method was demonstrated by intra-day and inter –day variation studies.

3.1 Intraday precision: In the intra –day studies, the standard solutions (1, 2,3,4,5 and 6 µg/ml) was analysed for five times in different time interval within day. %RSD was calculated presented in Table: 6.

3.2 Inter day precision: In the inter-day variation studies, the standard solution (1, 2,3,4,5 and 6µg/ml) was analysed for five times in different days. % RSD was calculated presented in Table: 7.

4. Sensitivity: The sensitivity of measuring of pristinamycin by use of the proposed method was estimated in terms of the limit of detection (LOD) and the limit of quantitation (LOQ).The LOD and LOQ were calculated by the use of the equation LOD=3.3 × σ/s and LOQ=10 × σ/s where σ is the standard deviation of response and S is the slope of the calibration curve and are given in Table: 8, 9.

5. Ruggedness: The solutions were prepared and analyzed with change in the analytical conditions like different laboratory conditions and different analyst and are given in Table: 10, 11.

6. Robustness: The solutions were prepared and analyzed with change in the analytical conditions like different wave lengths are given in Table: 12.

RESULTS AND DISCUSSION

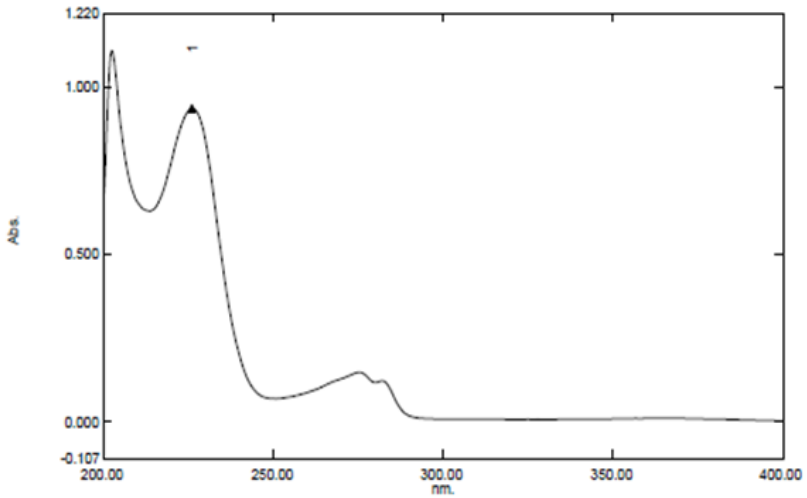

Fig.No:3. UV Spectrum for Pristinamycin

Table No: 3. Calibration data of pristinamycin

S. No.	Concentration(µg/ml)	Absorbance
1	0	0
2	1	0.11
3	2	0.215
4	3	0.32
5	4	0.418
6	5	0.52
7	6	0.61

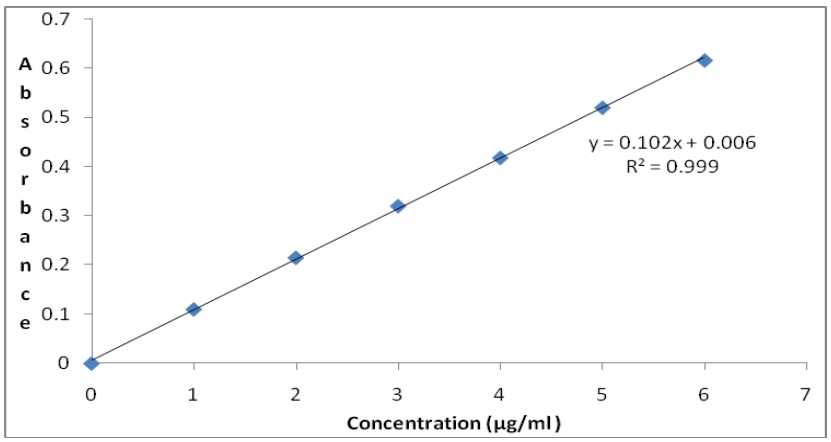

Fig: 4. Calibration curve of pristinamycin

Table No: 4. Optimum conditions, Optical characteristics and Statistical data of the Regression equation

Parameters	UV
Calibration range (µg/ml)	1-6(µg/ml)
Wavelength	230nm
Regression equation (y*)	0.102x+0.009
Slope	0.102
Intercept(a)	0.006
Correlation co efficient(r^2)	0.999
LOD (µg/ml)	0.04
LOQ (µg/ml)	0.14

*Y= b C + a where C is the concentration of Pristinamycin in µg / ml and Y is the absorbance at the respective $\lambda_{max.}$

**Average of six determinations.

Table No: 5. Accuracy results for Pristinamycin at 230nm by UV Spectroscopy

Sample (%)	Initial amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	%Recovery ±SD*	%RSD
80	0.5	4	4.42	98.2±0.002	0.43
100	0.5	5	5.46	99.27±0.002	0.35
120	0.5	6	6.43	98.9±0.004	0.60

*Average of three determination

Table No: 6. intraday precision results for Pristinamycin at 230nm by UVSpectroscopy

S. No	Conc µg/ml	Absorbance					AVG	SD	%RSD
		1	2	3	4	5			
1	1	0.114	0.115	0.116	0.117	0.116	0.114	0.0011	1
2	2	0.210	0.212	0.213	0.214	0.214	0.212	0.0014	0.53
3	3	0.320	0.324	0.325	0.322	0.322	0.322	0.0022	0.70
4	4	0.416	0.414	0.417	0.415	0.417	0.415	0.0013	0.31
5	5	0.525	0.520	0.521	0.519	0.521	0.521	0.0025	0.47
6.	6.	0.615	0.613	0.612	0.610	0.612	0.612	0.001	0.16

Table No: 7. Inter day precision results for Pristinamycin at 230nm by UVSpectroscopy

S. No	Conc µg/ml	Absorbance					AVG	SD	%RSD
		1	2	3	4	5			
1	1	0.112	0.19	0.110	0.108	0.114	0.11	0.002	1.81
2	2	0.216	0.217	0.214	0.212	0.218	0.217	0.0024	1.10
3	3	0.321	0.319	0.320	0.323	0.320	0.321	0.0015	0.46
4	4	0.415	0.412	0.410	0.412	0.417	0.415	0.0027	0.65
5	5	0.521	0.523	0.520	0.519	0.524	0.521	0.0020	0.38
6	6	0.610	0.612	0.608	0.614	0.615	0.61	0.0028	0.45

Table No: 8. Limit of Detection results for Pristinamycin at 230nm by UVSpectroscopy

S .No	Slope	SD of precision	LOD
1	0.102	0.0015	0.04

Table No: 9. Limit of quantitation results for Pristinamycin at 230nm by UV Spectroscopy

S. No	Slope	SD of precision	LOQ
1	0.102	0.0015	0.14

Table No: 10. Ruggedness results for Pristinamycin at 230nm by UV Spectroscopy

S. No	Analyst-1		Analyst-2	
	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
1	5	0.518	5	0.51
2	5	0.522	5	0.514
3	5	0.520	5	0.518
	AVG	0.520	**AVG**	0.51
	SD	0.002	**SD**	0.002
	%RSD	0.38	**%RSD**	0.39

Table No: 11. For Ruggedness (Instrument- 1 andInstrument-2) results for Pristinamycin at 230nm by UV Spectroscopy

S. No	Instrument-1		Instrument-2	
	Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
1	5	0.517	5	0.514
2	5	0.520	5	0.518
3	5	0.516	5	0.523
	AVG	0.51	**AVG**	0.518
	SD	0.002	**SD**	0.004
	%RSD	0.39	**%RSD**	0.77

Table No: 12. Robustness results for Pristinamycin at 230nm by UV Spectroscopy

S. No	Condition	Modification	Mean absorbance ± SD*	% RSD for absorbance
1	Wavelength (nm)	228(nm)	0.415±0.005	0.120
		232(nm)	0.42±0.001	0.23

*Average of the three determination.

The absorption spectra were recorded in the wavelength region of 200 - 400 nm in UV method. Pristinamycin showed linearity in the concentration range of 1 - 6 µg / ml in Spectroscopy methods. The spectra are presented as Fig No: 3. Beer's law range was confirmed by the linearity of the calibration curve of pristinamycin, which were represented in Fig No: 4.

The optical characteristics such as absorption maxima, Beer's law limits, Molar absorptivity, sensitivity, slope (b), intercept (C), correlation coefficient (r^2) obtained from different concentrations, percent relative standard deviation, LOD and LOQ values were presented in Table No .6.1. The results showed that these methods have reasonable precision.

The quantitative estimation was carried out on formulation. The quantitative results obtained were subjected to statistical analysis to find out standard deviation and standard error values. The % RSD values are less than 2 indicating the precision of the methodology and low standard error values indicates the accuracy of the method. The statistical data's are given in Table No: 5.

Results obtained for the proposed methods confirm the suitability of these methods for Pharmaceutical dosage forms. The other active ingredients and excipients usually present in the Pharmaceutical dosage forms did not interfere in the estimation, when commercial dosage forms were analyzed by these methods. The Accuracy of the methods was confirmed by the recovery studies, by adding known amount of the pure drug to the formulation and the

analytical data are presented in Table No: 5. the percentage recovery was found to be between 98.2-99.27 % shows that the method was free from the interference of excipients used in the formulation.

The Precision of an analytical method was calculated by performing intra-day and inter-day precision studies. The values were found to be precise and were presented in Table No: 6 and 7.

The results obtained in Ruggedness and Robustness test expresses the precision of the method. The Ruggedness results were listed in Table No: 10, 11 and the Robustness results were shown in Table No: 12.

The developed UV Spectrophotometric methods were found to be rapid, simple, precise, accurate and economic for routine estimation of Pristinamycin in commercial dosage forms. The method of derivative spectroscopy had shown the better results. It can be used for routine analysis of Pristinamycin in pure drug and Pharmaceutical dosage forms.

CONCLUSION

Development of methods to achieve the final goal of ensuring the quantity of drug substances and drug products is not a trivial undertaking. The capabilities of the four methods were complementary to each other. Hence they can be regarded as simple, specific and sensitive methods for the estimation of Pristinamycin in Bulk drug and Pharmaceutical dosage forms.

A very few analytical methods appeared in the literature for the determination of Pristinamycin. In view of the above fact simple sensitive, accurate, precise and economical analytical methods are planned to develop.

The UV Spectrophotometric method demonstrated applicable to the estimation of Pristinamycin in Bulk drug as well as in existing Pharmaceutical dosage form. In order to ensure that the data generated is accurate and precise. The experiments have been performed on calibrated equipments using suitable reference standards. The results found to be good and summarized in Table No: 3-12. In addition to positive requirements for analytical methods the striking advantage of all the presently developed methods are economical.

This method is validated in terms of accuracy, precision, repeatability, ruggedness and can be used for the routine determination of Pristinamycin in Bulk drug and Pharmaceutical formulations.

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