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Research Article

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NEW RP - HPLC METHOD DEVELOPMENT, AND VALIDATION FOR ANALYSIS AND ASSAY OF DAPTOMYCIN IN FORMULATION

B.Bhoomaiah*[1], A.Jaya shree[2], Danavena Rambabu[3], K Balamurali Krishna[4], K.Surendra Babu[5]

[1]Aurobindo Pharma, Hydrabad, A.P., India [2]CCST, Inistute of Science & Technology, JNTU, Hydrabad [3]Symed Laboratories Limited, Hydrabad, A.P., India [4]Dept of Chemistry, Acharyanagarjuna University,Nagarjuna Nagar, Guntur, A.P., India [5]S.V.R.M College,Nagaram A.P., India

Corresponding Author

bhoomesh.analyst@gmail.com

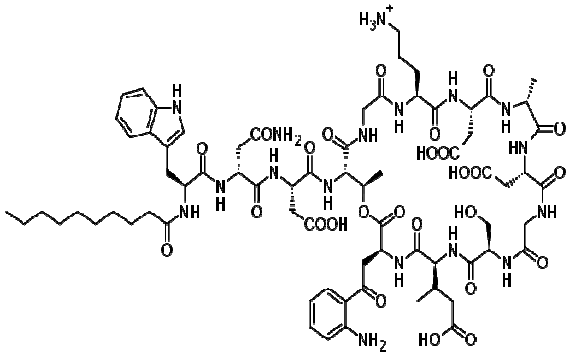
ABSTRACT

A simple, rapid and precise reverse phase high performance liquid chromatography method was developed for the analysis of Daptomycin in tablet. Chromatographic separation of Daptomycin was performed by using a kromosil C_{18} column (250 x 4.6mm, 5 µm) as stationary phase with a mobile phase comprising of 0.1% Ortho phosphoric acid : Acetonitrile: Methanol 30:45:25 (v/v) at a flow rate of 1.0ml/min and UV detection at 282nm. The linearity of Daptomycin is in the range of 0.2 mg/ml to 1.4 mg/ml. The limit of detection for Daptomycin was found to be 10 nano grams. The recovery was calculated by standard addition method. The proposed method was found to be accurate, precise and rapid for the analysis of Daptomycin.

KEY WORDS: Daptomycin, precise, recovery, linearity,282 nm

INTRODUCTION

Daptomycin Molecular formula $C_{72}H_{101}N_{17}O_{28}$ Molecular weight 1619.7086g/mol. IUPAC Name N-decanoyl-L-tryptophyl-L-asparaginyl-L-aspartyl-L-threonylglycyl-L-ornithyl-L-aspartyl-D-alanyl-L-aspartylglycyl-D-seryl-threo -3-methyl-L-glutamyl-3-anthraniloyl-L-alanine[egr]$_1$-lactone.



Structure of Daptomycin

Daptomycin is a novel lipopeptide antibiotic used in the treatment of certain infections caused by Gram-positive organisms. It is a naturally-occurring compound found in the soil saprotroph *Streptomyces roseosporus*. Its distinct mechanism of action means that it may be useful in treating infections caused by multi-resistant bacteria

MATERIALS AND METHODS

Methanol, Acetonitrile, Ortho phosphoric acid and Tetrahydrofuron are used analytical grade. Chromatographic separation was performed with PEAK high performance liquid chromatography having LC-P7000 isocratic pump, equipped with PEAK LC-UV7000 variable wavelength detector. Chromatograms and data were recorded by means of PEAK Chromatographic Software version 1.06.

PREPARATION OF STANDARD SOLUTION

10 mg of Daptomycin was taken in a 10ml volumetric flask and 10ml of mobile phase was added to obtain 1.0 mg/ml of Daptomycin standard solution.

CHROMATOGRAPHIC CONDITIONS

Mobile phase: Acetonitrile (45%)

Methanol (25%)

0.1% Orthophosphoric acid (30%)

PH **:** 6.5

Analytical Column: Kromosil C_{18} column (250mm x 4.6mm) 5µ

UV detection: 282 nm

Flow rate: 1.0ml/min.

Injection Volume: 20µl

Temperature: Ambient

Run time: 10 min.

Retention time: 3.3 min.

HPLC chromatogram was shown in figure 2

Linearity

In order to check the linearity for the developed method, solutions of seven different concentrations ranging from 0.2 mg/ml to 1.4 mg/ml were prepared. The chromatograms were recorded and the peak areas were given in table-1.A linear relationship between areas versus concentrations was observed in about linearity range. This range was selected as linear range for analytical method development of Daptomycin. Linearity graph was shown in figure: 3

PRECISION (REPEATABILITY)

0.8mg/ml standard solution was prepared to calculate the precision for the developed method. The prepared solution was injected into injector at same concentrations and same chromatographic conditions. The chromatograms were recorded. The values are given in table-2. R.S.D for the values calculated is 1.48 So, the developed method shows precision.

LIMIT OF QUANTIFICATION (LOQ) AND LIMIT OF DETECTION (LOD)

The LOQ and LOD were established at a signal to noise ratio. The LOD of Daptomycin is 15ng/ml. The LOQ of Daptomycin is 45ng/ml.

ANALYSIS OF DAPTOMYCIN FORMULATION

The formulation for Daptomycin (……-5mg) tablet was taken. This tablet is powdered and prepared 1.0 micro gram/ml sample solution. This solution was filtered through nylon membrane filter paper and the filtrate was collected into the flask.

At our developed Chromatographic conditions sample was injected and chromatogram was recorded. Chromatogram was shown in figure: 4

RESULTS AND DISCUSSIONS

The Reverse Phase High Performance Liquid Chromatography method was developed a stability indicating assay method. Pure drugs chromatogram was run in different mobile phases containing methanol, acetonitrile, THF, and different buffers like potassium dihydrogen phosphate, sodium dihydrogen phosphate,Ortho phosphoric acid in different volumes ratios. Different columns like C_8, C_{18}, phenyl, cyano with different dimensions were used. Then retention time and tailing factor were calculated. Finally 0.1% Orthophosphoric acid, methanol and acetonitrile in the volume of ratio 30:25:45: V/V and Kromosil C_{18} analytical column was selected which gave a sharp and symmetrical peak with 1.30 tailing. Calibration graph was found to be linear at range 0.2 mg/ml to 1.4 mg/ml. seven different concentrations of Daptomycin in range given above were prepared and 20µl of each concentration injected in HPLC. The slope (m) and intercept (c) obtained were found to be 390754.9 and 0.029.The correlation of coefficient (r^2) obtained was found to be 0.9993. It was observed that the concentration range showed a good relationship. The limit of detection for Daptomycin was found to be 10ng/ml and the limit of

quantification was found to be 45ng/ml. It proves the sensitivity of method. The % assay or average amount of Daptomycin in formulation CUBICIN was found to be 93.73%. The low values of standard deviation and coefficient of variation at each level of the recovery experiment indicate high precision of the method.

CONCLUSION

In this method there is no type of solid buffers. So the column does not spoil earlier. The RP-high performance liquid chromatographic method for the analysis of Daptomycin from their formulations was found to be accurate and precise. Thus, the proposed HPLC method can be successfully applied for the routine quality control analysis of Daptomycin formulations.

Tables

Linearity level	Concentration(mg/ml)	Area
1	0.2	76001.1
2	0.4	142422.3
3	0.6	219516.2
4	0.8	295425.0
5	1.0	373769.3
6	1.2	465371.4
7	1.4	5381693.1

Table-1

Day	Precession Area Mean	R.S.D.
Day - 1	273948.16	1.697
Day - 2	295096.32	1.743
Day - 3	285675.26	1.017

Table-2

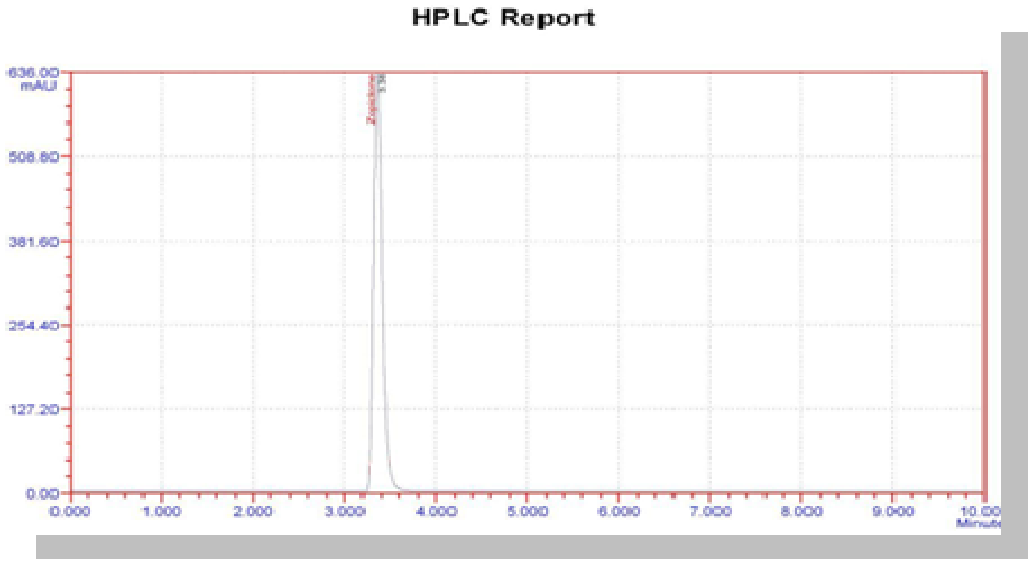
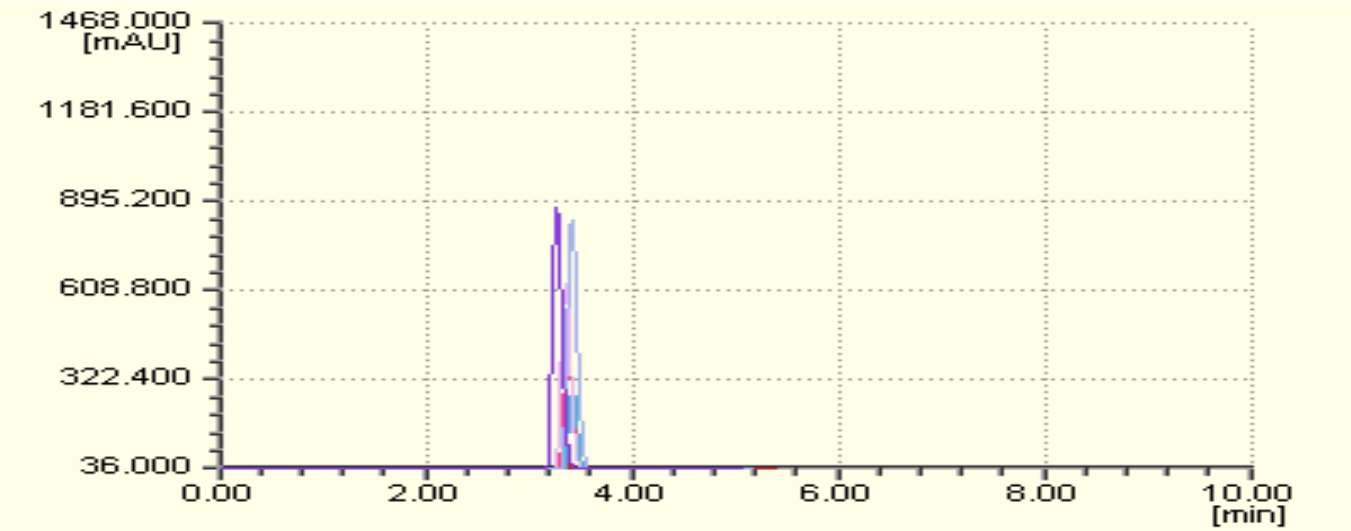


Figure: 2



Linearity Report

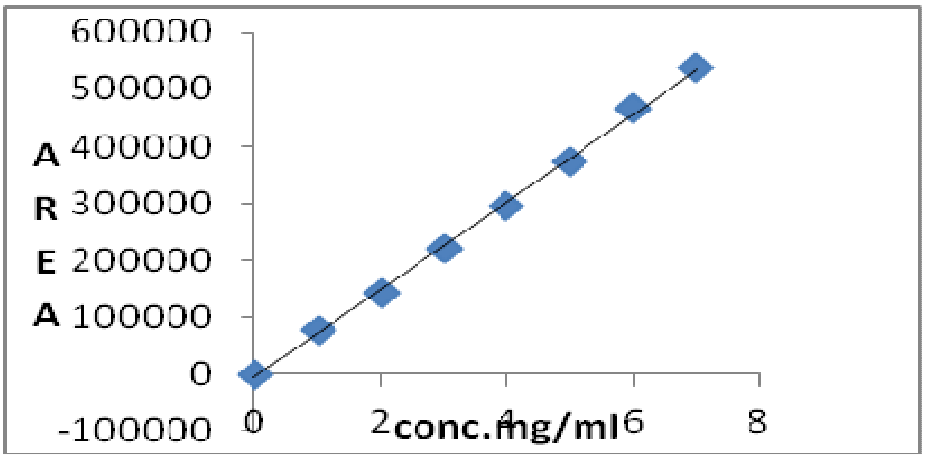


Figure: 3 Linearity graph for Daptomycin

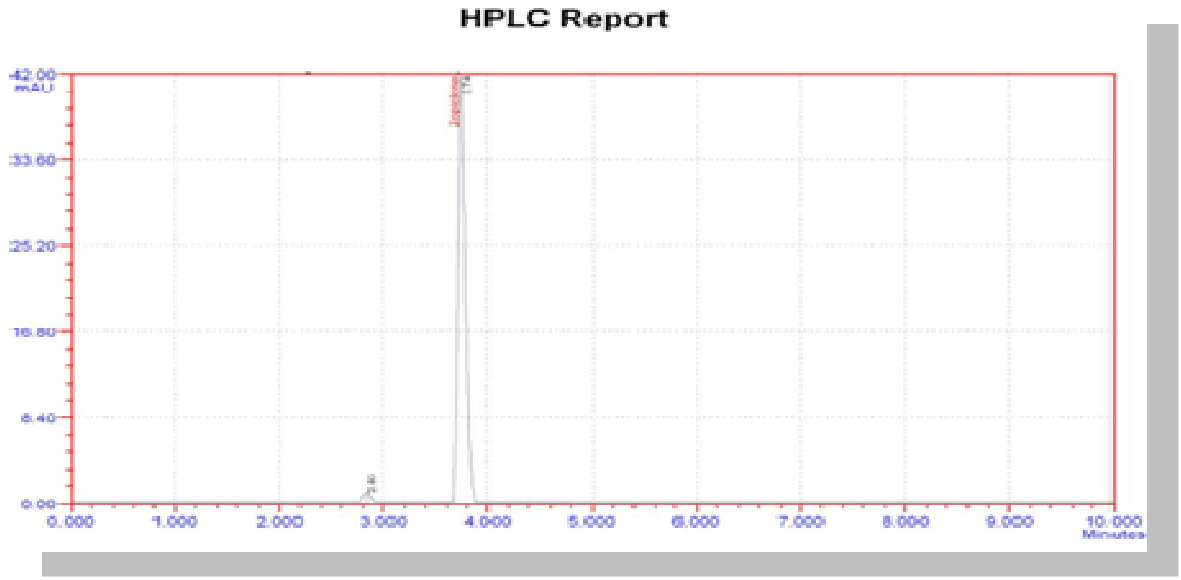


Figure: 4 Formulation report

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