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Analytical Method Development for Assay and Related Substances Test Parameters

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ABSTRACT

The proposed HPLC method for the related substance of Daptomycin in Daptomycin injection by HPLC is found further eligible to validate make eligible as a stability-indicating method for the quantification of Daptomycin the Injection formulation. Hence this method stands validated and can be used for routine analysis.

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ASSAY BY HPLC ANALYTICAL METHOD DEVELOPMENT

Table No.1: Chromatographic conditions:

HPLC column	**:**	Phenomenex,IB-Sil.C8 125A,250X4.6mm,5µm or Equivalent
UV detection	**:**	214 nm
Column oven temperature	**:**	25°C
Sample compartment temperature	**:**	25°C
Flow rate	**:**	1.5 mL/minute
Injection volume	**:**	15 µL
Run time	**:**	30minutes
Elution mode	**:**	Isocratic

Blank preparation: Use diluent as blank.

Standard preparation (200ppm):

Accurately weigh and transfer about 50.00 mg of Daptomycin standard into a 50 mL volumetric flask, add 30 mL of diluent, sonicate to dissolve completely then make up to the volume with diluent and mix well.

Transfer 5 ml of the above solution to a 25 mL volumetric flask and make up to the volume with diluent and mix well.

Note: the standard solution is stable at room temperature for about 41 hrs.

Sample preparation: 25 mg/mL – 1- mL fill 500 mg/20 mL (200 ppm):

Reconstitute 4 vials of Daptomycin injection each with 10 mL of diluent and transfer the whole contents to a 200 mL volumetric flask rinse each vial twice with 10 mL of diluent and transfer into the same volumetric flask, dilute and make up to the volume using diluent.

Further, dilute 2 mL of the above solution to 100 mL with diluent and mix well.

Note: sample solution is stable at room temperature for about 38 hrs.

Procedure:

1. Inject blank preparation (one), standard preparation (five replicates), and sample preparation into the chromatographic system.

2. From the chromatogram of standard and sample preparation, measure the response of Daptomycin peak.

System Suitability:

a. USP tailing factor/Asymmetry of Daptomycin peak from the first injection of standard preparation as recorded by software should not be more than 2.0.

b. USP Plate count/Theoretical plates of Daptomycin peak from the first injection of standard preparation as recorded by software should not be less than 2000.

c. %RSD of Daptomycin peak from replicate injections of the standard should be no more than 2.0.

Calculation: Calculate the % of the labeled amount of Daptomycin in the portion of Daptomycin for injection.

$$\% \text{ Assay of Daptomycin} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{N} \times \frac{P}{100} \times \frac{1}{LC} \times 100$$

Where,

AT: Area of peak response of Daptomycin in sample preparation

AS: Average Area of peak response of Daptomycin in standard preparation

WS: Weight of Daptomycin standard taken in mg

DS: Dilution of the standard preparation.

DT: Dilution of sample preparation.

N: No. of Vials taken for sample preparation.

P: Potency of Daptomycin standard on an as-is basis.

LC: Label claim of Daptomycin (mg/vial).

Note: Specimen Chromatograms are provided below

Figure No. 1: Chromatogram for Blank:

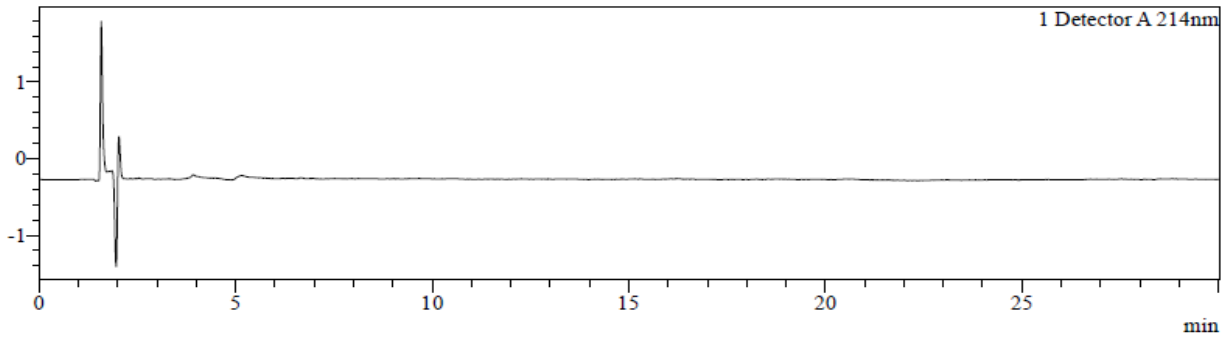


Figure No. 2: Chromatogram for Placebo:

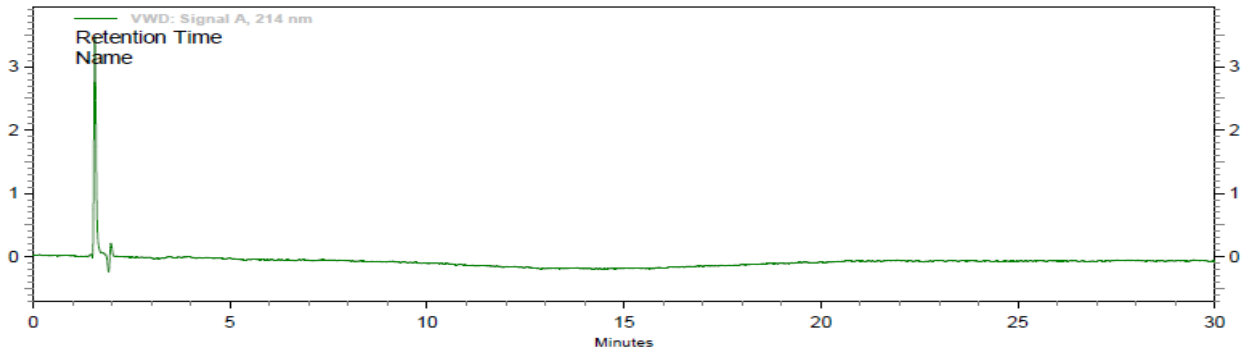


Figure No. 3: Chromatogram of the standard:

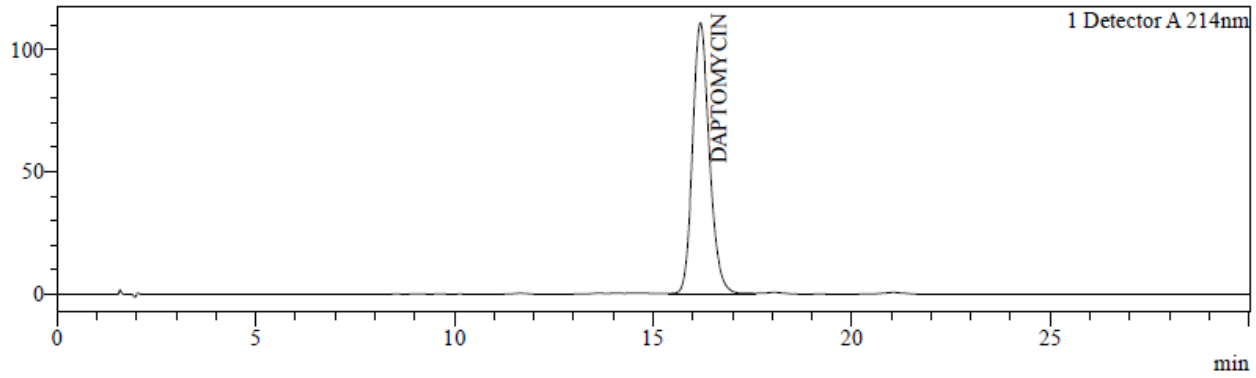
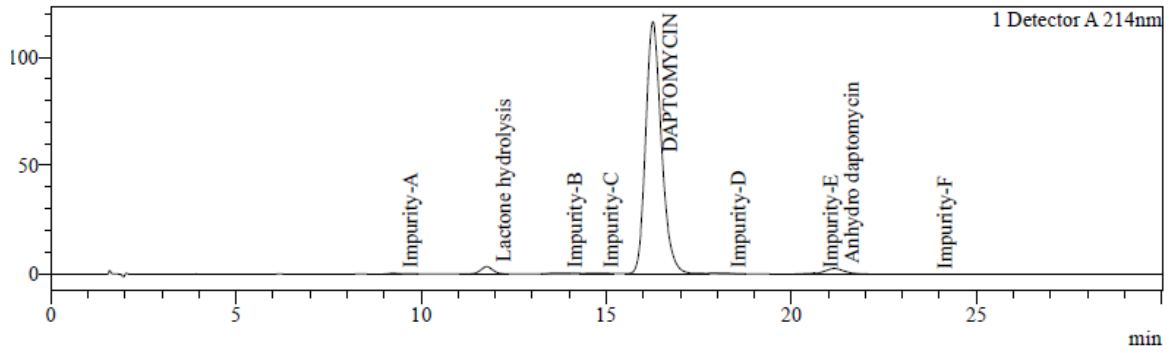


Figure No. 4: Chromatogram sample:



RS BY HPLC ANALYTICAL METHOD DEVELOPMENT

Table No.2: Chromatographic Conditions:

HPLC column	**:**	Phenomenex,IB-Sil.C8 125A,250X4.6mm,5µm or Equivalent
UV Detection	**:**	214 nm
Column Oven Temperature	**:**	25°C
Sample Temperature	**:**	5°C
Flow Rate	**:**	1.5 mL/minute
Injection Volume	**:**	20 µL
Run Time	**:**	75minutes
Elution Mode	**:**	Isocratic

Method Background:

Initial chromatographic conditions have been chosen based on the API Vendor Method (Daptomycin technical package-Version 201504 Volume I of II). Based on the UV maximum and API method wavelength is selected as 214nm.

Final Method:

Column Care: Before Use:

Flush the HPLC column with Acetonitrile and HPLC grade water in the ratio of 50:50 (v/v) for about 20 minutes. Then flush with Acetonitrile and HPLC grade water in the ratio of 30:70 (v/v) for about 20 minutes. Condition the column with mobile phase prior to measurement.

After Use:

Flush with Acetonitrile: HPLC grade water 10:90 (v/v) ratio for about 90 minutes at a column temperature of 35°C, Finally flush and store HPLC column in Acetonitrile: HPLC grade water 50:50 (v/v) ratio for about 60 minutes at a column temperature of 25°C.

Mobile phase preparation:

Buffer Preparation: Accurately weigh and transfer about 4.5g of Ammonium dihydrogen phosphate into 1000mL of HPLC grade water and mix well, then adjust the buffer to pH 3.2

with diluted Orthophosphoric acid and mix well. Then filter through a 0.45µm filter. (PVDF or Nylon).

Mobile phase: Mix 670mL of buffer and 330mL of Acetonitrile in the ratio of 67%: 33% (v/v) respectively.

(Adjust the Mobile Phase composition to get the Daptomycin Peak Retention Time between 34.5 min to 37.5 min)

Diluent: Mix 800mL of buffer and 200mL of Acetonitrile in the ratio of 80%:20% (v/v) respectively.

Blank Preparation: Use diluent as blank.

Standard preparation (2 ppm):

Accurately weigh and transfer about 20 mg of Daptomycin standard into a 20mL volumetric flask, add 10mL of diluent Sonicate to dissolve completely then make up to the volume with diluent and mix well.

Transfer 5mL of the above solution to a 100mL volumetric flask and make up to the volume with diluent and mix well.

Transfer 2 ml of the above solution to a 50mL volumetric flask and make up to the volume with diluent and mix well.

Note: Standard solution is stable at 2-8°C for about 72 hrs.

Sample preparation: 25 mg/mL – 1- mL fill 500 mg/20 mL (1000 ppm):

Reconstitute 2 vials of Daptomycin injection each with 10 mL of diluent and transfer the whole contents to a 100mL volumetric flask rinse each vial twice with 10mL of diluent and transfer into the same volumetric flask, dilute and make up to the volume using diluent.

Further, dilute 5mL of the above solution to 50mL with diluent and mix well.

Note: Sample solution is stable at 2-8°C for about 48 hrs.

Procedure:

(1) Inject blank preparation (one), standard preparation (Six replicates), and sample preparation (one) into the chromatographic system.

System Suitability:

a. USP tailing factor/Asymmetry of Daptomycin peak from the first injection of standard preparation as recorded by software should not be more than 2.0.

b. USP Plate count/Theoretical plates of Daptomycin peak from the first injection of standard preparation as recorded by software should not be less than 2000.

c. %RSD of Daptomycin peak from replicate injections of the standard should be no more than 5.0.

The calculation for Impurities:

Calculate the % of each impurity of Daptomycin in the portion of Daptomycin for Injection:

$$\text{Content of impurity in \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{N} \times \frac{P}{100} \times \frac{100}{LC} \times \frac{1}{RRF}$$

AT: Area of peak response of Known/Unknown impurity in the Sample preparation

AS: Average area of peak response of Daptomycin in the Standard preparation.

WS: Weight of Daptomycin standard taken in mg.

DS: Dilution for standard preparation

DT: Dilution for sample preparation

N: Number of Vials taken for reconstitution.

LC: Label claim of Daptomycin in mg/Vial

P: Potency of Daptomycin on an as-is basis

RRF: Relative Retention Factor

Total impurities: Sum of known impurities and unknown impurities.

Table No.: 3: Relative Retention Times for Daptomycin Impurities w.r.t. Daptomycin

Peak Names	RRT(About)
Impurity A	0.49
Lactone Hydrolysis Product	0.71
Impurity B	0.82
Beta Isomer	0.87
Impurity C (Appeared Split Peak, combined into peak)	0.92
Daptomycin	1.00
Impurity D	1.14
Impurity E	1.27
Anhydro-daptomycin	1.33
Impurity F	1.56

Table No.: 4: RRF of Known Impurities of Daptomycin

Name of Impurity	RRF Obtained
Lactone Hydrolysis Product	1.06
Daptomycin Beta Isomer	1.11
Anhydro Daptomycin	1.04

Specimen Chromatograms:

Figure No. 5: Chromatogram of Blank:

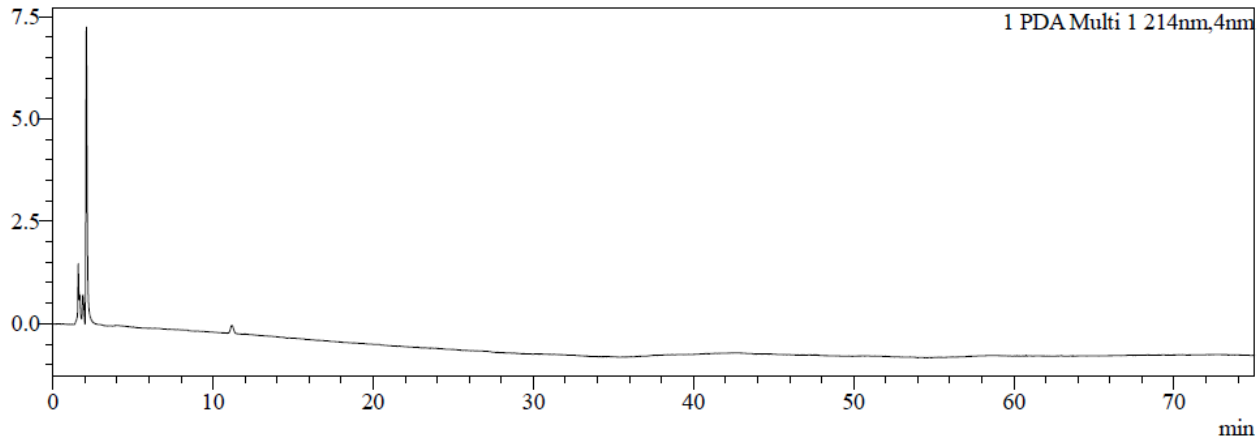


Figure No. 6: Chromatogram of Placebo:

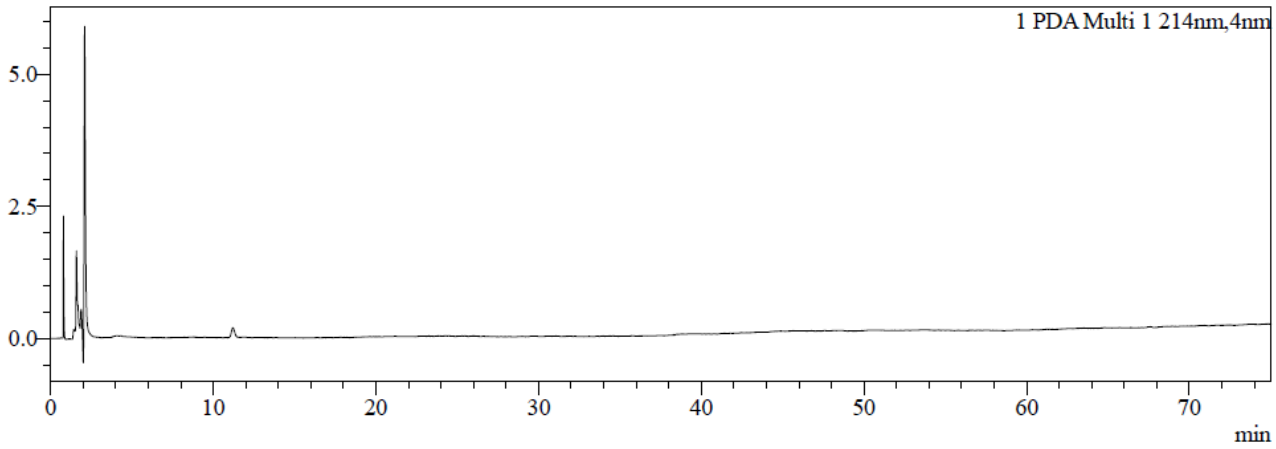


Figure No. 7: Chromatogram of Standard:

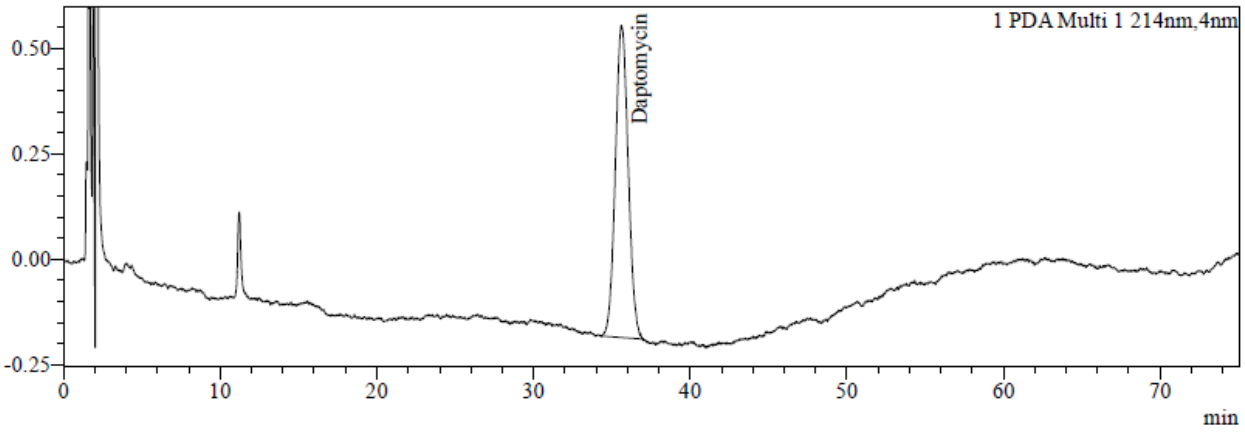
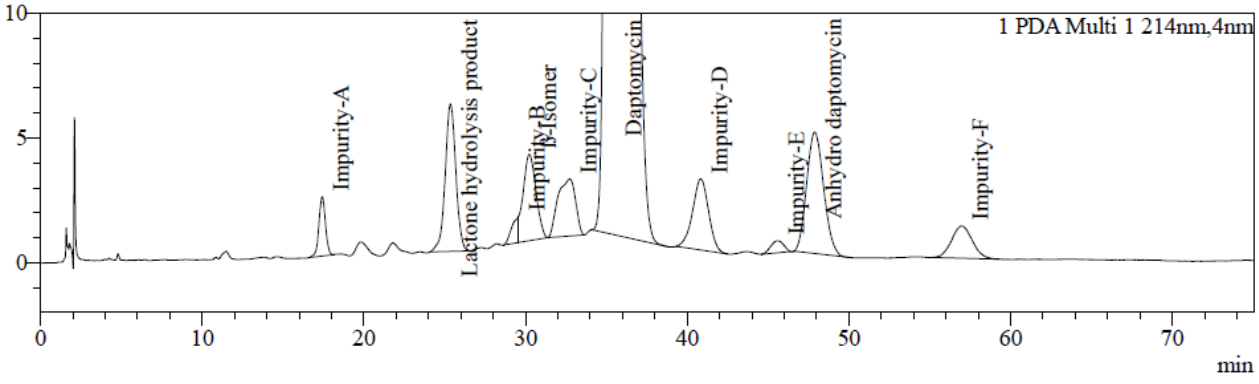


Figure No. 8: Chromatogram of Sample:



CONCLUSION:

The proposed HPLC method for the related substance of Daptomycin in Daptomycin injection by HPLC is found further eligible to validate make eligible as a stability-indicating

method for the quantification of Daptomycin the Injection formulation. Hence this method stands validated and can be used for routine analysis.

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