INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY ADVANCED RESEARCH TRENDS
ISSN : 2349-7408
VOLUME II, ISSUE IV, MAY 2015

DEVELOPMENT OF STABILITY INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF DORIPENEM IN PURE AND INJECTIONS FORMULATIONS

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ABSTRACT

A rapid reverse phase high pressure liquid chromatographic method has been developed for the estimation of Doripenem in injections. Chromatographic determination of Doripenem was performed on Phenomenex Prodigy C18 column (150 X 4.6 mm, 5µm) at 300 nm using 60:40 %, v/v of methanol and 10 mM potassium dihydrogen phosphate as mobile phase operated on isocratic mode with a flow rate of 0.7 ml/min. The injection volume is 20 µL. The retention time of doripenem was found to be 2.73 ± 0.10 min. LOD was found to be 0.5 µg/ml and LOQ 1.6 µg/ml. The number of theoretical plates were found to be 6656. The stress degradation studies were performed on doripenem and the results indicated complete degradation of the drug in alkaline medium. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The proposed method has been successfully used for the estimation of doripenem in dosage forms.

Keywords: Doripenem, RP- HPLC, forced degradation studies, validation.

1. Introduction

Doripenem [1] is a parenteral carbapenem that possesses antibacterial activities against a wide range of Gram-positive and gram-negative bacteria. Chemically it is (4R,5S,6S)-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-3-[(3S,5S)-5-[(sulfamoylamino)methyl]pyrrolidin-3-yl]sulfanyl-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (Fig-1). It is stable to human renal dehydropeptidase-I (DHP-I) and therefore does not require the co-administration of a DHP-I enzyme inhibitor. It can be used for bacterial infections such as: complex abdominal infections, pneumonia within the setting of a hospital, and complicated infections of the urinary tract including kidney infections with septicemia. It decreases the process of cell wall growth, which eventually leads to elimination of the infectious cell bacteria altogether. Literature survey reveals that doripenem was determined by using LC-MS and some spectrophotometric methods[2-9]. However, the LC-MS method is not appropriate for the routine quality control of doripenem in injections and spectrophotometric methods are less sensitive to HPLC. Traditionally, HPLC with

UV detection (LCUV) is the most widely used analytical technique for regular quality control of drugs. In the present study we report a validated stability indicating method for the determination of Doripenem in pharmaceutical dosage forms based on the need of newer sensitive analytical method for regular analysis.

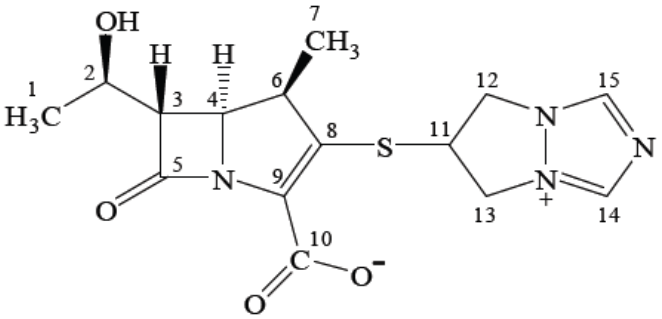

Fig-1: Structure of Doripenem

2. EXPERIMENTAL

Reagents and chemicals

Orthophosphoric acid (AR Grade, Merck ltd), Methanol (HPLC grade, Merck ltd), Milli-Q water, Doripenem (Gift sample from Aurobindo Pharma Ltd, Hyderabad). All other chemicals are of the highest grade commercially available unless otherwise specified.

Apparatus and chromatographic conditions

The chromatographic system consisted of a Shimadzu Class VP Binary pump LC-10ATVP, SIL-10ADVP Auto sampler, CTO-10AVP- Column Oven with SPD-10Avp UV-Visible Detector. All the components of the system are controlled using SCL-10Avp System Controller. Data acquisition was done using LC Solutions software.

The mobile phase consisted of 60:40 % (v/v) of Methanol and 10 mM potassium dihydrogen orthophosphate operated on isocratic mode. The flow rate is 0.7 ml/min. Chromatographic determination of Doripenem was performed on a Phenomenex prodigy C_{18} column (150 X 4.6 mm id, 5µm). The wavelength of detection is 300 nm. The injection volume is 20µL.

Preparation of standard solutions, Calibration Standards & Quality Control Samples

Stock solutions of Doripenem (5 mg/ml) were prepared separately in a volumetric flask and labeled accordingly. Suitable dilutions of Doripenem were prepared using 50:50 %, v/v methanol and Milli-Q water as diluent solution. A

linear calibration curve containing 8 non-zero standards were prepared using diluent solution in the concentration range of 5.04 – 100.80 µg/mL. The linear calibration standard sample is then transferred into the auto sampler for analysis. Samples for specificity, sample with drug, blank sample were also prepared accordingly.

For the preparation of quality control samples, a separate stock containing approximately the same concentration of the drug substance is prepared and labeled as quality control stock. From this stock, quality control samples were prepared at three concentration levels namely LQC (25.20 µg/mL), MQC (50.40 µg/mL) and HQC (75.60 µg/mL) so as to obtain low, median and high concentration quality control samples. The performance of the linear calibration curve is then evaluated using quality control samples.

Assay

The assay of injections containing doripenem is done using the procedure given in Indian Pharmacopoeia for injections. Five vials each containing 1000 mg of Doripenem as label claim were mixed together and weighed; a quantity of powder equivalent to 80.0 mg of Doripenem was weighed and transferred to a 20 mL volumetric flask. To this 10 mL of methanol was initially added and vortexed thoroughly. The final volume was made with methanol. The final solution was mixed well. This mixture is then carefully filtered using 0.22 µm membrane filter. The filtrate is then taken and suitably diluted sonicated for 10 min and injected in triplicate for analysis. The assay content was evaluated using the regression equation of linear calibration curve.

RESULTS AND DISCUSSION

Method development

The HPLC procedure was optimized with a view to develop a stability indicating assay method. Functional group analysis revealed the presence of acidic character to the molecule. Therefore the chromatographic behavior at different pH values ranging from pH 3.0 to pH 6.5 using various columns like Hypersil-BDS-C18, Symmetry C18, Ymc-pack C18, Ymc-pack pro, Spherisorb C18, Phenomenex C18 had been evaluated with different buffer salts such ammonium formate, ortho phosphoric acid, di-potassium hydrogen orthophosphate, in combination with acetonitrile, methanol and tetrahydrofuran. However less tailing and high theoretical plates are obtained with Phenomenex Prodigy ODS column 150 X 4.6 mm, 5 µm column. The wavelength of detection was set at 300 nm as the absorptivity was found to be high and reproducible. Mobile phase composition consisted of (60:40 v/v) of Methanol and 10 mM Potassium dihydrogen orthophosphate as opted for isocratic separation. The flow rate of the method was set at 0.7 ml/min to achieve shorter retention time. Calibration standards were prepared in diluent containing 50:50 % v/v of methanol and Milli-Q water. The column temperature is maintained at 25^0C. At the reported flow rate, peak shape was excellent, however increasing or decreasing the flow rate resulted in unacceptable tailing factor and poor peak shape with longer retention time. Hence 0.7 ml/min was optimized flow rate decreasing

the consumption of the mobile phase, which in turn proves to be cost effective for long term routine quality control analysis. Table 1 represents the optimized chromatographic conditions.

Table 1 Optimized Chromatographic conditions

S.No	Parameters	Value
1	Column	Phenomenex Prodigy C18 reverse phase column, 150 X 4.6mm, 5µm
2	Mobile phase	60:40 % v/v of Methanol and 10 mM Potassium dihydrogen phosphate
3	Flow rate	0.7 ml/min
4	Run time	4 minutes
5	Column temperature	Ambient at 25 ^{o}C
6	Volume of injection	20µl
7	Detection wavelength	300 nm
8	Retention time	2.73 min ± 0.1 min

Method Validation

System Suitability

The system suitability was assessed by six replicate analysis of the drug at a concentration of 40µg/ml. The % RSD of the peak area and the retention time of the drug was within the acceptable range (**Table-2**). The efficiency of the column was expressed as the number of theoretical plates for the six replicate injections was around 5517 ± 43 and the USP tailing factor was 1.31 ± 0.05.

Table-2: System Suitability test for Doripenem

Doripenem				
SR NO	Retention Time	Peak Area	Tailing Factor	Theoretical Plates
1	3.14	1297141	1.31	5786
2	3.16	1293131	1.30	5826
3	3.14	1287876	1.30	5744
4	3.14	1230609	1.30	5865
5	3.15	1273839	1.31	5785
6	3.14	1325749	1.30	5764
MEAN	3.145	1284724.167	1.303	5795.000
SD	0.0084	31511.15	0.01	43.83
% CV	0.27	2.45	0.40	0.76

Detection and Quantitation Limits (Sensitivity)

Limits of detection (LOD) and quantification (LOQ) were estimated from both linearity calibration curve method and signal to noise ratio method. The detection limit was defined as the lowest concentration level resulting in a peak area of three times the baseline noise. The method is found to be sensitive which can be determined from the data obtained from the (**Table-3)**.

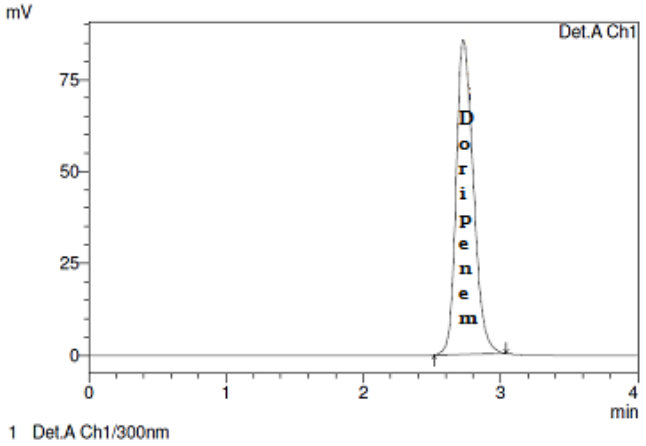

Fig-2: Chromatogram of Doripenem standard.

Linearity (Calibration Curve)

The calibration curve was constructed with eight concentrations ranging from 5.04 to100.80 µg/mL. The linearity was evaluated by linear regression analysis, which was calculated by least square method.

Table 3. Regression characteristics of the linearity plot of Doripenem

Parameter	Value
Linearity range [µg/mL]	10.08 – 100.80
Slope	23971
Intercept	-2840.5
Correlation coeffcient	0.9998
Regression equation	Y=23971.12x– 2840.5
LOD [µg/mL]	0.8
LOQ [µg/mL]	2.8
Theoretical plates	5982
Tailing factor	1.31

Accuracy and Precision

Accuracy of assay method was determined for both intra-day and inter-day variations using triplicate analysis of the QC samples. Precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day).

Repeatability refers to the use of the analytical procedure within the laboratory over the shorter period of the time that was evaluated by assaying the QC samples during the same day. Intermediate precision was assessed by comparing the assays on different days (3 days).

Table 4 Results of inter and intra-day accuracy & precision (% CV) for Doripenem by HPLC

	Nominal Concentration (µg/mL)		
	25.29	50.57	75.86
DAY 1			
MEAN	25.46	49.62	74.82
SD	0.07	1.28	3.14
% CV	0.29	2.58	4.20
DAY 2			
MEAN	25.21	50.28	75.92
SD	0.06	0.95	2.11
% CV	0.27	1.89	2.78
DAY 3			
MEAN	25.18	50.87	75.94
SD	0.11	0.95	1.38
% CV	0.44	1.87	1.82

Specificity

Specificity of the method was determined by comparing the Blank sample with that of the sample containing Doripenem. (**Fig-2**). A less than 20% interference of the peak area at the retention time of the drug in the blank sample is taken as acceptance criteria for the analyte. Sample specificity was also observed in the degradation study of the drug. None of the degraded products interfered with the quantification of the drug.

2.6.6 Stability

The stability of the drug is determined by placing the MQC samples for the short term stability by keeping at room temperature up to 12 hours and then comparing the obtained peak area with that of the similarly prepared fresh sample.

2.6.7 Stress Degradation Studies

For stress degradation analysis, 1 mL aliquots (in duplicate) of samples containing MQC level concentration are treated separately with 100 µL of 0.1N HCl (acid stress), 0.1N NaOH (alkaline stress), 5% v/v Hydrogen peroxide (oxidative stress), for 24 h. Samples for photolytic stress are placed in a transparent glass vial and kept in a UV chamber for 24 h. Samples are then injected for analysis. The results of analysis are then compared with similarly prepared fresh samples.

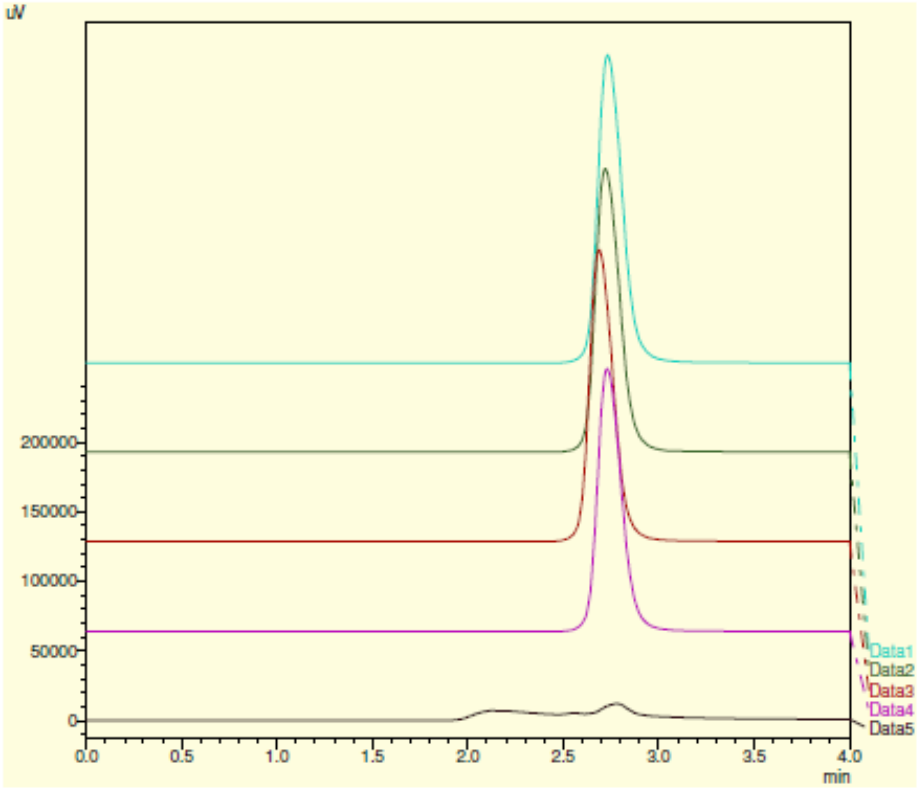

Fig-3: Overlay Chromatogram showing the influence of various stress conditions on Doripenem; Data 1 – Freshly prepared Sample; Data 2 – Photolytic Stress; Data 3 – Oxidative Stress; Data 4 – Acid Stress; Data 5 – Alkaline Stress. Data 3,4 and 5 clearly indicates the spectral degradation of Doripenem under oxidative, acid and alkaline stress conditions.

Table 5 Summary of forced degradation studies

Stress condition	Rt (min)	Peak area	%Purity
Acid Degradation	3.14	1287876	98.21
Base degradation	3.15	3839	15.21
Oxidative degradation	3.14	1325749	99.24
Thermal degradation	3.14	1230609	97.01
Photolytic degradation	3.15	1273839	97.36

Room Temperature Stability

Stability studies were done for short term stability up to 12 h on the bench top for the MQC levels. Stability is calculated as the ratio of the mean peak area of the stability sample to the mean peak area of the fresh sample and expressed as the percentage (n=6). The room temperature stability was found to be 105.61 %. .

Application of the method to dosage forms

The proposed HPLC method developed is sensitive and specific for the quantitative determination of Doripenem. Also the method was validated for different parameters, hence has been applied for the estimation of drug in pharmaceutical dosage forms. Doripenem injections of 1000 mg strength from two different manufacturers were evaluated. The amount of Doripenem in injections was found to be 99.05 ± 0.16. None of the excipients interfered with the analyte peak. The chromatogram of Doripenem extracted from the formulation was matching with that of standard Doripenem showing the purity of peak of Doripenem in the injections.

Table 6 Assay results of doripenem in injections

Parameter	**Value**
Label Claim	1000mg
Drug content%±SD*	99.67±0.45
%RSD**	0.86

*Standard deviation **Relative standard deviation.

4. Conclusions

A rapid sensitive and specific method for the determination of Doripenem in the pharmaceutical formulations has been developed and validated. Forced degradation studies were performed and the results proved the method is stable in acid medium, by photolytic stress and oxidative stress and unstable in alkaline medium. Results of statistical study proved the method is precise, accurate, sensitive and suitable for routine analysis without any interference by the degraded products.

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