



Stability Indicating HPLC Determination of Doripenem in Pharmaceutical Dosage Forms

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ABSTRACT

A simple, selective, precise and stability indicating high performance liquid chromatographic (HPLC) method of analysis of Doripenem in pharmaceutical dosage form was developed and validated. The chromatographic conditions comprised of a reversible phase C₁₈ column (250 × 4.6 mm, 5µm) with a mobile phase consisting of a mixture of acetonitrile and ammonium acetate (0.012M aqueous solution, pH:6.73) in the ratio of 15:85. Flow rate was 0.5ml/min. Detection was carried out at 295nm. The retention time of Doripenem was 7.13min. Doripenem was subjected to acid and alkali hydrolysis, oxidation, photochemical and thermal degradation. The linear regression analysis data for the calibration plot showed good linear relationship in the concentration range of 10 – 100 µg/ml. The value of correlation coefficient, slope and intercept were 0.99944, 48.36 and -25.026 respectively. The method was validated for precision, accuracy, ruggedness and robustness. The drug undergoes degradation under acidic, basic, photochemical and thermal degradation conditions. All the peaks of degraded products were resolved from the active pharmaceutical ingredient with significant different retention times. As the method could effectively separate the drug from its degradation products, it can be employed as a stability indicating one.

Keywords: RP-HPLC, Doripenem, stability indicating, dosage forms, validation.

INTRODUCTION

Doripenem is chemically (+)-(4R,5S,6S)-6-[(1R)-1-Hydroxyethyl]-4-methyl-7-oxo-3-[[[3S,5S)-5-[(sulfamoylamino)-methyl]-3-pyrrolidinyl]thio]-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid. It is a broad spectrum carbapenem antibiotic with antibacterial activity against a broad range of gram positive and gram negative bacteria, as well as against a variety of anaerobes^{1,2}. Doripenem appears to be a potent carbapenem with a spectrum resembling currently marketed antipseudomonal carbapenems, but with greater activity when tested against some non-fermentative bacillary strains. Furthermore, Doripenem has a 1-β-methyl group, hindering attack by human renal dehydropeptidase-1 and thus, unlike imipenem, its use does not require co-administration of a dehydropeptidase-1 inhibitor such as cilastatin. It has been recently approved in the European Union and the United States for clinical use in complicated intra-abdominal and urinary tract infections, joining imipenem, meropenem and ertapenem^{3,4}. In comparison to other carbapenems, it has equal or greater activity against β-lactam nonsusceptible Enterobacteriaceae, including strains with extended spectrum β-lactamases⁵. Doripenem was also found to be the most active carbapenem against *Pseudomonas aeruginosa*^{1,4}.

The Literature survey reveals few studies regarding determination of Doripenem in pharmaceutical dosage forms and biological fluids. These works include HPLC, UV spectrophotometric and potentiometric methods to determine Doripenem in pharmaceutical dosage forms⁶⁻⁹.

The assay of Doripenem in the human plasma and mouse serum are also reported by HPLC methods.¹⁰⁻¹⁴

Doripenem is not official in any Pharmacopoeia and there is no monograph containing methods to characterize or quantify Doripenem. Such methods could offer official parameters to guarantee the validity of the assay. Hence, there is a need for simple, rapid and reproducible method for the routine analysis of Doripenem in pharmaceutical dosage forms.

The present study describes the development and validation of an HPLC method for assay of Doripenem. The HPLC method was studied following official guidelines, evaluating the main parameters and the procedures that can be applied to consider a stability indicating assay. The International Conference on Harmonization (ICH) requires stability-indicating methods to be used for assay of a drug in stability test samples. Stability-indicating methods should be suitable for determination of the drug during hydrolysis (at various pH values), oxidation, photolysis and thermal degradation¹⁵⁻¹⁷. According to FDA guidance document, a stability indicating method accurately measures the active ingredients without interference from degradation products, process impurities, excipients or other potential impurities.

MATERIALS AND METHODS

Chemicals and Reagents

Doripenem monohydrate Reference Standard was supplied by Akorn India Private Limited, Himachal Pradesh, India.



Acetonitrile HPLC grade was procured from Avantor Performance Materials, Philipsberg, USA.

High purity water was prepared with Elix-milli-Q system.

Ammonium acetate AR procured from Merck, Germany.

Sodium hydroxide, hydrochloric acid and hydrogen peroxide were procured from Qualigens, India.

Instrumentation

The HPLC instrument used was AGILENT 1260 RRCL system equipped with a photodiode array detector PDA 1290 infinity.

Chromatographic Conditions

Chromatographic separation was achieved on a reverse phase column Phenomenax C₁₈ (250 × 4.6 mm, 5µm) at ambient temperature using a mobile phase consisting of a mixture of 0.012M ammonium acetate solution (pH 6.73) and Acetonitrile in the ratio of (85:15) at a flow rate of 0.5ml/min. Detection was carried out at 295nm. The mobile phase system after preparation was filtered through a membrane filter (0.22µm) and sonicated for 10 minutes. The pH of the mobile phase was set at 6.73. Injection volume used for assay and degradation studies was 20µl. The retention time of Doripenem was 7.13 min.

Standard Preparation

Stock solution of Doripenem (1mg/ml) was prepared by dissolving appropriate amounts of the compounds in methanol. The solution was stored in the dark under refrigeration at 4°C and was found to be stable for several weeks.

A series of working standard solutions of Doripenem were prepared by the appropriate dilution of the above mentioned stock solution in the mobile phase to get concentration range of 10.0 -100.0 µg/ml. Doripenem standard solutions were found to be stable during the analysis time. (Fig 1.)

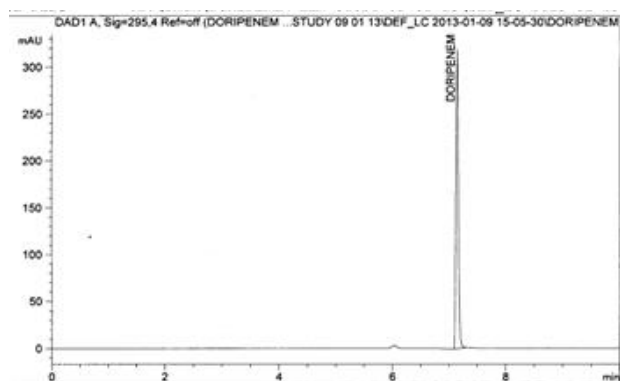


Figure 1: Chromatogram of Doripenem Standard

Sample Preparation

A quantity of Doripenem powder for injection (INAREM 500mg) equivalent to 20mg of Doripenem was dissolved in water and made up to volume in a 100ml volumetric flask. The solution was sonicated for 20 minutes. A 1ml aliquot was transferred to 10ml volumetric flask and

diluted to volume with water. 20µl of the solution was injected into the HPLC system. Peak area was measured at retention time of 7.13 min. (Fig 2.)

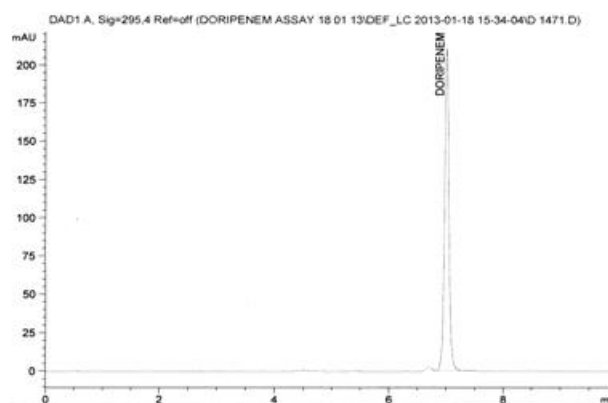


Figure 2: Chromatogram of Doripenem Injection assay.

Preparation of Calibration Graph

Linearity of response for Doripenem assay method was determined by preparing and injecting solutions having concentrations from 10 to 100µg/ml of Doripenem.

System Suitability

The system suitability test was performed to ensure that the HPLC method was suitable to the analysis intended. A standard solution containing 20µg/ml of Doripenem was injected in triplicate. Chromatographic parameters including peak area, retention time, theoretical plates, and tailing factors were measured and the relative standard deviation (RSD) for each parameter was determined.

Method Validation

The HPLC method was validated by evaluation of the analytical parameters including specificity, linearity, precision, accuracy and robustness. The stability indicating capability was determined by forced degradation conditions, including testing heat, light, oxidation and acid and basic degradation.

Specificity

Specificity was examined for the non-degraded and degraded samples. Since excipients are absent in the formulation, a placebo solution was not prepared. The accelerated degradation studies conducted were the following:

- Acidic degradation:** 20mg of Doripenem powder for injection was dissolved in 20ml water in a 100ml volumetric flask and then added 20ml of 0.1 N HCl solution, heated at 50°C for 30 min. Cooled, neutralized the solution and made up to 100ml with water. The solution was centrifuged at 2500 rpm for 10min. 1ml of the supernatant solution was diluted to 10ml with the mobile phase.
- Alkaline degradation:** 20mg of Doripenem powder for injection was dissolved in 20ml water in a 100ml volumetric flask, then added 20ml of 0.1 N NaOH

solution, after 3min neutralized the solution and made up to 100ml with water. The solution was centrifuged at 2500 rpm for 10min. 1ml of the supernatant solution was diluted to 10ml with the mobile phase.

- c) **Oxidative degradation:** 20mg of Doripenem powder for injection was dissolved in 20ml water, in a 100ml volumetric flask then added 60ml of 30% H₂O₂ solution, after 20 min. made up to 100ml with water. The solution was centrifuged at 2500 rpm for 10min. 1ml of the supernatant solution was diluted to 10ml with the mobile phase.
- d) **Thermal degradation:** 20mg of Doripenem powder for injection was dissolved in 20ml water in a transparent glass bottle and kept at 45°C for 48h. Transferred the contents into a 100 ml volumetric flask and made up with water. The solution was centrifuged at 2500 rpm for 10min. 1ml of the supernatant solution was diluted to 10ml with the mobile phase.
- e) **UV degradation:** 20mg of Doripenem powder for injection was exposed to sunlight for 48h. Then it was dissolved in water and made up to 100 ml with water. The solution was centrifuged at 2500 rpm for 10min. 1ml of the supernatant solution was diluted to 10ml with the mobile phase.

Linearity

Doripenem reference solutions were prepared at concentrations of 10, 20, 30, 40, 50 and 100µg/ml. Standard plots were constructed and linearity was evaluated statistically by linear regression analysis that was calculated by least-squares regression.

Precision

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day). In order to evaluate the repeatability of the methods, six sample solutions were determined during the same day for three concentrations of Doripenem. Intermediate precision was studied by comparing the assays performed on two different days.

Accuracy

The accuracy was determined by the recovery of known amounts of Doripenem reference standard added to the samples. The results were expressed as the percentage of Doripenem reference standard recovered from the sample. All solutions were prepared in triplicate and assayed.

Limit of Detection (LOD) and Quantification (LOQ)

The LOD and LOQ parameters were determined from the regression equation of Doripenem: $LOD = 3.3 S_y/a$, $LOQ = 10 S_y/a$; where S_y is the standard error and a is the slope of the corresponding calibration curve.

Robustness

The robustness of the procedure was evaluated after changing the following parameters: the composition of

the mobile phase (content of acetonitrile in the range %); the mobile phase flow rate (0.6-0.8ml/min) and temperature ($25 \pm 2^\circ\text{C}$). For each parameter change its influence on the retention time, resolution, peak (height, area and width) was evaluated.

RESULTS AND DISCUSSION

Method development

The chromatographic conditions were optimized with a view to develop a stability indicating assay method. The experimental studies revealed that the column, Phenomenax C₁₈ (250 × 4.6 mm, 5µm) was most suitable, since it produced best chromatographic performance and acceptable peak characteristics including high resolution and very good sensitivity. The Chromatographic conditions finally comprised of a mobile phase consisting of a mixture of 0.012M ammonium acetate solution (pH 6.73) and Acetonitrile in the ratio of (85:15) at a flow rate of 0.5ml/min using Phenomenax C₁₈ column (250 × 4.6 mm, 5µm) and detection was carried out at 295nm.

Calibration curve

Under the above mentioned experimental conditions, a linear relationship was established by plotting Doripenem concentrations against the corresponding peak areas. The response was linear over the range of 10 to 100µg/ml of Doripenem with a correlation coefficient, slope and intercept of 0.99944, 48.36 and -25.02 respectively. Under the applied chromatographic conditions, the LOD and LOQ of Doripenem were 0.5 and 1.5µg/ml respectively.

Validation of the method

Precision

The intraday and inter-day precision values were calculated for three concentrations of Doripenem. The RSD values were less than 1%, demonstrating that the method was precise.

Accuracy

The accuracy of the method was established by recovery studies. Results indicate that the individual recovery of Doripenem ranges from 99.42% to 101.62% with mean recovery of 100.36% and %RSD of 0.45%. The recovery of the Doripenem by proposed method is satisfactory as %RSD is not more than $\pm 2.0\%$ and mean recovery between 98.0 – 102.0%.

Ruggedness and Robustness

The method robustness and ruggedness was determined by changing the following parameters: composition of the mobile phase, mobile phase flow rate in the range of 0.4-0.8 ml/min, temperature in the range 23-27°C and pH in the range 6.3-7.0. With the deliberate aforementioned change in parameter, the effects on the retention time and peak resolution, peak shape and peak area (height and width) were evaluated. No significant changes in the resolution and shapes of the peak and retention time



were observed when temperature and flow rate were modified. Modifications of the composition of the mobile phase by changing the organic-to-inorganic ratio resulted in the essential changes of retention time and resolution in the determination of Doripenem.

Analysis of marketed formulation

The drug content was found to be 99.98% with %RSD of 0.32. It was noted that no degradation had occurred in the marketed formulation that were analyzed by this method. The low RSD value indicated the suitability of this method for routine analysis of Doripenem in pharmaceutical dosage forms.

Stability indicating property

The chromatogram of no stress treatment sample (as control) showed no additional peak (Fig 2). The chromatogram of acid degraded sample showed 87% degradation in the peak intensity at retention time of 7.13 min (Fig 3). The chromatogram of alkali degraded sample showed 100% degradation and shift of peak at

retention times of 4.65 min for the degraded component (Fig 4). The chromatogram of H₂O₂ degraded sample showed a 40% decrease in intensity of peak at 7.13 min and an additional small peak at retention time of 5.5 min corresponding to degraded component (Fig 5). There was no decrease in intensity of peak at 7.13 min in photo degraded sample (Fig 6) and thermal degraded sample (Fig 7). Thus Doripenem was found to be stable to dry heat and sunlight. In each forced degradation samples where additional peaks were observed, the response of the drug was changing from the initial control sample. This indicates that the drug is susceptible to acid-base hydrolysis degradation and H₂O₂ degradation. The lower retention times of the degraded components indicated that they were more polar than the analyte itself (Table 1).

Detection of the related impurities

The sample solution showed no additional peaks other than the principal peak. Hence related impurities are not present in the market sample.

Table 1: Degradation studies of Doripenem

Condition	Degradation	Additional peaks	Peak purity ^a	Tail peak ^b
Acid 0.1N HCl, heated at 50°C for 30 min	87%	-	99.8	1.13
Base 0.1N NaOH at room temp. for 3 min	100%	4.65 min	98.8	1.11
30% H ₂ O ₂ heated for 20 min	40%	5.50 min	98.9	1.11
Thermal, Heating at 45°C for 48 h	0%	-	99.8	1.13
Exposed to sunlight for 48 h	0%	-	99.5	1.20

^aPeak purity values in the range of 98-100 indicate a homogeneous peak; ^bTail peak <1.50 indicates symmetry of peak.

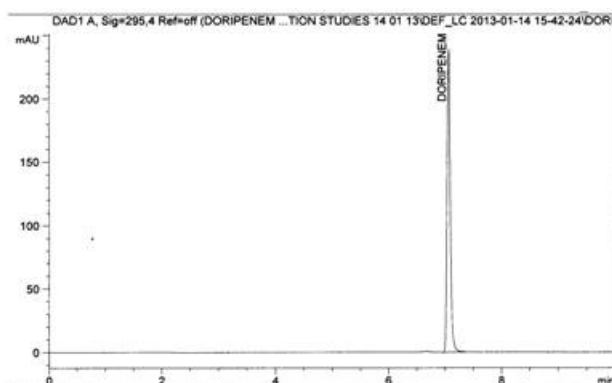


Figure 3: Chromatogram of acid degraded sample.

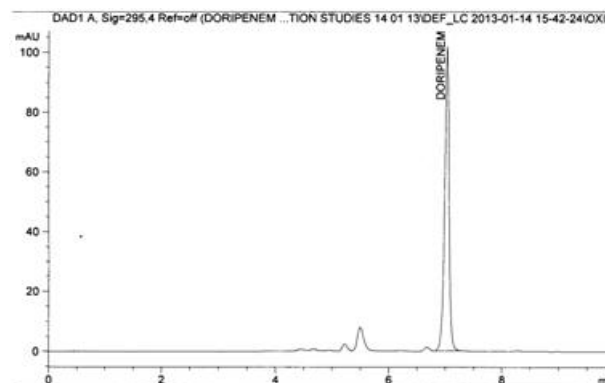


Figure 5: Chromatogram of peroxide degraded sample

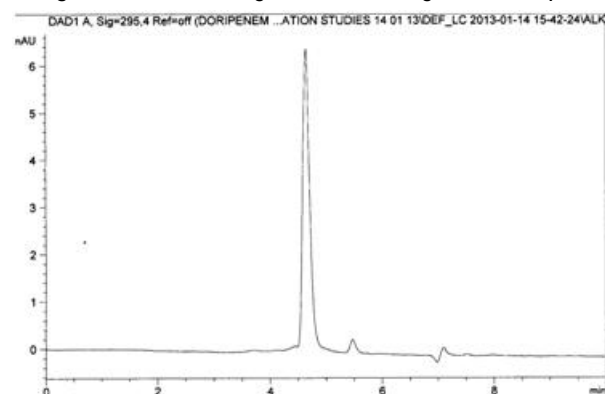


Figure 4: Chromatogram of alkali degraded sample

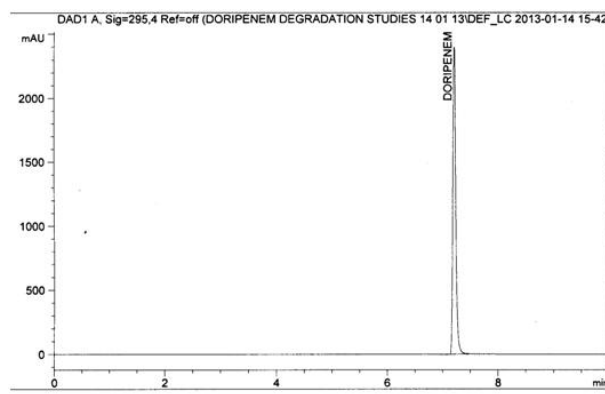


Figure 6: Chromatogram of photo degraded sample

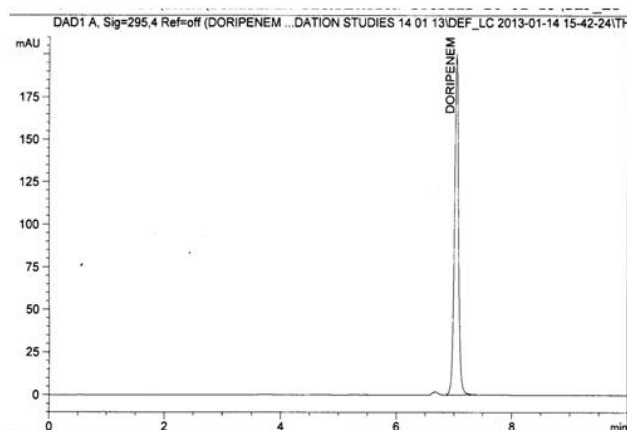


Figure 7: Chromatogram of heat degraded sample

CONCLUSION

The developed HPLC technique is simple, precise, accurate and stability indicating. As the carbapenems are sensitive to degradation, selectivity is an important validation parameter. Statistical analysis proves that the method is reproducible and selective for the analysis of Doripenem in pharmaceutical dosage forms. It can be used to determine the purity of the drug available from various sources. As the method separates the drug from its degradation products, under all stress conditions using HCl, NaOH, H₂O₂, heat and UV light, it can be employed as a stability indicating one.

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REFERENCES

- Jones RN, Huynh HK, Biedenbach DJ, Fritsche TR, Sader HS, Doripenem (S-4661), a novel carbapenem: comparative activity against contemporary pathogens including bactericidal action and preliminary in vitro methods evaluations, *J. Antimicrob. Chemother.*, 54,1, 2004,144-154.
- Matthews SJ, Lancaster JW, Doripenem monohydrate, a broad-spectrum carbapenem antibiotic, *Clin. Ther.*, 31,1, 2009, 42-63.
- Clock SA, Tabibi S, Alba L, Kubin CJ, Whittier S, Saiman L, In vitro activity of Doripenem alone and multi-agent combinations against extensively drug-resistant *Acinetobacter baumannii* and *Klebsiella pneumonia*, *Diagn. Microbiol. Infect. Dis.*, 76, 3, 2013, 343-346.
- Paterson DL and Depestel DD, Doripenem, *Clin. Infect. Dis.*, 49, 2, 2009, 291-298.
- Castanheira M, Jones RN, Livermore DM, Antimicrobial activities of doripenem and other carbapenems against *Pseudomonas aeruginosa*, other nonfermentative bacilli, and *Aeromonas* spp., *Diagn. Microbiol. Infect. Dis.*, 63, 2009, 426-433.
- Mantovani L, Sayago CTM, De Camargo VB, Silveira VF, Garcia CV, Schapoval EES, Mendez ASL, Stability-Indicating RP-HPLC method for analysis of the antibiotic Doripenem in pharmaceutical formulation-Comparison to UV Spectrophotometry and microbiological assay, *Acta Chromatogr.*, 24, 2012, 367-382.
- Al-Shaalan NH, Electroanalytical determination of Doripenem using a screen-printed electrode, *Am J Pharm & Toxicol.*, 7, 2012, 1-7.
- Piontek JC, Krause A, Zalewski P, Lunzer A, Jelinska A, Development and validation of stability-indicating LC- UV method for the determination of Doripenem and Biapenem in pharmaceutical dosage forms, *Acta Chromatogr.*, 24, 2012, 207-219.
- Al-Shaalan NH, Utilization of charge transfer and kinetic methods for the determination of Doripenem, *Am. J. Applied Sci.*, 9, 2012, 510-518.
- Ikeda K, Ikawa K, Morikawa N, Kameda K, Urakawa N, Ohge H, Sueda T, Quantification of Doripenem in human plasma and peritoneal fluid by HPLC with ultraviolet detection, *J. Chromatogr. B*, 867, 2008, 20-25.
- Sutherland C, Nicolau DP, Development of an HPLC method for the determination of Doripenem in human and mouse serum, *J. Chromatogr. B*, 855, 2007, 123-126.
- Dailly E, Bouquie R, Deslandes G, Jolliet P, Le Floch R, A liquid chromatography assay for a quantification of Doripenem, Ertapenem, Imipenem, Meropenem concentrations in human plasma: Application to a clinical pharmacokinetic study, *J. Chromatogr. B*, 879, 2011, 1137-1142.
- Piontek JC, Jelinska A, The UV-derivative spectrophotometry for the determination of Doripenem in the presence of its degradation products, *Spectrochimica Acta Part A: Mol Bimolecular Spectroscopy*, 77, 2010, 554-557.
- Mendez ASL, Mantovani L, Barbosa F, Sayago CTM, Garcia CV, Paula FR, Silva FEB, Denardin ELG, Characterization of the antibiotic Doripenem using physicochemical methods-chromatography, spectrophotometry, spectroscopy and thermal methods, *Quim. Nova*, 34, 2011, 1634-1638.
- ICH Guidance on analytical method validation, International convention on quality for the pharmaceutical industry, Toronto, Canada, 2002, 8-10.
- ICH, Q1B, Stability testing: photo stability testing of new drug substances and Products; International Conference on Harmonization, IFPMA, Geneva, Switzerland, 1996, 3-4.
- International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceutical for Human Use (2005) Validation of analytical procedures: text and methodology Q2 (R1), November, 2005, 1–13.

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