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THE ICH GUIDELINES IN PRACTICE: FORCED DEGRADATION STUDIES OF DORIPENEM BY AN LC-MS COMPATIBLE RP-HPLC METHOD

Ceema Mathew *, Shashikala Metri, Gorja Ashok and Devilal Jarpula

Gokaraju Rangaraju College of Pharmacy, Osmania University, Bachupally, Hyderabad - 500090, Telangana, India.

Keywords:

HPLC, Degradation, Stability-indicating, Doripenem

Correspondence to Author:
Dr. Ceema Mathew

Associate Professor,
Gokaraju Rangaraju College of Pharmacy, Osmania University, Bachupally, Hyderabad - 500090, Telangana, India.

E-mail: nirujose@gmail.com

ABSTRACT: In this manuscript, a fast, stability-indicating RP-HPLC method with UV detection was developed and validated to quantify doripenem in the bulk drug and its injectable formulation. The analysis was performed using Phenomenex (250 x 4.6mm, 5µm) ODS column with a flow rate of 1 mL min^{-1} and a SPD 20 A UV detector to monitor the eluate at 316 nm. The mobile phase was acetonitrile and 10 mM ammonium formate (pH=3.5) in the ratio 35:65, in isocratic mode. The linearity range is 25–250 µg mL^{-1} and the linear regression analysis data for the calibration plot showed good linear relationship with R^2 = 0.9999. The LOD and LOQ were 213 and 638 ng mL^{-1} respectively. The stress conditions employed include acid, alkali, water, hydrogen peroxide, dry heat and UV light. The drug peak was well resolved from the degradation products' peaks; hence, the method can be used to analyze stability samples also.

INTRODUCTION: Doripenem **Fig. 1,** a recently developed member of the carbapenem class of beta-lactam antibiotics is (+)-(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, with the empirical formula $C_{15}H_{24}N_4O_6S_2$ and molecular weight of 420.50. It has pKa values of 2.8 and 7.9 and poor lipid solubility (octanol/water partition coefficient = 0.002). Similar to meropenem and ertapenem, but unlike imipenem, doripenem has a 1-β-methyl side chain that provides resistance to the renal enzyme I-dehydropeptidase.

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Doripenem has broad-spectrum activity against Gram-negative and Gram-positive pathogens, including strains of Pseudomonas aeruginosa [1, 2]. Doripenem, like other carbapenems, was developed to treat hospitalized patients with moderate or severe bacterial infections [3]. The drug is marketed as a crystalline powder for reconstitution and injection without excipients in the formulation.

A thorough literature survey has shown the drug's characterization using different instrumental techniques [4], spectrofluorimetric methods [5, 6], Liquid–Liquid Microextraction [7], capillary electrophoresis [8, 9], and some LC studies for quantifying doripenem in biological fluids [10-13]. Various reported methods are concerned with the stability of doripenem [14-19]. The literature also reveals some of the LC-MS studies [20, 21] and NMR studies [22, 23]. As per the ICH guidelines entitled "Stability Testing of New Drug Substances and Products" the active substance's inherent stability characteristics can be elucidated through stress

testing [24]. Some stability-indicating LC methods reported for Doripenem 25-27 are more time consuming.

Hence, the objective of the present research work reported in this paper was to develop a simple, accurate, economical and less time consuming isocratic RP-HPLC method using an LC-MS compatible mobile phase that can characterize the antibiotic, doripenem in bulk and formulations even in the presence of degraded products and to validate the developed method to ensure compliance with ICH guidelines [28].

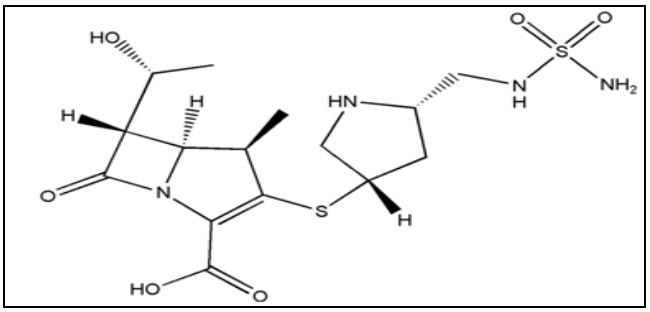

FIG. 1: CHEMICAL STRUCTURE OF DORIPENEM

MATERIALS AND METHODS:

Chemicals and Reagents: Doripenem was obtained as a gift sample from Mepromax Life Sciences Private ltd. Ammonium formate AR grade and acetonitrile (HPLC grade) were obtained from Merck (India). We purchased Doriglen 500 mg vials from a local pharmacy. Triple distilled water was prepared in-house by a distillation system.

HPLC Instrumentation: A Shimadzu Prominence binary gradient HPLC system equipped with LC 20 AD binary pump, SPD 20 A UV detector and Rheodyne manual Injector with 20µL loop and a Phenomenex ODS column (250mm X 4.6 mm i.d, 5 µm) were used for the chromatographic separation. We used LC Solution software for data acquisition.

Chromatographic Conditions: The LC system was operated isocratically at a 1.0 mL/min flow rate at room temp. The mobile phase was made up of acetonitrile, and 10 mM ammonium formate buffer of pH 4.72 (prepared with 632mg in 1000 mL of triple distilled water) in the ratio of 13:87 v/v, and the detection wavelength selected was 297 nm. The peak areas were integrated automatically by LC solution software. Peak identity was proved by retention time comparison.

Preparation of Stock Solution and Calibration Standards: 10 mg of the reference substance was dissolved in 10 mL of acetonitrile (1000 µg/mL) in a 10 mL volumetric flask to obtain the stock solution. From this, calibration standards were prepared by dilution using the mobile phase.

Preparation of Sample Solution: A quantity of powder equivalent to 10 mg of Doripenem was transferred to a 10 mL volumetric flask and dissolved in acetonitrile, with the help of an ultrasonic bath. The solution was clear due to the absence of excipients in the formulation and diluted using the mobile phase.

Forced Degradation Studies: The drug was subjected to forced degradation, and the resulting samples were analyzed by the developed method. It should be able to resolve the sample peak from the peak of the degradation product so that the technique will be stability indicating. Acidic and basic hydrolysis were performed in 0.1M hydrochloric acid and 0.1M sodium hydroxide. Neutral hydrolysis was performed in water. For oxidative degradation, we used 10 % hydrogen peroxide. Thermal degradation was done by heating the drug powder at 105^{o}C for 15 hrs. For photolytic degradation studies, pure drug powder was exposed to a UV lamp in a UV cabinet at 254 nm for 120 hrs.

Method Validation: The method validation was done as per ICH guidelines [28] concerning the following parameters: accuracy, precision, LOD, LOQ, specificity, robustness, stability and system suitability.

Precision: We evaluated the analytical method's precision by determining the repeatability of the technique (intraday precision) and intermediate precision (interday precision) of the sample solutions. We analyzed the repeatability by injecting three solutions of low, middle and higher concentrations (50, 100 and 200 µg/mL) in triplicate, prepared on the same day. Intermediate precision was calculated by injecting the same concentrations on three different days. The relative standard deviations of the areas of peaks were calculated.

Accuracy: The accuracy of the developed method was done by the standard addition method. To the

pre-analyzed sample solution (50 µg/mL), a known amount of standard drug was added at 80%, 100% and 120% levels and reanalyzed by the developed method in triplicate. The percentage recovery was calculated from the linear regression equation obtained in the linearity studies.

Sensitivity: The method's sensitivity can be estimated as LOD and LOQ and was estimated at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions of known concentration, and the respective formulae are given below:

$$LOD = 3.3 \times SD / S$$

$$LOQ = 10 \times SD / S$$

Where SD = standard deviation of the area of the lowest concentration, S= slope of calibration curve of the analyte.

Specificity: Method's specificity is the ability to measure analytical response in the presence of its potential impurities. For the stress degradation, the drug was subjected to oxidation, heat, hydrolysis (acidic, alkaline, neutral) and photolysis, followed by its analysis using the developed method.

Robustness: To determine the robustness, the experimental conditions, such as flow rate (1.0 mL/min), lambda max (297 nm) and percentage of acetonitrile (13), were changed at three levels (-1, 0, +1). We changed them one by one to analyze the impact of the change in the experimental conditions on the assay results. We noted the difference in the assay values and the retention time at each change in the analytical parameters.

RESULTS AND DISCUSSION:

Optimization of Chromatographic Conditions: A reverse-phase HPLC method was developed, optimized and validated to analyze the drug and its possible degradation products. A sharp and symmetric peak with good separation for the drug and its degradation products was obtained by an isocratic method using a mobile phase of acetonitrile and 10 mM ammonium formate (pH 4.72) in the ratio 13:87 (v/v), pumped at a flow rate of 1 ml/min. The detection was carried out at 297 nm, and the retention time was 3.809 min for Doripenem standard and 3.795 min for Doribax, the injectable formulation. **Fig. 2A** and **2B** represent the typical chromatograms of the drug and its injectable formulation, respectively.

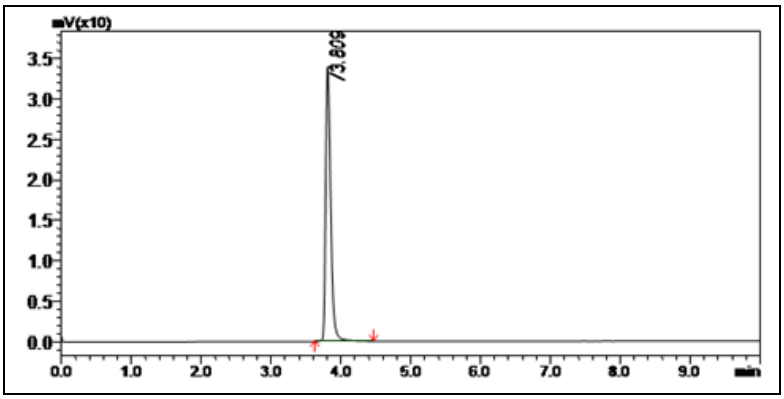

FIG. 2A: CHROMATOGRAM OF DORIPENEM STANDARD

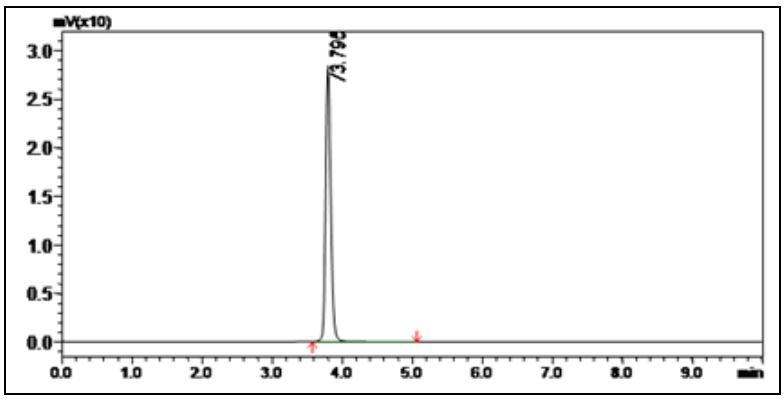

FIG. 2B: CHROMATOGRAMS OF THE INJECTABLE FORMULATION

Linearity: The results are linear in the range of 25–250 µg/mL, and the linearity equation is y=3198x + 2224 with a correlation coefficient of R^2 = 0.9999 that shows an excellent correlation between peak area and the concentration.

Precision: We evaluated the precision study by calculating % RSD values. The values were in the range of 0.18 to 0.80 and 0.24 to 0.81, respectively for intra-day and inter-day precision; the data is presented in **Table 1**.

The % RSD values are within the acceptance criteria (% RSD<2).

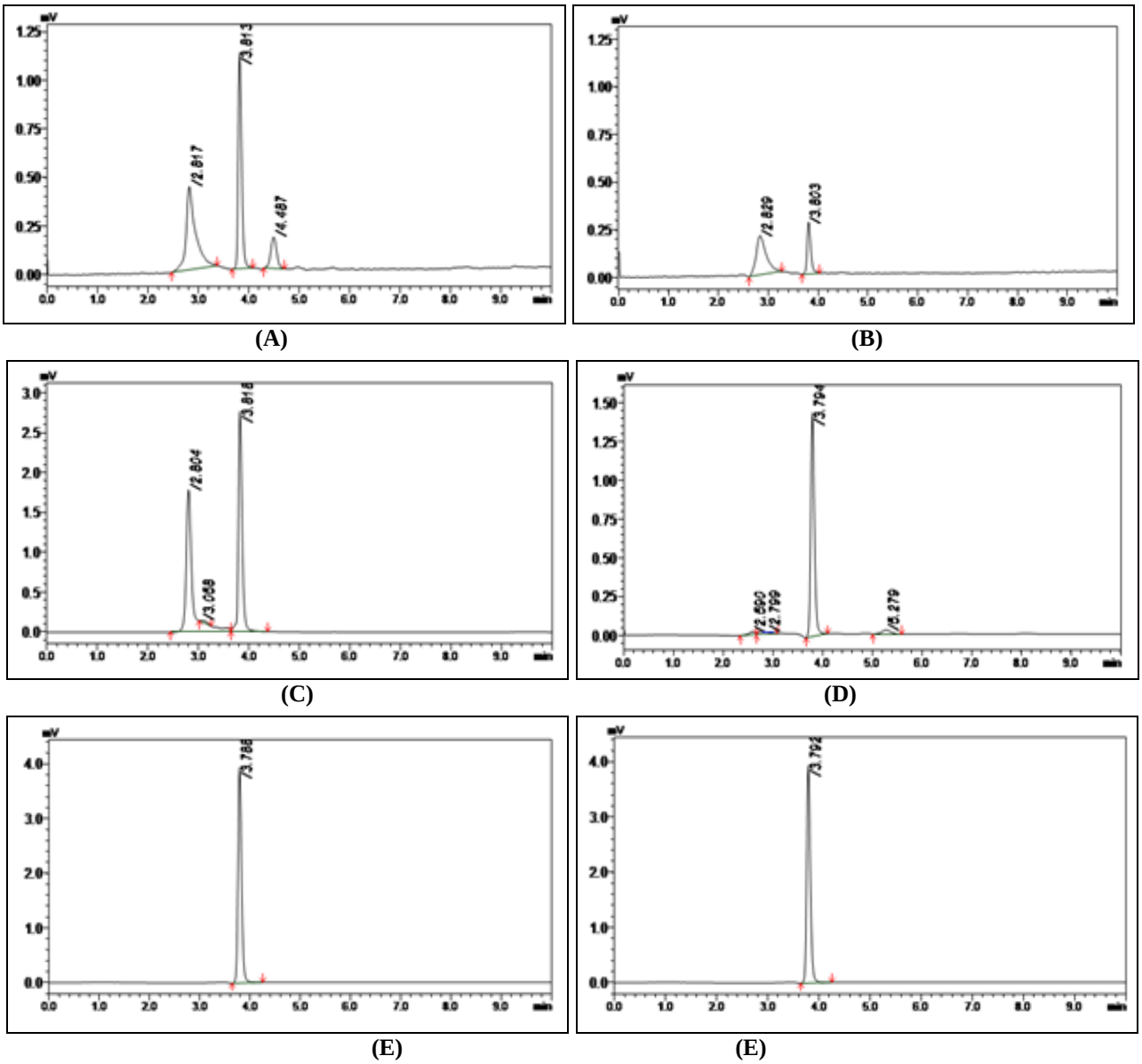

FIG. 3: (A) CHROMATOGRAM FOR ACIDIC (0.1N HCL) DEGRADATION B) CHROMATOGRAM FOR ALKALINE (0.1 N NAOH) DEGRADATION C) CHROMATOGRAM FOR OXIDATIVE DEGRADATION D)CHROMATOGRAM FOR HYDROLYTIC DEGRADATION E) CHROMATOGRAM FOR DRY HEAT DEGRADATION F) CHROMATOGRAM FOR PHOTOLYTIC DEGRADATION

TABLE 1: PRECISION RESULTS

Con.(µgmL $^{-1}$)	Intraday precision[a]			Interday precision[b]		
	Mean con.	SD	% RSD	Mean con.	SD	% RSD
50.0	50.59	0.40	0.80	50.51	0.41	0.81
100.0	100.11	0.18	0.18	100.53	0.45	0.44
200.0	200.40	0.57	0.29	199.94	0.49	0.24

[a]Mean concentration of six trials [b]Mean concentration of nine trials.

Accuracy: The quantitative recovery of Doripenem at three levels ranged from 100.13 - 101.95 % with a low % RSD value. The results of the recovery experiments, along with % RSD values, are given in **Table 2.**

TABLE 2: RECOVERY STUDIES

Excess drug added (µg mL $^{-1}$)	Drug Content in µg mL $^{-1}$		Recovery (%)	% RSD
	Theoretical	Practical [a]		
0	50	50.98	101.95	0.808
40	90	90.11	100.13	1.20
50	100	100.66	100.66	0.51
60	110	110.26	100.23	0.49

[a]Mean concentration of six trials.

Sensitivity: The proposed method found the LOD and LOQ values to be 213 and 638 ng mL-1, respectively.

Robustness: The robustness study, carried out by the deliberate changes in the chromatographic conditions (flow rate, % of acetonitrile in mobile phase, wavelength), indicated that the results are not much affected and are shown in **Table 3**. It suggests that the method is robust and reliable for regular usage.

TABLE 3: RESULTS OF ROBUSTNESS EVALUATION

Parameters for deliberate changes		Retention time(min)	Assay as %
Flow rate (mL min^{-1})	0.95	3.99	99.84
	1.05	3.62	98.99
% Acetonitrile	12	3.74	100.85
	14	3.56	99.13
Wavelength (nm)	296	3.67	99.48
	298	3.69	99.26

Assay of Doriglen: The developed method was applied for the assay of the marketed formulation (Doriglen 500 mg). There is a single sharp peak of similar retention time as that of standard (Rt of standard =3.809 min, Rt of formulation = 3.795 min). The assay results are tabulated in **Table 4.**

TABLE 4*:* ASSAY OF DORIGLEN

Formulation	Label claim	Amount found± SD*	Assay (%)	RSD (%)
Doriglen	500 mg	507.41 ± 0.40	101.48	0.39

*Mean concentrations of three trials.

Forced Degradation Studies: The results of the forced degradation study indicated that the developed method is specific due to the absence of the interfering peaks of degradants corresponding to the retention time of the drug (3.8 min). The drug undergoes hydrolysis in alkaline, acidic and neutral conditions. The drug was highly susceptible to alkaline degradation, shown by the complete absence of the drug peak after 20 min. All three hydrolytic reactions (acid, alkali and neutral) gave a degradation peak corresponding to a retention time of 2.8 min. Under oxidative stress, the drug undergoes degradation and gives an extra peak along with another due to hydrogen peroxide. Hydrolytic reactions were faster in alkaline and acidic conditions when compared to the neutral state. The drug was stable to dry heat and UV radiations **Fig. 3. (a), (b), (c), (d), (e)** and **(f)** represent the chromatograms of forced degradation studies

System Suitability: The system suitability was established by evaluating parameters like theoretical plates, tailing factor, retention time and resolution. We found the retention time as 3.80 min. The theoretical plates as 12113, the Tailing factor as 1.4, and the resolution was more than 2.

CONCLUSION: A new simple, fast, accurate, economic, LC-MS compatible isocratic RP-HPLC method is developed to analyze Doripenem bulk drug and its injectable formulation. The stress degradation studies proved that the drug undergoes faster degradation in alkaline and acidic conditions.

The degradation products' peaks are well resolved from the sample peak. Hence, one can use the method for the routine QC analysis of Doripenem samples and stability samples.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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