Research Article

Forced Hydrolytic Degradation Study of Doripenem by UV Spectrophotometric Method

V.Niraimathi*, A.Jerad Suresh, S.Jayapraba
Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai-600003, India.
***Corresponding author's E-mail:** vnm_anr@yahoo.co.in

Accepted on: 23-03-2013; **Finalized on:** 31-05-2013.

ABSTRACT

A forced degradation study of doripenem in bulk and injectable form was conducted under hydrolytic condition. The study was conducted as per available guidelines and main references. Stability indicating RP- HPLC method has been reported for analysis of doripenem injectable formulation in Brazil. Purposeful degradation can be a useful tool to predict the stability of a drug substance or a drug product with effects on purity, potency, and safety. Doripenem is a beta-lactam antibiotic belonging to the carbapenem group, with a broad antibacterial spectrum. Extensive degradation was observed under hydrolytic condition using acid and alkali, and the degraded products were analysed by standard comparison method of UV spectrophotometry. Forced degradation was performed in bulk drug and injectable formulation using 0.1 N Hydrochloric acid and 0.1 N sodium hydroxide. Complete degradation was observed after 90 mins using 0.1 hydrochloric acid, whereas 18 % of the drug was observed at the end of 90 minutes in alkali hydrolysis using 0.1 N sodium hydroxide. Complete degradation of doripenem was observed at the end of 1st day in both acid and alkali hydrolysis. It was concluded that doripenem was found unstable under acid and alkali condition.

Keywords: Doripenem, UV spectrophotometry, hydrolytic degradation, sodium hydroxide, hydrochloric acid.

INTRODUCTION

Doripenem is a novel, broad-spectrum parenteral carbapenem antimicrobial agent. Doripenem exerts its bactericidal activity by inhibiting bacterial cell wall biosynthesis. Doripenem inactivates multiple essential penicillin-binding proteins (PBPs) resulting in inhibition of cell wall synthesis with subsequent cell death. In *E. coli* and *P. aeruginosa,* doripenem binds to PBP 2, which is involved in the maintenance of cell shape, as well as to PBPs 3 and 4. It is approved for complicated intra-abdominal and complicated urinary tract infections (UTI) and for nosocomial pneumonia. All carbapenems (except for ertapenem) have very similar pharmacokinetics, including half-life (1 hour), protein binding (2-20%), distribution properties (0.23-0.35 L/kg), and temporal plasma profiles. Stability- Indicating RP- HPLC method has been reported for analysis of doripenem injectable formulation[1]. Determination of doripenem by Charge Transfer and Kinetic methods have been reported[2]. Antimicrobial activity of doripenem has been established[3]. It is very important to conduct the degradation study to understand the relative chemistry of the drug substance; also to determine the intrinsic stability of a drug substance. Doripenem powder for injection was subjected to hydrolytic dehydration using acid and alkali medium. The identification and evaluation of degraded product was conducted by standard comparison method of UV spectrophotometry.

MATERIAL AND METHODS

Materials and reagents

Hydrochloric acid and sodium hydroxide has been purchased from Rankem. Distilled water was used as a co-solvent. Doripenem powder for injection was obtained from reputed pharmaceutical company. The determinations were carried out at room temperature. All absorption spectra were measured using Shimadzu UV-1650PC (UV-visible) spectrophotometer with a scanning speed of 200 nm min^{-1} and a band width of 2.0 nm, equipped with 1 cm matched quartz cells.

Intraday-Hydrolytic Degradation using 0.1 N NaOH

Standard preparation: Doripenem was transferred to volumetric flask and dissolved in distilled water to achieve a concentration of 1 mg mL^{-1}. An aliquot quantity was diluted with distilled water to get a final concentration of 20 µg mL^{-1}. The solution was scanned in the UV region and the λ maximum was recorded at 298 nm.

Bulk drug preparation: 50 mg equivalent of Doripenem bulk drug was weighed and transferred into 50 mL volumetric flask. It was dissolved in 0.1 N sodium hydroxide solution to achieve a concentration of 1mg mL^{-1}. After 30 mins, an aliquot quantity was diluted with distilled water to get a final concentration of 20 µg mL^{-1}. The solution was scanned in the UV region and the λ maximum was recorded at 298 nm. The same procedure was repeated for 60 mins and 90 mins time intervals.

Sample preparation: 50 mg equivalent of Doripenem powder for injection was weighed and transferred into 50 mL volumetric flask. It was dissolved in 0.1 N sodium hydroxide solution to achieve a concentration of 1mg mL^{-1}. After 30 mins, an aliquot quantity was diluted with distilled water to get a final concentration of 20 µg mL^{-1}. The solution was scanned in the UV region and the λ maximum was recorded at 298 nm. The same procedure was repeated for 60 mins and 90 mins time intervals.

The procedure was repeated thrice. After the stipulated time, the absorbance of the resulting solutions showed maxima at 298 nm against reagent blanks treated in the same way. Three such determinations were made and the assay values were estimated. The values were concurrent.

Blank preparation: 50 mL of 0.1 N sodium hydroxide solution was taken in a 50 mL volumetric flak. The solution was kept at room temperature. After 30 mins, 2 mL of solution was pipetted out and it was diluted with 100 mL with distilled water and used as a blank solution.

Table 1: Results obtained from hydrolytic degradation - 0.1 N NaOH

Stress condition (Alkali hydrolysis)	Time	Bulk content (%)	Sample content (%)	Remarks
0.1 N Sodium hydroxide	30 mins	19.70	19.09	Degradation observed
	60 mins	19.25	18.64	Degradation observed
	90 mins	18.66	18.18	Degradation observed

Each value is the mean of 3 determinations

Table 2: Results obtained from hydrolytic degradation - 0.1 N HCl

Stress condition (Acid hydrolysis)	Time	Bulk content (%)	Sample content (%)	Remarks
0.1 N Hydrochloric acid	30 mins	16.67	18.13	Degradation observed
	60 mins	--	--	Complete degradation observed
	90 mins	--	--	Complete degradation observed

Each value is the mean of 3 determinations

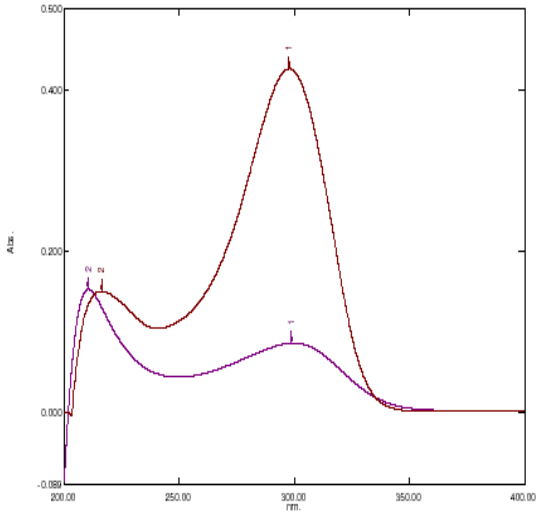

Figure 1: Overlay UV spectrum of Doripenem Sample with standard in 0.1 N NaOH after 90 mins

Intraday-Hydrolytic Degradation using 0.1 N HCl

Bulk drug preparation: 50 mg equivalent of Doripenem bulk drug was weighed and transferred into 50 mL volumetric flask. It was dissolved in 0.1 N hydrochloric acid to achieve a concentration of 1mg mL^{-1}. After 30 mins, an aliquot quantity was diluted with distilled water to get a final concentration of 20 µg mL^{-1}. The solution was scanned in the UV region and the λ maximum was recorded at 298 nm. The same procedure was repeated for 60 mins and 90 mins time intervals.

Sample preparation: 50 mg equivalent of Doripenem powder injection was weighed and transferred into 50 mL volumetric flask. It was dissolved in 0.1 N hydrochloric acid to achieve a concentration of 1 mg mL^{-1}. After 30 mins, an aliquot quantity was diluted with distilled water to get a final concentration of 20 µg mL^{-1}. The solution was scanned in the UV region and the λ maximum was recorded at 298 nm. The same procedure was repeated for 60 mins and 90 mins time intervals.

The procedure was repeated thrice. After the stipulated time, the absorbance of the resulting solutions showed maxima at 298 nm against reagent blanks treated in the same way. Three such determinations were made and the assay value was estimated. The obtained values were tabulated in table 2.

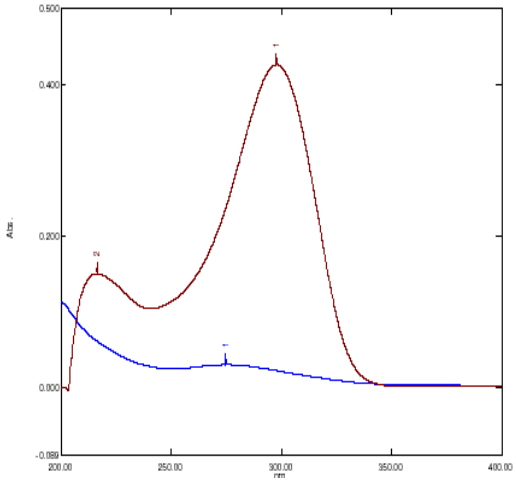

Figure 2: Overlay UV spectrum of Doripenem Sample with standard in 0.1 N HCl after 90 mins

Blank preparation: 50 mL of 0.1 N hydrochloric acid was taken in a 50 mL volumetric flak. The solution was kept at room temperature. After 30 minutes, 2 mL of solution was pipetted out and was diluted to 100 mL with distilled water. This was used as a blank solution.

Interday-Hydrolytic Degradation using 0.1 N NaOH & 0.1 N HCl

Standard preparation

The standard preparation was prepared in similar manner as in Intraday preparation.

Bulk drug preparation

Similar method was followed, but the final solution was scanned and absorbance was recorded at the end of 1st, 3rd & 5th day.

Sample preparation

Same method was followed, but the final solution was scanned and absorbance was recorded at the end of 1st, 3rd & 5th day.

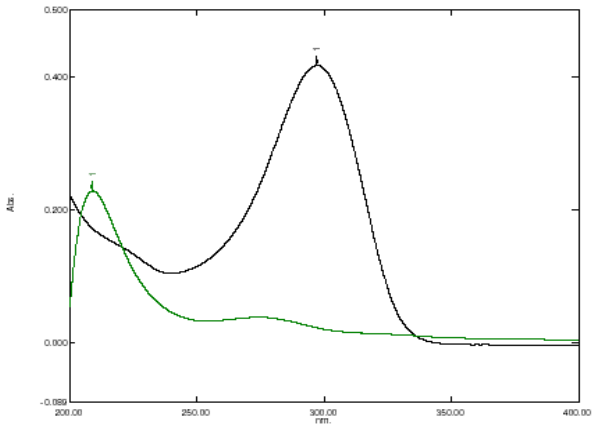

Figure 3: Overlay UV spectrum of bulk with standard in 0.1 N NaOH after 5th Day

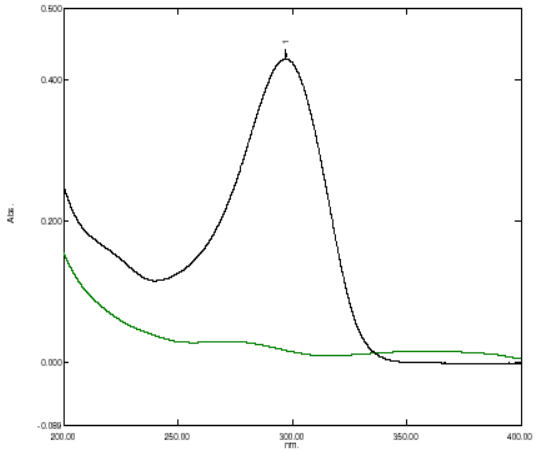

Figure 4: Overlay UV spectrum of bulk with standard in 0.1 N HCl after 5th Day

Blank preparation

Similar to intraday preparation.

The procedure was repeated thrice. After the stipulated time, the absorbance of the resulting solutions showed maxima at 298 nm against reagent blanks treated in the same way. Three such determinations were made and the assay value was estimated. The obtained values were concurrent.

RESULTS AND DISCUSSION

Doripenem was found to be unstable under alkali and acid degradation. It was found that only 18% w/w of Doripenem was present at the end of 30 minutes using 0.1 N hydrochloric acid and 19 % w/w at the end of 30 minutes using 0.1 N sodium hydroxide. Complete degradation was observed at the end of 90 minutes after exposure to 0.1 N hydrochloric acid; and 18 % w/w of Doripenem was present at the end of 90 minutes after exposure to 0.1 N sodium hydroxide. Complete degradation of the bulk and sample were observed on 1st, 3rd & 5th day. Therefore the drug doripenem has to be stored under such condition where the possibility of degradation would not arise.

CONCLUSION

The hydrolytic acid and alkali degradation of doripenem was studied by UV spectroscopy at various time intervals (30 mins, 60 mins & 90 mins; 1st, 3rd & 5th day); it was established that the drug doripenem is vulnerable to hydrolytic degradation.

REFERENCES

1. Mantovan.L, Sayago C.T.M., De Camargo V.B., Silveria V.F., Garcia C.V., Schapova E.E.S., and Mendez A.S.L. Stability-Indicating RP-HPLC Method for Analysis of the Antibiotic Doripenem in Pharmaceutical Formulation—Comparison to UV Spectrophotometry and Microbiological Assay. Acta Chromatographica, 24(3), 2012, 367–382.
2. Nora H. Al-Shaalan. Utilization of Charge Transfer and Kinetic Methods for the determination of Doripenem. American Journal of Applied Sciences 9(4), 2012, 510-518.
3. Jason Schafer J., Debra Goff A. and Julie Mangino E., Doripenem: A New Addition to the Carbapenem Class of Antimicrobials. Recent Patents on Anti-Infective Drug Discovery, 4, 2009, 18-28.
4. George Ngwa. Forced Degradation Studies. Drug Delivery Technology, 10(5), June 2010.

Source of Support: Nil, **Conflict of Interest:** None.