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A novel validated and stability indicating RP-HPLC method for the determination of doripenem in bulk and pharmaceutical formulations

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ABSTRACT

A simple, rapid, precise and accurate stability indicating reverse phase liquid chromatographic method (RP-HPLC) was developed for the determination of a beta-lactum antibiotic agent doripenem Doripenem (DPN) in pure and dosage forms. The degradation products formed under different stress conditions were successfully separated on a chromosil C18 column using a mobile phase of mixture of water, methanol and ortho phosphoric acid in the ratio 78:20:02 (v/v/v) at a rate of flow of 1.0 mL/min and were detected at a suitable wavelength of 290nm. The degradation products thus formed were well resolved with an acceptable retention time. The developed method was statistically validated for precision, accuracy, linearity, ruggedness, robustness, forced degradation, solution stability sensitivity and selectivity. Recovery studies of the dosage form were also carried out at three different concentration levels with in the linearity limits and the calculated percent of relative standard deviation (% RSD) was found to be acceptable. The response of the instrument (peak area) with concentration was observed to be linear in the range of concentration 5.0-40 μg mL^{-1}. Due to its simplicity, rapidity and accuracy the proposed method may be considered as an alternative method for routine quality control analysis in any pharmaceutical laboratory.

KEYWORDS

Doripenem;
Stability;
Degradation;
RP-HPLC;
Validation.

INTRODUCTION

Doripenem (DPN) is a recently developed member of carbapenem class of beta-lactum antibiotic. Doripenem was shown to have broad-spectrum activity against Gram-positive and Gram-negative pathogens, including strains of Pseudomonas aeruginosa[1]. It is similar to other carbapenems and was developed for the treatment of hospitalized patients with moderate or severe bacterial infections[2].

Doripenem is chemically known as (+)-(4R, 5S, 6S)-6-[(1R)-1-Hydroxy -ethyl] -4-methyl-7-oxo-3-[[(3S,5S)-5- [(sulfamoyl amino)- methyl]-3-pyrrolidinyl] thio]-1-azabicyclo [3.2.0] hept-2-ene-2-carboxylic acid with molecular formula and molecular weight $C_{15}H_{24}N_4O_6S_2$ and 420.50 g/mol respectively. The pharmaceutical preparations, sudopen 500 mg and 250 mg vials were injectables, each 500 mg vial contain 0.5 mg of doripenem as doripenem monohydrate. The chemical structure of the drug molecule was pre-

sented in Figure 1.

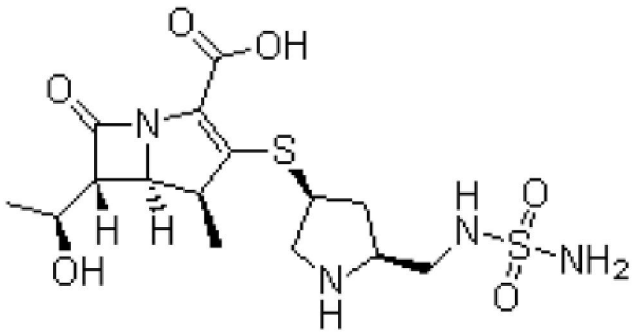

Figure 1 : Chemical structure of doripenem.

Literature survey reveals the use of ultraviolet spectrophotometer (first-derivative, first-derivative of ratio spectra and bivariate analysis) was used in the stability-indicating determination of ertapenem[3-5], meropenem[6,7] and doripenem[8]. A few high-performance liquid chromatographic methods for the determination of doripenem in intravenous solutions[9], in body fluids[10], in human plasma[11] were also reported. A liquid chromatography[12] assay for a quantification of doripenem, ertapenem, imipenem, meropenem concentrations in human plasma and simultaneous determination of eight β-lactam antibiotics in human serum by liquid chromatography-tandem mass spectrometry[13] were also presented in literature. Nora H and Al-Shaalan[14] reported visible spectrophotometric and kinetic methods for the determination of doripenem in pharmaceutical forms. From the entire literature it was noticed that no HPLC method reported for the determination of doripenem in pharmaceuticals, hence the authors were interested in developing a stability-indicating high performance liquid chromatographic method for the determination of Doripenem (DPN) in bulk drug and pharmaceutical formulations.

EXPERIMENTAL

Equipment

HPLC instrument equipped with a LC 20AT pump and variable wavelength programmable UV-visible detector SPD-10AVP, 20µL Hamilton syringe and a Chromosil C18 column (250 mm x 4.6 mm, 5µ) column was used for the present investigation. A Denwar balance and Loba ultrasonic bath sonicator were used for weighing the materials and degassing of the mobile phase respectively. ElicoSL 159 UV-Visible spectrophotometer was used for spectral studies. The data was analyzed by using peak software.

Materials and methods

HPLC grade Methanol, ortho phosphoric acid (Merck) and water were used for the preparation of mobile phase in the present investigation. Pure sample of Doripenem (standard) was gifted by Orchid, Chennai and its pharmaceutical formulations were purchased from local pharmacy.

Preparation of standard drug solution

10 mg of the pure DPN was accurately weighed and transferred into a clean and dry 10 mL volumetric flack, dissolved in methanol, made up to the mark, sonicated and filtered through a 0.45 µ nylon filter. Then stock solution of 100 µg mL^{-1} was prepared by transferring 1 mL of the above solution with a high precision pipette into 10 mL volumetric flask and made up to the mark with mobile phase, the solution was sonicated and filtered through a 0.45 µ nylon filter. A series of working standard solutions of different concentration ranging from 10 µg mL^{-1} to 40 µg mL^{-1} were prepared from the stock solution.

Preparation of sample solution

An amount of formulation (injection) equivalent to 10 mg of doripenem was transferred into a 10 mL volumetric flask, mixed with 5 mL of methanol, sonicated for 30 minutes and finally the contents of the flask were made up to volume with the mobile phase and filtered through a 0.45µ membrane filter. This solution was adequately diluted to obtain test solutions in the concentration range of 5-40 µg mL^{-1}.

Method development and chromatographic conditions

The mobile phase was allowed to pass through the column at a flow rate of 1.0 mL/min at ambient temperature for 30 min. to equilibrate the column. The optimum chromatographic conditions were obtained by performing different trails by injecting different volumes of working standard of different concentrations at different wavelengths using different composition of mobile phase and different columns. The best chromatographic conditions were found to be a chromosil column

of 250 mm length, 4.6 mm internal diameter and stationary phase with particle size 5µ, mobile phase was a mixture of water, methanol and 0.1 % aqueous ortho phosphoric acid in the ratio 78:20:02 (v/v/v), flow rate 1.0mL/min and detection wavelength at 290nm. A reasonable retention time and valid system suitability parameters such as number of theoretical plates, tailing factor and resolution were obtained under the above conditions. Typical chromatograms of blank and standard were presented in Figure 2 and Figure 3 respectively.

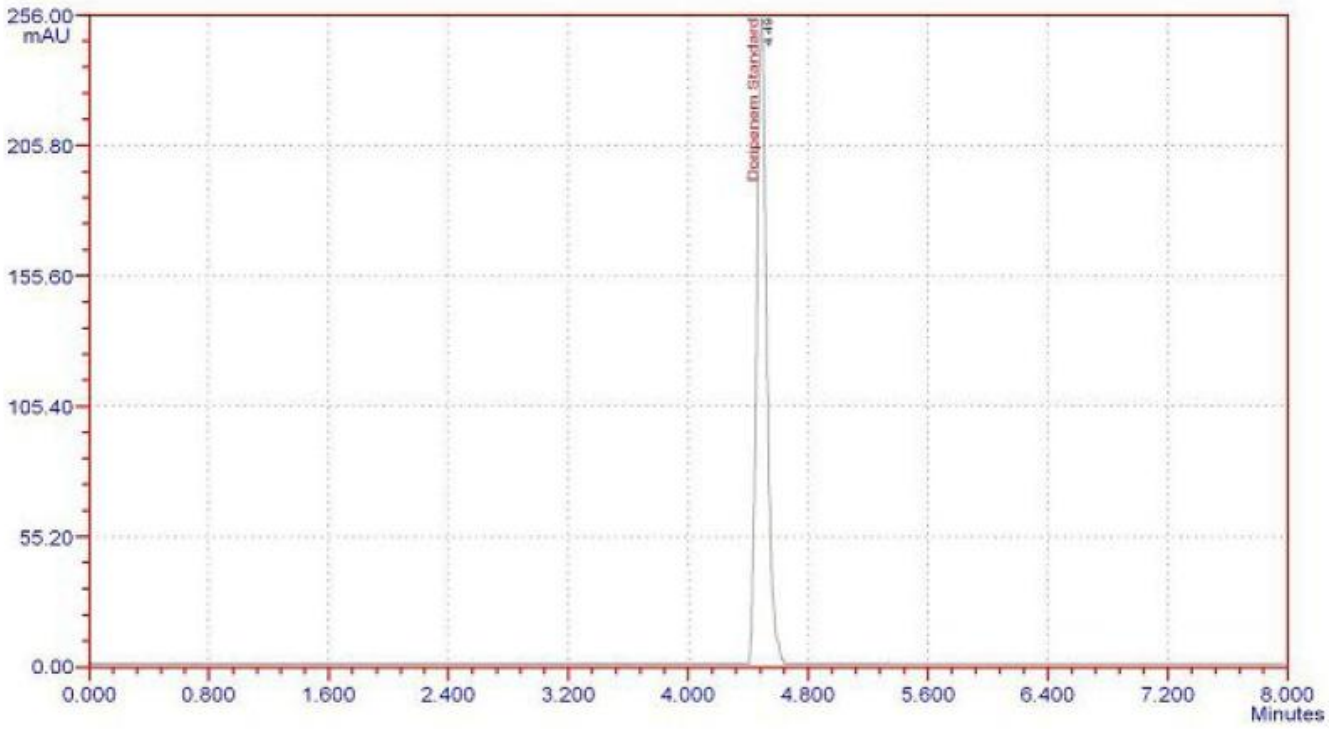

Figure 2 : A typical chromatogram of standard doripenem solution.

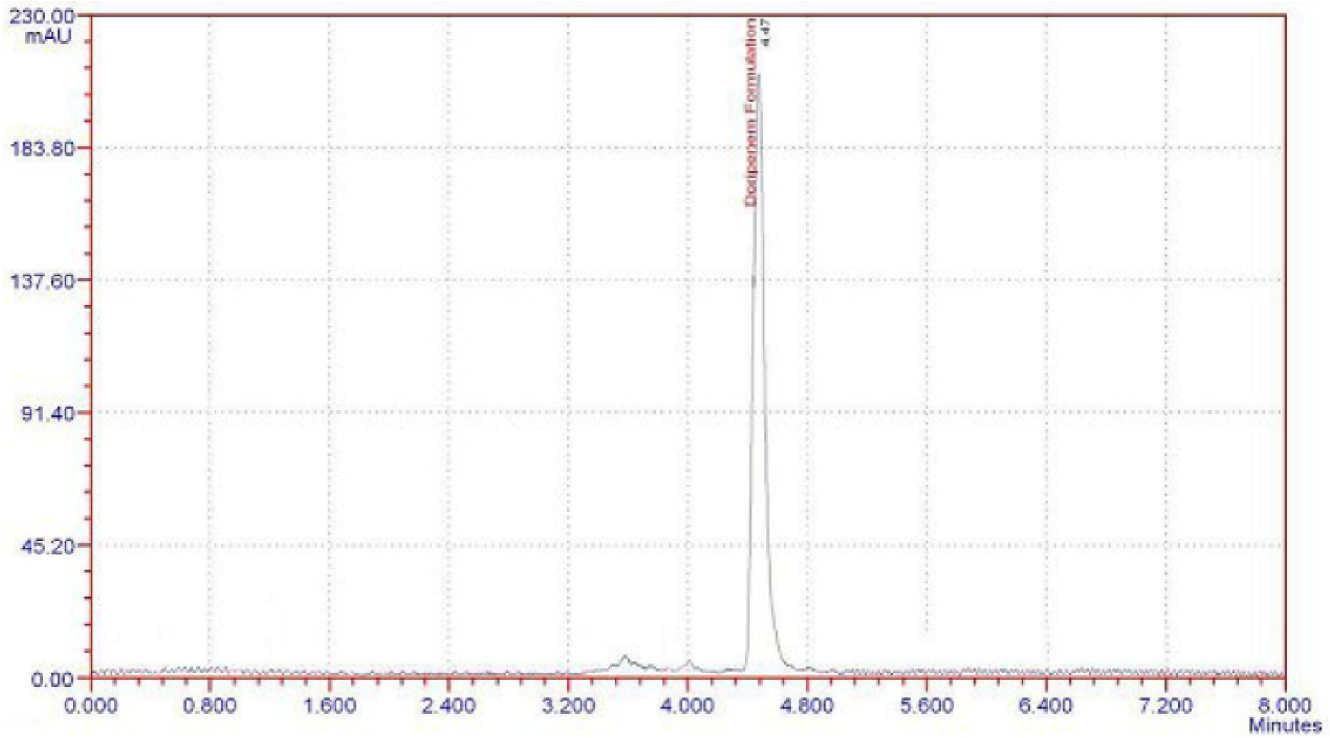

Figure 3 : A typical chromatogram of formulation of doripenem.

Procedure for method validation

(a) Precision

Method repeatability was evaluated by carrying out six replicate solutions against a reference standard. The intra day and inter day precision of the developed method was evaluated. The percent RSD values were found to be 0.735% and 0.527% respectively. The results as shown in TABLE 1 indicated the good precision of the developed method.

TABLE 1 : Intra day and Inter day precision of the proposed method.

Inter Day Precision		Intra Day Precision	
Sample ID	Peak area	Sample ID	Peak area
1	143290	1	143197
2	142120	2	143456
3	142349	3	144420
4	144798	4	142726
5	142042	5	144736
6	143302	6	143857
Mean	142983.50	Mean	143732.00
Standard Deviation	1050.95	Standard Deviation	757.82
%RSD	0.735	%RSD	0.527

(b) Linearity

The linearity between the response of the instrument and concentration of the drug was verified at seven concentration levels ranging from 5-40 μg mL^{-1}. The calibration curve (Figure 4) was constructed by plotting mean area against concentration. The regression equation obtained for Doripenem was y=4708x+2199 (r=0.9997). The results were shown in TABLE 2 which indicates that an excellent correlation existed between peak area and concentration of Doripenem within the concentration range tested.

(c) Accuracy

Accuracy of the method was studied by recovery experiments. The recovery was performed at three levels 15 μg mL^{-1}, 30 μg mL^{-1} and 45 μg mL^{-1} of the sample concentration. Calculated amount of Doripenem from stock solution was added to the fixed amount of pre-analyzed formulation solution to attain 15 μg mL^{-1}, 30 μg mL^{-1} and 45 μg mL^{-1} of sample concentration. Each sample was prepared in triplicate at each level. Blank and standard preparations were injected and the chromatograms were recorded. The recovery values for Doripenem were presented in TABLE 3 and ranged between 98.89 to 102.31 %.

TABLE 2 : Linearity between mean areas of the peaks and concentration of the drug.

Concentration (μg mL^{-1})	Area of the Peak*	Statistical Parameters	Values of parameters
5	25487	Correlation coefficient	0.9998
10	50201		
15	74809		
20	97849	Slope	4665.5
25	118861		
30	143197		
35	166866	Intercept	3406.7
40	189774		

***Mean of two measurements**

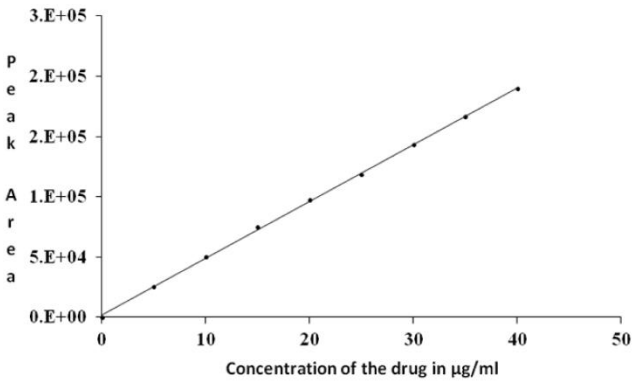

Figure 4 : A linearity plot of area of the peak against concentration of doripen.

(d) Assay analysis

The validated method was applied for the analysis of Doripenem in commercial formulations. The amount of Doripenem obtained in formulation was shown in the TABLE 4.

(e) Robustness

To determine the robustness of the developed method, experimental conditions were purposely altered. One factor at a time was changed to estimate the effect. Doripenem at 30 μg mL^{-1} concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The experimental results were presented in TABLE 5.

(f) Ruggedness

Ruggedness of the developed method was experi-

mentally determined by performing assay analysis in different laboratories, using different instruments and different columns under the same experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was found to be rugged (TABLE 6).

TABLE 3 : Accuracy of the proposed method.

15 µg mL^{-1}			30 µg mL^{-1}			45 µg mL^{-1}		
Sample ID	**Peak Area**	**% of Recovery**	**Sample ID**	**Peak Area**	**% of Recovery**	**Sample ID**	**Peak Area**	**% of Recovery**
1	74702	99.66	1	145349	101.29	1	167684	100.28
2	74262	99.07	2	146798	102.30	2	167729	100.31
3	75274	100.42	3	142042	98.99	3	167151	99.97
Mean	74746	99.71	Mean	144729	100.86	Mean	167521	100.19
SD	507.43	0.6769	SD	2437.73	1.6989	SD	321.50	0.1922
%RSD	0.68	0.68	%RSD	1.68	1.68	%RSD	0.1919	0.1919

TABLE 4 : Results of analysis of doripenem in formulations.

Formulation	Labeled amount mg/vial	Amount found (mg) ± SD	Percent Recovery ±% RSD
Vial - 1	Sudopen-500mg	499.33±5.71	99.86±1.143
Vial - 2	Sudopen-250mg	250.40±1.17	100.16±0.469

TABLE 5 : A report on study of robustness of the proposed method.

Parameter	Value of the Parameter	Retention Time (min)	Peak Area	Tailing Factor	Number of Theoretical Plates
Flow rate	0.8mL	4.726	142364.9	0.96	16641
	1.2mL	4.181	142651.4	0.76	11564
Mobile Phase Composition	72:25:2	4.410	140900.6	1.02	13831
	83:15:2	4.490	141285.9	1.11	13428
Wavelength	292nm	4.411	144604.3	0.84	16054
	288nm	4.399	144799.5	0.63	12496

TABLE 6 : A report on study of ruggedness of the proposed method.

Parameter	Retention Time	Peak Area	Tailing Factor	Number of Theoretical Plates
Laboratory-I	4.399	142131.4	1.04	12956
Laboratory-II	4.410	145545.9	0.76	12555
HPLC-System-I	4.473	142474.7	0.74	8893
HPLC-System-I	4.490	141910.7	1.07	13310
Column-I	4.399	141182.2	0.72	12960
Column-II	4.410	140089.1	0.88	13553

Procedure for forced degradation

The main objective of study of stability testing is to provide an experimental evidence of how much the quantity of a drug product that can be degraded in to other related substances under a variety of environmental conditions with time. Acidic, basic and peroxide degradation was studied using 0.1N HCl, 0.1 N NaOH and 3% hydrogen peroxide solution. Photo degradation study was performed for the same samples by exposing them to UV light. Thermal degradation was carried out by heating at 40^{0}C for 48 hours. All the degraded drug solutions after appropriate dilution with mobile phase were injected into the chromatographic system.

Hydrolytic studies

For the study of hydrolysis of drug in different medias1mg mL^{-1} solution of the drug was prepared by dissolving 10.0mg of drug in 10.0mL of 0.1N HCl, 0.1N NaOH and pure distilled water respectively and the solutions were refluxed for 12hours, 8hours and 10hours respectively, then 1mL of the each refluxed solution was diluted to 10mL with mobile phase so as to obtain 100 µg mL^{-1} stock solution. Further 3.5mL of the above solution (0.5mL in case of base hydrolysis and 2.5mL in case of aqueous hydrolysis) was again diluted to 10mL to get the final concentration 35 µg mL^{-1} (5 µg mL^{-1} for basic and 25 µg mL^{-1} for aqueous) and chromatogram was obtained under the similar chromatographic conditions. The amount of drug present after stressed conditions was calculated from the ratio of the peak area of the drug to that of total area of all the peaks and found to be 70.9%, 79.58 and 84.59 respectively. The degradation (29.1%) of the drug resulted in giving three additional peaks at 3.631, 4.276 and 4.739 min in acid hydrolysis, in case of base hydrolysis four additional peaks at 2.765, 2.873, 6.307 and 6.342 and in aqueous hydrolysis one peak at 3.724 min. The respective chromatograms were represented in Figure 5-Figure

7. This indicates that the drug was hydrolyzed under acid, base and aqueous conditions.

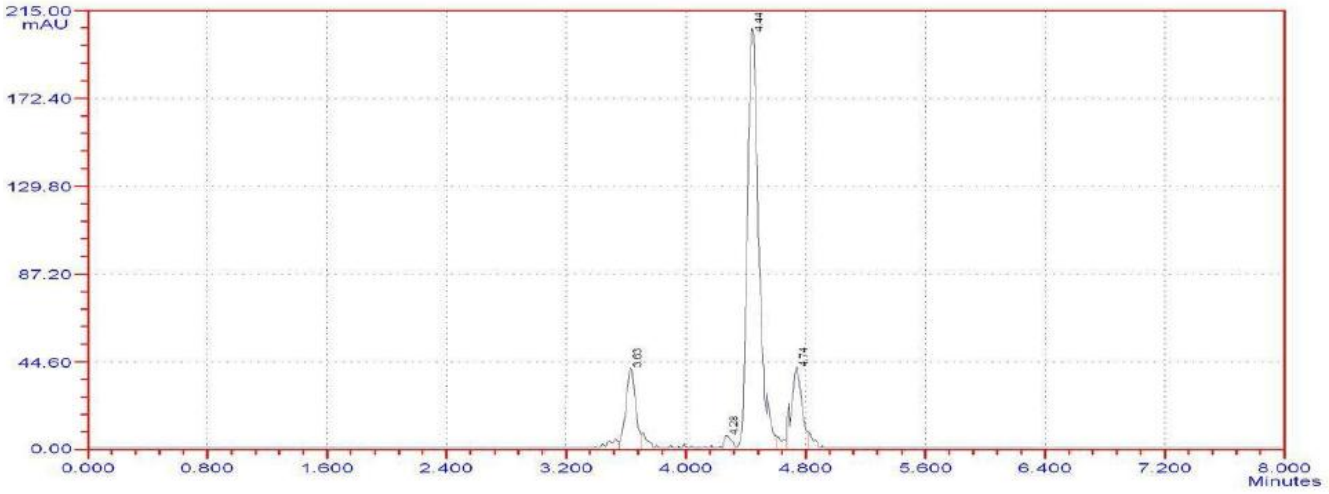

Figure 5 : A typical chromatogram of acid degradation of doripenem.

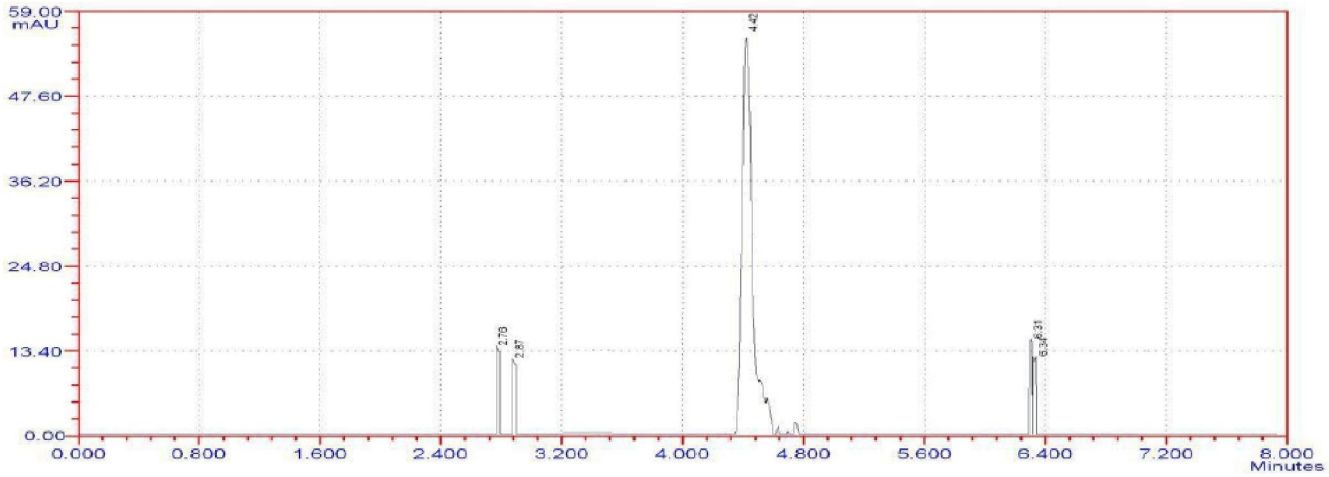

Figure 6 : A typical chromatogram of base degradation of doripenem.

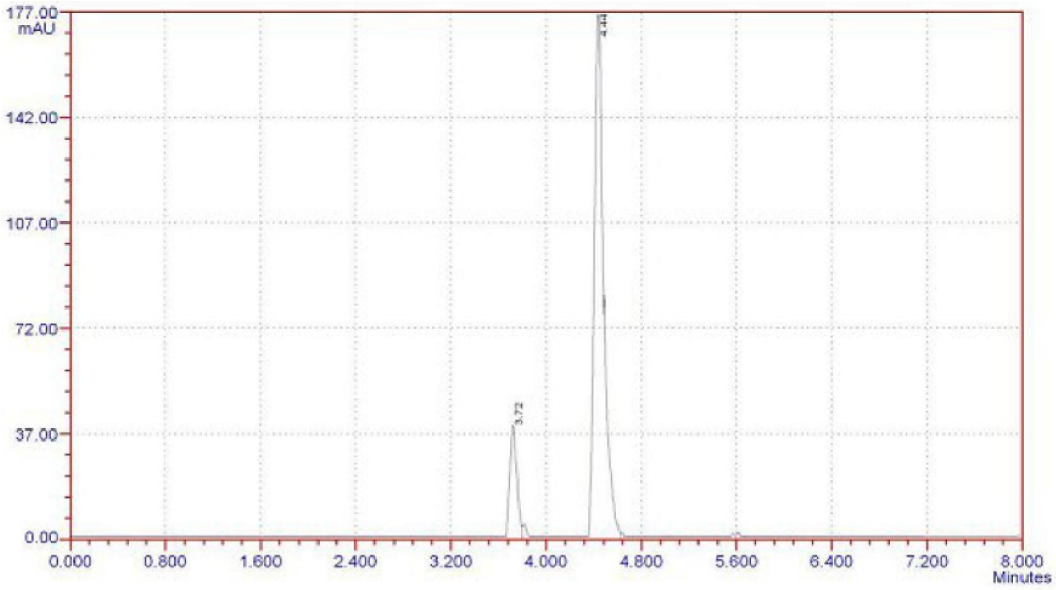

Figure 7 : A typical chromatogram of aqueous degradation of doripenem.

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Oxidative studies

For oxidative degradation study, initially 1 µg mL^{-1} strength of drug was prepared in 10% H_2O_2. The drug was kept for a period 12h under the conditions at room temperature and then for 24hours. The drug degradation was found to be very more (61.4%) when it was exposed to 10% H_2O_2 for 24h. Eleven degradation product peaks at measurable retention times were seen and there was significant rise in the height of the peak with time (Figure 8). This signifies that the drug was degraded in oxidative conditions to chromatographic compounds.

Thermal studies

Susceptibility of the drug to dry heat was studied by exposing the solid drug to 60 ^{0}C for 15 days in a hot air oven. Sampling was carried out every day to study its degradation behavior. Dorifenem was found to be degraded in negligible amount 19.33%. Two degradation product peaks at measurable retention times (2.200 and 2.642 min., Figure 9) were seen, this signifies that the drug was stable in thermal conditions.

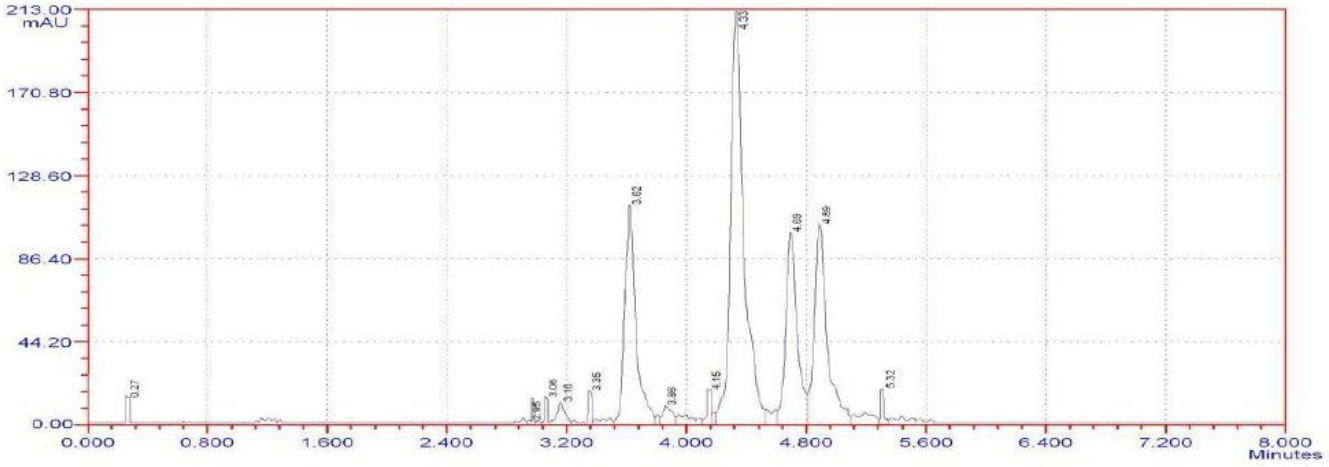

Figure 8 : A typical chromatogram of oxidative degradation of doripenem.

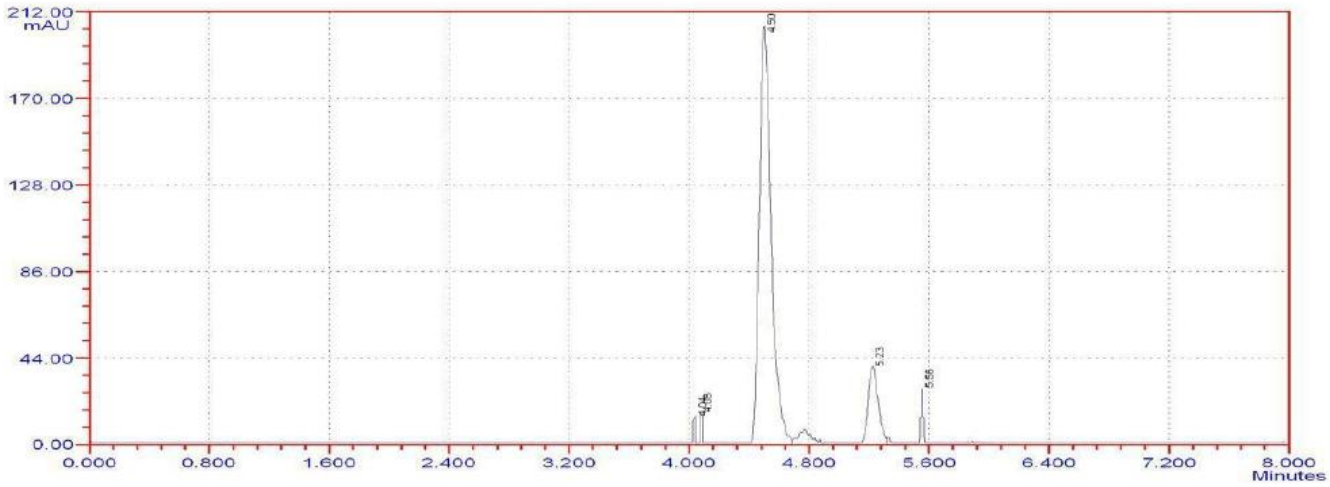

Figure 9 : A typical chromatogram of thermal degradation of doripenem.

Photolytic studies

The photochemical stability of the drug was studied by exposing the stock solution (1 µg mL^{-1}) as well as solid drug to direct sunlight for 10 days on a wooden plank and kept on terrace. Photolytic study was carried out in dry form. Here the drug was directly exposed to the sunlight for 10 days on a hot sunny day. Dorifenem was found to be degraded in negligible amount (19.28%) as four new peaks of degradation products were appear at 4.042, 4.078, 5.227 and 5.558 min respectively as shown in Figure 10. Only 19.28% of the drug was found to be degraded hence doripenem was almost stable after exposing the drug to 60 ^{o}C for 10 days.

TABLE 7 : A consolidated report on study of forced degradation of the doripenem.

S.No.	Degradation Parameter	Retention time	Area of the peak	Tailing factor	Plate count	Total Area	Percent of drug	
							Retained	Degraded
1	Acid	4.44	113541.50	1.22	13087	160123.70	70.91	35.00
2	Aqueous	4.44	98989.80	1.46	12588	117021.70	84.59	25.00
3	Base	4.42	31021.20	1.29	12572	38979.70	79.58	5.00
4	Heat	4.40	44527.50	1.25	13732	54454.30	81.77	10.00
5	Peroxide	4.34	134086.50	1.15	9384	346516.20	38.70	80.00
6	Sunlight	4.50	116273.40	1.43	12611	144053.10	80.72	30.00
7	UV-Light	4.44	127242.70	1.42	11920	181917.60	69.95	40.00

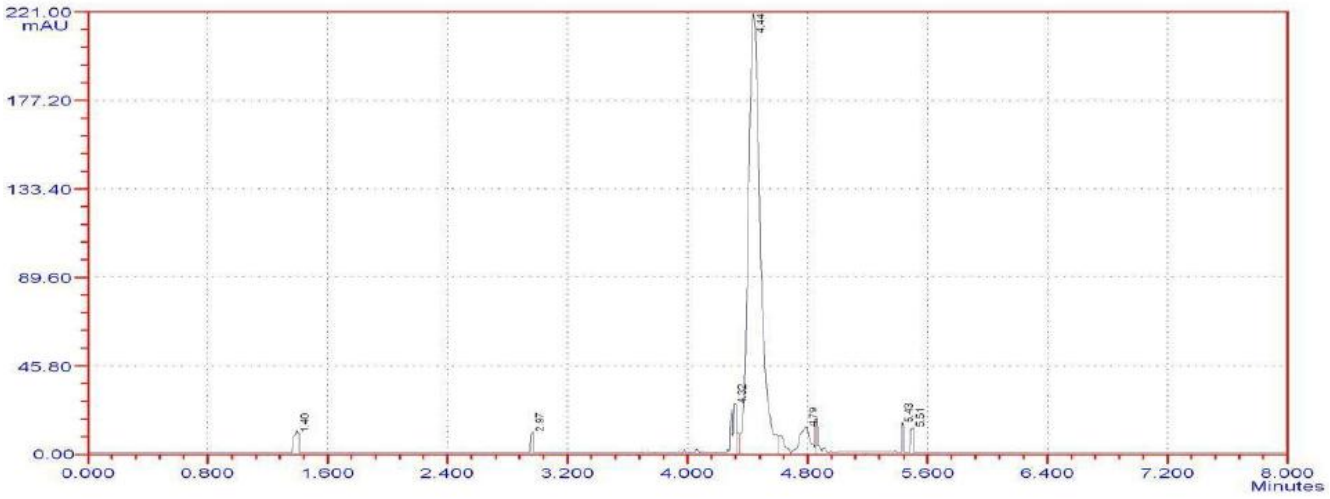

Figure 10 : A typical chromatogram of UV degradation of doripenem.

RESULTS AND DISCUSSION

The separation of the degradation products from Doripenem was achieved on chromosil C18 stationary phase and water, methanol and 0.1 % ortho phosphoric acid (78:20:02 v/v/v) as a mobile phase. The system suitability parameters such as number of theoretical plates, tailing factor and retention time were found to be 13072, 0.99 and 4.5 min for the main peak and less than 6.3min for the degradation products. The intra day and intermediate precision of the method were evaluated, the percent RSD values were found to be 0.735% and 0.527% for respectively. The drug had obeyed linearity between the response of the instrument and concentration of the drug in the ranging from 5-40 µg mL^{-1} with good correlation coefficient 0.9997. The recovery studies were performed at three different concentration levels between the linearity and the values were found to be between 98.89 to 102.31 %. A study of robustness and ruggedness was conducted and proved that the proposed method was robust and rugged. The study of degradation encompasses the influence of dilute acid (0.1N HCl), dilute base (0.1N NaOH) temperature (48°C), humidity, UV-light, oxidizing agent (10% H_2O_2). Drug at a concentration of 1 µg mL^{-1} was used in all degradation studies. The pH of the buffer was checked before and after reaction and no change was observed. Conditions employed for stability studies were as follows. The observed degradation of Doripenem was 29.1% in acidic conditions, 20.4% in basic conditions, 61.4% under oxidation conditions, 30.1% under UV light conditions and 19.3% under thermal conditions after 48 hours. High peak purity results indicate that the Doripenem peak was homogeneous in all stress conditions tested.

CONCLUSION

The proposed stability indicating method was found to be selective, rapid, precise, accurate, robust and rugged. This can be used for assessing the stability of

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Doripenem in bulk and pharmaceutical formulation.

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