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STABILITY INDICATING METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF DORIPENEM IN BULK AND IN SOLID DOSAGE FORM BY RP-HPLC

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ABSTRACT:

A robust and reliable high performance liquid chromatographic (HPLC) approach was developed and validated for the analysis of Doripenem in pharmaceutical dosage form. The method is characterised by its simplicity, selectivity, precision, and capacity to accurately determine the stability of Doripenem. The experimental setup included the use of a Hypersil BDS-C18 column (250 X 4.6 mm ID, 5 µm) as the stationary phase in a chromatographic system. The mobile phase consisted of a combination of methanol and potassium dihydrogen orthophosphate with a pH of 6.7, in a ratio of 20:80. The flow rate of the mobile phase was set at 1 ml/min. The detection of the eluents occurred at a wavelength of 290 nm. The observed retention time for Doripenem was 5.56 minutes. Doripenem underwent acid and alkali hydrolysis, oxidation, photochemical degradation, and heat degradation. The results obtained from the linear regression analysis of the calibration plot demonstrated a

strong linear connection within the concentration range of 70 – 130 µg/ml, as shown by a correlation coefficient value of 0.9995. The methodology was assessed to determine its precision, accuracy, ruggedness, and robustness. The medication experiences deterioration when exposed to environments characterised by acidity, alkalinity, photochemical reactions, and thermal stress. The active medicinal component exhibited distinct retention periods for each of its degradation product peaks, indicating successful resolution. The approach's ability to successfully isolate the medication from its degradation products renders it suitable for use as a stability-indicating method.

Keywords: Doripenem, HPLC, Stability-indicating method

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INTRODUCTION

Doripenem (DPM) is chemically (4R,5S,6S) – 6 - (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. The compound in research is a carbapenem antibiotic that has a wide spectrum of action against both gram-positive and gram-negative bacteria, in addition to many anaerobic microorganisms [1,2]. Doripenem has notable potency as a carbapenem, displaying a range of activity that is comparable to existing antipseudomonal carbapenems available on the market. However, it demonstrates enhanced efficacy when tested against certain nonfermentative bacillary strains. In addition, it should be noted that Doripenem has a 1-β-methyl group, which effectively impedes its susceptibility to human renal dehydropeptidase-1. Consequently, unlike imipenem, the administration of a dehydropeptidase-1 inhibitor such cilastatin is not necessary when using Doripenem [3,4]. Recently, the European Union and the United States have granted approval for the clinical use of this particular treatment in cases of difficult intraabdominal and urinary tract infections. This approval places it alongside imipenem, meropenem, and ertapenem as viable options for medical practitioners. When compared to other carbapenems, this particular compound exhibits comparable or enhanced efficacy against β-lactam nonsusceptibleEnterobacteriaceae, including strains that possess extended spectrum β-lactamases [5]. Doripenem had the highest level of activity among carbapenems against Pseudomonas aeruginosa.

The literature review indicates a limited number of research pertaining to the analysis of Doripenem in pharmaceutical formulations and biological specimens. Various analytical procedures, such as high-performance liquid chromatography (HPLC), ultraviolet (UV) spectrophotometry, and potentiometry, have been used to ascertain the presence and quantity of Doripenem in pharmaceutical formulations [6-9]. HPLC techniques have been used to determine the concentration of Doripenem in human plasma and mouse serum.Doripenem lacks formal recognition in any Pharmacopoeia, since it does not possess a monograph that

encompasses established methodologies for the characterization or quantification of this compound. These approaches have the potential to establish authoritative criteria that ensure the reliability of the test. Therefore, it is essential to develop a straightforward, efficient, and consistent technique for the regular assessment of Doripenem in pharmaceutical preparations. This paper presents the process of developing and validating a high-performance liquid chromatography (HPLC) technique for the quantitative analysis of Doripenem. The HPLC technique was examined in accordance with established protocols, assessing key parameters and methods to determine its suitability as a stability indicating test [10-14]. The use of stability-indicating methodologies for the analysis of medication samples in stability tests is mandated by the International Conference on Harmonisation (ICH). Stability-indicating methodologies must possess the necessary attributes to accurately determine the drug's concentration across processes such as hydrolysis (at different pH levels), oxidation, photolysis, and thermal degradation [15-17]. As per the guideline paper provided by the FDA, a stability indicating technique is capable of precisely quantifying the active components without being affected by degradation products, process contaminants, excipients, or any other possible impurities.

MATERIALS AND METHODS:

All solvents utilized in this study are HPLC grade. Methanol, potassium dihydrogen orthophosphate, HPLC grade water were acquired from Merck. RP-HPLC Shimadzu (LC 20) model with LC SOLUTIONS software was applied in this procedure. Analytical column utilized for the separation of analytes is Hypersil BDS-C18 column (250 X 4.6 mm ID, 5 µm).

METHOD:

Instrumentation:

The HPLC instrument used was Shimadzu (LC 20) model with LC SOLUTIONS software was applied in this procedure.

Chromatographic Conditions:

The chromatographic separation was conducted using a reverse phase column, specifically the Hypersil BDS-C18 column (250 X 4.6 mm ID, 5 µm), at room temperature. The mobile phase employed was a combination of Methanol and potassium dihydrogen orthophosphate with a pH of 6.7, in a ratio of 20:80. The flow rate was set at 1 ml/min, and detection of the separated components was performed at a wavelength of 290 nm. After preparation, the mobile phase system underwent filtration using a membrane filter with a pore size of 0.45µm, followed by sonication for a duration of 10 minutes. The pH of the mobile phase was adjusted to 6.7. The amount of injection used for both the test and degradation investigations was 20µl. The observed retention time for Doripenem was 5.56 minutes.

Standard Preparation:

A methanol solution containing Doripenem at a concentration of 1mg/ml was made by dissolving the required quantities of the chemical. A set of standard solutions of Doripenem for experimental use were created by diluting the previously described stock solution in the

mobile phase. This was done in order to achieve a concentration range of 70.0 - 130.0 µg/ml. The stability of the Doripenem standard solutions was observed throughout the duration of the investigation. (Figure 1.)

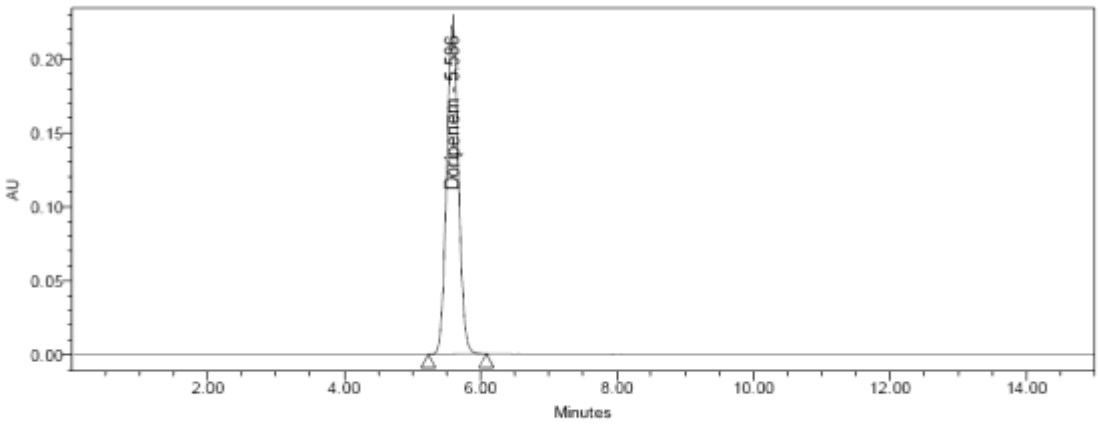

Figure 1Typical Standard Chromatogram

Sample Preparation:

An amount of doripenem monohydrate injection powder weighing 50 mg was measured and placed into a volumetric flask with a capacity of 50 ml. The volume was then adjusted to 50 ml by adding a diluent. A dilution process was performed on the aforementioned solution, resulting in a final volume of 50 ml, achieved by adding 5 ml of the solution. Doripenem was prepared at a concentration of 100 µg/mL.

RESULTS AND DISCUSSION:

METHOD DEVELOPMENT:

To improve separation and resolution, several chromatographic settings were used. The performance of the Hypersil BDS-C18 column (250 X 4.6 mm ID, 5 m) was evaluated and determined to be suitable. The UV detector was used to verify the peak purity of doripenem and the wavelength of 290 nm was found to be suitable for detecting the drug with sufficient sensitivity. Many various solvent and pH ratios were explored, but all of them resulted in poor peak form or resolution. The use of a C18 column in isocratic HPLC yielded excellent results in repeated attempts to get a nice, sharp peak. With a phosphate buffer (pH 6.7): methanol (80:20) mobile phase and a C18-ODS column, running at a flow rate of 1.0 ml/min with a detection wavelength of 290 nm, we obtained excellent retention time, symmetry, and sensitivity in an isocratic experiment.

SYSTEM SUITABILITY:

The chromatographic apparatus was then injected with standard solutions that had been produced in accordance with the testing procedure. Parameters such as theoretical plate count, resolution, and asymmetry factor were examined to determine the system's viability. Table 1 lists the parameters that should be considered while designing a system. It was determined that all of the criteria were suitable.

Table 1: System suitability study Results of DPM

Parameters	Acceptance limits	DPM
Retention time	-	5,58
Theoretical plates	NLT 5000	9643
Tailing factor	NMT 2.0	0.906

SPECIFICITY:

Acid hydrolysis: Upon performance of acid degradation studies, alkali degradation, peroxide degradation, thermal degradation and photolytic degradation studies15.3%, 18%, 17.3%. 16.5% and 19.4% respectively. Degradation studies results are tabulated in table no.2

Table 2: Degradation study Results of DPM

Degradation Study	DPM	
	Mean Area (n=5)	% Degradation
Acid	2378436	15.3
Alkali	2308068	18
Peroxide	2327771	17.3
Thermal	2350289	16.5
Photo	2268662	19.4

PRECISION:

The precision of the methodology Research conducted utilising the precision technique has validated the method's accuracy. Concentration sample solutions were generated for analysis at repeating concentrations. In accordance with the established experimental procedure, a total of six injections of sample solutions containing DPM were administered into the column. The precision results are shown in Table 3. The mean value was calculated, and the relative standard deviation (RSD) was provided. The findings obtained for the percentage relative standard deviation (% RSD) were found to fall within the acceptable range, indicating the method's precision.

Table 3: Precision study Results of DPM

Preparation	Retention Time	Peak area
Preparation 01	5.58	2814717
Preparation 02	5.59	2815345

Preparation 03	5.57	2816754
Preparation 04	5.57	2819876
Preparation 05	5.58	2814378
Preparation 06	5.59	2807856
Average	5.6	2814821
SD	0.0089	3957
% RSD	0.16	0.14

LINEARITY:

The assessment of the linearity of the assay technique included the preparation of test solutions with standard stock solutions of DPM at seven distinct concentration levels, spanning a range from 70% to 130% of the assay concentration. The association between peak area and concentration was examined by the use of least-squares linear regression analysis, as seen in figure 2. The findings obtained in this study indicate a significant association between peak regions and concentration within the concentration range of 70–130 µg/ml for DPM, as shown in Tables 4. The drug's correlation coefficients were found to be 0.9991, meeting the acceptance requirements for method validation. Therefore, it may be inferred that the testing technique demonstrates linearity for DPM.

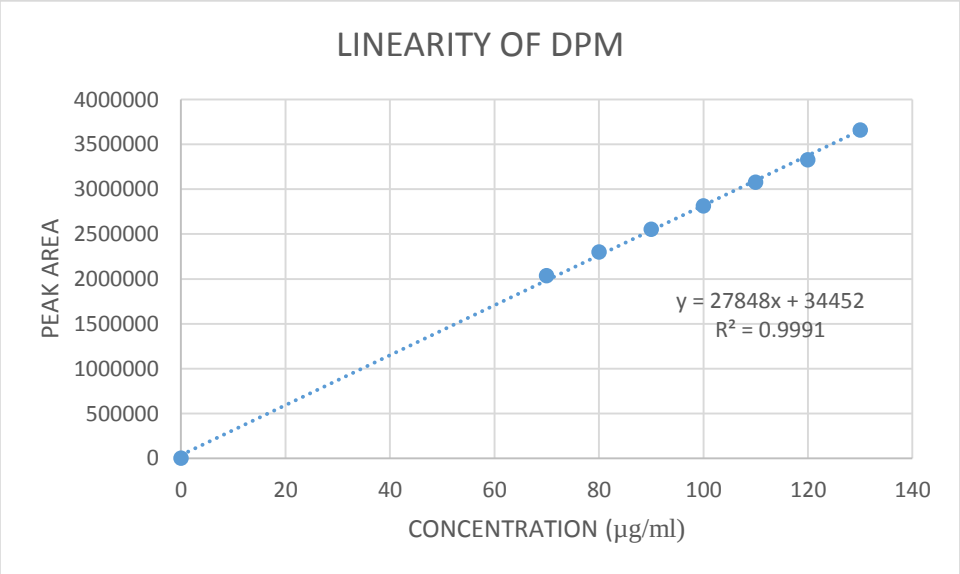

Fig. 2: Linearity chart for DPM

Table 4: Linearity study Results of DPM

S.No.	Concentration (µg/ml)	Peak Area
1	0	0
2	70	2036188

3	80	2298756
4	90	2554152
5	100	2814722
6	110	3077312
7	120	3328679
8	130	3659508
Correlation coefficient		0.9991
Slope (m)		27848
Intercept (c)		34452

ACCURACY:

The accuracy of the approach was evaluated by the implementation of recovery experiments, wherein the percentage mean recovery of both medications was determined at three unique concentration levels (70%, 100%, and 130%). Three determinations were performed for each level. Table 5 displays the computation of the percentage recovery and the mean percentage recovery for the medication. The observed data came inside the desired range, indicating that the developed technique is accurate, as seen by the exceptional recovery values.

Table 5: Accuracy study Results of DPM

Recovery level	µg/ml Added	µg/mL Recovered	% Recovery	Average % Recovery
70%	68.45	68.34	99.84	99.86
	68.47	68.35	99.82	
	68.45	68.40	99.93	
100%	96.27	96.31	100.04	99.88
	95.41	95.20	99.78	
	95.48	95.32	99.83	
130%	124.55	124.28	99.78	99.82
	125.83	125.70	99.90	
	124.58	124.31	99.78	

ROBUSTNESS:

To evaluate the robustness of the developed approach, deliberate adjustments were made to the experimental parameters, and an assessment of the system's suitable traits was performed. The solutions were prepared following the designated test protocol and subsequently introduced under different experimental conditions, encompassing diverse flow rates (0.8 and 1.2 ml/min), wavelengths (280 nm and 300 nm), concentrations of the mobile phase (10:90 & 30:70), temperatures (25°C and 35°C), and pH values (6.2 and 7.2). The system

appropriateness metrics derived from these injections were then evaluated against the accuracy of the approach. The results were documented and arranged in Table 6. The flow rate of 1.0 ml/min shows a prominent peak with excellent resolution, whereas the other flow rates were considered inadequate. The strategy effectively met all the necessary criteria for system suitability, indicating its resilience.

Table 6: Robustness study Results of DPM

Condition	**DPM**	
	Plate Count	**Tailing Factor**
Change in Flow rate		
Normal Condition (1.0 ml per minute)	9521	1.11
Flow rate (0.8ml per minute)	9534	1.08
Flow rate (1.2 ml per minute)	9543	1.02
Change in Wave Length		
Normal: Wave Length 290nm	9657	1.09
Wave Length 280nm	9754	1.12
Wave Length 300nm	9823	1.12
Change in organic concentration		
Normal: Methanol : Buffer (20:80)	9512	1.23
Methanol : Buffer (10:90)	9234	1.12
Methanol : Buffer (30:70)	9345	1.09
Change in temperature		
Normal: 30°C	9823	1.45
Temperature 25°C	9754	1.24
Temperature 35°C	9178	1.11
Change in pH		
Normal: pH 6.7	9865	1.09
pH 6.2	9923	1.05
pH 7.2	9145	1.07

CONCLUSION:

The proposed RP-HPLC technology exhibited many desirable qualities, including simplicity, specificity, accuracy, precision, robustness, speed, and cost-effectiveness. The aforementioned methodology exhibits a notable level of accuracy in discerning between the two pharmaceutical substances, while concurrently providing a quick analytical timeframe. The described RP-HPLC method has potential use for the regular analysis of Doripenem in both bulk form and pharmaceutical formulations.

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