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DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR DETERMINATION OF DORIPENEM IN HUMAN PLASMA AND PHARMACEUTICAL DOSAGE FORMS

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Abstract
Doripenem is drug that belongs to the class of carbapenems, and it is commonly used to treat the infections caused by bacteria. The main theme behind this study plan was to develop and validate a simple, economically suitable, sensitive, less time-consuming, and rapid HPLC method for the determination of doripenem in human plasma and pharmaceuticals. For UV spectroscopy, water was used as a solvent, and analysis was carried out at several wavelengths. Doripenem showed maximum absorbance at 298nm. In the HPLC method, a mixture of acetonitrile and water(40:60%v/v) was used at 1 mL/min for the determination of the drug. The stationary column used was Thermo Scientific ODS Hypersil C_{18} (250 × 4.6 mm, 5 µm) at room temperature and this method was developed using ICH guidelines. The proposed method showed accuracy, linearity, sensitivity, and it was also specific for stress degradation studies i.e., acidic (0.1 N HCl), basic (0.1 N NaOH), oxidative (3% H_2O_2), photolytic (200-800 nm, 3h) and thermal (70 °C, 2 h). The proposed method was successfully developed and validated for the determination of doripenem.

Keywords: Doripenem, Carbapenems, Method development, Plasma, ICH

Introduction
Doripenem is an antibiotic from the carbapenem class that is used to treat infections from the gram-positive and gram-negative types of bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Bacteroides fragilis*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*. The literature survey revealed that doripenem showed higher activity against Pseudomonas than any other drug of carbapenems. Doripenem was used for the treatment of urogenital infections, urinary tract, and complicated intra-abdominal.[1] Doripenem interacts with penicillin-binding protein of bacterial cell wall and breaks it.[2] For Gram-negative bacteria, doripenem functions similarly to meropenem, and for Gram-positive bacteria, it behaves like imipenem.[3]

There are very few reports available on doripenem method development and analysis. Most recently, Marwa et al. [4] published an analytical method that used phosphate buffer(0.05 M pH 3.0) acetonitrile: methanol (86:12:2 % v/v/v) as mobile phase. In the past Salman et al.[5] reported a simple UV technique to measure doripenem in the human plasma but it lacks specificity, Babu et al.[6] reported an RP-HPLC method, Kurien et al.[7] proposed a simple classical HPTLC method, Cielecka-Piontek et al.[8] published spectrophotometric determination of doripenem. Although the reported methods are very simple therefore less specific and sensitive or have other issues like mobile phase stability where we have to maintain certain pH and stability of solutions. In most of the cases limit of detection, limit of quantification was very poor. In contrary the method proposed in this manuscript is very simple, reliable, properly validated. Additionally, the stability was tested on plasma samples and under stressful conditions.

Materials and methods

Chemicals and reagents

Doripenem injection was purchased from Lahore Pharmacy, Hydrogen per oxide (H_2O_2), Hydrochloric Acid (HCl) 0.1 N, Sodium Hydroxide (NaOH) 0.1 N), Acetonitrile (ACN) used was of HPLC grade and distilled water was provided by chemistry department of university.

Preparation of mobile phase and standards

The mobile phase used for HPLC was prepared in several steps. First the water alone was used as a mobile phase but the peak was broad and distorted. In other analysis different percentages of acetonitrile and water was used. However, the shape and area of peak was fine in the composition of mobile phase described in **Table 1**. The mobile phase contains 600ml of water and 400ml of acetonitrile, then filtered with filtration assembly and sonicated.

Sr.No	Developed Conditions	HPLC Method
1	Mobile Phase	(H_2O: ACN) 60:40(%v/v)
2	Stationary Column	Thermo Scientific ODS Hypersil C_{18} (250 × 4.6 mm, 5 µm)
3	Wavelength	298 nm
4	Flow Rate	1 ml/min
5	Run Time	10 min
6	Injection Volume	20 µl
7	Concentration of stock	1 mg/ml
8	Diluent	Identical with mobile phase

Table 1. Chromatographic conditions.

The standard solution was prepared by weighing 100 mg of doripenem on a micro analytical balance and dissolved in 70 ml of water. After sonication of 5 minutes it was diluted up to 100 mL with water in the flask. Then, 25 ml of this aliquot was diluted to 100 ml in another flask to get the final concentration of 0.25 mg/l.

Working solution

First, the working solution 10 bottles were used and each of them contains 5 mg of doripenem in 10 ml of water. After this 5 ml of this solution was diluted with mobile phase in a 20 ml volumetric flask. Finally, 4 ml of above solution was diluted in a volumetric flask of 20ml. After making these concentrations a nylon-membrane syringe filter was used for filtration. The working solution of different concentrations was then analyzed by HPLC.

Chromatographic Conditions

The High-Performance Liquid Chromatography (HPLC) was of SHIMADZU, LC-20AT, SPD-M20A, CBM-20A and oven of CTO-20A. After the preparation of mobile phase using acetonitrile and water (40:60%v/v) it was pumped into the HPLC system. The flow rate was set at 1ml per minute

and run time was 10 minutes. The samples were prepared, and injected with the volume 20 µl. The injected sample then passed through the stationary column with the mobile phase. These eluents then entered the PDA detector which have the wavelength of 298 nm. All the analysis was carried out at room temperature. The comparison of above method with other methods is given in the **Table 2.**

Methods	Chromatographic Conditions	Results
1 HPLC-UV (2021)	(86 : 12 : 2% v/v/v) 0.05 M phosphate buffer: acetonitrile : methanol	Buffer is used to maintain the pH 3.00, Strict Conditions for peak, Other peaks are also shown with Doripenem
2 Ultra-Sensitive method (2019)	0.1 M borate buffer at pH 8.5 at 475nm and 70 ˚C	Lack of HPLC method, Lack of Specificity, Poor LOQ, High Temperature (Oven)
3 HPLC method	Methanol and 10 mM potassium dihydrogen orthophosphate, 60:40 % (v/v)	Buffer is used to maintain the pH, No sharp Peak, Poor LOD
4 HPTLC method (2014)	4:2:2% (v/v/v) butanol: water: glacial acetic acid	TLC method was used and instrumentation was complicated, No Sharp peak, Poor LOQ and LOD
5 UV method (2010)	UV derivative study at 324nm	Lack of HPLC method
6 RP-HPLC/UV Proposed method	(H_2O: ACN) 60:40(%v/v) UV at 298nm	Specific, No complicated instrumentation, Room temperature, Fine peak, Better LOD and LOQ, Stress degradation

Table 2. Comparison of proposed method with other methods

Method Validation

The different parameters were studied for method validation.

System Suitability

Six samples were prepared from standard solution and added to the HPLC system to test the suitability of the method developed. Subsequently, their retention time variability, area repeatability, tailing factor and theoretical plate count were analyzed.

Linearity

A solution of various dilutions was prepared to verify the linearity and a correlation line was plotted between the concentration and the area. A calibration curve was prepared by injecting 6 samples at 0.01 g/ml and 0.06 g/ml and a coefficient, an intercept, slope and a regression equation and slope were obtained.

Precision

If the method demonstrates the closeness of agreement in many samples under the same situation, it is called precise. It consists of two categories.

• ***Intra-day Precision***

In order to conduct the study, the same analyst prepared and injected six replicates at a concentration of 0.04 mg/ml one after the other in the same conditions during a short interval of time. By calculating the percentage RSD, the results were evaluated.

• ***Inter-day precision***

It was carried out by two analysts on different days and in different circumstances. per analyst injected three samples per day, and the percentage RSD was used to assess the outcomes. For inter-day precision, a total of six samples were injected each day.

Accuracy

For accuracy, doripenem recovery experiments were conducted. For accuracy, three distinct samples with concentrations of 80%, 100%, and 120% were examined. The conventional addition method was used to complete it. A 0.001 mg/ml standard solution was made with precision. Following this, 200 µl was diluted in a 10 ml volumetric flask to get different concentrations. Subsequently, 80 µl, 100

µl, and 120 µl of the mobile phase were added and filled to the mark. The HPLC equipment examined each sample three times before calculating the percentage of recovery.

Limit of quantification Limit of detection

Limit of quantification (LOQ) Limit of detection (LOD) were calculated by using the given formula according to the ICH guidelines.

Limit of quantification (LOQ) = 10 σ / S

Whereas, S = Calibration curve slope and σ = y-intercept

Limit of detection (LOD) = 3.3 σ / S

Robustness

The flow rate, temperature and wavelength (±2 nm) was varied to study the robustness of proposed method. Three replicates of samples were introduced into the HPLC system and these were analyzed at three different wavelengths of 296, 298, 300nm. The concentration of samples for suitability and robustness were the same.

Force degradation studies

The ability of the suggested approach to separate and quantify the analyte in the presence of additional mixtures, such as degradants, matrix, and contaminants.[4] Stress degradation studies were used to determine the suggested method's specificity. First, a mobile phase was used to create a standard doripenem solution at a dose of 1 mg/ml. Following that, three separate 10 mL volumetric flasks containing 1 mL of stock each were used to produce the solutions. Two milliliters of 0.1 N HCl and two milliliters of 0.1 N NaOH were then used for the acid and base degradation experiments, respectively. Likewise, two milliliters of 3% H2O2 were moved for investigations on oxidation. Following the solution's preparation, each of these flasks was sonicated for five to ten minutes before oven for an hour at 70 °C. After that, all the solutions were left to stabilize at room temperature for 12 to 15 minutes. All three solutions were neutralized and diluted following stabilization.

Result and Discussion

HPLC method development

The method was developed and chromatogram is shown in **Figure 1** and properties are shown in **Table 3**.

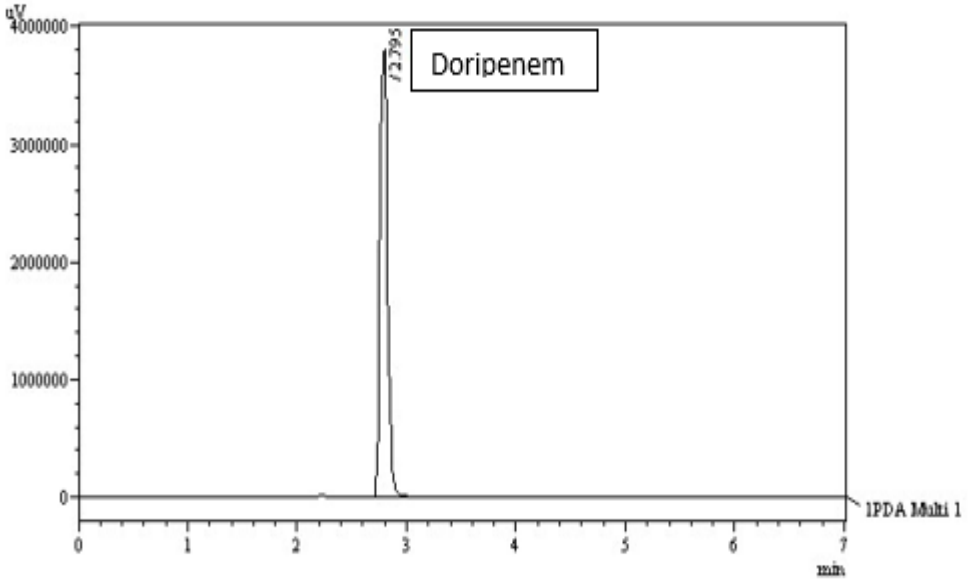

Figure 1. Chromatogram of doripenem at 298nm

Parameters	Results
Retention Time	3.00min
Flow rate	1mL/min
Run Time	10min
Area	993944
Theoretical Plates (N)	30441

Table 3. Properties of developed HPLC method.

Method Validation

The different parameters like linearity, robustness, accuracy, and specificity was validated by ICH guidelines.[9]

System precision or system suitability

Six standard samples were used to determine the precision and according to the guidelines %RSD values should be equal to or less than 2% as represented in **Table 4.**

Parameters	Results n=6
Retention Time	3.0 min
%RSD (Retention Time)	0.25%
Area	993944
Theoretical Plates (N)	30442
%RSD for replicate injections	1.76%

Table 4. Intra-day precision study of doripenem.

Intermediate precision

In this day-to-day and analyst-to-analyst precision was studied and sometimes it referred as ruggedness or Intra-Day precision.

			Inter-Assay		n=3	Inter-Assay (Day to Day) n=9		
	Preparations	**Peak Area**	**Mean**	**SD**	**% RSD**	**Mean**	**SD**	**% RSD**
Analyst 1 Day 1	Preparation 1 Preparation 2 Preparation 3	1263950 1260970 1262980	1262633.3	1241.03	0.098			
Analyst 1 Day 2	Preparation 1 Preparation 2 Preparation 3	1258964 1244972 1239973	1247969.6	8037.5	0.64	122240 6.7	4690 3.7	3.8
Analyst 1 Day 3	Preparation 1 Preparation 2 Preparation 3	1163944 1156952 1148956	1156617.3	6123.3	0.52			
Analyst 2 Day 1	Preparation 1 Preparation 2 Preparation 3	1262951 1258982 1272946	1264959.6	5875.05	0.46			
Analyst 2 Day 2	Preparation 1 Preparation 2 Preparation 3	1264972 1263896 1263928	1264265.3	499.85	0.039	122650 9.8	5388 5.9	4.3
Analyst 2 Day 3	Preparation 1 Preparation 2 Preparation 3	1162988 1138964 1148962	1150304.6	9853.60	0.856			
Analyst-to-Analyst n= 18								
Mean			**SD**			**RSD %**		
1224458.25			2051.5			0.16		

Table 5. Inter-day precision study of doripenem.

Linearity

By creating various working solutions from a standard doripenem solution and analyzing the results in an HPLC system, the linearity was investigated. The least amount of regression analysis was found using a calibration curve. By plotting a graph between area and concentration the regression equation was constructed and shown in **Fig. 2** and data is shown in **Table 6.**

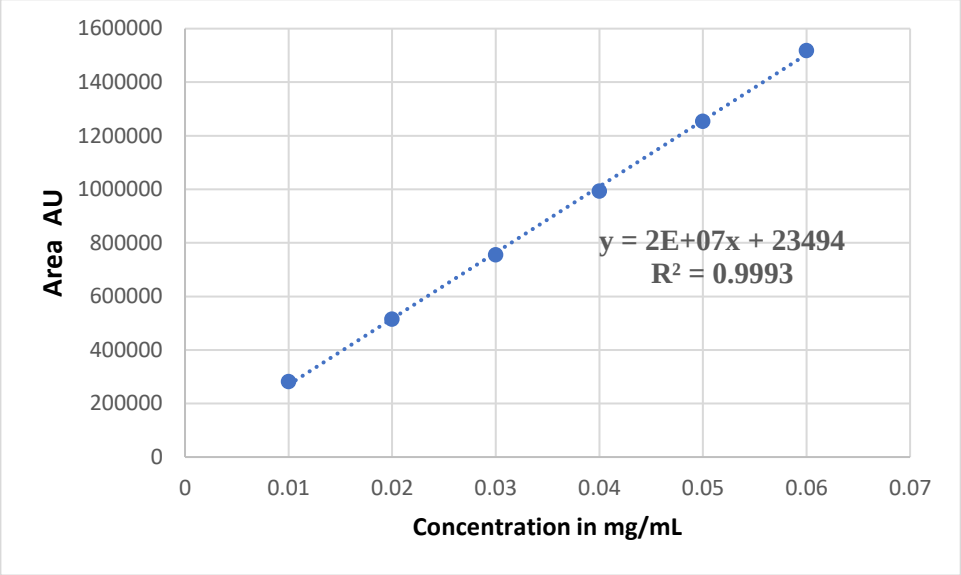

Figure 2. Calibration Curve of doripenem.

Regression equation (y)	y = 2E+07x + 23494
Correlation coefficient (R^2)	0.9993
Slope (m)	24669551
Y-Intercept(c)	23494

Table 6. Linearity study of doripenem.

Limit of quantification and detection limit (LOQ and LOD)

Based on the intercept's slope and standard deviation, LOQ and LOD were computed from the calibration curve. The calculated values for LOQ and LOD using the formula are 0.532 µg/ml and 0.175 µg/ml, respectively, indicating the sensitivity of the developed method.

Accuracy

Recovery studies were used to verify the suggested method's accuracy. Three distinct concentrations of the solution were prepared: 0.02 mg/ml, 0.04 mg/ml, and 0.06 mg/ml. After each of them was injected three times into the HPLC apparatus, the data collected was used to compute the percentage recovery and shown in **Table 7**.

Sr. No	**Concentration**	**Peak Area**	**%Recovery**	**Mean % recovery**
1	0.02mg/mL	806588	100.45	100.2
2		805780	100.3	
3		804568	100	
4	0.04mg/mL	1270158	99.7	99
5		1263944	99.25	
6		1262244	99	
7	0.06mg/mL	1756128	101.3	101
8		1749125	100.8	
9		1745225	100.5	

Table 7. Accuracy study of doripenem

Robustness

By changing wavelength, flow rate and temperature the %RSD was calculated which shows that the developed method is robust[4] shown in **Table 8.**

No. of Replicates	296 nm	298 nm	300 nm
1	1269122	1262958	1242346
2	1270058	1263944	1245158
3	1272186	1264962	1246282
Mean (n=3)	1270455	1263954	1244595
SD (n=3)	1282.03	818	1655
% RSD (n=3)	0.100	0.06	0.13
Mean (n=9)	1259668		
SD (n=9)	10983.6		
%RSD (n=9)	0.87		

Table 8. Robustness of the method.

Stress degradation

With the exception of sunlight, adding stress causes the doripenem deteriorate significantly. All of these findings demonstrated that the doripenem is stable in the presence of sunlight and does not require any particular storage conditions. The doripenem did not interfere with the peaks that were acquired through the application of stress degradations, thus demonstrating the stability indication of the approach. Results are shown in **Table 9** and chromatograms for HCl, NaOH, H_2O_2 and sunlight are given in **Fig. 3-6** respectively.

Stress condition	Peak Area	% Recovered	%Degradation
Acidic	311546	17.79	82.21
Basic	389347	22.4	77.6
Oxidative	668422	38.7	61.3
UV	1682446	96.4	3.6
Sunlight	1692428	96.1	3.1
Dry heat	1228224	70.3	29.7

Table 9. Results of Stress degradation studies of doripenem.

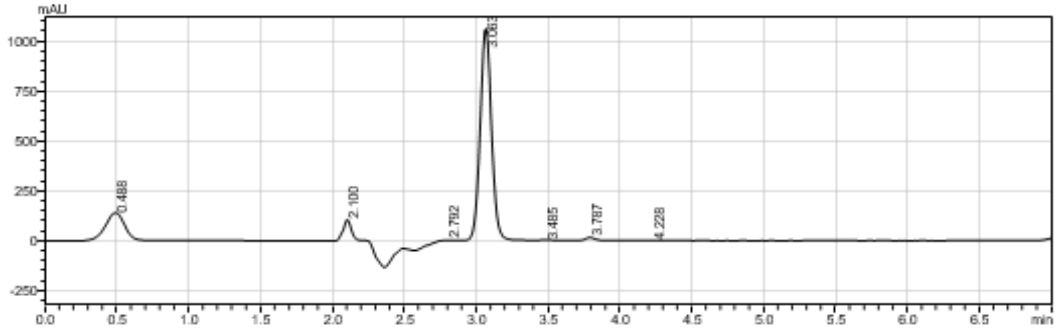

Figure 3. HPLC chromatogram for degradation in HCl.

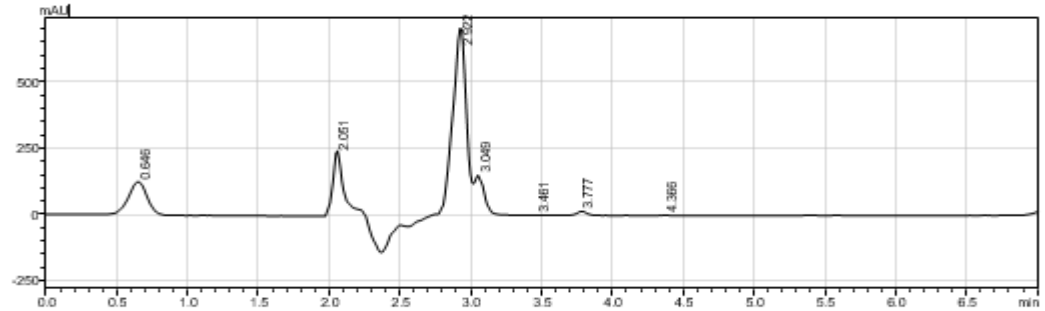

Figure 4. HPLC chromatogram for degradation in NaOH.

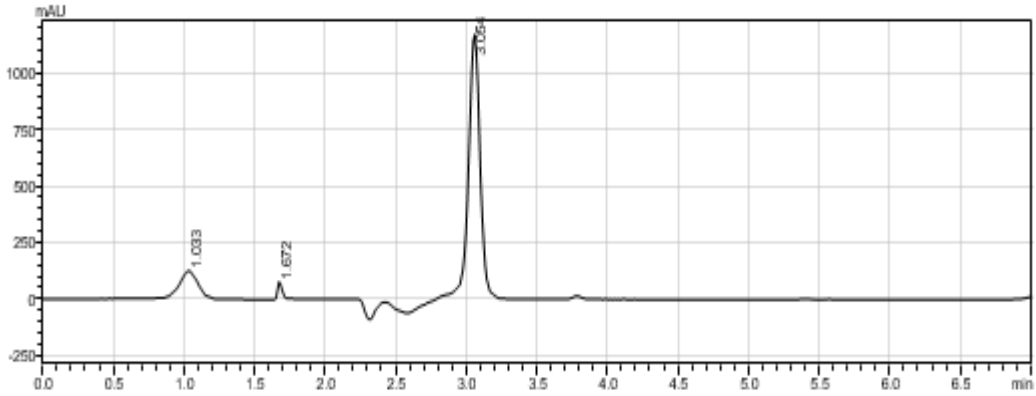

Figure 5. HPLC chromatogram for degradation in H_2O_2.

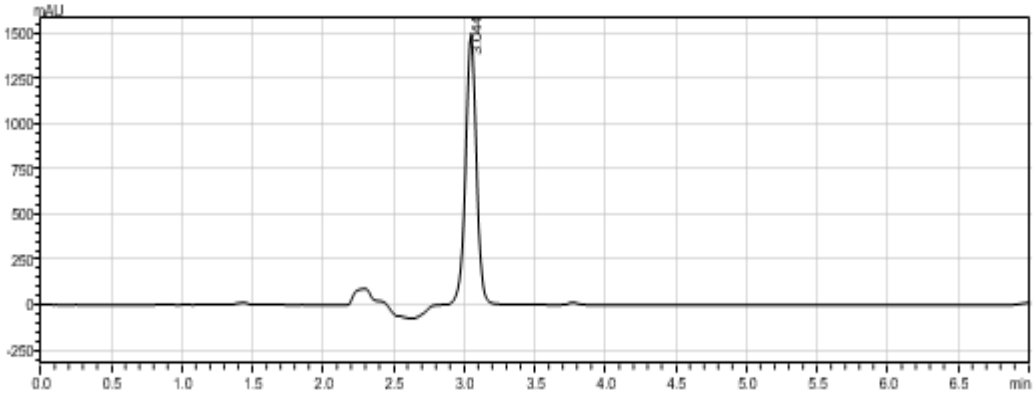

Figure 6. HPLC chromatogram in sunlight degradation.

Applications of the HPLC method

The amount of doripenem in pharmaceutical dosage forms was determined using the established HPLC method. %RSD < 2% was found when the findings were calculated. Therefore, this method is accurate and appropriate for determination of doripenem in dose forms.

Analysis of plasma samples

The developed method was utilized for the investigation of plasma which was isolated from human blood and spiked with doripenem. The RSD < 2% appeared that this method is precise and reasonable for the evaluation of doripenem in plasma. The results are following in **Table 10.**

Sample	Strength	Found	RSD %	Recovery %
Drug	2 mg	2.01 mg	1.02	100.5
Plasma	2 mg	1.97 mg	1.32	98.5

Table 10. Applications of the method for pharmaceutical dosage and plasma.

Conclusion

The planned study was carried out for the evaluation of doripenem and by using HPLC the method was developed and validated. This method was simple, rapid, precise, accurate, and robust and there is no need of any special conditions. All other methods described in literature need particular conditions and derivatization agents. The method was applied to analysis the ingredients of doripenem and the results were in the acceptable range. In the newly developed method, force degradation studies were applied to extract the ingredients of the drug. All the results revealed that this method is appropriate and authentic for the analysis of doripenem. Overall, it can be concluded that the HPLC method was successfully developed and validated and it may be applied to routine quality assessment.

Disclosure statement

The authors affirm that they have no conflicts of interest related to the work reported in the manuscript.

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