WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Stability Indicating Method Development and Validation for Estimation of Doripenem API and in Solid Dosage Form by RP-HPLC

Davuluri Prasanth*, Karavadi Thejomoorthy and Ch. Saibabu

Department of Pharmaceutical Analysis, Malineni Lakshmaiah College of Pharmacy, Kanumalla, Singarayakonda-523101

ABSTRACT

The present study describes a Stability Indicating Reversed Phase High Performance Liquid Chromatographic (RP-HPLC) method development and validation of Doripenem API. Inertsil ODS 3V column was used as stationary phase while mobile phase is Phosphate buffer pH 3.0 and Methanol in the ratio of 86:14 is used. Method was developed in isocratic mode with 15 minutes run time, at flow rate of 1.0 mL/min. Eluent was monitored at 300 nm. The method was validated for specificity, linearity, accuracy, precision, robustness and solution stability. Recovery was found to be in the range of 70-130%. The proposed method was successfully applied for the quantitative determination of Doripenem API and in formulations.

Keywords: Doripenem, Stability-indicating, method development, validation.

Introduction

Doripenem belongs to the carbapenem class of antimicrobials. 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. The serum concentration of Doripenem was increased by Probenecid. Co-administaration of Doripenem with Probenecid not recommended. Headache, nausea, diarrhea (may be serious; evaluate if occurs), rash, phlebitis, anemia, elevated hepatic enzymes; hypersensitivity reactions, *C.difficile*-associated diarrhea, seizures, pneumonitis with inhalation use. Chemically it is (4*R*,5*S*,6*S*)-6-(1-hydroxyethyl)-4-methyl-7-oxo-3-[(3*S*,5*S*)-5-(sulfamoylamino)methyl]pyrrolidin-3-yl]sulfanyl-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid. It is moderately soluble in water, slightly soluble in methanol, and virtually insoluble in ethanol. Chemical structure of doripenem was given in figure 1.

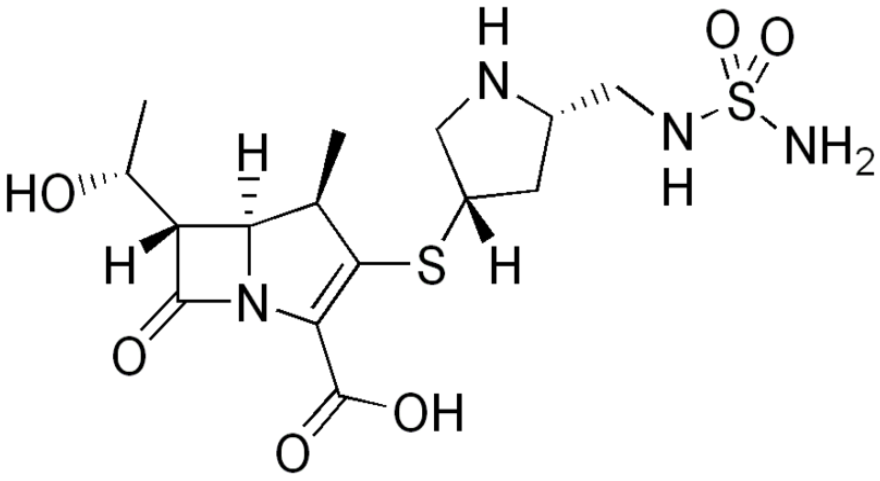

Fig 1: Structure of Doripenem

On literature survey it was found that Doripenem is estimated by HPLC, Spectrophotometric (UV and Colorimetry) LC-MS/MS, IR methods in human blood plasma. However no method was available for such estimation in the pharmacopoeia. In view of the need for a suitable method for routine analysis of Doripenem attempts are being made to develop simple, precise and accurate analytical methods for estimation of Doripenem and extend it for their determination in formulations.

The utility of the developed method to determine the content of all the drugs in commercial tablet is also demonstrated. Validation of the method was done in accordance with USP and ICH guidelines for the assay of active ingredients. The methods were validated for parameters like accuracy, linearity,

precision, specificity, ruggedness, robustness, and system suitability. This proposed method is suitable for the pharmaceutical analysis in analytical laboratories. The Chromatographic methods proposed in this paper presume that there is a linear relationship between absorbance and component concentration. This method have a calibration step followed by the prediction step, in which the results of the calibration step are used to estimate the component concentration from an unknown sample spectrum.

Materials and methods

Equipment

The Method development and Validation was carried out using Waters Alliance-HPLC system equipped with waters 1525 binary HPLC pump, 2695-separation module connected to 2996-photo diode array detector, and Waters 2707 auto sampler. The data was acquired by Empower® version 2. The other equipment used were Ascoset Electronic balance, ADWA pH meter, heating mantle. Ultrasonic bath was used for sonication of the samples. Hot air oven was used to carry out thermal degradation studies. UV cross linker, with series of 23400 model UV chamber, equipped with a UV fluorescence lamp with the wavelength range between 200 & 300 nm was used for photo degradation studies.

Chemicals and Reagents

Doripenem working standard was kindly given as gift sample by Mylan labs Pvt. Ltd, Hyderabad. HPLC grade solvents include acetonitrile, water and methanol. Analytical grade chemicals include sodium hydroxide, hydrochloric acid, 20% hydrogen peroxide, Ortho phosphoric acid, Triethyl amine and potassium dihydrogen phosphate were purchased from E. Merck Limited, Mumbai, India.

Chromatographic conditions

HPLC analysis was carried out on Waters Alliance-HPLC system equipped with 2695-separation module connected to 2996-photo diode array detector and the data was acquired by Empower® version 2. Separation was achieved using Inertsil ODS 3V (150*4.6) mm, 5µ as a column with mobile phase of pH 3.0 buffer and methanol in the ratio of 86;14. The samples were analyzed using 20 µL injection volume, Flow rate was maintained at 1.0mL/min with runtime of 15 min and the temperature was maintained at 30°C throughout the analysis. Detection and purity establishment of the drugs were achieved using PDA detector at 300 nm wavelength.

Preparation of Standard Solution:

Accurately weighed and transferred about 50 mg of Doripenem monohydrate working standard into a 50 mL volumetric flask and diluted to volume with diluent. 5 mL of the above solution was diluted to 50 mL with diluent. (Concentration of Doripenem 100 µg/ml)

Preparation of Sample Solution:

Weighed powder equivalent to 50 mg doripenem monohydrate and transferred into a 50 ml volumetric flask and dilutedt to 50 ml with diluent. 5 ml of above solution was diluted to 50 ml with diluent. (Concentration of Doripenem 100 µg/ml)

Method Validation

The developed and optimized RP-HPLC method was validated according to international conference on harmonization (ICH) guidelines Q2(R1) in order to determine the system suitability, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, ruggedness and robustness.

System suitability

System suitability parameters were evaluated to verify system performance. 20 µL of standard solution was injected five times into the chromatograph, and the chromatograms were recorded. Parameters such as number of theoretical plates and peak tailing were determined.

Specificity

The specificity of the analytical method was established by injecting the solutions of diluent (blank), placebo, working standards and sample solution individually to investigate interference from the representative peaks.

Precision

Repeatability/ method precision was performed by injecting six replicates of same concentrations of fingolimod, calculated % assay and %RSD. Reproducibility/ Ruggedness/ Intermediate precision was performed using different analysts and a different instrument in the same laboratory.

Accuracy

Accuracy of the proposed method was determined using recovery studies by spiking method. The recovery studies were carried out by adding known amounts (80%, 100% and 120%) of the working standard solutions of fingolimod to the pre-analysed sample. The solutions were prepared in triplicates to determine the accuracy.

Linearity

Linearity was evaluated by analyzing different concentrations of the standard solutions of fingolimod. Six working standard solutions ranging between 70µg/mL-130µg/mL were prepared and injected. The response was a linear function of concentration over peak area and were subjected to linear least-squares regression analysis to calculate the calibration equation and correlation coefficient.

Limit of detection and Limit of quantification: Limit of detection (LoD) and limit of quantification (LoQ) of fingolimod were determined by calibration curve method. Solutions of fingolimod were prepared in linearity range and injected (n = 3).

Robustness

To examine the robustness of the developed method, experimental conditions were deliberately changed, resolution, tailing factor, and theoretical plates of fingolimod peaks were evaluated. To study the outcome of the flow rate on the developed method, it was changed ± 0.2mL/minute. The effect of column temperature on the developed method was studied at ± 5°C, organic phase composition in mobile phase was changed ±10% and pH of the buffer is changed ±0.2. In all the above varied conditions, the composition of aqueous component of the mobile phase was held constant.

6.3.2.2 Degradation Studies:

A study was conducted to demonstrate the effective separation of Degradants from Doripenem sterile Drug substance. Samples were exposed to following stress conditions to induce degradation.

a) Acid Stress: Stressed with 1 mL of 1N HCl solution - Initial.

b) Alkali Stress: Stressed with 1 mL of 0.1N NaOH solution - Initial.

c) Peroxide stress: Stressed with 1 mL of 3% w/v H2O2 solution - Initial.

d) Water Stress: Stressed with 10 ml of water by heating in water bath at 80°C for 5 minutes.

e) Photolytic Stress:

a) Exposed to UV light for 309 hours (~315.18 watt hours/meter^2)

b) Exposed to Visible light for 309 hours (~2.07 million lux hours)

f) Heat stress: Exposed to heat at 85° C for 309 hours 30 minutes.

g) Humidity Stress: Exposed to humidity at 25°C / 97% RH for 310 hours.

Bench Top Stability of Standard and Test Solutions:

A study to establish the stability of Doripenem in standard and test solutions on bench top was conducted about 28 hours and 27 hours respectively. The assay of Doripenem in standard solution was estimated at initial and 28 hours against freshly prepared standard each time. The assay of Doripenem in test solution was estimated at initial and 27 hours against freshly prepared standard each time. The % difference in assay of Standard Solution from initial to 28 hours was calculated and found that standard solution is unstable on Bench top for 28 hours. The % difference in assay of test Solution from initial to 27 hours was calculated and found that test solution is unstable on Bench top for 27 hours. So that the standard solution and test solution prepared as per the test method and analyzed on hourly basis at Room temperature.

Refrigerator Stability of Standard and Test Solution:

A study to establish the stability of Doripenem in standard and test solutions on refrigerator condition was conducted about 28 hours and 27 hours respectively. The assay of Doripenem in standard solution was estimated at initial and 28 hours against freshly prepared standard each time. The assay of Doripenem in test solution was estimated at initial and 27 hours against freshly prepared standard each time. The % difference in assay of Standard Solution from initial to 28 hours was calculated and found that standard solution is unstable on Bench top for 28 hours. The % difference in assay of test Solution from initial to 27 hours was calculated and found that test solution is unstable on Bench top for 27 hours. So that the standard solution and test solution prepared as per the test method and analyzed on hourly basis at 5°C.

Results and Discussion

System Suitability

Separately inject 20 µL of the blank (diluent) and standard solution into the chromatograph using the given chromatographic parameters. Record the chromatograms and measure the peak responses. The system is suitable for analysis, if and only if, The USP tailing factor for Doripenem peak is not more than 1.5. The USP Theoretical Plates for Doripenem peak is not less than 2500. The % Relative Standard Deviation of the area counts of Doripenem Peak obtained from five replicate injections of standard solution is not more than 2.0.

Table No. 1: System suitability results for Doripenem

System Suitability Parameters	Observations	Acceptance Criteria
The **USP Tailing factor** for Doripenem peak in standard solution	1.1	**Not more than 1.5**
The **USP Theoretical Plates** for Doripenem peak in standard solution	5031	**Not less than 2500**
The **% RSD** of area counts of Doripenem peak from five replicate injections of Standard solution	0.45	**Not more than 2.0**

Specificity:

The specificity of the method was evaluated by injecting blank, Standard Solution and the sample solution prepared as per the proposed method to check for interference, if any, at the retention time of Doripenem peak from any peak due to blank. It was found that there was no interference of blank at the Doripenem peak RT

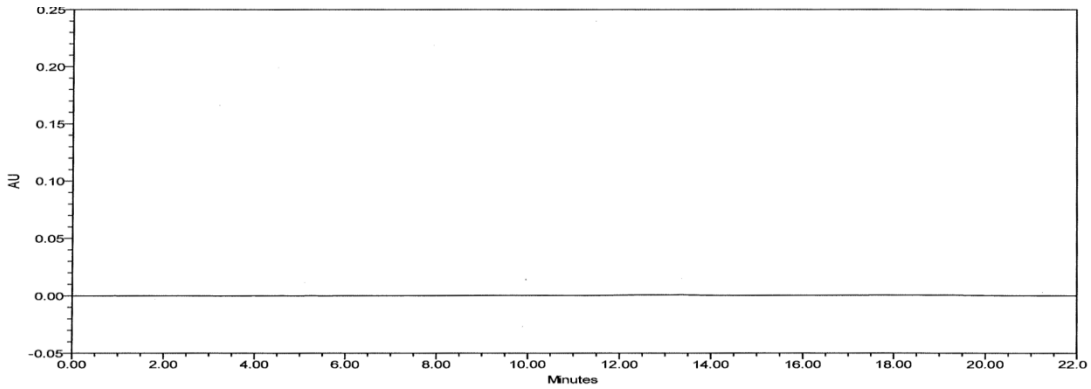

Fig. No. 2: Chromatogram of Blank (Diluent)

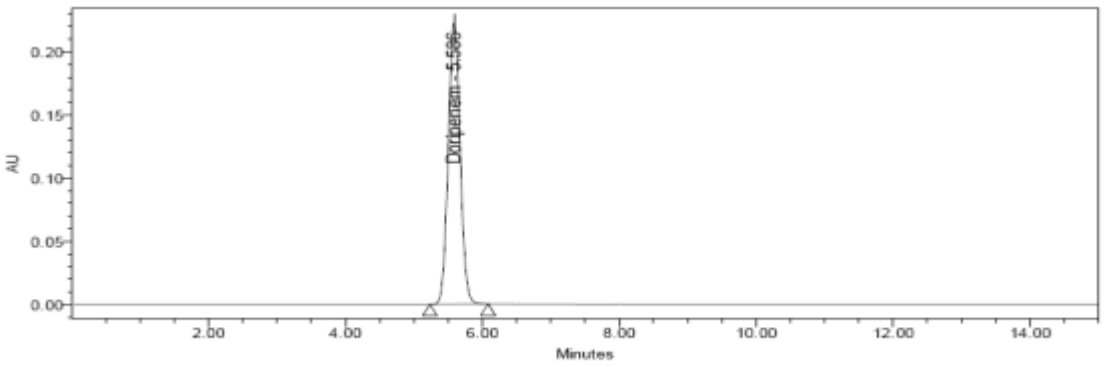

Fig. No. 3: Chromatogram of standard

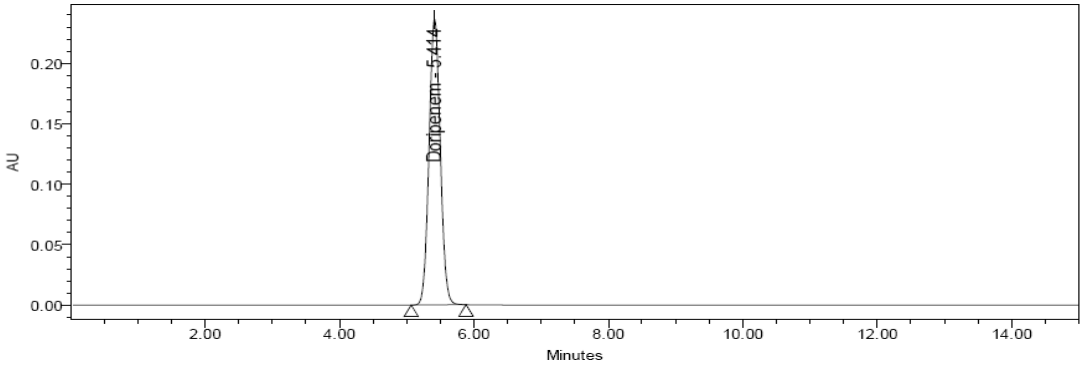

Fig. No. 4: Chromatogram of test sample

System precision

Standard solution was prepared as per the proposed method for system precision studies. Ten replicate injections were injected into the HPLC system. The % RSD for the peak responses of ten replicate injections should be NMT 1.0. It was observed from the data tabulated above that the system suitability parameters meet the predetermined acceptance criteria as per the test method and indicates the suitability of the selected system.

Table No: 2 system precision results for Doripenem

S.No	**Doripenem peak area (µV*Sec)**
1	2747175
2	2732272
3	2723509
4	2717501
5	2709318
6	2705644
7	2692852
8	2688842
9	2683385
10	2686199
Average	**2708670**
%RSD	**0.79**

Accuracy

The results were found within acceptance criteria. Hence the method is accurate throughout the selected range.

Table No. 3: Peak Results for Accuracy of Doripenem

Recovery level	**µg/mLAdded**	**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	

Method precision (Repeatability)

The precision of test method was evaluated by preparing six sample preparations of single batch of Doripenem Sterile Drug substance and analyzed as per the test method. The assay and the % relative standard deviation of assays were calculated and found to be within acceptance criteria.

Table No.4: Results for method precision

S. No	**Sample Weight (in mg)**	**Area of Injection 1**	**Area of Injection 2**	**Average Area**	**Assay**
1	50.12	2814717	2814756	2814737	100.41
2	50.18	2814524	2814539	2814532	100.11
3	50.09	2814602	2814619	2814611	100.30
4	50.16	2814582	2814576	2814579	100.25

5	50.21	2814692	2814705	2814699	100.38
6	50.05	2814535	2814567	2814551	100.13
Average					100.26
%RSD					0.12

Robustness

The % difference in Average assay value should not differ by more than 2.0 when compared with that of test method values. All system suitability parameters shall meet the requirements as per the test method. All individual assay values should meet the specification. (% w/w, on anhydrous basis). From the above study, it was established that the method is found to be sensitive with respect to shifting of retention time of Doripenem peak, when organic composition of mobile phase is varied to 98% as well as 102% of the test method. From the above study, it was established that the allowable variation in flow rate is from 0.8 mL/min to 1.2 ml/min of the test method. From the above study, it was established that the allowable variation in column oven temperature is from 27°C to 32°C.

Table No. 5: Robustness data

Variation in organic phase composition in mobile phase				
System Suitability Parameters	**Organic Composition of Mobile phase**			**Acceptance Criteria**
	100%*	**98%**	**102%**	
The **USP Tailing factor** for Doripenem peak in standard solution	1.1	1.1	1.1	Not more than 1.5
The **USP Theoretical Plates** for Doripenem peak in standard solution	4905	6231	4285	Not less than 2500
The **% RSD** of area counts of Doripenem peak from five replicate injections of Standard solution	0.40	0.96	0.75	Not more than 2.0
Variation in flow rate				
System Suitability Parameters	**Flow rate (ml/min.)**			**Acceptance Criteria**
	1.0*	**0.8**	**1.2**	
The **USP Tailing factor** for Doripenem peak in standard solution	1.1	1.1	1.1	Not more than 1.5
The **USP Theoretical Plates** for Doripenem peak in standard solution	4978	5887	4349	Not less than 2500
The **% RSD** of area counts of Doripenem peak from five replicate injections of Standard solution	0.25	0.29	0.29	Not more than 2.0
Variation in column temperature				
System Suitability Parameters	**Column Oven Temperature (°C)**			**Acceptance Criteria**
	30	27	32	
The **USP Tailing factor** for Doripenem peak in standard solution	1.1	1.0	1.0	Not more than 1.5
The **USP Theoretical Plates** for Doripenem peak in standard solution	4978	5107	5465	Not less than 2500
The **% RSD** of area counts of Doripenem peak from five replicate injections of Standard solution	0.25	0.35	0.90	Not more than 2.0

Ruggedness

All individual assay values should meet the specification. (% w/w, on anhydrous basis). The % relative standard deviation of Assay results should be not more than 2.0 by both the sets. Over all %Relative Standard Deviation should be not more than 2.0. Comparison of the results obtained from two sets shows that the assay method is rugged.

Table No. 6: Ruggedness – Intermediate Precision

System Suitability Parameters	Observations		Acceptance Criteria
	Set-1	Set-2	
The **USP Tailing factor** for Doripenem peak in standard solution	1.1	1.1	**Not more than 1.5**
The **USP Theoretical Plates** for Doripenem peak in standard solution	5031	4842	**Not less than 2500**
The **% RSD** of area counts of Doripenem peak from five replicate injections of Standard solution	0.45	0.30	**Not more than 2.0**

Table No. 7: Results of intermediate precision

S. No.	Assay (%w/w, on anhydrous basis)	
	Set-1	**Set-2**
1	100.41	100.38
2	100.11	100.22
3	100.30	100.26
4	100.25	100.37
5	100.38	100.42
6	100.13	100.48
Average	**100.26**	**100.36**
%RSD	**0.12**	**0.10**
Over all %RSD	**0.12**	
F Test Value	**3.00**	

Bench top stability:

The % difference in assay of Doripenem from initial value should be not more than 2.0. All system suitability parameters shall meet the requirements as per the test method. From the above study, it was established that the mobile phase for Assay of Doripenem Sterile Drug substance is stable for 48 hours on bench top. The Cumulative %RSD of Doripenem peak area from initial value should be not more than 1.0. All system suitability parameters shall meet the requirements as per the test method. From the above study, it was established that the standard solution and test solution is unstable on bench top.

Table No. 8: Ruggedness – Bench top Stability of Standard and Test Solutions

System Suitability Parameters	Observations	Acceptance Criteria
The **USP Tailing factor** for Doripenem peak in standard solution	1.1	**Not more than 1.5**
The **USP Theoretical Plates** for Doripenem peak in standard solution	5017	**Not less than 2500**
The **% RSD** of area counts of Doripenem peak from five replicate injections of Standard solution	0.24	**Not more than 2.0**

Table No. 9: Results for Bench top Stability of standard and test solution

Time in Hours	Area (µV*Sec) of Standard Solution	Cumulative %RSD	Area (µV*Sec) of Test Solution	Cumulative %RSD
Initial	2643653	NA	2645081	NA
1	2597517	1.24	2576800	1.85
2	2525004	2.31	2498115	2.86
3	2434453	3.58	2411950	3.97
4	2348450	4.78	2328530	5.07

Refrigerator Stability

The **Cumulative %RSD** of Doripenem peak area from initial value should be **not more than 1.0.** All system suitability parameters shall meet the requirements as per the test method**.** From the above study, it was established that the standard solution and test solution is stable in 5°C for 6 hours and 5 hours respectively.

Table No. 10: Refrigerator Stability of Standard and Test Solutions

System Suitability Parameters	Observations	Acceptance Criteria
The **USP Tailing factor** for Doripenem peak in standard solution	1.1	**Not more than 1.5**
The **USP Theoretical Plates** for Doripenem peak in standard solution	5017	**Not less than 2500**
The **% RSD** of area counts of Doripenem peak from five replicate injections of Standard solution	0.24	**Not more than 2.0**

Table No. 11: Results for Refrigerator Stability of standard and test solution

Time in Hours	Area (µV*Sec) of Standard Solution	Cumulative %RSD	Area (µV*Sec) of Test Solution	Cumulative %RSD
Initial	2643302	NA	2664828	NA
1	2650080	0.18	2644023	0.55
2	2623161	0.53	2629381	0.67
3	2614403	0.64	2624250	0.69
4	2608787	0.69	2612716	0.76
5	2592777	0.82	2595823	0.92
6	2581252	0.96	2586916	1.03
7	2573971	1.06	2574883	1.15
8	2561775	1.17	2563180	1.28
9	2548402	1.31	2549289	1.42
10	2536705	1.44	2538059	1.55

Conclusion

The objective of the proposed work was method development for the estimation of Doripenem in injectables by RPr-HPLC and to validate the developed method according to USP and ICH guidelines and applying the same for use in the quality control samples in pharmaceutical industry. As there is no official method for the estimation of Doripenem in formulation, so we tried to develop a method by which we can quantify the amount of drug present in the given sample. In RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate titled ingredients. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time. The system with Potassium Dihydrogen Phosphate with pH 3.0 buffer and methanol at Isocratic flow rate of 1.0 ml min^{-1} was found to be quite robust. The effect of degradation products on the main peak of Doripenem was determined by treating samples with different stress conditions like acid stress, alkali stress, peroxide stress, thermal stress and photolytic stress. The peak purity was found to be well below the purity threshold. Finally, it can be concluded that the assay values of formulation were the same as mentioned in the label claim with the RSD of < 1.0%. The proposed method was found to be accurate, precise, reproducible and stable, and can be successfully applied for the routine analysis of the drug in dosage forms.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Funding

According to authors, the research described in this paper did not receive any financial support.

References:

1] Willard HH, Merritt LL, Dean JA and Settle FA (2001). Instrumental Methods of Analysis. 7th ed. Delhi: CBS Publishers and Distributors. p3.

2] Skoog DA, West DM and Holler FJ (1996). Fundamentals of Analytical Chemistry. 7th ed. Philadephia: Saunders College Publishing. p. 1-3.

3] Sharma BK (2002). Instrumental Methods of Chemical Analysis. 21st ed. Meerut: Goel Publishing House, p. 3-5.

4] Skoog DA, Holler FJ, Timothy A and Nieman NW (2004). Principle of Instrumental Analysis. 5th ed. Bangalore: Eastern Press. p. 1-2, 678-688, 695-696.

5] Scott RPW (2003). Technique and Practice of chromatography. Marcel Dekker: Vol. 70. New York: p. 1-12.

6] Jeffery GH, Basset J, Mendham J and Denney RC (1996). Vogel's textbook of Quantitative Chemical analysis. 5th ed. England: Longman Publication. p. 647-649.

7] Connors KA (1999). A textbook of Pharmaceutical Analysis**.** 8th ed. New York: Wiley-Interscience. p. 408-421.

8] Hamilton RJ and Sewell PA (1982). Introduction to HPLC. 2nd ed. London: Chapman and Hall. p. 189.

9] Snyder LR, Kirkland JJ and Glajch JL (1997). Practical HPLC Method Development. 2nd ed. New York: Wiley. p. 1-20.

10] Sethi PD (2001). 'High Performance Liquid Chromatography', Quantitative Analysis of Pharmaceutical Formulations, 1st ed. New Delhi: CBS Publishers and Distributors. p. 3-11, 116-120.

11] Munson JW. (2001). Pharmaceutical Analysis: Modern Methods (Part B). New York: Marcel Dekker. p. 51-54,120,175.

12] Scott RPW. (1993). Liquid Chromatography for the Analyst. New York: Marcel Dekker. Vol. 67. p. 15-23, 265-272.

13] Chatwal GR and Anand SK (2004). Instrumental Methods of Chemical Analysis. 5th ed. Delhi: Himalaya Publishing House. p. 2.599-2.605.

14] Ewing's. (2005). Analytical Instrumentation Handbook, 3rd ed. New York. Cazes J, Marcel Dekker. p. 995-998.

15] Sharma BK (2003). Instrumental Methods of Chemical Analysis. 25th ed. Meerut: Goel Publishing House. p. 39-42, 96-104.

16] Parimoo P (1998). Pharmaceutical Analysis.1st ed. New Delhi: CBS Publication and Distributors. p. 151-152.

17] Schrimer RE (1991). Modern Method Pharmaceutical Analysis. 2nd ed. CRC Press. Vol -1. p. 75-76.

18] Beckett AH and Stenlake JB (2004). Practical Pharmaceutical Chemistry. Part 2. New Delhi: CBS Publishers and Distributors. p. 282-283

19] ICH Harmonised Tripartite Guideline (Nov 2005) Validation of Analytical Procedures: Text and Methodology Q2 (R1).

20] ICH, (October 1993). Q1A Stability testing of New Drug Substances and Products in Proceedings of the International Conference on Harmonization, Geneva.

21] The United States Pharmacopoeia, The Official Compendia of Standards, (2006), 29th ed., Rockville, MD, USP convention Inc.

22] Cielecka-Piontek J, Jelińska A. "The UV-derivative spectrophotometry for the determination of doripenem in the presence of its degradation products." Spectrochim Acta A Mol Biomol Spectrosc. 2010 Oct 1;77(2):554-7

23] V.Niraimathi, K.Sabarigirivasan "Estimation Of Doripenem In Pharmaceutical Formulation By Using Difference Spectroscopy" scientificipca.org/paper/2012/09/26/201209261221470A

24] V.Niraimathi*, A.Jerad Suresh, S.Jayapraba "Forced Hydrolytic Degradation Study of Doripenem BY UV Spectrophotometric method "Int. J. pharm. Sci. Rev. Res., 20(2), May – Jun 2013; n 26, 155-157

25] Venugopal V, Ramu G, Lakshmanarao P V and Rambabu C*
"Extractive spectrophotometric determination of doripenem using bromo cresol purple and methylene violet as color developing reagents" Der Pharma Chemica, 2013, 5(3):185-189.

26] K.Sabarigirivasan, V. Niraimathi*, A.Jerad Suresh " IR quantification of Doripenem in Pharmaceutical dosage forms" IJPT/April-2013/Vol.5/issue No.1/5199-5203.

27] Dhruv kumar Singh, Gunjan Maheshwari " Development and validation of spectrophotometric methods for carbapenems in pharmaceutical dosage forms" Medicinal Chemistry Research December 2013, Volume 22, Issue 12 pp 5680-5684.

28] Ohmori T, Suzuki A, Niwa T, Ushikoshi H, Shirai K, Yoshida S, Ogura S, Itoh Y. "Simultaneous determination of eight β-lactam antibiotics in human serum by liquid chromatography-tandem mass spectroscopy" J Chromatogr B Analyt Technol Biomed Life Sci. 2011 May 1;879(15-16)

29] SingaramKathirvel and Garikapati Devalarao "A validated new gradient stability LC method for the analysis of Doripenem Bulk and injection formulation" Chromatography Research International Volume 2013, Article ID 963595, 9 pages

30] Elfrides E. S. Schapoval "Characterization of the antibiotic Doripenem using physic chemical methods – Chromatography, Spectrophotometry, Spectroscopy and Thermal Analysis " Pub Med ID:17392039.

31] Sutherland C, Nicolau DP " Development of an HPLC method for the determination of doripenem in human and mouse serum" J Chromatogr B Analyt Technol Biomed Life Sci. 2007 Jun 15;853(1-2):123-6.

32] Kayo Ikeda, Kazuro Ikawa, Norifumi Morikawa, Keiko Kameda, Nami Urakawa, Hiroki Ohge, Taijiro Sueda " Quantification of doripenem in human plasma and peritoneal fluid by high performance liquid chromatography with ultraviolet detection" J Chromatogr BAnalyt Technol Biomed Life Sci. 2008 May 1;867 (1):20-5 18356120 Cit:4

33] E Dailly, R Bouquié, G Deslandes, P Jolliet, R Le Floch "A liquid chromatography assay for a quantification of doripenem, ertapenem, imipenem, meropenem concentrations in human plasma: application to a clinical pharmacokinetic study" J Chromatogr BAnalyt Technol Biomed Life Sci. 2011 May 1;879(15,16):1137 – 42 21474395