CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.7548273

Available online at: http://www.iajps.com *Research Article*

METHOD DEVELOPMENT AND VALIDATION FOR MEROPENEMAND VABORBACTAM BY RP-HPLC METHOD

D.Vaishnavi, Dr V.Kiran Kumar

Mother Teresa College of Pharmacy, Ghatkesar, NFC Nagar, Hyderabad, Telangana, India

Abstract:

A simple, Accurate, precise method was developed for the simultaneous estimation of the Meropenem and Vaborbactam in Tablet dosage form. Chromatogram was run through Inertsil ODS C185µm (4.6 x 250mm). Mobile phase containing Phosphate buffer and Acetonitrile in the ratio of 30:70 was pumped through column at a flow rate of 1ml/min. Buffer used at pH 4.6. Temperature was maintained at Ambient. Optimized wavelength for Vaborbactam and Vaborbactam was 260nm. Retention time of Meropenem and Vaborbactam were found to be 2.395min and 3.906min. The % purity of Meropenem and Vaborbactam was found to be 100.6% and 101.3% respectively. The system suitability parameters for Meropenem and Vaborbactam such as theoretical plates and tailing factor were found to be 1.3, 1012.4and 1.2, 1848.2 the resolution was found to be 9.0.The linearity study for Meropenem and Vaborbactam was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999 and 0.999, % mean recovery was found to be 100.1% and 100.4%, %RSD for repeatability was0.31 and 0.38, % RSD for intermediate precision was 0.12 and 0.15 respectively. The precision study was precise, robust and repeatable. LOD value was 2.94 and 3.03, and LOQ value was 9.87 and 10.1 respectively.

Keywords: *Meropenem, Vaborbactam, RP-HPLC*

Corresponding author:

D.Vaishnavi,

M. Pharmacy,

Mother Teresa College of Pharmacy, Department of pharmaceutical Analysis, Ghatkesar, NFC Nagar, Hyderabad, Telangana, India

E-mail: Vaishdasari22@gmail.com

QR code

Please cite this article in press D.Vaishnavi et al, ***Method Development And Validation For Meropenemand Vaborbactam By RP-HPLC Method..,*** *Indo Am. J. P. Sci, 2022; 09(12).*

INTRODUCTION:

Meropenem is for use as single agent therapy for the treatment of the following infections when caused by susceptible isolates of the designated microorganisms: complicated skin and skin structure infections due to Staphylococcus aureus (b-lactamase and non-b-lactamase producing, methicillin-susceptible isolates only), Streptococcus pyogenes, Streptococcus agalactiae, viridans group streptococci, Enterococcus faecalis (excluding vancomycin-resistant isolates), Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Bacteroides fragilis and Peptostreptococcus species; complicated appendicitis and peritonitis caused by viridans group streptococci, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacteroides fragilis, B. thetaiotaomicron, and Peptostreptococcus species. Also for use in the treatment of bacterial meningitis caused by Streptococcus pneumoniae, Haemophilus influenzae (b-lactamase and non-b-lactamase-producing isolates), and Neisseria meningitidis. The bactericidal activity of Meropenem results from the inhibition of cell wall synthesis. Vaborbactam readily penetrates the cell wall of most Gram-positive and Gram-negative bacteria to reach penicillin-binding- protein (PBP) targets. Its strongest affinities are toward PBPs 2, 3 and 4 of Escherichia coli and Pseudomonas aeruginosa; and PBPs 1, 2 and 4 of Staphylococcus aureus. IUPAC name (4R,5S,6S)-3-{[(3S,5S)-5-(dimethylcarbamoyl) pyrrolidin-3-yl] sulfanyl}-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo [3.2.0] hept-2-ene-2-carboxylic acid. Molecular formula is $C_{17}H_{25}N_3O_5S$. Molecular Weight is 383.4.

Vaborbactam is indicated in combination with Vaborbactam for the treatment of patients 18 years of age and older with complicated urinary tract infections (cUTI) including pyelonephritis caused by the following susceptible microorganisms: Escherichia coli, Klebsiella pneumoniae, and Enterobacter cloacae species complex. Vaborbactam is a cyclic boronic acid pharmacophore β-lactamase inhibitor that elicits potent inhibition of Klebsiella pneumoniae carbapenemase (KPC) enzymes and other Ambler class A and C enzymes such as serine β-lactamases that confer resistance to commonly-used antibiotics such as Carbapenems.1 Vaborbactam is a potent inhibitor of class A carbapenemases, such as KPC, as well as an inhibitor of other class A (CTX-M, SHV, TEM) and class C (P99, MIR, FOX) beta-lactamases. Vaborbactam interacts with β-lactamases of Ambler classes A and C via precovalent and covalent binding.[3] It exerts no inhibitory actions on class D or class B carbapenemases.[4] IUPAC Name 2-[(3R,6S)-2-hydroxy-3-[2-(thiophen-2-yl) acetamido]-1,2-oxaborinan-6-yl]acetic acid. Molecular Formula $C_{12}H_{16}BNO_5S$. Molecular Weight 2971.

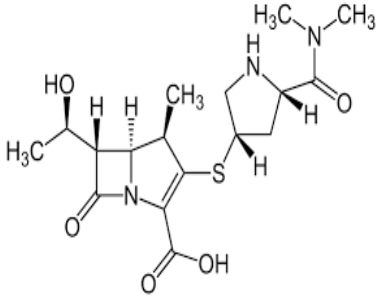

Figure 1: Structure of Meropenem

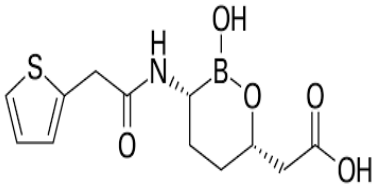

Figure 2: Structure of Vaborbactam

The literature survey revealed that There are very few methods reported in the literature for analysis of Meropenem and Vaborbactam alone or in combination with other drugs in the pure form and pharmaceuticals formulations by RP-HPLC.[3-9] In view of the need for a suitable, cost-effective RP-HPLC method for routine analysis of Meropenem and Vaborbactam Simultaneous estimation of in pharmaceutical dosage form. Attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Meropenem and Vaborbactam. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the Simultaneous estimation of Meropenem and Vaborbactam in pharmaceutical dosage form by using RP-HPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents: Meropenem and Vaborbactam were Purchased from market. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 235 nm with column Inertsil ODS C185µm (4.6 x 250mm), dimensions at 25^0C temperature. The optimized mobile phase consists of Phosphate buffer and Acetonitril in the ratio of 30:70. Flow rate was maintained at 1 ml/min.

Preparation of solutions:
Preparation of buffer:
Weighed 6.8 grams of KH_2PO_4 was taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water, adjusted the pH to 4.6 with ortho phosphoric acid.

Preparation of mobile phase:
A mixture of pH 4.6 Phosphate buffer 300 mL (30%), 700 mL of ACN (70%) are taken and degassed in ultrasonic water bath for 5 minutes. Then this solution is filtered through 0.45 µ filter under vacuum filtration.

The diluents:
The Mobile phase was used as the diluent.

Preparation of the individual Meropenem standard preparation*:*
10 mg of working standard was accurately weighed and transferred into a10 ml clean dry volumetric flask and about 2 ml of DMF is added. Then it is sonicated to dissolve it completely and made volume up to the mark with the diluant. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted up to the mark with diluant.

Preparation of the individual Vaborbactam standard preparation:

10mg of Vaborbactam working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and about 2ml of DMF is added. Then it is sonicated to dissolve it completely and made volume up to the mark with the diluant. (Stock solution). Further 10.0 ml from the above stock solution is pipette into a 100 ml volumetric flask and was diluted up to the mark with diluant.

Preparation of Sample Solution :(Tablet)

Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to 10 mg of Vaborbactam and Meropenem (marketed formulation) sample into a 10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume up to the mark with the same solvent. (Stock solution) Further 3 ml of above stock solution was pipetted into a10 ml volumetric flask and diluted up to the mark with diluant.

Procedure:
20µL of the standard, sample are injected into the chromatographic system and the areas for Meropenem and Vaborbactam peaks are measured and the %Assay are calculated by using the formulae.

METHOD:
The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.
System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 12 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Inertsil ODS C185µm (4.6 x 250mm), the mobile phase of composition Sodium Phospahte buffer 3.5 pH and Acetonitrile (30:70) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Meropenem and Vaborbactam in their tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:

Linearity: The linearity study was performed for the concentration of 100ppm to 500ppm and1ppm to 5ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Vaborbactam and Meropenem and calculate the individual recovery and mean recovery values. The results are shown in table 4,5.

Precision Studies: precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 6,7.

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in table 8,9.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.8 ml/min to 1.2 ml/min. The resulte are shown in table 10,11,12,13

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The resulte are shown in table 14.

LOD = 3.3σ/S and

LOQ = 10 σ/S, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

RESULTS AND DISCUSSION:

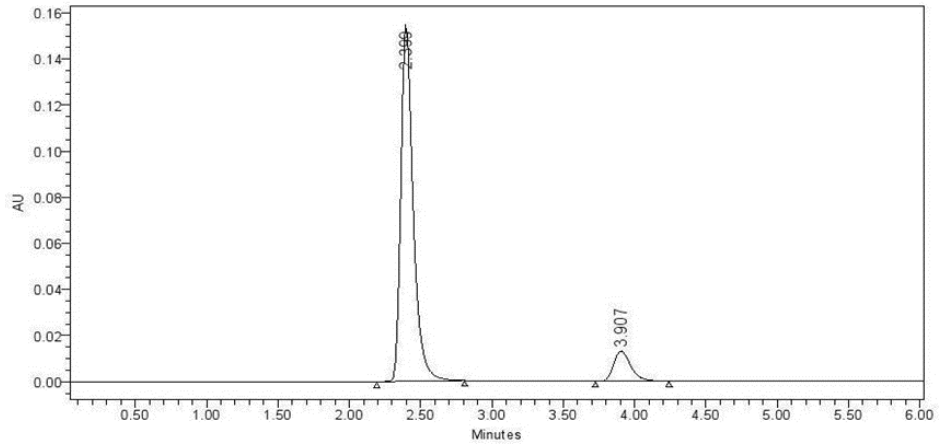

Figure 3: Standard chromatogram

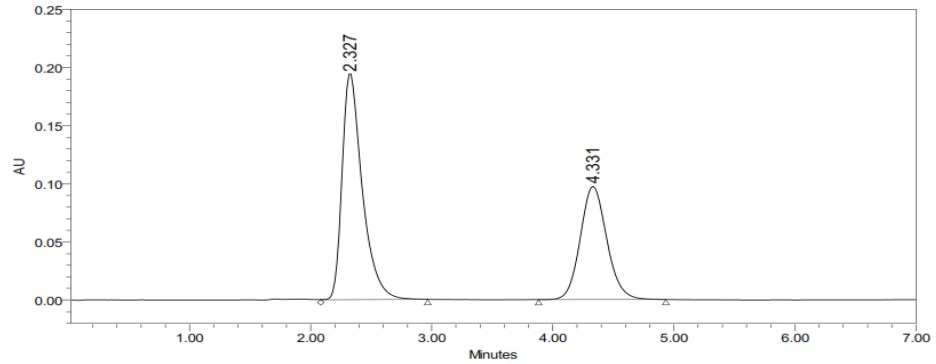

Figure 4: Sample chromatogram

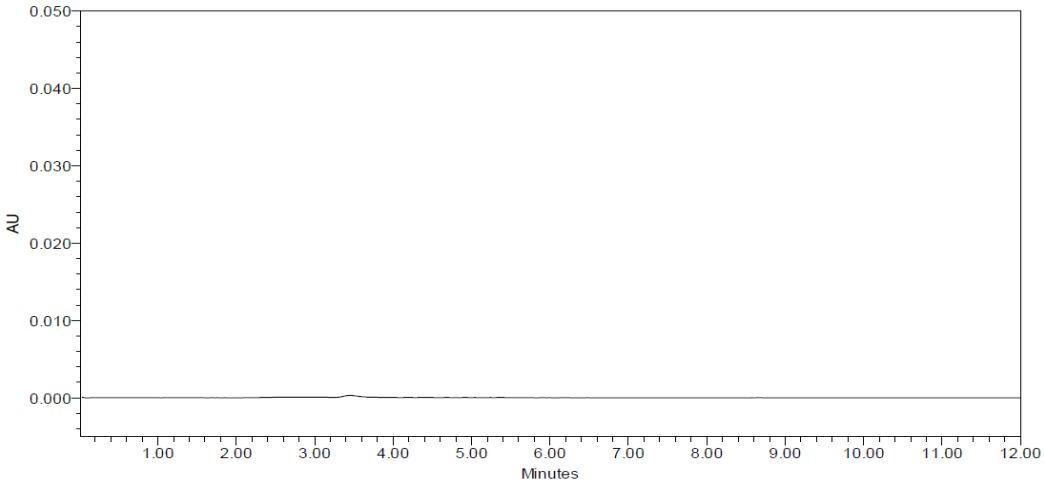

Figure 5: Blank chromatogram

Table 1: System suitability parameters

Parameters	Vaborbactam	Meropenem
Retention time	4.34	2.23
USP Plate count	2614	2632
USP Tailing	1.6	1.8

Table 2: Assay results for Vaborbactam and Meropenem

	Label Claim (mg)	% Assay
Meropenem	10	101.3
Vaborbactam	10	100.6

Table 3: Linearity results of Meropenem and Vaborbactam

S.NO	SAMPLE NAME	RT	AREA	HEIGHT	SAMPLE NAME	RT	AREA	HEIGHT
1	Linearty 1	2.309	1812101	145867	Linearty 1	4.304	1163273	74586
2	Linearty 2	2.322	2044373	176895	Linearty 2	4.323	1345955	87689
3	Linearty 3	2.324	2366122	206674	Linearty 3	4.214	1556574	101999
4	Linearty 4	2.336	2611248	228475	Linearty 4	4.524	1776565	117084
5	Linearty 5	2.340	2869662	259345	Linearty 5	4.218	1957821	129409

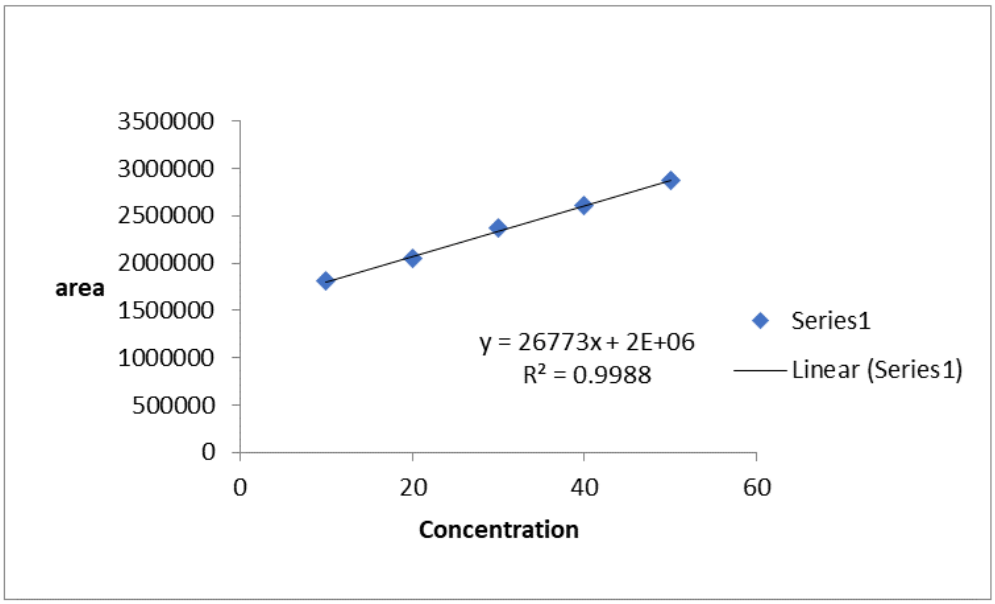

Figure 6: Linearity graph for Meropenem

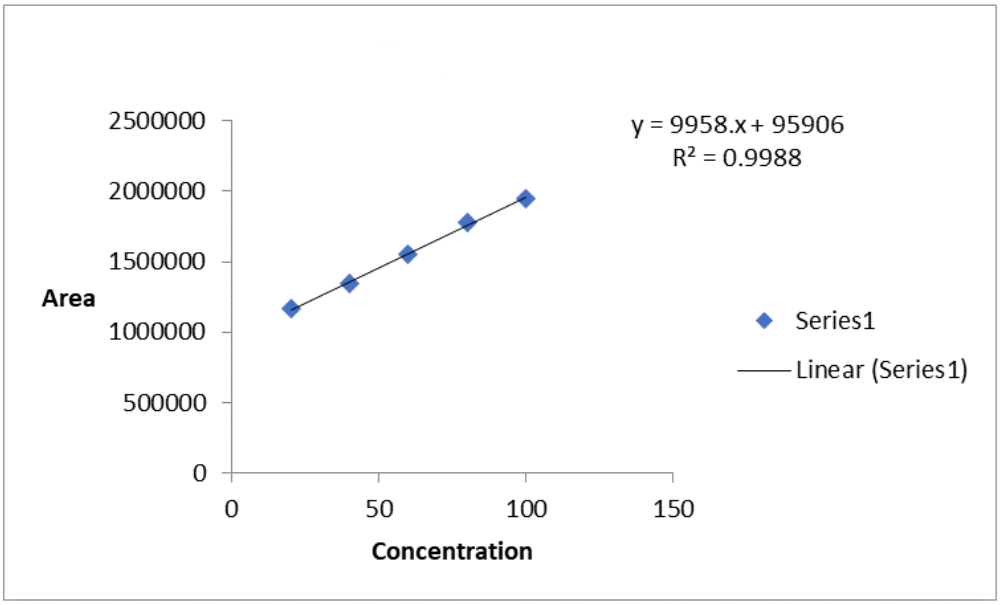

Figure 7: Linearity graph for Vaborbactam

Table 4: Showing accuracy results for Vaborbactam

%Concentration (at specification Level)	Area	Amount added(mg)	Amount found(mg)	% Recovey	Mean
50%	2331544	7.5	7.60	101.8%	100.4%
100%	3134597	15	14.8	99.9%	
150%	3917897	20	19.4	99.1%	

Table 5: Showing accuracy results for Meropenem

% Concentration (at specification level)	Area Area	Amount Added(mg)	Amount Found(mg)	% Recovey % Recovery	Mean Recovery
50%	353757	50	50.8	101.3%	
100%	4734988	100	99.4	99.4%	100.1%
150%	5911698	150	148.9	99.2%	

Table 6: Precision results for Vaborbactam

S.NO	Name	RT	Area	Height
1	Vaborbactam	4.302	1401375	100174
2	Vaborbactam	4.305	1401445	100068
3	Vaborbactam	4.325	1402315	98415
4	Vaborbactam	4.315	1404575	98155
5	Vaborbactam	4.312	1408514	98144
Mean			1491354	
Std.dev			5882.5	
%RSD			0.38	

Table 7: Precision results for Meropenem

S.NO	Name	RT	Area	Height
1	Meropenem	2.320	2267519	196958
2	Meropenem	2.341	2208588	197584
3	Meropenem	2.356	2275569	195874
4	Meropenem	2.344	2258841	194583
5	Meropenem	2.325	2257967	194587
Mean			2254401	
Std.dev			6535.5	
%RSD			0.31	

Table 8. Ruggedness results of Vaborbactam

S.NO	Name	RT	Area	Height
1	Vaborbactam	4.302	1401375	95613
2	Vaborbactam	4.305	1401442	95142
3	Vaborbactam	4.325	1402312	95158
4	Vaborbactam	4.315	1404673	95153
5	Vaborbactam	4.312	1408512	95143
Mean			1455158	
Std.dev			2344.5	
%RSD			0.15	

Table 9. Ruggedness results of Meropenem

S.NO	Name	RT	Area	Height
1	Meropenem	2.325	2165319	186958
2	Meropenem	2.315	2104788	187584
3	Meropenem	2.356	2147469	185874
4	Meropenem	2.325	2158641	184583
5	Meropenem	2.331	218957	184587
Mean			219556	
Std.dev			2559	
%RSD			0.12	

Robustness results

Table 10: Flow variation results for Vaborbactam

S.No	**Flow Rate(ml/min)**	**System suitability results**	
		USP Plate count	**USP Tailing**
1	0.8	1778.5	1.23
2	1.0	1547.2	1.2
3	1.2	1938.0	1.2

Table 11: Flow variation results for Meropenem

S.No	Flow Rate(ml/min)	System suitability results	
		USP Plate count	USP Tailing
1	0.8	882.3	1.56
2	1.0	1244.0	1.1
3	1.2	968.2	1.6

Table 12: System suitability results for Vaborbactam (Mobile phase)

S.No	Changein Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	1748.5	1.22
2	Actual	1548.2	1.2
3	10% More	1948.0	1.2

Table 13: System suitability results for Meropenem (Mobile phase)

S.No	Changein Organic Composition in the Mobile Phase	System suitability results	
		USP Plate count	USP Tailing
1	10% Less	878.3	1.56
2	Actual	1234.0	1.1
3	10% More	969.2	1.6

Table 14: LOD, LOQ of Vaborbactam and Meropenem

Drug	LOD	LOQ
Vaborbactam	3.03	10.1
Meropenem	2.94	9.87

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Meropenem and Vaborbactam in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Vaborbactam and Vaborbactam in pure and its pharmaceutical dosage forms.

REFERENCES:

1. Overington JP, Al-Lazikani B, Hopkins AL: How many drug targets are there? Nat Rev Drug Discov. 2006 Dec;5(12):993-6.
2. Imming P, Sinning C, Meyer A: Drugs, their targets and the nature and number of drug targets. Nat Rev Drug Discov. 2006 Oct;5(10):821-34.
3. Castanheira M, Rhomberg PR, Flamm RK, Jones RN: Effect of the beta-Lactamase Inhibitor Vaborbactam Combined with Vaborbactam against Serine Carbapenemase-Producing Enterobacteriaceae. Antimicrob Agents Chemother. 2016 Aug 22;60(9):5454-8. doi: 10.1128/AAC.00711-16. Print 2016 Sep. (PubMed ID 27381386)
4. Lomovskaya O, Sun D, Rubio-Aparicio D, Nelson K, Tsivkovski R, Griffith DC, Dudley MN: Vaborbactam: Spectrum of Beta-Lactamase Inhibition and Impact of Resistance Mechanisms on Activity in Enterobacteriaceae. Antimicrob Agents Chemother. 2017 Oct 24;61(11). pii: AAC.01443-17. doi: 10.1128/AAC.01443-17. Print 2017 Nov. (PubMed ID 28848018)
5. Negi V, Chander V, Singh R, Sharma B, Singh P, Upadhaya K. Method development and validation of Vaborbactam in pharmaceutical dosage form by Rp-HPLC. Indian J ChemTechnol 2017;24:441-6.
6. Casals G, Hernandez C, Hidalgo S, Morales B, Lopez Pua Y, Castro P, et al. Development and validation of a UHPLC diode array detector method for Vaborbactam quantification in human plasma. Clin Biochem 2014; 47:223-7.
7. Ikawa K, Kameda K, Ikeda K, Morikawa N, Nakashima A, Ohge H, et al. HPLC method for measuring Vaborbactam and biapenem concentrations in human peritoneal fluid and bile: application to comparative pharmacokinetic investigations.J Chromatogr Sci 2010;48:406-11.
8. Kloft C, Wicha SG. Simultaneous determination and stability studies of linezolid, Vaborbactam and vancomycin in bacterial growth medium by high-performance liquid chromatography. J Chromatogr B 2016; 1028:242-8.
9. Lobenhoffer JM, Bode-Boger SM. Quantification of Vaborbactam in human plasma by HILIC–tandem mass spectrometry.J Chromatogr B 2017;1046:13-7.
10. Frenich AG, Reyes RC, Gonzalez RR, Maresca MA, Vidal JL. Simultaneous analysis of antibiotics in biological samples by ultra high performance liquid chromatography-tandem mass spectrometry. J Pharm Biomed Anal 2014;89:203-12.
11. Rigo Bonnin R, Ribera A, Arbiol Roca A, Cobo Sacristan S, Padulles A, Murillo O, et al. Development and validation of a measurement procedure based on ultra-high performance liquid chromatography-tandem mass spectrometry for simultaneous measurement of β-lactam antibiotic concentration in human plasma. Clin Chim Acta 2017;468:215-24.