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Analytical method development and validation of meropenem and vaborbactam by RP-HPLC

Dr. R. Vani*, Sk. Salma Naheed

Department of Pharmaceutical Analysis, Shadan Women's College of Pharmacy, Khairatabad, Hyderabad.

***Corresponding Author:Dr. R. Vani**

ABSTRACT

A simple, accurate, economical, rapid, selective, reverse phase high performance liquid chromatography (RP-HPLC) was developed for simultaneous estimation of Meropenem and Vaborbactam in its bulk dosage form. The separation was carried out using a mobile phase of water: acetonitrile:methanol(20:30:50) pumped at a flow rate of 1 ml/min along with 297nm as a UV detection wavelength. The stationary phase used was column xterrac8 150x4.6 mm, 5µm. Meropenem and Vaborbactam were eluted at a retention time of Meropenem 2.82min and Vaborbactam 3.86 min. The method was developed and validated as per ICH guidelines by considering the parameters such as precision, accuracy, linearity, specificity, robustness and ruggedness. Linearity was 0.9991 for meropenem and 0.9997 for vaborbactam. The %RSD was less than 2%. The % recovery of meropenem was 99.2 and vaborbactam was 100.4. The developed RP-HPLC method can be used for routine analysis of Meropenem and Vaborbactam in combinational dosage form.

Keywords:RP-HPLC, method development, Validation, Meropenem, Vaborbactam.

INTRODUCTION

Meropenem, (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 [1-3].

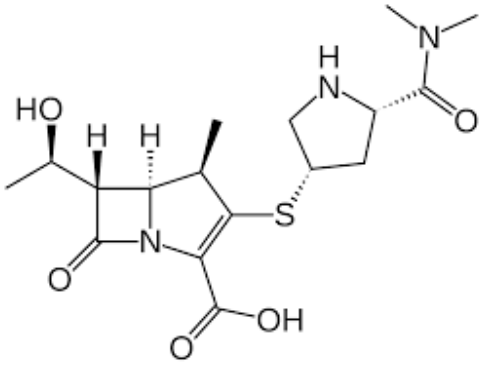


Fig.1: Chemical structure of Meropenem

Vaborbactam,2-[(3R,6S)-2-hydroxy-3-[2-(thiophen-2-yl)acetamido]-1,2-oxaborinan-6-yl]acetic acid [4-6].

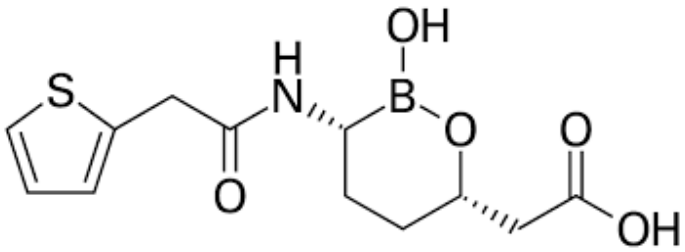


Fig. 2: Chemical structure of Vaborbactam

VALIDATION OF ANALYTICAL METHODS (USP/ICH)

Method validation, according to the United States Pharmacopeia (USP), is performed to ensure that an analytical methodology is accurate, specific, reproducible, and rugged over the specified range that an analyte will be analyzed. Regulated laboratories must perform method validation in order to be in compliance with FDA regulations [7-10]. In a 1987 guideline (Guideline for Submitting Samples and Analytical Data for Methods Validation), the FDA designated the specifications in the current edition of the USP as those legally recognized when determining compliance with the Federal Food, Drug and Cosmetic Act can be referred to as the "eight steps of method validation".

EXPERIMENTAL WORK

Table. 1: Optimized Conditions

Equipment	Source
High Pressure Liquid Chromatography (HPLC)	Shimadzu LC2010 CHT
Chromatographic data software	Empower
Column	Xterra RP-8 (150mm x 4.6 mm i.d., particle size 5 µm)
Detector	PDA
Injector	Automated
Electronic Balance	Eagle
Sonicator	Band Line Sonerex
p^H Meter	Thermo

Method Validation

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc. The described method extensively validated in terms of specificity, system suitability, linearity, accuracy, precision, limit of detection, limit of quantification and robustness.

Preparation of Phosphate bufferpH4.5

3.11gm of Ammonium hydrogen Phosphate Monobasic was weighed and dissolved in 1000 mL of water. Then adjust the pH to5.0±0.02 using diluted Sodium hydroxide solution. Buffer was filtered through 0.45µm filters to remove all fine particles and gases.

Mobile phase preparation for optimized trial

Take 20vol of water 30vol of acetonitrile and 50 vol of methanol in a beaker mix them and 15 min sonication done and subjected to vacuumfiltration.

Preparation of mobile phase

Take 20ml of water 30ml of acetonitrile and 50 ml of methanol in a beaker mix them and sonicated for 15 min and subjected to vacuumfiltration.

Preparation of Standard solution

About 10 mg of MPN and 10mg of VBB were weighed into volumetric flask of 50ml, to this 50 mL solvent was added, sonicated and subjected to volume make up up to the mark on the volumetric flask with the solvent.

Preparation of sample solution

Weigh about 10 mg of sample (MPN&VBB) and transfer in to 10ml volumetric flask, add 10ml of mobile phase sonicated and subjected to volume make up with the solvent.

Dilutions

Necessary dilutions are made from standard stock solutions (1ml of stock solution to 100ml) to get the concentration range of 10 µg/mL of MPN and 10 µg/mL of VBB.

RESULTS

Table. 2: Method precision results for MPN and VBB.

Injection	MPN		VBB	
	Area	%Assay	Area	%Assay
1	1026668	100.0	2029924	100.4
2	1032616	100.5	2018744	99.8
3	1025772	99.9	2030331	100.4
4	1017071	99.0	1995114	98.6
5	1016907	99.0	2018534	99.8
6	1011321	98.5	2013040	99.5
Average	1024346	99.5	2017615	99.7
SD	7906.21	0.8	12981.16	0.6
%RSD	0.77	0.8	0.64	0.6

Result

The %RSD of 6 determinations of MPN and VBB for System precision was in the limit i.e,< 2.0%.

Linearity and range

Preparation of standard stock solution

Standard stock solutions of MPN(1000µg/mL) andVBB (1000µg/mL) were prepared by dissolving 100 mg of MPN and 100 mg of VBB in 100 mL of mobile phase. After that filter it and Sonicated for 5 min further dilutions were given in the Table 3.

Table 3: Linearity Preparations.

Preparations	Volume from standard stock transferred in mL	Volume made up in mL (with mobile phase)	Conc. obtained (µg/mL)	
			MPN	VBB
Preparation 1	1.0	20	50	50
Preparation2	1.6	20	80	80
Preparation 3	2.0	20	100	100
Preparation 4	2.4	20	120	120
Preparation 5	3.0	20	150	150

Table. 4: Linearity data of MPN.

S.No	Concentration (µg/mL)	Area
1	50	558053
2	80	813525
3	100	1016907
4	120	1200288
5	150	1455360

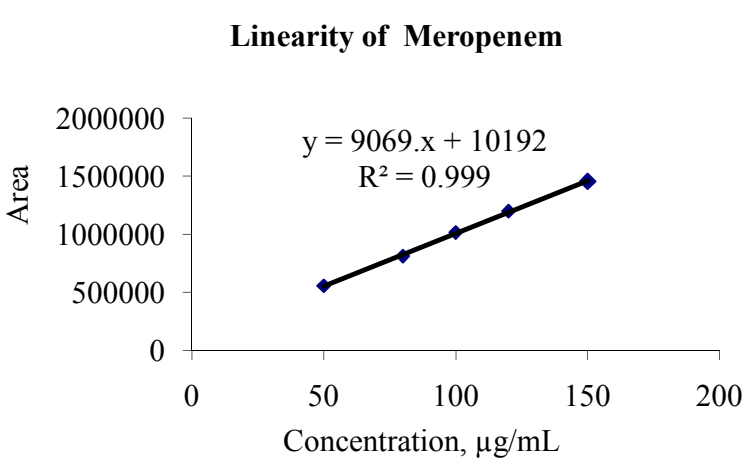


Fig.3: Graph for Linearity data of MPN.

Table. 5: Linearity data of VBB.

S.No	Concentration (µg/mL)	Area
1	50	999261
2	80	1634827
3	100	2018534
4	120	2422240
5	150	3067801

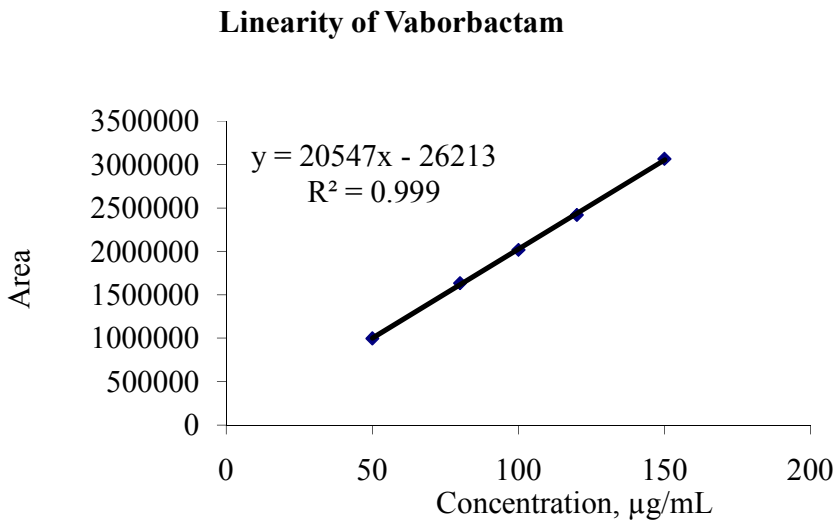


Fig.4: Graph for Linearity data of VBB.

Table 6: Results for Recovery of MPN.

%Recovery	Amount present (µg/mL)	Amount found (µg/mL) *	Percent Recovery *	% Mean Recovery
50%	50	49.64	99.3	
100%	100	99.17	99.2	99.2
150%	150	148.63	99.1	

Table. 7:Results for Recovery of VBB.

%Recovery	Amount present (µg/mL)	Amount found (µg/mL) *	Percent Recovery *	% Mean Recovery
50%	50	49.86	99.7	
100%	100	99.98	100.0	100.4
150%	150	152.08	101.4	

Acceptance criteria

The % recovery of MPN and VBB should lie between 98% and 102%.

Result

The % mean recovery of MPN and VBBwas founded between 98.0% to 102.0%

Limit of Detection (LOD)

LOD=3.3σ/S
= (3.3)*(3454.9)/9069
= 1.257µg/ml (MPN)
=(3.3)* (7825.5)/20547
=1.260µg/ml (VBB)
Where, σ = the standard deviation of the response
S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Observation

The LOD for this method was found to be 1.257µg/ml (MPN) and 1.26µg/ml (VBB)

Limit of Quantification (LOQ)

LOQ=10σ/S
= (10)*(3454.9)/9069
= 3.80µg/ml (MPN)
=(10)* (7825.5)/20547

=3.88µg/ml (VBB)
Where
σ = the standard deviation of the response
S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Observation

The LOQ for this method was found to be 3.80µg/ml (MPN) and 3.88µg/ml (VBB)

Robustness

The Robustness of the method was determined. It is obtained by variation in method parameters are summarized below.

Table. 8: Results for Robustness

Chromatographic changes		Theoretical Plates		Tailing factor		Resolution
		MPN	VBB	MPN	VBB	Between MPN&VBB
Flow rate (mL/min)	0.8	18377	24753	1.34	1.27	3.08
	1.2	16417	21569	1.36	1.22	2.85
Wavelength (nm)	295	17596	23533	1.35	1.26	2.96
	299	17377	23574	1.36	1.27	2.93

Ruggedness

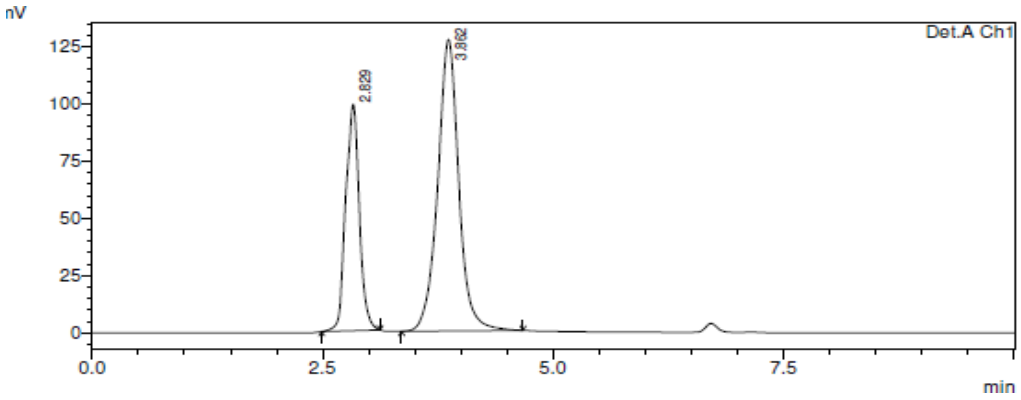
The ruggedness of the method was studied by performing the Assay by two different analysts

Table. 9: Results for Ruggedness

MPN	%Assay	VBB	%Assay
Analyst 01	100.8	Analyst 01	101.33
Analyst 02	98.56	Analyst 02	98.68
%RSD	0.58	%RSD	0.45

Observation

From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged.



Peak#	Ret. Time	Area	Height	heoretical Plates/met	Area %
1	2.829	1022197	98959	10935.306	33.467
2	3.862	2032157	127358	9555.738	66.533
Total		3054354	226317		100.000

Tailing Factor	Resolution
1.063	0.000
1.030	3.006

Figure 5: Standard Chromatogram

Table10:Results for MPN and VBB

S .NO	Name	RT	Area
1	MPN	2.768	1026668
2	VBB	3.634	2029924

Table 11:Results of assay

Drug	Label claim(mg)	Amount found(mg)	% Assay
MPN	1000	99.87	99.5
VBB	1000	99.746	99.7

Observation

Both drugs % assay was in limits. The percentage purity of both MPNand VBB was in limits that is 98-102%.

CONCLUSION

A new precise, accurate, rapid method has been developed for the simultaneous determination of MPN and VBB in pharmaceutical dosage form by RP-HPLC.

The optimum wavelength of MPN and VBB was selected at 297 nm on the basis of isosbestic point. Various trials were performed with different mobile phases in different ratios, but finally Water: Acetonitrile: Methanol (20:30:50) was selected.The Retention time of MPN and VBB was 2.829&3.862 min respectively. The Retention times for both the drugs were considerably less compared to the Retention time obtained for the drugs in the other mobile phase.

The different parameters of validation such as linearity, precision, accuracy, and specificity were determined according to International Conference on Harmonization ICH Q2B guidelines. The calibration curve was obtained by plotting peak area versus the concentration over the range of 50-150 µg/mL for MPN and50-150 µg/mL for VBB. From linearity the correlation coefficient R^2 value was found to be 0.9991 for MPN and 0.9997 for VBB. The proposed HPLC method was also validated for system suitability, system precision and method precision. The %RSD in the peak area of drug was found to be less than 2%. The number of theoretical plates was found to be more than 2000, which indicates efficient performance of the column. The percentage of recovery of MPN and VBB were found to be 99.2 and 100.4 respectively shows that the proposed method is highly accurate.

Hence the proposed method is highly sensitive, precise and accurate and it successfully applied for the quantification of API content in the commercial formulations of MPN and VBB in Educational institutions and Quality control laboratories.

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