WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 15, 783-795. Research Article ISSN 2277– 7105

A NOVEL UPLC METHOD DEVELOPMENT & VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF MEROPENEM AND VABORBACTAM IN BULK AND PHAMRACEUTICAL DOSAGE FORM

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Article Received on
10 October 2020,

Revised on 30 October 2020,
Accepted on 20 Nov. 2020

DOI: 10.20959/wjpr202015-18550

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ABSTRACT

Dar-us-salaam, aghapura, hyderabad-500 001 Telangana, India. A novel UPLC method was developed and validated for simultaneous estimation of Meropenem and Vaborbactam in bulk and pharmaceutical dosage form. Optimization is achieved by using the combination of methanol and water (70:30 v/v) in zodiac C18 column with a flow rate of 1.0ml/min at a wavelength of 270 nm. Meropenem and Vaborbactam were eluted at the retention time of 1.13 and 2.0 mins respectively. System suitability parameters were found to be within the limits. The method was shown to be specific, a s there is no interference of placebo peak with that of drug peak. The method to be linear in the concentration range of 50-150µg/ml for Meropenem and Vaborbactam, With correlation coefficient 0.9991 and 0.9997 respectively. The method was found to be accurate as the percentage recovery was 99.2 and 100.4 for MPN &VBB and was within the limits. The percentage RSD was determined to be 0.08 and 0.07 for MPN & VBB, which indicates that the method was precise. The LOQ for this method was found to be 3.80µg/ml (MPN) and 3.88µg/ml (VBB) The LOD for this method was found to be 1.257µg/ml (MPN) and 1.26µg/ml (VBB). The developed UPLC method can be used for routine analysis of Meropenem and Vaborbactam in bulk and pharmaceutical.

KEYWORDS: UPLC, Meropenem, Vaborbactam, LOD, LOQ.

INTRODUCTION

Meropenem is carabpenem class of antibiotic. whose IUPAC name is (4*R*,5*S*,6*S*)-3-(((3*S*,5*S*)-

5- (Dimethylcarbamoyl)pyrrolidin-3-yl)thio)-6-((*R*)-1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid. With a molar weight of 383.464 g/mol g·mol^{-1}. Meropenem is an carbapenem antibiotic. It is dynamic against Gram-positive and Gram-negative microbes. Meropenem applies its activity by entering bacterial cells promptly and meddling with the union of imperative cell divider segments, which prompts cell deathThe essential dimethyl- carbamoylpyrrolidinethio side chain at C2 on MEP upgrades action against gram-negative living beings. Carbapenems apply their bactericidal activity through penicillin-restricting proteins (PBPs) with resulting hindrance of cell divider combination. The MEP may give a more grounded anti-toxin spine contrasted with cephalosporins when joined with carbapenemase.

Vaborbactam is anon-β-lactam, cyclic boronic acid inhibitor of β-lactamases.the IUPAC name is 3R, 6S)- 2-hydroxy-3-[[2-(2-thienyl) acetyl] amino]-1, 2-oxaborinane-6acetic acid.with a molar weight of 297.13 g·mol^{-1}. Vaborbactam is a cyclic boronic corrosive pharmacophore β-lactamase inhibitor that evokes powerful restraint of Klebsiella pneumoniae carbapenemase (KPC) catalysts and other Ambler class An and C chemicals, for example, serine β-lactamases that present protection from usually utilized anti-infection agents, for example, Carbapenems. In blend with meropenem, varborbactam goes about as a non-self-destructive beta-lactamase inhibitor that shields meropenem from debasement interceded by serine beta-lactamases, for example, Klebsiella pneumoniae carbapenemase (KPC)literature review reveals that there are different methods of RP-HPLC and UV for the simultaneous estimation of Meropenem and Vaborbactam but that methods was found to be cost effective and time consuming. Hence our present plan is to develop a new, sensitive, robust& accurate method for its analysis in formulation, after a detailed study, a new UPLC method was decided to be developed and validated as per ICH norms.

Structures

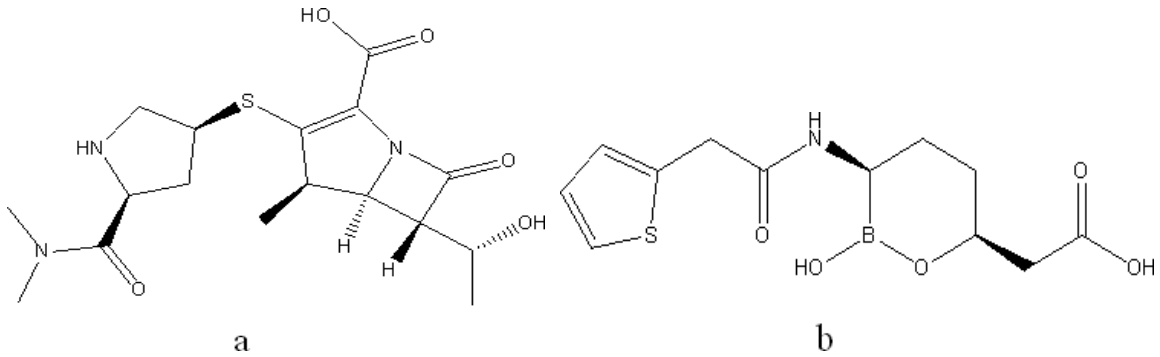

Fig. 1.a): Meropenem and b): Vaborbactam.

METERIALS AND METHOD

Intsruments used

UV-Visible Spectrophotometer-Thermo Electron co-orporation, UPLC-Agilent Infinity 1290, Ultra Sonicator-Citizen, Digital Ultrasonic Cleaner, pH meter-Thermo, Electronic balance-Mettler Toledo, UPLC Column-Zodiac column,C18(150x4.6 ID) 5µm.

Drug sample

Meropenem and Vaborbactam bulk drugs as Gift samples obtained from Madras pharmaceuticals, Chennai and marketed product VABOMERE from REMPEX pharmaceutical.

Reagent and Solutions

Methanol, water (uplc grade), sodium hydroxide, Ammonium hydrogen Phosphate Monobasic.

Determination of working wavelength (Λ_{MAX})

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard solution

About 10 mg of Meropenem and 10mg of Vaborabactum were weighed into a 50 mL volumetric flask, to this 50 mL of mobile phase was added, sonicated and the volume was made up to mark with the mobile phase.

Dilutions

Necessary dilutions are made from standard stock solutions to get the concentration range of 10 µg/mL of MEROPENEM and 10 µg/mL of VABORABACTUM.

The wavelength of maximum absorption (λ_{max}) of the solution of the drugs in mobile phase were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nmagainst mobile phase as blank. The absorption curve shows characteristic absorption maxima at 261 nm for MEROPENEM, 273 nm for VABORABACTUM and at 270 nm same absorbance for both the drugs, i.e., isobestic point. Thus, 270 nm was selected as detector wavelength for the UPLC chromatographic method.

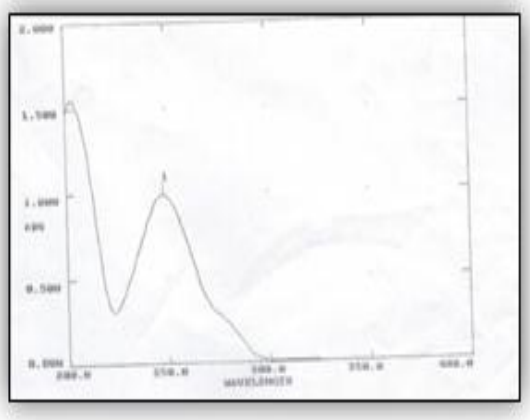

Fig. 1: UV-VIS Spectrum of Meropenem (261nm).

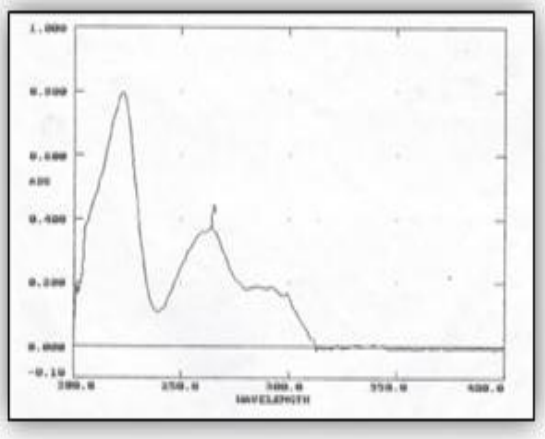

Fig. 2: UV-VIS Spectrum of Vaborabactum (273nm).

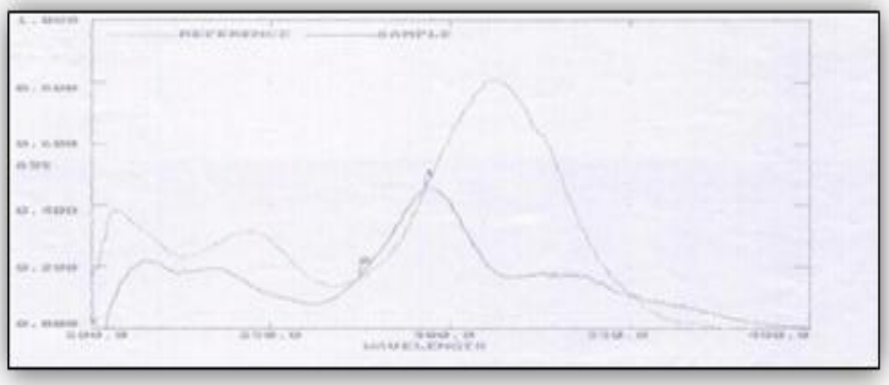

Fig. 3: UV-VIS Overlay Spectrum of Meropenem and Vaborabactum (270).

RESULT AND DISCUSSION

Method developement

Optimised method: Trials were performed for the method development and the best peak with least fronting factor was found to be with RT=1.45 min for MPP and 2.03 min for VBB.

Table 2: Optimized chromatographic conditions.

Mobile phase	**Methanol: Water (70:30V/V)**
Column	Zodiac column, C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Ambient Temperature
Wavelength	270nm
Injection volume	10 µl
Run time	5min
Retention time	About 1.13min for Meropenam, 2.0min for Vaborbactam.

Validation

1. System suitability

To verify that the analytical system is working properly and can give accurate and precise results were evaluated by 100µg/mL of MEROPENAM and 100µg/mL of VABORBACTAM. were injected six times and the chromatograms were recorded for the same.

Table 1: Results for system suitability of vaborbactam.

Inj	Retention time	Peak area	heoretical plates	Tailing factor
1	3.862	2032157	9555	1.03
2	3.823	2017044	9521	1.05
3	3.792	2015194	9584	1.09
4	3.749	2012644	9530	1.02
5	3.715	2008604	9547	1.07
6	3.695	2014157	9587	1.03
Mean	3.773	2016633	-	-
SD	0.065	8121	-	-
%RSD	1.7	0.4	-	-

Table 2: Results for system suitability of Meropenam.

Injection	RT	Peak area	Theoretical plates (TP)	Tailing factor (TF)
1	2.829	1022197	10935	1.06
2	2.820	1025670	10917	1.01
3	2.817	1041099	10901	1.05
4	2.829	1026496	10948	1.02
5	2.788	1006266	10961	1.06
6	2.790	1033915	10942	1.1
Mean	2.812	1025941	-	-
SD	0.019	11789	-	-
%RSD	0.7	1.1		

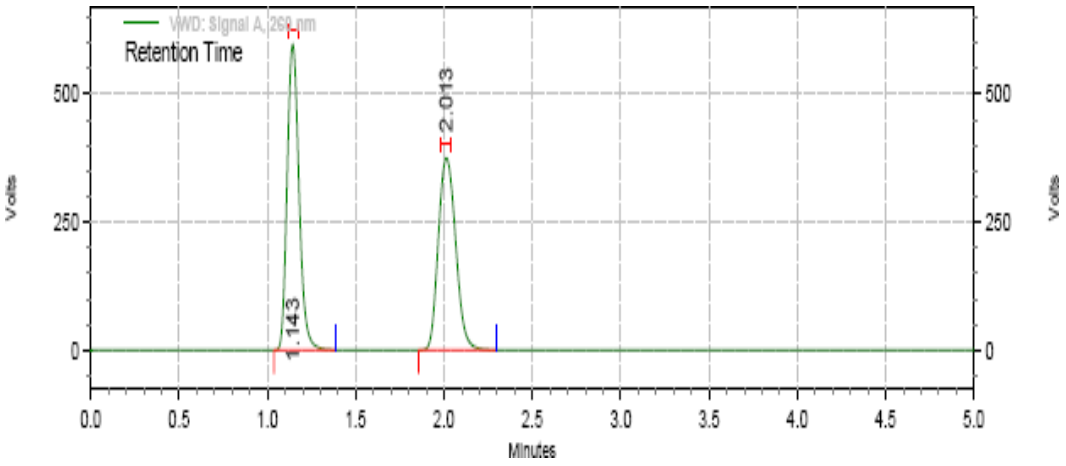

Fig. 2: System suitability chromatogram of meropenem and vaborbactam.

RESULT

The plate count and tailing factor results were found to be satisfactory and are found to be within the Limit.

2 Specificity: Blank solution was injected, and the chromatogram was recorded for the same as given in Fig. 9.17. Placebo solution was prepared, and it was injected, and the chromatogram was recorded for the same as given in Fig. 1,2.

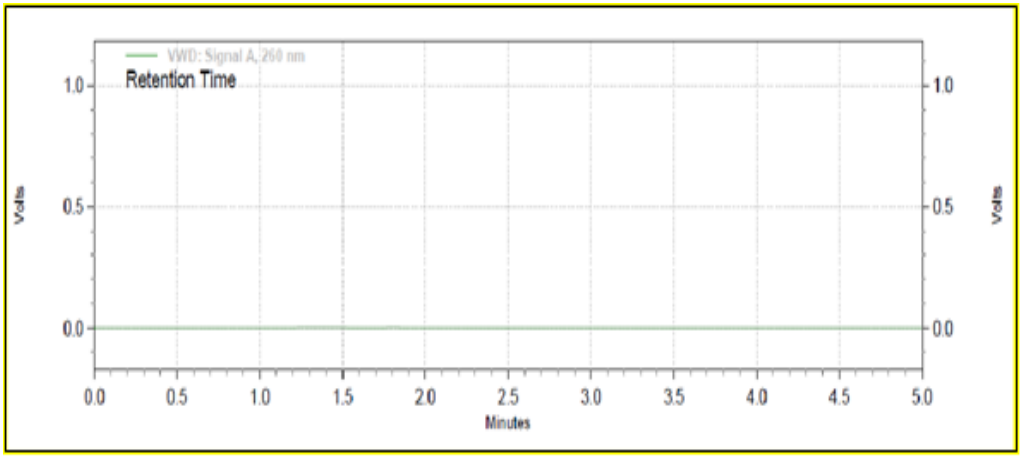

Fig. 1: **Chromatogram of blank.**

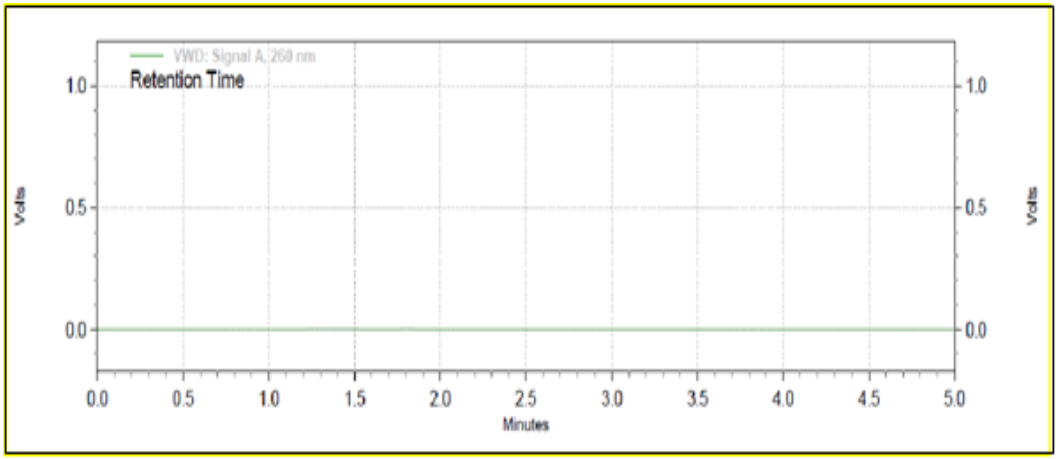

Fig. 2**: Chromatogram of placebo.**

RESULT

It was observed that diluents or placebo peaks was not interfering with the MPN and VBB peaks.

3. Linearity and Range

Preparation of standard stock solution**:** Standard stock solutions of MEROPENAM (1000µg/mL) and VABORBACTAM. (1000mg/mL) were prepared by dissolving 100 mg of

MEROPENAM and 100 mg of VABORBACTAM. in 100 mL of mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min further dilutions were given in the Table 1.

***Table 1*: Linearity preparations.**

Preparation s	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 2*: Linearity data of meropenam.**

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

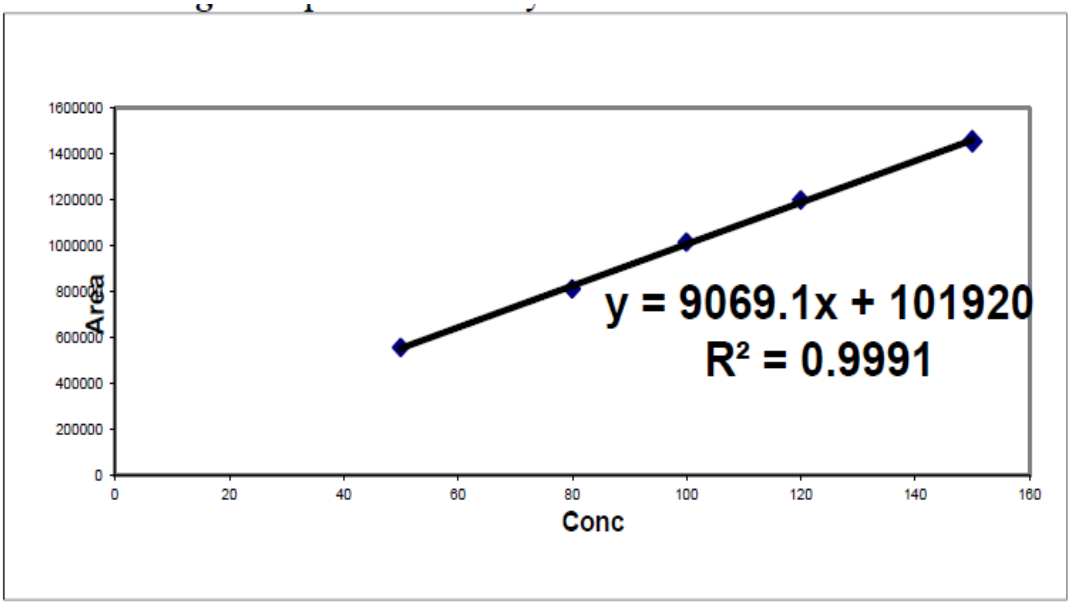

***Fig. 3:* Graph for linearity data of meropenam.**

***Table 3*: Linearity data of Vaborbactam.**

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

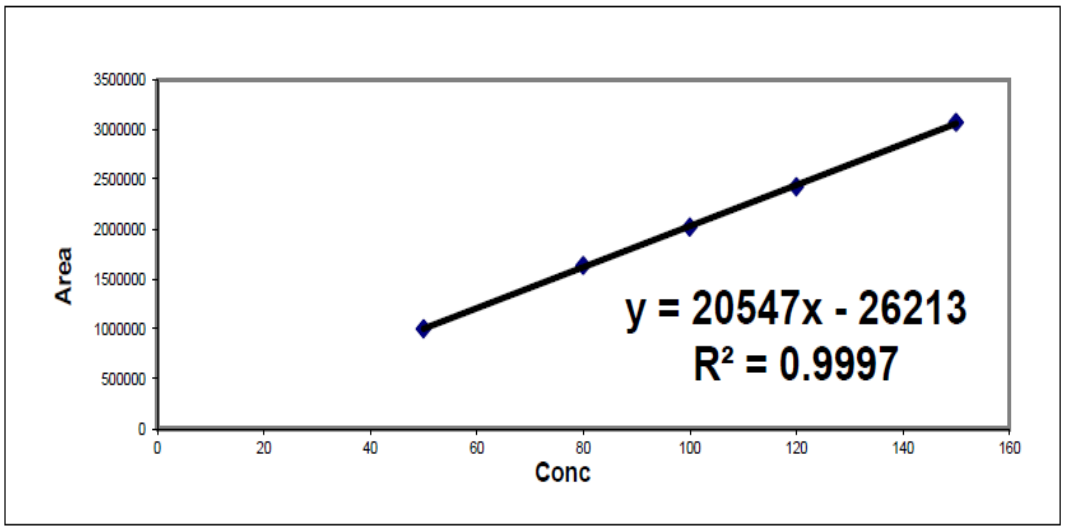

Fig. 4: Linearity graph of vaborbactam.

Table 4: Observation for linearity.

S. no	Parameter	MPN	VBB
1	Correlation coefficient	0.9991	0.9997
2	Slope	9069	20547
3	Intercept	101920	26213

RESULT

Cecorrelation coefficient for linear curve obtained between concentration vs. Area for standard preparations of MPN and VBB is 0.999 and 0.999 respectively.

4. Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (preanalysed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in Table.

***Table 1*: Results for recovery of MPM.**

%Recovery	Amount present (µg/mL)	Amount found (µg/mL)	Percent Recovery	Peak area
50%	50	49.64	99.3	5662
100%	100	99.17	99.2	5671
150%	150	148.63	99.1	5760
AVERAGE			99.2	5697.667
SD				54.16949
%RSD				0.950731

***Table 2*: Results for Recovery of VBB.**

%Recovery	Amount present (µg/mL)	Amount found (µg/mL)	Percent Recovery	Peak area
50%	50	49.86	99.7	2650
100%	100	99.98	100.0	2729
150%	150	152.08	101.4	2737
AVERAGE			100.2	2705.333
SD				48.08673
%RSD				1.777479

Acceptance criteria

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

RESULT

The % mean recovery of MPN and VBB was founded between 98.0 to 102..0.

5. Method precision

Method precision was determined by injecting six different solutions of sample solutions of MPN (100µg/mL) and VBB (100µg/mL) for six times are prepared separately. The chromatograms were recorded, and the results were summarized in Table.

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

Injection	MPN		VBB	
	Area	RT	Area	RT
1	5595	1.026	2823	4.727
2	5595	1.025	2823	4.722
3	5602	1.026	2825	4.721
4	5602	1.025	2825	4.721
5	5604	1.025	2825	4.729
6	5607	1.025	2824	4.721
Average	5600	1.025333	2824	4.7235
SD	4.875	0.000516	0.983	0.003564
%RSD	0.087	0.050364	0.03	0.075446

Result

The %RSD of 6 determinations of MPN and VBB for System precision found to be within the acceptance criteria of less than 2.0%.

6. Limit of detection (LOD).

Where, = 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.

= (3.3) *(3454.9)/9069

Observation

= 1.257µg/ml (MPN)

= (3.3) * (7825.5)/20547

=1.260µg/ml (VBB)

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

7. Limit of quantification (loq)

Where = 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.

= (10) * (3454.9)/9069

= 3.80µg/ml (MPN)

= (10) * (7825.5)/20547

= 3.88µg/ml (VBB

8. Robustness

The Robustness of the method was determined. The results obtained by deliberate variation in method parameters are summarized below in Table.

Table 1 **: Results for Robustness of MPN and VBB.**

Chromatographic changes		Theoretical Plates		Tailing factor	
		MP	VB	MP	VB
Flow rate (mL/min)	0.8	8377	8753	1.34	1.27
	1.0	9595	10987	1.28	1.21
	1.2	6417	8569	1.36	1.22
Wavelength (nm)	268	7596	9533	1.35	1.26
	270	9595	10987	1.28	1.21
	272	7377	6574	1.36	1.27

Result

The tailing factor and theoretical plates was found to be within the limits on small variation of flow rate and wavelength.

9. Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts.

Acceptance criteria

The % Relative standard deviation of Assay values between two analysts should be not more than 2.0%.

Table 1: Results for ruggedness.

Meropenam	%Assay	Vaborbactam	%Assay
Analyst 01	99.29	Analyst 01	100.78
Anaylst 02	99.45	Anaylst 02	100.24
% RSD	1.02	% RSD	0.85

RESULTS

The % Relative standard deviation of Assay values between two analysts found to be less than 2.0%.

ACKNOWLEDGEMENT

Most importantly I am thankful to the almighty, who is the creator and director of all that initial and final modes to destiny.

Finally, I consider this opportunity to express a deep sense of gratitude to my Parents who has always believed in my thoughts and supported me all through.

I take the opportunity to express my deep sense of gratitude and respect, Associate Prof. Dr S.H RIZWAN, M. Pharm (ph.D) (Department of pharmaceutical analysis) Deccan School Of Pharmacy for her guidance support and immense encouragement given during the course of seminar work.

I am grateful to Prof. Dr.Syed Abdul Azeez, M.Pharm, PGDPMIR, PCCRM, PH.D honorable Principal of Deccan School of Pharmacy, providing facilities to do this work and for his constant support and encouragement.

CONCLUSION

- The study focuses at developing stability indicating validated method for simultaneous estimation of Meropenem and Vaborbactam.
- The present study reveals the optimization is achieved by using the combination of methanol and water (70:30 v/v) in zodiac C18 column with a flow rate of 1.0ml/min at a wavelength of 270 nm.
- System suitability parameters were found to be within the limits.
- The method was shown to be specific, as there is no interference of placebo peak with that of drug peak
- The method to be linear in the concentration range of 50-150µg/ml for Meropenem and Vaborbactam,With correlation coefficient 0.9991 and 0.9997 respectively.
- The method was found to be accurate as the percentage recovery was 99.2 and 100.4 for MPN &VBB and was within the limits.
- The percentage RSD was determined to be 0.08 and 0.07 for MPN & VBB, which indicates that the method was precise.
- The LOQ for this method was found to be 3.80µg/ml (MPN) and 3.88µg/ml (VBB)
- The LOD for this method was found to be 1.257µg/ml (MPN) and 1.26µg/ml (VBB).
- Hence the method was developed and validated as per ICH guidelines by considering the parameters such as precision, accuracy, linearity, specificity, robustness& ruggedness.

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