THE MEROPENEM AND VEBORBACTAM ANALYSIS WAS DEVELOPED AND VALIDATED USING A HIGH -PERFORMANCE LIQUID CHROMATOGRAPHY METHOD

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Abstract

The goal was to develop a rapid, accurate, and repeatable liquid chromatography method for quantifying Meropenem (MEP) and vaborbactam (VAB) in bulk and pharmaceutical formulations. Methods: The chromatography was performed on a Kromasil C18 column (250?? 4,6 mm) with an acetonitrile mobile phase and a 10 mm phosphate buffer (pH 3,50) with a 30:70 v/v ratio and pumping rates of 1.0 ml/min with UV detection at 260 nm. During retention times of 2.29 and 3.10 minutes, MEP and VAB symmetrical and acute maxima were achieved, respectively. The chromatographic technique's linearity, detection and quantity limits, precision, accuracy, equipment compatibility, and robustness have all been investigated. Calibration curves were constructed at the 25-150 g/ml levels for the MEP and VAB levels. Stability testing, which included subjecting the analyser solution to a variety of stress scenarios, found no evidence of degradants in the HPLC process. The proposed method has been shown to be selective, dependable, linear, precise, and sensitive. For routine analysis, the method can be used to test bulk drug determination as well as mixed dosage kinds.

***Key words*:** *Meropenem, Vaborbactam, development, HPLC*

1. INTRODUCTION

Meropenem is a broad-spectrum carbapenem antibiotic. It is effective against gram-positive and gram-negative bacteria. Meropenem causes cell death by entering bacterial cells and interfering with the formation of key cell wall components. In August 2017, the FDA authorised vabomere,a combination antibiotic medication for the treatment of adult patients with severe urinary tract infections. Vabomere is an injectable drug made up of vaborbactam and meropenem. The goal of treatment when illnesses are known or highly suspected of producing susceptible bacteriais to alleviate the symptoms of infection and achieve negative urine cultivation. Vaborbactam is an inhibitor of cyclic boronic acid-lactamase. 2 Studies on the treatment of bacterial infections in people with varying degrees of renal insuffi ciency were used. The FDA approved a Vabomere antimicrobial therapy combination for the treatment of adult patients with severe urinary tract infections. (CUTI). Vabomere comprises vaborbactam and Meropenem for intravenous administration. Vaborbactam works by suppressing the target microbe's generation of serine beta-lactamases.

2. Materials and method

Chemicals and Reagents:

Methanol for HPLC (High-Performance Liquid Chromatography) Buffer for Hplc Grade (KH2PO4) Meropenem and Vaborbactam are the working standards.

Instrumentation

HPLC Conditions:

Compact HPLC system from Waters, model NO.2690/5 series, withInertsil- C18 ODS column.a state of electronic balance (SARTORIOUS) Sonicator is a word that has a lot of different meanings (FAST CLEAN)

Chromatographic Conditions:

The volumetric flow rate : 1.0ml / min
Column : Inertsil-C18, plate ODS Wave longitude detector : 252nm
The tempo of the column : Ambient Volume of injection : 20µl
Time to Finish : 6min

Preparation of Buffer (KH_2PO_4 0.1 M)

Weigh 3.8954 g di-sodium hydrogen phosphate dissolves completely in 1000 mL distilled water and 3.4023 g potassium dihydrogen phosphate. After that, the pH 2.5 is adjusted using orthophosphoric acid, and the solution is passed through a 0.45m membrane filter.

Phase of Mobility:

A sonicator was used to combine and degas a 90:10V/V mixtureof methanol and acetonitrile.

Method development

From the literature survey there are few analytical methods was established in combination of meropenam vebarobactam.but there no no common method in hplc.The present study was proposed a unique method was applicable hplc.In development process they have many trials were performed different buffers like water,phosphoric acid, formic acid finally di-sodium hydrogen phosphate was selected as buffer.In developmentprocess di hydrogen phosphate ratio gradually increasing at final stage we get good resolution and exists the system suitable conditions.

Method validation

The analytical method using HPLCwas validatedas per ICH guidelines for the parameters like system suitability,specificity,accuracy,precision,linearity,robustness,limit of detection(LOD), limit of quantification.

The first trial

Mobilephase: Methanol that has been degaussed to its purest form.

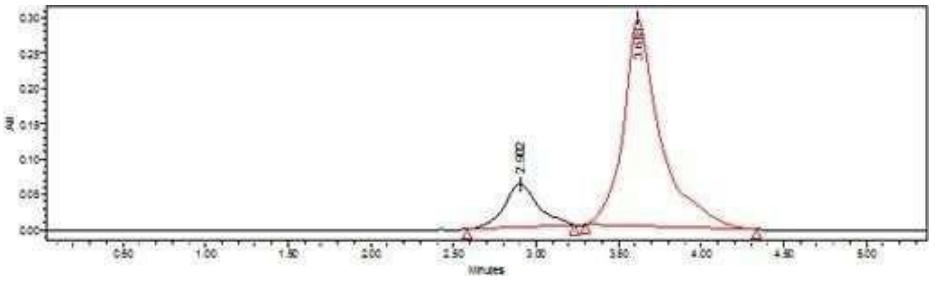


Fig.1:Test1 of chromatography

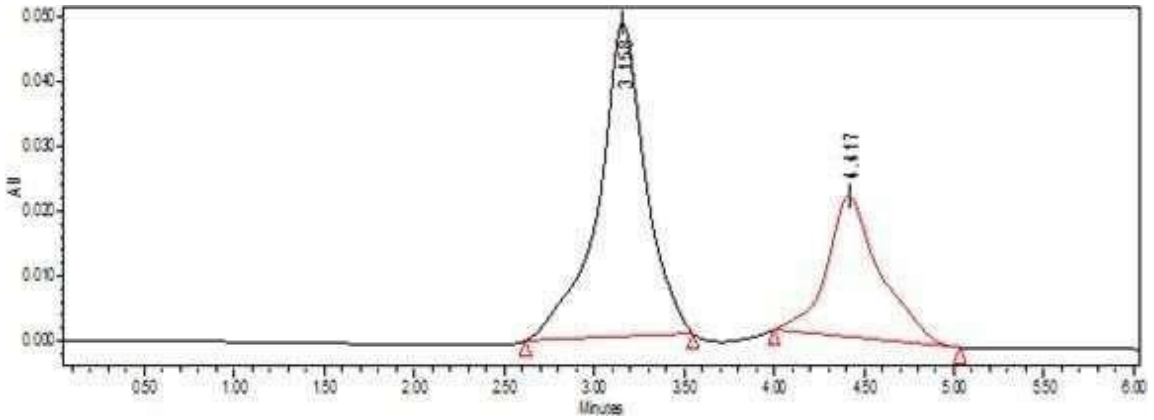
Inference: The baseline is unacceptable, and there are two clear peaks to be distinguished.

S.NO	The peak's name is	Time spent in retention (min)
1	**Meropenem**	2.902
2	**Vaborbactam**	3.618

The second trial

Phase of Mobility: A sonicator was used to combine and degas a 90:10V/V mixture of methanol and acetonitrile**.**

Fig. 2: Experiment 2 with chromatography:



Inference : It's only that we arrive at the apex first.

S.NO	The peak's name is	Time spent in retention (min)
1	**Meropenem**	3.158
2	**Vaborbactam**	4.417

Third trail: Methanol and degassed acetonitrile in an 80:20 V/V ratio in the mobile phase.

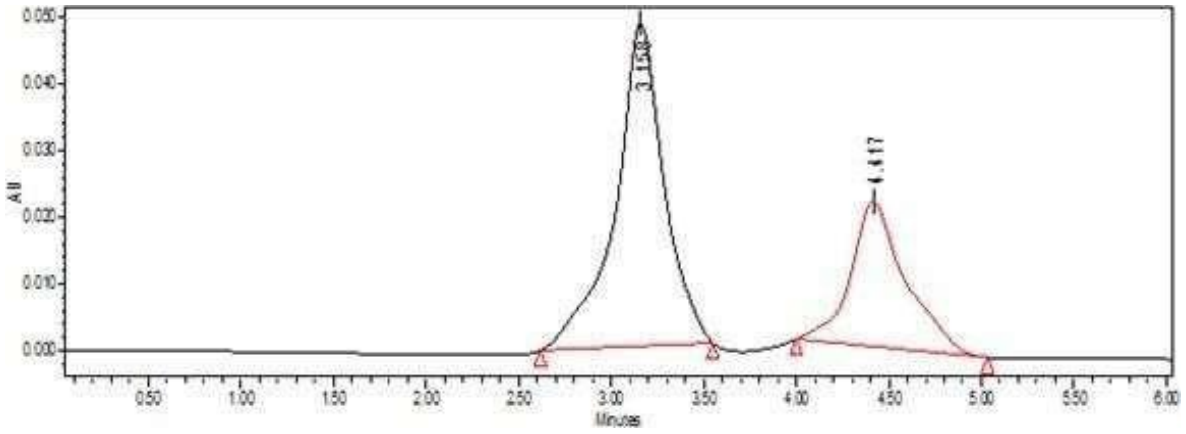


Fig. 3: Test of chromatography

Inference : The shape of the summits varies, but they are all unpleasant.

S.NO	The peak's name is	Retention time (in minutes)
1	**Meropenem**	2.618
2	**Vaborbactam**	3.602

OPTIMIZED METHOD:

Phase of Mobility: Methanol and buffer degassed in an 80:20 V/V ratio.

Preparation of Buffer(KH2PO4 0.1 M): Weigh 3.8954 g di-sodium hydrogen phosphate dissolves completely in 1000 mL distilled water and 3.4023 g potassium dihydrogen phosphate. After that, the pH 2.5 is adjusted using orthophosphoric acid, and the solution is passed through a 0.45m membrane filter.

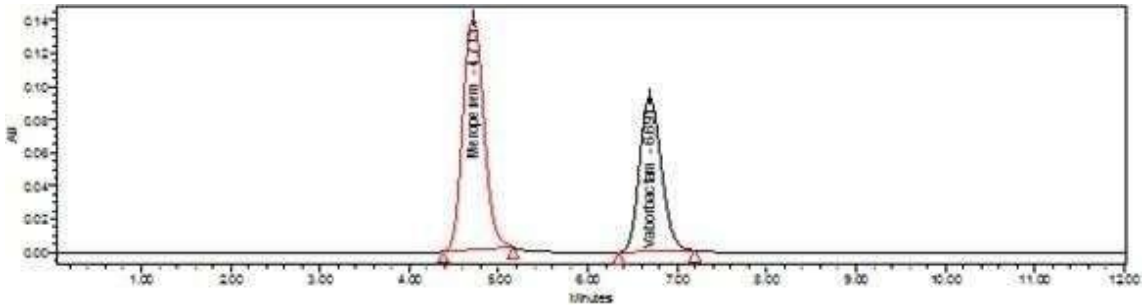


Fig 4: Chromatogram is a term that refers to a (standard) Inference: a 4.713min RT chromatogram to Meropenemand 6.691min to Vaborbactam

S.NO	The peak's name is	Time spent in retention (min)
1	**Meropenem**	4.713
2	**Vaborbactam**	6.691

3. METHOD VALIDATION:

SYSTEM OF THE SUITABILITY:

TABLE- 1(a): Information on the Meropenem System's Suitability

Injection	RT	Area of MaximumExpansion	Plate Count(USP)	Tailing (USP)
1	4.712	1412455	10023.845	1.158
2	4.707	1413987	10410.547	1.121
3	4.705	1418948	10236.874	1.141
4	4.706	1416555	10127.254	1.134
5	4.707	1412589	10184.658	1.145
Mean	4.704232	1414906	10336.8254	1.144
SD	0.00314	2796.31	-------	-------
% RSD	0.096627	0.197	-------	-------

TABLE-1(b): Information about the suitability of the Vaborbactam system

Injection	RT	Area of Maximum Expansion	Plate Count(USP)	Tailing (USP)
1	6.691	204258	8325.874512	1.036
2	6.684	204012	8384.547862	1.045
3	6.681	203902	8314.875424	1.056
4	6.680	204155	8372.784518	1.078
5	6.684	204365	8392.084512	1.033
Mean	6.686208	204138	8358.875421	1.047
SD	0.0.0507	185.59	-------	-------
% RSD	0.112138	0.090	-------	-------

Repeatability:

TABLE-2(i): Data on Meropenem Repeatability (System Precision)

	Injection	Meropenem peak areas	%Assay
40 parts per million	1	1413654	100.23
	2	1415782	100.38
	3	1412748	100.16
	4	1412320	100.13
	5	1414875	100.31
Analytical statistics	**Mean**	1415501	100.36
	SD	1414146	100.26
	% RSD	1454.28	0.103

TABLE-2(ii): Information on Vaborbactam Reliability (System Precise)

	Injection	Vaborbactam peak areas	%Assay
40 parts per million	1	204511	100.67
	2	204687	100.76
	3	204015	100.43
	4	204194	100.52
	5	204906	100.86
Analytical statistics	**Mean**	204735	100.78
	SD	204508	100.67
	% RSD	341.6513	0.167

Methodical precision:

TABLE-3 (I): Data on Meropenem Reproducibility (Method Precision)

	Injection	Meropenem peak areas	Assay percentage
40 parts permillion	1	1414856	100.31
	2	1413894	100.24
	3	1419876	100.67
	4	1418700	100.59
	5	1411845	100.10
	6	1410780	100.02
Analyticalstatistics	**Mean**	1414991	100.32
	SD	3646.13	0.258
	% RSD	0.257	0.257

TABLE-3(ii): Vaborbactam Repeatability (Method Precision) Data

	Injection	Vaborbactam's peak areas	Assay percentage
40 parts permillion	1	204324	100.58
	2	204578	100.70
	3	204689	100.76
	4	204714	100.77
	5	204177	100.51
	6	204866	100.85
Analyticalstatistics	**Mean**	204558	100.69
	SD	259.491	0.127
	% RSD	0.126	0.126

ACCURACY:

TABLE-4: Meropenem data

Percentage of spiking level concentration	**Amount increased (ppm)**	**Amount discovered (ppm)**	**% Recoverability**	**Percentage Recovery Statistical Analysis**	
50% Sample 1	20	20.05	100.28	**MEAN**	100.29
50% Sample 2	20	20.06	100.33		
50% Sample 3	20	20.05	100.26	**%RSD**	0.033
100 % Sample 1	40	40.05	100.14	**MEAN**	100.23
100 % Sample 2	40	40.09	100.23		
100% Sample 3	40	40.12	100.31	**%RSD**	0.083
150% Sample 1	60	60.09	100.01	**MEAN**	99.98
150% Sample 2	60	59.97	99.97		
150% Sample 3	60	59.99	99.98	**%RSD**	0.025

TABLE-5: Meropenem data

Percentage of spiking level concentration	Amount increased (ppm)	**Amount discovered (ppm)**	**% Recoverability**	**Percentage Recovery Statistical Analysis**	
50% Sample 1	20	20.17	100.88	**MEAN**	100.88
50% Sample 2	20	20.16	100.83		
50% Sample 3	20	20.18	100.92	**%RSD**	0.043
100 % Sample 1	40	40.24	100.62	**MEAN**	100.62
100 % Sample 2	40	40.21	100.54		

100% Sample 3	40	40.28	100.71	**%RSD**	0.084
150% Sample 1	60	60.28	100.47	**MEAN**	100.46
150% Sample 2	60	60.30	100.50		
150% Sample 3	60	60.24	100.40	**%RSD**	0.050

LINEARITY:

TABLE 6: Data on Linearity (Meropenem)

Concentration is required (ppm)	**AverageSurface Area**	**Analytical Statistics**	
0	0	Slope	35296
20	706445	y-Intercept	-1504
30	1059486	Correlation Coefficient	0.999
40	1412450		
50	1745687		
60	2119450		
70	2472451		
80	2825606		

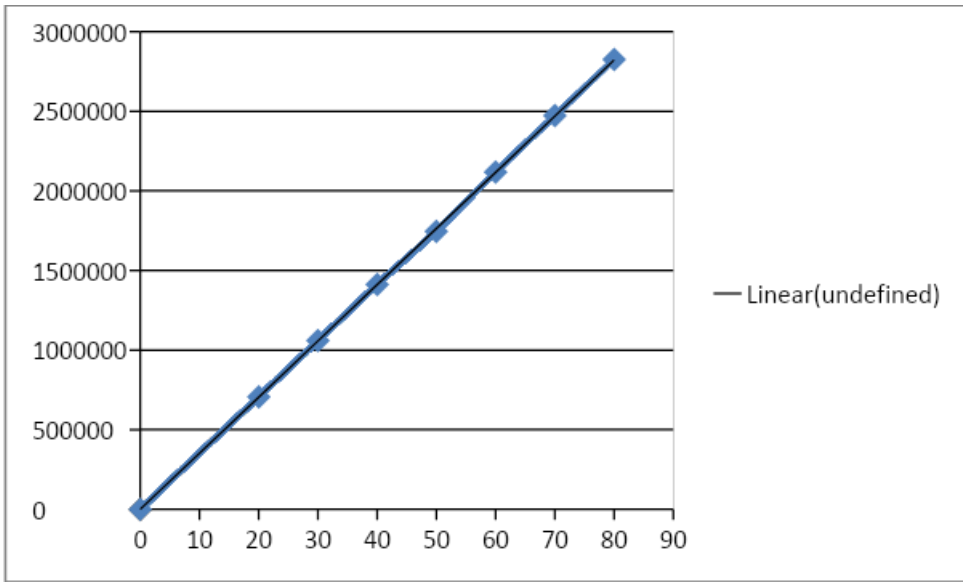
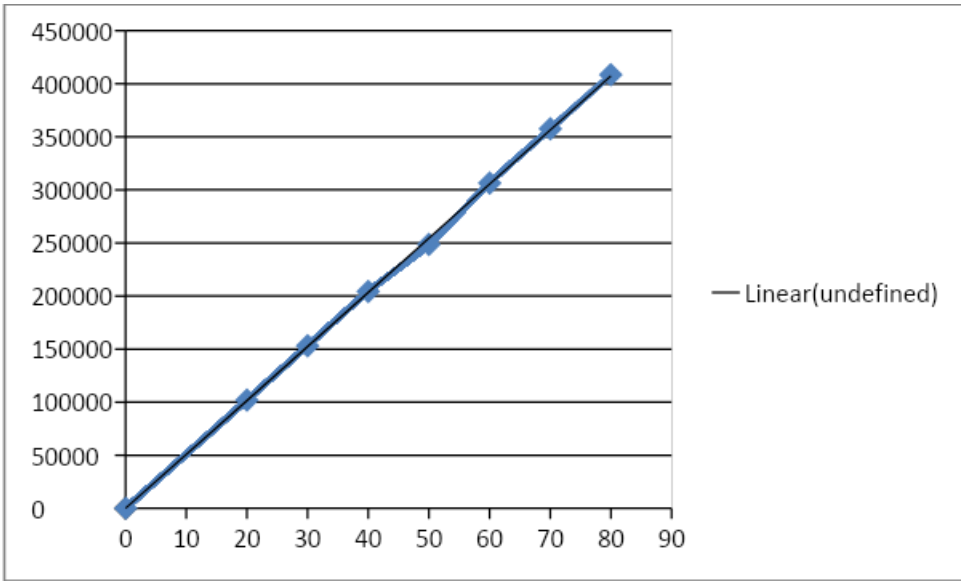


Fig: 5(a) Plot of Linearity (Concentration Vs Response)



Linearity information on vaborobactum

TABLE 7: Data on Linearity (Meropenem)

Concentration is required (ppm)	**Average SurfaceArea**	**Analytical Statistics**	
0	0	Slope	5099
20	102056	y-Intercept	-489.1

30	153287	Correlation Coefficient	0.999
40	204256		
50	248598		
60	306482		
70	357589		
80	408513		

Fig: 6(b) Plot of Linearity (Concentration Vs Response)

TABLE: 8(i) There's evidence that flow rate variability matters (Meropenem)

Flow 0.8 ml	Area of standard	a restraining element	1.0 mL Flow	Area of standard	a restraining element	1.2 mL Flow	Area of standard	restraining element
	1408784	1.011		1412118	1.051		1485978	1.089
	1408015	1.013		1415487	1.087		1483568	1.046
	1407009	1.011		1412689	1.056		1487521	1.084
	1407752	1.007		1412594	1.039		1485654	1.050
	1408329	1.016		1418745	1.045		1486895	1.064
Avg	1405452	1.010		1412290	1.035		1482058	1.086
SD	1407556	1.011	**Avg**	1413987	1.052	**Avg**	1485279	1.069
%RSD	1190.01	0.003	**SD**	2641.468	0.018	**SD**	2076.99	0.019

TABLE: 9(ii) Data on the influence of a change in flow rate (Vaborbactam)

Flow 0.8 ml	Area of standard	a restraining element	1.0 mL Flow	Area of standard	a restraining element	1.2 mL Flow	Area of standard	restraining element
	203145	1.078		204235	1.097		2022365	1.054
	203084	1.068		204298	1.078		2022984	1.036
	203198	1.058		204659	1.089		2022346	1.044
	203248	1.064		204879	1.086		2022684	1.024
	203265	1.044		204098	1.080		2022485	1.012
Avg	203942	1.048		204845	1.069		2022895	1.032
SD	203313	1.060	**Avg**	204502	1.083	**Avg**	2022626	1.033
%RSD	314.9785	0.015	**SD**	334.8275	0.009	**SD**	272.1358	0.014

4. Acknowledgment

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5. CONCLUSION:

To develop the analytical approach, various parameters were investigated. Meropenem and Vaborbactam, for example, have maximum absorbance at 244nm and 254nm, respectively. The most common wavelength will be 252nm, and the peaks will be extremely clear. The injection volume was set to 20 litres, which produced a great peak area. This piece used the Inertsil C18 column, and ODS chose a great peak shape. The temperature of the surrounding environment was assessed to be appropriate for the pharmaceutical solution type. The flow rate was set at 1.0ml/min due to the good peak area, adequate retention length, and good resolution. Different mobile phase ratios were explored, however due to its symmetrical peaks and good resolution, the mobile phase with the ratio of Methanol:Buffer (80:20) was chosen. As a result, this mobile phase was utilised in the intended investigation

6. REFERENCES

1. Organic Medicinal and Pharmaceutical Chemistry by Wilson and Gisvold, London, UK, 2004. 96-99 are the page numbers.

2. Indian Pharmacopoeia, Volume III, Controller of Publication, New Delhi, 2007. • P.1687 in Indian Pharmacopoeia, Volume III, Controller of Publication, New Delhi, 2007.

3. Controller of Publication, New Delhi, 2007, Indian Pharmacopoeia, volume III; pp. 143, 250, and 701

4. Merck laboratories, The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals, 14th ed., 2006; P. 385.

5. British Pharmacopoeia (British Pharmacopoeia) (British Pharmacopoeia) (British Pharmacopoeia) (Briti (1998). The Stationary Office of Her Majesty in London.

6 .DC Buss, AD Hutchings, S Scott, and PA Routledge (DC Buss, AD Hutchings, S Scott, and PA Routledge) (1990). A rapid liquid chromatographic approach can be used to quantify the amount of sumatriptan in human plasma. pp. 293-296 in Ther. Drug Monit., vol. 12, no. 2.