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Validated Contemporaneous Liquid Chromatographic Method for Quantification of Antibacterial drugs in Coformulation and Encompassing stress Degradation studies

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ABSTRACT

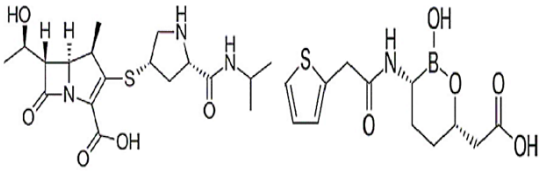
An unambiguous, precise, and accurate approach was created for the contemporaneous assessment of Meropenem (MPM) and Vaborbactam (VBM) in pharmaceutical dosage form and bulk. For the chromatogram, Standard Zorbax column C18 (4.6x150 mm, 5 µm) was utilized. Mobility Phase with buffer : One mL of 65 formic acid:35 acetonitrile was poured into a column at a time. 0.1% Formic acid buffer was utilized in this procedure. A constant temperature of 30°C was maintained. The ideal wavelength was chosen as 220 nm. Retention time of VBM and MPM were found to be 3.953, 2.364 minutes. MPM and VBM have respective RSD of 1.3 and 0.6. The recovery rates were 100.31% and 100.30% with MPM and VBM, respectively. LOD and LOQ values for MPM and VBM were 0.53, 1.62, and 0.52, 1.57, respectively, according to the regression models. The regression formula for MPM was y = 21101x + 10155, and for VBM was y = 21037x + 22037. The method was created in a simple and economical way, and it may be applied in many industries for regular quality control testing because the retention and run times were reduced.

Keywords: Meropenem, Vaborbactam, RP-HPLC.

INTRODUCTION

MBM is a broad-spectrum antibiotic of carbapenem. It invades bacterial cells and prevents cell wall formation. Experiments on subjects with normal renal function, subjects with bacterial infections, and those with Different Renal Insufficiency Levels. Adult patients with complex urinary tract infections (cUTIs) were given approval in August 2017 to receive treatment with a combined antibiotic drug known as vabomere. Intravenous administration of VBM and MPM is the mode of action for Vabomere Upon conducting a comprehensive literature review, it was discovered that a limited number of HPLC methods and a single LC-MS method[1] were available for the simultaneous quantification of MPM and VBM in parentals. There are less LC-MS and other analytical techniques available for measuring

MPM and VBM by themselves or in conjunction with other drugs[2–9]. Thus, the current work aimed to develop a uncomplicated, speedy, accurate, and verified stability-indicating RP-HPLC approach to contemporaneous evaluation of the parenteral as well as bulk dose forms of Meropenem and Vaborbactam.



(a) (b)

Fig. 1. Structure of Meropenem (a) and Vaborbactam (b)

EXPERIMENTAL

Chemicals

Aurobindo Laboratory, Hyderabad, India provided a free sample of the medications MPM and VBM. We bought a commercial VABOMERE injection from the neighbourhood market, which included both MPM and VBM. The components used are Methanol, Acetonitrile, Potassium dihydrogen Orthophosphate buffer, Orthophosphoric acid, Formic acid and AR-grade distilled water from Rankem.

Instruments

Denver Electronics Balance: pH meter and Ultrasonicator, Waters 2695 HPLC with Empower 2 software. UV-Vis spectrophotometer of UV Win 6 software, and bandwidth fixed of 2 mm and 10 mm.

Standard stock solution preparation

25 mg of each MPM and VBM were precisely taken into a 50 mL volumetric flask. Mixture of acetonitrile and water. (50:50) used as diluent and sonicated for ten minutes. End volume was made up to the mark (500 µg/mL VBM and 500 µg/mL MPM) with diluent labelled as "Standard stock solution".

Standard working solution (100% solution) preparation

End concentrations 50 µg/mL VBM and 50 µg/mL MPM were made by transferring 1 mL stock solution each into volumetric flask holding 10 mL capacity with Diluent.

Sample stock solution preparation

1 g of the dry powder each of VBM and MPM (for injection) were added to 500 mL volumetric flask, adding 5 mL of acetonitrile sonication was performed, Diluents were used to increase the volume to 500 mL. Finer porosity membrane filter was used to filter the mixture (500 µg/mL of MPM, 500 µg/mL of VBM).

Working sample solution (100% solution) preparation

50 µg/mL of MPM and 50 µg/mL of VBM were made by transferring 1 mL of filtered sample stock solution to 10 mL Volumetric flask. Dilutions were made with diluent.

Buffer Preparation

0.1% Formic acid: 1 mL Formic acid made up to 1000 mL with HPLC grade water.

Method validation

Method validation of was performed accordingly to standard ICH guidelines for Linearity, Accuracy, Precision, Sensitivity and Robustness.

Forced degradation studies

MPM and VBM powder, the API, was forced to a variety of stress to ascertain if the analytical method was stable. In order to evaluate MPM and VBM capacity to be separated from their breakdown products using the suggested procedure, deliberate degradation studies under stress conditions such as acidic (2N HCl), basic (2N NaOH), neutral (water), peroxide degradation (20% H_2O_2), photo stability studies, and thermal treatment (heated at 80°C) were conducted. To 1 mL of each stock solution 1mL of Hcl (2N) added, refluxed for 30 min with 60°C to achieve forced degradation in acidic medium. Diluting the results to produce 50 µg/mL, 10 µL solutions were injected, chromatograms were evaluated to check sample's stability. The drug's light stability was further investigated by introducing the 500 µg/mL solution to UV light. Using 200-watt hours per square meter in a photo stability laboratory or spending a day in a UV chamber. Chromatograms were obtained for the HPLC analysis by introducing 10 µL into the system after diluting the resulting solution to get solutions at 100 µg/mL.

Tests for alkali (NaOH), heat (80°C), peroxide (H_2O_2), and neutral degradation were carried out in a similar way.

RESULTS AND DISCUSSION

Several chromatographic experiments were examined while developing a novel HPLC approach to ascertain the ideal chromatographic conditions for the simultaneous detection of MPM and VBM. Numerous factors were thoroughly analyzed, including the optimum pH, columns, injection volumes, detector wavelength, flow velocity, ideal mobile phases with different ratios, and standard solution concentrations. To create the chromatographic separation, a Zorbax column C18 (4.6 x 150 mm, 5 µm) was ultimately employed. This procedure employed 65% formic acid buffer: 35% acetonitrile as the stage of mobility. It was conducted with10 µL injection volume, detection Wavelength 220 nm, flow rate 1 mL /min, and ambient Temp. (30°C). Chromatogram of the enhanced procedure was shown in (Fig. 2). The characteristics for system appropriateness were shown in Table 1.

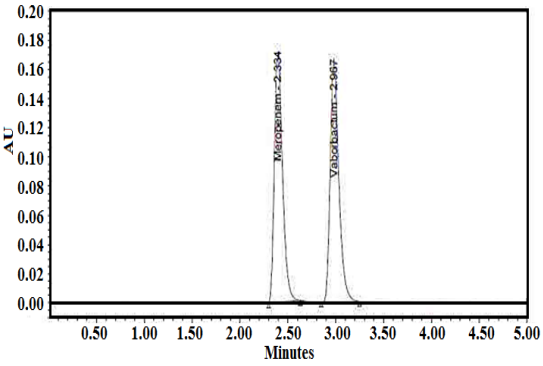


Fig. 2. Optimized chromatogram of MPN and VBM

Observation: Time of elution for MPM and VBM were 2.334 and 2.967 min, respectively, shows high resolution, with good plate count and tailing factor.

System suitability

Table 1: System suitability parameters of meropenem and vaborbactam

Sr. No	Meropenem			Vaborbactam			
INJ	Retention Time (Min)	Plate Count USP	T. F	Retention Time (min)	Plate Count USP	T. F	Resolution
1	2.364	3500	1.32	2.953	5000	1.36	3.5
2	2.380	3551	1.34	2.976	4898	1.37	3.6
3	2.389	3448	1.36	2.994	4897	1.35	3.5
4	2.393	3462	1.37	2.999	5036	1.35	3.6
5	2.398	3559	1.33	3.000	5089	1.36	3.5
6	2.405	3581	1.33	3.009	4937	1.35	3.6

Specificity

Meropenem and Vaborbactam had respective retention time of 2.364 mins and 3.953 minutes. In the blank and placebo, no contradictory peaks were found using this method.

Linearity

The least squares regression approach was used to assess the linearity. Six linear injection volumes of Vaborbactam (25-150 µg/mL) and Meropenem (25-150 µg/mL) were carried out in duplicate. Linearity equations y = 21101x + 10155 for Meropenem and y = 21037x + 22037 for Vaborbactam were found. The R^2 value was 0.999 for the two drugs.

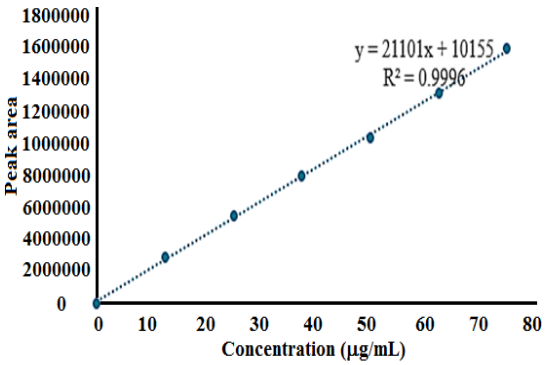


Fig. 3. Calibration Curve for Meropenum

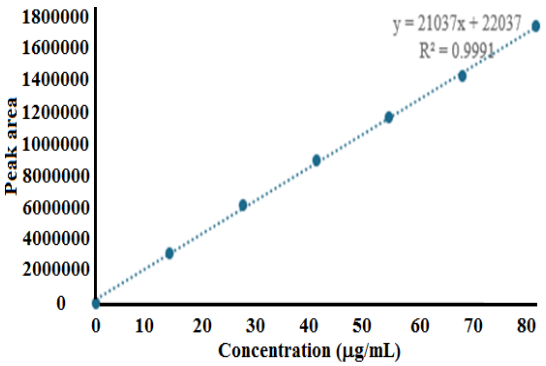


Fig. 4. Calibration Curve for Vaborbactam

Precision

The %RSD for Meropenem and Vaborbactam was determined to be 0.6 and 1.3 respectively.

Table 2: Precision of Meropenem and Vaborbactam

Sr. No	Area under Meropenem	Area under Vaborbactam
1	1030108	1028422
2	1032554	1041428
3	1045006	1010252
4	1035575	1031598
5	1039100	1023597
6	1042597	1048763
Mean	1037490	1030677
S. D	5791.2	13534.6
%RSD	0.6	1.3

Repeatability

Average area, SD, and RSD were determined as 1.0% and 0.9%, respectively For MPM and VBM.

Table 3: Repeatability with Meropenem and Vaborbactam

Sr.No	Area of Meropenem	Area of Vaborbactam
1	1048422	1042620
2	1041428	1041103
3	1030252	1019486
4	1041598	1022918
5	1023597	1032526
6	1048763	1025182
Mean	1039010	1030639
S. D	10104.6	9699.3
%RSD	1.0	0.9

Intermediate precision

After sample preparation, each working sample injection solution was administered the following day. Resulted areas are displayed in the above table. Six working sample solutions which has equal concentrations were created following sampling serially taken from a sample stock solution. Following the computation of the SD, average area and %RSD for the two drugs, MPM and VBM came out at 0.8% and 0.6%, respectively.

Table 4: Intermediate precision of Meropenem and Vaborbactam

Sr. No	Meropenem area	Vaborbactam area
1	1023367	1019437
2	1008277	1013962
3	1008668	1013668
4	1020929	1026095
5	1026801	1010335
6	1024889	1023754
Mean	1018822	1017875
S. D	8244.6	6235.9
%RSD	0.8	0.6

Accuracy

Three doses were administered for every accuracy and mean level. For MPM and VBM, the recoveries were 100.31% and 100.30%, respectively.

Table 5: Accuracy Meropenem

%Level	Spiked Amount (µg/mL)	Recovery Amount (µg/mL)	%Recovery	Mean %Recovery
50%	25	25.16494	100.66	
	25	25.33661	101.35	
	25	25.25602	101.02	
100%	50	49.37492	98.75	100.31%
	50	49.97285	99.95	
	50	49.82547	99.65	
150%	75	74.33919	99.12	
	75	75.6486	100.86	
	75	76.04757	101.40	

Table 6: Accuracy Vaborbactam

%Level	Spiked Amount (µg/mL)	Recovery Amount (µg/mL)	%Recovery	Mean %Recovery
50%	25	24.73737	98.95	
	25	25.36379	101.46	
	25	25.16552	100.66	
100%	50	49.98645	99.97	100.3%
	50	50.34915	100.70	
	50	49.82022	99.64	
150%	75	74.22741	98.97	
	75	75.27723	100.37	
	75	76.45444	101.94	

Sensitivity

LOD and LOQ values noted as 0.53 and 0.52 µg/mL^{-1} for MPM and 1.62 and 1.57µg/mL^{-1} for VBM, reported in Table 7, which indicates the sensitivity of the method.

Table 7: Sensitivity of Meropenem and Vaborbactam

Antibacterial drug	LOD (µg/mL)	LOQ (µg/mL)
Meropenem	0.53	0.52
Vaborbactam	1.62	1.57

Robustness

Samples were injected in duplicate, and different robustness conditions were maintained.

Assay studies

The Melanta therapeutics, bearing the label claim containing meropenem 1 g + Vaborbactam 1 g (Vabomere injection, sterile powder for reconstitution). The formulation mentioned above was used for the assay. Vaborbactam and

Meropenem yielded average assay percentages of 99.64 and 99.33, respectively.

Table 8: Robustness for Meropenem and Vaborbactam

Sr. No	Parameters	Meropenem %RSD	Vaborbactam %RSD
1	(-) 0.9 mL/min Flow rate	0.7	0.7
2	(+) 1.1 mL/min Flow rate	0.8	0.4
3	Mobile phase (-) 60B:40A	0.5	0.2
4	Mobile phase (+) 70B:30A	1.4	0.6
5	Temp (-) 25°C	1.3	0.1
6	Temp (+) 35°C	0.6	0.9

Table 9: Assay studies of Meropenem

Sr. No	Area under Standard	Area under Sample	%Assay
1	1030108	1048422	100.95
2	1032554	1041428	100.28
3	1045006	1030252	99.20
4	1035575	1041598	100.30
5	1039100	1023597	98.56
6	1042597	1048763	100.99
Avg	1037490	1039010	100.05
Stdev	5791.2	10104.6	0.97
% RSD	0.6	1.0	1.0

Table 10: Assay studies of Vaborbactam

Sr. No	Area of Standard	Area of Sample	% Assay
1	1028422	1042620	101.06
2	1041428	1041103	100.91
3	1010252	1019486	98.82
4	1031598	1022918	99.15
5	1023597	1032526	100.08
6	1048763	1025182	99.37
Avg	1030677	1030639	99.90
S. D	13534.6	9699.3	0.9401
%RSD	1.3	0.9	0.9

Forced Degradation Studies data

Table 11: Degradation studies for MPM and VBM

Degradation method	Meropenem			Vaborbactam		
	Area under curve	%Recovery	%Degrad ation	Area under curve	% Recovery	%Degrad ation
Acidic	984527	94.80	5.20	980913	95.08	4.92
Basic	1006077	96.88	3.12	998158	96.75	3.25
Peroxide	1010808	97.33	2.67	1008711	97.77	2.23
Thermal	1022946	98.50	1.50	1017546	98.63	1.37
Uv	1024714	98.67	1.33	1020511	98.91	1.09
Water	1035045	99.66	0.34	1026660	99.51	0.49

CONCLUSION

The approach that was devised was quick, easy to use, accurate, precise, and inexpensive. All validation metrics were found to be highly satisfactory based on the results. The suggested approach was used to examine the stress degradation studies of MPM and VBM, and the results showed that the degradation peaks were clearly isolated from the sample peak. The created technique was effectively used to quantify MPM and VBM simultaneously in parenteral dose form without interference.

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Conflicts of interests

According to the authors, there is no conflict of interest.

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