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Development and Validation of Stability Indicating UPLC Method for Simultaneous Estimation of Meropenem and Vaborbactam in Pharmaceutical Dosage Form

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Abstract
The objective of the method was to develop a simple, rapid, sensitive, precise, accurate and validated Ultra Performance Liquid Chromatographic (UPLC) method for the simultaneous estimation of Meropenem and Vaborbactam in pharmaceutical dosage form. Chromatographic separation was achieved on an acquity UPLC CHS C18 (2.1 x 100mm, 1.8µm) column with a mobile phase composed of 0.01N Potassium dihydrogen phosphate buffer and Acetonitrile in the ratio of 55:45 of 4.8pH at a flow rate of 0.2 ml/min and 1 µl injection volume. The effluents were detected at a wavelength of 260 nm using TUV detector. The retention times of Meropenem and Vaborbactam were found to be 0.665 and 0.875 min respectively. The method was validated with respect to specificity, accuracy, linearity, precision, robustness. The correlation coefficient for Meropenem and Vaborbactam were found to be 0.999 and 0.999 respectively. Recovery of Meropenem and Vaborbactam in formulation was found to be 99.93% and 99.62% respectively. Due to simplicity, high precision and rapidness the method can be successfully applied for simultaneous estimation of Meropenem and Vaborbactam in combined dosage form.

Keywords
Meropenem and Vaborbactam

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INTRODUCTION:
Meropenem, chemically 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 is a broad-spectrum carbapenem antibiotic It is active against Gram-positive and Gram-negative bacteria. Meropenem exerts its action by penetrating bacterial cells readily and interfering with the synthesis of vital cell wall components, which leads to cell death. The structure of Meropenem was shown in **Fig. 1**.

Vaborbactam has been used in trials studying the treatment of Bacterial Infections, Subjects with Normal Renal Function, and Subjects with Varying Degrees of Renal Insufficiency.

The chemical name of vaborbactam is 2-[(3R, 6S)-2-hydroxy-3-[2-(thiophen-2-yl) acetamido]-1,2-oxaborinan-6-yl] acetic acid. The structure of Vaborbactam was shown in **Fig. 2**. In August 2017, a combination antibacterial therapy under the market name vabomere was approved for treatment of adult patients with complicated urinary tract infections (cUTI). Vabomere consists of vaborbactam and Meropenem and is intravenously administered. The treatment aims to resolve infection-related symptoms and achieve negative urine culture, where the infections are proven or strongly suspected to be caused by susceptible bacteria.

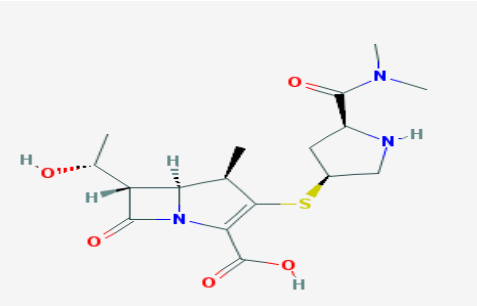

Fig. 1: Chemical Structure of Meropenem

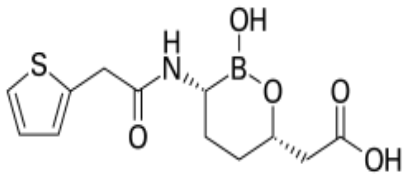

Fig. 2: Chemical Structure of Vaborbactam

The literature survey revealed that there are few stability indicating RP-HPLC1,2,3 , UV4 and LC-MS5 methods are available for the estimation of Meropenem . RP-HPLC6 method for Meropenem and vaborbactam combination have been reported. However, a stability indicating UPLC method was not available. Hence, present work focused on the development and validation of a simple, rapid, robust and economic stability indicating UPLC method. To the best of our knowledge the anticipated method is the first UPLC method to allow simultaneous estimation of Meropenem and vaborbactam in tablet dosage form.

MATERIALS AND METHODS

Instrumentation:

The separation was carried on Waters Acquity UPLC 2996 with Empower 2 software that consisted of a binary solvent manager equipped with automatic sampler. An acquity UPLC CHS C18, 2.1 ×100 mm, 1.8 µ column was used for separation of active ingredients. Analytes were monitored with TUV detector at a wavelength 260 nm. Ultrasonicator was used to remove dissolved gases and air bubbles in the mobile phase.

Chemicals and Reagents:

Meropenem and vaborbactam standard samples were obtained as gift samples from Spectrum Labs, Hyderabad. HPLC grade water and methanol were purchased from Merck Ltd., Mumbai. Analytical grade acetonitrile and orthophosphoric acid were obtained from Rankem, Avantor Performance Material India Ltd. Marketed formulation of combination was purchased from local market.

Chromatographic Conditions:

Separation of analytes was achieved with a mobile phase consisting of 0.01N potassium dihydrogen phosphate buffer and acetonitrile at a ratio of 55:45 delivered at a flow rate of 0.3ml/min through column kept at 25 ºC. The volume of injection was 1 µl and runtime was 2min. The eluents were detected at a wavelength 260 nm. Chromatograms of standard and optimized method were shown **Fig. 3** and **4**.

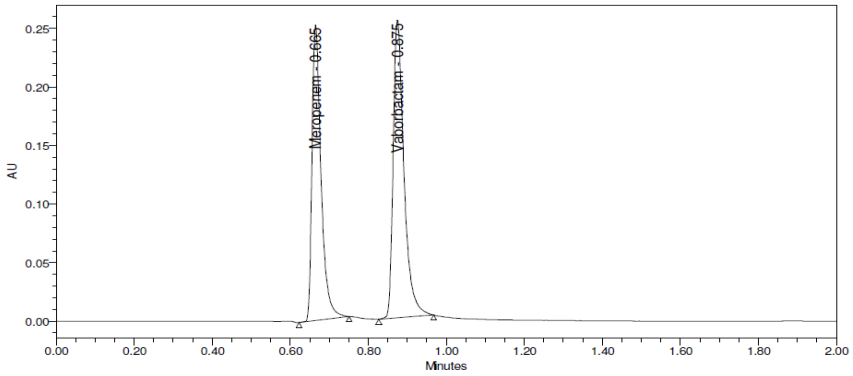

Fig. 3: Chromatogram of Optimized Method

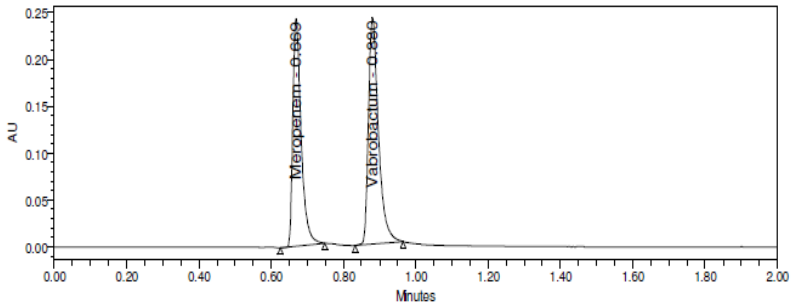

Fig. 4: Chromatogram of Standard Preparation

Preparation of Standard stock solutions:
Accurately weighed 50 mg of Meropenem, 50 mg of Vaborbactam and transferred to individual 50 ml volumetric flasks separately. 3/4 th of diluents was added to both of these flasks and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution 1and 2. (1000µg/ml of Meropenem and 1000µg/ml of Vaborbactam). 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (100µg/ml Meropenem)

Preparation of Sample stock solutions:
1g of dry powder (for injection) was weighed and transferred to 100 ml volumetric flask, to this 50 ml of diluent was added and sonicated. Volume was made upto 100 ml with diluents and filtered through 0.45 µm or finer porosity membrane filter (10000µg/ml of Meropenem and 10000µg/ml of Vaborbactam). 0.1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (100µg/ml of Meropenem and 100µg/ml of Vaborbactam.

Method Validation:

Table 1: System Suitability Results of Meropenem and Vaborbactam

S no	Meropenem			Vaborbactam			
Inj	RT (min)	USP Plate Count	Tailing	RT (min)	USP Plate Count	Tailing	Resolution
1	0.669	4358	1.57	0.879	5212	1.53	4.6
2	0.669	4338	1.57	0.879	5201	1.53	4.6
3	0.669	4342	1.57	0.879	5206	1.53	4.6
4	0.669	4328	1.57	0.880	5180	1.52	4.6
5	0.669	4338	1.57	0.880	5182	1.52	4.6
6	0.669	4338	1.57	0.880	5182	1.52	4.6

Table: 1 System suitability parameters for Meropenem and Vaborbactam

Specificity:
The specificity is the ability of an analytical method to assess unequivocally the analyte of interest in the presence of components that may be expected to be present, in the sample matrix.

Linearity:
The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a

given linearity. The linearity of the chromatographic method was tested by plotting peak area against concentrations (minimum of 5 concentration levels). The concentrations were prepared in a range of 25-150 ppm (25%-150%). Linear regression equation and correlation coefficient (R^2) were employed to statistically evaluate the linearity results. The calibration curves were shown in **Fig. 5** and **6**.

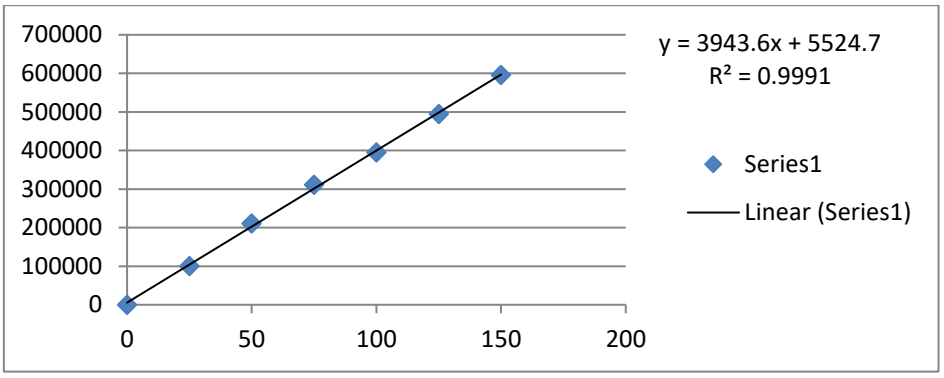

Fig No. 5 Calibration curve of Meropenem

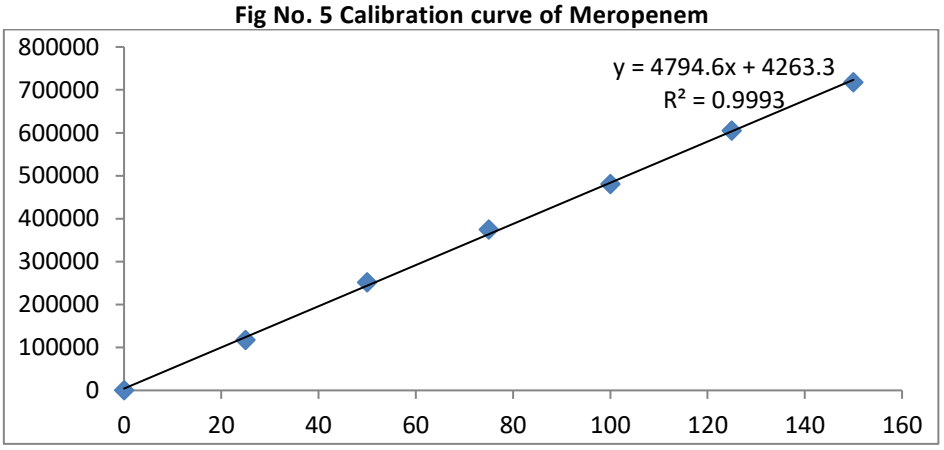

Fig No. 6 Calibration curve of Vaborbactam

Accuracy:

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of the method was carried by determining the recovery studies at three different concentration levels (50%, 100% and 150%) repeated three times. The percentage recovery and mean were calculated. The results of three concentration levels were shown in Table 2 & 3

Table 2. Accuracy table of Meropenem

% Level	Amount Spiked(µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery
50%	50	49.74	99.48	99.93%
	50	49.62	99.24	
	50	49.45	98.90	
100%	100	98.74	98.74	
	100	101.05	101.05	
	100	98.44	98.44	
150%	150	152.45	101.63	
	150	152.84	101.89	
	150	150.03	100.02	

Table 6.6: Accuracy table of Vaborbactam

% Level	Amount Spiked(µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery
50%	50	49.42	98.85	99.62%
	50	49.68	99.36	
	50	49.54	99.07	
100%	100	99.35	99.35	
	100	99.26	99.26	
	100	99.79	99.79	
150%	150	151.06	100.71	
	150	151.72	101.15	
	150	148.62	99.08	

Precision:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogenous sample. The precision of the analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of series of measurement. The precision of the proposed method was determined by intraday and inter day precision. For intra-day precision six replicates of test preparations were injected on the same day into chromatographic system and calculated the percentage assay and percentage RSD. For inter-day precision six replicate test samples of same concentrations were injected on two different days. The percentage assay and percentage RSD were calculated.

Limit of Detection and Limit of Quantification (LOD and LOQ):

The limit of detection is the point at which a measured value is larger than the uncertainty associated with it. It is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified. The limit of quantitation is the lowest injected amount that produces quantitative measurements in the target matrix with acceptable precision in chromatography. The quantitative limit is particularly used for the determination of impurities and degradation products. The results were shown in **Table 3**.

Table 3: Lod And Loq Results of Meropenem And Vaborbactam

Parameters	MEROPENEM	VABORBACTAM
Number of samples	6	6
Correlation range (µg /mL)	100-600 (µg/mL)	25-150 (µg/mL)
Regression coefficient	0.9992	0.9995
Limit of Quantification (µg/mL)	0.37	1.24
Limit of Detection (µg/mL)	0.12	0.41

Robustness:

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. The method was evaluated for robustness by changing different parameters like variation in temperature by ± 5% (20 °C and 30 °C) and variation in flow rate ± 0.1% (0.1 ml/min and 0.3 ml/min). The results implied that there was no marked change in system suitability parameters which makes the developed UPLC method a robust method.

Assay:

AstraZeneca pharmaceuticals (VABOMERE), bearing the label claim Meropenem 1000mg, Vaborbactam 1000mg. Assay was performed with the above formulation. Average % Assay for Meropenem and Vaborbactam obtained was 100.03% and 100.30% respectively. And shown in table 4.

Table 4. Assay Data of Meropenem and Vaborbactam

S.no	Standard Area Meropenem	Standard Area Vaborbactam	Sample area Meropenem	Sample area Vaborbactam	% Assay Meropenem	% Assay Vaborbactam
1	388408	470872	391638	473226	100.39	100.90
2	389101	467062	388395	465602	99.56	99.27
3	390788	467421	390564	472268	100.11	100.69
4	387946	467057	389897	471604	99.94	100.55
5	388329	471161	390242	475718	100.03	101.43
6	391525	464931	390755	464269	100.16	98.99
Avg	389350	468084	390249	470448	100.03	100.30
Stdev	1467.1	2438.1	1081.3	4512.3	0.28	0.96
%RSD	0.4	0.5	0.3	1.0	0.3	1.0

Forced Degradation Studies:

Forced degradation studies were conducted to know the stability of the method. The degradation studies were carried out by applying various stress conditions for the product like acid stress, base stress, UV stress, humidity stress, thermal stress and oxide stress. Degradation peaks were observed only in acid stress and peroxide stress and all degradation peaks were well resolved from analyte peaks. The results of forced degradation studies were shown in **Table 5**.

Table 5: Results of Forced Degradation Studies

Type of degradation	Meropenem			Vaborbactam		
	Area	%Recovered	% Degraded	Area	%Recovered	% Degraded
Acid	371193	95.15	4.85	446966	95.30	4.70
Base	374330	95.95	4.05	449323	95.80	4.20
Peroxide	368723	94.51	5.49	456073	97.24	2.76
Thermal	377886	96.86	3.14	460908	98.27	1.73
Uv	385858	98.91	1.09	463346	98.79	1.21
Water	388394	99.56	0.44	468889	99.97	0.03

RESULTS AND DISCUSSION:

Results of system suitability parameters shown uniformity and %RSD was 0.9 for both Meropenem and vaborbactam which implies the system is suitable for the proposed method. The specificity of the method was determined by standard chromatogram and formulation chromatogram. There was no interference of placebo or excipient peaks with standard or analyte peaks. Therefore, the developed method was specific. Accuracy of the method was determined by recovery studies. The mean recoveries of Meropenem and vaborbactam were found to be 98% to 100% (limit 98%-102%) which indicated a good accuracy for the analysis of two drugs. The linearity of the method was determined by plotting a calibration curve for concentration and area.

The correlation coefficient values for Meropenem and vaborbactam were 0.999 and 0.999 respectively. The precision was determined by carrying Intra-day and inter-day variations in terms of % RSD and the values were within limits (NMT 2%) which revealed that the method was precise. Robustness was determined by making small changes in chromatographic conditions and the results showed the method was robust. Degradation studies were performed under different conditions and there was no marked degradation except in acid stress. The degradation studies implied that there is no interference of degradants with the analytes peak.

CONCLUSION:

The developed UPLC analytical method provides an ecofriendly, reliable, reproducible, simple, rapid, sensitive, accurate, precise and specific assay method for the simultaneous estimation of Meropenem and vaborbactam in pharmaceutical formulations. Degradation studies reveal that the developed method was stability indicating. Hence the proposed method can be conveniently used for the routine analysis of Meropenem and vaborbactam in pure and pharmaceutical dosage forms.

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