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ANALYTICAL METHOD DEVELOPMENT VALIDATION OF MEROPENEM AND VABORBACTAM OF PURE AND DOSAGE FORMS USING RP-HPLC METHOD

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Meropenem and Vaborbactam in Tablet dosage form. Chromatogram was run through Agilent C18 (150 x 4.6 mm, 5µ) Mobile phase containing Buffer 0.01N KH_2PO_4: Methanol taken in the ratio 50:50 was pumped through column at a flow rate of 0.8 ml/min. PH adjusted to 5.0 with dil. Orthophosphoric acid solution. Temperature was maintained at 30°C. Optimized wavelength selected was 260 nm. Retention time of Meropenem and Vaborbactam were found to be 2.1119 min and 2.654 min. %RSD of the Meropenem and Vaborbactam were and found to be 0.7 and 0.7 respectively. %Recovery was obtained as 99.52% and 100.04% for Meropenem and Vaborbactam respectively. LOD, LOQ values obtained from regression equations of Meropenem and Vaborbactam were 0.07, 0.21 µg/ml and 0.07, 0.21 µg/ml respectively. Regression equation of Meropenem is y = 8848.x + 698.1, and y = 8748.x + 998.1of Vaborbactam. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

KEYWORDS: Meropenem, Vaborbactam, RP-HPLC.

INTRODUCTION

The quality of a drug plays an important role in ensuring the safety and efficacy of the drugs. Quality assurance and control of pharmaceutical and chemical formulations is essential for ensuring the availability of safe and effective drug formulations to consumers. Hence Analysis of pure drug substances and their pharmaceutical dosage forms occupies a pivotal role in assessing the suitability to use in patients. The quality of the analytical data depends on the quality of the methods employed in generation of the data[1] Hence, development of rugged and robust analytical methods is very important for statutory certification of drugs and their formulations with the regulatory authorities.

The quality and safety of a drug is generally assured by monitoring and controlling the assay and impurities effectively. While assay determines the potency of the drug and impurities will determine the safety aspect of the drug. Assay of pharmaceutical products plays an important role in efficacy of the drug in patients.

The wide variety of challenges is encountered while developing the methods for different drugs depending on its nature and properties. This along with the importance of achieving the selectivity, speed, cost, simplicity, sensitivity, reproducibility and accuracy of results gives an opportunity for researchers to come out with solution to address the challenges in getting the new methods of analysis to be adopted by the pharmaceutical industry and chemical laboratories. Different physico-chemical methods (1) are used to study the physical phenomenon that occurs as a result of chemical reactions. Among the physico-chemical methods, the most important are optical (refractometry, polarimetry, emission and fluorescence methods of analysis), photometry (photocolorimetry and spectrophotometry covering UV-Visible, IR Spectroscopy and nepheloturbidimetry) and chromatographic (column, paper, thin layer, gas liquid and high performance liquid chromatography) methods. Methods such as nuclear magnetic resonance (NMR) and para magnetic resonance (PMR) are becoming more and more popular. The combination of mass spectroscopy (MS) with gas chromatography is one of the most powerful tools available. The chemical methods include the gravimetric and volumetric procedures which are based on complex formation; acid-base, precipitation and redox reactions. Titrations in non-aqueous media and complexometry have also been used in pharmaceutical analysis. The number of new drugs is constantly growing. This requires new methods for controlling their quality. Modern pharmaceutical analysis must need the following requirements.

1. The analysis should take a minimal time.
2. The accuracy of the analysis should meet the demands of Pharmacopoeia.
3. The analysis should be economical.
4. The selected method should be precise and selective.

1.1 Chromatography

Chromatography (Chroma means 'color' and graphein means to 'write') is the collective term for a set of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a stationary phase,[2-4] which separates the analyte to be measured from other molecules in the mixture based on differential partitioning between the mobile and stationary phases. Differences in compounds partition coefficient results in differential retention on the stationary phase and thus changing the separation.

Different types of chromatographic techniques were summarized in

Table 1.1 Different types of chromatographic techniques.

Sl. No	Basic principle involved	Type of Chromatography
1.	Techniques by chromatographic bed shape	Column chromatography
		Paper chromatography
		Thin layer chromatography
2	Techniques by physical state of mobile phase	Gas chromatography
		Liquid chromatography
3	Affinity chromatography	Supercritical fluid chromatography
4	Techniques by separation mechanism	Ion exchange chromatography
		Size exclusion chromatography
5	Special techniques	Reversed phase chromatography
		Simulatedmoving-bed chromatography
		Pyrolysis gas chromatography
		Fast protein liquid chromatography
		Counter current chromatography
		Chiral chromatography

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for further use (and is thus a form of purification). Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportion of analytes in a mixture.

3. Drug Profile[25-39]

Meropenem

Meropenem 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.

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 meropenem and Vaborbactam and is intravenously admininstered. 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.

Structure

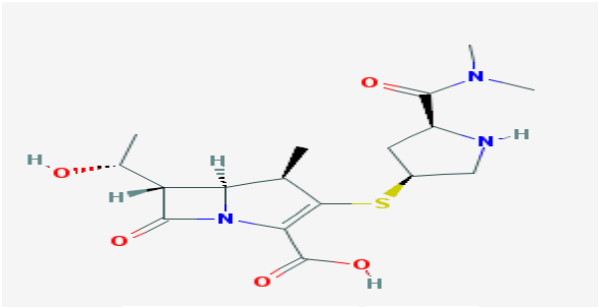


Fig. 2.1: Meropenem structure.

Vaborbactam

Description: 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.

Structure

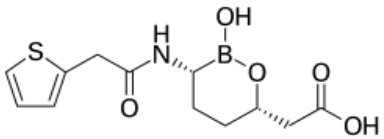


Fig.2.2: Vaborbactam.

5. MATERIALS AND METHODS

Materials

- Meropenem and Vaborbactam pure drugs (API) received from Aurobindo pharma ltd.

- Combination Meropenem and Vaborbactam Injection (**Vabomere**) Manufactured by: Facta Farmaceutici
- Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem

Instruments

- Electronics Balance-Denver
- p^H meter -BVK enterprises, India
- Ultrasonicator-BVK enterprises
- WATERS HPLC 2695 SYSTEM equipped with quaternary pumps, Photo Diode Array detector and Auto sampler integrated with Empower 2 Software.
- UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2 mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Meropenem and Vaborbactam solutions.

Methods

Diluent: Based up on the solubility of the drugs, diluent was selected, Methanol and Water taken in the ratio of 50:50

Preparation of Standard stock solutions: Accurately weighed 25mg of Meropenem and 25mg of Vaborbactam and transferred to 25ml volumetric flask. And 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (1000µg/ml of Meropenem and 1000µg/ml of vaborbactam).

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (100µg/ml Meropenem of and 100µg/ml of vaborbactam)

Preparation of Sample stock solutions: 1g of dry powder (for injection) was weighed and transferred to 500 ml volumetric flask, to this 5 ml of acetonitrile was added and sonicated. Volume was made upto 500 ml with diluents and filtered through 0.45 µm or finer porosity membrane filter (1000µg/ml of Meropenem and 1000µg/ml of Vaborbactam)

Preparation of Sample working solutions (100% solution): 0.5ml 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).

Preparation of buffer

0.01N KH_2PO_4 Buffer: Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then PH adjusted to 5.0 with dil. Orthophosphoric acid solution.

Validation

System suitability parameters

The system suitability parameters were determined by preparing standard solutions of Meropenem (100ppm) and Vaborbactam (100ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision

Preparation of Standard stock solutions: Accurately weighed 25mg of Meropenem and 25mg of Vaborbactam and transferred to 25ml volumetric flask. and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (1000µg/ml of Meropenem and 1000µg/ml of vaborbactam).

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (100µg/ml of Meropenem and 100µg/ml of Vaborbactam).

Preparation of Sample stock solutions: 1g of dry powder (for injection) was weighed and transferred to 500 ml volumetric flask, to this 5 ml of acetonitrile was added and sonicated. Volume was made upto 500 ml with diluents and filtered through 0.45 µm or finer porosity membrane filter (1000µg/ml of Meropenem and 1000µg/ml of Vaborbactam).

Preparation of Sample working solutions (100% solution): 0.5ml 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).

Linearity

Preparation of Standard stock solutions: Accurately weighed 25mg of Meropenem and 25mg of Vaborbactam and transferred to 50ml volumetric flask. and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (1000µg/ml of Meropenem and 1000µg/ml of vaborbactam).

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (25µg/ml of Meropenem and 25µg/ml of Vaborbactam)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (50µg/ml of Meropenem and 50µg/ml of Vaborbactam)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (75µg/ml of Meropenem and 75µg/ml of Vaborbactam)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (100µg/ml of Meropenem and 100µg/ml of Vaborbactam)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (125µg/ml of Meropenem and 125µg/ml of Vaborbactam)

150% Standard solution: 1.5ml each from two standard stock solutions was pipettede out and made up to 10ml (150µg/ml of Meropenem and 150µg/ml of Vaborbactam)

Accuracy

Preparation of Standard stock solutions: Accurately weighed 25mg of Meropenem and 25mg of Vaborbactam and transferred to 50ml volumetric flask. and 3/4 th of diluents was added to these flask and sonicated for 10 minutes. Flask were made up with diluents and labeled as Standard stock solution. (1000µg/ml of Meropenem and 1000µg/ml of vaborbactam).

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria

The % Recovery for each level should be between 98.0 to 102.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Meropenem and Vaborbactam solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Meropenem and Vaborbactam solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Degradation studies

Oxidation

To 1 ml of stock solution of Meropenem and Vaborbactam, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 100µg/ml&100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies

To 1 ml of stock s solution Meropenem and Vaborbactam, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c .The resultant solution was diluted to obtain 100µg/ml&100µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies

To 1 ml of stock solution Meropenem and Vaborbactam, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 100µg/ml&100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies

The standard drug solution was placed in oven at 105°C for 1 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 100µg/ml&100µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability studies

The photochemical stability of the drug was also studied by exposing the 1000µg/ml&1000µg/ml solution

to UV Light by keeping the beaker in UV Chamber for 1days or 200 Watt hours/m^2 in photo stability chamber For HPLC study, the resultant solution was diluted to obtain 100µg/ml&100µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 1hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 100µg/ml&100µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION

Determination of λ_{max} and Optimized wavelength

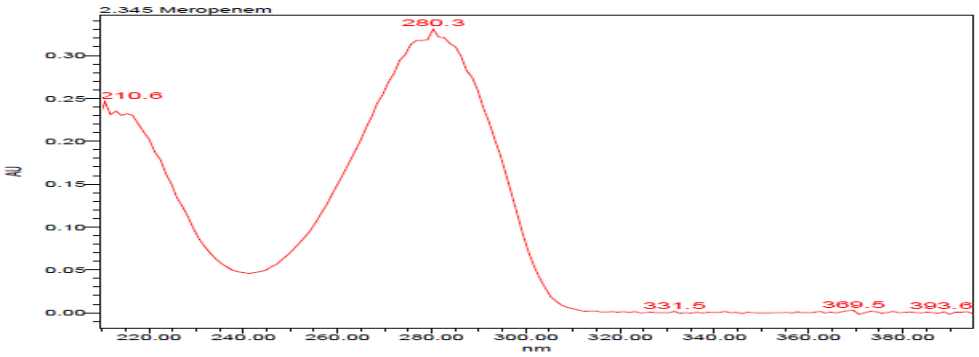


Fig. 6.1: Individual UV spectra of Meropenem and Vaborbactam.

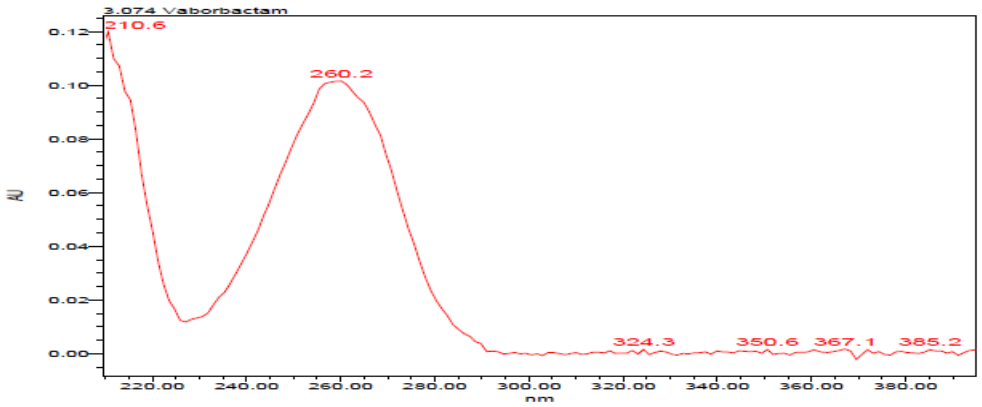
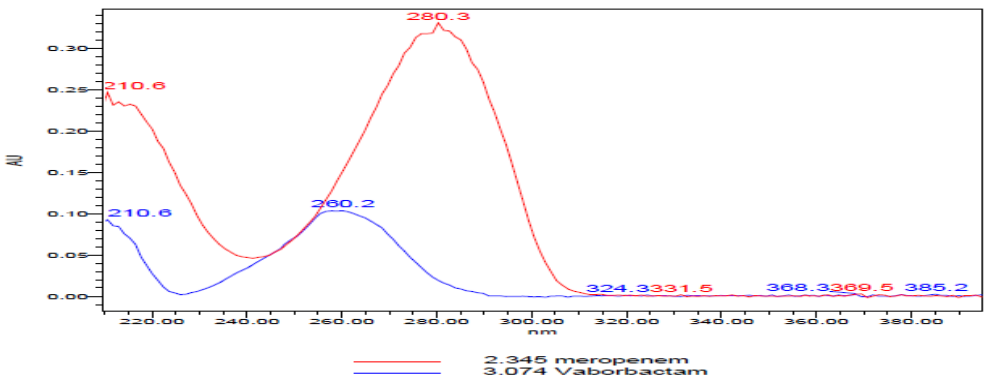


Fig. 6.2: Overlay UV spectra of Meropenem and Vaborbactam.



Optimized wavelength selected was 260nm.

Optimized method
Chromatographic conditions
Mobile phase: 50% 0.01N KH_2PO_4:50% Methanol
Flow rate: 0.8 ml/min
Column : Agilent C18 (4.6 x 150mm, 5µm)
Detector wave length: 260nm
Column temperature: 30°C
Injection volume: 10µL
Run time: 5min
Diluent: Water and Methanol in the ratio 50:50
Results: Both peaks have good resolution, tailing

Factor, theoretical plate count and resolution.

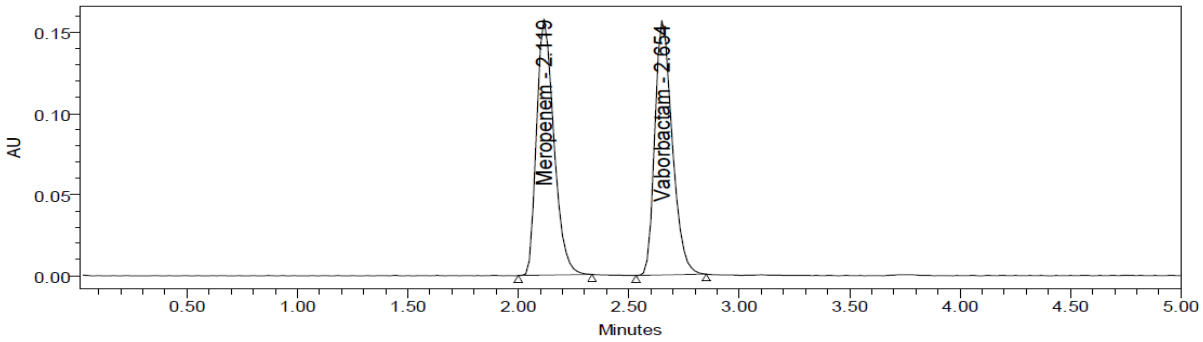


Fig. 6.8: Optimized Chromatogram.

Table 6.1: System suitability parameters for Meropenem and Vaborbactam.

S no	Meropenem			Vaborbactam			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resolution
1	2.109	3461	1.29	2.639	5476	1.29	3.5
2	2.111	3569	1.24	2.64	5727	1.27	3.6
3	2.114	3477	1.24	2.642	5583	1.29	3.6
4	2.115	3396	1.24	2.645	5319	1.27	3.6
5	2.119	3306	1.29	2.654	5422	1.26	3.6
6	2.122	3485	1.29	2.654	4771	1.31	3.5

Linearity
Table 6.2 Linearity table for Meropenem and Vaborbactam.

Meropenem		Vaborbactam	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
0	0	0	0
25	224279	25	224981
50	439298	50	430695
75	657021	75	647414
100	900728	100	891907
125	1106176	125	1102212
150	1322957	150	1302772

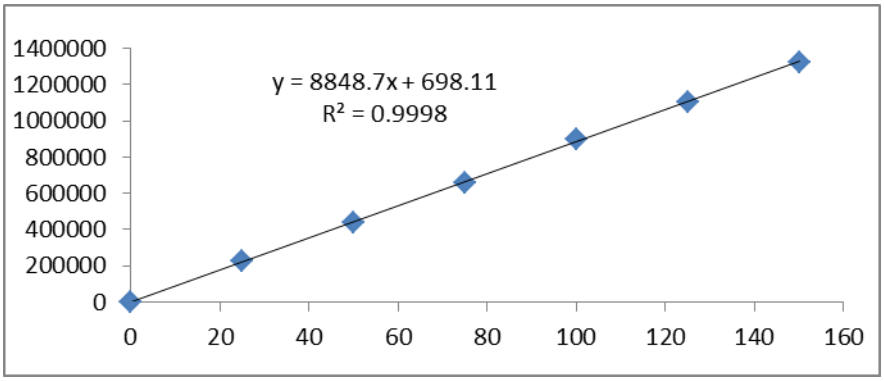


Fig. 6.13: Calibration curve of Meropenem.

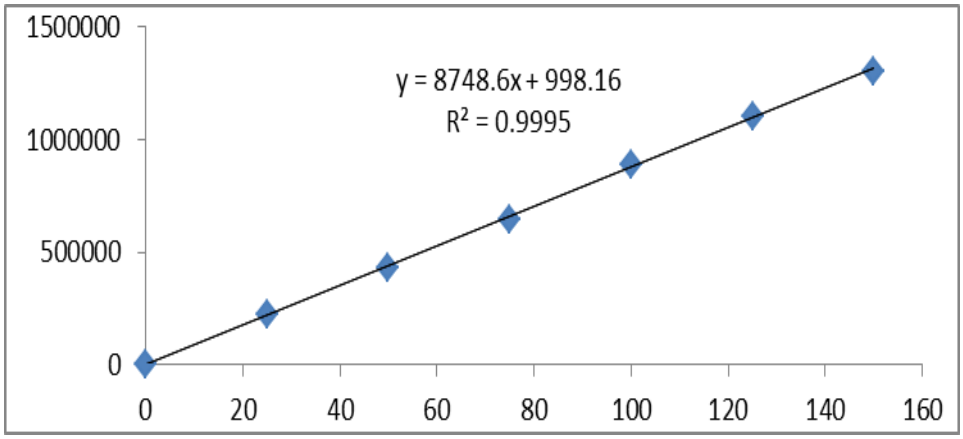


Fig. 6.14: Calibration curve of Vaborbactam.

Precision

System Precision

Table 6.3 System precision table of Meropenem and Vaborbactam.

S. No	Area of Meropenem	Area of Vaborbactam
1.	891526	890233
2.	900923	899278
3.	892902	894054
4.	892658	892473
5.	899923	895535
6.	881770	878965
Mean	893284	891756
S.D	6908.4	6965.4
%RSD	0.8	0.8

Repeatability

Table 6.4 Repeatability table of Meropenem and Vaborbactam.

S. No	Area of Meropenem	Area of Vaborbactam
1.	892702	887557
2.	893419	889201
3.	893353	892464
4.	909064	904282
5.	898243	897765
6.	894669	890771
Mean	896908	893673
S.D	6278.0	6270.3
%RSD	0.7	0.7

Intermediate precision (Day_ Day Precision)

Table 6.5: Intermediate precision table of Meropenem and Vaborbactam.

S. No	Area of Meropenem	Area of Vaborbactam
1.	809316	797098
2.	813755	792039
3.	809719	801354
4.	799339	797164
5.	818320	790217
6.	801625	791052
Mean	808679	794821
S.D	7172.1	4393.1
%RSD	0.9	0.6

Accuracy

Table 6.6: Accuracy table of Meropenem.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	50	49.12	98.23	99.52%
	50	49.44	98.87	
	50	50.25	100.49	
100%	100	99.35	99.35	
	100	100.08	100.08	
	100	100.08	100.08	
150%	150	149.44	99.63	
	150	149.00	99.33	
	150	149.46	99.64	

Table 6.7 Accuracy table of Vaborbactam.

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	50	49.72	99.45	100.04%
	50	50.26	100.52	
	50	50.34	100.68	
100%	100	98.62	98.62	
	100	98.59	98.59	
	100	98.55	98.55	
150%	150	152.12	101.42	
	150	151.99	101.33	
	150	151.81	101.21	

Sensitivity

Table 6.8 Sensitivity table of Meropenem and Vaborbactam.

Molecule	LOD	LOQ
Meropenem	0.07	0.21
Vaborbactam	0.07	0.21

Table 6.9: Robustness data for Meropenem and Vaborbactam.

S.no	Condition	%RSD of Meropenem	%RSD of Vaborbactam
1	Flow rate (-) 0.7ml/min	0.5	0.6
2	Flow rate (+) 0.9ml/min	0.6	0.9
3	Mobile phase (-) 45B:55A	0.6	0.4
4	Mobile phase (+) 55B:45A	0.5	0.1
5	Temperature (-) 25°C	0.4	0.2
6	Temperature (+) 35°C	0.3	0.4

Assay: The Medicines Company, bearing the label claim containing meropenem 1g + vaborbactam 1g (Vabomere (injection, sterile powder for reconstitution))Assay was performed with the above formulation. Average % Assay for Meropenem and Vaborbactam obtained was 100.00 and 99.81% respectively.

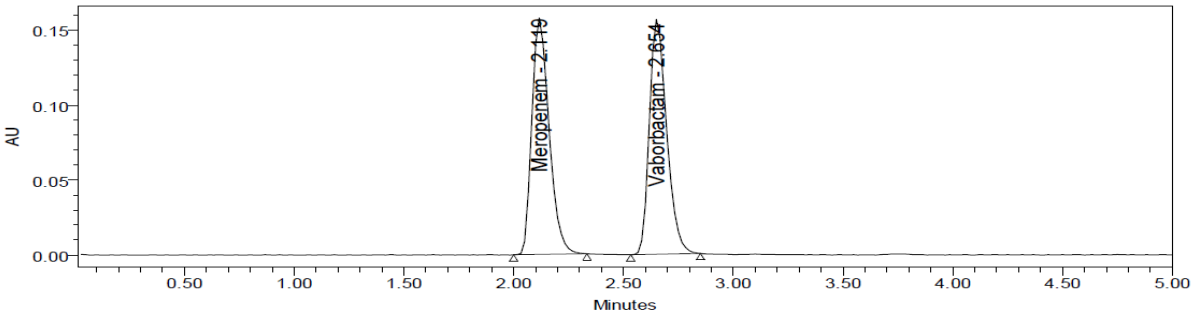


Table 6.10: Assay Data of Meropenem.

S. no	Standard Area	Sample area	% Assay
1	891526	892702	99.54
2	900923	893419	99.62
3	892902	893353	99.61
4	892658	909064	101.36
5	899923	898243	100.15
6	881770	894669	99.75
Avg	893284	896908	100.00
Stdev	6908.4	6278.0	0.70
%RSD	0.8	0.7	0.7

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Table 6.11: Assay Data of Vaborbactam.

S.no	Standard Area	Sample area	% Assay
1	890233	887557	99.13
2	899278	889201	99.31
3	894054	892464	99.68
4	892473	904282	101.00
5	895535	897765	100.27
6	878965	890771	99.49
Avg	892715	893673	99.81
Stdev	6965.4	6270.3	0.7
%RSD	0.8	0.7	0.7

6.8. Degradation data

Type of degradation	Meropenem			Vaborbactam		
	AREA	% Recovered	% Degraded	AREA	% Recovered	% Degraded
Acid	786747	87.72	12.28	784754	87.65	12.35
Base	843741	94.08	5.92	855121	95.51	4.49
Peroxide	846322	94.36	5.64	868169	96.97	3.03
Thermal	870857	97.10	2.90	883413	98.67	1.33
Uv	871110	97.13	2.87	881061	98.41	1.59
Water	891090	99.36	0.64	887046	99.07	0.93

Summary And Conclusion

Parameters		**Meropenem**	**Vaborbactam**	**LIMIT**
Linearity Range (µg/ml)		25-150 µg/ml	25-150 µg/ml	R< 1
Regressioncoefficient		0.999	0.999	
Slope(m)		8848	8748	
Intercept(c)		698.1	998.1	
Regression equation (Y=mx+c)		y = 8848.x + 698.1	y = 8748.x + 998.1	
Assay (% mean assay)		99.80%	99.90%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.8	0.8	NMT 2.0%
Method precision %RSD		0.7	0.7	NMT 2.0%
Accuracy %recovery		99.72%	100.04%	98-102%
LOD		0.07	0.21	NMT 3
LOQ		0.07	0.21	NMT 10
Robustness	**FM**	0.5	0.6	%RSD NMT 2.0
	FP	0.6	0.9	
	MM	0.6	0.4	
	MP	0.5	0.1	
	TM	0.4	0.2	
	TP	0.3	0.4	

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Meropenem and Vaborbactam in injection dosage form. Retention time of Meropenem and Vaborbactam were found to be 2.119 min and 2.654 min. %RSD of the Meropenem and Vaborbactam were and found to be 0.7 and 0.7 respectively. %Recovery was obtained as 99.52% and 100.04% for Meropenem and Vaborbactam respectively. LOD, LOQ values obtained from regression equations of Meropenem and Vaborbactam were 0.07, 0.21 µg/ml and 0.07, 0.21 µg/ml respectively. Regression equation of Meropenem is y = 8848.x + 698.1, and y = 8748.x + 998.1 of Vaborbactam. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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