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RESEARCH ARTICLE

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF MEROPENEM AND VABORBACTAM IN SYNTHETIC MIXTURE.

Ms Upasana K patel and Mr. Jaymin Ganshyambhai patel Sharda.
School of Pharmacy,Pethapur,Gandhinagar,Gujarat, A-25 hariom soc, near rajendrapark cross road,Ahmedabad.

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Abstract

A Simple, Rapid, Economical, Precise And Accurate Stability Indicating RP-HPLC Method for Simultaneous Estimation of Meropenem and Vaborbactam in their Combined Dosage Form has been Developed.

A Reverse Phase High Performance Liquid Chromatographic Method was Developed for the Simultaneous Estimation of Meropenem and Vaborbactam. In Their Combined Dosage Form has been Developed. The Separation was Achieved by LC- 20 AT C18 (250mm x 4.6 mm x 2.6 µm) column and Buffer (pH 6) : Methanol (70:30) as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 242 nm. Retention time of Meropenem and Vaborbactam were found to be 4.227 and 5.413 min, respectively. The method has been validated for Linearity, Accuracy and Precision. Linearity Observed for Meropenem 10-30 µg/ml and for Vaborbactam 10-30 µg/ml.

Developed method was found to be Accurate, Precise and Rapid for Simultaneous estimation of Meropenem and Vaborbactam in their combined dosage form.

The Drug was Subjected to Stress Condition of Hydrolysis, Oxidation, Photolysis and Thermal Degradation, Considerable Degradation was Found in Alkaline Degradation. The Proposed Method was Successfully Applied for the Simultaneous Estimation of Both the Drugs in Combined Dosage Form.

Corresponding Author:- Ms Upasana K patel.
Address:- School of Pharmacy,Pethapur,Gandhinagar,Gujarat, A-25 hariom soc, near rajendrapark cross road, Ahmedabad, Tiruvarur.

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Introduction:-

Meropenem is (4R,5S,6S)-3-(((3S,5S)-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. 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.

Meropenem is Sparingly soluble in water, practically insoluble in ethanol (96 per cent) and in methylene chloride.It is official in IP-2018, BP-2015, USP30-NF25.

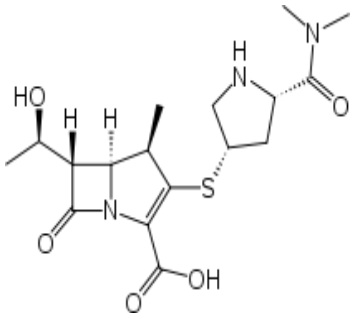

Fig 1:-Structure of Meropenem

Vaborbactam is {(3R,6S)-2-Hydroxy-3-[2-(thiophen-2-yl)acetamido]-1,2-oxaborinan-6-yl}acetic acid. Vaborbactam is a β-lactamase inhibitor based on a cyclic boronic acid pharmacophore It has been used in trials investigating the treatment of bacterial infections in subjects with varying degrees of renal insufficiency. Vaborbactam is Soluble in Dimethylsulfoxide, does not soluble in water. It is Not Official in any Pharmacopoeia.

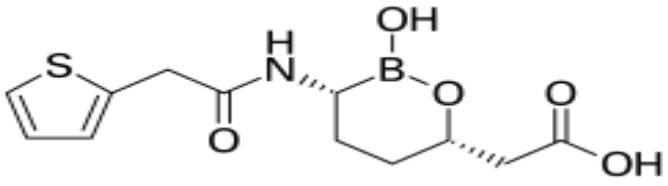

Fig 2:-Structure of Vaborbactam

Materials and methods:-

Instrumentation:

The chromatography was performed on a LC-20AT Instrument equipped with standard PDA Detector and Spinchrom software, BDS hypersil C18 column (25 cm × 0.46 cm) thermo scientific was used as stationary phase Injector, 20µL fixed loop, Electronic analytical balance Corning volumetric flasks and pipettes were used in the study.

Chemicals And Solvents:-

Vabomere Injection (Meropenem 1gm and Vaborbactam 1gm) was produced by Melinta therapeutics. Meropenem was procured as a gift samples from Aristo Pharmaceuticals pvt.ltd Vaborbactam was procured as a gift sample from Unnati Pharmaceuiticals pvt.ltd HPLC grades Acetonitrile, Methanol, distilled water (Finar Chemicals Ltd., Mumbai, India) were used and Potassium dihydrogenphosphate (Merck India Ltd. In Mumbai) were used.

Whatman Filter paper no. 41 (Whatman International Ltd., England) was used in the study.

Preparation of standard solutions :

Meropenem standard stock solution: (200 µg/mL)

A 20 mg of Meropenem was weighed and transferred to a 100 mL volumetric flask. volume was make up to the mark with methanol.

Vaborbactam standard stock solution: (200 µg/mL)

A 20 mg of Vaborbactam was weighed and transferred to a 100 mL volumetric flask was make up to the mark with methanol.

Preparation of standard solution of binary mixtures of Meropenem (20 µg/mL) and Vaborbactam (20 µg/mL)

Take 1 mL from the Meropenem stock solution and 1mL from Vaborbactam stock solution and transferred to 10 mL volumetric flask and volume make up to the mark by mobile phase which was used in particular trials.

Analytical Method Development:-

To optimize the HPLC parameters, several mobile phase compositions were tried. Satisfactory results were obtained from given chromatographic condition for Meropenem and Vaborbactam mentioned in Table no: 1

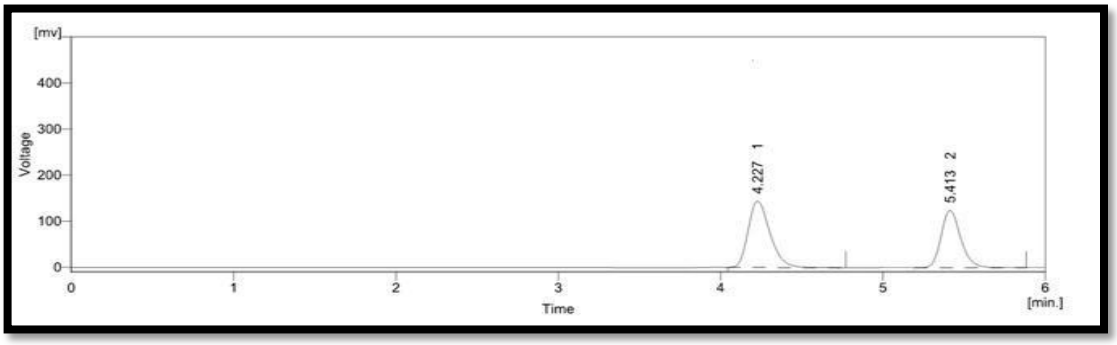

Fig. 3:-Chromatogram of Meropenem and Vaborbactam in Buffer: Methanol (70:30 v/v) (pH 6) (Flow rate-1.0 ml/min)

PARAMETER	CHROMETOGRAPHIC CONDITION
Mode of elution	Isocratic
Mobile Phase	Buffer: Methanol (70:30 v/v) (pH 6)
Column	BDS hypersil C18 column (25 cm × 0.46 cm)
Flow rate	1.0ml/min
Runtime	7 min
Injection volume	20 µL
Detection wavelength	242 nm

Table 1:-Method Development Parameters

Analytical Method Validation:-

The developed chromatographic method was validated as per ICH guideline for following parameters .it was found to ideally resolve the peaks with retention time (RT) 4.227 min and 5.413 min for Meropenem and Vaborbactam and respectively and the same is shown in fig.3.

Observed values for system suitability test: Table 2:

Results for system suitability test

Parameters	Data observed	
	Meropenem	Vaborbactam
Theoretical plates per column	4817	9606
Symmetry factor/Tailing factor	1.485	1.313

Resolution	5.109

Linearity And Range

The linearity for Meropenem and Vaborbactam were assessed by analysis of combined standard solution in range of 10-30µg/ml and 10-30 µg/ml. The results are shown in Figure: 9, 10 & 11 and Table 3.

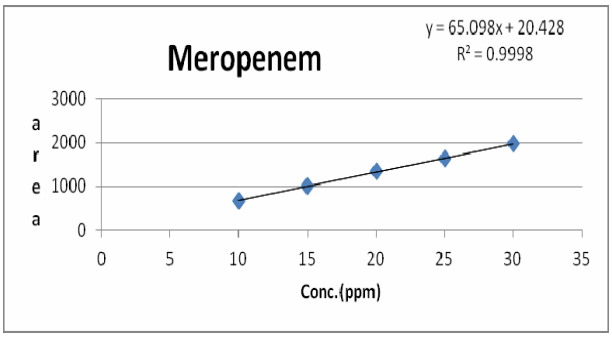

Fig 4:-Calibration Curve of Meropenem (10-30 µg/ml)

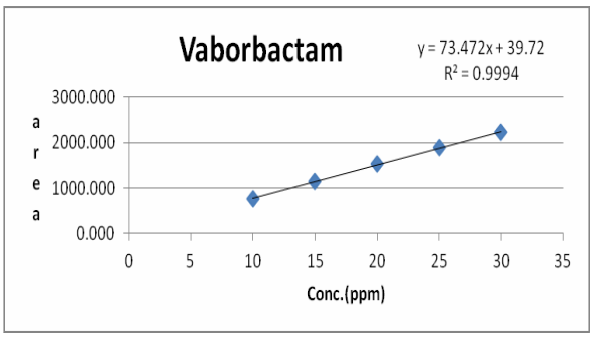

Fig 5:-Calibration Curve of Vaborbactam (10-30 µg/ml)

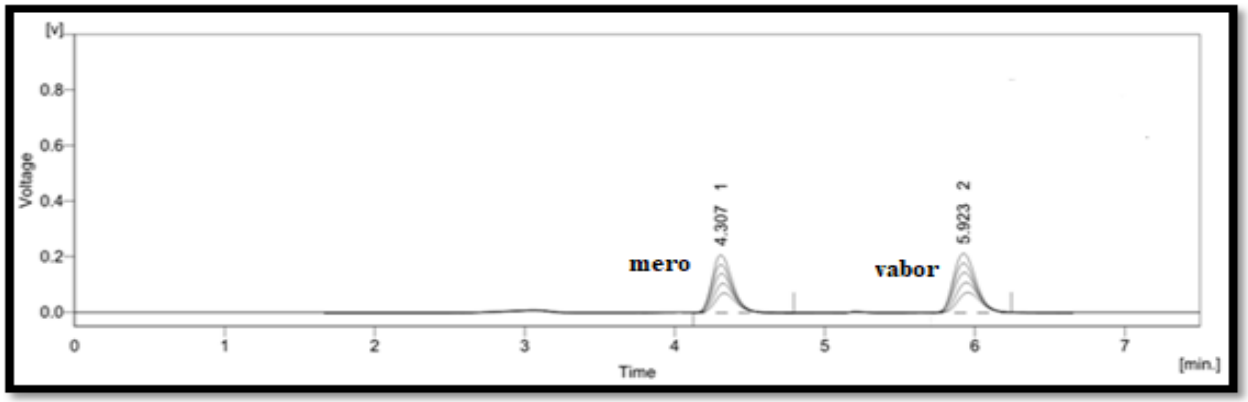

Fig 6:-Overlay chromatogram of different concentrations of binary mixtures of Meropenem and Vaborbactam

Accuracy:-

Good recoveries of Meropenem and Vaborbactam were obtained at various added concentrations By spiking standards like 80 %, 100 % and 120 %. Results are shown in Table 3.

Precision:-

The results of the repeatability, intra-day and inter-day precision experiments are shown respectively as given in Table 3. The developed method was found to be precise as the %RSD were< 2%.

Robustness:-

The robustness of an analytical procedure is a measure of its capacity to remain unaffected bysmall but deliberate variations in the analytical procedure parameters [pH (±0.2), Flow rate (±0.2 ml) and proportion of mobile phase (±2.0 v/v]. The standard deviation of the peak is calculated for each parameter and the %RSD was found to be less than 2%. Results are shown in Table 3.

Degradation Study:

Acid degradation

Acid decomposition studies were performed by Taking One ml of stock solution and transferred in to 10 ml of volumetric flask. Two ml of 0.1 N HCl solutions was added and mixed well and put for 1 hrs at 60ºC. Then Solution was neutralized with 2ml 1N NaOH and the volume was adjusted with diluent to get 20 µg/ml for Meropenem and 20 µg/ml for Vaborbactam.

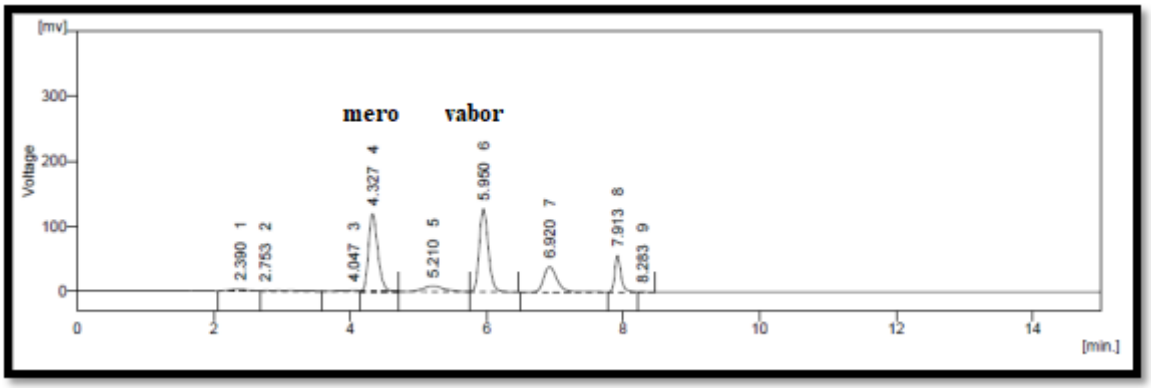

Fig 7:-Vaborbactam and Meropenem Acid Degradation Sample(1hr 60ºc)

Base degradation

Basic decomposition studies were performed by Taking One ml of stock solution and transferred in to 10 ml of volumetric flask. Two ml of 0.1 N NaOH solutions was added and mixed well and put for 1 hrs at 60ºC. Then the Solution was neutralize with 0.5n HCL and volume was adjusted with diluent to get 20 µg/ml for Meropenem and 20 µg/ml for Vaborbactam

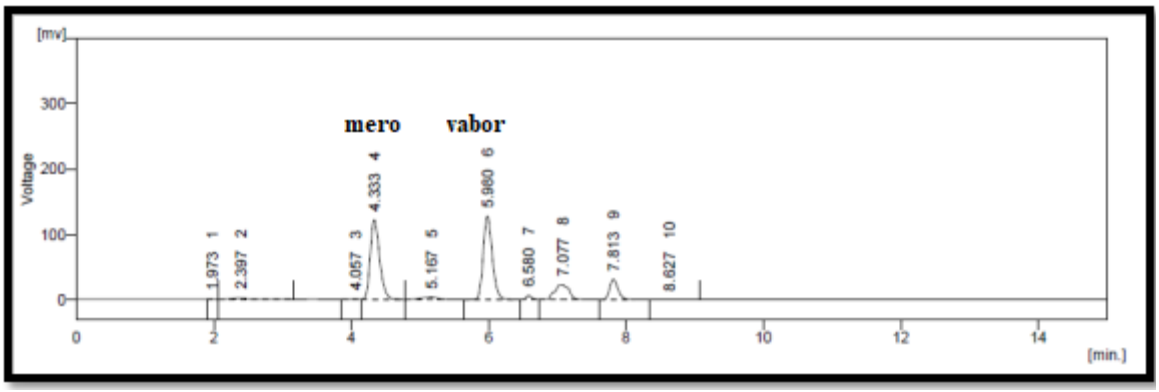

Fig 8:-Vaborbactam and Meropenem Base Degradation Sample(1hr 60ºc)

Oxidative degradation

Oxidative decomposition studies were performed by Taking One ml of stock solution and transferred in to 10 ml of volumetric flask. Two ml of 3% H_2O_2 solutions was added and mixed well and put for 2 hrs at RT. Then the volume was adjusted with diluent to get 20 µg/ml for Meropenem and 20 µg/ml for Vaborbactam.

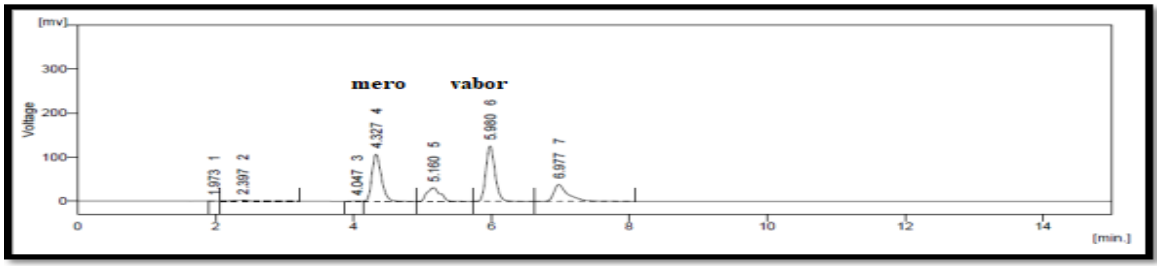

Fig. 9:-Vaborbactam and Meropenem Oxidation Degradation sample(2hr 60ºc)

Photo Degradation

Photo Degradation studies were performed by taking one ml of stock solution was transferred in to 10 ml of volumetric flask. The volumetric flask was kept under UV Light for 12hrs. Then the volume was adjusted with diluent to get 20µg/ml for Meropenem and 20µg/ml for Vaborbactam

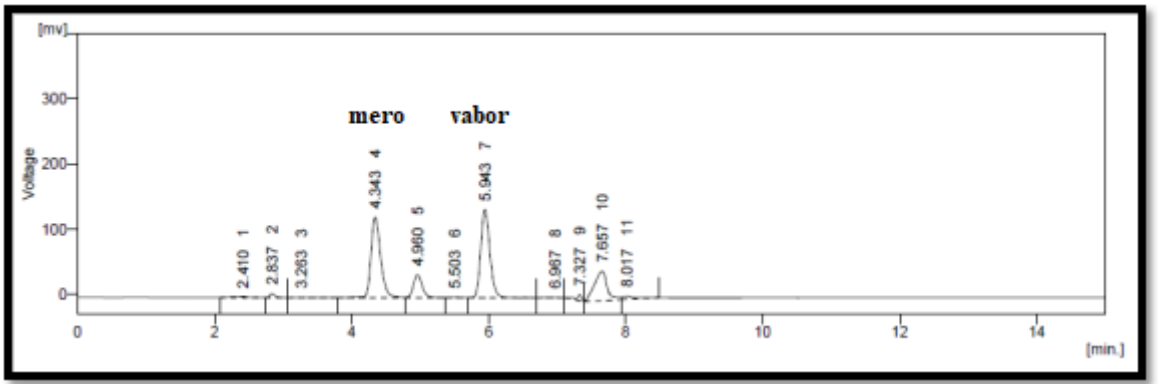

Fig. 10:-Vaborbactam and Meropenem Photo Degradation sample (UV light for 12hr)

Thermal degradation

A 20 mg of Meropenem and 20mg of Vaborbactam were taken in same petridish, petridish was put in oven for 2hrs at 80^0C temperature, than after petridish was removed and cool at RT, than this combined powder was transferred to 100ml volumetric flask and volume was made up with mobile phase, 1ml of this solution was transferred in 10ml volumetric and volume was made up with mobile phase to make 20µg/ml for Meropenem and 20µg/ml for Vaborbactam**.**

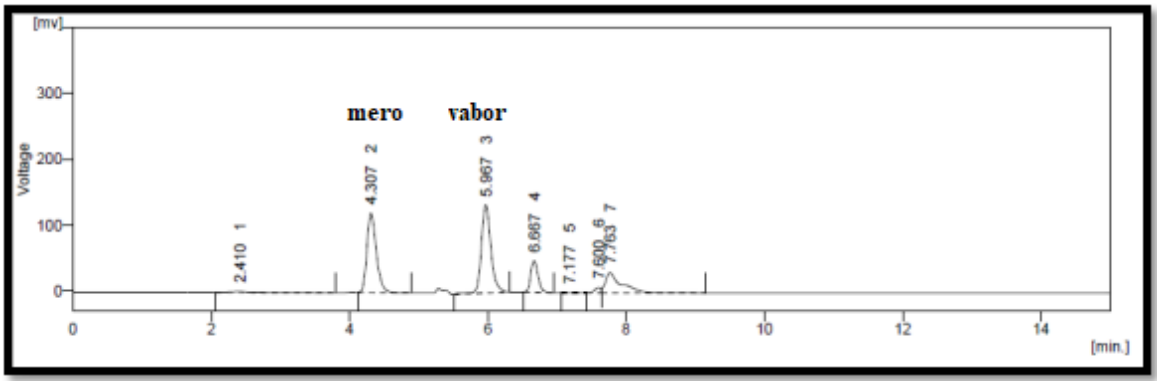

Fig. 11:-Vaborbactam and Meropenem Thermal Degradation sample(2hrs at 80ºc)

Results And Discussion:-

Validation Parameters:-

The method was validated in compliance with ICH guidelines

Force Degradation Studies:-

In the present investigation of the Meropenem and Vaborbactam were subjected to its stability studies as per ICH guideline[9]. The results of the forced degradation study of Meropenem and Vaborbactam summarized in Table 4 & 5**.**

Table 3:-Regression analysis data and summary of validation parameter

PARAMETERS	MEROPENEM	VABORBACTAM
Linearity Range(n=3) (µg/ml)	10-30	10-30
Regression Equation(R^2)	y= 5.098x + 20.428	y= 73.472x + 39.72
Co-relation Coefficient	0.999	0.999
LOD(µg/ml)	0.455	0.735

LOQ(µg/ml)		1.381	2.227
Recovery%		99.18-100.63 %	100.54-101.46 %
Repeatability(% RSD NMT 2)		0.44	0.36
Intra-day (n=3) Precision (% RSD NMT 2)		0.213-0.667	0.254-1.40
Inter-day (n=3) Precision (% RSD NMT 2)		0.717-1.408	1.24-1.74
	pH (± 0.2)	(-)0.666,(+)0.891	(-)1.172,(+)0.317
Robustness	Flow rate (± 0.2 ml)	(-)0.959,(+)0.815	(-)1.196,(+)0.303
	Mobile phase Ratio (± 2 ml)	(-)0.437,(+)1.003	(-)0.656,(+)0.211
Assay		100.65 ± 0.262	100.25 ± 0.672

Table 4:-Vaborbactam % Degradation

Vaborbactam				
Parameter	**Standard**		**Sample**	
	Area	%Degradation	Area	%Degradation
Acid	1175.974	18.994	1168.561	19.505
Base	1191.809	17.904	1194.659	17.707
Thermal	1190.408	18.413	1298.208	10.574
Oxidation	1147.508	20.955	1164.037	19.817
Photo	1270.226	12.502	1249.586	13.924

Table 5:-Meropenem % Degradation

Meropenem				
Parameter	**Standard**		**Sample**	
	Area	%Degradation	Area	%Degradation
Acid	1151.405	17.216	1142.063	17.888
Base	1192.729	14.245	1176.465	15.414
Thermal	1163.433	16.351	1138.876	18.117
Oxidation	1054.948	24.151	1024.116	26.368
Photo	1175.922	15.453	1216.380	12.545

Conclusion:-

The HPLC method developed for the analysis of Meropenem and Vaborbactam in their pharmaceutical preparations is simple, rapid and economic with less run time. The method has been validated and it has been shown that it is reliable, linear, accurate and precise as well as robust with minor variations in chromatographic parameters.

Therefore, it can be applied for both routine analytical and quality control assay and it could be a very powerful tool to investigate stability of Meropenem and Vaborbactam.

Reference:-

1. "Drug profile for Meropenem, August-2018 http://www.drugbank.ca/drugs/DB00760
2. "Drug profile for Meropenem, August-2018 https://aksci.com/item_detail.php?cat=J10087 3."Drug profile for Vaborbactam, August-2018 https://www.drugbank.ca/drugs/DB12107 4."Drug profile for Vaborbactam", August-2018, https://medkoo.com/products/7310
3. Indian Pharmacopoeia 2018, Government of India, Ministry of health and family welfare, ghaziyabad, pp2535-2536
4. United state pharmacopoeia-2007, USP30-NF25, United state food and drug administration, USP monograph Meropenem for Injection
5. British Pharmacopoeia 2015, The department of health, social services and public safety, London, Meropenem monograph