RESEARCH ARTICLE

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

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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.

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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.

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.

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 Pharmaceuticals 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 made 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 made up to the mark with methanol.

Preparation of standard solutions 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](image)

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

| PARAMETER       | CHROMATOGRAPHIC 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:

| Parameters          | Data observed |
|---------------------|---------------|
| Meropenem           | Vaborbactam   |
| Theoretical plates per column | 4817     | 9606     |
| Symmetry factor/Tailing factor | 1.485 | 1.313 |
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) 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 by small 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.

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

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.
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.

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°C 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.

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. 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(R²)             | y= 5.098x + 20.428         | y= 73.472x + 39.72        |
| Co-relation Coefficient             | 0.999                      | 0.999                     |
| LOD(μg/ml)                          | 0.455                      | 0.735                     |
Table 4: Vaborbactam % Degradation

| Parameter          | Standard | Sample |
|--------------------|----------|--------|
| Acid               | 1175.974 | 1168.561 |
| Base               | 1191.809 | 1194.659 |
| Thermal            | 1190.408 | 1298.208 |
| Oxidation          | 1147.508 | 1164.037 |
| Photo              | 1270.226 | 1249.586 |

Table 5: Meropenem % Degradation

| Parameter          | Standard | Sample |
|--------------------|----------|--------|
| Acid               | 1151.405 | 1142.063 |
| Base               | 1192.729 | 1176.465 |
| Thermal            | 1163.433 | 1138.876 |
| Oxidation          | 1054.948 | 1024.116 |
| Photo              | 1175.922 | 1216.380 |

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.

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