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RP- HPLC Method for Simultaneous Estimation of Meropenem and Vaborbactam in Bulk Samples

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Conflicts of Interest: Nil

Abstract

A simple, rapid reversed-phase high performance liquid chromatographic method had been developed and validated for estimation of meropenem and vaborbactam in tablet dosage form. The estimation was carried out on Phenomenex Luna C18 (25 cm x 4.60 mm, particle size 5 µm) column with a mixture of 10mM phosphate buffer (pH 6.8): Acetonitirle; 40: 60 (v/v) as mobile phase. UV detection was performed at 260 nm. The method was validated for linearity, accuracy, precision, specificity and sensitivity as per ICH norms. The developed and validated method was successfully used for the quantitative analysis of commercially available dosage form. The retention time was 2.95 and 3.53 min for meropenem and vaborbactam respectively and total run time was 10 min. at a flow rate of 1.0 mL/min. The calibration curve was linear over the concentration range of 40.00 - 240.00 µg/ mL for meropenem and 60.0 - 360.00 µg/mL for vaborbactam. The LOD and LOQ values were found to be 1.54 and 4.54 µg/ mL for meropenem and 4.60 and 13.65 µg/ mL for vaborbactam respectively. The low percentage coefficient of variance confirm the suitability of the method for the simultaneous estimation of meropenem and vaborbactam in bulk samples.

Keywords: Meropenem, Vaborbactam, RP-HPLC, Validation

Introduction

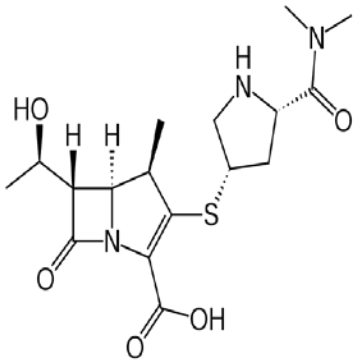
Due to counterfeiting, the drug quality has become a source of major concern worldwide, particularly in many developing countries. The most commonly counterfeited drugs are anti-infectives or antibiotics. Use of poor quality antibiotics bears serious health implications such as treatment failure, adverse reactions, drug resistance, increased morbidity, and mortality. Among antibiotics, penems are much recently introduced, widely prescribed and costlier. Therefore, incentive to produce their counterfeits because of profit margin increases considerably [1-2].

Meropenem is an ultra-broad spectrum injectable antibiotic used to treat a wide variety of infections, including meningitis and pneumonia. It is a beta-lactam and belongs to the subgroup of carbapenem, similar to imipenem and ertapenem. It is marketed in India by New Medicon Pharma with the brand name carbonem. It penetrates well into many tissues and body fluids including the cerebrospinal fluid, bile, heart valves, lung, and peritoneal fluid [3].

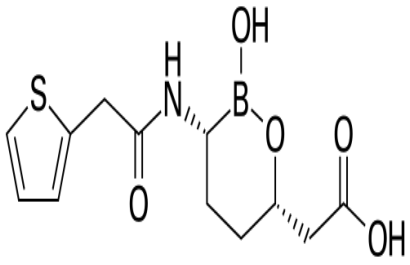
Meropenem is bactericidal except against Listeria monocytogenes where it is bacteriostatic. It inhibits bacterial wall synthesis like other beta-lactam antibiotics. In contrast to other beta-lactams, it is highly resistant to degradation by beta-lactamases or cephalosporinases.

Resistance generally arises due to mutations in penicillin binding proteins, production of metallo-betalactamases, or resistance to diffusion across the bacterial outer membrane [6-7].

Meropenem, present as a trihydrate (Fig.1.0) and it is official in Indian Pharmacopoeia (IP). It is a white to light yellow crystalline powder, with a molecular weight of 437.52. The chemical name for meropenem trihydrate is (4R,5S,6S)-3-[[(3S,5S)-5-(dimethylcarbamoyl)-3-pyrrolidinyl]thio]-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylicacid, trihydrate. The empirical formula of meropenem trihydrate is $C_{17}H_{25}N_3O_5S$ [1-4]. .



A



B

Fig.1.0: Chemical structures of A) Meropenem and B) Vaborbactam

Vaborbactam (Fig.1.0) is a new beta-lactamase inhibitor based on a cyclic boronic acid pharmacophore. Vaborbactam is a potent inhibitor of class A carbapenemases such as KPC as well as an inhibitor of other class A (CTX-M, SHV, TEM) and class C (P99, MIR, FOX) beta-lactamases. Vaborbactam does not inhibit class D or class B carbapenemases. When combined with meropenem, vaborbactam had the highest potency compared to combinations with other antibiotics in strains producing KPC beta-lactamase.

Vaborbactam is a white to off-white powder, with a molecular weight of 297.14. The chemical name for vaborbactam is (3R,6S)-2-hydroxy-3-[[2-(2-thienyl)acetyl]amino]-1,2-oxaborinane-6- acetic acid. Its empirical formula is $C_{12}H_{16}BNO_5S$ [4-5].

An extensive literature survey is carried out and found that pharmacokinetics studies [5], microbiology [6], HPLC [6, 8, 10-12], spectroscopy [9, 13-18, 20, 22], Capillary Electrophoresis [15], microdialysis [19], LC–MS/MS [22] were developed for meropenem and no methods were reported for simultaneous estimation of meropenem and vaborbactam by RP-HPLC.

Hence an attempt was made to develop simple and sensitive spectrophotometric method for the estimation of meropenem and vaborbactam in pure drug samples by RP-HPLC.

Experimental

Apparatus

RP-HPLC was performed with an Agilent chromatographic system equipped with 1200 series isocratic pump UV–visible and a Rheodyne universal loop injector of injection capacity 50 µL. The monitoring software was Ezichrome Elite. The equipment was controlled by a PC workstation. Compounds were separated on a 25 cm x 4.6 mm i.d, 5-µm particle, Phenomenex–Luna C18 column under reversed-phase partition chromatographic conditions. The flow rate was 1.0 mL/ min and injection volume was 20 µL, analyte were monitored at 260 nm and run time was 7 min.

Chemicals and reagents

Working Standards of pharmaceutical grade meropenem (MEP) and vaborbactam (VAB) were obtained as gift samples from Micro labs, Bangalore. All the chemicals and reagents used were of HPLC grade and purchased from Merck, Mumbai, India.

Preparation of standard stock solution

Standard stock solution of meropenem (MEP) and vaborbactam (VAB) pure drugs prepared by accurately weighing about 100 mg drugs and transferring in to 100 mL volumetric flask and dissolved in acetonitrile.

METHOD VALIDATION

The proposed method was validated as per ICH guidelines. The parameters studied for validation were system suitability, specificity, linearity, precision, ruggedness, robustness, limit of detection and limit of quantification, filter validation and solution stability [23].

Selectivity and Specificity

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix. If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective. It has been observed that there were no peaks of diluents and placebo at main peaks. Hence, the chromatographic system used for the estimation of meropenem (MEP) and vaborbactam (VAB) was very selective and specific. Specificity studies indicating that the excipients did not interfere with the analysis. The standard solution shown symmetric peak with retention times of 2.86 min for meropenem (MEP) and 7.40 min for vaborbactam (VAB). The results were depict in Fig.2.0.

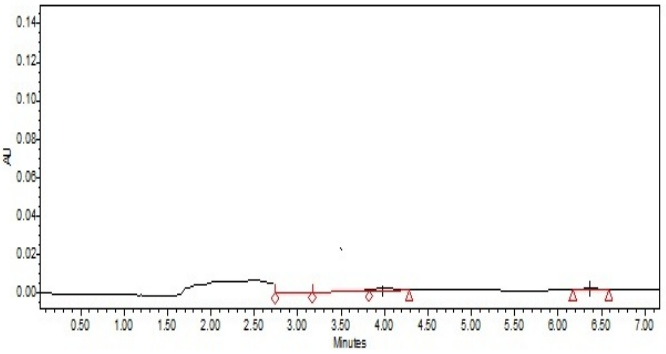


Fig.2.0. Chromatogram of blank sample

System suitability

Standard solution was prepared as per the proposed method and injected into the HPLC system in six replicates and the results were depicted in Table. 1.0 and Fig.3.0.

Table 1.0: System suitability results of MEP and VAB

Retention Time	Meropenem	2.95 min
	Vaborbactam	3.53 min
Peak Area	Meropenem	111223
	Vaborbactam	83213
Theoretical plates	Meropenem	8569
	Vaborbactam	9577
Tailing Factor	Meropenem	0.07
	Vaborbactam	0.32
Resolution	Meropenem	-
	Vaborbactam	5.61

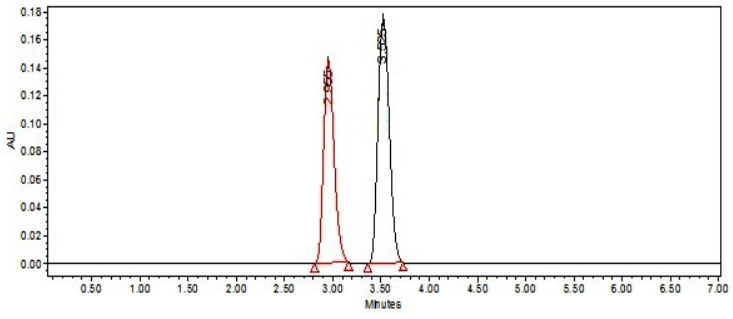


Fig.3.0. Chromatogram of Standard sample

Linearity & Range

A series of standard concentrations were prepared from 50 % to 150 % of the target concentration of meropenem (MEP) and vaborbactam (VAB). Linearity was assessed by performing single measurement at several analyte

concentration varying quantities of stock standard solution diluted with the mobile phase to get final concentrations of 40, 80, 120, 160, 200, 240 µg/mL of MEP and 60, 120, 180, 240, 300, 360 µg/mL of VAB. Injection was made at intervals of 10.0 min. Linearity of MEP was found to be exist between 40-240 µg/mL and for VAB was 60 - 360 µg/mL. The chromatograms were recorded and linearity graph was plotted by using peak area of drug against respective concentrations to obtain the linearity range. The results were depict in Table.2.0 and Fig.4.0 to 5.0.

Table.2.0: Linearity and range of MEP and VAB

S.No.	Concentration µg/mL	Area of meropenem	Concentration µg/mL	Area of vaborbactam
1	40	22988	60	16998
2	80	47605	120	31602
3	120	68028	180	50459
4	160	90704	240	67137
5	200	112120	300	84014
6	240	130654	360	101710
Concentration range	40-240 µg/mL		60-360 µg/mL	
Slope (m)	547		282	
Correlation coefficient (r^2)	0.9987		0.9995	

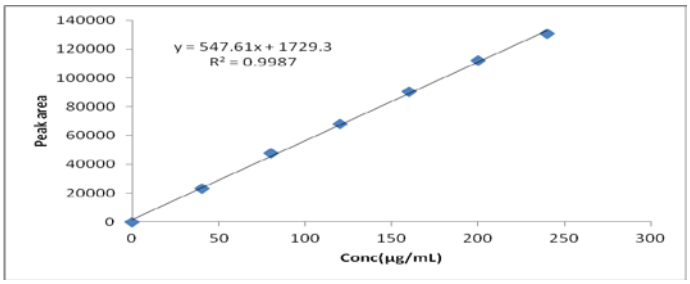


Fig.4.0: Linearity of Meropenem (MEP)

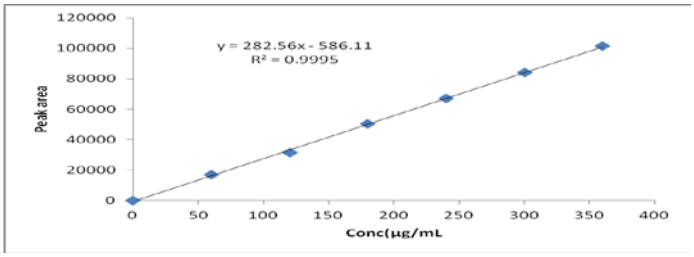


Fig.5.0: Linearity of Vaborbactam (VAB)

Precision

The intra-day and inter-day precision studies were carried out using a test sample assay method with six replicates on the same day and different days. The results were depicted in Table. No- 3.0 and 4.0.

Table. 3.0: Intraday precision data for MEP and VAB

Sample. No	Area of MEP	Area of VAB
1	110164	84015
2	110145	84037
3	111162	84052
4	111133	84062
5	110152	84034
6	111160	84014
Mean	110653	84036
SD	546.76	19.29
%RSD	0.49	0.02

Table.4.0: Interday precision data for MEP and VAB

Sample. No	Area of MEP	Area of VAB
1	110165	85031
2	110138	85137
3	110222	85012
4	110142	85034
5	110163	84511
6	110122	85038
Mean	110159	84961
SD	34.98	224.60
% RSD	0.03	0.26

Ruggedness

This is to prove the lack of influence of operational and environmental variables of the test results by using the method. Ruggedness is a measure of reproducibility of test results under the variation in conditions normally expected from system to system and from analyst to analyst. It was carried out by using a test sample assay method with six replicates using different analyst.

Robustness

Robustness was performed by change in mobile phase ratio, mobile phase flow rate and wavelength of the detector. The test was carried out by small variation in the chromatographic conditions at a concentration equal to standard concentrations 200 µg/mL for MEP and 300 µg/mL for VAB and % change was calculated. % Change in the results was calculated.

Limit of detection and Limit of quantification

The limit of detection (LOD) is defined as the lowest concentration of an analyte that can reliably be differentiated from background levels. Limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using the following equation as per ICH guidelines.

LOD = 3 .3 × σ / S; L OQ = 1 0 × σ / S;

Where σ is the standard deviation of y-intercepts of regression lines and S is the slope of the calibration curve.

Solution Stability

Solution stability was assed using standard and test stock solutions. These stocks were prepared and stored at room temperature and refrigerated conditions (2-8°C) for 36 h and % differences were calculated.

Filter validation

A study was conducted to determine the effect of filter on the assay, dissolution and impurities. Test solution was prepared as per the test method. Some portion of the above solution was filtered through three different filters namely 0.45µ PVDF filter, 0.45µ PTFE and 0.45µ Nylon filter and some portion was centrifuged and injected into the HPLC system. The % difference values between centrifuged and filtered sample were calculated.

Result and Discussion

In this RP-HPLC method, the conditions were optimized to obtain an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate analytes. The mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity or symmetry factor), run time and resolution. The system with 10 mM phosphate buffer (pH 6.8): acetonitirle (40: 60) (v/v) at flow rate of 1.0 mL/min was found to be robust method. The developed method was validated as per the ICH guidelines for the quantification of meropenem (MEP) and vaborbactam (VAB) in bulk samples.

A suitability test was applied to various system suitability parameters and the results obtained were within acceptable limits of tailing factor ≤ 2.0 and theoretical plates >2000. The calibration curve was constructed with series of concentration in the range of 40-240 µg/mL and 60-360 µg/mL for meropenem (MEP) and vaborbactam (VAB).

The correlation co-efficient of meropenem (MEP) and vaborbactam (VAB) was found to be >0.998. This concluded that the method was linear throughout the range selected. Specificity was studied for the quantification of impurities in meropenem (MEP) and vaborbactam (VAB). From the results it was indicated that none of impurities were interfere at analytes retention time. Hence the developed method was specific.

The precision of the method was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogenous sample with in the day (intraday) and next consequent three days for inter day precision. For each cases % RSD was calculated and results were the acceptable limits. The low values of RSD indicate that the method is precise.

Robustness test was carried out by small variation in the chromatographic conditions and % change was calculated. The % change in the results was calculated and it was found robust as % change was below 2.0 %.

A signal-to-noise ratio 2:1 is generally considered acceptable for estimating the detection limit. LOD is found to be 5.888 µg/mL for meropenem and 0.225 µg/mL for vaborbactam (VAB) and LOQ is found to be 17.841 µg/mL for meropenem (MEP) and 0.683 µg/mL for vaborbactam (VAB).

Sample and standard solution are stable at 5°C for 36 h as the % difference in the area was found to be less than 2.0 %. Filter interference was done on three types of 0.45 µ filters (Nylon, PVDF, PTFE) and the % difference was found to be below 2.0 % for sample solutions and standard solutions calculated against centrifuged samples and standard.

Conclusion

A new, reversed-phase HPLC method has been developed for simultaneous analysis of meropenem (MEP) and vaborbactam (VAB) in a tablet formulation. It was shown that, the method was linear, accurate, reproducible, repeatable, precise, selective and specific proving the reliability of the method. The run time is relatively short (7 min), which enables rapid determination of many samples in routine and quality control analysis.

Acknowledgement

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