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Original Article

Validation of Effective β-Lactam Antibiotics in Available Dosage Forms by RP-HPLC Developed Analytical Chromatographic Method

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ABSTRACT

Background: Vaborbactam and Meropenem are co-formulated in pharmaceutical tablets for enhanced antibacterial activity. A simple, economical, and reliable analytical method is required for their simultaneous quantification. **Materials and** Methods: A simultaneous HPLC method was developed and validated using a 996 PDA detector. Separation was achieved on a Phenomenex Gemini C18 column (150 × 4.6 mm, 5.0 µm) with an isocratic mobile phase consisting of methanol and triethylamine buffer (pH 4.8) in a 32:68 v/v ratio. The flow rate was 1 mL/min, and detection was performed at 260 nm. Calibration curves were constructed for both drugs over the 20-100 µg/mL concentration range. Method validation followed ICH guidelines. **Results:** Meropenem and Vaborbactam showed excellent linearity within the selected ranges, with recovery values of 99.92-100.36% and 100.56-100.74%**,** respectively. Precision studies demonstrated good repeatability with %RSD < 1% for both drugs. The LOD was 2.6 µg/mL for Meropenem and 3.4 µg/mL for Vaborbactam, while the LOQ was 7.8 µg/mL and 10.4 µg/mL, respectively. The method successfully quantified both active components in commercial tablet formulations. **Conclusion:** A simple, economical, and accurate HPLC method was developed and validated for the simultaneous determination of Vaborbactam and Meropenem in tablet dosage forms. The method meets ICH requirements and is suitable for routine quality-control analysis.

Keywords: Meropenem, Vaborbactam, RP-HPLC, Method Validation.

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INTRODUCTION

Meropenem and Vaborabactam are Anti-infective Agents, serves as first line β-lactam antibiotics[1] and are official in IP, BP.[2] The reported analytical approaches include an UV Spectrophotometric, HPLC assay methods.[3-11] The certain limitations with reported HPLC method like extensive retention time, Rapid mobile phase consumption, Elevated analysis time and non-economical approaches paved a way to validate and develop a new Liquid chromatographic technique which not only overcome above mentioned limitations but also offers to be with good ease, robustness, specificity, rapidity that allows an economical approach to quantify both the anti-infective drugs in their combined dosage forms.

MATERIALS AND METHODS

Reagents and Materials

The authentic drug standards of Meropenem and Vaborbactam are gifted by Sura labs, Hyderabad in India. Vabomere injection, sterile powder for reconstitution containing Meropenem (1 g) and Vaborbactam (1 g) were purchased from nearby pharmacy. The preparations were done on the same day of analysis by protecting the samples solutions from light by shielding.

Instruments

The HPLC System comprising of C18 column, WATERS Alliance 2695 separation module, a higher version LC solution 5.0 version Software to analyse and interpret the data recorded: Empower 2,996 using PDA as detector and Rheodyne injector connected

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with a loop of 20 µL. To record pH a Lab India pH meter is used and for weighing a Digital Microbalance model (CX-265) is used.

Experimental Conditions

The "Phenomenex Gemini C18 of particle size (4.6 mm×150 mm, 5.0 µm)" is used for chromatographic separation. The mobile phase with proportion of 32:68 v/v.20 µL and composition of Methanol: Triethylamine buffer of pH 4.8 was injected. The eluents were detected at 1 mL/min optimum flow rate at 260 nm. The system was maintained to run at 30°C room temperature.

Mobile Phase Preparation

An exact measure of 320 mL Methanol and 680 mL of Triethylamine buffer (68%) were homogenised in a sonicator for 15 min followed by filtration through 0.45 µ filter under vacuum condition.

Standard Solution Preparation

A stock solution of 1000 µg/mL of each drug are prepared in 10 mL standard flask by measuring 10 mg of Meropenem and Vaborabactam with an addition of 7 mL diluents. From the above stock solution, a 50 µg/mL working standard solution of are prepared for each drug with 0.5 mL aliquot into 10 mL standard flask respectively.

Preparation of Sample Solution

A 10 mg powdered drug was added to a 10 mL standard flask, and the volume was adjusted to 10 mL with diluent. Further an aliquot of 0.5 mL is added to 10 mL standard flask from above Meropenem and Vaborabactam stock solutions by diluting it with the diluents and passing them through a 0.45 millipore filter before analysis in the HPLC system.

Method Validation

The proposed approach validation is done in chord with the ICH recommendation (ICH, 2005) for the following validation parameters like "specificity, linearity, accuracy, precision, and robustness, as well system suitability tests".[12-15]

Sample Analysis

The combination dosage form containing Meropenem and Vaborbactam are analysed and established by this method. Results were satisfactory as per the label claims also the mean percentages for both drugs were in good accord for the three replica injections of standard and sample solutions the percentage assay is calculated for the drugs.

RESULTS

Method Development

Optimisation of Chromatographic Condition

UV spectra show that at 260 nm Meropenem and Vaborbactam exhibits a significant absorption; Hence 260 nm was used as detection wavelength. Eluent of composition in the desired ratio of 32:68 with Methanol and Tri Ethyl Amine buffer, "Phenomenex Gemini C18 (4.6 mm×150 mm, 5.0 µm) particle size" was chosen and further it was found that a flow rate of 1 mL/min was good to elute both medicines in less than 10 min. Figure 1 depicts the optimised chromatogram of Meropenem and Vaborabactam.

Table 1: System suitability tests results (*n*=6).

Parameters	Vaborabactam	Meropenem
Mean	5676.2	858082.4
*SD	16.2696	2024.409
*%RSD	0.286628	0.235922
Retention time	5.4	3.297
Tailing factor	1.38	1.26
Resolution	7	

*SD-Standard Deviation, %RSD-Percentage Relative Standard Deviation.

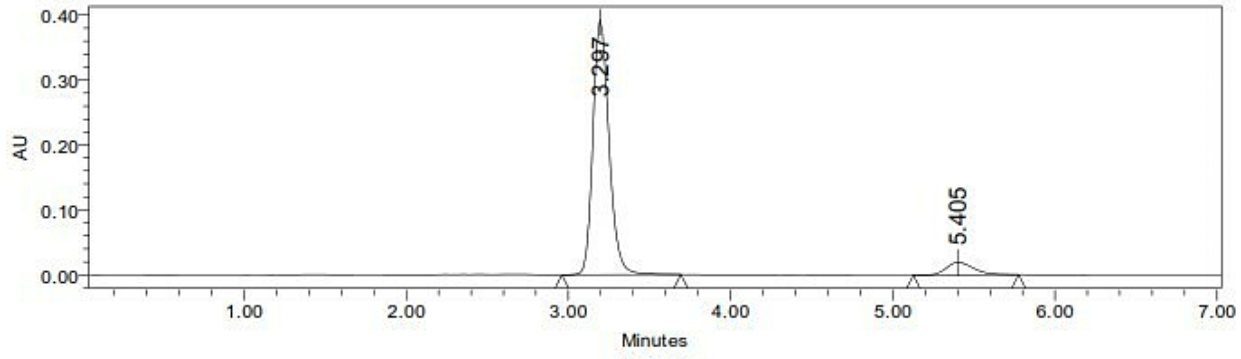

Figure 1: Optimised Chromatogram of Meropenem and Vaborabactam.

Method Validation

Test for system suitability

Table 1 represents system suitability parameters for the analysis carried out and it suggests that the developed analogy appropriate for the procedure and all the suitability criterion are within the suggested ranges.

Linearity

Linearity of Meropenem and Vaborabactam for the used concentration 20-100 µg/mL shows a correlation coefficient of 0.999 on regression analysis. Table 2 exhibits the Meropenem and Vaborabactam linearity data, Figures 2 and 3 depicts the calibration curve for Meropenem and Vaborabactam.

LOD and LOQ

The method sensitivity was assessed by applying Limits of Detection (LOD) and Quantitation (LOQ) formulas. It showed 2.6 µg/mL and 3.4 µg/mL of detection limit for Meropenem and Vaborabactam individually, whereas the Quantification values for Meropenem and Vaborabactam were found to be 7.8 µg/mL and 10.2 µg/mL individually.

Table 2: Linearity and statistical data of Meropenem and Vaborabactam by RP-HPLC.

	Meropenem	Vaborabactam
Concentration (µg/mL)	Average Peak Area	
20	325894	2038
40	615985	3859
60	877856	5698
80	1168594	7489
100	1445698	9218
*r^2	0.999	0.999
Regression equation	14312x+23400	14112 x+22400

*r^2 =Coefficient of determination.

Precision

To assess the intraday and inter-day precision repetitive injections of concentrations (50 µg/mL) for Meropenem and Vaborabactam were used, and the percent RSD was ascertained. The percent RSD in Table 3, shows that the approach was sufficiently applicable for both drugs with values less than 1 percent which confirmed the methods degree of agreement among individual test results.

Accuracy

The standard addition approach with sample drugs at three varied concentrations were examined in triplicate where the investigation findings for both Meropenem and Vaborbactam ranges between 99 to 110% as shown in Table 4.

Specificity

The % purity of combined dosage forms Meropenem and Vaborbactam is 99.82% the chromatogram of sample is depicted in Figure 4.

Robustness

i)Flow rate variations tested range was±0.1 mL/min (1.1 and 0.9 mL/min). We have observed slight changes in the retention time and no significant effect on Resolution, peak symmetry and %RSD was found to be within acceptance criteria.ii)Organic phase composition±5%, we have observed slight changes in the retention time and no effect on Resolution and peak symmetry.The method is said to be robust, because it can tolerate minor fluctuations without effecting the resolution, peak symmetry and can be

Table 3: Results of Repeatability and Intermediate Precision.

Repeatability(*n*=5)			Intermediate precision(*n*=6)			
Drugs (50 µg/mL)	Meropenem	Vaborabactam	Meropenem		Vaborabactam	
			Day-1	Day-2	Day-1	Day-2
Mean Area	858338	5682.2	867435.5	847434.3	5777.167	5876.667
±SD	1454.222	24.57031	2167.095	1201.345	18.40018	20.39281
%RSD	0.169423	0.432408	0.249828	0.141763	0.318498	0.347013

*SD- Standard Deviation, %RSD-Percentage Relative Standard Deviation.

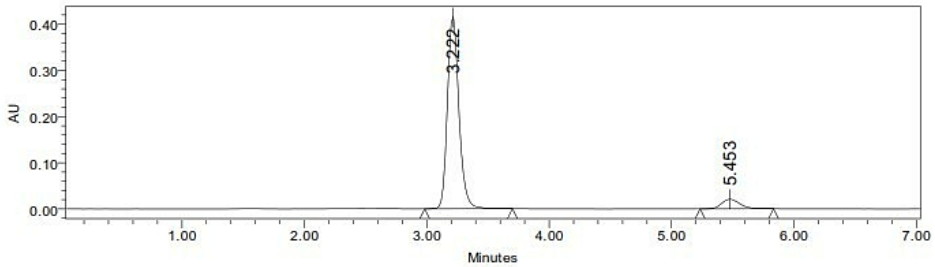

Figure 4: Sample Chromatogram.

successfully reliable for routine QC analysis. The data presented in Table 5 represents the robustness study involving the variations in various parameters.

DISCUSSION

Method Development

Optimisation of Chromatographic Condition

The mobile phase selection for obtaining desired peak symmetry, satisfactory retention time and good resolution was carried out taking various ratios of water and Acetonitrile, water and Methanol and also Methanol and TEA buffer, among all Methanol and Triethylamine buffer has efficiently separated the both the drugs over "Phenomenex Gemini C18 (4.6 mm×150 mm, 5.0 µm) particle size" at an outstanding flow rate of 1 mL/min with tested ranges varying in 0.5 to 1.2 mL/min in order to achieve adequate resolution and satisfactory peak at 260 nm detection wavelength. Phenomenex Gemini C18 column was chosen for this analysis because of robust performance over wide pH range and is more resistant to hydrolysis also. As Meropenem analyte is basic in nature, this column is chosen due to its pH stability.

Method Validation

Test for system suitability

For confirming the system performance, the working stock solution was injected five repetitive times and analysed %RSD, which is found to be within acceptance criteria from the reported Table 1. Thus proved the system performance.

Linearity

For the linearity study, five aliquots of standard solutions ranging between 20-100 µg/mL were prepared, plotted a line of best fit by using least square regression method for determining the correlation coefficient. The plotted curve proved that the developed method is obeying Beer's-Lamberts law with good correlation coefficient of 0.999.

LOD and LOQ

The calculated limit of detection and limit of quantitation values proved the method's sensitivity.

Precision

Minor fluctuations in the pump pressure, differences in temperature and humidity across the days may be the reasons for slight variations in the Precision. Though there are variations,

Table 4: The accuracy Data.

Drugs	Spike level	Peak Area	Amount Added (µg/mL)	Amount Found (µg/mL)	% Recovery	Mean Recovery
Meropenem	50%	451144.3	25	24.998	99.992%	100.1873%
	100%	897260.3	50	50.104	100.208%	
	150%	1344562	75	75.278	100.362%	
Vaborbactam	50%	2895	30	30.109	100.363%	100.34%
	100%	5685.333	60	60.100	100.166%	
	150%	8449	90	90.449	100.498%	

Table 5: Robustness Studies of Meropenem and Vaborabactam.

Parameter used for sample analysis	Drugs	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	Meropenem	859865	3.297	7895	1.25
	Vaborabactam	5698	5.405	6582	1.36
Less Flow rate of 0.9 mL/min	Meropenem	915847	3.639	7251	1.20
	Vaborabactam	6452	6.250	6785	1.32
More Flow rate of 1.1 mL/min	Meropenem	842564	2.859	7415	1.21
	Vaborabactam	5254	4.863	6365	1.34
Less organic phase	Meropenem	825498	3.460	7365	1.23
	Vaborabactam	5487	6.196	6254	1.38
More Organic phase	Meropenem	814578	3.022	7258	1.22
	Vaborabactam	5369	5.010	6298	1.33

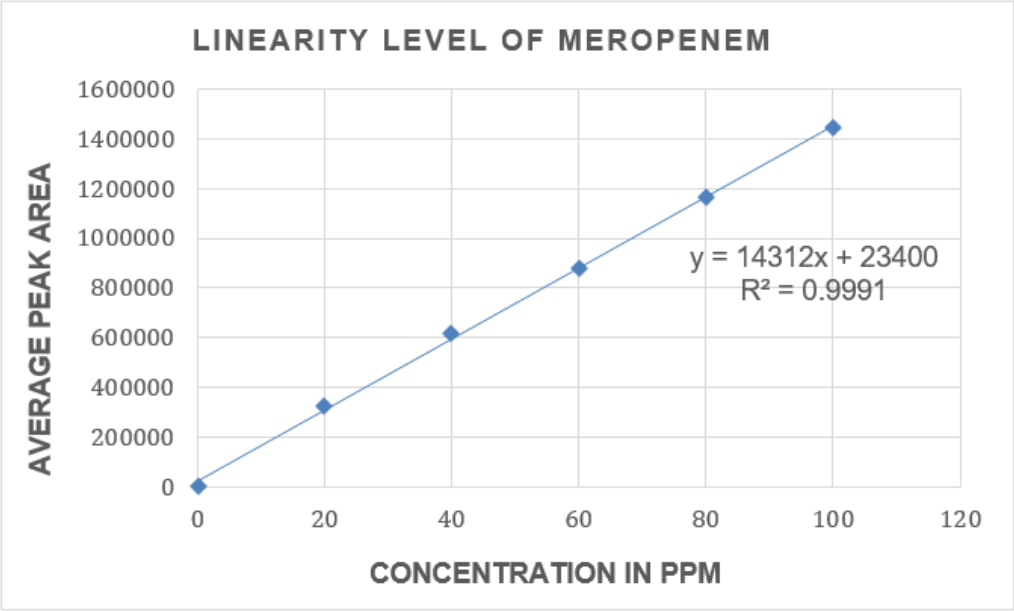

Figure 2: Calibration curve of Meropenem.

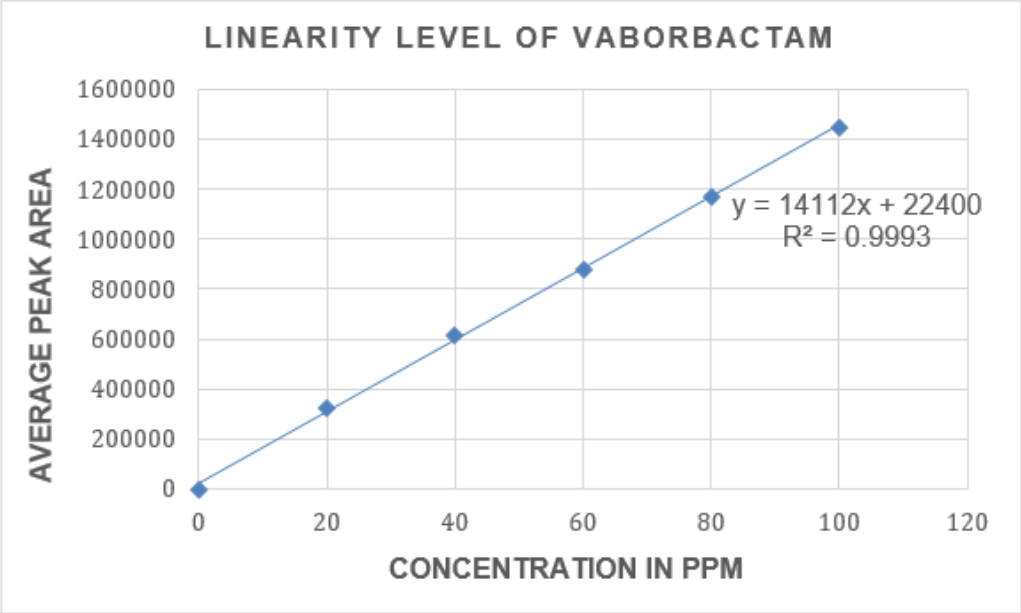

Figure 3: Calibration curve of Vaborabactam.

the %RSD values were found to be within the acceptance criteria indicating the methods high precision and reproducibility.

Accuracy

From the data reported in Table 4, the spiked samples were ascertained to be within the acceptance limits. Hence, the methods Accuracy.

Robustness

Even after making deliberate changes in the flow rate and mobile phase, which significantly cannot alter the response of analyte proved the robustness of current developed method.

CONCLUSION

A rational, fast, robust and specific HPLC approach that helps to measure Meropenem and Vaborbactam combined by developed analogy. The developed HPLC method can offer time and cost saving compared to other chromatographic methods. Shorter run time with good system suitability parameters reduced analysis time. Simple Mobile phase composition like MeOH: TEA buffer is inexpensive and easy to prepare compared to other methods using acetonitrile or gradient elution with multiple buffers. Thus the suggested technique shows a good ease to other developed techniques in terms of separation resolution, elution rate, Flow

time, simple mobile phase of appropriate ratio to assess the drugs in authentic and dosage forms and also to completely validate the parameters in chord with ICH guidelines which helps to be applied for all time quality control of mixtures through High Performance Liquid Chromatography technique with least detection and quantitation limits for combined dosage forms.

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ABBREVIATIONS

RP-HPLC: High Performance Liquid Chromatography; **ICH:** International Conference on Harmonization; **IP:** Indian Pharmacopoeia; **BP:** British Pharmacopoeia; **PDA:** Photo-Diode-Array; **TEA:** Triethylamine; **LOD:** Limit of detection; **LOQ:** Limit of Quantification; **SD:** Standard Deviation; **%RSD:** Percentage Relative standard deviation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

The authors received no financial support from any funding agency for this research work.

ETHICAL STATEMENT

Ethical approval was not sought for the present study as in vivo evaluation was not performed.

SUMMARY

A Reversed phase High performance chromatographic method was developed over "Phenomenex Gemini C18 of particle size (4.6 mm×150 mm, 5.0 µm)" column using eluent of Methanol (32 volumes) and TEA buffer (68 volumes) with a flow rate of 1 mL/min at 260 nm maximum absorbance. To assure the appropriateness of developed method for analytical purpose, it was validated as per ICH guidelines. The correlation coefficient obtained from linearity study by least square regression analysis proved that current method obeys Beer's- Lambert's Law. Precision studies showed percent RSD values <1% for both the drugs. The Detection limit of 2.6 µg/mL and 3.4 µg/mL and the Quantification values of 7.8 µg/mL and 10.2 µg/mL separately for Meropenem and Vaborabactam proved the sensitivity of the method. The obtained mean recovery values of 100.1873% and 100.34% proved the selectivity and the calculated %purity confirmed that the developed method can be routinely applicable for pharmaceutical dosage form Quality control analysis. From the less retention time for the analysis, confirmed that the present method can be readily applicable for checking the retest period of Active pharmaceutical ingredients.

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