WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 14, 906-920. Research Article ISSN 2277– 7105

DETERMINATION OF MEROPENEM AND VABORBACTAM IN DRUG SUBSTANCE AND DRUG PRODUCT DOSAGE FORMS – A VALIDATED CHROMATOGRAPHIC METHOD BY RP-UPLC USING ANALYTICAL QBD PRINCIPLES.

Vishnu Priya M.[1]*, P. Madhavan[1], Pramod Kumar[1] and Manoj Kumar Metta[2]

[1]Department of Chemistry, Jawaharlal Nehru Technology University, Hyderabad, India.

[2]United States Pharmacopeia India (P.) Ltd, IKP Knowledge Park, Genome Valley, Shamirpet, Turkapally (Village), Medchal (District), Hyderabad, Telangana – 500101.

Article Received on
21 September 2021,

Revised on 11 Oct. 2021,
Accepted on 01 Nov. 2021,

DOI: 10.20959/wjpr202114-22258

***Corresponding Author**
Vishnu Priya M.
Department of Chemistry, Jawaharlal Nehru Technology University, Hyderabad, India.

ABSTRACT

A parenteral drug product containing Meropenem and Vaborbactam is an antibacterial drug mainly to treat complicated urinary tract infection (cUTI). A RP-UPLC method was developed and validated per ICH (Q2) R1 for determining the identity and assay for its drug substance and drug product. The validation was performed on waters system equipped with the HSS C18 column, 0.1% Ortho phosphoric buffer: Acetonitrile (60: 40 v/v) as a mobile phase with a flow rate of 0.3 ml/min and λ max of 260 nm. The calibration plot was linear over the range of 25-150 µg/ml, LOD, LOQ were about 0.6 and 2.0 µg. The Accuracy by recovery was about 99%. The repeatability, robustness and solutions stability studies showed that the method is precise. Quality by design (ICH Q8-R1) requires a prospective summary of the desired quality characteristics of a drug substance and drug product. This is known as Quality Target Product Profile (QTPP), which forms the basis for the design and development of the method. Design space of the experiment was implemented to verify the robustness of the method using DOE.

KEYWORDS: Antibacterial; Method validation; ICH (Q2) R1; UPLC; Analytical Quality by Design (AQbD).

1. INTRODUCTION

Vaborbactam and Meropenem was approved by the Food and Drug Administration (FDA)

which is the first-in-class boronic acid-based β-lactamase inhibitor along with carbapenem a combination product.[1,2] This combination is used for the treatment of complicated urinary tract infections(cUTI) including pyelonephritis, intra-abdominal infection (cIAI), Hospital-acquired pneumonia (HAP) and ventilator associated pneumonia (VAP).[3] Vaborbactam (VBR) has been tested for treatment of bacterial infections in renal failure patients.[2] and Meropenem (MRP), is a broad spectrum carbapenem that is intravenously administered for severe bacterial infections.[4] Meropenem, present as a trihydrate, with a molecular weight of 437.5 g/mol, its chemical structure is given in Fig. 1. Vaborbactam with a molecular weight of 297.14 g/mol and its chemical structure is given in Fig.1.

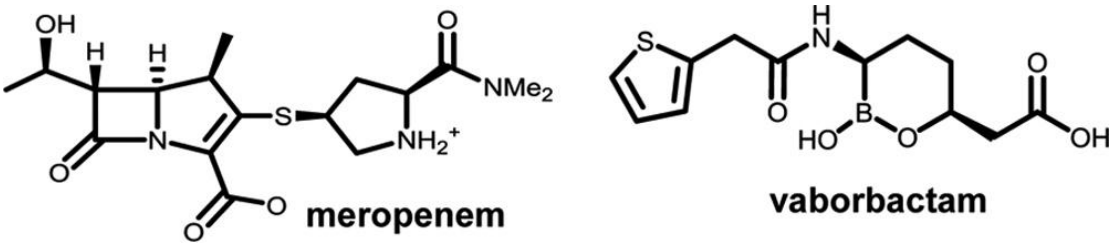


Fig. 1: Structure of Meropenem and Vaborbactam.

They two compounds are partially polaric in nature so freely soluble in ACN: Water (50:50, v/v). Vabomere is commercially available for adults with complicated urinary tract infections (cUTI), including a type of kidney infection, pyelonephritis, caused by specific bacteria. Vabomere is a combination of drug containing Meropenem, an antibacterial, and Vaborbactam, which inhibits certain types of resistance mechanisms used by bacteria.[5]

The increasing interest in Ultra-Performance Liquid Chromatography (UPLC) over the past couple of years is a demonstration that the potential power of this analytical methodology.[6,7] Analytical QbD is a science and risk based multivariate approach to identify the method performance with various method variability. It helps in development of a precise, accurate and cost-effective analytical method which can be implemented throughout the drug product life cycle.[8,9] USFDA also strongly recommended to implement analytical applications based on analytical QbD and method operable design region (MODR).[10] Robustness is a validation parameter which helps in understanding the stability of the drug product which is identified by small deliberate changes. QBD in robustness helps in developing models that describe the effect of parameters on the performance of the procedure, either through assessing performance against the ATP or other surrogate criteria which also enables the determination of procedure parameters and, if desired, a method operable design region (MODR).[11]

Several analytical methods were reported for the analysis of MRP in single or with the combination of other drugs in formulation combination and several biological fluids which includes human plasma using different sample preparation procedures: precipitation, liquid-or solid-phase extraction, generally followed by liquid chromatography coupled to UV detection or mass detection.[12-21] Spectrophotometric methods were also reported for the determination of MRP.[22-23] However, there is no stability indicating method for simultaneous estimation of MRP and VBR in a combined pharmaceutical dosage form along with Quality by Design concept for robustness studies. Hence, our present purpose of study is to develop and validate the Rp-UPLC method for the MRP and VBR stability studies, by performing forced degradation as per ICH guidelines along with QBD approach studies.

2. MATERIALS AND METHODS

2.1.Materials

The analytical method development and validation were performed on UPLC Make: Waters Separations module with Acquity TUV detector using Empower software (Version 2).

The Meropenem and Vaborbactam samples were obtained as a gift sample from **BMR chemicals and Spectrum laboratories**. The Solvents Distilled water (HPLC grade), Acetonitrile (HPLC grade), Methanol (HPLC grade), Ortho-phosphoric acid (AR grade). All the above chemicals and solvents are from **Merck specialties private limited, Mumbai**.

Buffer: (0.1%OPA).

Taken 1 ml of Ortho phosphoric acid solution in a 1000 ml of volumetric flask and added about 100 ml of milli-Q water and finally made up to 1000 ml with milli-Q water.

*Mobile phase***:** The Buffer and Acetonitrile are taken in the ratio of 60:40 ml.

*Diluent***:** The solvents Water and Acetonitrile are taken in the ratio of 50:50 ml.

2.2. Preparation of the Meropenem and Vaborbactam Standard and Sample Solution

Preparation of Solutions

Standard Preparation stock: Accurately Weighed and transferred 25mg of Meropenem and 25mg of Vaborbactam working Standards into 25 ml clean dry volumetric flasks, added 10ml of diluent, sonicated for 10 minutes and made up to the final volume with diluent.

Sample Preparation*:* Accurately weighed 2000 mg equivalent weight of the combination powder sample transfer into a 100 ml volumetric flask, 75ml of diluent was added and

sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (1000 µg/ml of Meropenem and 1000 µg/ml of Vaborbactam).

0.1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (100µg/ml of Meropenem 100µg/ml of Vaborbactam).

2.3. Method development

Method development is the process which is performed for a suitability of method to measure the API present in the dosage form. It is generally performed when there are no effective methods for new molecules or for improvisation of the existing methods.[24,25] The Meropenem and Vaborbactam have a high ratio of carbon to heteroatom and has a conjugated bond. Therefore, Meropenem and Vaborbactam can be separated on a C18 column mainly based on their overall hydrophobicity. However, to have a sound scientific judgement we did suitable method development studies. Prior to the method development the sample was injected in UV-Visible Spectrophotometry instrument to know the absorbance (λ-max value) of the molecules. From the spectrophotometry absorbance trails it was concluded that 260 nm was appropriate absorbance to achieve a good response (Fig.2). Hence, the development was started at 260 nm wavelength in UPLC instrument.

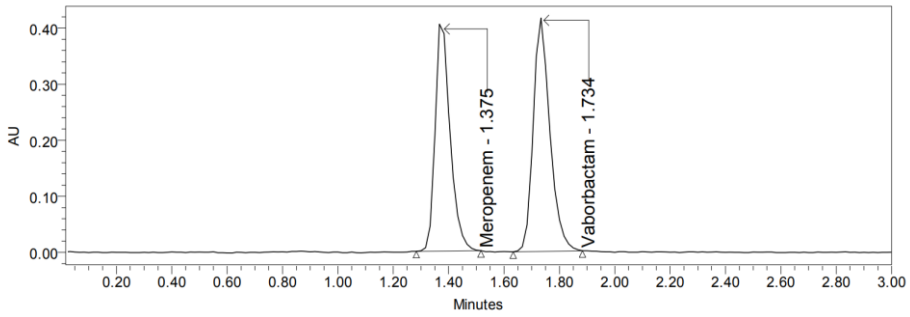


Fig. 2: Typical chromatogram of Meropenem and Vaborbactam.

Several chromatographic trails were performed to optimize the method and validate for the simultaneous estimation of Meropenem and Vaborbactam. The mobile phase and stationary phase play a vital role in theoretical plates, peak shape, symmetry, and resolution. To obtain symmetrical peaks with better resolution and peak purity, a variety of chromatographic conditions were investigated and optimized for the determination of Meropenem and Vaborbactam, such as mobile phases with different composition, pH and stationary phases with different packing material etc.

Finally, for the better efficiency of the method HSS C18 100 x 3 mm, 1.8µm, L1 packing, maintained at a 30^0 temperature was used. The optimum mobile phase used in this method was a mixture of buffer and Acetonitrile in the ratio of 60:40 v/v, with flow rate 0.3 mL/min. At 260 nm compounds were eluted and monitored.

Procedure

With the above optimized final method conditions 0.5µl of the standard and sample are injected into the chromatographic system and measured the areas for the Meropenem and Vaborbactam peaks to calculate the %Assay. The typical chromatogram with the final developed method is shown in the Fig.2.

3. RESULTS AND DISCUSSION

3.1. Method validation

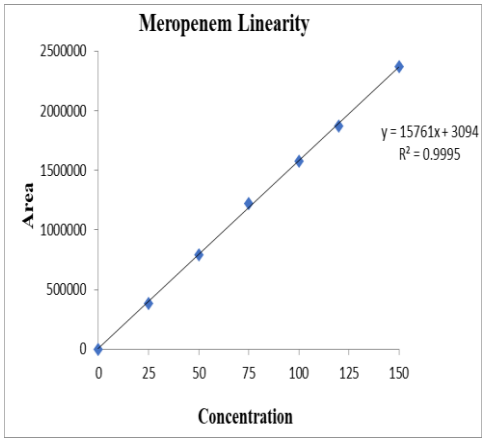
The method validation was performed as per ICH Q2 (R1) which does not cover specifications of validation parameters. The lack of validation guidance for these analytical data sets can lead to regulatory submissions with inadequate validation data, which can delay in application approval.[26] The below are the different validation parameters listed.

3.1.1. Specificity

Specificity of this assay was demonstrated by baseline or near baseline resolution of Meropenem and Vaborbactam main peaks from the other impurity peaks (Fig 2.). The absence of any UV absorbing components in the product excipients and baseline resolution was proved by obtaining the resolution of 3.5 (Limit: NLT 2.0) and Tailing factor 1.3 and 1.2 for Meropenem and Vaborbactam (Limit: NMT 2.0) within acceptable limits and also Peak Threshold of each analyte was more than the Peak angle.

3.1.2. Linearity and Range

Linearity of a sample of Meropenem and Vaborbactam showed that it contained 99% main peak (Fig.3) which was determined using three injections of six sample preparations ranging from 25 ppm – 150 ppm of the target sample concentration (100 ppm). The main peak area was plotted against the sample analyte concentration and the linearity of the assay was demonstrated by a Pearson correlation coefficient 0.99. Correlation coefficient for Meropenem and Vaborbactam were 0.9995 and 0.9992 respectively.



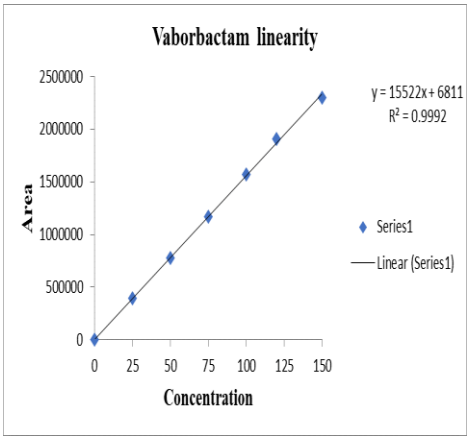


Fig. 3: Linearity for Meropenem. Linearity for Vaborbactam.

3.1.3. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The sensitivity of the method determined by LOD and LOQ. The LOD and LOQ for Meropenem and Vaborbactam were determined based on a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations. The LOD for Meropenem and Vaborbactam were 0.68 and 0.66 µg/ml, with s/n is 28.5 and 29.6. The LOQ for Meropenem and Vaborbactam were 2.06 and 2.00 µg/ml, with s/n is 67.9 and 68.4.

3.1.4. Accuracy/ Recovery

The recovery experiments were carried out to evaluate the accuracy,[27] of the method by injecting the spiked samples at three levels (50 %, 100 % and 150 %) of the target concentration. The obtained results were calculated and the mean recovery of 99.61 % for Meropenem and 99.29 % for Vaborbactam. The obtained results (Table 1) shows that there is no interference of the excipients.

Table 1: Accuracy results for Meropenem and Vaborbactam.

%Concentration (at specification Level)	% Recovery for Meropenem	Mean Recovery for Meropenem	% Recovery for Vaborbactam	Mean Recovery for Vaborbactam
50%	99.40	99.61	99.36	99.29
100%	99.59		99.46	
150%	99.85		99.84	

3.1.5. System Suitability

System Suitability is performed to know the condition of the instrument, by different parameters like resolution, tailing factor % RSD etc. to understand the reproducibility and detection sensitivity before and during the development and validation of the suggested work.

The resolution is obtained to be 3.0 for the specification of NLT 2.0, Tailing factor for Meropenem and Vaborbactam is 1.3 and 1.2 for the limit against NMT 2.0, Plate count for Meropenem and Vaborbactam is 3155 and 3986 for the limit of MLT 2000, and its precision i.e. the % RSD obtained is 0.8 and 0.3 for meropenem and Vaborbactam.[28,29]

3.1.6. Precision

The precision is performed at three level System precision, Method precision / Repeatability, Intermediate precision/ Ruggedness.[30]

For system precision known standard concentration of both Meropenem and Vaborbactam were checked and the % RSD obtained was 0.8 and 0.3 %. Intraday precision is injected using six preparations under same chromatographic conditions and the % RSD obtained is 0.2 and 0.3 % which is found to be less than 2.0% for Meropenem and Vaborbactam. Interday precision of the method was performed on different day by using different make column with same dimension with different analyst by injecting standard solution for six times on different day. The %RSD for the area of six replicate injections was found to be 0.4 and 0.7 % for Meropenem and Vaborbactam which is less than 2.0%.

3.1.7. Robustness

Robustness is the ability of an analytical method to remain unaffected by making some deliberate changes in the method parameters. This helps in the reliability of the method in regular usage. As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.27 ml/min to 0.33ml/min, the Organic composition in the Mobile phase was varied 10% and the column temperature was varied ± 5°C with the standard solution of 100ppm.The resolution obtained for all the variations was NLT 1.5.

3.1.8. Bench Top Stability of Test Solution

Benchtop solution stability was investigated to establish that of Meropenem and Vaborbactam is remaining stable in diluent i.e., Water: ACN (50:50). Test sample solutions were prepared and stored for a period of 24 hours at room temperature. Samples were analyzed after 24 hours. The results of bench top stability conclude that of Meropenem and Vaborbactam were 0.4 % and 0.7 % and the test sample solutions were stable at room temperature up to 24 hours.

3.2. Forced Degradation Studies

The stability of the drug substance or drug product is the critical parameter thus, the purity and behavior shall be checked. Changes in the drug stability can lead to the patient risk by formation of toxic substances or deliver lower dose of the drug than actually required and expected.[31-33] The FDA and ICH guideline requires that stress testing to be carried out to elucidate the inherent stability characteristics of the active substance but provided only little information on how much stress can be done has not been addressed. Thus, stress conditions should be done realistically but not excessively. Forced degradation was performed for Meropenem and Vaborbactam by exposing the drug substance and drug product to different stress conditions. Stressed samples were analyzed periodically and the presence of related peaks, retention time and peak purity for the active ingredients was checked.

No degradation was observed in Oxidative, thermal, photolytic and Temperature medium. Degradation was observed in acid with Peak purity Threshold and Purity angle are 0.52 and 0.36 for Meropenem and 0.57 and 0.36 for Vaborbactam and Basic medium with Peak purity Threshold and Purity angle are 0.59 and 0.39 for Meropenem and 0.58 and 0.38 for Vaborbactam (Fig.4.).

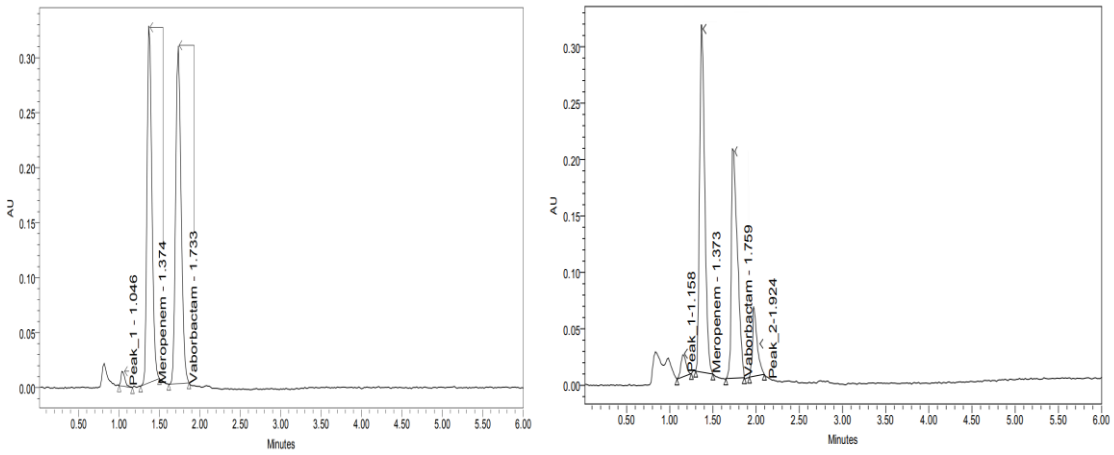


Fig. 4: Acid degradation. Base degradation.

However, no interference was observed due to degradation impurities for Meropenem and Vaborbactam. Peak purity angle value is less than peak purity threshold value of Meropenem and Vaborbactam in all degraded samples and within the acceptance criteria. So, there were no hidden peaks present masked by the main peak.

3.3. Design of Experiments

Most of the analytical method development screening done by a two-level full factorial design, a fractional factorial design, and a Plackett− Burman design. Prior to method development, the distributions of experiments in a three-dimensional (3D) space according to full factorial and fractional factorial designs are presented in Figure 5 to 6 respectively.

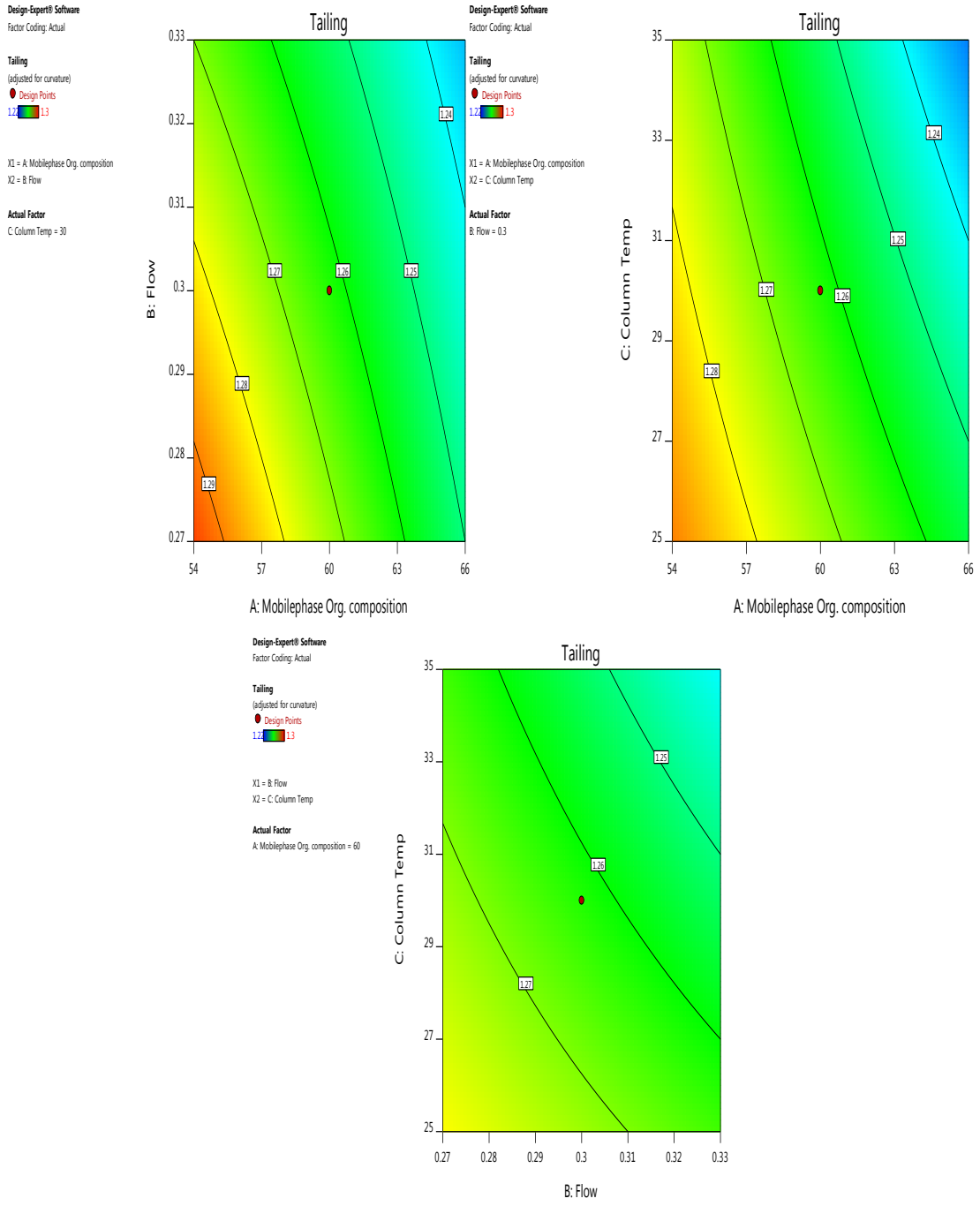


Fig. 5: Meropenem Factorial design between Flow, Column temperature and Mobile phase composition.

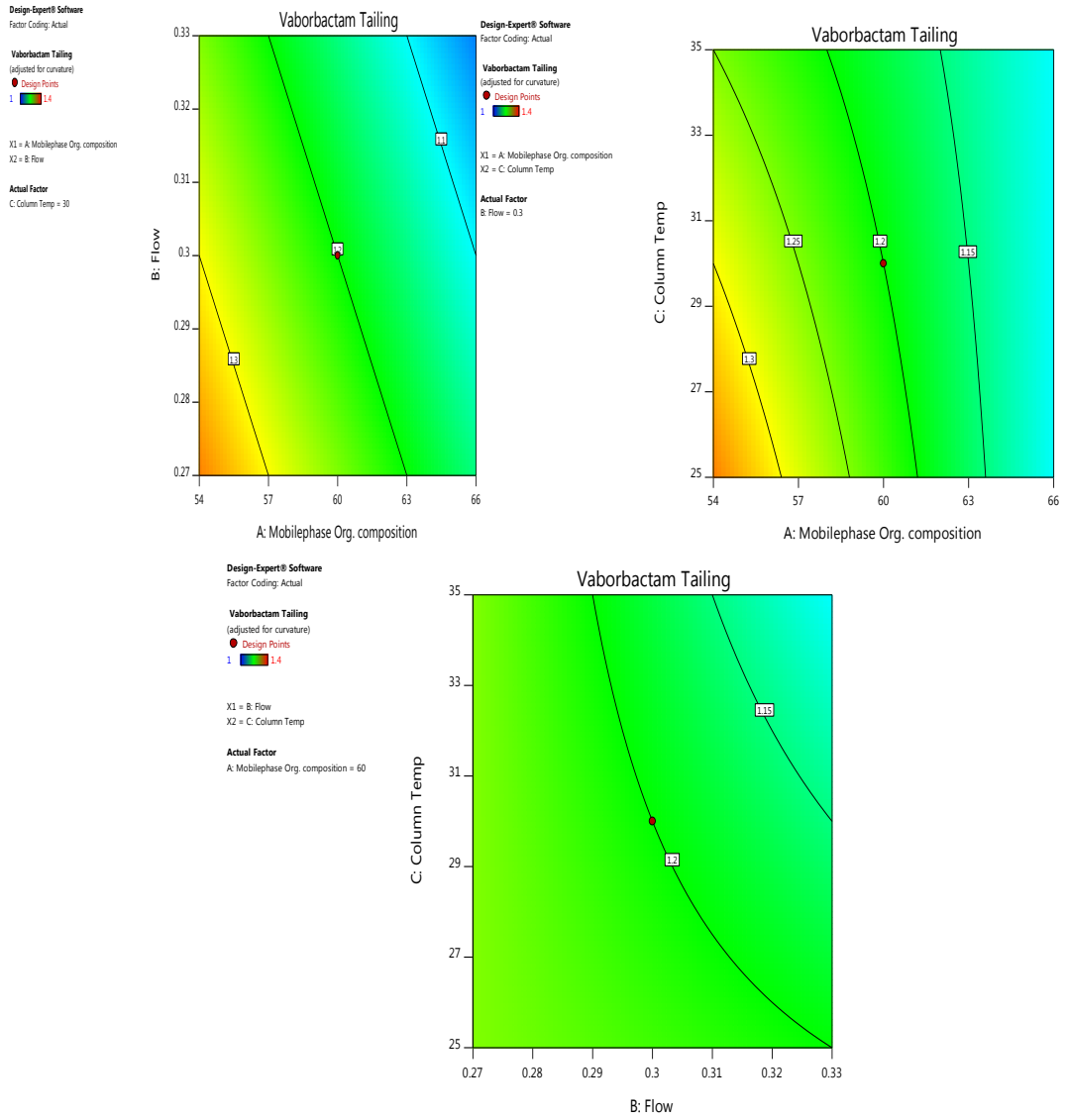


Fig. 6: Vaborbactam Factorial design between Flow, Column temperature and Mobile phase composition.

These designs allowed us to screen a high number of factors with fewer experiments, subsequently used for robustness testing. Design of experiments performed using three factors at low, center and high levels (Table 2 to Table 4) with Meropenem and Vaborbactam. We used three factors and two response factorial design. Factorial coding was performed by using Design expert software.

Table 2: Design of experiments using three factors at low, center, and high levels.

Name	Units	Type	Low	Center	High
Mobile phase Org. composition	%	Factor	54	60	66
Flow	mg/mL	Factor	0.27	0.3	0.33
Column Temp	^{o}C	Factor	25	30	35

Table 3: Design of experiments using three factors at low, center and high levels for Meropenem.

		Factor 1	Factor 2	Factor 3	Response 1	Response 2
Std	Run	A: Mobile phase Org. composition	B: Flow	C: Column Temp	Tailing	Meropenem %RSD
1	2	54	0.27	25	1.3	0.8
7	4	54	0.33	35	1.26	0.5
5	5	54	0.27	35	1.29	0.7
3	9	54	0.33	25	1.28	0.6
9	8	60	0.3	30	1.26	0.5
6	1	66	0.27	35	1.24	0.7
8	3	66	0.33	35	1.22	0.3
4	6	66	0.33	25	1.25	0.4
2	7	66	0.27	25	1.26	0.5

Table 4: Design of experiments using three factors at low, center and high levels for Vaborbactam.

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
Std	Run	A: Mobile phase Org. composition	B: Flow	C: Column Temp	Vaborbactam Tailing	Vaborbactam %RSD	Rs b/w Meropenem and Vaborbactam
1	2	54	0.27	25	1.4	0.7	3.8
7	4	54	0.33	35	1.2	0.4	3.6
5	5	54	0.27	35	1.3	0.6	3.7
3	9	54	0.33	25	1.3	0.5	3.7
9	8	60	0.3	30	1.2	0.3	3
6	1	66	0.27	35	1.2	0.6	2.5
8	3	66	0.33	35	1	0.3	2.6
4	6	66	0.33	25	1.1	0.4	2.7
2	7	66	0.27	25	1.1	0.4	2.6

Similar design of experiment as shown above figures also performed for the determination of the Meropenem and Vaborbactam %RSD. Meropenem and Vaborbactam %RSD factorial coding data not shown in this research article.

From the design space it iindicates that the tailing fcator for Meropenem and Vaborbactam is less than 1.5 which is well within acceptance criteria from all the experimental conditions.

As the intended purposed of this method is to check the related impurities and also as stability indicative method, we also established suitable resolution between meropenem and vaborbactam intact molecules. Again, fig 6 clearly indicated the plan of approach for robustness of this method.

4. CONCLUSION

A new sensitive UPLC method has been developed for the simultaneous determination of Meropenem and Vaborbactam in the drug substance and drug product formulations. The method was found to be capable for a good separation of all analytes (Meropenem and Vaborbactam, and degradation impurities) with acceptable resolution and tailing factor. The method was validated in accordance with ICH Q2 (R1) guidelines and found to be specific, precise, accurate, linear, robust, and rugged. Hence, the method can be used as part of a critical quality attribute in the pharmaceutical quality assessment of Meropenem and Vaborbactam Drug substance and Drug products.

Analytical QbD is a systematic approach for analytical method development that begins with setting the ATP, QTPP, determination of CMAs with their specifications, and identification of critical quality attributes. Though Meropenem and Vaborbactam final products are ANDA products, we tried to establish the design of experiments for robustness parameter with three factorial and two response factors for tailing, %RSD and resolution. Obtained experimental design was very supportive to investigate the effects of factors and their interactions on responses. These screening designs are used to identify significant factors, establishing a knowledge space during method validation. This research paper has demonstrated that analytical methods developed using the AQbD approach operate at their optimum and are highly robust, easily validated, and cost- and time effective because less experimental work is required for method development, and they can achieve maximum separation performance in a minimal analysis run time.

5. ACKNOWLEDGEMENTS

The authors would thank Jawaharlal Nehru Technology University, Hyderabad for the constant support and encouragement. The authors also thank to Spectrum labs and BMR Chemicals, Hyderabad for material donation. The author also thanks and grateful to Dr. Manoj Kumar Metta for persistent guidance and comments on the manuscript.

6. Disclosure Statement

The authors declare that there are no conflicts of interest.

REFERENCES

1. A. M. Hanretty, I. Kaur, A. T. Evangelista, W. S. Moore II, A. Enache, A. Chopra and J. J. Cies, Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 2018;

38: e87-e91.

2. A. Novelli, P. Del Giacomo, G. M. Rossolini and M. Tumbarello, Expert Review of Anti-infective Therapy, 2020; 18: 643-655.
3. S. C. J. Jorgensen and M. J. Rybak, Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, 2018; 38: 444-461.
4. M. A. Pfaller and R. N. Jones, Diagnostic microbiology and infectious disease, 1997; 28: 157-163.
5. S. Andrei, L. Valeanu, R. Chirvasuta and M.-G. Stefan, Discoveries (Craiova, Romania), 2018; 6: e81-e81.
6. M. E. Swartz, Journal of Liquid Chromatography & Related Technologies, 2005; 28: 1253-1263.
7. Cielecka-Piontek, J., Zalewski, P., Jelińska, A. et al. UHPLC: The Greening Face of Liquid Chromatography. Chromatographia, 2013; 76: 1429–1437.
8. M. K. Parr and A. H. Schmidt, Journal of Pharmaceutical and Biomedical Analysis, 2018; 147: 506-517.
9. G. Karen and F. Ludivine, Special, 2016; 29: 16–25.
10. P. Borman, Pharmacopeial Forum, 2019; 45.
11. USP-NF General chapter <1220> Analytical Procedure Life Cycle.
12. C. M. Rubino, S. M. Bhavnani, J. S. Loutit, E. E. Morgan, D. White, M. N. Dudley and D. C. Griffith, Antimicrobial Agents and Chemotherapy, 2018; 62: e02228-02217.
13. P. Mudassarahemadkhan and K. Ajay, International Journal of Pharmacy and Pharmaceutical Sciences, 2019; 11.
14. Negi V, Chander V, Singh R, Sharma B, Singh P, Upadhaya K. Method development and validation of Meropenem in pharmaceutical dosage form by Rp-HPLC. Indian J Chem Technol, 2017; 24: 441-6.
15. Casals G, Hernandez C, Hidalgo S, Morales B, Lopez Pua Y, Castro P, et al. Development and validation of a UHPLC diode array detector method for Meropenem quantification in human plasma. Clin Biochem, 2014; 47: 223-7.
16. Ikawa K, Kameda K, Ikeda K, Morikawa N, Nakashima A, Ohge H, et al. HPLC method for measuring meropenem and biapenem concentrations in human peritoneal fluid and bile: application to comparative pharmacokinetic investigations. J Chromatogr Sci., 2010; 48: 406-11.
17. Kloft C, Wicha SG. Simultaneous determination and stability studies of linezolid, meropenem and vancomycin in bacterial growth medium by high-performance liquid

chromatography. J Chromatogr B, 2016; 1028: 242-8.
18. Lobenhoffer JM, Bode-Boger SM. Quantification of meropenem in human plasma by HILIC–tandem mass spectrometry. J Chromatogr B, 2017; 1046: 13-7.
19. Frenich AG, Reyes RC, Gonzalez RR, Maresca MA, Vidal JL. Simultaneous analysis of antibiotics in biological samples by ultra high performance liquid chromatography-tandem mass spectrometry. J Pharm Biomed Anal, 2014; 89: 203-12.
20. Rigo Bonnin R, Ribera A, Arbiol Roca A, Cobo Sacristan S, Padulles A, Murillo O, et al.
21. Development and validation of a measurement procedure based on ultra-high performance liquid chromatography-tandem mass spectrometry for simultaneous measurement of β-lactam antibiotic concentration in human plasma. Clin Chim Acta, 2017; 468: 215-24
22. Babu KR, Kumari NA. Spectrophotometric determination of meropenem in bulk and injection formulations by 1, 2 naphtho quinone 4-sulphonic acid (NQS) reagent. Int J Pharm Sci Res., 2014; 5: 1963-7.
23. Marwada KR, Patel JB, Patel NS, Patel BD, Borkhatariya DV, Patel AJ. Ultraviolet spectrophotometry (dual wavelength and chemometric) and high performance liquid chromatography for simultaneous estimation of meropenem and salbactam sodium in pharmaceutical dosage form. Spectrochim Acta A Mol Biomol Spectrosc, 2014; 124: 292-9.
24. a) S. Sharma, S. Goyal and K. Chauhan, International Journal of Applied Pharmaceutics, 2018; 10: 8.
25. J. R. Lang and S. Bolton, Journal of Pharmaceutical and Biomedical Analysis, 1991; 9: 357-361.
26. J. Ermer, Journal of Pharmaceutical and Biomedical Analysis 2001, 24, 755-767; c) U. Nations, U. N. O. o. Drugs, C. Laboratory and S. Section, Guidance for the Validation of Analytical Methodology and Calibration of Equipment Used for Testing of Illicit Drugs in Seized Materials and Biological Specimens: A Commitment to Quality and Continuous Improvement, UN, 2009.
27. A. G. González, M. Á. Herrador and A. G. Asuero, Talanta, 2010; 82: 1995-1998.
28. D. E. Wiggins, Journal of Liquid Chromatography, 1991; 14: 3045-3060.
29. G. Maldener, Chromatographia, 1989; 28: 85-88.
30. J. Ermer and H.-J. Ploss, Journal of Pharmaceutical and Biomedical Analysis, 2005; 37: 859-870.
31. D. Reynolds, K. L. Facchine, J. F. Mullaney, K. M. Alsante, T. D. Hatajik and M. Motto,

Pharmaceutical technology, 2002; 26: 48-56.

32. M. Blessy, R. D. Patel, P. N. Prajapati and Y. K. Agrawal, Journal of pharmaceutical analysis, 2014; 4: 159-165.
33. J. Qureshi, R. Khanum, M. Chitneni, K. Mohandas and M. Rathbone, International Journal of Pharmacy and Pharmaceutical Sciences, 2014.