Research Article

Stability Indicating RP-UPLC Method Development and Validation for the Simultaneous Determination of Meropenem and Varobactum in Bulk and their Combined Dosage Form

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ABSTRACT

A stability indicating Ultra Performance Liquid Chromatography (UPLC) method was performed and validated for the simultaneous determination of Meropenem (MPM) and Vaborbactum (VBT) in bulk and their combined dosage form. The chromatographic development was performed on Aquity UPLC, Hibar C18 (100mm×2.1mm, 2µm) using isocratic elution of Acetonitrile and 0.01N Potassium dihydrogen ortho phosphate (KH_2PO_4) (50:50% v/v) at flow rate 0.3ml/min and at an ambient temperature of 30°c. Detection was carried out using UV detector at 250nm. The run time was 3min within which the compounds and their degradation peaks were separated. The developed method was validated and forced degradation was applied to identify the degradation behavior of the individual drugs under stress study. The retention times were found to be 0.858 and 1.218min for MPM and VBT respectively with a resolution 4.3. The method was found linear for MPM and VBT at ranges 25-150µg/ml and 25-150µg/ml respectively with the correlation coefficient 0.9995 and 0.9995 for MPM and VBT respectively. The method was found precise with the %RSD values of below 2% for replicate measurements. In the degradation study the result shows that the drugs degrade more in peroxide, base and least degraded in thermal, acid, photolytic and neutral. The developed UPLC method was stability indicating, successfully validated and yielded good results concerning range, linearity, accuracy, precision, specificity, system suitability, robustness, LOD and LOQ and is useful for the quantification and stability purpose.

Keywords: Meropenem, Vaborbactum, UPLC, Forced degradation, validation, tablet dosage form.

INTRODUCTION

Meropenem is a carbapenem antibiotic which has broad spectrum activity against gram positive and gram negative bacteria. It has the chemical name (1r,5s,6s)-2-[(3s,5s)-5-dimethylaminocarbonyl pyrrolidin-3-ylthio]-6-[(r)-1-hydroxyethyl]-1-methylcarba pen-2-em-3-carboxylic acid. It has a molecular formula $C_{17}H_{25}N_3O_5S$ and molecular weight 383.463 g/mol[1,2]. It has a molecular structure Fig.1.

Based on the cyclic boronic acid pharmacophore, Vaborbactum is a β-lactamase inhibitor. It has the chemical name 2-[(3R,6S)-2-hydroxy-3-[2-(thiophen-2-yl) acetamido]-1,2-oxaborinan-6-yl]acetic acid. It has a molecular formula $C_{12}H_{16}BNO_5S$ and molecular weight 297.13 g/mol[1]. It has a molecular structure Fig.2.

The only formulation with the combination of these two drugs was Vabomere which is available as injection and powder for solution, which is used in the treatment of adults with complicated urinary tract infections including pyelonephritis caused by Escherichia coli, *Enterobacter cloecia* species and klebsiella pneumonia[1-4].

Literature survey reveals that there are different analytical methods for the individual drug of Meropenem[5-8] and an RP-HPLC method for estimation of meropenem and vaborbactum using acetonitrile and OPA 50:50 ratio. There was no stability indicating method for the estimation of MPM and VBT in presence of their degradation products. Hence the authors made an attempt to develop a sensitive, accurate, precise validated stability indicating method for the estimation of MPM and VBT simultaneously by using UPLC in bulk and their combined dosage form. These methods were validated based on International conference of harmonization (ICH) guidelines[10].

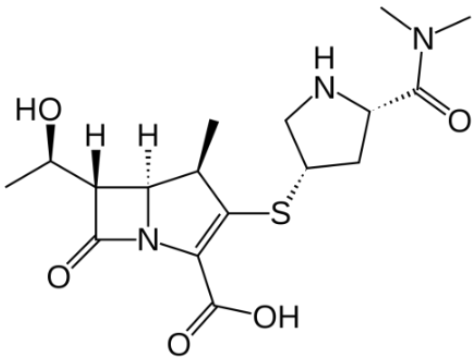

Figure 1: Structures of Meropenam

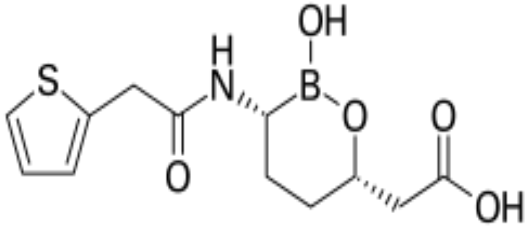

Figure 2: Structures of Vaborobactum

MATERIALS AND METHODS

Instrumentation

The UPLC used was Waters Aquity, equipped with UV detector. For data acquisition, Empower 2.0 version software is used for the system. The micro balance used was Sartorius. Sonicator used was ultra sonicator (Kshitij innovations). IR spectrophotometer used was bruker.

Chemicals

All the reference and pharmaceutical grade MPM (99.95) and VBT (99.93) was procured as a gift samples from Spectrum Pharma Pvt. Ltd., Hyderabad, India. All the HPLC graded solvents (MilliQ water, Acetonitrile, KH_2PO_4, Hydrogen peroxide, Sodium hydroxide, Hydrochloric acid) were obtained from Merck specialties private limited, Mumbai. The commercially available tablets of MPM and VBT were obtained from the nearby local pharmacy.

Preparation of buffer solution and Mobile phase

Weigh accurately about 1.36gm of Potassium dihyrogen ortho phosphate in a 1000ml of volumetric flask and add about 200ml of milli-Q water and sonicate. Finally make up the volume with water then added 1ml of Triethylamine then PH adjusted to 3.0 with dil. Orthophosphoric acid solution. Mobile phase was prepared by mixing 0.01% KH_2PO_4 and Acetonitrile HPLC Grade in the ratio of 50:50% v/v. The prepared mobile phase was sonicated for 15min and filtered through 0.22µm membrane filter to remove the impurities which may interfere with final chromatogram and solution was used as diluent.

Preparation of solutions

Preparation of standard solution

Accurately weighed and transferred 50 mg of Meropenem and 50mg of Vaborbactum working standards into a 50ml and 50ml clean dry volumetric flasks, add 25ml of diluent sonicated for 5mins and make up to the final volume with diluent and concentration was obtained 1000µg/ml. From the above stock solution 1ml was pipetted out into a 10ml volumetric flask and made up to 10 ml with diluent 100 µg/ml.

Preparation of sample solution:

Accurately weighed 20 tablets and crushed into powder by using mortar and pestle transferred equivalent quantity of 50 mg of Meropenem and 50mg of Vaborbactum working samples into a 50 ml clean dry volumetric flasks, add 25ml of diluent sonicated for 5mins and make up to the final volume with diluent and concentration was obtained 1000µg/ml. From the above stock solution 1ml was pipetted out into a 10ml volumetric flask and made up to 10 ml with diluent concentration was attained 100µg/ml.

Chromatographic conditions

The chromatographic analysis has been performed on Hibar C18 (100× 2.1mm, 2µm) column. Mobile phase consists of 0.01% KH_2PO_4: Acetonitrile (50:50% v/v) with 250nm UV detection and 30°c column oven temperature. Flow rate was adjusted at 0.3ml/min with 2µl injection volume. The total run time was 3min.

Method validation[10]

The proposed method was validated as per ICH guidelines (ICH Q2 R_1, 1995). The parameters observed for assay validation are accuracy, precision, linearity, range, specificity, limit of detection, limit of quantification [9].

RESULTS AND DISCUSSION

Method optimization

Several trails were made for accurate and precise method development. After using different buffers and columns, a good peak was obtained only in Aquity UPLC Hibar C18 (100× 2.1mm, 1.5µm) column with mobile phase consisting of Acetonitrile and 0.01N Potassium dihydrogen ortho phosphate (KH_2PO_4) (50:50% v/v). A simple isocratic program was applied for the analysis at flow rate of 0.3ml/min showed good resolution, which is greater than 3.00 between MPM and VBT. The retention times of the drugs under these conditions were 0.858 and 1.218min respectively for MPM and VBT. For wavelength selection the standard solution was screened over the range of 190-400nm using UV detector and 250nm was decided as detection wavelength which provides the maximum compatibility to the chromatographic method. The optimized chromatogram was shown in the Fig. 3. The assay results were shown in the Table no 1.

Table 1: Assay results

Drug	Labelled amount (mg/tab)	Estimated amount (mg/tab) (n=6)	% drug content
MPM	1000	998.89	99.88
VBT	1000	992.90	99.29

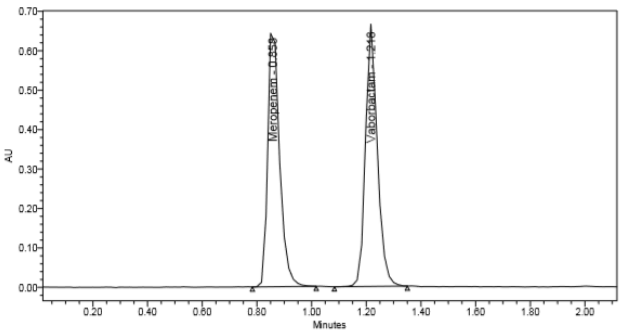

Figure 3: Optimized Spectra of MPM and VBT

System suitability

System suitability was checked for the conformation of the suitability and reproducibility of chromatographic system for the analysis. For suitability, six replicates of working standard samples were injected and the parameters like plate number (N), resolution, retention times (RT), tailing

factor (K) were evaluated. Results were showed in table no.2.

Table 2: System suitability results

Parameter	Results (n=6)	
	Meropenem	Vaborbactum
%RSD of peak area	0.3	0.3
%RSD of retention time	0.13	0.13
Tailing factor	1.26	1.165
Resolution	-	4.46
Plate count	2195	3460

Specificity

Specificity was exhibited by analyzing the blank and placebo to check the interference of the additional peaks in retention times of MPM and VBT from the other excipient compounds. The chromatograms were showed in figure no 4 & 5.

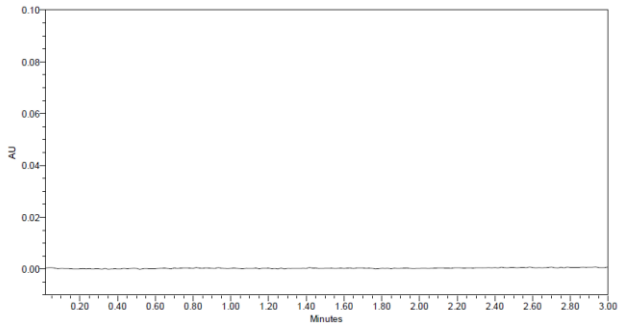

Figure 4: Chromatogram of Blank

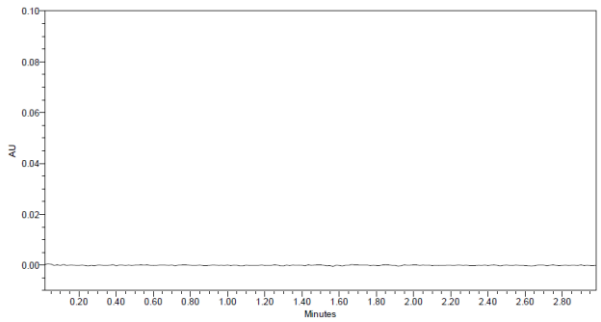

Figure 5: Chromatogram of Placebo

Linearity

For the linearity study, a series of aliquots were prepared in the range of 25-150µg/ml for both MPM and VBT, respectively from the working standard solution. Each concentration was injected 6 times into the column and each time the peak area and retention times were detected. Calibration curve was constructed by plotting concentration against mean peak area and generated by replicate analysis (n=6) at all concentrations and the regression equations were computed. These regression equations were used to estimate the drug content in commercial tablets. The calibration curves were showed in figure no 6 &7 and results were tabulated in table no 3.

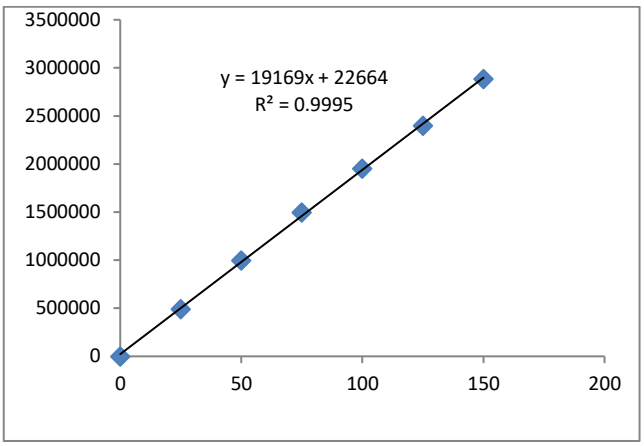

Figure 6: Calibration curve of MPM

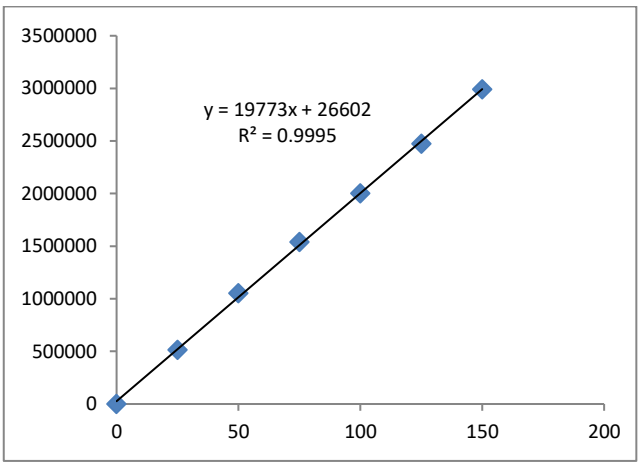

Figure 7: Calibration curve of VBT

Table 3: Optical characteristics

Parameter	Results(n=6)	
	Meropenem	Vaborbactum
Linearity range	25-150µg/ml	25-150 µg/ml
Correlation coefficient	0.9995	0.9995
Slope	19169	19773
Y-intercept	22664	26602
LOD	0.56µg/ml	1.68µg/ml
LOQ	0.73µg/ml	2.19µg/ml

Accuracy

Accuracy was performed on the basis of recovery studies were evaluated in triplicate at three levels 50, 100, 150% of test concentration (25, 50, 75µg/ml for both MPM and VBT) and by comparing the theoretical value and the actual value was found. The percentage mean recovery was calculated. Results were tabulated in table no 4 & 5.

Table 4: Accuracy results of MPM

S.No	Recovery level	Preanalysed concentration (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean	SD	% RSD
1.	50%	50	25	74.93	99.90	99.59	0.39	0.40
		50	25	74.94	99.92			
		50	25	74.91	99.88			
2.	100%	50	50	99.27	99.27			
		50	50	98.94	98.94			
		50	50	99.06	99.06			
3.	150%	50	75	124.59	99.67			
		50	75	124.82	99.85			
		50	75	124.73	99.78			

Table 5: Accuracy results of VBT

S.No	Recovery level	Preanalysed concentration(µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean	SD	% RSD
1.	50%	50	25	74.82	99.76	99.56	0.32	0.33
		50	25	74.74	99.65			
		50	25	74.92	99.89			
2.	100%	50	50	99.54	99.54			
		50	50	98.78	98.78			
		50	50	99.68	99.68			
3.	150%	50	75	124.69	99.75			
		50	75	124.25	99.40			
		50	75	124.51	99.60			

Table 7: Robustness results

Parameter	Variation	Chromatographic Conditions					
		Retention Time		Area		Tailing Factor	
		MPM	VBT	MPM	VBT	MPM	VBT
Flow change	0.2ml/min	0.879	1.421	1968545	2020071	1.23	1.19
	0.3ml/min	0.858	1.218	1875842	1945235	1.20	1.29
	0.4ml/min	0.822	1.021	1787073	1832045	1.15	1.18
Temperature	28	0.986	1.12	1953668	1999573	1.20	1.27
	30	0.858	1.21	1945248	1999245	1.29	1.18
	32	0.918	1.252	1948772	1998069	1.31	1.14
Mobile phase (%acetonitrile)	45	0.838	1.197	1931188	1990210	1.30	1.12
	50	0.855	1.210	1932254	1987841	1.31	1.15
	55	8.65	1.223	1922483	1975653	1.32	1.16

Precision

Precision was performed by multiple sampling of the same preparation. Six replicates were taken of same concentration and were analysed in triplicate on the same day. Fresh solutions were prepared and analysed for method precision. The percentage assay values have been calculated for each sample using the peak area of chromatogram. The relative standard deviation obtained from the assay value using UPLC is not more than 2%. Results were tabulated in table no.6

Table 6: Precision results of MPM and VBT

Parameter		Results (n=6)	
Name of drug		Meropenem	Vaborbactum
Concentration		100µg/ml	100µg/ml
Mean RT		0.898	1.27
Area	Mean	1959008	2056916
	SD	4684.1	8197.0
	%RSD	0.2	0.4

Robustness

The robustness was performed to study the method remained unaffected by small but deliberate changes in the analytical conditions. The variables evaluated were flow rate (±0.05ml/min), column oven temperature (±2°c), and % organic phase in mobile phase composition (±2%). The results were tabulated in table no 7.

Detection limit and quantification limit

LOD is comparing the measured signals from samples with those of blank samples and with known low concentrations of analytes and by establishing the minimum concentration at which the analyte can be reliably detected. The signal to noise ratio between 3 or 2:1 is generally acceptable for LOD.

LOQ is comparing the measured signals from samples with those of blank samples and with known low concentrations of analytes and by establishing the minimum concentration at which the analyte can be reliably quantified. Typically signal to noise ratio of LOQ was 10:1.

Forced degradation studies[9,11]

As per FDA guidelines, stability indicating test procedure is a validated quantitative analytical procedure that can detect changes in the quality attributes during the storage of any drug substance or product. The forced degradation study was performed by using acidic, alkali, oxidative, thermal, neutral and photolytic stress to the samples and using UPLC, the percentage of degradation was investigated[8,9]. The results were tabulated in table no 8.

Table 8: Degradation Studies of MPM and VBT

Stress condition	% assay of active ingredients after degradation (n=6)		% degradation	
	MPM	VBT	MPM	VBT
Acid	97.44	96.31	2.56	3.69
Base	92.72	93.71	7.28	6.29
Peroxide	93.70	93.96	6.30	6.04
UV	97.60	97.29	2.40	2.71
Thermal	98.11	98.44	1.89	1.56
neutral	99.23	99.19	0.77	0.81

Acid degradation studies

To 2.5ml of stock solution MPM and VBT, 2.5ml of 2N hydrochloric acid was added and refluxed for 30mins at 60^{0}c. Cool the solution to room temperature and neutralize it with 2N NaOH and make up to final volume in 50ml volumetric flask. Dilute the resultant solution to obtain 50µg/ml solution and 2µl sol ution was injected into the system and the chromatogra ms were recorded to assess the stability of sample. The ch romatogram was showed in figure no 6.

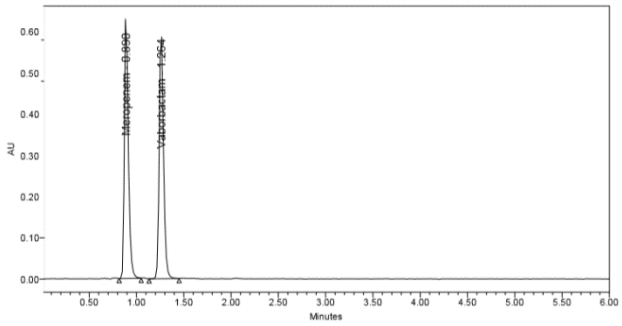

Figure 6: Chromatogram of acid degradation

Alkaline degradation studies

To 2.5 ml of stock solution of MPM and VBT, 2.5 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^{0}c. Cool the solution to room temperature and neutralize it with HCl and make up to final volume. Dilutethe resultant solution to obtain 100µg/mlsolution and 2µl solution was injected into the system and the chromatograms were recorded to assess the stability of the sample**.** The chromatogram was showed in figure no 7.

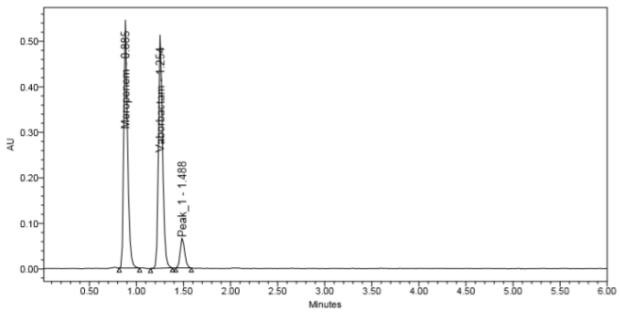

Figure 7: Chromatogram of alkali degradation

Oxidative degradation studies

To 2.5 ml of stock solution of MPM and VBT, 2.5ml of 30% H_2O_2 was added and heat for 30 mins at the temperature of 60°c. Cool the solution to room temperature and make up to final volume. Dilute the resultant solution to obtain 100µg/ml solution and 2µl solution was injected into the system and the chromatograms were recorded to assess the stability of the sample. The chromatogram was showed in figure no 8.

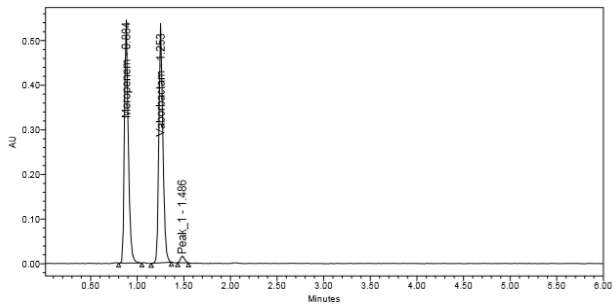

Figure 8: Chromatogram of peroxide degradation

Thermal degradation studies

Thermal degradation was performed by exposing the 50mg of MPM and VBT to 105°c for 6 hrs in hot air oven. Dissolve the drug to obtain 50µg/ml solution and 2µl solution was injected into the system and the chromatograms were recorded to assess the stability of the sample. The chromatogram was showed in figure no 9.

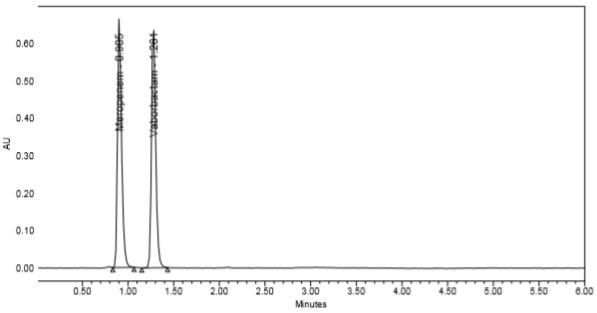

Figure 9: Chromatogram of Thermal degradation

Photo stability studies

For photolytic degradation, the standard stock solution was exposed to UV light for 7 days or 200 Watt hours/m2 in photo stability chamber. Dilute the resultant solution to obtain 100µg/ml solution and 2µl solution was injected into the system and the chromatograms were recorded to assess the stability of the sample. The chromatogram was showed in figure no 10.

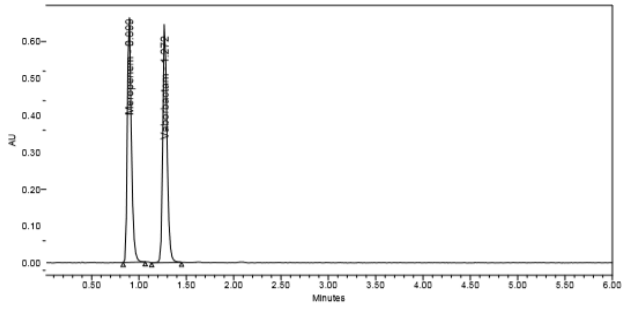

Figure 10: Chromatogram of UV degradation

Neutral degradation studies

For hydrolytic degradation, 10ml of water is added to the standard stock solution and refluxed for 6hrs at the temperature of 60°c. Dilute the resultant solution to obtain 100µg/ml solution and 10µl solution was injected into t he system and the chromatograms were recorded to stud y the stability of the sample**.** The chromatogram was showed in figure no 11.

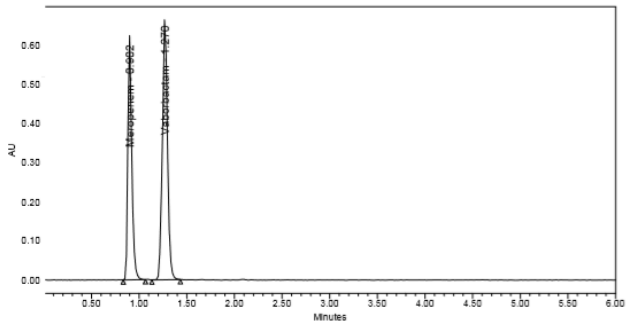

Figure 11: Chromatogram of UV degradation

Different parameters like linearity, accuracy, precision, system suitability, specificity, robustness, LOD and LOQ are performed and found to be within limits. System suitability was performed to evaluate the parameters like resolution, tailing factor, theoretical plates and %RSD for replicate injections. The results were within the limits and were shown in Table 2. The method was specific as there was no interference at the retention times of MPM and VBT from the other excipient compounds. Chromatograms of blank and placebo were given in Fig. 4 and 5. Linearity was established in the ranges of 25-150µg/ml for both MPM and VBT, respectively and the regression equation was computed. The correlation coefficient was found to be 0.9995 for both MPM and VBT, respectively. The summary of the parameters which are the correlation coefficient, slope, y-intercept were shown in Table 3 and the calibration plots of MPM and VBT were shown in the Fig. 6 and 7. Accuracy was established based on the recovery levels 50, 100, 150 % and the percentage recovery values were shown in the Tables 4 and 5. The percentage mean recovery values of MPM and VBT were found to be 99.59 and 99.56 respectively indicating that the method was accurate for the determination of MPM and VBT in pharmaceutical formulation. The precision of the method was performed by repeatability. Repeatability was performed by injecting the six individual preparations of sample containing same concentration of MPM and VBT which is 500 and 500µg/ml concentrations respectively. The %RSD was calculated for each sample and was found to be within acceptance criteria which confirm the good precision of the method. The precision values of MPM and VBT were given in the Table 6. LOD values of MPM and VBT were found to be 0.56µg/ml and 1.68µg/ml and LOQ values of MPM and VBT were 0.73µg/ml and 2.19µg/ml, respectively. The robustness of the method was evaluated by the method conditions such as, flow rate (±0.05ml/min), column oven temperature (±2°c), and % organic in mobile phase

composition (±2%) were altered and the influence of these changes on the assay, peak tailing, number of theoretical plates and peak area were evaluated. These system suitability parameters were found to be within the acceptance criteria. Thus the method was said to be robust with respect to the variability applied and results were shown in Table no.7. The method was showed ability to different stress conditions and proved as stability indicated.

CONCLUSION

The explored UPLC method was fast, simple, sensitive, accurate and precise for the estimation of Meropenem and Vaborbactum in bulk and their combined dosage form. The compounds were subjected to forced degradation applying some stress conditions. The proposed method successfully separates the two compounds with each other and also with degradants. The proposed method is specific and stability-indicating. Hence the developed method can be adapted to regular quality control analysis and stability studies.

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