DEVELOPMENT AND VALIDATION OF AN RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF MEROPENEM AND VABORBACTAM IN BULK AND DOSAGE FORM

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ABSTRACT

A analytical method employing reverse phase high performance liquid chromatography with PDA detection was developed to assay meropenem and vaborbactam in bulk and injection dosage form. The assay of meropenem and vaborbactam was achieved by Supelco C8 column (25 cm × 4.6 mm, 5 µm particle size) with isocratic elution. The mobile phase involved is methanol and potassium dihydrogen phosphate (0.1 Molar) mixed in ratio 65/35 (v/v). The eluted meropenem and vaborbactam are monitored at wavelength 235 nm. The procedure employed separated meropenem and vaborbactam within six min run time. Retention time of meropenem and vaborbactam were found to be 2.915 and 3.406. The parameters were tested by validating system suitability, linearity, sensitivity, selectivity, accuracy, robustness and precision. Linearity of meropenem and vaborbactam was in range of concentration 500 – 1500 µg/ml. The LOD and LOQ values were found to be 0.706 and 2.352 µg/ml for meropenem and 0.453 and 1.510 µg/ml for vaborbactam. For precision, relative standard deviation were found to be 0.102% (meropenem) and 0.050% (vaborbactam). The percent recoveries were 100.20 -100.36% and 99.60-99.67% for meropenem and vaborbactam respectively. The selectivity results proved the non interference from excipients. So this method is recommended for the routine analysis of meropenem and vaborbactam in laboratories of quality control.

Key words: Meropenem, Vaborbactam, RP-HPLC Method, Validation.

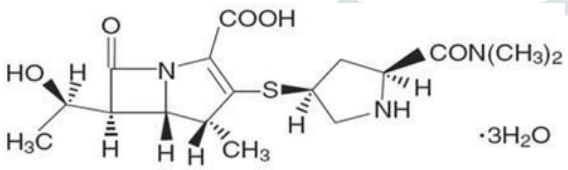
INTRODUCTION

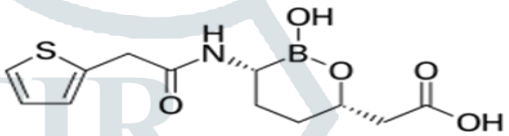
Meropenem, an antibacterial agent, belongs to carbapenems chemical category of drug. Meropenem chemical name is (4R,5S,6S)-3- (((3S,5S)-5-(Dimethylcarbamoyl)pyrrolidin-3- yl)thio)-6-((R)-1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene -2- carboxylic acid. It acts in opposition to gram positive or gram negative aerobic or and anaerobic organism that includes *Enterococcus, Klebsiella, Clostridium species, E.coli*, etc. Meropenem is more active against Enterobacteriaceae and less active against gram positive bacteria. It works against extended-spectrum beta lactamases, but may be more susceptible to metallo-β-lactamases. Meropenem is frequently given in the treatment of febrile neutropenia. This condition frequently occurs in patients with hematological malignancies and cancer patients receiving anticancer drugs that suppress bone marrow formation. Meropenem is indicated to treat patients (adult and pediatric with 3 or more than 3 months) suffering with bacterial meningitis, skin structure infections and intra abdominal infections. Half life is 1hr for adults and 1.5 hr for children with 3 months to 2 years age. 70% of administered meropenem is excreted over 12 hr in urine. It acts through penetrating the cells of bacteria, meropenem readily interferes with the formation of important components of bacterial cell wall. This leads to the bacterial cell death. The target of meropenem is penicillin binding protein in bacteria. Meropenem is administered intravenously as a white crystalline powder to be dissolved in 5% monobasic potassium phosphate solution. Dosing must be adjusted for altered kidney function and for haemofiltration.

Vaborbactam is a boronic acid, beta lactamase inhibitor with a high affinity for serine beta lactamases, including Klebsiella pneumoniae carbapenemase (KPC). Vaborbactam chemical name is {(3R,6S) -2- Hydroxy -3- [2-(thiophen-2-yl)acetamido]-1,2-oxaborinan -6- yl}acetic acid. Vaborbactam is not an antibiotic. The presence of vaborbactam increases the potency of other antibiotic drug belonging to beta lactam category. In combined form with other proper antibiotic vaborbactam is used for treating infections with gram negative bacteria. Half life is 1.68 hr after 2 gram dose administration and 2.25 hr after 3 gram dose administration. 75-90% of administered vaborbactam is excreted unchanged over 24-48 hr in urine. Vaborbactam renal clearance is 8.9 L/hr. Vaborbactam activity is based on its pharmacore, cyclic boronic acid pharmacophore. Vaborbactam inhibits carbapenemase of *Klebsiella pneumoniae* and Ambler class A & C enzymes like beta lactamases. These enzymes give resistance to normally used carbapenem antibiotics. By inhibiting these enzymes vaborbactam increases the potentiality of carbapenem antibiotic drugs.

In the United States, the combination drug meropenem/vaborbactam (Vabomere) is approved by the Food and Drug Administration for complicated urinary tract infections and polynephritis.

Fig 1: Meropenem Chemical structure **Fig 2: Vaborbactam Chemical structure**





MATERIALS AND METHODS

Chromatographic conditions:

HPLC system - Model 2695 Waters Alliance system with photodiode array detector. Column used for development and validation of method - Supleco, C8, 25 cm × 4.6 mm, particle size 5 µm.Vabomere sterile powder vials (Facta Farmaceutici, Teramo, Italy) were employed. Lara Drugs Private Limited, Hyderabad, Telangana, India provided gift samples of reference substances of meropenem and vaborbactam. Methanol of HPLC grade were used (Merck specialities Ltd, Hyderabad, Telangana, India.)

Preparation of mobile phase:

Prepared by combining methanol and 0.1 M potassium dihydrogen phosphate in the ratio 65:35 (v/v). The same is employed for preparing standard solutions.

Standard stock solution of meropenem and vaborbactam:

Stock solution of meropenem (10 mg/ml) and vaborbactam (10 mg/ml) was prepared by dissolving 1000 mg each of meropenem and vaborbactam in 100 ml of mobilephase in a 100 ml volumetric flask.

Standard working solutions of meropenem and vaborbactam:

Below mentioned working solutions of meropenem and vaborbactam are made through dilution of meropenem and vaborbactam stock solution using mobile phase.

- Standard calibration solutions: 500, 750, 1000, 1250 and 1500 µg/ml of meropenem and 500, 750, 1000, 1250 and 1500 µg/ml of vaborbactam.
- To validate the method, working standard of meropenem and vaborbactam with concentration 1000 µg/ml each is prepared from stock using mobile phase.

Injection sample solution preparation:

Five vials are made empty. Total weight was determined. Weight of powder that is equal to 1000 mg each of meropenem and vaborbactam was determined and transferred to volumetric flask (100 ml). Fifty ml mobile phase was transferred to the flask and was sonicated 20 min. The resulting solution was filtered and made to 100 ml with mobile phase. The concentration of resultant

solution is 10 mg/ml of meropenem and 10 mg/ml of vaborbactam. This stock injection solution was further diluted to a final test solution with concentrations 1000 µg/ml (vaborbactam) and 1000 µg/ml (meropenem) by mobile phase.

Meropenem and vaborbactam calibration curves:

To five volumetric flasks of 10 ml capacity, suitable aliquots of stock solution with concentration meropenem – 0.5, 0.75, 1.0, 1.25 and 1.5 mg/ml; vaborbactam – 0.5, 0.75, 1.0, 1.25 and 1.5 mg/ml, were pippeted. These aliquots were diluted by mobile phase to acquire calibration solutions of concentrations 500, 750, 1000, 1250 and 1500 µg/ml of meropenem and 500, 750, 1000, 1250 and 1500 µg/ml of vaborbactam. Ten µl of each concentration solution was injected into HPLC column. Both drugs were analyzed by employing above said HPLC conditions. The meropenem and vaborbactam peak areas were assessed at 235 nm. Meropenem and vaborbactam calibration graphs are constructed. Regression equations for drugs were worked.

RESULTS AND DISCUSSION

Method development:

For developing a method for estimating meropenem and vaborbactam in injection dosage form and bulk drug, firstly different parameters were tried to elute both drugs with good resolution, less tailing factor and greater plate count. Satisfactory results obtained from given chromatographic conditions for Meropenem and Vaborbactam given in Table: 1

Table 1: Parameters of method development

Parameters	Chromatographic Conditions
Mobile phase	KH_2PO_4 : Methanol (65:35, v/v)
Column	Supleco, C8, 25cm × 4.6mm, 5 µm
Flow Rate	1.0 ml/min
Temperature	25°C
Volume	10 µl
Run time	6min
Detector	235nm

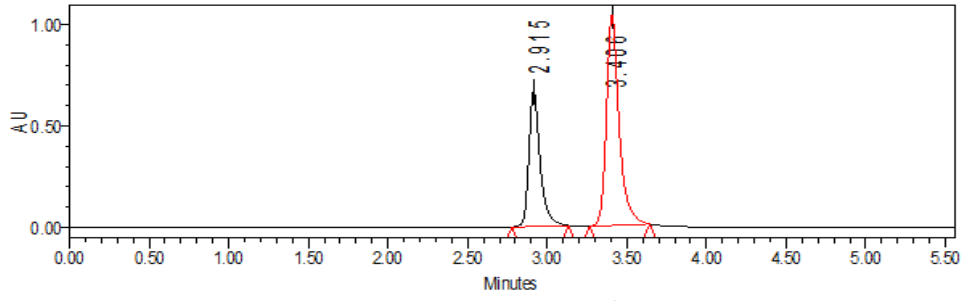


Fig 3: Chromatogram of Meropenem and Vaborbactam

Method validation:

Validation was performed in harmony to ICH guidelines. System suitability, selectivity, linearity, sensitivity, accuracy, precision, specificity and robustness were determined.

System suitability study:

The system suitability was established by six consecutive injections of the same working standard solution (1000 µg/ml of meropenem and vaborbactam). The parameters considered are: plate count, peak tailing, resolution, relative standard deviation and retention times of meropenem and vaborbactam. The values for the system suitability parameters of method, are presented in Table 2, are within acceptance limits.

Table 2: System suitability of meropenem and vaborbactam

Sample No.	RT	PA	PC	PT	RS
Meropenem (1000 µg/ml)					
1	2.909	3317180	8856	1.44	-
2	2.908	3307997	9233	1.49	-
3	2.905	3324951	9111	1.46	-
4	2.906	3303795	8944	1.48	-
5	2.903	3319644	9093	1.47	-
Mean, RSD (%)	**2.906, 0.082**	**3314713, 0.261**	**9047, 1.638**	**1.468, 1.310**	-
Vaborbactam (1000 µg/ml)					
1	3.396	5869937	9422	1.43	3.55
2	3.391	5849427	9689	1.48	3.58
3	3.388	5826482	9629	1.49	3.57
4	3.386	5877343	9177	1.5	3.49
5	3.383	5870984	9576	1.52	3.55
Mean, RSD (%)	**3.389, 0.147**	**5858835, 0.357**	**9499, 0.161**	**1.484, 0.265**	**3.548, 0.984**
Recommended limit	**RSD ≤ 2**	**RSD ≤ 2**	**> 2000**	**≤ 2**	**> 1.5**

RT-retention time; PC-plate count; PA-peak area; PT-peak tailing, Rs-resolution

Selectivity:

The selectivity of optimized RP-HPLC method was examined with working standard solution of meropenem (1000 µg/ml) and vaborbactam (1000 µg/ml) relative to the placebo solution, blank mobile phase and injection sample (meropenem - 1000 µg/ml and vaborbactam - 1000 µg/ml). No interference was observed.

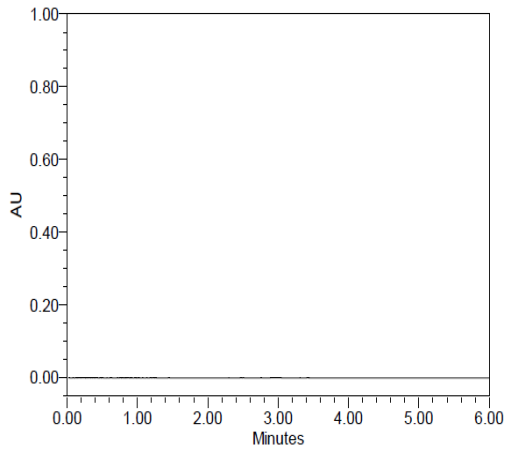


Fig 4: Blank chromatogram

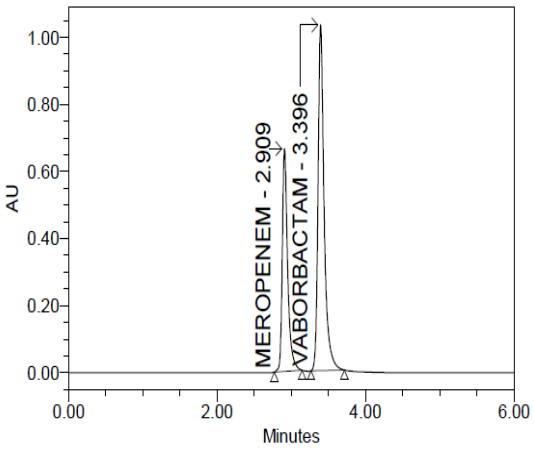


Fig 5: Standard solution chromatogram

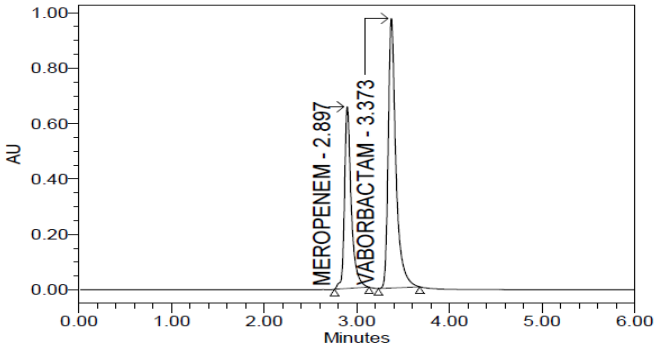


Fig 6: Injection sample chromatogram

Linearity:

Linearity was done by preparing five concentration levels (50-150% level of labeled claim) of meropenem and vaborbactam standard solutions. The linearity of detector response for meropenem and vaborbactam was verified by prepared solutions of over the concentration range of 500-1500 µg/ml. The correlation coefficient for both analytes was greater than >0.9990.

Table 3: Linearity data for meropenem and vaborbactam

Conc (%)	Meropenem		Vaborbactam	
	µg/ml	Area	µg/ml	Area
50	500	1659011	500	2929861
75	750	2481616	750	4394852
100	1000	3310690	1000	5853901
125	1250	4146493	1250	7323630
150	1500	4974605	1500	8785770

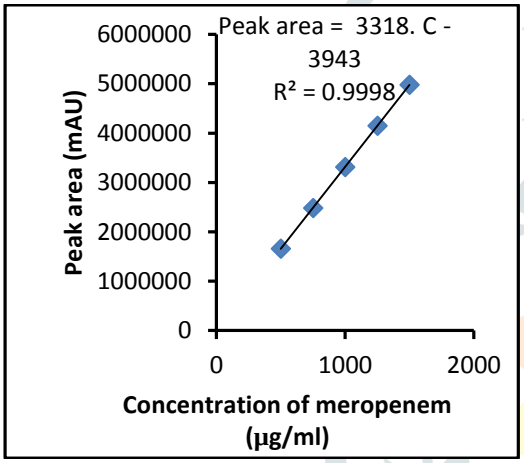


Fig 7: Meropenem linearity graph

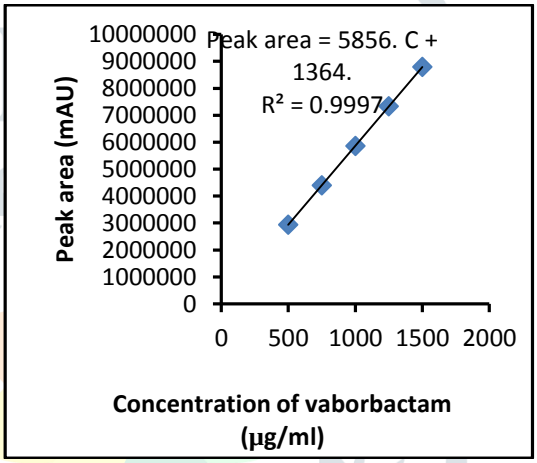


Fig 8: Vaborbactam linearity graph

Sensitivity:

Sensitivity of the method was represented as limits of detection (LOD) and quantitation (LOQ). These limits were determined based on the signal-to-noise ratio of 3:1 and 10:1, respectively. The determined LOD values are 0.706 and 0.453 for meropenem and vaborbactam, respectively. The LOQ values are 2.352 and 1.510 for meropenem and vaborbactam, respectively.

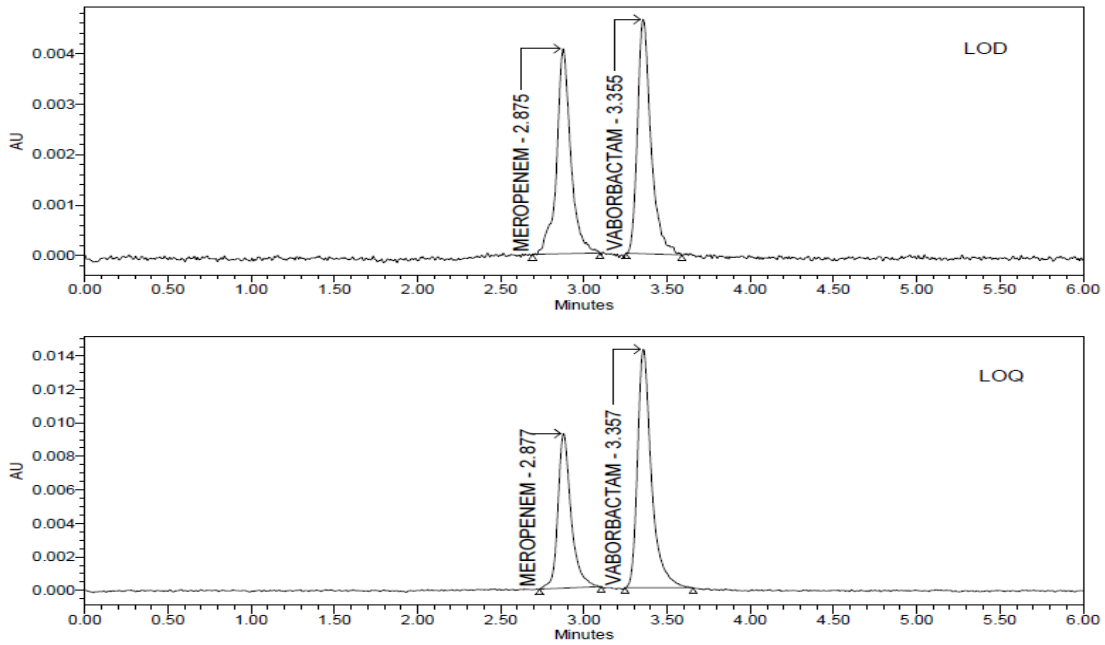


Fig 9: LOD and LOQ chromatograms of meropenem and vaborbactam

Precision:

For precision studies, the same standard solutions of meropenem and vaborbactam were injected 6 times into the HPLC system on the same day. The percentage RSD values calculated for meropenem and vaborbactam were less than 1.0 % indicating the precise assay with the developed HPLC method.

Table 4: Precision data of meropenem and vaborbactam

Injection	Meropenem	Vaborbactam
	Peak area	Peak area
1	3314492	5851156
2	3319449	5853861
3	3311112	5851891
4	3316617	5858974
5	3312906	5854769
6	3319243	5856373
Mean	3315637	5854504
%RSD	0.102	0.050

Recovery test:

The recovery experiments were performed by adding meropenem and vaborbactam standards to the preanalyzed injection sample for three times. The acquired results indicated that the developed HPLC method was accurate enough for simultaneous quantitative evaluation of meropenem and vaborbactam. There was no interference noticed from the excipients of the injection dosage form.

Table 5: Recovery of meropenem

Spiked	Concentration of meropenem (µg/ml)		Recovery (%)	Mean (%)
	Spiked	Determined		
50%	495	494.59	99.92	100.20
	495	496.96	100.40	
	495	496.43	100.29	
100%	990	994.38	100.44	100.36
	990	992.33	100.24	
	990	993.99	100.40	
150%	1485	1490.75	100.39	100.35
	1485	1490.19	100.35	
	1485	1489.77	100.32	

Table 6: Recovery of vaborbactam

Spiked	Concentration of vaborbactam (µg/ml)		Recovery (%)	Mean (%)
	Spiked	Determined		
50%	500	497.51	99.50	99.60
	500	498.49	99.70	
	500	498.05	99.61	
100%	1000	995.52	99.55	99.64
	1000	997.06	99.71	
	1000	996.69	99.67	
150%	1500	1494.28	99.62	99.67
	1500	1495.16	99.68	
	1500	1495.51	99.70	

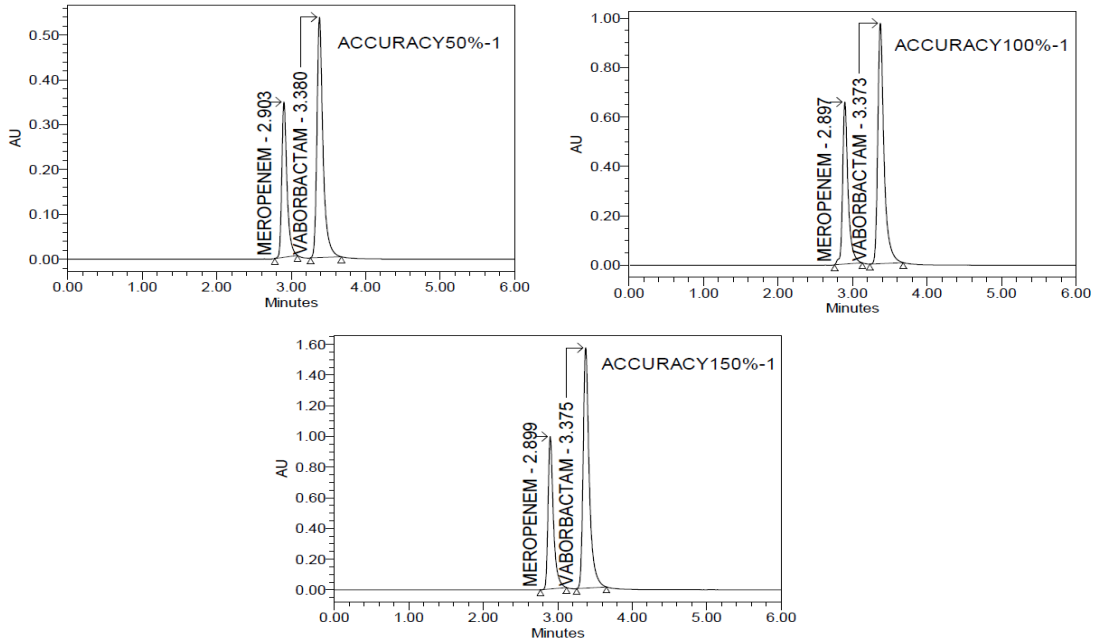


Figure 10: Accuracy chromatograms – 50%, 100%, 150% of meropenem and vaborbactam

Robustness:

Under the slightly varied chromatographic conditions (flow rate - ±0.1 ml/min; mobile phase ratio - ±5% organic phase; pH - ±0.1 unit; temperature - ±2 ºC), the meropenem and vaborbactam peaks were well separated and no significant change in the system suitability parameters, which illustrated the robustness of the method.

Table 7: System suitability values for meropenem and vaborbactam during robustness testing

Parameter	Tested values for meropenem and vaborbactam
Flow rate (ml/min)	1.0 - 0.1
	1.0 + 0.1
Temperature (°C)	25 – 2
	25 + 2
Mobile phase ratio (v/v)	70:30
	60:40
Detection wavelength (nm)	233
	237
pH value	3.7
	3.3

Assay of meropenem and vaborbactam in injection sample:

Injection sample solution with concentration 1000 µg/ml of meropenem and 1000 µg/ml of vaborbactam was analyzed thrice as described above. The quantity of meropenem and vaborbactam injection dosage form was calculated using calibration curve or regression equation of meropenem and vaborbactam.

Table 8: Assay data of meropenem and vaborbactam

Drug content (mg)	Determined quantity (mg)	Statistical readings	Drug content (mg)	Determined quantity (mg)	Statistical readings
Meropenem			Vaborbactam		
1000	1002.0	Average: 1003.03mg	1000	996.0	Average: 996.37mg
1000	1003.6	Deviation: 0.896	1000	996.4	Deviation: 0.351
1000	1003.5	Relative deviation: 0.089	1000	996.7	Relative deviation: 0.035

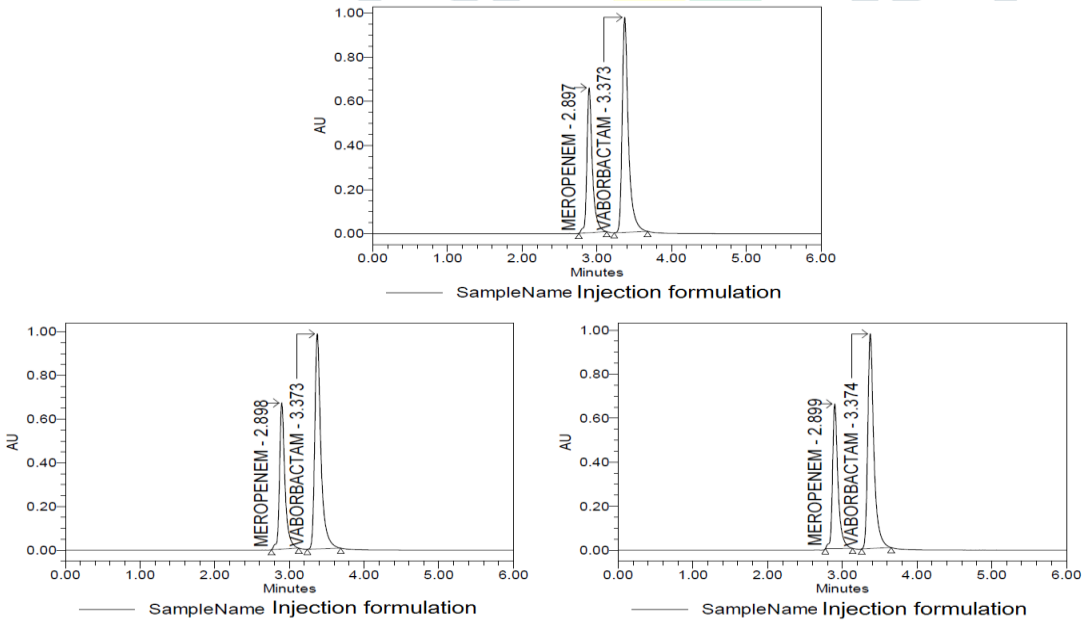


Figure 11 : Assay chromatograms of meropenem and vaborbactam

CONCLUSION

A RP-HPLC method has been developed and validated for simultaneous estimation of meropenem and vaborbactam bulk and in injection formulation. The method was validated in accordance with ICH guidelines. The proposed method was proved to be simple, economical, selective, accurate, precise and rapid. Good recoveries, absence of excipients interference and reproducible chromatograms were obtained. Therefore, the developed method can be used for the analysis of meropenem and vaborbactam in the injection formulation.

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