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Research

Reverse phase high performance liquid chromatography method development and validation for estimation of meropenem and vaborbactam in pure and pharmaceutical dosage form.

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Abstract

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Meropenem and Vaborbactam, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Phenomenex Gemini C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: Water (25:75% v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 240 nm. The retention time of the Meropenem and Vaborbactam was 2.256, 5.427 ±0.02min respectively. The method produce linear responses in the concentration range of 5-25mg/ml of Meropenem and 25-125mg/ml of Vaborbactam. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Meropenem and Vaborbactam, RP-HPLC, validation.

INTRODUCTION

'Health is wealth'. It is vital fact that a healthy body is desire of every human being. Good health is first condition to enjoy the life and all other things which mankind is having. Nowadays peoples are more concentrating towards health. Even governmental bodies of different countries and World health organization (WHO) are also focusing for health of human being. Health care is prevention, treatment and management of illness and preservation of mental and physical well being. Health care embraces all the goods and services designed to promote health including preventive, curative and palliative in interventions. The Health care industry is considered an industry or profession which includes people's exercise of skill or judgment or providing of a service related to the prevention or improvement of the health of the individuals or the treatment or care of individuals who are injured, sick, disabled or infirm. The delivery of modern health care depends on an Interdisciplinary Team.

The medical model of health focuses on the eradication of illness through diagnosis and effective treatment. A traditional view is that improvement in health results from advancements in medical science. Advancements in medical science bring varieties of medicines. Medicines are key part of the health care system. The numerous medicines are introducing into the world- market and also, that is increasing every year. These medicines are being either new entities or partial structural modification of the existing one. So, to evaluate quality and efficacy of these medicines is also important factor. Right from the beginning of discovery of any medicine quality and efficacy of the same are checked by quantification means. Quality and efficacy are checked by either observing effect of drug on various animal models or analytical means. The option of animal models is not practically suitable for every batch of medicine as it's require long time, high cost and more man-power. Later option of analytical way is more suitable, highly precise, safe and selective.

The analytical way deals with quality standards which are assigned for products to have desirable efficacy of the medicines. Sample representing any batch are analyzed for these standards and it is assumed that drug/medicine which is having such standards are having desire effect on use. Quality control is a concept, which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stage of production. The decision to release or reject a product is based on one or more type of control action.

Due to rapid growth of pharmaceutical industry during last several years, number of pharmaceutical formulations are enter as a part of health care system and thus, there has been rapid progress in the field of pharmaceutical analysis. Developing analytical method for newly introduced pharmaceutical formulation is a matter of most importance because drug or drug combination may not be official in any pharmacopoeias and thus, no analytical method for quantification is available. To check the quality standards of the medicine various analytical methods are used. Modern analytical techniques are playing key role in assessing chemical quality standards of medicine. Thus analytical techniques are required for fixing standards of medicines and its regular checking. Out of all analytical techniques, the technique which is widely used to check the quality of drug is known as 'Chromatography'.

History of chromatography and HPLC

In 1903 a Russian botanist Mikhail Tswett produced a colorful separation of plant pigments through calcium carbonate column. Chromatography word came from Greek language chroma = color and graphein = to write i.e. color writing or chromatography[1, 2]. Prior to the 1970's, few reliable chromatographic methods were commercially available to the laboratory scientist. During 1970's, most chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography, and thin-layer chromatography. However, these chromatographic techniques were inadequate for quantification of compounds and resolution between similar compounds. During this time, pressure liquid chromatography began to be used to decrease flow through time, thus reducing purification times of compounds being isolated by column chromatography. However, flow rates were inconsistent, and the question of whether it was better to have constant flow rate or constant pressure was debated[3]. High pressure liquid chromatography was developed in the mid-1970's and quickly improved with the development of column packing materials and the additional convenience of on- line detectors. In the late 1970's, new methods including reverse phase liquid chromatography allowed for improved separation between very similar compounds. By the 1980's HPLC was commonly used for the separation of chemical compounds. New techniques improved separation, identification, purification and quantification far above the previous techniques. Computers and automation added to the convenience of HPLC.

By the 2000 very fast development was undertaken in the area of column material with small particle size technology and other specialized columns. The dimensions of the General Introduction typical HPLC column are 100-300 mm in length with an internal diameter between 3-5 mm. The usual diameter of micro-columns, or capillary columns, ranges from 3 μm to 200 μm [4]. In this decade sub 2 micron particle size technology (column material packed with silica particles of < 2μm size) with modified or improved HPLC instrumentation becomes a popular with different instrument brand name like UPLC (Ultra Performance Liquid Chromatography) of Waters and RRLC (Rapid Resolution Liquid Chromatography) of Agilent.

MATERIALS AND METHOD

Meropenem -Sura labs, Vaborbactam-Sura labs, Water and Methanol for HPLC-LICHROSOLV (MERCK), Acetonitrile for HPLC-Merck.

HPLC METHOD DEVELOPMENT

TRAILS

Preparation of standard solution: Accurately weigh and transfer 10 mg of Meropenem and Vaborbactam working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.1ml of

the above Meropenem and 0.3ml of the Vaborbactam stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization: Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: Phosphate Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Acetonitrile: Phosphate Buffer in proportion 45:55 v/v respectively.

Optimization of Column: The method was performed with various columns like C18 column, Symmetry and Zodiac column. Phenomenex Luna C18 (4.6×250mm, 5µm) particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used	:	Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature	:	35ºC
Column	:	Phenomenex Luna C18 (4.6×250mm, 5µm) particle size
Buffer	:	Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.
pH	:	4.6
Mobile phase	:	Acetonitrile: Phosphate Buffer (45:55 v/v)
Flow rate	:	1ml/min
Wavelength	:	245 nm
Injection volume	:	10 µl
Run time	:	7 min

VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of Potassium dihydrogen Phosphate (KH2PO4) buffer (pH-4.6): Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 4.6 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.

Preparation of mobile phase: Accurately measured 450 ml (45%) of Methanol, 550 ml of Phosphate buffer (55%) were mixed and degassed in digital ultrasonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase	:	Methanol: Water (25:75% v/v)
Column	:	Phenomenex Gemini C18 (4.6×150mm, 5.0 µm)
Flow rate	:	1 ml/min
Wavelength	:	240 nm
Column temp	:	40ºC
Injection Volume	:	10 µl
Run time	:	10 minutes

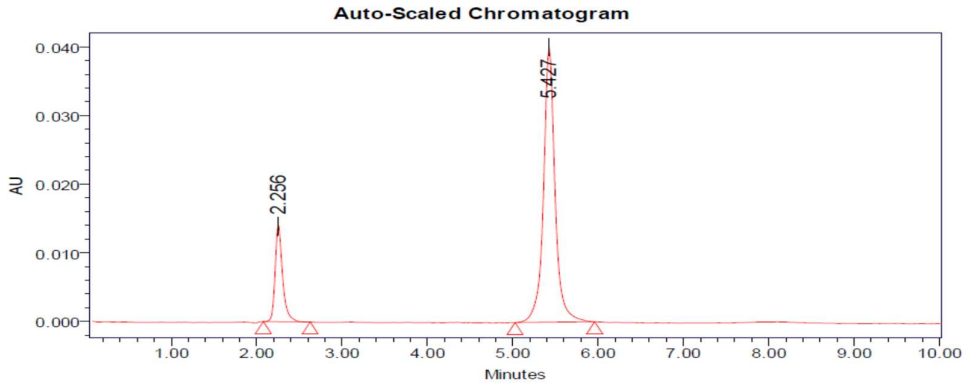

Fig 1: Optimized Chromatogram

Table 1: peak results for optimized

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Vaborbactam	2.256	84995	13906		1.33	5536
2	Meropenem	5.427	377907	39949	16.28	1.04	9102

From the above chromatogram it was observed that the Vaborbactam and Meropenem peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

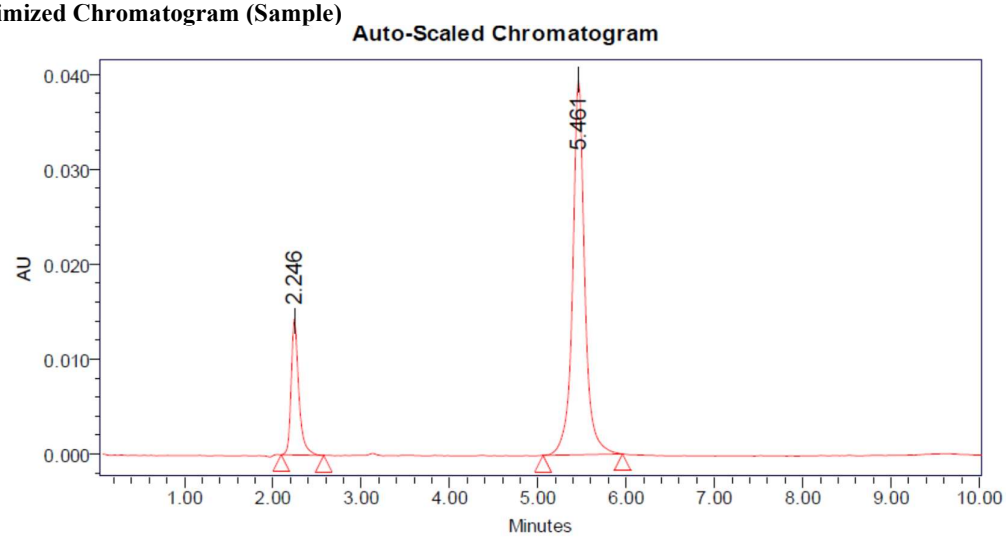

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Vaborbactam	2.246	86053	33062		1.33	5507
2	Meropenem	5.461	364679	39374	16.43	1.01	9148

- *Resolution between two drugs must be not less than 2*
- *Theoretical plates must be not less than 2000*
- *Tailing factor must be not less than 0.9 and not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

System Suitability

Table 3: Results of system suitability for Vaborbactam

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Vaborbactam	2.247	86093	14052	5507	1.36
2	Vaborbactam	2.246	85627	14026	5675	1.2
3	Vaborbactam	2.248	85558	14133	5299	1.2
4	Vaborbactam	2.252	86142	14307	5033	1.0
5	Vaborbactam	2.248	86558	14153	5811	1.33
Mean			85995.6			
Std. Dev			410.662			
% RSD			0.477538			

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Table 4: Results of system suitability for Meropenem

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Meropenem	5.452	376066	39374	9147	1.04	15.0
2	Meropenem	5.484	373326	39428	9025	1.5	15.5
3	Meropenem	5.491	373434	39404	9166	1.2	15.3
4	Meropenem	5.482	375114	39746	9077	1.1	15.1
5	Meropenem	5.491	373436	39404	9328	1.2	15.2
Mean			374275.2				
Std. Dev			1247.338				
% RSD			0.333268				

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 5: Peak results for assay standard

S.No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Vaborbactam	2.256	84995	13906		1.31	3536
2	Meropenem	5.427	377907	39949	16.28	1.04	9102
3	Vaborbactam	2.249	86395	14164		1.37	3702
4	Meropenem	5.430	376778	39936	16.14	1.06	9361
5	Vaborbactam	2.248	85871	14083		1.41	3685
6	Meropenem	5.443	375761	39608	16.18	1.06	9229

Assay (sample)

Table 6: Peak results for Assay sample

S.No	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Vaborbactam	2.247	86093	36066		1.36	9507	1
2	Meropenem	5.452	376778	37985	16.43	1.38	9512	1
3	Vaborbactam	2.246	86053	33062		1.32	9488	2
4	Meropenem	5.461	364678	39374	16.41	1.04	9147	2
5	Vaborbactam	2.243	84183	39538		1.03	9229	3
6	Meropenem	5.466	385424	39458	16.49	1.02	9248	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Vaborbactam and Meropenem in pharmaceutical dosage form was found to be 99.4 %.

LINEARITY

CHROMATOGRAPHIC DATA FOR LINEARITY STUDY OF VABORBACTAM:

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	5	51081
66.6	10	92209
100	15	139141
2133.3	20	180999
166.6	25	223921

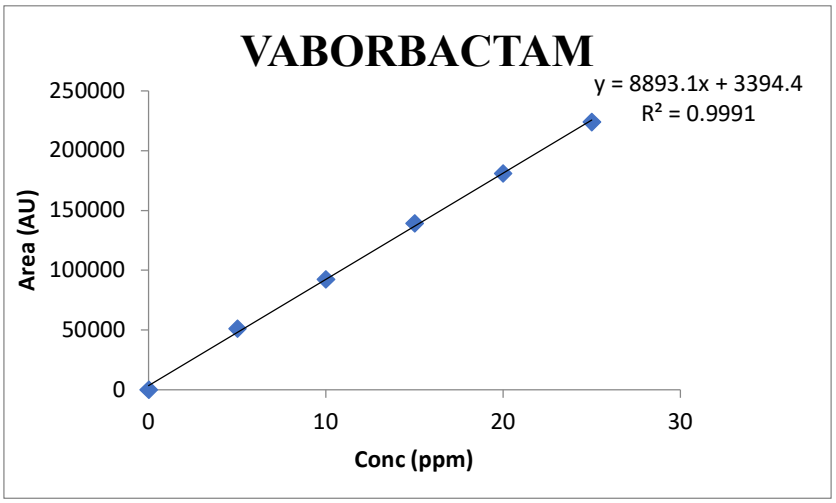

Fig 3: calibration graph for Vaborbactam

Meropenem

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	25	224574
66	50	441896
100	75	635378
133	100	842227
166	125	1041382

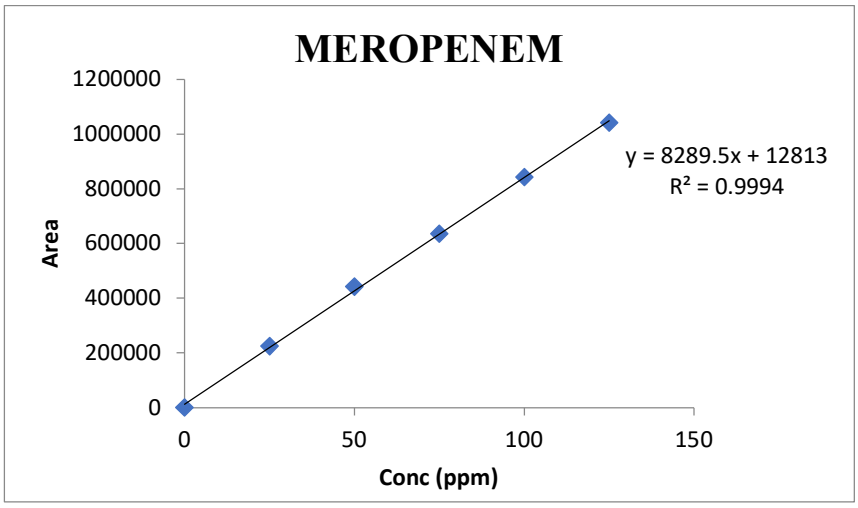

Fig 4: calibration graph for Meropenem

REPEATABILITY

Table 7: Results of repeatability for Vaborbactam

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Vaborbactam	2.269	85149	13803	3406.7	1.4
2	Vaborbactam	2.255	85368	13827	3338.4	1.4
3	Vaborbactam	2.252	85452	13798	3475.5	1.4
4	Vaborbactam	2.267	85813	13859	3423.2	1.4

5	Vaborbactam	2.260	87008	14017	3327.6	1.3
Mean		2.264	87210	13985	3417.4	1.4
Std. Dev			85998.6			
% RSD			881.5			
			1.1			

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 8: Results of method precession for Meropenem

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Meropenem	5.274	370077	40628	9076.5	1.1	15.4
2	Meropenem	5.266	370127	40936	9121.4	1.1	15.6
3	Meropenem	5.265	372485	41278	9213.4	1.1	15.3
4	Meropenem	5.278	376525	41455	8884.0	1.1	15.3
5	Meropenem	5.305	381813	41321	9042.5	1.1	15.3
Mean		5.319	374205.4	41134	8975.1	1.1	15.3
Std. Dev			4997.323				
% RSD			1.335449				

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Intermediate precision

Table 9: Results of Intermediate precision for Vaborbactam

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Vaborbactam	2.248	84028	13604	3518.3	1.4
2	Vaborbactam	2.245	84203	13521	3373.9	1.4
3	Vaborbactam	2.242	84746	13637	3412.8	1.4
4	Vaborbactam	2.239	85443	13776	3324.5	1.3
5	Vaborbactam	2.243	85536	13769	3434.4	1.4
6	Vaborbactam	2.246	85698	13738	3337.9	1.3
Mean			84942			
Std. Dev			720.3716			
% RSD			0.8			

- *%RSD of five different sample solutions should not more than 2*

Table 10: Results of Intermediate precision for Meropenem

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Meropenem	5.284	366832	40103	9181.2	1.1	15.8
2	Meropenem	5.293	368857	40465	9156.6	1.1	15.5
3	Meropenem	5.306	370175	39978	9038.6	1.0	15.5
4	Meropenem	5.319	370604	40749	9118.3	1.1	15.8
5	Meropenem	5.346	372579	39773	9184.9	1.1	15.6
6	Meropenem	5.352	376551	40084	9008.1	1.1	15.9
Mean			370933				
Std. Dev			3349.08				
% RSD			0.9				

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Table 11: Results of Intermediate precision Day 2 for Vaborbactam

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Vaborbactam	2.255	85443	40103	9181.2	1.4
2	Vaborbactam	2.260	85536	40465	9156.6	1.4

3	Vaborbactam	2.242	85698	39978	9038.6	1.4
4	Vaborbactam	2.245	84656	40749	9118.3	1.3
5	Vaborbactam	2.260	86755	39773	9184.9	1.4
6	Vaborbactam	2.255	85909	40084	9008.1	1.3
Mean			85665.84			
Std. Dev			682.4684			
% RSD			0.7			

- *%RSD of five different sample solutions should not more than 2*

Table 12: Results of Intermediate precision for Meropenem

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Meropenem	5.266	368857	39978	9038.6	1.0	15.5
2	Meropenem	5.265	370175	40749	9118.3	1.1	15.8
3	Meropenem	5.306	370604	39773	9184.9	1.1	15.6
4	Meropenem	5.293	369543	40084	9008.1	1.1	15.9
5	Meropenem	5.265	371266	56431	9024.8	1.2	15.1
6	Meropenem	5.266	378532	47653	9124.1	1.0	15.3
Mean			371496.2				
Std. Dev			3546.194				
% RSD			0.9				

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

ACCURACY

The accuracy results for Vaborbactam

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	69863.33	7.6	7.48	99.7	98.9%
100%	135468.7	16	14.9	98.7	
150%	199977	22.6	22.2	98.3	

The accuracy results for Meropenem

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	322955	37.6	38.4	98.7	99.8%
100%	632156	76	75.7	99.7	
150%	945871.3	113.5	113.5	101	

- *The percentage recovery was found to be within the limit (98-102%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Table 13: Results for Robustness

Vaborbactam

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	84995	2.256	5536	1.31
Less Flow rate of 0.9 mL/min	89988	2.505	5892	1.28
More Flow rate of 1.1 mL/min	80654	2.046	5084	1.21
Less organic phase	89988	2.505	5099	1.22
More organic phase	80655	2.046	5124	1.29

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Meropenem

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	377907	5.427	9102	1.01

Less Flow rate of 0.9 mL/min	397681	5.599	9408	1.03
More Flow rate of 1.1 mL/min	327898	4.576	9585	0.98
Less organic phase	396751	5.599	9406	1.02
More organic phase	339026	4.576	9585	0.99

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Meropenem and Vaborbactam in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Meropenem and Vaborbactam was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: Water (25:75% v/v) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Meropenem and Vaborbactam in bulk drug and in Pharmaceutical dosage forms.

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