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Research Article

Analytical method development and validation for simultaneous estimation of meropenem and vaborbactam in bulk and pharmaceutical dosage form by RP-HPLC

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination Meropenem and Vaborbactam in pharmaceutical dosage form. The column used was KromosilC_{18}(150mm x 4.6 mm, 5µm) in isocratic mode, with mobile phase containing phosphate buffer and acetonitrile (45:55v/v). The buffer is prepared by adding accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then pH adjusted to 5.0 with dil. Orthophosphoric acid solution. The flow rate was 1.0ml/ min and effluents were monitored at 260 nm. The retention times of Meropenem and Vaborbactam were found to be 2.299 min and 3.102 min, respectively. The linearity for Meropenem and Vaborbactam were in the range of 25-150µg/ml and 25-150 µg/ml respectively. Regression equation of Meropenem is y = 4826.x + 2593, and y = 4887.x + 6194 of Vaborbactam respectively. The proposed method was validated and successfully applied to the estimation of Meropenem and Vaborbactam in combined tablet dosage forms.

Keywords: Meropenem, Vaborbactam, Validation, Buffer and ICH Guidelines.

INTRODUCTION

Meropenem is a broad-spectrum carbapenem antibiotic. It is active against Gram-positive and Gram-negative bacteria. Meropenem exerts its action by penetrating bacterial cells readily and interfering with the synthesis of vital cell wall components, which leads to cell death. The bactericidal activity of meropenem results from the inhibition of cell wall synthesis. Meropenem readily penetrates the cell wall of most Gram-positive and Gram-negative bacteria to reach penicillin-binding- protein (PBP) targets. Its strongest affinities are toward PBPs 2, 3 and 4 of *Escherichia coli* and *Pseudomonas aeruginosa*; and PBPs 1, 2 and 4 of *Staphylococcus aureus*.[1]

Vaborbactam has been used in trials studying the treatment of Bacterial Infections, Subjects with Normal Renal Function, and Subjects with Varying Degrees of Renal Insufficiency. A minor pathway of meropenem elimination is hydrolysis of the beta-

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lactam ring (meropenem open lactam), which accounts for 22% of a dose eliminated via the urine. Vaborbactam does not undergo metabolism.[2]

Different analytical methods have been reported in the literature for the assay of Meropenemand Vaborbactamin pharmaceuticals and include spectrophotometry, HPLC and HPTLC [3–11]. The present study was to establish a simple, sensitive and low cost RP-HPLC method for simultaneous estimation of Meropenem and Vaborbactam in bulk as well as in other dosage forms. The developed method was validated as per ICH guidelines[12, 13].

EXPERIMENTAL

Reagents

Meropenem and Vaborbactam were kindly supplied by Aurobindo pharma ltd. Acetonitrile, water (HPLC grade, Merck) and all the other reagents of AR grade were purchased from M R Enterprisers. Combination Meropenem and Vaborbactam Injection (**VABOMERE**) Manufactured by: Facta Farmaceutici.

Instrumentation

The LC system consisted of a Waters model 515, PDA detector 2998 with 20 µL sample loop. The output signals were monitored and integrated using Empower 2 software.

Chromatographic conditions

The elution was isocratic and the mobile phase consisted of a mixture of buffer (accurately weighed 1.36g of Potassium dihyrogen Ortho phosphate in a 1000ml of volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water then pH adjusted to 5 with dil. orthophosphoric acid solution), and acetonitrile (70:30 v/v). The mobile phase was filtered through a 0.45-µm (HVLP, Germany) membrane filter prior to use. A Kromosil C_{18} (150mm x 4.6 mm, 5µm) was used for determination. The flow rate was 1.0 ml/min and the column was operated at ambient temperature (~30ºC). The volume of sample injected was 10 µL. Prior to injection of the solutions, column was equilibrated for at least 30min with mobile phase flowing through the system. The UV detector was set at wavelength of 260nm. A typical RP-HPLC chromatogram of Meropenem and Vaborbactam is shown in (Fig. 1).

Diluent: Acetonitrile and Water(50:50).

Standard Preparation

Accurately weighed and transferred 100mg of Meropenem and 100mg of Vaborbactam working Standards into a 100 ml clean dry volumetric flask, add 70ml of diluent, sonicated for 30 minutes and make up to the final volume with diluent. From the above stock solution, 1ml was pipetted out in to a 10ml volumetric flask and then make up to the final volume with diluent(1000µg/ml).

Sample Preparation

1g of dry powder (1 vial) was reconstituted (water for injection) and transferred to 100 ml volumetric flask, to this 5 ml of acetonitrile was added and sonicated. Volume was made upto 100 ml with diluents and filtered through 0.45 µm or finer porosity membrane filter (1000µg/ml of Meropenem and 1000µg/ml of Vaborbactam)

RESULTS

Method Validation

The developed method was validated as per ICH guidelines [16-17]for its accuracy, linearity, precision, specificity, robustness, ruggedness, limit of detection and limit of quantification by using the following procedures.

System suitability

System suitability and chromatographic parameters were validated such as asymmetry factor, tailing factor and number of theoretical plates were calculated.

Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of Meropenem and Vaborbactam at different concentration levels. Absorbance of resulting solutions was measured and the calibration curve was plotted between absorbance vs concentration of the drug (Figure: 2& 3). The response was found to be linear in the range 25-150µg/ml &25-150µg/ml for Meropenem and Vaborbactam. The data was given in table-1.

Accuracy

Accuracy was performed in triplicate for various concentrations of Meropenem and Vaborbactam equivalent to 50%, 100% and 150% of the standard amount were injected into the HPLC system per the test procedure. The average % recovery was calculated. The data was given in table-2.

Precision

A) Method Repeatability

Six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure. The results were given in table-3.

B) Intermediate Precision (Day to Day variability)

Two days as per test method conducted the study. For Day-1 and Day-2, six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure. The results were given in table-4.

Two instruments as per test method conducted the study. For Instrument-1 and Instrument-2, six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure. The results were given in table-5.

Limit of detection and Limit of Quantification

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines. The LOD and LOQ of Meropenemwere found to be 0.34µg/ml and 0.44µg/ml respectively. The LOD and LOQ of Vaborbactam were found to be 1.03µg/ml and 1.32µg/ml respectively.

Robustness

Robustness was done by small deliberate changes in the chromatographic conditions and retention time of Meropenem and Vaborbactamwere noted. The factors selected were flow rate and variation in the mobile phase composition. The results remained unaffected by small variations in these parameters as shown in table-6 and 7.

Assay

The assay and % purity were calculated for brand VABOMERE (Facta Farmaceutici)with label claim 1g and 1g. The observed value was compared with that of standard value without interference from the excipients used in the tablet dosage form. The results were given in table-8.

A reverse-phase column procedure was proposed as a suitable method for the simultaneous estimation of Meropenem and Vaborbactam dosage form. The chromatographic conditions were optimized by changing the mobile phase composition. Different ratios were experimented to optimize the mobile phase. Finally, buffer and acetonitrile in the ratio 50:50v/v was used as mobile phase, which showed good resolution of Meropenem and Vaborbactam peak. The wavelength of detection selected was 260nm, as the drug showed optimized absorbance at this wavelength. By our proposed method the retention time of Meropenem and Vaborbactam were about 2.267mins and 3.117mins and none of the impurities were interfering in its assay. Overall results were present in table 9.

Discussion

The statistical analysis of data and the drug recovery data showed that the method was simple, rapid, economical, sensitive, precise and accurate. It can thereby easily adopt for routine quality control analysis. The results of this analysis confirmed that the proposed method was suitable for determination of drug in pharmaceutical formulation with virtually no interference of additives. Hence the proposed method can be successfully applied in simultaneous estimation of Meropenem and Vaborbactam in marketed formulation.

CONCLUSION

The proposed method is rapid, accurate and sensitive. It makes use of fewer amounts of solvents and change of set of conditions requires a short time. This method can be suitably analyzed for the routine analysis of Meropenem and Vaborbactamin bulk and its pharmaceutical dosage forms. It does not suffer from any interference due to common excipients present in pharmaceutical preparation and can be conveniently adopted for quality control analysis.

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Table 1: Linearity data of Meropenem and Vaborbactam

S.No	Meropenem			Vaborbactam		
	Conc(µg/ml)	Rt(mins)	Area	Conc(µg/ml)	Rt(mins)	Area
1	25	2.367	125078	25	3.336	131950
2	50	2.343	243269	50	3.346	249912
3	75	2.354	369205	75	3.333	375757
4	100	2.365	483639	100	3.347	493927
5	125	2.355	603943	125	3.407	610263
6	150	2.354	726720	150	3.344	736931
	r = 0.9999			r = 0.9999		
	y = 4805x + 4842			y = 4827x + 10710		

Table 2: Accuracy data

S.No		Meropenem			Vaborbactam		
	Spiked level	Amount added (µg/ml)	Amount present (µg/ml)	Average %Recovery* ± %RSD	Amount added (µg/ml)	Amount present (µg/ml)	Average %Recovery* ± %RSD
1(n=6)	50%	25.00	24.95	99.99 ± 0.43	25.00	24.73	100.99 ± 0.46
2(n=6)	100%	50.00	50.03	100.21 ± 0.28	50.00	49.48	100.94 ± 0.55
3(n=6)	150%	75.00	55.06	101.19 ± 0.40	75.00	75.27	99.84 ± 0.59

*n=6 (Average of 6 determinations)

Table 3: Precision data of Meropenem and Vaborbactam

S.No	Meropenem			Vaborbactam		
	Conc(µg/ml)	Rt(mins)	Area	Conc(µg/ml)	Rt(mins)	Area
1	50	2.243	249147	50	3.142	249720
2	50	2.245	246404	50	3.145	243435
3	50	2.25	240156	50	3.146	245412
4	50	2.256	242466	50	3.154	240050
5	50	2.258	243861	50	3.155	241439
6	50	2.261	247924	50	3.163	246954
Mean			244993			244501.7
Std.dev			3432			3589
%RSD			1.40			1.47

Table 4: Intermediate Precision data relating to change of day

S.No	Inter-day precision					
	Meropenem			Vaborbactam		
	Peak area			Peak area		
	Conc (µg/ml)	Day-1	Day-2	Conc (µg/ml)	Day-1	Day-2
1	50	246934	239202	50	235638	245929
2	50	247283	240292	50	242833	244633
3	50	249474	243848	50	239383	244182
4	50	245849	244122	50	241132	242901
5	50	243938	235393	50	240293	240322
6	50	242384	243033	50	234842	243932
Mean		245977	240982		239020	243650
SD		2527	3381		3151	1906
%RSD		1.03	1.40		1.32	0.78

Table 5: Intermediate Precisison data relating to change of instrument

S.No	Instrument to Instrument					
	Meropenem			Vaborbactam		
		Peak area			Peak area	
	Conc (µg/ml)	Inst-1	Inst-2	Conc (µg/ml)	Inst-1	Inst-2
1	50	244847	244283	7.5	246282	243832
2	50	235838	238822	7.5	242837	242922
3	50	241934	242838	7.5	237927	241973
4	50	242931	243848	7.5	241983	240283
5	50	242482	241344	7.5	247282	242833
6	50	243013	243939	7.5	240484	235752
Mean		241841	242512		242799	241266
Std.dev		3100	2100		3521	2955
%RSD		1.28	0.87		1.45	1.22

Table 6: Robustness data relating to change in flow rate (1.0ml/min)

S.No		Meropenem			Vaborbactam		
	Flow rate (ml/min)	Average Peak Area*	Std.dev	%RSD	Average Peak Area*	Std.dev	%RSD
1	0.9ml/min	246364	1453	0.59	248606	1011	0.41
2	1.0ml/min	246108	1087	0.44	248242	865	0.35
3	1.1ml/min	246214	1233	0.50	246866	2723	1.10

*n=3 (Average of 3 determinations)

Table 7: Robustness data relating to change in mobile phase composition

S.No		Meropenem			Vaborbactam		
	Mobile phase variation (%)	Average peak area*	Std.dev	%RSD	Average peak area*	Std.dev	%RSD
1	M.P-1-(BUFFER:ACN:51:49)	246072	3048	1.24	247789	1720	0.69
2	M.P-2-(BUFFER:ACN::50:50)	246995	1237	0.50	247045	1356	0.55
3	M.P-3-(BUFFER:ACN::49:51)	246451	1751	0.71	247058	3622	1.47

*n=3 (Average of 3 determinations)

Table-8: Results of analysis of laboratory samples (Assay)

S.No	Sample	MEROPENEM			VABORBACTAM	
		Label	Amount found	%Purity ± RSD*	Amount found	%Purity ± RSD*
1	Brand-1 (VABOMERE)	1g/1g	0.99g	99.48 ± 0.30	0.96g	99.25 ± 0.73

*n=3 (Average of 3 determinations)

Table 9: System suitability parameters

Validation parameter	Results	
	Meropenem	Vaborbactam
Linearity range (µg/ml)	25 – 150	7.5 – 22.5
Regression equation	y = 4805x + 4842	y = 4827x + 10710
Correlation Coefficient(r)	0.9999	0.9999
Accuracy	98.58% to 100.71%	98.94% to 100.58%
Precision (%RSD)	1.40	1.47
Robustness (%RSD)		
Flow rate: (0.9ml/min & 1.1ml/min)	NMT 0.59	NMT 1.10
Mobile phase: Buffer : ACN::50:50	NMT 1.24	NMT 1.47
Intermediate Precision (%RSD)		
Interday – (Day 1 & Day 2)	NMT 1.32	NMT 1.40
Instrument to Instrument (Inst-1 & Inst-2)	NMT 1.45	NMT 1.22

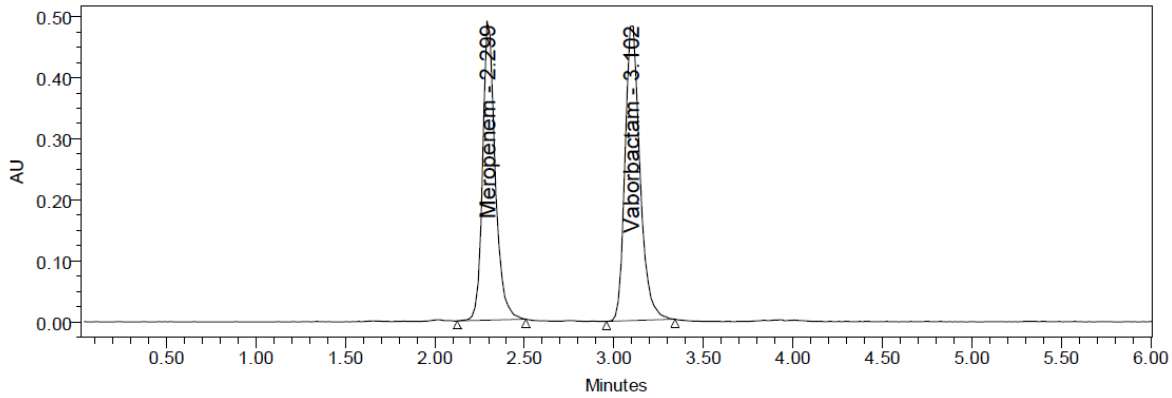

Fig - 1: HPLC chromatogram of Meropenem and Vaborbactam in optimized chromatographic conditions

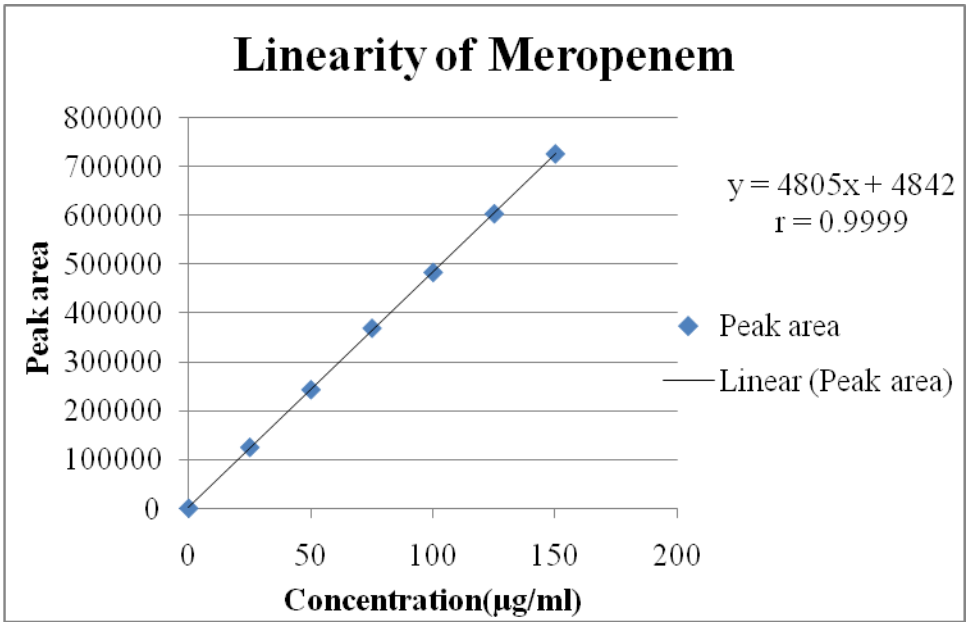

Fig – 2: Linearity of MEROPENEM in the range 25 to 150µg/ml.

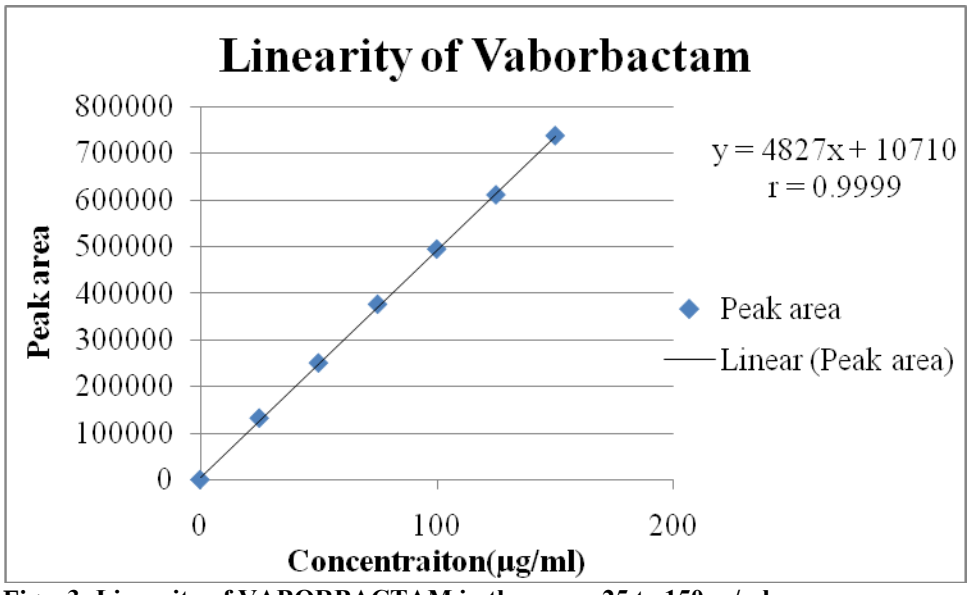

Fig – 3: Linearity of VABORBACTAM in the range 25 to 150µg/ml.

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