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DEVELOPMENT AND VALIDATION OF HPLC FOR SIMULTANEOUS ESTIMATION OF SULBACTAM AND MEROPENEM IN BULK AND IN COMBINED DOSAGE FORM

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ABSTRACT

A simple, accurate and precise HPLC method have been developed and validated for simultaneous estimation of Meropenem and Sulbactam Sodium in bulk and injectable dosage form. In HPLC method for simultaneous estimation of Meropenem and Sulbactam Sodium separation was achieved on Phenomenex C18H column (250 ×4.6 mm i.d., 5 µm), optimum mobile phase consisted of ratio of Pottasium dihydrogen phosphate buffer (pH-3.5 adjusted with orthophosphoric acid): Acetonitrile (90:10). The mobile phase at a flow rate of 1.0 ml/min, Injection volume 20µl and detection wavelength was kept at 265 nm. The retention time Meropenem and Sulbactam Sodium was 5.793±0.1min and 4.623±0.1min, respectively. The proposed conditions were successfully applied for the simultaneous estimation of both drugs in commercial injectable dosage form.

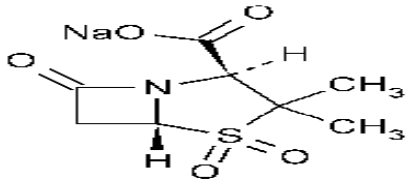
Key Words: HPLC, Sulbactam Sodium, Meropenem, Validation.

INTRODUCTION

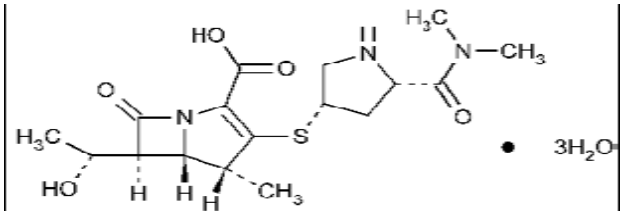
Meropenem is an Anti-bacterial agent for systemic use. It is similar to class of carbapenem. The bactericidal activity of meropenem results from the inhibition of cell wall synthesis. Meropenem readily penetrates the cell wall of most Gram-positive and Gram-negative bacteria to reach penicillin-binding- protein (PBP) targets, its solubility in Water, Methanol,0.1 N Sodium hydroxide, 0.1 N HCL (IP 2010, USP 2012, Anonymous 1)

Sulbactam is an irreversible inhibitor of beta-lactamase, It binds the enzyme and does not allow it to interact with the antibiotic, its solubility in Water, Methanol, 0.1 N Sodium hydroxide, 0.1 N HCl, CAN (BP 2009, USP 2012).

So, Meropenem and sulbactam combination is approved for use in indication of lower respiratory tract infection in adult (Anonymous 2; Anonymous 3).



Sulbactam



Meropenem

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MATERIALS AND METHODS

Meropenem and Sulbactam(as gratis sample from from Astral Pharmaceutical Indusry, Baroda.)
Methanol HPLC Grade, Acetonitrile HPLC Grade, Water HPLC Grade, KH_2PO, Ortho-phosphoric acid.

HPLC (Shimadzu) LC-20 AT, A Micro analytical balance (Shimadzu), Ultrasonic cleaner (EIE Instruments Pvt. Ltd. Ahmedabad), Nylon membrane filters (0.22 µm, 47 mm D). All instruments and glass wares were calibrated.

Meropenem standard stock solution (200 µg/ml)

A 200 mg of standard Meropenem was weighed and transferred to a 100 ml volumetric flask and dissolved in 100 ml mobile phase. The flask was shaken and volume was made up to the mark with mobile phase to give a solution containing 2000 µg/ml Meropenem From this solution 10 ml withdrawn diluted up to 100 ml with mobile phase to give 200 µg/ml.

For Linearity dilution from stock solution 5 ml, 7.5 ml, 10ml,12.5 ml and 15 ml take and dilute up to 100 ml with same solvent to get 10, 15, 20, 25, and 30µg/ml concentration solution.

Sulbactam standard stock solution: (100µg/ml)

A 200 mg of standard Sulbactam was weighed and transferred to a 100 ml volumetric flask and dissolved in 100 ml mobile phase. The flask was shaken and volume was made up to the mark with mobile phase to give a solution containing 1000 µg/ml Sulbactam. From this solution 10 ml withdrawn diluted up to 100 ml with mobile phase to give 100 µg/ml

For Linearity dilution from stock solution 5 ml, 7.5 ml, 10ml, 12.5 ml and 15 ml take and dilute up to 100 ml with same solvent to get 5, 7.5, 10, 12.5, and 15µg/ml concentration solution.

Sample preparation

Meromac plus containing 1000 mg of Meropenem and 500 mg of Sulbactam is available in market which is marketed by Macloeds Pharmaceutical. For preparation of stock solution,powder equivalent to 20 mg of Meropenem and 10 mg of Sulbactam was taken in 10 ml volumetric flask and diluted up to 10 ml with mobile phase. Then it was filtered through whatman filter paper (0.45µ). It was concentration of 2000 µg/ml of Meropenem and 1000 µg/ml of Sulbactam. From that 1 ml of aliquots was taken and diluted up to 10 ml for getting concentration of 200 µg/ml of Meropenem and 100 µg/ml of Sulbactam.

From this stock solution, working standard solution of 20 µg/ml of Meropenem and 10 µg/ml of Sulbactam was prepared by taking 1 ml and diluted it up to 10 ml with mobile phase. This solution was used for the estimation of Meropenem and Sulbactam in their combined dosage form.

Preparation of mobile phase

A mixture of 10 ml ACN and 90 ml 0.05 M Phosphate Buffer (pH 3.5 with orthophosphoric acid) of HPLC grade water filtered through 0.45 µm filter paper, Sonicate for 15 minutes to degas the mixture and used as mobile phase (Nanda RK *et al*., 2010).

Chromatographic conditions

Stationary phase : Octadecyl silane HPLC column (C18, 25 cm × 4.6 mm i.d.)
Mobile phase : Acetonitrile:Phosphate buffer(10:90v/v). The mobile phase was filtered through Millipore filter paper type HV (0.45 µm) and degassed by sonication.
Flow rate : 1.0 ml/min
Detection : By UV at 265 nm
Injection volume : 20 µl
Run time **:** 10 min

RESULTS

Validation Parameter

Linearity: (n=3)

The linearity of analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in sample within a given range. The range of analytical method is the interval between the upper and lower levels of analyte that have been demonstrated to be determined within a suitable level of precision, accuracy and linearity.

Accuracy (% Recovery Study)

Accuracy is the closeness of the test results obtained by the method to the true value. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80, 100 and 120 %) taking into consideration percentage purity of added bulk drug samples. It was determined by calculating the recovery of Meropenem and Sulbactam by standard addition method.

PRECISION

Repeatability

Six replicates of standard mixture solution having Meropenem (20 µg/ml) and Sulbactam (10µg/ml) were prepared and chromatograms were recorded and RSD was calculated.

Limit of Detection

Calibration Curve was repeated for 6 times and the SD of the Intercept was calculated then LOD was calculated as follow: From the formula LOD= (3.3*SD)/Slope, Where SD= the standard deviation of Y-intercept of 6 calibration curves, Slope= the Mean of the 6 calibration curves. (Table 9).

Limit of Quantitation

Calibration Curve was repeated for 6 times and the SD of the Intercept was calculated then LOQ was calculated as follow: From the formula LOQ= (10*SD)/Slope , Where, SD= the standard deviation of Y-intercept of 6 calibration curves, Slope= the Mean slope of the 6 calibration curves. (Table 10).

Robustness data for Meropenem and Sulbactam

Robustness study was performed in following altered chromatographic conditions:

• Variation in Mobile Phase (± 2)• Variation in pH (± 0.2)

Duplicate injections of a standard mixture solution having Meropenem (20 µg/ml) and Sulbactam (10 µg/ml) were analyzed as per the procedure in each altered condition and chromatograms were recorded. % RSD of Meropenem and Sulbactam was calculated. No particular change was found to be critical for the method of analysis.

Ruggedness data for Meropenem and Sulbactam

Ruggedness was performed in following altered experimental conditions (Table 11):

• 2 different analyst

• 2 different column(Phenomenex and Inertsil)

Forced Degradation Study

From each standard stock soln of Meropenem (200 µg/ml) and Sulbactam (100 µg/ml) 1 ml were taken in in separate container,2 ml of stress solution were added and diluted upto 10 ml with mobile phase to give Meropenem (20 µg/ml) and Sulbactam (10 µg/ml) . Three different sets were prepared for acid, base and peroxide degradation respectively. The above solutions were kept under acid/base hydrolytic and oxidative stress conditions as mention below. Same treatment was applied to dosage form. 1) For acid hydrolysis: 2ml 0.1 N HCl was added separately in acid hydrolysis flask and mixed and reflux at 70ºC for 30 mins. 2) For basic degradation: 2 ml 0.1N NaOH was added separately in base hydrolysis flask and mixed and kept at 70ºC for 1 hrs. 3) For peroxide degradation: 2 ml 3% H_2O_2 was added separately in peroxide hydrolysis flask and mixed and kept in reflux for 30 min at 70ºC. 4) For Thermal: Drug substances were exposed to dry heat at 70°C for 4 hr. Then prepared as in normal condition. 5) UV degradation: For UV degradation solid drugs were spread in 1 mm thickness uniform layer on a petridish and exposed in UV stability chamber at 254 -366 nm(energy 1.2 lux) for 4 hr. Then prepared as in normal condition. At specific interval measurement was done for each condition.

Tabale 1. System Suitaibility Parameter

Parameter	Drug	
	Sulbactam	**Meropenem**
Retention time (min)	4.623	5.793
Capacity Factor (K')	0.53	0.9
Selectivity (α)	0.58	
Resolution (Rs)	4.641	
Tailing Factor (T)	1.367	1.405
Numbers of Theoretical Plates	6661	6970
HETP	3.75 * 10-3 cm	3.58 * 10-3 cm

Table 2. Assay results

Drug	Level claim (mg)	Conc. taken for Assay (µg/mL)	Avg. Peak Area of Sample (n=3)	Avg. Conc. Found ± SD (µg/mL, n=3)	Assay ± SD (%, n=3)
Meropenem	1000	20	2405.25	20.01 ± **0.65**	100.04 ± **0.55**
Sulbactam	500	10	1315.05	9.99 ± **1.03**	99.91 ± **0.92**

Table 3. Linearity

Sr No	Concentrations (µg/ml) Sulbactam	Peak area of Sulbactam	Concentrations (µg/ml) Meropenem	Peak area of Meropenem
1	5	661.12	10	1206.53
2	7.5	987.73	15	1802.88
3	10	1318.29	20	2406.55
4	12.5	1600.21	25	2915.4
5	15	1973.46	30	3602.94

Table 4. % Recovery of Meropenem

Level of Spiking (%)	Amount spiked (µg/ml)	Total amount (µg/ml)	Amount recovered (µg/ml)	Recovery (%)	Mean recovery ± SD (%, n =3)
80	16	36	15.95	99.71	100.03 ± 0.32
	16	36	16.06	100.30	
	16	36	16.00	100.03	
100	20	40	20.11	100.54	99.87 ± 0.84
	20	40	19.78	98.94	
	20	40	20.02	100.13	
120	24	44	23.98	99.92	100.24 ± 0.46
	24	44	24.18	100.77	
	24	44	24.01	100.03	

Table 5. % Recovery of Sulbactam

Level of Spiking (%)	Amount spiked (µg/ml)	Total amount (µg/ml)	Amount recovered (µg/ml)	Recovery (%)	Mean recovery ± SD (%, n =3)
80	8	18	7.97	99.62	99.96±0.32
	8	18	8.02	100.27	
	8	18	7.99	99.98	
100	10	20	10.05	100.50	99.82±0.84
	10	20	9.88	98.87	
	10	20	10.01	100.09	
120	12	22	11.98	99.84	100.18±0.47
	12	22	12.08	100.7	
	12	22	11.99	99.99	

Table 6. Repeatability

Standard Drug	Target Concentration (µg/ml)	Peak Area of Sample	Found Concentration (µg/ml)	Mean	SD	% RSD
Meropenem	20	2430.66	20.22	19.99	0.18	o.9
	20	2382.52	19.82			
	20	2407.33	20.03			
	20	2382.56	19.82			
	20	2397.02	19.99			
	20	2425.70	20.18			
Sulbactam	10	1305.12	9.91	10.01	0.1	1.00
	10	1328.77	10.09			
	10	1315.63	9.99			
	10	1331.47	10.11			
	10	1305.12	9.91			
	10	1320.92	10.03			

Table 7. Interday precision data for Meropenem and Sulbactam(n=3)

Standard Drug		Target Concentration (µg/ml)	Average peak area	Found Mean Concentration (µg/ml)	%RSD
Meropenem	Day-1	10	1205.36	9.99	0.86
	Day-2	20	2406.83	20.16	1
	Day-3	30	3605.53	30.31	0.6
Sulbactam	Day-1	5	660.67	4.99	0.9
	Day-2	10	1319.17	10.08	1
	Day-3	15	1976.74	15.16	0.61

Intraday precision

Table 8. Intraday precision data for Meropenem and Sulbactam(n=3)

Standard Drug	Target Concentration (µg/ml)	Average peak area	Found Mean Concentration (µg/ml)	%RSD
Meropenem	10	1204.92	9.99	1.22
	20	2401.76	20.12	0.91
	30	3595.44	30.23	1.06
Sulbactam	5	660.68	4.99	1.2
	10	1316.51	10.06	0.9
	15	1970.83	15.12	1.02

Table 9. Limit of Detection

Parameter	Sulbactam	Meropenem
Mean Slope (n=5)	118.4	129.53
SD of Y-intercept (n=5)	19.67	4.33
LOD (µg/ml)	0.54	0.11

Table 10. Limit of Quantitation

Parameter	Sulbactam	Meropenem
Mean Slope (n=5)	118.4	129.53
SD of Y-intercept (n=5)	19.67	4.33
LOQ(µg/ml)	1.66	0.33

Table 11. Robustness data for Meropenem and Sulbactam

Parameter	Variation	Average area		% RSD	
		Sulbactam	Meropenem	Sulbactam	Meropenem
Mobile phase (ACN : Buffer) (10 : 90)	12 : 88	2400.45	1315.20	1.16	1.15
	8 : 92	2400.04	1315.22	1.17	1.16
pH (3.5)	+0.2	2394.58	1312.13	1.21	1.20
	-0.2	2403.04	1316.68	1.04	1.04

Table 12. Ruggedness data for Meropenem and Sulbactam

Parameter	Variation	Average area		% RSD	
		Sulbactam	Meropenem	Sulbactam	Meropenem
Analyst	1	2404.45	1316.20	1.16	1.15
	2	2403.04	1314.22	1.17	1.16
Column	Phenomenex	2399.58	1314.13	1.21	1.20
	Inertsil	2404.05	1316.68	1.04	1.04

Table 13. Validation Parameter

Parameter		Drug	
		Sulbactam	Meropenem
Linearity		r^2= 0.998 y= 129.4x + 13.30	r^2=0.998 y= 118.1x + 24.73
Accuracy		5 - 15	10 - 30
Precision		0.11	0.54
LOD (µg/ml)		0.33	1.66
LOQ (µg/ml)		0.523	0.532
Ruggedness			
Analyst	1	1.15	1.16
	2	1.16	1.17
Column	Phenomenex	1.20	1.21

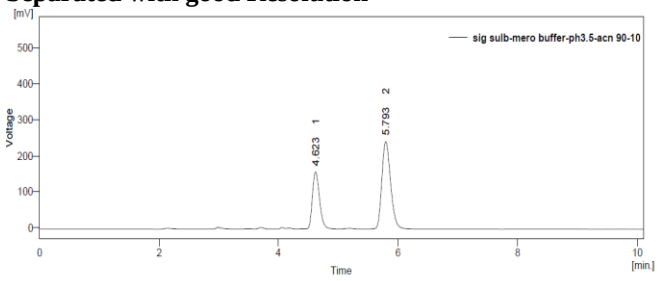
	Inersil	1.04	1.04
Robustness			
Mobile phase (ACN:Buffer) (10 : 90)	12:88	1.16	1.15
	8:92	1.17	1.16
pH (3.5)	+0.2	1.21	1.20
	-0.2	1.04	1.04

Table 14. Forced degradation study

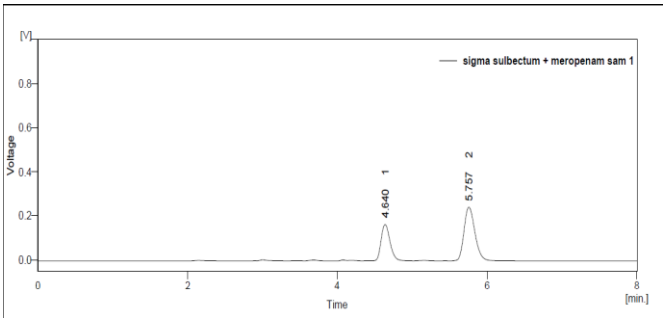
Degradation Parameter	Time Interval	Peak Height		Percentage Undegraded		Percentage Degraded	
		Meropenem	Sulbactam	Meropenem	Sulbactam	Meropenem	Sulbactam
Acid	10 min	200.87	132.62	84.48	83.45	15.52	16.55
	20 min	120.62	76.20	50.73	47.95	49.27	52.05
	30 min	5.28	2.49	2.22	1.57	97.78	98.43
Base	10 min	198.48	129.51	83.47	81.49	16.53	18.51
	20 min	116.87	72.90	49.15	45.87	50.85	54.13
	30 min	3.38	1.28	1.42	0.81	98.58	99.19
Peroxide	10 min	194.92	130.51	81.97	82.12	18.03	17.88
	20 min	113.04	73.75	47.54	46.41	52.46	53.59
	30min	4.30	1.50	1.81	0.94	98.19	99.06
Thermal	1	236.80	158.41	99.59	99.68	0.41	0.32
	2	233.69	156.42	98.28	98.43	1.72	1.57
	3	231.29	155.53	97.27	97.87	2.73	2.13
	4	229.45	154.16	96.50	97.00	3.50	3.00
UV Light	1	236.68	158.22	99.62	99.56	0.38	0.44
	2	234.57	156.65	98.65	98.57	1.35	1.43
	3	231.29	154.44	97.27	97.18	2.73	2.82
	4	230.59	153.82	96.98	96.79	3.02	3.21

Height of Sulbactam -std : 158.92
Height of Meropenem -std : 237.78

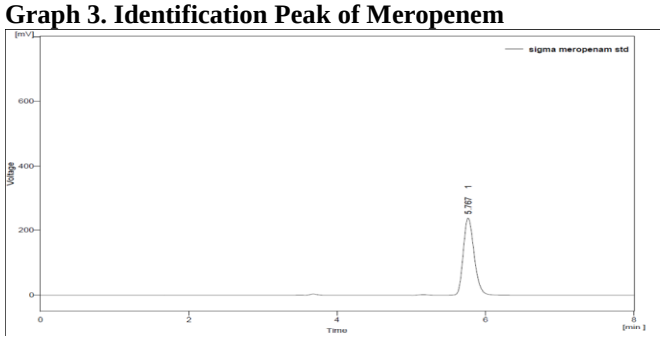
Graph 1. Acetonitrile : buffer pH :3.5(90:10) Both Peaks Separated with good Resolution



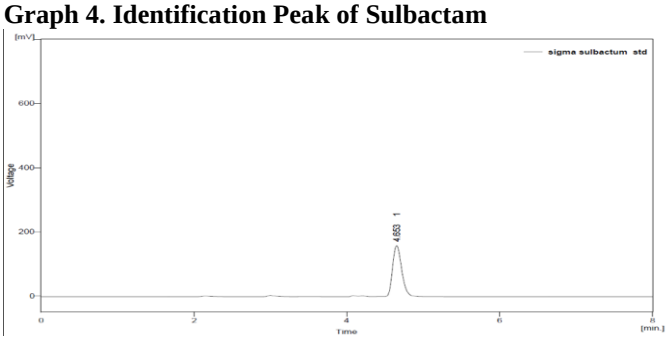
Graph 2. Chromatogram for Assay of Meropenem and Sulbactam



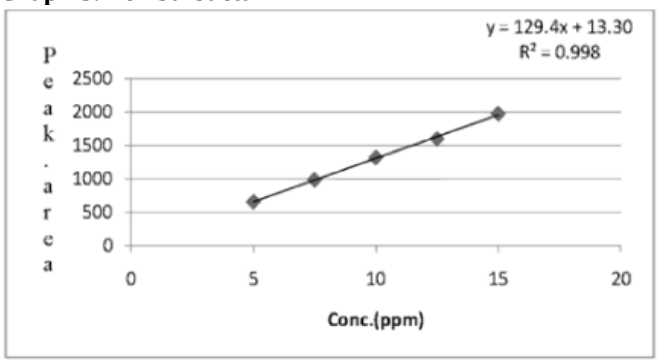
Graph 3. Identification Peak of Meropenem



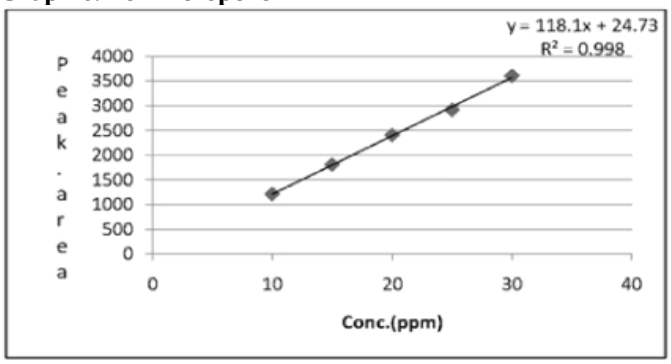
Graph 4. Identification Peak of Sulbactam



Graph 5. For Sulbactam



Graph 6. For Meropenem



DISCUSSION

From table no 1 & 2 and graph 1 to 4 % Assay of Meropenem and Sulbactam was found in an acceptance limit so this method could be used for analysis of this combination

From table no 3 and graph 5 & 6 Linearity range for Meropenem was found to be 10-30 µg/ml in Mobile Phase. Regression Equation for Meropenum at 265 nm: Y= 118.1x + 24.73. r^2 value: 0.998 Linearity range for Sulbactam was found to be 5-15 µg/ml in mobile phase. Regression Equation for Sulbactam at 265 nm: Y = 129.4x + 13.30. r^2 value: 0.998.

From table no 4 & 5 result obtained reveals that % recovery of Meropenem and Sulbactam ware within acceptance criteria given in ICH i.e. 98-102%.

From Table 6, 7 & 8 the % RSD for Repeatability of both the drugs was found to be less than 2. So, it was concluded that proposed method for estimation of Meropenem and Sulbactam is précised in nature.

From table no 9 the proposed method can detect Diclofenac Sodium and Tramadol HCl at very low level. So, it was concluded that the proposed method is very sensitive in nature.

From table no 10 the proposed method can quantify small amount of drugs with precisely. So, it was concluded that the proposed method is very sensitive in nature.

CONCLUSION

Stability indicating HPLC methods for estimation of Meropenem and Sulbactam in their combine dosage form was established and validated as per the ICH Guidelines (Anonymous 4). The forced degradation study confirmed that there was no merging between peaks of active ingredients and any other degradation products as well as other additives and shows that both drugs are very stable in thermal and photolytic condition, but less stable in acidic, basic and oxidative condition. The developed method is recommended for routine and quality control analysis of the investigated drugs in combined dosage form.

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