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Original Article

Development and Validation of Analytical Methods for Simultaneous Determination of Meropenem and Sulbactam Sodium in Combined Pharmaceutical Dosage Form.

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Abstract

Meropenem and Sulbactam sodium as combination injection is used for the treatment of lower respiratory tract infection caused by gram negative bacteria in adults only. The aim of this research work to developed and validate simple, specific and sensitive RP-HPLC method for estimation of Meropenem and Sulbactam sodium in combined pharmaceutical dosage form. In RP-HPLC, method was carried out by isocratic technique on a reversed-phase. HyperSil C18 column kept at ambient temperature and UV detection at 225 nm with mobile phase containing a mixture of water with 0.2% triethylamine and Acetonitrile pH 6.0 adjusted with Ortho-phosphoric acid (80:20 v/v), at a flow rate of 1.0 ml/min. Calibration curves were linear in the concentration range of 8-18 µg/ml for MPM (r^2 = 0.9998) and SUL 4-9 µg/ml for SUL (r^2 = 0.9995).the method was validated in terms of accuracy, precision ,interday, intraday and robustness as per ICH guideline. Thus all three methods were found to be simple, sensitive, accurate, precise so these methods applicable for routine analysis.

Keywords: *Meropenem, Sulbactam sodium, RP-HPLC.*

1. Introduction

Meropenem is an ultra-broad spectrum Injectable antibiotic used to treat a wide variety of infections including meningitis and pneumonia. It is a beta- lactam and belongs to the subgroup of carbapenem. Meropenem (MPM) is chemically (4R, 5S. 6S) – 3 - [(2S, 5S) – 5 – (Dimethyl Carbamoyl) Pyrrolidin -2 yl] Sulfanyl -6– (1-hydroxy ethyl) – 4 – methyl – 7- Oxo – 1 – azabicyclo [3.2.0] hept – 2 ene – 2 carboxylic acid.

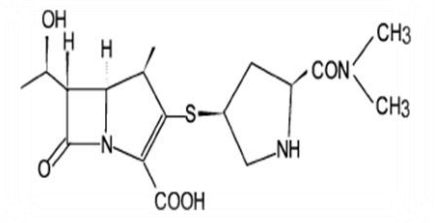

Fig. 1. Meropenem.

Sulbactam sodium (SUL) is chemically (2S,5R)-3,3-Dimethyl-7-oxo-4-thia-1- azabicyclo[3.2.0] heptane -2-carboxylic acid 4,4-dioxide.It is a penicillanic acid sulphone with Glactamase inhibitory properties.

It generally has only weak antibacterial activity, except against *N.gonorrhoea* and *N.meningitides* but it is an irreversible inhibitor of several beta-lactamases 1,2. Sulbactam sodium may therefore enhance the activity of many beta lactam antibiotics against bacteria that are normallyresistant because of the production of beta-lactamases, such as *staphylococci sp., N.gonorrhoea* and some enterobacteriaceae.

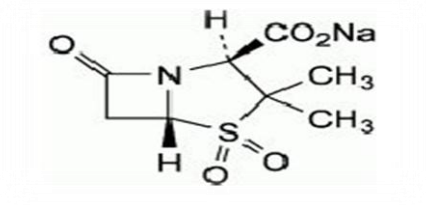

Fig. 2. Sulbactam sodium.

Meropenem is official in United state pharmacopoeia and Indian Pharmacopoeia. Literature survey reveals that UV spectroscopy and HPLC methods are reported for estimation of Meropenem in single as well as combined dosage form with other drugs.Sulbactam sodium is official in United state pharmacopoeia and Japanese pharmacopoeia. Literature survey reveals UV spectroscopic and HPLC methods for the estimation of Sulbactam sodium individually as well as in combination. Hence we attempted to develop a simple, precise, and economical RP-HPLC method.

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Meropenem and Sulbactam sodium in combination is approved by CDSCO in year 2011. The objective of the present work is to develop and validate suitable high precision and accurate analytical methods for the estimation of drugs in parenteral dosage form by reverse phase high performance liquid chromatography (RP-HPLC) that can be effectively applied for routine analysis.

2. Materials and Methods

2.1. Reagents and chemicals

Meropenem API obtained from U square lifescince, Ahemdabad. Sulbactam sodium API obtained from Microlab, Banglore. Distilled water (HPLC),Triethylamine (HPLC),Orthophosphoric acid (HPLC), Acetonitrile (HPLC) were obtain from Spectrochem and E-Merck Limited respectively.Dosage form of these drugs combination (Meromac plus, Mecloeds Pharma) was procured from local market

2.2. HPLC Instrument specifications

Model : Hitachi
Pump : L-2130
Detector : L-2400
Hamilton syringe : 50µl
Data processor : EZ start

2.3. Optimized HPLC condition

Parameter	Specification
Stationary phase	C_{18} ODS HyperSil
Mobile phase	Water with 0.2% triethylamine pH(6) : Acetonitrile (20:80)
pH of Mobile phase	6.0
Flow rate (ml/min)	1.0 ml/min
Column Temperature(˚C)	Ambient
Volume of injection(µl)	20 µl
Detection wavelength	225nm

2.4. Preparation of Mobile phase

The mobile phase containing water and acetonitrile were mixed in the ratio (80:20v/v) and 2 ml trimethylamine and pH adjusted up to 6.0 using of orthophosphoric acid. The standard solution of MPM and SUL were run on HPLC C18 HyperSil column using different mobile phases in order to get a good separation and stable peak. The mobile phase was filtered through (0.45µm) membrane filter and degassed.

2.5. Preparation of standard stock solution

Accurately weighed quantity of MPM 50 mg and SUL 25 mg were transferred into same 50 ml volumetric flask, dissolved and diluted up to mark with mobile phase to get a stock solution having strength of 1000 µg/ml of MPM and 500 µg/ml SUL.

2.6. Preparation of working stock solution

From the standard stock solution 5 ml was pipetted out in 50 ml volumetric flask and dilute with mobile phase upto mark to get the 100 µg/ml of MPM and 50 µg/ml of SUL.

2.7. Preparation of calibration range

From the working stock solution 0.8, 1.0, 1.2, 1.4, 1.6 and 1.8 ml were pipetted out in separate 10 ml volumetric flask and diluted to 10 ml with mobile phase to get the concentration range of 8-18 µg/ml for MPM and 4-9 µg/ml SUL respectively.

2.8. Sample preparation

The powder equivalent to 50mg was transferred into 50ml of volumetric flask. Add about 30 ml of mobile phase and placed in an ultrasonic bath at room temperature for 20 min. Adjusted volume up to the mark then filtered through nylon filter paper. The aliquot portion of the filtrate was further diluted to get final concentration of 14 µg/ml of MPM and 7 µg/ml of SUL. The % assay of the drugs was calculated.

Results and Discussion

The proposed HPLC method required fewer reagents and materials, and it is simple and less time consuming. This method could be used in quality control test in pharmaceutical industries. The chromatograms of Meropenem and Sulbactam sodium were shown in figure no. 5. There was clear resolution between Meropenem and Sulbactam sodium with retention time of 4.31 and 2.01 and minutes respectively.

Validation

Linearity and range

The linearity range for MPM was found to be in the range of 8-18 µg/ml and for SUL 4-9 µg/ml. Linearity data for MPM and SUL are depicted in table 8.10. Correlation co-efficient, regression line equation of MPM and SUL figure 3 and 4 respectively.

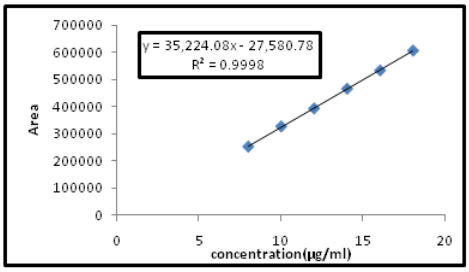

Fig. 3. Calibration curve of MPM by HPLC method

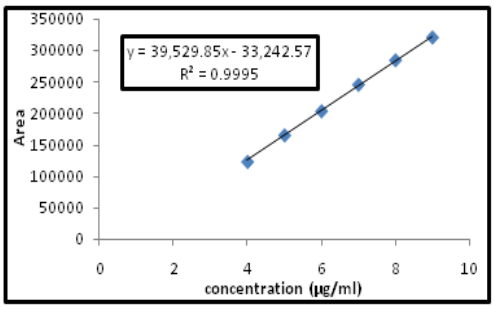

Fig. 4. Calibration curve of SUL by HPLC method

Precision

Intraday precision

The % R.S.D. for Intraday precision was found to be 0.307-0.792 for MPM and 0.194-0.629 for SUL respectively. % RSD is not more than 2. This indicates that method is precise.

Interday precision

The % R.S.D for Intraday precision was found to be 0.94-1.58 for MPM and 0.348-1.25 for SUL respectively. % RSD is not more than 2. This indicates that method is precise.

Repeatability

The % R.S.D for Repeatability was found to be 0.847 for MPM and 0.622 for SUL respectively. % RSD is not more than 2. This indicates that method is precise.

Accuracy

Accuracy of method was determined by standard addition method at three different concentrations of MPM and SUL in the range of calibration. The percentage recovery was found to be 99.73-100.04 % and 98.66-99.69 % of MPM and SUL, as depicted in Table 1 respectively. This indicates that method is accurate.

Table1.
Accuracy data of MPM and SUL.

Drug	Spiking level	Amount present in mixture (µg/ml)	Amount Added (µg/ml)	Total Amount recovered (mean ± SD) (n=3)	% Recovery ± SD
MPM	**80 %**	18	14.4	32.38 ± 0.452	99.93 % ± 0.17
	100 %	18	18	35.90 ± 0.245	99.73% ± 0.919
	120 %	18	21.6	39.61 ± 0.958	100.04%± 0.11
SUL	**80 %**	9	7.2	16.15 ± 0.754	99.69% ± 0.54
	100 %	9	9	17.74 ± 0.965	98.66 % ± 1.13
	120 %	9	10.8	19.71 ± 1.24	99.54% ± 1.65

Limit of Detection (LOD)

The limit of detection for MPM and SUL were found to be 0.2599 and 0.113µg/ml respectively.

Limit of Quantification (LOQ)

The limit of detection for MPM and SUL were found to be 0.7877 and 0.344µg/ml respectively.

Robustness

Keeping the ratio of mobile phase constant (water: acetonitrile) (pH: 6) in the ratio of 80:20% v/v) and the chromatograms of drug solution was recorded with different flow rates such as 0.8 ml/min, 1.0 ml/min and 1.1 ml/min.

By changing in the flow rate, we are getting satisfactory results, it was observed that there were no marked change in chromatogram which is indicates that proposed method was robust.

Keeping the flow rate constant (1 ml/min) and the chromatograms of drug solution were recorded by changing mobile phase ratios such as water: acetonitrile (pH: 6) 82:18, 80:20 and 78:22 v/v. By changing in the mobile phase ratio, we are getting satisfactory results, it was observed that there were no marked change in chromatogram which is indicates that proposed method was robust.

System Suitability Parameters

The data for system suitability parameters of developed HPLC method are presented in table 2.

Table 2.
System suitability parameters.

Parameter	Ideal condition	MPM (result ± SD)(n=6)	SUL (result± SD)(n=6)
Retention time	ABOVE 2.0	4.31 ± 0.0056	2.03 ± 0.0082
Theoretical plates	NLT 2000	2453 ± 39.5	2654 ± 42.9
Asymmetry	NMT 2.0	1.92 ± 0.052	1.34 ± 0.039
Resolution	NLT 2.0	6.5 ± 0.045	

Table 3.
Assay of Pharmaceutical dosage form.

Sample No.	Labelled claim		Amount Found (n=3)		% Found	
	MPM (mg)	SUL (mg)	MPM (mg)	SUL (mg)	%MPM	%SUL
1	1000	500	993.98	498.83	99.39	99.76
2	1000	500	996.16	490.014	99.61	98.00
3	1000	500	997.76	494.034	100.09	98.80
Mean			995.97±1.89	497.03±1.90	99.59±0.644	98.85±0.882

Table 4.
Summary of Validation parameters.

Sr. No.	Parameters	Results	
		MPM	SUL
1.	**Linearity & Range**		
	a) Range (µg/ml)	8-18(µg/ml)	4-9(µg/ml)
	b) Correlation coefficient	0.9998	0.9995
2.	**Precision**		
	a)Repeatability %RSD	0.847	0.622
	b) Intra-day % RSD	0.307-0.792	0.19-0.68
	c) Inter-day % RSD	0.39-1.12	0.35-1.25
3.	**Accuracy (% Recovery)**	99.73-100.04	98.66-99.69
4.	**LOD (µg/ml)**	0.259	0.113
5.	**LOQ (µg/ml)**	0.787	0.344
6.	**Assay**	99.59 ± 0.644	98.85 ± 0.882
7.	**Robustness**	Robust	
8.	**System suitability**	Suitable	

Simultaneous estimation of MPM and SUL in pharmaceutical dosage form

The proposed method was applied to analyze the combined parenteral dosage form of MPM and SUL. Marketed preparation was analyzed by the proposed method. The amount of MPM and SUL was found to be 99.59 % and 98.85 % of the labelled amount respectively. Thus, the developed RP-HPLC method is sensitive, precise, accurate and economical. It can be applied for routine

analysis of MPM and SUL combination dosage forms.

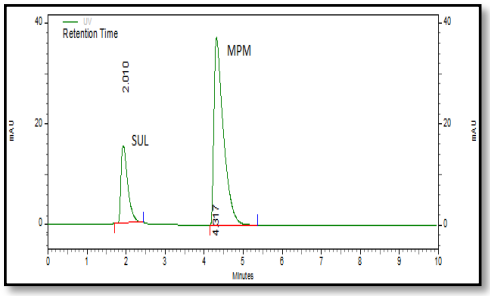

Fig. 5. Chromatogram of marketed formulation

Conclusion

The proposed method was found to be simple, precise, accurate and rapid for simultaneous determination of Meropenem and Sulbactam sodium and from pure pharmaceutical formulations. The mobile phase is simple to prepare and the run time was less than 5min which consumes only less than 5ml of mobile Phase shows that the method was economical. The sample recoveries in all formulations were in good agreement with their respective label claims suggested non-interference in the estimation. Hence, the method can be easily and conveniently adopted for routine analysis of Meropenem and Sulbactam sodium in combined dosage forms .The simplicity ensures that the RP-HPLC method can be applied for estimation of Meropenem and Sulbactam sodium in Parenteral dosage forms. Since the good separation and resolution of the chromatographic peaks, the method was found to be accurate, precise, linear, robust and rugged.

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