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Method Development and Validation for the Simultaneous Estimation of Meropenem and Sulbactam Sodium.

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Abstract
A simple and sensitive spectrophotometric method has been developed for simultaneous determination of Meropenem and Sulbactam in a binary mixture. In the proposed method, the absorbances were measured at 296.0 nm and 258.0 nm corresponding to the absorbance maxima of Meropenem and Sulbactam in 0.1 N Sodium Hydroxide respectively. Linearity range was observed in the concentration range of 5-25 µg/ml for Meropenem and 2.5-12.5µg/ml for Sulbactam. Concentration of each drug was obtained by using the absorptivity values calculated for both drugs at two wavelengths, 296.0 nm and 258.0 nm and solving the simultaneous equation. Developed method was applied to laboratory mixture. The method was validated statistically and recovery study was performed to confirm the accuracy of the method. The method was found to be rapid, simple, accurate and precise.

Key Words
Meropenem, Sulbactam, Simultaneous equation, Sodium Hydroxide (NaOH).

Introduction
Meropenem, chemically 3-[5-(dimethylcarbamoyl) pyrrolidin-2-yl] sulfanyl-6-(1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo [3.2.0] hept-2-ene-2-carboxylic acid, is an Anti-bacterial agent for systemic use. 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. Literature survey reveals, there are UV and HPLC methods reported for the estimation of Meropenem in Pharmaceutical formulations. Sulbactam is (*R*)-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid 4, 4-dioxide is a Beta-lactamase inhibitor. Sulbactam is an irreversible inhibitor of beta-lactamase; it binds the enzyme and does not allow it to interact with the antibiotic. Literature survey reveals, there are UV and HPLC methods reported for the estimation of Sulbactam with other drugs in Pharmaceutical formulations. Extensive literature survey reveals, none of the method is available that is based on estimation of Meropenem and Sulbactam by simultaneous equation method.

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Aim of present work was to develop simple, precise, accurate and economical Spectrophotometric methods for simultaneous determination of Meropenem and Sulbactam. The proposed method was optimized and validated in accordance with International Conference on Harmonization (ICH) guidelines[20]

Materials and Method
Apparatus
SHIMADZU-UV Spectrophotometer with UV-Detector (C-1800).
Reagent
0.1N Sodium hydroxide, Meropenem and Sulbactam (API).
Study of overlain spectra and selection of wavelength
Meropenem & Sulbactam 100 mg each were accurately weighed and dissolved separately in 100 ml 0.1 N NaOH Shake it up to the 15 min until clear solution appeared. From the above solution 10ml were diluted upto 100ml with 0.1 N NaOH to make concentration of Meropenem & Sulbactam of 100 µg/ml which is used as a stock solution. The stock solution of Meropenem dilute with 0.1 N NaOH to obtain 5-25µg/ml of Meropenem. The stock solution of Sulbactam dilutions of 2.5-12.5µg/ml were made with 0.1 N NaOH. Absorbances of both dilutions were determined (Table 1). Calibration curve were

plotted of both that is Meropenem (Fig 1, 2) and Sulbactam (fig 3,4) as absorbance Vs Concentration. From the overlain spectra (Fig 5) of two wave lengths, 296.0 nm and 258.0 nm were selected and absorptivity values E (1%, 1cm) of both the drugs at both wavelengths were determined for formation of simultaneous equation (Table 1and 2).

C1 = (A2ay1 – A1ay2)/ (ax2ay1- ax1ay2) --- (1)
C2 = (A1ax2 – A2ax1)/ (ax2ay1- ax1ay2) --- (2)

Analysis of Matrix

Preparation of standard solution

Stock solution of Meropenem (100 µg/ml), Sulbactam (100 µg/ml) and their mixtures were prepared in 0.1N sodium hydroxide. From the respective stock solutions, different concentrations of Meropenem (5-25 µg/ml), Sulbactam (2.5-12.5 µg/ml) and mixtures of Meropenem and Sulbactam were prepared and scanned in UV region.

Preparation of sample solution

Quantity equivalent to 100 mg of Meropenem and 50 mg of Sulbactam were weighed, transferred to a 100 ml volumetric flask, extracted and made up to volume with 0.1N sodium hydroxide and filtered with Whatmann filter paper (no. 42). From this solution, appropriate aliquot 1.5 ml was transferred to 100 ml volumetric flask and volume was adjusted up to the mark with same solvent to get concentration 15µg/ml of Meropenem and 7.5 µg/ml of Sulbactam. Suitable aliquots were prepared, scanned in UV region and absorbances were noted at selected wavelengths (Table: 3).

Validation of the Method

The developed method was validated in terms of parameters like Accuracy, Precision, Linearity and Stability studies.

Accuracy

In order to ensure the suitability and reliability of proposed method, recovery studies were carried out. A known quantity of standard Meropenem and Sulbactam added at 80%, 100% and 120% level of nominal concentration and the contents were re-analysed by the proposed method. The % recovery was calculated (Table 4).

Recovery was calculated by using following formula

$$\%\text{Recovery} = \frac{\text{Experimental Value}}{\text{Actual Value}} \times 100$$

Precision

Precision studies were performed by preparing the standards six times and measuring the absorbances of drugs at 296 nm and 258 nm. Low %RSD shows that the method has good precision (Table 5).

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines[20].

$$\text{LOD} = 3.3 \times \sigma/S$$
$$\text{LOQ} = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Results and Discussion

In this simultaneous equation method, the overlain spectra of drugs showed the λmax of 296.0 nm and 258.0 nm for Meropenem and Sulbactam respectively. Both the drugs obeyed linearity range 5-25 µg/ml and 2.5-12.5 µg/ml respectively and correlation coefficient (r2) were found to be <1 in both cases. The absorptivity values were calculated and along with absorbances, these values were submitted in equation (1) and (2) to obtain concentration of drugs. The percentage purity of drugs in combined matrix was found to be 98.67%for Meropenem and 97.50 % for Sulbactam. The accuracy of the method was determined by performing recovery study by standard addition method. The % recoveries were found near to 100 % for Meropenem and Sulbactam. The experiment was repeated six times in a day for precision. The method was found to be precise as % RSD for precision were < 2.

Conclusion

The proposed method is simple, precise, and accurate for the rapid for simultaneous determination of Meropenem and Sulbactam in combined Matrix and this method may be successfully applied in control laboratories for their determination in combined dosage form.

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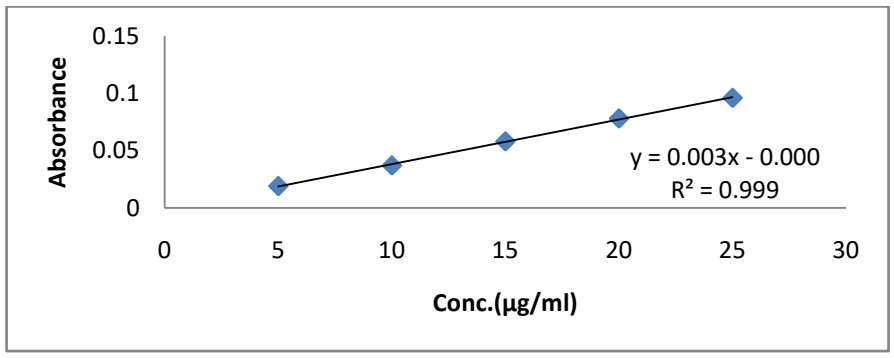

Fig. 1: Calibration graph of Meropenem at 296 nm.

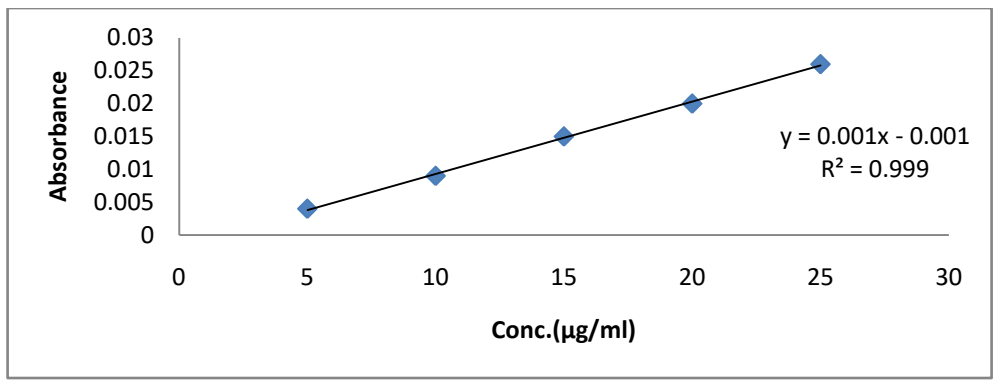

Fig. 2: Calibration graph of Meropenem at 258 nm.

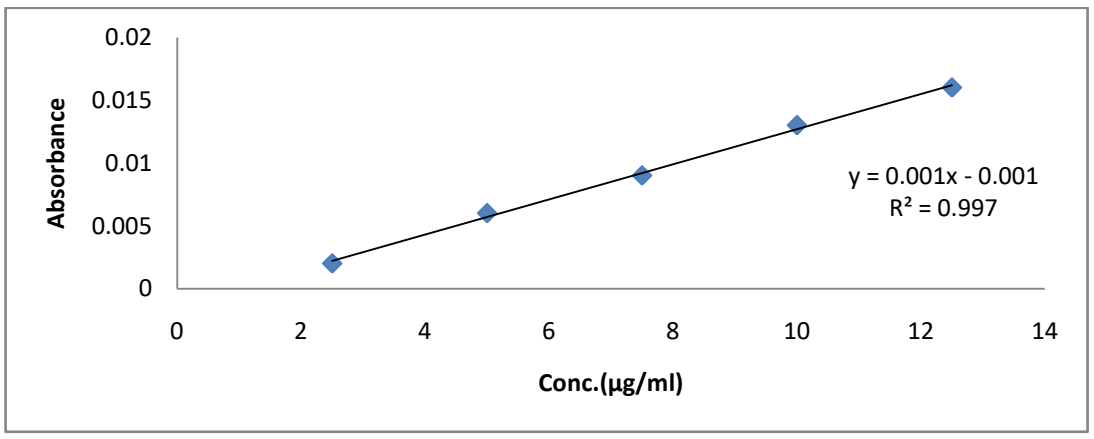

Fig. 3: Calibration graph of Sulbactam at 296 nm.

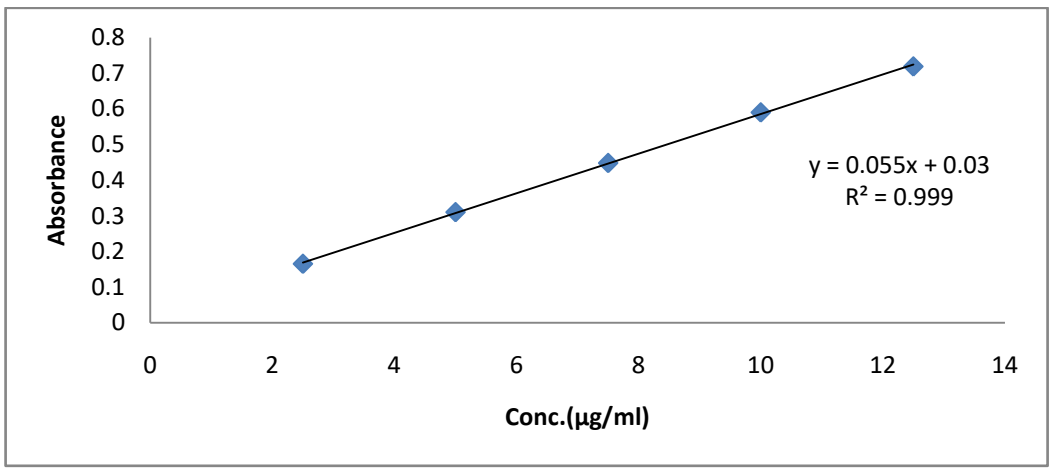

Fig. 4: Calibration graph of sulbactam at 258 nm.

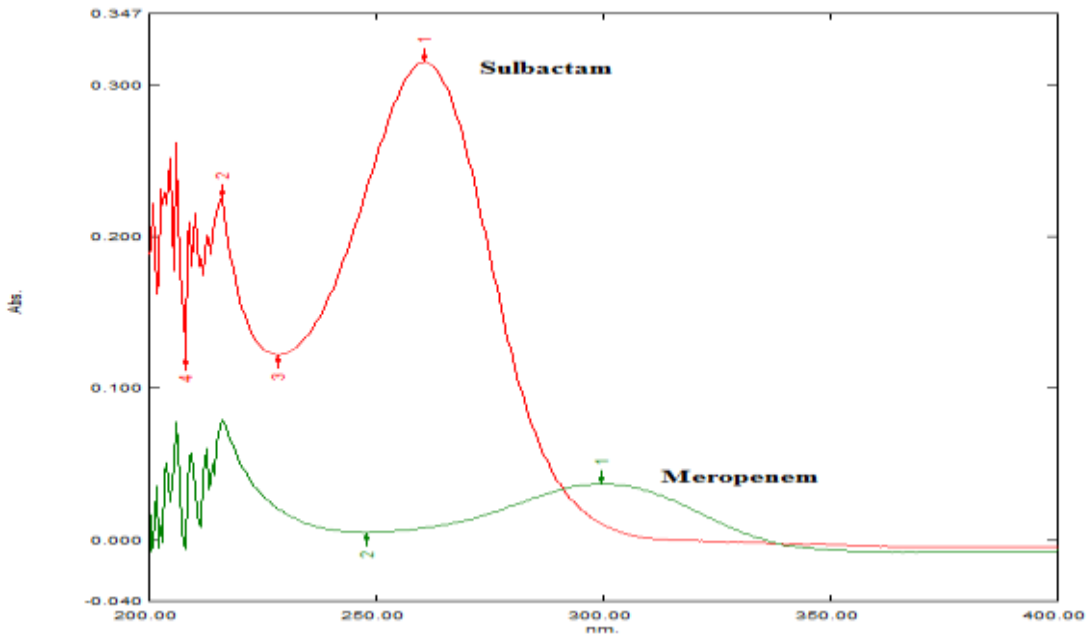

Fig. 5: Overlain UV spectra of Meropenem and Sulbactam in 0.1N sodium hydroxide.

Table 1: Absorbances and absorptivities of Meropenem at selected wavelengths.

Conc. (µg/ml)	296 nm			258 nm		
	Abs.	**Absorpti- vities**	**Avg. Absorpti-vities**	**Abs.**	**Absorpti-vities**	**Avg. absorpti-vities**
5.0	0.019	0.0038	0.0038	0.004	0.0008	0.0009
10.0	0.037	0.0037		0.009	0.0009	
15.0	0.058	0.0038		0.015	0.0010	
20.0	0.078	0.0039		0.02	0.0010	
25.0	0.096	0.0038		0.026	0.0010	

Table 2: Absorbances and absorptivities of Sulbactam selected wavelengths.

Conc. (µg/ml)	296 nm			258 nm		
	Abs.	**Absorpti- vities**	**Avg. Absorpti-vities**	**Abs.**	**Absorpti-vities**	**Avg. absorpti-vities**
2.5	0.002	0.0008	0.0011	0.165	0.066	0.060
5.0	0.006	0.0012		0.310	0.062	
7.5	0.009	0.0012		0.448	0.059	
10.0	0.013	0.0013		0.590	0.059	
12.5	0.016	0.0012		0.719	0.057	

Table 3: Analysis of Matrix

Drug	Amount(µg/ matrix)		% label claim	%RSD*
	Labeled	Found		
Meropenem	15	14.800	98.67	0.4532
Sulbactam	7.5	7.3125	97.50	0.3467

Table 4: Recovery studies

Level	% Recovery	
	Meropenem	Sulbactam
80%	99.47	99.23
100%	99.16	98.93
120%	99.53	99.20

Table 5: Precision studies.

Level	Absorbances		% RSD	
	Meropenem	Sulbactam	Meropenem	Sulbactam
	296 nm	258 nm	296 nm	258 nm
100%	0.058	0.447	1.19	0.20
	0.057	0.447		
	0.058	0.448		
	0.058	0.449		
	0.059	0.447		
	0.057	0.448		
	0.058	0.449		

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