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RP- HPLC and Visible Spectrophotometric methods for the Estimation of Meropenem in Pure and Pharmaceutical Formulations

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Abstract : A validated method for the determination of Meropenem has been developed by using reverse phase high performance liquid chromatography and visible spectrophotometry in pharmaceutical dosage forms. Spectrophotometric determination was carried out at an absorption maximum of 520 nm using 2,2'- bipyridyl in the presence of ferric chloride and orthophosphoric acid. The linearity over the concentration range of 2-12 µg/ml with correlation coefficient 0.9998 are obtained. Chromatographic separation was carried out using a mobile phase of methanol and 0.01 M potassium dihydrogen phosphate (pH adjusted to 3.0 with orthophosphoric acid) in proportion of 2:1 (V/V) ratio on kromosil C18 column (250 X 4.6 mm, 5µm) in an isocratic mode at a flow rate of 1.0 ml/min. with detection at 290 nm using a UV detector. The linearity over the concentration range of 20 – 100 µg/ml with correlation co-efficient 0.9997 are obtained. The developed methods were found to be precise and accurate for the estimation of Meropenem in pharmaceutical dosage forms.

Key words: Meropenem, RP-HPLC, Spectrophotometry, Recovery experiments.

Introduction:

Meropenem is chemically (4R, 5S, 6s) – 3- [(2S, 5S)- 5- (Di methyl carbamoyl) pyrrolidin-2-yl] sulfanyl – 6 – (1- hydroxy ethyl) – 4 – methyl – 7 –oxo – 1- aza bicyclo [3.2.0] hept -2 ene-2-carboxylic acid. It is a broad–spectrum carbapenem antibiotic and is active against Gram-positive and Gram – negative bacteria, exerts its action by penetrating bacterial cells readily and interfering with the synthesis of vital cell wall components, which leads to cell death.

Literature survey reveals a few HPLC[1-7] and Spectrophotometric methods [8-9] for the estimation of Meropenem. In the present study new RP-HPLC and visible spectrophoto metric methods have therefore been developed for the estimation of Meropenem in pure and pharmaceutical dosage forms.

Experimental:

a) **Instrumentation:**
UV- Visible spectrophotometer Techcomp UV-2301 was used for spectroscopic determination and PEAK LC – P 7000 Isocratic pump equipped with UV detector was used for HPLC analysis.

b) **Chemicals and Reagents**:
HPLC grade methanol and water, A.R. grade potassium dihydrogen phosphate (0.01 M), Orthophosphoric acid (0.2 M), Ferric Chloride (0.003M), 2,2'- bipyridyl (0.01M) were used in this study.

c) **Chromatographic conditions**:
HPLC chromatographic separation was carried out in an isocratic mode utilizing kromosil C18 column with dimensions (5µ, 250mm x 4.6mm) as stationary phase with injection volume of 20µl.

The mobile phase composed of methanol and 0.01 M potassium dihydrogen phosphate (pH adjusted to 3.0 with ortho phosphoric acid) in the ratio of 2:1 at a flow rate of 1.0 ml/min. with UV-detection at 290 nm.

d) **Spectrophotometric conditions:**
In this method Meropenem was oxidized with ferric chloride, followed by complex formation with 2,2'- bipyridyl that was an orange red coloured chromogen which showed the absorption maximum at 520 nm.

Preparation of standard solutions:

Spectrophotometry :
About 100mg of Meropenem was accurately weighed and transferred into a 100 ml volumetric flask and diluted to volume with methanol to get the stock solution (1mg /ml). From this suitable dilutions were made to obtain a final working concentration of 100 µg/ml.

HPLC:
An accurately weighed quantity of Meropenem (50mg) was taken in a 100 ml volumetric flask, dissolved in mobile phase to obtain a stock solution containing 500 µg/ml of Meropenem. From this suitable dilutions were made to obtain the concentrations ranging from 0-150 µg/ml.

Sample preparation:

Spectrophotometry:
Accurately weighed formulation powder equivalent to 100 mg of Meropenem was transferred to a 100 ml volumetric flask. About 20ml of methanol was added and sonicated for 10 min. finally made up the volume with methanol and mixed thoroughly. The resulting solution was filtered through a Whatman filter paper. From this, suitable dilutions were made to obtain the concentration of 100 µg/ml.

HPLC:
The formulation powder equivalent to 50 mg was accurately weighed and transferred to a 100 ml volumetric flask. About 20ml of mobile phase was added and sonicated for 10min. filtered through 0.45 µm membrane filter and the volume was made to the mark with mobile phase to get the stock solution. From this, suitable dilutions were made to obtain the concentrations ranging from 0-150 µg/ml.

Procedure:

Spectrophotometry :
Aliquots of standard drug solution of Meropenem ranging from 0.5 to 5ml (100 µg/ml) were added to a series of heating tubes. To each tube 1ml of ferric chloride and 1ml of 2,2'- bipyridyl were added and heated for 15 min. at 100^0C on a water bath and then cooled to room temperature and 2ml of ortho phosphoric acid was added. The contents of the tubes were transferred to a series of 25 ml standard flasks, then diluted to the mark with distilled water. The absorbance of each solution was measured at 520 nm against the reagent blank. The amount of Meropenem was computed from the calibration curve.

HPLC:
Various standard concentrations of Meropenem ranging from 0-150 µg/ml were prepared in mobile phase. The contents of the mobile phase were filtered before use through 0.45 µm membrane filter, degassed with a helium sponge for 15 min. and pumped from the respective solvent reservoirs to the column at a specified flow rate. Prior to injection of the drug, the mobile phase was pumped for about 30 min. to saturate the column there by to get the base line corrected, then 20 µl of each of the drug solution was injected for five times. Quantitative determinations were made by comparison of the peak area from a standard injection. The amount of Meropenem present in the sample was calculated through the calibration curve.

Fig. 1. Linearity Curve of Meropenem (Spectrophotometry)

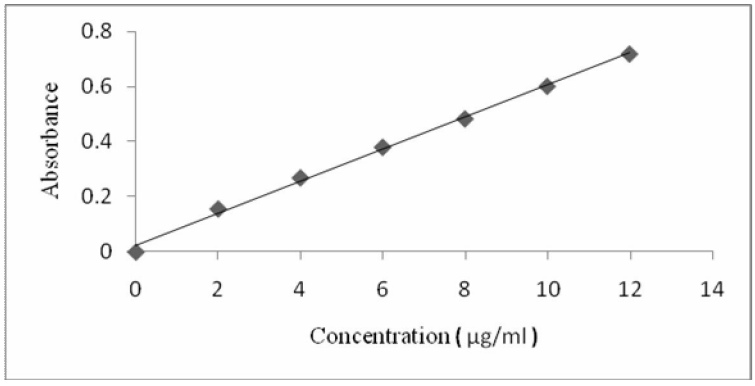

Fig. 2. Linearity Curve of Meropenem (RP-HPLC)

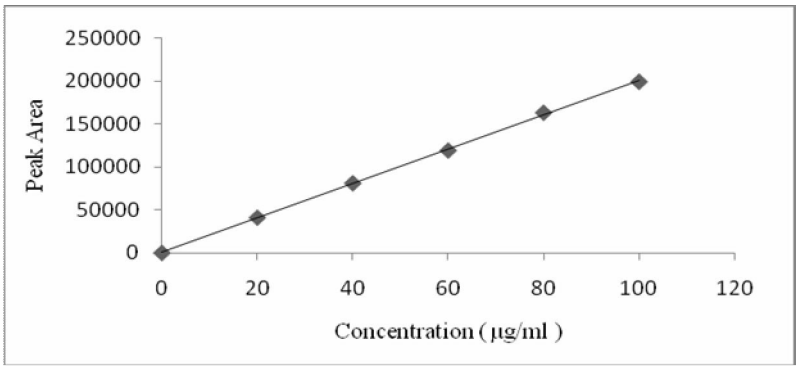

Table- 1 : Analytical parameters of Meropenem

S.No.	Parameter	Spectrophotometry	RP-HPLC
1	Linearity range (µg /ml)	2-12	20 – 100
2	Slope (m)	0.0557	1991.945
3	Intercept (b)	0.0449	1219.9
4	Correlation coefficient (r)	0.9998	0.9997

Fig. 3. Chromatogram of Meropenem

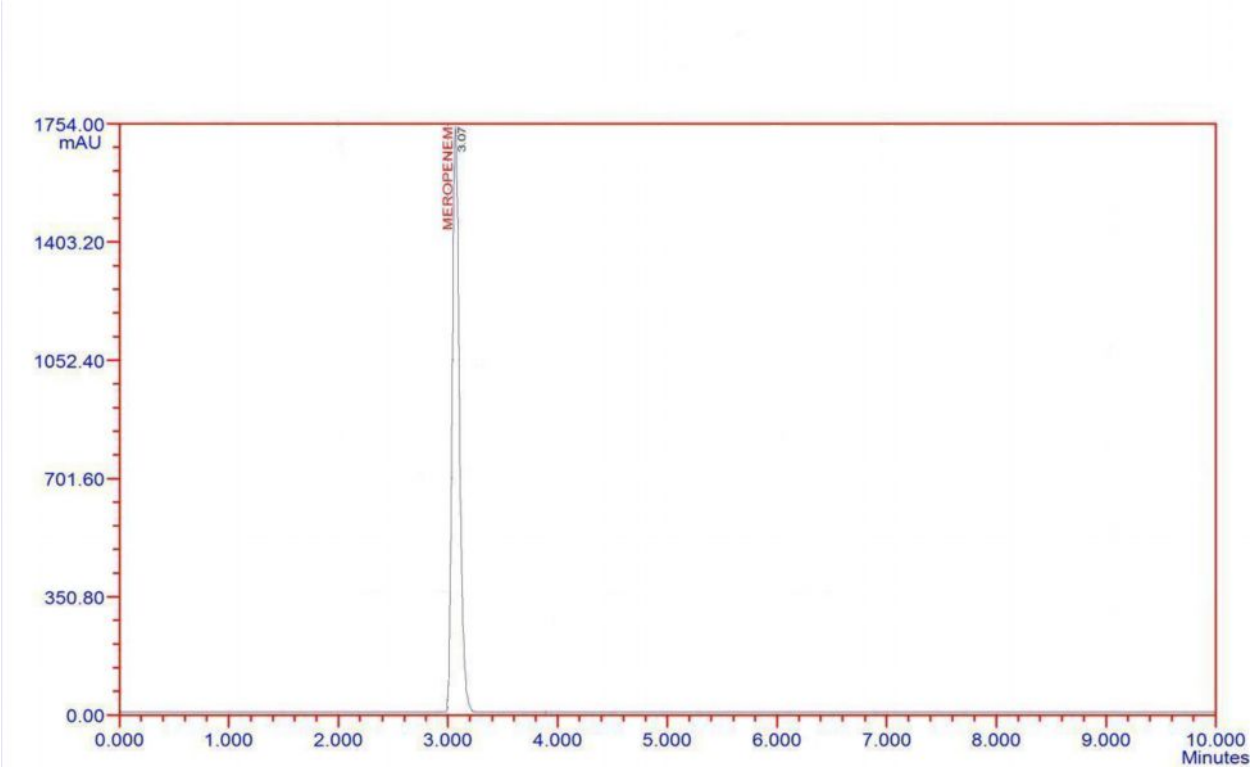

Table -2: System suitability parameters

S.No.	**Parameter**	**RP- HPLC**
1	Retention time (min.)	3.07
2	Theoretical plates	8284
3	Tailing factor	1.68
4	Calibration range (µg/ml)	20 – 100
5	Limit of detection	2.9528
6	Limit of quantification	9.8426

Table – 3 : Assay of commercial formulations by proposed methods

Formulation	Labeled amount mg/vial	**Spectrophotometry**		**RP-HPLC**	
		Amount found*	% Recovery **	Amount found*	%Recovery **
A	500	499.95 ± 0.109	99.86 ± 0.106	499.90 ± 0.053	99.23 ± 0.445
B	500	499.89 ± 0.029	98.00 ± 0.179	499.93 ± 0.037	99.51 ± 0.155
C	500	499.91 ± 0.038	99.70 ± 0.127	499.90 ± 0.042	99.92 ± 0.035

*Mean of five determinations

** Mean of three determinations

Results:

Linearity:

Calibration curve for spectrophotometric method was constructed by plotting absorbance Vs concentration of solution. For chromatographic method it was constructed by plotting peak area against concentration of solution. Figs. 1 and 2 show spectrophotometric and HPLC linearity curves of Meropenem. Linearity ranges and correlation coefficients obtained from these methods are presented in Table – 1. The chromatogram of Meropenem was showed in Fig. 3.

System suitability parameters:

The system suitability tests were carried out on freshly prepared standard stock solution of Meropenem under the optimized chromatographic conditions. The parameters that were studied to evaluate the suitability of the system were: a) No. of theoretical plates b) tailing factor c) retention time d) calibration range e) LOD and LOQ. These values are presented in Table-2.

Assay and recovery study:

To determine the accuracy of the proposed methods, recovery experiments were carried out by standard addition method. The values of recovery experiments and assay of commercial formulations are presented in Table-3.

Discussion :

The linearity was obeyed in the range of 2-12 µg/ml for spectrophotometric method and 20 – 100µg/ml for chromatographic method. Quantitative estimation of formulations showed average percentage purity of 99.97 to 99.99 with mean recovery percentage of 99.23 to 99.92 for these methods. System suitability indicates that the developed method has acceptable accuracy and precision.

Conclusion:

The developed methods are simple, accurate and reproducible, so these methods are suitable to determine Meropenem in formulations.

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