EXTRACTIVE SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF MEROPENEM PURE AND IN MARKETED FORMULATIONS USING ACIDIC DYES (BTB &BCP)

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Abstract:

Two simple, sensitive and extractive spectrophotometric methods have been developed and validated for the determination of meropenem in pure and in marketed formulations. The proposed methods were based on the formation of ion-pair complex between meropenem and Bromothymol Blue and Bromocresol Purple at pH 3.0 ± 0.01 which was extracted into chloroform and the absorbance of yellow ion-pair complex was measured at 420nm and 418nm repectively. Under optimized conditions, the Beer's law was obeyed over 10-50µg/mL for BTB and BCP and 12.5-62.5µg/mL respectively, and the corresponding molar absorptivity values were $1.018x10^3$L/mol/cm and $1.43x10^3$L /mol/cm. The Sandell sensitivity values of 0.6548 and 0.7854µg/cm^2 for BTB and BCP respectively. Application of the proposed methods to commercial formulations of meropenem was validated according to ICH guidelines.

Keywords: Meropenem, Ion association complex

1.Introduction:

Meropenem[1-3][**Figure.I**] (4R, 5S. 6S) – 3 - [(2S, 5S) – 5 – (Dimethyl Carbamoyl) Pyrrolidin -2 yl] Sulfanyl -6 – (1-hydroxy ethyl) – 4 – methyl – 7- Oxo – 1 – azabicyclo hept – 2 ene – 2 carboxylic acid, used to treat a wide variety of bacterial infections, including meningitis and pneumonia. It inhibits bacterial wall synthesis like other beta-lactam antibiotics

To date, several methods have been described for measuring meropenem concentrations in two types of biological samples that include microbiological assay[4] and liquid chromatography– tandem mass spectroscopy (LC–MS–MS) method[5] in peritoneal fluid; and a different microbiological assay[6] and a high-performance liquid chromatography (HPLC) method using solid-phase extraction for sample pretreatment[7] in bile. However, visible spectrophotometric methods involving ion-pair complexes of acidic dyes viz, Bromothymol blue (BTB) and Bromocresol purple (BCP) were not yet reported with this drug and this prompted the authors to develop simple extractive spectrophotometric methods for the determination of meropenem using the above mentioned dyes. In this paper two simple and sensitive extractive spectrophotometric methods were developed and validated for meropenem assay in pure and formulations basing on ion–pair complexation of the meropenem with acidic dyes {Bromothymol blue (BTB) and Bromocresol purple (BCP)} and subsequent extraction of colored complexes into chloroform, and measuring the absorbance of color complexes respectively.

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2.Experimental:

2.1.Instruments:

A Elico (Model UV-SL-159) digital spectrophotometer with matched 1.0cm quartz cells was used for absorbance measurements. A digital pH meter Model Elico L1 120 was used for pH measurements.

2.2.Chemicals and Reagents:

Chemicals used were of analytical grade. The solvents used were of the analytical grade and double distilled water was used throughout the investigation. Solution of Bromothymol Blue (0.1%v/v) and Bromocresol Purple solution (0.1%v/v) were prepared by weighing and dissolving 100mg of appropriate dyes (BTB and BCP) separately in 100ml of double distilled water. Buffer solution, (pH 3.0) was prepared by mixing 50mL of 0.2M Glycine acetate solution with 22.4mL of 0.2 M HCl solution and diluted to 200mL with doubly distilled water. The pH of the solution was adjusted to an appropriate value with the aid of a pH meter.

Meropenem was received as a gift sample from cipra Lab Limited, sanath nagar, Hyderabad, India. Standard stock drug solution(1000µg/ml) of meropenem was prepared by dissolving 100mg of pure meropenem into 100ml volumetric flask with to double distilled water from this stock solution desired concentrations 200µg/ml for BTB and 250µg/ml for BCP were prepared respectively.

Injection powder of meropenem(1.0gm were weighed are analytically weighted, triturated and analytically transferred with 50 mL of double distilled water to 100mL volumetric flask.The content of the flask is filtered through analytical filter paper in a 100 mL volumetric flask, the filter paper is washed with another 40mL of double distilled water and latter the volumetric flask is completed to the mark double distilled water with to give the stock sample solution of concentration 1000µg/ml. This working solution is then filtered using whatmann filter paper No.45and from this filtrate, appropriate dilutions were made in double distilled water to obtain the desired concentration of 200µg/ml for BTB and 250µg/ml for BCP and was subjected to analysis by the procedure given below.

3.Proposed procedure:

Different aliquots of drug solution were transferred into a series of 100ml separating funnels. To this add 5.0ml of glycine -acetate buffer , 5.0ml of various dye solutions((BTB or BCP), were added and total volume was made upto 15ml with distilled water. To this 10ml of chloroform was added, and the contents were shaken for 5 minutes. The organic layer was separated and the absorbance of yellow colored solution is measured spectrophotometrically (420nm for BTB and 418nm for BCP against blank similarly prepared) which is stable for 24hrs. For the two proposed methods, standard calibration plots were prepared by plotting the absorbance versus drug concentration, and the concentration of the unknown was read from the plotted calibration graphs or computed from the respective regression equations derived using the absorbance concentration data.

4.Results and Discussion:

The proposed methods discussed in the present paper provided a convenient and reliable way for quantitative determination of meropenem in pure and in formulations. The drug meropenem in its protonated form reacts with the acidic dyes viz, BTB and BCP in aqueous solution at pH 3.0 ± 0.01 forming yellow colored ion pair extractable complex. Wavelength of maximum absorbance for colored ion-pair complexes of meropenem were selected at 420nm for BTB and 418nm for BCP and were used for the quantitative determination. Linearity for meropenem was observed in the concentration ranges and the regression analysis of the Beer's law data indicated a linear relationship between absorbance and concentration (**Table.I&Figures II&III**) which is corroborated by high values (close to unity) of the correlations coefficients respectively, for all two methods. The calculated molar absorptivity and Sandell sensitivity values are summarized in **Table.I**. The high values of ε and low values of Sandell sensitivity indicate the high sensitivity of the proposed methods. Precision studies for the proposed methods were carried out by one fixed concentration six times on the same day and the results of this study were summarized in **Table.I**. The percentage relative standard deviation (%RSD) values indicating high precision of the proposed methods respectively. The accuracy of the proposed methods was determined by the percent mean deviation from known concentration, at one fixed concentration and these results are also presented in **Table.I**. The Percent relative error (%RE) values demonstrated the high accuracy of the proposed methods. The proposed methods were applied for the quantification of meropenem in

marketed formulations(injection powder) and the results of statistical analysis(**Table-II**) of did not detect any significant difference between the proposed method and reference method[6] with respect to accuracy and precision as revealed by the Students t-value and variance ratio F-value.

5. Conclusion:

The present research work demonstrated the feasibility of the use of visible spectroscopy and ion complexation reaction for the determination of meropenem in pure and its dosage formulations. The proposed methods make use of simple reagents and were found to be simple, precise, economical and less time consuming, which an ordinary analytical laboratory can afford. The proposed methods were statistically evaluated and results obtained are accurate, precise, sensitive and free from the interferences of other additives present in formulations. The proposed extractive visible spectrophotometric methods can be applied for determination of meropenem in pure and dosage forms with high precession and good accuracy in quality control laboratories.

6. Acknowledgements:

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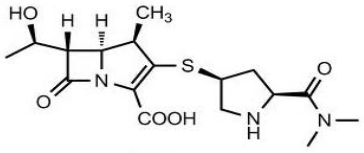
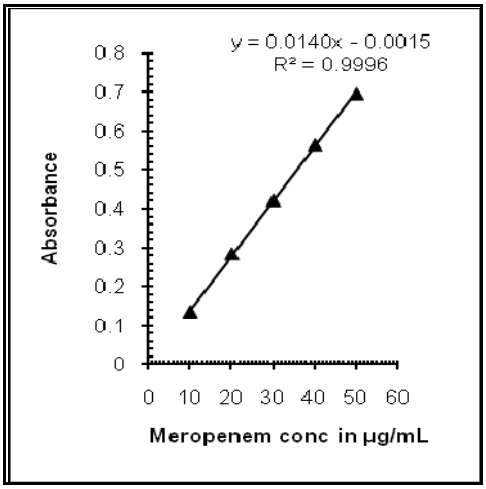


Fig,I. Structure of Meropenem

Fig,II. Linearity curve of meropenem with BTB



Fig,III. Linearity curve of meropenem with BCP

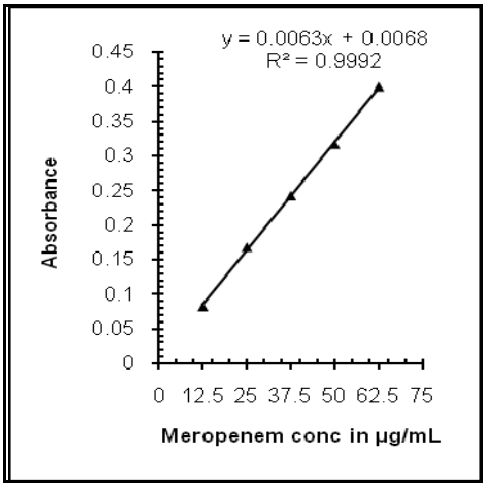


Table.I: Results of optical characteristics and precision of the proposed methods for meropenem assay

Parameter	BTB	BCP
λ_{max} (nm)	420	418
Beer's law limits (µg/ml)	10 –50	12.5–62.5
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	$1.018x10^3$	$1.43x10^3$
Sandell's sensitivity (µg.cm^{-2}/0.001 absorbance unit)	0.6548	0.7854
Optimum photometric range (µg/ml)	12 – 48	15 – 60
Regression equation (Y=a+bc) ;slope (b)	0.0140	0.0063
Standard deviation on slope (S_b)	0.000167	0.000099
Intercept (a)	-0.0015	0.0068
Standard deviation on intercept (S_a)	0.00555	0.00414
Standard error on estimation (S_e)	0.00530	0.00394
Correlation coefficient (r)	0.9996	0.9992
Relative standard deviation (%)*	1.118	1.118
% Range of error (confidence limits)		
0.05 level	0.935	0.935
0.01 level	1.383	1.383
LOD(µg/ml)	1.188	1.986
LOQ(µg/ml)	3.96	6.57

* Average of six determinations considered

Table.II: Assay and recovery of meropenem in pharmaceutical formulations (injection)

Method	Pharmaceutical Formulation	Labelled Amount (mg)	Proposed Method			Found by reference method[6]±S.D	% Recovery by proposed methods**±S.D
			Amount found** (mg) ±S.D	t (value)	F (Value)		
BTB	**MEROCRIT**	500	499.91 ± 0.16	0.769	1.562	499.99 ± 0.20	99.98 ± 0.603
BCP		500	499.89 ± 0.22	0.824	1.214		99.97 ± 0.346

** Average of six determinations