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Reverse Phase HPLC and Visible Spectrophotometric Methods for the Determination of Meropenem in Pure and Pharmaceutical Dosage Form

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Abstract: In the present investigation a reverse phase high performance liquid chromatographic method and a visible spectrophotometric method were developed for the quantitative determination of meropenem, a highly active antibiotic in powder for injection. The chromatographic analysis was carried out by reversed phase technique on an ultra sphere XL-ODS, 75mm x 4.6mm I.D.,5µm column with a mobile phase composed of ammonium acetate buffer of pH =4.0 and acetonitrile in the ratio 95:5 v/v, at a detection wavelength 298nm. In spectrophotometric method meropenem was reacted with oxidized form of 3-methyl 2-benzothia zolinone hydrazone (MBTH) in the presence of ferric chloride (Fe (III)) which forms an orange red colored chromogen giving absorption maximum at 600 nm. Linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) were studied according to International conference Harmonization guidelines. The developed methods were found to be simple, sensitive, precise and accurate and can be used for the reliable quantization of meropenem in pure and pharmaceutical dosage form.
Key words: Meropenem, RP-HPLC, Visible spectrophotometry, Validation and Assay.

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, similar to imipenem and ertapenem. It is marketed in india by New Medicon Pharma with the brand name Carbonem. It penetrates well into many tissues and body fluids including the cerebrospinal fluid, bile, heart valves, lung, and peritoneal fluid.[1] Meropenem is bactericidal except against Listeria monocytogenes where it is bacteriostatic. It inhibits bacterial wall synthesis like other beta-lactam antibiotics. In contrast to other beta-lactams, it is highly resistant to degradation by beta-lactamases or cephalosporinases. Resistance generally arises due to mutations in penicillin binding proteins, production of metallo-beta-lactamases, or resistance to diffusion across the bacterial outer membrane.[2] Meropenem 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

and the chemical structure of the drug is given in Fig.1.

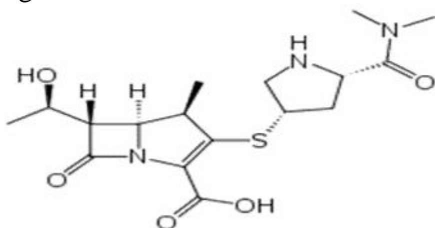

Fig.1 Structure of meropenem drug molecule

An extensive literature survey is carried out and found that there are a few spectrophotometric[3-4] and some HPLC[5-11] for the estimation of Meropenem. A capillary electrophoresis method[12] by direct injection of serum samples without any pretreatment is also reported.Stability-indicative determination of meropenem[13] in the presence of its open-ring degradation product, the metabolite, is investigated. Selective quantification of the drug, singly in bulk form, pharmaceutical formulations and/or in the presence of its major degradate is demonstrated. A reverse phase high performance liquid chromatographic method and a visible spectrophotometric method are developed[14] for the determination of meropenem in pharmaceutical dosage forms. In the present study, an RP-HPLC and a visible spectrophotometric method are developed for the estimation of meropenem in pure and pharmaceutical dosage forms in which the chromatographic method is found to be more sensitive than the reported ones.

EXPERIMENTAL

Agilent 1100 series HPLC system having tunable absorbance detector and injector with 20 µl loop volume agilent chemstation software was used for data collecting and processing. Chromatographic system equipped with pump, sample, column, U.V. detector and recorder. Elico-SL159 model, wavelength range190-1100nm, 2nm high resolution, double beam, 1cm length quartz coated optics was used for all the spectral measurements.

Materials and methods

Acetonitrile and ammonium acetate of HPLC grades were purchased from Merck, India Ltd. MBTH and ferric chloride of analytical grade were obtained from Fluka and Qualigens respectively. Meropenem was received as gift sample from cipra lab, sanathnagar, Hyderabad, India. For chromatographic analysis about 25mg of the meropenem working standard was accurately wieghed and transfered into a 100ml volumetric flask, to this about 70ml of mobile phase was added, sonicated, make up to the volume with mobile phase and filtered through 0.45µm membrane filter. Then working standard solution was prepared by diluting 1ml of stock solution to 10ml with mobile phase. In the analysis of pharmaceutical formulations an amount of the powder equavalent to 25mg was accurately weighed, transfered into a 100 ml volumetric falsk, dissolved in 70ml of mobile phase, sonicated, make up to the volume with mobile phase and filtered through 0.45µm membrane filter and then 1ml of above solution was make up to 10ml with mobile phase. In spectrophotometric analysis about 100 mg of meropenem was accurately weighed and transferred into a 100ml volumetric flask, dissolved in distilled water and diluted to 100ml to get the stock solution of concentration 1mg/ml. Further 50.0ml of the stock solution was diluted to 100ml to obtain a working standard solution of concentration 500µg/ml.

Experimental conditions

In chromatographic analysis a mixture of ammonium acetate (10.53mmol/L) and acetonitrile in the ratio of 95:5 v/v was used as mobile phase. It was filtered through 0.45 µm membrane filter and degassed. The mobile phase was pumped at 1.0ml/min. The Column was maintained at ambient temperature. The detection of the drug was monitored at 298nm. The injection volume of sample and standard were 20 µl. Under these optimized chromatographic conditions the retention time obtained for the drug was 7.897 minutes. A typical chromatogram showing the separation of the drug was given in Fig.2. The percentage relative standard deviation for meropenem peak area calculated from six replicate injections of standard solution was found to be not more than 2.0.

In spectrophotometric analysis aliquots of standard drug solution of meropenem ranging from 0.5 to 3.0 ml. (500µg/ml) were transferred into a series of 25ml calibrated tubes. To each tube 0.5ml of MBTH (8.56×10^{-3}M) solution was added and kept aside for 5min. After that 2.0ml (1.58×10^{-2} M) of ferric chloride solution was added and kept aside for 10min. The volume was made up to the mark with distilled water. Maximum absorbance was determined by measuring absorbance against a similar reagent blank from 380nm to 800nm and found to be 601nm. The absorbance of each solution was measured at wavelength of maximum absorbance against a similar reagent blank. The amount of the drug was calculated from its

calibration graph drawn by plotting absorbance against weight of the drug in µg/ml (Fig. 4). Optimum conditions in this method were fixed based on the study of the effects of various parameters such as effect of acid, nature and volume of oxidant, volume of MBTH, order of addition of reagents, solvents for final dilution, among the various oxidants tried, Fe (III) is found to be superior.

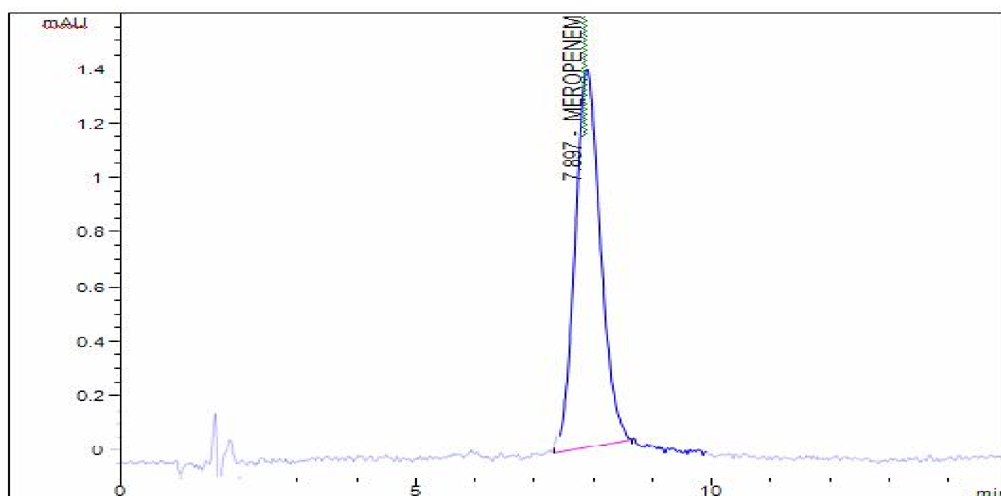

Fig.2 A typical chromatogram of the standard meropenem

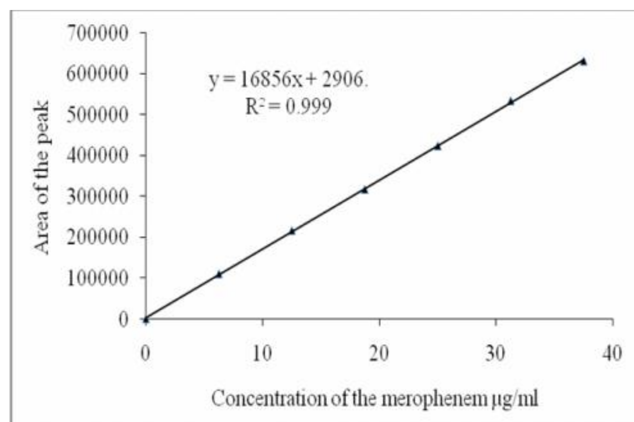

Fig.3. Linearity plot of peak area against the concentration of meropenem in µg/ml

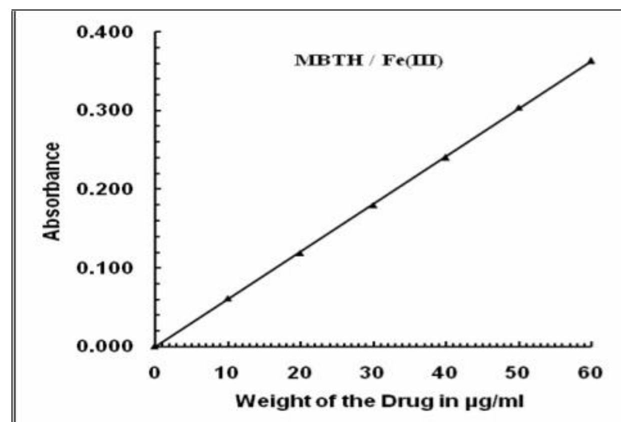

Fig.4. Linearity plot of absorbance against the concentration of meropenem in µg/ml

RESULTS AND DISCUSSION

Linearity and Range

Linearity was studied by preparing a series of six different concentration levels (6.25-37.5 µg/ml and 10-60µg/ml for chromatographic and spectrophotometric analysis respectively. Linearity plot of peak area against the concentration of meropenem in µg/ml is presented in Fig.3. Calibration curves were constructed by plotting peak area or absorbance against the amount of drug in µg/ml. Linearity range, slope intercept and correlation coefficient were presented in the Table - 1. Optical characteristics and statistical parameters of the proposed spectrophotometric method are included Table-5.

Precision

Precision refers to the reproducibility of measurements within a set of measurements. One of the most common statistical terms employed to explain precision was the standard deviation. The precision of each method was ascertained from the peak area or absorbance values for six replicates of a fixed amount of meropenem within the range of linearity. The percent relative standard deviation was calculated and presented in Table-2.

Table-1: Table showing linearity range, slope, intercept and correlation coefficient in chromatographic and spectrophotometric analysis

Chromatographic analysis			Spectrophotometric analysis	
S.No.	**Weight of meropenem μg/ml**	**Area of the peak**	**Weight of meropenem μg/ml**	**Absorbance**
1	6.25	110193	10	0.061
2	12.50	216148	20	0.119
3	18.75	317017	30	0.180
4	25.00	423820	40	0.240
5	31.25	534013	50	0.304
6	37.50	631491	60	0.363
	Slope	16856	Slope	0.006
	Intercept	2906	Intercept	0.00134
	Correlation Coefficient	0.9998	Correlation Coefficient	0.9999
	LOD μg/ml	0.430	LOD μg/ml	0.6029
	LOQ μg/ml	1.435	LOQ μg/ml	2.0098

Table-2: Precision of the chromatographic and spectrophotometric methods

Chromatographic analysis			Spectrophotometric analysis		
S.No.	**Standard**	**Area**	**S.No.**	**Standard**	**Absorbance**
1	25	421140	1	100	0.241
2	25	419905	2	100	0.245
3	25	418956	3	100	0.244
4	25	419446	4	100	0.241
5	25	423281	5	100	0.243
6	25	421235	6	100	0.244
Mean		420661	Mean		0.243
S.D.		1573.41	S.D.		0.001673
%R.S.D*.		0.37403	%R.S.D*.		0.688609

*The percent of R.S.D. was calculated for six replicate measurements

Table-3: Accuracy of the chromatographic and spectrophotometric methods

Chromatographic analysis				Spectrophotometric analysis			
Spike Level	**50%**	**100%**	**150%**	**Spike Level**	**50%**	**100%**	**150%**
Mean	213671	420911	637902	Mean	0.1201	0.2406	0.3636
S.D.	3293.63	1528.06	3329.01	S.D.	0.0011	0.0015	0.0005
%R.S.D**.	1.541	0.363	0.522	%R.S.D.	1.2661	0.6347	0.1587
%Assay	100.53	99.02	100.04	%Assay	98.87	99.15	99.88

**The percent of R.S.D. was calculated for three replicate measurements at each spike level.

Table-4: Assay analysis of meropenem in pharmaceutical formulations

Formulation	Method	Amount present (mg/injection)	Amount found (mg/ injection)	Percent of drug recovered
Meromac, Macleods Pharmaceutical Ltd.,	Chromatography	500	498	99.60†
	Spectrophotometry	500	497	99.40†

†Mean percent of recovery was calculated for four replicate determinations

Table-5: Optical characteristics and statistical parameters of the proposed spectrophotometric method

S.No.	Parameter	Value
1	Maximum Wavelength max	6.01E+02
2	Beer's Law Limits µg/ml	10---60
3	Sandell's Sencitivity (µg/cm2/0.001 Absorbance	1.64E-01
4	Molar absorptivity lt/mole/cm	2.33E+03
5	Standard Deviation on slope(Sb)	3.13E-05
6	Standard Deviation on intercept(Sa)	1.22E-03
9	Standard Error on Estimation(Se)	1.71E-03
7	0.05 level confidence limit	0.2147

Accuracy

Accuracy is a measurement of exactness of the analytical method and it can be determined at three different concentration levels 50%, 100% and 150% of the target the concentration of Meropenem standard, thrice at each concentration. Percent of assay of meropenem is calculated by using the formula (A_T / A_S) x (W_1/W_2) x100 x LC. Where, A_T is average peak area count / absorbance of meropenem in the sample preparation, A_S is average peak area count /absorbance of meropenem in the standard preparation, W_1 is weight of the meropenem in mg, W_2 is weight of the meropenem sample in mg and LC is label claim. The percent of recovery was found to be between 98.0 and 102.0% with percentage of RSD not more than 2.0 at each concentration. The results are given in Table-3.

Limit of detection (LOD) and limit of quantification (LOQ)

Limit of detection (LOD) and Limit of quantification (LOQ) were calculated based on the standard deviation of the response and the slope of the calibration curve at levels approximating the LOD according to the formulae LOD=3.0x(standard deviation / slope) and LOQ=10.0(standard deviation / slope). The results of LOD and LOQ are summarized in Table-1.

Assay

The assay of meropenem in the injection powder formulation was calculated by comparing area of the sample with that of standard. The percentage assay of individual drug was calculated and presented in Table-4.

CONCLUSION

The developed methods are simple, accurate and reproducible, so these methods are suitable to determine meropenem in pure and pharmaceutical dosage form. Chromatographic method was found to be more sensitive, precise and accurate than spectrophotometric method. The percent of recovery of the chromatographic method was found to be more reliable than spectrophotometry.

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