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Spectrophotometric Determination Of Penems In Bulk And Injection Formulations By MBTH reagent

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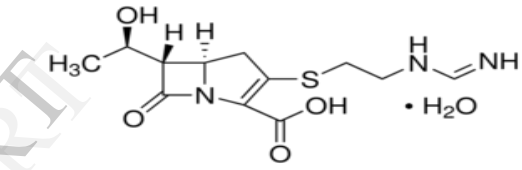
Abstract

Three simple and cost effective spectrophotometric methods were described for the determination of Imipenem, Meropenem and Biapenem in pure form and in pharmaceutical formulations. The method is based on the formation of green colored chromogen when the drug reacts with 3-methyl-2-benzothiazolinone hydrazone (MBTH) in presence of an oxidizing agent $FeCl_3$. The colored species has an absorption maximum at 608 nm for Imipenem (Method A), 397 nm for both Meropenem (Method B), Biapenem (Method C) and obeys beer's law in the concentration range 0.02 – 0.012 mg/mL of Imipenem, 0.04 – 0.012 mg/mL Meropenem and 0.04 – 0.16 mg/mL of Biapenem. The apparent molar absorptivities were 0.034, 0.0106 and 0.0144 and sandell's sensitivity were 2.5x10-5, 2.96x10-3 and 1x10-3 respectively for Imipenem, Meropenem and Biapenem. The slopes were 0.4379 ± 0.01829, 0.2048 ± 0.003599 and 0.2008 ± 0.001171 and intercept of the equation of the regression line are 0.06607 ± 0.03298, -0.0009786 ± 0.006970, 0.003963 ± 0.002950 for Imipenem, Meropenem and Biapenem respectively. The optimum experimental parameters for the reaction have been studied and the validity of the described procedure was assessed. Statistical analysis of the results has been carried out revealing high accuracy and good precision. The proposed method was successfully applied for the determination of Imipenem, Meropenem and Biapenem in pharmaceutical formulations.

Key words: *Imipenem, Meropenem, Biapenem, MBTH, Spectrophotometry*.

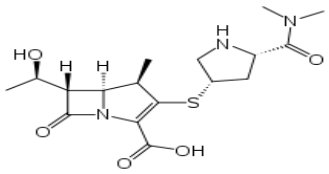
1.Introduction

Imipenem[1] is a broad spectrum beta-lactam antibiotic belonging to the carbapenem class. Chemically it is (5R,6S)-6-[(1R)-1-hydroxyethyl]-3-({2-[(iminomethyl)amino]ethyl}thio)-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid.



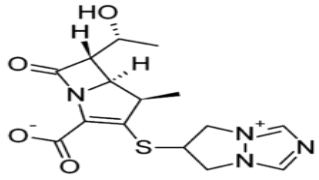
Imipenem acts by interfering with their ability to form cell walls, and therefore the bacteria break up and die. It is a broad spectrum antibiotic with activity against many aerobic and anaerobic gram-positive and gram-negative organisms.

Meropenem[2] is an ultra-broad spectrum injectable antibiotic used to treat a wide variety of infections. It is a beta-lactam and belongs to the subgroup of carbapenem, similar to imipenem and ertapenem. It penetrates well into many tissues and body fluids including the cerebrospinal fluid, bile, heart valves, lung, and peritoneal fluid. Chemically it is 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.



In contrast to other beta-lactams, it is highly resistant to degradation by beta-lactamases or cephalosporinases.

Biapenem[3] is a carbapenem antibiotic. It has in vitro activity against anaerobes.



Biapenem is a new parenteral carbapenem antibacterial agent with a broad spectrum of in vitro antibacterial activity encompassing many Gram-negative and Gram-positive aerobic and anaerobic bacteria, including species producing beta-lactamases.

Literature survey reveals that the drugs were determined by using HPLC and some spectrophotometric methods for Imipenem[4-9] and Meropenem[10-23]. According to the literature survey there is no method reported for Biapenem with MBTH reagent by visible spectrophotometry. Hence an attempt made to develop simple and sensitive spectrophotometric methods for the estimation of the above named penems in pure drug and in pharmaceutical formulations. The method uses the well known oxidation reaction involving MBTH reagent and penems resulting in the formation of a blue /green chromogen that could be measured at 300 – 800 nm.

2. Experimental

2.1 Apparatus

All spectral characteristics and absorbance measurements were made on Perkin Elmer, LAMBDA 25 double beam UV-Visible spectrophotometer with 10 mm matched quartz cells. All chemicals used were of analytical reagent grade and double distilled water was used throughout. MBTH supplied by SD Fine chemicals ltd., India, was used and 0.2% MBTH solution was prepared by dissolving 200 mg MBTH in 100 mL distilled water. $FeCl_3$ solution (0.5%) was prepared by dissolving 500 mg of $FeCl_3$ in 100 mL double distilled water. 10 mg/mL stock reference solution was freshly prepared from pure sample of penems by dissolving 100 mg in 100 mL of double distilled water.

2.2 General procedure

2.2.1 Method A

Into 10 mL volumetric flask, different aliquots of working standard solution (0.5 – 3.0 mL) of Imipenem were transferred to provide final concentration range of 0.02 – 0.12 mg/mL. To each flask, 2 mL of freshly prepared $FeCl_3$ and 2 mL of MBTH reagent were successively added and kept aside for 5 minutes. The solutions were made up to volume with distilled water. The absorbance of each solution was measured at 608 nm against the reagent blank. The calibration graph was then prepared by plotting the absorbance versus the concentration of the drug. The concentration of the unknown was read from the calibration graph or computed from the regression equation.

2.2.2 Method B

Into 10 mL volumetric flask, different aliquots of working standard solution (1.0 – 3.0 mL) of Meropenem were transferred to provide final concentration range of 0.04 – 0.12 mg/mL. To each flask, 2.5 mL of freshly prepared $FeCl_3$ and 2.5 mL of MBTH reagent were successively added and kept aside for 5 minutes. The solutions were made up to volume with distilled water. The absorbance of each solution was measured at 397 nm against the reagent blank. The calibration graph was then prepared by plotting the absorbance versus the concentration of the drug. The concentration of the unknown was read from the calibration graph or computed from the regression equation.

2.2.3 Method C

Into 10 mL volumetric flask, different aliquots of working standard solution (1.0 – 4.0 mL) of Biapenem were transferred to provide final concentration range of 0.04 – 0.16 mg/mL. To each flask, 1.5 mL of freshly prepared $FeCl_3$ and 2.0 mL of MBTH were successively added and kept aside for 5 minutes. The solutions were made up to volume with distilled water. The absorbance of each solution was measured at 397 nm against the reagent blank. The calibration graph was then prepared by plotting the absorbance versus the concentration of the drug. The concentration of the unknown was read from the calibration graph or computed from the regression equation.

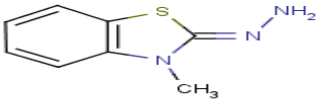
3. Procedure for Injections

An amount of powder equivalent to 100 mg of penems were weighed into a 100mL volumetric flask, 50 mL of distilled water was added and shaken thoroughly for about 10 minutes, then the volume was made up to the mark with the distilled water, mixed well and filtered. Further dilutions were made and the assay of injections was completed according to general procedure.

4. Results and Discussion

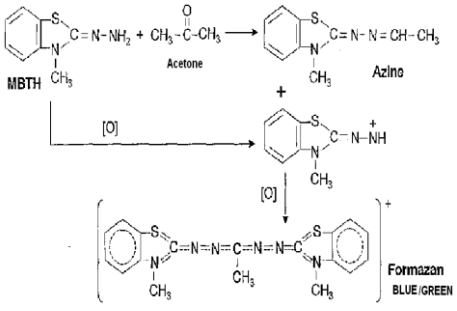
3-methylbenzthiazolinone-2(3H)-hydrazone (MBTH) originally introduced as a reagent for aldehydes and ketones. Later its use was extended to a variety of organic compound (example: Phenols,

aryl amines and different N- and S- heterocyclic compound).



Aldehydes and ketones are partially oxygenated organic compounds containing carbonyl group. An aldehyde functional group consists of a carbon atom bonded to a hydrogen atom and double-bonded to an oxygen atom (O=CH-). Whereas a ketone functional group contains a carbonyl group (C=O) bonded to two other carbon atoms.

MBTH reacts with aldehyde/ketones first to form an azine. Only if there is remaining MBTH, it is oxidized to another species which combines with the azine to form formazan. However, if there is enough aldehyde/ketone, all the MBTH is converted to azine and there is no formation of blue color. Thus, by using the limiting agent MBTH to test the amount of aldehyde/ketone around the point of interest, then less aldehyde/ketone would produce more blue /green color and more aldehyde/ketone would produce less blue/green color. The end color may be different depending upon the order of addition of the reactants. For example, if an oxidizing agent and MBTH are mixed before adding the aldehyde/ketone, a light green to green/blue color results. This method could be used could be used for measurements with a device or instrument such as a color reader and used in combination with a second aldehyde/ketone tester and a pH tester. With Phenols under reaction condition MBTH loses two electrons and one proton to form the electrophilic intermediate, which has been identified as the active coupling species that undergoes electrophilic substitution with phenol and other groups to form the colored product.



Optimization of conditions on absorption spectrum of the reaction product

The Conditions under which reaction of penems with MBTH fulfills the essential requirements was investigated. All conditions studied were optimized at room temperature (32 ± 2^0C).

5. Selection of reaction medium

To find a suitable medium for the reaction, different aqueous bases were used, such as Sodium meta periodate, Ammonium Ceric Sulphate, Ferric chloride. The best results were obtained when Ferric chloride was used. In order to determine the optimum concentration of Ferric chloride, different volumes of 0.5% Ferric chloride solution (1 – 2.5 mL) were used to a constant concentration of Imipenem (25 mg/mL), (1 – 3.0 mL) were used to a constant concentration of both Meropenem and Biapenem (25 mg/mL) and the results of the observation were plotted. From the figure it is evident that 2 mL of 0.5% Ferric chloride solution for Imipenem, 2.5 mL of 0.5% Ferric chloride solution for Meropenem and 1.5 mL of 0.5% Ferric chloride solution for Biapenem were found optimum. Larger volumes had no effect on the absorbance of the colored species.

6. Effect of order of addition of reactants

Few trials were performed to ascertain the influence of order of addition of reactants on the color development and the results are presented in Table 1. The order of addition of serial number (ii) is recommended for all the three samples.

Table 1. Effect of order of addition of drug reactants on color development.

S. No.	Drug		Order of Addition	Absorbance	Recommended order of Addition
1.	Imipenem[a]	i	D+MBTH+ $FeCl_3$	0.50	
		ii	D + $FeCl_3$+ MBTH	0.70	ii
		iii	$FeCl_3$+ MBTH + D	0.10	
2.	Meropenem[a]	i	D + MBTH + $FeCl_3$	0.41	
		ii	D + $FeCl_3$+ MBTH	0.57	ii
		iii	$FeCl_3$+ MBTH + D	0.32	
3.	Biapenem[a]	i	D + MBTH + $FeCl_3$	0.25	
		ii	D + $FeCl_3$+ MBTH	0.38	ii
		iii	$FeCl_3$+ MBTH + D	0.22	

[a]For 40 µg/mL of Drug samples

7. Effect of MBTH concentration

Several experiments were carried out to study the influence of MBTH concentration on the color development by keeping the concentration of drug and Ferric chloride to constant and changing reagent concentration. It was apparent that 2.0 mL of reagent gave maximum color for Imipenem, 2.5 mL for Meropenem and 2.0 mL for Biapenem.

8. Reaction time and stability of the colored species

The color reaction was not instantaneous. Maximum color was developed within 5 minutes of mixing the reactants and was stable for 60 minutes thereafter.

9. Absorption spectrum and calibration graph

Absorption spectrum of the colored complex was scanned at 400-900 nm against a reagent blank. The reaction product showed absorption maximum at 608 nm for Imipenem, 397 nm for both Meropenem and Biapenem. Calibration graph was obtained according to the above general procedure. The linearity replicates for six different concentrations of Imipenem and Meropenem, seven different concentrations of Biapenem with MBTH were checked by a linear least - squares treatment. All the spectral characteristics and the measured or calculated factors and parameters were summarized in Table 2.

Fig 1. Calibration graph of Imepenem

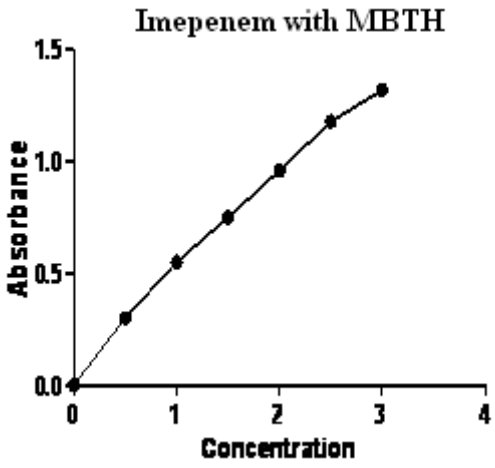


Fig 2 Calibration graph of Meropenem

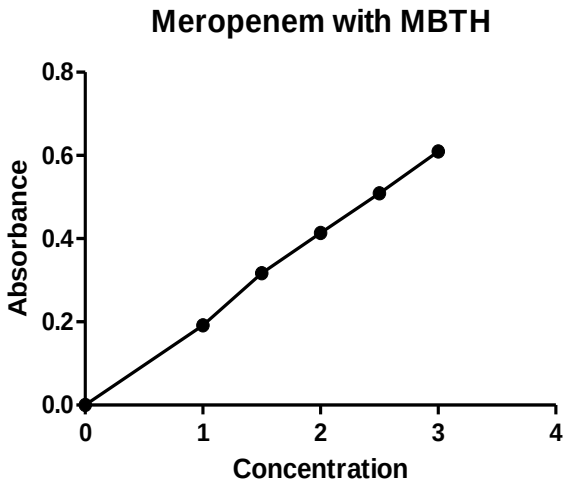
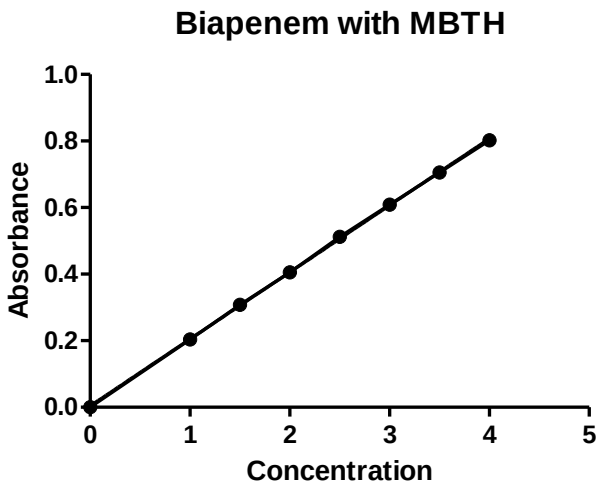


Fig 3 Calibration graph of Biapenem



10. Sensitivity, accuracy and precision

Sandell's sensitivity, molar absorptivity, precision and accuracy were found by performing eight replicate determinations containing 3/4th of the amount of upper Beer's law limits. The measured standard deviation (S.D), relative standard deviation (RSD), and confidence limits (Table 2) were considered satisfactory.

Table 2. Optical and regression characteristics, precision and accuracy of the proposed method for penems.

Parameters	Values		
	Imipenem	**Meropenem**	**Biapenem**
λmax nm	608 nm	397 nm	397 nm
Beer's law limits, µg/mL	0.02– 0.03	0.04 – 0.12	0.04 – 0.16
Molar absorptivity, L/mol.cm	0.034	0.0106	0.0144
Sandell's sensitivity (µg/cm2/0.001 absorbance unit)	$2.5x10^{-5}$	$2.96x10^{-3}$	$1x10^{-3}$
Regression equation			
Slope(b)	0.4379 ± 0.0182	0.2048 ± 0.0035	0.2008 ± 0.001171
Intercept	0.066 ± 0.0329	-0.0009 ± 0.0069	0.003963 ± 0.002950
r^2	0.9913	0.9988	0.9998
Limit of Detection	0.3653	0.1385	0.0678
Limit of Quantification	1.1071	0.42	0.2056

11. Interference

Aldehydes and ketones are partially oxygenated organic compounds containing carbonyl group. An aldehyde functional group consists of a carbon atom bonded to a hydrogen atom and double-bonded to an oxygen atom (O=CH-). Whereas a ketone functional group contains a carbonyl group (C=O) bonded to two other carbon atoms. MBTH reacts with aldehyde/ketones first to form an azine. Only if there is remaining MBTH, it is oxidized to another species which combines with the azine to form formazan. However these substances are seldom present in the reagents and used in the pharmaceutical formulations. Hence, the method is devoid of error due to above substances.

12. Application to formulation

The proposed procedures were applied for the determination of penems in commercially available injections. Table 3 summarized the results.

Table 3. Results of analysis of injection formulations containing penems

Injection	Imi penem	Mero penem	Bia penem
Company Name	Troika Pharma	Neon Pharma	Novachem
Formulation	Inj	Inj	Inj
Labeled amount, mg	1000	1000	1000
% Recovery	99.8	99.56	98.92

13. Conclusion

The proposed methods were found to be simple, rapid and inexpensive, hence can be used for routine analysis of penems in bulk and in injection formulations.

14. Acknowledgement

We wish to thank Aribindo labs, Hyd. for providing gifted samples of Penems, Dept., of Engineering chemistry, AUCE(A), Andhra University, Visakhapatnam, India, the research lab, Dept., of Analysis, GIET School of Pharmacy, Rajahmundry, India, for the kind provision of equipment.

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