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Research Article

SPECTROPHOTOMETRIC DETERMINATION OF PENEMS IN BULK AND INJECTION FORMULATIONS BY POTASSIUM FERRI CYANIDE AND FERRIC CHLORIDE

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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 blue colored chromogen when the drug reacts with Potassium ferri cyanide reagent in presence of an oxidizing agent like $FeCl_3$. The colored species has an absorption maximum at 725 nm for Imipenem (Method A) / Meropenem (Method B) and 745 nm for Biapenem (Method C) and obeys beer's law in the concentration range 0.02 – 0.12 mg/mL of Imipenem, 0.02 – 0.1 mg/mL of Meropenem and 0.02 – 0.1 mg/mL of Biapenem. The apparent molar absorptivities were 0.0625, 0.0151 and 0.0197 and sandell's sensitivity were $7x10^{-3}$ for Imipenem, $6x10^{-4}$ for both Meropenem and Biapenem respectively. The slopes were 0.7479 ± 0.0423, 0.3486 ± 0.0195 and 0.4446 ± 0.0105 and intercept of the equation of the regression line are 0.1690 ± 0.07637, 0.04971 ± 0.02964, 0.02295 ± 0.01604 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.

Keywords: Imipenem, Meropenem, Biapenem, Potassium ferri cyanide, Spectrophotometry.

INTRODUCTION

Imipenem

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.

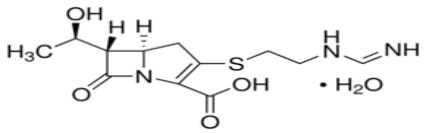

Fig.1: Structure of Imipenem

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

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. It is marketed in India by FHC with the brand name merofit & outside India by Astrazeneca with the brand names Monan and Meronem. 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.

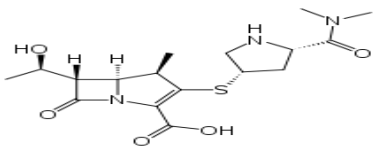

Fig.2: Structure of Meropenem

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

Biapenem

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

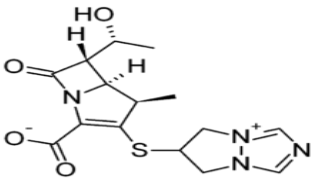

Fig.3: Structure of Biapenem

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 literature survey there is no method reported for Biapenem with Potassium ferri cyanide 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 reduction reaction involving Potassium ferri cyanide reagent and penems resulting in the formation of a blue chromogen that could be measured at 725 nm for both Imipenem and Meropenem, 745 nm for Biapenem.

Experimental

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. Potassium ferri cyanide reagent 0.1% supplied by BDH Fine chemicals Ltd., India, was used by dissolving 100 mg in 100 mL distilled water. Ferric chloride solution (0.054%) was prepared by dissolving 54 mg of Ferric chloride in 100 mL double distilled water. 10 µg/mL stock reference solution was freshly

prepared from pure sample of penems by dissolving 100 mg in 100 ml of double distilled water.

General procedure

Method A

Into 10ml 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, 2.5 mL of Ferric chloride and 2 mL of Potassium ferri cyanide 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 725 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.

Method B

Into 10 ml volumetric flask, different aliquots of working standard solution (0.5 – 3.0 mL) of Meropenem were transferred to provide final concentration range of 0.02 – 0.12 mg/mL. To each flask, 2.5 mL of Ferric chloride and 2.5 mL of Potassium ferri chloride 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 725 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.

Method C

Into 10ml volumetric flask, different aliquots of working standard solution (0.5 – 3.0 mL) of Biapenem were transferred to provide final concentration range of 0.02 – 0.12 mg/mL. To each flask, 2.5 mL of Ferric chloride and 2.5 mL of Potassium ferri chloride 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 745 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.

An amount of powder equivalent to 100 mg of penems were weighed into a 100 mL volumetric flask, 50 mL of distilled water was added and shaken thoroughly for about 10 min, 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.

RESULTS AND DISCUSSION

Potassium ferri cyanide[24]

Potassium ferricyanide is the chemical compound with the formula $K_3[Fe(CN)_6]$. Molar mass 329.24 g/mol, appears as deep red crystals, sometimes small pellets, orange to dark red powder This bright red salt contains the octahedrally coordinated $[Fe(CN)6]^{3-}$ ion.[25] It is soluble in water and its solution shows some green-yellow fluorescence.

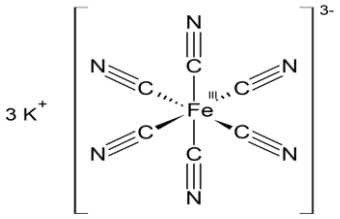

Fig.4: Structure of Potassium ferri cyanide

Antibiotic + Fe^{3+} → Antibiotic^{+} + Fe^{2+}

The chemical formula of insoluble Prussian blue is $Fe_7(CN)_{18}{\cdot}xH_2O$, where x = 14–16.

Preliminary studies were designed to examine the reaction between the selected antibiotics and chromogenic reagents at room temperature. The obtained results confirmed that no consider able interactions occurred under these condition. In this method (potassium ferricyanide method) was based on reduction of the Fe^{3+} in $FeCl_3$ to Fe^{2+} by penems in the presence of K_3 [Fe (CN_6)]. Subsequently, the in situ formed Fe^{2+} reacts with K_3 [Fe $(CN)_6$] under acidic conditions to form soluble prussian blue (KFeIII [FeII $(CN)_6$]) whose absorbance was measured at 650-800 nm against the corresponding reagent blank.

Optimization of conditions on absorption spectrum of the reaction product

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

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 Ferric chloride solution (0.5 – 3.0 mL) were used to a constant concentration of imipenem, meropenem and biapenem (25 mg/mL), and the results of the observation were plotted. From the figure it is evident that 2.5 mL of Ferric chloride solution for imipenem, meropenem and biapenm was found optimum. Larger volumes had no effect on the absorbance of the colored species.

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 both imipenem and meropenem, (i) for biapenem.

Table.1*:* Effect of order of addition of reactants on color development.

S. No.	Drug		Order of Addition	Absorbance	Recommended order of Addition
		i	D + $K_3[Fe(CN)_6]$ + $FeCl_3$	0.213	
1.	Imipenem[a]	ii	D + $FeCl_3$ + $K_3[Fe(CN)_6]$	1.245	ii
		iii	$FeCl_3$ + $K_3[Fe(CN)_6]$ + D	0.986	
		i	D + $K_3[Fe(CN)_6]$ + $FeCl_3$	0.457	
2.	Meropenem[a]	ii	D + $FeCl_3$ + $K_3[Fe(CN)_6]$	0.524	ii
		iii	$FeCl_3$ + $K_3[Fe(CN)_6]$ + D	0.392	
		i	D + $K_3[Fe(CN)_6]$ + $FeCl_3$	0.749	
3.	Biapenem[a]	ii	D + $FeCl_3$ + $K_3[Fe(CN)_6]$	0.691	i
		iii	$FeCl_3$ + $K_3[Fe(CN)_6]$ + D	0.490	

[a]*For 40 µg/mL of Drug samples*

Effect of Potassium ferri cyanide concentration

Several experiments were carried out to study the influence of Potassium ferri cyanide 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 both Imipenem, 2.5 mL for both Meropenem and Biapenem.

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.

Absorption spectrum and calibration graph

Absorption spectrum of the colored complex was scanned at 550-850 nm against a reagent blank. The reaction product showed absorption maximum at 725 nm for Imipenem/Meropenem and at 745 nm for Biapenem. Calibration graph was obtained according to the above general procedure. The linearity replicates for six different concentrations of Imipenem, seven different concentrations of Meropenem and five different concentrations of Biapenem 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.

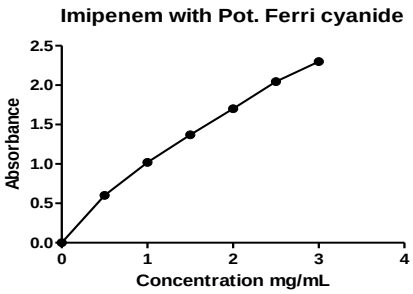

Fig.5: Calibration graph of Imipenem

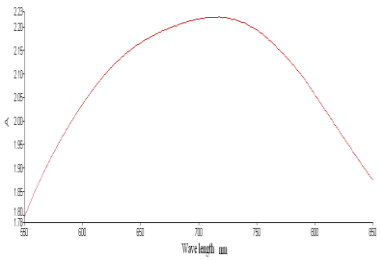

Fig.6: Absorption spectra of Imipenem

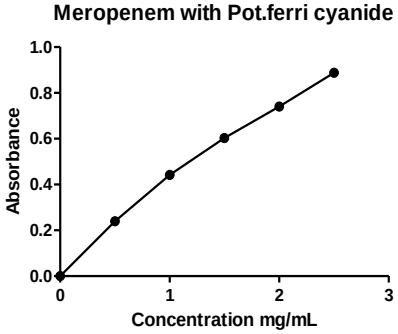

Fig.7: Calibration graph of Meropenem

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.

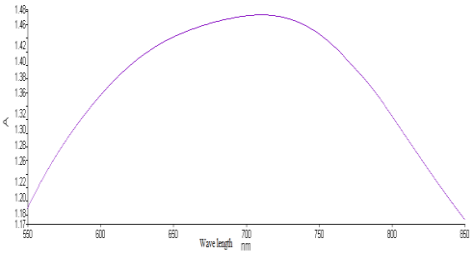

Fig.8: Absorption spectra of Meropenem

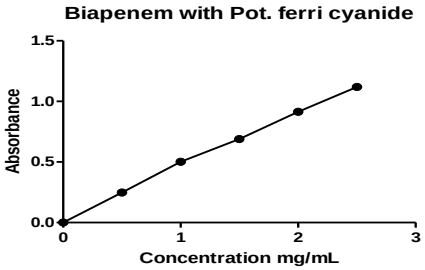

Fig .9: Calibration graph of Biapenem

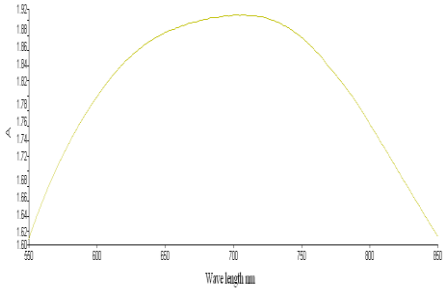

Fig.10: Absorption spectra of Biapenem

Interference

The chemical reaction between potassium ferricyanide and the selected penems was based on reduction of the Fe^{3+} in $FeCl_3$ to Fe^{2+} by penems in the presence of $K_3[Fe(CN_6)]$. Subsequently, the in situ formed Fe^{2+} reacts with K_3 [Fe $(CN)_6$] under acidic conditions to form soluble prussian blue (KFeIII [FeII $(CN)_6$]). However these substances are seldom present in the reagent and used in the pharmaceutical formulations. Hence, the method is devoid of error due to above substances.

Quantification

The limits of the Beer's law, the molar absorptivity and the Sandell's sensitivity values were evaluated and are given in Table 2.The regression analyses of the Beer's law plots at their respective λmax values revealed a good correlation. Graphs of absorbance versus concentration showed zero intercept, and are described by the regression equation, Y = bX + c (where Y is the absorbance of a 1 cm layer, b is the slope, c is the intercept and X is the concentration of the drug in µg/ml) obtained by the least-squares method. The results are summarized in Table 2.

Table.2: Optical and regression characteristics of the proposed method for penems.

Parameters	Values		
	Imipenem	**Meropenem**	**Biapenem**
λ_{max} nm	725 nm	725 nm	745 nm
Beer's law limits, mg/mL	0.02 – 0.12	0.02 – 0.1	0.02 – 0.1
Molar absorptivity, L/mol.cm	0.0625	0.0151	0.0197
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	$7x10^{-3}$	$6x10^{-4}$	$6x10^{-4}$
Regression equation			
Slope(b)	0.7479 ± 0.0423	0.3486 ± 0.0195	0.4446 ± 0.0105
Intercept	0.1690 ± 0.0763	0.0497 ± 0.0296	0.0229 ± 0.0160
r^2	0.9842	0.9875	0.9977
Limit of Detection	0.4961	0.3884	0.1641
Limit of Quantification	1.506	1.1771	0.4973

Analytical Validation

The validity of the methods for the assay of penems was examined by determining the precision and accuracy. These were determined by analyzing six replicates of the drug within the Beer's law limits. The low values of the relative standard deviation (R.S.D.) indicate good precision of the methods. To study the accuracy of the methods, recovery studies were carried out by the standard calibration curve method. For this, known quantities of pure penems were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed as before. The total amount of the drug was then determined and the amount of the added drug was calculated by difference. The results are given in Table 3. The average percent recoveries obtained. were quantitative indicating good accuracy of the methods.

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	**Imipenem**	**Meropenem**	**Biapenem**
Company Name	**Troika Pharma**	**Neon Pharma**	**Novachem**
Formulation	Inj	Inj	Inj
Labeled amount, mg	1000	1000	1000
% Recovery	99.8	99.56	98.92

CONCLUSION

The reagents utilized in the proposed methods are cheap, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. Moreover, the methods are free from interference by common additives and excipients. The wide applicability of the new procedures for routine quality control was well established by the assay of penems in pure form and in pharmaceutical preparations, hence can be used for routine analysis of penems in bulk and in injection formulations.

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