XVII International Conference on Recent"Trends in Engineering, Applied Science and Management"
(IETE) Institution of Electronics and Telecommunication Engineers,Chandigarh TEAM-18
24th June 2018 www.conferenceworld.in ISBN: 978-93-87793-31-6

SPECTROPHOTOMETRIC DETERMINATION OF BIAPENEM IN BULK AND INJECTION FORMULATIONS BY CHLORAMINE – T AND GALLOCYANINE

Dr.G.Venkata Ramana[1], Dr.N.Aruna Kumari[2]

[1]Reader in Chemistry,SKVT College,Rajamahendravam,E.G (district)

[2]Assoc.Prof of Engineering Chemistry,GIET(A),Rajamahendravam,E.G (district)

ABSTRACT

A simple and cost effective spectrophotometric method was described for the determination of Biapenem in pure form and in pharmaceutical formulations. The method is based on the formation of colored chromogen when the drug reacts with Chloramine – T and Gallocyanine in acidic medium. This method was applied for the determination of drug contents in pharmaceutical formulations and enabled the determination of the selected drug in microgram quantities (0.5 to 3.0 mL). No interferences were observed from excipients and the validity of the method was tested against reference method. The colored species has an absorption maximum at 542 nm for Biapenem and obeys beer's law in the concentration range 0.02-0.12 mg/mL of Biapenem. The apparent molar absorptivity was $1x10^{-3}$ and sandell's sensitivity was $7x10^{-2}$. The slope is 0.2050 ± 0.0021, the intercept of the equation of the regression line is 0.0245 ± 0.0137. 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 Biapenem in pharmaceutical formulations.

Keywords- Biapenem, Chloramine - T, Gallocyanine, HCl, Spectrophotometry.

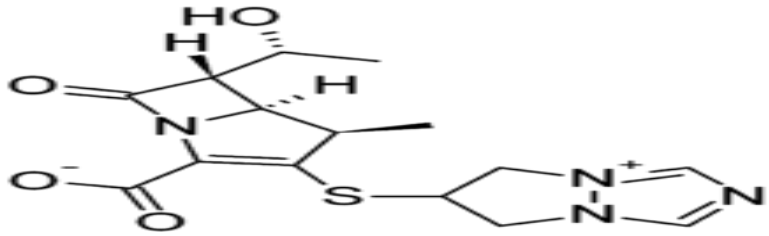
1. INTRODUCTION

Due to counterfeiting, the drug quality has become a source of major concern worldwide, particularly in many developing countries. The most commonly counterfeited drugs are anti-infective or antibiotics. Use of poor quality antibiotics bears serious health implications such as treatment failure, adverse reactions, drug resistance, increased morbidity, and mortality[1]. Among antibiotics, penems are much recently introduced, widely

prescribed and costlier. Therefore, incentive to produce their counterfeits because of profit margin increases considerably.

Biapenem[2] (BAP) is a_parenteral carbapenem that possesses antibacterial activities against a wide range of Gram-positive and -negative bacteria. It has a broad spectrum of in vitro antibacterial activity.

1.1 Drug Profile

Name	:	Biapenem (BAP)
Chemical Name	:	(4R,5S,6S)-3-(6,7-dihydro-5H-pyrazolo[1,2-a][1,2,4]triazol-8-ium-6-ylsulfanyl)-6-(i-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate
Structure	:	
Molecular formula	:	$C_{15}H_{18}N_4O_4S$
Empirical formula	:	$C_{15}H_{18}N_4O_4S$
Molecular weight	:	350.39 gm/mol
Color	:	White to beige
p^{Ka}	:	3.22
Solubility	:	Soluble in water and slightly soluble in methanol
Pharmacodynamic / Chemotherapeutic category	:	Antibacterial Agent

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

Literature survey reveals that a few analytical methods have been reported for the determination of BAP in pure drug, pharmaceutical dosage forms and in biological samples using UV spectroscopy and RP-HPLC methods. Most of the HPLC[3-6] based on analytical techniques reported earlier demonstrated the absence of buffer capacity leading to instability of the sample and/or its solutions. Apart from providing better detection

and improve peak shape and high resolution, the proposed method has demonstrated a low noise and better signal-to-noise ratio during the detection process.

According to literature survey there is no method reported for Biapenem with CAT and GC by visible spectrophotometry. Hence in the present investigation, an attempt was made to develop simple and sensitive spectrophotometric method for the estimation of Biapenem in pure and in pharmaceutical formulations. The method uses the well known oxidation reaction between the reagent and oxidisable centers present in Biapenem resulting in the formation of a coloured chromogen that could be measured at 542 nm for Biapenem.

2. EXPERIMENTAL

2.1 Apparatus and Chemicals

All spectral characteristics and absorbance measurements were made on Perkin Elmer, LAMBDA 25 double beam UV-Visible spectrophotometer with 10 mm matched quartz cells. A systronics digital pH meter 361 was used for pH measurements.

Preparation of standard drug solution

The stock solution (1mg/mL) of drug was prepared by dissolving 100 mg of BAP in 100 mL of distilled water. A portion of this stock solution was diluted stepwise with the distilled water to obtain the working standard solution of 100 µg/mL for the proposed method.

Preparation of reagents:

CAT Solution (Loba; 0.02%, $7.1x10^{-4}$M)	:	Prepared by dissolving 20 mg of CAT in 100 mL of distilled water and standardized iodometrically.
GC solution (Chroma; 0.01%, $2.969X10^{-4}$M)	:	Prepared by dissolving 50 mg of GC in 500 mL of distilled water.
Hydrochloric acid (E.Merck,5M)	:	Prepared by diluting 217.5 mL of con. HCl to 500 mL with distilled water and standardized.

2.2 General procedure

Different aliquots of working standard solution (0.5 to 3.0 mL) of BAP were transferred into a series of 25 mL volumetric flask, to provide final concentration range of 2 – 12 µg/mL. To each flask, 2.0 mL of 5 M HCl (5M) and 2.5 mL of CAT (0.02%) were successively added and the volume was made up to 15 mL with distilled water. After 20 min, 10 mL of GC was added and mixed thoroughly and the absorbance was measured after 15 min. at 542 nm against distilled water. Blanks were prepared appropriately. The decrease in absorbance corresponding to consumed CAT, which in turn to the drug quantity was obtained by subtracting the absorbance

of the test solution from that of the blank solution. The calibration graph was drawn by plotting the decrease in the absorbance of the dye GC, against the amount of the drug.

2.3 Procedure for Injections

An amount of powder equivalent to 100 mg of Biapenem was 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.

3. RESULTS AND DISCUSSION

Chemistry of the coloured species in the present investigation

BAP possesses different functional moieties such as β-lactum ring in which there is a carboxylic acid, Tertiary nitrogen, Vulnerable oxidising centers, Hetero Sulphur and Double bonds.

An attempt has been made to indicate the nature of coloured species formed in each proposed method for the determination of selected BAP tentatively based on analogy. In the present investigation, the coloured species formation in the method for the assay of Biapenem appears to be the formation of an oxidation reaction analogy. In the present investigation, Biapenem has been estimated with the above method, the reaction pathway has shown in the following Scheme.

Step - 1

The first step in the method below is the oxidation of DRUG with the oxidant.

DRUG + CAT → oxidation products + reduced CAT + unreacted CAT

Step – 2

CAT	+	GC	→	GC (unreacted, colored)	Reduced form of CAT	+ Mixture of compounds with rupture of conjugated system in dye reproducible, (colorless) but not stoichiometric as several alternate pathways possible.

4. Optimization of the conditions on absorption spectrum of the reaction product

The condition under which the reaction of Biapenem with Chloramine - T and Gallocyanine fulfills the essential requirements was investigated. All conditions studied were optimized at room temperature (32 ± 2^0C).

4.1 Selection of reaction medium

To find a suitable medium for the reaction, different acids have been used. The best results were obtained when HCl was used. In order to determine the optimum concentration of HCl, different volumes of HCl solution (0.5 – 2.5 mL) were used to a constant concentration of Biapenem (1mg/mL) and the results were observed. From the absorption spectrum it was evident that 2.0 mL of HCl solution was found optimum. Larger volumes had no significant effect on the absorbance of the colored species.

Parameter	Range of study	Optimised condition in procedure	Remarks
λ_{max} (nm)	350-650	542	
Effect of volume of CAT required for Charge transfer complex formation (mL)	0.5-3.5	2.5	Volume above 2.5 mL gave high optical densities in blanks (>2.5), which resulted in deviations from Beers law.
Effect of volume of HCl (mL)	0.5-2.5	2.0	To speed up the reaction stage in color development, 2.0 mL of HCl (5.0M) was found necessary for maximum color development.
Effect of volume of GC (mL)	10.0	10.0	To speed up the reaction stage in color development, 10.0 mL of GC was found necessary for maximum color development.
Effect of reaction time (min)	15-30	15	The minimum time required for complete oxidation was found to be 15 min.
Effect of temperature (^{0}C)for Charge transfer complex formation	20-40	32 ± 2 Lab. Temp	At low temperatures (<30^{0}C) the reaction time was found to be more and at high temperatures (>34^{0}C) no added advantage was found.
Standing time (min)	15	15	A minimum amount of time, i.e., 1 min was necessary for undergoing Charge transfer complex

			formation and beyond 15 min results in low sensitivity.
Stability period after final dilution (min)	5-40 min	40 min	The absorbance of the colored product decreases slowly with time after 40 min.

4.2 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 (i) is recommended for Biapenem

Tab: 1. Effect of order of addition of reactants on color development

S.No.	Drug		Order of Addition	Absorbance	Recommended order of Addition
1.	Biapenem[a]	i	D+HCL+CAT+GC	0.188	i
		ii	D+CAT+GC+HCL	0.124	
		iii	HCL+CAT+GC+D	0.06	

[a]*For 40 µg/mL of Drug sample*

4.3 Effect of Chloramine - T concentration

Several experiments were carried out to study the influence of CAT concentration on the color development by keeping the concentration of drug and HCL to constant and changing reagent concentration (0.5-3.5). It was apparent that 2.5 mL of CAT gave maximum color for Biapenem

4.4 Effect of Gallocyanine concentration

Several experiments were carried out to study the influence of GC. To speed up the reaction stage in color development, 10.0 mL of GC was found necessary for maximum color development.

Tab: 2 RESULTS OF METHOD OPTIMISATION FOR BIAPENEM – CHLORAMINE – AND GALLOCYANINE

5. 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.

6. ABSORPTION SPECTRUM AND CALIBRATION GRAPH

Absorption spectrum of the colored complex was scanned at 350-650 nm against a reagent blank. The reaction product showed absorption maximum at 542 nm for Biapenem. Calibration graph was obtained according to the above general procedure. The linearity replicates for six different concentration of Imipenem was checked by a linear least - squares treatment. All the spectral characteristics and the measured or calculated factors and parameters were summarized in Table 3.

Fig 1. Calibration graph of Biapenem

BAP(0.5 - 3 mL)+ HCl(2mL)+ CAT(2.5mL) + GC(10mL)

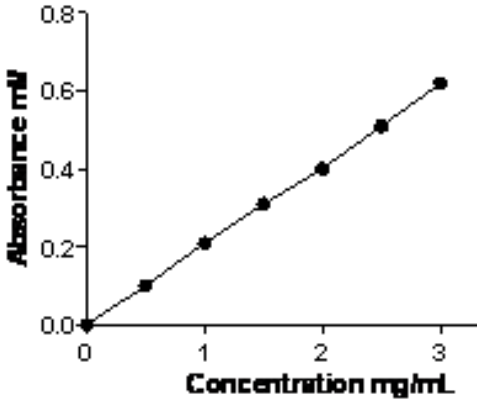
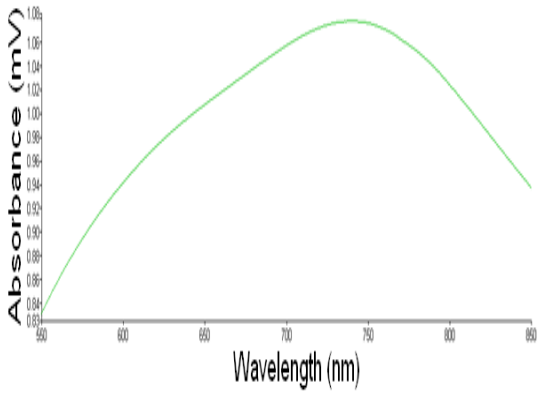


Fig 2 Absorption spectra of Biapenem

HCl (5M), CAT(0.02%), GC (0.01%)



6.1 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 3) were considered satisfactory.

6.2 Interference

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.

Tab: 3 Optical and regression characteristics of the proposed method for Biapenem

PARAMETER	VALUE
λ_{max} nm	542
Beer's law limits, µg/mL	2-12
Molar absorptivity, L/mol.cm	$1x10^{-3}$
Sandell's sensitivity µg/cm^2/0.001 absorbance unit	$7x10^{-2}$
Regression equation (Y = a + bc)	
Slope(b)	0.2050 ± 0.0021
Standard deviation of slope (Sb)	0.0057
Intercept	-0.0003 ± 0.0039
r^2	0.9994
Limit of Detection	0.006
Limit of Quantification	0.018
Standard deviation of intercept (Sa)	0.0011
Standard error of estimation (Se)	0.0027
Correlation coefficient ®	0.9998
Relative standard deviation (%)*	0.0199
% Range of error (Confidence limits)*	
Precision	
0.05 level	0.2231
0.01 level	0.3196
Accuracy	

Bulk sample	Amount found (µg)	Amount found (µg)
50	49.79	49.79
75	74.89	74.89
100	99.95	99.95

7. APPLICATION TO FORMULATION

The proposed procedure was applied for the determination of Biapenem in commercially available injections. Table 4 summarized the results.

Tab: 4 Results of analysis of injection formulations containing Biapenem

Injection	Biapenem
Company Name	Troika Pharma
Formulation	Inj
Labeled amount, mg	1000
% Recovery	99.6

8. CONCLUSION

The proposed method was found to be simple, rapid and inexpensive, hence can be used for routine analysis of Biapenem in bulk and in injection formulations.

9. ACKNOWLEDGEMENTS

We wish to thank Aurobindo labs, Hyd. for providing gifted samples of Penems; Research lab, Dept., of Engineering chemistry, AUCE(A), Visakhapatnam, India, Dept., of Analysis, GIET School of Pharmacy, Rajahmundry, India.

10. REFERENCES

1. United States Pharmacopeia Drug Quality and Information Program. 2004. A review of drug quality in Asia with focus on anti-infectives, United States Pharmacopoeia, Drug Quality and Information Program 1-46.

2. Singaram Kathirvel and Garikapati Devalarao International Chromatography Research Volume 2013 (2013), Article ID 963595, 9 pages.

3. Sean C. Sweetman, Martindale Extra Pharmacopoeia 36(1), 286, Pharmaceutical Press, 2009.

4. Venugopal V, Ramu G, Lakshmanarao P V and Rambabu C* Der Pharma Chemica, 2013, 5(3):185-189.

5. Cielecka-Piontek J, Jelińska A, Spectrochimica Acta Part A Molecular and Biomolecular Spectroscopy (Impact Factor: 1.98). 10/2010; 77(2):554-7. DOI:10.1016/j.saa.2010.06.019

6. The United States Pharmacopeia (USP 30), National Formulary (NF25), The United States Pharmacopeial Convention, Inc., Rockville 2007, pp. 2334-2336.