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RESEARCH ARTICLE

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

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ABSTRACT

A simple and cost effective spectrophotometric method was described for the determination of Imipenem 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 540 nm for Imipenem and obeys beer's law in the concentration range 0.02-0.12 mg/mL of Imipenem. The apparent molar absorptivity was $162X10^{-5}$ and sandell's sensitivity was $7x10^{-4}$. The slope is 0.2200 ± 0.0022, the intercept of the equation of the regression line is 0.0042 ± 0.0039. 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 in pharmaceutical formulations.

Keywords: *Imipenem, Chloramine - T, Gallocyanine, HCl, Spectrophotometry.*

INTRODUCTION

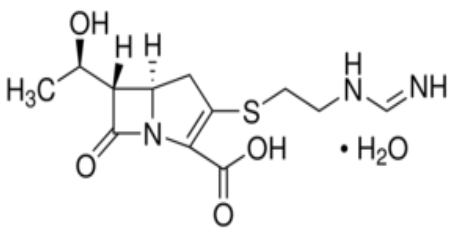
Due to counterfeiting, the drug quality has become a source of major concern worldwide, particularly in many developing countries. The most commonly counterfeited drugs are anti-infectives or antibiotics. Use of poor quality antibiotics bears serious health implications such as treatment failure, adverse 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. Imipenem[2] is a broad spectrum beta-lactam antibiotic belonging to the carbapenem class.

1.1 Drug Profile

Name : Imipenem (IMP)

Chemical Name : (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

Structure :



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Molecular formula	:	$C_{12}H_{17}N_3O_4S$
Empirical formula	:	$C_{12}H_{17}N_3O_4S \cdot H_2O$
Molecular weight	:	240.28 g/mol
Color	:	Off-white
p^{Ka}	:	3.2
Solubility	:	Soluble in water and slightly soluble in methanol
Pharmacodynamic/ Chemotherapeutic category	:	Antibacterial Agent

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

Literature survey reveals that the drugs were determined by using HPLC and some spectrophotometric methods for Imipenem[3-8]. According to literature survey there is no method reported for Imipenem with Brucine reagent by visible spectrophotometry. Hence an attempt was made to develop simple and sensitive spectrophotometric method for the estimation of the above drug in pure and in pharmaceutical formulations. The method uses the well known oxidation reaction between the reagent and Imipenem resulting in the formation of a coloured chromogen that could be measured at 540 nm for Imipenem.

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.

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 IMP were transferred into a series of 10 mL volumetric flask, to provide final concentration range of 0.02 – 0.12 µg/mL. To each flask, 1.5 mL of HCl (5M) and 2.0 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 (0.01%) was added and mixed thoroughly and the absorbance was measured after 15 min. at 540 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 Imipenem 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

In the present investigation, Imipenem 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 Imipenem 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 Imipenem (1mg/mL) and the results were observed. From the absorption spectrum it was evident that 1.5 mL of HCl solution was found optimum. Larger volumes had no significant effect on the absorbance of the colored species.

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 Imipenem

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

S. No.	Drug		Order of Addition	Absorbance	Recommended order of Addition
1.	Imipenem[a]	i	D+HCL+CAT+GC	0.185	i
		ii	D+CAT+GC+HCL	0.122	
		iii	HCL+CAT+GC+D	0.04	

[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 (0.5-2.5). It was apparent that 2.0 mL of CAT gave maximum color for Imipenem

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.

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 40 minutes thereafter.

Parameter	Range of study	Optimised condition in procedure	Remarks
λ_{max} (nm)	350-650	540	
Effect of volume of CAT required for Charge transfer complex formation (mL)	0.5-2.5	2.0	Volume above 2.0 mL gave high optical densities in blanks (>2.0), which resulted in deviations from Beers law.
Effect of volume of HCl (mL)	0.5-2.5	1.5	To speed up the reaction stage in color development, 1.5 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 temp. (^{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	40	The absorbance of the colored product decreases slowly with time after 40 min.

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

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 540 nm for Imipenem. 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 Imipenem
IMP(0.5-3mL)+HCl(1.5mL)+ CAT(2mL)+GC(10mL)

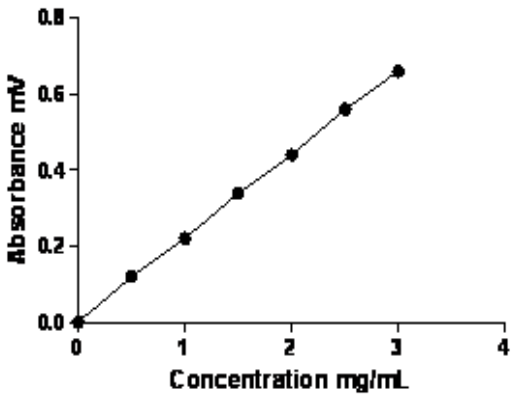
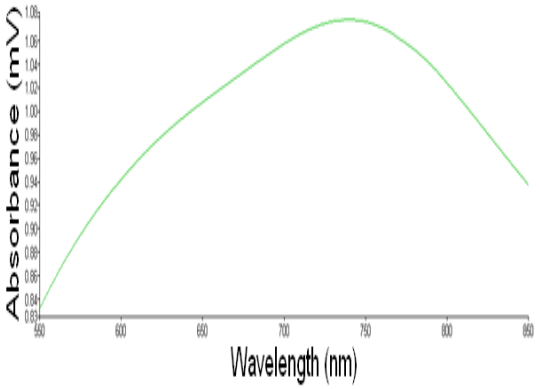


Fig. 2: Absorption spectra of Imipenem
HCl (5M), CAT (0.02%), GC (0.01%)



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 Imipenem

PARAMETER	VALUE	
λ_{max} nm	540	
Beer's law limits, µg/mL	0.02-0.12	
Molar absorptivity, L/mol.cm	$162X10^{-5}$	
Sandell's sensitivity µg/cm^2/0.001 absorbance unit	$7x10^{-4}$	
Regression equation (Y = a + bc)		
Slope(b)	0.2200 ± 0.0022	
Standard deviation of slope (Sb)	0.0058	
Intercept	0.0042 ± 0.0039	
r^2	0.9995	
Limit of Detection	0.00652	
Limit of Quantification	0.0195	
Standard deviation of intercept (Sa)	0.0012	
Standard error of estimation (Se)	0.0029	
Correlation coefficient ®	0.9999	
Relative standard deviation (%)*	0.0179	
% 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	49.78	49.78
75	74.89	74.89
100	99.74	99.74

7. APPLICATION TO FORMULATION

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

Tab: 4 Results of analysis of injection formulations containing Imipenem

Injection	Imipenem
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 Imipenem in bulk and in injection formulations.

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