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RESEARCH ARTICLE

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

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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 haematoxylin and chloramine - T in presence of a buffer potassium hydrogen phosphate and disodium hydrogen phosphate having pH 7.0. 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 555 nm for Imipenem and obeys beer's law in the concentration range 2-12 µg/mL of Imipenem. The apparent molar absorptivity was $22x10^{-4}$ and sandell's sensitivity was $7x10^{-2}$. The slope is 0.0276 ± 0.0012, the intercept of the equation of the regression line is 0.0047 ± 0.0022. 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, Haematoxylin, Chloramine - T, Buffer, 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 (United States Pharmacopeia Drug Quality and Information Program. 2004). Among antibiotics, penems are much recently introduced, widely prescribed and costlier. Therefore, incentive to produce their counterfeits because of profit margin increases considerably. Imipenem (Sean et al., 2009) is a broad spectrum beta-lactam antibiotic belonging to the carbapenem class.

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
Molecular : $C_{12}H_{17}N_3O_4S$

Structure :

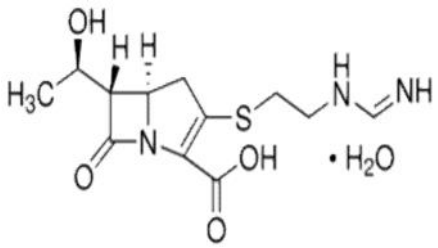

Fig: 2.2.1

Empirical formula	:	$C_{12}H_{17}N_3O_4S•H_2O$
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 (Forsyth and Ip, 1994; Gravallese et al., 1984; Myers and Blumer, 1984; Garcia- Capdevila et al., 1997; Irene et al., 2006; Chaudhary et al., 2010). According to literature survey there is no method reported for Imipenem with Haematoxylin and Chlormaine - T 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 Charge transfer complex formation between the reagent and hetero sulphur present in Imipenem resulting in the formation of a coloured chromogen that could be measured at 555 nm for Imipenem.

Experimental

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. All chemicals used were of analytical reagent grade and double distilled water was used throughout.

Preparation of reagents:

Haematoxylin (HAE) (Aldrich, 0.2%, 6.605×10^{-3} M)	:	Prepared by dissolving 200 mg of Haematoxylin in 100 mL of methanol.
Chloramine-T (CAT) (BDH 0.4%, 1.42×10^{-2} M)	:	Prepared by dissolving 400 mg of Chloramine-T in 100 mL of distilled water.
Buffer solution (pH=7.0)	:	Prepared by mixing 390 mL of 0.067 M potassium hydrogen phosphate (BDH) and 610 mL of 0.067M disodium hydrogen phosphate (BDH) and the pH of the solution was adjusted to 7.0.

General procedure

To a series of 25 mL graduated test tubes, 1 mL each of HAE (0.2%) and CAT (0.4%), 15 mL of buffer (pH 7.0) solutions were added successively. The mixture was set aside for 20 min. then added aliquots of drug with in Beer's law limits (0.5-3.0 mL), to provide final concentration range of 2 – 12 µg/mL and kept in a water bath at 70^0C for 20 min. the test tubes were removed from the water bath, cooled to the room temperature. The contents in tube were diluted to 25 mL with distilled water and the absorbance read at 555 nm within the stability period (immediate 40 min). The amount of drug was deduced from its standard calibration curve.

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.

Results and Discussion

IMP possesses different functional moieties such as, secondary amine, β-lactum ring in which there is a carboxylic acid, Tertiary nitrogen, Vulnerable oxidising centers, Hetero Sulphur, Double bonds and Active methylene group. An attempt has been made to indicate the nature of coloured species formed in the proposed method for the

determination of Imipenem tentatively based on analogy.

This method appears to be due to the formation of charge-transfer complex involving in situ formed haematin (oxidation product of haematoxylin with CAT) which is electron acceptor due to the presence of enolic form of o-quinone moiety and penems electron donor (due to the existence of hetero sulphur with lone pair of electrons shown in Scheme) as in the case of quinone and organic sulphur compounds.

Optimization of the conditions on absorption spectrum of the reaction product

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

Selection of reaction medium

To find a suitable medium for the reaction, a buffer of pH-7 containing potassium hydrogen phosphate and disodium hydrogen phosphate is used to a constant concentration of Imipenem (1mg/mL) and the results were observed. From the absorption spectrum it was evident that, 15.0 mL of Buffer was found necessary to speed up the reaction and for maximum color development. Larger volumes had no significant 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 (iii) is recommended for Imipenem.

Effect of Haematoxylin concentration

Several experiments were carried out to study the influence of haematoxylin concentration on the color development by keeping the concentration of drug and Chloramine - T to constant and changing the reagent concentration (0.5-2.0). It was apparent that 1.0 mL of haematoxylin gave maximum color for Imipenem and volume above 1.0 mL gave high optical densities in blanks (>1.0), which resulted in deviations from Beers law.

Effect of Chloramine - T concentration

Several experiments were carried out to study the influence of Chloramine - T concentration on the color development by keeping the concentration of drug, haematoxylin to constant and changing Chloramine - T concentration (0.5-2.0). It was apparent that 1.0 mL of reagent gave maximum color for Imipenem.

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 450-650 nm against a reagent blank. The reaction product showed absorption maximum at 555 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 2.

Sensitivity, accuracy and precision

Sandell's sensitivity, molar absorptivity, precision and accuracy were found by performing eight replicate determinations containing $3/4^{th}$ 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.

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.

Scheme1. Existence of hetero sulphur with lone pair of electrons

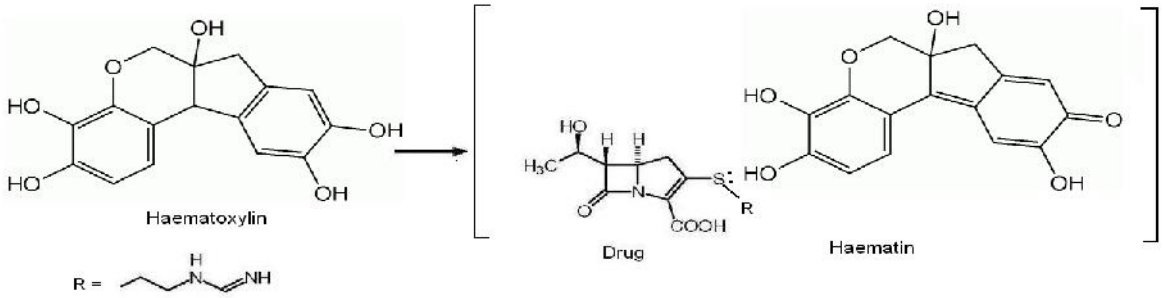

Table 1. Effect of order of addition of reactants on color development.
[a]*For 40 µg/mL of Drug samples*

S.No.	Drug	Order of Addition	Absorbance	Recommended order of Addition
1.	Imipenem[a]	i D+HAE+CAT+Buffer	0.124	iii
		ii D+CAT+HAE+Buffer	0.04	
		iii HAE+CAT+Buffer+D	0.199	

Table 2. Results of method optimisation for Imipenem –- HAE & CAT

Parameter	Range of study	Optimised condition in procedure	Remarks
λ_{max} (nm)	450-650	555	
Effect of volume of HAE required for Charge transfer complex formation (mL)	0.5-2.0	1.0	Volume above 1.0 mL gave high optical densities in blanks (>1.0), which resulted in deviations from Beers law.
Effect of volume of CAT (mL)	0.5-2.0	1.0	To speed up the reaction, 1.0 mL of CAT was found necessary for maximum color development.
Effect of volume of Buffer (mL)	15	15	To speed up the reaction, 15.0 mL of Buffer was found necessary for maximum color development.
Effect of reaction time (min)	15-30	15	The minimum time required for complete the reaction 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)	1-3	2	A minimum amount of time, i.e., 1 min was necessary for undergoing charge transfer complex formation and beyond 3 min results in low sensitivity.

Figure 1. Calibration graph of Imipenem

HAE(1mL)+CAT(1mL)+buffer(15mL)+IMP(0.5 - 3mL)

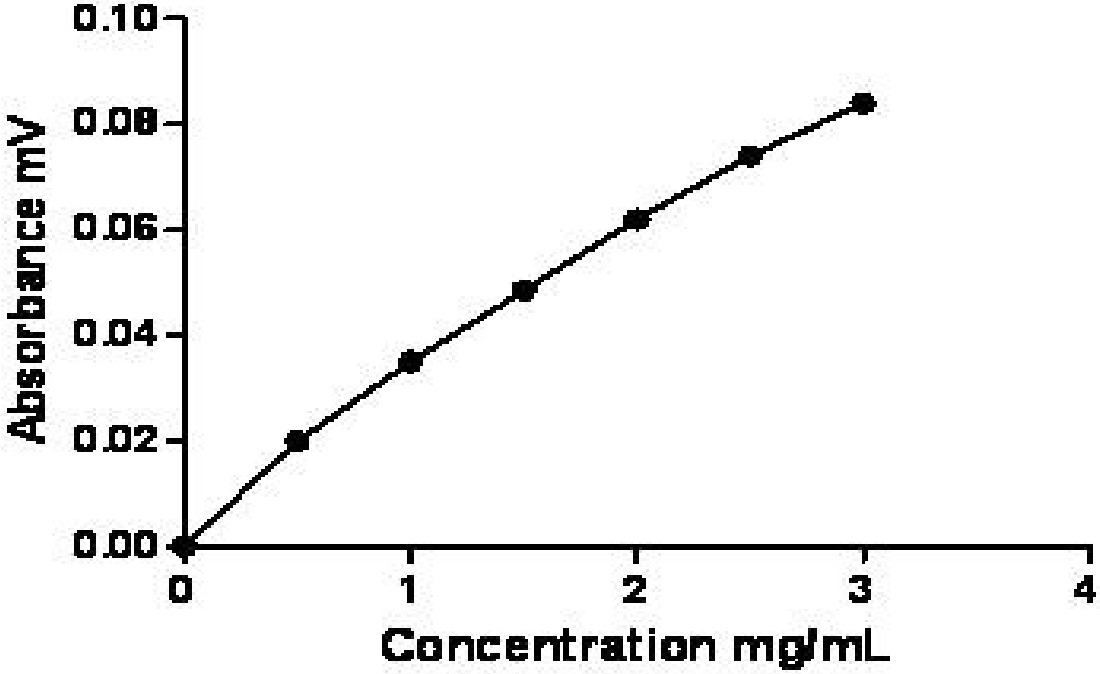

Figure 2. Absorption spectra of Imipenem

HAE (0.2%), CAT (0.4%), Buffer (pH 7.0)

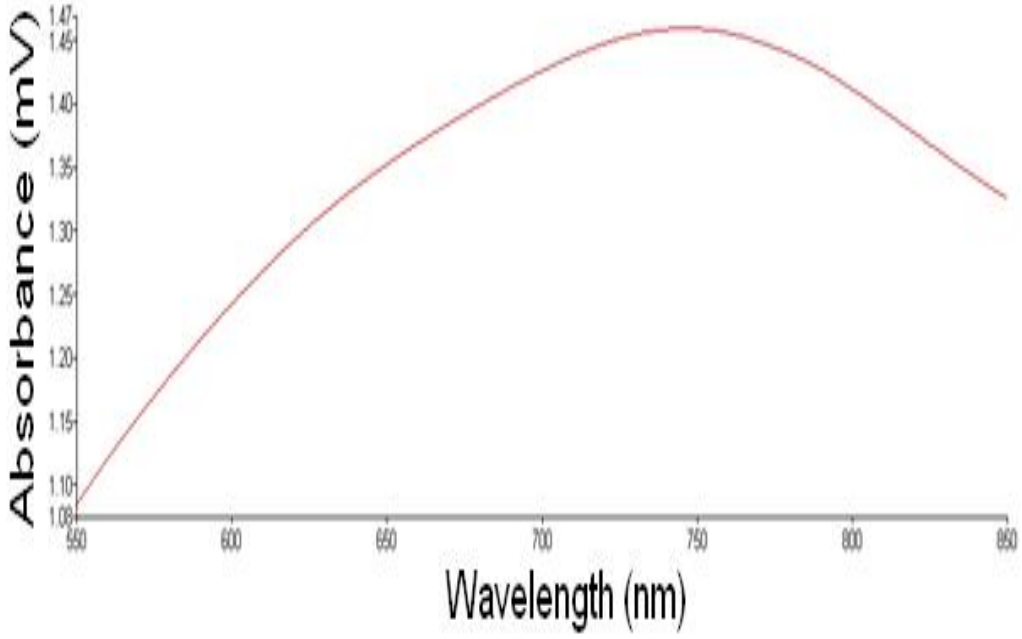

Table 3. Optical and regression characteristics of the proposed method for Imipenem.

λ_{max} nm	555	
Beer's law limits, µg/mL	2-12	
Molar absorptivity, L/mol.cm	$22x10^{-4}$	
Sandell's sensitivity µg/cm^2/0.001 absorbance unit	$7x10^{-2}$	
Regression equation (Y = a + bc)		
Slope(b)	0.0276 ± 0.0012	
Standard deviation of slope (Sb)	0.003287	
Intercept	0.0047 ± 0.0022	
r^2	0.9900	
Limit of Detection	0.0067	
Limit of Quantification	0.0197	
Standard deviation of intercept (Sa)	0.0026	
Standard error of estimation (Se)	0.0029	
Correlation coefficient ®	0.9997	
Relative standard deviation (%)*	0.0163	
% Range of error (Confidence limits)*		
Precision		
0.05 level	0.2214	
0.01 level	0.3124	
Accuracy		
Bulk sample	Amount found (µg)	Amount found (µg)
50	49.79	49.79
75	74.88	74.88
100	99.98	99.98

Table 4. Results of analysis of injection formulations containing Imipenem

Injection	Imipenem
Company Name	Troika Pharma
Formulation	Inj
Labeled amount, mg	1000
% Recovery	99.89

Application to formulation

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

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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