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SPECTROPHOTOMETRIC DETERMINATION OF IMIPENEM IN BULK AND INJECTION FORMULATIONS BY *P* - CHLORANILIC ACID

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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 chloranilic acid. 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 518 nm for Imipenem and obeys beer's law in the concentration range 3-50 µg/mL of Imipenem. The apparent molar absorptivity was $115x10^{-5}$ and sandell's sensitivity was $175x10^{-3}$. The slope is 0.1703 ± 0.0046, the intercept of the equation of the regression line is -0.0155 ± 0.0084. 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, Chloranilic acid, Chromogen, Molar absorptivity, Sandell's sensitivity 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-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.

Imipenem[2] 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

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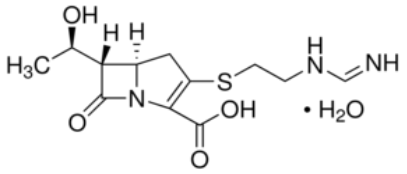
Structure	:	 **Fig: 2.2.1**
Molecular formula	:	$C_{12}H_{17}N_3O_4S$
Empirical formula	:	$C_{12}H_{17}N_3O_4S{\bullet}H_2O$
Molecular weight	:	240.28 g/mol
Color	:	Off-white
p^{Ka}	:	3.2
Solubility	:	Soluble in water and slightly soluble in methanol
Pharmacodynamic / Chemotherapeuticcategory	:	Antibacterial 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 Chloranilic acid 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 518 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:

CA solution (Sd-fine;0.1%,7.25X10^{-3}M)	:	Prepared by dissolving 100 mg of p-chloranilic acid initially in 20 mL of isopropyl alcohol followed by dilution to 100 mL with chloroform.

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 5 – 30 µg/mL. To each flask, 2.0 mL of CA (0.1%) was added and kept aside for 30 min at lab temperature. The volume in each flask was made up to the mark with chloroform. The absorbance of the colored species was measure at 518 nm against a 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.

Procedure for Injections

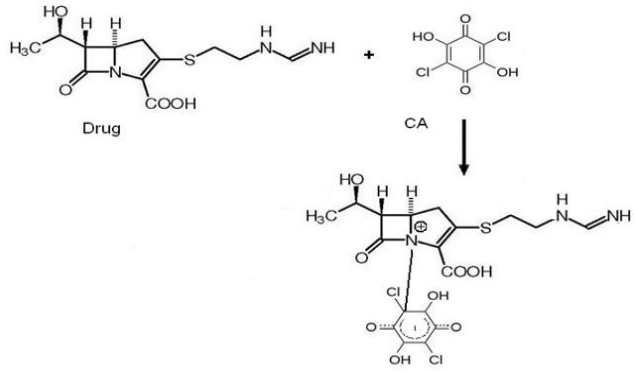
An amount of powder equivalent to 100 mg of Imipenem was weighed into a 100 mL volumetric flask, 50 mL of distilled water was added and shaken thoroughly for about 10 min, then the volume was made up to the mark with the distilled water, mixed well and filtered. Further dilutions were made and the assay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

In the present investigation, the coloured species formation in the method (chloranil, electron acceptor) for the assay of Imipenem (electron donor) appears to be the formation of radical ion through analogy.



Optimization of the conditions on absorption spectrum of the reaction product

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

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.

Effect of Chloranilic acid concentration

Several experiments were carried out to study the influence of chloranilic acid concentration on the color development by keeping the concentration of drug to constant and changing the reagent concentration (0.5-2.5). It was apparent that 2.0 mL of Chloranilic acid gave maximum color for Imipenem and volume above 2.0 mL gave high optical densities in blanks (>2.0), which resulted in deviations from Beers law.

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.

Absorption spectrum and calibration graph

Absorption spectrum of the colored complex was scanned at 400-650 nm against a reagent blank. The reaction product showed absorption maximum at 518 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.

Application to formulation

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

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 + CA	0.199	
1.	Imipenem[a]	ii	CA + D	0.06	i

[a]*For 40 µg/mL of Drug samples*

Table 2. Results of Method Optimisation For Imipenem –- P-Chloranilic Acid

Parameter	Range of study	Optimised condition in procedure	Remarks
λ_{max} (nm)	400-650	518	
Effect of volume of CA 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 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)	20-40	32 ± 2 Lab. Temp	At low temperatures (<30^0C) the reaction time was found to be more and at high temperatures (>34^0C) no added advantage was found.
Standing time (min)	1-3	2	A minimum amount of time, i.e., 2 min was necessary for Chloranilic acid to undergo Charge transfer complex formation and beyond 3min 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.

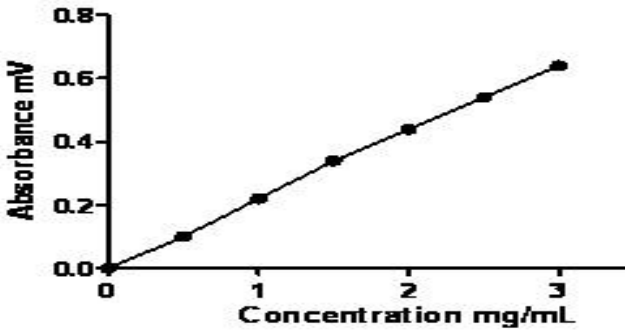
Table 3. Optical and regression characteristics of the proposed method for Imipenem

Parameter	Value	
λ_{max} nm	518	
Beer's law limits, µg/mL	5 – 30	
Molar absorptivity, L/mol.cm	$115x10^{-5}$	
Sandell's sensitivity µg/cm^2/0.001 absorbance unit	$175x10^{-3}$	
Regression equation (Y = a + bc)		
Slope(b)	0.1703 ± 0.0046	
Standard deviation of slope (Sb)	0.01237	
Intercept	-0.0155 ± 0.0084	
r^2	0.9962	
Limit of Detection	0.0067	
Limit of Quantification	0.0197	
Standard deviation of intercept (Sa)	0.0014	
Standard error of estimation (Se)	0.0025	
Correlation coefficient ®	0.9994	
Relative standard deviation (%)*	0.0261	
% Range of error (Confidence limits)*		
Precision		
0.05 level	0.2135	
0.01 level	0.3218	
Accuracy		
Bulk sample	**Amount found (µg)**	**Amount found (µg)**
50	49.78	49.78
75	74.96	74.96
100	99.79	99.79

Table 4. Results of analysis of injection formulations containing Imipenem

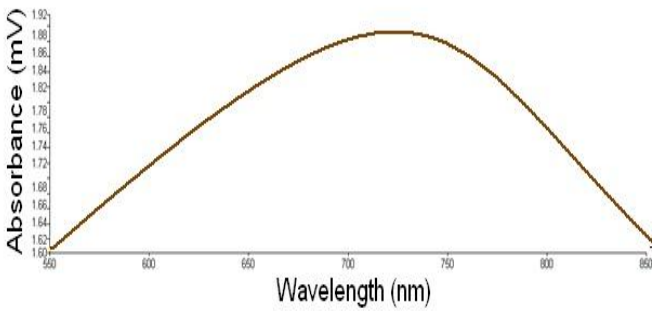
Injection	Imipenem
Company Name	Troika Pharma
Formulation	Inj
Labeled amount, mg	1000
% Recovery	99.89

Fig 1. Calibration graph of Imipenem



IMP(0.5-3mL)+buffer(4mL)+NH(2mL) +AA(1mL)

Fig 2. Absorption spectra of Imipenem



pH 5.0 Buffer, NH (0.1%), AA (0.1%)

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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