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SPECTROPHOTOMETRIC DETERMINATION OF IMIPENEM IN BULK AND INJECTION FORMULATIONS BY NINHYDRIN AND ASCORBIC 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 ninhydrin and ascorbic acid in presence of a buffer having pH 5.0, prepared by a mixture of citric acid and NaOH solutions 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 560 nm for IMP and obeys beer's law in the concentration range 2-12 µg/mL of IMP. The apparent molar absorptivity was $157x10^{-6}$ and sandell's sensitivity was $7x10^{-2}$. The slope is 0.2157 ± 0.0035, the intercept of the equation of the regression line is 0.0021 ± 0.0064. 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 IMP in pharmaceutical formulations.

Keywords: Imipenem, ninhydrin, Ascorbic acid, 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 [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.

IMP 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 IMP [3-8]. According to literature survey there is no method reported for IMP with ninhydrin and ascorbic 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 pharmaceutical formulations. The method uses an intermolecular oxidation and reduction of the ninhydrin (NH) in the presence of ascorbic acid (AA) between the reagent and amino group present in IMP

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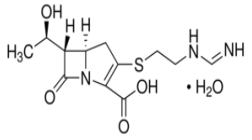
resulting in the formation of a coloured chromogen that could be measured at 560 nm for IMP.

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	:	 **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 Chemotherapeutic category	:	Antibacterial Agent

MATERIALS AND METHODS

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:

Ninhydrinsolution (BDH; 0.1%, $5.605X10^{-5}M$)	:	Prepared by dissolving 1 g of Ninhydrin in 100 mL of acetone.
Ascorbic acid (BDH; 0.1%, 5.678×10^{-3} M)	:	Prepared by dissolving 100 mg of Ascorbic acid in 100 mL of distilled water.
Buffer solution	:	Prepared by diluting a mixture of 200 mL of 0.5 M citric acid and 200 mL of 1.0 M NaOH solutions to 500 mL with distilled water and the pH was adjusted to 5.0

General procedure

Into a series of 25 mL of volumetric flasks, aliquots of working standard solution (0.5 to 3.0 mL) of IMP were transferred to provide final concentration range of 2 – 12 µg/mL were delivered. To each flask 4.0 mL of pH 5.0 buffer solution 2.0 mL of NH solution (0.1%) and 1.0 mL of AA (0.1%) were added and the flask is heated on a water bath for 15 min. and then cooled in an ice bath and the total volume of was made up to the mark with distilled water. The absorbance was measured immediately at 560 nm against a similar reagent blank. The colored species was stable for 1 h. The amount of IMP present in sample solution was calculated from its calibration graph.

Procedure for Injections

An amount of powder equivalent to 100 mg of IMP 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.

Ammonium salts, dilute ammonia solutions, and some amines give a blue colour under certain conditions, apparently because of an intermolecular oxidation and reduction of the ninhydrin(NH) in the presence of ascorbic acid (AA). In the present investigation, the selected penem possesses amino group in their moiety, when heated with ninhydrin in the presence of AA forms a blue violet colour product. The reaction pathway can be represented in Scheme.

Optimization of the conditions on absorption spectrum of the reaction product

The condition under which the reaction of IMP with ninhydrin and ascorbic acid 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-5 prepared by diluting a mixture of 200 mL of 0.5 M citric acid and 200 mL of 1.0 M NaOH solutions to 500 mL with distilled water is used to a constant concentration of IMP (1mg/mL) and the results were observed. From the absorption spectrum it was evident that, addition of 4.0 mL of Buffer is necessary for

proceeding 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 (i) is recommended for IMP.

Effect of Ninhydrin concentration

Several experiments were carried out to study the influence of ninhydrin concentration on the color development by keeping the concentration of drug and ascorbic acid to constant and changing the reagent concentration (0.5-3.0). It was apparent that 2.0 mL of Ninhydrin gave maximum color for IMP and volume of Ninhydrin above 2.0 mL gave high optical densities in blanks (>2.0), which resulted in deviations from Beers law.

Effect of Ascorbic acid concentration

Several experiments were carried out to study the influence of ascorbic acid concentration on the color development by keeping the concentration of drug, ninhydrin to constant and changing ascorbic acid concentration (0.5-3.0). It was apparent that 1.0 mL of reagent gave maximum color for Imipenem and hence, it is observed that, to speed up the condensation stage in color development, 1.0 mL of ascorbic acid was found necessary for maximum color development.

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 400-600 nm against a reagent blank. The reaction product showed absorption maximum at 560 nm for IMP. 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.

Table 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 + Buffer + NH + AA	0.189	
		ii	D + AA + NH + Buffer	0.04	I
		iii	NH+AA+Buffer + D	0.121	

[a]*For 40 µg/mL of Drug samples*

Table 2. Results of Method Optimisation for Imipenem –- NH & AA

Parameter	Range of study	Optimized condition in procedure	Remarks
λ_{max} (nm)	400-600	560	
Effect of volume of NH required for Condensation (mL)	0.5-3.0	2.0	Volume of NH above 2.0 mL gave high optical densities in blanks (>2.0), which resulted in deviations from Beers law.
Effect of volume of AA (mL)	0.5-3.0	1.0	To speed up the condensation stage in color development, 1.0 mL of AA was found necessary for maximum color development.
Effect of volume of Buffer (mL)	4.0	4.0	Addition of 4.0 mL of Buffer is necessary for proceeding the reaction
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 condensation	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 condensation and beyond 3 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.

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

Parameter	Value	
λ_{max} nm	560	
Beer's law limits, µg/mL	2-12	
Molar absorptivity, L/mol.cm	$157x10^{-6}$	
Sandell's sensitivity µg/cm^2/0.001 absorbance unit	$7x10^{-2}$	
Regression equation (Y = a + bc)		
Slope(b)	0.2157 ± 0.0035	
Standard deviation of slope (Sb)	0.009411	
Intercept	0.0021 ± 0.0064	
r^2	0.9986	
Limit of Detection	0.0065	
Limit of Quantification	0.0195	
Standard deviation of intercept (Sa)	0.0024	
Standard error of estimation (Se)	0.0121	
Correlation coefficient ®	0.9998	
Relative standard deviation (%)*	0.0414	
% 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	49.84	49.84
75	74.98	74.98
100	99.68	99.68

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)

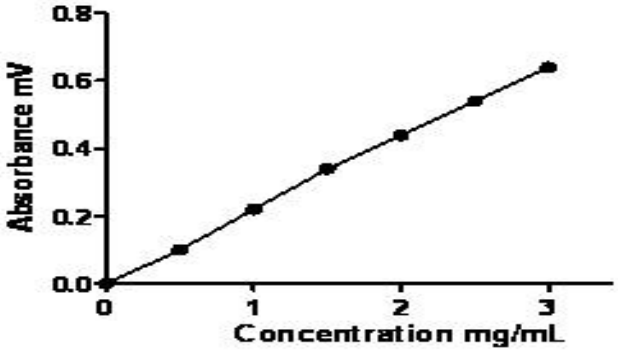
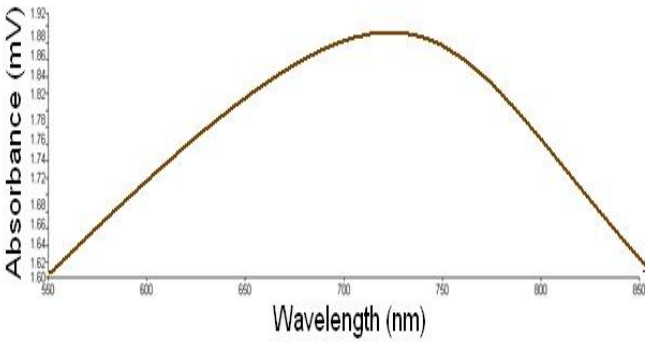


Fig 2 Absorption spectra of Imipenem pH 5.0 Buffer, NH (0.1%), AA (0.1%)



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.

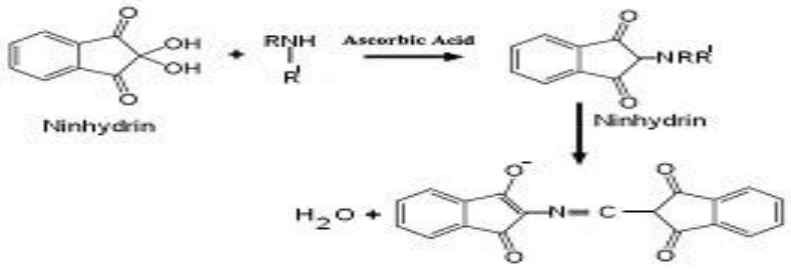
Interference

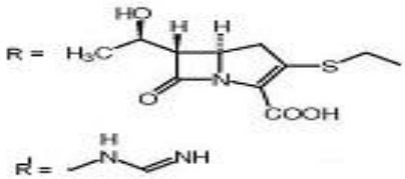
These substances are seldom present in the reagents and used in the pharmaceutical formulations. Hence, the method is devoid of error due to above substances.

Application to formulation

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

Scheme





CONCLUSION

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

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