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Spectrophotometric determination of imipenem in bulk and injection formulations by brucine

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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 brucine and sodium periodate 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 520 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 $61X10^{-5}$ and sandell's sensitivity was $7x10^{-4}$. The slope is 0.0230 ± 0.0008, the intercept of the equation of the regression line is 0.0230 ± 0.0008. 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, Brucine, Sodium periodate, 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 :

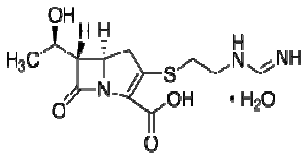


Fig: 2.2.1

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

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 oxidative coupling reaction between the reagent and Imipenem resulting in the formation of a coloured chromogen that could be measured at 520 nm for Imipenem.

MATERIALS AND METHODS

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. Brucine supplied by Loba chemicals ltd., India, was used by diluting 200.0 mg to 100 mL with distilled water. $NaIO_4$ supplied by BDH chemicals ltd., India, was used by dissolving 200.0 mg of Sodium meta periodate to 100 mL with distilled water. H_2SO_4 (18 M) supplied by Qualigens was used as it is. 10 mg/mL stock reference solution was freshly prepared from pure sample of Imipenem by dissolving 100 mg in 100 mL of double distilled water.

2.2 General procedure

Method

Different aliquots of working standard solution (0.5 to 3.0 mL) of Imipenem 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, 2.5 mL of BCN (0.2%), 2.5 mL of $NaIO_4$ (0.2%) and 2.0 mL of H_2SO_4 were successively added and kept aside for 5 min. The solutions were made up to volume with distilled water. The absorbance of each solution was measured at 520 nm against the 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.

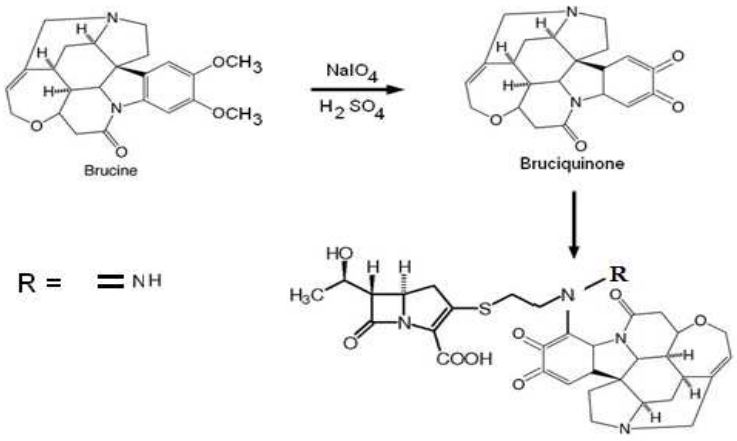
2.3 Procedure for Injections

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.

RESULTS AND DISCUSSION

The dimethoxy benzene nucleus of brucine is attacked by IO_4^- with the formation of O-quinone (bruciquinone) which in turn undergoes nucleophilic attack on the most electron rich position of the coupler i.e., proton bearing amino group (secondary amine in Imipenem), to give1-mono substituted bruciquinone derivative. In the present study, Imipenem was successfully determined by this method. The reactions of the Imipenem with brucine in the presence of IO_4^- are described in the Scheme.



Scheme

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

The condition under which the reaction of Imipenem with Brucine 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 acidic mediums have been used. The best results were obtained when H_2SO_4 was used. In order to determine the optimum concentration of H_2SO_4, different volumes of H_2SO_4 solution (0.5 – 3.0 mL) were used to a constant concentration of Imipenem (1mg/mL) and the results were observed. From the absorption spectrum it was evident that 2.0 mL of H_2SO_4 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.

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 + BCN + $NaIO_4$	0.189	i
		ii	D + $NaIO_4$ + BCN	0.04	
		iii	BCN + $NaIO_4$ + D	0.145	

[a]*For 40 µg/mL of Drug samples*

4.3 Effect of Brucine concentration

Several experiments were carried out to study the influence of Brucine concentration on the color development by keeping the concentration of drug and Sodium meta periodate to constant and changing reagent concentration. It was apparent that 2.5 mL of Brucine gave maximum color for Imipenem. The results of the method optimization have given in Table. 2

Tab: 2 RESULTS OF METHOD OPTIMISATION FOR IMIPENEM – BRUCINE

Parameter	Range of study	Optimised condition in procedure	Remarks
λ_{max} (nm)	400-600	520	
Effect of volume of BCN required for oxidative coupling (mL)	0.5-3.0	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 H_2SO_4 (mL)	0.5-3.0	2.0	To speed up the reaction in color development, 2.0 mL of H_2SO_4 (18M) was found necessary for maximum color development.
Effect of volume of $NaIO_4$ (mL)	0.5-3.0	2.5	Addition of 2.5 mL of $NaIO_4$ was found optimum for oxidizing the drug.
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 oxidative coupling	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., 2 min was necessary for undergoing oxidative coupling and beyond 3 min results in low sensitivity.
Stability period afterfinal dilution (min)	5-40	40	The absorbance of the colored product decreases slowly with time after 40 min.

4.4 Effect of Sodium meta periodate concentration

Several experiments were carried out to study the influence of Sodium meta periodate concentration on the color development by keeping the concentration of drug, BCN to constant and changing Sodium meta periodate concentration. It was apparent that 2.5 mL of reagent gave maximum color for Imipenem.

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 450-600 nm against a reagent blank. The reaction product showed absorption maximum at 520 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 - 3 mL) + BCN (2.5mL) + $NaIO_4$ (2mL)

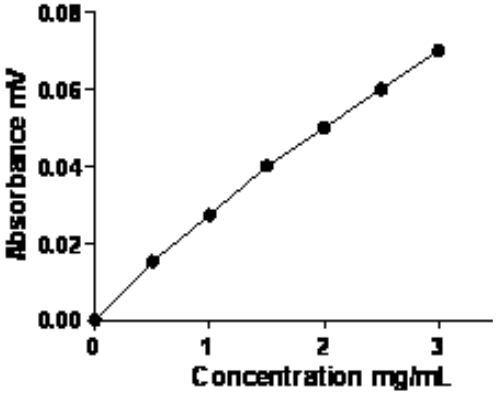
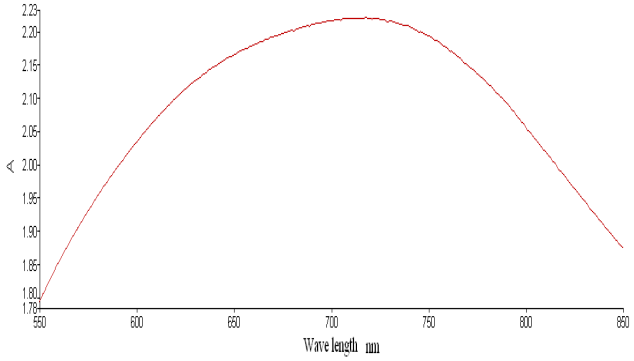


Fig 2 Absorption spectra of Imipenem



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.

PARA,ETER	VALUE	
λ_{max} nm	520	
Beer's law limits, µg/mL	0.02-0.12	
Molar absorptivity, L/mol.cm	$61X10^{-5}$	
Sandell's sensitivity µg/cm^2/0.001 absorbance unit	$7x10^{-4}$	
Regression equation (Y = a + bc)		
Slope(b)	0.0230 ± 0.0008	
Standard deviation of slope (Sb)	0.0021	
Intercept	0.0029 ± 0.0014	
r^2	0.9939	
Limit of Detection	0.00667	
Limit of Quantification	0.0998	
Standard deviation of intercept (Sa)	0.0023	
Standard error of estimation (Se)	0.0121	
Correlation coefficient ®	0.9998	
Relative standard deviation (%)*	0.0480	
% Range of error (Confidence limits)*		
Precision		
0.05 level	0.2231	
0.01 level	0.3196	
Accuracy		
Bulk sample	Amount found (µg)	% error
50	49.54	0.92
75	74.95	0.06
100	99.54	0.46

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	99.8

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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