

# Spectrophotometric Determination of Imipenem in Bulk and Injection Formulations by 1,2 Naphtho Quinone 4-Sulphonic Acid (NQS) Reagent

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## ABSTRACT

A simple and cost effective spectrophotometric method is described for the determination of Imipenem (IMP) in pure and pharmaceutical formulations. The method is based on the formation of dark yellow colored chromogen when the drug reacts with 1,2 naphtho quinone 4-sulphonic acid sodium salt (NQS) reagent in alkalline medium. The method involves the addition of excess NQS of known concentration in the presence of 2.0 mL NaOH and the unreacted NQS was determined by the measurement of the  $\lambda_{\max}$  449 nm, which was found to be the most suitable of several tests. This method was applied for the determination of drug contents in pharmaceutical formulations and enabled the determination of the drug in microgram quantities (0.5 to 3.0 mL). No interference is observed from excipients and the validity of the method was tested against reference method. The colored species has an absorption maximum at 449 nm for IMP (Method A) and obeys beer's law in the concentration range 0.02 – 0.12 mg/mL of IMP. The apparent molar absorptivity is 0.0190 and sandell's sensitivity is  $7 \times 10^{-4}$ . The slope is  $0.2284 \pm 0.0118$  and intercept of the equation of the regression line is  $0.04971 \pm 0.02144$ . 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, 1,2 naphthoquinone 4-sulphonic acid sodium salt (NQS), Spectrophotometry.

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## 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<sup>1</sup>. Among antibiotics, penems are much recently introduced, widely prescribed and costlier. Therefore, incentive to produce their counterfeits because of profit margin increases considerably.

Imipenem<sup>2</sup> is a broad spectrum beta-lactam antibiotic belonging to the carbapenem class. Chemically it is (5*R*, 6*S*)-6-[(1*R*)-1-hydroxyethyl]-3-({2-[(imino-methyl) amino]ethyl}thio)-7-oxo-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylic acid.

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.

Literature survey reveals that the drugs were determined by using HPLC and some spectrophotometric methods for Imipenem<sup>3-8</sup>. 1,2-naphthoquinone-4-sulphonic acid sodium salt (NQS) has been used as a chromogenic reagent for the spectrophotometric determination of many pharmaceutical amines<sup>9-13</sup>. However, the reaction between NQS and IMP has not been investigated so far. The present study describes the evaluation of NQS as a chromogenic reagent in the development of simple and rapid spectrophotometric method for the determination of IMP in its injection formulation. According to literature survey there is no method reported for IMP with NQS reagent by visible spectrophotometry. Hence an attempt was made to develop simple and sensitive spectrophotometric

method for the estimation of IMP in pure drug and in pharmaceutical formulations. The method uses the well known nucleophilic displacement reaction involving NQS reagent and IMP resulting in the formation of a dark yellow colored chromogen that could be measured at 449 nm.

## EXPERIMENTAL

### Perkin Elmer Uv-Visible Spectrophotometer

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 are of analytical reagent grade and double distilled water was used throughout. NQS supplied by SD Fine chemicals Ltd., India, was used by diluting 500.0 mg to 100 mL with distilled water. NaOH supplied by SD Fine chemicals Ltd., India, was used by diluting 20 gm in 100 mL distilled water. 10 mg/mL stock reference solution was freshly prepared from pure sample of IMP by dissolving 100 mg in 100 mL of double distilled water.

### General Procedure

Into 10 ml volumetric flask, different aliquots of working standard solution (0.5 to 3.0 mL) of IMP were transferred to provide final concentration range of 0.02 – 0.12 mg/mL. To each flask, 2.0 mL of NaOH, 1.5 mL of NQS were successively added and kept aside for 5 minutes. The solutions were made up to volume with distilled water. The absorbance of each solution was measured at 449 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.

#### Procedure for Injections

An amount of powder equivalent to 100 mg of IMP is 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

### 1,2 naphtho quinone 4-sulphonic acid sodium salt (NQS)

Molecular Formula:  $C_{10}H_5NaO_5S$

Molecular Weight: 260.19

IUPAC Name: sodium 3,4-dioxo-naphthalene-1-sulfonate.

In developing the method, a systematic study of the effects of various relevant parameters in the concerned were under taken by varying one parameter at a time and controlling all other parameters to get maximum color development, minimum blank color, reproducibility and reasonable period of stability of final courted species formed.

### Nature of Coloured Species

The reaction of 1,2-naphthaquinone-4-sulfonic acid (NQS) with aromatic amines was discovered by Boniger as far back as 1894. The colored species formed from IMP in this method can be explained based on the analogy of previous reports<sup>[14-15]</sup>. As IMP possesses amino groups, it involves in yielding coloured produced by nucleophilic displacement of the sulfonic acid group of 1,2-naphthaquinone-4-sulfonic acid in alkaline conditions.

## OPTIMIZATION OF CONDITIONS ON ABSORPTION SPECTRUM OF THE REACTION PRODUCT

The condition under which the reaction of IMP with NQS fulfills the essential requirements was investigated. All conditions studied were optimized at room temperature ( $32 \pm 2^\circ\text{C}$ ).

### Selection of Reaction Medium

To generate the nucleophile from IMP and activate the nucleophilic substitution reaction, alkaline medium was necessary. Different inorganic bases were tested, sodium hydroxide, disodium hydrogen phosphate, and sodium bicarbonate, all prepared as aqueous solution of a concentration range of  $1-25 \times 10^{-3}\text{M}$ . Best results were obtained in case of sodium hydroxide where with other bases either precipitation of white colloid occurred upon diluting the reaction solution with organic solvent, high blank readings, non reproducible results, and/or weak sensitivity were observed. In order to determine the optimum concentration of Sodium hydroxide, different volumes of 5.0M Sodium hydroxide solution (0.5 – 3.0 mL) were used to a constant concentration of IMP (1mg/mL) and the results were observed. From the absorption spectrum it was evident that 1.5 mL of 5.0M Sodium hydroxide solution was found optimum. Larger volumes had no significant effect on the absorbance of the colored species. This was possibly due to the fact that the -NH group of IMP exists in the form of hydrochloride amine salt, thus, it loses the nucleophilic substitution capability. This was attributed probably to the increase in the amount of hydroxide ion that holds back the condensation reaction between IMP and NQS.

### 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.

#### Effect of NQS Concentration

Several experiments were carried out to study the influence of NQS concentration on the color development by keeping the concentration of drug and Sodium hydroxide to constant and changing reagent concentration (0.5 – 3.0 mL). It was apparent that 1.5 mL of NQS gave maximum color.

#### 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 450-850 nm against a reagent blank. The reaction product showed absorption maximum at 449 nm for IMP. Calibration graph was obtained according to the above general procedure. The linearity replicates for six different concentrations of IMP were 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<sup>th</sup> of the amount of upper Beer's law limits. The measured standard deviation (S.D), relative standard deviation (RSD), and confidence limits (Table 2) 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 procedures were applied for the determination of IMP in commercially available injections. Table 3 summarized the results.

#### CONCLUSION

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

#### ACKNOWLEDGEMENTS

We wish to thank Aurobindo labs, Hyd. for providing gifted samples of Penems; Research lab, Dept., of Engineering chemistry, AUCE(A), Visakhapatnam, India, Dept., of Analysis, GIET School of Pharmacy, Rajahmundry, India.

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**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 + NQS + NaOH	0.662	
1.	Imipenem <sup>a</sup>	ii	D + NaOH + NQS	0.514	i
		iii	NaOH + NQS + D	0.483	

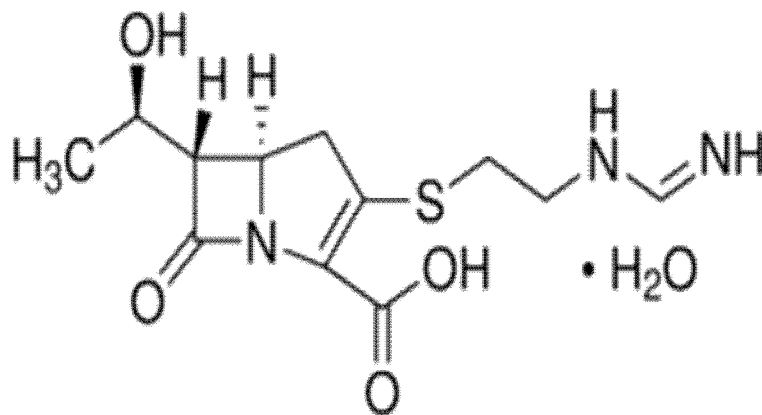
For 40 µg/mL of Drug samples

**Table 2.** Optical and regression characteristics of the proposed method for penems

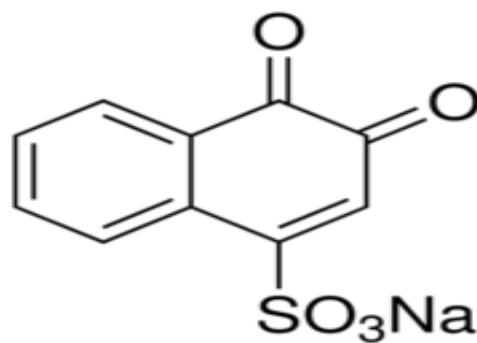
Parameters	Results
	Imipenem
$\lambda_{\max}$ nm	449 nm
Beer's law limits, mg/mL	0.02 – 0.12
Molar absorptivity, L/mol.cm	0.0190
Sandell's sensitivity (µg/cm <sup>2</sup> /0.001 absorbance unit)	7x10 <sup>-4</sup>
Regression equation	
Slope(b)	0.2284 ± 0.01189
Intercept	0.04971 ± 0.02144
r <sup>2</sup>	0.9866
Limit of Detection(µg/mL)	14.2928
Limit of Quantification(µg/mL)	43.3117

**Table 3.** Results of analysis of injection formulations containing Imipenem

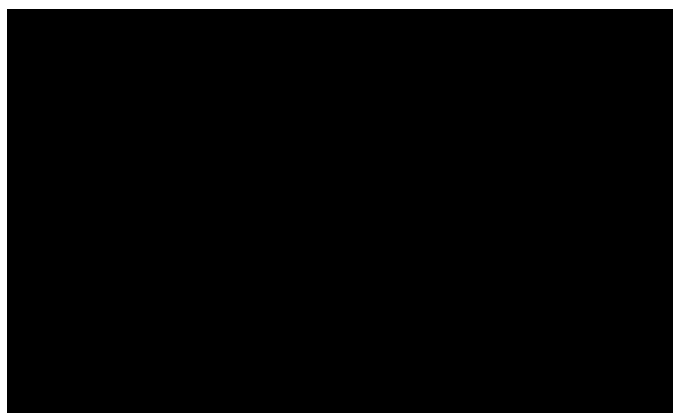
Injection	Imipenem
Company Name	Troika Pharma
Formulation	Inj
Labeled amount, mg	1000
% Recovery	99.8



**Figure 1.** Structure of Imipenem



**Figure 2.** Structure of NOS



**Figure 3.** Calibration graph of Imipenem

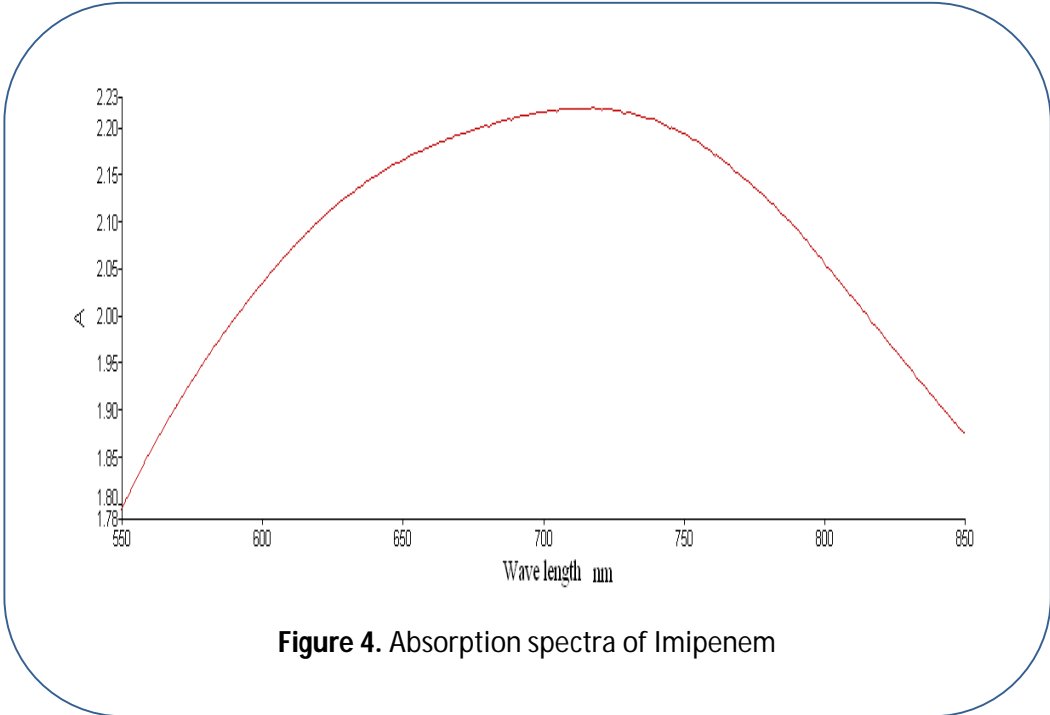


Figure 4. Absorption spectra of Imipenem