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SPECTROPHOTOMETRIC DETERMINATION OF MEROPENEM 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 Meropenem (MP) 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 1.5 mL NaOH and the unreacted NQS is determined by the measurement of the λ_{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 MP (Method A) and obeys beer's law in the concentration range 0.02 – 0.12 mg/mL of MRP. The apparent molar absorptivity is 0.007 and sandell's sensitivity is $7x10^{-4}$. The slope is 0.1556 ± 0.0029 and intercept of the equation of the regression line is 0.0057 ± 0.0052**.** 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 MP in pharmaceutical formulations.

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].

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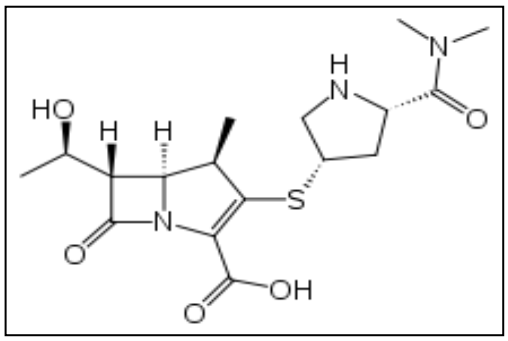
Among antibiotics, penems are much recently introduced, widely prescribed and costlier. Therefore, incentive to produce their counterfeits because of profit margin increases considerably.

Meropenem [2] is an ultra-broad spectrum injectable antibiotic used to treat a wide variety of infections. It is a beta-lactam and belongs to the subgroup of carbapenem, similar to imipenem and ertapenem. It penetrates well into many tissues and body fluids including the cerebrospinal fluid, bile, heart valves, lung, and peritoneal fluid. Chemically it is 3-[5-(dimethylcarbamoyl) pyrrolidin-2-yl] sulfanyl-6-(1-hydroxyethyl)-4-methyl-7-oxo- 1-azabicyclo [3.2.0] hept-2-ene-2-carboxylic acid.



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 Meropenem [3-8]. 1, 2-naphthoquinone-4-sulphonic acid sodium salt (NQS) has been used as a chromogenic reagent for the spectrophotometric determination of many pharmaceutical amines [9-13]. However, the reaction between NQS and MRP 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 MP in its injection formulation. According to literature survey there is no method reported for MRP with NQS reagent by visible spectrophotometry. Hence an attempt was made to develop simple and sensitive spectrophotometric method for the estimation of MRP in pure drug and in pharmaceutical formulations. The method uses the well-known nucleophilic displacement reaction involving NQS reagent and MRP resulting in the formation of a dark yellow colored chromogen that could be measured at 449 nm.

EXPERIMENTAL:

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 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 MRP by dissolving 100 mg in 100 mL of double distilled water.

GENERAL PROCEDURE:

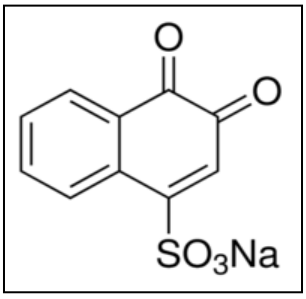
Method A: Into 10 ml volumetric flask, different aliquots of working standard solution (0.5 to 3.0 mL) of MRP were transferred to provide final concentration range of 0.02 – 0.12 mg/mL. To each flask, 1.5 mL of NaOH, 1.5 mL of NQS were successively added and kept aside for 5 minutes. The solutions were 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 MRP 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 is 260.19 and IUPAC Name: sodium 3, 4-dioxonaphthalene-1-sulfonate.

Structure:



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 colored species formed.

Nature of colored species: The reaction of 1, 2–napthaquinone–4-sulfonic acid (NQS) with aromatic amines was discovered by Boniger as far back as 1894.The colored species formed from MRP in this method can be explained based on the analogy of previous reports [14-15]. As MRP possesses amino groups, it involves in yielding colored 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 MP with NQS fulfills the essential requirements was investigated. All conditions studied were optimized at room temperature (32 ± 2^0C).

Selection of reaction medium: To generate the nucleophile from MRP 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} 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 MRP (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 MRP 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 MRP 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.

TABLE 1*:* EFFECT OF ORDER OF ADDITION OF REACTANTS ON COLOR DEVELOPMENT.

S. No.	Drug		Order of Addition	Absorbance	Recommended order of Addition
1.	Meropenem[a]	i	D + NQS + NaOH	0.662	i
		ii	D + NaOH + NQS	0.514	
		iii	NaOH + NQS + D	0.483	

[a]For 40 µg/mL of Drug samples

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 MRP. Calibration graph was obtained according to the above general procedure. The linearity replicates for six different concentrations of MRP 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.**

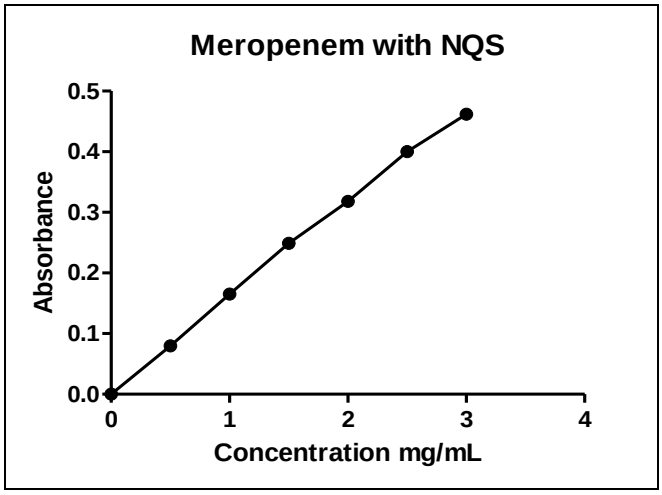


FIG 1: CALIBRATION GRAPH OF MEROPENEM

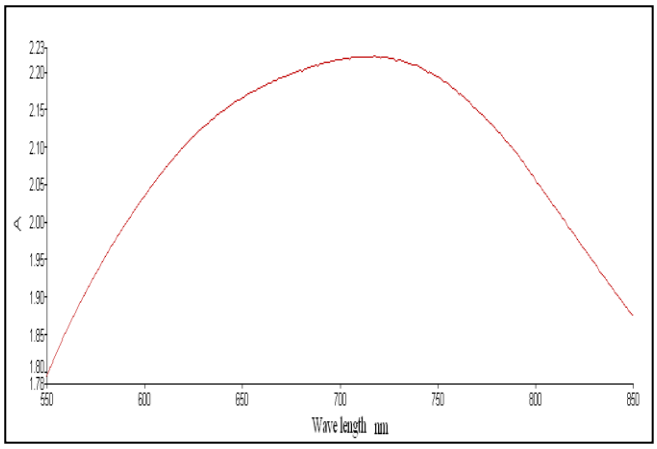


FIG. 2: ABSORPTION SPECTRA OF MEROPENEM

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 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.

TABLE 2: OPTICAL AND REGRESSION CHARACTERISTICS OF THE PROPOSED METHOD FOR MEROPENEM.

Parameters	Results
	Meropene
λ_{max} nm	449 nm
Beer's law limits, mg/mL	0.02 – 0.12
Molar absorptivity, L/mol.cm	0.007
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	7x10^{-4}
Regression equation	
Slope (b)	0.1556 ± 0.0029
Intercept	0.0057 ± 0.0052
r^2	0.9982
Limit of Detection (µg/mL)	0.5190
Limit of Quantification (µg/mL)	0.4950

Application to formulation: The proposed procedures were applied for the determination of MRP in commercially available injections. **Table 3** summarized the results.

TABLE 3: RESULTS OF ANALYSIS OF INJECTION FORMULATIONS CONTAINING MEROPENEM

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

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.

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