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Der Pharma Chemica, 2013, 5(6):312-316
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Spectrophotometric determination of doripenem in bulk and injection formulations by 1,2-naphthoquinone 4-sulphonic acid sodium salt (NQS) reagent in alkalline medium

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ABSTRACT

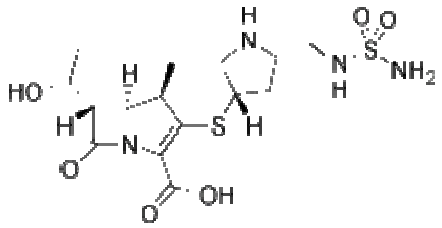
A simple and cost effective spectrophotometric method was described for the determination of Doripenem(DP) in pure form and in pharmaceutical formulations. The method is based on the formation of dark yellow colored chromogen when the drug reacts with 1,2 naphtho quinone 4-sulphonic acid sodium salt (NQS) reagent in alkalline medium. The method involves the addition of excess NQS of known concentration in the presence of 0.5 mL NaOH for DP (Method A), 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 for DP. 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 DP (Method A) and obeys beer's law in the concentration range 0.02 – 0.12 mg/mL of the drugs The apparent molar absorptivity is 0.0047, sandell's sensitivitt is $8x10^{-4}$. The slope is 0.0997 ± 0.0017 and intercept of the equation of the regression line is ***-0.0003*** *± 0.0033. 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 Doripenem in pharmaceutical formulations.*

Keywords: Doripenem, 1,2 naphthoquinone 4-sulphonic acid sodium salt (NQS), Spectrophotometry.

INTRODUCTION

Doripenem[1] is an ultra-broad spectrum injectable antibiotic. It is a beta-lactam and belongs to the subgroup of carbapenems. It is particularly active against Pseudomonas aeruginosa.

Fig I the structure of Doripenem



(4R,5S,6S)-6-(1-hydroxyethyl)-4-methyl-7-oxo-3-[(3S,5S) [(sulfamoylamino)methyl]pyrrolidin-3-yl]sulfanyl-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid

Doripenem can be used for bacterial infections such as complex abdominal infections, pneumonia within the setting of a Hospital, and complicated infections of the urinary tract including kidney infections with septicemia. Primarily, doripenem decreases the process of cell wall growth, which eventually leads to elimination of the infectious cell bacteria altogether. It is recommended that those allergic to doripenem or to any type of beta-lactam antibiotics such as cephalosporin or other Carbapenems not receive doripenem. The carbapenems are beta-lactam-type antibiotics with an exceptionally broad spectrum of activity.

Literature survey reveals that the drugs were determined by using HPLC[2-7] and some spectrophotometric methods[8]. According to literature survey there is no method reported for these penems with NQS reagent by visible spectrophotometry. Hence an attempt made to develop simple and sensitive spectrophotometric methods for the estimation of the above named penems in pure drug and in pharmaceutical formulations. Thees methods involves in the nucleophillic displacement of the sulfonic acid group of 1,2-naphthaquinone-4- sulfonic acid in alkaline conditions with the amine groups of the penems resulting in the formation of an yellow coloured chromogen, that could be measured at 449nm.

MATERIALS AND METHODS

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

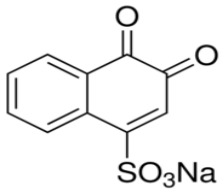
General procedure:
Method A:
Into a series of 10ml volumetric flask, 0.5 mL of sodium hydroxide and 1.0 mL of NQS reagent were successively added and to each flask, different aliquots of working standard solution (0.5 – 3.0 mL) of Doripenem were transferred to provide final concentration range of 0.02 – 0.12 µg/mL 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 DP were weighed into a 100 mL volumetric flask, 50 mL of distilled water was added and shaken thoroughly for about 10 minutes, 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-dioxonaphthalene-1-sulfonate

Fig II the structure of NQS:



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 colour development, minimum blank colour, reproducibility and reasonable period of stability of final coured species formed.

NATURE OF COLOURED 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 DP in this method can be explained based on the analogy of previous reports [9-10]. As DP possesses amino groups, it involves in yielding coloured produced by nucleophillic 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 Conditions under which reaction of DP with NQS reagent 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 DP 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 – 2.5 mL) were used to a constant concentration of DP (1mg/mL) and the results were observed. From the absorption spectrum it was evident that 0.5 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 DP 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 DP 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 (iii) is recommended for both.

Table I. 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	0.1756	iii
		ii	D + NaOH + NQS	0.1519	
		iii	NaOH + NQS + D	0.1935	

[a]*For 40 µg/mL of Drug sample*

Effect of NQS reagent 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 350-550 nm against a reagent blank. The reaction product showed absorption maximum at 449 nm for Doripenem. Calibration graph was obtained according to the above general procedure. The linearity replicates for six different concentrations of Doripenem were checked by a linear least - squares treatment. All the spectral characteristics and the measured or calculated factors and parameters were summarized in Table II.

Table II. Optical and regression characteristics, precision and accuracy of the proposed method for penems

Parameters	Results
	Doripenem
λ_{max} nm	449 nm
Beer's law limits, mg/mL	0.02 – 0.12
Molar absorptivity, L/mol.cm	0.0047
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	8x10^{-4}
Regression equation	
Slope(b)	0.0997± 0.0017
Intercept	-0.0003743 ± 0.00339
r^2	0.9988
Limit of Detection(µg/mL)	0.139
Limitof Quantification(µg/mL)	0.4231

Fig III the Calibration graph of Doripenem.

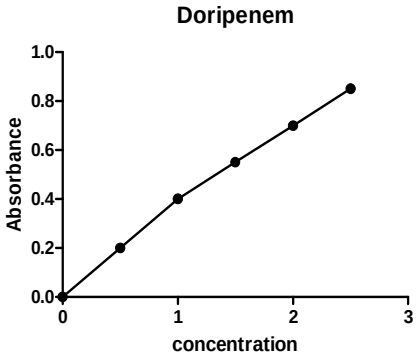
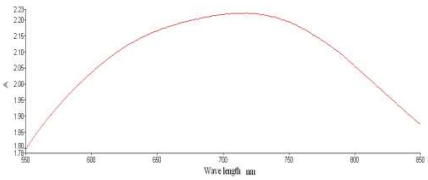


Fig. IV the absorption spectra of Doripenem.



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.

Application to formulation
The proposed procedures were applied for the determination of penems in commercially available injections. Table III summarized the results.

Table III. Results of analysis of injection formulations containing penem

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

CONCLUSION

The proposed methods were 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., for their kind provision of equipment.

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