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Research Article

Spectrophotometric Determination of Doripenem, Ertapenem in Bulk and Injection Formulations by NBS Reagent

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Abstract

Two simple and cost effective spectrophotometric methods were described for the determination of Doripenem(DRP) and Ertapenem(ERP) in pure and pharmaceutical formulations. The method is based on the formation of pink colored chromogen when the drugs react with N-bromo succinamide(NBS) reagent and celestin blue(CB) in acidic medium. The method involves the addition of excess NBS of known concentration in the presence of 5.0M HCl and the unreacted NBS is determined by the measurement of the decrease in the absorbance of the dye celestine blue (λ_{max} 535 nm), which was found to be the most suitable of several dyes tested. This method was applied for the determination of drug contents in pharmaceutical formulations and enabled the determination of the drugs 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 535 nm for both DRP (Method A) / ERP (Method B) and obeys beer's law in the concentration range 0.02 – 0.12 mg/mL of DRP, ERP. The apparent molar absorptivities were 0.0074, 0.0057 and sandell's sensitivity was $7x10^{-4}$ for both DRP, ERP. The slopes were 0.1534± 0.0104 for DRP and 0.1372± 0.0076 for ERP. Intercept of the equation of the regression line are 0.0434 ± 0.0188, 0.0292 ± 0.0138 for DRP, ERP respectively. 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 DRP, ERP in pharmaceutical formulations.

Keywords: Doripenem(DRP), Ertapenem(ERP), N-bromo succinamide (NBS), Celestin blue (CB), Spectrophotometry

1. 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.

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.

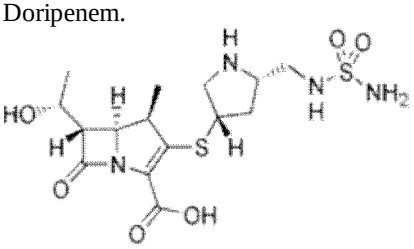


Fig:1 Structure of Doripenem

(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.
Ertapenem[2] is a carbapenem antibiotic marketed by Merck as Invanz. It is structurally very similar to meropenem in that it possesses a 1-β-methyl group.

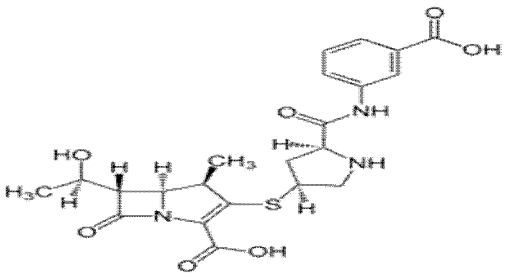


Fig:2 Structure of Ertapenem

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

The carbapenems are beta-lactam-type antibiotics with an exceptionally broad spectrum of activity. Ertapenem is a new carbapenem developed to address the pharmacokinetic shortcomings (short half-life) of Imipenem and Meropenem. Ertapenem shares similar structural features with Meropenem, including its stability to dehydropeptidase-1, allowing it to be administered without a dehydropeptidase-1 inhibitor. Ertapenem, like Imipenem and Meropenem, demonstrates broad-spectrum antimicrobial activity against many Gram-positive and -negative aerobes and anaerobes and is resistant to nearly all beta-lactamases, including extended-spectrum beta-lactamases. However, it differs from both Imipenem and Meropenem in demonstrating limited activity against Enterococcusspp., Pseudomonasaeruginosa and other nonfermentative Gram-negative bacteria commonly associated with nosocomial infections. The extensive protein binding of Ertapenem extends the half-life and allows for once-daily dosing. Prospective, multicenter, randomized, double-blind, comparative clinical studies demonstrate similar clinical efficacy of Ertapenem compared with other agents.
Literature survey[3-8] reveals that the drugs were determined by using HPLC and some spectrophotometric methods[9]. According to literature survey there is no method reported for these penems with NBS reagent by visible spectrophotometry. It's more important application is as reagent for a number of interesting substitution, addition and oxidation reactions in a polar medium because of its high selectivity under a variety of experimental conditions.The method uses the well known reduction reaction involving reagent and penems resulting in the formation of a pink chromogen that could be measured at 535 nm. 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.

2. Experimental:

2.1 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 were of analytical reagent grade and double distilled water was used throughout. NBS reagent supplied by SD Fine chemicals ltd., India, was used by diluting 10 mg to 100mL with distilled water. CB prepared by dissolving 5.0 mg in 100 mL double distilled water. 5.0M HCl is prepared by diluting 43.5 mL to 100 mL with distilled water.10 µg/mL stock reference solution was freshly prepared from pure sample of penems by dissolving 100 mg in 100 ml of double distilled water.

2.2 General procedure:

Method A:

Into 10ml volumetric flask, different aliquots of working standard solution (0.5 – 3.0 mL) of DRP were transferred to provide final concentration range of 0.02 – 0.12 µg/ml. To each flask 2.5 mL of HCl, 1.5 mL of NBS and 4.0 mL of CB reagent 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 535 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.

Method B

Into 10 ml volumetric flask, different aliquots of working standard solution (0.5 – 3.0 mL) of ERP were transferred to provide final concentration range of 0.02 – 0.12 µg /ml. To each flask, 3.5 mL of HCl, 1.5 mL of NBS and 3.5 mL of CB reagent 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 535 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 penems 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.

3. Results and discussion

N-bromo succinamide (NBS)

N-bromo succinamide (NBS) IUPAC name 1-Bromo-2, 5-pyrrolidinedioneis a chemical reagent used in radical substitution and electrophilic addition reactions in organic chemistry. NBS can be a convenient source of cationic bromine. Molecular Formula is $C_4H_4BrNO_2$, Molar mass 177.98 g/mol, Density 2.10 g/cm³, Melting point 175 °C and easily soluble in water.

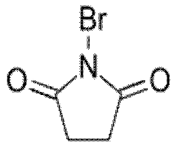


Fig 3: Structure of N-bromo succinamide

Celestine blue: It is a dark blue colored powder. It also called as celestin blue, celestine blue, mordant blue 4, coreine blue b, mordant blue 14, celestin blue b, celestine blue b, gallo sky blue b having the molecular formula $C_{17}H_{18}ClN_3O_4$, molecular weight 363.8, soluble in water.

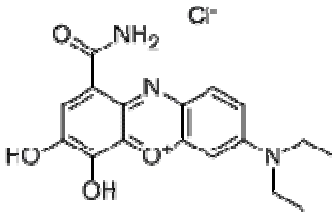


Fig 4: Structure of Celestine Blue

Mordant dyes include azo dyes, which are converted into their final, insoluble form on the fibres. A mordant is a metal, most commonly chromium, aluminium, copper or iron. The dye forms together with a mordant, an insoluble metal-dye complex and precipitates on the natural fiber.

NBS + CB → Oxidation products of dye + Unreacted CB
(Coloured)

In the recent years, there has been growing interests in the role of N-bromo succinamide (NBS) as an analytical reagent in the determination of organic compounds[10]. NBS was basically developed for use as a synthetic reagent and in view of the high yield of the reaction products obtained in its reactions; it has been adopted for use in the determination of many organic compounds. It's more important application is as reagent for a number of interesting substitution, addition and oxidation reactions in a polar medium because of its high selectivity under a variety of experimental conditions. When the reaction is stoichiometric and fast, the solution of NBS is useful in titrimetric[10-11] determinations in which the end-point is determined with either a visual indicator or an electrometric technique like potentiometry. In some instances the reaction may proceed slowly, in which case a back or indirect procedure has been suggested. An excess of standard NBS is added to the analyte solution and is allowed to react for a given time. The excess NBS present in the acidic medium is determined either by titrimetry e.g.iodometry (determination of compounds containing olefinic double bonds) or spectrophotometry[12-20]. Titrimetric procedures are not suitable for the determination of compounds at microgram levels, but indirect spectrophotometry gave reasonable sensitivity, although it required close control of pH and gave ales stable final charge-transfer complex. Even though the coloured dyes(azine and oxazine dyes) are well known for their intensity and λ_{max} , they have not been utilized for estimating excess NBS in the indirect determination of bio-active compounds. The

present investigation proposes a rapid and sensitive indirect spectrophotometric method for the determination of penems, the antibacterial agents in their bulk and pharmaceutical formulations. The principle involved is quantitative decolorization of celestine blue (CB), an oxidizable dye (1-aminocarbonyl-7-diethylamino-3,4-dihydroxy-phenoxazin-5-ium chloride) by NBS.

NBS is an oxidizing agent and perhaps the most important positive bromine containing organic compound, used for spectrophotometric determination of many pharmaceutical compounds [21–23] close examination of the literature search presented in the introduction reveals that NBS has not yet been used for the spectrophotometric determination of these drugs. The present work involves the bromination of the investigated drugs by NBS followed by determination of surplus NBS after allowing the bromination reaction to complete. The unreacted NBS is determined by reacting with a fixed amount of CB dye and measured at 535 nm.

It is uncommon, but possible for NBS to selectively oxidize secondary alcohols in the presence of primary alcohols using NBS in presence of acidic medium.

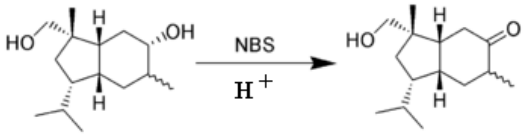


Fig 5: Oxidation process by using NBS

3.1 Optimization of conditions on absorption spectrum of the reaction product:

The condition under which the reaction of penems with NBS fulfills the essential requirements was investigated. All conditions studied were optimized at room temperature (32 ± 2^0C).

3.2 Selection of reaction medium:

To find a suitable medium for the reaction, different aqueous acids were used, such as HCl H_2SO_4 and H_3PO_4. The best results were obtained when hydrochloric acid was used. In order to determine the optimum concentration of hydrochloric acid, different volumes of 5.0M HCl solution (0.5 – 3.0 mL) were used to a constant concentration of DP (25µg/mL); (0.5 – 4.5 mL) were used to a constant concentration of EP (25µg/mL) and the results were observed. From the absorption spectrum it was evident that 2.5 mL of 5.0M HCl solution for DP; 3.5 mL of 5.0M HCl solution for EP were found optimum. Larger volumes had no significant effect on the absorbance of the colored species.

3.3 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 I. The order of addition of serial number (iii) is recommended for both DRP and ERP.

3.4 Effect of NBS concentration

Several experiments were carried out to study the influence of NBS concentration on the color development by keeping the concentration of drug and Hydrochloric acid to constant and changing reagent concentration. It was apparent that 1.5 mL of NBS gave maximum color for both DRP and ERP.

3.5 Effect of Celestin Blue concentration

Several experiments were carried out to study the influence of CB concentration on the color development by keeping the concentration of drug, Hydrochloric acid and NBS to constant and changing CB concentration. It was apparent that 4.0 mL of CB gave maximum color for DRP and 3.5 mL for ERP.

4.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.

5. 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 535 nm for both DP and EP. Calibration graph was obtained according to the above general procedure. The linearity replicates for six different concentrations of both DP and EP 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

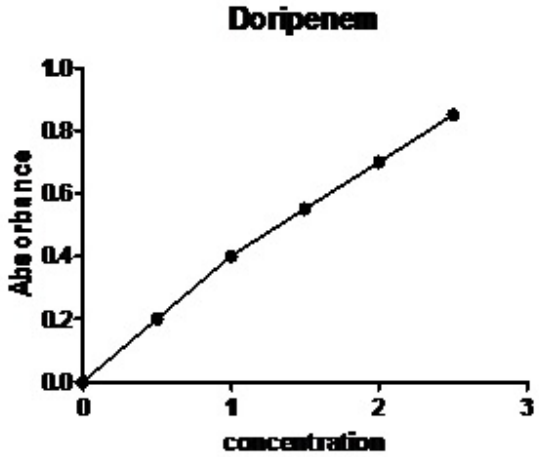
6. Application to formulation

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

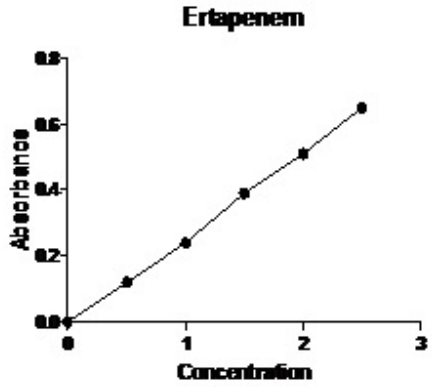
7. Conclusion:

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.

8. **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.



Graph I. Calibration graph of Doripenem



Graph II. Calibration graph of Ertapenem

Table I. Effect of order of addition of reactants on color development.

S.No.	Drug		Order of Addition	Absorbance	Recommended order of Addition
		i	D + NBS + CB+ HCl	0.072	
1.	Doripenem[a]	ii	D + CB +NBS + HCl	0.0192	iii
		iii	D + HCl + NBS+ CB	0.15	
		iv	NBS + HCl + CB+ D	-0.0004	
		i	D + NBS + CB+ HCl	0.090	
2.	Ertapenem[a]	ii	D + CB +NBS + HCl	0.050	iii
		iii	D + HCl + NBS+ CB	0.157	
		iv	NBS + HCl + CB+ D	-0.0004	

[a]For 40 µg/ml of Drug sample

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

Parameters	Values		
	Doripenem	Ertapenem	
λmax nm	535 nm	535 nm	
Beer's law limits, µg/mL	0.02-0.12	0.02-0.12	
Molar absorptivity, L/mol.cm	0.0074	0.0057	
Sandell's sensitivity (µg/cm2/0.001 absorbance unit)	$7x10^{-4}$	$7x10^{-4}$	
Regression equation			
Slope(b)	0.1534± 0.0104	0.1372± 0.0076	
Intercept	0.0434 ± 0.0188	0.0292 ± 0.0138	
r^2	0.9773	0.9845	
Limit of Detection	0.5964	0.4897	
Limit of Quantification	1.8074	1.4841	

Table III. Results of analysis of injection formulations containing penem

Injection	Doripenem	Ertapenem
Company Name	Troika Pharma	Neon Pharma
Formulation	Inj	Inj
Labeled amount, mg	1000	1000
Recovery amount	99.8	99.56

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