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Spectrophotometric Determination of Doripenem, Ertapenem in Bulk and Injection Formulations by F-C Reagent

K. Raghu Babu[1], N. Aruna Kumari[2*], R. Vijaya Lakshmi[3]

[1]*Department of Engg. Chem, Andhra University, India*
[2]*Department of HBS, GIET, Rajahmundry, India*
[3]*Department of Analysis, GIET School of Pharmacy, Rajahmundry, India*

ABSTRACT
Two simple and cost effective spectrophotometric methods were described for the determination of Doripenem and Ertapenem in pure form and in pharmaceutical formulations. The method is based on the formation of blue colored chromogen when the drug reacts with Folin-Ciocalteu (F-C) reagent in alkaline medium. The colored species has an absorption maximum at 725 nm for Doripenem (Method A); 848 nm for Ertapenem (Method B) and obeys beer's law in the concentration range 0.02-0.1 mg/mL for both Doripenem and Ertapenem. The apparent molar absorptivities were 1.26×10^{-5}, 7.86×10^{-6} and sandell's sensitivity was 6×10^{-4} respectively for Doripenem and Ertapenem. The slopes were 0.3371 ± 0.01245, 0.2611 ± 0.004327 and intercept of the equation of the regression line are 0.02857 ± 0.01885, -0.008095 ± 0.006550 for Doripenem and Ertapenem 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 Doripenem and Ertapenem in pharmaceutical formulations.

Keywords: Doripenem, Ertapenem, F-C reagent, Spectrophotometry.

INTRODUCTION

Doripenem [1] (common name doripenem monohydrate) is an ultra-broad spectrum injectable antibiotic. It is a beta-lactam antibiotic and belongs to the subgroup of carbapenems. It is particularly active against *Pseudomonas aeruginosa*. Chemically it is (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.

Ertapenem [2] is a carbapenem antibiotic marketed by Merck as Invanz. It is structurally very similar to meropenem in that it possess a 1-β-methyl group. Chemically it is (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. Prospective, multicenter, randomized, double-blind, comparative clinical studies demonstrate similar clinical efficacy of ertapenem compared with other carbapenems.

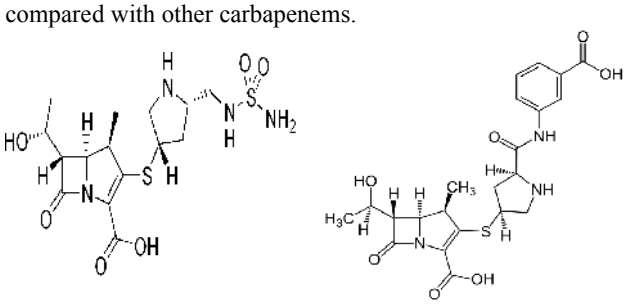

Fig. 1 : Chemical Structure of Doripenem and Ertapenem

Literature survey revealed that the drugs were determined by using HPLC [3-8] and some spectrophotometric methods. [9] According to literature survey there is no method reported for these penems with F-C reagent by visible spectrophotometry. The aim of this study was to make an attempt to develop simple and sensitive spectrophotometric methods for the estimation of the above named penems in pure drug and in pharmaceutical formulations. The method uses the well known oxidation reaction involving F-C reagent and penems resulting in the formation of a green chromogen that could be measured at 725 nm for doripenem and 848 nm for ertapenem.

***Corresponding author: Mrs. N. Aruna Kumari,** Department of HBS, GIET, Rajahmundry-533296, East Godavari Dist., Andhra Pradesh, India;
E-mail: arunanakkella@yahoo.co.in

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 were of analytical reagent grade and double distilled water was used throughout. F-C reagent 2N supplied by SD Fine chemicals ltd., India, was used by diluting 50 mL to 100 mL with distilled water. Sodium carbonate solution (10%) was prepared by dissolving 10 g of sodium hydroxide in 100 mL double distilled water. 1 mg/mL stock reference solution was freshly prepared from pure sample of doripenem/ertapenem by dissolving 100 mg in 100 ml of double distilled water.

General procedure

Method A

Into 10ml volumetric 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 mg/mL. To each flask 2.0 mL of sodium carbonate and 1.0 mL of F-C reagent 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 725 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 25 mL volumetric flask, different aliquots of working standard solution (0.5-2.5 mL) of Ertapenem were transferred to provide final concentration range of 0.02-0.1 mg/mL. To each flask, 5 mL of sodium carbonate and 2.0 mL of F-C reagent 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 848 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

A volume of the injection equivalent to 100 mg of Doripenem/Ertapenem 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

The F-C reagent was used in the determination of many phenolic compounds, amines and a large number of substances of pharmaceutical interest. The proposed method is based on the formation of a blue colored chromogen, following the reduction of phospho-molybdo tungstic mixed acids of the F-C reagent by Doripenem/Ertapenem in the presence of sodium carbonate, which could be measured at 725 nm and 848 nm respectively. The mixed acids in the F-C reagent were the final chromogen and involve the following chemical species:

$3H_2O$, P_2O_5, $13WO_3$, $5MoO_3$, $10\ H_2O$

And

$3H_2O$, P_2O_5, $14\ WO_3$, $4MoO_3$, $10H_2O$

Both drugs probably effect reduction of oxygen atoms from tungstate and / or molybdate in the F-C reagent, there by producing one or more possible reduced species which have characteristic intense blue color. The effect of different variables such as nature and strength of alkali, optimum volumes of sodium carbonate and F-C reagent, reaction time and order of addition of reactants were studied and optimized for attainment of maximum color and stability of colored species.

Optimization of conditions on absorption spectrum of the reaction product

The condition under which reaction of both drugs with F-C reagent fulfills the essential requirements was investigated. All conditions studied were optimized at room temperature (32±2°C).

Selection of reaction medium

To find a suitable medium for the reaction, different aqueous bases were used, such as borax, sodium hydroxide, sodium carbonate or bicarbonate, sodium acetate and sodium hydrogen phosphate. The best results were obtained when sodium carbonate was used. In order to determine the optimum concentration of sodium carbonate, different volumes of 10% sodium carbonate solution (2.0-7.0 mL) were used to a constant concentration of Doripenem (1 mg/mL); (3.0-7.0 mL) were used to a constant concentration of Ertapenem (1mg/mL) and the results were observed. From the absorption spectrum it was evident that 2.0 mL of 10% sodium carbonate solution for Doripenem; 5.0 mL of 10% sodium carbonate solution for Ertapenem were found optimum. Larger volumes had no significant effect on the absorbance of the colored species.

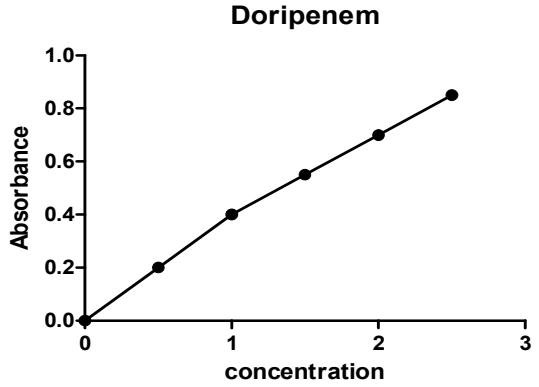

Fig. 2 : Calibration graph of Doripenem

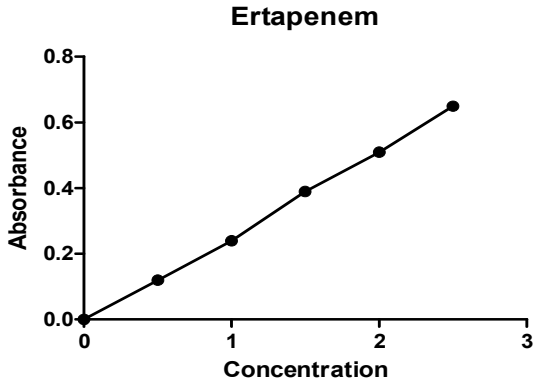

Fig.3: Calibration graph of Ertapenem

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 both Doripenem and Ertapenem.

Effect of F-C reagent concentration

Several experiments were carried out to study the influence of F-C reagent concentration on the color development by keeping the concentration of drug and sodium carbonate to constant and changing reagent concentration. It was apparent that 1.0 mL of F-C reagent gave maximum color for both Doripenem and 2.0 mL for Ertapenem.

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.

Table 1: Effect of order of addition of reactants on color development

S. No	Drug		Order of Addition	Absorbance	Recommended order of Addition
1.	Doripenem[a]	i	D + Na_2CO_3 + F.C	0.36	i
		ii	D + F.C + Na_2CO_3	0.36	
		iii	F.C + Na_2CO_3 + D	-0.003	
2.	Ertapenem[a]	i	D + Na_2CO_3 + F.C	0.18	i
		ii	D + F.C + Na_2CO_3	0.18	
		iii	F.C + Na_2CO_3 + D	-0.005	

[a]For 40µg/mL of Drug samples

Table 2: Results of optical and regression characteristics of the proposed method for doripenem and ertapenem

Parameters	Values	
	Doripenem	Ertapenem
λ_{max} nm	725 nm	848 nm
Beer's law limits, µg/mL	0.02-0.1	0.02-0.1
Molar absorptivity, L/mol.cm	1.26×10^{-5}	7.86×10^{-6}
Sandell'ssensitivity (µg/cm^2/0.001absorbance unit)	6×10^{-4}	6×10^{-4}
Regression equation		
Slope(b)	0.3371± 0.0124	0.2611±0.0043
Intercept	0.0285 ± 0.0188	-0.0080± 0.0065
r^2	0.9946	0.9989
Limit of Detection	0.2553	0.1697
Limit of Quantification	0.7737	0.5143

Table 3: Results of analysis of injection formulations containing penem

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

Absorption spectrum and calibration graph

Absorption spectrum of the colored complex was scanned at 550-850 nm against a reagent blank. The reaction product showed absorption maximum at 725 nm for Doripenem and 848 nm for Ertapenem. Calibration graph was obtained according to the above general procedure. The linearity replicates for six different concentrations of Doripenem, five different concentrations of Ertapenem 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 and Fig. 2 & 3 show the Calibration graph of Doripenem and Ertapenem.

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

Reducing ions, tryptophan, hydroxyproline, 2-and 3-hydroxypyridines, ascorbic acid, uric acid also reduce F-C reagent to molybdenum blue. However 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 3 summarized the results.

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.

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