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Extractive spectrophotometric determination of doripenem using bromo cresol purple and methylene violet as color developing reagents

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ABSTRACT

Two simple extractive spectrophotometric methods were developed for the determination of doripenem in pure and pharmaceutical formulations. Doripenem, a recently developed member of carbapenem class of beta-lactum antibiotic has been shown to have broad-spectrum activity against Gram-positive and Gram-negative pathogens, including strains of Pseudomonas aeruginosa. A Tech-comp model UV-2301 UV-Visible spectrophotometer with 1 cm matched quartz cells was used for all spectral measurements. Aqueous solutions of 0.1% bromo cresol purple (Method-A) and 0.2% methylene violet (Method-B) were used in the present investigation. Freshly prepared working standard solution of doripenem of concentration 200µg/mL and 300µg/mL were used for method A and method B respectively. These methods were based on the formation of colored ion-ion association complex between protonated drug molecule (doripenem having positive charge) and dissociated bromo cresol purple or methylene violet (i.e. reagent molecular ion having negative charge) in the aqueous phase at a suitable pH extractable into dichloromethane or chloroform in Method A and Method B respectively. The absorption spectra were recorded for the colored products and it was evident that the wavelength of maximum absorbance at 417nm and 560nm for methods A and B respectively. The molar absorbitivity and Sandell's sensitivity of the proposed methods were found to be 6.032x10^3 &3.843x10^3 and 0.0697 &0.1094 respectively. Limit of detection and limit of quantitation were observed to be 0.10&0.70 and 0.165&2.31 µg/mL. The developed methods had been statistically validated and applied to pharmaceutical formulations without any interference from excipients.

Key words: Doripenem, Bromo Cresol Purple, Methylene Violet, Extractive Spectrophotometry

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INTRODUCTION

Doripenem is a recently developed member of carbapenem class of beta-lactum antibiotic. Doripenem has been shown to have broad-spectrum activity against Gram-positive and Gram-negative pathogens, including strains of Pseudomonas aeruginosa [1]. It is similar to other carbapenems and was developed for the treatment of hospitalized patients with moderate or severe bacterial infections [2]. It is chemically known as (+)-(4R, 5S, 6S)-6-[(1R)-1-Hydroxy -ethyl] -4-methyl-7-oxo-3- [[(3S, 5S)-5- [(sulfamoyl amino)- methyl]-3-pyrrolidinyl] thio]-1-azabicyclo [3.2.0] hept-2-ene-2-carboxylic acid with molecular formula $C_{15}H_{24}N_4O_6S_2$ and molecular weight 420.50426g/mol. It is available in brand names like finibax and doribax. Molecular structure of doripenem was presented in Fig.1.The literature survey reveals that one spectrophotometric [3] and a few HPLC [4-9] methods have been reported. Analytically active functional moieties that were present in doripenem were not fully exploited and there is a scope to develop new spectrophotomeric methods for the determination of doripenem. Different active pharmaceutical ingredients were determined by different authors [10-14] by extractive spectrophotometry. Therefore in the present investigation the author made some attempts for the development extractive spectrophotometric methods for the determination of doripenem in formulations and succeeded.

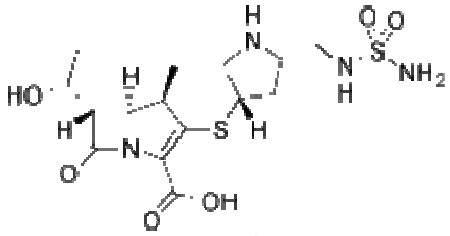

Fig.1 Molecular structure of Doripenem molecule

MATERIALS AND METHODS

Instrumentation: A Tech-comp model UV-2301 UV-Visible spectrophotometer with 1 cm matched quartz cells was used for all spectral measurements and a systronics digital pH meter was used for pH measurements.

Chemicals and reagents: Analytical grade chemicals and reagents were used for the present investigation. Doripenem was obtained from local pharmaceutical laboratory and its pharmaceutical formulations were obtained from commercial sources.

Preparation of solutions: Stock solution of concentration 500µg/mL was prepared by dissolving accurately weighed amount of 50mg of doripenem in 100mL of double distilled water. Working standard solutions of concentration 80µg/mL for method-A and 120µg/mL for method-B were prepared by diluting 16mL or 24mL of the stock solution to 100mL. Aqueous solutions of 0.1% bromo cresol purple and 0.2% methylene violet were prepared by dissolving 100mg or 200mg of the respective reagents in 100mL with distilled water. 0.1M Hydrochloric acid was prepared by mixing 8.6 mL of conc. HCl with 1000 mL distilled water. Buffer solution of pH 9.8 was prepared by mixing 100 mL of 0.025 M borax and 30 mL of 0.1M sodium hydroxide and diluted to 200 mL with distilled water.

Method development and Optimization

Method development in the present investigation was carried out based on the nature of the drug molecule. Doripenem contains both acidic (carboxylic acid) and basic (amino) moieties; therefore it forms an ion-ion association complex with basic dyes like methylene violet (tertiary nitrogen) and acidic dyes such as bromo cresol purple (due to phenolic –OH group). The colored product formed between drug and dye was extractable into less polar organic solvents from aqueous phase at a suitable pH. The author made some different trails to optimize the method by systematic study of parameters such as concentration of drug and dye, pH, temperature, choice of organic solvent for the extraction of the colored species and the order of addition of reagents. The optimized experimental conditions for getting maximum absorbance were followed through out the analysis.

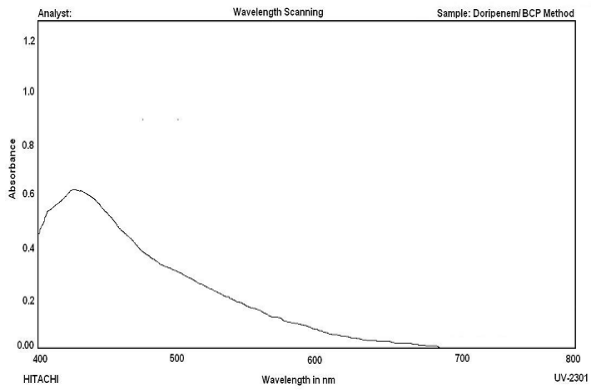

Fig. 2 Absorption spectrum of Doripenem with bromo cresol purple

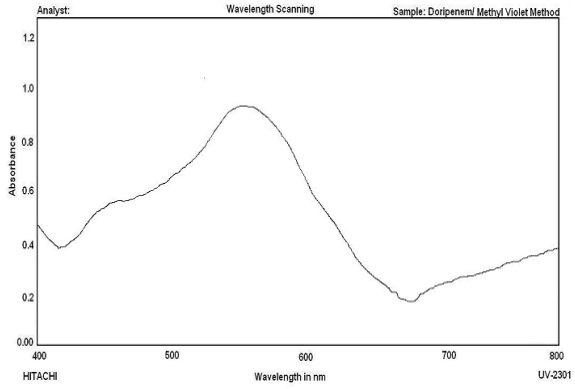

Fig. 3 Absorption spectrum of Doripenem with methylene violet

Method-A: 1.5mL of working standard solution 80µg/mL was accurately transferred into a 125mL separating funnel, 0.5mL of HCl solution and 2.0 mL of bromo cresol purple solution were added and the total volume of aqueous phase was adjusted to 15 mL with double distilled water. About 10mL of dichloromethane was added; the contents were shaken for 5 min. and allowed to separate. The organic layer was separated and the absorption spectrum was recorded against reagent blank and presented in Fig.2. Wavelength 417nm was found to be a wavelength of maximum absorbance.

Method-B: Into a 125mL separating funnel, 2.5mL working standard solution 120µg/mL was accurately transferred, 1.0 mL of buffer solution of pH 9.8 and 3.0mL of methylene violet solution were added. Then the total volume of aqueous phase in each funnel was adjusted to 15mL with distilled water and 10mL of chloroform was added. The contents were shaken for 5min. and allowed to separate. The absorbance of organic layer was measured at 560nm against a reagent blank and the absorption spectrum was given in Fig.3.

RESULTS AND DISCUSSION

Validation

Proper validation of analytical methods is important for pharmaceutical analysis when ensurance of the continuing efficacy and safety of each batch manufactured relies solely on the determination of quality. The ability to control this quality is dependent upon the ability of the analytical methods, as applied under well-defined conditions and at an established level of sensitivity, to give a reliable demonstration of all deviation from target criteria. The most widely applied validation characteristics are precision (repeatability and intermediate precision), accuracy, detection limit (LOD), quantitation limit (LOQ), linearity, range and stability of analytical solutions.

Precision: The intra-day precision (repeatability) and inter day precision (reproducibility) of the developed method were evaluated in terms of statistical parameters such as standard deviation and %RSD. The %RSD was calculated from the standard deviation and mean area of six replicate measurements of the working standard solution of concentration 12µg/mL in method-A and 30µg/mL in method-B. The results were presented in Table-1

Accuracy: The accuracy (closeness of the experimentally determined value to the most probable value or true value) of the method was determined at three different concentration levels 50%, 100% and 150% (4, 8 and 12 µg/mL) in terms of recovery by standard addition method by adding a known amount of sample (8µg/mL) at different spike levels and determined the amount of individual recovery and mean recovery. The experimental results were presented in Table-2

Linearity and Range: Linearity of the proposed method was demonstrated in terms of measuring absorbance values as a function of concentration of the drug. According to Beer's law, the absorbance was linearly proportional to the drug concentration. The correlation between concentration of the drug and absorbance was expressed in terms of correlation coefficient. Slope and intercept are evaluated by linear regression analysis using linear least square method and the values were given in Table-1

Table 1: Optical, regression characteristics and precision of proposed methods

Parameter		Method A	Method B
λ max, nm		417.0	560.0
Beer's law limits (µg/mL)		2.0-16.0	6.0-30.0
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)		6.0320×10^3	3.8430×10^3
Sandell's sensitivity (µg/cm^2/0.001 abs.unit)		0.0697	0.1094
Slope		0.0556	0.0318
Intercept		0.0069	0.0019
Correlation coefficient		0.9996	0.9999
% RSD*	Intra day	0.432	0.320
	Inter day	0.934	0.650
Limit of detection (µg/mL)		0.100	0.700

Table 2: Recovery studies for determination of Doripenem by proposed methods

	Amount taken, (µg/mL)		Amount obtained, (µg/mL)		% Recovery	
Spike level	Method A	Method B	Method A	Method B	Method A	Method B
50%	12	12	11.85	11.84	98.75	98.67
	12	12	11.79	11.77	98.25	98.08
	12	12	11.89	11.96	99.08	99.67
100%	16	16	15.69	15.89	98.06	99.31
	16	16	16.07	15.87	100.44	99.19
	16	16	15.71	15.92	98.18	99.50
150%	20	20	20.31	19.78	101.55	98.90
	20	20	19.94	20.36	99.70	101.30
	20	20	20.09	19.90	100.45	99.50

Method-A: Different aliquots of the drug (0.25-2.5mL, 80µg/mL) were taken in a series of 125mL separating funnels, 0.5mL of 0.1M HCl solution and then 2mL of 1% BCP solution were added. The volume of aqueous phase was adjusted to 15mL with double distilled water. Then the colored product was extracted into 10.0mL of

dichloromethane by shaking the contents for five minutes. The absorbance of the organic layer was measured at 417nm against a reagent blank. Calibration curve was constructed by plotting absorbance against concentration and was presented in Fig.4

Method B: Aliquots of standard doripenem solution (0.5-2.5mL, 120µg/mL), 1.0mL of buffer solution and 3.0mL of methylene violet solution were placed into a series of 125mL separating funnels. The total volume of aqueous phase in each funnel was adjusted to 15mL with distilled water and 10mL of chloroform was added. The contents were shaken for 5min. and allowed to separate. The absorbance of organic layer was measured at 560nm against a reagent blank. Linearity plot was constructed by plotting absorbance against concentration and was given in Fig.5

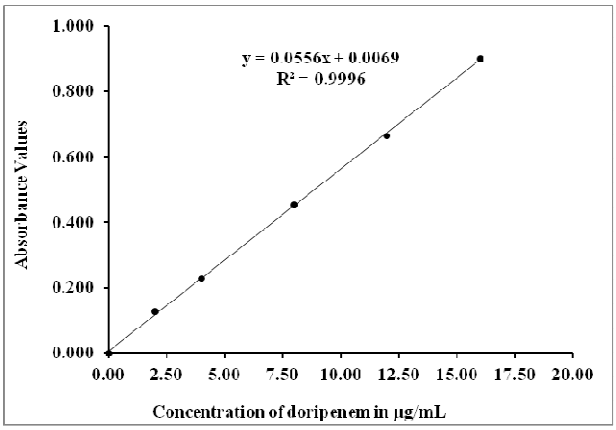

Fig. 4 Calibration curve of Doripenem with bromo cresol purple

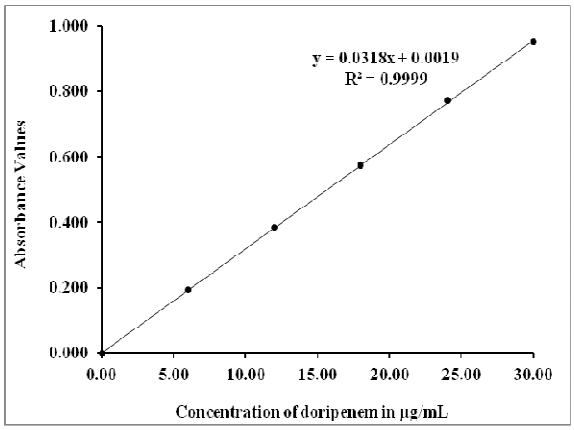

Fig. 5 Calibration curve of Doripenem with methylene violets

Limit of Detection and Limit of Quantification: The Limit of Detection (LOD) is the smallest concentration of the analyte that gives the measurable response where as limit of quantification (LOQ) is the smallest concentration of the analyte, which gives response that can be accurately quantified. The LOD and LOQ of the proposed method were calculated by using standard deviation of the intercept (σ) and slope (s) of the calibration curve. These were calculated by using the formulae LOD=3σ/s and LOD=10σ/s and are presented in Table-1

Assay of pharmaceutical formulations

Sample stock solution of concentration 500 µg/mL was prepared by taking an amount of injection powder equivalent to 50mg, dissolved in 50mL of double distilled water, sonicated, filtered through 0.45µm filter and made up to 100mL. This stock solution was further diluted to obtain the working standard solutions of concentration 80 µg/mL for method A and 120 µg/mL for method B. Color was developed by following the above procedure in two methods and absorbance was obtained, the percent of drug present in test solution was determined by comparing the absorbance of test and standard. The experimental results were presented in Table-3

Table 3: **Assay of Doripenem in pharmaceutical formulations**

Sample	Labeled amount (mg/injection)	Amount obtained, mg		% Assay	
		Method A	Method B	Method A	Method B
1	500	492.08	496.83	98.42	99.37
2	500	500.12	497.64	100.02	99.53

CONCLUSION

These methods described in the present study were simple, rapid and accurate and were successfully applied to the determination of doripenem in commercial formulations. Therefore these can be applied in routine quality control of doripenem in raw material samples and pharmaceutical formulations.

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