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Development and Validation of UV Spectrophotometric Area Under Curve Method for Quantitative Estimation of Piperacillin and Tazobactam

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Abstract

The aim of the present work was to develop an accurate, precise, reproducible and economical UV spectrophotometric Area under Curve method for the estimation Piperacillin and Tazobactam. The UV– spectrophotometric method was carried out using methanol as a solvent. Area under the Curve in the range of 203-218nm and 208-216nm was selected for the analysis of Piperacillin and Tazobactam respectively. The method was validated according to International Conference on Harmonization guidelines and successfully applied to marketed pharmaceutical formulations. The method was found to be linear in the concentration range of 10-50 µg/ml (r2 =0.999) and 5-25µg/ml (r2 =0.998) with the regression equation y = 0.040x +0.012 and y =0.031x-0.006 for Piperacillin and Tazobactam, respectively. Satisfactory values of Percent relative standard deviation for the intra-day and inter-day precision studies indicated that method is precise. The developed method can be used for routine estimation of Piperacillin and Tazobactam in formulation.

Keywords

Area under curve, Piperacillin, Tazobactam, UV spectrophotometry, Quantitative estimation.

1. Introduction

Piperacillin/tazobactam is a combination antibiotic containing the extended-spectrum penicillin antibiotic piperacillin and the b-lactamase inhibitor tazobactam and is used to reduce the development of drug-resistant bacteria. Piperacillin [2S - [2a,5a,6b(S*)]] - 6 - [[[[(4 - ethyl - 2,3 - dioxo-1-piperazinyl) carbonyl] amino] phenyl-acetyl] amino-3, 3dimthyl - 7 - oxo - 4 - thia- 1 - azabicyclo - [3.2.0] heptanes - 2 - carboxylicacid belongs to the ureidopenicillin class and it is used for the treatment of serious infections caused by susceptible strains of microorganisms **(1)**. Tazobactam(2S,3S,5R) -3 -methyl-7-oxo-3 - (1H - 1,2,3-triazolylmethyl) - 4 - thia - 1- azabicyclo [3.2.0] heptanes-2-carboxylic acid - 4, 4 - dioxide is used in combination with beta-lactamase antibiotic as antibacterial **(2)**. Literature review revealed enormous analytical methods were reported for the estimation of Piperacillin and Tazobactam individually or in combination with

other drugs **(3-16)**. There is no UV spectrophotometric Area under Curve method available in the literature for Piperacillin and Tazobactam. So our aim is to develop and validate a new simple, rapid, accurate, specific and highly sensitive and economical UV spectrophotometric Area under Curve method for estimation of Piperacillin and Tazobactam.

2. Chemicals and reagents

Piperacillin and tazobactam was a gift sample from MPC division of CDRI. All chemicals used were analytical grade. Methanol (Loba chem Ar grade), in house mill Q water was used throughout the study.

3. Instrumentation

The UV visible (UV-Vis) spectrophotometric method was developed on a Perkin Elmer Lamba 25 UV-Vis double beam spectrophotometer, with spectral width of 1 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cells. Analytical balance (Shimadzu AUW-120D, Japan) was used for all weightings.

4. Preparation of piperacillin and tazobactam stock and sample solution

Accurately weighed 100 mg of Piperacillin and tazobactam were separately dissolved in methanol to give concentration of 50 µg/mL. The above solutions were used for further analysis.

5. Sample solution for UV

Sterile preparation of 4 gm of Piperacillin and 0.5 gm of Tazobactam was taken and diluted with 20 mL of sterile water. From above solution 10 mL was taken in 100 mL volumetric flask and make up with 100 mL by methanol. Sonicated for 15 min and filtered through 0.45 µm membrane filter. Further diluted to obtain a concentration of 50 µg/mL.

6. Selection of wavelength by area under curve method

It involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths λ_1-λ_2 and λ_3-λ_4. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The solutions of drugs were scanned in the range of 200-400 nm. For Area under Curve method, the sampling wavelength ranges selected for estimation of Piperacillin and Tazobactam were 203-218 nm (λ_1-λ_2) and 208-216 nm (λ_3-λ_4) respectively **(Figures 1 and 2)**. For determining the concentration of drugs by AUC method, the following equation was used. Amount of each drug was calculated using following formula.

A = absorbance of sample

A = molar absorptivity of sample

B = path length

C = concentration of sample

$$a = A/bc$$

Concentration of sample 0.05mg

By substituting mean absorptivity values in the equation given below,

$$A_1 = ax_1+ay_1$$

$$A_2 = ax_2+ay_2$$

A_1 = 93.2pip+512.6taz----------(i) at 203-218nm

A_2 = 146.2pip+410taz---------- (ii) at 208-216nm

Where, 93.2 and 146.2 are mean molar absorptivity values of PIP at (λ_1-λ_2) and (λ_3-λ_4) respectively. 512.6 and 410 are mean absorptivity values of TAZ at (λ_1-λ_2) and (λ_3-λ_4) respectively.

Where, 0.1184 and 0.1119 are the absorbance of mixed standards A_1 and A_2.

Then substitute value in A_1 and A_2 in above equation (i) and (ii)

0.1184 = 93.2pip+512.6taz

0.1119 = 146.2pip+410taz

7. Results and discussion

7.1. Method validation

The newly developed method was validated according to the International Conference on Harmonisation guidelines with respect to accuracy, precision (repeatability and reproducibility), linearity and range, Limit of Detection (LOD) and Limit of Quantitation (LOQ) and recovery studies.

7.2. Recovery

The recovery experiment was carried out at three different levels, i.e. 80, 100, and 120%. The percentage recovery was found to be in the range 93.84 - 97.45 for Piperacillin and 94.52 - 97.64 for Tazobactam. The low values of % relative standard deviation (RSD) are indicative of the accuracy and reproducibility of the method **(Table 1).**

7.3. Precision

Precision of the method was evaluated in terms of intra- and inter-day precision. Inter-day precision studies were performed in a same day, but different time intervals. The % RSD values of Piperacillin and Tazobactam were found to be 0.1669% and 0.5777%. Intra-day precision was carried out similarly but in two different days and the %RSD was calculated, the % RSD for Piperacillin and Tazobactam were found to be 1.357% respectively. The % RSD values indicate a good degree of precision within the specified range. This indicates the method is precise **(Tables 2 and 3)**.

7.4. Precision

It was observed that the optimized method was linear within a specific concentration and range of the drug. The calibration curve was plotted, the linearity range for Piperacillin and Tazobactam were found to be 10 to 50µg /ml and 5-25µg /ml. Linear regression equations and correlation coefficient (r_2) were, Piperacillin Y = 0.040x + 0.012 (r_2 = 0.999) and Tazobactam Y = 0.031x + 0.006 (r_2 = 0.998) **(Tables 4 and 5) (Figures 3 and 4)**.

7.5. Limit of detection and limit of quantitation

LOD and LOQ determination is based on the standard deviation of the response and slope. LOD of Piperacillin and Tazobactam was found to be 52.206µg/mL and 55.66µg/mL respectively, LOQ of Piperacillin and Tazobactam was found to be 158.2µg/mL and 168.66µg/mL respectively **(Table 6)**.

Figure (1): Overlain spectra of Piperacillin.

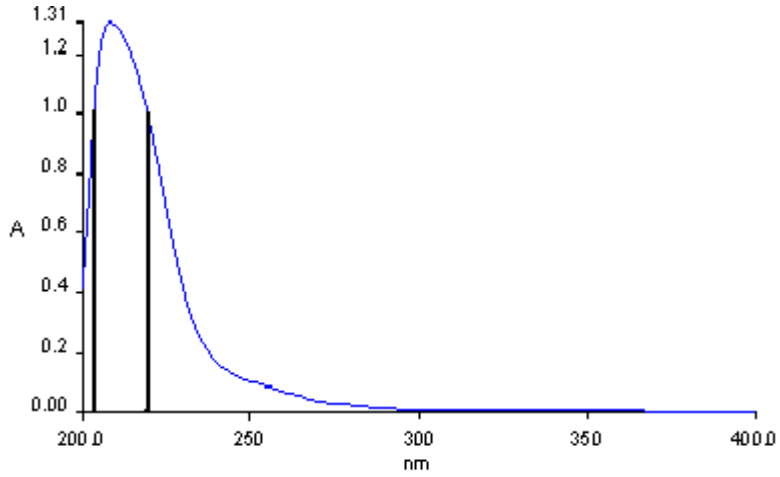

Figure (2): Overlain spectra of Tazobactam.

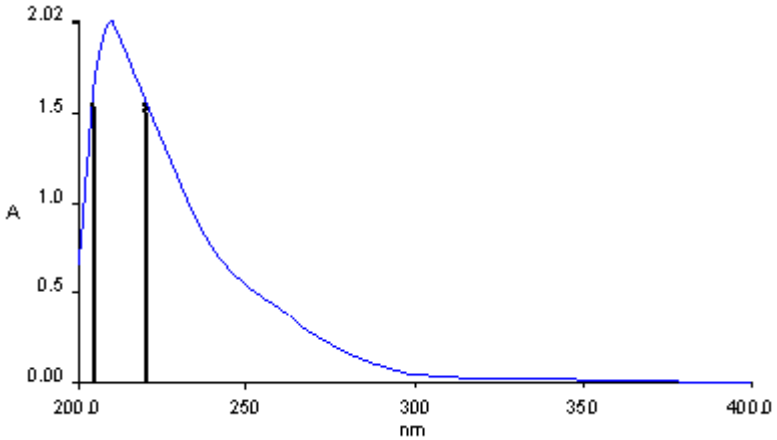

Table (1): Recovery studies for Piperacillin and Tazobactam.

Level	Amount Added	Mean Absorbance		Amount Recovery		% Recovery	
		Piperacillin	Tazobactam	Piperacillin	Tazobactam	Piperacillin	Tazobactam
80%	2.5	0.9985	0.398	24.34	24.98	96.45	97.64
100%	5	1.9971	0.796	49.98	49.63	97.9	96.63
120%	7.5	2.9955	1.194	74.76	74.78	93.84	94.52

Table (2): Intraday precision studies for Piperacillin and Tazobactam.

S.No	Absorbance	
	Piperacillin	Tazobactam
1	1.991	0.794
2	1.989	0.792
3	1.992	0.794
Mean	1.99	0.793
SD	0.001528	0.001155
%RSD	0.0767	0.1456

Table (3): Inter-day precision studies for Piperacillin and Tazobactam.

S.No	Absorbance					
	Piperacillin			Tazobactam		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day3
1	1.984	1.974	1.97	0.794	0.782	0.778
2	1.982	1.972	1.972	0.794	0.788	0.772
3	1.989	1.976	1.969	0.792	0.779	0.775
Mean	1.985	1.974	1.97	0.793	0.783	0.775
SD	0.00361	0.002	0.00153	0.00116	0.00458	0.003
%RSD	0.1816	0.1013	0.0775	0.1456	0.5853	0.387

Table (4): Linearity studies for Piperacillin.

Concentration(µg/mL)	Absorbance of Piperacillin	Statistical Analysis of Piperacillim
10	0.412	Slope = 0.040 Correlation coefficient = 0.999
20	0.822	
30	1.245	
40	1.652	
50	1.997	

Figure (3): Linearity of Piperacillin.

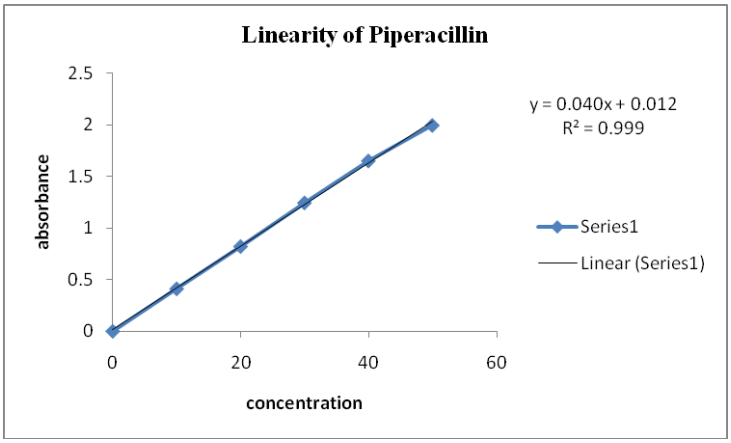

Table (5): Linearity studies for Tazobactam.

Concentration(µg/mL)	Absorbance of Tazobactam	Statistical Analysis of Tazobactam
5	0.154	
10	0.301	Slope = 0.015
15	0.465	Correlation coefficient=0.998
20	0.612	
25	0.798	

Figure (4): Linearity of Tazobactam.

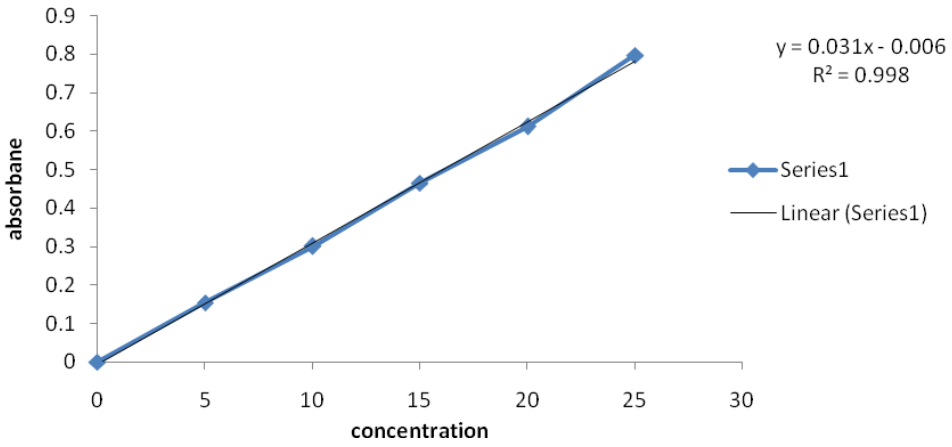

Table (6): LOD and LOQ for Piperacillin Tazobactam.

S. No	Concentration (µg/mL)	Absorbance	
		Piperacillin	Tazobactam
1	10	0.412	0.154
2	20	0.822	0.301
3	30	1.245	0.465
4	40	1.652	0.612
5	50	1.997	0.798
SD (σ)		0.6328	0.253
Slope (m)		0.04	0.015
LOD (3.3 × σ / Slope)		52.206 µg/mL	55.66 µg/mL
LOQ (10 × σ / Slope)		158.2 µg/mL	168.66 µg/mL

8. Conclusion

The results of the present study indicated that the developed method is simple, precise and cost effective for routine quality control analysis in bulk and pharmaceutical formulation.

The developed and validated UV method outlined is very obvious, affordable, dynamic, low cost, rapid easy to perform with small sample volume and good repeatability. It can be adopted for the routine quality control analysis of simultaneous determination of Piperacillin and Tazobactam.

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