METHOD DEVELOPMENT AND VALIDATION OF AMOXICILLIN TRIHYDRATE IN BULK AND SOLID DOSAGE FORM BY UV SPECTROSCOPY

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ABSTRACT:

Objective: A new, simple, sensitive, precise and reproducible UV spectroscopic method was developed for the estimation of Amoxicillin Trihydrate in bulk and Solid Formulation.

Methods: The UV spectrum of Amoxicillin Trihydrate in 0.1% TFA showed λ max at 228 nm. Beer's law is valid in the concentration range of 10-50µg/ml. This method was validated for linearity, accuracy, precision, ruggedness and robustness.

Results: The method has demonstrated excellent linearity over the range of 10-50µg/ml with regression equation y = 0.021-0.122 and regression correlation coefficient r^2 = 0.999. Moreover, the method was found to be highly sensitive with LOD (1.57µg/ml) and LOQ (4.76µg/ml).

Conclusion: Depending on results the given method can be successfully applied for assay of Amoxicillin Trihydrate in Solid formulation.

Keyword: Amoxicillin Trihydrate, UV spectroscopy, method development and validation, 0.1% TFA, Solid Formulation.

INTRODUCTION:

Amoxicillin Trihydrate is an antibiotic used to treat a number of bacterial infections. These include middle ear infection, strep throat, pneumonia, skin infections, and urinary tract infections among others .It is taken by mouth, or less commonly by injection.

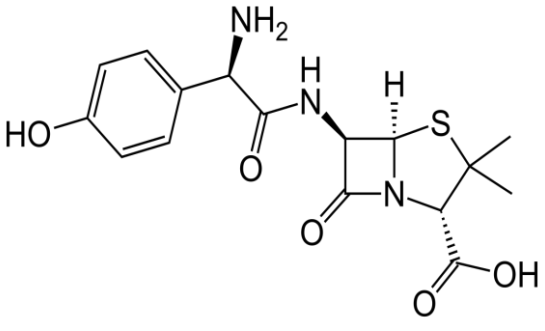

Figure1: Structure of Amoxicillin Trihydrate

Amoxicillin Trihydrate is soluble in methanol: water, TFA and Ethanol. The aim of this study is to give a new, simple, sensitive, precise and reproducible UV spectroscopic method was developed for the estimation of Amoxicillin Trihydrate in bulk and Solid formulation[1-3].

MATERIALS AND METHOD:

Materials

Amoxicillin Trihydrate was taken as gift sample from, Medcef Pharma, Delhi. 0.1% TFA was taken from local market.

Instruments:

Analytical balance (Labmann), Sonicator (Microclean-1103), UV-Visible spectrophotometer (Systronics 2201).

Experimental:

Preparation of standard stock solution:

Accurately weighed 10mg of Amoxicillin Trihydrate transferred to 100ml volumetric flask. It was dissolved in 0.1% TFA& sonicated for 5 minutes. The volume was made up to mark with same diluent to make up final strength.

Procedure for plotting calibration curve:

For calibration curve in a series of 10 ml volumetric flasks, 1-5 ml of standard solution was pipetted out separately. The volume was completed to the mark using0.1% TFA. The absorbance was measured at wavelength 228 nm against blank solution.

Analysis of Amoxicillin Trihydrate in Tablet Formulation:

10 mg equivalent Amoxicillin Trihydrate tablet was weighed and transferred to the 100ml volumetric flask and dissolved in 0.1% TFA as a solvent. After that sonicated for 5min and vortex for 2min. 4 ml of above solution was pipetted out and transferred to the 10ml volumetric flask and make up the volume upto the mark with same solvents and analysed at 228nm. Calculate the % purity of Amoxicillin Trihydrate.

RESULTS AND DISCUSSION:

The absorption spectrum shows λ max of Amoxicillin Trihydrate at 228nm.

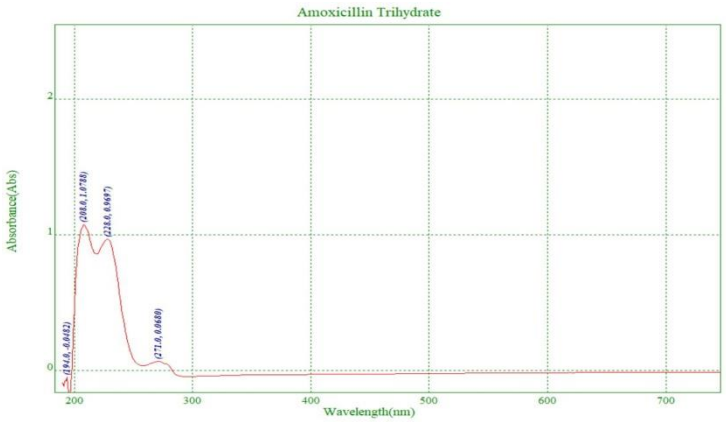

Figure 2: UV spectrum of Amoxicillin Trihydrate

The proposed method was validated according to ICH Q28 R1 guidelines for validation of analytical procedure.[4-8]

Linearity:

Five different concentrations of Amoxicillin Trihydrate were prepared and analysed at wavelength 228nm. The regression coefficient was found to be 0.999. The absorbance was found in limit i.e. 0-2. Hence the analysed parameter was found to be validated (table 1).

Table 1: Results of Linearity

Sr.no.	Concentration(µg/ml)	Absorbance
1	10	0.1042
2	20	0.3021
3	30	0.523
4	40	0.7401
5	50	0.9697

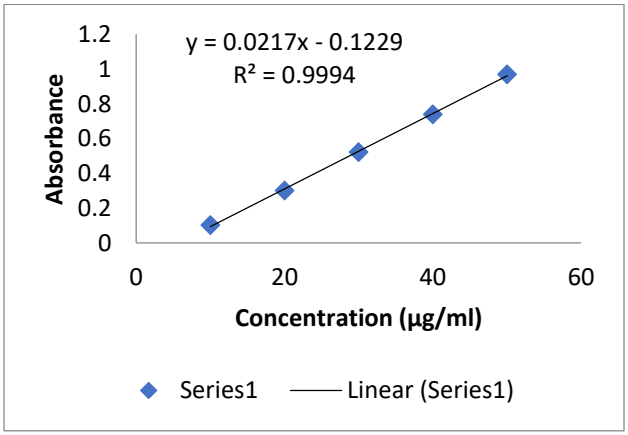

Figure 3: Calibration curve for Amoxicillin Trihydrate (Conc. vs. Abs.)

Table 2: Optimization parameters of Amoxicillin Trihydrate

Parameters	Method values
Maximum Wavelength	228nm
Beer's Law	10-50µg/ml
Correlation Coefficient (r^2)	0.999
Regression Equation	y = 0.0021x - 0.122
Slope (m)	0.0021
Intercept (c)	0.122

Accuracy:

The concentration 40, 50, 60µg/ml was taken as 80,100,120% and % recovery was found to be in range 99%-101%. Hence the parameter was found to be validated.

Table 3: Results of Accuracy

% Conc.	Reps	Spiked Conc. (ug/ml)	Abs	Amt Recovered (ug/ml)	% recovery	AVG	STDEV	RSD
80	Rep 1	39.88	0.7401	22.83	57.24	57.27	0.02	0.04
	Rep 2	39.88	0.7405	22.84	57.27			
	Rep 3	39.88	0.7406	22.84	57.28			
100	Rep 1	49.85	0.9697	49.85	100.00	99.96	0.04	0.04
	Rep 2	49.85	0.9694	49.83	99.97			
	Rep 3	49.85	0.9689	49.81	99.92			
120	Rep 1	59.82	1.1230	69.28	115.81	115.85	0.04	0.04
	Rep 2	59.82	1.1238	69.33	115.89			
	Rep 3	59.82	1.1234	69.30	115.85			

Range: Range is an interval between highest and lowest concentration limit of the analyte i.e. 10-50µg/ml.

Precision:

In precision intra-day and inter-day precision were performed at concentration (50µg/ml). The obtained results were found within limit i.e. less than 2%RSD.

Table 4: Results of Intra-day Precision

Sr. no.	Concentration	Absorbance
1	(50µg/ml)	0.9697
2		0.9699
3		0.9692
4		0.9691
5		0.9689
6		0.9695
	SD	0.000422
	%RSD	0.04

Table 5: Results of Inter-day precision

Sr.no.	Concentration	Absorbance (Day1)	Absorbance (Day2)
1	(60µg/ml)	0.9697	0.9695
2		0.9699	0.9697
3		0.9692	0.9695
4		0.9691	0.9692
5		0.9689	0.9697
6		0.9695	0.9694
	SD	0.000422	0.00019
	%RSD	0.04	0.019571

Limit of Detection (LOD):

The limit of detection was found to be 1.57µg/ml (table 6).

Limit of Quantification (LOQ):

The limit of quantification was found to be 4.76µg/ml (table 6).

Table 6: Results of LOD and LOQ

LOD	1.57 µg/ml
LOQ	4.76µg/ml

Ruggedness:
The change in analyst with same concentration and environmental condition didn't affect the results.

Table 7: Results of Ruggedness

Concentration	Absorbance (Analyst1)	Absorbance (Analyst2)
50µg/ml	0.9697	0.9701
	0.9699	0.9703
	0.9692	0.9705
	0.9691	0.9702
	0.9689	0.9701
	0.9695	0.9705
Average	0.969383	0.970283
SD	0.000422	0.000183

Robustness:
The change in wavelength (228nm and 232nm) and concentration (50µg/ml) didn't affect the results.

Table 8: Results of Robustness

Wavelength	228nm	232nm
Concentration	50µg/ml	50µg/ml
Absorbance	0.9697	0.9691
	0.9699	0.9693
	0.9692	0.9689
	0.9691	0.9691
	0.9689	0.9687
	0.9695	0.9689
Average	0.969383	0.969
SD	0.000422	0.00021

Assay:
The assay was performed by using amoxytor tablet at concentration 40µg/ml. The % purity was found to be 99.73%.

Table 9: Results of Assay

Formulation	Absorbance	Amount obtained	% purity
amoxytor tablet	0.9697	0.9671	99.73

CONCLUSION:
An analytical UV Spectrophotometric method was developed & validated thoroughly for quantitative determination of Amoxicillin Trihydrate in bulk drug and tablet formulation. The presented method was found to be simple, precise, accurate, rugged, reproducible and gives an acceptable recovery of the analyte, which can be directly easily applied to the analysis of pharmaceutical tablet formulation of Amoxicillin Trihydrate.

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