***Original Article***



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**NEW VISIBLE SPECTROPHOTOMETRIC METHODS FOR THE DETERMINATION OF AMOXICILLIN TRIHYDRATE IN**

**BULK DRUG & THEIR FORMULATIONS**

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## Abstract

A simple, sensitive and economical spectrophotometric method was developed for the determination of Amoxicillin trihydrate in pharmaceutical formulations. This method is based on the formation of pink colored chromogen complex by the reaction of drug with ferric chloride and 1,10-Phenanthroline, which absorbs maximally at 510 nm. Beer’s law is obeyed at a concentration range of 2-20 mcg/ml for method. This method has been successfully applied for the assay of the drug in pharmaceutical formulations.

**Keywords:** Amoxicillin trihydrate, Ferric chloride, 1, 10-Phenanthroline, Spectrophotometry.

## Introduction

Amoxicillin is susceptible to degradation by β- lactamase-producing bacteria, which are resistant to a broad spectrum of β-lactam antibiotics, such as penicillin. Chemically it is a (2S,5R,6R)- 6-

{[(2R)-2-amino- 2-(4-hydroxyphenyl)- acetyl] amino}- 3,3-dimethyl- 7-oxo- 4-thia- 1- azabicyclo[3.2.0] heptane- 2-carboxylic acid. This drug acts by inhibiting the synthesis of bacterial cell walls. It inhibits crosslinkage between the linear peptidoglycan polymer chains that make up a major component of the cell walls of both Gram-positive and Gram-negative bacteria. It has two ionizable groups in the physiological range (the amino group in alpha-

position to the amide carbonyl group and the carboxyl group).

The drug has been determined by variety of analytical techniques such as high performance liquid chromatography assay with 1,2,4 triazole and mercury chloride[Jun Haginaka and Junko Wakai Analyst, 1985, 110, 1277-1281] , spectroflourimetric study catalyzed by metal ions[P. Gutiérez Navarro, A. El Bekkouri and E. Rodriguez Reinoso *Analyst*, 1998, 123, 2263- 2266], Determination in fermentation media by high-performance liquid chromatography using pre-column derivatisation with 1-hydroxy benzotriazole [Ajit J.Shah, Maxwell W. Adlard and Geoffrey Holt Analyst, 1988, 113,

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1197-1200], Study of spectrophoto metric and mercurimetric methods[B. Nowak and H. Wollmann Pharmazie**,** 1987, 42(12), 862-863], Determination of cefixime in the presence of cloxacillin[A. O. Akanni and J. S. K. Ayim**,**Department of Pharmaceutical Chemistry, University of Ibadan, Ibadan, Nigeria], Simultaneous spectrophotometric and volumetric determinations[Qureshi SZ, Qayoom T, Helalet MI,Department of Chemistry, Analytical Research Laboratory, Aligarh Muslim University, India.]

The estimation of cefixime was carried out using different methods like spectrophoto -metric determination of cefixime [J. W. G. Smith, G. E. de Grey and V. J. Patel Analyst, 1967, 92, 247- 252], quality control assay [L.A.Okoro E.N.Ejike], Copper(II) complexation with cefixime [S.V.Lapshin and V.G.Aleksee], determination of spectro -photometric method with pyrocatechol violet [Amin AS], department of Chemistry, Faculty of Science, Benha University, Benha, Egypt.],Spectrophotometric determination of some cephalosporins with ammonium vanadate.[ Ibrahim el-SA, Beltagy YA, El-Khalek ], Studies on readymix suspension of cefixime trihydrate[Jafar m.\*, Aejaz a. Vol 2, Suppl 2, 2010].Different spectrophotmetric have been recommended which include Reaction of hydrochloric acid and potassium iodate followed by na2so4[analytical abstracts 1997], reaction of borate buffer with methanolic chloranil[analytical abstracts 1998], analytical investigation using paramolybdate anion[P.B.Issopoulos, J.Pharm, Biomed,analysis, 1998], spectrophotometric method by the reaction of Ce(III) ions complexed with arsenazo III [analytical abstracts 2001], This paper describes simple and sensitive spectrophotometric method. it includes formation

of pink colored complex by the reaction of drug with ferric chloride and 1,10-phenanthroline, which absorbs maximally at 510 nm.

## Materials and Methods

### Apparatus

Ultraviolet-Visible-Spectrometer SHIMADZU- 1700 with 1 cm matched quartz cells was used for all spectral measurements.

### Reagents and standards

All the chemicals used were of analytical grade.

1. **1,10-Phenanthroline AR grade (0.1008M)**: 2g of 1, 10 phenantroline is dissolved in 100 ml of methanol ARgrade.
2. **Ferric chloride hexahydrate AR grade** (0.2%W/V): 405mg of ferric chloride is dissolved in 100 ml of distilled water.

### Procedure

**Preparation of standard solution of amoxicillin trihydrate**:

Standard stock solution was prepared by dissolving 10mg of amoxicillin trihydrate in 100 ml of distilled water, sonicate for 15 min from these aliquots of standard solution taken to prepare 1,2,3,4,5,6,7,8,9,10µg/ml with dilution.

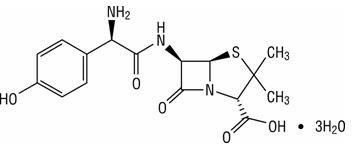
**Method: Recommended procedure for the determination of amoxicillin trihydrate in bulk drug -** Aliquots of working sample of drug containing 1-10 ml (1ml=100µg/ml) is transferred into a series of 10ml graduated test tubes. To each test tube 0.5ml of (0.1008M) solution of 1,10-phenanthroline and 0.3 ml of (0.012 M) solution of ferric chloride is added. These test tubes along with the blank were heated at a temperature of 700 c for 15 minutes. After heating these test tubes are cooled at room temperature and the volume is made up to 10ml using distilled water. The absorbance of the pink

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colored chromogen was measured at a maximal wavelength of 510nm against a reagent blank and the concentration was measured using calibration curve.



**FeCl3 1,10-phenanthroline**

**Procedure for the assay of amoxicillin trihydrate in pharmaceutical formulations** The methods was extended for the determination of amoxicillin Trihydrate from novamox formulations. The total contents of 20 amoxicillin trihydrate capsules were powdered and the powder equivalent to 10mg was dissolved in 100 ml of distilled water. The above solution was further diluted and analyzed as described in the above mentioned method for bulk drug. The procedure was repeated three times with novamox formulations.

## Results and Discussion

Iron(Fe) exihibits variable valency and exisists as ferrous (FeII) and ferric (FeIII) salts. Ferrous (Fe II) salts acts as a reductant and involved in

complex formation with 1,10-phenanthroline



### Pink colored complex Here, M = Fe

**Table 01: Optical characteristics & precision Parameters Method**

 max (nm) 510

Beer’s law limits 2-20 mcg/ml

Molar absorptivity 1.528 x 103(l/mol.cm) 0.109(mcg/ml/cm2/0.001

which have a tendency to get oxidized.

Sand ell’s sensitivity

Regression Equation\* (Y)

absorbance unit)

Drug when reacted with known amount of iron (FeIII) undergoes oxidation to give reduced form of ferric iron i.e. ferrous (FeII) ion which has a

Slope (m) 0.026

Intercept (c) 0.1311

Correlation Coefficient(r) 0.998

Precision\*\*

tendency to give coloured complex with 1,10-

phenanthroline.

(%Relative Standard Deviation)

0.37771

### Reaction Mechanism

The ferrous (FeII) ion formed by the oxidation of drug undergoes reaction with 3 molecules of 1,10-phenanthroline to form light pink coloured tris complex.

Standard error of mean 0.157471

Y= bC + a

Where C is the concentration of Amoxicillin trihydrate in mcg/ml and Y is the absorbance at the respective lambda max,\*\*for eight measurements.

**Table 02: Evaluation of Amoxicillin trihydrate in pharmaceutical dosage forms**

**Formulation (Brand)**

**Labeled Amount (mg/cap)**

**Amount Obtained By Proposed method**

**% Recovery\*\***

**±S.D**

Amoxicilin

|  |  |  |  |
| --- | --- | --- | --- |
| Amoxicilin | 250 | 250.2 | 100.1+/-0.023 |
| Amoxicilin | 250 | 249.5 | 99.8+/-0.013 |

250 251.5 100.6+/-0.037

trihydrate

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## Conclusion

A simple visible spectrophotometric method for the determination of amoxicillin trihydrate in pure and its dosage forms was developed. The absorbance of the chromogen was measured at maximum absorbance of 510nm against the corresponding reagent blank. The method is found to be simple, precise, economic, and less time consuming. The method has also been statistically evaluated and the results obtained are accurate, precise and free from the interferences of other additives present in the formulation.

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