



Estimation and validation of visible spectrophotometric method for confirmation studies on amoxicillin trihydrate of expired and non-expired in pharmaceutical dosage form

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ABSTRACT

Amoxicillin trihydrate is that the 4-hydroxy analog of ampicillin significantly used for the infections caused by pathogenic bacteria. The present investigation states that the study of antibacterial activity and spectrophotometric analysis with both expired and non-expired medication. The microorganisms were used for the antibacterial activity which was done by agar well diffusion method. *Escherichia coli*, *Pseudomonas fluorescence* bacteria were given the high cytotoxic effect in both expired and non-expired drugs of amoxicillin trihydrate were *Streptococci aureus* given the less inhibition zone once compare with gram -positive bacterium. The easy and precise spectrophotometric method was done to develop and validate the estimation of both expired and non-expired amoxicillin trihydrate in the dosage of 250mg. The calibration curves were formed in the concentration range of 10-80 μ g/ml. The correlation coefficient, linearity, LOD, LOQ and RSD values were found for both expired and non-expired drugs.

Key words: Amoxicillin trihydrate, antibacterial activity, spectrophotometry.

INTRODUCTION

Amoxicillin is first become introduced in 1972 [1]. It is one of the world health organization's list of essential medications the foremost necessary medications needed in the basic health system and its most typically prescribed antibiotics in youngsters [2]. Amoxicillin is an antibiotic helpful for the treatment of a variety of infections. And it is used for treatment for middle ear infections, strep throat, pneumonia, gonorrhea, skin infections, *acute otitis*, eubacterium raw throat, urinary tract infections, enteric bacterial infections, *Lyme* disorder and *Chlamydia* infections [3]

This is a moderate-spectrum, lysis, β -lactam antibiotic among the aminopenicillin family, with chemical formula, $C_{16}H_{19}N_3O_5S$. It's well absorbed, following oral administration, as compared to totally different β -lactam antibiotics. Amoxicillin is at a risk of degradation by the β -lactamase-producing microorganism that is proof against a slender spectrum of β -lactam antibiotics, like an antibiotic drug [4] [5] [6].

An antimicrobial is an agent that kills organisms or inhibits their growth which will be sorted in line with the microorganisms they act against. Antibiotics are used to combat bacteria and are classified in step with their operation. Antimicrobials that kill microbes are known as microbicidal and people inhabit their growth are known as microstatic. Antibiotics may be biological process and bacteriocidal [3]. These agents, together with antibiotics and alternative substances that kill (bactericidal activity) or inhibit the expansion (bacteriostatic activity) of microorganism.

The drug remains effective for a period of time and retains its real effect or potency. The cycle starts from producing the drug and its span time lies between producing date and expiry date [4] [5]. Drug demise period is indicated on

most medication labels, as well as prescription, over-the-counter (OTC) and dietary (herbal) supplements. It is mandatory by law that pharmaceutical producers should indicate demise dates on prescribed product and prior to marketing [7]

Drugs that are passed their termination date might not be toxic, however could have lost their efficiency or impact. Intake of such medicine won't be as effective because it is meant to be and should lead to infection-causing microbes resistant to the drug. If identical drug is run once more in future, there will be very little or no effect. The surroundings conditions during which the drugs are keep is additionally a determinant think about the time period of a drug. The drug should be stored in a cool and dry place but it is not exposed to heat, high humidness, or strong light. Prolonged exposure to light could induce chemical reactions, fixing the property of the drug, resulting in degradation. A decrease within the efficiency of a drug will cause antimicrobial resistance. The later additionally results from the inaccurate of the drug. Antimicrobial resistance has currently become a significant world drawback [8] [9]. Therefore, it's vital to require the precise drug with the specified efficiency at the acceptable time of storage to compact microorganism and fungous infections.

EXPERIMENTAL SECTION

Chemicals

All the chemicals used throughout this study were of analytical grade were made from Hi media laboratories.

Strain Solution

Streptococci aureus, *Pseudomonas fluorescense*, *Escherichia coli* was gifted by Marina Labs Pvt.Ltd and Chennai. Amoxicillin trihydrate (250mg) Non-Expired and Expired (within seven month of expired drugs) were obtained/collected from medical shops in Tindivanam.

Medium

The strain was grownup in liquid broth medium, ready exploitation 2g of tryptone, 1g of yeast extract and 2g of NaCl of Hi-media, dissolved in water. Autoclaved and keep at room temperature.

Agar Well Diffusion Method

The MIC (Minimum Inhibitory Concentration) test was performed [10]. The broth culture of *Pseudomonas fluorescense*, *Escherichia coli* and *Streptococci aureus* were fully grown till they had an absorbance or O.D of 0.8-1.0 at 600nm wavelength. Then they were spreaded on LB agar to the petriplates containing bacterial cells. Amoxicillin trihydrate of termination and non expired drugs in different concentration of 1mg/ml, 0.75mg/ml, 0.25mg/ml and 0.5mg/ml were added in the four wells and control created by gel puncture method at appropriate distance on LB agar in the petriplates. The plates were incubated at 37°C for incubated at 24hrs. The zone of inhibition (cm) was observed and further it was measured.

ESTIMATION OF AMOXICILLIN TRIHYDRATE

Preparation of reagent and standard stock solution

Accurately weighed 500mg of ninhydrin powder was transferred to a 100ml of conical flask, dissolved in 100ml of distilled water. 250mg of amoxicillin trihydrate (stock solution) was transferred to a 100ml of conical flask, dissolved in 10ml of distilled water

Methodology

2ml of standard stock solution of amoxicillin trihydrate of Expired and Non Expired Drugs were transferred to 10ml test tubes. 1.5ml of ninhydrin reagent was added and mixed. The test tubes were immersed in a water bath at 92±1°C for 20 minutes and it cooled to room temperature and the volumes in each test tube was adjusted to 10ml with distilled water. The absorbances of the coloured solution was scanned in the range of 400-800nm. The Maximum absorbance was obtained at 575nm.

OPTIMIZATION OF DIFFERENT CONCENTRATIONS

Effect of concentration of ninhydrin reagent

2ml of stock solution of amoxicillin trihydrate of Expired and Non Expired Drugs were transferred to 10ml test tubes. To each test tubes, different volumes of ninhydrin reagent (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0ml) were added and mixed. The test tubes were immersed in a water bath at 92±1°C for 20 minutes and it cooled to room temperature and the volumes in each test tube was adjusted to 10ml with distilled water. The absorbances were obtained at 575nm. [11]

Effect of temperature

2ml of stock solution of amoxicillin trihydrate of Expired and Non Expired Drugs were transferred to 10ml test tubes. To each test tube, 1.5ml of ninhydrin reagent was added and mixed. The test tubes were immersed in a water bath at different temperatures (40°C, 50°C, 60°C, 70°C, 80°C and 90°C) for 20minutes and it cooled to room temperature and the volumes in each test tube was adjusted to 10ml with distilled water. The absorbances were obtained at 575nm. [11]

Effect of Time

2.0ml of stock solution of amoxicillin trihydrate of Expired and Non Expired Drugs were transferred into 10ml test tubes. To each test tube, 1.5ml of ninhydrin reagent was added and mixed. The test tubes were immersed in a water bath at 92±1°C for different time intervals (5,10,15,20,25,30,35,40,45,50,55 and 60minutes and it cooled at room temperature and therefore the volume in every tubes were adjusted to 10ml with water. The absorbances of the ensuing solutions were measured at 575nm. [11]

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [11]

Linearity

Calibration curve were plotted over a concentration range of 10-80µg/ml for amoxicillin trihydrate of Expired and Non Expired drugs. Accurately measured Standard stock solution of amoxicillin trihydrate (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0ml) was transferred into 10ml test tubes. To each test tube, 1.5ml of ninhydrin reagent was added and mixed. The test tubes were immersed in a water bath at 92±1°C for 20minutes and it cooled to room temperature and the volumes in each test tube was adjusted to 10ml with water. The absorbances of the resulting solutions were measured at 575nm.

Method precision (% Repeatability)

The precision of the instrument were checked by repeated scanning and measurement of the absorbance of solutions (n=6) of amoxicillin trihydrate (250mg) without changing the parameters for the method. The repeatability was expressed in terms of relative standard deviation (%RSD).

Intermediate Precision (Reproducibility)

The intraday and interday precision of the proposed method were performed. By analyzing the corresponding responses three times on the same day and on three different days over a period of one week for different concentrations of standard solutions of amoxicillin trihydrate of expired and non-expired drugs.

Limit of Detection (LOD)

Detection limit is the smallest concentration of a solution of an element that can be detected with 95% certainty [12] [13]. This is the quantity of the element that gives a reading equal to twice the standard deviation of a series of any least ten determinations taken with solutions of concentrations which are close to the level of the blank. Based on the standard deviation of the reagent blank and the slope of the calibration curve of the analyte, the detection limit (DL) may be expressed as:

$$D_L = 3.3\sigma/S$$

Where σ =the standard deviation of the reagent blank

S = the slope of the calibration curve

Limit of Quantification (LOQ)

The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte with those of blank samples and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision [14] [15]. Based on the standard deviation of the reagent blank samples and the slope of the calibration curve of the analyte, the quantification limit (QL) may be expressed as:

$$Q_L = 10\sigma/S$$

Where σ =the standard deviation of the reagent blank

S = the slope of the calibration curve

RESULTS AND DISCUSSION

An antibiotic act on several microbes and disrupt their cell wall and their components and also inhabit their protein synthesis and finally kill the bacteria. Most of the β -lactams are the antibacterial agents. A minimum inhibitory concentration is the method which is used to estimate the drug sensitivity against the bacteria or microorganisms. Amoxicillin trihydrate is oral semi synthetic penicillin structurally related to ampicillin and the presence of a benzyl ring in the side chain extends the antibacterial activity to gram negative bacteria [16]. Antibacterial activity for both expired and non expired drugs were determined using agar well diffusion method. A gram negative of *Pseudomonas fluorescence*, *Escherichia coli* highest zone of inhibition in both expired and non expired amoxicillin trihydrate (Fig: 1, 2) where *Streptococci aureus* given lowest zone of inhibition when compare with gram positive bacteria.

Fig 1: graph showing antibacterial activity different concentration of Non-Expired drugs of amoxicillin trihydrate

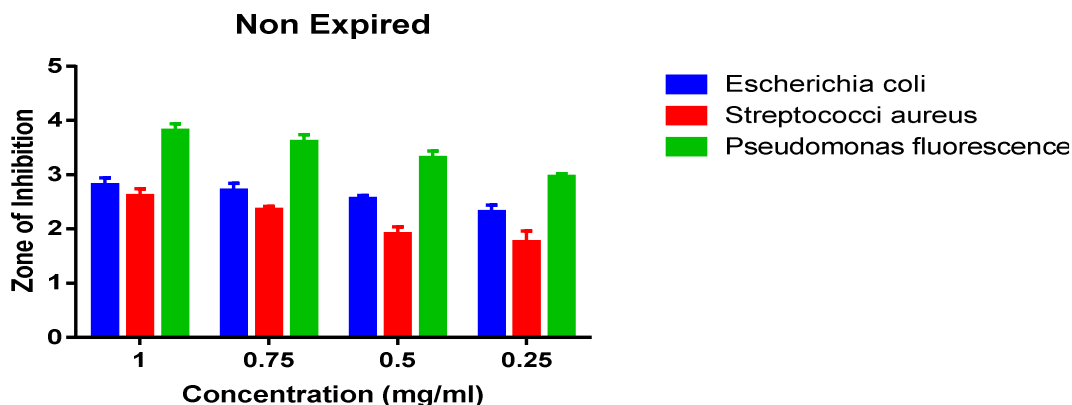


Fig 2: graph showing antibacterial activity different concentration of Expired drugs of amoxicillin trihydrate

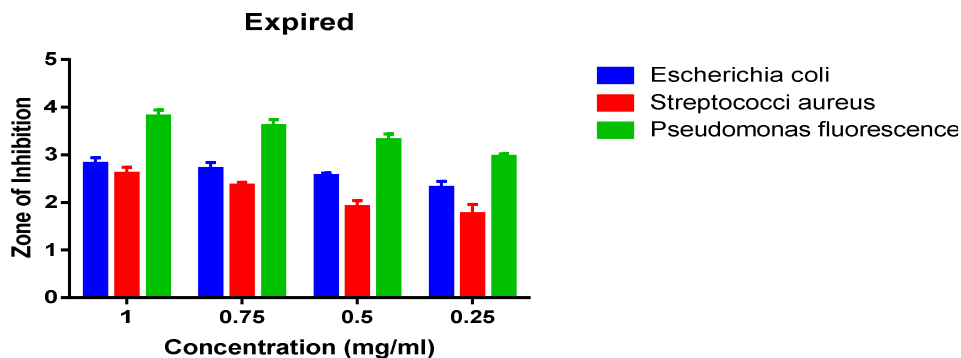


Fig 3: Overlain absorption spectra amoxicillin trihydrate in both Expired and Non Expired drugs in distilled water

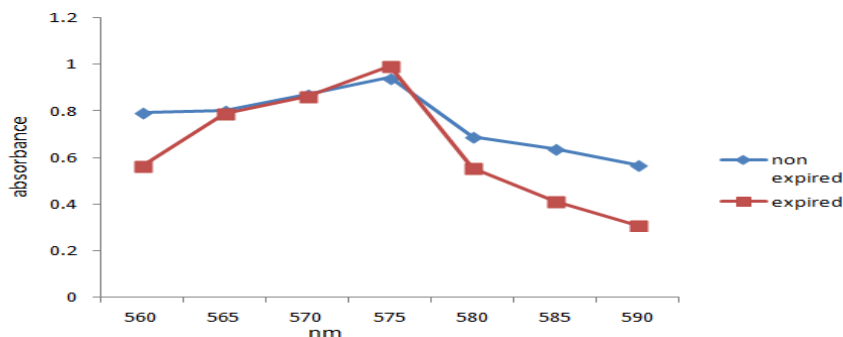


Fig 4: Spectra of amoxicillin trihydrate in Expired and Non Expired drugs with ninhydrin reagent in distilled water at 575nm

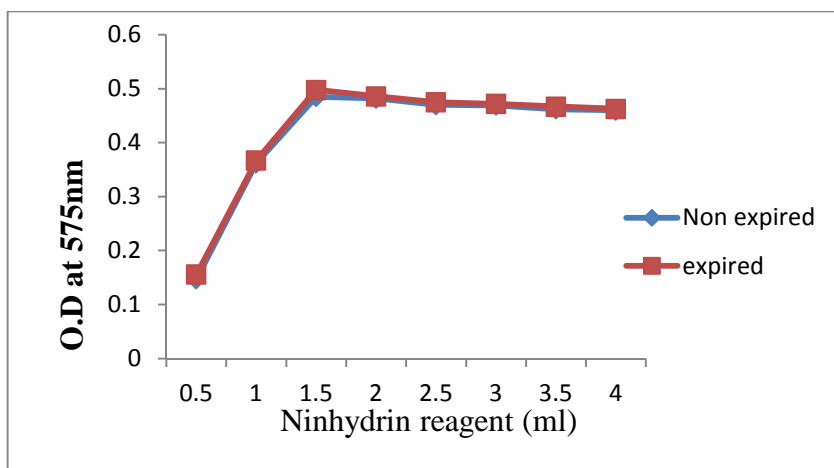


Fig 5: Optimization of heating temperature (°C) of amoxicillin trihydrate at 575nm with distilled water in Expired and Non Expired drugs

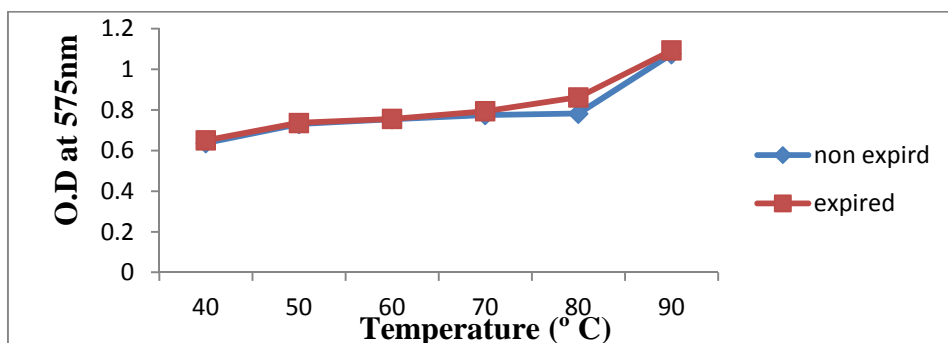


Fig 6: Optimization of heating time (minutes) at 575nm with distilled water in Expired and Non Expired

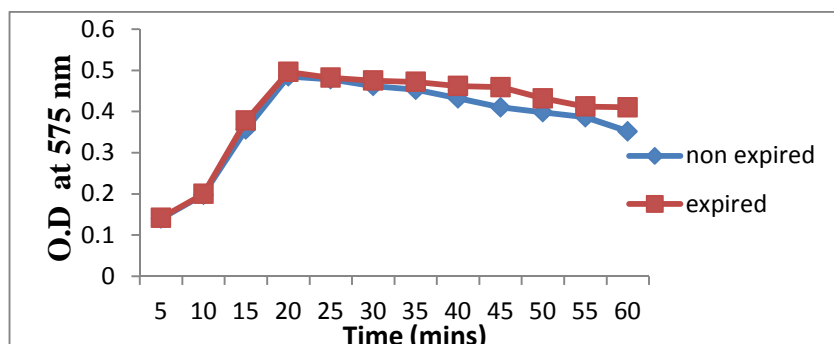


Fig 7: Calibration curve of amoxicillin trihydrate in Expired and Non Expired drugs at 575nm

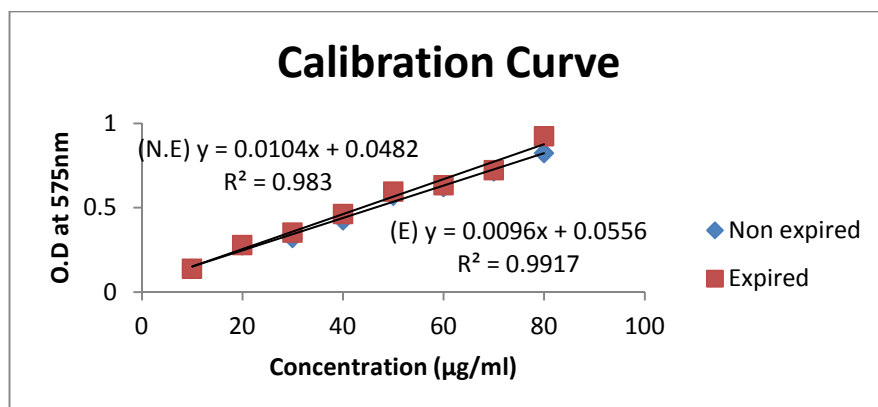


Table:1 Regression analysis data and summary of validation parameters for the proposed method

S.NO	PARAMETER	AMOXICILLIN TRIHYDRATE	
		EXPIRED	NON EXPIRED
1	Wavelength (nm)	575nm	575nm
2	Beer's law limit ($\mu\text{g/ml}$)	10-80	10-80
3	Regression equation	Y=0.0104x+0.0482 Slope=0.0104 Intercept=0.0482	Y=0.096+0.0556 Slope=0.0960 Intercept=0.0556
4	Correlation Coefficient (r^2)	0.9830	0.9917
5	Limit of detection (LOD) (μgml^{-1})	0.0312	0.0300
6	Limit of quantification (LOQ) μgml^{-1}	0.0102	0.0010
7	Repeatability (n=6) %RSD	1.058	0.963
8	Reproducibility Intraday (n=3) (S.D \pm %RSD)	0.09 \pm 0.22	0.058 \pm 0.25
		0.26 \pm 0.30	0.11 \pm 0.31
		1.13 \pm 0.86	0.14 \pm 0.33
9	Interday (n=3) (S.D \pm %RSD)	0.39 \pm 0.89	0.09 \pm 0.42
		0.80 \pm 0.91	0.18 \pm 0.53
		1.59 \pm 1.20	0.40 \pm 0.83

The detection of free amino and carboxyl groups in proteins and peptides were founded by ninhydrin reagent, it can be recognized appearing purple colour [17]. The amino group containing molecules of amino acids, protein and peptides when reacted with ninhydrin form ammonia, carbon dioxide it turned into purple blue complex when it is heated at proper condition [18][19]. So it suggested that ninhydrin reagent used to estimation of amino group containing drugs like amoxicillin trihydrate.

The working standard solutions of Amoxicillin trihydrate were prepared separately in distilled water. They were scanned in the wavelength range of 400-800 nm. The maximum absorbance was obtained at 575nm for amoxicillin trihydrate in both expired and non expired drugs respectively (Figure 3). These two wavelengths were employed for the determination of amoxicillin trihydrate.

In the proposed method, the amoxicillin trihydrate both Expired and Non Expired; it was found that 1.5ml of 0.5% w/v ninhydrin reagent (Figure 4), 90 \pm 1 $^\circ$ C heating temperature (figure 5) and 20minutes heating time was sufficient for the development of maximum colour intensity. Stability study of the developed chromogen was carried out by measuring the absorbance values at a time intervals of 20minutes (Figure 6).

Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curves were linear over the concentration range of 10-80 $\mu\text{g/ml}$ for Amoxicillin trihydrate. Absorbances values were plotted against respective concentrations and linear regression analysis was performed on the resultant curves. Correlation coefficient were found to be ($R^2 = 0.9917$) and ($R^2 = 0.983$) for both Expired and Non Expired drugs respectively (Figure 7). The method precision (Repeatability), the RSD values of Amoxicillin trihydrate in both expired and non expired drugs were found to be 1.058 and 0.963 at 575nm respectively. Low value of RSD indicates that proposed method is repeatable (Table 1).

The low RSD values of interday (0.39-0.89, 0.80-0.91 and 0.1.59-1.20) for amoxicillin trihydrate in Expired drugs, and (0.09-0.42, 0.18-0.53 and 0.40-0.83) Amoxicillin trihydrate in Non Expired drugs at 575nm. And intraday (0.09-0.22, 0.26-0.30 and 0.1.13-0.86 for Amoxicillin trihydrate in expired dugs, and (0.05-0.25, 0.11-0.31 and 0.14-0.33) for amoxicillin trihydrate in non expired drugs at 575nm (Table 1).

The LOD of amoxicillin trihydrate both Expired and Non Expired was found to be 0.0312 $\mu\text{g/ml}^{-1}$ and 0.0300 $\mu\text{g/ml}^{-1}$, and LOQ was found to be 0.0102 $\mu\text{g/ml}^{-1}$ and 0.0010 $\mu\text{g/ml}^{-1}$ respectively (Table 1).

CONCLUSION

The present investigation revealed that the study of antibacterial activity and spectrometric method with both expired and non-expired drugs. The cell protection and their development of resistance to antibiotics is increasing, demanding the need for the continual cycles of discovery and development of new antibiotics. Every company manufacturing the antibiotics should ensure that the risk to benefit ratio is less. The efficacy of the antibiotics after the expiry will lead to lose its chemical integrity and also play a role in the development of antibiotic resistance .So, the Government should take proper measures that the expired drugs are not in use. The proposed visible spectrophotometric method was found to be, simple sensitive, precise, LOD, LOQ, Correlation coefficient for

determination of amoxicillin trihydrate in expired and non-expired tablet dosage form. Hence this method can be easily used for routine quality control of the drug in pharmaceutical dosage form.

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