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Spectrophotometric Determination of Ampicillin Using Chromogenic Reagents (MBTH and PDAC) and their Application in Pharmaceutical Formulations

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ABSTRACT

The present work highlights the spectrophotometric method development and validation for the assay of ampicillin, a broad-spectrum β-lactam antibiotic. Two methods have been proposed by employing popular chromogenic reagents viz 3-methyl 2-benzothiazolinone hydrazone (MBTH) and para dimethyl amino cinnamaldehyde (PDAC). Method A involves the coupling of ampicillin with MBTH in the presence of ferric chloride through the oxidative mechanism, resulting in the formation of green coloured solution with a maximum absorption peak at 620 nm. In method B, the condensation reaction of ampicillin with an acidic solution of PDAC gave a coloured reddish-brown complex, showing a maximum absorption peak at 514nm. Linear calibration curve for MBTH and PDAC has been obtained while obeying Beer's law in the concentration range of 0.5 -1 and 5-15 µg/ml having a good correlation coefficient of 0.9985 and 0.9974 respectively. The sensitivity of the submitted methods was found to be more than the reported methods. Both methods were successfully applied to a pharmaceutical formulation for quantifying the ampicillin effectively. Therefore, the developed spectrophotometric methods can serve as a good analytical assay procedure for ampicillin to undergo quality control in pharmaceutical setups.

Keywords: *Ampicillin, Spectrophotometry, 3-methyl 2-benzothiazolinone hydrazone (MBTH), Para dimethyl amino cinnamaldehyde (PDAC), Method Development.*

Introduction

Ampicillin (Fig 1), chemically named as 2S,5R,6R)-6-([(2R)-2-amino-2-phenylacetyl] amino) -3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2- carboxylic acid, belongs to β- lactam, a broad spectrum antibiotic. It acts as a bactericidal by inhibiting the third and final stage of cell wall synthesis. Because of its broad-spectrum action, it is effective against infections involving the middle ear, the urinary bladder, kidney, sinuses and also gonorrhoea (1). Besides ampicillin can penetrate the cerebrospinal fluid and exert its action against meningitis (2).

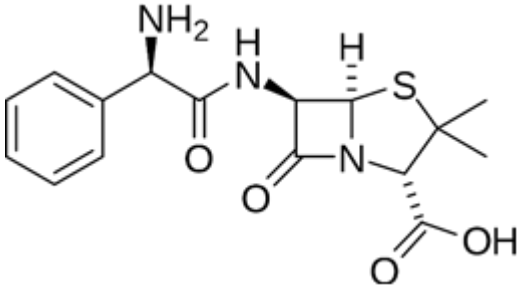


Figure 1: Structure of ampicillin.

Recently, various ampicillin derivatives and their metal ion complexes have been developed for enhancing their biological properties (3). Various analytical techniques such as; HPLC (4), voltammetry (5), titrimetric (6), and fluorimetry (7) have been used for the determination of ampicillin. Among several techniques, spectrophotometric methods are the preferable choice due to the ease, sensitivity, cost-effectiveness and reliability for the determination of drugs in pharmaceutical analysis. Chromogenic agents are chemical compounds used to detect or measure the presence of the analyte in a sample. These reagents work by producing a visible colour change when it comes into contact with the analyte under investigation. The colour changes detect the analyte effectively both qualitatively and quantitively. Literature survey revealed that ampicillin has been determined previously using various chromogenic reagents namely; follin ciocalte (FC) (8), and 1,2-naphthoquinone-4-sulfonic (NQS) (9). However, two very important chromogenic reagents viz 3-Methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) & p-dimethylamino cinnamaldehyde (PDAC) have not been reported yet.

MBTH is a reagent which determines the carbonyl compounds sensitively and is also utilized for the detection of phenols, polyhydroxy aldehydes and aromatic amines. MBTH has been used for the estimation of drugs containing several heterocyclic rings such as acyclovir (10), ganciclovir (11), and cefadroxil (12). Similarly, PDAC is also a popular chromogenic reagent used for the estimation of drugs containing hetero nitrogen moieties such as sympathomimetic amines like methyldopa and noradrenaline (13) and also zolmitriptan in bulk and pharmaceutical formulation (14). Besides MBTH and PDAC are greener reagents because they do not belong to PBT (Persistent, bioaccumulative, and toxic) or Hazardous list and are therefore less toxic (15).

Therefore, the present work aimed to develop a sensitive spectrophotometric method for the estimation of ampicillin using two important chromogenic reagents viz., PDAC and MBTH. Further, to apply the developed method for quantifying ampicillin in a finished marketed formulation.

Materials and Methods

Instrument

UV-Visible Spectrophotometer (UV-1875, Shimadzu corporation, Kyoto, Japan) equipped with two similar quartz cells was used for absorbance measurements. Precision weighing balance (GF-3002A, Haryana, India), involved in weighing the sample.

Reagents and standards

Each of the analytical grade chemicals and double distilled water was used throughout the experiment. MBTH and PDAC (AR Grade) were purchased from Sisco Research Laboratory (SRL) Pvt. Ltd., Mumbai, India. MBTH was accurately weighed (0.2 gm) and transmitted into a 100 mL calibrated volumetric flask. Distilled water was added in a small amount to make up the volume with 0.2% w/v concentration. PDAC (0.5 % w/v solution) was weighed as 0.5gm and 100 mL of methanol was added using a 100 mL calibrated volumetric flask. To make ferric chloride (0.5% w/v) solution, 0.5gm of ferric chloride was dissolved in 100 mL of water.

Ampicillin trihydrate as a reference standard was obtained as a gift sample from Hetero Labs Ltd., Hyderabad, India.

Standard stock solution (1mg/ml): 100 mg of ampicillin was dissolved in 30 mL of 0.1 M HCl using a 100 ml calibrated volumetric flask. Distilled water was used for volumes made up to 100 ml.

Proposed procedure for the analysis of ampicillin

Method A: Different aliquots of the working standard were prepared to range between 0.2-1 µg/ml in a 10 mL volumetric flask and 2 mL of freshly prepared ferric chloride was added in each of the flasks followed by the addition of 2 mL of MBTH reagent in each of the tubes. The samples were successfully prepared and kept aside for 5 minutes. Distilled water was used to make the final volume up to 10 mL, the green colour of the sample obtained was observed to show absorbance at 620 nm when treated against the blank.

Method B: Aliquots of working standard of ampicillin were prepared using stock solution varying from 2-10 µg/ml. In each of the 10 ml of the volumetric flask, 0.5 ml of PDAC reagent was added followed by the addition of 1 ml HCl. The solution was kept aside for 20 minutes. Distilled water was used to make up the volume of up to 10 ml. The reddish-yellow-coloured sample was observed showing absorbance at 514 nm against a reagent blank prepared similarly except without the drug.

Procedure for the analysis of ampicillin in the finished formulation: A quantity equivalent to 100 mg of the drug from the 5 capsules was weighed accurately and transferred to a 100 mL of volumetric flask. The drug

content was dissolved in 50 mL of 0.1M HCl and the remaining volume was makeup with distilled water. The filtrate of the resultant solution was used for further reaction with selected chromogenic reagents as described under methods A & B and was analysed at 620 nm and 514 nm respectively.

Results

Validation protocol

The ICH Q2R1 guideline was used to assess the reliability of the proposed method and also the results obtained. The calibration curve of ampicillin using method A (MBTH) and method B (PDAC) are shown in figures 2 and 3 respectively and the regression parameters are summarized in Table 1. The linearity range of ampicillin was found to be 0.5 -1 and 5-15 µg/mL for methods A & B, respectively. The limit of detection (LOD) for the above-mentioned methods A and B were found to be 0.11 & 1.42 µg/mL while the limit of quantification (LOQ) was found to be 0.33 & 4.31 µg/mL. Besides, the high values of correlation coefficient (> 0.99) of both methods represented excellent linearity.

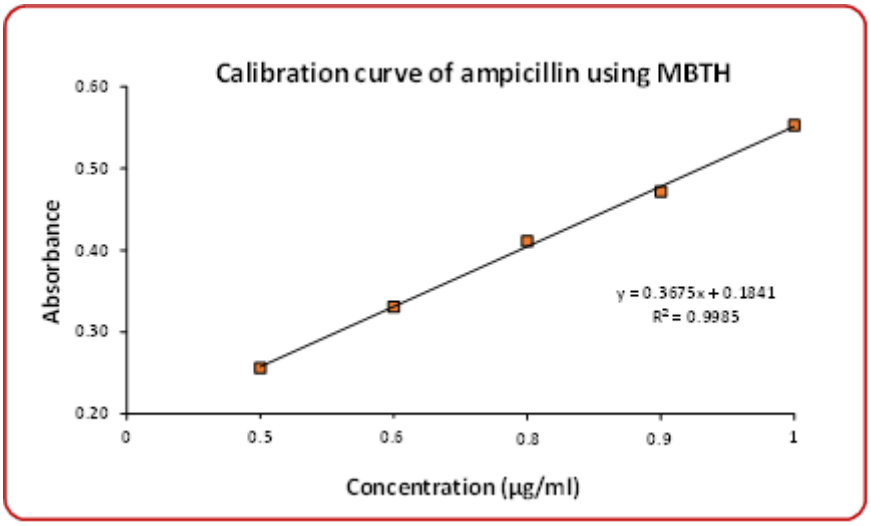


Figure 2: Calibration curve of ampicillin using MBTH at 620 nm.

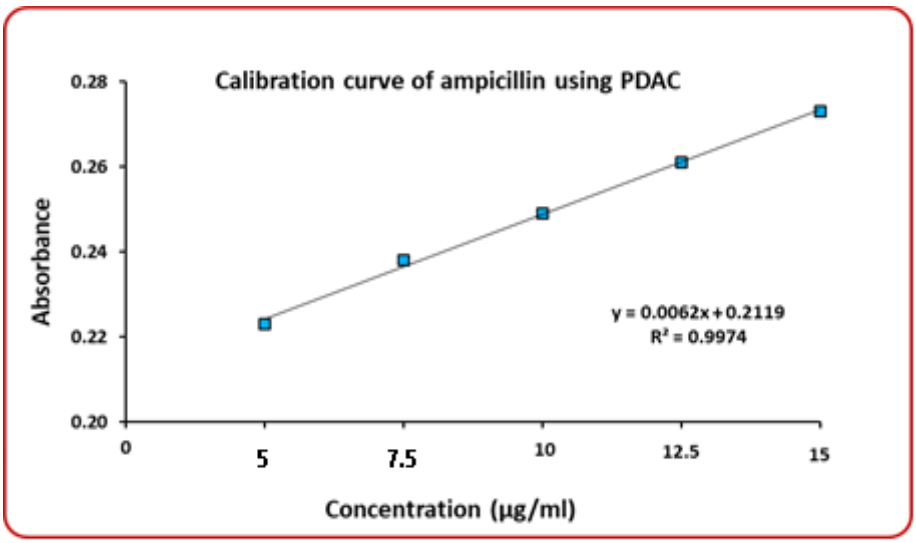


Figure 3: Calibration curve of ampicillin using PDAC at 514 nm.

Table 1: Analytical parameters of ampicillin using MBTH and PDAC.

Analytical parameters	Method A	Method B
λ_{max} (nm)	620	514
Linearity range (µg/mL)	0.2- 1	2-10
slope	0.3675	0.0062
y-intercept	0.1841	0.2119
Correlation coefficient	0.9985	0.9974
Detection limit (µg/mL)	0.11	1.42
Quantitation limit (µg/mL)	0.33	4.31
Range	0.5-1	5-15
LOD: Limit of detection; LOQ: Limit of quantification; RSD: Relative standard deviation; λ_{max}: Wavelength at maximum absorbance		

Recovery: Recovery studies are an essential parameter for validating the performance of the analytical method. Recovery studies have performed the determination of the accuracy of the result obtained. The recovered percentage of ampicillin for both methods A and B are summarized in Tables 2 & 3. The mean recoveries for methods A & B were found to be 96.51± 1.58 and 98.63 ±1.41, respectively.

Table 2: Percent recovery study of ampicillin in the proposed method A (MBTH).

Concentration taken (µg/ml)	Found concentration (µg/ml)	% Recovery
0.2	0.190	95.00
0.4	0.382	95.50
0.6	0.578	96.33
0.8	0.773	96.65
1.0	0.991	99.10
Mean; 96.51, Standard Deviations; 1.58; %RSD 0.13		

Table 3: Percent recovery study of ampicillin in the proposed method B (PDAC).

Concentration taken (µg/mL)	Found concentration (µg/mL)	% Recovery
2	1.923	96.15
4	3.958	98.95
6	5.948	99.13
8	7.942	99.27
10	9.968	99.68
Mean; 98.63, Standard Deviation; 1.41 %RSD; 0.42		

Precision: The essential feature in analysis is to avoid small, but deliberate variations in the process thereby. The inter-day and intraday precision were calculated by determining ampicillin, five times within the same day and alternative day at three different concentrations (Table 4). The % RSD for the inter-day of proposed methods A and B were in the ranges of 0.07 – 0.36 and 0.06 -0.43, respectively. Similarly, the %RSD for intra-day was in the ranges of 0.08 – 0.55 and 0.07 – 0.32 for methods A and B, respectively.

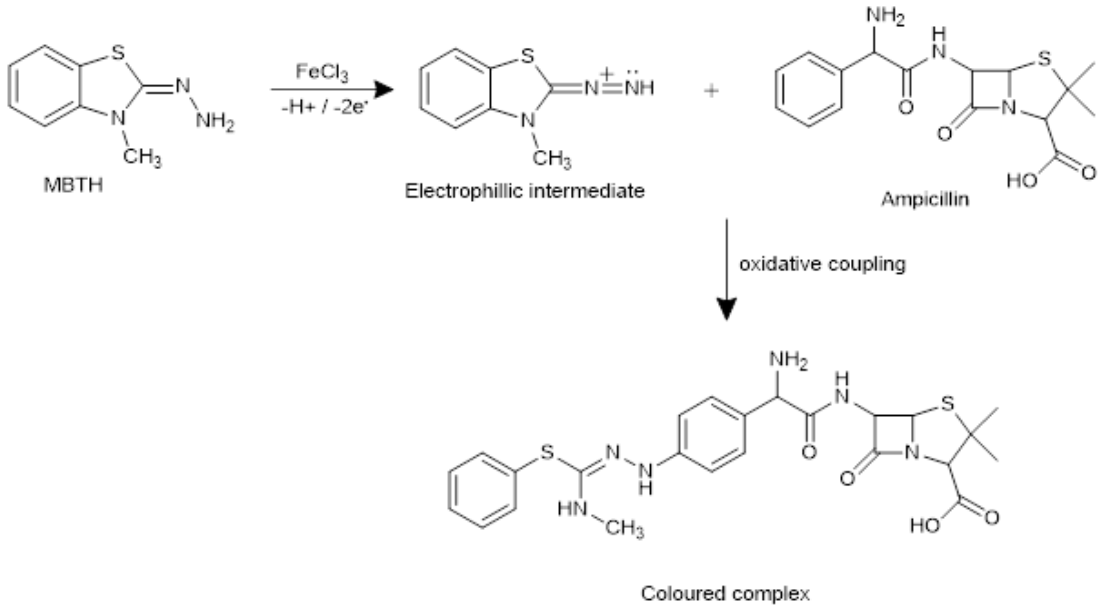
Table 4: Summary of the precision results of the proposed methods.

Proposed Methods	Concentration (µg/mL)		RSD (%)
	Taken	Measured ± SD	
Method A			
Inter-day assay	0.3	0.34 ± 0.02	0.36
	0.7	0.69 ± 0.03	0.52
	1	1.01 ± 0.08	0.07
Intra-day assay	0.3	0.33 ± 0.02	0.35
	0.7	0.71 ± 0.03	0.55
	1	1.02 ± 0.04	0.08
Method B			
Inter-day assay	5	4.98 ± 0.04	0.43
	10	9.82 ± 0.02	0.06
	15	14.88 ± 0.08	0.15
Intra-day assay	5	5.02 ± 0.03	0.13
	10	9.93 ± 0.05	0.32
	15	15.01 ± 0.06	0.07

Discussion

The method developed and validated was proved effective in the spectrophotometric estimation of ampicillin. The scan mode of spectrophotometry gives spectral bandwidth of 1 nm resolution, having a wide wavelength range of (190 to 1100 nm). The experiment was performed while maintaining the required criteria, especially the purity of the drug, storage conditions, and solubility and it was made sure that the solvent used did not react with the solute or took part in the reaction.

The mechanistic understanding of the reaction between ampicillin and the selected chromogenic reagents is shown in schemes 1 & 2. MBTH undergoes an oxidative coupling reaction with the aromatic ring of ampicillin via a two-step process. In the first step, MBTH forms an electrophilic intermediate which acts as an active coupling agent by losing two electrons and one proton. In the presence of iron (III), in the second step, coloured products are formed by the electrophilic attack of the intermediate on the aromatic ring of ampicillin (Scheme 1). While PDAC undergoes condensation reaction resulting in the formation of Schiff's base on reaction with the amino group of ampicillin. A loss of water molecule (dehydration process) occurs when a condensation reaction takes place between the primary amine and aldehyde group of PDAC resulting in a coloured product (Scheme 2).

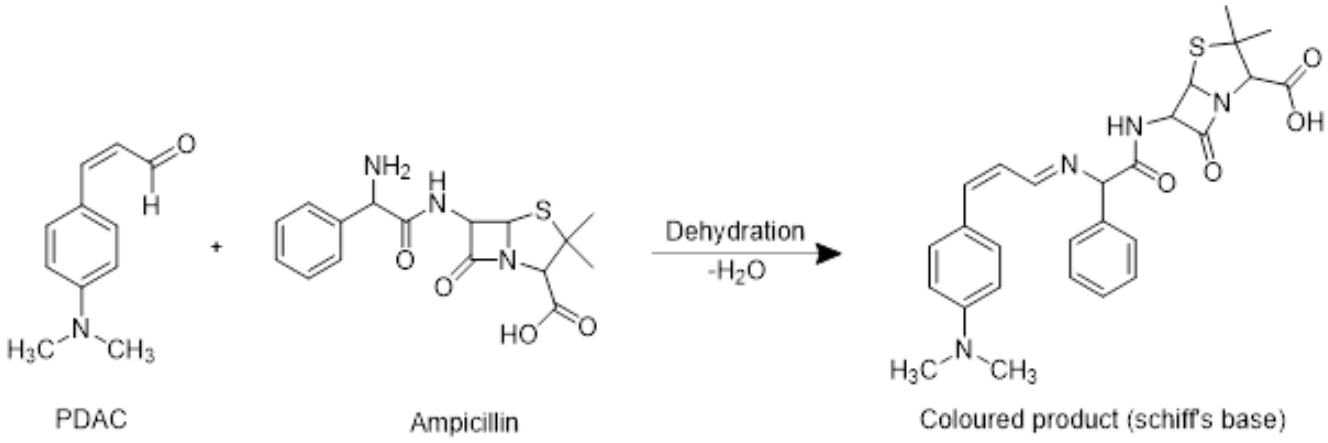


Scheme 1: Reaction involving the formation of coloured complex between MBTH & ampicillin.

As per the ICH Q2R1 guidelines, the above method was validated and was able to estimate the drug efficiently. The proposed method was successfully applied for the determination of ampicillin in the marketed pharmaceutical formulations and then compared with the reference concentration. The amount of ampicillin was found to be 98.63 ±1.42 and 97.51 ±1.63 using methods A and B, respectively (Table 5). The difference in the ranges of the drug sample using MBTH and PDAC revealed that the response function of the UV spectrophotometer for both the reagents had an acceptable level of precision along with accuracy and linearity. The overall error present in a particular experiment is considered minimum when the absorbance lies in the range of 0.2 to 0.9, hence, obeys the Beers-Lambert law satisfactorily.

Table 5: Determination of ampicillin using the marketed formulation of Ampicillin trihydrate.

Method	Labelled concentration (mg)	Amount found(mg)
A	100	98.63±1.42
B	100	97.51±1.63



Scheme 2: Reaction involving the formation of coloured complex between PDAC & ampicillin.

The sensitivity of the developed method was assessed by comparing the detection limit of proposed methods A & B with the reported methods. It was found that method A was more sensitive (LOD, 0.11 µg/mL) than method B (LOD, 1.42 µg/mL), vis-à-vis reported methods. On the other hand, proposed method B was shown to have a near equal detection limit with the reported NQS method while higher than the FC-based method (Table 6).

Table 6: Comparative assessment of the sensitivity of developed methods using different chromogenic reagents.

Chromogenic reagent	Detection limit (µg/mL)
3-Methyl-2-Benzothiazolinone Hydrazone (MBTH)	0.11 (present work)
Para-Di-amino cinnamaldehyde (PDAC)	1.42 (present work)
Folin-Ciocalteu (FC)	1.22 [8]
1,2-Naphthoquinone-4-sulfonate (NQS)	1.50 [9]

Conclusion

Two spectrophotometric methods were developed and validated as per the ICH Q2R1 guideline and were able to efficiently estimate the selected drug ampicillin. The statistical evaluation showed good sensitivity of the developed methods over the reported method. Besides, the selected chromogenic reagents (MBTH & PDAC) are found to be safer and less toxic. Hence the methods are sustainable and applicable to the assay of pharmaceutical formulations containing ampicillin.

Conflicts of Interest

There are no conflicts to declare.

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