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Spectrophotometric determination of penicillins in pure and pharmaceutical formulations using Folin-Ciocalteu reagent

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A new combination of time, temperature, and alkali is described for the spectrophotometric determination of amoxicillin and ampicillin using Folin-Ciocalteu reagent. The method is based on the development of blue-coloured product due to the reduction of tungstate and/or molybdate in Folin-Ciocalteu reagent by amoxicillin and ampicillin in alkaline medium. The chromogenic reaction has λ_{max} at 720 and 740 nm with molar absorptivity 1.6295×10^4 and 0.1085×10^4 l mol^{-1} cm^{-1} in the Beer's Law range 2–10 μg mL^{-1} and 10–70 μg mL^{-1} for amoxicillin and ampicillin, respectively. The method is reproducible, quick, inexpensive, and particularly helpful in determining the drug content in commercial dosage forms.

Keywords: amoxicillin; ampicillin; Folin-Ciocalteu reagent; pharmaceutical formulations

Introduction

Penicillin is a unique molecule having a fused β-lactam thiazolidine ring system, wherein the strained β-lactam ring is susceptible to cleavage by a variety of reagents as well as some enzymes.[1] Amoxicillin (AMX) and ampicillin (AMP) belong to a class of antibiotics called penicillins. Chemically, AMX and AMP are (2S, 5R, 6R)-6-[(R-)(−)-2-amino-2-(p-hydroxyphenyl) acetamido]-3,3-dimethyl-7-oxo-4-thia-1-aza-bicyclo [3.2.0] heptane-2-carboxylic acid trihydrate and (2S, 5R, 6R)-6-[[(2R)-2-amino-2-phenylacetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-aza-bicyclo [3.2.0] heptane-2-carboxylic acid trihydrate, respectively.

Penicillins undergo catalytic kinetic degradation in methanol in the presence of Zn^{2+} and Cd^{2+} ion[2–4] and it was observed that Cd^{2+} degrades penicillin at a greater velocity than Zn^{2+}. These antibiotics also undergo degradation in aqueous solutions by the UV/ZnO photocatalytic process.[5]

The methods reported for the determination of AMX and AMP included spectrophotometric[6–9] and high performance liquid chromatography (HPLC) methods.[10,11] Folin-Ciocalteu reagent (FCR) has been reported in the determination of alkaloids (ajmaline and brucine) in alkaline medium,[12] phenolic compound,[13] and penicillins in acidic medium.[14] Some of these methods are less sensitive, time-consuming, and involve tedious experimental procedure. To overcome these limitations in the existing methods, there is a need for a sensitive and cost effective method for the determination of these drugs.

The developed spectrophotometric method is based on the reduction of tungstate and/or molybdate in FCR by AMX and AMP in alkaline medium resulting in a blue-coloured product having λ_{max} 720 and 740 nm, respectively. This work was undertaken to study the analytical aspects of the reaction and to test the sensitivity, accuracy, and selectivity of the method towards studied drugs. The developed method is superior to the reported Pd (II) chloride[9] method (high cost and less sensitivity), the Mo-SCN reagent method[8] (extraction with methylene chloride), and FCR in acidic medium[14] (less sensitive for AMX). FCR has a higher sensitivity towards AMX in alkaline medium (2–10 μg mL^{-1}). The developed method is selective, accurate, and sensitive, particularly for AMX than the reported methods.

Experimental

Materials and solutions

Reference standard of pure amoxicillin trihydrate and ampicillin trihydrate (CDH, Delhi India) were used. AMX and AMP drugs were purchased from the market. FCR (Sisco Research Laboratory, Mumbai, India) was used. All other reagents were of AR grade.

Standard drug solutions

Stock solutions of AMX (2 mg mL^{-1}) and AMP (2 mg mL^{-1}) were prepared by dissolving 200 mg of each drug in the least volume of methanol and diluted up to mark in a 100-mL volumetric flask with bidistilled water. The working solution of AMX (100 μg mL^{-1}) and AMP (1000 μg mL^{-1}) were prepared by further dilution of the stock solutions.

Sample preparations

In a 100-mL standard flask, an accurately weighed amount (mixed content of the capsules) equivalent to 500 mg of the respective drug, was dissolved in 5.0 mL of methanol, completed to volume with bidistilled water and filtered. Appropriate aliquots of the drug solutions were taken and the standard procedure was followed for analyzing the drug content.

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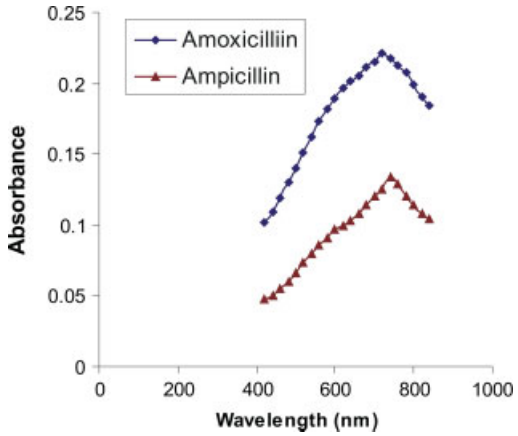


Figure 1. Absorption spectra of the reaction products of Folin Ciocalteu reagent with amoxicillin (5 μg mL^{-1}) and ampicillin (50 μg mL^{-1}).

Table 1. Optical characteristics and statistical data for the regression equation of the proposed method

Parameters	Amoxicillin	Ampicillin
λ_{max}, nm	720	740
Colour	Blue	Blue
ε (l mol^{-1} cm^{-1})	1.6295×10^4	0.1085×10^4
Beer's Law range (μg mL^{-1})	2–10	10–70
Regression equation (Y^a)*		
Slope (b)	0.0388	0.00269
Intercept (a)	0.0161	0.0153
Correlation coefficient (r)	0.9997	0.9966
Standard deviation (S_o)	0.0019	0.0010
Variance (S_o^2)	3.61×10^{-6}	1.0×10^{-6}
RSD**	0.81	0.69
LOD (μg mL^{-1})	0.16	1.22
LOQ (μg mL^{-1})	0.49	3.70

* $Y = a + bX$, where X is the conc. in μg mL^{-1}.
** Six replicate analysis of these drugs.

Apparatus

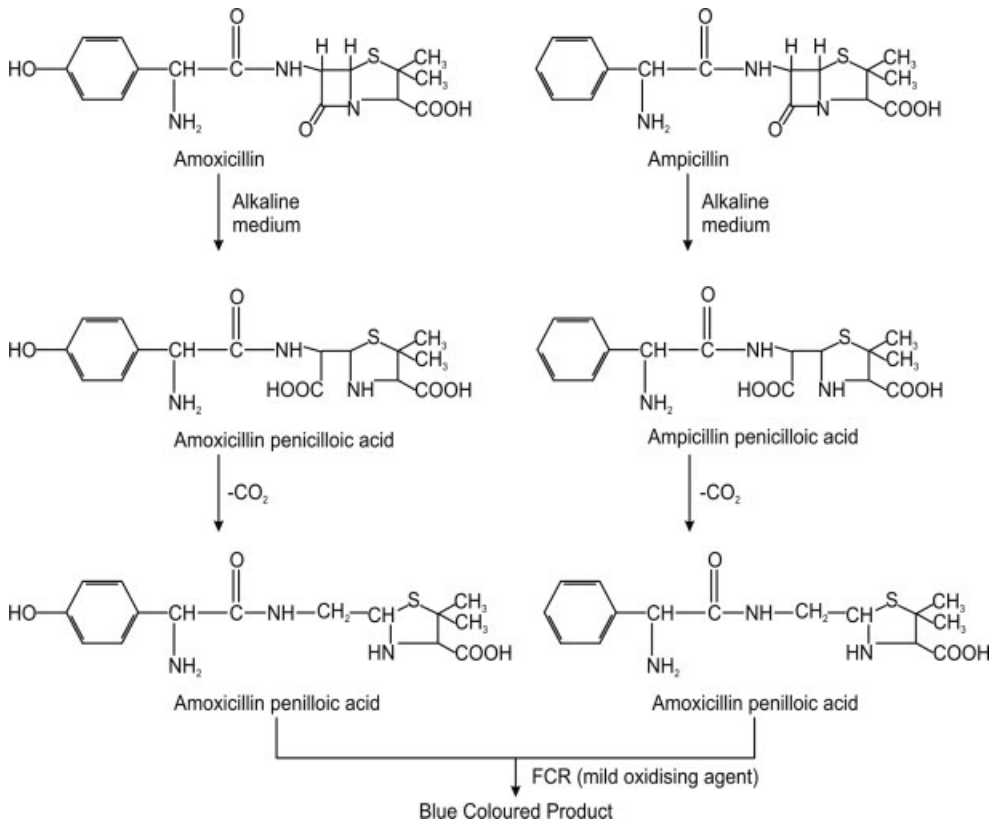
A thermostat water bath and Bausch and Lomb spectronic-20 with quartz cells of 1-cm optical path length were used for heating and absorbance measurements, respectively.

Determination of AMX and AMP

Accurately measured volumes of drug solutions equivalent to 2–10 μg mL^{-1} of AMX and 10–70 μg mL^{-1} of AMP were transferred into a series of 10-mL volumetric flasks. Sodium carbonate solution (2.5 mL, 10% w/v) was added to each followed by FCR (3.5 mL, 20% v/v). The flask and its contents were swirled, diluted to the mark with bidistilled water, and placed in a water bath maintained at 98 ± 2 °C for 40 min. After cooling, the absorbance was measured at λ_{max} 720 and 740 nm for AMX and AMP, respectively against the reagent blank.

Results and discussion

Absorption spectra of AMX and AMP were recorded with λ_{max} 720 and 740 nm, respectively (Figure 1). The reaction was carried



Scheme 1. Reaction Mechanism.

Table 2. Determination of amoxicillin and ampicillin in the presence of excipients

Excipients	Amount taken (μg mL^{-1})	Amoxicillin	Ampicillin
		% Recovery ± % RSD*	
Dextrose	30	100.90 ± 1.03	100.4 ± 0.67
Starch	50	99.02 ± 1.19	99.92 ± 0.93
Ascorbic Acid	10	101.7 ± 0.45	101.74 ± 0.90
Talc	50	100.2 ± 0.69	100.2 ± 0.59
Magnesium Stearate	60	99.3 ± 0.77	99.58 ± 0.77

10 μg mL^{-1} and 20 μg mL^{-1} of amoxicillin and ampicillin were used respectively.
* Average of five determination.

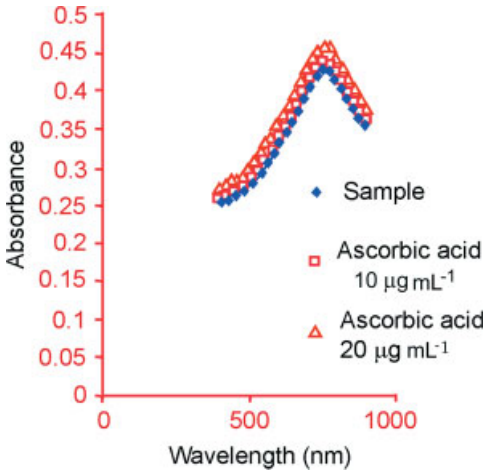


Figure 2. Effect of concentration of ascorbic acid on absorption spectra of amoxicillin (10 μg mL^{-1}).

out at different temperatures (40–100 °C) and periods ranging from 5–60 min. Heating for 40 min at 98 ± 2 °C was found to be the most suitable condition for maximum absorbance and stability of the colour. Maximum and constant absorbance was found with 2.5 mL of Na_2CO_3 solution. The effect of varying volumes of FCR on the absorbance was also studied. 3.5 mL of FCR was found to be the optimum volume to produce maximum absorbance. The sensitivity of the method for AMX is higher than AMP due to greater hydrolysis of AMX in alkaline medium (pH ~9). Penicillins (viz. AMX and AMP) undergo degradation rapidly in alkaline condition (pH 7.5–9.0) and the amide bond gets open to give penicilloic acid. The carboxyl group present on penicilloic acid after bond opening undergoes decarboxylation giving rise to penilloic acid. The β-carboxyl group of thiols is particularly susceptible to oxidation by mild oxidizing agents. Depending upon the reaction conditions, 2–8 electrons are released in the reaction medium during oxidation of the thiols. In this process, the release of electrons resulting in a reduction of the phosphomolybdate (giving a characteristic yellow colour to the FCR) and phosphotungstate of the FCR to produce a stable and intense blue complex. The blue colour that develops is attributable to the heteropolyanions of Mo(VI) and W(VI), commonly known as heteropolyblues, which are classified as mixed valence complexes of Mo(V) and Mo(VI) or W(V) and W(VI).[15] The probable reaction mechanism is proposed in Scheme 1.

In order to test Beer's Law, the absorbance of a series of solutions containing varying amounts of AMX and AMP were recorded against the reagent blank. Optical characteristics such as Beer's Law limit, molar absorptivity, etc., are given in Table 1. Data of the regression analysis using the least squares method made for the calibration curves are also given (Table 1). The accuracy and precision of the method were checked by analyzing six replicate samples within the Beer's Law range containing the same amount of drug. The lower RSD values indicate good precision and reproducibility of the method.

The validity of the proposed procedure for the determination of AMX and AMP in their pure state was checked by analyzing these drugs using the proposed method. The results obtained for pure drugs were reproducible with low RSD. The limits of detection (LOD) and quantitation (LOQ) were calculated using the following relation:[16]

$$LOD = 3.3 \times S_0/b \quad \text{and} \quad LOQ = 10 \times S_0/b$$

LOD is well below the lower limit of the Beer's Law range. Where, S_0 is the standard deviation of the calibration curve and b is the slope. The small value of variance suggested negligible scatter of experimental data point around the line of regression.

In order to investigate the interference of the excipients, varying amounts of the excipients were added to the fixed amounts of AMX

Table 3. Comparison of proposed method with the reference method in pharmaceutical formulations

Drug and formulations[a]	Amount taken (μg mL^{-1})	Recommended method		RSD[b] (%)	Reference method		RSD[b] (%)	t[c]	F[d]
		Amount found (μg mL^{-1})	% Recovery ± SD		Amount Found (μg mL^{-1})	% Recovery ± SD			
Amoxicillin									
Almox 250 mg/tab	5.0	5.01	100.18 ± 1.05	0.41	4.99	99.74 ± 1.82	0.73	1.43	3.16
Mymox 250 mg/tab	5.0	5.00	100.05 ± 1.07	0.43	4.96	99.17 ± 1.98	0.86	2.31	1.98
Ampicillin									
Campicillin 500 mg/tab	10.0	9.97	99.66 ± 0.80	0.16	9.95	99.58 ± 1.1	0.22	2.29	1.90

* Reference method used for AMX[17] and AMP[8].
[a] Preparations were in capsule form.
[b] Five independent analysis.
[c] Theoretical t-value at 95% confidence level was 2.776.
[d] Theoretical f-value at 95% confidence level was 6.39.

and AMP. It was observed that dextrose, starch, ascorbic acid, talc, and magnesium stearate do not interfere up to the concentration 30, 50, 10, 50 and 60 μg mL^{-1}, respectively (Table 2). The positive error in the case of ascorbic acid is due to its reducing property. However, ascorbic acid concentration above 10 μg mL^{-1} resulted in significant error (Figure 2). The sample absorption spectra of AMX and AMP with and without excipients overlap each other and show same λ_{max}. Thus the proposed method is free from interference by the investigated foreign substances.

The applicability of the method for the assay of pharmaceutical preparations was examined. The results of available tablets of AMX and AMP are summarized in Table 3. These results were reproducible. The results of the assay of tablets were crosschecked by the reported method for AMX[17] and AMP.[8]

Conclusion

From an analytical point of view, it is concluded that the described procedure allows for the determination of AMX and AMP in pure and pharmaceutical dosage forms. The investigated procedure is simple, faster, and more sensitive than most of the reported methods. The reagents are cheaper and the procedure does not involve any toxic solvent or critical conditions.

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