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Original Article

OPTIMIZED AND VALIDATED SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF AMPICILLIN IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Objective: A simple, precise, and accurate spectrophotometric method has been developed to determine Ampicillin in pharmaceutical formulations.

Methods: The proposed method, based on the carboxylic acid group reaction, present in Ampicillin with a mixture of KIO_3 and KI, form a yellow-colored product in an aqueous medium. The response was allowed to proceed at 25±1 °C, and absorbance measured after 5 min against a reagent blank prepared simultaneously using a UV-Vis spectrophotometer. The parameters verified were specificity, linearity, linearity range, accuracy, precision, detection limit, quantitation limit, robustness, and ruggedness.

Results: The yellow-colored product was measured at 352 nm against the reagent blank using UV–Vis spectrophotometer. The linear dynamic range of concentration was 0.25–2.5 µg/ml with a correlation coefficient of 0.9999. The LOD, LOQ values to be 0.086 and 0.261 µg/ml, respectively, for the proposed method. The percentage of recoveries was 98.27–100.89% with an acceptable relative standard deviation (±2%). The robustness and ruggedness values were excellent.

Conclusion: The ICH guidelines for pharmaceuticals and human use were followed and applied to validate the proposed method. The method was compared with available literature and found similar results that confirmed the reliability and effective way for Ampicillin's determination.

Keywords: Ampicillin, Potassium iodate, Potassium iodide, Spectrophotometer, Method development, Validation, Tablets

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INTRODUCTION

Ampicillin is semi-synthetic antibiotics with broad antimicrobial properties., chemically known 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 (fig. 1). It is an amphoteric compound and acts as an aliphatic amino acid. It has been a therapeutic drug against many gram-positive microorganisms. It is still more active with gram-negative microorganisms due to its enhanced ability to penetrate the bacteria through the outer membrane.

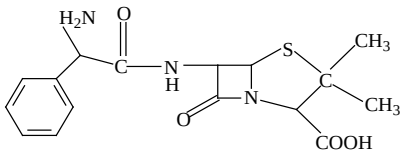


Fig. 1: Structure of penicillin-type antibiotic drug, Ampicillin

Ampicillin is available in tablets, capsules, powder for oral suspension and injection as commercial usage since 1961 [1]. The extensive literature survey revealed that several analytical techniques, likely as high–performance liquid chromatography (HPLC) [2, 3], capillary electrophoresis (CE) [4, 5], Fourier–transform infrared spectroscopy (FTIR) [6] were available for the determination in bulk and pharmaceutical formulations. It can be quantified with liquid chromatography-mass spectrophotometry (LCMS) [7] and ultra-performance liquid chromatography-mass spectrophotometry (UPLC–MS) [8] in biological fluids. Most of the techniques are involved with the complicated procedure, extraction with toxic solvents, and expensive instrumentation and pretreatment of the sample before analysis. However, Ultraviolet-Visible (UV-Vis) spectrophotometer is a convenient technique for the study of pharmaceuticals. Generally, the quantification without any pretreatment procedure before analysis [9–13]. Ampicillin as well can be quantified in bulk and drugs [14, 15]. The presented paper describes a fast, sensitive, and economical method for determining Ampicillin in pharmaceutical formulations. The procedure was based on the reaction of a carboxylic acid group of Ampicillin with a mixture of potassium iodate (KIO_3) and potassium iodide (KI) to form a yellow-colored product in an aqueous medium at room temperature (25 °C), followed spectrophotometrically by measuring the absorbance at 352 nm.

MATERIALS AND METHODS

Apparatus

The spectrophotometer, Cecil (CE 7400, UK), with a 1 cm glass cell was applied to carry out all the spectral runs.

Materials and reagents

The pharmaceutical formulation products of Ampicillin such as Ampicyn 500 (Cipla Limited, India), Campicillin 250 (Cadila Pharmaceuticals, India), and Ampiclox 500 (GlaxoSmithKline, UK) were brought from the local pharmacy. Potassium iodate and potassium iodide purchased from Sigma Aldrich, USA, and prepared solutions in distilled water with 2.5×10^{-3} and 2.5×10^{-2} M, respectively.

Extraction of ampicillin from the dosage form

Ampicillin (500 mg/capsule) powder materials were transferred into a 250 ml volumetric flask and dissolved with distilled water. The separation was continued with column chromatography using silica gel as a stationary phase and water: methanol: glacial acetic acid (1.2: 8.5: 0.3 v/v/v) as mobile phase. The target compound was separated and dried as solid Ampicillin.

Standard Ampicillin solutions

The standard Ampicillin solution (50 µg/ml) was prepared in distilled water and diluted as per the requirement.

Optimization of variables

The parameters were thoroughly studied related to the color development of the product. Therefore, several conditions were optimized during quantification and maintained their optimum value throughout the determination process.

Potassium iodate (KIO_3) concentration

The effect of volume of potassium iodate (2.5×10^{-3} M) was investigated in the range of 0.1–1.5 ml, keeping ampicillin (2.5 µg/ml) and KI (1.25×10^{-3} M) as constant. The absorbance was increased with the increasing volume of KIO_3 and became steady at 1.1 ml. Further addition of work does not have any impact on the color development of the product. Therefore, 1.3 ml used as an optimum volume throughout the experiment for KIO_3.

Potassium iodide (KI) concentration

The effect of volume of potassium iodide (2.5×10^{-2} M) was investigated for product's color development in the range of 0.2–2.2 ml, keeping ampicillin (2.5 µg/ml) and KIO_3 (7.5×10^{-5} M) as constant. The maximum intensity of the colored product gave rise to 1.8 ml of KI, and a further increase in the volume did not affect the absorbance. Thus, the proposed procedure used 2 ml as constant throughout the experiment for KI.

The proposed procedure for the determination of Ampicillin

Into a series of 50 ml volumetric flasks, different volumes of Ampicillin (50 µg/ml) corresponds to 0.25–2.50 µg/ml added with 2 ml of KI (2.5×10^{-2} M) and 1.3 ml of KIO_3 (2.5×10^{-3} M). The final volume was made up of distilled water and shook the mixture well enough to mix them properly. The reaction's equilibrium achieves after 5 min; therefore, 5 min is used as the equilibrium time for its determination. Absorbance was recorded for all standard samples at 352 nm and applied to construct a regression equation against Ampicillin's initial concentrations [16]. The intraday and interday precision of the proposed methods were established by measuring Ampicillin's content at three different concentration levels (low, medium, and high) at 0.5. 1.25 and 2 µg/ml within one day and five consecutive days, respectively. Ampicillin, with a known amount (0.5 µg/ml) in dosage form, was spiked with 100%, 200%, and 300% of additional Ampicillin pure drug, respectively, and determine the accuracy, precision of the proposed method following the recommended procedure as ICH guidelines [17–19].

Analysis of pharmaceutical formulations

Two ampicillin (label claim: 250 mg) capsules were mixed with 250 ml distilled water and kept for 30 min after stirred. After filtration to recover the drugs completely, repeated the washing steps several times. This solution was used for further process and applied the above procedure to determine the Ampicillin in pharmaceutical formulations.

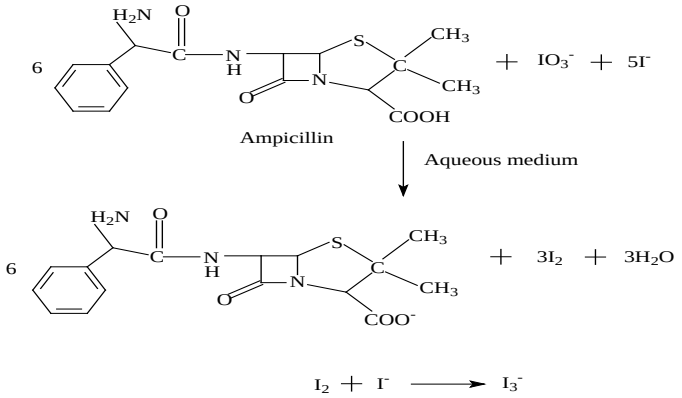
RESULTS AND DISCUSSION

Reaction with a mixture of iodide and iodate

The literature reported [20] iodine formed by the interaction between potassium iodate and potassium iodide due to organic and inorganic acid reaction by the below equation.

$$5\ I^- + IO_3^- + 6\ H^+ \rightarrow 3\ H_2O + 3\ I_2$$

The liberated iodine reacted with excess iodide ions to yield a triiodide ion ($I_2+I^-\rightarrow I_3^-$), which absorbs maximally at 352 nm. It was the fundamental mechanism to develop a spectrophotometric method for Ampicillin as it contains–COOH group in its moiety. The reaction mechanism suggested in Scheme 1 as per the above reaction. The communicated process corresponds to Ampicillin (0.25–2.50 µg/ml) was reacted with 1.3 ml KIO_3 (fig.2) and 2 ml KIO_3 (fig. 3) mixture and formed yellow-colored triiodide ions.



Scheme 1: Formation of triiodide ion that measures spectrophotometrically at 352 nm

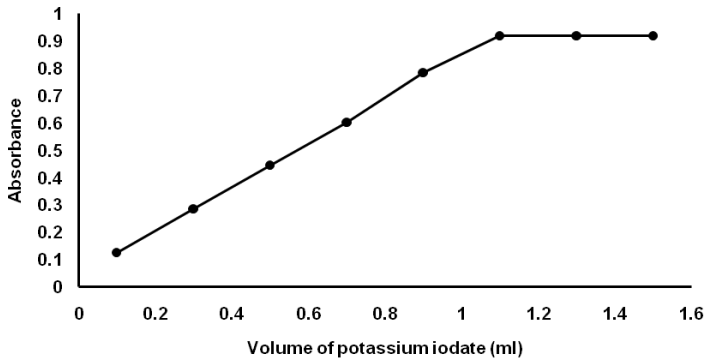


Fig. 2: Effect of the volume of potassium iodate (2.5×10^{-3} M) on the absorbance of the product, Ampicillin (2.5 µg/ml), and KI (1.25×10^{-3} M)

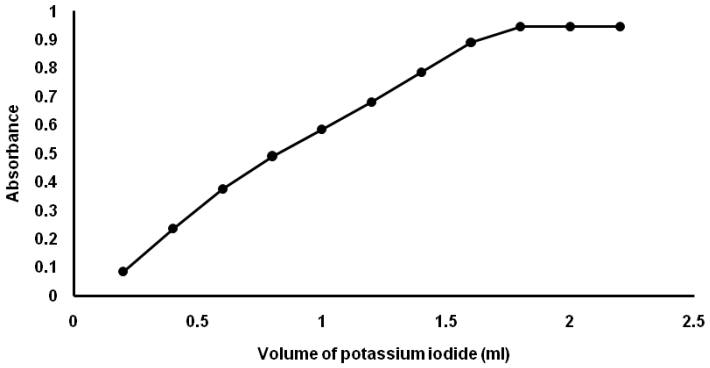


Fig. 3: Effect of the volume of potassium iodide (2.5×10^{-2} M) on the absorbance of the product, Ampicillin (2.5 µg/ml), and KIO_3 (7.5×10^{-5} M)

Validation of proposed methods

Specificity

The proposed method's specificity was evaluated by preparing a synthetic mixture of bulk drugs with common excipients used in the tablet formulations. It observed that no interference of excipients during the quantification drug.

Linearity and linear range

Under the optimized experimental conditions, the absorbance and concentration were linear within the range of 0.25–2.5 µg/ml (fig. 4). The statistical treatment of the data yielded the linear regression equation A = 0.0035+0.4227 C. The statistical regression data provided the slope, intercept, detection limits, quantitation, and results summarized in table 1. All the parameters were important as per the guidelines and considered during the validation of the developed method.

Accuracy and precision

The intraday and interday precision have resulted in an Ampicillin concentration of 0.5, 1.25, and 2 µg/ml (table 2). The % RSD was in the range of 0.24–1.81 % within±2%. The recovery studies of each concentration level established the accuracy of the method. The procedure's % recovery was in the range of 99.32–100.65 % (table 3) within the acceptable limit of 98–102%, as per ICH guidelines [17–19].

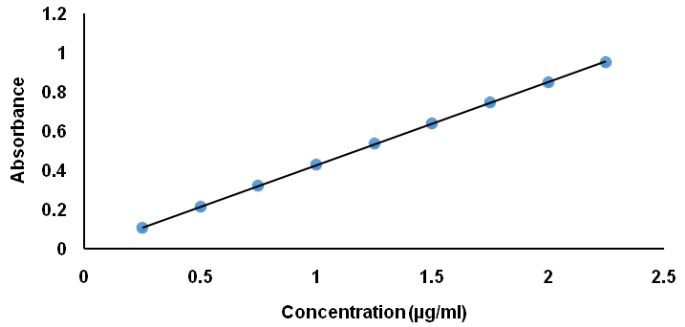


Fig. 4: Linearity of the proposed method

Table 1: Optical and regression characteristics of the proposed method

Parameters	Values
λ_{max}	352 nm
Calibration equation	A = 0.0035+0.4227 C
Linear dynamic range (µg/ml)	0.25–2.5
Standard Deviation (S_0)	1.1×10^{-2}
Slope (m)	4.22×10^{-1}
Correlation coefficient (r^2)	0.9999
Detection limit (µg/ml)	0.086
Quantitation limit (µg/ml)	0.261

Table 2: Summary of accuracy and precision results of the proposed method

Proposed method	Amount (µg/ml)		% Recovery	% RSD
	Taken	Found±SD[a]		
Intraday	0.75	0.756±0.014	100.89	1.81
	1.50	1.497±0.004	99.81	0.24
	2.25	2.234±0.006	99.27	0.27
Interday	0.75	0.741±0.014	98.78	1.84
	1.50	1.502±0.005	100.12	0.328
	2.25	2.231±0.01	99.17	0.441

[a]Mean for five independent analyses, SD, standard deviation, RSD, relative standard deviation

Table 3: **Summary of accuracy and precision results of the proposed method for ampicillin pharmaceutical formulations**

Proposed method	Amount (µg/ml)		% RSD	% Recovery
	Taken	Found±SD[a]		
Ampicyn-500	1.0	0.993±0.014	1.375	99.32
Ampicyn-500	1.5	1.508±0.004	0.239	100.65
Ampicyn-500	2.0	2.000±0.002	0.118	100.01
Campicilin-250	1.0	0.993±0.014	1.375	99.32
Campicilin-250	1.5	1.508±0.007	0.471	100.54
Campicilin-250	2.0	2.001±0.001	0.068	100.05
Ampiclox–500	1.0	0.993±0.014	1.375	99.32
Ampiclox–500	1.5	1.508±0.004	0.239	100.65
Ampiclox–500	2.0	2.000±0.002	0.118	100.01

[a]Mean for five independent analyses, SD, standard deviation, RSD, relative standard deviation, [b]SAE, standard analytical error, [c]C. L., confidence limit at 95 % confidence level and 4 degrees of freedom (t = 2.776)

Robustness

The robustness study was performed at different laboratories. The due reason recorded the absorbance after 4.5 min and well continued for 5.5 min of reaction. The investigation resulted at 351 nm and 353 nm to check the method's robustness. The procedure's % recovery was in the range of 98.32–99.97 % and % RSD between 0.352–0.755 %, respectively, within the acceptable limit.

Ruggedness

The ruggedness test was conducted at three different concentration levels within the linearity range. The test was expressed in terms of %RSD, applied the same procedure using another UV-Vis spectrophotometer. The procedure's % recovery was within 98.55–99.72 % and % RSD between 0.023–0.325 % within the acceptable limit of±2%.

Application of the proposed method

The proposed method was successfully developed and validated for the determination of Ampicillin in pharmaceutical formulations. The proposed method was compared with the reference method [3] using point and interval hypothesis tests. The calculated t-(paired) and F-values at 95% confidence level do not exceed the theoretical ones indicating any significant differences between the developed method's performances with the reference method (table 4). Table 4 showed that all samples of the drug's actual bias were smaller than ±2% and thus confirming that the proposed method was reliable with acceptable recovery.

Table 4: Point and interval hypothesis tests: comparing the proposed methods with the reference method at 95% confidence level

Formulations	Proposed method				Reference method	
	% Recovery	% RSD	t-and F [b]	$\theta_{L \text{ and }} \theta_U$ [c]	% Recovery	% RSD
Ampicyn-500	99.91	0.25	t =0.016 F= 1.022	θ_L=0.989 θ_U=1.004	99.94	0.35
Campicilin-250	99.93	0.16	t =0.121 F= 1.83	θ_L=0.984 θ_U=1.015	99.92	0.31
Ampiclox–500	99.91	0.22	t =0.461 F= 1.48	θ_L=0.992 θ_U=1.003	99.81	0.55

Theoretical t-(ν = 8) and F-values (ν = 4, 4) at 95 % confidence level are 2.306 and 6.39, respectively. θ_L and θ_U are within the acceptable limits of ±2%.

CONCLUSION

The proposed UV-Vis technique is a simple, low-cost method and can easily be applied to Ampicillin's routine quality control sample analysis in bulk and pharmaceutical formulations. It has a more comprehensive dynamic range for the study with excellent accuracy and precision value. The proposed method does not require any laborious cleanup procedure before analysis and simple methodology for its determination. Therefore, it can easily accommodate in the laboratories of research, hospitals, and pharmaceutical industries for the quantification of Ampicillin in pure and pharmaceutical dosage forms.

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Nil

AUTHORS CONTRIBUTIONS

Single author and contributed all.

CONFLICT OF INTERESTS

The author report no conflicts of interest.

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