**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 ruggedness values were excellent. Therefore, ultraviolet-Visible (UV-Vis) spectrophotometry is a convenient technique for the study of pharmaceuticals. Generally, the quantification without any pretreatment procedure before analysis [9–15]. 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 (KIO₃) 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 pre pared 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 (1:2.85: 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 (KIO₃) concentration

The effect of volume of potassium iodate \((2.5 \times 10^{-3} \text{ M})\) was investigated in the range of 0.1 –1.5 ml, keeping ampicillin \((2.5 \mu\text{g/ml})\) and KI \((1.25 \times 10^{-3} \text{ M})\) as constant. The absorbance was increased with the increasing volume of KIO₃ 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 KIO₃.

Potassium iodide (KI) concentration

The effect of volume of potassium iodide \((2.5 \times 10^{-2} \text{ M})\) was investigated for product's color development in the range of 0.2 –2.2 ml, keeping ampicillin \((2.5 \mu\text{g/ml})\) and KIO₃ \((7.5 \times 10^{-5} \text{ M})\) 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 \((50 \mu\text{g/ml})\) corresponds to 0.25–2.50 µg/ml added with 2 ml of KI \((2.5 \times 10^{-2} \text{ M})\) and KIO₃ \((2.5 \times 10^{-1} \text{ M})\). 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 \((0.5 \mu\text{g/ml})\) 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 \((0.25–2.50 \mu\text{g/ml})\) was reacted with 1.3 ml KIO₃ (fig.2) and 2 ml KIO₃ (fig. 3) mixture and formed yellow-colored triiodide ions.

![Scheme 1: Formation of triiodide ion that measures spectrophotometrically at 352 nm](image)

Fig. 2: Effect of the volume of potassium iodate \((2.5 \times 10^{-3} \text{ M})\) on the absorbance of the product, Ampicillin \((2.5 \mu\text{g/ml})\), and KI \((1.25 \times 10^{-3} \text{ M})\).
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. 3: Effect of the volume of potassium iodide ($2.5 \times 10^{-2}$ M) on the absorbance of the product, Ampicillin (2.5 µg/ml), and KIO₃ (7.5×10⁻⁵ M)](image)

![Fig. 4: Linearity of the proposed method](image)

### Table 1: Optical and regression characteristics of the proposed method

| Parameters                  | Values                  |
|-----------------------------|-------------------------|
| $\lambda_{max}$ (nm)        | 352 nm                  |
| Calibration equation        | $A = 0.0035 + 0.4227 C$ |
| Linear dynamic range (µg/ml)| 0.25–2.5                |
| Standard Deviation (S+)     | 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  |       |
| 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±0.001 | 99.17  | 0.441 |

*Mean for five independent analyses, SD, standard deviation, RSD, relative standard deviation*
**AUTHORS CONTRIBUTIONS**

Single author and contributed all.

**CONFLICT OF INTERESTS**

The author report no conflicts of interest.

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