

# Spectrophotometric Determination of Ampicillin with Sulfanilic Acid by Oxidative Coupling Reaction

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## Abstract

Sensitive, economic, simple and accurate spectrophotometric method for estimation of ampicillin in bulk and dosage form is described. Ampicillin was oxidized by sodium hypochlorite and coupling with sulfanilic acid in the presence of sodium hydroxide to obtain a stable yellow colored chromogen which exhibit a maximum absorption ( $\lambda_{\max}$ ) at 400 nm. The optimum conditions were carefully evaluated .

Plot of absorbance against concentration was linear over the range (50-300  $\mu\text{g}\cdot\text{ml}^{-1}$ ). This method was applied to the estimation of ampicillin in pure drug and commercial formulations successfully.

**Key words:** Spectrophotometric determination, Ampicillin, Sulfanilic acid, Oxidation reaction.

## Introduction

Ampicillin is a drug from penicillin family. It is used to treat a broad range of bacterial infections produced by haemophilus influenza, streptococcus and escherichia. Ampicillin is used in the treatment of urinary and genital tract infections, otitis media in children and upper respiratory tract infections.

Ampicillin is a crystalline powder with white color with chemical formula, 6-[(Amino phenyl acetyl) amino]-3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0] heptane-2-carboxylic<sup>(1)</sup>.

A survey of literature revealed that several analytical methods such as high performance liquid chromatography<sup>(2-4)</sup>, flow injection<sup>(5,6)</sup>, atomic spectroscopy<sup>(7)</sup>, capillary electrophoresis<sup>(8,9)</sup>, iodometric titration<sup>(10)</sup>, potentiometric methods<sup>(11,12)</sup> and spectrophotometric methods<sup>(1, 13-16)</sup> have been reported for the determination of ampicillin.

The present study involves the development of accurate, sensitive and reproducible spectrophotometric method based on oxidation of ampicillin by sodium hypochlorite and coupling with sulfanilic acid in presence of sodium hydroxide as basic medium. This method is successfully applied to estimation the ampicillin in its commercial formulations.

## Experimental

### Instruments

The absorption spectra and all spectrophotometric measurements were carried out on a double-beam 1800 Shimadzu spectrophotometer with 1 cm matched quartz cells.

### Materials and reagents

Ampicillin standard powder received was provided in pure form (99.99%) from the State Company for Drug Industries and Medical appliance-(SDI) Sammara-Iraq and all chemicals used were of analytical reagent grade.

### Reagents solution

-Sulfanilic acid [ $6.35 \times 10^{-4}$  M]: 0.11 g of sulfanilic

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acid was dissolved in 0.5 mL of 4 M NaOH and complete dilution to 100 mL with distilled water.

**-Sodium hypochlorite [1% (v/v)]:** prepared by dilution of 10 mL of 5% sodium hypochlorite to 50 mL by distilled water in volumetric flask.

**-Sodium hydroxide [4 M]:** 16 g of sodium hydroxide was dissolved in 100 mL distilled water.

**-Standard ampicillin solution 1000  $\mu\text{g}\cdot\text{mL}^{-1}$ :** 0.1 g of ampicillin was dissolved in 0.5 mL of 4 M NaOH and further diluted to 100 mL with distilled water.

### Preparation of drug in dosage form

Ten capsules and ten vials of ampicillin were weighed, and an accurately weighted portion of the powder equivalent to 250 and 500 mg of ampicillin were dissolved in a 0.5 mL of 4 M NaOH and mixed well and then filtered by using Whatman filter paper number 41. Then volume was diluted to 100 mL with distilled water and analyzed as given under the assay procedures for bulk samples.

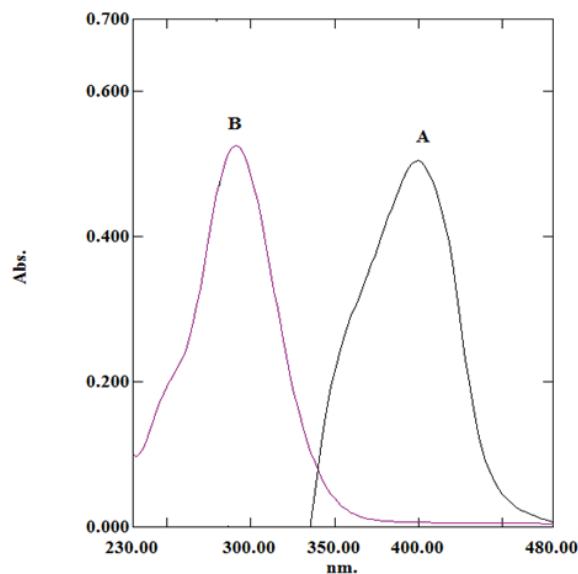
### General standard procedures

Aliquots (0.5- 3.0 ml) of standard 1000  $\mu\text{g}\cdot\text{mL}^{-1}$  ampicillin solution were transferred into a series of 10 ml volumetric flasks. To each 0.5 mL of  $6.53 \times 10^{-4}$  M sulfanilic acid solution, 2 ml of 1% sodium hypochlorite and 1.5 ml of 4 M sodium hydroxide were added. The volumes were made up to the mark with distilled water. The absorbance of yellow colored compound was recorded at 400 nm against the blank solution.

## Results and Discussion

### Absorption spectra

When ampicillin react with sulfanilic acid in the presence of sodium hypochlorite in basic medium of sodium hydroxide, a yellow colored oxidizing coupling product with an absorption maximum at 400 nm is formed. The absorption spectra of product and blank solution were recorded. The results were graphically represented in Figure 1.



**Figure (1): Spectrum of: (A) 200  $\mu\text{g}\cdot\text{mL}^{-1}$  ampicillin complex, (B) blank solution under optimization conditions.**

### Optimization of reaction variables

#### The influence of different type of bases

When some bases (NaOH, KOH and  $\text{NH}_4\text{OH}$ ) that the presence led to increase the intensity of the produced product were tested for the basic medium of reaction, it was found that NaOH was the most suitable for a maximum absorbance for method.

#### The influence of sulfanilic acid volume

Volume of sulfanilic acid effect on the color development was studied. It is evident that 0.5 mL sulfanilic acid give a maximum color intensity and a minimum absorbance of the blank.

#### The influence of sodium hypochlorite volume

The study of volume of sodium hypochlorite as an oxidant agent was evaluated. Maximum absorbance was observed when the volume of sodium hypochlorite was 2 mL. Above this volume the absorbance was decreased.

#### The influence of sodium hydroxide volume

The effect of the volume of NaOH on the color intensity of product was studied. 1.5 ml of 4M NaOH which was give high sensitivity.

#### Order of addition influence

Different orders of component addition influence on chromogen formation was investigated by changing the sequence of addition of drug, reagent, oxidizing agent and base. The sequence was {ampicillin – sulfanilic acid – NaOCl and NaOH} for the best intensity.

**The influence of reaction time**

The effect of coupling reaction time on color of chromogen development was examined. The yellow

product formed due to the oxidation coupling of ampicillin with sulfanilic acid by sodium hypochlorite in presence of base, attained the maximum color at 0 minute, that means the reaction was very fast.

**Linearity and analytical data**

Emplying the conditions described under procedure, a linear calibration graph, Figure 2 for ampicillin obtained shows that Beer’s law is obeyed over the concentration range 50-300 µg.mL<sup>-1</sup>. Linear regression and analytical parameters are listed in Table 1.

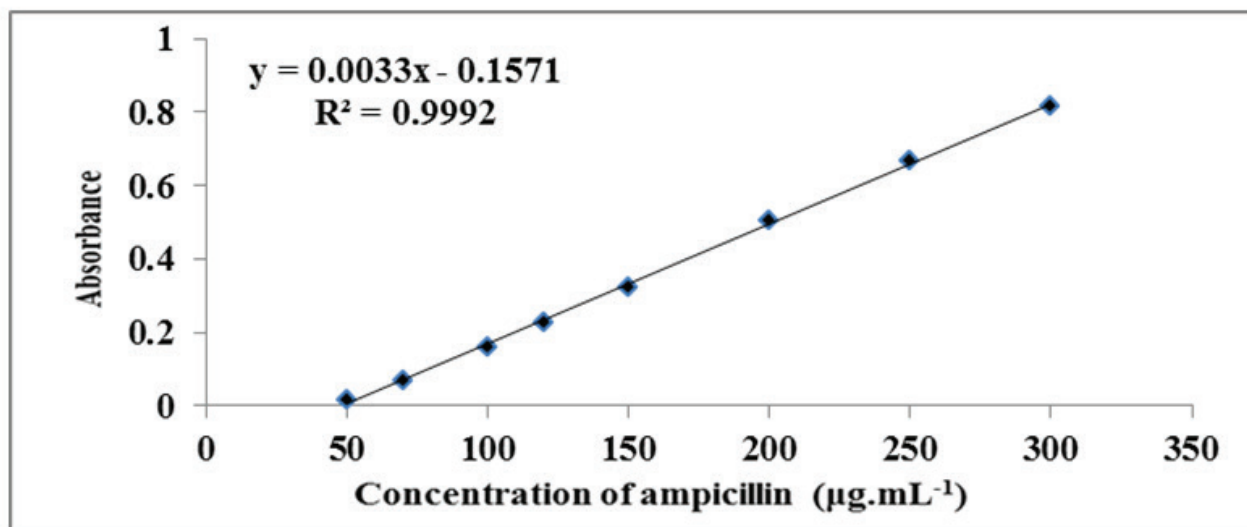


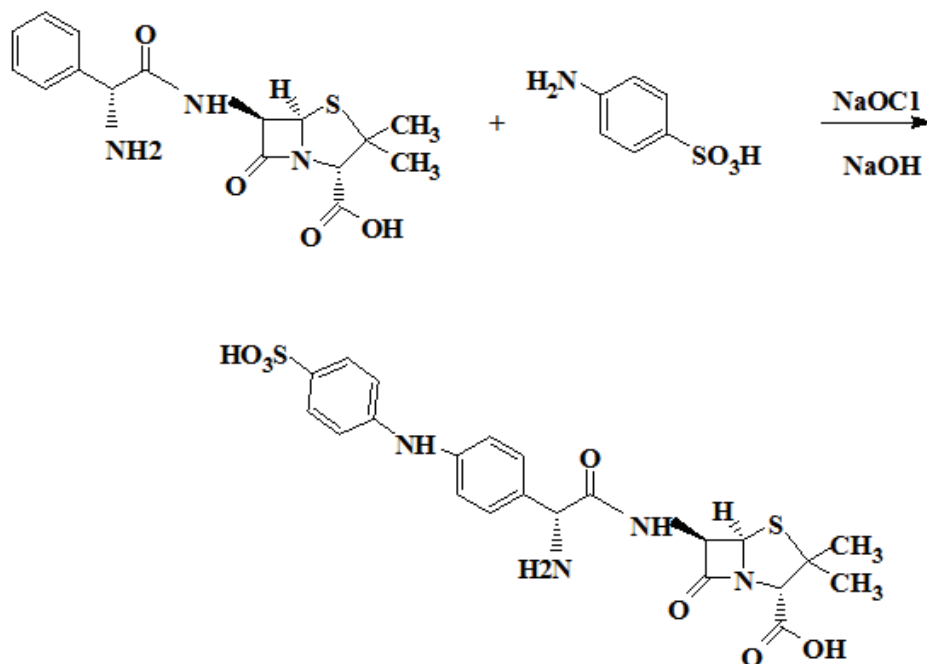
Figure (2): Spectrophotometric calibration curve for estimation of ampicillin.

Table (1): Linear regression and analytical parameters for estimation of ampicillin.

Parameter	Value
λ <sub>max</sub> (nm)	400
Regression equation	Y = 0.0033 [Ampicillin µg.mL <sup>-1</sup> ] - 0.1571
Calibration sensitivity(mL.µg <sup>-1</sup> )	0.0033
Linearity range (µg.mL <sup>-1</sup> )	50-300
Correlation coefficient (R)	0.9996
Molar absorptivity (ε) (L. mol <sup>-1</sup> .cm <sup>-1</sup> )	1331.55
LOD (µg.mL <sup>-1</sup> )	0.2405
Sandell’s sensitivity (µg.cm <sup>-2</sup> )	0.3030

### Composition of complex

Stoichiometry of the product formed from the reaction of ampicillin with sulfanilic acid was studied applying the Job's method by using equimolar solutions ( $1.24 \times 10^{-3}$  M) of ampicillin and sulfanilic acid. The results obtained show that 1:1 drug to reagent complex was formed at 400 nm. The reaction may proceed as given in Scheme 1.



**Scheme (1): The suggested mechanism for oxidation reaction.**

### Accuracy and precision

Accuracy was done by relative error % and precision was evaluated by relative standard deviation (RSD) %. Three different concentration levels of ampicillin were analyzed in three times. Table 2 list the results of accuracy and precision.

**Table (2): Accuracy and precision of the method for estimation of ampicillin.**

Sample	Conc. of ampicillin ( $\mu\text{g.mL}^{-1}$ )		Relative error %	RSD* %
	Taken	Found*		
Ampicillin	50	50.355	0.710	0.6330
	200	200.74	0.370	0.3724
	300	299.592	-0.136	0.2302

\*Average of three determinations.

### Application to Pharmaceutical Preparations

The application of the method for the assay of ampicillin in drugs has been done successfully and the results obtained are listed in Table 3 for each sample in three replicates.

**Table (3): Application of the ampicillin concentration measurements in pharmaceutical formulations.**

Sample	Labeled amount of ampicillin (mg)	*Found amount of ampicillin(mg)	Recovery %	RSD* %
Ampicillin Caps S. D. I.- Iraq	250	252.42	100.96	0.9431
	500	498.70	99.74	0.5260
Ampicillin Vials S. D. I.-Iraq	250	491.33	98.27	1.0980
	500	502.00	100.40	0.4122

\*Average of three determinations.

### Conclusions

The oxidative coupling reaction with sulfanillic acid and sodium hypochlorite in the presence of sodium hydroxide was rapid, sensitive, simple and precise spectrophotometric method was developed for estimation of ampicillin. The proposed method was applied successfully in pharmaceutical formulations.

**Acknowledgement :** Thanks for everyone who helped us accomplish this research

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** Non

**Funding:** Self-funding

### References

- Mohammed Q Y. Optimization of Ampicillin Oxidation Reaction with Hydrogen Peroxide and Potassium Dichromate in Different Media. *International Journal of ChemTech Research*. 2015;8(4):1689-1694.
- Tuani YT, Kissi FA, Sackey J, Gordon AJ and Akanji O. Development and Validation of RP-HPLC Method for the Simultaneous Determination of Ampicillin and Cloxacillin in Oral Suspension Dosage Form. *International Journal of Pharmaceutical Sciences Review and Research*. 2014;28(14): 64-68.
- Tótolí E G and Salgado H R N. Development and Validation of an Economic, Environmental Friendly and Stability-Indicating Analytical Method for Determination of Ampicillin Sodium for Injection by RP-HPLC. *World Journal of Pharmacy and Pharmaceutical Sciences*. 2014;3:1928-1943.
- Badgajar M A and Mangaonkar K V. Simultaneous Estimation of Ampicillin Sodium and Sulbactam Sodium in Injectable Dosage Form by High Performance Liquid Chromatography. *Oriental Journal of Chemistry*. 2011;27(4):1659-1664.
- Chivulescu A I, Doni M B, Cheregi M C and Danet A F. Determination of Amoxicillin, Ampicillin and Penicillin G Using a Flow Injection Analysis Method with Chemiluminescence Detection. *Revue Roumaine de Chimie*. 2011;56(3):247-254.
- Ma L, Kang W, Xu X, Niu L, Shi H and Li S. Flow-injection chemiluminescence determination of penicillin antibiotics in drugs and human urine using luminol-Ag(III) complex system. *Journal of Analytical Chemistry*. 2012;67(3):219-225.
- Chudobova D, Dostalova S, Blazkova I, Michalek, Petr R N, Branislav S, Matej N, Lukas K, Jiri G, Jaromir T, Katerina K, Marie V, Marketa H, David M, Michal K, Jindrich K R and Adam V. Effect of Ampicillin, Streptomycin, Penicillin and Tetracycline on Metal Resistant and Non-Resistant *Staphylococcus aureus*. *International Journal of Environmental Research and Public Health*. 2014;11:3233-3255.

- 8- Simon B, Hancu G and Gyéresi Á. Application of capillary electrophoresis to the simultaneous determination and stability study of four extensively used penicillin derivatives Brazilian Journal of Pharmaceutical Sciences. 2014;50(3):521-527.
- 9- Kowalski P and Konieczna L. Determination of Penicillin Antibiotics in Poultry Muscle by Capillary Electrophoresis. Bull Vet Inst Pulawy. 2007;51:595-598.
- 10- Ramezania Z, Rahbara N, Shadmani M and Toosi Y E. The Effect of Beta-Cyclodextrin on the Iodometric Determination of Some Penicillins Reported in USP and BP. Iranian Journal of Pharmaceutical Sciences. 2012;8(4):227-231.
- 11- Nestor Z, Alberto N A, Conceição B S M, Ricardo P O. Sequential Injection Analysis of Ampicillin in Pharmaceuticals by Using Potentiometric Detectors Based on PVC and Sol-Gel Membranes. American Journal of Analytical Chemistry. 2011;2:491-499.
- 12- Tomassetti M, Conta G, Campanella L, Favero G, Sanzò G, Mazzei F and Antiochia R. A Flow SPR Immunosensor Based on a Sandwich Direct Method. Biosensors. 2016; 6(22):1-13.
- 13- Khan A A P, Mohd A, Bano S, Siddiqi K S and Asiri A M. Spectrophotometric methods for the determination of ampicillin by potassium permanganate and 1-chloro-2,4-dinitrobenzene in pharmaceutical preparations. Arabian Journal of Chemistry. 2015;8:255-263.
- 14- Hapse S A, Mane A R, Kadam S D, Hajare P P and Gulve S A, Spectrophotometric Estimation of Ampicillin and Cloxacillin in Pure and Capsule Dosage Form by Using Different Methods. International Journal of Pharmaceutical Sciences Review and Research. 2012;14(2):67-70.
- 15- Ezeanokete C C, Ngwoke K G, Okoye F B C and Osadebe P O. Spectrophotometric Determination of Ampicillin and Cloxacillin in Pure and Fixed Dosage Forms Through Charge Transfer Complexation. Eur. Chem. Bull. 2013;2(12):1009-1012.
- 16- Abdelrahman M M, Naguib I A, Elsayed M A and Zaaza H A. Three Spectrophotometric Methods for Simultaneous Determination of Ampicillin and Dicloxacillin in Presence of Their Major Impurity 6-Aminopenicillanic Acid. Austin Journal of Analytical and Pharmaceutical Chemistry. 2015;2:1-7.