Synthesis, quantification and characterization of 1,3,4-thiadiazole derivatives of ampicillin by HPLC

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Abstract

In this study synthesized new Ampicillin derivative and diagnosed using FTIR and HNMR Technique. Also, in this study, an accurate, rapid, and sensitive chromatographic method was developed to separate the prepared derivative from the standard drug, and then this method was applied to pharmaceutical preparations and This method was linear, accurate, and specific. and The optimal conditions used in this method were is Mobile phase (%80 H2O containing 0.1% acetic acid + %20 Acetonitrile v/v), Nucludar C18-DB column, particle size 3 µm (50 x 4.6 mm I.D), flow rate 2 mL/min, pH = 4.5. The injection volume was 20 L, the wavelength was 210 nm, the run time was 6.00 minutes, and the linearity ranged from 0.1 to 10 µg/ml. Ampicillin has a retention time of 4.105 minutes. R^2 was found to be 0.9988 for the standard medication, and the LOD and LOQ for Amp were determined to be (0.01 µg/ml and 0.033 µg/ml) respectively**.**

Keywords: Ampicillin , HPLC , FTIR ,HNMR and 1,3,4-thiadiazole

Introduction

Ampicillin[1]is white crystals in color. has a molecular mass of (349.41) $g.mol^{-1}$ and a melting point ranging from (199 - 202) C˚ . low soluble in water or the colorless, its defining characteristics and has a specific rotation angle (287.9+)˚. the molecular formula for Ampicillin is $\mathbf{C_{16}H_{19}N_3O_4S}$ and the chemical name for this

drug is [2] (2S,5R,6R)-6- [(R)-2-amino-2-phenylacetamido]-3,3-dimehtyl-7-oxo-4-thia-1- zabicyclo[3.2.0]heptane-2-carboxylic acid [3]

As for its many commercial names, including the following: - Amcill (Park, Davis), Ampilag (Lagap), Ampilar (Lagap), Alpen (Lederle Amblosin), the chemical structure show in fig 1.

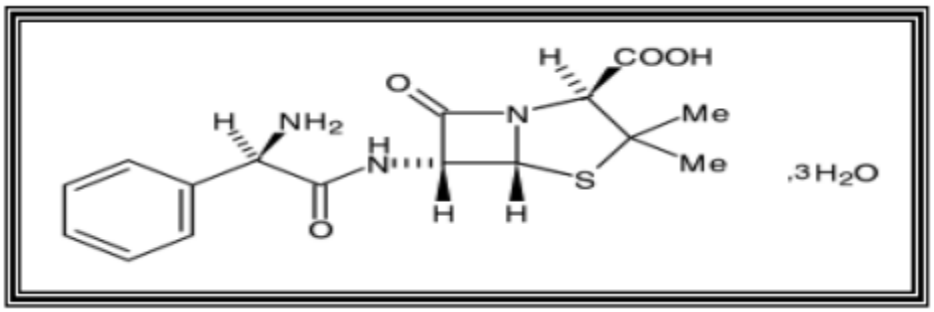


Fig 1: the chemical structure for Ampicillin[3].

Ampicillin is a broad-spectrum antibiotic used to treat a variety of infections, including bacterial diseases since 1961, and it is of the aminopenicillin family, mainly used in the treatment of a multitude of bacterial illnesses, including endocarditis, meningitis, urinary tract infections, and salmonellosis (lining of the heart). It is given orally, or by injection into a muscle or into a vein. Rash, nausea, and diarrhea are some of its frequent side effects , and may also include colitis or anaphylaxis; Therefore, patients who are allergic to penicillin should not use it. Its use during pregnancy and lactation is also generally safe ,It may also be used to prevent group B streptococcal infection in newborns and It is also used in prevention; When patients who have already been infected with rheumatic heart disease , and subject to dental procedures or uterine eradication . Ampicillin belongs to the aminopenicillin family and belongs to the beta-lactam penicillin antibiotic class [4] .

Ampicillin was determined using a variety of chromatographic and spectroscopic techniques. A variety of methods for determining Ampicillin have been documented in the References. *Chromatographic procedures include RP-HPLC (5-9,18), UV-VIS (1,3,10,11), spectrofluorimetric (12) and flow injection(13-16). In this study, Ampicillin 1,3,4-thiadiazole derivatives were synthesized, separated, identified, and their biological activity was investigated. In addition, a simple and accurate method for determining Ampicillin in pharmaceutical formulations was developed in this work. The proposed method was validated and found to be useful in analysis.*

2.MATERIALS AND METHODS

2.1.Chemical and Solvent

Ampicillin (Amp) as pure standard was provided from Samara Drug Industries (SDI) in Iraq, thiosemicarbazid, POCl3, water and KOH . Also, several different pharmaceutical companies were used that available in the Iraqi market, which were in the form of tablets, capsules and vial. The experiment made use of deionized water that had just been prepared and Acetonitrile that was HPLC grade (BDH).

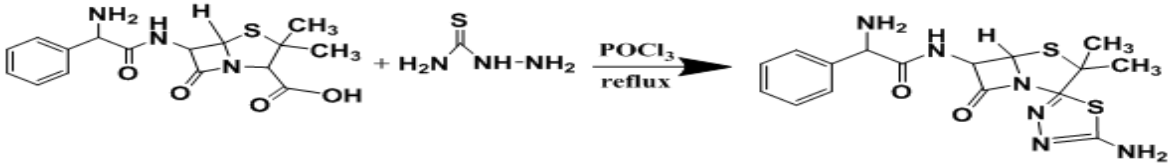
2.2.Equipment and chromatographic conditions

Used in this study, *HPLC LC-10A Shimadzu, (Japan), Sartorius balance (Germany), Ultrasonic bath (Branson Sonifier, USA), Shaking water bath (Taiwan) and oven (Memmert, Germany), FT-IR 8400 Shimadzu, Japan) and H-NMR (Bruker,500 MHz). Under optimal conditions, the primary compound was separated on an FLC (Fast Liquid Chromatographic) column. Column: nucludar C18-DB, 3m particle size (50 x 4.6 mm I.D) column, isocratic mobile phase (%80 H2O with acetic acid 0.1% +%20 ACN v/v), pH=4.5, flow rate 1.1 ml/min, and wavelength 260 nm*

The separation took place on a Shimadzu 10AV-LC fitted with a binary delivery pump model LC-10A Shimadzu, and the eluted peaks were examined using a UV-Vis 10 A- SPD spectrophotometer.

2.3.Synthesis of 1,3,4-thiadiazole derivatives of Ampicillin

(*1o mmol , 5ml) of phosphorous oxychloride ($POCl_3$) was added gradually and very carefully to a mixture consisting of (3g, 10 mmol) of Ampicillin with(0.91 g, 10 mmol) of thiosemicarbazide, and the mixture was raised for 3 hours, then the solution was left to cool. 25 mL of distilled water was added to it slowly and very carefully with stirring, and the mixture rose for 4 hours, and then the solution was neutralized using potassium hydroxide solution, then the precipitate was filtered and washed with distilled water several times. It was recrystallized using ethanol (V:V). The purity of the compound was confirmed using TLC technology.*



Scheme 1: the derivative of Ampicillin

Fig 2: The Ampicillin derivative that were prepared are in the form of precipitate

2.4.Optimum conditions selection for the drug and its derivatives

This method was used to estimate the property under study. This method was implemented by injecting 20 microliters of standard Ampicillin solution using manual injection through the injection valve. The drug solution dissolved in the appropriate solvent that is filtered using special filters of the type 2.5 micrometers is injected into a stream of the liquid mobile phase on Nucludar C18-DB column , 3µm particle size (50 x 4.6 mm I.D) column by reverse phase liquid chromatography technique. The drug was detected and the signal recorded for the reagent used as a UV detector. A number of factors were studied for the purpose of obtaining a uniform peak of the estimated material with a short retention time, where the type of mobile phase, pH function, velocity of the mobile phase and wavelength of measurement were studied. The best conditions that were chosen for the separation and quantification of the standard drug and their pharmaceutical preparations and their new derivatives prepared are Mobile phase: isocratic technique, combination of deionized water acidified with acetic acid 0.1% and acetonitrile in the ratio (80: 20 v/v), flow rate 1.1 ml/min UV detection at 260 nm with a PH of 4.5.

2.5.Standard series for drug

The drug solution that was used for the purpose of preparing the standard series of the drug was prepared with a concentration of 100 µg / ml. The concentrations of the standard series were prepared by conducting the appropriate dilution using the solvent %80 H2O with acetic acid 0.1% + %20 Acetonitrile by applying the dilution law for solutions, and the concentrations ranged between 0.1 -10 µg/mL, to determine the range of concentrations subject to Lambert-Beer law. 20 µL of the drug solution was injected using manual injection through the injection valve. The area under the top of the drug concentration series was measured and recorded by applying the best conditions.

2.6. Method for medication assay in pharmaceutical tablets

Each Ampicillin(Amp) pharmaceutical formulation sample had three pills that were properly weighed and pulverized into a powder. Weighing 0.1 g, dissolving it in mobile phase (%80 H2O with acetic acid 0.1% +%20 Acetonitrile), transferring it to a 100 mL volumetric flask, and then completing the reaction to the mark using the same solvent. A known volume containing the right quantity of each drug in line with the calibration curve's range was then placed into a 25 mL container. The straight line equation was used to determine medication concentration.

3. Results and Discussion

3.1.Estimation of drugs Ampicillin and their derivatives

These tests were conducted to choose the appropriate conditions for measuring the drug Ampicillin according to the working method, here many practical tests were conducted, including the selection of the best conditions, Table 1.

Four mobile phases were studied, which are: %80 H2O with acetic acid 0.1% + %20 Acetonitrile, 80% water and 20% methanol, 50% water and 50% methanol and 25% water, 25% Acetonitrile, 50% ammonium acetate.

The best mobile phase was %80 H2O with acetic acid 0.1% + %20 Acetonitrile) depending on the time of detention and the area under the peak, and its pH was 4.5 with the flow rate of 1.1 ml/min, which gave an appropriate time to separate the substance and not interfere with the peak of the solvent that appears soon from the beginning of the peak, this separation at a wavelength of 260 nm which gave the best area under the peak. Figure3.

Table 1: Summary of the practical results obtained for the working conditions and the optimal conditions for the HPLC method

Parameter	Use Factor	Area	Retention Time, min
Mobile phase	%80H_2O+%20MeOH	49464	4.456
pH	7		
Flow rate, ml/min	1		
Wavelength, nm	270		
Mobile phase	%50H_2O+%50MeOH	60615	2.977
pH	3		
Flow rate, ml/min	1		
Wavelength, nm	270		
Mobile phase	50AA+25ACN+25H2O	12989	1.658
pH	3		
Flow rate, ml/min	1		
Wavelength, nm	270		
Mobile phase	%80H_2O with acetic acid (0.1 %) +%20 ACN	341282	4.105
pH	4.5		
Flow rate, ml/min	1.1		
Wavelength, nm	260		

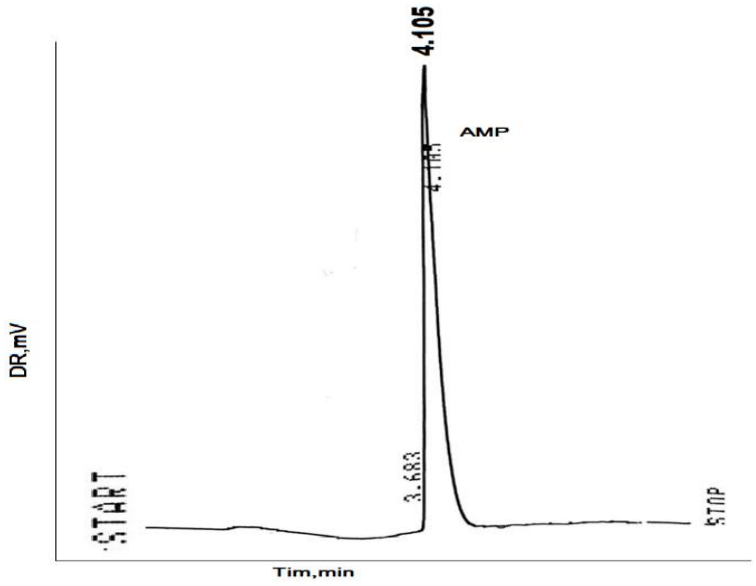


Fig 3: Chromatogram of standard Ampicillin solution at the best conditions

3.2.Validation of analytical method

Validation of developed method was carried out as per ICH Q_2 R_1 guideline . Parameters such as Linearity , Accuracy , Precision , Specificity, LOD , LOQ , were taken up as test for analytical method validation.

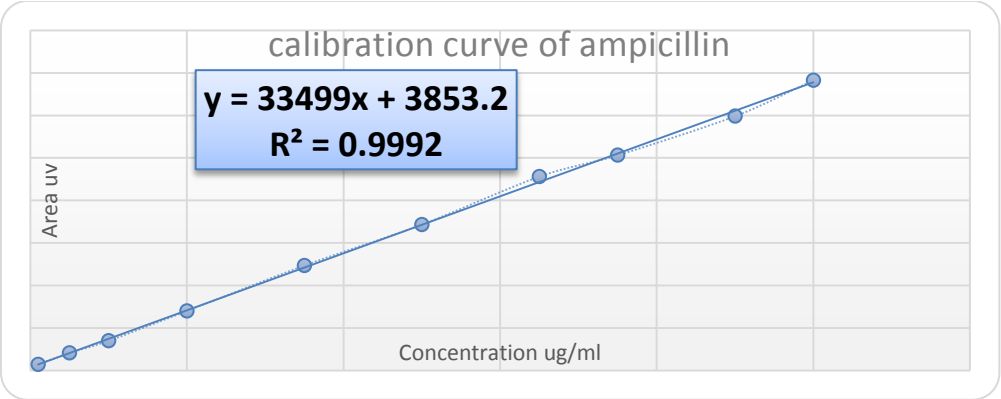


Fig 4: Calibration curve of Ampicillin using (RP-HPLC)

Table 2: The statistical results obtained from the standard calibration curve of Ampicillin

Statistical factors	Value Ampicillin
Linear equation	y = 33499x + 3853.2
Linearity range µg/ml	0.1-10
Slope, m	33499
Intercept	3853.2
Correlation of Determination, R^2	0.9988
Percentage linearity, R^2%	% 98.88
Correlation Coefficient	0.9993
"R.S.D."	0.16
"LOD" µg/ml	0.01
"LOQ" µg/ml	0.033

3.3.Method precision and accuracy

This approach was implemented to determine the suggested method's accuracy and precision. Table.3 summarizes the results obtained, which show that the approach has good accuracy and control, where the accuracy was stated in terms of the relative standard deviation percentage, which ranged (0.13% - 0.22%), as well as in relation to the accuracy expressed by the percentage. For the medicine utilized in the study, the relative error varied from (-0.25% to 0.75%). The recovery rate for three reference drug concentrations (8, 4, 2 µg/ml) was (100.333), and the standard deviation was (0.52041).

Table 3:proposed method accuracy of drugs determination

Amp µg/mL		% Recovery		% Error	R.S.D n =3
Taken	Found				Taken
8	7.98	99.75	Mean=100.333 S.D. =0.52041	-0.25	0.13
4	4.03	100.75		0.75	0.22
2	2.01	100.5		0.5	0.18

3.4.Derivative Diagnosis via FTIR and H-NMR

The FT-IR spectrum for Der-Amp

The infrared spectrum[17] of the derivative showed an absorption band at 3448-3224 for NH_2, an absorption band at 3163 due to stretching the NH bond , an absorption band at 3062 due to stretching the CH aromatic , and an absorption band at 2891-2769 returning to stretching the bond Aliphatic CH, while the N-C=O bond stretching frequency is amide at 1666, and an absorption band of 1613 is due to CH=N bonding and an absorption band returning to N=N bonding is at 1504 as shown in the figure 5.

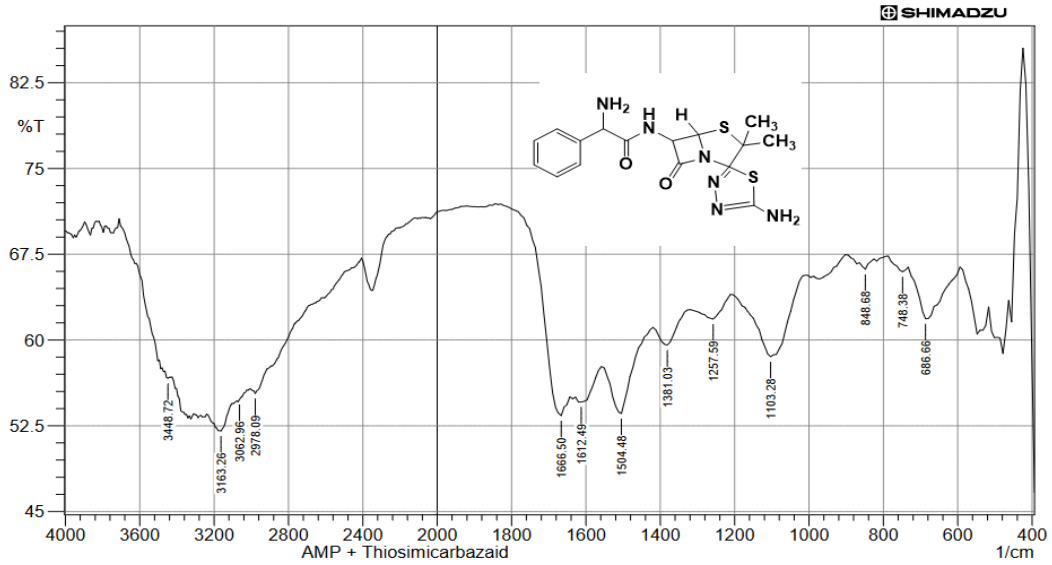


Figure 5: FTIR spectrum for Der (Ampicillin with thiosemicarbazid)

The ^{1}H-NMR spectrum for Der-Amp

The ^{1}H-NMR analysis of the Der-1 derivative showed a multiple signal between 7.8-5.6 ppm due to CH_{Ar} in the aromatic rings, a single signal at 8.8 ppm due to 2H of the NH_2 group, a single signal at 8.1 ppm due to 1H of the NH group , a single signal at 4.4 ppm due to CH-S group, a signal between 3.3-3-1 ppm due to 3H of the CH_3 , Figure 6.

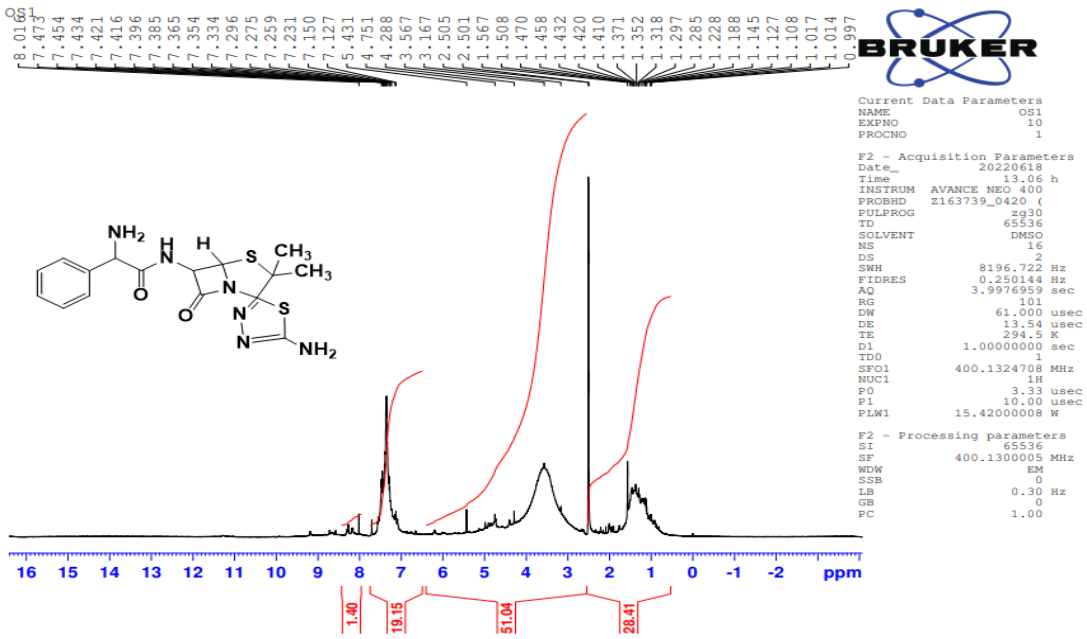


Figure 6: H-NMR Spectrum for Der-Amp (Ampicillin with thiosemicarbazid)

3.5.Separation the derivatives of the Ampicillin from the standard Ampicillin

The Ampicillin derivative were separated from the standard Ampicillin drug according to the optimal separation conditions %80 H2O with acetic acid 0.1% + %20 ACN، pH=4.5 , Flow rate 1.1 ml/min and Wavelength 260 nm. The results showed that the standard Ampicillin drug in minute 4.02 and the derivative appeared in minute 4.935 respectively. Figure 7 shows the peaks of each of Ampicillin and its derivative.

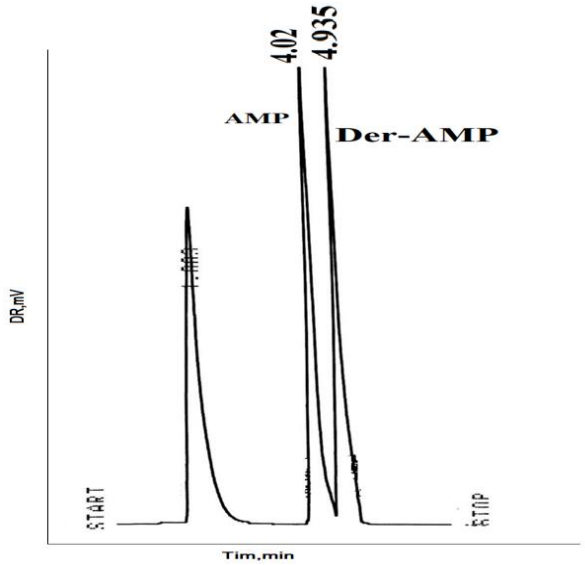


Fig 7: Chromatogram of standard Ampicillin and derivative at optimal conditions

3.6.Determination of Ampicillin pharmaceutical preparation

The appearance of the pharmaceutical peaks for Ampicillin, which are AJANTA and KANGBO, using the optimal separation conditions; Mobile phase (%80 H2O with acetic acid 0.1% + %20 ACN، pH=4.5 , Flow rate 1.1 ml/min and Wavelength 260 nm) were at 4.118 and 4.133 minutes, respectively. Figures 8 show the peaks of pharmaceuticals.

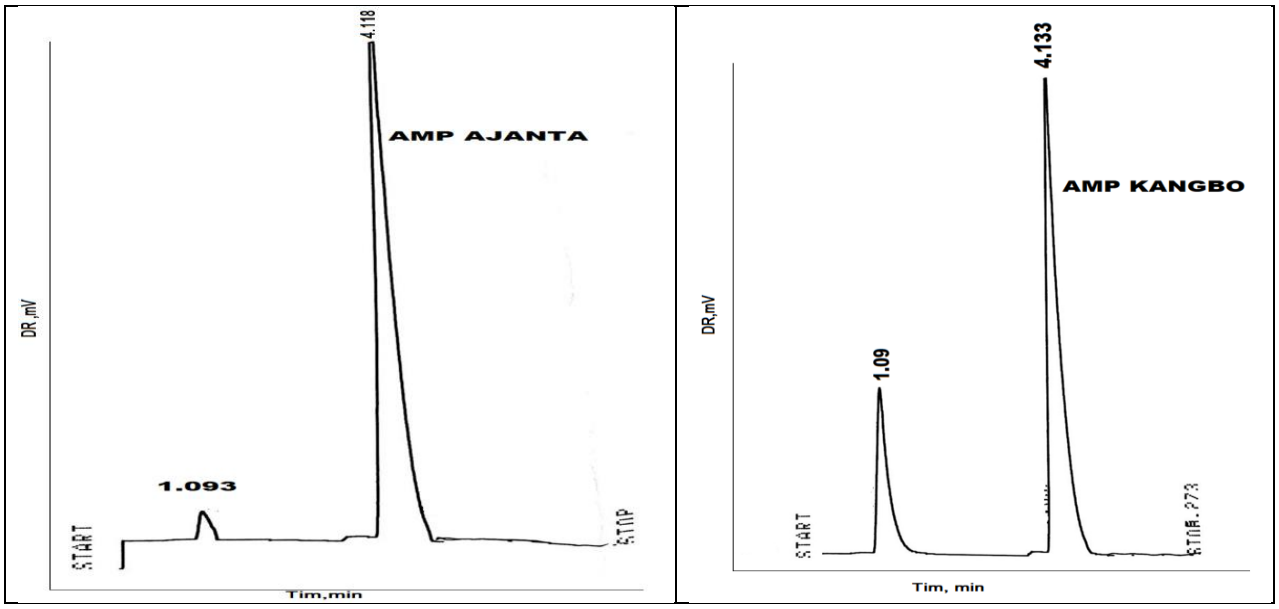


Fig. 8: Chromatogram of pharmaceutical preparation (AJANTA , KANGBO company).

Conclusion

The validated UV – spectroscopy and RP – HPLC methods employed here proved to be simple, fast, accurate, precise and robust to determin AMP and AMP-Der , thus can be used for the routine analysis of Ampicillin in pharmaceutical samples and in aqueous solution. The advantages of this method are being rapid, sensitive, selective and relatively low cost for that we could utilize it for the analysis in quality control units.and in this study new derivative from ampicillin containing a ring 1,3,4-thiadiazole was synthesized.

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Authors' contributions

All the authors have contributed equally

Conflict of interests

Declared none

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