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MOLECULAR, ATOMIC ABSORPTION, FTIR SPECTROPHOTOMETRY AND HPLC METHODS FOR DETERMINATION OF AMOXICILLIN

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Abstract

In this study, there are three techniques, namely, involved molecular, flame atomic absorption, supporting with FTIR and HPLC have been used to determine the concentration of amoxicillin. This process can occur by reacting with $HAuCl_4$ in strong acidic media. The results obtained were (2-100, 0.5-12 and 5-120µg/ml) linearity, (0.722, 0.196 and 1.358µg/ml) detection limits, [(99.070-98.910), (99.160-99.150) and (98.850-98.750)%] recoveries, [(-0.930--1.090), (-0.840--0.850) and (-1.150 to -1.250)%] relative errors, [(2.748-3.923), (1.842-3.021) and (2.371-4.132)%] relative standard deviations for (capsule and suspension) with used molecular, atomic and HPLC methods respectively. These methods successfully implemented for determination of amoxicillin in bulk and pharmaceutical formulations.

Key words: Amoxicillin, Molecular, Atomic absorption, HPLC, FTIR, determination.

Introduction

Amoxicillin, a semi synthetic antibiotic drug, an analog of ampicillin, with a broad spectrum of bacterial activity against many both Gram-positive and negative microorganisms. Chemically name, it is (2S, 5R, 6R) -6-[(R)-(-) -2-amino -2- (p-hydroxyphenyl) acetamido] -3, 3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-carboxylic acid trihydrate. The structure represented in Fig. 1.

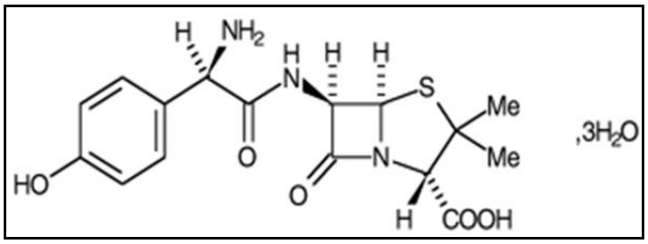

Fig. 1: Chemical structure of amoxicillin trihydrate.

It is widely used in prevention and relief of nausea and vomiting as well as in combination with chemotherapy, where drugs such as cisplatin and other cytotoxic agents, are highly emetic, Due to their wide use for the medical and clinical treatments, it is found that there are enormous research publications been dedicated to estimating these drugs in the pharmaceuticals and to a little in bio-samples using different instrumental techniques (Zuhair., 2016).

Several analytical methods used for determination of amoxicillin such as High-Performance Liquid Chromatographic (Nelis, 1992; Luo, 1997; DeAbreu., 2003; Xie, 2012; Chandera, 2016, Marcel, 2018). Electrophoresis (Hancu., 2016), Kinetic (Belal., 2000), Spectrophotometric (Al_Abachi, 2005; Qader., 2017), Fluorometric (Rezaei, 2019), Colorimetric (Akhond, 2015) and Voltammetric (Rezaei., 2009; Fouladgar., 2011).

The aim for this work is directed towards designing a new approach by implementation three methods for determination of amoxicillin involved molecular, flame atomic absorption, supporting with FTIR spectrophotometry and HPLC were proposed after reaction with $HAuCl_4$ in strong acidic media. The results showed analysis of amoxicillin in bulk, capsule and suspension by these methods successfully implemented.

Materials and Methods

Instrumentations

The main techniques used in this study were Flame

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AAS (Jenway PFP7/UK) Flame atomic absorption Spectrometry (Jenway PFP7/UK) was used for absorbance measurements, Air Acetylene Flame. Double-beam UV-Visible spectrophotometer: Varian Gary 100 UV-Vis spectrophotometer. Analytical balance: DENVER Instrument Max 220 gm, d = .0001g. HPLC Shimadzu LC 2010 A, UV-VIS detector, Kyoto Japan. Fourier Transform Infra-Red FTIR spectrometer, Shimadzu 8400S.

Reagent and Chemicals

All reagents and chemicals were of analytical grade. Amoxicillin monohydrate standard material was supplied from the State Company for Drug Industries and Medical Appliances (Samara-IRAQ-SDI). Au standard solution (1000ppm) for AAS (Fluka). Hydrochloric acid HCl (BDH), Sodium Hydroxide NaOH (Fluka).

Standard solution

Ultra-high-purity grade Chemicals was used for preparation process, 0.1g of Amoxicillin monohydrate standard dissolved in 100 ml distilled water as stock solution (1000ppm) other standard solutions were prepared by subsequent dilution of stock solution. Amoxicillin solution 100 ppm was used for recording UV-Vis spectrum. 1000 ppm standard solution $HAuCl_4$ was used for analysis in atomic absorption spectroscopy, other standard solutions were prepared by subsequent dilution of stock solution. Hydrochloric acid 0.1M, diluting 1.17ml of 36%, specific gravity 1.19 to 100 ml. 0.4g of pure sodium hydroxide dissolved in 100 ml distilled water in order to make 0.1M.

Preliminary studies and Absorption spectra

For the molecular and flame atomic absorption spectrophotometry primary testing of absorption spectra were studied following the procedure; three 10ml volumetric flask, first containing 2.5ml of Amoxicillin 1000 ppm (pH=4.26) and 2.5ml of $HAuCl_4$ 1000 ppm (pH=0.41) after mixing (pH=0.70), second in addition to above solutions added 1ml of 0.1M NaOH solution and third containing 2.5ml of gold solution 1000 ppm and completing the volumes of three volumetric flask to mark with distilled water the deference in color and appearance of any particles was monitoring and recorded the absorbance after heating these solution to 50ºC for 5 minutes.

Atomic Absorption preliminary investigation, Three 10ml volumetric flask, first containing 2.5ml of amoxicillin 100ppm and 2.5ml of gold ion 100ppm, second in addition to above solutions added 1ml of 0.1M NaOH solution,and third containing 2.5ml of gold ion solution 100ppm and completing the volumes of three volumetric flask to mark with distilled water the deference in color and appearance of any particles was monitoring and recorded the absorbance after heating these solution to 50ºC for 5 minutes, using air: acetylene and gold Hallow Cathode Lamp HCL at λ resonance radiation 242.8nm.

The two FTIR spectra for amoxicillin alone and for reaction product of amoxicillin with gold ion were recorded using cesium bromide for preparation disks.

The HPLC method applied for showing differences in two chromatograms for reaction product of amoxicillin with gold ion was recorded. Acetonitrile: buffer solution consists of 15mM H_2SO_4 and 7.5mM sodium dodecyl sulphate SDS (33:67) was used as a mobile phase with flow rate 1ml/min., 150×4.65mlm supelcosil LC-18, injection volume 200µl and detector wavelength at 214nm.

Results and Discussion

Ultraviolet-Visible spectra

The molecular spectrum of 50ppm Amoxicillin in aqueous solutions, is shown in Fig. 2. The maximum absorption peaks were at λ 230, 265nm correspond mainly to $\pi \rightarrow \pi^*$ transfer transitions (Robert., 2000).

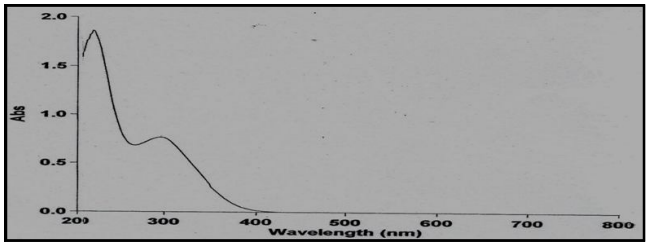

Fig. 2: Molecular spectrum of 50 ppm Amoxicillin solution.

Fig. 3 represent UV-Vis spectrum of 50 ppm $HAuCl_4$ yellow solution. Two absorption bands showed up at 240, 325nm and a low intensity band at 385nm.

When amoxicillin mixed with $HAuCl_4$ changes in

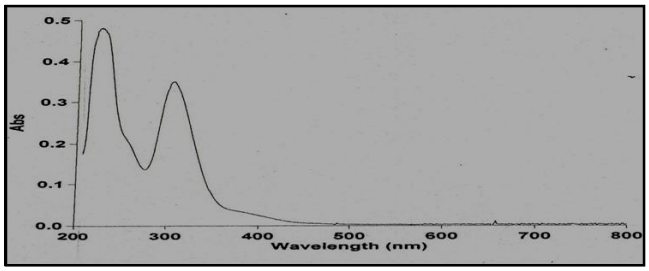

Fig. 3: Molecular spectrum of 50 ppm $HAuCl_4$.

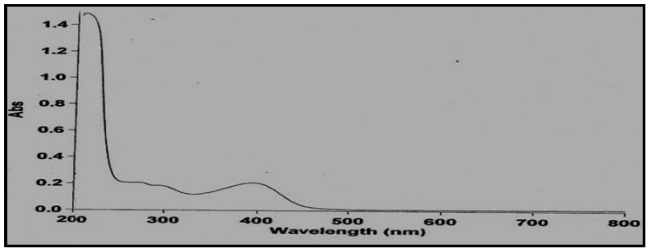

Fig. 4: Molecular spectrum for 50ppm Amoxicillin with $HAuCl_4$.

color and absorption were noticed which indicate to formation of new compound. Fig. 4 shows the absorption spectrum of new product. Comparison the spectrum of amoxicillin in Fig. 3 and amoxicillin with the $HAuCl_4$ in Fig. 4 exhibited hypsochromic shifts of the $\pi \rightarrow \pi^*$ transition bands for new absorption band in the visible region with maximum absorption at wavelength (395nm) for pale yellow solution (Edward., 1999).

FTIR spectra

Fig. 5 and 6 for two FT-IR spectra for amoxicillin alone and for reaction product of amoxicillin with $HAuCl_4$

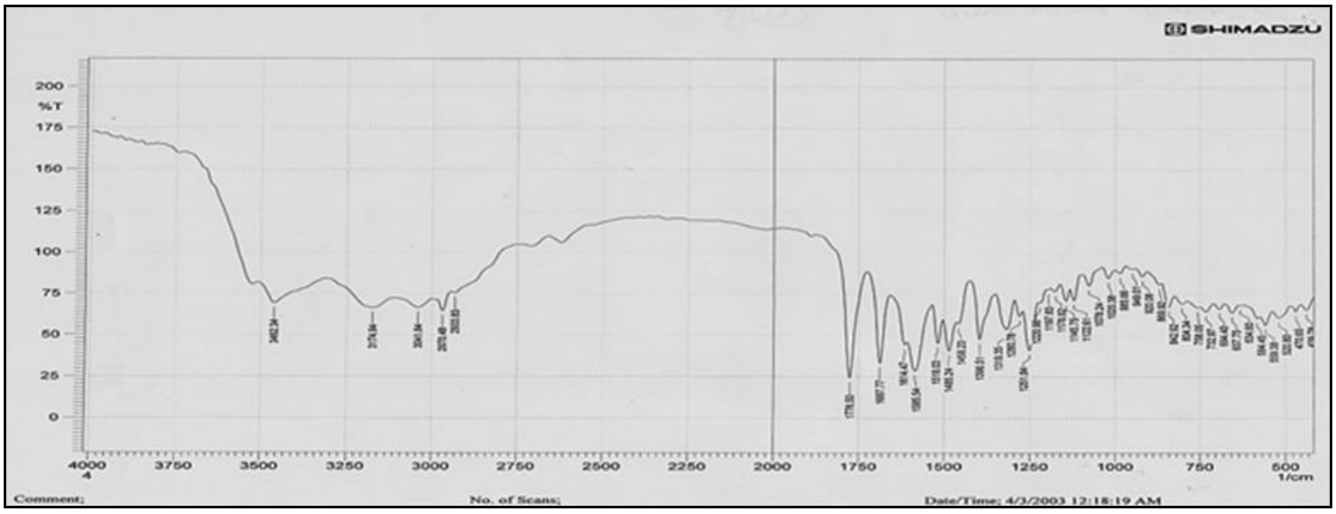

Fig. 5: FT-IR spectrum for pure Amoxicillin.

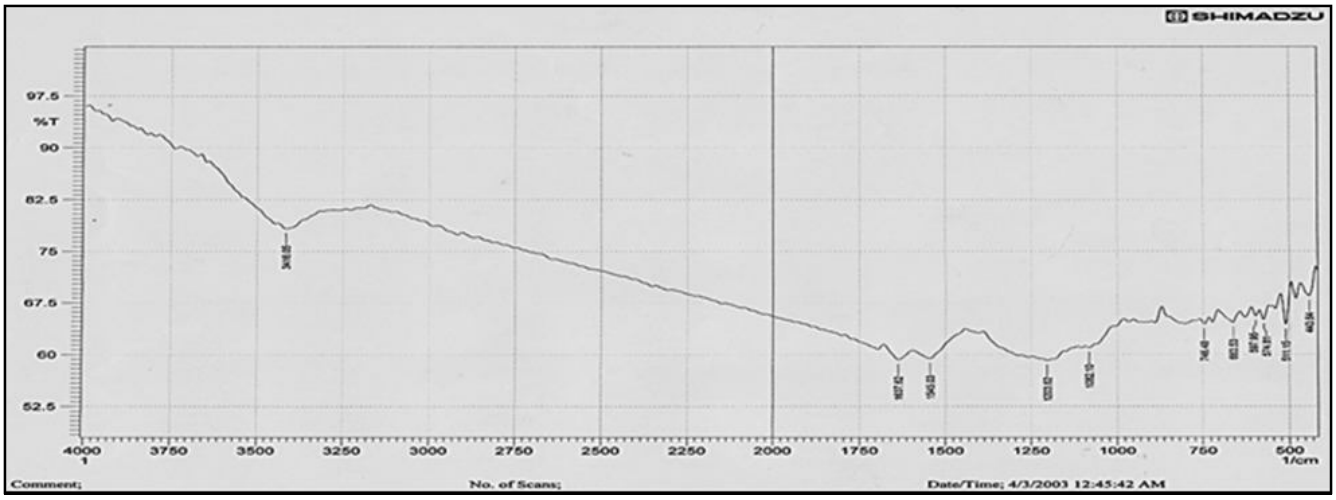

Fig. 6: FT-IR spectrum for product of reaction Amoxicillin with $HAuCl_4$.

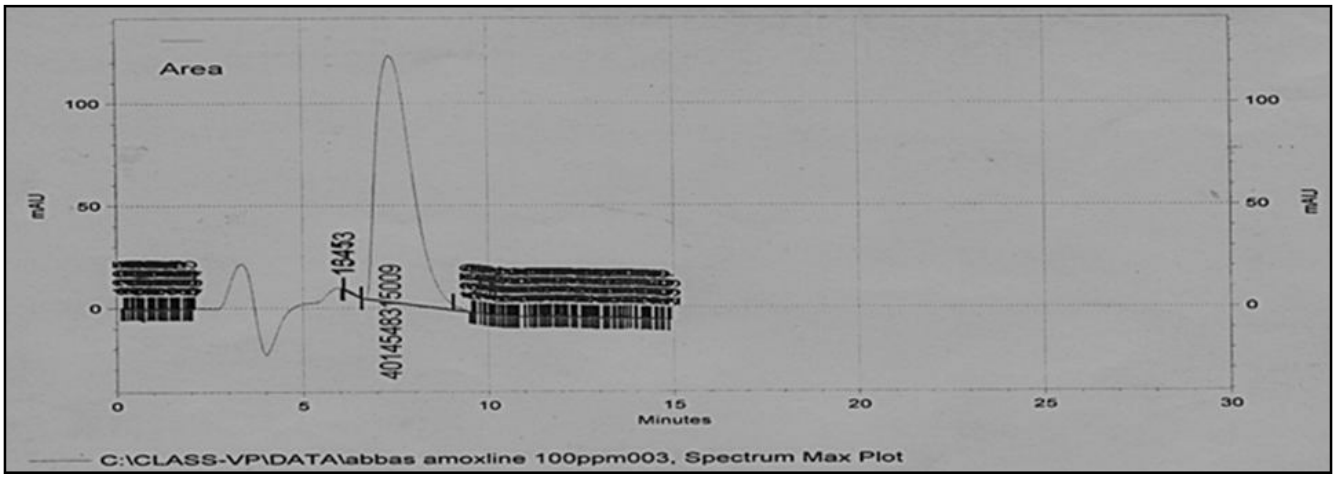

Fig. 7: Chromatogram of pure Amoxicillin.

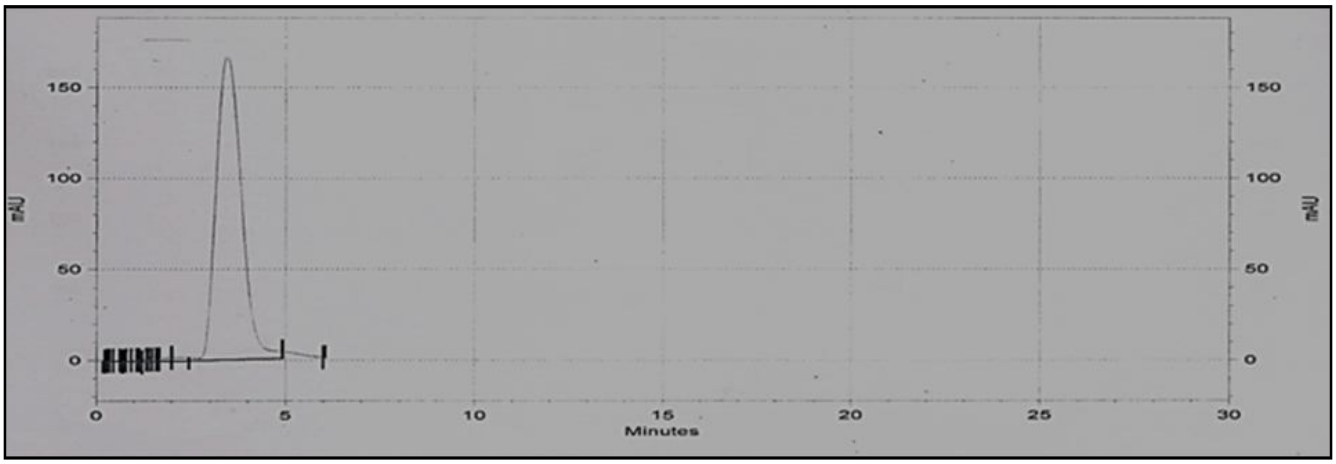

Fig. 8: Chromatograms for reaction product of Amoxicillin with $HAuCl_4$.

were recorded explain disappearance and, or reduce intensity of few origin absorption peaks and increase intensity or and appearance of few new absorption peaks.

HPLC measurements

The HPLC method was utilized to determination of amoxicillin and its reduction reaction formed by reaction with gold ion. Fig. 7 and 8 show chromatograms of amoxicillin alone and for reaction with $HAuCl_4$ with retention time (7.7, 3.9 min.), respectively at maximum absorption 214 nm.

Optimization Conditions

In order to study the optimum conditions, number of significant experimental parameters which mainly effect the absorbance of product reduction reaction such as; $HAuCl_4$ concentration, pH, temperature and heating time have been systematically optimized.

The Effect of gold ion concentration

The effect of $HAuCl_4$ concentration was investigated first, 2.5ml of 100ppm solution of amoxicillin was added to five volumetric flask 10ml containing 2.5ml of (1000, 500, 100, 50 and 10ppm) of gold ions, a little drops of 0.1M NaOH or HCl were added in order to adjust the pH of mixture until the pH is adjusted to 0.70, then diluted to mark with water and the atomic absorption of each solutions was measured after heated to 50ºC for 5 minutes. The effect the of $HAuCl_4$ concentration represented in Fig. 9. Absorbance a solution 100ppm concentration of gold ion considered optimum concentration.

The effect of pH

Solution pH was studied by mixing 2.5ml of 100ppm Amoxicillin solution with 2.5ml of 100ppm gold ion in eight 10 ml volumetric flasks the pH was adjusted to (0.7, 2.0, 4.0, 6.0, 7.0, 8.0, 10.0 and 12.0) by adding a little drops of 0.1M NaOH or HCl solution, completed the volume with

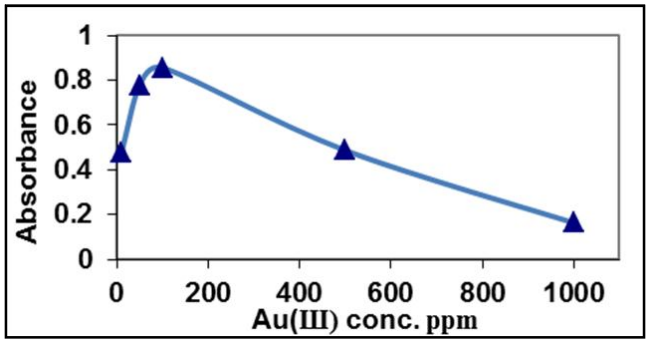

Fig. 9: $HAuCl_4$ concentration.

distilled water then heated to 50ºC for 5 min and measured the recorded UV-Vis absorption.

Reduction reaction between amoxicillin and gold ion prefer higher acidic media (pH=0.7), because possess higher absorbance the absorbance value was decrease with increasing pH because gold hydroxide may be formed in neutral and basic media (Robert., 2000) (Edward., 1999) and the color changes from light yellow to pink to darker pink to light pink to lighter pink to light violet to light red and to colorless, as in Fig. 10.

The effect Temperature

Many temperature values were studied on the mixing of amoxicillin and gold ion solutions after adjusted gold ion and amoxicillin concentration, pH value, heat the samples (20, 35, 50, 65, 80 and 95ºC) in water bath and

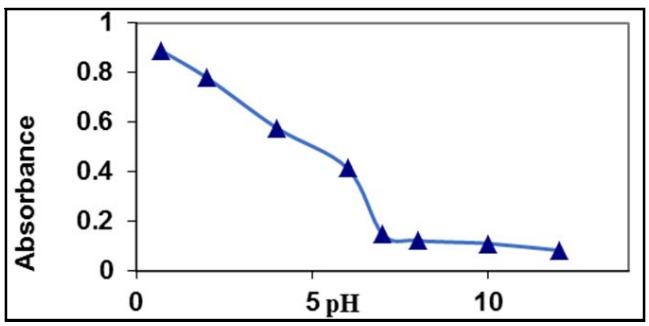

Fig. 10: Effect of pH.

measured the molecular UV-Vis absorption.

Fig. 11 showed the absorbance of product reduction reaction increases with temperature increases and remained constant above 50ºC.

Effect of heating time

After adjusting optimum conditions above (gold ion and amoxicillin concentration, pH and temperature), the effect of heating period was studied for (1, 5, 10, 15, 20, 25, 30 min) then recorded the molecular UV-Vis absorption. The absorbance of reaction product increases with time until above 5 minutes approximately constant as shown in Fig. 12.

Analytical figures of merit

Fig. 13, 14 showed constriction of standard calibration curve for determination of amoxicillin by molecular and atomic spectrophotometry methods, respectively.

Table 1 contained data of percentage recovery and percentage relative error studied on prepared standard solutions of concentrations (20, 40 and 60 µg/ml) and (2, 4 and 6 µg/ml) by molecular and atomic spectrophotometry methods, respectively.

The analytical data obtained (Regression equation, linearity, correlation coefficient and detection limit) listed in table 2.

The constriction of calibration curve for determination of Amoxicillin by HPLC illustrated in Fig. 15.

Table 3 contained data obtained by HPLC method of percentage recovery and percentage relative error studied on prepared standard solutions of concentrations (20, 40 and 60 µg/ml) and (2, 4 and 6 µg/ml) by molecular and atomic spectrophotometry methods respectively.

Table 1: Percentage recovery and relative error for spectrophotometric methods.

Molecular Absorption spectrophotometry					
Taken µg/ml	**Found µg/ml**	**Percentage Recovery%**	**Relative Error%**		**Percentage Relative Standard Deviation RSD % (n = 3)**
20	19.912	99.560	Mean = 99.076	-0.440	1.576
40	39.654	99.140		-0.870	2.783
60	59.119	98.530		-1.470	3.931
Atomic Absorption spectrophotometry					
Taken µg/ml	**Found µg/ml**	**Percentage Recovery%**	**Relative Error %**		**Percentage Relative Standard Deviation RSD % (n = 3)**
2	1.994	99.700	Mean = 99.437	-0.300	0.974
4	3.973	99.330		-0.680	1.589
6	5.957	99.280		-0.720	2.893

Table 2: Analytical data for spectrophotometric methods.

Molecular Absorption spectrophotometry								
preparation type	**Found µg/ml**	**Recovery % (Rec.%)**	**RE%**	**RSD%**	**Regression Equation**	**Linearity µg/ml**	**correlation coefficient**	**Detection Limit DL (µg/ml)**
Capsules(250mg)	247.684	99.070	-0.930	2.748	Y =0.0158 X+0.1381	2-100	0.9939	0.772
Suspension(250mg)	247.264	98.910	-1.090	3.923				
Atomic Absorption spectrophotometry								
Capsules(250mg)	247.905	99.160	-0.840	1.842	Y =0.0981 X+0.0038	0.5-12	0.9986	0.196
Suspension(250mg)	246.879	99.150	-0.850	3.021				

Table 3: Percentage Recovery and Relative Error for HPLC method.

High Performance Liquid Chromatography					
Taken µg/ml	**Found µg/ml**	**Percentage Recovery%**		**Relative Error%**	**Percentage Relative Standard Deviation RSD% (n=3)**
20	19.852	99.260	Mean = 98.897	-0.740	1.784
40	39.556	98.890		-1.110	3.223
60	59.124	98.540		-1.460	4.183

RSD = Percentage Relative Standard Deviation, RE = Relative Error, R = Percentage Recovery.

The analytical data obtained by HPLC method (Regression equation, linearity, Correlation coefficient and Detection limit) are listed in table 4.

Acknowledgements

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Table 4: Analytical data for HPLC method.

High Performance Liquid Chromatography								
Preparation type	**Found µg/ml**	**Recovery% (Rec.%)**	**RE%**	**RSD%**	**Regression equation**	**linearity µg/ml**	**Correlation coefficient**	**DetectionLimit DL (µg/ml)**
Capsules(250mg)	247.125	98.850	-1.150	2.371	Y=0.0147 X+0.0938	5-120	0.9977	1.358
Suspension(250mg)	246.873	98.750	-1.250	4.132				

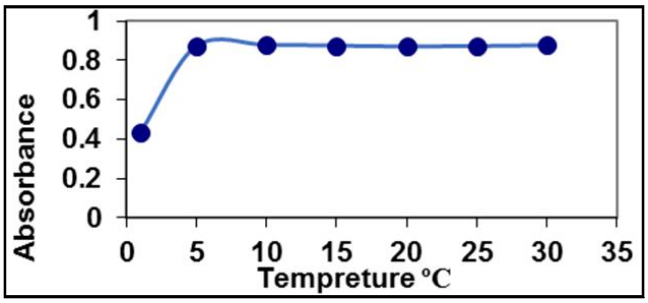

Fig. 11: Effect of temperature.

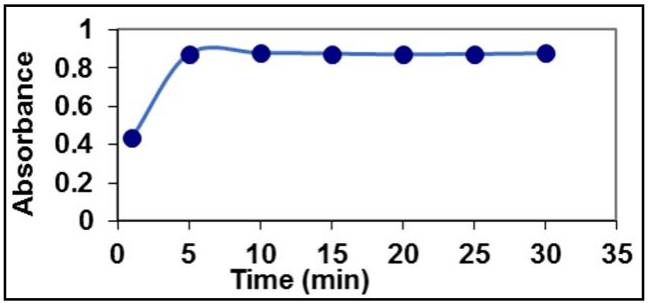

Fig. 12: Effect of time heating.

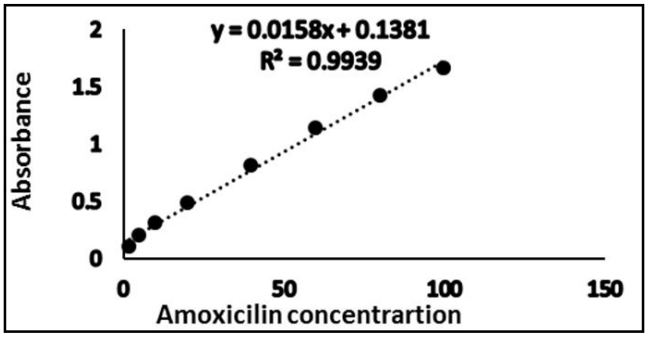

Fig. 13: Molecular spectrophotometry calibration curve for analysis of Amoxicillin.

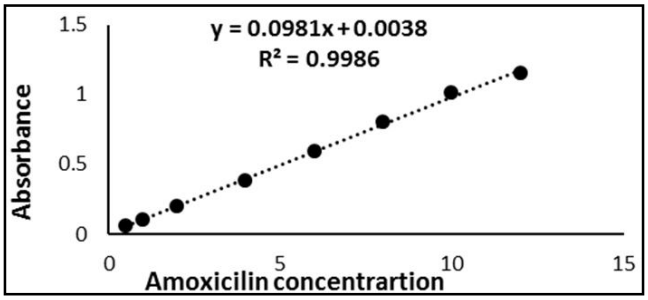

Fig. 14: Atomic spectrophotometry calibration curve for analysis of Amoxicillin.

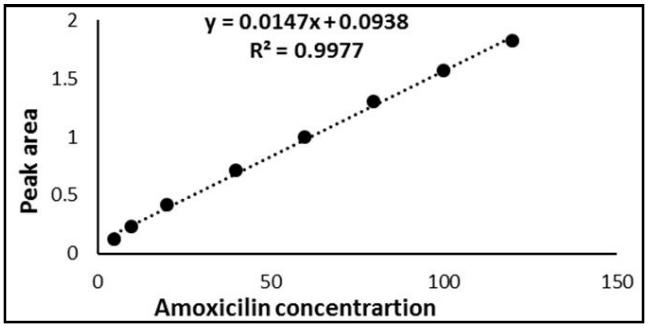

Fig. 15: HPLC peck area against amoxicillin concentration.

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