Simultaneous Estimation of Amprolium Hydrochloride and Sulfaquinoxalin using Simultaneous Equation Method

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Abstract: *The objective of the study is to develop simple, precise, authentic and cost effective analytical method for the estimation of antiprotozoal drugs. Accurate and precise analytical methods were developed for the analysis of amprolium hydrochloride and sulfaquinoxalin sodium in bulk and formulation. The drugs are antiprotozoal drugs which are active against Coccidiosis. 234 nm and 248 are the λmax or the absorption maximum of Amprolium hydrochloride and Sulfaquinoxalin sodium respectively. The isobestic point was 242 nm. The UV methods are developed by using 2-10µg/ml of AMP. Hcl and 1.5- 7.5µg/ml SQS. In simultaneous equation method correlation coefficient of AMP.Hcl was 0.99928.Sulfaquinoxalin shows correlation coefficient of 0.99780. Developed methods are validated as per ICH guidelines.*

Keywords: Amprolium hydrochloride, sulfaquinoxalin, antiprotozoal, method validation

1. Introduction

The word protozoa was coined by zoologist George August Gold form[1].Free living protozoans are present in fresh,brackish and salt water as well as other moisture environments such as soil and mosses. All protozoans require a most habit howeversome can survive forlong periods without water by forming resting cysts[2].

Numerous drugs and drug combinations have been used with some success.

Sulfadimethoxine: 50-60 mg/kg daily for 5-20 days (D, C)

Sulfadimethoxine / Ormetoprim: 55 mg/kg of sulfadimethoxine and 11 mg/kg of ormetaprim for 7-23 days (D)

Quinacrine: 10 mg/kg daily for 5 days (C) Amprolium: 300 to 400 mg (total) for 5 days (D); 110-200 mg (total) daily for 7-12 days (D); 60- 100 mg/kg (total) daily for 7 days (C); 1.5 tbsp (23 cc)/gal (sole water source) not to exceed 10 days (D)

Amprolium/Sulfaquinoxalin: 200 mg/kg of amprolium and 150 mg/kg of sulfaquinoxalin for 14 days (D)[3]

Analytical method development is the key element of pharmaceutical development program. and it is the process of proving that the developed method can be used to detect the amount /concentration of API in various formulations [4].

UV-Visible spectroscopy is based on the measurement of intensity of absorption of near uv and visible light by a sample and wavelength, In July 1941, Arnold Beckman introduced DU UV-Visible spectrophotometer. The absorption or reflectance in the visible reagion based on the colour of chemicals. In this region atoms and molecules undergo electronic transition[5].

If a sample contains two absorbing drugs(x and y) each of which absorbs at the λmax of the other, it may possible to determine both the drugs by the technique of simultaneous equation (Vierordt's method) provided that certain criteria apply.Absorbance was measure at the maximum wavelength of the drugs and apply to simultaneous equation[6] .

The absorbance of the mixture is the sum of the individual absorbance of x and y.Simultaneous equation need algebraic skills. They are called simultaneous because they are solved at the same time.This is the simple, precise, accurate, reproducible and efficient method.

Let CX and CY the concentration of x and y respectively in the diluted samples.

$$A1 = ax1 \ b \ Cx + Ay1 \ b \ Cy \dots\dots\dots\dots (1)$$
$$A2 = ax2 \ b \ Cx + Ay2 \ b \ Cy \dots\dots\dots\dots (2)$$

Where , A1 and A2 = absorbance of the diluted samples at λ1 and λ2 respectively.
ax1 and ax2 = absorptivities of x at λ1 and λ2 respectively
ay1 and ay2 = absorptivities of y at λ1 andλ2 Cx and Cy = concentration of x and y respectively in the diluted samples.
Rearrange equation (2)
cX = A2aY1 – A1aY2/ aX2aY1 – aX1aY2
cY = A1aX2 – A2aX1/aX2aY1 – aX1aY2

Experimental Apparatus
1) Electronic Balance Tandem TJ series
2) UV Spectrophotometer Shimadzu, UV1700, Pharmaspec, Japan (attached to acomputer software UV probe 2.0,

with a spectral width of 2 nm,wavelength accuracy of 0.5 nm and pair of 1 cm matched quartz cells.)

Reagents and Materials:
1) AMPROLIUM HYDROCHLORIDE TCI. Chemicals Chennai
2) SULFAQUINOXALIN SODIUM Sigma aldrich
3) METHANOL Finar chemicals
4) ACETIC ACID Nice chemicals pvt ltd, Mumbai
5) WATER (HPLC GRADE) Research lab fine chem. Industries, Mumbai

Experimental Procedure

Selection of wavelength range for estimation
Both AMP and SQS were dissolved seperately in methanol ,And appropriate dilutions were prepared by taking aliquots from the stock solution .The drug solutions were scanned from 200-400 nm and from that wavelength ranges are selected for estimation of drugs.

Preparation of standard stock solutions (1000µg/ml)
An accurately weighed quantity of AMP (50 mg) and SQS (50 mg) were transferred to a separate 50 ml volumetric flask.Methanol is used to dissolve the drugs and the volume was made up to the mark with methanol to get the solution having a concentration of 1000µg/ml . And the solution is used as the first stock, from that further dilutions carried out.

Preparation of working standard solutions
From the above prepared stock solutions of amprolium hydrochloride and sulfaquinoxalin sodium 1 ml were transferred separately to 10 ml volumetric flask to obtain working standard solutions having a concentration of 100 µg/ml.

Preparation of calibration curve
From the above working standard solutions of both AMP (0.2,0.4,0.6,0.8,1 ml) and SQS (0.15,0.3,0.45,0.6,0.75 ml) aliquots were transferred separately in a series of 10 ml volumetric flask.The volume was adjusted to the mark with methanol.To get a concentration range of 2-10µg/ml of Amprolium hydrochloride and 1.5-7.5µg/ml of sulfaquinoxalinsodium. The absorbance of all the solutions were calculated by scanning from 200-400 nm,Against methanol as the blank.

2. Methodology

The working standard solutions of AMP and SQS were scanned in uv from the range of 200-400 nm.Where amprolium shows 234 and sulfaquinoxalin sodium shows 248 nm as the wavelength having maximum absorbance.And this wavelengths are selected for the quantitative estimation of amprolium and sulfaquinoxalin. A set of two simultaneous equations were framed using absorptivity coefficient at selected wavelengths, from the overlain spectra of drugs, Based on the equations the concentration of the two components in the mixture were calculated.

Method Validation
Linearity and Range: Different dilutions of concentration 2, 4, 6, 8, 10µg/ml of amprolium and 1.5,3,4.5,6,7.5µg/m of sulfaquinoxalin were prepared. The calibration curve was plotted and interpreted in terms of correlation coefficient and equation of line. Method: Absorbance of each solutions were noted down at their respective wavelengths (234,248).

Method precision (Repeatability): The precision of the instrument was checked by repeated scanning and measuring the absorbance of solution of (n = 6) AMP (4 µg/ml) and SQS (3 µg/ml) without changing the parameters of developed methods.

Reproducibility: The intraday and interday precision was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of AMP (4, 6, 8 µg/ml) and SQS (3, 4.5, 6 µg/ml). Relative standard deviation (% RSD) was used to report the results.

Accuracy (% Recovery): Accuracy can be reported in terms of % recovery. The percentage spiking levels are 80,100 and 120%, About 4µg of amprolium hydrochloride and 3µg of sulfaquinoxalin sodium were used for the study.

Limit of detection and Limit of quantification(LOD & LOQ): The LOD and LOQ were calculated by the equation method. LOD = $3.3 \times \sigma/S$ LOQ = $10 \times \sigma/S$ Where, σ = the standard deviation of the response S = slope of the calibration curve.

3. Results and Discussion

In this method the dilute solutions of AMP and SQS were scanned from 200-400 nm. Two wavelengths 234 nm and 248 nm are selected for amproliumhydrochloride and sulfaquinoxalin respectively. Here 234 nm is the absorbance maxima of AMP and 248 nm is the λmax of sulfaquinoxalinsodium.

A set of two simultaneous equations were framed using absorptivity coefficient at selected wavelengths. The concentrations of two drugs in the mixture were calculated using the following equations.

$$Cx = \frac{A1\ ay2 - A2\ ay1}{ax1\ ay2 - ax2\ ay1} \text{--------------Eq. (i)}$$

$$Cy = \frac{A1\ ax2 - A2\ ax1}{ay1\ ax2 - ay2\ ax1} \text{--------------Eq. (ii)}$$

Simultaneous equation was developed using the following set of equations:
At 234 nm A1 = ax1bcx + ay1bcy ----------- (1)
At 248 nm A2 = ax2bcx + ay2bcy ----------- (2)
Where Cx and Cy are concentration of MSL and PRD, respectively.

A1 and A2 are absorbance of sample at 234 nm and 248 nm respectively; ax1 and ax2are absorption coefficient of AMP at 234nm and 248nm respectively; ay1 and ay2 areabsorption coefficient of SQS at 234nm and 248nm

respectively. And the amount of sample solution were calculated from the simultaneous equation.

Method Validation

Linearity: Different dilutions of concentration 2, 4, 6, 8, 10µg/ml of amprolium and1.5,3,4.5,6,7.5µg/m of sulfaquinoxalin were used to record the Absorbance of eachsolutions at their respective wavelengths (234,248) the calibration curve was recorded (Fig3 and Fig 4).

Precision (Repeatability): Here the % RSD is less than 2 indicates the method is repeatable (Table2).

Reproducibility (Intermediate Precision): Here the percentage rsd was found to be below 2% indicates the reproducibility of thedeveloped analytical method.

Accuracy: Here the recovery result indicates the accuracy of the proposed method. Theaccuracy was calculated by recovery studies in various levels (Table 3).

LOD and LOQ: According to ICH guideline there are several methods for the determination of LOD and LOQ in the present study the LOD and LOQ were calculated by equation .TheLOD and LOQ of sulfaquinoxalin was found to be 0.3773 & 1.132 respectively. For amprolium it is 0.2811 & 0.8518 µg/ml (Table 1).

Table 1: Regression analysis data and summary of validation parameters from the calibration plot.

Parameter	Amprolium Hydrochloride	Sulfaquinoxalin Sodium
Absorption maximum	234 nm	248 nm
Linearity range(µg/ml)	2-10	1.5-7.5
Correlation coefficient	0.99928	0.99780
Regression equation	Y=0.0324x+0.01140	Y=0.06773x+0.00300
slope	0.0324	0.06773
Y intercept	0.01140	0.00300
RSD	0.00754	0.03240

Table 2: Precision data for Amprolium hydrochloride and Sulfaquinoxalin

Concentration AMP:SQS (4&3) n=6	Absorbance	
	Amprolium Hydrochloride (234nm)	Sulfaquinoxalin Sodium (248nm)
1	0.137	0.21
2	0.138	0.211
3	0.136	0.212
4	0.135	0.21
5	0.137	0.21
6	0.137	0.212
MEAN	0.136	0.21
SD	0.001	0.0009
RSD (%)	0.735	0.428

Table 3: Results of recovery studies by developed method

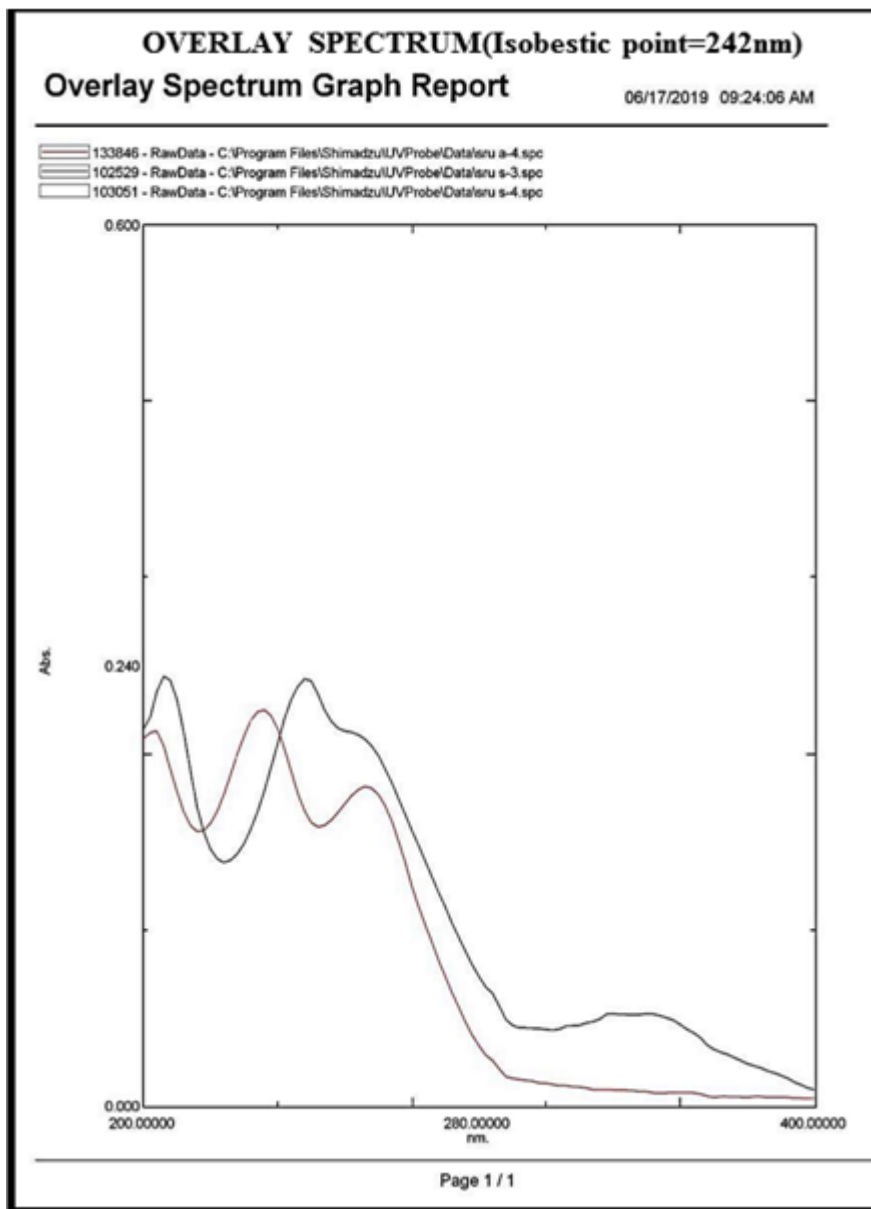| Drug	Accuracy Level (%)	Amount			% Recovery	Mean ± SD	% RSD
		actual (µg/)	added (µg/)	found (µg/)			
AMP. Hcl	80%	4	3.2	7.1	98.6	98.82±0.2029	0.205
	100%	4	4	7.92	99		
	120%	4	4.8	8.7	98.86		
SQS	80%	3	2.4	5.39	99.8	98.86±0.819	0.828
	100%	3	3	5.9	98.3		
	120%	3	3.6	6.5	98.48		

Figure 1: Overlain zero order absorption spectra of Amprolium hydrochloride and sulfaquinoxalin
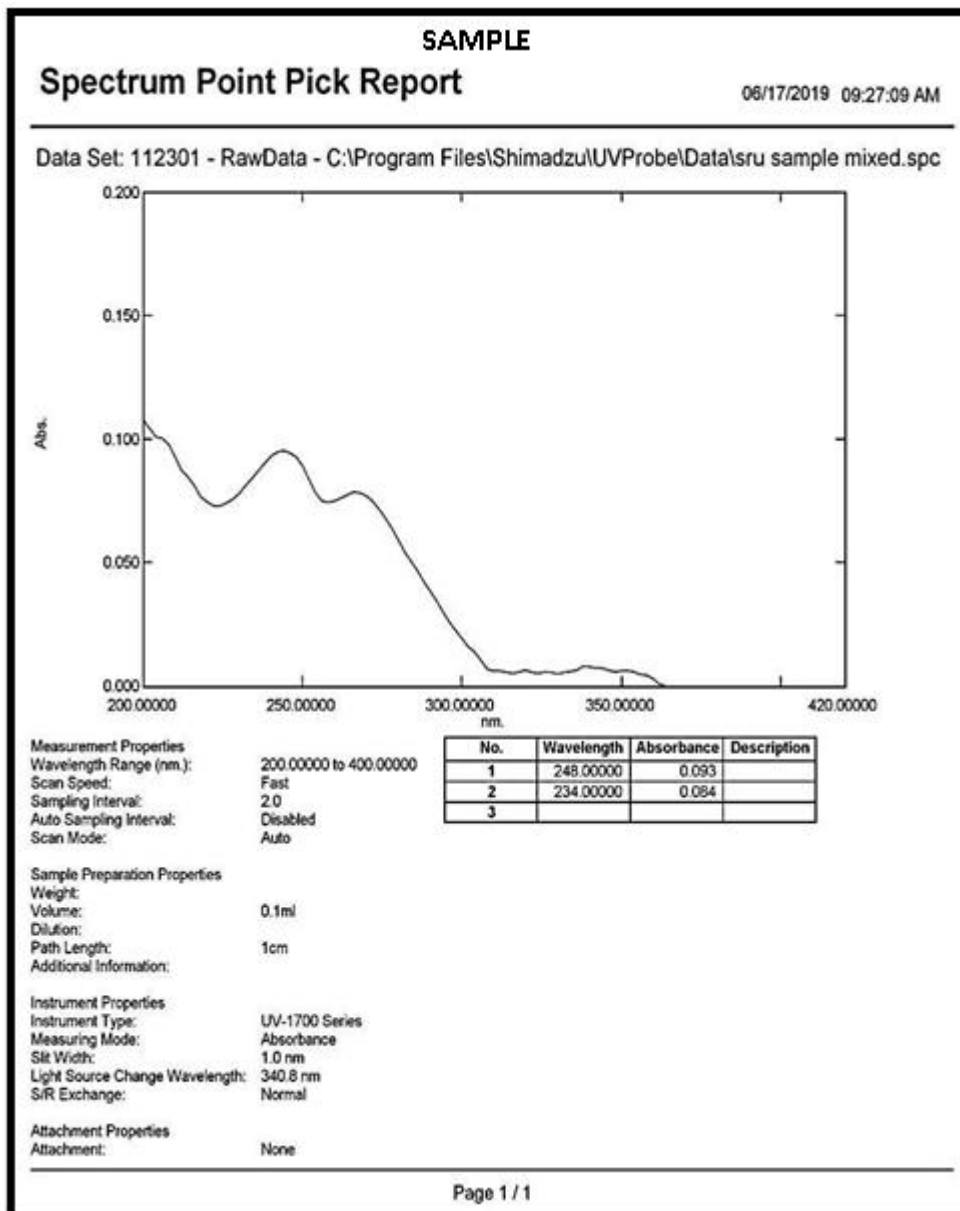
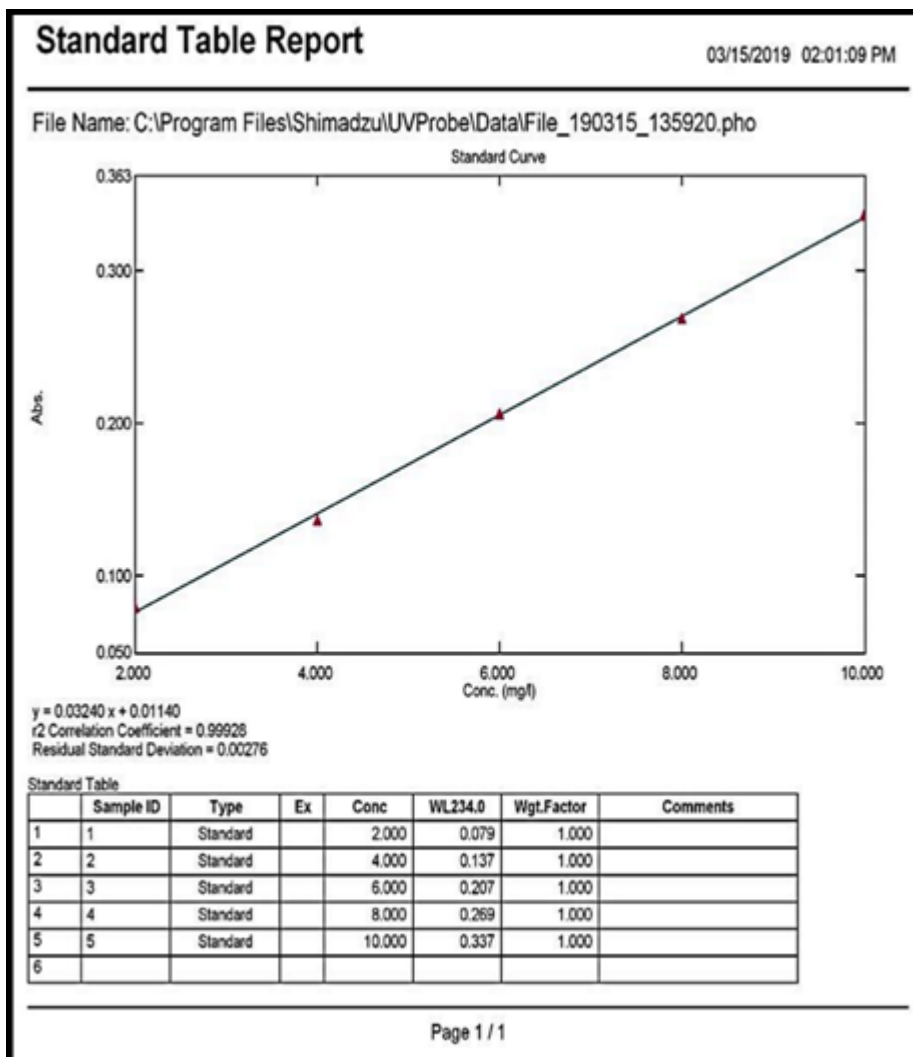**Figure 2:** Zero order absorption spectra of Samples

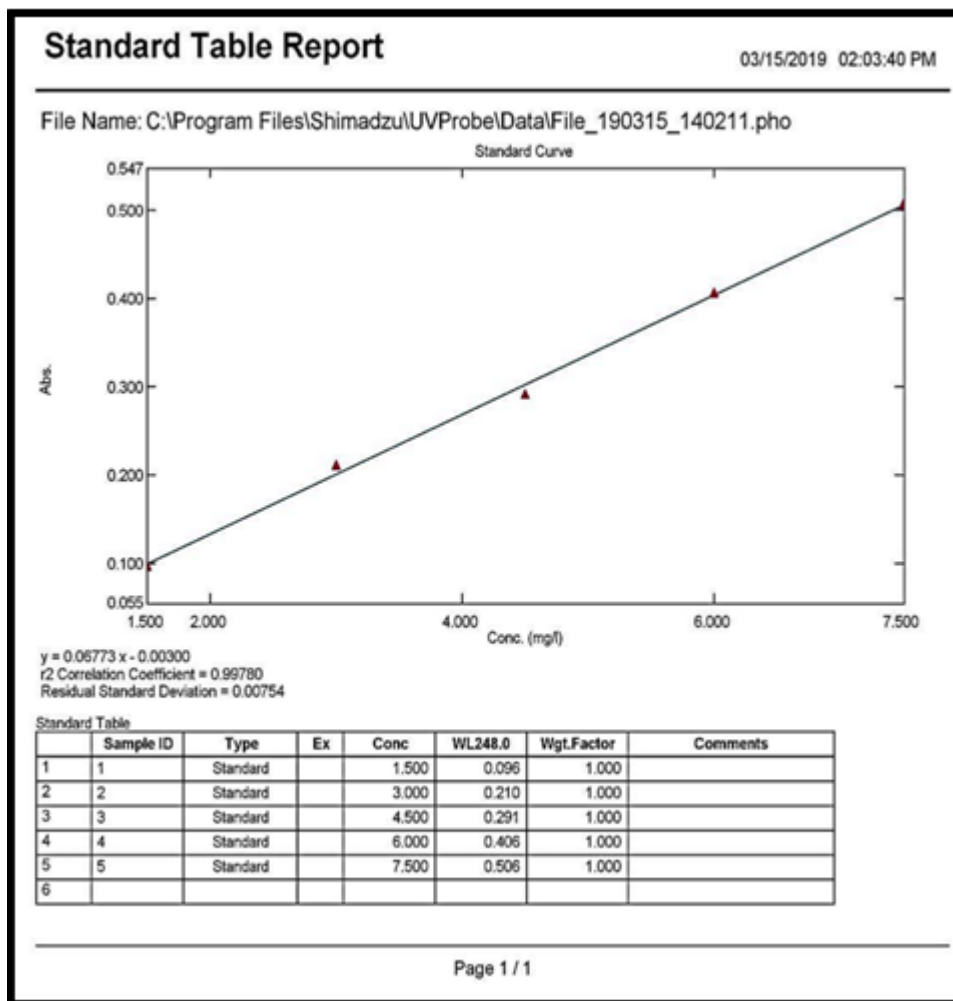**Figure 3:** Calibration curve of amprolium)

Figure 4: Calibration curve of Sulfaquinoxalin

4. Conclusions

A simple as well as precise analytical method were developed for the estimation of Amprolium hydrochloride and Sulfaquinoxalin in bulk and formulation.

- The methods were developed by using UV-Visible spectrophotometer.
- The absorption maxima of amprolium hydrochloride was found to be 234 nm and 248 is the λmax of sulfaquinoxalin sodium. The isobestic point was 242 nm.
- The developed method was simultaneous equation method, and for AMP.Hcl method shows linearity from 2-10μg/ml. And a correlation coefficient of 0.99928.

Sulfaquinoxalin shows linearity from 1.5-7.5μg/ml along with an correlation coefficient of 0.99780.

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