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SIMULTANEOUS ESTIMATION OF AMPROLIUM HYDROCHLORIDE AND SULFAQUINOXALIN SODIUM USING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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ABSTRACT

HPTLC was a method developed for the Simultaneous estimation of amprolium hydrochloride and sulfaquinoxalin sodium . The developed high performance thin layer chromatographic method was a suitable method for the analysis of drugs and here the calibration curve was plotted by using 2-10µg/ml of AMP.Hcl and 6-14µg/ml of SQS .Drugs shows good correlation coefficient and linearity. The Methods Shows good accuracy at a spiking levels of 80,100 and 120%. The developed methods can routinely be used for the estimation of amprolium hydrochloride and sulfaquinoxalin sodium in bulk and formulation.

KEYWORDS: Amprolium hydrochloride, Sulfaquinoxalin sodium, HPTLC, chromatography, validation.

INTRODUCTION

Sulfaquinoxaline sodium(IUPAC name: sodium;(4-aminophenyl)sulfonyl-quinoxalin-2ylazanide) Is a veterinary medicine which can be given to cattle and sheep to treat coccidiosis. Amprolium hydrochloride (IUPAC name:5-[(2-methylpyridin-1-ium-1-yl)methyl]-2- propylpyrimidin-4-amine;chloride) is an anti protozoal drug. Here we propose an additional method for the quantification of sulfaquinoxaline and Amprolium in bulk as well as formulation by HPTLC.The new and modern version of Thin layer chromatography is known as HPTLC and are the most modern and simple technique used for the analysis of compounds.[1] Using HPTLC we can collect a large amount of data in a short time.[2] In thin layer chromatography the separation is carried out in an stationary phase by the movement of an mobile phase. and the stationary phase consisting of an suitable material spread on an supporting medium, The separation is mainly due to adsorption, partition and ion exchange.[3] It is also known as planar chromatography or flat-bed chromatography, Because the mobile phase is liquid and the stationary phase is solid.[4] The mobile phase is moved due to capillary forces across the layer of the stationary phase. And the separation is based on the strength of stationary phase and relative solubility of solute in mobile phase and here retarding action of sorbent acts as the driving force. Finally the sample compound migrate a certain distance from the point of application.[5,6]

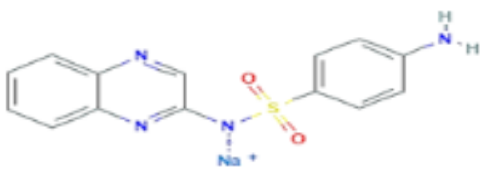

Fig.1: Chemical structure of sulfaquinoxalin sodium.

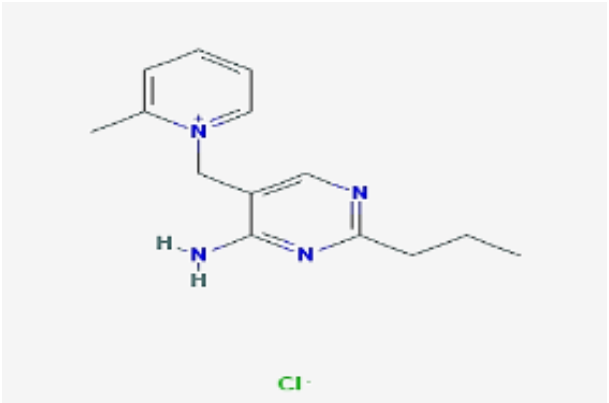

Fig.2:Chemical structure of amprolium Hydrochloride.

MATERIALS AND METHODS

Calibration curve for standard drugs

The standard solution of amprolium hydrochloride (2,4,6,8,10µg/spot) and sulfaquinoxalin sodium (6,8,10,12,14µg/ml) were applied on TLC plates and further spot development and scanning were carried out as per the given chromatographic condition .The calibration curve were plotted using peak area against concentration.

Analysis of marketed formulation

The selected formulation contains about 200mg of amprolium hydrochloride and 150 mg of sulfaquinoxalin sodium. For analysis the drugs solution were appropriately diluted and were spotted on HPTLC plate. The amount of drugs were calculated by comparison of the areas measured for standard solutions.

Steps involved in HPTLC analysis

Selection of stationary phase: Analysis is carried out in pre coated plates, Selection of stationary phase is depending upon the nature of analyte. HPTLC plates precoated with silica gel 60F254 on aluminum sheets (0.2 mm thickness) Are employed in many research work because these plates are flexible, easy to handle and can be cut in desired sizes. The plates are heat resistant and compatible with most organic solvents. It may also contain UV indicator.

Prewashing and activation of plates: It is mainly to remove the impurities including water vapours and volatile compounds. silica gel60F is the most widely used solvent .The major dis advantage s the presence of iron as impurity, So prewashing is carried out using methanol: water in 9:1 ratio. Plate activation is mainly by heating at 1200c.

Selection of mobile phase: Depending upon the nature of sample mobile phase is selected. The solvent should be of adequate purity at reasonable cost.

Sample application: This is the most important step in HPTLC. We can apply the sample manually or by using automatic sample. Now a days we are going for automatic samplers . Because manual application is time consuming and skilled work. Automatic sample application is accurate and precise and it will completely transfer the sample .It will not damage the sorbent layer. Sample can be applied as spots or bands.

Development: Generally the development is carried out in developing chambers. Different modes can be used for development like ascending, descending, 2-dimensional, horizontal, multioverrun, gradient, radial, anti radial, multimodel, forced flow. The spotted plates were placed in developing chambers After development plates were removed from chamber.

Drying: Drying can be done by using vaccum desicators. Air drying may leads to evaporation of volatile components. If we go for hand drying it will leads to contamination.

Derivatization: Scan speed – 20 mm/s.
Center of scan beam and of sample band should overlap. Always record spectra of all samples, except when not required in method.

- By immersion technique.
- Dip speed.
- Dip time

Visualization and detection: UV detection is the non destructive method. Fluorescent compounds can be seen at 254 nm (short wave length) or at 366 nm (long wave length).

Scanning densitometry: Densitometric scanning can be used for quantification and it contain uv,visible and fluorescent lamps. With range of 190-90 nm for identification and purity check.

Documentation: Digital camera based documentation is generally used. Everything should be documented including type of chamber, plate, mobile phase, running time, etc.

METHOD VALIDATION

According to ICH Q2(R1) analytical methods are validated. The validation parameters are:

- Linearity/ Range
- Accuracy

Linearity / Range

Through linearity we are checking the ability of an analytical method to give test results proportional to the concentration. The interval between upper and lower concentration of an analyte in an sample is known as range. Linearity can be evaluated by visual inspection. The correlation coefficient, y-intercept, slope of the regression line and residual sum of squares must be submitted along with the linearity plot. A minimum of five concentration is recommended for linearity.

Accuracy

It is the closeness of agreement between the true value and the reference value.

RESULTS AND DISCUSSION

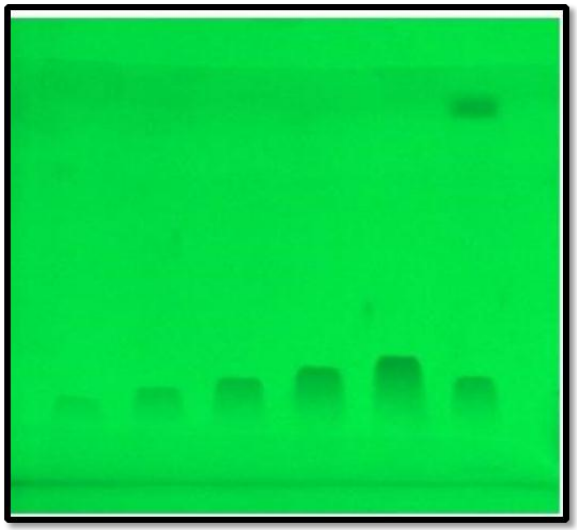

Figure 3: HPTLC chromatogram of amprolium hydrochloride at 254nm.

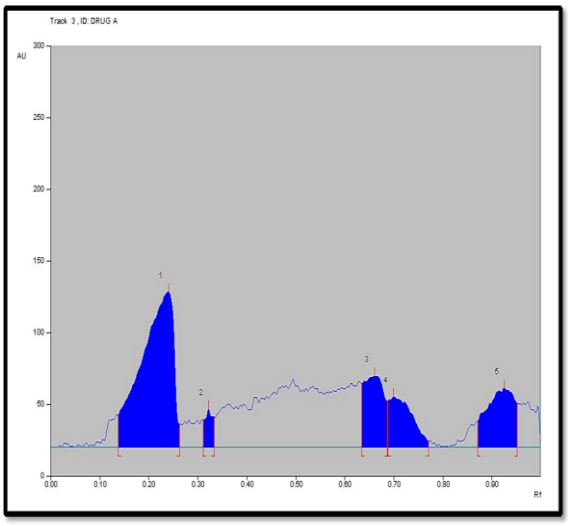

Figure 4: chromatogram of standard.

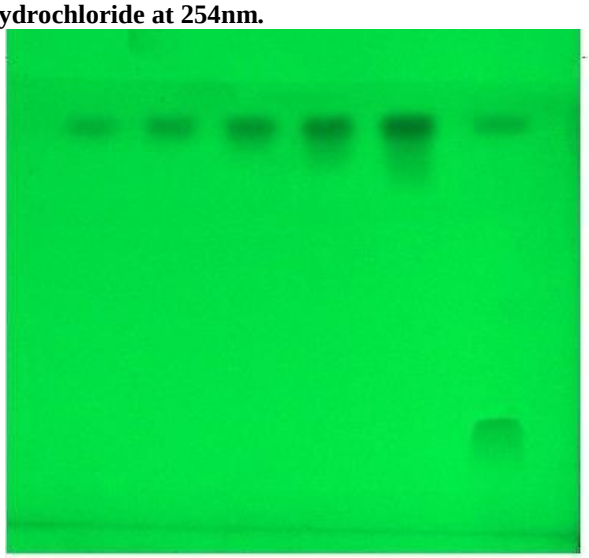

Figure 5: HPTLC chromatogram of Sulfaquinoxalin sodium.

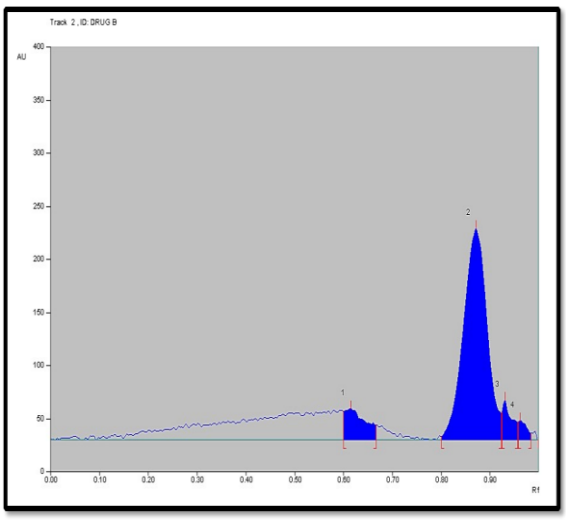

Figure 6: chromatogram of standard sulfaquinoxalin sodium.

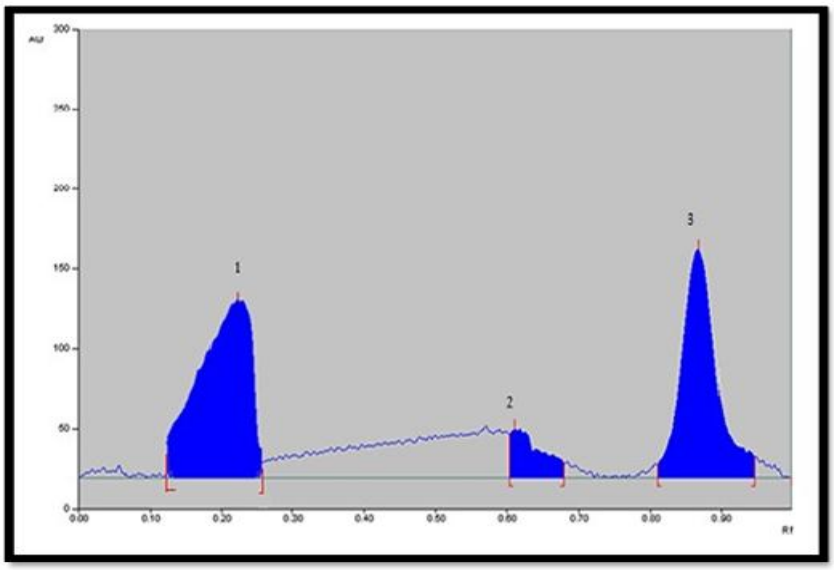

Figure 7: chromatogram of sample.

Analysis of marketed formulation

Table 1: Analysis of marketed formulation.

Formulation	Drug	Label Claim (Mg)	Sample Solution Concen-tration (µg/ml)	Amound Found (µg/ml)	Drug Content (%)
1	AMP. Hcl	200 Mg	8	7.926	99.0750
	SQS	150 Mg	6	5.618	93.6333

Method validation

Linearity

The linearity was checked according to ICH guidelines Linear correlation was obtained between peak area Vs concentrations of amprolium hydrochloride and sulfaquinoxalin sodium.Both drugs showed good linearity over a concentration range. Amprolium hydrochlorie shows linearity from 2-10µg/ml. And sulfaquinoxalin shows linearity from 6-14µg/ml and the correlation coefficients are 0.979 for AMP.Hcl and 0.9849 for SQS.

Table 2: calibration data for AMP. Hcl.

SL.NO	CONCENTRATION(µg/ml)	AREA(Au)
1	2	2752.6
2	4	5129.9
3	6	6530.5
4	8	9807.4
5	10	10807.2

Table 3: calibration data of SQS.

SL.NO	CONCENTRATION(µg/ml)	AREA(Au)
1	6	6349.3
2	8	8977.0
3	10	13874.7
4	12	18633.9
5	14	24533.4

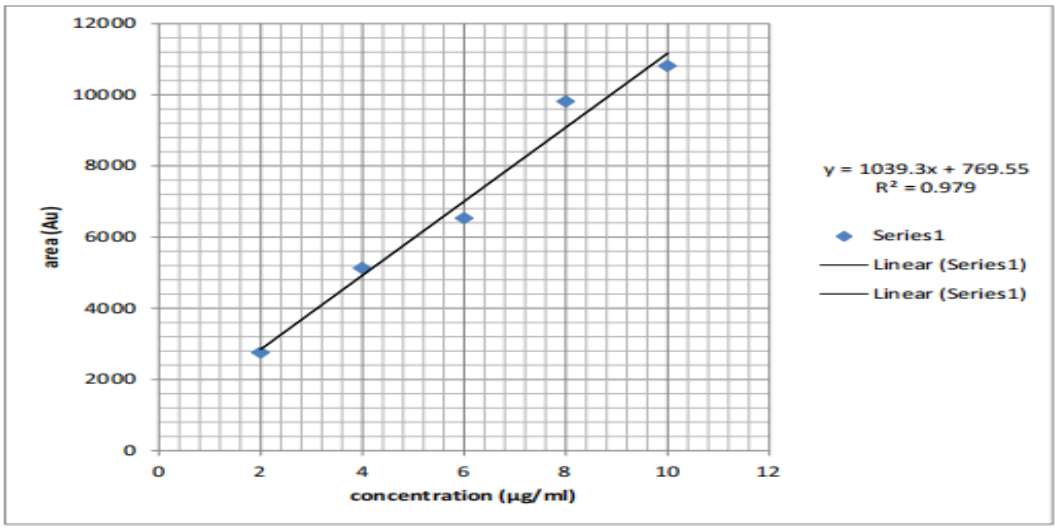

Figure 8: Calibration curve of Amprolium hydrochloride.

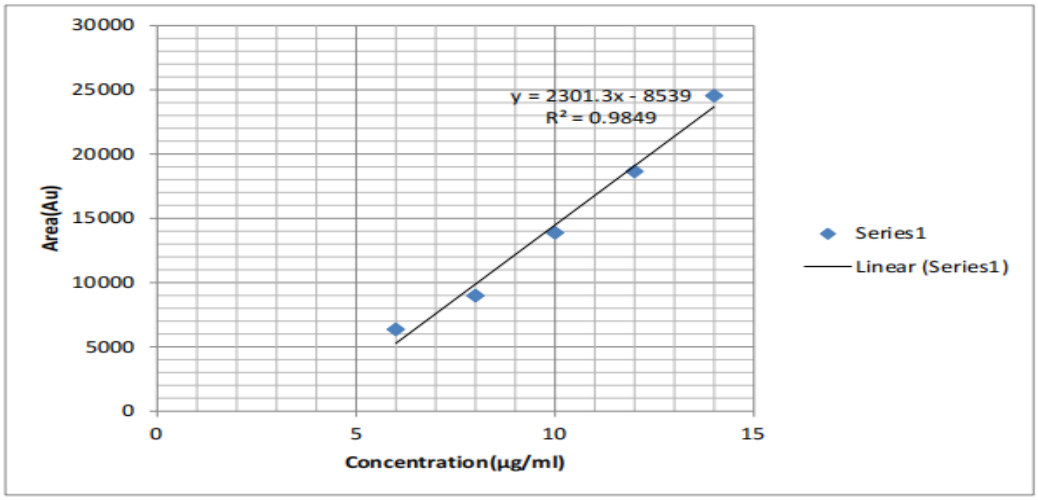

Figure 9: Calibration curve of Sulfaquinoxalin sodium.

Accuracy

Table 4: Accuracy.

DRUG	ACCU-RACY LEVEL (%)	AMOUNT			% RECO-VERY	MEAN±SD	%RSD
		actual (µg/ml)	added (µg/ml)	found (µg/ml)			
AMP	80	8	6.4	6.37	99.5	99.46±0.446	0.4484
	100	8	8	7.92	99.00		
	120	8	9.6	9.59	99.89		
SQS	80	6	4.8	4.75	98.95	99.52±0.514	0.5164
	100	6	6	5.98	99.66		
	120	6	7.2	7.197	99.95		

CONCLUSION

Simple as well as precise analytical methods were developed for the estimation of Amprolium hydrochloride and Sulfaquinoxalin in bulk and formulation.

The developed high performance thin layer chromatographic method was an suitable method for the analysis of drugs and here the calibration curve was plotted by using 2-10µg/ml of AMP. Hcl 6-14µg/ml of SQS .Drugs shows good correlation coefficient and linearity.

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