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Estimation of Piracetam in Bulk and Formulation Using High Performance Thin- Layer Chromatography

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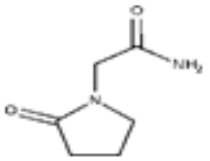
Abstract:- A rapid, accurate and precise HPTLC method has been developed for the estimation of Piracetam in bulk and Pharmaceutical formulation. In this method standard and sample solutions of Piracetam were applied on pre-coated 6 x 10 silica gel 60F254 TLC plate, and developed using chloroform: methanol:glacial acetic acid: triethyl amine (16:4:0.2:0.2) as mobile phase. A Camag HPTLC system comprising of Camag Linomat -5-applicator, Camag twin trough chamber, Camag TLC-3 scanner was used for the analysis. The drugs on the plate were scanned at 254 nm. The dynamic linearity range was 2µl, 4µl, 6µl, 8µl & 10µl for Piracetam. The method was validated for precision, accuracy and recovery and the results were satisfactory.Precision (%RSD-0.31), Accuracy (%RSD-0.59) values are under the limit. According to ICH guidelines the values of accuracy and precision should be in the limit less than 2.

Keywords: *Estimation, HPTLC, Piracetam.*

I. INTRODUCTION

Piracetam is a nootropic drug in the racetam group, with the chemical name 2-oxo-1-pyrrolidine acetamide. It shares the same 2- oxopyrrolidone base structure with pyroglutamic acid and is a cyclic derivative of the neurotransmitter γ-aminobutyric acid (GABA). However its mechanism of action differ from that of endogenous GABA. Piracetam has neuroprotective and anticonvulsant properties and is reported to improve neural plasticity.Its efficacy is documented in cognitive disorders and dementia, vertigo, cortical myoclonus.Literature survey reveals that various analytical methods like UV and HPLC were reported for the estimation of piracetam individually, but no HPTLC method was reported for the individual estimation and also only one simultaneous HPTLC method was reported for piracetam and citicoline. Hence the objective of the present work is to develop and validate simple, sensitive, accurate and reproducible HPTLC method for the estimation of piracetam in bulk and pharmaceutical dosage form.

- *Structure of Piracetam:*



II. MATERIALS AND METHODS

- *Standard Preparation:*

The pure drug Piracetam was weighed 10 mg and dissolved in 10 ml of methanol (1mg/ml). Hence, the above concentration is 1mcg/µl.

- *Sample Preparation:*

5 tablets (NOOTROPIL 800 mg) were weighed and weight equivalent to 1mg of Piracetam was weighed and dissolved in 1ml of methanol. The solution contains 1µg equivalent of piracetam in 1µl Methanol.

- *Sample Loading:*

A series of 1µl, 2µl, 4µl, 6µl, 8µl, 10µl Standard of Piracetam solutions were loaded as an 8mm band length in the 20 x 10 Silica gel 60F**254** TLC plate using a 25µl Hamilton syringe and CAMAG-LINOMAT-5 instrument.

- *Spot Development:*

The sample and standard loaded plate were kept in a Twin trough chamber 20 x 10cm with respective mobile phase up to 15min for chamber saturation. After completion of chamber saturation, the plate was kept in the mobile phase for development up to 70mm.

- *Photo-Documentation:*

The developed plate was dried by hot-air to evaporate solvents from the plate and the plate was kept in Photo-documentation chamber. The images of developed plate were captured at visible light, UV 254nm and UV 366nm

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using CAMAG TLC Scanner instrument.

➢ *Scanning:*

The developed plate was scanned before derivatization in UV 254nm wavelength for Piracetam using CAMAG-TLC SCANNER-3 instrument. The Baseline display, Peak densitogram and Peak table of each track were obtained.

➢ *Spectrum Scanning:*

The assigned peaks of standards and sample were scanned in the spectrum of UV region (200nm-300nm) and found the λmax value of Piracetam.

➢ *Mobile Phase:*

Chloroform: Methanol : Glacial acetic acid : Triethyl amine(16:4:0.2:0.2)

➢ *Detection:*

Black coloured quenching zone at UV 254nm mode (Rf- 0.50) before derivatization appeared in the given standard track indicates the presence of Piracetam.

➢ *Validation of Developed Method:*

Method validation refers to the process of proving the developed method whether it is acceptable for its intended purpose.The method was validated for linearity, accuracy, limit of detection, limit of quantification, repeatability of mea- surement and repeatability of sample application. Samples applied on the plate were developed with the mobile phase and the peak areas were noted. The mobile phase chloroform**:** methanol : glacial acetic acid : triethyl amine (16: 4: 0.2: 0.2 v/v) gave Rf value of 0.50 ± 0.02 for Piracetam.

➢ *Linearity and Regression:*

Linearity is one of the validation parameter used to check the linear response when graph is plotted between concentration and peak area. A good linear relationship was obtained over the concentration range of 1µl, 2µl, 4µl, 6µl, 8µl and 10µl/spot of piracetam. The linear regression data showed a regres- sion coefficient of 0.9992 for Piraceta m.

➢ *Sensitivity:*

The LOD with signal/ noise ratio were found to be 1µl/spot for Pira ceta m. The LOQ with signal/ noise ratio was found to be 2µl/spot for Piracetam.

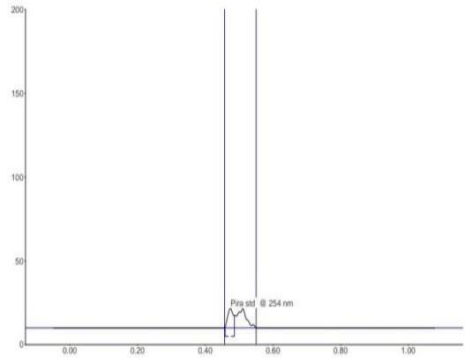
➢ *Accuracy:*

The recovery study was carried out at three levels, 80%, 100 % and 120%. To the powdered formulation, the standard drug of Piracetam were added at 80%, 100% and 120% levels, dilutions were made and analyzed by the method. The % recovery and % RSD were calculated and found to be within the limit.

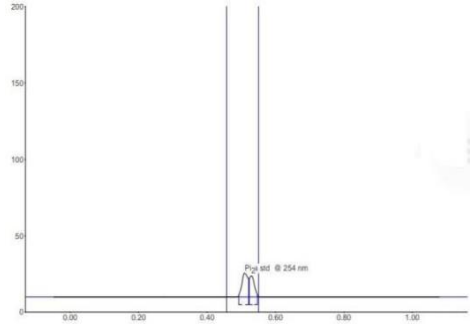
➢ *Precision:*

Precision was found by analysis of standard drug by three times for the concentration 2µl,6µl,10µl and percentage relative standard deviation (%RSD) was calculated. The RSD was found to be less than 2.

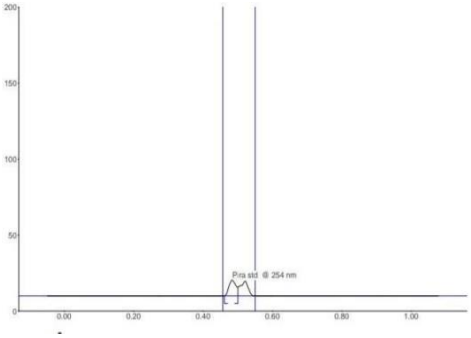
➢ 2µl



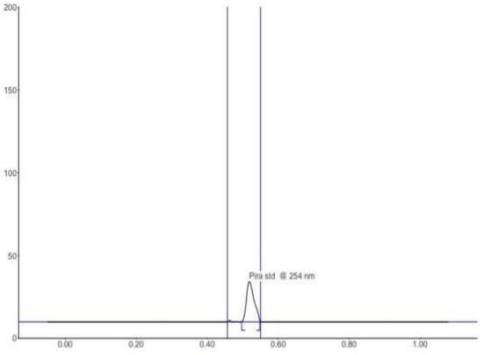
➢ 6µl



➢ 4µl



➢ 8µl



➢ 10µl

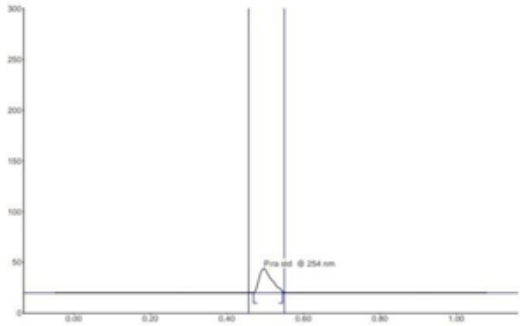


Fig 1 HPTLC Chromatogram for Concentration of 2µl, 4µl, 6µl, 8µl and 10µl

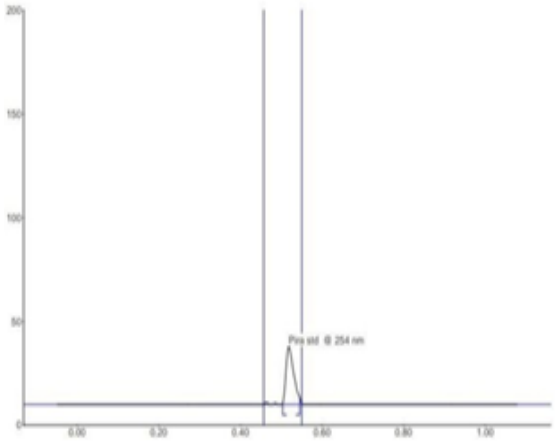


Fig 2 HPTLC Chromatogram of Formulation (Nootropil 800mg)

Formulation solution containing Piracetam 8**µl** showing Rf value- 0.52

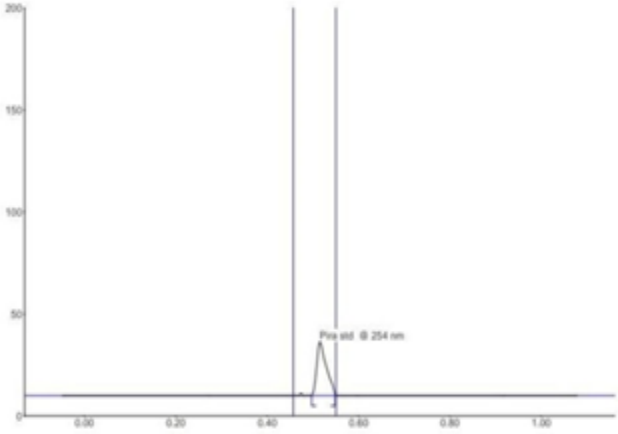


Fig 3 HPTLC Chromatogram of Formulation (Nootropil 800mg)

Formulation solution containing Piracetam 9**µl** showing Rf value- 0.51

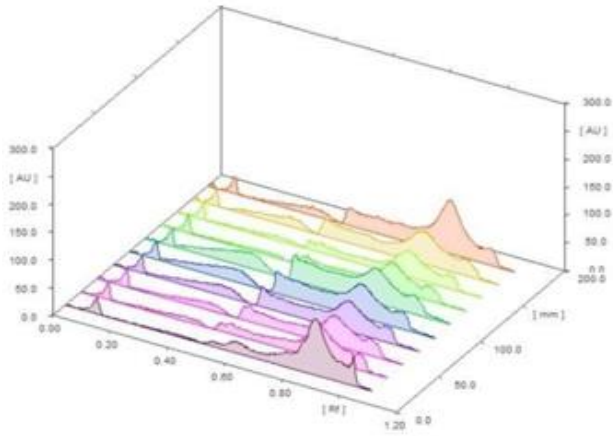


Fig 4 3D Display of Piracetam Calibration Samples and Formulation

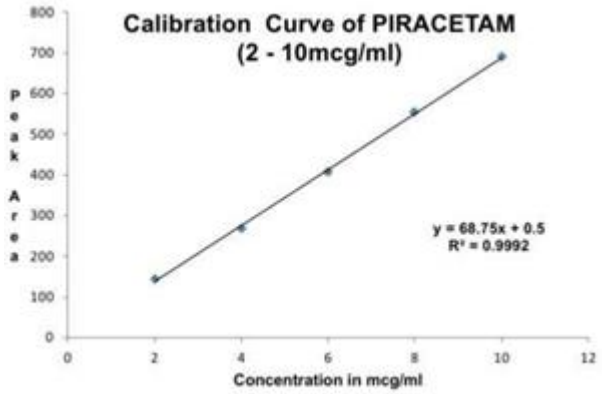


Fig 5 Linearity Graph Of Piracetam

Table 1 Result of Analysis of Formulation

Tablet formulation	Peak area	% Recovery	Mean (n=3)	SD	%RSD
NOOTROPIL (800mg)	530	95%	529.3	0.81	0.15
	599	94%	598.3	0.81	0.13

Table 2 Validation Parameters

1. Linearity concentration	2µl, 4µl, µl, 8µl, 10µl
2. Rf value	0.50 ± 0.02
3. R2 value	0.9992
4. LOD	1µl
LOQ	2µl
5. Accuracy %RSD	0.59
6. Precision %RSD	0.31

Table 3 Recovery Data

Level	Amount added (mcg)	Amount found(mg)*	% Recovery*	% RSD *
80%	4.8	4.57	95.25	0.50
100%	6	6.14	102.4	0.21
120%	7.2	7.48	103.97	0.59

III. RESULTS AND DISCUSSION

During the stage of method development different mobile phases were tried and the mobile phase comprising of chloroform: methanol: glacial acetic acid: triethyl amine in the proportion of (16: 4: 0.2: 0,2) was confirmed. The Rf value was found to be 0.50 Piracetam. Linearity of the drug was determined by the calibration curve and the linearity based on the peak area was in the range of 2 - 10mcg . The regression coefficient value for Piracetam was 0.9992. The limit of quantification was determined by injecting minimum concentration of the drugs. The limit of quantification (LOQ) was found as 2 m c g/ spot. The recovery was less than 104% for 80,100 and120% Piracetam samples(table.3) and the repeatability showed excellent % RSD less than 0.35(table.2). The method passes all the validation parameter limits and proves to be selective, sensitive and precise. Hence the proposed method can be used for the routine assay of Piracetam using HPTLC.

IV. CONCLUSION

Since, the developed HPTLC method is rapid, precise and accurate; the statistical analysis proved that the method is repeatable and selective for the analysis of Piracetam in bulk drugs and in pharmaceutical dosage forms without any interference from the excipients.

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