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VALIDATED STABILITY-INDICATING HPTLC METHOD FOR QUANTIFICATION OF NORTRIPTYLINE

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ABSTRACT
The examination was conducted using HPTLC plates of Silica gel 60F254 (20×10 cm, 250 µm) by using Toluene, Ethyl acetate, methanol, and Glacial acetic acid in the proportion of 4:4:1.6:0.4 v/v, which made up the mobile phase. After being prewashed with methanol, the plates were allowed to air dry before being activated for five minutes at 110°C. Samples were administered with a Camag micro syringe (150 nl/s) in 8 mm strips spaced 5 mm apart. At 26 ± 2 °C and 50 ± 5% RH, gradual development was carried out in a saturated twin-compartment chamber (20 min development, 15 min saturation). The chromatogram was 80 mm in length. Using Camag winCATS (v1.4.5) in reflectance absorbance mode, with a deuterium lamp (190–400 nm), a 6 × 0.3 mm slit, and a scan speed of 20 mm/s, the plates are air-dried after development and checked at 279 nm. Peak area using linear regression served as the basis for quantification. The correlation coefficient r^2= 0.00083 and linearity (200–600 ng/band) were demonstrated by the technique. The range of Nortriptyline recovery at 80%, 100%, and 120% was 101.45% to 101.97%. In order to conduct degradation investigations, Nortriptyline was refluxed with either 1N HCl or 1N NaOH for three hours at 80°C. After neutralisation, the solutions were diluted to 5000 ng/spot. Base degradation produced peaks at Rf 0.40, 0.45, 0.49, 0.58, and 0.62, whereas acid-degraded samples had peaks at Rf 0.42, 0.49, 0.53, 0.55, 0.62, and 0.82. Rf 0.39, 0.48, 0.57, and 0.73 were the locations of further degradation peaks that demonstrated the compound's vulnerability to both basic and acidic environments.
Keywords: Impurity Profile, Force Degradation, Densitogram, Nortriptyline, HPTLC.
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INTRODUCTION

Planar chromatography, also known as HPTLC, is an advanced technique that is well-known throughout the world and stands out for its adaptability, dependability, and affordability. It is a member of the micro-analytical techniques, which are crucial in both routine and research labs, along with HPLC and GC. The simplest separation method currently accessible to analysts is HPTLC. It can be thought of as a time machine that speeds up work and enables you to accomplish multiple tasks at once, which is typically not achievable with conventional analytical methods. Instrumental Thin-Layer Chromatography frequently provides a better answer and is employed as a substitute or confirmation method.[1,2] Since quantitative TLC is an offline approach, automation is challenging, and due to its open nature, it is heavily influenced by environmental conditions, making TLC/HPLC more problematic than GLC/HPLC. Therefore, it is crucial that each step be properly tested to identify any potential sources of error, and this may call for a unique approach.[3]

EXPERIMENTAL

Drug Profile

As seen in Fig.-1, Nortriptyline has the molecular formula C19H21N and molecular weight 263.38. It inhibits the norepinephrine's presynaptic receptors, preventing absorption of the neurotransmitter and raising its levels in the central nervous system's synaptic cleft. Nortriptyline also binds to cholinergic, histaminergic, and alpha-adrenergic receptors. Because Nortriptyline increases the activation of adrenergic receptors, long-term use of the drug causes these receptors to be downregulated.[4,5]

Instruments

HPTLC was carried out using a CAMAG Linomat 5 sample applicator to ensure precise and consistent sample application. Pre-coated silica gel plates (20 × 10 cm) 60 F_{254}were used as the stationary phase.

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Chromatographic development was performed in a CAMAG twin trough glass chamber (20×10cm) that was pre-saturated with the mobile phase to ensure uniform development.

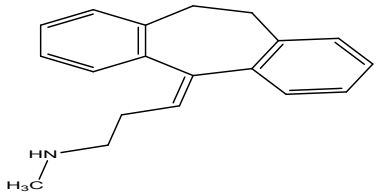

Fig.-1: Chemical Structure of Nortriptyline

After development, the plates were analyzed using a (CAMAG) TLC Scanner IV equipped with a UV detector for at 239nm densitometric scanning. Data acquisition, processing, and documentation were conducted using WinCATS software (CAMAG, Switzerland), ensuring accurate and reproducible evaluation of the chromatographic data. These are the Chromatographic parameters selected for further study.

RESULTS AND DISCUSSION

Method Validation[6-8]

Linearity

A stock solution of 0.5N NaOH containing 100µg/ ml of Nortriptyline was made. To get 200, 300, 400, 500, and 600ng/band of Nortriptyline, respectively, different amounts of stock solution—2, 3, 5, and 6 µl—were spotted on a TLC plate. Figure-2 displays the calibration curve standard plotted against the peak area vs. concentration.

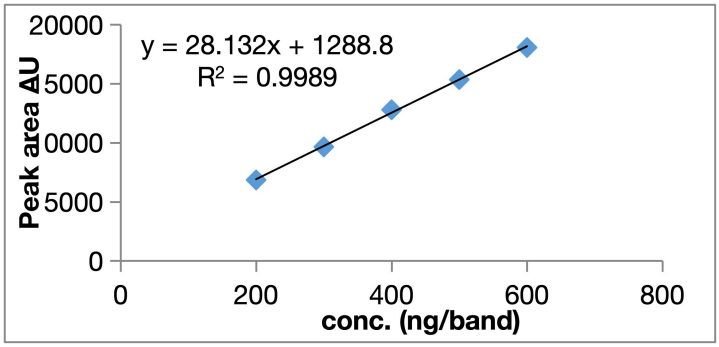

Fig.-2: Calibration Curve of Nortriptyline

Precision

Studies of fluctuation within and between days showed how accurate the system was. Three distinct concentrations of the standard stock solution—200, 400, and 600 ng/band—were observed in triplicate and subjected to analysis during the intraday investigations. Tables-1 and 2 provide the results of the calculation of the % RSD.

Table-1: Nortriptyline Bulk Drug Intra-Day Precision

Conc. in ng/band	Area			Mean	Standard Deviation	%RSD
	Trial I	Trial II	Trial III			
200	6850	6839	6759	6816	± 49.66	0.7285
400	12792	12716	12781	12763	± 41.07	0.3218
600	18066	18403	18090	18186	± 188.02	1.0338
Average % RSD= 0.6947						

Table-2: Nortriptyline Bulk drug Inter Day Precision

Conc. in ng/ban)	Area			Mean	Standard Deviation	% RSD
	Day I	Day II	Day III			
200	6857	6825	6801	6827	± 28.095	0.411
400	12790	12804	12802	12798	± 7.5718	0.059
600	18054	18051	18048	18051	± 3	0.016
Average % RSD=0.162						

Limit of Detection (LOD)-The measured LOD was 151.0984ng/band.

Limit of quantification (LOQ)-LOQ was found to be 457.8741ng/band

Range- Nortriptyline: 200 to 600ng/band.

Accuracy

Recovery trials were conducted by adding standard drug solution to a pre-analysed sample solution at 80%, 100% and 120%, three different levels, in order to assess the method's accuracy. Through over spotting, 240,300 and 360ng/band of Nortriptyline sample added to the initial concentration of 300ng/band of the Nortriptyline bulk medication. The regression equation was used to get the Nortriptyline concentrations. The results are shown in Table-3. 80% is equal to 6ml from the sample Stock solution plus 4.80 ml from the bulk drug stock solution or 240ng/band. 100% is equal to 6ml of the sample Stock solution plus 6 ml of the bulk drug stock solution, or 300ng/band. 120% is equal to 6ml of the sample stock solution plus 7.2 ml of the bulk drug stock solution, or 360ng/band.

Table-3: Nortriptyline Recovery Studies

Level in %	Conc. (ng/band)	Area	Mean	Recovered conc.	% Recovery
80	300+240	16480	16473	539.81	99.96
		16470			
		16470			
100	300+300	17940	17917	591.14	98.52
		17810			
		18000			
120	300+360	19470	19467	646.24	98.15
		19480			
		19450			

Specificity

We looked for any interference at the Rf of Nortriptyline in the densitogram. The method's specificity was demonstrated by the deficiency of overlapping peaks in the blank at the Rf of Nortriptyline. There were no extra interferences at the relevant Rf, as evidenced by the identical spectra of the standard and the sample.

Robustness

The effects on the results were investigated by making minor adjustments to the composition of the mobile phase, as indicated in Table 4. Chromatograms were performed after experimenting with mobile phases of varying composition. There were variations of 20, 40, and 60 minutes between spotting, chromatography, and development.

Table-4: Robustness Study of Nortriptyline Bulk Drug

Composition of Dichloromethane	Peak Area			Mean	Std. Deviation	% RSD
	Trial I	Trial II	Trial III			
9.4	9648	9652	9650	9650	± 2	0.020%
9.5	9650	9652	9650	9650	±1	0.010%
9.6	9649	9650	9652	9650.33	±1.5275	0.015%
Average % RSD=0.015						

Degradation Studies[9-11]

Acidic Hydrolysis

Two millilitres of hydrochloric acid at concentrations of 0.5N, 1N, 1.5N, and 2N, respectively, were combined with six millilitres of the stock solution in four different 10-millilitre volumetric flasks. 0.5N sodium hydroxide was used to get the total volume down to 10 ml (0.3µg/5µl). At room temperature, this combination was left to stand for three hours. After loading 5µl of the resultant solution (300ng/band) onto a TLC plate next to the matching blank in a nearby lane, the densitogram depicted in Fig.-3 was

created. Under the conditions with 2N hydrochloric acid, degradation was seen. 1.5N, 1.6N, and 1.7N hydrochloric acid concentrations were used to repeat the procedure. At a concentration of 1.7N hydrochloric acid, degradation was observed.

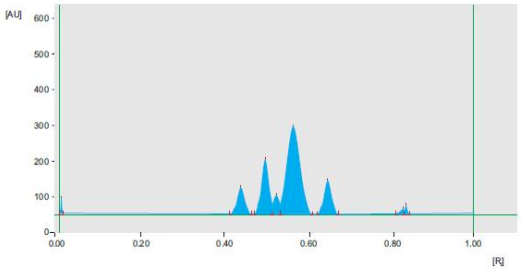

Fig.-3: Densitogram of Acidic degradation of Nortriptaline

Alkaline Hydrolysis

Two millilitres of sodium hydroxide at concentrations of 0.5N, 1N, 1.5N, 2N, and 2.5N were combined with six millilitres of the stock solution in five separate 10-millilitre volumetric flasks. After that, 0.5 N sodium hydroxide (0.3µg/5µl) was added to get the total volume down to 10 ml. At 30°C, the resultant mixture was allowed to stand for three hours. Densitograms were created using this solution by applying 5µl (300ng/band) to a TLC plate next to the matching blank on a nearby track, as seen in Fig.-3. In the 2.5 N sodium hydroxide solutions, degradation was noted. 2.1 N, 2.2 N, 2.3 N, and 2.4 N concentrations were used in a rerun of the experiment. At the 2.2 N sodium hydroxide solution, degradation was observed.

Oxidative Degradation

2 ml of hydrogen peroxide at concentrations of 3%, 4%, 5%, and 6% was mixed with 6 ml of a stock solution in four distinct volumetric flasks. 0.5 N sodium hydroxide (0.3µg/5µl) was used to get the total volume down to 10 ml. After that, this mixture was left to stand at RT for 3 hours. Densitograms were created by applying a 5µl aliquot of the final solution (300ng/band) on a Thin Layer Chromatography plate next to the matching blank in the adjacent track. The 6% hydrogen peroxide solution showed signs of degradation.

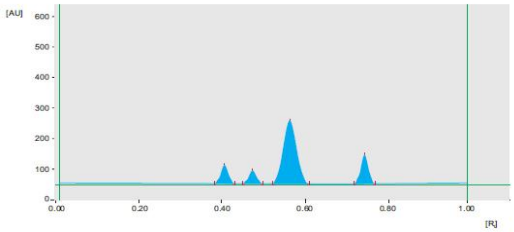

Fig.-4: Densitogram of OxidativeDegradation of Nortriptyline

Thermal Degradation

The drug sample was placed in an oven that was heated to 500°C in order to do thermal studies. A concentration of 100µg/ml was achieved by dissolving 10 mg of the treated medication in 0.5N NaOH in a 100ml capacity volumetric flask after it had been precisely weighed and transferred. A working standard solution (0.3 µg/5 µl) was then made by diluting a 6 ml standard stock solution of Nortriptyline in 10 ml of 0.5N NaOH. The densitogram was created by applying 5 µl of the final solution (300ng/band) to a Thin Layer Chromatography plate along with the corresponding blank in the nearby track. For 48 hours, same procedure was carried out again, but no degradation was observed.

Photo Degradation

The drug sample was exposed to UV light in order to investigate its photo stability. A concentration of 100µg/ml was obtained by precisely weighing 10 mg of medication dissolved in 100 ml of 0.5N NaOH after an interval of one hour. The working standard solution (0.3µg/5µl) was then obtained by diluting the 6 ml standard stock solution of the Nortriptyline in 10 ml of 0.5N NaOH. After that, 5µl of the final solution (300ng/band) was applied to the TLC. From the above study, the results are obtained as shown in Table-5.

Table-5: Degradation Studies of Nortriptyline Bulk Drug

No	Parameters of Degradation	Area	Degradation	Conc. of degraded Nortriptyline (ng/band)
1	Initial	9650	-	
2	Acidic Degradation	8420	14.57%	43.73
3	Alkali degradation	8514	13.46%	40.39
4	Oxidative degradation	8571	12.78%	38.35
5	Thermal degradation	9646	0.04%	0.14
6	Photo degradation	9651	0.13%	0.39

CONCLUSION

The recommended method is simple, precise, accurate, and repeatable. The method's excellent sensitivity and simplicity in sample preparation make it appropriate for regular analysis. The results of the analysis were validated in compliance with ICH standards. For a wide pH range, stability investigations include the effects of Acid, alkali, oxidative, and Photo degradation studies. The degraded chemical has been successfully isolated and characterised using this technique. Additionally, it helps isolate further deterioration in the future. This process can be used to study the quality of the drug formulation, preserve the active ingredient, change the bioavailability, and prevent harmful degradation products.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to ***SDG-3: Good Health and Well-Being***. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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