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Original Article

ANALYSIS OF GABAPENTIN BY HPTLC WITH DENSITOMETRIC MEASUREMENT AFTER DERIVATIZATION

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ABSTRACT

Objective: Development and validation of a simple, specific, precise and accurate HPTLC method for the analysis of gabapentin in bulk drug and tablet formulation using metformin as internal standard.

Methods: The separation was performed on a TLC plate precoated with silica gel $60F_{254}$ using n-butanol–acetic acid–water (5:2:2, v/v/v) as mobile phase. Densitometric analysis was performed first at 254 nm for IS and 550 nm for gabapentin after derivatization using ninhydrin solution.

Results: The method was validated for linearity, accuracy, precision and robustness according to ICH guidelines. The R_f values were found to be 0.45 ± 0.025 for IS and 0.63 ± 0.03 for gabapentin. Regression analysis showed good linearity over the concentration range of 25-150 ng/band. The recovery studies showed good recoveries in the range of 99.89-100.13%. **Conclusion:** The proposed method was simple, accurate, precise and robust. Statistical analysis showed that the method was used for routine analysis of gabapentin tablets.

Keywords: Gabapentin, Metformin, HPTLC, Method validation.

INTRODUCTION

Gabapentin (GBP) [1-(amino methyl) cyclo hexane acetic acid] an antiepileptic agent, is a structural analogue of gamma amino butyric acid (GABA) (Figure 1). It is orally active and acts by irreversible inhibition of GABA transaminase enzyme and thus preventing the degradation of GABA in the brain. Studies have shown that the antihyperalgesic and antiallodynic effects of gabapentin are mediated by descending noradrenergic system, resulting in the activation of spinal alpha-2 - adrenergic receptors. Gabapentin has also been shown to bind and activate the adenosine A_1 receptor. It has no significant UV, visible or fluorescence absorption due to the lack of chromophore [1].

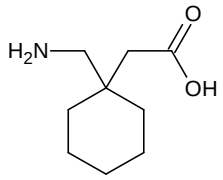

Fig. 1: Structure of Gabapentin

The literature survey revealed few methods like UV [3], Colorimetry [2], HPLC [4], HPTLC [6-7], and LC-MS [5]. The objective of the present study was to develop a simple, accurate and precise HPTLC method for estimation of gabapentin in bulk drug and formulation in the presence of an internal standard. HPTLC is a widely used analytical technique due to its advantages of low operating cost, high sample throughput, and need of minimum sample preparation [8-10]. The internal standard metformin was structurally similar to gabapentin, was stable in mobile phase and didn't interfere with gabapentin. The proposed method was validated for linearity, accuracy (recovery studies), specificity, precision, robustness, ruggedness, limit of detection (LOD), limit of quantitation (LOQ), and repeatability according to the ICH guidelines and its updated international convention [11].

MATERIALS AND METHODS

Materials and Reagents

Pharmaceutical grade gabapentin was obtained as gift sample from Micro Labs, Bangalore, India. The internal standard metformin was obtained from Sun Pharmaceuticals Ltd., Mumbai, India. The other chemicals such as methanol, n-butanol, acetic acid, toluene and ammonia of AR grade were obtained from Merck Chemicals, Mumbai, India. The tablet dosage forms (labeled to contain 100 mg gabapentin) were procured from local pharmacy.

Instrumentation and Chromatographic conditions

The samples were spotted on Merck TLC plates precoated with silica gel $60F_{254}$ (10.0 × 10.0 cm with 250 µm layer thickness), in the form of bands with a CAMAG 100 µL sampler (Hamilton, Switzerland) syringe using CAMAG Linomat 5 automatic TLC Sampler. The plates were prewashed with methanol and activated at 110 °C for 5 min prior to chromatography. A constant application rate of 10 µL/sec was employed using a nitrogen aspirator. The space between two bands was 10 mm. The slit dimension was kept at 2 mm × 0.1 mm, with scan rate of 4 mm/sec. The monochromator band width was set at 30 nm. The mobile phase consists of n-butanol: acetic acid: water (5:2:2 v/v/v) and 10 mL of mobile phase was used per chromatography. Ascending development was carried out in a 10 cm × 10 cm horizontal glass chamber saturated with mobile phase. The optimized sample saturation time was 20 min at room temperature. The length of chromatogram run was 80 mm. After development the plates were dried at 60 °C for 4 min to eliminate mobile phase. Densitometric scanning was performed in absorbance/ reflectance mode at 254 nm for IS and then ninhydrin solution was sprayed. The plates were again dried and scanned at 550 nm for gabapentin using CAMAG TLC scanner 3 with winCATS software for scanner control and data processing. The source of radiation utilized was deuterium and tungsten lamp. Concentrations of the compounds chromatographed were determined from diffusely reflected light.

Standard solutions and calibration graphs

Standard stock solutions of gabapentin and IS were prepared separately by dissolving 10 mg each of the drug in 100 mL methanol to get a concentration of 100 µg/mL. A mixture of these solutions which contain 10 µg/mL each of gabapentin and IS was prepared by transferring appropriate aliquots from standard stock solutions and diluted with methanol. From this 2.5 µL - 15.0 µL were applied to the HPTLC plates to get a concentration of 25 - 150 ng/band of both drugs and was developed on previously described mobile phase. The procedure was repeated three times and peak area was noted. The calibration plot was constructed by plotting the relative retention

factor, R.R.F value (i.e. ratio between peak area of gabapentin and IS) against respective concentration.

Sample Preparation

Ten tablets were accurately weighed, pulverized to fine powder and powder equivalent to 100 mg of gabapentin (label claim) was weighed, transferred into a 100 mL volumetric flask, dissolved in methanol and sonicated for 5 min. Then the volume was adjusted with methanol and filtered through Whatman filter paper No.41. From this solution, 1 mL was transferred to a 10 mL volumetric flask and 1 mL of standard stock solution of IS (100 µg/ mL) was added, diluted with methanol to obtain a solution containing 10 µg/ mL each of gabapentin and IS. 10 µL of this solution was applied to HPTLC plate to furnish a concentration of 100 ng/band of both gabapentin and IS. The plate was developed using previously described chromatographic conditions and densitometric scanning was done at 254 nm for IS. Then ninhydrin solution was sprayed, dried and scanned at 550 nm for gabapentin. Quantification was done using R.R.F value and procedure was repeated five times for each brand.

Method Validation

The method was validated according to the ICH guidelines for the following parameters [9].

Linearity and Sensitivity

A series of solutions ranging from 2.5 - 15.0 µL from mixed solution containing 10 µg/mL of gabapentin and IS were spotted to TLC plate to get a concentration of 25 - 150 ng/band and was developed using above mobile phase. The R.R.F value was plotted against corresponding concentration to obtain calibration curve (Figure 2). The limit of detection (LOD) and quantification (LOQ) were calculated on the basis of equation, LOD = 3.3 × S/B and LOQ = 10 × S/B. where, S is SD of peak areas of drugs taken as a measure of noise and B is the slope of corresponding calibration curve.

Precision

The intra-day and inter-day precision were evaluated by using three different aliquots of standard solutions (initial, medium and high concentrations) in triplicate in a day and on three consecutive days. For each solution, triplicate analysis was performed by a single analyst at the same time under same conditions.

Accuracy

The accuracy of the method was examined by performing recovery studies in triplicate using standard addition method. Accurately known amount of sample were added to a known amount of pre-analyzed tablet powder and the powder was analyzed.

Robustness

Robustness of the method was determined by introducing small changes in the mobile phase composition, time of spotting and detection wavelength. The effect on the results was examined by triplicate.

RESULTS AND DISCUSSION

Optimization of HPTLC-Densitometric method

Various solvent systems were evaluated to arrive at an optimum resolution of drug and IS using TLC technique. Initially petroleum ether, toluene, butanol, ethyl acetate, ammonia and methanol were tried in different ratios. But the resolution was not satisfactory and some systems gave necklace effects. Finally the mobile phase consisting n-butanol–acetic acid–water (5:2:2, v/v/v) gave better resolution and found to be optimum. In order to reduce the edge effect, the chamber was saturated for 30 min using saturation pads. Figure 3 shows the typical chromatogram of pure gabapentin with R_f value 0.63 ± 0.03.

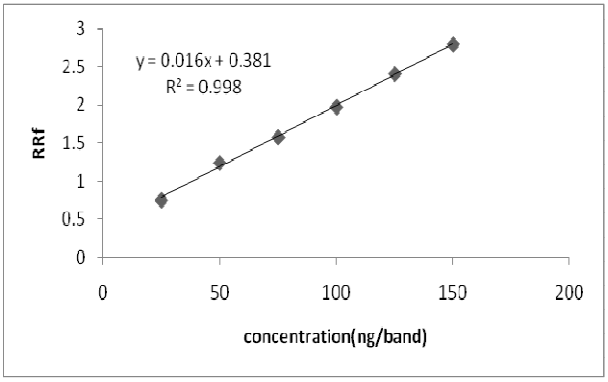

Fig. 2: Linearity graph of Gabapentin

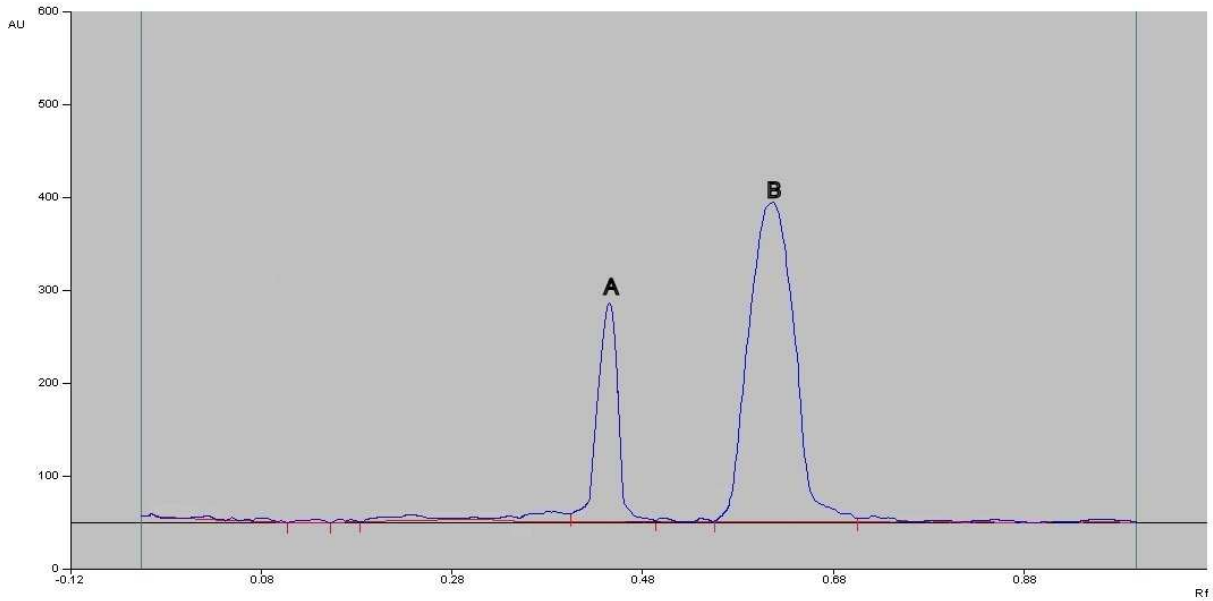

Fig. 3: Typical HPTLC Chromatogram of standard A) IS (R_f = 0.45 ± 0.025) and B) gabapentin (R_f = 0.63 ± 0.03)

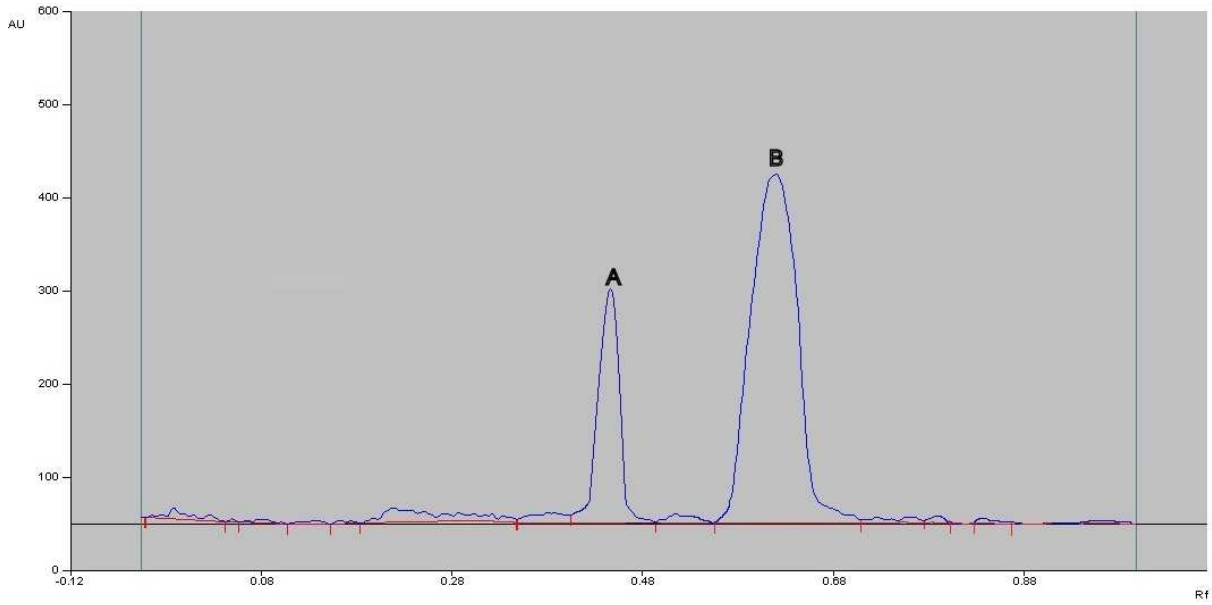

Fig. 4: Typical HPTLC Chromatogram of sample A) IS (R_f = 0.42 ± 0.02) and B) gabapentin (R_f = 0.59 ± 0.04)

Linearity and sensitivity

Gabapentin showed good correlation coefficient in the concentration range of 25-150 ng/band with r = 0.998. Linearity was evaluated by determining six working standard solutions containing 25-150 ng/band of gabapentin in triplicate. The linearity of calibration graph and adherence of system to Beer's law was validated by high value of correlation coefficient and standard deviation of intercept value which was less than 2%. No significant difference was observed in the slopes of standard curves. The LOD and LOQ were found to be 15.81 and 47.92 ng/band. Results are shown in table 1.

Precision and Recovery studies

The intra and inter day precisions were calculated using initial, medium and final concentrations of gabapentin. The low RSD value indicates the sensitivity and repeatability of the method. The results are shown in table 2. The results of recovery studies showed good recoveries in the range of 99.89%-100.13%. This shows that the method was accurate (Table 3).

Table 1: Linearity parameters for calibration curve

Parameter	GBP
Retention factor (R_f)	0.63 ± 0.03
Linearity range (ng/band)	25-150
Regression equation (y = mx + c)	y = 0.016x + 0.381
Correlation coefficient (r)	0.998
Limit of detection (ng/band)	5.81
Limit of quantification (ng/band)	14.92
Standard deviation of slope (S_a)	0.0024
Standard deviation of intercept (S_b)	0.0115
Regression coefficient (r^2)	0.9973

Table 2: Results of precision studies of proposed method

Drug Concentration (ng/band)	Intraday precision			Inter day precision		
	Calculated amount ± SD[a] (ng/band)	SEM	%RSD	Calculated amount ± SD[a] (ng/band)	SEM	%RSD
25	25.01 ± 0.0418	0.37	0.17			
100	100.13 ± 0.4726	0.74	0.47	24.86 ± 0.0528	0.48	0.21
150	151.452 ± 1.897	0.93	1.25	100.08 ± 0.4673	0.69	0.47
				150.43 ± 0.9327	1.02	0.62

[a]Mean of three replicates

Table 3: Accuracy of the proposed method

Brand	Initial amount (ng/band)	Fortified amount (ng/band)	Amount recovered[a] ± SD (ng/band)	Mean recovery (%)	SEM	%RSD
I	50	25	75.063 ± 0.816	100.08	0.34	1.09
	50	100	100.07 ± 0.783	100.08	0.67	0.78
	50	125	125.15 ± 0.579	100.13	1.34	0.48
II	50	25	74.973 ± 0.586	99.96	0.53	0.78
	50	100	99.869 ± 0.614	99.87	0.64	0.61
	50	125	125.08 ± 1.536	100.06	0.95	1.23

[a]Mean of three replicates

Robustness

Results of robustness study was shown in table 4. %RSD value in all robustness parameters were examined and found to be < 2%. The result showed that proposed method was robust.

Analysis of marketed formulations

Experimental results showed good agreement with the expressed label claim, suggesting that there was no interference from any of the excipients, which was commonly present in tablets. The low

value of %RSD indicated that the method was suitable for routine analysis in pharmaceutical dosage form (Table 4).

Table 4: Results from robustness study

Condition		Retention factor (R_f)	Assay (%)	%RSD
Mobile phase composition (v/v/v)				
n-butanol–acetic acid–water	(5:2:2)	0.63	98.90	0.89
	(6:1:2.5)	0.70	99.40	0.63
	(5:3:1)	0.68	98.70	0.97
Development distance (cm)	6	0.54	98.70	0.71
	9	0.85	99.60	1.03
Time of spotting to chromatogram (min)	9	0.75	100.40	1.35
	11	0.64	99.20	0.56
Detection wavelength (nm)	244 & 530	0.55	98.40	1.54
	274 & 524	0.55	97.60	1.21

Table 5: Results of HPTLC quantification of Gabapentin in tablets

Sample	Label claim (gm)	Amount present (gm)	SD[a]	%RSD
Brand I	0.100	0.10313	0.73	0.42
Brand II	0.100	0.1018	1.05	0.64

[a]Mean of five replicates

CONCLUSION

The proposed HPTLC method provides simple, accurate and reproducible quantitative analysis of gabapentin in tablets. The method was validated as per ICH guidelines. Statistical analysis indicated that the method was suitable for routine determination of gabapentin in pharmaceutical formulations.

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