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STABILITY-INDICATING HPTLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF GABAPENTIN AND NORTRIPTYLINE IN BULK DRUG AND MARKETED FORMULATION

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ABSTRACT

Gabapentin and nortriptyline are co-formulated (100 mg/10 mg and 200 mg/10 mg strengths) and used for the management of neuropathic pain, yet no stability-indicating high-performance thin-layer chromatographic (HPTLC) method for their simultaneous estimation has been reported to date. The present work describes the development and validation of a simple, rapid, precise and stability-indicating HPTLC method for the simultaneous determination of gabapentin and nortriptyline in bulk drug and in a marketed tablet formulation, in accordance with ICH Q1A(R2) and Q2(R1) guidelines. Chromatographic separation was achieved on pre-coated silica gel 60 F254 aluminium plates using methanol:acetonitrile:ethyl acetate:ammonia (5:3:3:0.1, v/v/v/v) as the mobile phase, with densitometric scanning at 236 nm for nortriptyline and, after post-chromatographic derivatization with 2% ninhydrin reagent, at 487 nm for gabapentin. The retention factor (Rf) values of nortriptyline and gabapentin in the mixture were 0.372 and 0.235, respectively. The method was linear over 200–450 ng/band (r^2 = 0.9956) for nortriptyline and 6000–8500 ng/band (r^2 = 0.991) for gabapentin. Mean recoveries ranged from 98.9–100.5%, and intra-day/inter-day precision (%RSD) was below 2% for both analytes. The limit of detection/limit of quantification were 176.89/536.05 ng/band for nortriptyline and 281.70/853.63 ng/band for gabapentin. The method was robust to small deliberate changes in mobile-phase composition

and chamber saturation time, and was specific in the presence of degradation products generated under acid, base, oxidative, thermal and photolytic stress. Assay of the marketed formulation (Gabapin NT 300) gave 99.0% and 99.25% of the labelled claim for gabapentin and nortriptyline, respectively. The validated method is suitable for routine quality control and stability testing of gabapentin–nortriptyline combination products.

KEYWORDS: Gabapentin; Nortriptyline; HPTLC; Stability-indicating method; Method validation; ICH Q2 (R1); Forced degradation.

1. INTRODUCTION

Neuropathic pain arises from a lesion or disease affecting the somatosensory nervous system and is commonly associated with diabetes, autoimmune disorders, nerve trauma and compression.[1] Unlike nociceptive pain, it responds poorly to conventional analgesics such as ibuprofen and paracetamol, and is instead managed with agents originally developed for depression and epilepsy.[2] Tricyclic antidepressants (TCAs) such as nortriptyline relieve neuropathic pain by inhibiting the reuptake of serotonin and noradrenaline at presynaptic terminals, independent of their antidepressant action, while gabapentin - a structural analogue of γ-aminobutyric acid (GABA) - binds to the α2δ subunit of voltage-gated calcium channels and reduces calcium influx and the release of excitatory neurotransmitters.[1,2] The rational combination of gabapentin and nortriptyline is now marketed in India (as, for example, Gabapin NT 300 and Gabator NT 200) for the management of neuropathic pain.

Analytical method development and validation are integral, interdependent activities in pharmaceutical development, providing streamlined and reliable procedures to confirm the identity, purity and content of an active pharmaceutical ingredient in bulk and finished dosage forms.[3,4] High-performance thin-layer chromatography (HPTLC) is a modern, instrumentalised extension of classical TLC that combines the simplicity, low cost and visual transparency of planar chromatography with the sensitivity, precision and reproducibility of automated sample application, chamber saturation control and densitometric scanning.[5-7] Several samples can be analysed in parallel on a single plate, sample and mobile-phase consumption are minimal, and the method allows re-scanning or derivatization of the same plate after data acquisition, making HPTLC a cost-effective alternative to HPLC and GC for routine quality control.[5-7]

A stability-indicating method (SIM) is a validated analytical procedure capable of accurately measuring the change in concentration of the active ingredient without interference from degradation products, process impurities or excipients, and is developed through forced (stress) degradation studies conducted under conditions more severe than accelerated storage conditions.[8] Such studies elucidate degradation pathways, establish the intrinsic stability of the drug substance, and support the design of a regulatory-compliant stability programme.[8] A review of the literature shows several reported methods for gabapentin and nortriptyline individually, and in combination with other drugs, by UV-spectrophotometry, RP-HPLC and HPTLC [9-30] (Table 1). However, no stability-indicating HPTLC method for the simultaneous estimation of gabapentin and nortriptyline in combination has been reported to date. The present study was therefore undertaken to develop and validate a simple, precise, accurate and stability-indicating HPTLC method for the simultaneous estimation of gabapentin and nortriptyline in bulk drug and in a marketed tablet formulation, as per ICH Q1A (R2) and Q2 (R1) guidelines,[31] and to apply the method to a forced degradation study encompassing acidic, alkaline, oxidative, thermal and photolytic stress conditions.

Table 1: Representative reported analytical methods for gabapentin and nortriptyline.

Analyte(s)	Technique	Key conditions	Ref.
Gabapentin (IP assay)	LC	C18, ACN: phosphate buffer, 210–215 nm	[11-13]
Nortriptyline (IP assay)	TLC / LC	Silica gel, cyclohexane:diethylamine (85:15); C18 column, 239 nm	[14,15]
Gabapentin + Nortriptyline (bulk)	RP-HPLC	C18, methanol:0.1 M ammonium acetate (20:80), 254 nm	[16]
Gabapentin + Nortriptyline (bulk & tablet)	RP-HPLC	C18, 0.2% triethylamine buffer:ACN (50:50), 210 nm	[17]
Gabapentin + Pregabalin	HPTLC	Silica gel 60 F254, ethyl acetate:ammonia:methanol (6:0.1:4)	[20]
Gabapentin + Amitriptyline	HPTLC	Silica gel 60 F254, methanol:ethyl acetate:ACN:ammonia (5:2:3:0.1)	[21]
Nortriptyline + Pregabalin	HPTLC	Silica gel 60 F254, toluene:methanol:ethyl acetate (6:1:2)	[28]

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents: Working standards of gabapentin and nortriptyline hydrochloride (>99% purity) were procured as gift samples from Perfect Medicare Pharma (Godhra, India) and Sunvij Drugs Pvt. Ltd. (Waghodia GIDC, Vadodara, India), respectively. Methanol, acetonitrile, ethyl acetate, chloroform, ninhydrin and ammonia solution, all of

analytical/HPLC grade, were purchased from Merck India Pvt. Ltd. High-purity water was obtained from a Merck Milli-Q system. The marketed formulation (Gabapin NT 300, gabapentin 300 mg + nortriptyline hydrochloride equivalent to nortriptyline 10 mg, Intas Pharmaceuticals Ltd.) was procured from the local pharmaceutical market.

2.2. Instrumentation: HPTLC analysis was performed using a CAMAG HPTLC system (Switzerland) consisting of a Linomat 5 automatic sample applicator, a twin-trough glass developing chamber (20 × 10 cm), a TLC Scanner III operated in absorbance/reflectance mode with a deuterium (D_2) lamp and controlled by winCATS software, and a CAMAG Visualizer for plate documentation. Chromatographic separation was carried out on pre-coated silica gel 60 F_{254} aluminium TLC plates (10 × 10 cm, Merck). The optimized chromatographic separation was achieved using a mobile phase comprising methanol:acetonitrile acetate (5:3:3:0.1, v/v/v/v). The developing chamber was saturated with 20 mL of the mobile phase for 20 min at ambient temperature before plate development. Standard and sample solutions (1 µL) were applied as 6 mm bands, positioned 10 mm from the lower edge of the plate, using the Linomat 5 applicator under a stream of nitrogen. Plates were developed to a migration distance of 80 mm, air-dried for 5 min, and subjected to densitometric scanning. Nortriptyline was directly quantified at 236 nm, whereas gabapentin was derivatized with 2% ninhydrin reagent followed by heating and quantified at 487 nm. The optimized chromatographic conditions are summarized in Table 2.

Table 2: Optimized HPTLC Chromatographic Conditions.

Parameter	Optimized condition
Stationary phase	Pre-coated silica gel 60 F254 aluminium plate, 10 × 10 cm
Mobile phase	Methanol : Acetonitrile : Ethyl acetate : Ammonia (5:3:3:0.1, v/v/v/v)
Chamber saturation time	20 min
Development distance	8.0 cm
Diluent	Methanol
Derivatizing reagent	2% Ninhydrin solution (in acetone)
Drug ratio in mixture (Gabapentin : Nortriptyline)	30 : 1
Detection wavelength	Nortriptyline: 236 nm; Gabapentin (post-derivatization): 487 nm
Rf (individual standards)	Gabapentin: 0.274; Nortriptyline: 0.385
Rf (in mixture)	Gabapentin: 0.235; Nortriptyline: 0.372

2.3. Preparation of Standard Solutions: A standard stock solution of gabapentin (3000 µg/mL) was prepared by accurately weighing 30 mg of gabapentin, transferring it into a 10 mL volumetric flask, dissolving in methanol, and diluting to volume with the same solvent. Similarly, a standard stock solution of nortriptyline (100 µg/mL) was prepared by dissolving 1 mg of nortriptyline in methanol and making up the volume to 10 mL. A mixed standard stock solution containing gabapentin and nortriptyline in the marketed formulation ratio (30:1, w/w) was prepared by dissolving 30 mg of gabapentin and 1 mg of nortriptyline in methanol and diluting to 10 mL. Working standard solutions for calibration and method validation were prepared by appropriate dilution of the stock solutions with methanol. The derivatizing reagent was prepared by dissolving 2 g of ninhydrin in acetone and diluting to 100 mL to obtain a 2% (w/v) ninhydrin solution.

2.4. Analytical Method Validation: The developed HPTLC method was validated in accordance with the International Council for Harmonisation (ICH) Q2(R2) guideline for analytical procedure validation with respect to linearity and range, accuracy, precision (repeatability and intermediate precision), specificity, robustness, limit of detection (LOD), and limit of quantification (LOQ).

2.4.1. Working Range (Linearity): The calibration curve was plotted over the concentration range of 6000–8500 ng/spot for Gabapentin and 200–450 ng/spot for Nortriptyline. Accurately measured Standard working solutions of Gabapentin and Nortriptyline (1 µl) were spotted on the precoated TLC plate under Using the Camag Linomat 5 (Switzerland) automatic sample spotter, spots were applied to pre-coated silica gel 60 F254 TLC plates (E. Merck). The plate was dried in air and developed up to 8.0 cm using a mixture of methanol. ACN: Ethyl Acetate: Ammonia (5:3:3:0.1 v/v/v/v) as a mobile phase in a Camag twin-through chamber, previously saturated with the mobile phase for 20 minutes. The plate was removed from the chamber dried in air, and standard zones were scanned and quantified at 236 nm for Nortriptyline, and after degravitation with ninhydrin, Gabapentin was quantified at 487nm. The calibration curves were constructed by plotting peak areas vs. concentration (ng/spot) corresponding to each spot.

2.4.2. Accuracy (% Recovery): The accuracy of the method was determined by calculating recoveries of Gabapentin and Nortriptyline by the standard addition method. Known amounts of standard solutions of Gabapentin and Nortriptyline (80, 100, and 120% levels) were added to previously analysed sample solutions of bulk powder. The amount of 1 µl injected sample

of Gabapentin and Nortriptyline were analysed by applying these values to the regression equation of the calibration curve.

2.4.3. Precision

a. Precision (% Repeatability) The precision of the instrument was checked by repeatedly spotting and scanning (n = 6) solution of Gabapentin and Nortriptyline at concentration levels (7000 ng/band & 300 ng/band) and peak area were determined. The results were reported in term of % coefficient of variance.

b. Precision (Interday & Intraday) It expresses within laboratory variations as on different days analysis or equipment within the laboratory. Precision was evaluated in terms of intraday and interday precision. The intraday precision was investigated using three different concentrations of sample solutions prepared as 6500, 7000, 7500 ng/band for Gabapentin & 250,300 & 350 for Nortriptyline from stock solution. The intraday and interday precision of the proposed method was determined by analysing the corresponding concentration 3 times on the same day and on different days over a concentration for both Gabapentin and Nortriptyline.

2.4.4. Accuracy (% Recovery): Recovery studies performed at 80%, 100% and 120% spiking levels gave mean recoveries of 100.15 ± 0.88% (%RSD 1.07–1.37) for nortriptyline and 98.99–100.26% (%RSD 0.56–1.38) for gabapentin, all within the ICH-recommended acceptance range of 97–103%, confirming the accuracy of the method for both analytes in the presence of formulation matrix.

2.4.5. Robustness: The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The determination of robustness requires that methods characteristic is assessed when one or more operating parameter varied. The approach remained unaffected by the minor changes, since the percentage RSD for both parameters was less than 2%.

2.4.6. Limit of Detection (LOD) and Limit of Quantification (LOQ): LOD and LOQ were calculated from the standard deviation of the y-intercept (σ) and the slope (S) of the respective calibration curve, as per ICH Q2(R1)

$LOD = 3.3 \times \sigma / S$ $LOQ = 10 \times \sigma / S$

2.4.7. Specificity and Selectivity: The specificity of the method was established through separation of the drug peak from the nearest resolving peak and also among all other peaks. Selectivity was confirmed through peak purity data. To assess the method specificity, chromatogram of bulk powder mixture was compared for Rf value and purity with respective Gabapentin and Nortriptyline standard to evaluate specificity of the method.

2.5. Analysis of Marketed Formulation: Twenty tablets of the marketed formulation (Gabapin NT 300; label claim 300 mg gabapentin + 10 mg nortriptyline) were weighed and finely powdered. A quantity of powder equivalent to 30 mg gabapentin and 1 mg nortriptyline was accurately weighed, dissolved in methanol with the aid of sonication (15 min), filtered through Whatman filter paper, and diluted to volume with methanol in a 10 mL volumetric flask. The resulting solution was applied to the HPTLC plate alongside the standard, developed and scanned under the optimized chromatographic conditions, and the amount of each drug was calculated from the respective regression equation. The % label claim and % RSD were determined from six replicate determinations.

2.6. Forced Degradation Studies: Stress (forced) degradation studies were performed in accordance with ICH Q1A (R2) guidance[8,31] under acidic, alkaline, oxidative, thermal and photolytic conditions, using the same standard weighing of gabapentin (30 mg) and nortriptyline (1 mg) employed for the stock solutions. Each stressed sample was analysed under the optimized chromatographic conditions, and % degradation was calculated by comparing the peak area of the stressed sample with that of the corresponding freshly prepared (unstressed) standard.

2.6.1. Acid Hydrolysis: Acidic hydrolysis degradation for both drug standard was performed by accurately weighing 30 mg of Gabapentin in 1 ml & 1 mg of Nortriptyline in 1 ml then Add 2.5 ml of 1 N HCl and heated for 2 Hr. at 70-80 ºC. After that allow it to cool down then neutralize it with 1N NaOH and dilute up to the mark with methanol in 10ml volumetric flask.

2.6.2. Alkaline Hydrolysis: Alkaline hydrolysis degradation for both drug standard was performed by accurately weighing 30 mg of Gabapentin in 1 ml & 1 mg of Nortriptyline in 1 ml then Add 2.5 ml of 1 N NAOH and heated for 2 Hr. at 70-80 ºC. After that allow it to cool down then neutralize it with 1N HCL and dilute up to the mark with methanol in 10ml volumetric flask.

2.6.3. Oxidative Degradation: Oxidative hydrolysis degradation for both drug standard was performed by accurately weighing 30 mg of Gabapentin in 1 ml & 1 mg of Nortriptyline in 1 ml then Add 2.5 ml of 3% H2O2 and heated for 2 Hr. at 70-80 ºC. After that allow it to cool down then dilute up to the mark with methanol.

2.6.4. Thermal Degradation: Gabapentin (30 mg) & Nortriptyline (1 mg) was kept in the Hot air oven at 90 1000C for 3 hrs. Allowed to cool down then diluted up to the mark in 10 ml volumetric flask.

2.6.5. Photolytic Degradation: Standard and accuratly weighted Gabapentin (30 mg) & Nortriptyline (1 mg) transferred in to the petri dish. The petri plate was kept in the UV chamber under the 254 nm wavelength for 5 Hrs. After that transferred it to the 10 ml of volumetric flask and diluted up to the mark with methanol in 10ml volumetric flask.

3. RESULTS AND DISCUSSION

3.1. Optimization of the Chromatographic Method: Preliminary trials with ethyl acetate: methanol: ammonia (6.0:4.0:0.1, v/v/v) and ethyl acetate:methanol: ammonia: toluene mixtures resulted in either poor migration of gabapentin, streaking, or unresolved bands, and were judged unsatisfactory (Table 4). Since gabapentin lacks a chromophore, its detection required post-chromatographic derivatization with 2% ninhydrin, which forms a purple-coloured Ruhemann's-purple adduct with the primary amino group of gabapentin, permitting visualisation and densitometric quantification at 487 nm. A mobile phase of methanol:acetonitrile:ethyl acetate:ammonia (5:3:3:0.1, v/v/v/v) produced compact, symmetrical bands with good resolution and reproducible Rf values for both analytes and was therefore selected as optimal (Rf 0.274 for gabapentin and 0.385 for nortriptyline as individual standards; 0.235 and 0.372, respectively, in the combined mixture, Table 3).

Table 3: Optimization of the HPTLC mobile phase.

Trial	Mobile phase (ratio, v/v/v/v)	Observation	Remark
1.	Ethyl acetate : Methanol : Ammonia (6.0:4.0:0.1)	Gabapentin not observed; nortriptyline spot observed	Not satisfactory
2.	Ethyl acetate : Methanol : Ammonia : Toluene (5.0:0.5:4.5:0.1)	Gabapentin observed but did not migrate; nortriptyline spot observed	Not satisfactory
3.	Ethyl acetate : Methanol : Ammonia : Toluene (5.0:1.0:4.0:0.1)	Gabapentin observed only after ninhydrin derivatization; nortriptyline spot observed	Not satisfactory

4.	Methanol : ACN : Ethyl acetate : Ammonia (5:3:2:0.1)	Both spots observed; resolution not clear	Not satisfactory
5.	Methanol : ACN : Ethyl acetate : Ammonia (5:3:3:0.1)	Good, clear separation with distinct Rf values	Satisfactory (selected)

3.2. Validation

3.2.1. Linearity: The linearity of calibration curve for Gabapentin & Nortriptyline established in five the concentration range. For determination of linearity each standard was taken in volumetric flask and diluted up to the mark with methanol to get the linearity stock solution. The linearity stock solution was spotted on TLC plate with Linearity range of (6000-8500ng/band) & (200-450ng/band). The calibration curve was plotted against area of peak verses concentration of standard. Correlation of coefficient value for Gabapentin found 0.991 & Nortriptyline 0.995.(Figure 1, Figure 2 and Table 4)

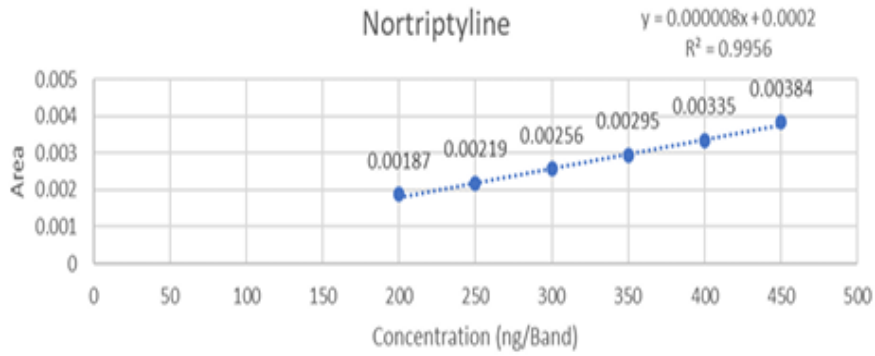

Figure 1 Nortriptyline calibration curve.

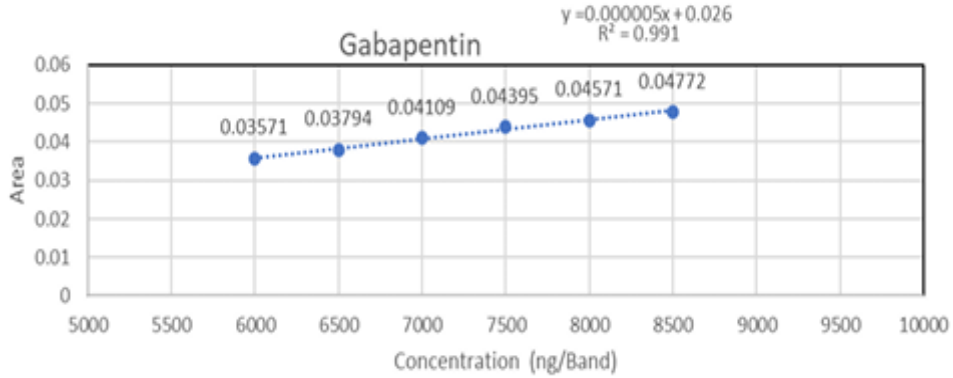

Figure 2 Gabapentin calibration curve.

Table 4: Linear Regression Data for Gabapentin and Nortriptyline.

Parameter	Gabapentin	Nortriptyline
Linearity range (ng/band)	6000–8500	200–450
Regression equation	y = 0.000005x + 0.026	y = 0.000008x + 0.0002
Correlation coefficient (r^2)	0.991	0.9956
Slope	0.000005	0.000008
Intercept	0.026	0.0002

3.2.2. Accuracy (% Recovery): Recovery studies performed at 80%, 100% and 120% spiking levels gave mean recoveries of 100.15 ± 0.88% (%RSD 1.07–1.37) for nortriptyline and 98.99–100.26% (%RSD 0.56–1.38) for gabapentin (Table 5), all within the ICH-recommended acceptance range of 97–103%, confirming the accuracy of the method for both analytes in the presence of formulation matrix.

Table 5: Accuracy (% recovery) of gabapentin and nortriptyline (mean of triplicate determinations at each level).

Drug	Spiking level (%)	Mean recovery (%)	SD	% RSD
Nortriptyline	80	100.15	0.876	0.876
Nortriptyline	100	100.48	1.070	1.065
Nortriptyline	120	100.00	1.002	1.002
Gabapentin	80	98.99	0.557	0.563
Gabapentin	100	100.14	1.378	1.376
Gabapentin	120	100.26	1.111	1.109

3.2.3. Precision: Instrumental repeatability (n = 6) gave % RSD of 1.73% (nortriptyline, 300 ng/band) and 0.91% (gabapentin, 7000 ng/band). Intra-day and inter-day precision (% RSD) at all tested concentration levels were below 2% for both drugs (0.89–1.61% for nortriptyline and 0.05–0.13% for gabapentin) (Table 6), demonstrating that the method is precise and reproducible within a single laboratory.

Table 6: Precision (repeatability and intermediate precision, expressed as % RSD).

Drug	Repeatability (n = 6)	Intra-day precision (% RSD range)	Inter-day precision (% RSD range)
Nortriptyline	1.73%	0.89–1.61%	0.89–1.61%
Gabapentin	0.91%	0.05–0.13%	0.05–0.13%

3.2.4. Robustness: Small, deliberate variations in mobile-phase composition and chamber saturation time produced no significant change in Rf value or band compactness; % RSD for both parameters remained below 2% for each analyte (Table 7), confirming that the method is robust and unaffected by minor, intentional variations likely to occur during routine use.

Table 7: Robustness of the HPTLC method.

Parameter varied	Condition	Rf (Gabapentin)	Rf (Nortriptyline)	% RSD (Gabapentin)	% RSD (Nortriptyline)
Mobile-phase composition	5:3:3:0.1 (v/v/v/v)	0.279	0.385	1.537	0.368
Mobile-phase composition	5:3:3:0.5 (v/v/v/v)	0.273	0.383	—	—

Chamber saturation time	20 min	0.279	0.385	1.021	0.183
Chamber saturation time	22 min	0.275	0.386	—	—

3.2.5. Limit of Detection and Limit of Quantification: The LOD and LOQ, calculated from the calibration data (Section 2.5.5), were 281.70 ng/band and 853.63 ng/band for gabapentin, and 176.89 ng/band and 536.05 ng/band for nortriptyline, respectively (Table 8), confirming that the method is sufficiently sensitive for the intended purpose.

Table 8: LOD and LOQ of gabapentin and nortriptyline.

Parameter	Gabapentin (ng/band)	Nortriptyline (ng/band)
LOD	281.70	176.89
LOQ	853.63	536.05

3.2.6. Specificity and Selectivity: Peak-purity correlation values (R(s,m) and R(m,e)) for both drugs, in standard and in formulation samples, exceeded 0.99 (Table 9), confirming that the gabapentin and nortriptyline peaks were homogeneous and free from interference by excipients present in the marketed formulation, establishing the specificity of the method.

Table 9: Specificity/selectivity- peak purity data.

Sample	Assigned Rf	R (s, m)	R (m, e)	Purity
Gabapentin (standard)	0.249	0.999851	0.995995	OK
Gabapentin (formulation)	0.242	0.999789	0.993191	OK
Nortriptyline (standard)	0.325	0.999821	0.999268	OK
Nortriptyline (formulation)	0.317	0.999782	0.998868	OK

3.3. Analysis of Marketed Formulation: Application of the validated method to the marketed formulation (Gabapin NT 300) gave a well-resolved, sharp chromatogram for both analytes, free from interference by tablet excipients. The content found was 99.0% (% RSD 0.48) for gabapentin and 99.25% (% RSD 1.78) for nortriptyline (Table 10), both within the acceptable assay range (98–102%) of the labelled claim, confirming that the method can be applied for routine quality control of the combined dosage form.

Table 10. Assay of the marketed formulation (Gabapin NT 300) by the developed HPTLC method.

Drug	Label claim (mg)	Amount found (mg)	% Content found	% RSD
Gabapentin	300	297	99.0	0.48
Nortriptyline	10	9.92	99.25	1.78

3.4. Forced Degradation Study: Gabapentin and nortriptyline were separately subjected to acidic, alkaline, oxidative, thermal and photolytic stress and analysed by the developed method. Additional degradation-product peaks, well resolved from the intact drug peaks, appeared in the chromatograms under each condition, confirming that the method is stability-indicating and capable of resolving the parent drugs from their degradation products. Nortriptyline showed extensive degradation under acidic (79.60%), alkaline (85.86%), oxidative (85.02%) and photolytic (86.13%) conditions, and comparatively limited degradation under thermal stress (20.11%), suggesting that nortriptyline is highly susceptible to hydrolytic, oxidative and photolytic degradation but relatively stable to dry heat. Gabapentin, in contrast, showed the greatest susceptibility to oxidative (72.70%) and thermal (65.28%) stress, moderate degradation under acidic (31.08%) and alkaline (21.33%) conditions, and the least degradation under photolytic exposure (16.28%) (Table 11). These differential degradation profiles indicate that the two drugs degrade through distinct pathways and stress susceptibilities, information that is directly relevant to formulation development, packaging selection and storage-condition recommendations for the combination product.

Table 11. Summary of forced degradation study for gabapentin and nortriptyline.

Stress condition	Conditions	% Degradation — Gabapentin	% Degradation — Nortriptyline
Acid hydrolysis	1 N HCl, 80–90 °C, 2 h	31.08	79.60
Alkaline hydrolysis	1 N NaOH, 80–90 °C, 2 h	21.33	85.86
Oxidative degradation	3% H2O2, 80–90 °C, 2 h	72.70	85.02
Thermal degradation	90–100 °C, dry heat, 3 h	65.28	20.11
Photolytic degradation	UV 254 nm, 5 h	16.28	86.13

4. CONCLUSION

A simple, rapid, sensitive, precise, accurate and stability-indicating HPTLC method was successfully developed and validated for the simultaneous estimation of gabapentin and nortriptyline in bulk drug and in a marketed tablet formulation, using methanol:acetonitrile:ethyl acetate:ammonia (5:3:3:0.1, v/v/v/v) as the mobile phase on silica gel 60 F254 plates, with densitometric detection at 236 nm for nortriptyline and, after ninhydrin derivatization, at 487 nm for gabapentin. The method was linear, accurate (recovery 97–103%), precise (% RSD < 2%) and robust as per ICH Q2(R1) guidelines, and successfully resolved both drugs from degradation products generated under acidic, alkaline,

oxidative, thermal and photolytic stress, confirming its stability-indicating nature. As no HPTLC method for the simultaneous estimation of this combination has previously been reported, the validated method can be conveniently and reliably applied for routine quality control, stability testing and regulatory submission of gabapentin–nortriptyline combination formulations.

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