Research Article

Stability-Indicating Method Development and Validation of Gabapentin and Nortriptyline in Bulk And Pharmaceutical Dosage Forms Using LC–MS And HPTLC

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ABSTRACT:

Background: Gabapentin and nortriptyline are used in combination-oriented therapeutic strategies for neuropathic pain, but their markedly different physicochemical properties create analytical challenges for simultaneous estimation and stability assessment. This study developed and evaluated complementary LC–MS and HPTLC approaches for identification, quantitative analysis, method validation, and forced-degradation assessment of gabapentin (GAB) and nortriptyline (NOR) in bulk materials and pharmaceutical dosage forms.

Methods: LC–MS method development was investigated through systematic mobile-phase and column trials, followed by optimization using a Sciex API 4000 mass detector with a Turbo IonSpray source. The optimized LC–MS conditions used water:methanol (5:95, v/v) containing 1 mL ammonia solution, a flow rate of 1.0 mL/min with 50:50 splitter configuration, and a CHIRALCEL OZ-3R column (4.6 × 150 mm, 3 µm). HPTLC was performed on silica gel 60 GF254 plates using chloroform:methanol:formic acid (8.2:1.5:1, v/v/v), with 15 min chamber saturation and approximately 15 min development. Validation included selectivity/specificity, linearity, accuracy, precision, sensitivity, robustness, and assay of formulations. Forced-degradation studies included oxidative, thermal and photolytic conditions for gabapentin and acid, alkaline, wet/thermal and photolytic conditions for nortriptyline, as documented in the thesis.

Results: The thesis data demonstrated satisfactory precision and accuracy for the validated procedures. For gabapentin, the reported LOQ was 0.509 ng/mL, with 97.5% accuracy and 4.2% precision at the LOQ. For nortriptyline, the reported LOQ was approximately 49.10 pg/mL, with 95.6% accuracy and 6.3% precision. Gabapentin showed intraday precision of 0.9–2.0% CV and interday precision of approximately 0.9–3.8% CV, with intraday accuracy of 100.0–105.2% and interday accuracy of 98.0–105.2% for the reported quality-control levels. Robustness testing by wavelength variation showed minimal changes in retention factor and peak area. In oxidative stress testing, gabapentin showed three additional degradation peaks and an approximately 35.66% reduction in parent peak intensity. Under dry-heat stress, no detectable degradation was reported for gabapentin under the tested conditions. The HPTLC assay of three formulations produced gabapentin and nortriptyline contents within the ranges reported in the thesis.

Conclusion: The combined LC–MS/HPTLC strategy provides complementary capabilities for sensitive analytical characterization and routine stability-indicating quality assessment of gabapentin and nortriptyline. LC–MS is particularly useful for sensitive detection and degradation-product investigation, whereas HPTLC offers a comparatively rapid and practical platform for simultaneous assay and stability screening. The analytical procedures described in the thesis provide a foundation for further journal-level validation and independent confirmation using complete chromatographic, statistical and degradation-product datasets.

Keywords: Gabapentin; Nortriptyline; HPTLC; LC–MS; Stability-indicating method; Forced degradation; Method validation; Pharmaceutical analysis.

1. INTRODUCTION

Pharmaceutical quality control requires analytical procedures capable of accurately identifying and quantifying active pharmaceutical ingredients in the presence of excipients, impurities and degradation products. The analytical challenge becomes more pronounced for combination products containing compounds with substantially different polarity, ionization behavior and degradation pathways [1]. Gabapentin is a highly polar, zwitterionic anticonvulsant/neuropathic-pain agent, whereas nortriptyline is a tricyclic antidepressant with substantially different chromatographic and mass-spectrometric behavior. Consequently, an analytical procedure intended for their simultaneous assessment should provide adequate selectivity while remaining sufficiently sensitive for low-level measurements and degradation studies [2].

The supplied thesis describes a dual-platform strategy using liquid chromatography–mass spectrometry (LC–MS) and high-performance thin-layer chromatography (HPTLC). LC–MS was selected for sensitive detection and characterization-oriented work, while HPTLC was used as a practical quantitative and stability-indicating platform.

This orthogonal strategy is scientifically relevant because regulatory analytical validation requires demonstration that an analytical procedure is fit for its intended purpose, including specificity/selectivity, linearity, range, accuracy, precision, detection/quantitation capability and robustness. ICH Q2(R1) describes these principal validation characteristics, while ICH Q1A(R2) establishes the role of stability testing in demonstrating how drug quality changes under environmental stresses [3], [4].

Previous literature demonstrates the analytical difficulty of gabapentin and nortriptyline. Gabapentin has been quantified by derivatization-based HPLC because of its limited native UV response, while nortriptyline has been determined by chromatographic and spectrophotometric procedures. cite turn0search9 turn0search11 HPTLC has also been successfully validated for simultaneous estimation of nortriptyline with related neuropathic-pain drugs, demonstrating the continuing relevance of planar chromatography for pharmaceutical analysis [5,6].

The objective of the present work was therefore to develop and validate complementary LC–MS and HPTLC procedures for gabapentin and nortriptyline in bulk and dosage forms and to evaluate their stability-indicating capability through forced degradation studies.

2. MATERIALS AND METHODS

2.1 Study Design

The experimental design followed the workflow documented in the supplied thesis: preliminary analytical method development; optimization of chromatographic conditions; validation of the selected procedure; application to pharmaceutical formulations; and forced-degradation/stability-indicating assessment. The present manuscript preserves the reported experimental conditions and results and does not introduce unreported numerical observations [7].

2.2 Chemicals, standards and formulations

Gabapentin and nortriptyline reference materials and pharmaceutical formulations were analyzed as described in the thesis. Methanol, water, ammonia solution, chloroform and formic acid were used for chromatographic development. Three formulations/brands were evaluated for stability and/or assay. The thesis reports formulation strengths including a 250-mg product, a 200-mg product and a formulation reported on a per-teaspoon basis; exact commercial product names and batch details should be inserted from the original laboratory records before submission [8] (Table 1).

Table 1: LC–MS instrumentation and optimized chromatographic conditions

Parameter	Optimized condition reported in thesis
Mass detector	Sciex API 4000
Ion source	Turbo IonSpray
Pump	Shimadzu LC-10ADvp
Autosampler	Shimadzu SIL-HTC
Degasser	Shimadzu DGU-20As
Column oven	Shimadzu CTO-10AS VP
Column	CHIRALCEL OZ-3R, 4.6 × 150 mm, 3 µm
Mobile phase	Water:methanol (5:95, v/v) + 1 mL ammonia solution
Flow rate	1.0 mL/min; splitter 50:50

Method development involved comparison of several water: methanol compositions and column configurations. The thesis reports progressive trials from 40:60 to 5:95 water: methanol and different stationary phases, culminating in the CHIRALCEL OZ-3R configuration described above.

Table 2: HPTLC Method

Parameter	Condition reported in thesis
Stationary phase	Silica gel 60 GF254, precoated
Mobile phase	Chloroform:methanol:formic acid (8.2:1.5:1, v/v/v)
Chamber saturation	15 min

Development time	15 min

The HPTLC method was applied to simultaneous estimation of gabapentin and nortriptyline in formulations and to stability-indicating studies. The thesis reports that the developed system resolved the analytes in the presence of formulation matrices and degradation products [9].

2.3 Validation procedure

Validation was performed using the characteristics reported in the thesis and framed against ICH Q2(R1): specificity/selectivity, linearity, accuracy, precision, sensitivity (LOD/LOQ), robustness and application to dosage forms. ICH Q2(R1) identifies specificity, linearity, range, accuracy, precision, detection limit and quantitation limit as core validation characteristics, with robustness addressed as an indicator of the reliability of an analytical procedure under deliberate variation [10].

2.4 Linearity

For gabapentin, the thesis reports nine nominal concentration levels ranging from approximately 0.560 to 550.868 ng/mL. Back-calculated concentrations were assessed in replicate. The reported percent accuracy across the levels ranged from 98.5% to 112.4%, with the individual values presented in the thesis. The corresponding nortriptyline calibration dataset included multiple concentration levels; the complete regression statistics should be transferred from the original thesis tables into the final journal submission because the scanned source did not yield all regression coefficients reliably in machine-readable form [11].

2.5 Accuracy and recovery

Accuracy/recovery was evaluated using replicate quality-control or spiked measurements. For gabapentin, the thesis reports acceptable recovery performance and quality-control accuracy generally within the predefined acceptance range. Detailed recovery values should be reproduced from the original validated worksheet when preparing the final submission, rather than reconstructed from OCR text [12].

2.6 Precision

Repeatability and intermediate precision were assessed. For gabapentin, intraday precision across low, dilution, middle and high quality-control levels ranged from 0.9% to 2.0% CV, while interday precision was reported at approximately 0.9–3.8% CV. At the LOQ quality-control level, intraday precision was 3.7% and interday precision was 5.2%. The corresponding intraday accuracy range was 100.0–105.2%, and interday accuracy was 98.0–105.2%; LOQ quality-control accuracy was 99.1% intraday and 96.2% interday (Table 3) [13].

Table 3: Sensitivity

Analyte	Reported LOQ	Accuracy at LOQ	Precision at LOQ
Nortriptyline	≈49.10 pg/mL	95.6%	6.3% CV
Gabapentin	0.509 ng/mL	97.5%	4.2% CV

The thesis states that six replicate injections were used for LOQ evaluation. For gabapentin, the back-calculated concentrations ranged from 0.465 to 0.526 ng/mL, with a mean of 0.4963 ng/mL. For nortriptyline, the reported back-calculated values were approximately 46.4–54.1 pg/mL, with a mean of 49.6415 pg/mL. Signal-to-noise observations were also recorded in the thesis [14].

2.7 Robustness

Robustness was assessed by deliberate variation of detection wavelength. The thesis reports wavelength levels of 206, 207 and 209 nm. For gabapentin, the reported Rf value was approximately 0.59 and for nortriptyline approximately 0.21 across the tested conditions. Peak-area responses showed only small variation, supporting the robustness of the chromatographic measurement under the evaluated wavelength change [15].

2.8 Forced degradation and stability-indicating assessment

Forced degradation was performed under acidic, alkaline, oxidative, thermal and photolytic conditions where documented. The study was intended to demonstrate separation of parent analytes from degradation products and

thereby establish stability-indicating capability, consistent with the general purpose of stability testing described by ICH Q1A(R2) [16].

2.8.1 Gabapentin

The thesis reports oxidative stress producing three additional chromatographic peaks and an approximately 35.66% reduction in the gabapentin parent peak intensity. UV spectral evaluation supported retention of gabapentin in the stressed sample together with degradation-product formation. Under dry-heat exposure, no detectable degradation was reported, including extended exposure at 60 °C for 48 h. Photostability testing was conducted using short-wavelength UV exposure, although the complete quantitative degradation dataset is not reliably recoverable from the scanned source and should therefore be inserted from the original laboratory record before submission [17].

2.8.2 Nortriptyline

Nortriptyline was subjected to acid, alkaline, wet/thermal, dry-heat and photolytic stress. The thesis reports substantial susceptibility to acid hydrolysis, with complete degradation under the described 1 N HCl reflux conditions and disappearance of the parent Rf signal accompanied by multiple degradation peaks. Alkaline stress used 1 N NaOH at 60 °C for 3 h. Dry-heat studies included 100 °C for 3 h and 60 °C for 24 h. Photostability testing used UV radiation at 254 nm for 24 h [18].

3. RESULTS

3.1 LC–MS method development

Systematic chromatographic trials were undertaken by varying the water: methanol ratio and stationary phase. The final method used a highly organic mobile phase (5:95 water: methanol) with ammonia modifier and the CHIRALCEL OZ-3R column. The thesis identifies the Sciex API 4000 platform with Turbo IonSpray as the mass-detection system. The selected configuration provided the basis for sensitive analysis and subsequent method-performance assessment (Table 4, 5) [19].

Table 4: Validation performance

Validation characteristic	Gabapentin	Nortriptyline
LOQ	0.509 ng/mL	≈49.10 pg/mL
Accuracy at LOQ	97.5%	95.6%
Precision at LOQ	4.2% CV	6.3% CV
Intraday precision	0.9–2.0% CV (QC levels)	Detailed values should be taken from original thesis table
Interday precision	≈0.9–3.8% CV	Detailed values should be taken from original thesis table
Intraday accuracy	100.0–105.2%	Detailed values should be taken from original thesis table
Interday accuracy	98.0–105.2%	Detailed values should be taken from original thesis table
Robustness	Wavelength variation; minimal response change	Wavelength variation; minimal response change

Table 5: HPTLC assay of formulations

Formulation	Reported gabapentin content	Reported nortriptyline content
Brand 1	174.5 mg/unit; 0.698%	16.15 mg/unit; 0.646%
Brand 2	14.74 mg/unit; 0.737%	0.586 mg/unit; 0.293%
Brand 3	~31.8 mg/tsp; 0.758%	~21.12 mg/tsp; 0.704%

The results states that the HPTLC method was applied to simultaneous estimation in traditional and modern dosage forms and that matrix components did not materially alter the peak shape. The exact product descriptions and label

claims should be verified against the original product records before publication, particularly because OCR of the scanned thesis introduced ambiguity in some units and formulation descriptions [20].

Table 6: Stability of marketed formulations

Analyte / formulation	4 °C: 24/48/72 h (%)	25 °C: 24/48/72 h (%)
Gabapentin – Brand 1	99.02 / 98.79 / 98.07	98.87 / 98.15 / 97.54
Gabapentin – Brand 2	98.22 / 97.83 / 97.46	98.01 / 97.87 / 97.01
Gabapentin – Brand 3	98.01 / 97.54 / 96.91	97.89 / 97.07 / 96.50
Nortriptyline – Brand 1	99.45 / 98.99 / 97.07	97.91 / 97.09 / 96.11
Nortriptyline – Brand 2	98.22 / 97.99 / 96.37	97.33 / 96.82 / 96.09
Nortriptyline – Brand 3	97.89 / 97.01 / 95.99	97.11 / 96.06 / 95.74

The reported short-term stability study showed progressive decreases in measured content with time for the tested formulations, with greater decreases generally observed at 25 °C than at 4 °C. These results are appropriate as comparative short-term stability observations and should not be interpreted as shelf-life data; formal stability conclusions require an ICH-compliant long-term and accelerated design appropriate to the product and intended market (Table 6) [21].

Table 7: Forced-degradation findings

Analyte	Stress condition	Principal finding reported in thesis
Gabapentin	Oxidative	Three additional peaks; ~35.66% reduction in parent peak intensity
Gabapentin	Dry heat	No detectable degradation under reported extended exposure
Gabapentin	UV exposure	Photostability study performed; complete quantitative dataset requires source-table verification
Nortriptyline	1 N HCl reflux	Complete degradation reported; multiple degradation peaks; parent Rf absent
Nortriptyline	1 N NaOH, 60 °C, 3 h	Alkaline degradation study performed
Nortriptyline	Dry heat, 100 °C, 3 h	Thermal stress study performed
Nortriptyline	Dry heat, 60 °C, 24 h	Thermal stress study performed
Nortriptyline	UV 254 nm, 24 h	Photostability study performed

4. DISCUSSION

The principal outcome of this study is the demonstration that gabapentin and nortriptyline can be approached through complementary chromatographic platforms rather than relying on a single analytical technique for all analytical questions. The LC–MS procedure provides a sensitive platform suitable for low-level measurement and degradation-product investigation, while HPTLC provides a practical planar-chromatographic approach for simultaneous assay and stability screening [22, 23].

The very low reported LOQs demonstrate the sensitivity achieved in the LC–MS-oriented procedure. Importantly, sensitivity alone does not establish a stability-indicating method; the ability to resolve the parent drug from degradation products is central. The thesis therefore appropriately combines validation with forced-degradation experiments. For gabapentin, oxidative stress generated three additional peaks and reduced the parent response by approximately 35.66%, providing direct evidence that the HPTLC procedure could detect chemical change. Conversely, the absence of detectable degradation under the reported dry-heat condition indicates that stress response is condition-dependent rather than universally rapid [24, 25].

Nortriptyline showed a different degradation profile. The thesis reports pronounced acid sensitivity, with disappearance of the parent Rf signal and formation of multiple degradation peaks under 1 N HCl reflux. Such orthogonal stress behavior reinforces the importance of evaluating multiple stress pathways when establishing specificity. A method that is stable under one stress condition cannot automatically be considered stability-indicating under all conditions [26].

The HPTLC assay results further demonstrate applicability to formulations. However, journal submission should preserve a clear distinction between assay of marketed formulations and formal stability testing. The short-term 4 °C and 25 °C observations reported here are useful comparative findings, but they do not replace long-term/accelerated stability programs designed according to ICH expectations [27].

The analytical approach is consistent with the broader pharmaceutical literature. Gabapentin analysis often requires specialized chromatographic strategies because of its physicochemical characteristics, while validated HPTLC procedures have been successfully applied to nortriptyline-containing combinations. The present study adds value through its combination of LC–MS sensitivity and HPTLC stability-indicating application to the specific gabapentin–nortriptyline analytical problem described in the thesis [28, 29].

5. CONCLUSION

A LC–MS and HPTLC analytical strategy was developed and evaluated for gabapentin and nortriptyline in bulk and pharmaceutical dosage forms. The reported validation data demonstrate sensitive quantification, acceptable accuracy and precision, and robustness to the tested wavelength variation. Forced-degradation studies established useful stability-indicating behavior, particularly the detection of oxidative degradation products of gabapentin and pronounced acid degradation of nortriptyline. HPTLC additionally demonstrated applicability to simultaneous formulation assay and short-term comparative stability testing. The work provides a practical analytical framework for pharmaceutical quality control, while the complete original validation datasets and chromatographic records should be incorporated before submission to a specific Scopus-indexed journal.

Declarations:

Ethics approval and consent to participate:

Not applicable to this analytical pharmaceutical study.

Consent for publication:

Not applicable.

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