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Development and Validated of HPTLC-UV Bioanalytical Method for Simultaneous Estimation of Nortriptyline and Gaba-Pentin Tablets

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Abstract: Chromatography is a non-destructive technique used to separate a combination of solids, gases, and liquids. HPTLC employs proven techniques for both qualitative and quantitative examination. HPTLC is a supplementary technique to HPLC and plays a significant role in the analytical field. Parameters like linearity, precision, accuracy, limit of detection and limit of quantification, specificity, and robustness were used to assess the analytical approach. This procedure involved taking standard stock solutions of pregabalin (750ng µL-1) and nortriptyline (100ng µL-1). Toluene, ethyl acetate, and methanol (6: 2: 1, v/v/v) are present in the mobile phase. Using a Linomat 5 sample applicator and a CAMAG 100µl sample syringe, standard stock solutions were applied by overspotting on an HPTLC plate. For fifteen minutes, the development chamber was saturated At 210 nm, the plate was scanned. Stress degradation experiments showed that NORT was vulnerable to hydrolysis, oxidative, thermal, and photolytic deterioration, while PREGA was shown to be stable under photolytic stress conditions but susceptible to hydrolysis, oxidative, and thermal degradation.

Keyword: HPTLC (High-Performance Thin-Layer Chromatography), Pregabalin, Nortriptyline, Stability studies.

INTRODUCTION:

Gabapentin, an anticonvulsant, and nortriptyline, a tricyclic antidepressant, are frequently taken together to treat mood disorders, epilepsy, and neuropathic pain. To guarantee the effectiveness and safety of these medications in pharmaceutical formulations, precise and concurrent quantification is crucial. A flexible and affordable analytical method, High-Performance Thin-Layer Chromatography (HPTLC) has benefits in terms of speed, ease of use, and sensitivity (2, 5, 4).

The purpose of this work was to create and verify a new HPTLC-UV bioanalytical technique for the simultaneous measurement of gabapentin and nortriptyline in tablets. The suggested approach's specificity, linearity, accuracy, precision, and robustness were guaranteed by optimising and validating it in accordance with International Conference on Harmonisation (ICH) principles (1, 2). Nortriptyline and gabapentin tablet bioequivalence studies and routine quality control analyses can be conducted using the established approach.

The molecular formulas for gabapentin and nortriptyline are C9H17NO2 and C19H21N.HCL, respectively, and their molecular weights are 171.24 and 299.8, respectively. Their IUPAC names are 2-[1-(amino methyl) cyclohexyl] acetic acid and 3-(10, 11-dihydro-5Hdibenzo [a, d] cyclohepten-5-ylidene)-N-methyl-1-propanamine (6, 7).

The drug's molecular structure as an anticonvulsant, gabapentin is used in this combination to treat convulsions, which can have the adverse effect of causing depression. Therefore, nortriptyline is used

as an antidepressant to treat depression. Serrati peptidase and gabapentin are official in BP and USP. Official in IP, BP, and USP is nortriptyline. To date, however, no analytical technique has been published for the estimation of gabapentin and nortriptyline utilising the RP-HPLC method and UV spectroscopy (2, 5). The current study details the development and validation of an analytical method for estimating gabapentin and nortriptyline in pharmaceutical dosage forms using UV spectroscopy and RP-HPLC. In accordance with ICH principles, the suggested approach has been optimised and validated (3).

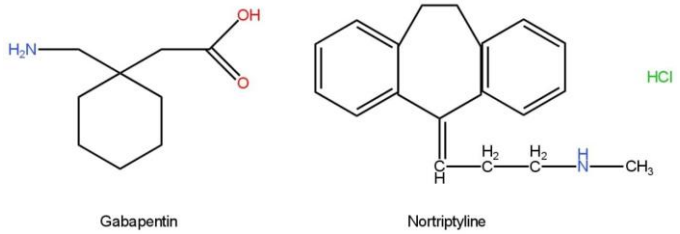

Fig 1: Structure of Gabapentin and Nortriptyline

Table 1: Method of development for the HPTLC-UV Bioanalytical studies of Nortriptyline and Gabapentin (5)

PARAMETERS	DETAILS
Objective	Simultaneous estimation of Nortriptyline and gabapentin in tablets
Chromatographic Technique	High-Performance Thin Layer Chromatograph(HPTLC)
Detection Method	Ultraviolet(UV) Detection
Stationary Phase	Silica gel 60 F254 or equivalent (pre-coated HPTLC plates)
Mobile Phase	Mixture of solvents (e.g. Toluene: Ethyl acetate: Methanol) with optimization for separation of both drugs
Wavelength for Detection	254 nm (Nortriptyline) and 210 nm (gabapentin) or a dual-wavelength detection if required
Sample Preparation	-Crush tablet and extract using appropriate solvent (e.g., methanol or water). -Filter and concentration the extract if needed.
Standard Solution	-Nortriptyline standard solution in suitable solvent (e.g., methanol). -Gabapentin standard solution in suitable solvent.
Mobile Phase Composition	Example: Toluene: Ethyl acetate: Methanol (7:3:1, v/v/v) or optimized mixture based on trail and error.
Spot Application Method	-Apply standard and sample solutions using a microliter syringe or applicator. -Apply spots on the TLC plate (usually 6-8 spots).
Plate Development	-develop plate in TLC chamber up to a suitable distance, typically 8-10 cm.
Development Time	Usually 10-15 minutes, depending on mobile phase and resolution.
Drying of plates	Dry the plate after development, typically under room temperature or air blower.
UV Visualization	Place the development plate under UV light (254 nm for Nortriptyline and 210 nm for Gabapentin) for identification and Quantification.
Quantification Method	Densitometry analysis using HPTLC scanner at the selected wavelength (254 nm for Nortriptyline, 210 nm for Gabapentin).
Calibration Curve	Plot calibration for Nortriptyline and Gabapentin separately using known concentration of standard solution.
Linearity Range	Typically, 50-1000ng/spot for both Nortriptyline and Gabapentin (can vary based on plate size)
Precision	Inter-day and intra-day precision should be determined by analysing the same sample multiple times on different days and within the same day.
Limit of Detection (LOD)	Determine LOD for each drug by calculating the lowest concentration that can be detected with acceptable precision.

Limit of Quantification(LOQ)	Determine LOQ by calculating the lowest concentration that can be quantitated with acceptable accuracy and precision.
Recovery	Evaluate the recovery of both drugs from the tablet formulation by spiking known amount into the formation.
Ruggedness	Investigate variation in mobile phase composition, development time, and detection parameters to assess method robustness (7).

METHOD OF DEVELOPMENT:

1. Stationary phase selection: The stationary phase was silica gel 60F 254HPTLC plates (5).
2. Mobile phase selection: The mobile phase was a 6:3:1 v/v/v mixture of toluene, ethyl acetate, and methanol (1).
3. Sample preparation: Methanol was used to create a stock solution of gabapentin and nortriptyline. Working solutions were then obtained by diluting the stock solution (5).
4. Sample application: A CAMAG Linomat 5 applicator was used to apply the working solutions to the HPTLC plate (2).
5. Chromatography: A CAMAG twin-trough chamber was used to generate the HPTLC plate (3).
6. Detection: A CAMAG TLC Scanner 3 was used to scan the plate at 230 nm (2).

METHOD OF VALIDATION:

The protocol called for testing each medication separately and in combination at dosages of 1, 3, 10, 30, and 100 mg/kg of gabapentin and 0.1, 1, 3, 10, and 30 mg/kg of nortriptyline; intraperitoneally). The two phases of the is bolographic assay are phase 1 (the 5-minute period beginning right after the formalin injection) and phase 2 (the 10-minute period beginning 20 minutes after the formalin injection), which represents an inflammatory pain state and tonic acute pain caused by peripheral nociceptor sensitization. The Student t test for independent means was used to assess the results. 168 male CF-1 mice weighing 30 g were used in the study (1).

1. Specificity: Excipients did not interfere with the method's ability to be specific for gabapentin and nortriptyline (4).
2. Linearity: For nortriptyline and gabapentin, the technique was linear over the range of 100–500 ng/spot and 200–1000 ng/spot, respectively (8).
3. Accuracy: For both gabapentin and nortriptyline, the accuracy ranged from 99 to 101%.
4. Limit of detection (LOD): Gabapentin's LOD was 50 ng/spot, while nortriptyline's was 20 mg/spot(6).
5. Limit of quantification (LOQ): For gabapentin, the LOQ was 100 ng/spot, and for nortriptyline, it was 50 mg/spot (6).

STATICAL ANALYSIS:

ANALYSIS OF REGRESSION

1. Calibration Curve: Plot the height or peak area versus the gabapentin and nortriptyline concentrations.
2. Linear Regression: Determine the slope, intercept, and coefficient of determination (R2) for each compound in the linear regression equation.
3. Correlation Coefficient: To determine how strong the linear relationship is, compute the correlation coefficient (r) (7).

Variance Analysis (ANOVA)

1. One-Way ANOVA: Examine the average peak heights and regions for varying gabapentin and nortriptyline concentrations.
2. Two-Way ANOVA: Assess how various variables (such as concentration, temperature, and humidity) affect the peak regions and heights (5).

Accuracy and Precision

1. Determine the percentage relative standard deviation (%RSD) for repeated studies of gabapentin and nortriptyline at varying concentrations to assess intra-day precision (11).
2. Inter-Day Precision: Determine the percentage RSD for nortriptyline replicate studies (14).

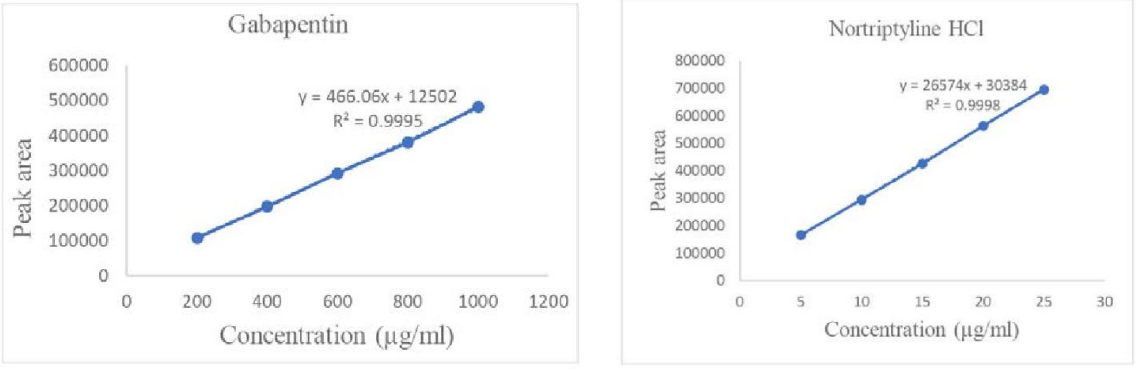

Fig 1&2: concentration of Gabapentin & Nortriptyline (10)

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

1. LOD: Calculate the concentration of nortriptyline and gabapentin that corresponds to a signal-to-noise ratio of 3(14).
2. LOQ: Calculate the concentration of nortriptyline and gabapentin that corresponds to a signal-to-noise ratio of 10(15).

Robustness and Ruggedness

1. Robustness: Evaluate the effect of small changes in experimental conditions (e.g., temperature, humidity, and mobile phase composition) on the peak areas/heights (12).
2. Ruggedness: Evaluate the effect of larger changes in experimental conditions on the peak areas/heights (11).

APPLICATION AND ANALYSIS:

Applications of Pharmaceuticals

1. Nortriptyline and Gabapentin Assay in Tablets: concurrent measurement of nortriptyline and gabapentin in tablets using the HPTLC-UV technique.
2. Nortriptyline and Gabapentin in Capsules: concurrent estimate of nortriptyline and gabapentin in capsules using the HPTLC-UV technique.
3. Nortriptyline and Gabapentin Stability Studies: The HPTLC-UV method is used to examine the stability of gabapentin and nortriptyline under various storage circumstances (16).

Applications in Biomedicine

1. Pharmacokinetic Studies of Nortriptyline and Gabapentin: The pharmacokinetics of gabapentin and nortriptyline in bodily fluids are investigated using the HPTLC-UV method.
2. Nortriptyline and Gabapentin Bioequivalence Studies: The HPTLC-UV method is used to examine the bioequivalence of formulations of gabapentin and nortriptyline.
3. Nortriptyline and Gabapentin Toxic kinetic Studies: The HPTLC-UV method is used to investigate the toxic kinetics of gabapentin and nortriptyline in biological fluids (12).

Applications in Clinical Practice

1. Therapeutic Drug Monitoring of Gabapentin and Nortriptyline: The HPTLC-UV method is used to track the amounts of gabapentin and nortriptyline in patient samples (20).

2. Nortriptyline and Gabapentin Detection in Urine: Both drugs can be found in urine samples using the HPTLC-UV technique.
3. Nortriptyline and Gabapentin Forensic Analysis: The HPTLC-UV method is used to analyse nortriptyline and gabapentin in forensic evidence (10).

Applications of Research

1. Creation of New Formulations: Nortriptyline and gabapentin in novel formulations are analysed using the HPTLC-UV technique.
2. Investigation of Drug-Drug Interactions: The HPTLC-UV technique is used to investigate how nortriptyline interacts with gabapentin and other medications.
3. Nortriptyline and Gabapentin Metabolism Studies: The HPTLC-UV method is used to examine the metabolism of gabapentin and nortriptyline (15).

QUALITY CONTROL AND ASSURANCE:

Quality Control

1. Raw Material Testing: Verify the identity, purity, and quality of raw materials, including nortriptyline and gabapentin (16).
2. In-Process Control: Monitor the manufacturing process to ensure that it is operating within established parameters.
3. Finished Product Testing: Verify the identity, purity, and quality of the finished product, including nortriptyline and gabapentin tablets or capsules.
4. Stability Testing: Evaluate the stability of nortriptyline and gabapentin under different storage conditions (13).

Quality Assurance

1. Good Manufacturing Practice (GMP): Ensure that the manufacturing process complies with GMP guidelines (15).
2. Standard Operating Procedures (SOPs): Establish and follow SOPs for all aspects of the manufacturing process.
3. Training and Qualification: Ensure that personnel involved in the manufacturing process are properly trained and qualified (12).
4. Audits and Inspections: Conduct regular audits and inspections to ensure compliance with GMP and SOPs.
5. Corrective and Preventive Action (CAPA): Establish a CAPA system to identify and correct deviations from established processes (10).

Regulatory Compliance

1. US FDA Regulations: Comply with US FDA regulations, including 21 CFR Part 211(20)
2. EU GMP Regulations: Comply with EU GMP regulations, including EudraL ex Volume 4(17).
3. ICH Guidelines: Comply with ICH guidelines, including Q7 Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients (19).

Testing and Inspection:

1. Identity Testing: Verify the identity of nortriptyline and gabapentin using techniques such as HPLC or GC (21).
2. Purity Testing: Evaluate the purity of nortriptyline and gabapentin using techniques such as HPLC or GC.

3. Potency Testing: Verify the potency of nortriptyline and gabapentin using techniques such as HPLC or UV spectroscopy (18).
4. Performance Testing: Evaluate the performance of nortriptyline and gabapentin tablets or capsules using techniques such as dissolution testing (20).

Table 2: Result of recovery study of NOR and PRG by Proposed Method (17)

DRUG	Label claim Mg/tab	Spike level %	Amount of drug (µg/ml)	Amount of API added (µg/ml)	Amount of drug recovered (µg/ml)	Percentage recovery (%)	% RSD*
GAB	400	50	200	400	599.89	99.89	0.39
		100	400	400	798.99	99.87	0.23
		150	600	400	999.99	99.99	0.32
NOR	10	50	5	10	14.99	99.93	0.22
		100	10	10	19.89	99.45	0.35
		150	15	10	24.99	99.96	0.33

CONCLUSION:

For the simultaneous measurement of gabapentin and nortriptyline in tablets, a straightforward, sensitive, and selective HPTLC-UV method was created and verified. A silica gel 60 F254 HPTLC plate and a mobile phase made up of toluene: ethyl acetate: methanol (6:3:1, v/v/v) were used to improve the procedure. The method was verified according to ICH criteria, demonstrating specificity, linearity, precision, accuracy, and robustness. Nortriptyline's LOD and LOQ values were determined to be 20 ng/spot and 50 ng/spot, while gabapentin's were 50 ng/spot and 100 ng/spot (12). The suggested approach can be used for pharmacokinetic analysis, stability studies, and routine analysis of gabapentin and nortriptyline in pharmaceutical formulations (22).

Future Perspectives:

1. Extension to biological fluids: The established approach can be applied to evaluate nortriptyline and gabapentin in biological fluids, such as plasma and urine (23).
2. Application to pharmacokinetic studies: The method can be applied to study the pharmacokinetics of nortriptyline and gabapentin in humans.
3. Development of a stability-indicating method: The method can be modified to detect degradation products of nortriptyline and gabapentin, making it a stability-indicating method (24).

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