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REVIEW ARTICLE **OPEN ACCESS**

Analytical Method Development and Validation of Gabapentin and Nortriptyline: A Review Article

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ABSTRACT
Analytical technique development and validation are essential parts for drug discovery process since they are a constant and interdependent effort involved in pharmaceutical development and production. The process of demonstrating that an analytical technique is appropriate to identifying the presence of an active pharmaceutical ingredient in a specified compounded dosage form is known as method development. It enables the use of streamlined procedures to confirm that an analysis procedure reliably & accurately delivers a measurement of an active ingredient in a compounded preparation. Effective technique development and validation can lead to considerable reductions in bias errors and gains in precision. Additionally, it might help in avoiding time-consuming and expensive exercises. Gabapentin and Nortriptyline are currently approved combination with 100 &10/200&10mg by CDSCO new combination approval list for treatment of neuropathic pain. Gabapentin individually also having anti-epileptic action. And Nortriptyline is TCA (Tricyclic amines) class of drugs in combination of both widely used for neuropathic pain. the purpose of review is to discuss determination of these both raw drug and pharmaceutical formulations alone or in combination.
Keywords: Gabapentin, Nortriptyline, HPLC, HPTLC, Lc-MS/MS, UV Spectrophotometry.

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Non-standard Abbreviations:
HPLC-High Performance Liquid Chromatography, HPTLC-High Performance Thin Layer Chromatography, UV-Ultra Violet, LC MS-Liquid Chromatography Mass Spectroscopy

INTRODUCTION

Pain which originates from neurological pathology is usually referred to as neuropathic pain. Examples of disorders that may result in neuropathic pain include with diabetes, autoimmune disorders, trauma & nerve compression. An expansion of interest has resulted from the creation of new pharmaceutical approaches as well as animal models.

Neuropathic pain is caused due to injured nerves. It differs from pain signals that come from damaged tissue (such as a fall, cut, or knee inflammation(arthritis)) and are sent along healthy nerves. Different medications than those used to treat pain from damaged tissue are utilized to treat neuropathic pain. Ibuprofen and paracetamol are not typically useful for neuropathic pain, however some persons with neuropathic pain respond very well to medications are occasionally used to treat depression & epilepsy.

Both peripheral and central sensitization pathways are reflected in neuropathic pain. In addition to the damaged axons, the undamaged nociceptors that share the wounded nerve's innervation region can also send out abnormal signals. This review focuses on how the processes behind these surprisingly prevalent illnesses are being clarified through both human studies and animal models. The fast expansion of our understanding of aberrant signaling portends significant advances in the treatment of continuously disabling diseases. **[1]**

The drug Gabapentin, IUPAC name 1- (amino methyl) cyclohexane acetic acid, is a structure of Gabapentin found to be widely used to treat neuropathic pain is related to postherpetic neuralgia (PHN), post poliomyelitis neuropathy, and reflex sympathetic dystrophy.[2]

The FDA has approved the use of Nortriptyline to treat depression & another use, it can be used o to treat disorders like myofascial pain, post-herpetic neuralgia, diabetic neuropathy and chronic pain. Official USFDA Approval [3]

MECHANISM OF ACTION: -

Gabapentin accelerates the action and release of alpha2delta-1 receptors, that decrease density of pre-synaptic voltage-gated ca+ channels and consequent release of excitatory neurotransmitters. It's likely that this inhibition also underlies gabapentin's anti-epileptic effects. [4]

Tricyclic amines (TCAs), include the antidepressant Nortriptyline. It is generally agreed that nortriptyline prevents serotonin and norepinephrine from being taken up again from presynaptic membrane(neuronal), increasing the concentration of neurotransmitters in the synapses. The presented mechanism of Nortriptyline in neuropathic pain is an increase level of noradrenaline functioning on 2-adrenoceptors expressed by non-neuronal satellite cells within the dorsal root ganglia.

This activates β2-adrenoreceptors reduce the neuropathy and used to produces TNFα, that reduce the neuropathic pain. [6]

Table 1. DRUG CHEMICAL PROFILE: -

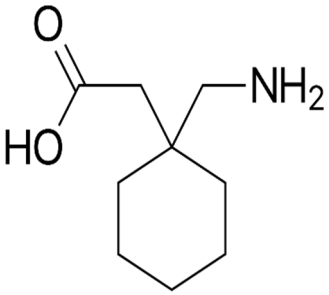
Drug	**Gabapentin**[4]	**Nortriptyline** [5]
IUPAC Name	(2- [1- (amino methyl) cyclohexyl] acetic acid)	3-(10,11-Dihydro-5H-dibenzo [a, d] cyclohepten-5-ylidene)-N-methyl-1-propanamine
Structure		
Molecular formula	C9H17NO2	C19H21N
Molecular weight	171.24g/mol	263.4 g/mol
Category	Anticonvulsants, Calcium Channel Blockers	Tricyclic antidepressants. (TCA)
Melting point	162-166˚C	>215˚C

Table 2. OFFICIAL INDIAN PHARMA COPEIA ANALYTICAL METHOD FOR GABAPENTIN: AVAILABLE METHODS FOR ASSAY OF GABAPENTIN: -

Serial No.	**Drug name**	**Analytical techniques**	**Description**	
1.	Gabapentin	LC	**Stationary phase:** 5µm Column (25 cm x 4.6mm) **Mobile phase:** Acetonitrile: buffer solution of sodium perchlorate, ammonium dihydrogen phosphate & water **Ratio:** 24:76 %v/v **Flow Rate:** 1.0 ml per min **Wavelength of detection:** 215nm. **Time of Retention:** 1.0 min	[7]
2.	Gabapentin capsules (Assay) (Dissolution)	LC	**Stationary phase:** 5µm Column (25 cm x 4.6mm) **Mobile phase:** potassium hydroxide; Buffer solution of potassium dihydrogen orthophosphate with water (6.9pH): Acetonitrile **Ratio:** 40:60%v/v **Flow Rate:** 1.2 ml per min **Wavelength of detection:** 210 nm.	[8]

3.	Gabapentin Tablets	LC	**Stationary phase:** 5µm Column (25 cm x 4.6mm) **Mobile Phase A:** 40 6.9pH buffer of 1.2g potassium dihydrogen orthophosphate with water and potassium hydroxide: Acetonitrile **Mobile Phase B:** 6.9pH buffer of 1.2g potassium dihydrogen orthophosphate with water and potassium hydroxide: Acetonitrile **Ratio:** 70:30%v/v **Flow Rate:** 1.5 ml per min **Wavelength of detection:** 210 nm	[9]

Table 3. OFFICIAL INDIAN PHARMACOPEIA ANALYTICAL METHOD FOR NORTRIPTYLINE: -

Serial No.	Drug name	Analytical technique	Description	REF. NO.
1.	Nortriptyline	TLC	**Stationary phase:** silica gel coated plate **Mobile phase:** cyclohexane**:** Diethyl amine **Ratio:** 85:15 %v/v **Wavelength of detection:** UV detection at 365 nm	[10]
2.	Nortriptyline tablets	LC	**Stationary phase:** 10µm Column **(**20 cm x 4.6 mm) **Mobile phase**: Acetonitrile:0.56 %w/v sodium hexane sulphonate with water (pH 4.5) **Ratio:** 50:50 %v/v **Flow Rate:** 2 ml per min **Wavelength of detection:** 239 nm.	[11]

Table 4. REPORTED METHODS FOR QUANTITATIVE & QUALITATIVE ANALYSIS OF GABAPENTIN AND NORTRIPTYLINE COMBINATION: -

Serial.NO	Drug name	Analytical technique	Description	REF. NO
1.	Gabapentin & Nortriptyline Bulk drug	Reverse Phase HPLC	**Stationary phase:** C18 column (5µm, 250 mm x 4.6 mm) **Mobile phase:** methanol: 0.1M ammonium acetate **Ratio:** 20:80 %v/v **Flow Rate**: 1.0 ml per min **Wavelength of detection:** 254 nm. **Time of Retention:** Gabapentin: -2.66min Nortriptyline: - 3.58 min	[12]
2.	Gabapentin and Nortriptyline Bulk drug & Tablet formulation	Reverse Phase HPLC	**Stationary phase**: C18 Column (5µm;250 × 4.60 mm) **Mobile phase:** buffer 0.2 % Triethylamine**:** Acetonitrile **Ratio:** 50:50% v/v **Flow Rate:** 1.2 ml per min **Wavelength of detection:**210 nm **Time of Retention:** Gabapentin: -1.96min Nortriptyline: - 4.54 min	[13]

Table 5. REPORTED METHODS FOR QUANTITATIVE & QUALITATIVE ANALYSIS OF GABAPENTIN

S..NO	Drug name	Analytical technique	Method Description	REF. NO
1.	Bulk & solid dosage form Gabapentin	Reverse Phase HPLC	**Stationary phase:** C18 column (5µm, 250 mm x 4.6 mm) **Mobile phase:** Acetonitrile: Water **Ratio:** 30:70 % v/v **Flow Rate**: 1.0 ml per min	[14]

			Wavelength of detection: 240 nm **Time of Retention:** 2.790 min	
2.	API & Pharmaceutical Formulations Gabapentin	UV	**Range of concentration:** 0.25 - 3.5 µ g/ml **Wavelength of detection:** 210nm	[15]
3.	Gabapentin and pregabalin drug dosage form	HPTLC	**Stationary phase:** pre-coated Silica Gel G^{60} F^{254} aluminum sheet; thickness(0.2mm) **Mobile phase:** Ethyl Acetate**:** Ammonia: Methanol **Ratio: -** (6.0: 0.1:4.0) % v/v **R_f:** 0.24 & 0.48	[16]
4.	Gabapentin and Amitriptyline hydrochloride	HPTLC	**Stationary phase:** pre-coated Silica Gel(pre-coated) G^{60} F^{254} aluminum sheet; thickness(0.2mm) **Mobile phase:** Methanol**:** Ethyl acetate**:** acetonitrile**:** ammonia **Ratio: -** 5:2:3:0.1%v/v **Range: -** **Amitriptyline**: 40-80 ng per band **Gabapentin**:1200-2400 ng per band **R_f:** **Amitriptyline:** 0.55 **Gabapentin:** 0.35	[17]
5.	Methyl cobalamin Gabapentin in bulk and tablet	UV	**Solvent: -** distilled water **Wavelength:** **Gabapentin:** 405nm **Methyl cobalamin:** 351nm **Range: -** 50-300µg/ml	[18]
6.	Gabapentin in Pure drug and its Pharmaceutical Formulations	Reverse Phase HPLC	**Stationary phase:** Phenomenex cyano column **Mobile phase:** ethanol: acetonitrile:20 mm KH2PO4 (pH 2.2) **Ratio: -** 5:5:90%(v/v/v) **Flow Rate:** 1.25 ml per min **Wavelength of detection:**210 nm **Time of Retention:** **Gabapentin: -** 1.25 min	[19]
7.	Gabapentin, Methyl cobalamin and Alpha lipoic acid by Simultaneous estimation.	UV Spectrophotometry	**Range of concentration taken:** **Alpha lipoic acid:** 100–500 µg/ml **Gabapentin:** 100–500 µg/ml **Methyl cobalamin:** 0.5–2.5 µg/ml **Wavelength of detection:** **Alpha lipoic acid:** 242.21 nm **Gaba:** 731.10 nm **Methyl cobalamin:** 768.53 nm	[20]
8.	Gabapentin Metformin as internal standard {Gabapentin after derivatization using ninhydrin solution}	HPTLC	**Stationary phase:** HPTLC F_{254} Plates (Silica gel coated) **Mobile phase**: acetic acid: water: n-butanol **Ratio**: - (2:2:5%v/v/v) **Wavelength of detection:** **1st:** 254nm **2nd:** 550nm	[21]

Table 6. REPORTED METHODS FOR QUANTITATIVE & QUALITATIVE ANALYSIS OF NORTRIPTYLINE

Serial. NO	Drug name	Analytical technique	Description	REF. NO
1.	Nortriptyline Hydrochloride in bulk and tablet dosage form	Reverse Phase HPLC	**Stationary phase:** Waters C-18 column (5µm) **Mobile phase:** acetonitrile: methanol: phosphate buffer (PH 3.0) **Ratio: -** 40: 10: 50% V/V/V **Flow Rate:** 1.0 ml per min **Wavelength of detection:**235nm **Time of Retention:** **Nortriptyline: -** 3.0 min	[22]
2.	Nortriptyline in Tablets With stability	Reverse Phase HPLC	**Stationary phase:** Waters C-18 column, (250×4.6mm;5µm) **Mobile phase:** Methanol: phosphate buffer **Ratio: -**70:30% V/V **Flow Rate:** 1.0 ml per min **Wavelength of detection:** 220nm **Time of Retention:** **Nortriptyline: -** 3.8 min	[23]
3.	Nortriptyline and pregabalin in tablet(solid) dosage form	HPTLC	**Stationary phase:** Silica Gel(pre-coated) G^{60} F^{254} Aluminum Sheet; Thickness Layer(0.2mm) **Mobile phase:** Toluene**:** Methanol**:** Ethyl acetate **Ratio:** (6: 1: 2, % v/v/v)	[24]
4.	Nortriptyline HCL and Fluphenazine HCL	Reverse Phase HPLC	**Stationary phase:** C_8 (5 µm ;250 mm × 4.6 mm) column **Mobile phase:** 0.1 M formic acid: methanol **Ratio:** 67: 33%v/v **Flow Rate:** 1.1 ml per min **Wavelength of detection:**251 nm **Time of Retention:** **Nortriptyline: -** 5.11 min **Fluphenazine:** - 8.05min	[25]
5.	Amitriptyline and its metabolite Nortriptyline (rat plasma)	HPLC–MS/ESI	**Sample preparations:** extraction(liquid–liquid) with t-butyl ether after alkalified with NaOH (0.5 mol/l). **Stationary phase:** XB-C4 (5 µm;4.6 mm × 250 mm) column **Mobile phase:** acetonitrile: ammonium acetate (0.6% formic acid) **Ratio: -** (40: 60% v/v) **Flow Rate:** 1.0 ml per min	[26]

Table 7. Reported Analytical methods for the estimation by drug:

Serial No	Drug name	Analytical technique	Description	REF. no
1.	Gabapentin and Nortriptyline Hydrochloride (derivatives)	UV	**Solvent**: - Methanol **Detection Wavelength**: Gabapentin: 335nm Nortriptyline: 241 nm	[27]

CONCLUSION

In conclusion, this review article provides a comprehensive overview of the developing and validation of analytical methods for the raw drugs & pharmaceutical dosage form Gabapentin and Nortriptyline. It emphasizes the importance of method development and validation in the pharmaceutical industry & highlights the potential benefits it can bring. The article also discusses the mechanism of action and approved uses of gabapentin and nortriptyline. It includes a summary of official Indian Pharmacopeia

analytical methods for these drugs, as well as other reported methods like HPLC/UV/HPTLC for Gabapentin individual and in combination with Nortriptyline, Pregabalin& Methyl cobalamin for their estimation. Also, for Nortriptyline Hydrochloride bulk drug and in combination with Gabapentin, Amitriptyline etc. derivatization method by UV spectrophotometry for Gabapentin and Nortriptyline pharmaceutical formulation. Overall, this article provides valuable information for research and analysis for analytical methods Gabapentin & Nortriptyline in the field of pharmaceutical analysis.

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CONFLICT OF INTEREST:
Authors listed into the article suggest no conflict of interest.

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Each author contributed in the work is mentioned.

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