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DEVELOPMENT AND VALIDATION OF HPTLC METHODS FOR SIMULTANEOUS ESTIMATION OF GABAPENTIN AND AMITRIPTYLINE HYDROCHLORIDE IN ITS MARKETED FORMULATION

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ABSTRACT

A simple, selective, linear, precise, and accurate HPTLC method was developed and validated for the simultaneous estimation of Gabapentin & Amitriptyline hydrochloride.HPTLC method was developed using stationary phase pre-coated silica gel G60 – F_{254} aluminium sheet and Methanol: acetonitrile: ethyl acetate: ammonia (5:3:2:0.1) as a mobile phase. In HPTLC method, the method was found to be linear in the range of 1200-2400 ng/band for GABA and 40-80 ng/band for AMI. R_f value obtained was 0.35 for Gabapentin and 0.55 Amitriptyline Hydrochloride. The developed method was validated as per ICH guidelines Q2 (R1). The proposed HPTLC method is accurate, precise, sensitive, selective and rapid and can be used for the routine simultaneous estimation of Gabapentin and Amitriptyline Hydrochloride in combination.

Introduction

Gabapentin (GABA) is an antilipemic agent and chemical it is 2-[1-(aminomethyl)cyclohexyl]acetic acid (MolWt- 171.23), Gabapentin increases the synaptic concentration of G,ABA, enhances GABA responses at non-synaptic sites in neuronal tissues, and reduces the release of mono-amine neurotransmitters and it is used Anti- epilepsy, Anti-anxiety Agent, Antiparkinson Agents. Amitriptyline(AMI) is an Antidepressant and chemically it is 3-(10,11-dihydro-5H-dibenzo[a.d]cyclohept-5-ylidene) propyldimethylamine hydrochloride (Mol Wt- 313.9). It inhibits the reuptake of the neurotransmitter serotonin at the neuronal membrane or acts at beta-adrenergic receptors It is used as an Antidepressant, Norepinephrine-Reuptake Inhibitors. [1,2] From the literature review it is found that there is no official HPTLC

method in Pharmacopoeia for the simultaneous estimation of Gabapentin and Amitriptyline HCl in pharmaceutical tablet dosage form. A very few analytical methods are available for estimation of Gabapentin and amitriptyline HCl alone and its combination with other drugs such as UV and HPLC.[3,17] The combination

therapy is used for Neuropathy. The combination of gabapentin and Amitriptyline HCl decreases pain more than either medication alone in the treatment of neuropathic pain from diabetic polyneuropathy or postherpetic neuralgia. Hence, here is an attempt to develop a more precise and accurate HPTLC method for the simultaneous determination Gabapentin and Amitrptyline HCl of in pharmaceutical solid dosage forms. Various validation aspects of the analysis accuracy, precision, recovery, the limits of detection and quantification have been measured as per ICH guidelines.[18]

Materials and Methods

Instrumentation

- Camag Linomat 5: Semiautomatic application, band application by spray on technique (2 - 500µl).
- Camag twin trough glass chamber (10 x 10 and 20 x 10)
- Camag TLC scanner 3: Scanning speed up to 100mm/s, Spectral range 190 – 800nm
- CamagReprostar 3 with digital camera: For 254nm, 366nm and with light.
- Camag UV cabinet with dual wavelength UV lamp: Dual wavelength 254 / 366nm
- Stationary Phase: Silica gel G60 F254 coated on aluminum sheet.
- Hamilton 100µl HPTLC syringe.
- Data Resolution: 100µm/step

Material and Reagents

Gabapentin and Amitriptyline hydrochloride were provided by Intas pharmaceutical Pvt Ltd. Ahmedabad, India. Methanol was supplied by Merck Pvt. Ltd, Mumbai. Acetonitrile, ethyl acetate, ammonia was supplied by S. D. Fine Chemicals Pvt Ltd, Mumbai. All other reagents used in this study were of AR grade and were supplied by S.D Fine Chemical Pvt. Ltd, Mumbai.

Methodology

Selection of detection wavelength the sensitivity of HPTLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected. In the present study, individual standard drug solution of Gabapentin and Amitriptyline HCl 10 µg/ml each were prepared in Methanol. Instrumentation and Chromatographic Conditions The chromatographic estimations were performed using precoated Silica Gel G60 F254 aluminium sheet (10 x 10 cm), with a thickness layer of 0.2 mm as stationary phase. The plate was pre-washed with methanol and allowed to dry in oven at 50°C for 15 minutes and allowed to come to room temperature and used immediately. Methanol: ACN: Ethyl acetate: Ammonia (5:3:2:0.1 v/v) was used as a mobile phase. Mobile phase was saturated in TLC chamber for 20 minutes before used for sample analysis at temperature of 27 ± 30 C. Five spot (5mm) were spotted at the height of 8 mm and side age 10 mm on TLC plate with 0.1µl/second speed with 5mm length. The distance between the spot were adjusted automatic by using win- CATS software. Mobile phase were run up to the distance of 80 mm approximately. The plate was scanned at 217 nm wavelength with slit dimension of 5 x 0.45 mm, scanning speed of 1 mm/second. The scanned data were analyzed by using win-CATS software.

Solvent Selection

Methanol was selected as a solvent, because both the drugs are freely soluble in Methanol.

Selection of Chromatographic Layer

Pre-coated TLC plates (Pre-coated silica gel G60 – F254 Aluminium sheet (E. Merck, Germany) (100×100 mm, thickness layer 0.2 mm); pre-washed with methanol prior to use were selected for the detection of drugs.

Optimization of Mobile phase

Mobile phase optimization was done initially by developing thin layer chromatogram of the drugs separately in various neat solvents of different polarities and then being proceed with the combination of solvents which was analyzed qualitatively by UV radiation having the wavelength of 217 nm.

Preparation of stock solution

Accurately weighed 150 mg GABA and 5 mg AMI were dissolved in methanol in two different 50 ml volumetric flask and made up the volume up to the mark with methanol to obtain stock solution of (3000 µg/ml for GABA and 100 µg/ml for AMI) of both the drugs in same volumetric flask.

Preparation of Working Standard Solution

From the above-prepared stock solution, aliquot of 1 ml was transferred into 10 ml volumetric flask and diluted upto the mark to prepare working standard solution of 300 µg/ml and 10 µg/ml.

Selection of Detection Wavelength

Sensitivity of HPTLC method depends on the selection of wavelength. From the overlay spectra as shown in, analytical wavelength was selected for simultaneous estimation of GABA and AMI.

Instrumentation and Chromatographic Condition

Samples were applied as 6 mm of the bands and 15 mm away from the left edge of the plate and 10 mm above from the bottom of the plate under the presence of nitrogen gas. Development of chromatogram was performed using optimized mobile phase in twin trough chamber which was previously saturated with the 10 ml of mobile phase for 20 min. After development, densitometric scanning was performed by winCATS planer chromatography software (CAMAG, Muttenz, Switzerland). The deuterium lamp was used as the radiation source and bends were scanned at 217 nm. The slit dimensions were 5 mm length and 0.45 mm width and scanning rate of 20 mm/s.

Preparation of Solutions for Calibration Curve

From the working standard solution aliquot of 4-8 µl were applied using Linomat V sample applicator which gave band from 1200-2400 ng/band for GABA and 40-80 ng/band for AMI. The plate was developed and scanned using optimized chromatographic conditions. For the each concentration peak area was recorded and calibration curve was plotted as concentration vs peak area.

Method Validation

The method was validated as per ICH guideline Q2 (R1) for method validation. The parameters were linearity, range, Precision, accuracy, LOD and LOQ and robustness.

Linearity and Range

Linearity was evaluated by constructing calibration curve over a range of 1200–2400 ng/band for GABA and 40-80 ng/band for AMI. The calibration curves were developed by plotting concentration vs peak area (n=5). The correlation coefficient and regression line equation was calculated.

Precision

Repeatability study was carried out by applying a 6 µl of stock solution (1800 ng/band) for GABA and (60 ng/band) for AMI on the TLC plate and injection repeatability study and scanner repeatability study were performed. In scanner repeatability, one spot was scanned six times and in injection repeatability one concentration was applied six times on the plate. The % RSD was calculated. Intra-day precision was determined using three different concentrations (lowest, medium and highest) three times on the same day and Inter-day precision using three different concentrations (lowest, medium and highest), three times on three different days . The % RSD was determined.

Accuracy (Recovery)

The recovery study was carried out by spiking standard GABA (960, 1200 & 1440 ng/band) and AMI (32, 40 & 46 ng/band) into prequantified sample solution of 1200 ng/band & 40 ng/band GABA and AMI, respectively at 80, 100 & 120% levels.

LOD and LOQ

Limit of detection was carried out to determine the lowest concentration of analyte and limit of quantification was the lowest amount of analyte that can be quantitatively determined. The LOD and LOQ were calculated by using the Equation as per ICH guidelines.

$LOD = 3.3 \times \sigma / S$

$LOQ = 10 \times \sigma / S$

Where, σ = standard deviation of y intercepts of regression line

S = slope

Robustness

Robustness was carried out by making small deliberate changes in chromatographic conditions like mobile phase composition, chamber saturation time and results were examined. The samples were applied in triplicate and % RSD was calculated.

Specificity

Specificity was carried out to determine the analyte in the presence of components which may include impurities, degradants and matrix. Specificity of GABA and AMI were analysed by using excipients like Avicel PH 101 (49 %), talc (2 %) and magnesium stearate (1.1 %) for preparation of synthetic mixture. Interference of excipients was noted.

Assay

Assay of GABA and AMI was done in a synthetic mixture. Excipients like Avicel PH 101 (49%), talc (2%) and magnesium stearate (1.1%) were used for preparation of synthetic mixture. Amount of drug recovered was calculated.

Result and Discussion

Selection of Detection Wavelength

Individual standard drug solution of Gabapentin and Pregabalin 10 µg/ml each were prepared in Acetonitrile. These standard drug solutions were scanned over wavelength of 200 to 400 nm by using UV-Visible spectrophotometer and 217 nm was selected as analytical wavelength for analysis of gabapentin and Amitriptyline HCl in pharmaceutical dosage form as depicted in Figure 3.

Optimization of Mobile Phase

Method development for separation of Gabapentin and Amitriptyline HCl in combination was started with combinations of various solvent like Chloroform, Toluene, Methanol, Ethanol, Acetone and Ethyl acetate. Eventually Methanol: ACN: ethyl acetate: Ammonia (5:3:2:0.1 v/v) gave optimum separation and resolution of Gabapentin and Pregabalin. The retention factor for Gabapentin and Amitriptyline HCl were 0.35 and 0.55 respectively. Finally Methanol: ACN: ethylacetate: Ammonia (5:3:2:0.1 v/v) gave satisfactory separation as depicted in Table 1 and Figures 4, 5, 6.

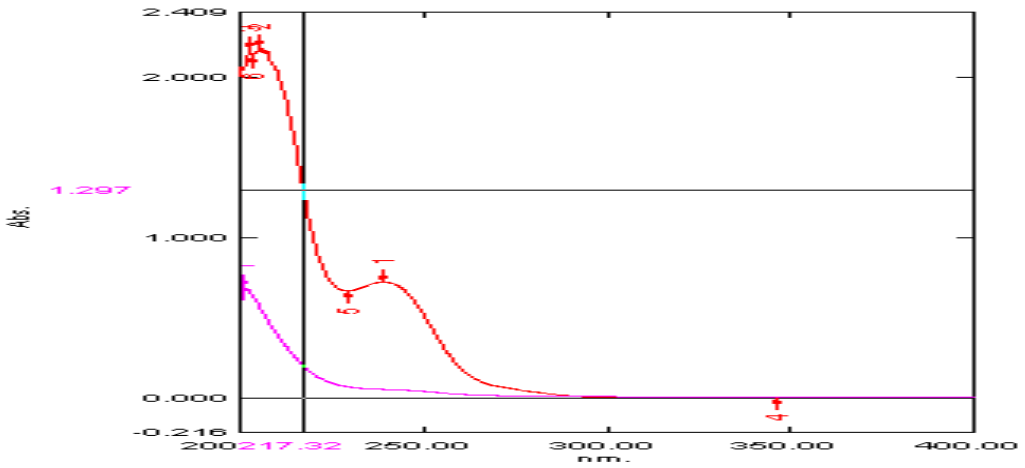

Figure1: Overlay Spectra of Gabapentin and Amitriptyline HCl (in Methanol)

Table 1: Optimization of mobile phase for separation of Gabapentin and Amitriptyline HCl

S. No.	Mobile Phase	Separation
1.	Methanol:ethyl acetate (8:2)	Gabapentin = 0.78 ,Amitriptyline Hydrochloride = 0.58 Proper separation was observed
2.	Methanol:ethyl acetate:Ammonia (7:3:0.1)	Gabapentin=0.65,Amitriptyline Hydrochloride = 0.5 Proper separation was there but tailing was observed for both drugs
3.	Methanol:ACN:Ethyl acetate:Ammonia(4:4:2:0.1)	Gabapentin=0.21,Amitriptyline Hydrochloride = 0.52 Proper separation was observed for both drugs
4.	Methanol:ACN:Ethyl acetate:Ammonia(5:3:2:0.1)	Gabapentin=0.35,Amitriptyline Hydrochloride = 0.55 Batter separation of both drugs obtained

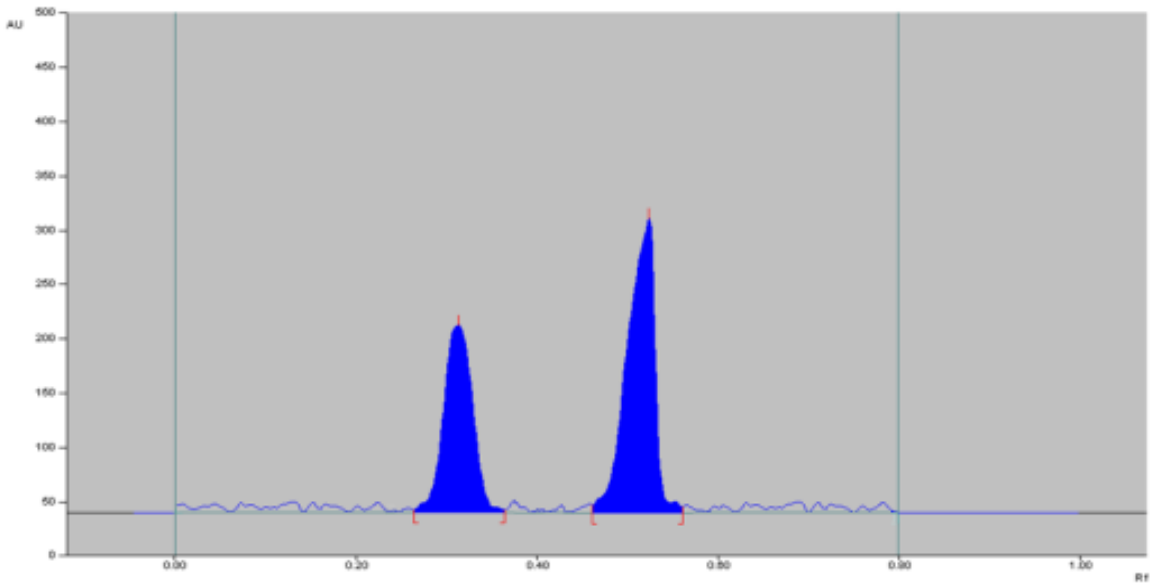

Figure 2: Chromatogram of mixed standard solution containing 1200 ng/spot of Gabapentin and 4ng/spot of Amitriptyline HCl using mobile phase as Methanol: ACN: Ethylacetate: Ammonia (5:3:2:0.1)

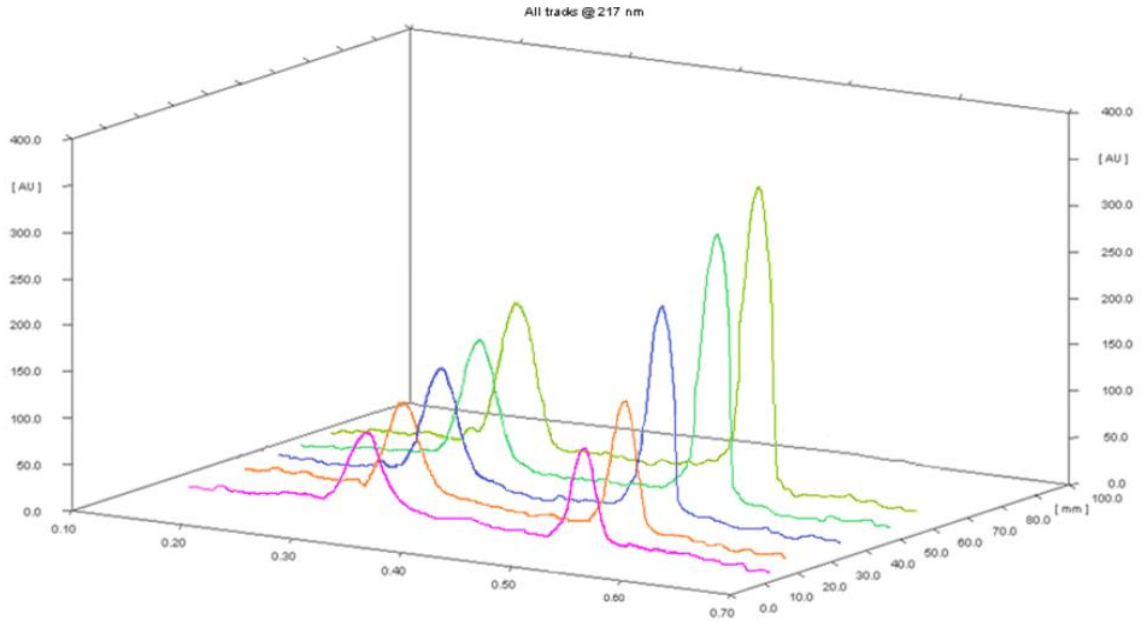

Figure 3: 3-D overlay of mixture of calibration

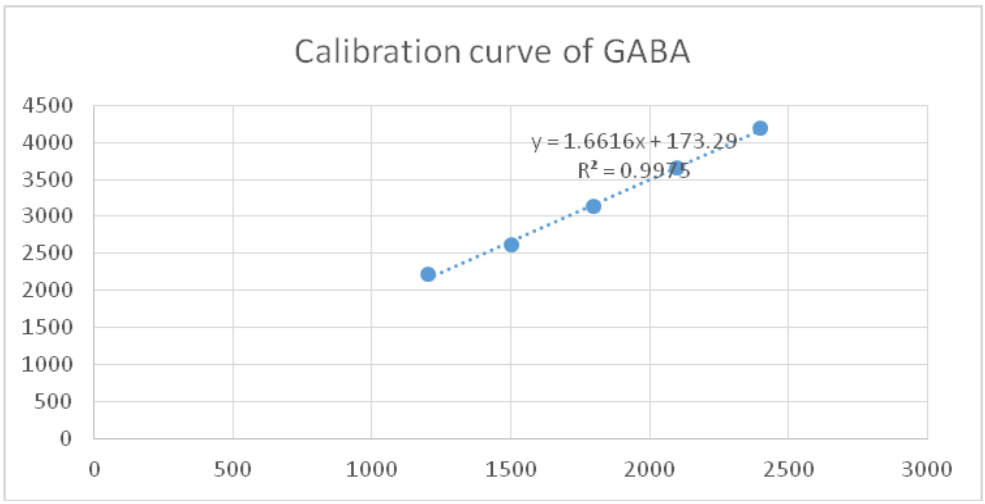

Figure 4: Calibration curve of GABA

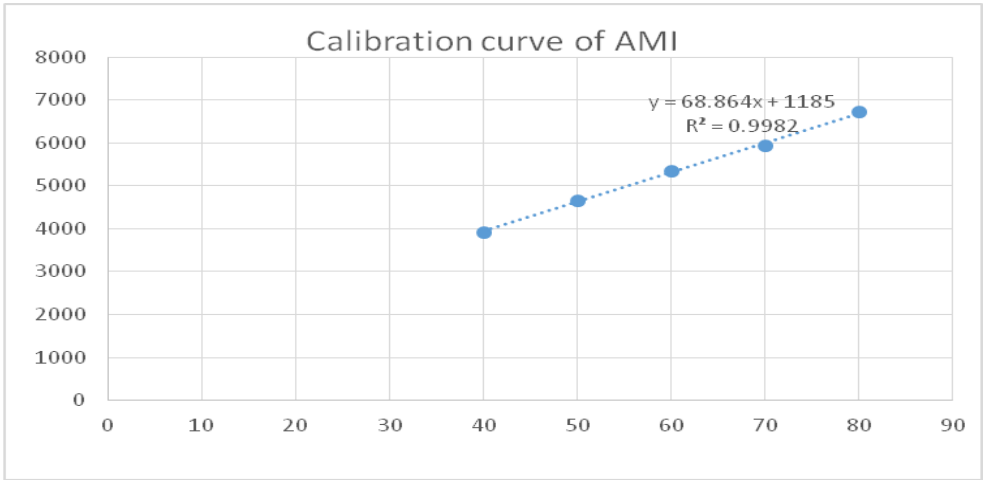

Figure 5: Calibration curve of AMI

Summary of Validation Parameters

Summary of validation parameters for simultaneous estimation of GABA and AMI in combination has been mentioned in Table 2.

Table 2: **Summary of validation parameters by HPTLC method**

Parameters		GABA	AMI
Concentration range (ng/band)		1200-2400 ng/band	40-80 ng/band
Regression equation		y = 1.6616x + 173.29	y = 68.864x + 1185
Correlation Coefficient (r^2)		0.9975	0.9982
Precision (% RSD)			
Repeatability (n=6)	Scanner	0.97	1.16
	Injection	0.62	0.79
Intra-day Precision (n=3)		1.24-1.90 %	0.89-1.22 %
Inter-day Precision (n=3)		0.38 – 0.75 %	0.76 – 0.90 %
Accuracy (% Recovery) (n=3)		98.61 - 101.30 %	98.95 - 100.81%
LOD (ng/band)		85.85	6.94
LOQ (ng/band)		258.43	21.04
Robustness		Robust	

Conclusion

The results of the analysis of pharmaceutical dosage form by the proposed method were found to be highly reproducible, reliable and precise. The percentage recoveries were found to be 98.61-101.30% for Gabapentin and 98.95-100.81 for Amitriptyline HCl indicating high degree of accuracy of the developed method. Peak purity data and standard drug peaks indicates no interference from the other peaks in chromatograms. Lower value of SD indicates that developed method is precise. The proposed HPTLC method is accurate, precise, sensitive, selective and rapid and can be used for the routine simultaneous estimation of Gabapentin and Amitriptyline hydrochloride in combination.

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