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Research Article

SIMULTANEOUS HPTLC METHOD FOR ESTIMATION OF GABAPENTIN AND PREGABALIN

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ABSTRACT

Objective: A simple, sensitive, accurate, precise HPTLC method developed for the simultaneous estimation of gabapentin and pregabalin in pharmaceutical dosage forms.

Method: The chromatographic estimations were performed using pre-coated Silica Gel G60 F_{254} aluminum sheet (10 x 10 cm), with a thickness layer of 0.2mm as stationary phase. Ethyl Acetate: Methanol: Ammonia (6.0: 4.0: 0.1 v/v) was used as a mobile phase.

Results: The method is validated for linearity, range, accuracy, precision, LOQ, system suitability etc., Linearity exhibits at a concentration ranges from 2-12 ng / ml with r^2 value 0.993 and 0.992 for gabapentin and pregabalin respectively. Limit on Quantitation values ranges from 0.4609 and 1.624 for gabapentin and pregabalin respectively. The method was found to be accurate with % recovery 99.13% - 101.83% for Gabapentin and 99.00% - 101.00% for Pregabalin. The method was found to be precise with % CV 1.08 – 2.89 for intraday (n=3) and % CV 2.54 – 3.96 for interday (n=3) for Gabapentin and % CV 1.45 – 3.16 for intraday (n=3) and % CV 1.95 – 4.84 for interday (n=3) for Pregabalin.

Conclusion: The proposed HPTLC method is accurate, precise, sensitive, selective and rapid and can be used for the routine simultaneous estimation of Gabapentin and Pregabalin in combination.

Keywords: Gabapentin, Pregabalin, HPTLC method, Validation.

INTRODUCTION

Pregabaline (PRG; (3S)-3-(aminomethyl)-5-methylhexanoic acid) is a widely used Anticonvulsant and Analgesic for Epilepsy [1]. The chemical structure of Pregabaline as shown in Figure1. Gabapentin (GBP), 1-(amino methyl) cyclohexane acetic acid, is a structural analogue of the inhibitory neurotransmitter *g*-amino butyric acid as shown in Figure 2. GBP is also used in the treatment of neuropathic pain [2, 3]. Several methods for determination of GBP in pharmaceutical dosage forms as well as in biological fluids were found in literature surveys. Literature suggested methods such as LC/MS/MS [3-7], GC/MS [8-9], HPTLC [10], HPLC[11-13], GLC. Several reports are there in literature for PRG determination based on chromatographic methods, i.e., gas chromatography-mass spectrophotometry (GC-MS), LC-MS-MS [14,15] HPLC [16] coupled with varying detection techniques like tandem mass spectrometry, fluorometry and enantiospecific analysis [17, 18]. But there was no method available for simultaneous estimation of both the drugs in combined dosage forms. Thus present study aims to estimate gabapentin and pregabalin simultaneously by using HPTLC method.

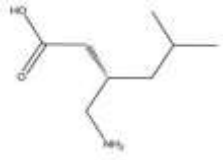

Fig.1: Chemical Structure of Pregabalin

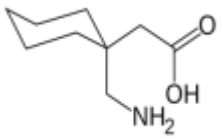

Fig.2: Chemical structure of Gabapentin

MATERIALS AND METHODS

Instrumentation

1. Camag Linomat 5: Semiautomatic application, band application by spray on technique (2 - 500µl).
2. Camag twin trough glass chamber (10 x 10 and 20 x 10)
3. Camag TLC scanner 3: Scanning speed up to 100mm/s, Spectral range 190 – 800nm
4. CamagReprostar 3 with digital camera: For 254nm, 366nm and with light.
5. Camag UV cabinet with dual wavelength UV lamp: Dual wavelength 254 / 366nm
6. Stationary Phase: Silica gel G_{60} F_{254} coated on aluminum sheet.
7. Hamilton 100µl HPTLC syringe.
8. Data Resolution: 100µm/step

Material and reagents

Gabapentin and Pregabalin were provided by Zydus Cadila Health Care, Ahmedabad, India. Blank Human Serum samples were kindly supplied form Prathama Blood Centre, Research Foundation, Vasna, Ahmedabad, India. Methanol was supplied by Merck Pvt. ltd, Mumbai. Orthophosphoric acid was supplied by S. D. Fine Chemicals Pvt Ltd, Mumbai. HPLC grade distilled water and all other reagents used in this study were of AR grade and were supplied by Merck 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 Pregabalin12 µg/ml each were prepared in Acetonitrile.

Instrumentation and Chromatographic Conditions

The chromatographic estimations were performed using precoated Silica Gel G60 F_{254} aluminium sheet (10 x 10 cm), with a thickness layer of 0.2mm as stationary phase. The plate was pre-washed with methanol and allowed to dry in oven at 50°C for 15minutes and allowed to come to room temperature and used immediately. Ethyl acetate: Methanol: Ammonia (6.0: 4.0: 0.1 v/v) was used as a mobile phase. Mobile phase was saturated in TLC chamber for 25 minutes before used for sample analysis at temperature of 27 ± 3^0 C. Eight 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 70 mm approximately. The plate was scanned at 210 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.

Optimization of mobile phase

Method development for separation of Gabapentin and Pregabalin in combination was started with combinations of various solvent like Chloroform, Toluene, Methanol, Ethanol, Acetone and Ethyl acetate. The mixed standard solution containing 6000ng/µl of Gabapentin and Pregabalin was chromatographed with mobile phase of different ratios, Ethyl acetate: Methanol: Ammonia (5.0:5.0:0.1, 5.5:4.5:0.1, 6.0:4.0:0.1 v/v) were also tried and chromatograms were recorded.

Preparation of mobile phase

AR grade solvents Ethyl acetate, Methanol and Ammonia in the ratio of (6.0:4.0:0.1 v/v) were used as mobile phase. Properly Mixed solvents were poured in TLC chamber and kept for saturation up to 30 minutes.

Preparation of Standard stock Solution of Gabapentin and Pregabalin

Accurately weighed 10 mg Gabapentin and 10 mg Pregabalin were transferred into 100 ml volumetric flask individually, dissolved in sufficient amount of acetonitrile and diluted up to mark with acetonitrile to give concentration of 1000 µg/ml of Gabapentin and 1000 µg/ml of Pregabalin. From this solution 0.2 ml was taken and further diluted to 10 ml with methanol to get 20 µg/ml of Gabapentin and 20 µg/ml of Pregabalin respectively.

Selection of analytical wavelength for measurement

After chromatographic development bands were scanned over the range of 200-400 nm. From the overlain spectra it was observed that both the drug showed considerable absorbance at 210 nm. So, 210 nm was selected as the wavelength for measurement.

Preparation of sample solution

Accurately weighed quantity equivalent to 100 mg of Gabapentin tablet powder and 100 mg of Pregabalin capsule powder were mixed well for the preparation of synthetic mixture. A quantity of powder equivalent to 20 mg of Gabapentin and 20 mg of Pregabalin was transferred to a 25 ml volumetric flask containing acetonitrile (15 ml) and sonicated for 20 minutes. After complete dissolution the volume was making up to the mark with acetonitrile. The final solution was filtered through Whatmann filter paper No. 41 and appropriate aliquotes 1.5ml was transferred to a 10 ml volumetric flask and diluted to the mark with same to obtain final working solution with Gabapentin (120 µg/ml) and Pregabalin (120 µg/ml).

Analysis of sample solution

50µl of the above prepared sample solution was applied on pre-washed TLC plate, developed under the same chromatographic conditions described in the above section using Ethyl acetate, Methanol and Ammonia in the ratio of (6.0:4.0:0.1 v/v) as mobile phase. The developed plates were dried in air and photo metrically analyzed as described above. From the peak area obtained in the chromatogram, the amounts of both the drugs were calculated.

Method Validation

Linearity and range

The calibration curve was plotted over the concentration range of 2 to 12 ng/spot for Gabapentin and Pregabalin. Accurately measured standard working solutions of Gabapentin and Pregabalin (1, 2, 8, 12, 16, 20 and 30 µl) were spotted on precoated TLC plate under nitrogen stream using Linomat V semiautomatic spotter. The plate was dried in air and developed up to 7.0 cm using mixture of Ethyl acetate: Methanol: Ammonia (6.0:4.0:0.1 v/v) as mobile phase in a Camag twin-through chamber previously saturated with the mobile phase for 30 minutes. The plate was removed from the chamber, dried in air and standard zones were scanned and quantified at 210 nm in absorbance mode with Camag TLC Scanner III using win-CATS software. The calibration curves were constructed by plotting peak areas versus concentration (ng/spot) corresponding to each spot.

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of Gabapentin and Pregabalin by the standard addition method. Known amounts of standard solutions of Gabapentin and Pregabalin (50, 100, and 150 % level) were added to previously analyzed sample solutions of bulk powder. The amount of Gabapentin and Pregabalin were analyzed by applying these values to the regression equation of the calibration curve.

Precision (% Repeatability)

The precision of the instrument was checked by repeatedly spotting and scanning (n = 6) solution of Gabapentin and Pregabalin at three concentration levels (4µg/spot, 6µg/spot and 8 µg/spot) and peak area were determined. The results were reported in term of % coefficient of variance (% CV).

Intermediate precision

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 discussed above, from stock solution. The intraday and interday precision of the proposed method was determined by analyzing the corresponding concentration 3 times on the same day and on different days over a concentration of (4µg/spot, 6µg/spot and 8 µg/spot) for both Gabapentin and Pregabalin.

Robustness

Method robustness was performed by applying small changes in the ratio of mobile phase, spotting volume and wave length. Robustness of the method was done at three different concentration levels of 4µg/spot, 6µg/spot and 8 µg/spot. The results were expressed in terms of % coefficient of variance (% CV).

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 R_f value and purity with respective Gabapentin and Pregabalin standard to evaluate specificity of the method.

RESULTS AND DISCUSSION

Selection of detection wavelength

Individual standard drug solution of Gabapentin and Pregabalin12 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 210 nm was selected as analytical wavelength for analysis of gabapentin and pregabalin in pharmaceutical dosage form as depicted in Figure 3.

Optimization of mobile phase

Method development for separation of Gabapentin and Pregabalin in combination was started with combinations of various solvent like Chloroform, Toluene, Methanol, Ethanol, Acetone and Ethyl acetate. Eventually Ethyl Acetate: Methanol: Ammonia (6.0: 4.0: 0.1 v/v) gave optimum separation and resolution of Gabapentin and Pregabalin. The retention factor for Gabapentin and Pregabalin were 0.24 and 0.48 respectively. Finally Ethyl acetate: Methanol: Ammonia (6.0:4.0:0.1 v/v) gave satisfactory separation as depicted in Table 1 and Figures 4, 5, 6.

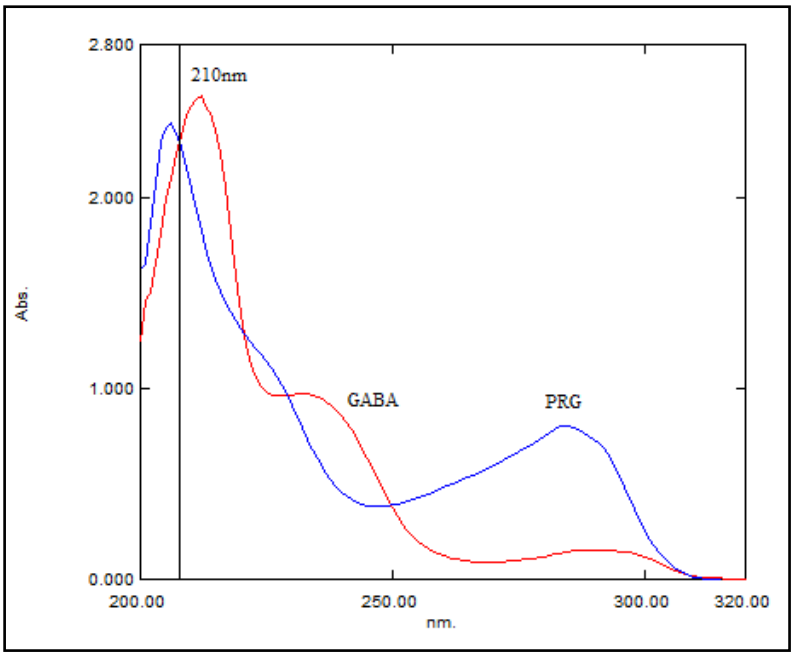

Fig. 3: Spectra of gabapentin and pregabalin in pharmaceutical dosage form corresponding at 210 nm by UV-Visible spectrophotometer

Table 1: Optimization of mobile phase for separation of Gabapentin and Pregabalin

Mobile phase	Ratio	Results
Methanol: Chloroform	6: 4	GABA spot was good but PRG not properly run and tailing observed ⇨ Replace Chloroform with more polar solvent.
Methanol: Ethyl acetate	6: 4	GABA spot was good but PRG not properly run and tailing observed ⇨ Tailing to be removed.
Methnol: ethanol: butanol	5: 3: 2	GABA spot also not good and PRG peak merging was obtained ⇨ Use of ethyl acetate gave good resolution free from merging.
Methanol: Ethyl acetate	7: 3	GABA near run to solvent front and PRG also run with good Rf ⇨ So use Ammonia
Ethyl Acetate: Methanol: Ammonia	6.0: 3.0:1.0	Optimum resolution with good peak symmetry but GABA run near to solvent front ⇨ Ratio change to decrease Ammonia content.
Ethyl Acetate: Methanol: Ammonia	6.0: 4.0: 0.1	Optimum result obtained.

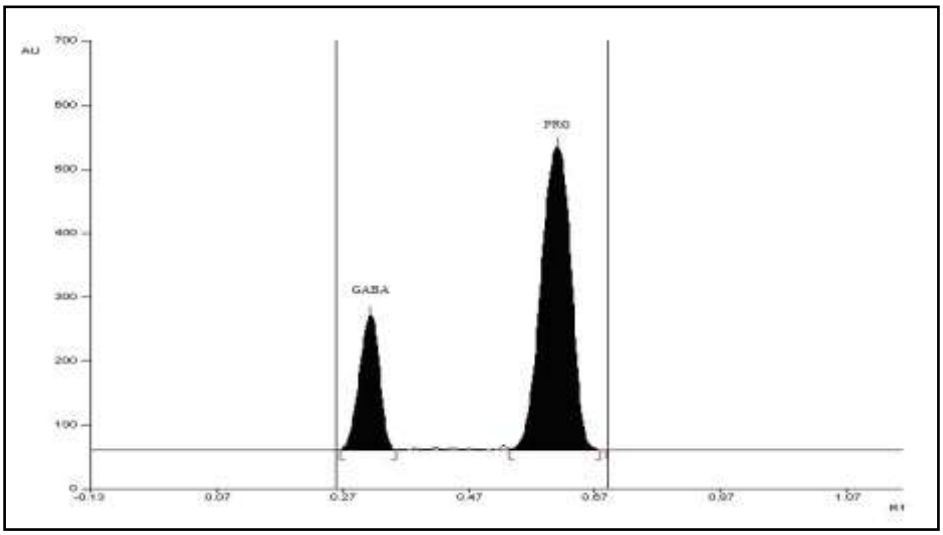

Fig. 4: Chromatogram of mixed standard solution containing 2µg/spot of Gabapentin and Pregabalin using mobile phase as Ethyl Acetate: Methanol: Ammonia (6.0:4.0:0.1 v/v)

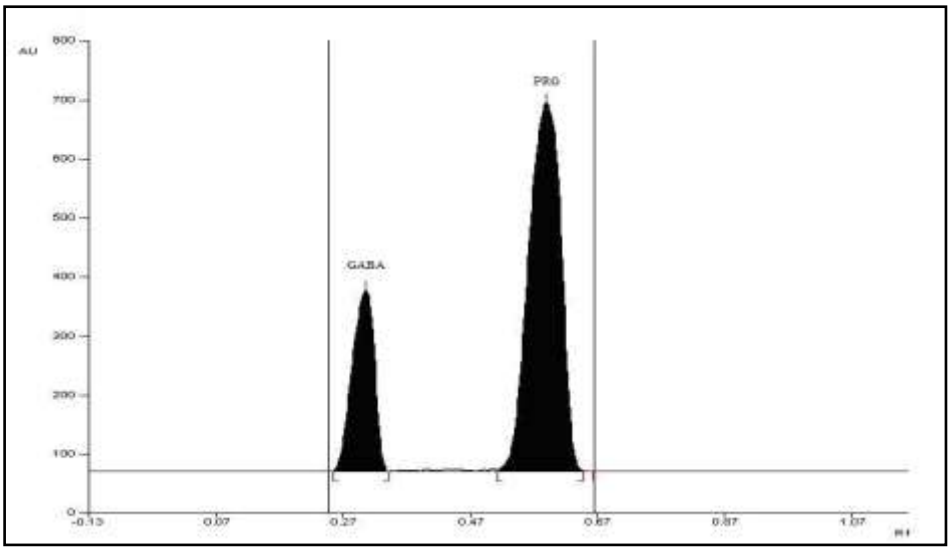

Fig. 5: Chromatogram of mixed standard solution containing 4µg/spot of Gabapentin and Pregabalin using mobile phase as Ethyl Acetate: Methanol: Ammonia (6.0:4.0:0.1 v/v)

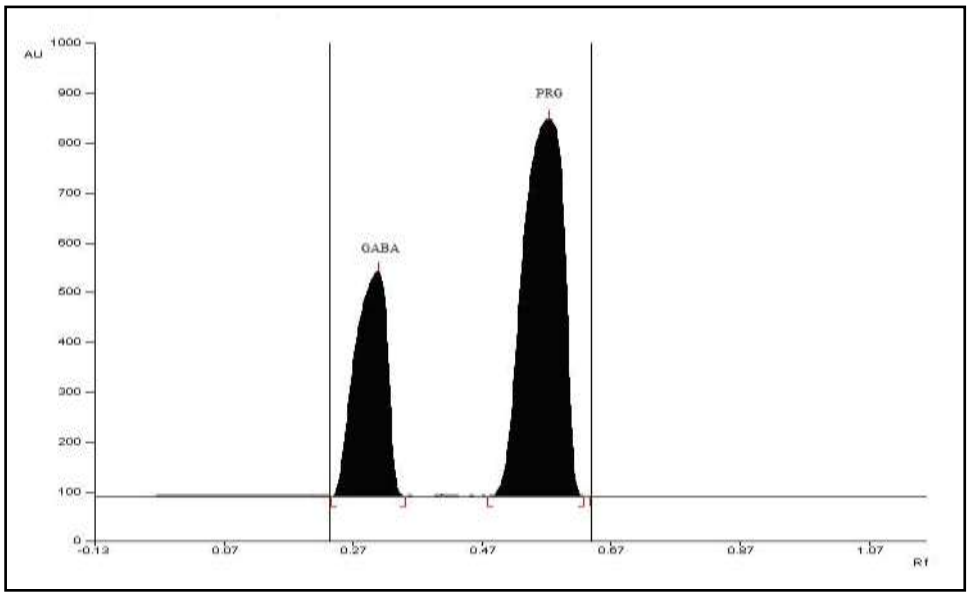

Fig. 6: Chromatogram of mixed standard solution containing 12µg/spot of Gabapentin and Pregabalin using mobile phase as Ethyl Acetate: Methanol: Ammonia (6.0:4.0:0.1 v/v)

Linearity and range

The calibration curve was plotted over the concentration range of 2 to 12 ng/spot for Gabapentin and Pregabalin. The calibration curves were constructed by plotting peak areas versus concentration (ng /spot) corresponding to each spot. Results were as shown in Table 2, 3 , 4 and Figure 7, 8.

Accuracy (% Recovery)

The accuracy of the method was determined by calculating recoveries of Ganapentin and Pregabalin by the standard addition method. The method was found to be accurate with % recovery 99.13% - 101.83% for Gabapentin and 99.00% - 101.00% for Pregabalin. Results are indicated in Table 5.

Table 2: Result of calibration Curve for Pregabalin at 210nm by HPTLC method

Concentrations (µg/spot)	Area Mean ± S.D. (n=6)	Coefficient of variation (%CV)
2	17967.13 ± 337.54	1.8786
4	22177.28 ± 425.18	1.9171
6	26952.15 ± 555.34	2.0601
8	30617.13± 524.64	1.7135
10	34524.52 ± 507.07	1.4687
12	38919.80 ± 668.20	1.7168

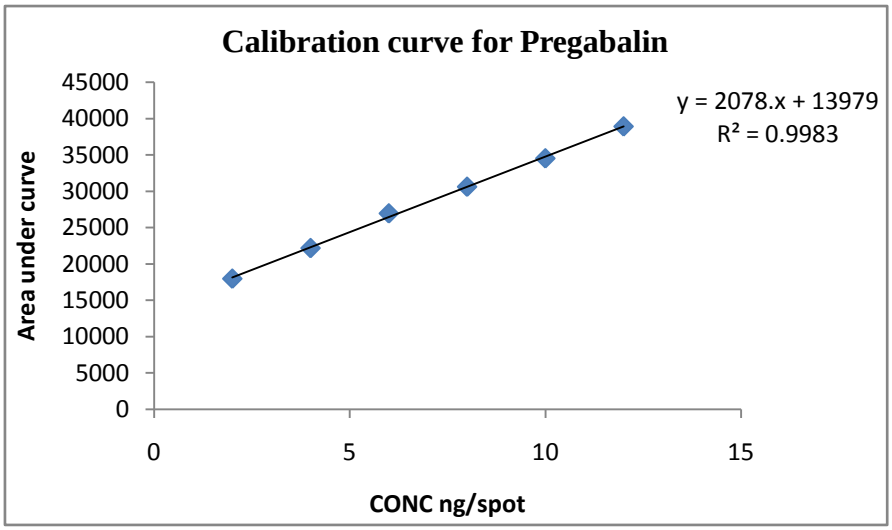

Fig.7: Calibration curve of Pregabalin

Table 3: Result of calibration curve for Gabapentin at 210nm by HPTLC

Concentrations (µg/spot)	Area Mean ± S.D. (n=6)	Coefficient of variation
2	5229.50 ± 58.59	1.1203
4	7620.28 ± 110.56	1.4508
6	10254.16 ± 217.47	2.0270
8	12648.83 ± 264.45	2.0907
10	15253.33 ± 219.76	1.4407
12	17974.76 ± 229.11	1.2746

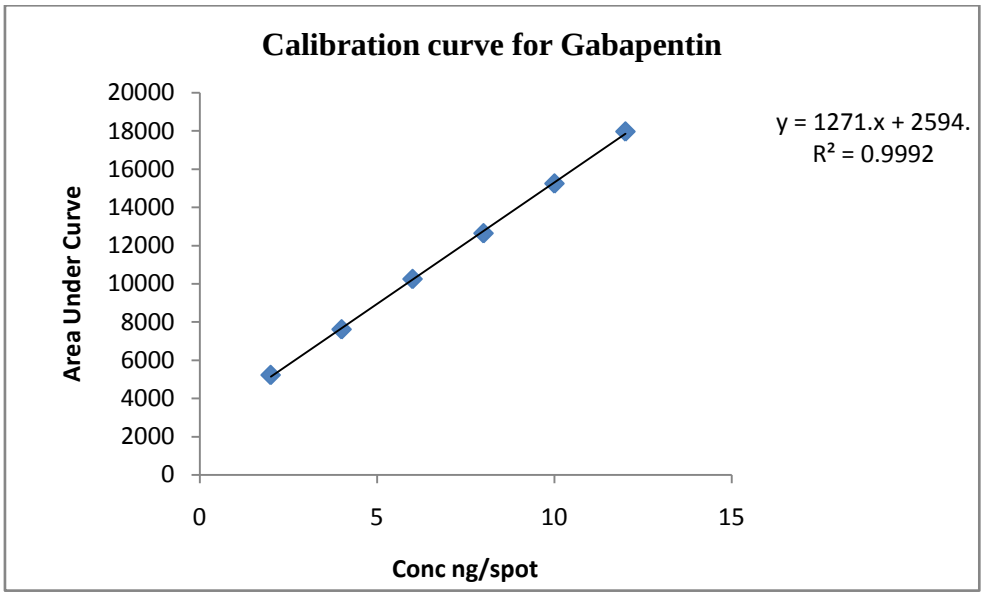

Fig. 8: Calibration curve of Gabapentin

Table 4: Statistical data for Gabapentin and Pregabalinby HPTLC method

Parameter	Gabapentin	Pregabalin
Linear Range(µg/spot)	2-12	2-12
Slope	1271	2078
Intercept	2594	13979
Limit of Detection (µg/spot)	0.1521	0.5360
Limit of Quantitation (µg/spot)	0.4609	1.6243

Table 5: Determination of Accuracy

Amt of sample		Amt. of drug added		Amt. recovered		% Recovery (n=3)	
GABA (µg/spot)	PRG (µg/spot)	GABA (µg/spot)	PRG (µg/spot)	GABA µg/spot)	PRG (µg/spot)	GABA	PRG
6	6	3	3	9.12	8.90	101.33	99.00
6	6	6	6	12.10	12.08	100.83	100.67
6	6	9	9	14.87	15.15	99.13	101.00

Precision (% Repeatability)

The precision of the instrument was checked by repeatedly spotting and scanning (n = 6) solution of Gabapentin and Pregabalin at three concentration levels (4µg/spot, 6µg/spot and 8 µg/spot) and peak area were determined. Precision was evaluated in terms of intraday and interday precision. The results were reported in terms of % coefficient of variance (% CV). The method was found to be precise with % CV 1.08 – 2.89 for intraday (n=3) and % CV 2.54 – 3.96 for interday (n=3) for Gabapentin and % CV 1.45 – 3.16 for intraday (n=3) and % CV 1.95 – 4.84 for interday (n=3) for Pregabalin as shown in Table 6 -11.

Table 6: Repeatability data for Pregabalin

Concentration	4(µg/spot)	6(µg/spot)	8(µg/spot)
Area	22789.3	26964.3	30658.9
	21703.5	25989.7	30499.6
	20704.3	27613.5	31023.5
	21839.6	26907.3	30287.3
	22184.7	27364.5	29879.6
	22842.3	26873.6	31353.9
Mean.	22011.82	26958.20	30611.2
Std. Dev.	394.78	255.34	324.64
RSD	1.7934	0.9471	1.0605

Table 7: Repeatability data for Gabapentin

Concentration	4(µg/spot)	6(µg/spot)	8(µg/spot)
Area	7650.3	10209.3	13016.6
	7531.8	10016.7	12889.6
	7489.6	10104.4	13247.1
	7697.3	10179.5	12565.2
	7783.8	10403.5	12407.8
	7568.9	10611.6	12666.7
Mean	7621.34	10431.4	12970.3
Std. Dev.	110.56	144.92	226.35
RSD	1.4507	1.3892	1.7451

Table 8: Statistical Validation Data for Intra-day Precision of Pregabalin

Pregabalin	Mean Area*	Standard Deviation*	Co-efficient of Variation*
4µg/spot	22011.82	394.78	1.7934
6µg/spot	26958.20	255.34	0.9471
8µg/spot	30611.2	324.64	1.0605

*n = 6

Table 9: Statistical Validation Data for Inter-day Precision of Pregabalin

Pregabalin	Mean Area*	Standard Deviation*	Co-efficient of Variation*
4µg/spot	21530.83	414.27	1.9240
6µg/spot	27150.47	229.14	0.8439
8µg/spot	30630.8	137.55	0.4490

*n = 3

Table 10: Statistical Validation Data for Intra-day Precision of Gabapentin

Gabapentin	Mean Area*	Standard Deviation*	Co-efficient of Variation*
4µg/spot	7621.34	110.56	1.4507
6µg/spot	10431.4	144.92	1.3892
8µg/spot	12970.3	226.35	1.7451

*n = 6

Table 11: Statistical Validation Data for Inter-day Precision of Gabapentin

Gabapentin	Mean Area*	Standard Deviation*	Co-efficient of Variation*
4µg/spot	7641.23	147.30	2.54
6µg/spot	10244.28	321.15	3.15
8µg/spot	12576.37	434.75	3.96

*n = 3

Specificity and selectivity

To assess the method specificity, chromatogram of bulk powder mixture was compared for R_f value and purity with respective Gabapentin and Pregabalin standard to evaluate specificity of the method. Results are as shown in Table 12- 14. Specificity values ranges from 99.87% and 100.46% for gabapentin and pregabalin respectively.

Analysis of sample solution

50µl of the prepared sample solution was applied on pre-washed TLC plate, developed under the same chromatographic conditions**.** From the peak area obtained in the chromatogram, the amounts of both the drugs were calculated. Results were indicated in Table 15.

Table 12: Specificity and Selectivity study

Study	Gabapentin	Pregabalin
Specificity	99.87 %	100.46 %
Selectivity	Selective	Selective

Table 13: Solvent Suitability Study

Time	Area		%	
	Pregabalin	Gabapentin	Pregabalin	Gabapentin
09.00am	26955.33	10489.23	99.42	100.51
01.00pm	26963.12	10467.75	99.61	99.78
05.00pm	26988.09	10455.11	100.3	99.68
09.00am	26933.87	10508.44	99.36	100.62

Table 14: System Suitability Test Parameters

System Suitability Parameters	Pregabalin	Gabapentin
Peak Purity	0.9998	0.9995
Rf	0.48	0.24

Table 15: Assay Results of Sample Mixture

Formulation	Actual concentration µg/spot		% Gabapentin Obtained	% Pregabalin Obtained
	Gabapentin	Pregabalin		
Synthetic Mixture	6	6	100.67	99.16

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 100.43 for Gabapentin and 100.22 for Pregabalin 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 S.D and %CV 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 Pregabalin in combination.

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