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Research Article

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DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF ACECLOFENAC AND PREGABALIN IN BULK DOSSAGE FORM

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Abstract

The present study reports simultaneous estimation of Aceclofenac and Pregabaline. A new simple, accurate, precise and selective stability-indicating high performance thin layer chromatographic (HPTLC) method has been developed and validated for the determination of Aceclofenac and Pregabaline in bulk and in formulation. Chromatographic separation was performed on aluminum plate precoated with Silica Gel 60 F254 using Toluene: Methanol: Formic acid (7: 3: 0.2 v/v/v) as the mobile phase with saturation time 20 min, followed by densitometric scanning at 210 nm. This system was found to give compact spot for Aceclofenac and Pregabaline (Rf value = 0.68 ± 0.03 and 0.27 ± 0.03 respectively) and specificity in accordance with international conference on harmonization (ICH) prescribed stress conditions. The calibration curve of Aceclofenac was found to be linear between 100-600 ng/band whereas Pregabaline was found to be linear between 75 - 450 ng/band. The % mean recovery found to be of Aceclofenac 99.67 ± 1.92 and Pregabaline is 100.80 ± 1.08. The limit of detection of Aceclofenac 19.53 ng/ band and Pregabaline 15.87 ng/band, whereas limit of Quantitation were found to be of Aceclofenac 59.17 ng/band and Pregabaline is 48.10 ng/band, respectively. The proposed method was found to be accurate, precise, reproducible, specific and sensitive and applicable for the determination of Aceclofenac and Pregabaline in bulk and in formulation. The drug was subjected to stress condition of hydrolysis (acid, base and neutral), oxidation, photolysis and thermal degradation.

Key Words: Aceclofenac, Pregabaline, Simultaneous estimation, HPTLC

INTRODUCTION

Aceclofenac is a white crystalline solid which is insoluble in water, freely soluble in alcohol. Aceclofenac is phenyl acetic acid derivative chemically 2-[2-[2-[(2,6 dichloro phenyl)amino] phenyl]acetyl] oxy acetic acid. It has anti-inflammatory and analgesic action.It blocks the action of cyclooxygenase (COX) that is involved in the production of prostaglandins (PG) which is accountable for pain, swelling, inflammation and fever.

Pregabalin is chemically (S)-3-(aminomethyl)-5-methylhexa-noic acid, is an antiepileptic and structurally related to the inhibitory neurotransmitter gamma aminobutyric acid (GABA) It was recently approved for adjunctive treatment of partial seizures in adults in United States and Europe and for the treatment of neuropathic pain from post therapeutic neuralgia and diabetic neuropathy1,2. Different methods were described for the determination of PRG in pharmaceutical formulations and biological fluids. [1]

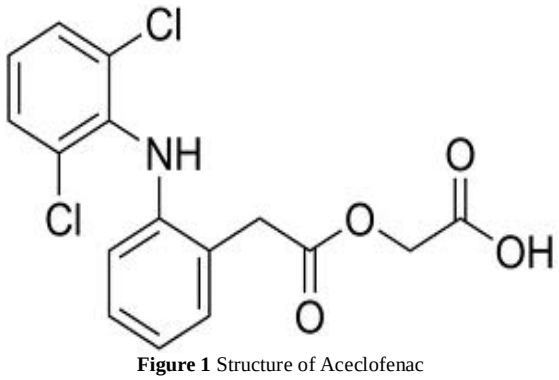

Figure 1 Structure of Aceclofenac

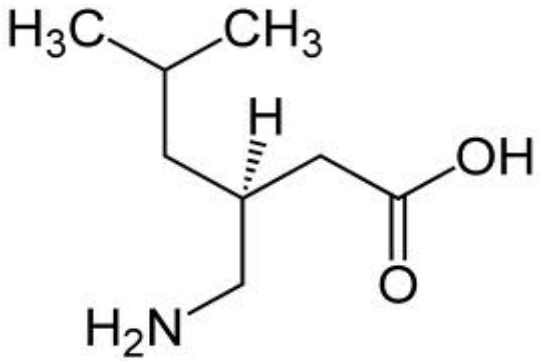

Figure 2 Structure of Pregabaline

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The literature survey reveals that several UV-VIS Spectrophotometric2,3, HPLC4-6, and HPTLC8-10 methods have been reported for the analysis of Aceclofenac and pregabaline as a single drug or in combination with other drugs in pharmaceutical dosage form available. No reports were found for stability-indicating HPTLC Simultaneous Estimation of Aceclofenac and Pregabalin in bulk and in formulation. This paper describes simple, precise, accurate and sensitive HPTLC Simultaneous method development and validation as well as stability study (hydrolysis, oxidation, photo-degradation and thermal degradation) as per international conference on harmonisation guidelines.

Experimental

Reagents and Chemicals

Authentic sample of Aceclofenac and Pregabaline were obtained from Inventia Healthecare Pvt. Ltd. East, Mumbai. Purified water prepared using Milipore Milli-Q water purification system. Methanol, Toluene, Formic acid (all AR grade) were purchased from Merck Limited (Mumbai, India).

Instruments and Chromatographic Conditions

Chromatographic separation of drug was performed on Aluminium plates precoated with silica gel 60 F254, (10 cm × 10 cm with 250 µm thickness) purchased from E-Merck, Darmstadt, Germany. Samples were applied on the plate as a band with 6 mm width using Camag 100 ☐l sample syringe (Hamilton, Switzerland) with a Linomat 5 applicator (Camag, Switzerland). Linear ascending development was carried out in a twin trough glass chamber (for 10 x 10 cm) and a densitometric scanning was performed using Camag TLC scanner 3 at 210 nm, operated by winCATS software (Version 1.4.3, Camag). Chamber saturation time was 20 min. Migration distance was 90 mm, slit dimensions were 5.00 x 0.45 mm and Deuterium lamp was used as a radiation source.

Table 1 Optimized Chromatographic Conditions

Sr. No.	Parameter	Conditions used for Analysis
1	Stationary phase	TLC aluminum plate precoated with silica gel 60 F_{254}
2.	Mobile phase	Toluene: Methanol: Formic acid (7: 3: 0.2 v/v/v)
3.	Detection Wavelength	210 nm
4.	Saturation time	20 min
5.	Solvent front	85mm
6.	Band length	6 mm
7.	Slit dimension	5 mm × 0.45 mm
8.	Source of radiation	Deuterium
9.	Rf value of Aceclofenac	0.68 ± 0.03
10.	Rf value of Pregabalin	0.27 ± 0.03

Preparation of Standard Stock Solution

Standard stock solution of ACF and PRB were prepared separately by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg/ml. From the respective standard stock solution, working standard solution was prepared containing 100 µg/ml (100 ng/µl) of ACF and 75 µg/ml (75 ng/µl) of PRB separately in methanol.

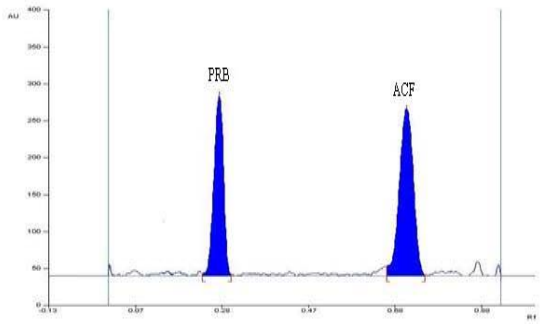

Figure 3 Standard chromatogram of ACF (200 ng/band) and PRB (150 ng/band) Recording of the chromatogram

Recording of the chromatogram

With the fixed chromatographic conditions standard solutions were applied on the plate, dried, developed, analyzed photo metrically and chromatogram were recorded. The retention factor of Aceclofenac and Pregabaline were found to be 0.68 ± 0.03 and 0.27 ± 0.03 respectively. This was done by applying sample solution from the formulation.

Analysis of Tablet Formulation

Twenty tablets each containing 100 mg of Aceclofenac and 75 mg of Pregabaline were weighed and powdered, An average weight was taken. Powder equivalent to 10 mg of Aceclofenac and 7.5 mg of Pregabaline were transferred to 10 ml volumetric flask and was diluted with methanol and volume made to 10 ml (1000 µg/ml of Aceclofenac and 750 µg/ml of Pregabaline) with methanol. Solution was filtered and further dilutions were made with mobile phase to get the final concentration of 100 µg/ml of Aceclofenac and 75 µg/ml of Pregabaline. 2 µl volume was applied on TLC plate to get concentration 200 ng/band of Aceclofenac and 150 ng/band of Pregabalin.

Validation of Method

The method was validated by establishing linearity, accuracy, intraday and interaday precision of measurement, robustness, and repeatability of sample application. The limit of detection and limit of quantification were also determined.

Table 2 Analysis of Formulation

Sr. No.	Aceclofenac			Pregabalin		
	Peak area	Amount recovered (ng/band)	% recovery	Peak area	Amount recovered (ng/band)	% recovery
1	4370.5	204.35	102.17	1945.5	152.80	101.86
2	4317.5	197.61	98.80	1919.4	148.55	99.03
3	4325.8	198.66	99.33	1928.7	150.06	100.04
4	4310.2	196.68	98.34	1935.2	151.12	100.75
5	4365.8	203.75	101.88	1942.8	152.36	101.57
6	4297.4	195.05	97.52	1942.5	152.31	101.54
Mean	4331.20	199.35	99.67	3985.50	151.20	100.80
% RSD	0.70	1.92	1.92	0.52	1.08	1.08

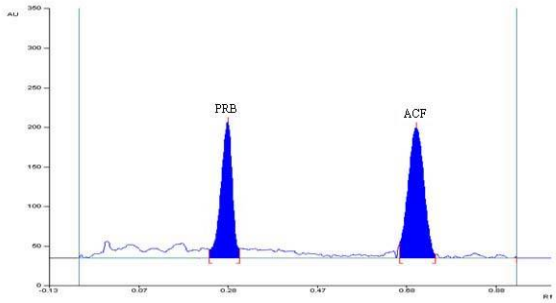

Figure 4 Chromatogram of Aceclofenac (200 ng/band) and Pregabaline (150 ng/band) in formulation

Linearity and range

The linearity of an analytical method is its ability to obtained the results which are directly proportional to the concentration of an analyte in the sample. Six replicates per concentration were applied. The linearity (relationship between peak area and concentration) was determined over the concentration range 100-600 ng/band for Aceclofenac and 75-450 ng/band for Pregabaline. The calibration curves were drawn by plotting peak area verses concentrations with the help of wincats software which are shown in the graph-I and graph-II .

Precision

Intraday Precision

Intraday precision was found by carrying out the analysis of standard drugs at three different concentrations in the linearity range of the drugs for the three times on the same day. Each concentrations were applied in a six replicates and % RSD was calculated.

Interday Precision

Interday precision was found by carrying out the analysis of standard drugs at three different concentrations in the linearity range of the drugs for the three days. Each concentrations were applied in a six replicates and % RSD was calculated.

Repeatability

Repeatability of sample application

The repeatability of measurement of peak area was assumed by spotting 150 ng/ml of drug solution, six times TLC plates followed by development of plate and recording the peak area for six spots and % RSD was calculated.

Repeatability of sample measurement

The repeatability of measurement of peak area was determined by spotting standard drug solution on TLC plate and developing the plate. The spot was scanned six times without changing the position of the peak develop and % RSD was calculated.

Accuracy

To check accuracy of the method, recovery studies were carried out by adding standard drug to sample solution at three different levels 50, 100 and 150 % and % RSD was calculated.

Limit of detection (LOD) and limit of quantification (LOQ)

The sensitivity of measurements of Aceclofenac and Pregabaline by the used of proposed method was estimated in terms of limit of quantitation (LOD) and limit of detection (LOQ). These were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot.

Standard and Sample solution stability Stability studies were also carried out by keeping the standard and sample solution prepared at room temperature for several hours and was spotted every time on fresh plate. After development and scanning plates were observed for change in peak areas and appearance of additional peak. The RSD was calculated.

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The purity of drug peak was ascertained by analyzing the spectrum at peak start, middle and peak end. The purity was determined on TLC scanner three in the range of 200-400nm by using WinCats software.

Robustness

Robustness of an analytical method is measure of its capacity to remain unaffected by small, but deliberate variations in methods parameters provides an indication

of its reliability during normal usage. Robustness of the method was determined by carrying out the analysis under conditions during which Mobile phase composition and Chamber saturation period were altered and the effects on the area were noted.

Results and Discussion

Method Development

It was observed that both drugs showed considerable absorbance at 210 nm. So 210 nm was selected for as the wavelength for detection. Method Development for resolution of Aceclofenac and Pregabalin were started with the development of densitogram with neat solvents in different ratios and combination of Toluene: Methanol: Formic acid (7: 3: 0.2 v/v/v) was selected as the mobile phase with good resolution of at Rf value = 0.68 ± 0.03 and 0.27 ± 0.03 for Aceclofenac and Pregabalin respectively.

Validation

Linearity and Range

The linear regression data showed a good relationship over a concentration range 100-600 ng/band for Aceclofenac and 75-450 ng/band for Pregabalin. The slope intercept and correlation coefficient values of Aceclofenac and Pregabalin were found to be 7.92, 2748 and 0.998 respectively and 6.05, 1021and 0.995 respectively for Pregabalin. The results are shown in Table 3

Table 3 Regression analysis of the calibration curves for Aceclofenac and Pregabalin

Parameters	Aceclofenac	Pregabalin
Linear Range (ng/band)	100-600 ng/band	75-450 ng/band
Slope	7.92	6.05
Intercept	2748	1021
Regression Coefficient (r^2)	0.998	0.995

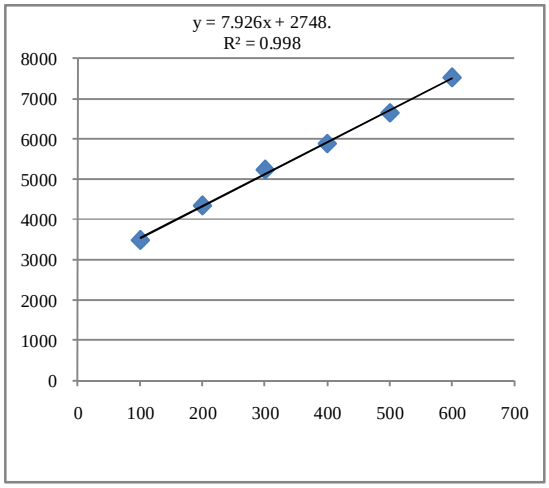

Figure 5 Calibration Curve for Aceclofenac

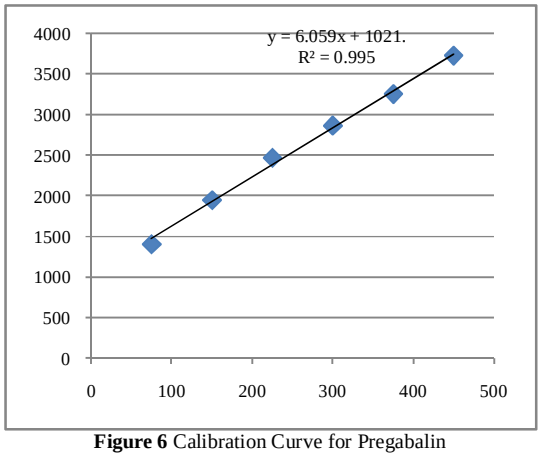

Figure 6 Calibration Curve for Pregabalin

Precision

Precision was calculated as interday and intraday variations. In the Intra-day studies, 6 replicates of ACF (200 ng/band) and 6 replicates of PRB (150 ng/band) were analyzed in a day and percentage RSD was calculated The RSD (Relative Standard Deviation) was found to be not more than 2 % for both interday and intraday precision.

Table 4 Intraday precision study of Aceclofenac

Concentration (ng/band)	Area	% Recovery	Mean % Recovery ± SD	% RSD (n = 6)
200	4358.6	101.42	100.74	
200	4321.5	99.06	±	
200	4385.24	103.11	1.52	1.51
200	4328.5	99.5		
200	4335.7	99.96		
200	4358.5	101.41		

Table 5 Intraday precision study of Pregabalin

Concentration (ng/band)	Area	% Recovery	Mean % Recovery ± SD	% RSD (n = 6)
150	1924.5	99.59		
150	1945.27	101.84		
150	1950.5	102.41	100.31	
150	1917.5	98.83	±	
150	1929.8	100.16	1.49	1.48
150	1919.5	99.05		

Table 6 Interday precision study of Aceclofenac

Concentration (ng/band)	Area	% Recovery	Avg % Recovery ± SD	Mean % Recovery ± % RSD
300	5157.3	101.49	101.24 ± 0.97	100.11 ± 1.22
	5126.23	100.17		
	5170.8	102.06		
400	5977.24	102.20	100.27 ± 1.71	
	5897.5	99.66		
	5874.7	98.94		
500	6658.23	99.09	98.81 ± 0.49	
	6625.49	98.26		
	6658.23	99.09		

Table 7 Interday precision study of Pregabaline

Concentration (ng/band)	Area	% Recovery	Avg % Recovery ± SD	Mean % Recovery ± % RSD
300	5157.3	101.49	101.24 ± 0.97	100.11 ± 1.22
	5126.23	100.17		
	5170.8	102.06		
400	5977.24	102.20	100.27 ± 1.71	
	5897.5	99.66		
	5874.7	98.94		
500	6658.23	99.09	98.81 ± 0.49	
	6625.49	98.26		
	6658.23	99.09		

The percentage recovery for Aceclofenac was found to be 100.76 ± 1.41(at 50%) 99.69 ± 0.67 (at 100%) 99.67 ± 0.80 (at 150%) for Pregabalin 98.52 ± 1.84 (at 50%) 99.15 ± 0.53 (at 100%) 99.72 ± 0.81 (at 150%)

Table 8 Recovery study of Aceclofenac

Level	Conc. (ng/band) Sample	Conc. (ng/band) Std.	Area	% Recovery	Mean % Recovery ± SD
50 %	200	100	5130.5	101.71	100.76 ± 1.41
			5128.2	101.42	
			5110.2	99.13	
100 %	200	200	5910	100.45	99.69 ± 0.67
			5889.8	99.16	
			5894.4	99.46	
150 %	200	300	6695.8	100.30	99.67 ± 0.80

Table 9 Recovery study of Pregabalin

Level	Conc. (ng/band) Sample	Conc. (ng/band) Std.	Area	% Recovery	Mean % Recovery ± SD
50 %	150	75	2398.5	100.39	98.52 ± 1.84
			2389.7	98.48	
			2381.5	96.70	
100 %	150	150	2855.4	99.74	99.15 ± 0.53
			2845.8	98.70	
			2848.8	99.02	
150 %	150	225	3314	99.64	99.72 ± 0.81
			3326.8	100.57	
			3304.5	98.96	

LOD and LOQ

Limit of Detection was found to be 19.53 ng/band and 15.87 ng/band for Aceclofenac and Pregabalin respectively. Where as Limit of Quantification was found to be 59.17 ng/ band and 48.10 ng/band for Aceclofenac and Pregabalin respectively.

Specificity

The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 0.995, indicating the non interference of any other peak of degradation product or impurity

CONCLUSION

The developed method is stability indicating and can be used for assessing the stability of drug in bulk drug and pharmaceutical dosage form. The developed method is accurate specific, selective, robust, rugged and precise.

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