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Research Article

Simultaneous Estimation of Aceclofenac and Pregabalin in Combined Dosage Form by Solubility Based Separation Method

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Abstract

Objective: A simple, rapid and extraction free spectroscopic method was developed and validated for simultaneous estimation of aceclofenac and pregabalin in tablet dosage form. The method was validated as per ICH guidelines and profitably used for the quantitative analysis of commercially available tablet.

Methodology: The both the drugs are separated based upon the solubility, in which the aceclofenac was insoluble in water and whereas pregabalin is soluble in water. The method was validated with respect to linearity, robustness, precision and accuracy and was favorably applied for the simultaneous Estimation of aceclofenac and pregabalin from the combined dosage formulation. The % amount for both the drugs was found to be within limits in the tablet dosage form for selected method.

Results and Discussion: The calibration curve was linear over all the concentration range of 10 - 50µg/ml with wavelength of 276 nm for aeclofenac and 50 - 500µg/ml for pregabalin with the wavelength of 406 nm. The LOD was found to be 0.50µg/ml for aceclofenac and 0.6µg/ml for pregabalin and LOQ was found to be 2µg/ml for aceclofenac and 4µg/ml for pregabalin indicated good sensitivity for developed method.

Conclusion: Aceclofenac and pregabalin was assayed a simple, sensitive and precise UV - Spectroscopic method. The developed method was validated according to the ICH guidelines. The method can be used for routine quality control experiments for simultaneous estimation of aceclofeanc and pregabalin in their pharmaceutical dosage form.

Keywords: Aceclofenac; Pregabalin; Solubility Separation Method; ICH Guidelines for Validation

Introduction

Aceclofenac: Aceclofenac (ACF) Chemically 2-[2-[2 -[(2,6-dichlorophenyl) amino] phenyl] acetyl] oxy acetic acid (Figure 1). It is a NSAID, used in the management of osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis [1]. The favorable mainstay of pain relief due to its synergistic effects, multiple actions, quick relief, and patient acceptance [2]. Aceclofenac relieves pain by stimulating cartilage synthesis [3].

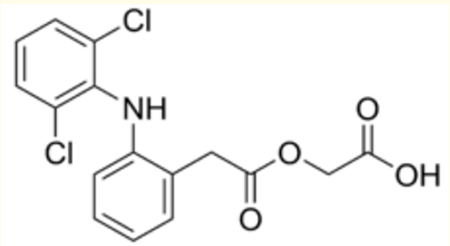

Figure 1: Chemical Structure of Aceclofenac.

Pregabalin: Pregabalin (PGB) chemically S - 3 - (amino methyl) - 5 - methylhexanoic acid (Figure 2). It is white crystalline solid, soluble in water and in both basic and acidic aqueous solutions and used as analgesic medication [4], anticonvulsant drug for neuropathic pain, adjunct for partial seizures, generalized anxiety disorders [5], antiepileptic and structurally related to the inhibitory neurotransmitter amino butyric acid (GABA) [6,7].

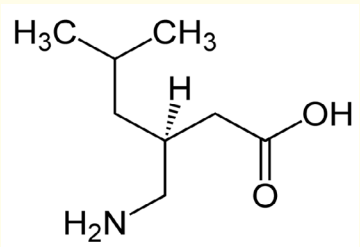

Figure 2: Chemical Structure of Pregabalin.

Use of combination of above two drugs (Zerodol - PG). Mixed pain syndrome is related with conditions like, radiculopathy, low back pain, chronic sciatica and neuropathic pain are managed by ACF and PGB respectively [8].

The various analytical and bioanalytical methods are already developed for single combination of both the drugs. Few of the combination's methods include, RP - HPLC [9-19], LC - MS [20], UV Spectroscopy [21-26], Colorimetric method [27], Bioanalytical method [29,30], HPTLC [31] etc. From the review of literature, it was found that all the methods were a bit complicated and were not cost effective methods. Most of the methods were of HPLC which required multiple steps in setting up the systems which was time consuming and also costly, but our developed method does not consume any costly solvent. As there were no simultaneous estimation of aceclofenac and pregabalin in combined (dosage form by solubility based separation method the in literature, hence we employed simultaneous estimation of aceclofenac and pregablin in combined dosage form.

Methodology

Equipment's used

A double beam UV - VIS spectrophotometer (UV - 1700, Shimadzu) with a pair of matched quartz cell of 1cm width was used for measuring absorbance. Electronic balance AW 120 (Shimadzu) was used for weighing of chemicals and ultra - so nicator instruments used for sonicating the drug and sample solution.

Reagents and Materials

Aceclofenac and Pregabalin Pure drug, received as a gift sample from Shreechem Pharmaceuticals Private Ltd, Mumbai. The commercial fixed dose combination product ZERODOL - PG consists of Aceclofenac 200 mg and pregablin 75mg was marketed by IPCA Laboratories Ltd., Mumbai. All chemicals and reagents were of analytical grade and were purchased from Merck chemicals Pvt. ltd, Mumbai, India.

Selection of solvent

ACF and PGB both are soluble in methanol. PGB soluble in water where as ACF is insoluble in water. Therefore, both the drugs are separated based upon the solubility parameter. Overlain spectrum of ACF in methanol as a solvent was recorded for concentration ranges from 10 - 50µg/ml. The overlain spectra of PGB in water and methanol (25:75 v/v) as a solvent using ninhydrin as a derivatizing reagent and recorded for the concentration ranges from 50 - 500µg/ml. Therefore, methanol was selected as solvent for ACF and water was selected as solvent for PGB.

Preparation of standard stock solution

Aceclofenac (ACF) standard stock solution (100 µg/ml)

A standard solution of ACF was prepared by dissolving 100mg of the drug in 100 ml volumetric flask using methanol and sonicated for 10 minutes. The flask was shaken, and volume was made up to the mark with methanol to give a solution containing 100µg/ml ACF.

Pregabalin (PGB) standard stock solution (100 µg/ml)

A standard solution of PGB was prepared by dissolving 100mg of the drug in 100 ml volumetric flask Using 25 ml of distilled water and sonicated for 10 minutes. The flask was shaken, and volume was made up to the mark with methanol to give a solution containing 100 µg/ml PGB.

Preparation of ninhydrin solution (derivatizing reagent)

The 0.2% solution of ninhydrin was prepared by dissolving 200mg of ninhydrin in 100ml of ethanol and was kept in an amber colored bottle.

Estimation of aceclofenac and pregabalin in their Combined Dosage

Sample preparation (formulation)

Twenty tablets were weighed and finely powered. Powder equivalent to 100 mg of PGB was accurately weighed and trans-

ferred to 100 ml volumetric flask. Approximately 25 ml of distilled water was transferred to this volumetric flask and sonicated for 10 minutes. The flask was shaken, and the solution was filtered through Whatman filter paper (0.45µ) to another 100ml volumetric flask. Filtered solution was made up to the mark by methanol which contains PGB. Later, 0.5ml of this solution was transferred to 10 ml volumetric flask. Then the volume was made up to the mark with methanol to give a solution containing 50 µg/ml (PGB). The remaining ACF residue which is insoluble in water is dissolved in methanol and make up to 100ml in volumetric flask. The solution was filtered through Whatman filter paper (0.45µ). From this solution 0.1ml was transferred to 10 ml volumetric flask and volume was made up to the mark with methanol.

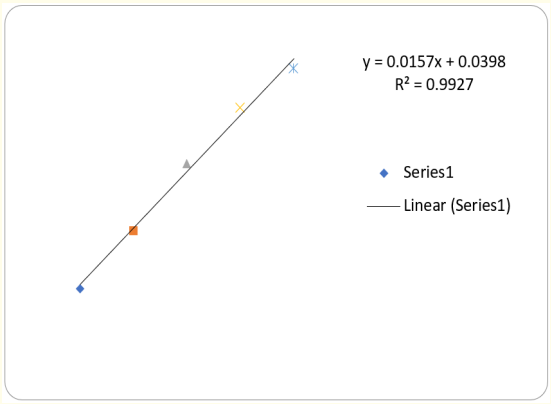

Figure 3: Calibration Curve of Aceclofenac at 276 nm.

Results and Discussion

Selection of Analytical Wavelength

10 - 50 µg/ml solutions of ACF were prepared in methanol and spectrum was recorded between 200 - 400 nm. Spectrums for above concentration were obtained. Similarly, 50 - 500 µg/ml solutions of PGB were prepared in water and methanol (25:75 v/v) and spectrum was recorded between 400 - 800 nm. ACF showed λ max at wavelength 276 nm and PGB showed λ max at wavelength at 406nm. The Wavelength, for detection of ACF was 276 and PGB was 406 selected.

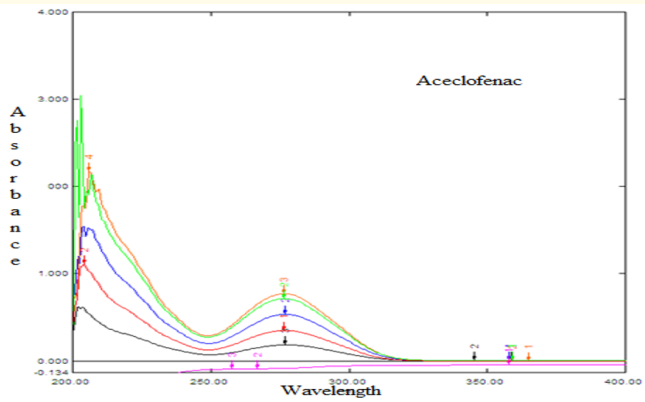

Figure 4: Overlain spectra of Aceclofenac at 276 nm.

Calibration Curve for ACF and PBG

Calibration curve for the ACF

From the ACF working standard solutions 0.1, 0.2, 0.3, 0.4, and 0.5ml were pipette out into a series of 10 mL volumetric. The volumes were made up to the mark using methanol to obtain the concentration of 10 to 50µg/mL. Absorbance of each solution was measured at 276 nm with methanol as blank. The overlain spectrum of absorbance was recorded and was shown in figure 3 and the plot of absorbance vs. concentration was plotted as shown in figure 4.

Calibration curve for the PGB

From the PGB working standard solutions, 0.5,1,2,3,4 and 5 mL were pipetted out into a series of 10 mL volumetric flask to obtain the concentration of 5 to 500 µg/ml. To these aliquots 1 ml of ninhydrin reagent (0.2% w/v) was added and heated on a water bath at a temperature of 70 - 75˚C for 20 minutes. After cooling to room temperature, the absorbance of each solution was recorded at 406 nm with methanol as blank. The overlain spectra of absorbance were recorded and were shown in figure 5 and calibration graph with straight - line equation was shown in figure 6.

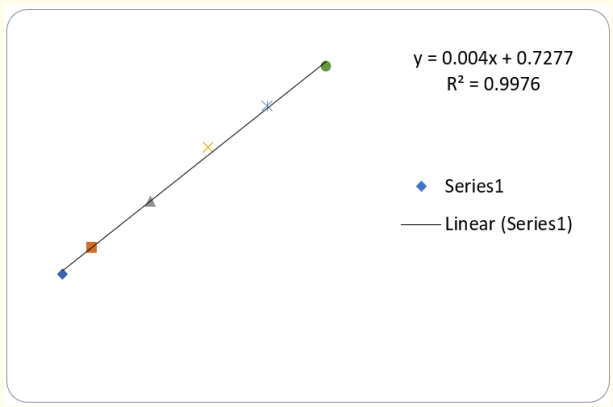

Figure 5: Calibration Curve of Pregablin at 406 nm

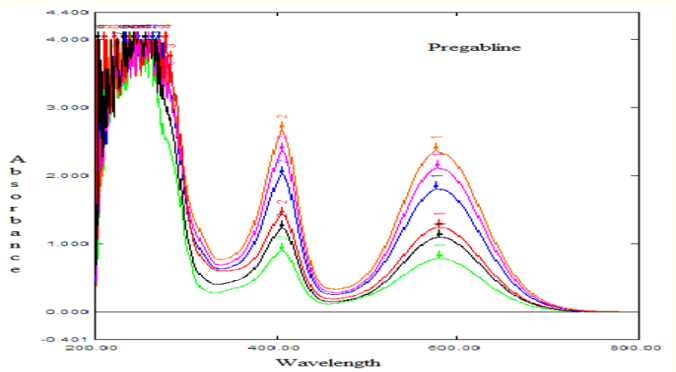

Figure 6: Overlain Spectra of Pregablin at 406 nm.

Method validation

The developed method was validated as per ICH guidelines for the consecutive parameters. Linearity and Range, Limit of detection (LOD), Limit of Quantitation (LOQ), Precision, Accuracy and Robustness, Ruggedness.

Linearity and Range

Aceclofenac

From the above ACF working standard solutions 0.1, 0.2, 0.3, 0.4, and 0.5ml were pipette out into a series of 10 mL volumetric flask. The volumes were made up to the mark using methanol to obtain the concentration of 10 to 50µg/mL. Absorbance of each solution was measured at 276 nm with methanol as blank. The optical parameters of the developed methods were shown in table 1.

Sl.no	Concentration (µg/ml)	Absorbance at 276nm
01	10	0.184
02	20	0.348
03	30	0.533
04	40	0.688
05	50	0.789

Table. 1: Linearity Study of ACF at 276 nm (λ max).

Linear regression data from the calibration curve indicates good linear relationship between concentration and absorbance at the concentration range of (10 - 50 µg/mL for ACF) respectively. The linear equation for the calibration curve was y = 0.015x - 0.039. The r2 value was 0.992 which nearly equals to unity.

Pregabalin

From the above PGB working standard solutions 0.5,1, 2, 3, 4 and 5 mL were pipetted out into a series of 10 mL volumetric flask to obtain the concentration of 5 to 500µg/ml. To these aliquots 1 ml of ninhydrin reagent (0.2%) was added and heated on a water bath at a temperature of 70 - 75˚C for 20 minutes. After cooling to room temperature, the absorbance of each solution was recorded at 406 nm with methanol as blank. The optical parameters of the developed methods were shown in table 2.

Sl.no	Concentration (µg/ml)	Absorbance in nm
1	50	0.902
2	100	1.135
3	200	1.532
4	300	1.998
5	400	2.361
6	500	2.708

Table 2: Linearity Study of PGB at 406 nm (λmax).

Linear regression data from the calibration curve indicates good linear relationship between concentration and absorbance at the concentration range of (50 - 500µg/mL for PGB) respectively. The linear equation for the calibration curve was y = 0.004x - 0.727. The r^2 value was 0.997 which nearly equals to unity.

Accuracy

Accuracy of the method was confirmed by the recovery studies in three levels that is 80, 100 and 120% were shown in table 3.

Level of recovery	Amount of formulation (µg/ml)		Amount of pure drug (µg/ml)		Total amount of drug (µg/ml)		%Recovery	
ACF PGB	ACF	PGB	ACF	PGB	ACF	PGB	ACF	PGB
80%	30	200	24	160	54	360	99.65	100.40
100%	30	200	30	200	60	400	98.31	101.78
120%	30	200	36	240	66	440	101.40	99.14

Table 3: Results of Recovery Study.

The mean % recovery found was 99.77 and 100.22 (ACF and PGB) by ZERODOL - PG while % RSD was NMT more than 2 for both the methods indicating that the developed methods were found to be sufficiently accurate.

Precession

Precision of the method was by performing, intraday precision and interlay precision. In intraday precision, six replicates of three concentrations were analyzed at short interval of time. In inter day precision, six replicates of the concentrations were analyzed at three consecutive days were shown in table 4.

	Drug	Concentration (µg/ml)	Absorbance (nm)	SD	% RSD
Intra - day (n=6)	ACF	10	0.181	0.00254	1.39911
		30	0.521	0.00611	1.17276
		50	0.774	0.00972	1.25578
	PGB	50	0.886	0.01683	1.89959
		200	1.499	0.01645	1.09768
		500	2.626	0.05229	1.99069
Inter - day (n = 6)	ACF	10	0.179	0.00264	1.47807
		30	0.517	0.00566	1.09477
		50	0.784	0.00822	1.04923
	PGB	50	0.883	0.01687	1.91007
		200	1.498	0.01737	1.15961
		500	2.625	0.05113	1.94791

Table 4: Results of Precision Studies Intra and Inter Day.

The precision is always expressed as the % RSD around mean value. Here by both the methods % RSD for Intra-Day and Inter - Day precision for the estimation (ACFandPGB) is NMT 2%. Hence the developed methods were found to be sufficiently precise.

LOD and LOQ

Limit of Detection

Limit of detection is the minimum concentration of drug that can be detected by the developed method. It is calculated by using the below formula,

$$LOD = \frac{3.3 \text{ X Standard deviation from concentration}}{\text{Slope from caliberation curve}}$$

Limit of Detection

Limit of detection is the minimum concentration of drug that can be detected by the developed method. It is calculated by using the below formula,

$$LOQ = \frac{10 \text{ X Standard Deviation from Concentration}}{\text{Slope from calibration curve}}$$

Where 'SD' is the standard deviation of Y - intercept of 3 calibration curve. Slope is the mean slope of the 3 calibration curve was shown in Table 5 and 6.

Precession

Combined standard solutions of Aceclofenac (30µg/ml) and pregabline (200µg/ml) were prepared and analysed changing wavelength by measuring the corresponding response 3 times was shown in Table 5 and 6.

Ruggedness

Combined standard solutions of Aceclofenac (30µg/ml) and pregabline (200µg/ml) were prepared and analysed changing analyte and instrument by measuring the corresponding response 3 times were shown in table 5 and 6.

Parameters	Values
Linearity range (µg/ml)	10 - 50
Regression Equation	Y = 0.015x + 0.0039
Slope	0.015
Intercept	0.0013
Correlation coefficient (R^2)	0.992
LOD (µg/ml)	0.50234
LOQ (µg/ml)	2.0678
Robustness	0.839097
Ruggedness	0.304526 0.588364
Precision: Intraday Inter day	1.275888 1.207366
Accuracy (% Recovery)	99.7915%

Table 5: Optical parameters of the developed method for aceclofenac.

Parameters	Values
Linearity range (µg/ml)	50 - 500
Regression Equation	Y = 0.004x + 0.727
Slope	0.004
Intercept	0.727
Correlation coefficient (R^2)	0.997
LOD (µg/ml)	0.6545
LOQ (µg/ml)	4.0326
Robustness	0.2834
Ruggedness	0.3891 0.3511
Precision: Intraday Inter day	1.6626 1.6725
Accuracy (% Recovery)	100.4487%

Table 6: Optical parameters of the developed method for pregabalin.

Conclusion

Aceclofenac and pregabalin was assayed a simple, sensitive and precise UV - Spectroscopic method. The developed method was validated according to the ICH guidelines. The method can be used for routine quality control experiments for simultaneous estimation of aceclofeanc and pregabalin in their pharmaceutical dosage form.

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Conflict of Interest

There is no conflict of interest among the authors.

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Volume 2 Issue 12 December 2018