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Simultaneous Determination of Aceclofenac and Pregabalin in Combined Dosage form by using RP-HPLC Method

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ABSTRACT

A simple, rapid reverse phase high performance liquid chromatographic method (RP- HPLC) has been developed and validated for simultaneous estimation of Aceclofenac and Pregabalin in tablet dosage form. Chromatographic separation was achieved C-18 (250 mm × 4.6 mm, 5.0µ) as stationary phase and mobile phase containing phosphate (pH adjusted to 5.0 ± 0.05 using NaOH.) Buffer: Acetonitrile (30:70 v/v) at flow rate of 1 ml/min using UV detection at 210 nm. The retention time for Aceclofenac and Pregabalin was found to be 3.177 and 5.530 min respectively. The method was validated as per International Conference on Harmonization guideline and successfully used for the quantitative analysis of commercially available tablet. The calibration curve was linear over the concentration range of 20-60µg/ml for Aceclofenac and 15-45µg/ml for Pregabalin.. Lower values of Limit of Detection (0.60µg/ml for Aceclofenac and 0.88µg/ml for Pregabalin) and Limit of Quantification (1.84µg/ml for Aceclofenac and 2.68µg/ml for Pregabalin) indicated good sensitivity of the method. The method was validate with respect to linearity, robustness, precision and accuracy and was successfully applied for the simultaneous determination of aceclofenac and pregabalin from the combined dosage formulation. The percent amount for both the drugs were found to be within limits in the tablet dosage form for both the methods.

Keywords: Aceclofenac, Pregabalin, RP-HPLC, validation.

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INTRODUCTION

Aceclofenac has higher anti-inflammatory action than conventional NSAIDs. It is a cytokine inhibitor. Aceclofenac works by blocking the action of a substance in the body called cyclo-oxygenase. Cyclo-oxygenase is involved in the production of prostaglandins (chemicals in the body), which cause pain, swelling and inflammation. Chemical structure is given in figure 1.

Pregabalin is a potent ligand for the alpha 2- delta subunit of voltage –gated calcium channels in the central nervous system that exhibits potent anticonvulsant ,analgesic, and anxiolytic activity in a range of animal models. In addition, pregabalin has been shown to be a highly effective adjunctive therapy for partial seizures in clinical trials, potent binding to the alpha-2 delta site reduces depolarization –induced calcium influx with a consequential modulation in excitatory neurotransmitter release. Chemical structure is given in figure 2.

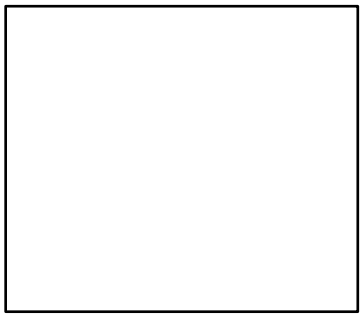

Figure 1: Structure of Aceclofenac

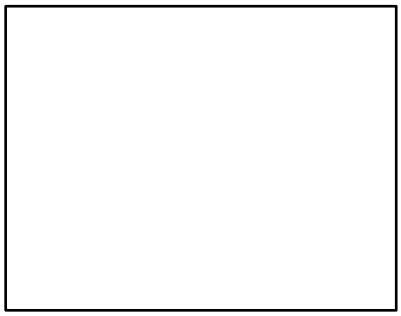

Figure 2: Structure of Pregabalin

Use of combination of above two drug (ACENAC N) .Mixed pain syndrome is associated with conditions like low back pain, sciatica, radiculopathy (cervical & lumbar) & chronic post operative pain. Both component of mixed pain syndrome nociceptive (inflammatory) & neuropathic pain are taken care by aceclofenac & pregabalin respectively

By literature survey there are some official and reported methods are available for the estimation of pregabalin and aceclofenac in the single or combination with another drugs .[6-16] but no official and reported methods are available for the simultaneous estimation of aceclofenac and pregabalin in their combination dosage form. So thought of my interest to develop and validate analytic methods for simultaneous estimation of marketed combine formulation of Aceclofenac and Pregabalin.

MATERIALS AND METHODS

Instruments and Apparatus

YL 9100 HPLC Gradient system (Young lin instruments Co. Inc., korea) was used for the RP-HPLC method. Digital pH. meter (Systronic pH 361) was used for measuring the pH.of mobile

phase. Electronic balance (Sartorius make, model – GD 603 OCE, Gottingen, Germany, 0.1mg sensitivity) was used for the accurate weighing of the chemicals. Ultrasonicator (PCI make, model- 1.5L(H)) was used for the sonication process

Chemical and materials

Aceclofenac (dishman.pharma.), Pregabalin (Gitar Laboratories ,Ahmedabad) (Pfizer), Potassium Dihydrogen Phosphate (AR Grade, SD Fine Chem Ltd. , Mumbai) ,Acetonitrile (HPLC Grade, SD Fine Chem Ltd. , Mumbai) , Triethylamine (AR Grade, SD Fine Chem Ltd. , Mumbai) ,Water (HPLC Grade, SD Fine Chem Ltd. , Mumbai), ACENAC N (Medley pharma)

Chrom tographic Condition:

Column –C18 Column(250mm × 4.6mm, 5µ) as stationary phase

Mobile Phase–Phosphate Buffer:Acetonitrile (pH5.0±0.05)(30:70%v/v)

Buffer consisted of 0.02 M Potassium Dihydrogen Phosphate, pH adjusted to 0.1N using sodium hydroxide. Buffer was prepared using HPLC Grade water.

Flow Rate – 1ml/min

Injection volume: 20 µL

Detection Wavelength: 210nm

Preparation of Mixed Stock Solutions

An accurately weighed quantity of standard aceclofenac (40 mg) and pregabalin (30 mg) were transferred to 100 ml volumetric flasks and volumes were made up to mark with mobile phase to get 400 µg/ml of Aceclofenac and 300 µg/ml of Pregabalin.

Preparation of Sample Solution

Twenty tablets were weighed accurately. Powder equivalent to 40 mg of Aceclofenac and 30 mg of Pregabalin was weighed and transferred in a 100 ml volumetric flask and mobile phase was added. This solution was sonicated for 15 minutes and final volume was made to the mark with mobile phase. The solution was filtered through Whatman filter paper No. 41. The filtrate (1ml) was transferred in a 10 ml volumetric flask and diluted to the mark with mobile phase to obtain sample solution.

RESULTS AND DISCUSSION

Linearity

The calibration curve was plotted for both the drugs, taking Concentration on X-axis and Peak Area on Y-axis chromatogram is given figure 3. For the linearity study, 0.5, 0.75, 1.0, 1.25, 1.5 ml of Aceclofenac and Pregabalin Stock solutions were mixed in five different 10 ml volumetric

flasks and volume was made up to the mark using mobile phase to obtain the final concentration 20, 30, 40, 50, 60 µg/ml of aceclofenac and 15, 22.5,30, 37.5,45 µg/ml pregabalin respectively. Linearity curve is given figure 4 & 5.

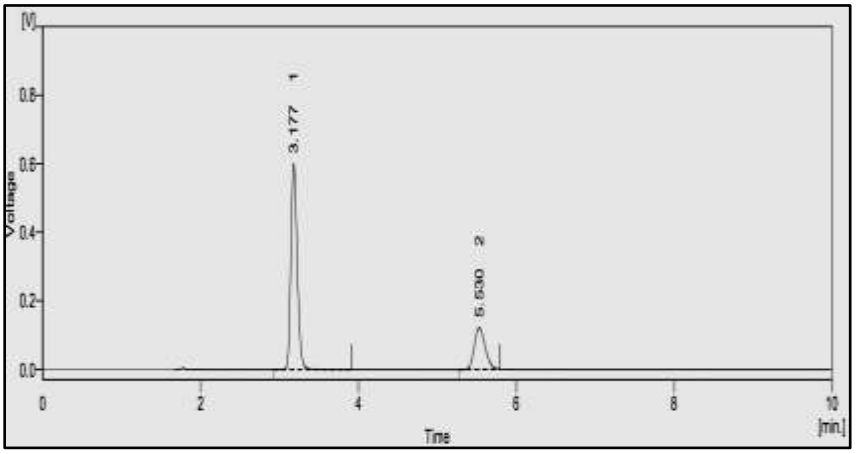

Figure 3: Chromatogram showing peaks for Aceclofenac(40 µg/ml)and Pregabalin (30µg/ml) with Buffer (0.02 M Potassium Dihydrogen Phosphate pH 5.0) and Acetonitrile as mobile phase (30:70)

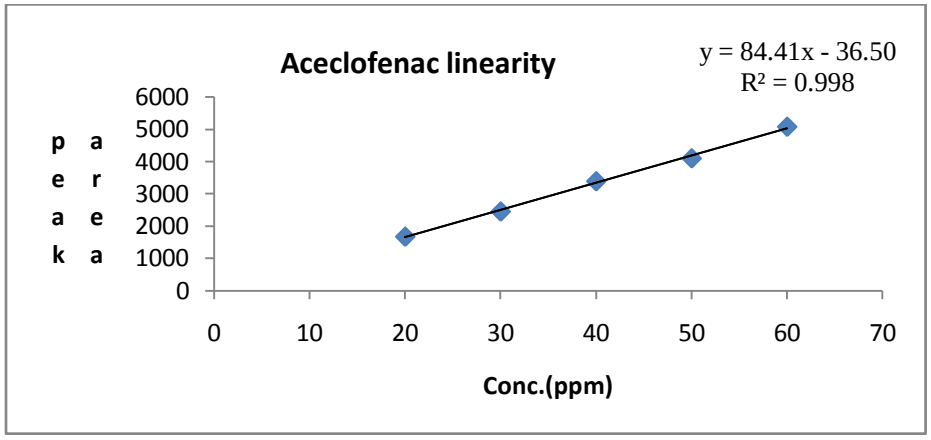

Figure 4: Calibration curve of Aceclofenac for RP-HPLC Method

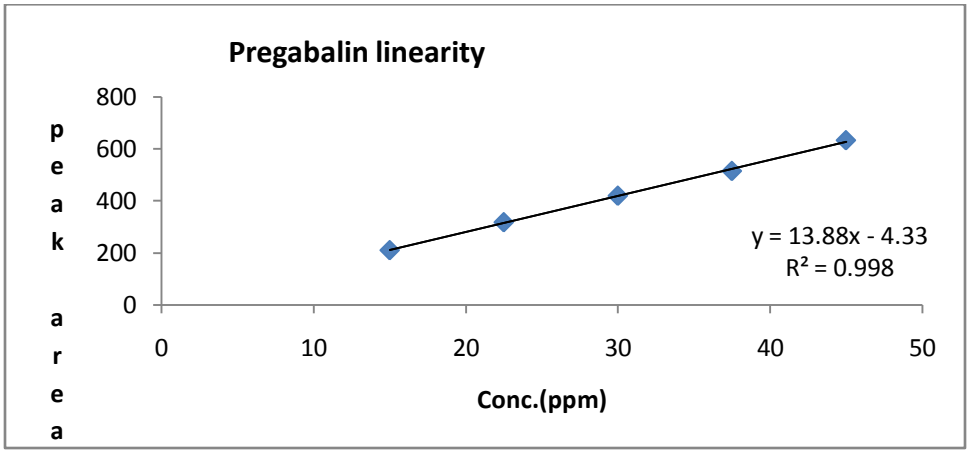

Figure 5: Calibration curve of Pregabalin for RP-HPLC

Accuracy (% Recovery)

The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

Accuracy of the method was determined in terms of % recovery by the method of standard addition. Recovery studies were carried out by the addition of drug standard solution at the level of 80%, 100% and 120% to the pre-analyzed sample (20 µg/ml of aceclofenac& 15 µg/ml of Pregabalin) accuracy data are shown in table 1.

Table 1: Result of Recovery Studies (Accuracy) for RP-HPLC method

Drug	Amount Taken (µg/ml)	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery ± SD* (n = 3)
Aceclofenac	20	16	35.75	99.33±0.83
	20	20	39.89	99.47±0.44
	20	24	43.70	99.27±0.21
Pregabalin	15	12	27.12	100.44±1.26
	15	15	29.56	98.54±1.04
	15	18	33.18	100.54±0.77

n= no. of repetition, * Standard Deviation

Limit of detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value. LOD data is Shown in table 2.

Limit of quantification (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. LOQ data is shown in table 2.

Table 2: LOD & LOQ for Aceclofenac and Pregabalin

Sr.no	Drug	LOD (µg/ml)	LOQ (µg/ml)
1	Aceclofenac	0.60	1.84
2	Pregabalin	0.88	2.68

Optical Regression Characteristics & Validation Parameter

Various compositions of mobile phase were used. The best results were obtained with 0.02 M potassium dihydrogen phosphate (pH adjusted to 5.0 ± 0.05 using sodium hydroxide),

acetonitrile in a proportion of 30:70 (v/v) at 1.0 ml/min flow rate which gave the retention times were 3,243 min for Aceclofenac and 5.127 min for Pregabalin. The optimum wavelength for detection was set at 210 nm at which much better detector responses for both drugs were obtained. The optical regression characteristics and validation parameters are shown in table 3

Table 3: Regression Analysis Data for RP-HPLC method

Parameter	Aceclofenac	Prgabalin
Detection Wavelength (nm)	210nm	
Concentration Range (µg/ml)	20 - 60ug/ml	15 – 45 ug/ml
Regression Equation (Y = mx + c)	y = 84.41x - 36.50	y = 13.88x - 4.33
Slope (m)	84.41	13.88
Intercept (c)	-36.50	-4.33
Correlation Coefficient (r)	0.998	0.998
Intraday (%RSD, n=3)	0.75 – 0.93	0.47-1.06
Interday (%RSD*, n=3)	0.52 – 0.68	0.99-1.68
Detection limit	0.60	0.88
Quantitation limit	1.84	2.68
Accuracy (n=3)	%Recovery± S.D	%Recovery± S.D
(80%)	99.33±0.83	100.44±1.26
(100%)	9947±0.44	98.54±1.04
(120%)	99.27±0.21	100.54±0.77

n= no. of repetition

***:**Relative standard deviation

Application of pharmaceutical dosage form.

Applicability of the proposed method was tested by analyzing the tablet formulation (ACENAC N). The results are shown in Table 4

Table 4:Analysis of Pharmaceutical Dosage form

Formulation (Film coated table)	Label claim (mg)	Amount found (n=3)	% Amount found ±SD *
Aceclofenac	100	99.21	98.63±0.63
Pregabalin	75	75.80	100.20±1.76

n= no. of repetition

*** Standard Deviation**

CONCLUSION

A new, accurate, precise, sensitive, specific and robust RP-HPLC method was developed which can be employed for routine simultaneous analysis of Aceclofenac and Pregabalin in combined dosage form. The method was successfully applied to the available marketed formulation without any interference due to the excipients and can have an application in the industry.

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