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Research Article

METHOD DEVELOPMENT AND VALIDATION OF ACECLOFENAC AND PREGABALIN IN MARKETED FORMULATION BY UPLC METHOD

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ABSTRACT

A Simultaneous estimation of UPLC method was established and validated for the estimation of Pregabalin and aceclofenac in tablet dosage form using Aquity UPLC HSS C18 column (100mm x 2.1mm, 1.8µ) 0.05 M phosphate buffer (pH- 6.2): Methanol : Acetonitrile (55 : 30 :15 v/v) with a flow rate of 0.3ml/min and UV detection at 218nm. Recovery was perceived 98.78% to 100.69 %.Accuracy of the method was observed to be within the limits of 98% to 102% by mean of 3 determinations. Precision of drugs was found to be less than 2.0 of %RSD from the mean of six preparations. Linearity was observed in the concentration range 100–600 µg/mL for Aceclofenac and 38–225 µg/mL for pregabalin. LOD and LOQ were found to be 9.85 and 3.03 µg/mL for Aceclofenac and Pregabalin, 29.86 µg/mL and 9.18 µg/mL for Aceclofenac and pregabalin respectively. The method was validated as per ICH guidelines.

Keywords :- Pregabalin, Aceclofenac, UPLC.

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INTRODUCTION

Pregabalin (PRG) is a novel antiepileptic drug. PRG is chemically (S) - 3- (aminomethyl) - 5- methylhexanoic acid shown in fig 1. And it is permitted in the US and Europe for adjunctive therapy of partial seizures in adults, and also has been permitted for the treatment of pain from diabetic Neuropathy or post- herpetic neuralgia in adults. Recently, it has been approved for treatment of anxiety disorders in Europe. Pregabalin is structurally associated to the antiepileptic drug Gabapentin and the site of action of both drugs is similar, the alpha2-delta protein, an ancillary subunit of voltage gated calcium channels [1].

Aceclofenac, 2-[(2, 6-dichlorophenyl) amino] phenyl] acetyl] oxyacetic acid is used as anti-inflammatory drug shown in fig 1. Aceclofenac is a non-steroidal anti-inflammatory drug (NSAID). Aceclofenac has advanced anti-inflammatory action than conventional NSAIDs. It is a cytokine inhibitor. Aceclofenac works by blocking the action of a substance in the body called cyclooxygenase. Cyclooxygenase is involved in the

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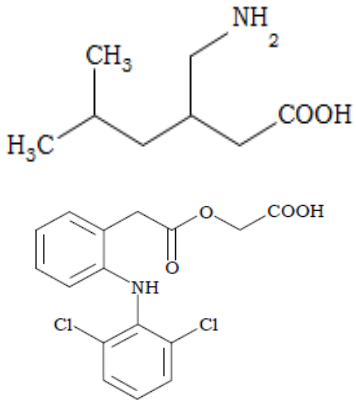
production of prostaglandin which origins pain, swelling and inflammation. Aceclofenac is the glycolic acid ester of Diclofenac [2].

Fig 1. Structure of Pregabalin and Aceclofenac



The combination of the Pregabalin and Aceclofenac is used for the relief of pain and inflammation in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. This combination is also used as an adjunctive in the treatment of partial seizures, epilepsy, fibromyalgia and neuropathic pain. The literature survey revealed that Several HPLC, UV methods were reported for estimation of aceclofenac individually and also with other combinations of drugs in different pharmaceutical dosage forms [3-6].

Many HPLC based methods have been developed for Pregabalin alone and with different formulations also reported [7-10]. Literatures shows that UPLC method for Simultaneous estimation of Aceclofenac with combinations has developed [11]. HPLC method for Simultaneous estimation of Aceclofenac with pregabalin also reported [12].

According to literature survey, there was no method reported in UPLC for the simultaneous estimation of Pregabalin and Aceclofenac. Hence the UPLC method was developed and validated as per ICH guidelines [13].

MATERIALS AND METHODS

Standards and chemicals

The standard drug Pregabalin was obtained from Cipla, Himachal Pradesh, India. Aceclofenac was obtained from Wockhardt Ltd., Aurangabad, India. Milliq water HPLC grade used was obtained from Rankem chemicals, HPLC grade methanol Merck Ltd., India, HPLC-grade acetonitrile, Merck Ltd., India. Buffering agent's potassium dihydrogen ortho phosphate, tri ethylamine was procured from Fisher scientific, Mumbai. India. Ortho phosphoric acid was obtained from SD fine, Mumbai. India.

Chromatographic conditions

Ultra performance liquid chromatography was performed on Waters Acquity UPLC system with 2996 PDA Detector and UPLC HSS C18 column (100mm x 2.1mm, 1.8µ) column with a Injection volume 2µL injected by Auto sampler, Selected wavelength 218 nm for analysis. System was equipped with Empower-3 software for data acquisition. The mobile phase components are 0.05 M phosphate buffer (pH- 6.2): Methanol : Acetonitrile (55 : 30 :15 v/v) at a flow rate of 0.3 mL/min in isocratic mode. The mobile phase was filtered through a 0.22 µm. The retention time of Aceclofenac and Pregabalin was around 2.1 and 4.2 min, respectively and the total run time was 8 min.

Diluent preparation

Mixed Water: Methanol: Acetonitrile in the ratio of 50:30:20, degassed well

Preparation of Working Standard Solution

Accurately weighed quantity of Aceclofenac WRS 20mg and Pregabalin WRS 7.6mg was transferred into 50mL volumetric flasks, dissolved with diluent and diluted up to mark with diluent to acquire strength of 400 - µg/mL of Aceclofenac and 150 - µg/mL of Pregabalin.

Method Validation

Specificity: Chromatograms of standard, sample and diluent of Aceclofenac and Pregabalin were compared.

Precision

Precision of the method was determined by performing repeatability and inter day study. In repeatability study, six individual preparations of sample analysed. In inter day precision, six individual preparations analysed on different day from repeatability study.

Accuracy: Recovery studies were performed by spiking the of standard drug solution at the level of 50%, 100%, and 150% to the sample. In this method the known concentration standard drug was added to the assay sample.

LOD and LOQ

The LOD calculated from LOD = 3.3 × N/S

The LOQ calculated from LOD = 10 × N/S

Where, N is the standard deviation of the peak area of the drug and S is the slope of the

Calibration curve obtained from linearity.

Robustness

Analyzed by changing pH of the mobile phase and flow rate by measured the corresponding responses from three individual preparations.

Assay of Pharmaceutical Formulation

Five numbers of tablets were transferred into 500mL volumetric flasks. Added 350 ml of diluent.Sonicated for 20 minutes with intermittent shaking. Introduced in mechanical shaking for 20 minute with 200 RPM. Diluted up to the volume with diluent and mixed well.

Further centrifuged at 6000 RPM for fifteen minutes. Filtered the supernatant liquid by using 0.22µ PDVF filter.Further diluted 10mL to 50mL with diluent to produce of 400 - µg/mL of Aceclofenac and 150 - µg/mL of Pregabalin.

RESULTS AND DISCUSSION

Method Development and Optimization of Chromatographic Conditions

The mobile phase components are phosphate buffer (pH-6.2): Methanol: Acetonitrile (55: 30:15 v/v) was found to be satisfactory and gave two symmetric and well-resolved peaks for Aceclofenac and Pregabalin (Figure 3). The retention time for Aceclofenac and Pregabalin was 2.12 and 4.11 min, respectively. The resolution between Aceclofenac and Pregabalin was found to be 9.779, which indicates good separation of both of the compounds. The asymmetric factors for Aceclofenac and Pregabalin were 1.02 and 1.01, respectively. The mobile phase flow rate was maintained at 0.3 mL/min. From the literature review 218.0nm was selected as a detection wavelength.

Specificity

In specificity study there was absence of any other peaks and no interference at the retention time of pregabalin and aceclofenac. The purity angle is lesser than the purity threshold shows the specificity of the method shown in Table 1.

Table 1. Specificity

Name	Results
Aceclofenac	2.2 Minutes (Retention time)
Pregabalin	4.2 Minutes (Retention time)
Blank	Absence in the RT of Aceclofenac and Pregabalin
Purity threshold (Aceclofenac)	1.678
Purity Angle (Aceclofenac)	1.256
Purity threshold (Pregabalin)	1.798
Purity Angle (Pregabalin)	1.134

Precision

Assay % RSD was found to be 1.01 and 0.86 for Aceclofenac and Pregabalin, respectively. For interday precision, Assay % RSD was found to be 0.89 and 1.11 for Aceclofenac and pregabalin, respectively shown in Table 2 and 3.

Table 2. Precision (Repeatability)

Name	% Assay Results for Aceclofenac	% Assay Results for Pregabalin
Preparation-01	99.45	100.58
Preparation-02	98.20	99.89
Preparation-03	99.87	100.3
Preparation-04	100.5	100.89
Preparation-05	98.34	100.23
Preparation-06	100.45	98.45
% RSD	1.01	0.86

Table 3. Precision (Interday)

Name	% Assay Results for Aceclofenac	% Assay Results for Pregabalin
Preparation-01	100.59	100.67
Preparation-02	100.98	100.14
Preparation-03	99.99	98.34
Preparation-04	100.34	99.76
Preparation-05	98.56	98.1
Preparation-06	99.34	98.34
% RSD	0.89	1.11

Accuracy/Recovery

In accuracy study obtained results for Aceclofenac and pregabalin lies within the the range of 98.78 – 100.32 and 99.22 – 100.69 respectively shown in Table 4.

Table 4. Accuracy

Name	% Recovery for Aceclofenac	% Recovery for Pregabalin	% Average		% RSD	
			For Aceclofenac	For Pregabalin	For Aceclofenac	For Pregabalin
Accuracy-50%-01	98.67	99.34	99.78	99.22	0.30	0.30
Accuracy-50%-02	98.56	99.44				
Accuracy-50%-03	99.12	98.89				
Accuracy-100%-01	100.34	100.99	100.32	100.69	0.22	0.33
Accuracy-100%-01	100.54	100.34				
Accuracy-100%-01	100.09	100.75				
Accuracy-150%-01	98.23	99.54	99.14	99.52	0.81	0.21
Accuracy-150%-02	99.76	99.32				
Accuracy-150%-03	99.43	99.73				

Linearity

The linearity curve for Aceclofenac and Pregabalin was found to be linear in the concentration range of 100–600 µg/mL and 38–225 µg/mL, respectively in Table 5.

Table 5. Linearity

Name	Concentration for Aceclofenac (µg/ml)	Concentration for Pregabalin (µg/ml)	Peak area	
			Aceclofenac	Pregabalin
Linearity-25%	100	38	313886	194145
Linearity-50%	200	75	647778	388289
Linearity-100%	400	150	1255555	776578
Linearity-125%	500	188	1569444	980723
Linearity-150%	600	225	1883333	1164867
Slope	3120.287006	5198.892	-	-
Intercept	10695.62791	-930.233	-	-
Steyx	9318.686	4782.072	-	-
Correlation Co efficient	0.9999	0.9998	-	-

LOD and LOQ

LOD was found to be 9.85 and 3.03 µg/mL for Aceclofenac and Pregabalin, respectively.
LOQ was found to be 29.86 µg/mL and 9.18 µg/mL for Aceclofenac and Pregabalin respectively

Robustness

In Robustness study, Variation in the pH and flow rate of the mobile phase has been changed to the analytical method to assess the ability of the method to remain unaffected by such variations. The average assay was found with in the acceptance criteria shown in Tables 6 and 7.

Table 6. Robustness (Flow rate change)

Parameter	Name	% Assay Results for Aceclofenac	% Assay Results for Pregabalin
Flow rate Plus (0.33 ml)	Preparation-01	100.01	100.67
	Preparation-02	99.75	100.14
	Preparation-03	100.45	98.34
	Average	100.07	99.72
Flow rate Minus (0.27 ml)	Preparation-01	100.34	99.76
	Preparation-02	99.89	99.26
	Preparation-03	99.34	99.12
	Average	99.86	99.38

Table 7. Robustness (pH Change)

Parameter	Name	% Assay Results for Aceclofenac	% Assay Results for Pregabalin
pH plus (pH-6.4)	Preparation-01	100.12	100.67
	Preparation-02	100.45	100.45
	Preparation-03	100.23	100.56
	Average	100.27	100.56
pH Minus (pH-6.0)	Preparation-01	99.76	99.98
	Preparation-02	99.42	99.49
	Preparation-03	99.83	100.56
	Average	99.67	100.01

Assay of Marketed Formulation

Assay for of Aceclofenac and pregabalin was found to be 100.66% and 100.48% for Aceclofenac and pregabalin respectively in Table 8.

Table 8. Assay of Marketed formulation

Name	% Assay Results for Aceclofenac	% Assay Results for Pregabalin
Preparation-01	100.43	100.98
Preparation-02	100.89	100.34
Preparation-03	100.66	100.12
Average	100.66	100.48

CONCLUSION

The proposed UPLC chromatographic method was found to be Specific, Accurate, Precise, Robust and Rugged for determination of Aceclofenac and pregabalin in combined dosage form. The proposed method is advanced and less time consumption, Injection volume, mobile phase quantity compare than previously reported chromatographic method. This method having ability to full fill the regulatory requirements as per ICH guidelines. Hence this method can adapt to routine analysis of Aceclofenac and pregabalin.

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Nil

CONFLICT OF INTEREST

No interest

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