

Research Article

Practical Implication of Chromatographic Method for Estimation of Aceclofenac and Pregabalin in Bulk and Pharmaceutical Dosage Forms

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Background. Aceclofenac and Pregabalin in combination significantly reduce pain as compared to individual drug in chronic low back pain. Literature reveals that all the reported spectrophotometric methods either need tedious extraction procedures, do not offer high sensitivity, use nonspecific reagent, or recommend the measurement of absorbance in the near UV region where interference most probably occurs that does not offer suitable linearity range. **Result.** A selective, sensitive, accurate, and precise, high performance liquid chromatographic method with UV detector analysis of Aceclofenac and Pregabalin was investigated. Good chromatographic separation was achieved using an ODS-BP hypersil C₁₈ column (250 mm × 4.6 mm, i.d., 5 μm) and a mobile phase consisting of 0.05 M phosphate buffer (KH₂PO₄) (pH 6.0) : methanol (60 : 40, v/v) at a flow rate 1 mL/min. The ultraviolet detector was set at wavelength 218 nm. Retention time for Aceclofenac and Pregabalin was found to be 3.220 and 5.910 min, respectively. Rectilinear relationship with good regression coefficients 0.999 and 0.999 was found over the concentration ranges of 5–25 μg/mL and 3.75–18.75 μg/mL for ACF and PGB, respectively, with detection limits 0.64 and 0.35 μg/mL and quantitation limits 1.95 and 1.06 μg/mL. **Conclusion.** The mean percentage recoveries were in the range of 98.45–100.08 and 99.69–100.48 for ACF and PGB, respectively. The developed method was successfully applied to the analysis of the drugs in their commercial tablets.

1. Introduction

ACF is 2-[2-[2-[(2,6-dichlorophenyl) amino] phenyl] acetyl] oxy acetic acid. It is a nonsteroidal anti-inflammatory drug with good analgesic effect [1] (Figure 1). PGB is S-3-(amino methyl)-5-methylhexanoic acid. It is an anticonvulsant drug for neuropathic pain and adjunct for partial seizures. It can be used in generalised anxiety disorders [2] (Figure 2). ACF is official in British Pharmacopoeia, 2009, Indian Pharmacopoeia, 2010, and European Pharmacopoeia, 2005 [1, 3, 4]. PGB is official in Indian Pharmacopoeia, 2010 [1]. The literature survey revealed that few analytical methods have been published concerning the simultaneous estimation of ACF and PGB either alone or in combination with other drugs, namely, spectrophotometric [5, 6] and chromatographic [7–9] methods for ACF and spectrophotometric [10], chromatographic [11], and also spectrofluorimetric [12,

13] methods for PGB. Spectrofluorimetries are not available in many labs. Regarding spectrophotometric methods for determination of PGB, some of them do not offer high sensitivity or need tedious extraction procedures. Meanwhile, some of the spectrophotometric methods recommended the measurement of absorbance in the near UV region where interference most probably occurs or use nonspecific reagent (potassium iodide/potassium iodate) that does not offer suitable linearity range. Therefore, our target was to develop a rapid, simple, efficient, and selective method for the analysis of ACF and PGB in pharmaceutical formulation.

2. Experimental

2.1. Reagents and Materials. ACF and PGB pure API were procured as a gratis sample from West Coast Pharmaceutical

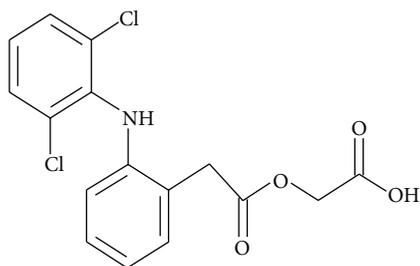


FIGURE 1: Structure of ACF.

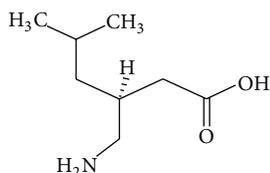


FIGURE 2: Structure of PGB.

Works, Ahmedabad. All employed chemicals were of HPLC grade.

2.2. Instruments. The instruments were HPLC (Analytical Technologies), S 1122 series pump, 2203 UV-Visible detector, and Rheodyne injector (20 μ L). Swisher electronic balance was used for weighing the samples.

2.3. Chromatographic Conditions. A hypersil C₁₈ (250 \times 4.6 mm) chromatographic column and mobile phase consisting of phosphate buffer (pH-6.0): methanol (60:40) were used. Flow rate was maintained at 1 mL/min and effluents were monitored at 218 nm. The sample was injected using 20 μ L Rheodyne injector. Freshly prepared samples were used at the time of use.

2.4. Preparation of Standard Stock Solution. Accurately weighed quantity of ACF (100 mg) and PGB (75 mg) was transferred into two separate 100 mL volumetric flasks, dissolved, and diluted up to mark with methanol to get strength of 1000 μ g/mL of ACF and 750 μ g/mL of PGB.

2.5. Preparation of Working Standard Solution. Transfer 10 mL of stock solution of ACF and PGB into two separate 100 mL volumetric flasks and dilute up to mark with methanol to get strength of 100 μ g/mL of ACF and 75 μ g/mL of PGB.

2.6. Preparation of Combined Standard Solution of Aceclofenac and Pregabalin. Accurately weighed quantities of Aceclofenac (100 mg) and Pregabalin (750 mg) were transferred into 100 mL volumetric flasks. They were dissolved and diluted up to the mark with methanol to give a combined stock solution (1000 μ g/mL) of Aceclofenac and (750 μ g/mL) of Pregabalin. Stock solution (10 mL) was transferred in 100 mL volumetric flask and diluted up to mark with methanol to obtain combined working standard solution (100 μ g/mL) of

Aceclofenac and (75 μ g/mL) of Pregabalin. This solution was used to prepare standard solution for linearity.

2.7. Calibration Curve for ACF and PGB. The combined solution of Aceclofenac and Pregabalin ranging from 5 to 25 μ g/mL and 3.75 to 18.75 μ g/mL was prepared by pipetting out 0.5, 1.0, 1.5, 2.0, and 2.5 mL of the combined working standard solution of Aceclofenac (100 μ g/mL) and Pregabalin (75 μ g/mL) into series of 10 mL volumetric flasks and the volume was adjusted to mark with mobile phase.

Chromatogram of each solution was recorded. The graph of area versus respective concentration was plotted.

3. Method Validation

3.1. Specificity. Chromatograms of standard and sample solutions of Aceclofenac and Pregabalin were compared.

3.2. Linearity and Range. The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 5–25 μ g/mL and 3.75–18.75 μ g/mL for ACF and PGB, respectively.

3.3. Accuracy. Accuracy of the method was determined in terms of % recovery of standard. Recovery studies were carried out by addition of standard drug solution at the level of 50%, 100%, and 150% to the preanalyzed sample. In this method the known concentration standard drug was added to the assay sample.

3.4. Precision. Precision of the method was determined by performing repeatability, intraday precision, and interday precision. In repeatability study, one concentration of both drugs was analysed six times. In intraday precision, three replicates of three concentrations were analyzed at short interval of time. In interday precision, three replicates of three concentrations were analyzed at three consecutive days.

3.5. LOD and LOQ. The LOD and LOQ may be calculated as

$$\text{LOD} = 3.3 \times \left(\frac{\text{SD}}{\text{Slope}} \right),$$

$$\text{LOQ} = 10 \times \left(\frac{\text{SD}}{\text{Slope}} \right),$$
(1)

where SD is the standard deviation of Y-intercept of 5 calibration curves. Slope is the mean slope of the 5 calibration curves.

3.6. Robustness. Combined standard solutions of Aceclofenac (15 μ g/mL) and Pregabalin (7.5 μ g/mL) were prepared and analyzed changing mobile phase, flow rate, and pH by measuring the corresponding responses 3 times.

3.7. Assay of Pharmaceutical Formulation. Twenty tablets were weighed and powdered. The tablet powder equivalent to 100 mg of Aceclofenac or 75 mg of Pregabalin was transferred to a 100 mL volumetric flask, dissolved, and diluted

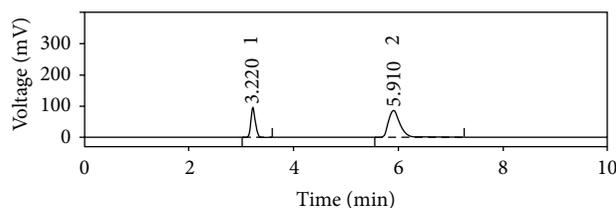


FIGURE 3: Chromatogram of Aceclofenac (15 $\mu\text{g}/\text{mL}$) and Pregabalin (11.25 $\mu\text{g}/\text{mL}$) in buffer (pH-6.0) : methanol (60 : 40).

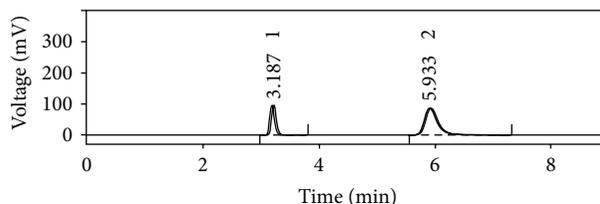


FIGURE 4: Overlain chromatograms of standard and sample solutions of Aceclofenac and Pregabalin.

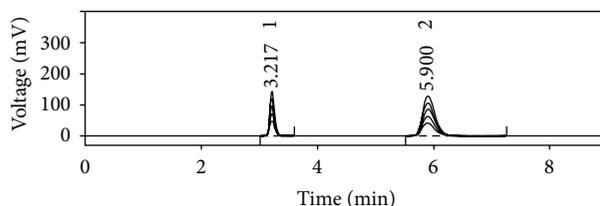


FIGURE 5: Overlain chromatogram of Aceclofenac (5–25 $\mu\text{g}/\text{mL}$) and Pregabalin (3.75–18.25 $\mu\text{g}/\text{mL}$).

up to mark with methanol to get strength of 1000 $\mu\text{g}/\text{mL}$ Aceclofenac or 750 $\mu\text{g}/\text{mL}$ Pregabalin (stock solution). The solution was filtered through Whatman filter paper number 41 and first few mL of filtrate was discarded. From stock solution, 10 mL solution was transferred to 100 mL volumetric flask and volume is adjusted to the mark with methanol to get strength of 100 $\mu\text{g}/\text{mL}$ Aceclofenac and 75 $\mu\text{g}/\text{mL}$ Pregabalin (working solution). From working solution, 1.0 mL solution was transferred to 10 mL volumetric flask and volume is adjusted to the mark with mobile phase to get strength of 10 $\mu\text{g}/\text{mL}$ Aceclofenac and 7.5 $\mu\text{g}/\text{mL}$ Pregabalin. Chromatogram of this solution was taken and the concentration of each drug was calculated using regression equation.

4. Result and Discussion

4.1. Method Development and Optimization of Chromatographic Conditions. The mobile phase phosphate buffer (pH-6.0) : methanol (60 : 40 v/v) was found to be satisfactory and gave two symmetric and well-resolved peaks for ACF and PGB (Figure 3). The retention time for ACF and PGB was 3.220 and 5.910 min, respectively. The resolution between ACF and PGB was found to be 9.779, which indicates good separation of both of the compounds. The asymmetric factors for ACF and PGB were 1.333 and 1.569, respectively. The mobile phase flow rate was maintained at 1 mL/min. From the literature review 218.0 nm was selected as a detection wavelength.

5. Method Validation

5.1. Specificity. Chromatograms of standard and sample solutions of Aceclofenac and Pregabalin were compared (Figure 4).

5.2. Linearity and Range. The calibration curve for ACF and PGB was found to be linear in the concentration range of 5–25 $\mu\text{g}/\text{mL}$ and 3.75–18.75 $\mu\text{g}/\text{mL}$, respectively (Figure 5).

5.3. Accuracy (Standard Addition Method). Result obtained reveals that % recovery of ACF and PGB was found to be 98.45–100.08 and 99.69–100.48, respectively (Table 1).

5.4. Precision. For repeatability, % CV was found to be 1.32 and 1.55 for ACF and PGB, respectively. For intraday precision, % CV was found to be 0.91–1.29 and 0.72–1.07 for ACF and PGB, respectively. For interday precision, % CV was found to be 1.32–1.98 and 0.94–1.77% for ACF and PGB, respectively.

5.5. Robustness. Variation in the flow rate, mobile phase, and pH has been made to the analytical method in order to evaluate and measure the capacity of the method to remain unaffected by such variations. The % CV was found to be less than 2 (Tables 2, 3, and 4).

TABLE 1: Accuracy (% recovery study) ($n = 3$).

Conc. ($\mu\text{g/mL}$)	ACF			PGB			
	% of std. drug added	% recovery (mean \pm SD)	% CV	Conc. ($\mu\text{g/mL}$)	% of std. drug added	% recovery (mean \pm SD)	% CV
10	50	98.89 \pm 0.68	0.69	7.5	50	100.48 \pm 1.81	1.81
	100	98.45 \pm 0.77	0.78		100	99.69 \pm 1.74	1.75
	150	100.08 \pm 0.73	0.72		150	99.99 \pm 1.40	1.40

TABLE 2: Change in flow rate ($n = 3$).

Drug	Flow rate (mL)	Mean area \pm SD	% CV
Aceclofenac (15 $\mu\text{g/mL}$)	0.95	1362.80 \pm 10.54	0.77
	1.05	1281.76 \pm 14.59	1.14
Pregabalin (11.25 $\mu\text{g/mL}$)	0.95	577.58 \pm 5.31	0.92
	1.05	543.88 \pm 6.70	1.23

TABLE 3: Change in mobile phase combination ($n = 3$).

Drug	Mobile phase composition	Mean area \pm SD	% CV
Aceclofenac (15 $\mu\text{g/mL}$)	58.8 : 41.2	1340.04 \pm 23.94	1.79
	61.2 : 38.8	1275.57 \pm 21.35	1.67
Pregabalin (11.25 $\mu\text{g/mL}$)	58.8 : 41.2	569.37 \pm 7.93	1.39
	61.2 : 38.8	542.44 \pm 7.80	1.44

TABLE 4: Change in pH ($n = 3$).

Drug	pH	Mean area \pm SD	% CV
Aceclofenac (15 $\mu\text{g/mL}$)	5.9	1341.40 \pm 24.68	1.84
	6.1	1254.06 \pm 19.24	1.53
Pregabalin (11.25 $\mu\text{g/mL}$)	5.9	572.18 \pm 6.12	1.07
	6.1	532.47 \pm 4.58	0.86

TABLE 5: Assay results of marketed formulation ($n = 5$).

Label claim (mg)	ACF		Label claim (mg)	PGB	
	Amount found (mg)	% assay \pm SD		Amount found (mg)	% assay \pm SD
100	98.50	98.79 \pm 1.41	75	74.25	99.39 \pm 1.68

TABLE 6: Summary of validation parameters.

Parameter	Aceclofenac	Pregabalin
Linearity		
Regression equation	$y = 64.93x + 306.8$	$y = 36.05x + 133.4$
Regression coefficient (R^2)	0.999	0.999
Range ($\mu\text{g/mL}$)	5–25	3.75–18.75
Accuracy (% mean recovery) ($n = 3$)	98.45–100.08	99.69–100.48
Precision (% CV)		
Repeatability ($n = 6$)	1.32	1.55
Intraday precision ($n = 3$)	0.91–1.29	0.72–1.07
Interday precision ($n = 3$)	1.32–1.98	0.94–1.77
LOD ($\mu\text{g/mL}$)	0.64	0.35
LOQ ($\mu\text{g/mL}$)	1.95	1.06
Specificity	Complied	Complied
Robustness	Complied	Complied

5.6. *LOD and LOQ.* LOD was found to be 0.64 and 0.35 $\mu\text{g/mL}$ for ACF and PGB, respectively. LOQ was found to be 1.95 and 1.06 $\mu\text{g/mL}$ for ACF and PGB, respectively.

5.7. *Assay of Marketed Formulation.* Percentage purity of ACF and PGB was found to be 98.79% and 99.39% for ACF and PGB, respectively (Table 5). A summary of validation parameters can be found in Table 6.

6. Conclusion

The proposed chromatographic method was found to be simple, sensitive, accurate, and precise for determination of ACF and PGB in combined dosage form. The common excipients and additives which are usually present in the combined dosage form do not interfere in the analysis of ACF and PGB in the method; hence it can be conveniently adopted for routine quality control analysis of the drugs in combined dosage form.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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