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# Development and validation of a novel stability indicating RP-HPLC method for simultaneous determination of aceclofenac and misoprostol in bulk and from their combined dosage form

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**ABSTRACT**

*The present work is a method development and validation for the simultaneous determination along with stability studies for the bulk and combined tablet formulation of Aceclofenac and Misoprostol by using reverse phase High performance liquid chromatography (HPLC) with isocratic elution where the stationary phase used was Luna C18 250x4.6 mm, 5µ column, mobile phase was 30:70 (v/v) acetonitrile: aqueous 0.01M triethylamine buffer (pH 2.5 adjusted with 2% v/v o-phosphoric acid) , flow rate 1 ml/minute, eluent was monitored by UV detector wavelength at 227 nm. Retention time was found to be 2.541 minutes and 3.831 minutes, correlation coefficient 0.998 and 0.999, LOD 0.125 and 0.127 nm, LOQ 0.250 and 0.255 nm for Aceclofenac and Misoprostol, respectively. Linearity range was designed 0.5-7.52 µg/ mL for Aceclofenac and 0.51-7.56 µg/mL for Misoprostol. Accuracy study revealed percentage recovery 100.1%-100.8% and 100.0%-100.4%, repeatability results in terms of relative standard deviation (%RSD) 0.21 and 0.28 for Aceclofenac and Misoprostol respectively. The developed method was validated as per ICH guideline and was found to be an ideal and optimal one for regular analysis in the laboratory.*

**Keywords:** Aceclofenac, Misoprostol, Stability Indicating Studies, RP-HPLC, UV detector

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## INTRODUCTION

Aceclofenac (ACF) is a non steroidal anti inflammatory cytokine inhibitor which is broadly used for the symptomatic treatment of pain and inflammation specifically in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis with the recommended dose of 100 mg twice daily [1-4]. The drug works by inhibiting the action of cyclooxygenase (COX) that is involved in the production of prostaglandins (PG) which is accountable for pain, swelling, inflammation and fever [5]. Aceclofenac (C16H13Cl2NO4), chemically [(2-{2,6-dichlorophenyl) amino} phenylacetooxyacetic acid], is a crystalline powder with a molecular weight of 354.19 [6-9]. It is practically insoluble in water with good permeability [8, 9]. It is metabolized in human hepatocytes and human microsomes to form [2-(2', 6'-dichloro-4'-hydroxy- phenylamino) phenyl] acetoxyacetic acid as the major metabolite, which is then further conjugated [10]. Figure 1 represents the structure of Aceclofenac.

Misoprostol Misoprostol (MP), chemically, Methyl 7-((1R, 2R, 3R)-3-hydroxy-2-((S, E)-4-hydroxy-4-methyloct-1-enyl)-5-oxocyclopentyl) heptanoate is a synthetic analogue of natural prostaglandin E19. It produces a dose-related inhibition of gastric acid and pepsin secretion, enhances mucosal resistance to injury. It is an effective anti-ulcer agent and also has oxytocic properties. It is on the World Health Organization's List of Essential Medicines, the most important medications needed in a basic health system [11,12]. Misoprostol is a prostaglandin E1 (PGE1) analogue used for the treatment and prevention of stomach ulcers. Misoprostol seems to inhibit gastric acid secretion by a direct action on the parietal cells through binding to the prostaglandin receptor[13]. Figure 2 represents structure of Misoprostol.

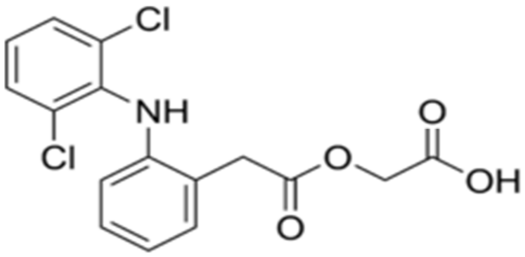


**Figure-1**

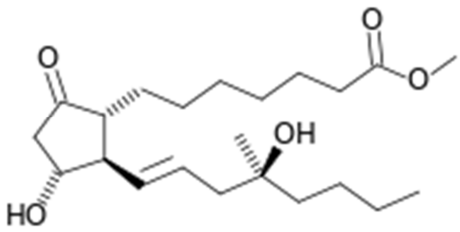


**Figure-2**

After thorough literature survey [14-18] it was observed that after the launching of these drugs continuous effort has been laid by numerous scientists to establish several methods for the determination of these compounds either individually or in different combinations. Authors like A Bose et al proposed a spectrophotometric method for aceclofenac alone. Susmitha A et al innovated spectrophotometric along with method validation for aceclofenac. S.M. Ashraful Islam et al carried out Validation of UV-Spectrophotometric and RP-HPLC methods for the simultaneous analysis of Aceclofenac in combination with paracetamol. Simões SS et al innovated an UPLC-Tandem MS method for estimation of misoprostol acid in whole blood and Manoj S.Charde et al developed a HPLC method for simultaneous estimation of misoprostol in combination with other drug; But so far no method was developed for determination of combined formulation of aceclofenac and misoprostol. Hence we made a sincere attempt to come out with a simple and precise method for the estimation of above mentioned combination by HPLC.

## MATERIALS AND METHODS

**Instruments:** HPLC system Waters equipped of a quaternary pump with Auto sampler and Auto injector , UV-Vis detector at a wavelength 266 nm. The software used was Empower software, Digital balance (shimadzu), PH meter (Thermo scientific).

**Chemicals:** All chemicals and reagents used were of Analytical grade. Sufficient amounts of concentrations were picked while choosing various chemicals [19,20].various substances were Reference standard, Tablet samples, ortho phosphoric acid and potassium ortho dihydrogen phosphate from LOBA Chemie Pvt. Ltd. And Milli Q water (Rankem).

**Chromatographic conditions**: Mobile phase - 30:70 (v/v) mixture of Acetonitrile and triethyl amine buffer (pH 2.5 adjusted with 2 % v/v o-phosphoric acid).Stationary phase- Luna C18 250x4.6 mm, 5 µm particle size C18 column. Flow rate - 1.0 ml/ min. Temperature- 30 °C, Detecting wave length was 266 nm.

**Experimental :**

**Preparation of Standard**

Accurately weighed and transferred 10mg each of Misoprostol and Aceclofenac working standards in to 10ml clean dry volumetric flasks, added 3/4$^{th}$ volume of diluents and were sonicated and made up to the volume with diluents. From the above stock solutions 1ml was pipetted out in to a 10ml volumetric flask and made up to final volume with diluents.

**Preparation of Sample:**

Ten capsules were weighed and average weight of each tablet was calculated and then the weight equivalent to 10mg of Aceclofenac and misoprostol was transferred in to 10ml volumetric flasks,7ml of diluents was added ,thoroughly sonicated and was made up with diluents and filtered. From the above solution, 1ml was pipetted out in to a 10ml volumetric flask and made the volume up to 10ml with diluents. Mobile phase is used as diluent.

**System suitability:** The method was developed after checking out various chromatographic parameters [21]. Aceclofenac and misoprostol got eluted in reasonable time and were acceptable for regular analytical work and suitable when a $C_{18}$ column was used with organic mobile phase in the ratio 30:70.the column length was also sufficient for efficient separation. All the other parameters like tailing factor, resolution results also were found satisfactory.

**Validation parameters**: The method was validated as per ICH guidelines [22].The validation parameters considered were accuracy, precision, system precision, linearity, limit of detection, limit of quantification and robustness studies.

Accuracy: Accuracy was determined by calculating recoveries of Aceclofenac and Misoprostol by standard addition method. Accurate amounts of Standard solutions of Aceclofenac and Misoprostol (each 50%, 100% and 150%) were spiked to pre analysed sample solutions. The amounts of each compounds recovered were estimated.

Precision : The instrumental repeatability or Precision was assessed at two levels, i.e. repeatability (intra-day precision) and intermediate precision, in accordance with ICH recommendations. Six injections, of three different concentrations, were given on the same day and the percent relative standard deviations (% RSD) were calculated to determine repeatability. These studies were also repeated on six consecutive days to determine inter-day precision. Retention time, Number of theoretical plates, peak symmetry and peaks resolution were under observation.

Linearity : Linearity tests were conducted for both the molecules having concentration range
Aceclofenac: 0.5-7.52ppm
Misoprostol: 0.51-7.65ppm

Calibration curve: Results of correlation coefficient(r), Slope of regression, SD of slope, Regression intercept and %RSD intercept were under observation.

LOD and LOQ: Limit of detection and Limit of quantification were calculated using following equations. LOD =3.3×σ/S and LOQ = 10×σ/S, where σ indicates the standard deviation of y intercepts of regression lines and S indicates the slope of the calibration curve.

Robustness: Robustness: Robustness test was performed by varying different chromatographic parameters like temperature, flow rate, mobile phase composition etc.

**Assay of marketed formulation**: The formulation (Tablet-MozaMPS) was purchased from local medical store. Ten tablets were taken, weighed, triturated to powder and collected a quantity equivalent to Aceclofenac 10 mg and Misoprostol 10 mg in a 10 ml volumetric flask. Dissolved in diluent, sonicated and made the volume with diluent. Pipetted out 1 ml of the solution into a 10 ml volumetric flask, made the volume with diluent. 10 µl was injected to the column and result was compared with standard in terms of %RSD .

**Stability studies:** Forced degradation studies [23] were conducted by providing varying physico-chemical environment. The procedure for stability studies are explained below. Figure to Figure represents the chromatograms due to stress degradation of the compounds. 11.24 mg of Aceclofenac and Misoprostol were weighed and dissolved in 10 ml of diluent to obtain solutions of 1000 µg /ml concentration. These stock solutions were used for forced degradation studies.

**Oxidative Degradation studies:** 0.1ml of 3% v/v solution of Hydrogen peroxide was added to 0.1ml of stock solution of Aceclofenac and misoprostol .these solutions were heated separately on water bath for 10 minutes at 70°C in the dark (to exclude the possible degradative effect of light).After cooling, it was made up to the volume with diluent.

**Acidic Degradation studies:**
To 0.1 ml of stock solution of Aceclofenac and Misoprostol, 0.1ml of 5N HCl was added and heated on water bath for 10mins at 70°C in the dark (to exclude the possible degradative effect of light).After cooling, it was made up to the volume with diluent.

**Alkaline Degradation studies:**
To 0.1 ml of stock solution of Aceclofenac and Misoprostol, 0.1ml of 5N NaoH was added and heated on water bath for 10mins at 70°C in the dark (to exclude the possible degradative effect of light).After cooling, it was made up to the volume with diluent.

**Neutral(water) Degradation studies:**
Stress testing under neutral water was studied by refluxing the drug in water for 6hrs at a temperature of 70°C. For the HPLC study, After cooling, it was made up to the volume with diluent.

**Reduction Degradation studies:**
To 0.1 ml of stock solution of Aceclofenac and Misoprostol, 0.1ml of 10% Sodium bisulphate was added and heated on water bath for 10mins at 70°C in the dark (to exclude the possible degradative effect of light).After cooling, it was made up to the volume with diluent.

**Thermal Degradation**

To 0.1 ml of stock solution of Aceclofenac and Misoprostol, 3 ml diluent was added. and heated on water bath for 20 mins at 70°C in the dark (to exclude the possible degradative effect of light).After cooling, it was made up to the volume with diluent.

**Photochemical Degradation**

10 ml of stock solutions of Aceclofenac and Misoprostol were separately subjected under sunlight for 3 hrs to study the effect of photo degradation.

## RESULTS

**Method Development**

To develop an optimized method for estimation and degradation studies several trials were conducted, to achieve most suitable chromatographic conditions with good separation. During the optimization of HPLC method, two columns (Luna C18 250x4.6 mm, 5µ; Luna CN 250x4.6 mm,5µ), organic solvent ( acetonitrile) were tested. Aceclofenac and Misoprostol exhibited quite a similar behavior as both of them contain polar functional groups. Amongst the stationary phases tried, Luna C18 250x4.6 mm, 5µ gave the best results in terms of resolution, peak shape and analysis time. Reasonable retention time, number of theoretical plates, value of tailing factors and all were found within the validation limit by using optimized chromatographic condition. Figure 3 represents a typical chromatogram of Aceclofenac and Misoprostol.

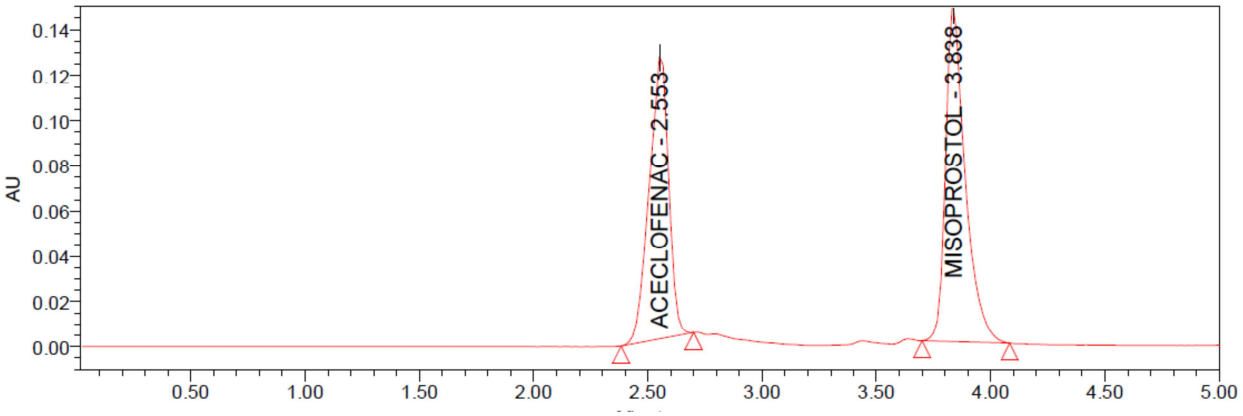


**Fig. 3 A typical chromatogram of Aceclofenac and Misoprostol**

This method has many advantages like isocratic conditions rather than gradient RP-HPLC which requires more sophisticated instrumentation and analysis time was also shorter. However, stability indicating methods are reported for quantitation of Aceclofenac and Misoprostol individually, there is no published method so far for study of the stability of the drugs simultaneously under conditions of forced degradation and quantitation hence, there is a need for a new analytical method for the combined dosage form of Aceclofenac and Misoprostol .

**Regression Analysis:** Test for linearity showed that all the mean values of slope, Y intercept, correlation coefficient, Tailing factor and theoretical plates were with in the limit. The linearity range was 0.5-7.52 µg/ml and 0.51-7.65 µg/ml for Aceclofenac and Misoprostol respectively. Table 1 contains details of results for regression studies.

**Table 1: Regression analysis of calibration curve**

| Parameters (Units) | Linearity range (µg/mL) | N | Slope | Intercept | $R^2$ | Retention time R (min) | Tailing factor (T) | Number of theoretical plates (N) |
|---|---|---|---|---|---|---|---|---|
| Aceclofenac | 0.5-7.52 | 6 | 177978.94 | -19167.96 | 0.9983 | 2.546 | 0.89 | 3276 |
| Misoprostol | 0.51-7.65 | 6 | 176874.29 | -3548.11 | 0.9999 | 3.826 | 1.4 | 8113 |

**Accuracy Results:**

Recovery of Aceclofenac and Misoprostol standards 50%,100% and 150% were 100.3,100.5 and 100.4 and 100.1, 100.4 and 100.8 in terms of percentage respectively. Table 2. describes all the details of recovery studies.

**Table 2: Accuracy results**

| Spike (%) | Misoprostol | | | | Aceclofenac | | | |
|---|---|---|---|---|---|---|---|---|
| | Amount taken(mg) | Amount Recovered(mg) | % Recovery | % RSD* | Amount taken(mg) | Amount Recovered(mg) | % Recovered | % RSD* |
| 50 | 2.903 | 2.906 | 100.1 | 0.200 | 2.91 | 2.92 | 100.3 | 0.340 |
| 100 | 5.35 | 5.37 | 100.4 | 0.370 | 5.62 | 5.65 | 100.5 | 0.270 |
| 150 | 7.86 | 7.923 | 100.8 | 0.070 | 8.44 | 8.47 | 100.4 | 0.410 |

**Precision:**
Precision was assessed at two levels, i.e. repeatability (intra-day precision) and intermediate precision, in accordance with ICH recommendations. Six injections, of three different concentrations, were given on the same day and the percent relative standard deviations (% RSD) were calculated to determine repeatability. These studies were also repeated on six consecutive days to determine inter-day precision. The % RSD values for the intra-day precision study for Aceclofenac and Misoprostol were 0.23 and 0.3 and for the inter-day study 0.4 and 0.25 respectively. Since the values were less than 2 %, it proved that the method was sufficiently precise.

**LOD, LOQ :** For sensitivity test LOD of Aceclofenac and Misoprostol were 0.255 and 0.127. LOQ of Aceclofenac and Misoprostol were 0.250 and 0.125 respectively. Table 3 Represents results of precision and sensitivity studies.

**Table 3: Precision and sensitivity**

| Parameters | Aceclofenac | Misoprostol |
|---|---|---|
| Retention time(min) | 2.553 | 3.833 |
| LOD | 0.255 | 0.027 |
| LOQ | 0.628 | 0.082 |
| Accuracy % | 100.1-100.8 | 100.3-100.5 |
| Intraday precision RSD% | 0.23 | 0.3 |
| Interday precision RSD% | 0.4 | 0.25 |

**Robustness studies :** Robustness is the measure of capacity of analytical methods to remain unaffected by small but deliberate variation of the operating conditions. By making changes like slight increase or decrease in flow rate and PH of the Mobile phase composition, effect was observed. All the methods were with in the limit and method was found robust.

**Results of degradation studies:** Stability studies were conducted in different physicochemical conditions like acid, alkali, peroxide, UV radiation, elevated temperature and neutral conditions. Solutions of the drugs shown that they were stable enough under stress environment. Very minor degradation was observed in acid, alkali and oxidative conditions. Figure 4 to 9 represent chromatogram after different stress condition. Tables 4 to 8 describes results of force degradation.

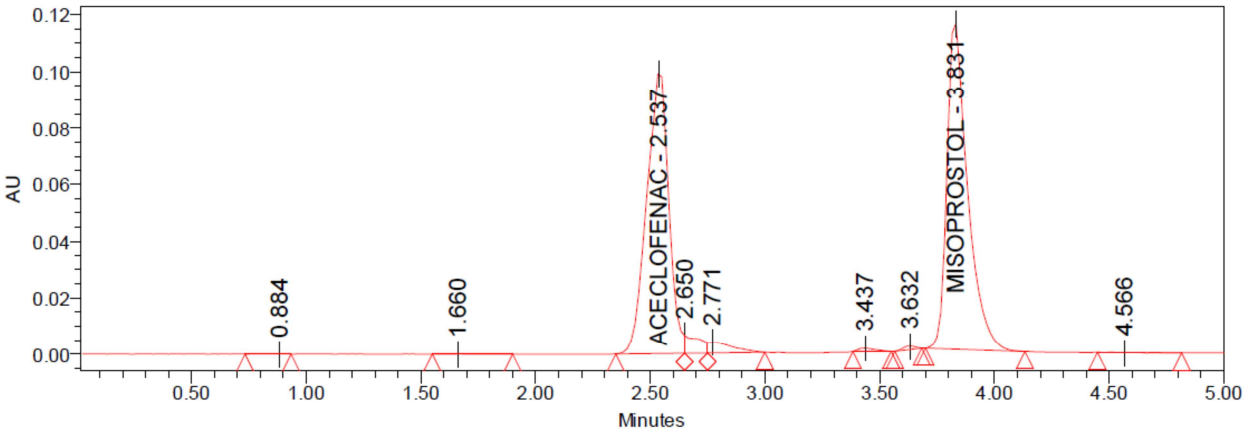


**Figure 4: Chromatogram after acid stress**

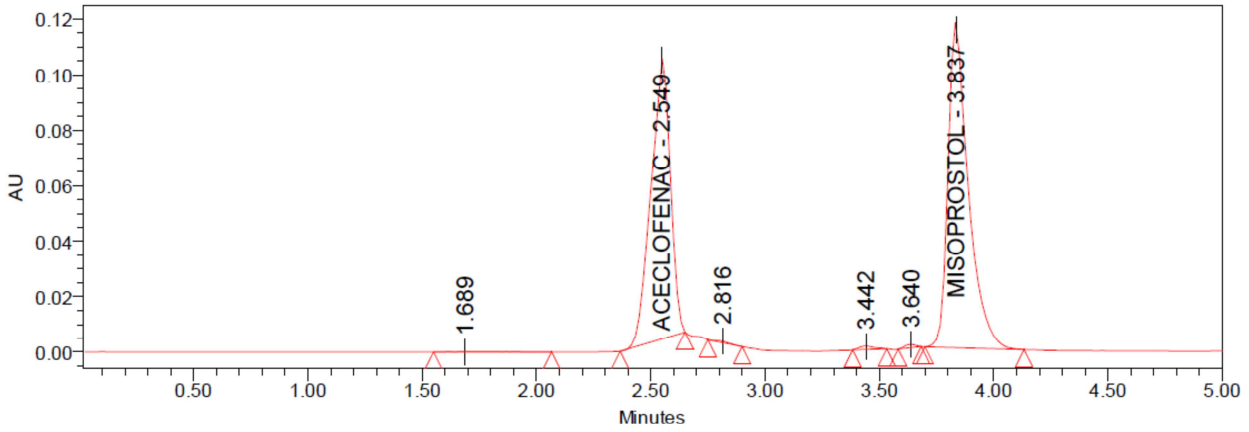


**Figure 5: Chromatogram after alkali stress**

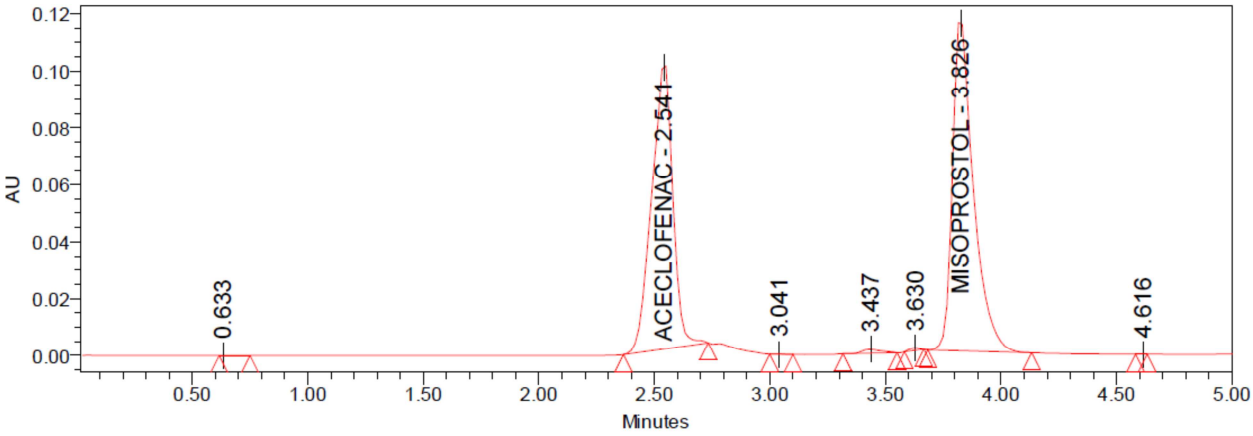


**Figure 6: Chromatogram after oxidative stress**

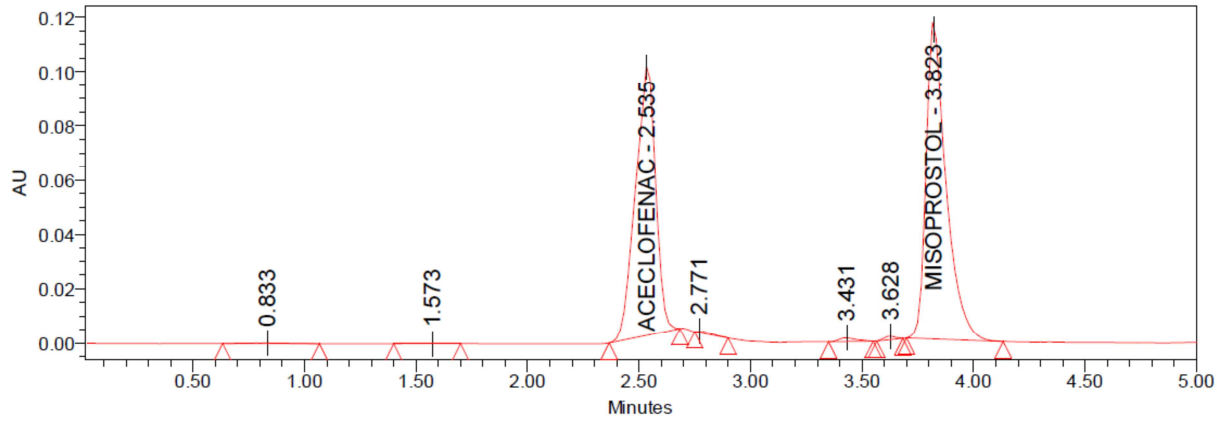


**Figure 7: Chromatogram after UV radiation stress**

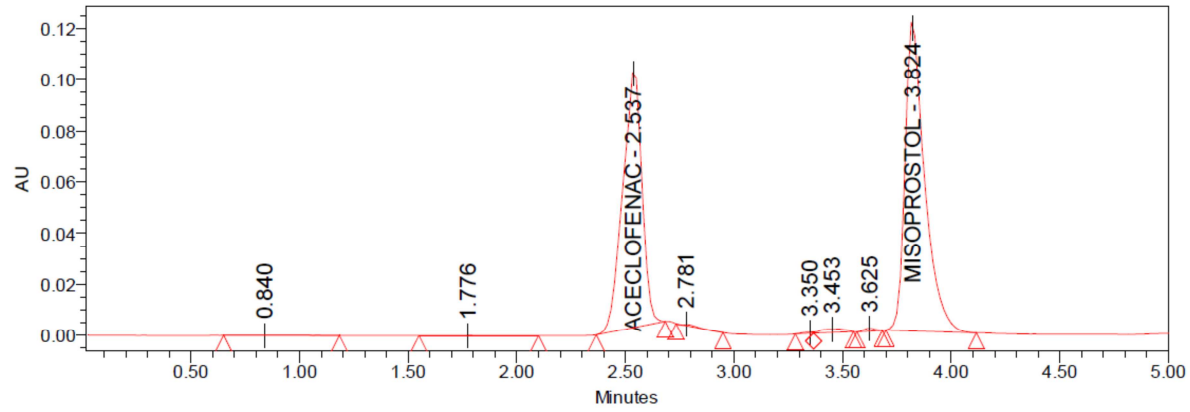


**Figure 8: Chromatogram after Thermal stress**

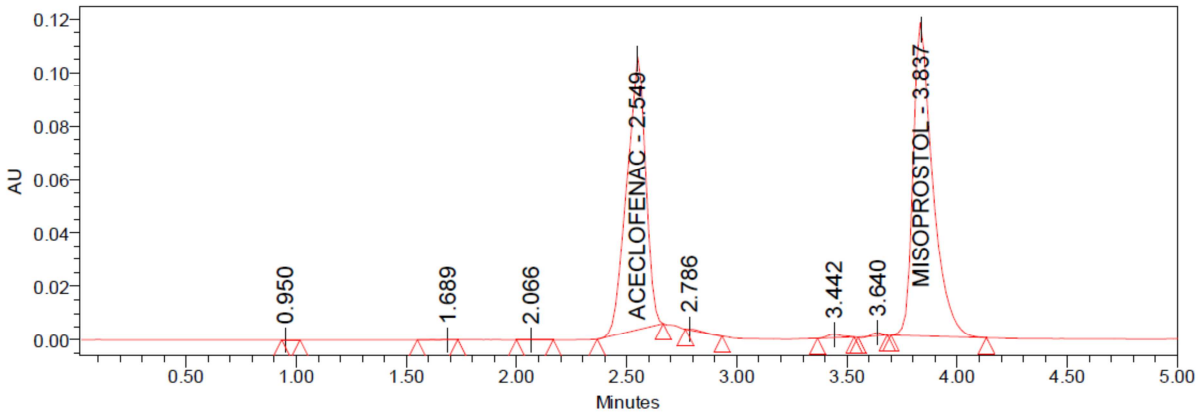


**Figure 9: Chromatogram after Hydrolytic stress**

**Table 4: Acid degradation results**

| Sl.no | Sample name | Retention time | Area | USP tailing | USP plate count | USP resolution |
|---|---|---|---|---|---|---|
| 1 | Aceclofenac | 2.537 | 663203 | | 2844 | |
| 2 | Misoprostol | 3.831 | 722702 | 1.51 | 8818 | 1.47 |
| 3 | Peak1 | 2.650 | 30076 | | | |

**Table 5: Alkali degradation results**

| Sl.no | Sample name | Retention time | Area | USP tailing | USP plate count | USP resolution |
|---|---|---|---|---|---|---|
| 1 | Aceclofenac | 2.549 | 595644 | 0.85 | 3161 | 4.60 |
| 2 | Misoprostol | 3.837 | 723684 | 1.53 | 9207 | 1.49 |
| 3 | Peak1 | 1.69 | 1183 | 1.08 | 106 | |

**Table 5:Oxidation degradation results**

| Sl.no | Sample name | Retention time | Area | USP tailing | USP plate count | USP resolution |
|---|---|---|---|---|---|---|
| 1 | Aceclofenac | 2.541 | 633530 | 0.86 | 3144 | 17.67 |
| 2 | Misoprostol | 3.826 | 729502 | 1.57 | 8837 | 1.56 |
| 3 | Peak1 | 3.437 | 8341 | 1.08 | 6195 | 2.98 |

**Table 6 : UV degradation results**

| Sl.no | Sample name | Retention time | Area | USP tailing | USP plate count | USP resolution |
|---|---|---|---|---|---|---|
| 1 | Aceclofenac | 2.535 | 620541 | 0.88 | 2969 | 3.95 |
| 2 | Misoprostol | 3.823 | 723653 | 1.58 | 9106 | 1.44 |
| 3 | Peak1 | 3.431 | 7641 | 1.35 | 7643 | 4.52 |

**Table 7 : Thermal degradation results**

| Sl.no | Sample name | Retention time | Area | USP tailing | USP plate count | USP resolution |
|---|---|---|---|---|---|---|
| 1 | Aceclofenac | 2.537 | 616579 | 0.87 | 3203 | 1.46 |
| 2 | Misoprostol | 3.824 | 757704 | 1.55 | 8915 | 1.48 |
| 3 | Peak1 | 3.350 | 1679 | | | |

**Table 8 : Hydrolytic degradation results**

| Sl.no | Sample name | Retention time | Area | USP tailing | USP plate count | USP resolution |
|---|---|---|---|---|---|---|
| 1 | Aceclofenac | 2.549 | 607474 | 0.85 | 3124 | 1.77 |
| 2 | Misoprostol | 3.837 | 723684 | 1.53 | 9207 | 1.51 |
| 3 | Peak1 | 3.442 | 6123 | 1.15 | 10187 | 4.99 |

## DISCUSSION

The chromatographic conditions were optimized by different means i.e. using different buffers, Organic modifiers, different flow rate, different columns, different wave lengths and different diluents. The proposed method found to be linear in the concentration range of 0.5-7.25 µg/mL, 0.51-7.65 µg /mL for Aceclofenac and Misoprostol respectively. The method was specific since degradants are not interfering in the estimation of above four compounds. Accuracy of the method indicated by recovery values from 100 to 100.5 for Aceclofenac and Misoprostol.. Precision is reflected by %RSD values less than 2.The LOQ values for Aceclofenac and Misoprostol were found 0.628 & 0.082 µg/mL respectively. These low values suggest sensitivity of the developed method.

## CONCLUSION

The newly developed method was found to be best method as it was stability indicating, less time consuming, highly accurate as results of recovery studies showed low values of percentage RSD, indicating method is more precise and robust. The above method is more suitable to use for the estimation of combined formulation of Aceclofenac and misoprostol.

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