International Standard Serial Number (ISSN): 2319-8141
*International Journal of Universal Pharmacy and Bio Sciences 8(2): March-April 2019*
***INTERNATIONAL JOURNAL OF UNIVERSAL PHARMACY AND BIO SCIENCES***
*IMPACT FACTOR 4.018****
*ICV 6.16****
**Pharmaceutical Sciences** **RESEARCH ARTICLE……!!!**

# SIMULTANEOUS DETERMINATION AND VALIDATION OF ACECLOFENAC AND CYCLOBENZAPRINE HYDROCHLORIDE BY ZERO ORDER AND FIRST ORDER DERIVATIVE METHODS IN BULK AND PHARMACEUTICAL FORMULATIONS

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**KEYWORDS:**

Aceclofenac, Cyclobenzaprine Hydrochloride, Zero Order method, First Order derivative.

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## ABSTRACT

Two simple UV Spectrophotometric methods have been developed & Validated for simultaneous estimation of Aceclofenac & Cyclobenzaprine Hydrochloride in bulk drug & tablet dosage form by Zero order & First order derivative methods using methanol as solvent. The analytical wavelengths for Aceclofenac and Cyclobenzaprine Hydrochloride were 276nm and 225nm respectively for Zero order derivative method and for First order derivative method the 295nm and 230nm respectively. The Beer's law was obeyed in the concentration range of 6-30µg/ml for Aceclofenac and 0.45-2.25µg/ml for Cyclobenzaprine Hydrochloride with correlation coefficient 0.999 in both the methods. LOD and LOQ was found to be 0.1201µg/ml and 0.3641µg/ml for Cyclobenzaprine hydrochloride and 0.1169µg/ml and 0.3542µg/ml for Aceclofenac respectively in Zero order method and for first order derivative method LOD and LOQ was found to be 0.0931µg/ml and 0.2823µg/ml for Cyclobenzaprine Hydrochloride and 0.2484µg/ml and 0.7527µg/ml for Aceclofenac respectively. In both the methods the % RSD for intra-day and inter-day precision was within 2%. Both the methods were found to be rapid specific , precise and accurate. Hence these methods can be applied for routine analysis of Aceclofenac and Cyclobenzaprine hydrochloride in combined dosage form. The above methods are validated as per ICH guidelines.

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## INTRODUCTION:

Aceclofenac [1-4] chemically, a phenylacetic acid derivative, has anti-inflammatory and analgesic properties. It is Non- steroidal anti-inflammatory drug (NSAIDs) used in various commercial pharmaceutical formulations for the treatment of fever, relief of pain and inflammation in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis and reported to have good anti-rheumatic activity. ACECLO is the glycolic ester of Diclofenac. It is inhibitor of cytokine and works by blocking the action of a substance in the body called cyclooxygenase which involved in the production of prostaglandins and responsible for the generation of pain, swelling and their inflammatory conditions.

Cyclobenzaprine [5-7] exhibits anticholinergic activity, potentiation of norepinephrine, and antagonism of reserpine. Cyclobenzaprine does not directly act on the neuromuscular junction or the muscle but relieves muscle spasms through a central action, possibly at the brain stem level. Cyclobenzaprine binds to the serotonin receptor and is considered a 5-HT2 receptor antagonist that reduces muscle tone by decreasing the activity of descending serotonergic neurons. It is an official drug in USP. Several analytical techniques like RP – HPLC, HPTLC, UPLC and Spectrophotometric method for the estimation of Cyclobenzaprine HCL individually and in other combinations have been reported.

The combination of Aceclofenac and Cyclobenzaprine hydrochloride is used to relieve pain and relax the muscle [8].

On literature survey, it has been found that several methods have been reported for the estimation of Aceclofenac and Cyclobenzaprine individually and in combination with other drugs. In the view of the need for a suitable method for routine analysis in combined formulations, attempts are being made to develop simple, precise and accurate analytical methods for simultaneous estimation of titled drugs and extend it for their determination in formulation.

## MATERIALS AND METHODS:

### Instrument

For UV-Visible Spectroscopy methods, Shimadzu model 1800 double beam UV-Visible Spectrophotometer with spectral band width of 1 ± 0.2nm, wavelength accuracy of ± 0.3nm and a pair of quartz cuvettes having 1cm path length was used. .[9-10]

### Chemicals

Standard Aceclofenac and Cyclobenzaprine hydrochloride was obtained as gift sample from micro labs, Bangalore. Methanol of AR grade, procured from S D FINE CHEM ltd. It was used throughout the experimental work.

**Methods**

**Preparation of standard solutions**

**Preparation of standard solution of Aceclofenac (ACF) And Cyclobenzaprine Hydrochloride (CBP)**

100mg each of Aceclofenac and Cyclobenzaprine Hydrochloride were weighed separately and transferred in two different 100ml volumetric flask. Both the drugs were dissolved in 50ml of methanol by ultrasonication and then volume was made up to the mark with methanol to obtain a concentration of 1000µg/mL of each component (stock –A).

From the above stock A solutions, 10 mL of aliquot was pipetted out in a 100 mL volumetric flask for both the drugs and the volume was made upto the mark with the methanol to obtain a concentration of 100µg/ml of each component( stock-B).

From the above stock –B solution further dilutions were made to get the concentration range 6-30µg/mL and 0.45-2.25µg/mL for Aceclofenac and Cyclobenzaprine hydrochloride respectively.

**Preparation of sample solution**

20 tablets which contains both ACF and CBP were weighed and powdered. The tablet powder equivalent to 100mg ACF was weighed accurately and dissolves in 70 ml methanol and sonicated for 15mins. The solution was filtered through Whatmann filter paper No. 41, finally the volume was made up to the mark with methanol. Further dilutions were made to bring the concentration of the drugs within the range.

**Methods of Estimation:**

**Method A (Zero Order method )**

The above standard solutions were scanned in the wavelength range of 400-200nm using UV – Spectrophotometer. Zero order derivative method involves the measurement of absorbences at selected analytical wavelengths, i.e 276nm and 225nm for estimation of Aceclofenac and Cyclobenzaprine hydrochloride respectively and amount of drugs present was calculated by using calibration plot.

**Method B (First order derivative)**

For the estimation of Aceclofenac and Cyclobenzaprine hydrochloride by first order derivative spectroscopy, zero crossing point for both drugs were obtained and the wavelengths were selected in manner such that, at the zero crossing of one drug, the other drug should show substantial absorbance. From the first order derivative spectra of standard Aceclofenac and Cyclobenzaprine hydrochloride, zero crossing point of Aceclofenac was found at 276nm and zero crossing point of Cyclobenzaprine

Hydrochloride was found at 225nm and wavelength selected for their estimation was 295nm for ACF and 230nm for CBP.[11-13]

**VALIDATION PARAMETRE:** [14]

**Linearity**

In Method A (Fig. 3 to 5) overlay spectra of mixtures were shown. Fig.3 shows the linearity of both the drugs in their respective wavelengths. The responses for both drugs shows linear concentration range of 6-20µg/ml and 0.45-2.25µg/ml for ACE and CBP respectively. The regression equation calculated by least square method was y =0.0027x+0.001 and y = 0.0667x-0.004 with correlation coefficient of both drugs was $r^2$ = 0.999 and $r^2$ = 0.999.

In Method B (Fig. 6 to 8) overlay spectra of both drugs and their mixtures were shown. Fig.6 shows the linearity of both the drugs in their respective wavelengths. The responses of first derivatives both drug shows linear concentration range of 6-30µg/ml and 0.45-2.25µg/ml for ACF and CBP respectively. The regression equation calculated by least square method was y = -0.008x+0.002 and y = -0.341x-0.009 with correlation coefficient of both drugs was $r^2$= 0.999 and $r^2$ = 0.999. Summary of validation parameters by developed methods as shown in Table no 1.

**Accuracy**

Recovery studies was carried out by applying the method to drug sample to which known amount of Aceclofenac and Cyclobenzaprine hydrochloride corresponding to 80, 100, 120% of label claim has been added (standard addition method). The results are tabulated in Table no 2.

**Precision:**

Precision was studied to find out intra-day and inter-day variations in the test method of ACF and CBP. Intra-day assay precision was found by analysis of standard drug thrice on the same day in different intervals of time. Inter- day assay precision was carried out on three different days and percentage relative standard deviation (%RSD) was calculated. The % RSD were found to be less than 2.0%. Statistical validation data for Intra-day and Inter-day precision methods as shown in Table no 3 and Table no 4.

**LOD and LOQ**

LOD is the lowest amount of the analyte can be detected but not quantified. LOQ is the lowest amount of analyte that can be detected and quantified with an acceptable accuracy, precision. In this study, LOD and LOQ were determined based on the standard deviation of the response and the slope of the corresponding curve using the following equations.

The LOD & LOQ were calculated from the followings formulas

LOD = 3.3 SD/Slope and LOQ = 10 SD/Slope.

SD= Standard Deviation of y- intercept

**RESULTS AND DISSCUSION:**

The selected drugs Aceclofenac and Cyclobenzaprine hydrochloride in Bulk and Formulations were estimated by using both UV spectrophotometric methods and validated as per ICH guidelines. In both the methods linearity response for ACF and CBP was 6-30µg/ml and 0.45-2.25µg/ml respectively. The % RSD for intraday and inter-day precision was found to be less than 2%. The accuracy of the methods were validated by recovery studies and was found to be significant and under specification limits, with % recovery 99-100%. The assay results were found to be within the acceptable limits. Hence developed methods was found to be precise and sensitive.[15]

**Fig 1 :Chemical structure Aceclofenac**

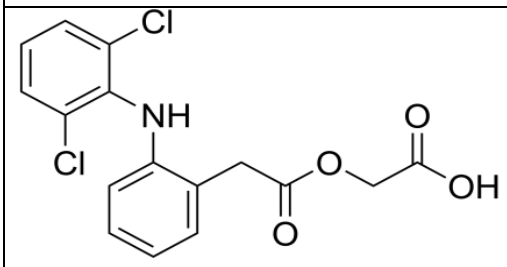


**Fig 2: Chemical structure of Cyclobenzaprine**

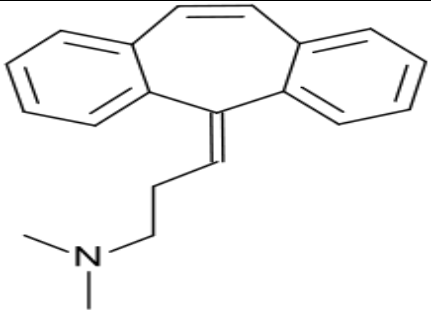


**Fig 3: Overlay spectra of standard drug mixture**

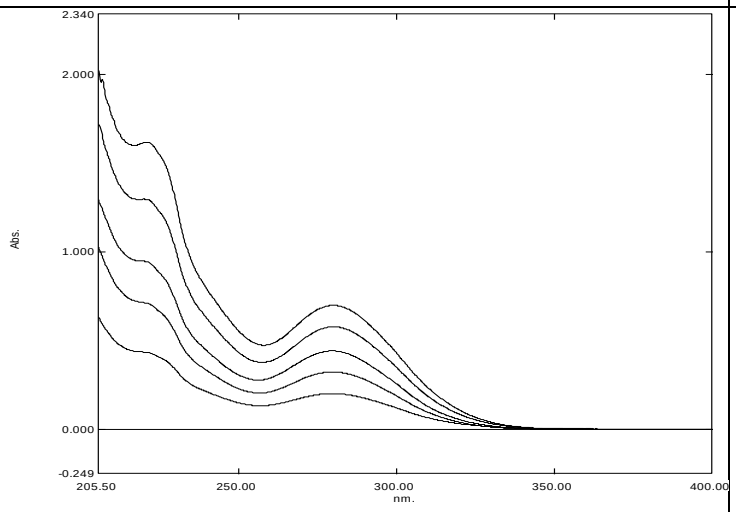


**Fig. 4: Calibration Curve for ACF at 276 nm**

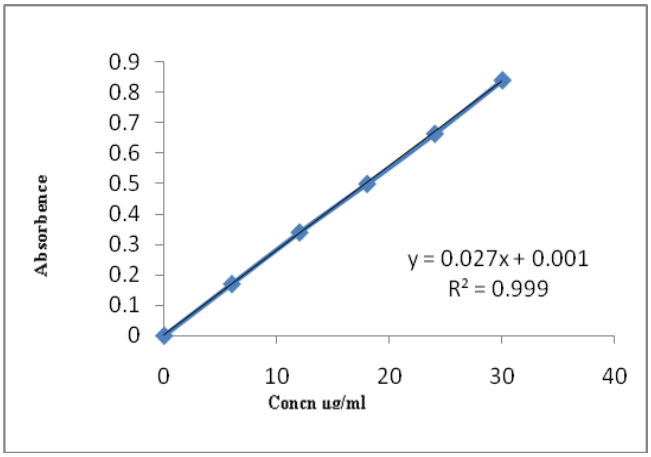

**Fig. 5: Calibration Curve for CBP at 225 nm**

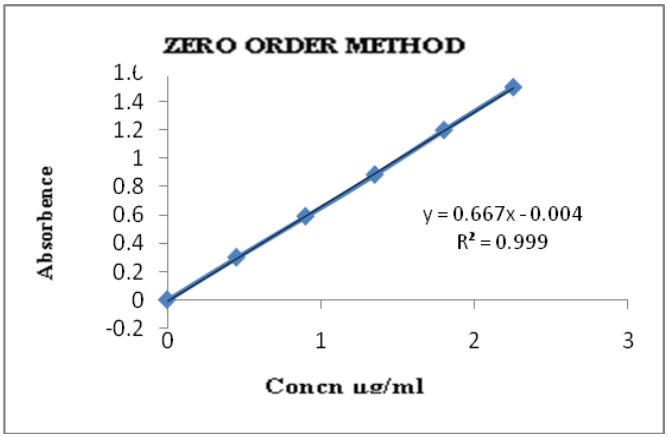


**Fig 6: overlay spectrum of Standard drug mixture**

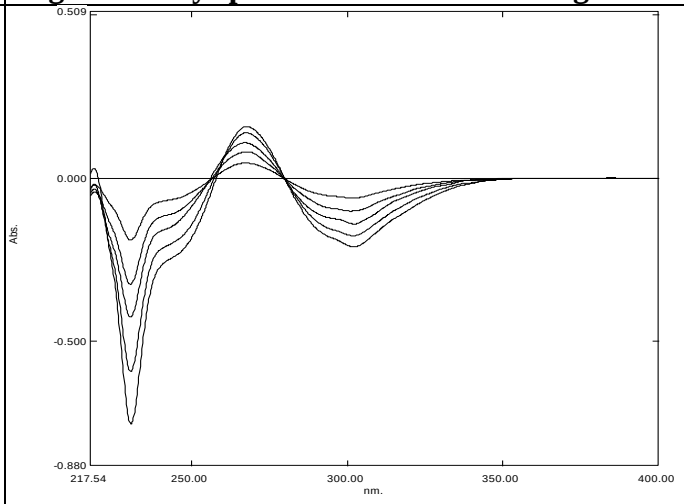


**Fig 7: Calibration curve of ACF at 295nm**

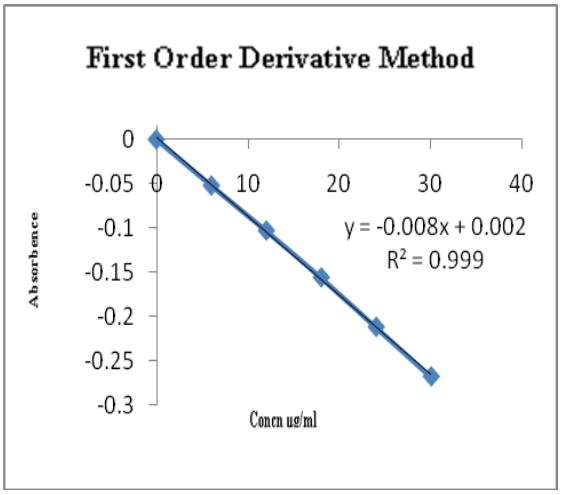


**Fig 8 : calibration curve of CBP at 230nm**

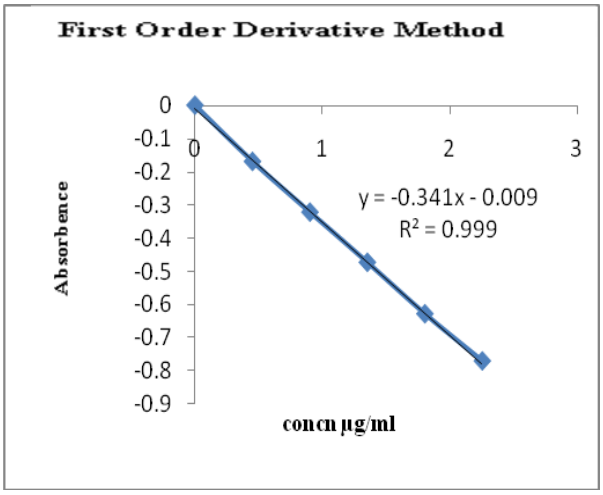


**Table 1: Summary of Validation Parameters by Developed Methods**

| Parameter | Method A | | Method B | |
|---|---|---|---|---|
| | ACF | CBP | ACF | CBP |
| Wavelength (nm) | 276 | 225 | 295 | 230 |
| Linearity Range (µg/ml) | 6.0-30.0 | 0.45-2.25 | 6.0-30.0 | 0.45-2.25 |
| Regression equation (y = a + bc) | y=0.0274x+ 0.001 | y = 0.667x-0 .004 | y = -0.008x + 0.002 | y = 0.341x - 0.009 |
| Slope (b) | 0.027 | 0.667 | 0.008 | 0.341 |
| Intercept (a) | 0.001 | 0.004 | 0.002 | 0.009 |
| Correlation Coefficient ($r^2$) | 0.999 | 0.999 | 0.999 | 0.999 |
| LOD (µg/ml) | 0.1169 | 0.1201 | 0.248 | 0.0931 |
| LOQ (µg/ml) | 0.3542 | 0.3641 | 0.752 | 0.2823 |

**Table 2 : Statistical Validation Data for Accuracy Determination**

| Level of % Recovery | Components | Amount present (µg/ml) | Amount of Standard drug added (µg) | Method A | | | Method B | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Total amount recovered (µg) | % Recovery | RSD | Total amount recovered (µg) | % Recovery | RSD |
| 80% | ACF | 6 | 4.8 | 10.79 | 99.9 | 0.3186 | 10.78 | 99.8 | 0.2096 |
| | CBP | 0.45 | 0.36 | 0.749 | 99.8 | 0.4251 | 0.749 | 99.8 | 0.2787 |
| 100% | ACF | 6 | 6 | 11.9 | 99.1 | 0.1675 | 11.9 | 99.1 | 0.2791 |
| | CBP | 0.45 | 0.45 | 0.899 | 99.8 | 0.2227 | 0.899 | 99.5 | 0.3411 |
| 120% | ACF | 6 | 7.2 | 13.19 | 99.8 | 0.0803 | 13.18 | 99.6 | 0.0805 |
| | CBP | 0.45 | 0.54 | 0.989 | 99.6 | 0.1851 | 0.986 | 99.5 | 0.107 |

**Table 3: Statistical Validation Data for Intra-day Precision**

| Components | Method A | | Method B | |
|---|---|---|---|---|
| | ACF | CBP | ACF | CBP |
| Mean | 99.8 | 99.71 | 99.52 | 99.55 |
| Standard Deviation | 0.0070 | 0.0070 | 0.2453 | 0.1987 |
| Relative Standard Deviation | 0.0070 | 0.0070 | 0.2464 | 0.1996 |
| Standard Error | 0.0028 | 0.0029 | 0.1005 | 0.0814 |

**n*=6**

**Table 4: Statistical Validation Data for Inter-day Precision**

| Components | Method A | | Method B | |
|---|---|---|---|---|
| | ACF | CBP | ACF | CBP |
| Mean | 99.95 | 99.87 | 99.4 | 99.54 |
| Standard Deviation | 0.0070 | 0.0070 | 0.2286 | 0.3531 |
| Relative Standard Deviation | 0.0070 | 0.0071 | 0.2300 | 0.3548 |
| Standard Error | 0.00288 | 0.00289 | 0.0937 | 0.1447 |

**n*=3**

## CONCLUSION:

The proposed methods were validated as per ICH guidelines. The standard deviation and % RSD calculated for the proposed methods were low, indicating high degree of precision of the method. The

results of the recovery study performed show the high degree of accuracy of proposed methods. Hence, these methods can be employed successfully for the simultaneous estimation of Aceclofenac and Cyclobenzaprine Hydrochloride in routine analysis.

**ACKNOWLEDGEMENT:**

Authors are grateful to Micro labs ltd, Bangalore for providing the gift sample of Aceclofenac and Cyclobenzaprine hydrochloride. We are also thankful to the Principal and department of Pharmaceutical analysis of National College of pharmacy, Shimoga for guidance, encouragement and providing the necessary facilities to carry out this work.

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