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Pharmaceutical Sciences **RESEARCH ARTICLE……!!!**

SIMULTANEOUS DETERMINATION AND VALIDATION OF ACECLOFENAC AND CYCLOBENZAPRINE HYDROCHLORIDE BY RP-HPLC METHOD IN BULK AND PHARMACEUTICAL FORMULATIONS

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KEYWORDS:

Aceclofenac, Cyclobenzaprine Hydrochloride, HPLC Method, Validation.

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ABSTRACT

The objective of the current study was to develop a simple, accurate, precise and rapid RP-HPLC method with subsequently validate as per ICH guidelines for the simultaneous determination of Aceclofenac (ACF) and Cyclobenzaprine Hydrochloride (CBP) using mobile phase [Mixture of Methanol and 0.02M Phosphate buffer at pH 3 in the ratio of 60:40v/v] . The proposed RP-HPLC method utilizes a C18 column, 5µm, 250mm × 4.6mm i.d and UV detection at 280nm. The retention times were 7.381 min and 4.370 min for ACF and CBP at flow rate of 1ml/min .The described method was linear over the range of 12-60 µg/ml (r^2=1) for ACF and 0.9-4.5 µg/ml (r^2=0.999) for CBP respectively. The percentage mean recovery was found to be 99.91 for ACF and 99.85 for CBP. The method was statistically validated for its linearity, accuracy and precision. Both inter-day and intra-day variation was found to be showing less % RSD (Relative Standard Deviation) value indicating high grade of precision of the method.

INTRODUCTION:

Aceclofenac [1-10] 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 [1-10] 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.

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. [9-13].

MATERIALS AND METHODS:

INSTRUMENT

A high performance liquid chromatographic system (SHIMADZU Corporation, LC-20 AD), a Shimadzu SPD-20A UV/VIS detector was used for analysis. The data was recorded using Lab Solutions Software.

CHEMICALS AND REAGENTS:

Methanol (HPLC grade) was procured from S D FINE CHEM ltd, distilled water (HPLC grade), potassium di-hydrogen orthophosphate and all other chemical reagents were of analytical grade.

DRUG SAMPLE:

Standard Aceclofenac and Cyclobenzaprine Hydrochloride was obtained as gift sample from micro labs, Bangalore.

PREPARATION OF MOBILE PHASE:

Mobile phase was prepared by mixing 600 ml of Methanol and 400 ml 0.02M KH_2PO_4. The pH was adjusted to 3 using O-phospharic acid. Solution was filterd through whatman filter paper(0.45µm) and sonicated for 10 min and this solution was used as mobile phase.

PREPARATION OF BUFFER SOLUTION:

2.72 gm of KH_2PO_4 was weighed and dissolved in 1000 ml of HPLC water to prepare 0.02M KH_2PO_4 buffer.

Method

Preparation of Standard solutions

Preparation of standard solution of Aceclofenac (ACF) and Cyclobenzaprine Hydrochloride (CBP)

100 mg each of ACF and CBP were weighed separately and transferred in two different 100 mL volumetric flasks. Both the drugs were dissolved in 50 mL of mobile phase by sonication and then volume was made up to the mark with mobile phase to get a concentration of 1000 µg/mL of each component (stock A and A' solution).

From the above stock A and A' solution, 10 ml of aliquot was pipetted out in a 100ml volumetric flasks and the volume was made up to the mark with mobile phase to obtain the final concentration of 100µg/ml of each component (stock B and B' solution)

From the above stock-B solution further dilutions were made to get the concentration range of 12-60 µg/ml and 0.9-4.5 µg/ml for Aceclofenac and Cyclobenzaprine Hydrochloride respectively.

Sample preparation:

Twenty tablets of ACF and CBP in combination were weighed and their average weight was determined. The tablets were crushed to fine powder and a tablet powder equivalent to 100 mg of ACF was weighed which also contains 7.5 mg of CBP and transferred to 100 ml volumetric flask, dissolved in sufficient quantity of mobile phase. The solution was filtered through 0.4 µm membrane filter paper. The contents were sonicated for 20 minutes and the final volume was made up to the mark with mobile phase (sample stock A solution).

From the above stock solution A pipette out 10 ml to 100ml volumetric flask and volume was made upto the mark with mobile phase(Sample stock B)(100µg/ml)**.**

From the above stock B solution further dilutions were made to bring the concentration of the drugs within the range.

RESULTS AND DISCUSSION:

The developed method for determination of Aceclofenac and Cyclobenzaprine Hydrochloride was further validated by using the following parameters:

Linearity:

Linearity was established by least square regression analysis of the calibration curve. The constructed calibration curve was linear over the concentration range of 12-60 µg/ml for ACF and 0.9-4.5 µg/ml for CBP. Peak area of ACF and CBP were plotted versus their respective concentration and linear regression analysis was performed on the resultant curves (fig.3 & fig.4). The regression equation was found to be y = 10278x-3.809 (r^2 = 1) for ACF and y = 11823+553.0 (r^2 = 0.999) for CBP. Summary of validation and system suitability parameters of Aceclofenac and Cyclobenzaprine Hydrochloride as shown in Table 1.

Accuracy:

Accuracy of the method was calculated by recovery studies. It is carried out by preparing the samples at the level of 80%, 100% and 120% of target concentration. The samples were prepared in triplicate for each level. The results of studies are tabulated in Table 2.

Precision:

The precision of the method was verified by repeatability, inter-day and intra-day precision. Repeatability studies were performed by analysis of three different concentrations of the drug in six times on the same day. Intraday precision was determined by analyzing sample solutions at different time intervals on the same day and on different day for inter-day precision. Statistical validation data for Intra-day and Inter-day precision methods are shown in Table 3 and 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**.**

LOD and LOQ for Aceclofenac was found to be 0.6231 µg/ml and 1.80 µg/ml respectively**.**

LOD and LOQ for Cyclobenzaprine Hydrochloride was found to be 0.1245 µg/ml and 0.6258 µg/ml respectively.

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness of the method was investigated under a variety of conditions including changes of flow rate and wavelength. The Robustness results for variation in flow rate (ml/min) and wavelengths as shown in table 5 and table 6.

Ruggedness:

The evaluation of robustness should be considered during the development phase and depends upon the type of procedure under study. It should show the reliability of analysis with respect to deliberate variations in analyst or instrument. The solution containing 36µg/ml of Aceclofenac and 2.7µg/ml of Cyclobenzaprine Hydrochloride was injected into sample injector of HPLC two times by different analysts. Ruggedness result for variations in Analyst is shown in table (7)

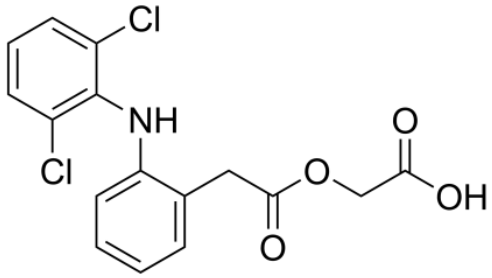

Fig 1 : Chemical structure of Aceclofenac

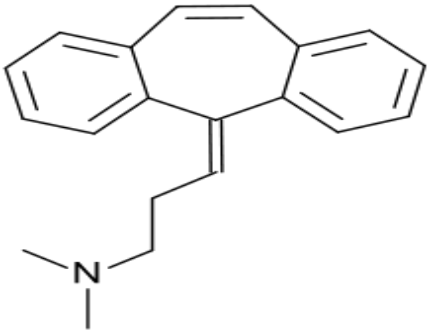

Fig 2: Chemical structure of Cyclobenzaprine

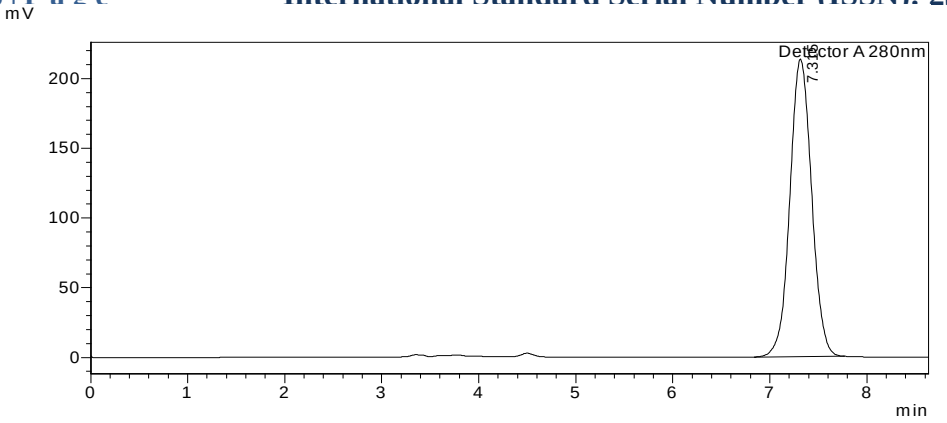

Fig.3 : Chromatogram showing Retention Time of Aceclofenac

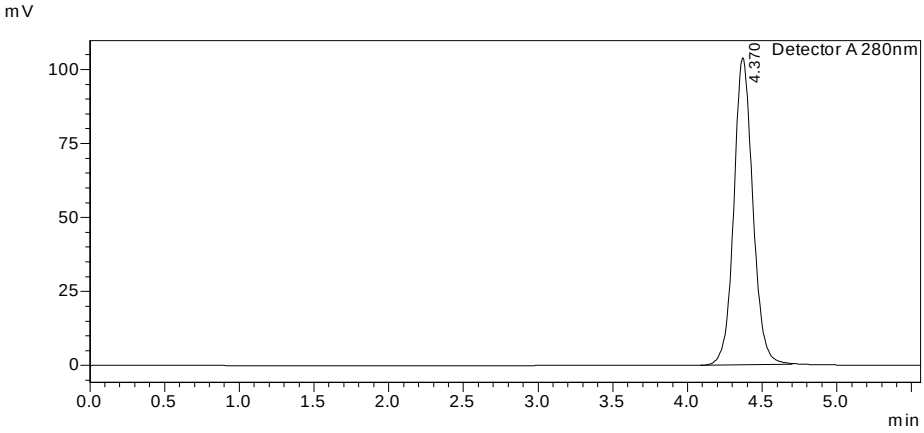

Fig 4: Chromatogram showing Retention Time of Cyclobenzaprine Hydrochloride

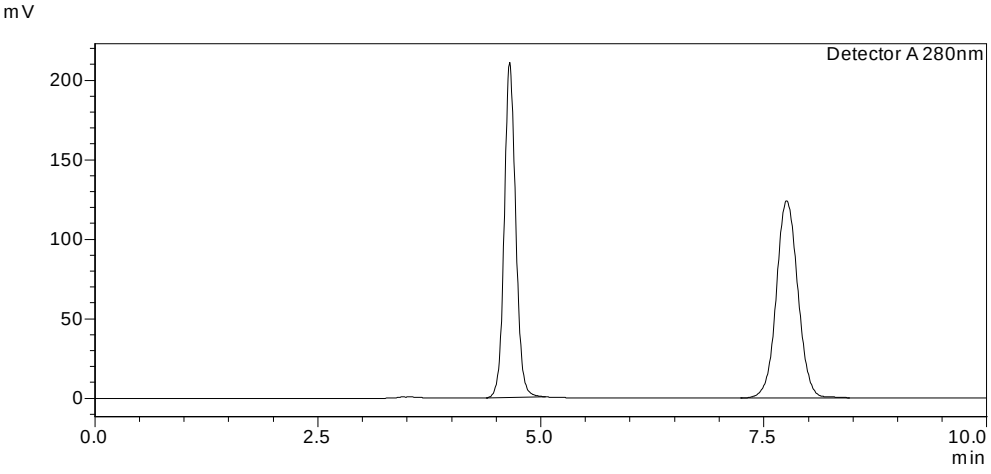

Fig 5 : Chromatogram of Aceclofenac and Cyclobenzaprine Hydrochloride at 280nm

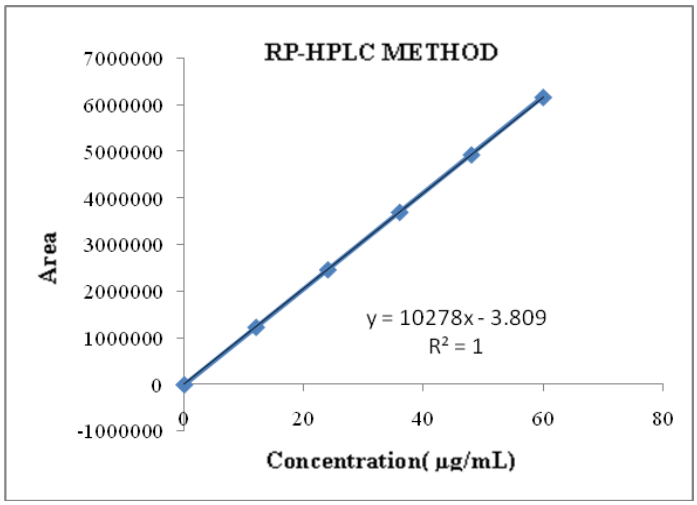

Fig. 6: Calibration Curve of ACF at 280 nm by RP – HPLC Method.

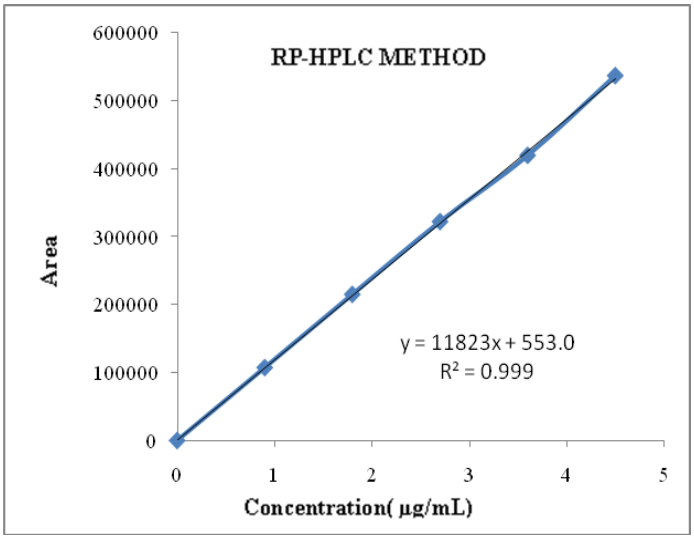

Fig. 7: Calibration Curve of CBP at 280 nm by RP-HPLC Method

Table 1: Summary of Validation and System Suitability Parameters of Aceclofenac and Cyclobenzaprine Hydrochloride.

Parameters	ACF	CBP
Linear Range (µg/ml)	12.0-60.0	0.9-4.5
Slope	10208	11919
Intercept	11237	1.904
Regression coefficient (r^2)	1.000	0.9990
Limit of Detection (µg/ml)	0.6231	0.1245
Limit of Quantification (µg/ml)	1.80	0.6258
Retention time (min)	7.381	4.370
Tailing Factor	1.04	1.083
Resolution Factor	9.195	
Theoretical Plate	5154	5388

Table 2 : Statistical Validation Data for Accuracy determination.

Level of % Recovery	Components	Amount present (µg/ml)	Amount of Standard drug added (µg)	Total amount recovered (µg)	% Recovery	RSD
80%	ACF	36	28.8	64.78	99.96	0.03212
	CBP	2.7	2.16	4.858	99.95	0.04284
100%	ACF	36	36	71.90	99.86	0.03858
	CBP	2.7	2.7	5.39	99.81	0.0386
120%	ACF	36	43.2	79.18	99.97	0.0163
	CBP	2.7	3.24	5.938	99.96	0.01684

***n=3**

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

Component	Mean*	Standard Deviation*	Relative Standard Deviation*	Standard Error*
ACF	99.90	0.0336	0.0336	0.0137
CBP	99.49	0.1839	0.1848	0.0753

***n=6**

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

Component	Mean*	Standard Deviation*	Relative Standard Deviation*	Standard Error*
ACF	99.86	0.0856	0.0857	0.0351
CBP	99.55	0.4402	0.4421	0.1804

***n=3**

Table 5: Robustness for Variation in Flow Rate (ml/min)

Method Parameter	Level	Retention Time		Tailing factor	
Flow Rate (ml/min)		ACF	CBP	ACF	CBP
0.9	-1	7.442	4.420	1.035	1.095
1.0	0	7.381	4.370	1.040	1.080
1.1	+1	7.325	4.335	1.042	1.071

Table 6 : Robustness result for variations in wavelength(nm)

Method Parameter	Level	Retention Time		Tailing factor	
Wavelength (nm)		ACF	CBP	ACF	CBP
278	-2	7.375	4.346	1.045	1.072
280	0	7.381	4.370	1.040	1.080
282	+2	7.386	4.357	1.038	1.084

Table 7: Ruggedness result for variations in analyst.

Method Parameter	Retention Time		Tailing factor	
Analysts	ACF	CBP	ACF	CBP
Analysts 01	7.381	4.370	1.040	1.080
Analysts 02	7.375	4.362	1.046	1.072

CONCLUSION:

An accurate, sensitive and precise HPLC method with UV detection for the simultaneous estimation of Aceclofenac and Cyclobenzaprine Hydrochloride was developed and validated for quality control analysis in combined tablets. The proposed method is rapid, where the total analytical run time for both drugs are less than 10 min and shows high degree of accuracy and precision. It is convenient for laboratory quality control of tablet dosage forms containing both substances.

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