(RESEARCH ARTICLE)

Simultaneous determination and validation of Aceclofenac and cyclobenzaprine hydrochloride by area under curve and q-absorbance ratio methods in bulk and pharmaceutical formulations

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Abstract

A simple, accurate, rapid and UV spectrophotometric methods has been developed for the simultaneous estimation of Aceclofenac (ACF) and Cyclobenzaprine hydrochloride (CBP) in bulk and combined tablet dosage form. The methods employed were(A) Area under curve and (B) Q-absorbance ratio method. Method- A involves measurement of peak area in the wavelength range 271-281 nm and 220-230 nm for ACF and CBP respectively. In Method B two wavelengths 276 nm, λ max of Aceclofenac and 257 nm, the isobestic point were selected. In both the methods linearity was found in the concentration range of 6-30 μg/ml for Aceclofenac and 0.45-2.25 μg/ml Cyclobenzaprine hydrochloride. 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 without any interference by the excipients. The above methods are validated according to ICH guidelines.

Keywords: Aceclofenac; Cyclobenzaprine Hydrochloride; Area Under Curve; Q-Absorbance Ratio

1. Introduction

Aceclofenac 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 [1-4].

Cyclobenzaprine 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. [5-7].

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

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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.

2. Materials and methods

2.1. 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]

2.2. 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.

2.3. Methods

2.3.1. 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 into 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.

2.3.2. 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.

2.4. Methods of Estimation:[10-11]

2.4.1. Method A (Area Under Curve method)

In this method the overlain spectra of both drugs wavelength range of 271 nm to 281 nm for Aceclofenac and 220 nm to 230 nm for Cyclobenzaprine Hydrochloride was selected for the analysis. The calibration curve of area v/s concentration was plotted. By applying Cramer's rule and matrix method, concentration of Aceclofenac and Cyclobenzaprine Hydrochloride can be calculated as,

$$CACF = \frac{XCBP\,\lambda1 - \lambda2\,AUC\,\lambda3 - \lambda4 - XCBP\,\lambda3 - \lambda4\,AUC\,\lambda1 - \lambda2}{XCBP\,\lambda1 - \lambda2\,XACF\,\lambda3 - \lambda4 - XCBP\,\lambda3 - \lambda4\,XACF\,\lambda1 - \lambda2}$$

$$CCBP = \frac{XACF\,\lambda1 - \lambda2\,AUC\,\lambda3 - \lambda4 - XACF\,\lambda3 - \lambda4\,AUC\,\lambda1 - \lambda2}{XCBP\,\lambda1 - \lambda2\,XACF\,\lambda3 - \lambda4 - XCBP\,\lambda3 - \lambda4\,XACF\,\lambda1 - \lambda2}$$

2.4.2. Method B (Q-Absorbance Ratio method)

In this method absorbances are measured at two wavelengths. One being the λmax of Aceclofenac and other being a wavelength of absorptivity of the Cyclobenzaprine Hydrochloride. Then absorbance of both drugs was recorded on

selected wavelengths. Concentrations of Aceclofenac & Cyclobenzaprine hydrochloride were calculated by using following equations.

$$C_{ACF} = Q_m-Q_y \,/\, Q_x-Q_y \text{ x } A_1/ax_1$$

$$C_{CBP} = Q_m-Q_y \,/\, Q_y-Q_x \text{ x } A_1 \,/ay_1$$

Where, Q_m is ratio of absorbances A1 and A2 of mixture at λ1 and λ2 (isobestic point wavelength) Q_x is ratio of absorptivities ax1 and ax2 at λ1 and λ2. Q_y is ratio of absorptivities ay1 and ay2 at λ1 and λ2. C_{ACF} and C_{CBP} are concentrations of Aceclofenac & Cyclobenzaprine Hydrochloride.

2.5. Validation parametre: [12]

2.5.1. 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.

2.5.2. 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.

2.5.3. 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.

2.5.4. 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

$$\text{LOD} = 3.3 \text{ SD/Slope and } \text{LOQ} = 10 \text{ SD/Slope.}$$

SD= Standard Deviation of y- intercept

3. Results and discussion

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
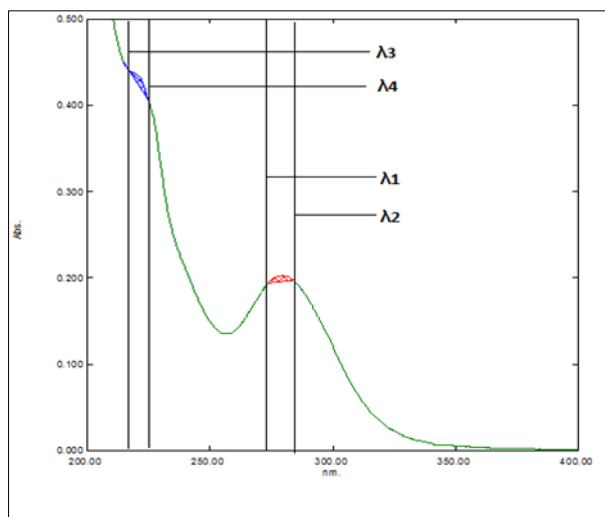
significant and under specification limits, with % recovery 99-100%. The assay results were found to be within the acceptable limits. Hence developed methods was found to be precise and sensitive. [13]
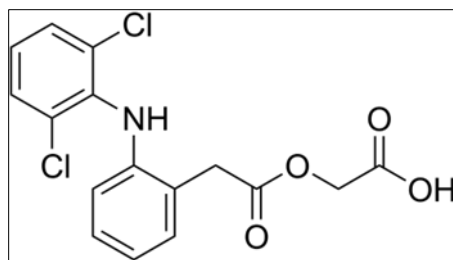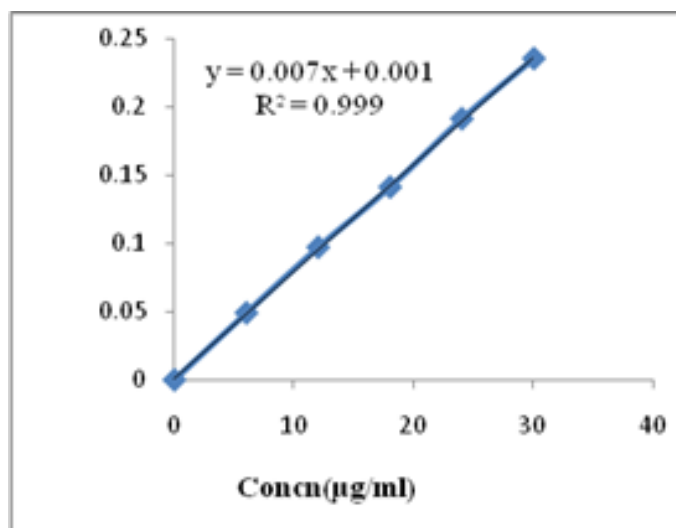

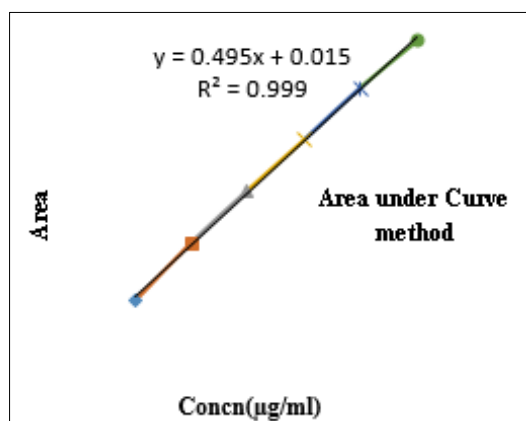**Figure 1** Chemical structure Aceclofenac
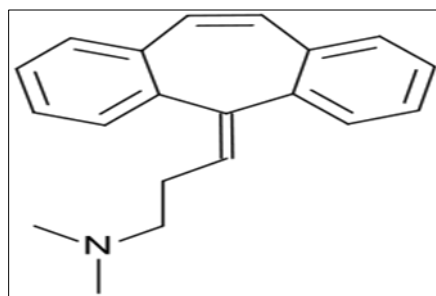
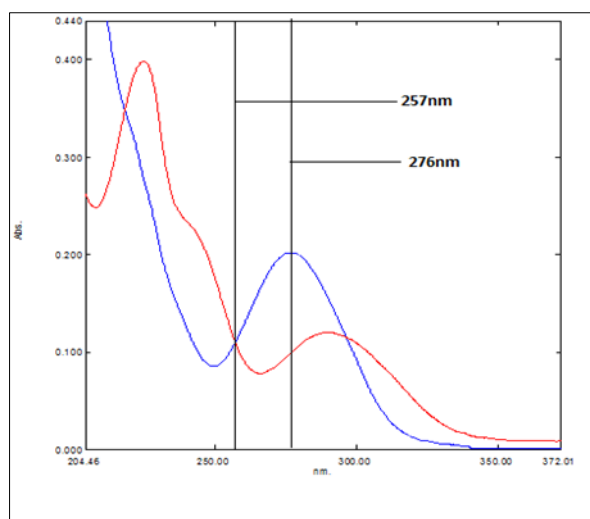**Figure 2** Chemical structure of Cyclobenzaprine

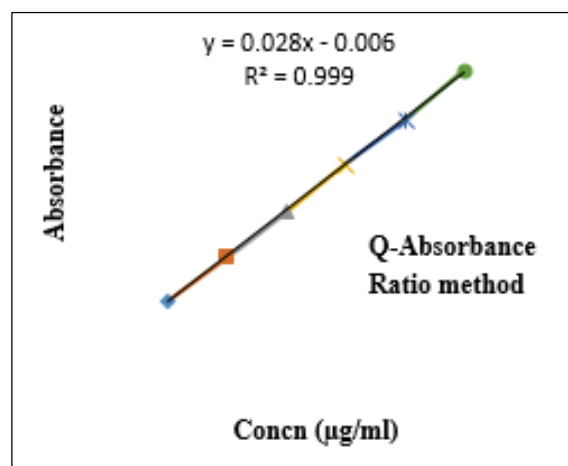**Figure 3** Spectra of ACF and CBP for Area Under Curve method at 271-281nm and 220-230nm

Figure 4 Calibration Curve for ACF between 271-281nm by Area under curve method
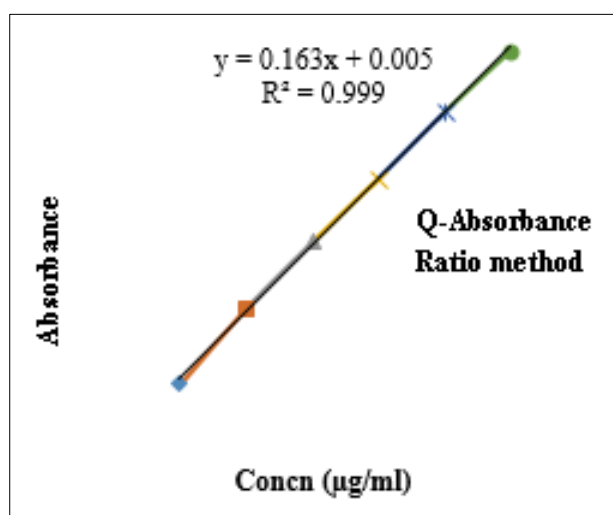

Figure 5 Calibration Curve for CBP between 220-230nm by Area Under Curve Method

Figure 6 Isoabsorptive point of ACF and CBP at 257nm

Figure 7 Calibration curve for ACF at 276nm by Q-Absorbance Ratio Method

Figure 8 Calibration curve for CBP at 257nm by Q-Absorbence Ratio Method

Table 1 Summary of Validation Parameters by Developed Methods

Parameter	Method A		Method B	
	ACF	CBP	ACF	CBP
Wavelength (nm)	271-281	220-230	276	257
Linearity Range (µg/ml)	6-30	0.45-2.25	6-30	0.45-2.25
Regression equation (y = a + bc)	y = 0.007x + 0.001	y = 0.495x+ 0.015	y = 0.028x-0.006	y = 0.163x+ 0.005
Slope (b)	0.007	0.495	0.028	0.163
Intercept (a)	0.001	0.015	0.006	0.005
Correlation Coefficient (r^2)	0.999	0.999	0.999	0.999
LOD (µg/ml)	0.1893	0.0693	0.1632	0.0929
LOQ (µg/ml)	0.5737	0.2102	0.4948	0.2815

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	99.90	0.1390	10.76	99.62	0.2321
	CBP	0.45	0.36	0.749	99.86	0.1338	0.747	99.6	0.2358
100%	ACF	6	6	11.9	99.16	0.2791	11.96	99.66	0.2791
	CBP	0.45	0.45	0.899	99.88	0.1705	0.898	99.77	0.2046
120%	ACF	6	7.2	13.19	99.92	0.0760	13.17	99.72	0.0902
	CBP	0.45	0.54	0.989	99.89	0.1548	0.986	99.59	0.1802

Table 3 Statistical Validation Data for Intra-day Precision

Components	Method A		Method B	
	ACF	CBP	ACF	CBP
Mean	99.73	99.61	99.65	99.71
Standard Deviation	0.0070	0.0070	0.0070	0.0070
Relative Standard Deviation	0.0070	0.0070	0.0071	0.0070
Standard Error	0.0028	0.0029	0.0029	0.0029

*n=6

Table 4 Statistical Validation Data for Inter-day Precision

Components	Method A		Method B	
	ACF	CBP	ACF	CBP
Mean	99.95	99.47	99.76	99.48
Standard Deviation	0.0070	0.0070	0.00707	0.00707
Relative Standard Deviation	0.007	0.0071	0.0070	0.0071
Standard Error	0.0028	0.0028	0.0028	0.0028

*n=3

4. 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.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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