

Development and Validation of RP-HPLC and UV-Spectrophotometric Absorptivity Method for Simultaneous Estimation of Cyclobenzaprine hydrochloride and Aceclofenac in Pharmaceutical dosage form

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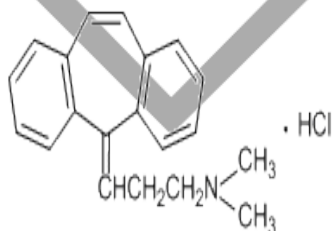
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Abstract: The objective of present research is to develop a simple, sensitive, linear, precise and accurate RP-HPLC and UV-Spectrophotometric method for simultaneous estimation of Cyclobenzaprine hydrochloride and Aceclofenac in bulk and tablet formulation as developed and validated. UV-Spectrophotometric method Calibration plot were linear $R^2=0.9996$ over the concentration range 1-5 μ g/ml for Cyclobenzaprine hydrochloride, $R^2= 0.9995$ for the Aceclofenac 13-65 μ g/ml. And Chromatographic conditions used are stationary phase Inertsil ODS column (250 \times 4.6 mm \times 5 μ) (5 μ m particle size) the mobile phase Methanol: 10mm KH₂PO₄ Buffer (Ph-3) (70:30) and flow rate was maintained 0.9ml/min, detection wavelength was 220nm. The retention times were 4.7min and 2.9 min for Cyclobenzaprine hydrochloride and Aceclofenac respectively. Calibration plot were linear $R^2 = 0.9996$ over the concentration range 3-15 μ g/ml for Cyclobenzaprine hydrochloride, $R^2 = 0.9994$ for the Aceclofenac 40-200 μ g/ml. No interference from any component of pharmaceutical dosage form was observed. The proposed method has been validated as per ICH guidelines, validation studies revealed that method is specific, rapid, reliable and reproducible. The developed method successfully employed for routine quality control analysis in the combined pharmaceutical dosage form.

Keywords: Cyclobenzaprine hydrochloride and Aceclofenac, UV- Spectrophotometric, RP-HPLC Method.

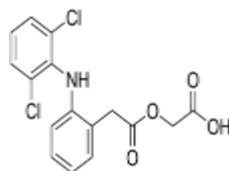
I. INTRODUCTION:-

Cyclobenzaprine hydrochloride is a once daily extended release skeletal muscle relaxant which relieves muscle spasm of local origin without interfering with muscle function. The exact mechanism of action is unknown. The IUPAC name is 3-(5H-Dibenzo [A, D] Cyclohepten-5-Ylidene) N, N-Dimethyl-1-Propanamine. **Absorption:** Immediate-release (IR) mean oral bioavailability ranges from 33% to 55. **Distribution:** About 93% is plasma protein-bound. **Metabolism:** During first pass through GI tract and liver, drug and metabolites undergo enterohepatic recycling. Cyclobenzaprine is eliminated quite slowly, with an effective half-life of 18 hours (range 8-37 hours; n=18); plasma clearance is 0.7 L/min. Metabolites excreted in the urine are likely water-soluble glucuronide conjugates.



Cyclobenzaprine Hydrochloride

Aceclofenac is a non-steroidal agent with marked anti-inflammatory and analgesic properties. The mode of action of aceclofenac is largely based on the inhibition to prostaglandin synthesis. Aceclofenac is a potent inhibitor of the enzyme cyclooxygenase, which is involved in the production of prostaglandins. The IUPAC name is [(2, 6-dichlorophenyl) amino] phenylacetoxyacetic acid. After oral administration, aceclofenac is rapidly absorbed and the bioavailability is almost 100%. Peak plasma concentrations are reached approximately 1.25 to 3 hours. Aceclofenac is highly protein-bound (> 99.7%). Aceclofenac penetrates into the synovial fluid where the concentrations reach approximately 60% of those in plasma. Aceclofenac is probably metabolized via CYP2C9 to the main metabolite 4-hydroxyaceclofenac. The mean plasma elimination half-life is 4-4.3 hours. Approximately two-thirds of the administered dose is excreted via the urine, analysis conjugated hydroxymetabolites.



Aceclofenac

The present research invention that there are many methods for the individual determination of Cyclobenzaprine hydrochloride and Aceclofenac but few methods are cited for determination of combined dosage form so, it was proposed to develop an economical, rapid and Absorptivity UV Spectrophotometric and RP-HPLC method for simultaneous estimation of these drugs in combined dosage form.

2. MATERIAL AND METHODS:-

2.1 Reagent and chemicals

Cyclobenzaprine hydrochloride (Titan Lab Mahad) and Aceclofenac (Cipla Mumbai) were received as gift sample. Marketed formulation (Cyclobenzaprine hydrochloride and aceclofenac 2 FLEXABENZ PLUS) containing 15 mg of Cyclobenzaprine hydrochloride and aceclofenac 200 mg was procured from local market. HPLC grade acetonitrile and purified grade potassium dihydrogen phosphate were purchased from Fischer Chemical LTD, India and Merck, India respectively. All other reagents employed were of high purity analytical grade. All weighing was done on a calibrated analytical balance. Calibrated glass wares were used throughout the work. Double distilled water and Milli-Q water were used in the UV method and RP-HPLC method respectively.

2.2. UV spectrophotometric method

2.2.1. Instrumentation

The UV method was performed on SHIMADZU double beam spectrophotometer (Model: UV-1800) with 2 nm spectral bandwidth using 10 mm matched quartz cuvettes. Data acquisition was done by using UV-probe software version 2.42. The absorption spectra of reference and test solution were carried out over the range of 200–400 nm.

2.2.2. UV- Spectrophotometric Method- Determination of Absorptivity of drugs at selected wavelengths

Aliquot portions of CBP-H from stock solution were transferred to 10 ml volumetric flasks; volume was adjusted to mark to obtain the concentration of 1 µg/ml. Similarly, aliquot portions from ACF stock solution was transferred to 10 ml volumetric flasks; volume was adjusted to mark to obtain Concentration of 13 µg/ml. Absorbance of these solutions was recorded at two wavelength 290 nm and 274 nm. A (1%, 1 cm) values of drugs were calculated using following formula equation no 1 and 2

2.2.3. Simultaneous estimation of drugs in standard laboratory mixture

In order to study the practicability of proposed method for simultaneous estimation of cyclobenzaprine HCL and aceclofenac in marketed pharmaceutical formulations, the method was first tried for estimation of drugs in standard laboratory mixture. Accurately weighed 10 mg CBP-H and 10mg ACF were transferred to 10 ml volumetric flask with HCL. Sonicated for 10 minute and the volume was adjusted to the mark with the same solvent. Appropriate aliquot portion of these solutions were mixed to get the concentration 1000 µg/ml of CBP-H and ACF. Absorbance was measured at 290 nm and 274 nm against Methanol as blank. Amount of each drug was estimated using I and II equation as follows

$$C_x = \frac{A_{2y1} - A_{1y2}}{a_{x2y1} - a_{x1y2}} \quad \text{----- 1}$$

$$C_y = \frac{A_{1x2} - A_{2x1}}{a_{x2y1} - a_{x1y2}} \quad \text{----- 2}$$

Where,

A1 and A2 are the absorbance of the sample solution measured at 257 nm and 271 nm respectively. Cx and Cy are concentration of TPE-H and PHE-H respectively. ax1 and ax2 are absorptivity's of TPE-H at 272 nm and 290 nm respectively; ay1 and ay2 are absorptivity's of PHE-H at 272 nm and 290 nm respectively.

2.2.4. Simultaneous estimation of drugs in tablets

Five tablets were weighed and ground to fine powder. An accurately weighed 365 mg (equivalent to 15mg of CBP-H And 200mg of ACF) of tablet powder was transferred to 100 ml volumetric flask containing about 10ml methanol sonicated for 10 min and volume was made up to the mark with the 90 ml water solvent to get concentration of 2 µg/ml CBP-H and 13 µg/ml of ACF and filtered through Whatman filter paper (No. 41). The absorbances were recorded. The concentrations of two drugs in sample were determined using equation no. I and II Brand Name: Flexabenz plus Average Wt.: 365 mg. Composition: CBP-H 15mg and ACF 200mg

2.2.5. Selection Of Wavelength: -

The UV spectrum of Cyclobenzaprine Hydrochloride and aceclofenac in 10 mg both drug dissolved in 10 ml methanol has maximum absorption (mix), at 290 nm and 274nm. The absorbance of excipients in tablet solution did not interfere with

Cyclobenzaprine Hydrochloride and aceclofenac at 290nm & 274nm respectively. As a result, the wavelength was selected for quantitative analysis

2.2.6. Preparation of calibration curve

The stock solutions of Cyclobenzaprine Hydrochloride and Aceclofenac were used to prepare two different sets of diluted standards. Appropriate aliquots of CBP and ACF stock solutions were taken in different 10 ml volumetric flasks and diluted up to the mark with water to obtain final concentrations in the range of 1, 2, 3, 4, 5, µg/ mol of CBP and 13, 26, 39, 52, 65, µg/ ml of ACF respectively. Spectra of solutions were scanned between 200 –400 nm and Calibration curves were constructed relating the peak amplitude at 290nm of CBP and at 274nm of ACF to the corresponding concentrations and regression equations were computed for CBP and ACF.

2.2.7. Preparation of the sample solution

These were labelled to contain 15 mg of Cyclobenzaprine HCL and 200 mg of Aceclofenac as an active substance per tablet. 5 tablets containing 365.5mg Cyclobenzaprine HCL and of Aceclofenac were accurately weighed and powdered. The powder equivalent to 200mg of Aceclofenac and 15mg Cyclobenzaprine Hcl as weighed 18.25mg and transferred to a 100 mL volumetric flask; 10 MI Methanol was added and sonicated for 20 min. The volume was adjusted to 90mL with water. The solution was filtered through Whatman filter paper. In order to obtain the final concentration of Cyclobenzaprine HCL Aceclofenac 100µg/ml 200µg/ml respectively

UV spectrum of pure drug Cyclobenzaprine hydrochloride at 290(λmax)

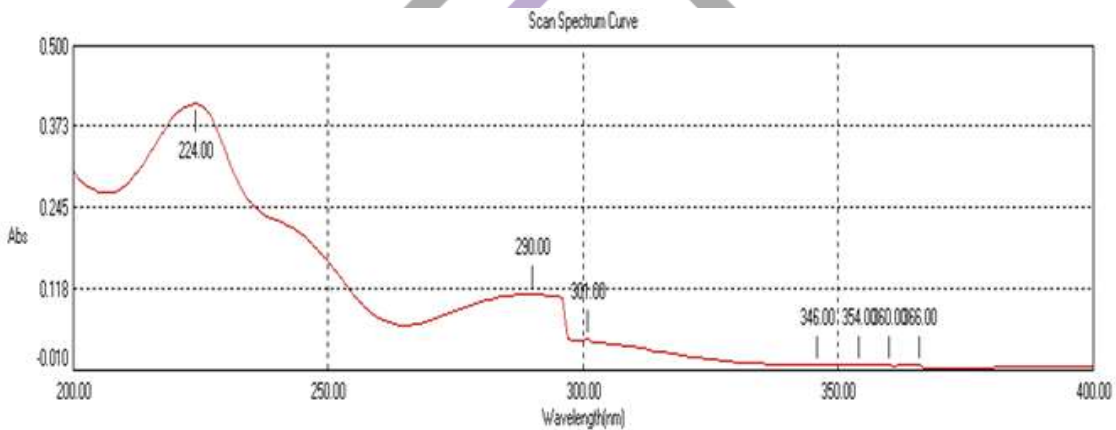


Fig.No.1 UV spectrum of pure drug cyclobenzaprine hydrochloride at 290 (λmax)

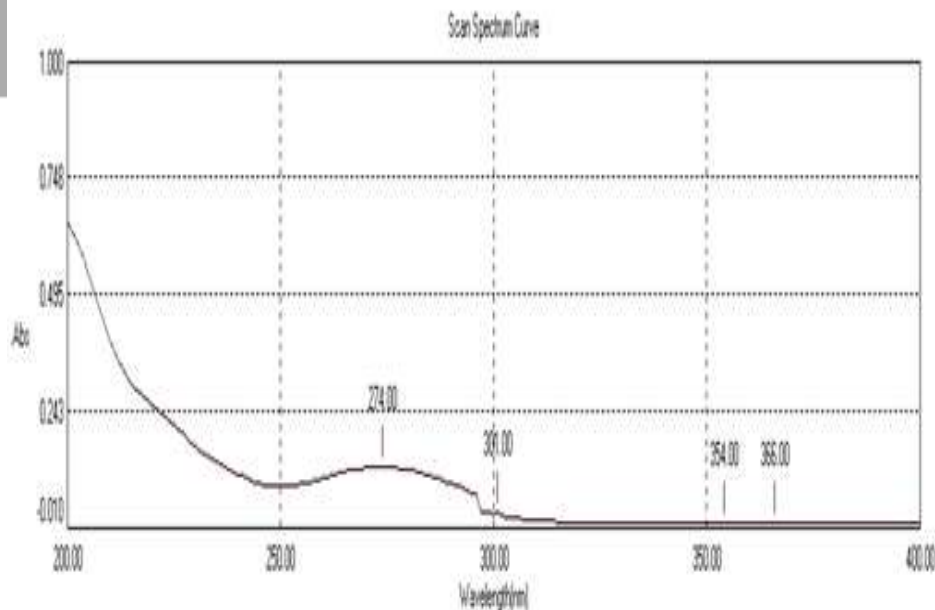


Fig. No. 2 UV spectrum of pure drug Aceclofenac at 274 (λmax)

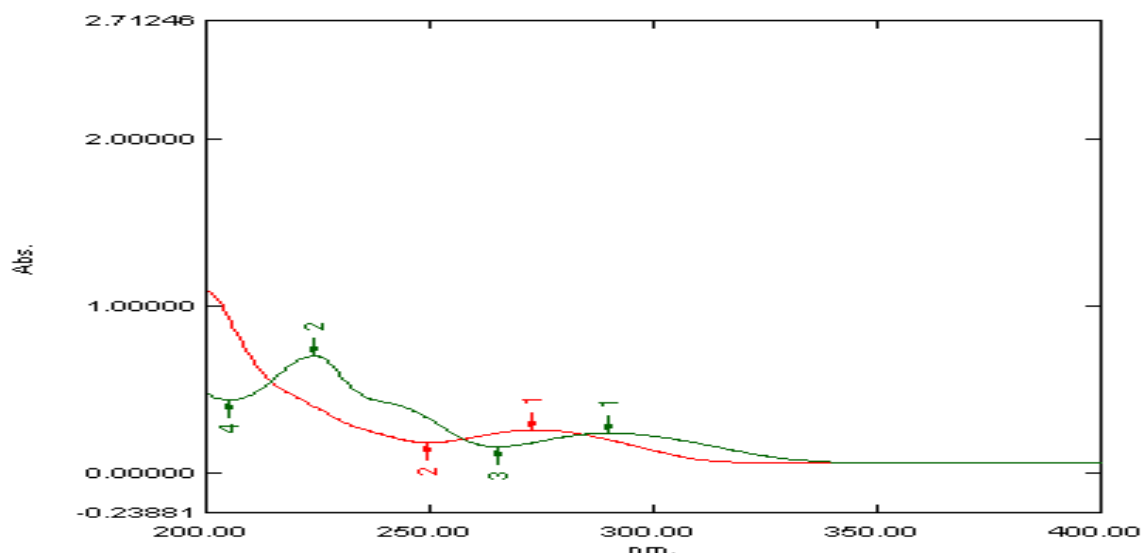


Fig. No. 2 overlay UV spectra Cyclobenzaprine Hydrochloride and Aceclofenac

2.3 RP-HPLC METHOD

2.3.1 Instrumentation

HPLC (Water 600 controller) instrument equipped with a model code 6CE In Line Degasser Af, Reciprocating pump, Rheodyne 7725i manual injector with a 20 μ l fixed loop and HPLC syringe of 100 μ l and with UV-Vis detector. Separations and quantitation were made on RP-18, Inertsil ODS column (250 \times 4.6 mm \times 5 μ) (5 μ m particle size). Analytical balance used for weighing standard and sample was SHIMADZU AUX 220. The flow rate was set to 0.9 ml/min and detection of both drugs was carried out at 290 nm and 274 nm nm by UV detector.

2.3.2. Chromatographic condition

The optimal composition of the mobile phase was determined to be potassium dihydrogen phosphate buffer pH 5.3: Acetonitrile (55:45 v/v). The mobile phase was filtered through nylon 0.45 μ m membrane filter and was degassed before use (30 min). Stock solution was prepared by dissolving CBP-H and ACF (10 mg each) that were weighed accurately and separately transferred into 100 ml volumetric flasks. Both drugs were dissolved in 25 ml of mobile phase to prepare standard stock solutions. After the immediate dissolution, the volume was made up to the mark with mobile phase. These standard stock solutions were observed to contain 100 μ g/ml of CBP-H and ACF. Appropriate volume from this solution was further diluted to get appropriate concentration levels according to the requirement. From the above stock solutions, dilutions were made in the concentration range of 1–6 μ g/ml of both drugs. A volume of 50 μ l of each sample was injected into column.

2.3.3. Preparation of buffer

0.02 M potassium dihydrogen phosphate buffer of pH 5.3 was used for method development. Buffer was prepared by dissolving 13.6 g of potassium dihydrogen phosphate by diluting with Milli-Q water to 1000 ml. Then stock solution was further diluted to get 0.02M Phosphate buffer. The pH was adjusted by ortho-phosphoric acid using pH meter (Eutech Instruments, Singapore). The prepared buffer was passed through 0.45 μ m membrane filter (Milipore, USA) and the same was used for mobile phase preparation.

2.3.4. Preparation of Mobile Phase

Mobile phase was prepared by mixing Methanol and 0.02 M Phosphate Buffer in ratio [70:30], filter through 0.45 μ m nylon membrane filter and degassed. Mixture was shaken vigorously and sonicated for 30 min prior to use.

2.3.5. Preparation of stock solutions and test solutions (CBP-H, ACF and binary mixture)

Aqueous solution (100 μ g/ml) of CBP-H, ACF and its binary mixture was prepared by adding accurately weighed 10 mg of CBP-H and ACF and binary mixture of both drugs in 50 ml of mobile phase, then sonicated for 10 min and diluted up to 100 ml. Series of test solutions were prepared in the concentration range of 1–6 μ g/ml. by diluting appropriate volume of the stock solution (100 μ g/ml) with mobile phase. The dilutions were first vortexed and then used for further analysis.

2.3.6. Preparation of calibration curve

The calibration curve was prepared by injecting concentration of 1–6 μ g/ml for CBP-H, ACF and binary mixture solutions manually in triplicate to the HPLC system at detection wavelength of 290 and 273 nm. Mean of n = 6 determinations was plotted as

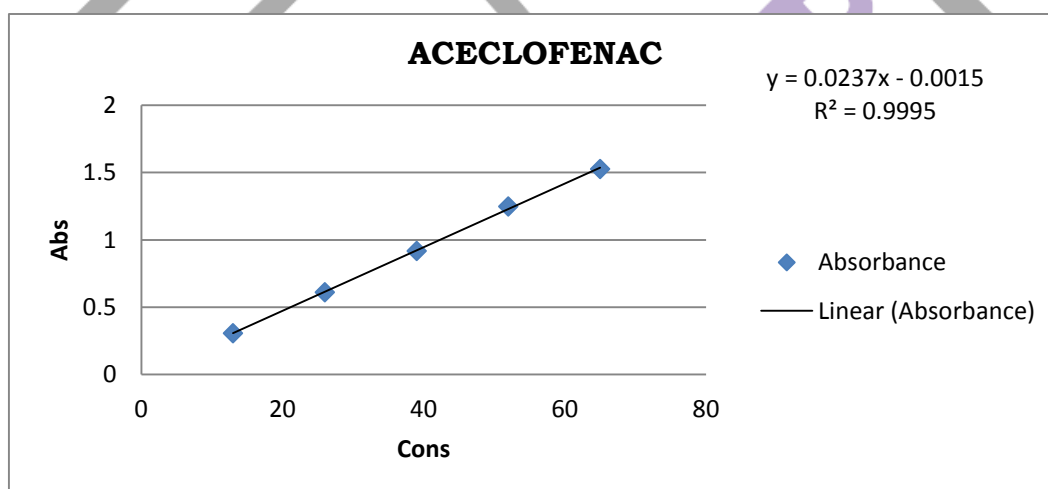
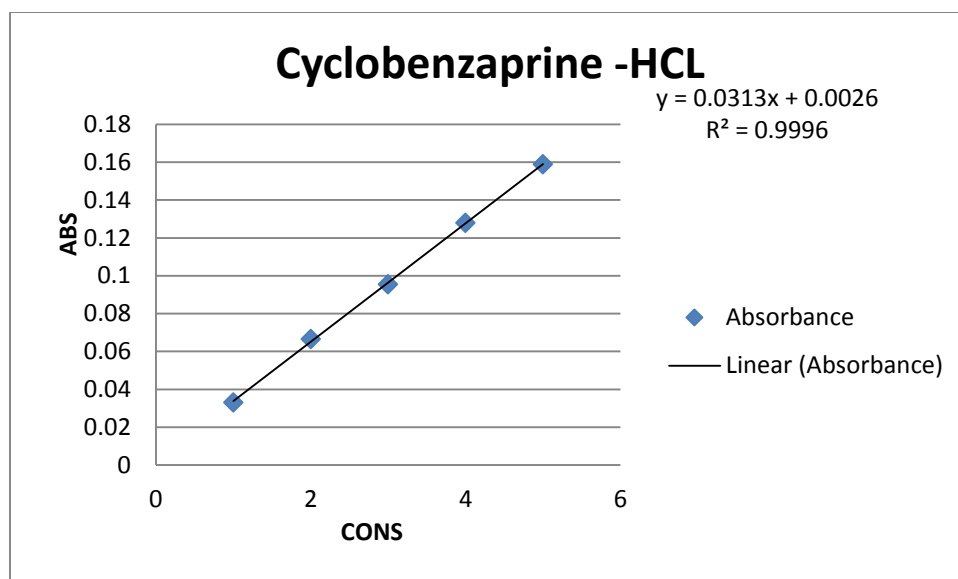
the standard curve. The calibration curve was tested by validating it with inter-day and intra-day measurements. Linearity, accuracy and precision were determined for both interday and intra-day measurements.

2.4 Method Validation:-

The RP- HPLC method was validated as per ICH guidelines.

2.4.1. Linearity

The methods were validated according to International Conference on Harmonization Q2B guidelines (2005) for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for each analyte. Calibration curves were generated with appropriate volumes of working standard solutions for both UV and HPLC with the range of 1-15 and 40-200 µg/ml respectively. The linearity was evaluated by the least square regression method using unweighted data.



2.4.2. Precision and accuracy

Both precision and accuracy were determined with standard quality control samples (in addition to calibration standards) prepared in triplicates at different concentration levels covering the entire linearity range. Precision is the degree of repeatability of an analytical method under normal operational conditions. The precision of the assay was determined by repeatability (intra-day) and intermediate precision (interday) and reported as %R.S.D. for a statistically significant number of replicate measurements.

The intermediate precision was studied by comparing the assays on 3 different days and the results documented as standard deviation and %R.S.D. Accuracy is the percent of analyte recovered by assay from a known added amount. For the measurement of accuracy data from nine determinations over three concentration levels covering the specified range were determined.

2.4.3. Specificity

The method specificity was assessed by comparing the chromatograms (HPLC) and scans (UV) obtained from the drug and the most commonly used excipient mixture with those obtained from blank (excipient solution in water without drug).

The excipients chosen are the ones used commonly in tablet formulation, which included di-calcium phosphate (DCP), lactose, starch, micro-crystalline cellulose (MCC), polyvinyl pyrrolidone (PVP), sodium starch glycolate (SSG) and magnesium stearate. The drug to excipient ratio used was similar to that in the commercial formulations.

2.4.4. LOD and LOQ

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability (ICH guideline Q2B, 2005). The LOD and

LOQ were calculated as

$$\text{LOD} = \frac{3}{S} \sigma$$

$$\text{LOQ} = \frac{10}{S} \sigma$$

Where σ is the standard deviation of the lowest standard concentration and S is the slope of the standard curve.

2.5. Analysis of marketed tablet formulation (CBP-H ACF)

Twenty tablets of marketed formulation (CBP-H ACF) were weighed and grounded to obtain fine powder. Accurately weighed powder sample equivalent to **5 mg of ACF and 10 mg of CBP** was dissolved in a 100 ml volumetric flask containing 0.01 N HCl. The solution was kept for sonication for 20 min, filtered through Whatmann filter paper No. 41. Aliquot of this solution was diluted to produce the concentration of 5 $\mu\text{g/ml}$ for AM and 10 $\mu\text{g/ml}$ for BZ ($n=6$). The absorbance of sample solutions at 237 and 366 nm was measured and the amount of drug present in the sample solution was calculated in the same manner as that of pure mixed standard solution.

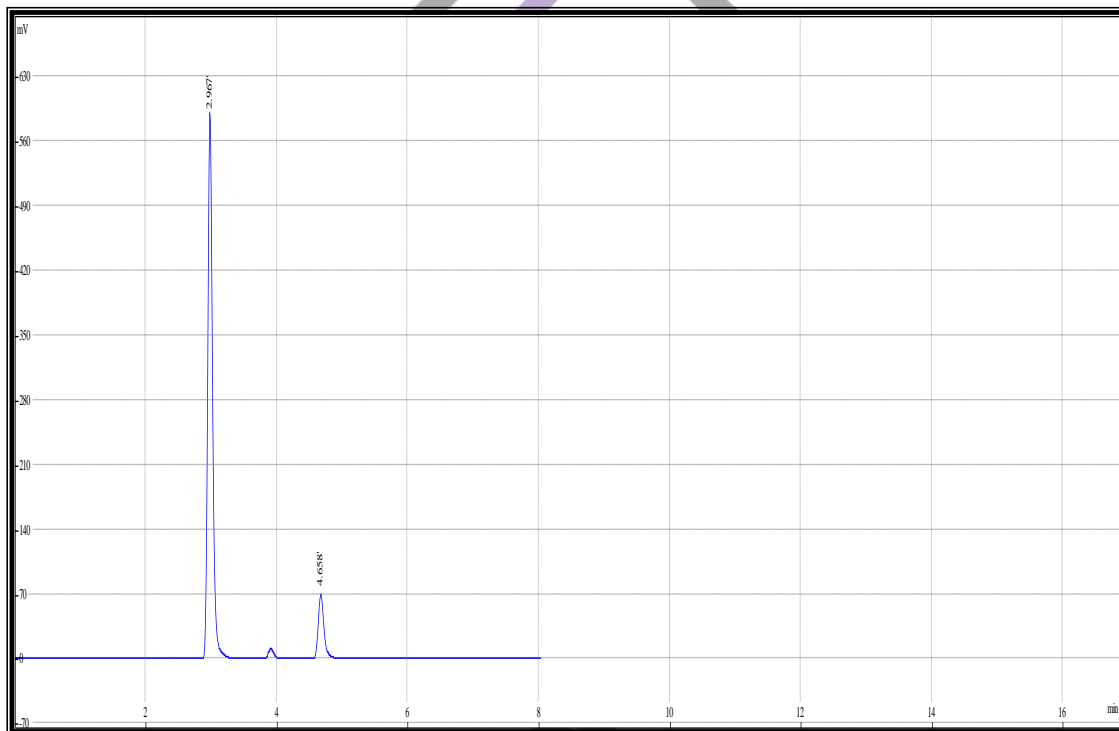


Fig. No.5 Chromatogram of Cyclobenzaprine & Aceclofenac

3 RESULT AND DISCUSSION

3.1 RP-HPLC and UV-method validation

RP-HPLC and UV-spectrophotometric methods were developed for Cyclobenzaprine hydrochloride and Aceclofenac which can be conveniently employed for routine analysis in pharmaceutical dosage forms and will eliminate unnecessary tedious sample preparations. The Chromatographic conditions were optimized in order to provide a good performance of the assay. The retention time (Rt) of Cyclobenzaprine hydrochloride and Aceclofenac were 4.7 and 2.9 min respectively are shown in fig. no. The chromatograms have been shown in fig. 5 A seven point calibration curve was constructed with working standard and was found linear (0.999) for each of the analysts over their calibration ranges. The Slopes were calculated using the plot of drug concentration versus area of the chromatogram. The developed RP-HPLC method was accurate, precise, reproducible and very sensitive. Fig. 2 shows overlay spectra of both drugs of the UV-Spectrophotometric method. The regression coefficient of the correlation equation curve was greater than 0.999 and the method was validated by using binary mixture of both drugs with less than 2% RSD (Table 4). All the method validation parameters are well within the limits as specified in the ICH Q2B guidelines as shown in Table 2.

The intra- and inter-day precision (%R.S.D.) at different concentration levels was found to be less than 2% (Table 3). Table 4 lists the percent recovery (content uniformity) of both drugs in the commercial formulations by HPLC and UV methods. Moreover the %R.S.D. (less variation) shows good precision of both developed methods. The calculated LOQ and LOD concentrations confirmed that the methods were sufficiently sensitive. The methods were specific as none of the excipients interfered with the analytes of interest (Table 4). Hence, the methods were suitably employed for assaying both the drugs in commercial marketed formulation (Table 3).

Table No.1: Absorptivity values of CBP-HCL and ACF at 290 nm and 274 nm

CBP-HCL					ACF				
Conc	abs at $\lambda 1$	ax1	abs at $\lambda 2$	ax2	conc	abs at $\lambda 1$	ay1	abs at $\lambda 2$	ay2
1	0.032	0.0320	0.021	0.020	13	0.210	0.0161	0.310	0.02384
2	0.065	0.0320	0.041	0.0205	26	0.412	0.0158	0.612	0.02353
3	0.096	0.0323	0.615	0.0201	39	0.620	0.0158	0.918	0.02359
4	0.129	0.0322	0.0789	0.0190	52	0.810	0.0157	1.249	0.02401
5	0.159	0.03122	0.102	0.020	65	1.020	0.0156	1.527	0.02349
	Mean	0.03296	Mean	0.02093		Mean	0.01580	Mean	0.02369

TableNo.2: Result of Analysis of CBP-HCL and ACF in Standard Laboratory Mixture

Sr.no	Drugs	Conc. of std ($\mu\text{g}/\text{ml}$)	Amount found ($\mu\text{g}/\text{ml}$)	% Amount found
1	CBP-HCL	2	2.04	102.2
2	ACF	26	26.22	100.84

Table No. 3: Application of Proposed Method for Analysis of Tablet

Sr.no	Drugs	Conc. of tab ($\mu\text{g}/\text{ml}$)	Mean Amount found ($\mu\text{g}/\text{Ml}/\text{tab}$)	%Amount found
1	CBP-HCL	2	1.98	99.89
2	ACF	26	25.90	99.61

3. Statistical comparison of HPLC and UV methods

Statistical comparison was done on assay results obtained from UV and HPLC methods for marketed formulation (CBP-H ACF) by using student's t-test. Calculated values for t-test were 1.98 and 1.90 for CBL-HCL and ACF respectively which is less than table value (2.306) indicating that there was no significant difference between the HPLC method and UV method.

Table no4: Linear regression data of UV - Method

Sr. No	Parameters	Results	
		Cyclobenzaprine Hydrochloride	Aceclofenac
1	λ_{max} (nm)	290nm	274nm
2	Beer's law limit($\mu\text{g}/\text{mL}$)	1-35	1-200
3	Correlation coefficient(r^2)	0.999	0.999
4	Regression equation	$Y=0.031x+0.002$	$Y=0.023x-0.001$
5	Slope (m)	0.031	0.023
6	Intercept (c)	0.002	0.001
7.	Assay%	99.89%	99.61%

Sr. No.	Parameters	Results			
		Cyclobenzaprine hydrochloride		Aceclofenac	
1	Mix (nm)	220nm		220nm	
a.	Beer's law limit($\mu\text{g/mL}$)	1-35 $\mu\text{g/ml}$		1-200 $\mu\text{g/ml}$	
b.	Correlation coefficient(r^2)	0.999		0.999	
c.	Regression equation	Y=20287x+31921		Y=2309.x- 32532	
d.	Slope (m)	20287		2309	
e.	Intercept (c)	31921		32532	
2.	Precision	Intraday Precision (Morning)		Intraday Precision (Morning)	
		S.D.	R.S.D.	S.D.	R.S.D.
		427.1783	0.0139	309.49205	0.0724
		Intraday Precision (Evening)		Intraday Precision (Evening)	
		S.D.	R.S.D.	S.D.	R.S.D.
		1767.300	0.0344	1762.837	0.4121
3.	Accuracy	R.S.D.		R.S.D.	
		0.07111		0.25235	
4.	LOD	0.01210		0.05905	
5.	LOQ	0.03667		0.17894	
6.	Robustness	S.D.	%RSD	S.D.	%RSD
		1307.8	0.10527	1659.99	1.0935
7.	% Assay	100.016%		100.015%	

CONCLUSION

Simple, rapid, accurate and precise RP-HPLC as well as spectrophotometric methods have been developed and validated for the routine analysis of Cyclobenzaprine hydrochloride and Aceclofenac in API and tablet dosage forms. Both methods are suitable for the simultaneous determination of Cyclobenzaprine hydrochloride and Aceclofenac in multi-component formulations without interference of each other. The developed method is recommended for routine and quality control analysis of the investigated drugs in two component pharmaceutical preparations. The amount found from the proposed methods was in good agreement with the label claim of the formulation. Also the value of standard deviation and coefficient of variation calculated were satisfactorily low, indicating the suitability of the proposed methods for the routine estimation of tablet dosage forms. The developed method can also be conveniently adopted for dissolution testing of Cyclobenzaprine hydrochloride and Aceclofenac in commercial formulation.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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AG, MM determined the architectural and contents of the manuscript. RG and PB carried out the data collection and drafted the manuscript. All the authors read and approved the final manuscript.

REFERENCES:

- [1] Rajendra Patil, Review On Analytical Method Development And Validation, Research And Reviews: Journal Of Pharmaceutical Analysis 2014.
- [2] Skoog, Holler, Fundamentals of Analytical Chemistry; Thomson Asia Pt. Ltd Singapore, 2004; 788-807, Page No. 973-992.
- [3] A.H. Beckett, J. B. Stenlake, the Practical Pharmaceutical Chemistry; CBS Publishers and Distributors, New Delhi, Part II, 1997; Page No. 255-346.
- [4] Katz, E., Eksteen, R., Schoenmakers, P. and Miller, N., and Handbook of HPLC, Volume 78, and USA: CRC Press, 1 Christian G. D In: Analytical Chemistry, 4th Edn. John Wiley and Sons, United Kingdom, (1986) Page No 1-6.
- [5] P.D. Seth High Performance Liquid Chromatography, CBS Publication. Vol:1-4 Analysis of Pharmaceutical Formulations; CBS Publishers and Distributor Pvt. Ltd.; 1st ed.; Volume. I; 2010; Page No.50-63.

- [6] Ravisankar A Review on Analytical Method Development, Indian Journal of Research in Pharmacy and Biotechnology ISSN 2321-5674.
- [7] Lough W.J., Wainer I.W.W., HPLC fundamental principles and practices, Blackie Academic and professional, (1991),Page No .52-67.
- [8] Mendham, J., Denny, R. C., and Thomas, M.,In Vogel's text book ofQuantitative Analysis, 6th Ed., Pearson Education Limited, (2004) 1-12.
- [9] M.S. Tswett, on the new form of adsorption phenomena and its application in biochemical analysis, Proc. Warsaw Natural Biol. Society 1903, 14, 20.
- [10] M. S. Tswett, Chromophils in Animal and Plant World, Doctor of Science Dissertation, Warsaw, 1910, 379.
- [11] Indian Pharmacopoeia, Volume II, Government of India, Ministry of Health and Family Welfare, Published by The Indian Pharmacopoeia Commission, Ghaziabad 2007, Page no.893-894.
- [12] ICH, Q2A, Text on Validation of Analytical Procedures, International Conference on Harmonization, Geneva, October 1994, 1-5.
- [13] ICH, Q2B, Guidelines, Validation of Analytical Procedures: Methodology, recommended on November by the ICH steering committee, 1996; 1-International Conference on Harmonization (1999) Specifications,Q6A: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products. Chemical substances 65(146): page No. 6748.
- [14] Validation of Analytical Procedures: Text and Methodology Q2 (R1), ICH Harmonised Tripartite Guideline, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 30th ed. London: European Medicines Agency, 2005.
- [15] Roshni D Patel, Usmangani K Chhalotiya, Falgun A Mehta et.al, Liquid Chromatographic Estimation Of Cyclobenzaprine Hydrochloride And Aceclofenac In Pharmaceutical Formulation,Research And Reviews: Journal Of Pharmacy And Pharmaceutical Sciences ,Volume 3 (2014), Page No.36-42.
- [16] S.M. Ashraful Islam, Sharmi Islam, Mohammad Shahriar And Irin Dewan, Comparative In Vitro Dissolution Study Of aceclofenac Marketed Tablets In Two Different Dissolution Media By Validated Analytical Method, Journal Of Applied Pharmaceutical Science, Volume 01 (09); (2011) Page No.87-92.
- [17] S.M. Ashraful Islam, Sharif Md. Abuzar and Pijush Kumar Paul, Validation of UV-Spectrophotometric and RP-HPLC Methods For the Simultaneous Analysis Of Paracetamol And Aceclofenac In Marketed Tablets, Int. J. of Pharm. & Life Sci. (IJPLS), Volume 2, (2011), Page No.1267-1275.
- [18] P. P. Dash, MK Saho, Simple Spectrophotometric Methods for estimation of Aceclofenac From Bulk And Formulations, Pharmaceutical Methods Volume 2, (2010) Page No.58-65.
- [19] Rachana R Joshi, Maithili K Golhar, Krishna R Gupta et.al, Development And Validation of spectrophotometric Methods For determination Of Aceclofenac In Tablets, Int. J. Chemtech Volume 3(2), (2011) Page No.780-790
- [20] Sneha J. Joshi,Pradnya A. Karbhari, et.al.RP-LC Gradient Elution Method For Simultaneous Determination Of Thiocolchicoside, Aceclofenac and Related Impurities In Tablet Formulation, Journal Of Pharmacy And Bio-allied Sciences, Journal Of Pharmacy And Bio-allied Sciences ,Volume 6 (2014),Page No-36.
- [21] P. N. Patel, G. Samanthula, V. V. Shrigod , Validated Stability-Indicating Assay Method For Simultaneous Determination Of Aceclofenac and thiocolchicoside Using RP-HPLC,Volume1, (2013),Page No.429-435,
- [22] Atul Singh Rathore, Preeti Chandra, Sathiyarayanan Lohidasan et.al, Application Of HPLC For The Simultaneous Determination of Aceclofenac, Paracetamol And Tramadol Hydrochloride In Pharmaceutical Dosage Form,Scientific Pharmaceutical, (2012),Page No.337-351.
- [23] Falgun A Mehta, Usmangani K Chhalotiya, Falgun A Mehta et.al, Liquid Chromatographic Estimation Of Cyclobenzaprine Hydrochloride And Aceclofenac In Pharmaceutical Formulation,Research And Reviews: Journal Of Pharmacy And Pharmaceutical Sciences ,Volume 3 (2014), Page No.36-42.
- [24] M. Constanzer, C. Chavez, B. Matuszewski,Development and Comparison of High-Performance Liquid Chromatographic methods with tandem mass spectroscopic and UV absorbance detection for the determination of cyclobenzaprine in human plasma and urine, Journal of chromatography, B 666 (1995) , Page No. 117-126.
- [25] Ashok R. Parmar, Dharmishtha N. Bhaskhar et.al,Simultaneous Estimation Of Aceclofenac And Serratiopeptidase In Tablet Dosage Form By Absorbance Ratio Method Using Visible Spectrophotometry,Pelagia Research Library,Der Pharmacia Sinica, 2012, (3) Page No.326.
- [26] Minal T. Harde A, Sagar B. Wankhede B, Praveen D. Chaudhari A, A Validated Inherent Stability Indicating HPTLC Method For Estimation Of Cyclobenzaprine hydrochloride In Tablets And Use Of MS-QTOF In characterization Of Its Alkaline Stress Degradation Product, Bulletin Of Faculty Of Pharmacy, Volume No.3, (2016) Page No.3-4.
- [27] Pravin D. Chaudhari, Chetna Ramchandra Madke, Hitesh Motilal Jain et.al, Development And Validation Of Simple UV Spectrophotometric Method For The Estimation Of Cyclobenzaprine Both In Bulk & In Marketed Dosage formulation, World Journal Of Pharmacy And Pharmaceutical Sciences, Volume 3,(2014.) Page No. 1954-19604.
- [28] Jian-Chun Lil, Fei-Hu Chen1, Jun-Dong Zhang, Hai-Jun Dong And Shu Gao, A Sensitive, Fast And Accurate Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry (LC-MS/MS) Method For The Pharmacokinetic Study Of Cyclobenzaprine Tablets, African Journal Of Pharmacy And Pharmacology Volume. 6(10), (2012) Page No. 708-716.
- [29] Wenhong Yu, Xiaojing Yang, Wenwen Sui, et.al,Rapid and Sensitive Analysis of Cyclobenzaprine byLCMS/MS: Application To A Pharmacokinetic Study Of Cyclobenzaprine In Dog, Assian Journal Of Pharmaceutical Science, (2014) Page No 117-122.
- [30] Ting Hui Ching, Tatianemaria De, Lima Souza Brioschi, et.al, Pharmacokinetics And Bioequivalence Evaluation Of Cyclobenzaprine Tablets, Biomed Research International, (2013) ,Page No.1-5.

- [31] Deepali Garge, Pandurang Dhabale, Simultaneous Estimation Of Aceclofenac And Paracetamol In Solid Dosage Form By RP-HPLC Method, Int. J. Chemtech, Volume 2, (2010), Page No. 942-946.
- [32] Mehta, Usmangani K. Chhalotiya, Development And Validation Of First Order Spectrophotometric Method For The Estimation Of Cyclobenzaprine Hydrochloride And Aceclofenac In Its Pharmaceutical Dosage Form, International Journal Of Pharmacy And Integrated Life Sciences, Volume 2(5) (2014) Page No. 80-85.
- [33] Madhuri D, Srujani Ch Sraavathi et.al, Validated UV Spectrophotometric Methods For The Estimation Of Aceclofenac In Bulk and Pharmaceutical Formulation, Sch. Acad. J. Pharm., 2014; 3(6): Page No. 471-476
- [34] Chromatographic Methods With Tandem Mass Spectrometric And Ultraviolet Absorbance Detection For The Determination of cyclobenzaprine In Human Plasma And Urine, Journal Of Chromatography B, Volume 666 (1995), Page No. 117-126.

