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Water Soluble Method Development and Validation of Cyclobenzaprine Hydrochloride and Aceclofenac in Bulk and Tablet Dosage form By UV-Visible Spectroscopy.

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ABSTRACT

A Water Soluble UV spectrophotometric method development using simultaneous equation and Q- absorbance ratio method was developed for determination of Cyclobenzaprine hydrochloride and Aceclofenac in a binary mixture. In the proposed method, the signals were measured at 233.0 nm and 268.9 nm corresponding to absorbance maxima of Cyclobenzaprine hydrochloride and Aceclofenac in mixture of 20% urea and sodium benzoate. Isobestic point was found at 245 nm. Linearity range was observed in the concentration range of 2-10 µg/ml for Cyclobenzaprine hydrochloride and 5-25 µg/ml for Aceclofenac. Concentration of each drug was obtained by using the absorptivity values calculated for both drugs at two wavelengths, 233.0 nm and 268.9 nm and solving the simultaneous equation. For Q-Absorbance ratio method Concentrations of both drug was found at Isobestic point 245 nm. Developed method was applied to laboratory mixture and its pharmaceutical formulation. The method was validated statistically and recovery study was performed to confirm the accuracy of the method. The method was found to be rapid, simple, accurate and precise.

Key words: Cyclobenzaprine hydrochloride, Aceclofenac, Simultaneous estimation method, Q-absorbance ratio method, water soluble method development, UV-Visible spectrophotometer.

INTRODUCTION:

Today, there is a demand for the development of non-toxic methods, without pollution and without danger to the environment, of bulk medicines and pharmaceutical preparations. The use of organic solvents in the development of analytical methods may increase the cost of analysis. It has the potential to affect the cost of the formulation. Cost-effective and safe methods of analysis for pharmaceutical formulations must therefore be developed. Hydrotropy is the best option for developing a safe and cost-effective method of analysis.

Hydrotropy is a Solubilization phenomenon in which the addition of a large amount of the second solute increases the aqueous solubility of another solute. Hydrotropy term has been used in the literature to designate non-micelle-forming substances. To insoluble substance to make soluble; liquid or solid, organic or inorganic would be used. [1] Hydrotropy is the term used for the enhancement of the solubility of an insoluble solute in water by adding the agent called hydrotrope. The formation of molecular

structure in the form of complexes can be a reason for the solubility enhancement. An increase in solubility is an important factor in determining the therapeutic effect of the drug. [2]

Mixed hydrotropic solubilization is a unique technique that deals with the application of more than one hydrotropic mixture. 'Mixed hydrotropic solubilization technique' is the phenomenon to increase the solubility of poorly water-soluble drugs in the aqueous solution containing mixture of hydrotropic agents, which may give a synergistic enhancement effect on the solubility of poorly water-soluble drugs and reduce concentrations of each hydrotropic agent to minimize their toxic effects due to high concentration of hydrotropic agents. In mixed hydrotropy instead of using one single hydrotropic agent in a larger concentration, this method prefers to use of a combination of two or more hydrotropic agents in a smaller concentration; which gives better resolution in solubility study. [3]

Hydrotropism refers to the salting in of nonelectrolytes which are highly soluble in water. The mechanism involved in hydrotropy is related to complexation which involves interaction between lipophilic drugs and a hydrotropic agent such as urea, nicotinamide, sodium alginate, sodium benzoate, sodium citrate etc. those salts or additives which increases solubility in given solvents are referred as "salt in" and which decreases solubility are referred to as"salt out"[4, -9]

Several methods were used to increase the water solubility of the drugs that are, including the hydrotropic method, micellar solubilization, pH adjustment, complexation, solid dispersion, co-solvency, chemical modification, and micronization. Hydrotropes are water-soluble and surface-active compounds that can significantly enhance the solubility of organic solutes such as esters, alcohols, aldehydes, ketones, hydrocarbons, and fats.[10] All are non-reactive and non-toxic and do not produce any temperature effect when dissolved in water.[11] The solvent character being independent of pH, high selectivity, and the absence of emulsification are the other properties.[12]

The formulation development of an orally administered drug with poorly aqueous solubility should consider the solubility enhancement as an important parameter. Drug absorption, sufficient and reproducible bioavailability, and/or pharmacokinetic profile in humans depend upon the solubility of a drug in an aqueous medium & they are recognized today as one of the major challenges in oral delivery of new drug substances.[13] Cyclobenzaprine hydrochloride and Aceclofenac has very low water solubility, so this combination have been selected as a model drug for mixed Hydrotropy technique.

Cyclobenzaprine hydrochloride and Aceclofenac were slightly soluble in water, and freely soluble in organic solvents.

Cyclobenzaprine Hydrochloride

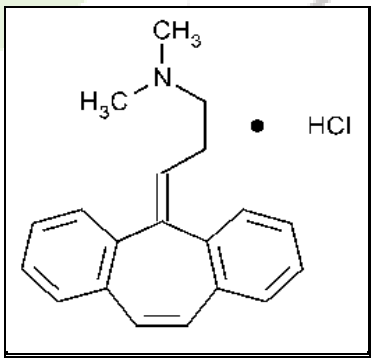

Figure no. 1 structure of Cyclobenzaprine hydrochloride

Cyclobenzaprine is a skeletal muscle relaxant that works on the brainstem to treat muscle spasms of local origin. [14] IUPAC name is dimethyl(3-{tricyclo[9.4.0.0^{3,8}]pentadeca-1(15),3,5,7,9,11,13-heptaen-2-ylidene}propyl)amine. Water solubility 0.00689 mg/mL[15] Cyclobenzaprine is used with rest, physical therapy, and other measures to relax muscles and relieve pain and discomfort caused by strains, sprains, and other muscle injuries. Cyclobenzaprine is in a class of medications called skeletal muscle relaxants. It works by acting in the brain and nervous system to allow the muscles to relax. [16]

Aceclofenac:

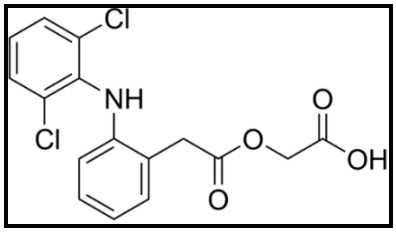

Figure No. 2 structure of Aceclofenac

Aceclofenac is a non-steroidal anti-inflammatory drugs (NSAID). It works by blocking the release of certain chemical messengers that cause pain and inflammation (redness and swelling).[17] Aceclofenac is used for pain relief. It relieves pain and inflammation in conditions like rheumatoid arthritis, ankylosing spondylitis, and osteoarthritis. IUPAC name is 2-[2-[2-[(2, 6-dichlorophenyl) amino] phenyl] acetyl] oxyacetic acid. [18]

Few hydrotropic methods were reported to enhance aqueous solubility of Cyclobenzaprine hydrochloride and Aceclofenac by UV–Visible Spectroscopy, RP-HPLC, LC, HPTLC, LCMS/MS. [19-34] but not yet any method reported by mixed hydrotropic technique using Urea and Sodium Benzoate mixture. The proposed work has main aim to improve aqueous solubility of Cyclobenzaprine hydrochloride and Aceclofenac and develop analytical method and its validation by using UV-Visible spectrophotometer. For assay purpose Simultaneous estimation and Q-absorbance ratio method have been used. Due to increase in aqueous solubility the applicability of method is also improved.

MATERIAL AND METHOD:

Material:

Cyclobenzaprine hydrochloride and Aceclofenac was procured form local market also hydrotropes Urea and sodium benzoate of AR grade procured from local market.

Instruments:

Table No. 1 List of Instruments/equipment

Sr. no.	Instrument/ Equipment	Make
1	Digital Balance	Wenstar electronics 1mg sensitivity
2	Ultrasonicator	Citizen digital ultra-sonic cleaner
3	UV-Visible spectrophotometer	Shimadzu UV-1800

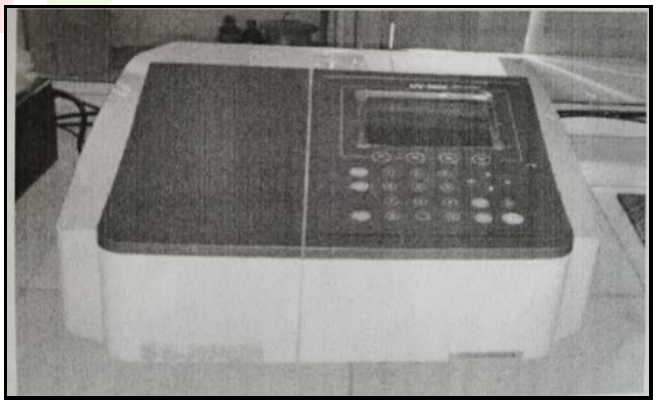

Figure no. 3 Double beam UV-Visible spectrophotometer Shimadzu1800

EXPERIMENTAL WORK:

1. Selection of common Solvent:

After assessing the solubility of a drug in different hydrotropic solvents mixture of 20% Urea and sodium benzoate has been selected as a common solvent for developing spectral characteristics.

Solubility Study: The Cyclobenzaprine Hydrochloride and Aceclofenac has been subjected to the solubility study by dissolving in different hydrotropes; results were shown in Table no.2

Table no.2 Selection of Hydrotropic mixture

Solvent	Solubility
40% Urea	++
50%Sodium benzoate	++
10% Urea + 10% Sodium bicarbonate	--
10% Urea + 10% Sodium Citrate	--
5% Urea+ 5% Sodium benzoate	--
10% Urea +10% Sodium benzoate	++

(--) symbolized insoluble

(++) symbolized soluble

2. Preparation of hydrotropic solvent:

Weighed accurately 10 gm urea and sodium benzoate separately, transferred in 100 ml volumetric flask. Water was slowly added to solubilize all hydrotropes then made volume up to 100 ml mark and prepared 20% solution.

3. Selection of wavelength:

A representative overlain spectrum of Cyclobenzaprine Hydrochloride and Aceclofenac in mixture of 20% Urea and sodium benzoate as shown in figure no. 6. The dilution was obtained to the concentration of 10 µg/ml for both Cyclobenzaprine Hydrochloride and Aceclofenac Solution. Both the solutions were scanned in UV (200 – 400 nm) range against reagent blank. The study of spectrum revealed that Cyclobenzaprine hydrochloride and Aceclofenac show a well-defined λ_{max} at 233 nm and 268.9 nm respectively as shown in figure no. 4 and 5. These two wavelengths selected for development of simultaneous equation. From the overlain isobestic point at 245 nm was selected.

4. Preparation of standard stock solution and study of Beer – Lambert's law:

The standard stock solution of Cyclobenzaprine Hydrochloride and Aceclofenac were prepared by dissolving 50 mg of each drug in mixed hydrotropic solution of 20% urea and sodium benzoate. Add this hydrotropic solution in sequence of 0.5 ml and dissolved by keeping in ultrasonicator for a minute. Add this solution when both drug completely soluble. Both drug completely soluble in 25 ml mixed hydrotropic solution and makeup final volume up to 50 ml with distilled water. Final concentration of each drug solution was 1000 µg/ml. From this solution pipette out 1 ml and makeup volume up to 10 ml with distilled water. Concentration of this solution was 100 µg/ml. From this solution prepare Concentration in range of Cyclobenzaprine Hydrochloride 2-10 µg/ml and Aceclofenac 5-25 µg/ml. The absorbance of resulting solution were measured at their respective λ_{max} and Isobestic point. A calibration curve as concentration vs. absorbance (figure no.7 and 8) was constructed to study Beer- Lambert's law. Calculate absorptivity value of both drugs at respective wavelengths.

5. Study Beer-Lambert's Law:

The aliquot portion of standard stock solution of Cyclobenzaprine Hydrochloride and Aceclofenac were diluted approximately with water to get series of concentrations from 2-10 µg/ml and 5-25 µg/ml respectively.

6. Method I (Simultaneous equation method):

If sample contain two drug each of which absorb at the λ_{max} of the other, it may be possible to determine both drugs by the technique of simultaneous equation.

Two wavelengths were selected for the development of the simultaneous equation are 233nm and 268.90nm. The absorptivity values determined for Cyclobenzaprine Hydrochloride are 0.0370 (ax_1), 0.0072(ax_2) and for Aceclofenac are 0.015 (ay_1), 0.0635 (ay_2) at 233 nm and 268.90 nm respectively. These valued are mean of three estimations. These absorbance and absorptivity values at these wavelengths were substituted in equation 1 and 2 to obtain the concentration of both drugs.

$$C_x = \frac{A_2 \times 0.015 - A_1 \times 0.0635}{-0.0022} \dots\dots\dots\dots (1)$$

$$C_y = \frac{A_1 \times 0.0072 - A_2 \times 0.0370}{-0.0022} \dots\dots\dots\dots (2)$$

Where C_x and C_y are concentrations of Cyclobenzaprine Hydrochloride and Aceclofenac respectively in 1µg/ml and 13 µg/ml solution.A1 and A2 are the absorbance of the mixture at 233nm and 268.90 nm respectively.

7. Method II (Q absorbance ratio method):

Q-Absorbance ratio method of analysis is based on the absorbance at two selected wavelengths, one of which is an isobestic point and the other being the wavelength of any one drug. From overlein spectra (Figure no. …) 233nm (λ_{max} of Cyclobenzaprine Hydrochloride) and 245 nm (Isobestic point) are selected for the formation of Q absorbance equation (equation 3 and 4). The absorptivity values determined for Cyclobenzaprine Hydrochloride are 0.0370 (ax_1), 0.0207 (ax_2) and for Aceclofenac 0.0115 (ay_1), 0.0124 (ay_2) at 233 nm and 245 nm respectively. These values are mean of three estimations. The absorbance and absorptivity values at these wavelengths were substituted in equation 3 and 4 to obtain the concentration of drugs.

$$C_x = \frac{Q_m - 1.0782}{-0.5188} \times \frac{A_1}{0.0370} \dots\dots\dots\dots (1)$$

$$C_y = \frac{Q_m - 1.0782}{0.5188} \times \frac{A_2}{0.0115} \dots\dots\dots\dots (2)$$

Where C_x and C_y are concentrations of Cyclobenzaprine Hydrochloride and Aceclofenac respectively. A1 and A2 are the absorbance of the mixture at 233 nm and 245nm respectively.

8. Analysis of tablet formulation:

Twenty tablets of marketed formulation were accurately weighed and powdered. A quantity of powder equivalent to 100 mg of Aceclofenac was transferred to 100 ml volumetric flask. Dissolved powder in 25 ml mixed hydrotropic solution and made up the volume up to mark by distilled water. The sample solution was then filter through Whatman filter paper No. 41. From the resulting solution 1 ml of solution was taken and diluted to 10 ml with water to get a solution containing 100µg/ml of Aceclofenac and corresponding concentration of Cyclobenzaprine Hydrochloride. From this solution 1 ml of solution was diluted with water in 10 ml volumetric flask to get final concentration of Aceclofenac 13 µg/ml and Cyclobenzaprine Hydrochloride 1 µg/ml. Analysis procedure was repeated three times with tablet formulation. The result of Tablet analysis reported in table no. …..

VALIDATION OF DEVELOPED METHOD:

1. Linearity:

The linearity was determined by analyzing 3 independent levels of linearity curve in the range of 5-25 µg/ml and 2-10µg/ml. Absorbance of each solution was recorded. Absorbance vs. Concentration graph was plotted and correlation coefficient and regression line equation for Aceclofenac and Cyclobenzaprine Hydrochloride was determined. The linearity was calculated by the linear regression equation y= mx + c, where y represents the absorbance and x represents drug concentration in µg/mL. A correlation coefficient of approximately 0.999 or more was considered as desirable for all calibration curves.

2. Accuracy:

Accuracy was determined by performing recovery studies within the analytical concentration range of proposed method at three different set at level of 80%, 100% and 120%. The amount of Cyclobenzaprine hydrochloride and Aceclofenac and % recoveries was calculated at each level.

3. Precision:

Interday precision was determined by analysing Cyclobenzaprine hydrochloride(2-10µg/ml) and Aceclofenac (5-25 µg/ml)at three different time of the same day and Intra-day was determined at different day and %RSD was calculated.

4. Repeatability:

Repeatability was carried out by using a minimum of 6 determinations at one of the test concentration.

5. LOD and LOQ:

The limit of detection (LOD) was determined by lower concentrations of Aceclofenac and Cyclobenzaprine Hydrochloride. The limit of Quantification (LOQ), which is the lowest quantifiable concentration, was also determined from range of concentrations analyzed for the LOD determination. LOD and LOQ were estimated from the set of 3 linearity curves used to determine method.

LOD= 3.3*s/S and LOQ= 10*s/S

Where,

s = the standard deviation of y- intercepts of regression lines

S= the slope of the linearity curve

6. Ruggedness:

The evaluation of ruggedness should be considering during the development phase and depends on the type of procedure under study. It should show reliability of an analysis with respect to external factors in method parameters such as instrument, analyst variation.

RESULT AND DISCUSSION:

1. Selection of common Solvent:

After assessing the solubility of a drug in different hydrotropic solvents mixture of 10% Urea and 10% Sodium benzoate has been selected as a common solvent for developing spectral characteristics.

2. Selection of wavelength:

The study of spectrum revealed that Cyclobenzaprine Hydrochloride and Aceclofenac show a well-defined λ_{max} at 233 nm and 268.9 nm respectively. These two wavelengths selected for development of simultaneous equation. From the overlain Isobestic point at 245 nm was selected.

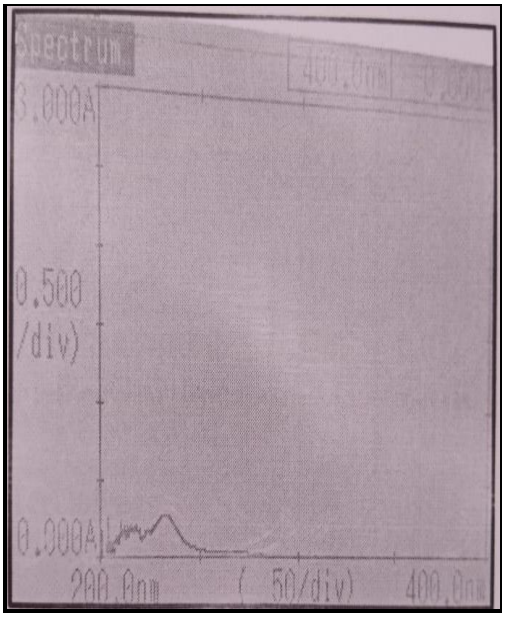

Figure. no. 4. Spectra of Cyclobenzaprine HCl

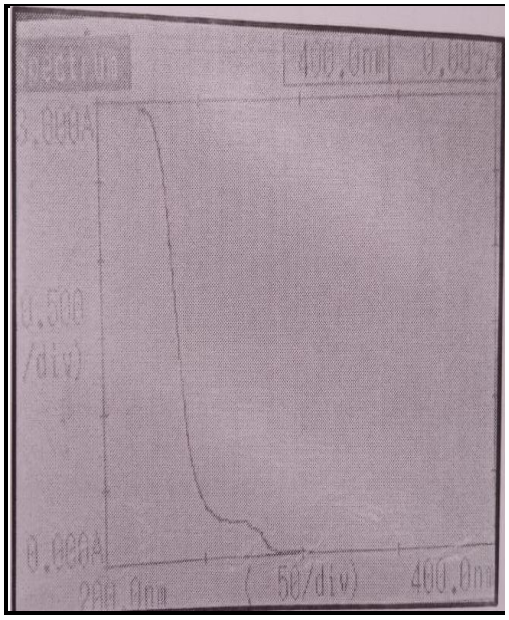

Figure no.5. Spectra of Aceclofenac

Figure no. 6. Overlain of Cyclobenzaprine hydrochloride and Aceclofenac

3. Study Beer-Lambert's Law:

Linearity range for Cyclobenzaprine Hydrochloride and Aceclofenac are2-10µg/ml and 5-25 µg/ml at respective wavelength as shown in figure no.7, 8 and Table no.3and 4.

Table No. 3 Linearity curve of Cyclobenzaprine Hydrochloride at 233nm and 245 nm

Conc	Abs (233nm)	245nm
0	0.005	0.005
2	0.095	0.05
4	0.155	0.093
6	0.225	0.13
8	0.3	0.175
10	0.37	0.207

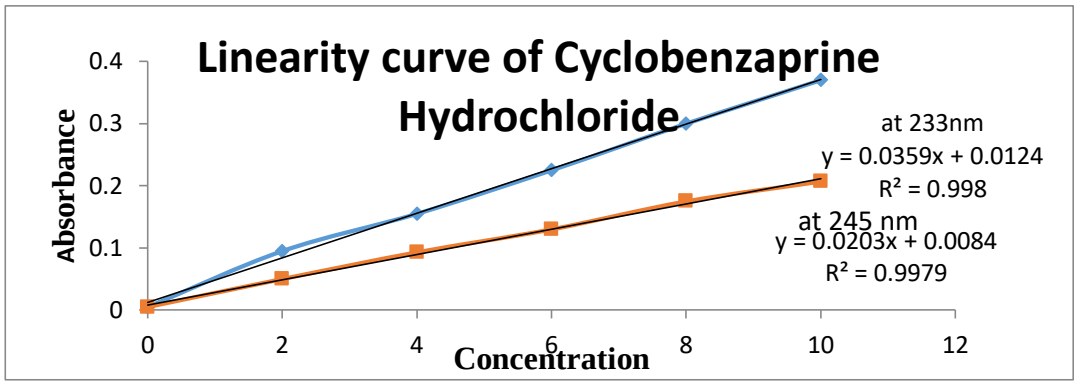

Figure No. 7 Linearity curve of Cyclobenzaprine Hydrochloride at 233nm and 245 nm

Table No. 4 Linearity curve of Aceclofenac at 268.9 and 245 nm

Conc.	Abs (268.9nm)	245nm
0	0.005	0.005
5	0.322	0.05
10	0.635	0.124
15	0.936	0.2
20	1.315	0.275
25	1.635	0.325

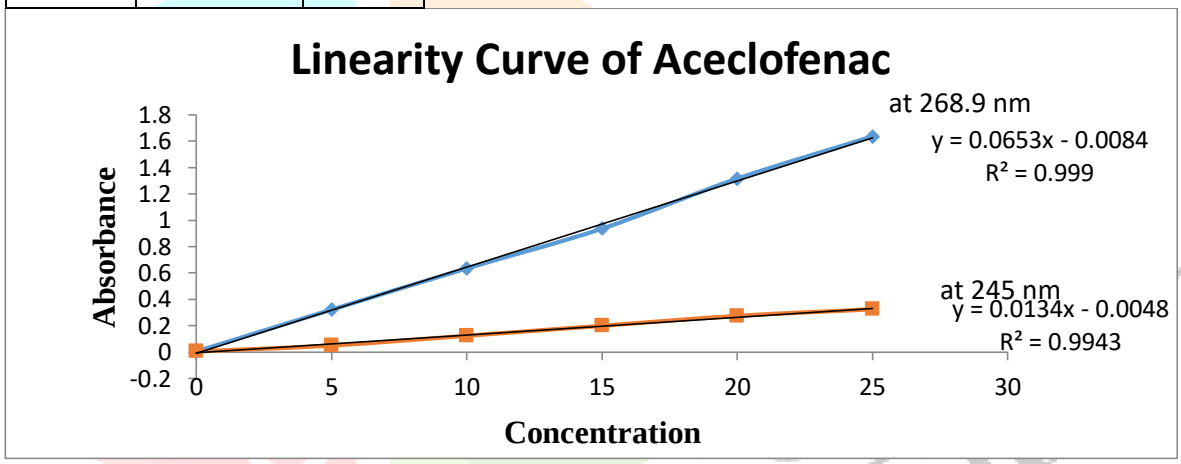

Figure No. 8 Linearity curve of Aceclofenac at 268.9 and 245 nm

4. Assay of Cyclobenzaprine hydrochloride and Aceclofenac tablet

The average absorbance of the drug was computed from the spectrophotometer and the amount of cyclobenzaprine hydrochloride and Aceclofenac in tablet was found by using linearity curve. The results of Simultaneous equation method obtained are shown in table no.5 and the results of Q absorbance ratio method obtained are shown in table no. 6

Table no.5 Assay of Cyclobenzaprine hydrochloride and Aceclofenac tablet by Method I Simultaneous equation method

Absorbance reading at selected wavelength			Concentration (µg/ml)		% amount found	
Wavelength (λ max)	233 nm (λ_1)	268.9 nm (λ_2)	Cyclo	Aceclo	Cyclo	Aceclo
Cyclobenzaprine Hydrochloride	0.370	0.072	1	13	99.5	98.5
Aceclofenac	0.115	0.635	1	13	99.49	99.1
			Average		99.49	99.03
			SD		0.001	0.1

Table no.6 Assay of Cyclobenzaprine hydrochloride and Aceclofenac tablet by Method II

Q Absorbance ratio method

Absorbance reading at selected wavelength			**Concentration (µg/ml)**		**% amount found**	
Wavelength (λ max)	**233 nm (λ_1)**	**245nm (λ_2)**	Cyclo	Aceclo	Cyclo	Aceclo
Cyclobenzaprine Hydrochloride	0.370	0.207	1	13	99.9	99.5
Aceclofenac	0.115	0.165	1	13	99.9	99.5
			Average		99.9	99.5
			SD		0.001	0.001

Validation of developed method as per ICH guidelines

The method was validated according to the validation of analytical procedures provided in the ICH guidelines and draft guidance for the industry.

1. Linearity:

A linear relationship was obtained between the absorbance for the drug and corresponding concentration. The Summary of linearity study presented in table no. 7. The linearity curves in Fig. no.9 exhibit linearity of Cyclobenzaprine Hydrochloride over the concentration range of 2-10 µg/mL with regression coefficient 0.998 at 233nm and at 245 nm it was 0.0.9979. The linearity curves in Fig. no.10 exhibit linearity of Aceclofenac over the concentration range of 5-25 µg/mL with regression coefficient 0.999 at 268.9nm and at 245 nm it was 0.9943. The methods (R^2=0.999) provided a good correlation between absorbance and drug concentration.

Table no. 7: Summary of linearity study of Cyclobenzaprine hydrochloride and Aceclofenac

Parameters	**Cyclobenzapril at 233 nm**	**Cyclobenzapril at 245 nm**	**Aceclofenac at 268.9 nm**	**Aceclofenac at 245 nm**
Linearity range	2-10 µg/mL	2-10 µg/mL	5-25 µg/mL	5-25 µg/mL
Regression equation	y = 0.0359x + 0.012	y = 0.0203x +0.0084	y = 0.0653x - 0.0084	y = 0.0203x + 0.0084
Correlation coefficient	R^2 = 0.998	R^2 = 0.9979	R^2 = 0.999	R^2 = 0.9943

Table no. 8: linearity study of Cyclobenzaprine hydrochloride at 233 nm

Conc	**I**	**II**	**III**	**Avrg**	**SD**	**%RSD**
0	0.005	0.005	0.005	0.005	0.000	0.00
2	0.095	0.094	0.095	0.095	0.001	0.61
4	0.155	0.155	0.154	0.155	0.001	0.37
6	0.225	0.224	0.223	0.224	0.001	0.45
8	0.3	0.299	0.3	0.300	0.001	0.19
10	0.37	0.371	0.37	0.370333	0.00	0.16

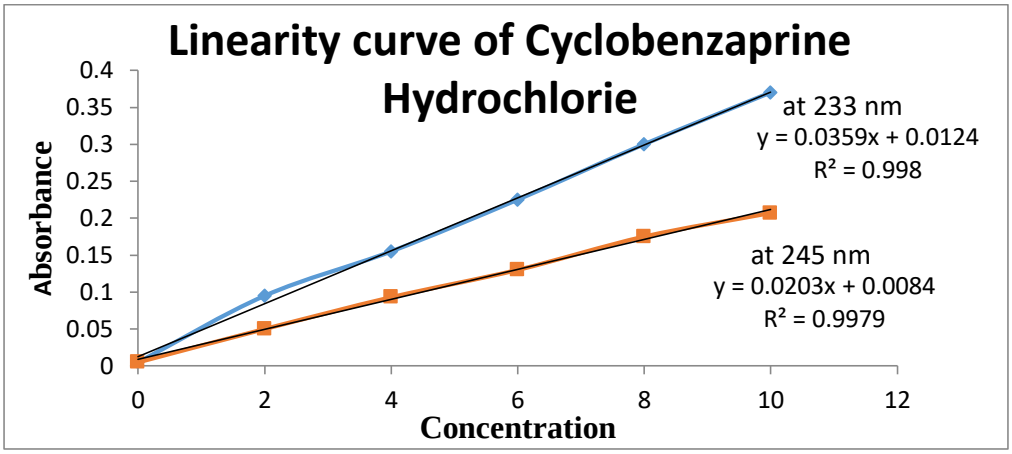

Figure no.9 Linearity curve of Cyclobenzaprine Hydrochloride

Table no. 9: linearity study of Aceclofenac at 268.9 nm

Conc.	I	II	III	Avrg	SD	%RSD
0	0.005	0.005	0.005	0.005	0.000	0.00
5	0.322	0.321	0.322	0.322	0.001	0.18
10	0.635	0.635	0.636	0.635	0.001	0.09
15	0.936	0.936	0.937	0.936	0.001	0.06
20	1.315	1.314	1.315	1.315	0.001	0.04
25	1.635	1.635	1.634	1.6346667	0.00	0.04

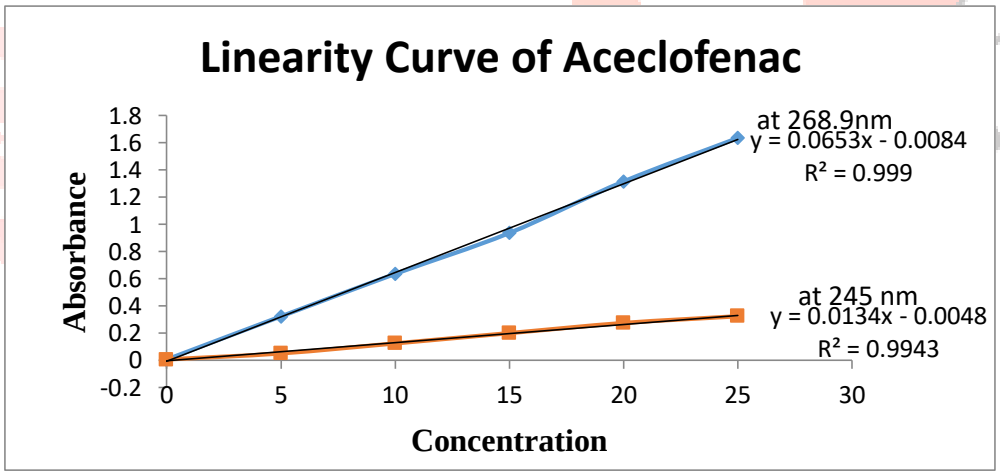

Figure no.10 Linearity curve of Aceclofenac

2. Accuracy:

The amount of Cyclobenzaprine Hydrochloride was calculated at each level and % recoveries were computed. The percentage recovery study was carried out for 80%, 100%, and 120%. The results are shown in Table no10, 11 and 12 respectively. The amount of Aceclofenac was calculated at each level and % recoveries were computed. The percentage recovery study was carried out for 80%, 100%, and 120%. The results are shown in Table no13, 14 and 15 respectively.

Table no. 10 Accuracy study of Cyclobenzaprine Hydrochloride

		80%			
Conc.	**Amount added**	**Abs**	**Amount found**	**Amount recovered**	**% recovery**
6	4.8	0.391	10.74	4.74	98.81
6	4.8	0.392	10.77	4.77	99.40
6	4.8	0.39	10.77143	4.77	99.40
		Mean	10.76	4.76	99.21
		SD	0.02	0.02	0.34
		%RSD	0.15	0.35	0.35

Table no. 11 Accuracy study of Cyclobenzaprine Hydrochloride 100%

		100%			
Conc.	**Amount added**	**Abs**	**Amount found**	**Amount recovered**	**% recovery**
6	6	0.4340	11.97	5.97	99.52
6	6	0.4360	12.03	6.03	100.48
6	6	0.4350	12.00	6.00	100.00
		Mean	12.00	6.00	100.00
		SD	0.03	0.03	0.48
		%RSD	0.24	0.48	0.48

Table no. 12 Accuracy study of Cyclobenzaprine Hydrochloride 120%

	120%				
Conc.	**Amount added**	**Abs**	**Amount found**	**Amount recovered**	**% recovery**
6	7.2	0.476	13.17	7.17	99.60
6	7.2	0.475	13.14	7.14	99.21
6	7.2	0.476	13.17	7.17	99.60
		Mean	13.16	7.16	99.47
		SD	0.02	0.02	0.23
		%RSD	0.13	0.23	0.23

Accuracy study of Aceclofenac

Table no. 13 Accuracy study of Aceclofenac at 80%

		80%			
Conc.	**Amount added**	**Abs**	**Amount found**	**Amount recovered**	**% recovery**
15	12	1.746	26.98	11.98	99.87
15	12	1.745	26.97	11.97	99.74
15	12	1.745	26.96923	11.97	99.74
		Mean	26.97	11.97	99.79
		SD	0.01	0.01	0.07
		%RSD	0.03	0.07	0.07

Table no. 14 Accuracy study of Aceclofenac at 100%

		100%			
Conc.	**Amount added**	**Abs**	**Amount found**	**Amount recovered**	**% recovery**
15	15	1.9450	29.92	14.92	99.49
15	15	1.9460	30.06	15.06	100.41
15	15	1.9440	30.03	15.03	100.21
		Mean	30.01	15.01	100.03
		SD	0.07	0.07	0.48
		%RSD	0.24	0.48	0.48

Table no. 15 Accuracy Study of Aceclofenac at 120%

	120%				
Conc.	**Amount added**	**Abs**	**Amount found**	**Amount recovered**	**% recovery**
15	18	2.129	32.88	17.88	99.32
15	18	2.132	32.92	17.92	99.57
15	18	2.130	32.89	17.89	99.40
		Mean	32.90	17.90	99.43
		SD	0.02	0.02	0.13
		%RSD	0.07	0.13	0.13

3. Precision:

The intra-day and inter-day precision of the assay method were studied by analyzing replicates at 3 different concentration levels: 4,6and 8 µg/mL for cyclobenzaprine hydrochloride shown in table no. 16 and 17. For Aceclofenac concentration levels selected were 10,15and 20 µg/mL shown in Table no. 18 and 19. The precision of this method reflected by relative standard deviation (%RSD) of replicates was not more than 2% for interday and intraday precision study.

3.1 Interday study of Cyclobenzaprine hydrochloride

Table No. 16 Inter day study of Cyclobenzaprine hydrochloride

	Absorbance							
Conc.	**I**	**II**	**III**	**Mean**	**Amt Found**	**% Amt Found**	**SD**	**RSD**
4	0.155	0.156	0.156	0.156	4.02	100.48	0.001	0.001
6	0.225	0.225	0.524	0.225	6.01	100.16	0.001	0.001
8	0.300	0.300	0.299	0.300	8.13	101.67	0.000	0.000

3.2 Intraday study of Cyclobenzaprine hydrochloride

Table No. 17 Intraday study of Cyclobenzaprine hydrochloride

	Absorbance							
Conc	**I**	**II**	**III**	**Mean**	**Amt Found**	**% Amt Found**	**SD**	**RSD**
4	0.154	0.153	0.154	0.154	3.96	99.05	0.001	0.001
6	0.224	0.225	0.223	0.224	5.97	99.52	0.001	0.001
8	0.298	0.299	0.298	0.712	8.10	101.19	0.001	0.001

Precision study of Aceclofenac

3.3 Interday study of Aceclofenac

Table No. 18 Inter day study of Aceclofenac

	Absorbance							
Conc	**I**	**II**	**III**	**Mean**	**Amt Found**	**% Amt Found**	**SD**	**RSD**
10	0.635	0.635	0.636	0.635	9.90	98.97	0.001	0.001
15	0.948	0.949	0.949	0.949	14.72	98.12	0.001	0.001
20	1.313	1.313	1.314	1.313	20.33	101.64	0.000	0.000

3.4 Intraday study of Aceclofenac

Table No. 19 Intraday study of Aceclofenac

	Absorbance							
Conc	**I**	**II**	**III**	**Mean**	**Amt Found**	**% Amt Found**	**SD**	**RSD**
10	0.637	0.636	0.636	0.636	9.91	99.13	0.001	0.001
15	0.950	0.949	0.949	0.949	14.73	98.19	0.001	0.001
20	1.310	1.309	1.310	1.310	20.27	101.36	0.000	0.000

4. Repeatability:

The results of repeatability of Cyclobenzaprine Hydrochloride and Aceclofenac are shown in table no.20 and 21, which predicted that the relative standard deviation (%RSD) was found to be not more than 2%.

Table No. 20 Repeatability study of Cyclobenzaprine hydrochloride

Sr No.	Concentration	Absorbance	Amount Found	%Amount Found
1	6	0.225	6.00	100.00
2	6	0.225	6.00	100.00
3	6	0.224	5.97	99.52
4	6	0.224	5.97	99.52
5	6	0.224	5.97	99.52
6	6	0.224	5.97	99.52
7	6	0.224	5.97	99.52
8	6	0.224	5.97	99.52
9	6	0.223	5.94	99.05
10	6	0.223	5.94	99.05
		Mean	5.97	99.52
		SD	0.01807	
		%RSD	0.30261	

Table No. 21 Repeatability study of Aceclofenac

Sr. No.	Concentration	Absorbance	Amount Found	%Amount Found
1	15	0.965	14.97	99.79
2	15	0.965	14.97	99.79
3	15	0.965	14.97	99.79
4	15	0.965	14.97	99.79
5	15	0.967	15.00	100.00
6	15	0.966	14.98	99.90
7	15	0.966	14.98	99.90
8	15	0.966	14.98	99.90
9	15	0.966	14.98	99.90
10	15	0.966	14.98	99.90
		Mean	14.98	99.90
		SD	0.009851	
		%RSD	0.065761	

5. LOD and LOQ:

The limit of detection (LOD) was evaluated by determining the minimum level of concentration for Cyclobenzaprine hydrochloride and Aceclofenac that could be detected using this analytical method. The limit of quantification (LOQ) was studied by estimating the minimum concentration that could be quantified with acceptable accuracy and precision. The LOD for Cyclobenzaprine hydrochloride was found to be 0.63µg/mL and LOQ was found to be 1.91µg/mL, and for Aceclofenac LOD and LOQ was found to be 0.034 µg/mL and 1.030 µg/mL respectively shown in table no. 22.

Table No. 22: LOD & LOQ Study.

Parameter	Cyclobenzaprine Hydrochloride	Aceclofenac
LOD	0.63	0.034
LOQ	1.91	1.030

RESULT SUMMARY

Table no. 23 Result summary

Sr. No.	Validation parameters		Result	
			Cyclobenzaprine Hydrochloride	**Aceclofenac**
1	UV detection wavelength (nm)		233nm	268.9nm
2.	Isobestic point wavelength (nm)		245nm	
2	Linearity range (µg/ml)		2-1091µg/mL	5-2591µg/mL
3	Standard regression equation		y=0.035x-0.015	y=0.065x-0.008
4	Correlation coefficient (R^2)		R^2= 0.998	R^2= 0.998
5	Precision (% RSD) Interday Intraday		 0.1 0.1	 0.1 0.1
6	Repeatability (n=10)		0.065761	0.30261
7	% Recovery (Accuracy, n=9)	80	99.91	99..71
		100	100	100.03
		120	99.47	99.43
8	LOD (µg/ml)		0.63	0.034
9	LOQ (µg/ml)		1.91	1.030
10	Assay (% Label claim) Method I (Simultaneous equation) Method II (Q absorbance ratio method)		 99.49 99.90	 99.03 99.5

CONCLUSION:

The solubility of Cyclobenzaprine hydrochloride and Aceclofenac in distilled is very low. To improve solubility of drugs in distilled water, mixed hydrotropic solubilization technique had been employed. It was found that solubility of both drugs in distilled water increased by 20 fold as compared to other methods. The proposed method has been new, simple, cost effective, accurate, sensitive, free from toxicity, environment safe and precise. This developed method can be adopt for routine analysis of Cyclobenzaprine hydrochloride and Aceclofenac in tablet dosage form. Presence of hydrotropic agent do not show any significant interference in the spectrophotometric assay. Thus further confirming the applicability and reproducibility of the developed method.

REFERENCES:

1. Kapadiya Nidhi, Singhvi Indrajeet, Hydrotropy: A Promising Tool For Solubility Enhancement, International Journal of Drug Development and Research, 2011,3 (2),
2. Sunil Jayant Kulkarni, Ajaygiri Kamalgiri Goswami, Research and Application of Hydrotropy: A Review, International Journal of Science, Engineering and Technology, 2014, 3(10): 2617.
3. R. K. Maheshwari, Harshal Wadhwani, Anuj Jain And Pawan Jat , Employment of Hydrotropic and Mixed Hydrotropic Solutions as Mobile Phase to Carry Out TLC Precluding The Use of Organic Solvents, World Journal Of Pharmacy And Pharmaceutical Sciences, 2020, 9(11):1377-1385
4. Badwan A.A, El-Khordagui L.K, Saleh A.M. The Solubility of Benzodiazepines in Sodium Salicylate Solutions and a Proposed Mechanism for Hydrotropic Solubilisation. Int. J. Pharm.1982; 13(1): 67- 74.
5. Pandit A, Sharma M.M. Intensification of Heterogeneous Reactions through Hydrotropy. Alkaline Hydrolysis of Esters and Oximation of Cyclododecanone, Chem. Eng. Sci.1987; 42: 2519-2523.
6. Chen X, Micheu J.C. Hydrotrope Induced Auto Catalysis in the Biphasic Alkaline Hydrolysis of Aromatic Esters. J.Colloid Interface Sci. 2002; 249(1):172-179.
7. Akhilesh Kumar Jain. Solubilization of Indomethacin Using Hydrotropes for Aqueous Injection, European Journal of Pharmaceutics and Biopharmaceutics,2008; 68(3):701-714
8. Md. Ali, Vicky Choudhary, Solubility Enhancement Methods with Importance of Drug Delivery an Therapeutics, Journal of Drug Delivery and Therapeutics, 2012, 2(6): 96-101.
9. Prof. Satyanand Tyagi, Patel Chirag J, Dadarwal Poonam, Mangukia Dhruv, A Novel Concept for Enhancement of Solubilization and Bioavailability of Poorly Soluble Drugs: Hydrotropy : A Review, International Journal Of Pharmaceutical Research And Bioscience, 2013,
10. Kumar V. S., C. Raja, C. Jayakumar, A Review On Solubility Enhancement Using Hydrotropic Phenomena International Journal Of Pharmacy And Pharmaceutical Sciences, 2014, 6(6):4
11. Kulkarni S. J., Goswami A. K., Research On Application Of Hydrotropy: A Review, International Journal of Science, Engineering and Technology Research, 2014, 3(10): 2617
12. Kumar V. S., C. Raja, C. Jayakumar, A Review On Solubility Enhancement Using Hydrotropic Phenomena International Journal Of Pharmacy And Pharmaceutical Sciences, 2014, 6(6):1-7
13. Pawar P. H., Dr. Khutle N. And Dalvi H., "Solubility Enhancement Of Poorly Water Soluble Drug By Using Various Solubility Enhancement Technique", World Journal Of Pharmaceutical Research Review Article, 2018, 7(7):1704-1721
14. *https://go.drugbank.com/drugs/db00924*
15. *https://go.drugbank.com/salts/dbsalt000479*
16. *https://medlineplus.gov/druginfo/meds/a682514.html#:~:text=cyclobenzaprine%20is%20used%20with%20rest,medications%20called%20skeletal%20muscle%20relaxants.*
17. *https://www.1mg.com/generics/aceclofenac-209313*
18. *https://en.wikipedia.org/wiki/aceclofenac*
19. Shailendraj Suryawanshi Sanjay, Zaranappal,Chaluvaraju K C, Veena M K. Rajani, Development and Validation of Uv-Spectrophotometric Method for Simultaneous Estimation of Aceclofenac and Pantoprazole in Bulk and Tablet Dosage Forms Using Hydrotropic Solvent, International Journal of Pharmacy and Pharmaceutical Research, 2016, 6(3): 332-344.
20. S.M. Ashraful Islam, Sharif Md. Abuzar ,Pijush Kumar Paul, Validation of UV Spectrophotometric and RP-HPLC Methods for The Simultaneous Analysis of Paracetamol and Aceclofenac in Marketed Tablets International Journal of Pharmacy & Life Sciences, 2011, 2(12): 1267 1275
21. Rajesh Sharmal, Pandurang Gaikwad, Rupali Joshi, A Novel Application of Hydrotropic Solubilization for Simultaneous Estimation and Validation of Acetaminophen, Chlorzoxazone and Aceclofenac in Tablet Dosage Form, Der Pharma Chemica, 2010, 2 (3):90-99
22. Srujani. Ch, Sravanthi.B, Madhuri D, Validated UV Spectrophotometric Methods For The Estimation of Aceclofenac in Bulk And Pharmaceutical Formulation, Scholars Academic Journal of Pharmacy , 2014, 3(6): 471-476

23. Rohit Shah, Chandrakt Magdum, Shital Kumar Pail, Validated Spectroscopic Method for Estimation of Aceclofenac from Tablet Formulation, Research Journal Pharmacy and Technology , 2008, 1(4): 430-432
24. Rajasekhar Tulasi Barul, Prasanth Bitla Cyclobenzaprine Drug Assay and Cyclobenzaprine Excipient Interaction Study by Chromatography, Thermal and Spectral Analysis. Journal Pharm Chem Sci. 2017;1(1):1-9
25. Roshni Patel, Usmangani K Chhalotiya, Falgun A Mehta, Dimal A Shah, And Kashyap K Bhatt, Liquid Chromatographic Estimation of Cyclobenzaprine Hydrochloride and Aceclofenac in Pharmaceutical Formulation, Journal of Pharmacy and Pharmaceutical Sciences, 2014, 3(3):37-44
26. Bhumika K Patel, Ankit B. Chaudhary, Shweta M. Bhalani And Bhoomi D. Patel, Development And Validation Of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Aceclofenac and Cyclobenzaprine Hydrochloride in Pharmaceutical Dosage Form, World Journal Of Pharmacy And Pharmaceutical Sciences, 2022, 7(5):874-888
27. Sunil R Dhaneshwar, Kanchan O Raut , Vidhya K Bhusari, Validated Hplc Method For Simultaneous Estimation Of Paracetamol, Aceclofenac and Thiocolchicoside in Bulk Drug And Formulation, Research Journal Of Pharmaceutical, Biological And Chemical Sciences, 2011, 2(2): 1-7.
28. Wenhong Yu, Xiaojing Yang, Wenwen Sui, Haiyan Xu, Xinyi Luan, Xiangjun Wang, Yi Jin, Bo Yuan Rapid and Sensitive Analysis of Cyclobenzaprine By LCMS/MS: Application to a Pharmacokinetic Study Of Cyclobenzaprine In Dog, Asian Journal of Pharmaceutical Sciences,2014,9(2): 118-122
29. Minal T. Harde , Sagar B. Wankhede , Praveen D. Chaudhari, A Validated Inherent Stability Indicating HPTLC Method For Estimation of Cyclobenzaprine Hydrochloride in Tablets and Use of Ms-Qt of in Characterization of Its Alkaline Stress Degradation Product, Bulletin Of Faculty Of Pharmacy, Cairo University 2016, 54: 145-156.
30. Mv. Bhure, A.T. Hemke ,K.R. Gupta, UV-Spectrophotometric Methods for Determination of Aceclofenac and Diacerein in Pharmaceutical Formulation, Journal Of Pharmaceutical Research And Sciences. 2010, 2 (7): 426-432.
31. Susmitha A, Hepcy Kalarani D, Venkatesh P. Ravindra Reddy K., Analytical Method Development and Validation of Aceclofenac in Pharmaceutical Dosage Form By UV Spectroscopy Technique, International Journal of Pharmacy and Pharmaceutical Sciences, 2013, 5(3):150-153.
32. Rajan V. Rele, Simultaneous Spectrophotometric Estimation of Paracetamol and Aceclofenac By Second Order Derivative Method in Combined Dosage Form, Journal of Chemical and Pharmaceutical Research, 2015, 7(6):512-517
33. Ashok R. Parmar, Dharmishtha N. Bhakhar, Dolita K. Shah And Kinjal V. Vekariya Simultaneous Estimation of Aceclofenac and Serratiopeptidase in Tablet Dosage Form by Absorbance Ratio Method Using Visible Spectrophotometry, Der Pharmacia Sinica, 2012, 3(3):321-326.
34. Rajani N. Jinde, Pathan Azhar Khan, Manadar Abhyankar, Megha Jadhav, Development and Validation of Reverse Phase-HPLC Method for Estimation of Aceclofenac and Rabeprazole Sodium in Bulk and Combined Dosage Form Journal of Innovations of Pharmaceutical and Biological Sciences, 2015, 2 (1):17-23,