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A Novel Approach For Separation Of Cyclobenzaprine Hcl And Aceclofenac Using Mixed Hydrotropic Thin Layer Chromatography

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ABSTRACT: This study explores the novel application of mixed hydrotropic Thin Layer Chromatography (TLC) to separate Cyclobenzaprine Hydrochloride (HCl) and Aceclofenac. Traditional methods employ organic solvents, which pose environmental and health risks. This research uses a combination of hydrotropic agents such as sodium benzoate, sodium citrate, and urea to enhance the aqueous solubility of the drugs, facilitating a green and cost-effective chromatographic method. The developed method demonstrated clear separation with an optimized mobile phase composition.

Keywords: Cyclobenzaprine HCl, Aceclofenac, Thin Layer Chromatography, Hydrotropy, Mixed Hydrotropic Solubilization

1. INTRODUCTION

Chromatography was first discovered in 1906 by a botanist, Tswett, who was trying to separate colored plant pigments [1]. Chromatography is a separation and purification technique used for organic and inorganic compounds. Two or more compounds or ions are separated by the distribution of the mobile and stationary phases. These two phases can be solid-liquid, liquid-liquid or gas-liquid [2]. The term "chromatography" was created by M. Tswett in 1906. Chromatography is a set of laboratory techniques to separate a mixture into its components [3]. TLC is performed on a solid support such as glass, plastic, or aluminum foil, coated with a thin adsorbent material, usually silica gel. This adsorbent layer is called the stationary phase. After the sample is applied to the plate, capillary action draws the mobile phase into the plate. Since different analytes appear on the TLC plate at different rates, separation is achieved [4]. Thin layer chromatography (TLC) relies on the principle of separation. Separation depends on the relative affinity of the two compound phases, i.e. the stationary phase and mobile phase respectively. The sample is placed on an extension plate with a mobile phase. This solvent then rises the plate by capillary action [5]. The Rf value is a physical constant used to compare and identify compounds [6].

Rf = distance travelled by centre of a component from origin/Distance travelled by solvent front from origin

There are various types of TLC techniques like: Partition TLC, Adsorption TLC, Ion-exchange TLC, TLC on Dextran Gels, Preparative TLC, Two-Dimensional TLC, TLC on Polyamides, Reversed Phase Partition TLC (RPPTLC), Thin Layer Ionophoresis and Thin Layer Electrophoresis [7]. Normal phase chromatography, Reverse phase chromatography [8]. Stationary phase in a chromatography is a solid or liquid phase coated on the surface of a solid support. Silica gel, Silica gel H, Silica gel G, Silica gel GF,

Alumina, Alumina G, Cellulose powder, Kieselguhr G, Polyamide powder etc. [9] The mobile phase in chromatography is a liquid or gas that passes through the stationary phase and carries mixed components with it. The components of the mixture are separated at a different rate by adsorption onto the stationary phase [10]. Mixture of two or three solvents of different polarity offer fundamentally different and improved separation as compared to chemically homogeneous solvents [11, 12]. Hydrotropy is a technique that enhances the aqueous solubility of poorly water-soluble drugs. The agents used to increase the solubility of poorly water-soluble drugs are known as "Hydrotropes" [13]. Some examples of hydrotropes: Urea, tosylate, sodium benzoate, sodium citrate, nicotinamide, sodium acetamide, sodium salicylate, niacinamide, cumenesulfonate, xylene sulfonate [14].

Advantages of Hydropic Technique

1. Hydrotropy is the method which increases the solubility of the poorly water soluble drugs.
2. It only requires mixing the drug with the Hydrotropes in water.
3. It is less toxic than organic solvents.
4. It is cheap.
5. It is non-corrosive.
6. It is environment friendly [15].

Advantages of Hydropic Technique

1. There are issues related to toxicity associated with excess use of hydrotropic agents.
2. There are chances of weak interaction between hydrotropic agent and drugs.
3. Use of water as a solvent, complete removal of water cannot be achieved [16].

Properties of Hydrotropes:

1. Hydrotropes can increase the solubility of various organic solvents like esters, alcohols, aldehydes, ketones, hydrocarbons and fats.
2. These are nonreactive and non-toxic.
3. They do not affect the solubility of the drug in water by the interference of temperature.
4. P^H, high selectivity and absence of emulsification are properties of Hydrotropes that do not affect solvent properties [17].

Mixed hydrotropic solubilization is a technique used to increase the solubility of poorly water soluble drugs in the mixtures of hydrotropic agents, which gives synergistic effect on solubility of poorly water soluble drugs. Single hydrotropic agent in high concentration may produce toxic effect; mixed Hydrotropy is the best choice to overcome this problem [18].

Advantages of Mixed Hydrotropy:

1. It reduces the concentration of individual Hydrotropes.
2. It is simple and cost effective.
3. It produces additive effect on solubility [19].

The need of the research work is that Cyclobenzaprine HCL and Aceclofenac are water insoluble, so for solubilization purposes, non-polar mobile phase is generally selected. But non-polar solvents are toxic, corrosive, carcinogenic and costly. Hydrotropic agents are generally used to make insoluble samples water soluble. Cyclobenzaprine HCL and Aceclofenac are easily made water soluble by using a mixed hydrotropic solution. Mixed hydrotropic solutions are non-toxic, non-corrosive, cost effective and environment safe [20,21,22,23]. Few methods were found for analysis of Cyclobenzaprine HCL and Aceclofenac by UV [24,25,26,27], HPTLC [28,29], HPLC [30,31]. But not yet any TLC development method reported for separation of Cyclobenzaprine HCL and Aceclofenac by using mixed hydrotropic method. The objective of the work is that Cyclobenzaprine HCL and Aceclofenac make a water-soluble solution by using a mixed hydrotropic solution. To separate Cyclobenzaprine HCL and Aceclofenac by TLC using Silica Gel G as a stationary phase and mixed hydrotropic solutions as mobile phase.

2. MATERIAL

2.1 Material

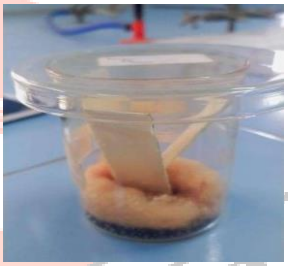
- **Drugs:** Cyclobenzaprine HCl and Aceclofenac (procured from a local supplier)
- **Tablet Formulation:** Flexabenz Plus Tablet (procured from the local market)
- **TLC Plate:** Silica gel G-coated plates
- **Mobile Phase:** 10% Urea, 5% Sodium Benzoate, and 5% Sodium Citrate (Analytical Reagent Grade)
- **Detection Equipment:** Iodine Chamber, UV Cabinet

2.2 INSTRUMENTS/ EQUIPMENTS:

Table No. 01. Instruments and Equipment

Sr.No.	Instruments /Equipment	Make
1	Digital balance	AN ISO 9001:300COMPANY, GB 600
2	Ultrasonic sonicator	BIO- TECHNICS INDIA
3	UV cabinet	LAB HOSP™
4	Hot air oven	LABLINE™

Fig No. 01. UV Cabinet



No. 02. Iodine Chamber

2.3 DRUG PROFILE:

2.3.1 CYCLOBENZAPRINE HCL -

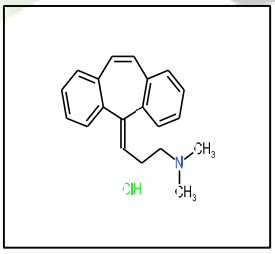


Fig. No.3 Structure of Cyclobenzaprine HCl

IUPAC Name- N,N-dimethyl-3-(2-tricyclo[9.4.0.03,8] pentadeca 5),3,5,7,9,11,13heptaenylidene) propane-1-amine;hydrochloride

Molecular Formula: $C_{20}H_{22}N_1HCl$

Molecular Weight:311.9

Solubility-Ethanol (25 mg/ml), Dimethylformamide (25mg/ml), insoluble in water

Category-Muscle Relaxant

Aceclofenac

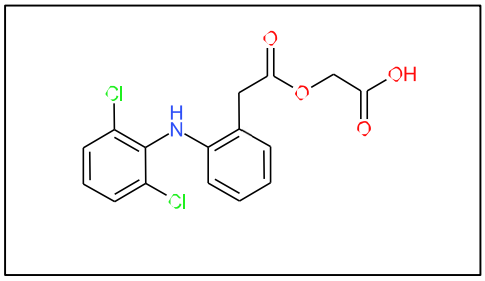


Fig. No. 04. Structure of Aceclofenac

IUPAC Name - 2-[2-[2-(2,6-dichloroanilino) phenyl] acetyl] oxyacetic acid **Molecular Formula-** $C_{16}H_{13}Cl_2NO_4$

Molecular Weight- 354.2

Solubility- Ethanol (10mg/ml), Dimethyl Sulfoxide (30mg/ml), Insoluble in water

Category- Non-steroidal anti-inflammatory agent

FLEXABENZ PLUS TABLET

Fig. No. 05. Flexabenz plus table

3. EXPERIMENTAL METHOD:

Step 1: Preparation of chromatographic plate

The stationary phase is applied onto the glass slides uniformly by the pouring method.

Step 2: Activation of TLC plate

The glass slides are allowed to dry in air (5-10 min) and it is further dried and activated by heating at about 100 ^{0} C for 30 minutes. Glass slides made with volatile organic liquid may not require this further drying. By removing the liquids associated with the layer completely, the adsorbent layer is activated. Glass slides may be kept for a short period in a desiccator but long storage is not recommended.

Step 3: Solvent system

The choice of mobile phase depends on the solubility of drugs, a mixed hydrotropic system was used.

Solubility study

The Cyclobenzaprine HCL and Aceclofenac have been subjected to the solubility study by dissolving in different Hydrotropes.

Table No.02: Solvent selection

Sr. No.	Solvents	Solubility	
		Cyclobenzaprine HCL	Aceclofenac
1	Water	(-)	(-)
2	Ethanol	(++)	(++)
3	Sodium benzoate	(-)	(-)
4	Sodium citrate	(-)	(-)
5	Urea	(-)	(-)
6	Sodium benzoate (5%) +Urea (10%)	(+)	(+)

7	Sodium benzoate (5%) +Urea (10%) + Sodium citrate (5%)	(++)	(++)

(-) sign indicates poorly soluble
(+) sign indicates slightly soluble
(++) sign indicates freely soluble

Preparation of mobile phase (hydrotropic solvents):

Weigh accurately 10 gm of urea, 5 gm of sodium benzoate, and 5 gm of sodium citrate separately, transfer into 100 ml volumetric flask. Add sufficient amount of water and mix the solution properly. Make up the volume to 100 ml by using distilled water

Step:4 Preparation of standard solution

The standard stock solutions of Cyclobenzaprine HCL and Aceclofenac were prepared by dissolving 10 mg of each drug in a mixed hydrotropic solution of 10 % urea, 5% sodium benzoate, and 5% sodium citrate separately. Add this hydrotropic solution in sequence of 1 ml and dissolve by keeping in an ultrasonicator for 1 minute. Aceclofenac was completely soluble in 1 ml of mixed hydrotropic solution and made a final volume up to 10 ml by using distilled water.

Cyclobenzaprine HCL was completely soluble in 6 ml of mixed hydrotropic solution and make a final volume up to 10 ml by using distilled water. Final concentration of each drug solution was 1000 µg/ml.

Step:5 Preparation of sample solution

Take 20 tablets of Flexabenz plus and make fine powder of them. Weigh accurately equivalent weight of 10 mg of Aceclofenac and add it into the 10 ml of volumetric flask. Add sufficient amount of hydrotropic solution until drug gets completely soluble. Make up the volume up to 10 ml with distilled water.

Step:6 Application of sample

The area of application should be kept as small as possible for sharper and greater resolution. The spot was applied on the TLC plate at the origin line by using a capillary. Three spots were applied at the origin line. First of standard Aceclofenac, second of standard Cyclobenzaprine HCL, and third of stock solution of marketed tablet

Step 7: Development of chamber

The TLC slides were placed vertically in a rectangular chromatographic chamber or tank. The type and size of the chamber also decide the success and Rf value. The development should be carried out at room temperature. In diffuse daylight by covering a chamber with aluminum foil.

Step 8: Development of chromatogram

Ascending development: The sample spotted plate was placed in the chromatographic chamber containing solvent at the bottom. Direction of flow of solvent or mobile phase from bottom to top by capillary rise action.

Step 9: Location of spot

Physical and chemical methods are used for the location of spots. we used a UV cabinet and an iodine chamber.

Step 10: Evaluation of the chromatogram

After locating the spots on the plate and making their position and size, they are evaluated either qualitatively or quantitatively.

4. OBSERVATION AND CALCULATION

Rf value is defined as the ratio of the distance moved by the solute to the distance moved by the solvents along the stationary phase.

Rf value = distance travelled by a solute/distance travelled by a solvent

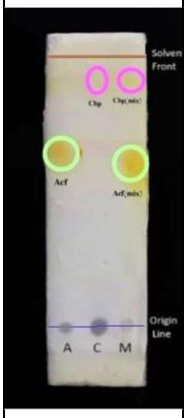
Selection of Mobile phase:

Table no. 03: Optimization of mobile phase

Sr No.	Hydrotropes	Solubility (Cyclobenzaprine HCL and Aceclofenac)	Separation
1	1% Urea	Not soluble	No separation
	5% Urea	Not soluble	No separation
	10 % Urea	Sparingly soluble	No separation
2	1% Sod. benzoate	Not soluble	No separation
	3%Sod. benzoate	Not soluble	No separation
	5%Sod. benzoate	Sparingly soluble	No separation
3	1%Sod. citrate	Not soluble	No separation
	3%Sod. citrate	Not soluble	No separation
	5%Sod. citrate	Sparingly soluble	No separation
4	Jrea + 5% sod. benzoate	Partially soluble	No separation
5	0% Urea + 5% sod .benzoate	Partially soluble	No separation
6	Jrea+5%Sod. citrate	Partially soluble	No separation
7	10 % Urea + 5% sod. citrate	Partially soluble	No separation
8	10 % Urea + 5% od. citrate+ 5% sod. benzoate	Completely soluble	Separation found

Separation of Components:

Table No. 04: Separation of Components

Plate . No.	Mobile Phase	Component	Solvent Front	Distance travelled by solute	Rf value	Detection method	FIGURE
1.	Water	benzaprine HCl	7	6	0.85	Iodine chamber	
		Aceclofenac	7	4.5	0.64		
		Component1(mix)	7	6	0.85		
		Component2(mix)	7	4.3	0.61		
2.	ydrotropic Solution	benzaprine HCl	7.1	6	0.84	Iodine chamber	
		Aceclofenac	7.1	4	0.56		
		Component1(mix)	7.1	5.8	0.82		
		Component2(mix)	7.1	4	0.56		
3.	ydrotropic Solution	benzaprine HCl	6.4	6.2	0.96	Iodine chamber	
		Aceclofenac	6.4	5.1	0.79		
		Component1(mix)	6.4	6.2	0.96		
		Component2(mix)	6.4	5.2	0.81		

6. RESULT AND DISCUSSION:

Various hydrotropic solutions were used for solubilization of Cyclobenzaprine HCl and Aceclofenac in 3% Urea, 5% Urea, 10% Urea, 1% Sod. Benzoate, 3% Sod. Benzoate, 5% Sod. Benzoate, 1% Sod citrate, 3% Sod citrate, and 5% Sod citrate, but the drugs were not soluble.

1. By using a mixed hydrotropic solution (5% Sod citrate, 10% Urea and 5% Sod benzoate) Cyclobenzaprine HCl and Aceclofenac were completely soluble so this mixed hydrotropic solution was used as a mobile phase as well standard and sample solution also prepared in this mobile phase.
2. As the same mobile phase and solvent were used for preparing both the standard and sample solutions,

some separation issues arose. This problem was resolved by adding a drop of ethanol to the standard and sample solutions, which subsequently led to clear separation.

3. The calculated RF value of standard Cyclobenzaprine HCl and Aceclofenac was matched with separated spots of the sample mixture. Hence need for study was achieved.

7.SUMMARY:

The separation of Cyclobenzaprine HCl and Aceclofenac was successfully achieved using TLC. Optimization of the mobile phase was performed using a trial-and-error method. The best separation was obtained using a combination of 10% Urea, 5% Sodium Benzoate, and 5% Sodium Citrate. The calculated Rf values of standard Cyclobenzaprine HCl and Aceclofenac matched with separated sample spots, confirming the success of the method.

8. CONCLUSION

The mixed hydrotropic TLC method offers a fast, simple, cost-effective, and environmentally friendly alternative for separating Cyclobenzaprine HCl and Aceclofenac.

9. FUTURE PROSPECTIVE

This method can be extended to HPLC and HPTLC applications. Mixed hydrotropic solutions could revolutionize analytical techniques by significantly reducing the use of organic solvents.

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