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Eco-friendly Thin-Layer Chromatography of Poorly Water-Soluble Drugs Using Mixed Hydrotropy: A Green Alternative to Organic Solvents

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Research Article

Keywords: Mixed Hydrotropy, Thin-Layer Chromatography (TLC), Green Chemistry, Poorly Water-Soluble Drugs, Solubility Enhancement

Posted Date: March 19th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9127187/v1

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Additional Declarations: No competing interests reported.

Abstract

Thin-layer chromatography (TLC) is commonly used in pharmaceutical quality control, but the analysis of poorly water-soluble drugs often requires harmful and expensive organic solvents. This study presents a green and cost-effective alternative by developing a TLC method based on the principles of hydrotropy and mixed hydrotropy. Hydrotropy involves the use of concentrated aqueous solutions of hydrotropic agents to enhance drug solubility. In this work, combinations of agents such as sodium benzoate, sodium citrate, sodium acetate, phenol and urea were used to prepare environmentally friendly mobile phases. These hydrotropic blends successfully replaced organic solvents in TLC, showing improved Rf values and reduced tailing. The developed method demonstrated superior performance over conventional systems, highlighting the potential of hydrotropy as a sustainable solution in pharmaceutical analysis. This approach is aligned with the principles of green chemistry and offers a promising platform for wider application in analytical and formulation research.

1.INTRODUCTION

Thin-layer chromatography (TLC) has long been recognized as one of the simplest, most cost-effective, and rapid analytical techniques for the separation and identification of pharmaceutical compounds based on their physicochemical properties. [1, 2] However, a major challenge remains in analyzing poorly water-soluble drugs, which often results in inadequate separation and weak detection when using conventional TLC methods. [3, 4, 5]. Standard protocols, as outlined in official pharmacopeias, typically involve organic solvents such as methanol, toluene, chloroform, and dioxane. These solvents not only pose health and environmental risks but also often fail to provide satisfactory solubilization and chromatographic performance for poorly soluble compounds.

To address these limitations, the application of hydrotropy and mixed hydrotropy has emerged as a promising green alternative. Hydrotropy refers to the use of concentrated aqueous solutions of hydrotropic agents—such as sodium benzoate, sodium salicylate, sodium citrate, niacinamide, and urea—to significantly enhance the aqueous solubility of otherwise poorly water-soluble drugs. [6, 7, 8, 9, 10, 11, 12]. Unlike surfactants or co-solvents, hydrotropes operate through mechanisms such as π–π stacking interactions (between the aromatic systems of hydrotropes and hydrophobic drug moieties), hydrogen bonding, dispersion forces, electrostatic interactions, and reduction of interfacial tension [13, 14, 15, 16]. These interactions disrupt the crystalline structure of the solute, thereby improving solubility in aqueous systems.

Mixed hydrotropy, a synergistic advancement of this concept, involves combining two or more hydrotropic agents to further enhance solubility beyond the capacity of individual agents [17, 18]. This technique allows for flexible tailoring of the solvent environment, enhancing both solubilization and separation of complex drug mixtures [19]. The use of such eco-friendly, non-toxic solubilizing systems in TLC thus represents a novel and sustainable approach for pharmaceutical analysis, especially for poorly water-soluble drugs [20, 21].

The present study aims to optimize and develop a green TLC method for the analysis of ofloxacin, mefenamic acid, and acyclovir, using hydrotropic and mixed hydrotropic mobile phases [22]. Among these, mefenamic acid and acyclovir are classified as poorly water-soluble (approximately 0.006 mg/mL and 1.3 mg/mL at 25°C, respectively), while ofloxacin is moderately soluble in water (33 mg/mL at 25°C) [23, 24, 25]. By evaluating different hydrotropic agents and their combinations, this work seeks to establish robust, reproducible, and eco-friendly TLC protocols for these drugs.

Furthermore, conventional mobile phases used in TLC analysis of these drugs—as per pharmacopeial standards—employ harmful organic solvents. For example, the European Pharmacopoeia (2002) recommends a mixture of concentrated ammonia, methanol, and methylene chloride for acyclovir; [26] the British Pharmacopoeia (2003) prescribes a mobile phase of glacial acetic acid, dioxane, and toluene for mefenamic acid; [27] and a combination of glacial acetic acid, water, and ethyl acetate for ofloxacin.[30] These compositions highlight the urgent need for sustainable alternatives. In contrast, the present study explores the compatibility and efficiency of various hydrotropic blends to achieve effective separation with minimal tailing and enhanced resolution—entirely free of organic solvents. [28, 29]

2. MATERIALS AND METHODS

2.1 Materials

1. **Drugs Used:**
Crude drug samples of acyclovir and ofloxacin were procured from BLD Pharma Ltd and mefenamic acid was obtained as a gift sample from Micro Labs, Bangalore.

2. **Instruments and Equipment:**
Thin-layer chromatography (TLC) chamber, capillary tubes, TLC plates, plate cutter, petri dishes, UV chamber, and hot air oven.

3. **Solvents and Chemicals:**
Hydrochloric acid, ethyl acetate, and chloroform were used as solvents throughout the study.

2.2 Methods

1. **Preparation of TLC Plates:**
A suspension of silica gel G was prepared and uniformly spread on 7 cm long glass slides to achieve a coating thickness of 0.25–0.30 mm. The coated plates were air-dried and subsequently activated by heating in a hot air oven at 100–105 °C for 1 hour.

2. **Preparation of Sample Solutions:**
Sample solutions were prepared by dissolving a known quantity of each drug in an appropriate solvent shown in table-II; (hydrochloric acid, ethyl acetate, or chloroform), depending on solubility characteristics.

Table I. Solutions used for dissolving drugs for Thin Layer Chromatography

DRUG	SOLVENT
Acyclovir	Hydrochloric acid
Mefenamic acid	Ethyl Acetate
Ofloxacin	Chloroform

3. **Preparation of Mobile Phases**

Mobile phases were prepared by dissolving various inorganic components—such as sodium benzoate, sodium acetate, sodium citrate, urea, and phenol—in distilled water to form hydrotropic or mixed hydrotropic solutions.

4. **Thin-Layer Chromatography (TLC) Studies**

TLC analysis of the drug samples was performed using glass slides coated with a thin layer of silica gel F254. Small sample spots were applied using fused capillary tubes. Various combinations of hydrotropic agents solution were used as mobile phases, including:

· Sodium benzoate + sodium acetate

· Sodium benzoate + sodium citrate

· Sodium benzoate + urea

· Phenol + sodium benzoate etc.

After spotting, the TLC plates were placed in a saturated TLC chamber to allow for chromatographic development. Following development, the plates were dried in a hot air oven. The dried plates were then visualized under a UV chamber to detect the sample spots. TLC experiments were repeated with different hydrotropic or mixed hydrotropic solvent systems given in table-III. After each development, the plates were again dried in a hot air oven and visualized under UV light.

The **retention factor (Rf)** for each drug was calculated using the following formula:

Retention factor= Distance travelled by the solute (sample spot)/ Distance travelled by the solvent front

Rf is the retention factor (a unitless ratio), The **distance traveled by the solute** is measured from the baseline (origin) to the center of the spot, The **distance traveled by the solvent front** is measured from the baseline to the furthest point reached by the solvent.

Table II. Compositions of various hydrotropic blends for TLC of Acyclovir, Mefenamic acid, and Ofloxacin.

1.	Blend 1	10 gm sodium benzoate and 10 gm of sodium acetate dissolved in distilled water and volume made up to 100ml
2.	Blend 2	10 gm of sodium benzoate and 10 gm of sodium citrate dissolved in distilled water and volume made up to 100ml
3.	Blend 3	10 gm of sodium benzoate and 10 gm of urea dissolved in distilled water and volume made up to 100 ml
4.	Blend 4	30 gm of sodium benzoate and 10 gm of urea dissolved in distilled water and volume made up to 100 ml
5.	Blend 5	30 gm of sodium benzoate dissolved in distilled water and volume made up to 100 ml
6.	Blend 6	2.5 gm Phenol and 2 gm of Sodium Benzoate dissolved in distilled water and volume made up to 10 ml

3. RESULTS AND DISCUSSION

The Rf values for acyclovir, mefenamic acid, and ofloxacin were evaluated using both standard pharmacopeial mobile phases and various hydrotropic and mixed hydrotropic solvent blends. The data are summarized in Table I.

Table III

TLC studies of the selected drugs

S.no.	Drug	Mobile phase	Rf value
1.	Acyclovir	Standard (EP) {0.2 ml of concentrate ammonia + 2 ml of methanol+8ml of methylene chloride}	0.26
		Blend 1 (10 gm of SB + 10 gm of SA)	Nil
		Blend 2 (10gm of SB+10gm of SC)	Nil
		Blend 3 (10gm of SB + 10gm of Urea)	Nil
		Blend 4 (30 gm of SB + 10 gm of Urea)	Nil
		Blend 5 (30gm of SB)	Nil
		Blend 6 (2.5 gm Phenol + 2 gm SB)	0.86
2.	Mefenamic Acid	Standard (BP) {1ml of glacial acetic acid+ 2.5ml of 1,4-Dioxan+9ml of toluene)	0.60
		Blend 4 (30gm of SB + 10gm of Urea)	0.70
		Blend 5 (30gm of SB)	0.70
		Blend 6 (2.5 gm Phenol + 2 gm SB)	0.87
3.	Ofloxacin	Standard (BP) {2 volumes of glacial acetic acid + 2 volumes of water + 2 volumes of ethyl acetate}	0.34
		Blend 4 (30gm of SB + 10 gm of Urea)	0.58
		Blend 5 (30gm of SB)	0.60
		Blend 6 (2.5 gm Phenol + 2 gm SB)	0.95
*SB-Sodium Benzoate SA-Sodium Acetate SC-Sodium Citrate			

Acyclovir exhibited an Rf value of **0.26** with the standard European Pharmacopoeia (EP) mobile phase composed of 0.2 mL concentrated ammonia, 2 mL methanol, and 8 mL methylene chloride. However, no distinct Rf values were observed when using blends containing sodium benzoate (SB) with sodium acetate (SA), sodium citrate (SC), or urea (Blends 1 to 5), indicating a lack of effective migration or spot development. In contrast, Blend 6 (2.5 g phenol + 2 g SB) produced a significantly higher Rf value of **0.86**, suggesting improved solubilization and migration of acyclovir under these conditions.

Mefenamic acid showed an Rf value of **0.60** with the British Pharmacopoeia (BP) standard mobile phase (1 mL glacial acetic acid, 2.5 mL 1,4-dioxane, and 9 mL toluene). Among the hydrotropic solvent systems, Blend 4 and Blend 5 (SB + urea and SB alone, respectively) yielded slightly higher Rf values of **0.70**, indicating moderate mobility. Notably, Blend 6 again demonstrated the highest Rf value of **0.87**, emphasizing the enhanced elution power of phenol in combination with SB.

Ofloxacin, when analyzed with the BP standard mobile phase (a ternary mixture of glacial acetic acid, water, and ethyl acetate in equal volumes), had an Rf value of **0.34**. This increased to **0.58** and **0.60** with Blends 4 and 5, respectively. The most significant increase was observed with Blend 6, which produced an Rf value of **0.95**, indicating excellent migration and interaction with the mobile phase.

Overall, the results clearly demonstrate that the hydrotropic solvent system comprising **phenol and sodium benzoate (Blend 6)** consistently provided superior chromatographic performance across all three drugs. This suggests that phenol, possibly due to its strong hydrogen bonding and solubilizing ability, plays a key role in enhancing the mobility of poorly water-soluble drugs on silica gel plates. The study supports the potential utility of mixed hydrotropic systems, particularly those involving phenol and SB, as effective alternative mobile phases in TLC analysis for drug identification and separation.

4. CONCLUSION

This study demonstrates the effectiveness of environmentally friendly, mixed hydrotropic solvent systems as viable alternatives to conventional organic solvents in the thin-layer chromatography (TLC) of poorly water-soluble drugs. Among the various blends, **Blend 6**—a mixture of phenol and sodium benzoate—consistently provided the highest Rf values for **acyclovir, mefenamic acid, and ofloxacin**, indicating significantly improved solubility and migration. This enhanced performance is likely due to the strong hydrogen bonding and solubilizing capability of phenol within the hydrotropic system.

In comparison, other hydrotropic blends that excluded phenol (Blends 1 through 5) either failed to produce any migration or yielded only moderate Rf values, particularly for acyclovir. While standard pharmacopoeial mobile phases were effective, they rely on harmful organic solvents such as methylene chloride, toluene, and ethyl acetate, which pose environmental and health risks.

The results strongly support **Blend 6 as a green, efficient alternative mobile phase** for TLC analysis. The use of non-toxic and biodegradable hydrotropic agents not only enhances chromatographic performance but also aligns with the principles of green chemistry. This study underscores the potential of mixed hydrotropy—especially systems containing phenol and sodium benzoate—as a sustainable strategy for the chromatographic analysis of poorly soluble pharmaceutical compounds.

GLOSSARY

Hydrotropy: It is a molecular solubilization process in which high concentrations of small, structurally simple organic or inorganic compounds increase the aqueous solubility of hydrophobic molecules through non-micellar aggregation.

Mixed Hydrotropy: A cooperative hydrotropic system formed by two or more hydrotropic agents that generate enhanced solubilization via synergistic aggregation or interaction networks.

Hydrotropic Agent: A low-molecular-weight compound capable of forming dynamic, reversible assemblies in water that promote solubilization of poorly soluble substrates (e.g., sodium benzoate, urea, phenol).

Thin-layer Chromatography (TLC): A planar chromatographic technique using a stationary adsorbent layer (silica gel) and a mobile phase to separate analytes based on differential partitioning.

Retention Factor (Rf)

A dimensionless ratio representing the relative migration distance of an analyte to the migration distance of the solvent front.

Stationary Phase: The immobilized solid phase silica gel on TLC plates, providing adsorption sites for analyte-surface interactions.

Mobile Phase: the solvent system responsible for analyte transport along the stationary phase; in this work, composed of hydrotropic or mixed hydrotropic aqueous blends.

Solubility Enhancement: Improvement in aqueous solubility resulting from hydrotropic-induced disruption of solute-solute interactions and formation of transient solubilizing domains.

Non-micellar Aggregation: Formation of loosely organized, low cooperatively molecular clusters characteristic of hydrotropic systems, distinct from surfactant micelles.

π-π Interaction: A non-covalent interaction involving hydrogen donors and acceptors that support hydrotrope-drug association and mobility in the TLC system.

Hydrogen Bonding: Directional interactions involving hydrogen donors and acceptors that support hydrotrope-drug association and mobility in the TLC system.

Eco-friendly solvent system: Aqueous, non-toxic solvent blend replacing hazardous organic solvents while maintaining chromatographic performance.

Poorly water soluble drugs: Pharmaceutical agents with limited aqueous solubility requiring solubilization strategies for analytical or formulation applications.

Declarations

Author contributions

All the authors have contributed to the development and completion of this study and have diligently been a part of the assessment of the outcomes and writing of the manuscript

Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this research work.

Funding Sources

No funds were received for the completion of this work.

ACKNOWLWDGEMENTS:

The research was conducted primarily at the KLE COLLEGE OF PHARMACY, BANGALORE, KARNATAKA, INDIA. The corresponding author is affiliated with the JSS COLLEGE OF PHARMACY, MYSURU, KARNATAKA, INDIA and wishes to thank them for general support during the final preparation of manuscript.

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Figures

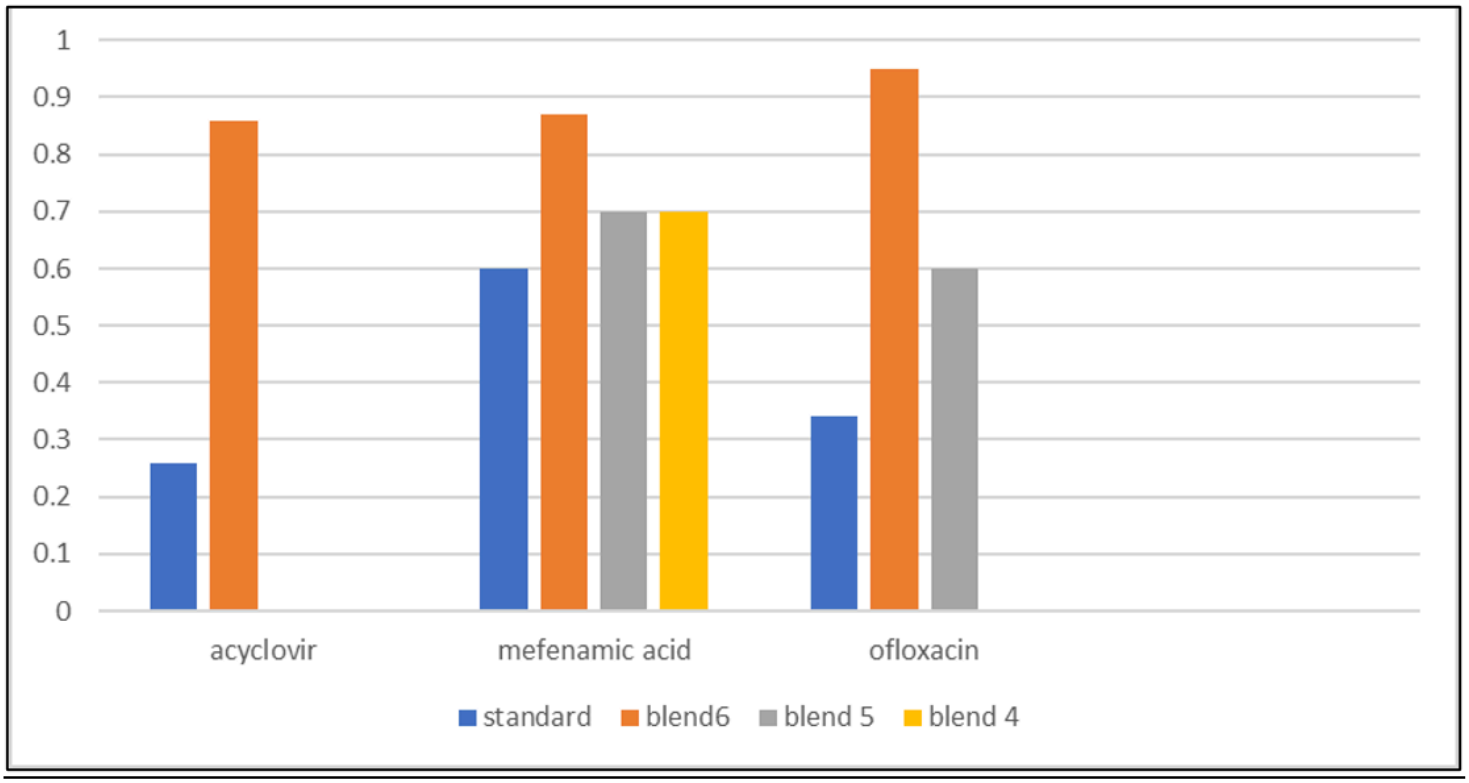


Figure 1

Comparison of Rf value of three drugs with standard mobile phase and blend 4,5 and 6

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