

Application of Mixed Hydrotropy to Carryout Thin Layer Chromatography of Paracetamol, Nifedipine, Indomethacin & Lamotrigine

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Abstract

The extensive use of volatile organic solvents in thin-layer chromatography (TLC) poses significant environmental and health concerns, making it necessary to develop eco-friendly analytical approaches for their replacement. This study investigated the application of mixed hydrotropy as an alternative solvent system for the TLC of paracetamol, nifedipine, indomethacin, and lamotrigine. Four different mixed hydrotropic blends were evaluated for their ability to produce satisfactory chromatographic separations. The chromatographic performance was assessed based on spot appearance, tailing behaviour, and retention factor (R_f). Among the investigated blends, Blend 1 produced distinct chromatographic spots for paracetamol, indomethacin, and lamotrigine, whereas Blend 4 was suitable for nifedipine. The remaining blends resulted in tailing or failed to produce well-defined spots on the TLC plates. The results demonstrate that appropriately selected mixed hydrotropic systems can provide satisfactory chromatographic performance while reducing the dependence on hazardous organic solvents. This study highlights the potential of mixed hydrotropy as a simple, economical, and environmentally compatible alternative for TLC analysis and contributes to the advancement of greener chromatographic methodologies.

Keywords: Mixed hydrotropy, thin-layer chromatography, green analytical chemistry, Paracetamol; Nifedipine; Indomethacin; Lamotrigine.

Introduction-

Pharmaceutical analysis plays a significant role in drug development and routine quality control. It helps ensure the identity, purity, and safety of pharmaceutical substances. Accurate and reliable analytical methods are indispensable for the regulatory compliance and quality assurance of APIs and finished pharmaceutical products^{1,2}. Among the various analytical techniques, chromatographic techniques are valued because of their high resolving power, sensitivity, reproducibility, and versatility in separating complex mixtures^{3,4}. Thin-layer chromatography (TLC) is a chromatographic technique that involves the distribution of analytes between the stationary and mobile phases. The affinity of the analyte with the mobile and stationary phases enables effective separation, making TLC one of the simplest and most reliable chromatographic techniques⁵⁻⁷.

Despite its various advantages, the application of thin-layer chromatography is accompanied by the use of volatile organic solvents as the mobile phase⁸. Methanol, chloroform, toluene, acetone, ethyl acetate, and hexane are commonly employed solvents for effective separation; however, many of these solvents are associated with considerable health hazards, including toxicity, flammability, and occupational exposure risks⁹⁻¹¹. They also contribute to environmental pollution and increase the economic burden on analytical laboratories. To minimize

the use of organic solvents, the application of mixed hydrotropy has been extensively explored for solubility enhancement, spectrophotometric analysis, and pharmaceutical formulation development.¹²⁻¹⁴

Hydrotropy is an approach to enhance the aqueous solubility of poorly water-soluble drugs using high concentrations of water-soluble substances, known as hydrotropic agents.¹⁵⁻¹⁷ Hydrotropic agents such as sodium benzoate, sodium salicylate, sodium citrate, nicotinamide, urea etc. facilitate solubilization without causing significant chemical modification of the solute, thereby offering a simple, economical, and eco-friendly alternative.¹⁸ To further enhance solubility, the concept of mixed hydrotropy was introduced, wherein two or more hydrotropic agents are combined to produce synergistic solubilization while reducing the concentration of each individual component¹⁹. Owing to these advantages, the application of hydrotropic systems in chromatographic techniques as mobile phases can be employed as a potential strategy to reduce the dependence on hazardous organic solvents while maintaining satisfactory analytical performance.^{8, 20, 21}

In this study, paracetamol, nifedipine, indomethacin, and lamotrigine were used as model pharmaceutical compounds to investigate the applicability of mixed hydrotropic systems in thin-layer chromatography²². These drugs were selected because they represent therapeutically diverse classes and exhibit distinct physicochemical characteristics, particularly with respect to aqueous solubility, polarity, and chromatographic behaviour of the drugs^{4, 23, 24}. Paracetamol is a widely used analgesic and antipyretic with relatively high aqueous solubility, whereas nifedipine and indomethacin are classified as poorly water-soluble drugs, which pose analytical and formulation challenges²². Lamotrigine, an antiepileptic agent with moderate aqueous solubility, further broadens the physicochemical diversity of the selected compounds. The inclusion of drugs with varying solubility profiles provides an opportunity to comprehensively evaluate the effectiveness of mixed hydrotropic systems as alternative mobile phase components for chromatographic separation^{25, 26}.

Material-

All drugs, hydrotropic agents, and solvents, including Paracetamol, Nifedipine, Indomethacin, Lamotrigine, sodium citrate, sodium benzoate, sodium acetate, sodium salicylate, urea, ethanol, acetone, and dimethyl sulfoxide (DMSO), were procured from the Department of Pharmacy, Shri G.S. Institute of Technology and Science, Indore, Madhya Pradesh, India. Precoated silica gel GF254 TLC plates were purchased from a local scientific supplier and used for chromatographic analysis.

Method-

1. Preparation of mobile phase

Four mixed hydrotropic blends designated as blend 1, blend 2, blend 3 and blend 4 were prepared by mixing selected hydrotropic agents in predetermined ratio. Each drug was chromatographed with all the blends individually to assess the suitability of blend for chromatographic separation. Composition of blends are presented in table 1.

Blend 1- was prepared by dissolving accurately weighed 10gm of sodium benzoate, 10gm sodium citrate, 10gm urea in sufficient quantity of distilled water and the volume was made up to 100 ml with distilled water.

Blend 2- was prepared by dissolving accurately weighed 10gm sodium citrate, 10gm sodium acetate and 10gm sodium salicylate in water and the volume of the solution was made up to 100 ml with distilled water.

Blend 3- was prepared by dissolving accurately weighed 10gm sodium citrate, 10gm sodium acetate and 5gm sodium salicylate and 5gm of urea in water and the volume of the solution was made up to 100 ml with distilled water.

Blend 4- was prepared by dissolving accurately weighed 15gm sodium benzoate, 10gm sodium citrate and 5gm of urea in water and the volume of the solution was made up to 100 ml with distilled water.

Table 1-Composition of various mobile phases

S. no.	Blend	Composition (%w/v)
1.	Blend 1	10% Sodium Benzoate + 10% Sodium Citrate + 10% Urea
2.	Blend 2	10% Sodium Citrate + 10% Sodium Acetate + 10% Sodium Salicylate
3.	Blend 3	10% Sodium Citrate + 10% Sodium acetate + 5% Urea + 5% Sodium Salicylate
4.	Blend 4	15% Sodium Benzoate + 10% Sodium Citrate + 5% Urea

2. Preparation of Drug Solutions

Sample solution (1%w/v) was prepared by dissolving drug in suitable solvent based on solubility as listed in table 2.

Paracetamol - The sample solution for paracetamol was prepared by dissolving 100mg of accurately weighed paracetamol in 10ml of water.

Nifedipine - The sample solution for nifedipine was prepared by dissolving 100mg of accurately weighed nifedipine in 10ml of acetone.

Indomethacin - The sample solution for indomethacin was prepared by dissolving 100mg of accurately weighed indomethacin in 10ml of DMSO.

Lamotrigine - The sample solution for lamotrigine was prepared by dissolving 100mg of accurately weighed lamotrigine in 10ml of ethanol.

Table 2- Solutions of drugs employed for application of the spots on TLC plates.

S. no.	Drug sample	Solvent	Solution concentration(w/v)
1.	Paracetamol	Water	1%
2.	Nifedipine	Acetone	1%
3.	Indomethacin	DMSO	1%
4.	Lamotrigine	Ethanol	1%

3. Preparation of TLC chamber

A clean and dried beaker was used as developing chamber. Beaker was filled with mobile phase (0.5- 1 cm), ensuring that the solvent level remained below the sample application line on TLC plate. Inner wall of beaker was lined with filter paper, and the beaker was covered using a petri dish to facilitate chamber saturation with mobile phase prior to the chromatographic development as illustrated in fig.2.

4. Spotting of Sample on TLC Plate

An origin line was drawn approximately 0.5-1cm above the bottom edge of the TLC plate using a pencil. Sample solution was spotted at the centre of the line through a fused glass capillary tube. The plate was then kept for drying in oven allowing the solvent to evaporate before development.

5. Development of Chromatogram

Spotted plate was placed vertically into saturated chamber. The mixed hydrotropic solution was allowed to migrate upto an appropriate distance. The plate was removed immediately and solvent front was marked with pencil and the plate was air dried before visualisation.

6. Visualisation and calculation of R_f value

Air dried precoated TLC plate was placed into UV chamber and examined for the presence of spot. The distance travelled by the analyte and the distance travelled by mixed hydrotropic blend front from the origin line was measured as demonstrated in fig.1 and R_f value was calculated using the formula-

$R_f = \text{Distance travelled by the solvent front} / \text{Distance travelled by the analyte spot}$

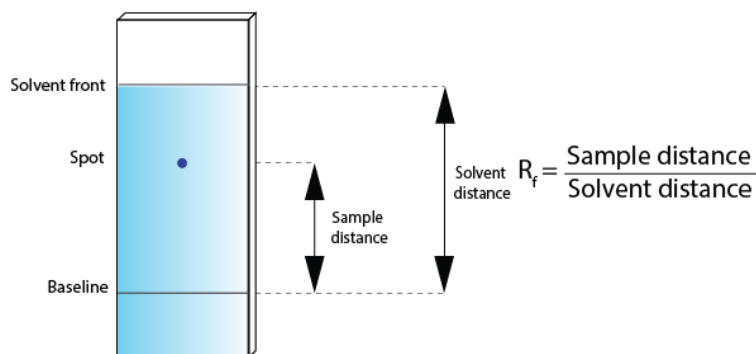


Fig. 1: Calculation of R_f value

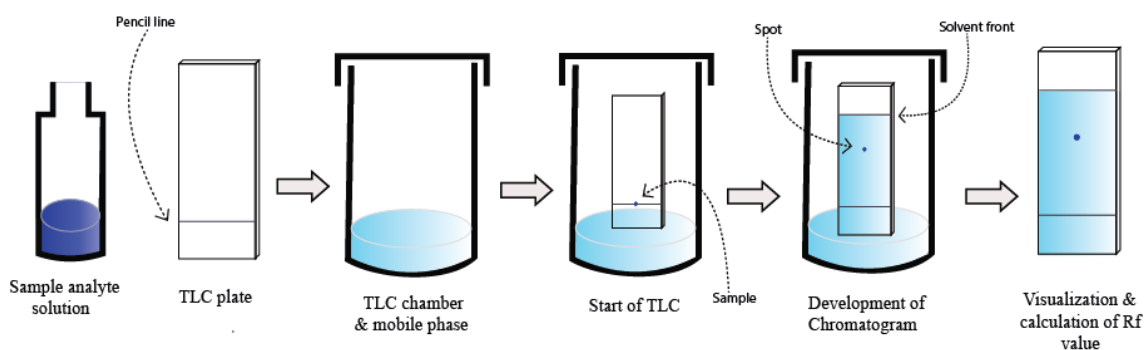


Fig. 2: Schematic representation of Thin Layer Chromatography (TLC) procedure

Results-

Different mixed hydrotropic blends were evaluated as alternative mobile phase for carrying out thin layer chromatography of paracetamol, nifedipine, indomethacin, and lamotrigine. The chromatographic performance

of each blend was assessed based on spot appearance and tailing behaviour and Rf value was calculated in table 3.

Table 3 - TLC studies of lamotrigine, paracetamol, nifedipine and indomethacin

S. no.	Drug	Blend	Observation	No. of spots	Rf value
1.	Paracetamol	Blend 1	Spot visible	01	0.89
		Blend 2	Tailing	-	-
		Blend 3	Tailing	-	-
		Blend 4	Tailing	-	-
2.	Nifedipine	Blend 1	Tailing	-	-
		Blend 2	Tailing	-	-
		Blend 3	Tailing	-	-
		Blend 4	Spot Visible	01	0.85
3.	Indomethacin	Blend 1	Spot Visible	01	0.71
		Blend 2	No spot visible	-	-
		Blend 3	No spot visible	-	-
		Blend 4	Tailing	-	-
4.	Lamotrigine	Blend 1	Spot Visible	01	0.72
		Blend 2	Tailing	-	-
		Blend 3	Tailing	-	-
		Blend 4	Tailing	-	-

Among the four blends that are investigated for paracetamol, Blend 1 produced a distinct and well-resolved chromatographic spot with an Rf value of 0.89 (Fig. 3). In contrast, Blends 2, 3, and 4 resulted in tailing, indicating inadequate chromatographic separation. The satisfactory performance of Blend 1 suggests that its hydrotropic composition provided an appropriate balance between analyte solubilization and migration on the silica gel stationary phase.

For nifedipine, only Blend 4 yielded a clearly visible chromatographic spot with an Rf value of 0.85 (Fig. 3), whereas Blends 1, 2, and 3 exhibited tailing. The selective effectiveness of Blend 4 indicates that the chromatographic behaviour of nifedipine is highly dependent on the composition of the mixed hydrotropic system, emphasizing the importance of optimizing hydrotropic combinations for individual analytes.

In the case of indomethacin, Blend 1 demonstrated superior chromatographic performance by producing a well-defined spot with an Rf value of 0.71 (Fig. 3). No visible spot was obtained with Blends 2 and 3, while Blend 4 resulted in tailing. These findings indicate that Blend 1 was capable of maintaining an appropriate balance between drug solubilization and interaction with the stationary phase, leading to efficient chromatographic migration.

Similarly, lamotrigine showed satisfactory chromatographic separation only with Blend 1, producing a distinct spot with an Rf value of 0.72 (Fig. 3). The remaining blends exhibited tailing, indicating less favourable chromatographic performance.

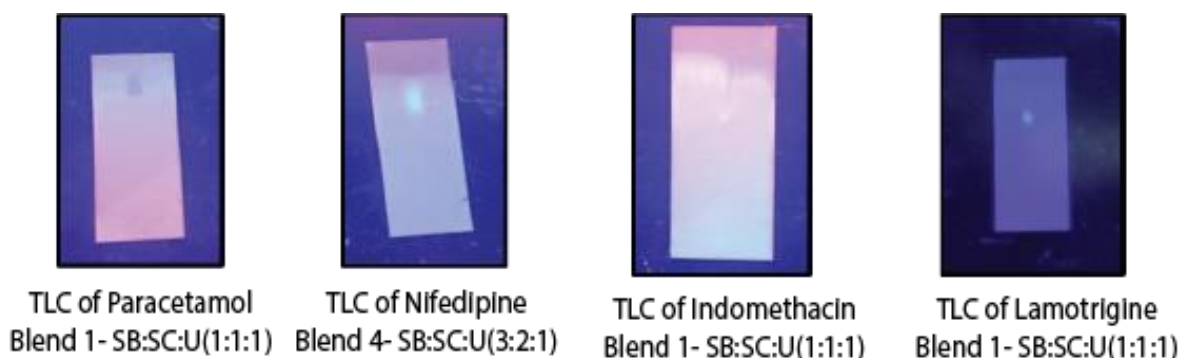


Fig. 3: Representative TLC chromatogram of paracetamol, nifedipine, indomethacin and lamotrigine developed using mixed hydrotropic blends of sodium benzoate (SB), sodium citrate (SC) and urea (U) in different ratios.

Overall, the results demonstrate that the chromatographic performance of mixed hydrotropic systems is drug dependent, with each analyte exhibiting different migration characteristics depending on the hydrotropic blend employed. Among the investigated blends, Blend 1 exhibited the broadest applicability, successfully separating paracetamol, indomethacin, and lamotrigine, whereas Blend 4 proved to be the most suitable for nifedipine. The occurrence of tailing or the absence of visible spots with certain blends may be attributed to unfavourable interactions between the analyte, hydrotropic components, and the silica gel stationary phase, which influenced analyte migration and spot resolution. These findings suggest that carefully optimized mixed hydrotropic systems can serve as potential alternatives to conventional organic solvent-based mobile phases for TLC, supporting the development of eco-friendly chromatographic methodologies.

Conclusion-

The present study successfully demonstrated the feasibility of employing mixed hydrotropic systems as alternative mobile-phase solvent systems for thin-layer chromatography of selected pharmaceutical compounds. Among the four hydrotropic blends investigated, sodium benzoate (SB), sodium citrate (SC) and urea (U) in different ratios was found to be most suitable for paracetamol, indomethacin, lamotrigine and nifedipine. The remaining blends exhibited tailing or failed to produce distinct chromatographic spots, highlighting the importance of selecting an appropriate hydrotropic composition for each analyte. The findings indicate that mixed hydrotropy can effectively facilitate chromatographic migration while reducing the reliance on conventional organic solvents. Thus, the proposed approach offers a simple, economical, and environmentally friendly alternative for TLC analysis and supports the development of eco- friendly chromatographic methodologies. Further optimization and evaluation of different hydrotropic combinations may broaden the applicability of this technique for the separation and identification of a wider range of pharmaceutical compounds.

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