



A Review: Green Analytical Application of Hydrotropy and Mixed Hydrotropy in the Field of TLC and HPLC

Ashwinee Parihar¹, Rajesh Kumar Maheshwari²

^{1,2}Shri G. S. Institute of Technology and Science, 23, Park Road, Indore, M.P., India – 452003.

ABSTRACT

Solvents account for more than half of the raw materials utilised in the active pharmaceutical sector. Benzene, 1,2-dichloroethane, benzene, 1,2-dichloroethene, carbon tetrachloride, 1,1,1-trichloroethane, acetonitrile, methyl cyclo hexane, chlorobenzene, chloroform, methylene chloride, nitro methane, ethylene glycol, pyridine, formamide, toluene, hexane, sulfolane, methanol, tetrahydrofuran, 2-methoxy ethanol, tetralin, 1,4-dioxane, 1,2-dimethoxy ethane, N,N-dimethylformamide, N,N-dimethylacetamide, trichloroethylene and cyclohexane are commonly employed organic solvents in various field of science. A large number of organic solvents are used to perform thin layer chromatography (TLC), UV spectrophotometric estimation, HPLC and titrimetric analysis. The majority of these organic solvents are very harmful to human being. Some of them are cancer-causing. The majority of organic solvents are expensive. Most of the organic solvents are also responsible for environmental contamination. Furthermore, the disposal of organic solvents after their use necessitates rigorous processes, making the process both costly and troublesome. Hydrotropic and mixed hydrotropic solutions were used as mobile phases to perform TLC and HPLC of the drugs, eliminating the need for organic solvents. TLC and HPLC technologies suggested here are novel, simple, cost-effective, environmentally friendly, and safe. Hydrotropic solutions will be a boon in the TLC, high performance liquid chromatography (HPLC) and high performance thin layer chromatography (HPTLC) analysis of a wide range of pharmaceuticals in the future, significantly reducing the consumption of organic solvents.

Keywords: Hydrotropy, Mixed Hydrotropy, Thin layer chromatography, High performance liquid chromatography, organic solvents.

1. Introduction

Hydrotropic solubilization is the increase in the aqueous solubility of a poorly water-soluble drug in the presence of a large concentration of an agent, i.e., a hydrotropic agent. Newberg (1916) coined the term "hydrotropic agent," which he defined as "a metallic salt of organic acid whose relatively high concentration in water might augment the aqueous solubility of organic compounds that are just weakly soluble in water." Salts that improve solubility are said to "salt in" the solute, while salts that decrease solubility are said to "salt out."^{1,2}

Additives can either enhance or reduce a solute's solubility in a given solvent. Salts that increase solubility are said to 'salt in' the solute, while salts that decrease solubility are said to 'salt out' the solute. The effect of an additive is heavily influenced by its influence on the configuration of water or its ability to interact with the solvent water molecules. Salts with large anionic or cationic groups that are very soluble in water cause salting in non-electrolytes and are known as 'Hydrotropic Salts,' and the phenomenon is known as 'HYDROTROPISM.'^{3,4}

1.1 The advantages of hydrotropic solubilization

- It is a revolutionary, cost-effective, safe, accurate, precise, and environmentally friendly method for analysing pharmaceuticals that are poorly water-soluble.
- This method is said to be superior to others such as micellar solubilization and co solvency since the solvent character is independent of pH, has good selectivity, and does not require emulsification.
- Hydrotropy is a simple procedure because it only requires the drug to be mixed with the hydrotrope in water.
- This method does not involve the chemical modification of hydrophobic drug, nor does it require the use of organic solvents or the development of an emulsion system.

Hydrotropic solubilization has been employed to enhance the aqueous solubility of several poorly water- soluble drugs.⁵⁻²⁰

1.2 MIXED HYDROTROPY

The mixed hydrotropic solubilization approach was proposed by Dr R.K. Maheshwari in 2008. It is an innovative and advanced method for enhancing the solubility of drugs that are poorly water soluble. The use of low concentrations of two or more hydrotropic agents to boost the solubility of poorly

water-soluble drugs by many folds is known as mixed hydrotropic solubilization. The increase in solubility can be quantified using the solubility enhancement ratio, which is the ratio of drug solubility of drug in hydrotropic solution to drug solubility in water.

Mixed hydrotropic solubilization has been employed to enhance the aqueous solubility of several poorly water-soluble drugs.²¹⁻⁴³

Solubility Enhancement Ratio = Solubility of drug in mixed hydrotropic solution / Solubility of drug in water.

1.2.1 THE BENEFITS OF MIXED HYDROTROPIC SOLUBILIZATION

1. Large concentration of single hydrotropic agent may be the reason of toxicity in pharmaceutical dosage forms. However, mixed hydrotropic solutions containing two or more hydrotropic agents in safe concentrations may be employed to formulate a marketable pharmaceutical dosage form solving the problem of toxicity issue of hydrotropic agent.
2. It is a new, easy, cost-effective, safe, accurate, precise, and environmentally friendly approach for analysing poorly water-soluble drugs (trimetric, spectrophotometric, and TLC analysis) without the use of organic solvents.
3. It prohibits the use of organic solvents, avoiding the problems of residual toxicity, inaccuracy due to volatility, pollution, cost, and so on.

1.2.2 APPLICATIONS OF HYDROTROPY IN THIN LAYER CHROMATOGRAPHY

Various organic solvents have been used for thin layer chromatographic studies of poorly water-soluble drugs, including methanol, chloroform, dimethyl formamide, acetonitrile, benzene, hexane, pyridine, tetralin, sulfolane, methyl cyclo hexane, methyl butyl ketone, toluene, methylene chloride, chlorobenzene, nitro methane. Organic solvents have a number of disadvantages, including toxicity, increased cost, and pollution. In these circumstances, hydrotropic solubilization is a better option than using organic solvents.

Maheshwari et al.⁴⁴ employed atenolol, paracetamol, ibuprofen, diclofenac sodium, and caffeine as model poorly water-soluble compounds. TLC studies were carried out on these compounds using hydrotropic solutions of sodium benzoate and urea in distilled water (Table-1). This approach avoids the use of organic solvents, which are both toxic and expensive. The proposed TLC methods are novel, simple, cost-effective, environment friendly, and safe.

Silica gel G plates were used to perform TLC. Iodine chamber was used for spot detection.

Table 1 TLC of atenolol, paracetamol, ibuprofen, diclofenac sodium, and caffeine by hydrotropic method

S. No.	Drug	IP method	Rf value	Hydrotropic method	Rf value
1.	Atenolol	99 volumes methanol, 1 volume strong ammonia solution	0.67	5.0 M Urea solution, 1.0 M Sodium benzoate solution	0.77 0.63
2.	Paracetamol	A mixture of 65 volumes of chloroform, 25 volumes of acetone and 10 volumes of toluene	0.65	5.0 M Urea solution, 0.5 M Sodium benzoate solution	0.86 0.68
3.	Ibuprofen	A mixture of 75 volumes of n-hexane, 25 volumes of ethyl acetate and 5 volumes of glacial acetic acid	0.60	5.0 M Urea solution, 2.0 M Sodium benzoate solution	0.90 0.82
4.	Diclofenac sodium	A mixture of 100 volumes of toluene, 10 volumes of hexane and 10 volumes of anhydrous formic acid	0.87	5.0 M Urea solution, 2.0 M Sodium benzoate solution	0.87 0.64
5.	Caffeine	A mixture of 40 volumes of 1-butanol, 30 volumes of chloroform, 10 volumes of strong ammonia solution and 3 volumes of acetone	0.70	5.0 M Urea solution, 2.0 M Sodium benzoate solution	0.89 0.87

Mangal et al.⁴⁵ used poorly water-soluble drug omeprazole as model drug, and 5 M sodium benzoate was utilised as model hydrotropic solution to develop the TLC plate (Table-2). Silica gel-G plates were employed in this study.

Table 2 TLC of omeprazole drug by hydrotropic method

S. No.	Drug	IP method	Rf value	Hydrotropic method	Rf value
1.	Omeprazole	A mixture of 5 volumes of benzene, 30 volumes of ethyl acetate and 10 volumes of methanol. (IP)	0.69	5.0 M Sodium Salicylate solution.	0.75

Sonali et al.⁴⁶ employed, hydrotropic solutions as mobile phases for estimation of poorly aqueous soluble drugs by TLC technique. Erythromycin, ciprofloxacin and norfloxacin were selected as model drugs for estimation by TLC and sodium benzoate, urea, and sodium salicylate were taken as hydrotropic agents for mobile phase in place of solvents like dichloromethane, menthol, acetonitrile, and toluene, diethyl amine, propanol, and ethyl acetate most of which are much expensive and toxic (Table-3). TLC of selected model drugs were also performed by using proposed method given in Indian Pharmacopoeia (Table-3). The proposed hydrotropic TLC methods are new, simple, cost-effective, environment friendly and safe. In future, hydrotropic solutions shall prove a boon in TLC and high performance thin layer chromatography (HPTLC) analysis of a vast number of drugs thereby limiting the use of organic solvents to a great extent.

Table 3 TLC of erythromycin, ciprofloxacin and norfloxacin by hydrotropic method

S. No.	Drug	Method	Rf value	Hydrotropic method	Rf
1.	Erythromycin	A mixture of solvent containing 45 volumes of ethyl acetate, 40 volumes of 15% w/v of ammonium acetate and 20 volumes of 2-propanol	0.87	2.0 M Urea solution, 2.0 M Sodium benzoate solution	0.92 0.93
2.	Ciprofloxacin	A mixture of solvent containing 40 volumes of dichloromethane, 40 volumes of methanol, 20 volumes of strong ammonia and 10 volumes of acetonitrile	0.63	2.0 M Sodium benzoate solution, 2.0 M Sodium salicylate solution	0.53 0.68
3.	Norfloxacin	A mixture of solvent containing 40 volumes of dichloromethane, 40 volumes of methanol 20 volumes of toluene, 14 volumes of diethyl amine and 8 volumes of water	0.88	2.0 M Urea solution, 2.0 M Sodium benzoate solution	0.81 0.90

1.2.3 APPLICATIONS OF MIXED HYDROTROPY IN THIN LAYER CHROMATOGRAPHY

Maheshwari et al.⁴⁷ used poorly water soluble drugs norfloxacin, diazepam, metronidazole, naproxen, and nimesulide as model drugs, and sodium benzoate, urea, sodium citrate, sodium acetate, and niacinamide were utilised as model hydrotropic agents in various combinations in distilled water (Table-4). This process eliminates the need for organic solvents, which are both expensive and dangerous to one's health. As a result, hydrotropic solutions can be used for TLC studies replacing hazardous organic solvents.

Silica gel-G was used for the preparation of TLC plates. The detection of the spot was done using iodine chamber.

Table 4 TLC of norfloxacin, diazepam, metronidazole, naproxen, and nimesulide using hydrotropic method

S. No.	Drug	IP method	Hydrotropic method	Rf value
1.	Metronidazole	A mixture of 80 volumes of chloroform , 20 volumes of dimethylformamide and 5 volumes of a 90 percent v/v solution of formic acid (IP)	30 % Sodium benzoate solution	0.78
2.	Diazepam	A mixture of 100 volumes of chloroform and 10 volumes of methanol	30 % Sodium benzoate solution	0.63
3.	Nimesulide	-	15% Niacinamide and 15% sodium benzoate 15% Sodium benzoate and 15% sodium acetate 15% Sodium benzoate and 15% sodium citrate	0.89 0.75 0.82

4.	Naproxen	-	15% Sodium benzoate and 15% sodium acetate	0.42
5.	Norfloxacin	A mixture of 40 volumes of dichloromethane, 40 volumes of methanol, 20 volumes of toluene, 14 volumes of Diethyl amine and 8 volumes of water.	15% Sodium benzoate and 15% sodium citrate 15% Sodium benzoate and 15% urea 7.5% Sodium benzoate and 7.5% urea	0.50 0.64 0.35

Salunke P.A. et al.⁴⁸ used mixed hydrotropic methods to perform thin layer chromatography, which is used to improve the water solubility of drugs which are poorly water soluble. Bromocresol green and phenol red were chosen as the model samples. Both dyes are soluble in mixed hydrotropic solutions like 10% sodium citrate and 10% urea as well as optimal single hydrotropic solutions like 20% niacinamide, 25% niacinamide, and 10% sodium benzoate. Better results are obtained when the mobile phase is a mixed hydrotropic solution of 10% sodium citrate and 10% urea. In a mixed hydrotropic solution, the R_f values (Table-5) for phenol red and bromocresol green were determined to be 0.82 and 0.51, respectively. While dyes are entirely soluble in a single hydrotropic solution, chromatographic separation is not properly obtained. A mixed hydrotropic solution is used to improve dye aqueous solubility. When the same solution was employed as a mobile phase, appropriate separation of colours was observed. Using thin layer chromatography, the rate of separation in mixed hydrotropic mobile phase was faster.

Table 5 TLC for bromocresol green and phenol red dye by mixed hydrotropy method

S. No.	Dye	Hydrotropic method	R _f value
1.	Bromocresol green + Phenol red	10% Sodium citrate and 10% urea	0.51 (Bromocresol green) 0.82 (Phenol red)

Maheshwari et al.⁴⁹ used hydrotropic solutions and mixed hydrotropic solutions as mobile phases to perform thin layer chromatography nine drugs. Propranolol hydrochloride, guaifenesin, ciprofloxacin hydrochloride, pyridoxine hydrochloride, lidocaine hydrochloride, thiamine hydrochloride, and metformin were selected as compounds for TLC. The hydrotropic agents used in mobile phases were sodium benzoate, sodium citrate, sodium acetate, and niacinamide. These TLC (hydrotropic procedures) proposed were safe, easy, new, environmentally benign, and cost effective. Eight blends (hydrotropic and mixed hydrotropic) were employed to carry out of all nine compounds. The R_f values for good spots (without tailing effects) only are reported in table-6. In this study silica gel-G plates were employed and spot identification was done using iodine chamber.

Table 6 Results of TLC studies using hydrotropy and mixed hydrotropy⁴⁷

S. no.	Drug	Blend	R _f value
1.	Propranolol HCl	7.5% Niacinamide + 7.5% Sodium benzoate	0.80
2.	Guaiphenesin	5% Sodium benzoate + 5% Sodium acetate	0.64
		5% Sodium benzoate + 5% Niacinamide	0.70
		10% Sodium benzoate	0.82
		5% Sodium acetate + 5% Sodium citrate	0.66
3.	Ciprofloxacin HCl	5% Sodium citrate + 5% Niacinamide	0.47
4.	Vitamin B6 HCl	5% Sodium acetate + 5% Sodium citrate	0.84
5.	Lidocaine HCl	5% Sodium benzoate + 5% Sodium acetate	0.42
		5% Sodium benzoate + 5% Niacinamide	0.42
		5% Sodium benzoate + 5% Sodium citrate	0.45
		10% Sodium benzoate	0.41
		5% Sodium acetate + 5% niacinamide	0.37
		5% Sodium acetate + 5% Sodium citrate	0.37
6.	Vitamin B1 HCl	5% Sodium benzoate + 5% Niacinamide	0.48
		10% Sodium benzoate	0.33
7.	Metformin HCl	5% Sodium benzoate + 5% Niacinamide	0.48
		5% Sodium acetate + 5% Sodium citrate	0.37
8.	Piperazine HCl	5% Sodium benzoate + 5% Sodium acetate	0.67
		5% Sodium benzoate + 5% Niacinamide	0.65
		5% Sodium benzoate + 5% Sodium citrate	0.60
		5% Sodium acetate + 5% Sodium citrate	0.65
9.	Losartan	5% Sodium benzoate + 5% Sodium acetate	0.73

	Potassium	5% Sodium benzoate + 5% Sodium citrate	0.63
		10% Sodium benzoate	0.80
		5% Sodium acetate + 5% Niacinamide	0.60
		5% Sodium citrate + 5% Niacinamide	0.56

1.2.4 STUDIES ON HPLC BY MIXED HYDROTROPY CONCEPT

Remi et al⁵⁰. Drugs are typically analysed using high performance liquid chromatography using toxic and expensive organic mobile phases such as acetonitrile, methanol, etc. In this study, hydrotropic solution was used as the mobile phase for RP-HPLC estimation of the drug cefixime, which is poorly soluble in water. The study was done on the Agilent 1220 Infinity LC (G4288C) model, which consists of a Rheodyne injector (20 microliters), a variable wavelength detector, and an Agilent 1220 Infinity LC pump. Agilent Eclipse Plus C18 with 3.5micron particle size was the analytical column that was employed. Aqueous sodium acetate solution at a concentration of 6% with a pH adjusted to 6.2 by acetic acid was used as the mobile phase, and the drug was validated in accordance with ICH requirements. Cefixime's retention time was found to be 2.34 minutes. With a correlation coefficient of 0.999, linearity was seen in the concentration range of 40-240 micrograms/ml. Six replicate measurements were found to have a relative standard deviation percentage of 0.0055. This shows that the suggested procedure was accurate. Recovery investigations were carried out within the limits of linearity at three distinct concentration limit levels, and the average percentage in tablet dosage form was found to be 99.54 percent. As a result, the developed approach using hydrotropic solution as the mobile phase was new, easy to use, exact, economical, environmentally friendly, and safe. It can also be successfully used to estimate cefixime in pharmaceutical dosage forms.

This study uses a hydrotropic solution as the mobile phase, eliminating the need for an expensive and potentially harmful organic solvent. Therefore, the suggested method can be successfully used for the routine analysis of cefixime in pharmaceutical dosage dosage forms. It is also new, straightforward, precise, economical, safe, and easy to use.

HPLC Operating conditions

Column: Agilent eclipse Plus C18 (4.6x 100 with 3.5 µm particle size)

Detector: Variable wavelength detector

Injection volume: 20µL

Flow rate: 1.0 ml/minute

Temperature: Ambient

Run time: 10 minutes

Mobile phase: 6% Sodium acetate (pH 6.2) solution in HPLC grade water

Remi et al.⁵¹ used hydrotropic solutions as mobile phases to perform High performance liquid chromatography on poorly water-soluble drug. Drugs are typically analysed using high performance liquid chromatography using hazardous, pricey organic mobile phases such as acetonitrile, methanol, etc. In the current work, a hydrotropic solution (5 percent urea in HPLC grade water) that was both eco-friendly and cost-effective was used as the mobile phase for the RP-HPLC quantification of the poorly aqueous soluble medicines diclofenac sodium and paracetamol. Shimadzu LC6AD dual pumps with PDA Detector SPD-M20A, Rheodyne injector, and 20-L loop were used for the analysis. The analytical column utilised was a Shimadzu shim packC18 column with 4.6250mm dimensions and a 5µg particle size. Drugs were detected at 268nm at room temperature using urea solution (5%) flowing at a rate of 1.0 mL/min as the mobile phase. It was discovered that a new, environmentally friendly mobile phase that contained 5% urea solution gave sharp peaks for paracetamol and diclofenac sodium with retention times of 3.272 and 1.772 min, respectively. ICH guidelines were followed in the method's validation. In the range of 100–500 µg/mL for paracetamol and 10–50 µg/mL for diclofenac sodium, linearity for detector response was seen. The accuracy of the suggested procedure is demonstrated by the percent recovery for paracetamol and diclofenac sodium, which were 99.97 and 99.79, respectively. A high degree of precision is indicated by the percent RSD for both tablet analysis and recovery studies being less than 2%. The suggested approach is sensitive, as evidenced by the LOD and LOQ values. As a result, the devised approach using hydrotropic solution as the mobile phase was unique, straightforward, exact, economical, safe, and can be successfully applied.

HPLC Operating conditions

Column : Agilent eclipse Plus C18 (4.6x 100 with 3.5 µm particle size)

Detector : Variable wavelength detector

Injection volume: 20µL

Flow rate : 1.0 ml/minute

Temperature : Ambient

Run time : 10 minutes

Mobile phase: 5% urea solution

Remi et al.⁵² For the estimation of the drug ornidazole, which is poorly water soluble, a novel eco-friendly, safe, and economical RP-HPLC method was developed. The mobile phase in the bulk of RP-HPLC analyses is made up of toxic, costly, and volatile organic solvents. In the current investigation, an environmentally friendly, cost-effective hydrotropic solution (5 percent urea in HPLC grade water) was used as the mobile phase and solubilizing agent for the RP-HPLC estimation of the poorly aqueous soluble medication ornidazole. Shimadzu LC6AD dual pump with PDA Detector SPD-M20A, Rheodyne injector (20-l loop), and analysis were used. Shimadzu shim pack C, a column with 5 μ particle size and dimensions of 4.6 x 250 mm, was the analytical column that was employed. The drug was detected at 320 nm at room temperature using urea solution (5%) and a flow rate of 1.0 ml/minute as the mobile phase. An innovative, environmentally friendly mobile phase that contains 5% urea solution was shown to be effective and to provide a sharp peak for ornidazole with a retention time of 3.996 minutes. ICH guidelines were followed in the method's validation. Regression equation $y=49321+33223x$ showed linearity over the concentration range of 10- 50 μ g/ml ($r^2=0.9990$). Ornidazole's percentage recovery was 99.36, proving the proposed method's accuracy. A good degree of precision is indicated by the percent RSD for both tablet analysis and recovery studies being less than 2%. The LOD and LOQ values of 0.015771 μ g/ml and 0.047793 μ g/ml, respectively, demonstrated the sensitivity of the proposed approach.

HPLC Operating conditions

Column : Agilent eclipse Plus C18 (4.6x 100 with 3.5 μ m particle size)

Detector : Variable wavelength detector

Injection volume : 20 μ L

Flow rate : 1.0 ml/minute

Temperature : Ambient

Run time : 10 minutes

Mobile phase : 5 % urea solution in HPLC grade water

Matungi et al⁵³ Using the concept of hydrotropy, an eco-friendly, precise, and accurate RP-HPLC technique for the detection of gatifloxacin was established in this study. Hydrotropy is the process of increasing a solute's solubility in water by adding an agent known as a hydrotrope. This concept decreases the usage of organic solvents in drug analysis, making it more environmentally friendly and less expensive. The method was created with 3% sodium benzoate (pH 6.5) as the mobile phase and a Shimadzu ODS-C18 column as the stationary phase. The flow rate was kept constant at 1.4 ml/min, and detection was done at 293 nm with a diode array detector. The retention period was 2.5 minutes, and the technique was shown to be linear with correlation coefficients of 0.999 for a concentration range of 0.5-30 μ g/ml. The developed approach was validated according to the ICH Q2 (R1) guidelines and used to estimate gatifloxacin in eye drop formulation.

A precise, accurate reversed phase liquid chromatographic method for estimating gatifloxacin from eye drop formulation has been developed. The method employs an aqueous 3% solution of sodium benzoate as the mobile phase, reducing the need for organic solvents. The approach takes less time, with a retention time of 2.5 minutes. Organic solvents, which are hazardous to the environment and promote pollution, are used in the reported analytical methods. The current method is environmentally friendly, cost effective, sensitive, and precise. The method was successfully employed for gatifloxacin estimation in its formulation, and it can be used for gatifloxacin analysis in bulk and quality control samples.

HPLC Operating conditions

Column : ODS-C18 column

Detector : Diode array detector

Flow rate : 1.4 ml/minute

Temperature : Ambient

Run time : 2.5 minutes

Mobile phase : 3% sodium benzoate (pH 6.5)

CONCLUSION

It is, thus, well-observed that the R_f values obtained employing the proposed methods using the hydrotropic solutions and mixed hydrotropic solutions as mobile phases were quite satisfactory. The proposed TLC methods were mostly devoid of the tailing effect. The hydrotropic resolution also showed an advantage of the absence of tailing effect. Time effectiveness was also observed. Thin layer chromatography of all compounds (both water soluble and water insoluble compounds) is possible using hydrotropic solutions and mixed hydrotropic solutions as mobile phases.

This study uses hydrotropic solutions as mobile phases for RP-HPLC studies which eliminates the need for a more expensive and corrosive organic solvent as a mobile phase. As a result, the suggested approach is unique, easy, exact, cost-effective, environmentally friendly, and safe, and it may be successfully employed for regular TLC and HPLC of poorly water-soluble compounds.

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