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# Spectrophotometric and HPLC Methods for Poorly Water Soluble Drugs



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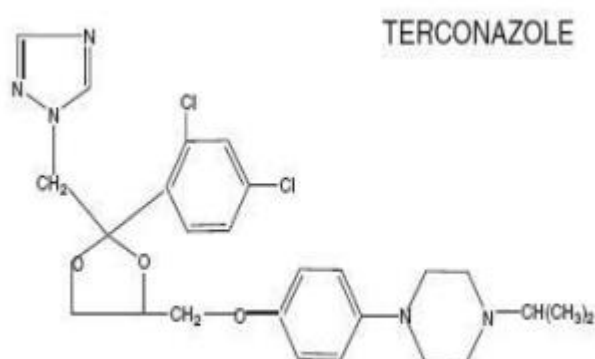
## ABSTRACT

Several techniques are used to increase the aqueous solubility of poorly water-soluble drugs. Hydrotropic solubilization technique is one of them. A simple, efficient and new spectrophotometric method has been developed for spectroscopic estimation of Terconazole in bulk form. In the present study, a very slightly soluble drug, Terconazole has been solubilizing in water using the hydrotropic agent of urea and scanned between 200-400nm taking respective reagent blanks in a double beam spectrophotometer. The  $\lambda$  max was found to be 286nm and Terconazole was found to be linear in the range of 50-250  $\mu$ g/ml. In this proposed method, organic solvents are not used which makes it eco-friendly. Thus, hydrotropic solubilization technique, a novel approach can be used not only for estimation but also used to increase solubility and release of poorly water-soluble drugs. The hydrotropic agent used is cost-efficient, safe and non-harmful to the environment, hence it can be employed for routine analysis of Terconazole in bulk form. Hydrotropic agent urea did not interfere in spectrophotometric determination. From the results, it was justified that hydrotropic solubilization technique is one of the best method used to increase the solubility of the Terconazole as it shows gradual linearity values with increase in the amount of urea and so finally this method is used for routine quantitative analysis of terconazole samples.

## INTRODUCTION

In the pharmaceutical analysis and formulation development fields it is most often required to increase the aqueous solubility of poorly water-soluble drugs. Most of the newly developed drug molecules are lipophilic in nature and poor solubility is one of the most difficult problems of these drugs. In general, in order for a drug to exert its biologic effect, it must be soluble in and transported by the body fluids, traverse the required biologic membrane barriers, etc. It is well known that drug efficacy can be severely limited by poor aqueous solubility. It is also known that the side effects of some drugs are the result of their poor solubility. The ability to increase aqueous solubility can thus be a valuable aid to increase efficacy and/or reducing side effects for certain drugs. Various organic solvents like methanol, chloroform, alcohol, dimethylformamide, and benzene have been employed for the solubilization of poorly water-soluble drugs for spectrophotometric estimations. Drawbacks of organic solvents include higher cost, toxicity, pollution, and error in the analysis due to volatility. There are various approaches for solubilization of poorly water-soluble drugs. "Hydrotropy" is a solubilization technique in which addition of a large amount of a second solute results in an increase in the aqueous solubility of another solute. The hydrotropic agents are defined as non-micelle-forming substances, either liquids or solids, organic or inorganic, capable of solubilizing insoluble compounds. Hydrotropic agents consist generally of two essential parts, an anionic group and a hydrophobic aromatic ring or ring system. The anionic group is obviously involved in bringing about high aqueous solubility, which is a prerequisite for a hydrotropic substance. On the other hand, the planarity of the hydrophobic part has been emphasized as an important factor in the mechanism of hydrotropic solubilization. Review of literature shows that a large number of poorly water-soluble drugs have been analyzed *viz.* frusemide [1], cefixime [2], salicylic acid [3], ketoprofen [4], tinidazole [5], aceclofenac [6], amoxicillin [7], ofloxacin [8], hydrochlorothiazide [9], metronidazole [10], nalidixic acid [10], ibuprofen [11], naproxen [11], flurbiprofen [11], aspirin [12], cephalixin [13], paracetamol [14], and piroxicam [15] using hydrotropic solubilizing agents. Concentrated aqueous solutions of a large number of hydrotropic agents like sodium benzoate, sodium salicylate, urea, sodium ascorbate, niacinamide, and sodium citrate have been employed to enhance aqueous solubility of various poorly water-soluble drugs. Terconazole is an anti-fungal medication, primarily used to treat vaginal fungal infections. It is commercially available in several dosage forms for topical administration. Topical terconazole formulations have been found to be effective in controlling vaginal infections without producing significant adverse reactions. Terconazole is an odorless,

white powder having a molecular weight of 532.47g and melting point of 126.3°C. Its chemical formula is  $C_{26}H_{31}Cl_2N_5O_3$ . The structural formula of terconazole is as follows Figure 1:



**Figure 1: Terconazole**

The IUPAC name of Terconazole is 1-(4-{[(2R, 4S)-2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4yl] methoxy}phenyl)-4-(propan-2-yl)piperazine. It is sparingly soluble in ethanol, soluble in Butanol and insoluble in water. It is available as Terazol, Terazol 3, Terazol 7 and Tercospor. Terconazole acts by disrupting normal fungal cell membrane permeability. It inhibits cytochrome P450 14- $\alpha$ -demethylase in susceptible fungi, which leads to a decrease in ergosterol concentration. Depletion of ergosterol will disrupt the structure and function of the fungal cell and cell growth. Literature survey reveals no validated method was found for its quantitative determination in bulk and pharmaceutical dosage forms, but it was used as an internal standard in estimation of some other drugs like ketoconazole, Itraconazole, and in some enantiomer separations, also in detection of some anti-fungal agents in illegal shampoo by HPLC.

**OBJECTIVE:** The objective of this work was to develop sensitive and efficient analytical methods for quantitative determination of terconazole in bulk forms. In this study, efforts were made to develop a simple, easy, safe and economic UV spectrophotometric method using hydrotropic solubilization technique for the determination of terconazole in the bulk form. The hydrotropic agent (urea) used is cost-efficient, safe, and non-harmful to the environment; hence it can be employed for routine analysis of Terconazole in bulk forms.

## MATERIALS AND METHODS:

**Instruments:** UV-Visible Double Beam Spectrophotometer (Thermo Scientific, Evolution 201) with 1cm matched Quartz cells, micropipette of variable volume and Digital Balance. **Chemicals:** Standard Solution of Terconazole, 0.1N HCl, distilled water. Terconazole was procured from our college i.e. Nirmala college of pharmacy. Phase solubility studies (UV method for analysis) Standard Terconazole of 10mg was accurately weighed and transferred to the 10ml volumetric flask. It was dissolved properly by using 2ml of 0.1N HCl and diluted to mark with distilled water to obtain a concentration of 1mg/ml. This solution was used as working standard solution. From this 0.5,1,1.5,2 and 2.5 ml of this solution was taken in 10ml of volumetric flask and make up the volume up to the mark with distilled water, and UV absorbance of each concentration was measured at 286 nm with UV-VIS Spectrophotometer (Thermoscientific-evolution 201). The data was indicated in Table-1 and graph of absorbance was plotted against the concentrations to give the standard curve (Fig 2). The calibration curve for the terconazole method was linear over the range of 50-250 $\mu$ g/ml ( $r^2 = 0.998$ ). The UV-Vis spectrophotometer was previously calibrated according to the method mentioned in Indian Pharmacopoeia (I.P.) 1996, i.e. control on absorbance test, in which absorbance of potassium dichromate solution was checked at the wavelengths indicated in I.P. 1996. The A (1%, 1 cm) for each wavelength was measured and found in the permitted limits according to I.P. 1996.

**Calibration Data of Standard Terconazole (Table-1)**

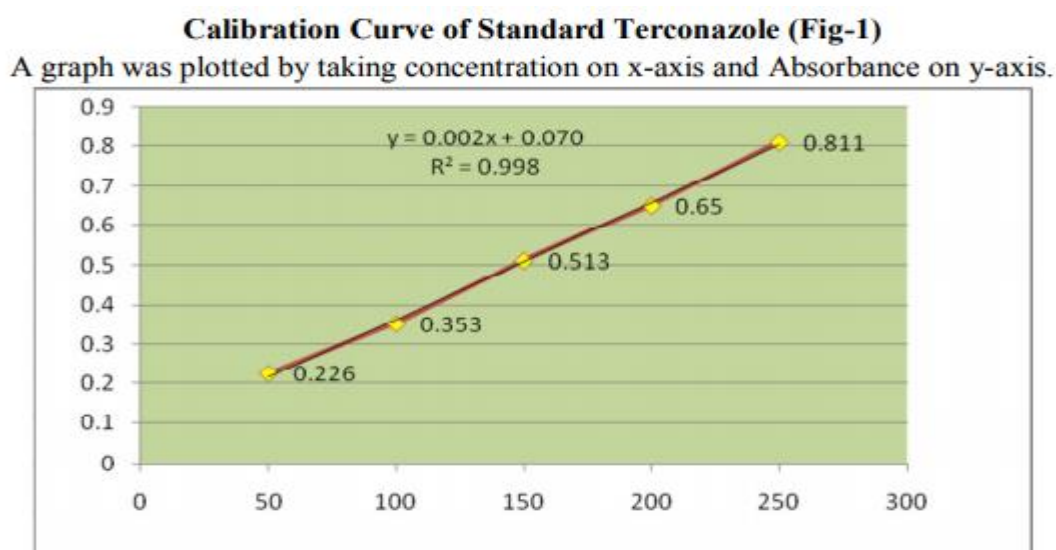
S. No	Concentration ( $\mu$ g/ml)	Absorbance
1.	50	0.226
2.	100	0.353
3.	150	0.513
4.	200	0.650
5.	250	0.811

### Solubility Analysis with Variation in Amount of Urea:

Solubility was determined with the hydrotropic substance of different amounts (10, 20, 30, 40, 50 and 60mg of urea). 10 mg of STD Terconazole was weighed into series of glass vials and increasing amounts of 10, 20, 30, 40, 50 and 60mg of urea were added and made the volume up to 10ml with distilled water. The samples were sonicated for 4 hrs and kept at 25°C for 24 hrs and passed through a 0.45 µm filter. Then clear solutions were analyzed spectrophotometrically at 286 nm using UV-Vis Spectrophotometer (UV-201, Thermo scientific). Absorbance was extrapolated on the calibration curve to determine the unknown concentration and the solubility of each sample was calculated by using the following formula.

**Drug solubility = unknown conc. from graph \* Dilution factor**

Then from obtained values % drug solubility was calculated. Results are presented in Table-2.



The recovery studies were carried out at a different level of concentration by spiking a known concentration of standard drug to the reanalyzed sample and contents were reanalyzed by proposed methods. The methods were validated statistically as per International Conference of Harmonization (ICH)/United State Pharmacopeia (USP) guidelines for a parameter like an accuracy, precision, ruggedness, linearity, and range. Accuracy was ascertained on the basis of recovery studies. Precision was studied by analyzing five replicates of the sample solution and Standard Deviation (SD) values were determined. Ruggedness was established by carrying out an experiment at various conditions like different time within a day (intraday), different day (interday) and by a different analyst. Linearity and range were determined by analyzing drug solution at 80-120% of label claim of the drug. Results of solubility studies indicate that the

enhancement in the solubility of RZM was more than 14 folds in 6M urea solution as compared to its solubility in distilled water and other hydrotropic agents. Therefore, this hydrotropic solution was selected to study the effect on enhancement in solubility of RZM. Part A solution of drug in 6M urea solution was kept at room temperature for 48 h. There was no precipitation of drug in Part A solution within 48 h. In addition, drug contents of Part A solutions (after 48 h) were same as those of Part B solutions (fresh solutions). This study reveals that the estimations can be done within 48 h at least, without having any detrimental effect on drug stability.

**TABLE 1: RESULTS OF RZM TABLET FORMULATION AND RECOVERY STUDIES**

Tablet analysis			Recovery study	
Tablet formulation	Label claim (mg)	% Drug estimation*	Pure drug added (mg)	% Recovery* (Mean±SD)
Brand A	4	98.40	6	98.65±0.6521
Brand B	8	99.46	12	98.12±1.0547

\*Results are the mean of five determinations

**TABLE 2: RESULTS OF THE VALIDATION STUDY**

Validation parameter	Observation with S.D.
Accuracy	98.65±3.6521 <sup>a</sup> 98.1±1.0547 <sup>b</sup>
Precision	1.109 <sup>a</sup> 0.084 <sup>b</sup>
Ruggedness	
Intraday	98.05%±0.14
Interday	97.52%±0.72
Different analyst	98.13%±1.31
Linearity and range	80%-120%

The table gives results of the validation parameters of the method. The results are mean of five determinations. The "a" and "b" indicates brand A and brand B of the tablets of RZM, respectively.

From Table 1, it is evident that there is good agreement between the amounts estimated and those claimed by the manufacturers. Percent label claims were very close to 100%, with low values of standard deviation. The recovery study shows the accuracy of the method. On observing the validation parameters the method was found to be accurate and precise (Table 2). From this study, it is obvious that there was no interference of urea solution in the estimation of rosiglitazone maleate. Urea is cheaper than most of the organic solvents and can thus substitute expensive methanol, dimethylformamide, chloroform and carbon tetrachloride. Drawbacks of organic solvents include toxicity, error due to volatility, pollution, and cost. Thus, hydrotropic solutions of urea to solubilized poorly water-soluble drug RZM, from tablets to carry out spectrophotometric analysis precluding the use of organic solvents. The tablets containing RZM were analyzed successfully. From the present investigation, it is concluded

that the proposed method is new, simple, cost-effective, accurate, safe, and precise. Thus, it can be successfully employed in the routine analysis of RZM in bulk drug and its pharmaceutical tablet dosage form.

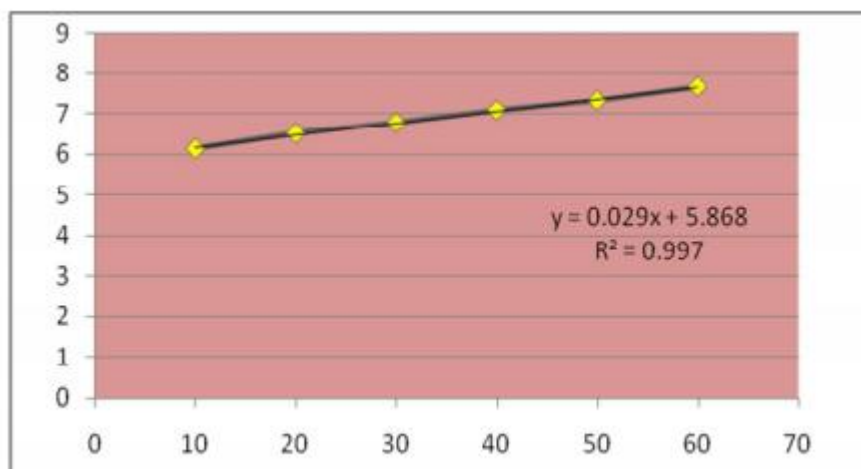
## RESULTS AND DISCUSSION:

Hydrotropes are amphiphilic in nature i.e. composed of hydrophilic as well as lipophilic portions. These molecules are generally used as solubility enhancer (solubilizers). This method is commonly known as micellar solubilization since they form micelles, which are an association, segregate of surfactants. Hydrotropic agents have been used to enhance aqueous solubility of hydrophobic drugs. In many instances, the aqueous solubility was increased by orders of magnitude simply by mixing with hydrotropic agents in water. Hydrotropy is a collective molecular phenomenon describing an increase in the aqueous solubility of a sparingly water-soluble drug by addition of a relatively large amount of a second solute. Hydrotropic agents self-associate into loose non-covalent assemblies of non-polar microdomains to solubilize hydrophobic solutes. However, the detailed mechanisms of Hydrotropy have not been fully understood. Currently, the most widely used method for increasing the aqueous solubility is to add surfactants to the aqueous release media. However, this method is not applicable for polymeric micelle systems because even a small amount of surfactants could destroy their micellar structure and distort their release profiles. A hydrotropic agent could be a good alternative to increasing the aqueous solubility. The aqueous solubility of terconazole is 0.00116mg/ml at 25°C. In the present investigation, solubility enhancement caused by urea was studied. The solubility of terconazole at 25°C in the presence of different amounts of urea was given in following Table No-3.

### Solubility study with increase in amount of urea (Table-2)

S. No	Amount of urea added (mg)	Drug solubility (mg)	% Drug Solubility
1	10	0.613	6.13
2	20	0.650	6.5
3	30	0.677	6.77
4	40	0.708	7.08
5	50	0.732	7.32
6	60	0.766	7.66

A graph was plotted by taking the amount of urea on x-axis and % drug solubility on the y-axis (Fig-2).



From this study, it is obvious that there was no interference of urea solution in the estimation of terconazole. Urea is cheaper than most of the organic solvents and can thus substitute expensive methanol, dimethylformamide, chloroform and carbon tetrachloride. Drawbacks of organic solvents include toxicity, error due to volatility, pollution, and cost. This study indicates that the increase in terconazole solubility was due to the addition of Hydrotropes. Increase in amount of urea increased the solubility of the terconazole in distilled water. The present study describes the increase in solubility by Hydrotropes as well as the increase in solubility with an increase in the number of hydrotropic agents. This experimental method using a hydrotropic agent provides an alternative tool for an increase in the release of poorly soluble drugs in aqueous solution. Thus hydrotropic solubilization can be used for quantitative analysis, dissolution study and increase in bioavailability. This method provides the dynamics of the Hydrotropes insolubilization of terconazole. From the present investigation, it is concluded that the proposed method is new, simple, cost-effective and safe. Thus, it can be successfully employed in the routine analysis of Terconazole in bulk drug. In the future research I want to focus on visible spectrophotometry and HPLC, since till now this drug does not have any validated methods and in all cases, it was used just as an internal standard.

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