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A NOVEL SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF TENOFOVIR DISOPROXIL FUMERATE USING HYDROTROPIC SOLVENTS.

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ABSTRACT:

Ultraviolet-Visible spectrophotometric method for estimation of poorly water soluble drug like Tenofovir Disproxil Fumerate, has been developed. Aqueous solubility of drug has been enhanced to great extent (15folds) in 10%Urea + 30% Sodium benzoate blend. The primary objective of the present investigation was to employ these hydrotropic solutions to extract the drugs from their dosage forms, precluding the usage of costlier organic solvents. The selected λ_{max} 300nm, Urea and Sodium benzoate did not show any interference during estimation. The results of analysis have been validated statistically according to ICH guidelines, and by recovery studies. The proposed methods are new, simple, easy, economic, co-friendly, accurate, safe and precise.

Key Words; Tenofovir Disproxil Fumerate, Urea, Sodium Benzoate, Hydrotrophic, ICH Guidelines,

Introduction:

There are a variety of factors affecting the GIT drug absorption such as poor aqueous solubility and poor membrane permeability of the drug molecule. When a drug is administered orally, before it penetrates the membrane of GIT, it must first dissolve in gastric or intestinal fluids. Therefore the solubility and dissolution rate of poorly water soluble drugs should be enhanced. So for this, the drug should be available at the proper site of action within optimum doses. Bioavailability and solubility of drug molecules may affect the therapeutic effectiveness of a drug. The most important parameter to achieve desired concentration of drug in the systemic circulation for pharmacological response is the solubility. Due to the poor bioavailability, 40% of the lipophilic drug candidate fails to reach our markets. Therefore, they had a high dose to attain the proper pharmacological action. Different solubilization[2,3] techniques can be used to increase the solubility of the poorly water soluble drugs. Hydrotropy[1] is generally used to enhance the solubility of the drug agents by mixing a sufficient amount of alkali metal salts. Nevertheless, this term describes non micelle-developing substances having an aptitude for solubilizing insoluble compounds. Moreover, these substances might be of an organic or inorganic type and in either state (i.e., solid or liquid).

The Hydrotropes[4] are known to self-assemble in solution. The chemical structure of the conventional Neuberg's hydrotropic salts (prototype, sodium benzoate) consists 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. The type of anion or metal ion appeared to have a minor effect on the phenomenon. On the other hand, the planarity of the hydrophobic part has been emphasized as an important factor in the mechanism of hydrotropic solubilization. Additives or salts that increase the solubility in a given solvent are said to be “salt in” the solute and salts that decrease the solubility are “salt out” the solute. Several salts with large anions or cations that are themselves very soluble in water result in “salting in” of non-electrolytes called “hydrotropic salts” a phenomenon known as “hydrotropism”. Hydrotropic solutions don’t show colloidal properties and involve a weak interaction between the hydrotropic agent and solute.

It is evident from the literature survey that more is the concentration of hydrotrope[13-16] more is the aqueous solubility of poorly water-soluble drugs. Therefore, highly concentrated solutions of hydrotropic agents were used in the present investigation. Distilled water was used in making hydrotropic solutions. 2 M sodium benzoate (2 M SB), 2 M niacinamide (2 M NM), 2 M sodium salicylate (2 M SS), 4 M sodium acetate (4 M SA), 10 M urea (10 M UR) and 1.25 M sodium citrate (1.25 M SC) were employed as hydrotropic solutions.
Tenofovir disoproxil fumarate (Tenofovir DF) is an oral prodrug, fumaric acid salt form of tenofovir,a nucleoside reverse transcriptase inhibitor analog of adenosine. Tenofovir disoproxil fumarate is prescribed to treat HIV and chronic hepatitis B virus (HBV) in adults.

The aim of the study was to determine the suitable mixture of hydrotropic agent for solubilizing the drug Tenofovir Disproxil Fumerate.To develop and validate UV-visible spectrophotometric method for determination of Tenofovir Disproxil Fumerate by using hydrotropic mixture.The present work focuses on the enhancement of solubility of poorly water soluble drug[7,8], the enhancement of drug’s dissolution rate[10], and there by to increase the rate of bioavailability of drug[11].

Materials and Methods[17,18,19]:

LAB-INDIA UV-VIS spectrophotometer 3000+ with matched quartz cells was employed for spectrophometric analysis. The drug Tenofovir Disoproxil Fumarate was Laurus Laboratories Limited, Hyderabad,(India).Tablets of Tenofovir Disoproxil Fumarate (Cipla Ltd.) were procured from Local drug stores. Other chemicals and solvents were of analytical grade.

Preliminary solubility studies of drug (Tenofovir Disproxil Fumerate):

Initially excess amount of Tenofovir disoproxil fumarate was added to 4 screw capped 10ml of volumetric flasks containing different aqueous systems viz 10% Urea(U) +30% Sodium benzoate(B); 10% Urea(U) + 30%Sodium acetate(A); 10% Urea(U) + 30% Sodium citrate (C); 10% Urea+ 10% Sodium acetate +20% Sodium citrate using distilled water as a solvent. The volumetric flasks were mechanically shaken for 12hrs at room temperature in a mechanical

shaker. This solution were allowed to equilibrate for next 24hrs and centrifuged for 5min at 2000 rpm. The supernatant of each flask was filtered through Whatmann filter paper No.41. The filtrates were suitably diluted with distilled water and analyzed spectrophotometrically against corresponding blank solution. After analyzing the results it was found that the blend U+B in the ratio of 10:30 was suitable for the study of drug.

PREPARATION OF SOLUTIONS:

Preparation of 10% w/v Urea solution:

10gm of Urea was weighed and dissolved in distilled water in a 100ml volumetric flask and make the volume up to 100ml with distilled water.

Preparation of 30% w/v Sodium benzoate solution:

30gm of sodium benzoate was weighed and dissolved in distilled water in a 100ml volumetric flask and make the volume up to 100ml with distilled water.

Preparation of stock solution in urea+ sodium benzoate (10:30) hydrotropic mixture:

Accurately weigh 30mg Tenofovir disoproxil fumarate and transfer into 100ml volumetric flask and dissolve with 10%urea + 30% sodium benzoate solution and volume was made up to 100ml with 10%U+30%B solution to get a concentration of 300µg/ml (stock A).

Scanning for absorption maxima (ƛ max):

Study of spectral characteristic of Tenofovir disoproxil fumarate(TDF) in Urea+sodium benzoate(10:30) solution:

For study of spectral characteristic of tenofovir disoproxil fumarate in urea+sodium benzoate, concentration of 300µg/ml solution of TDF in urea +sodium benzoate (10:30) was selected randomly. After blank correction the drug solution was scanned in the UV range 400 to 200 nm. Absorption maxima were found to be at 300nm. (Figure No-1)

Validation:

Linearity:

The stock solution of TDF in Urea + sodium benzoate (10:30) (stock A) suitably diluted with water. From stock A varying concentrations of 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50 µg/ml, 60µg /ml, 70µg /ml, 80µg /ml, 90 µg/ml and 100 µg/ml were taken in 10 different 10ml volumetric flasks were prepared and scanned at selected wave length 300nm and the absorbances were plotted against concentration.

From the graph it was found that the Beer's law limit lies between 10-50 µg/ml for TDF in U+B(10:30). The regression analysis was carried out for calibration graph to find out correlation coefficient(r), intercept and slope of the regression line. (Figure No-2)

Accuracy:

Accuracy, Specificity of the proposed method was performed by conducting recovery studies. Recovery studies were carried out by mixing a known quantity of standard drug in three levels to pre-analyzed sample solution and the contents were re-analyzed by the proposed method and the percentage was calculated by using the following formula and results were tabulated in Table no: 6

Precision:

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Repeatability Studies:

Repeatability is given by inter-day and intra-day precision. Intra-day precision was determined by analyzing, the six sets of selected concentration of drug in the same day. Inter-day precision was determined by analyzing the drug for three days in a week and the results were presented in Table no: 5

Analysis of tablet formulation of drug by proposed method:

Twenty tablets of Tenofovir Disproxil Fumerate were weighed, and grounded to fine powder. Accurately powder sample was weighed which is equivalent to 100mg of TDF into a 100ml volumetric flask. 50ml of solvent solution was added, and the flask was sonicated for 30min, and the volume was made upto the mark with solvent solution. The resultant was filtered with Whatmann filter paper No.41. Suitable concentration of the sample was transferred into 10ml volumetric flask and to this a mixture of 10%Urea +30%Sodium benzoate solution was added and the concentration of each component was obtained by analysis of the spectral data of sample solution using the absorbance of sample. The amount in the formulation was calculated.

$$\text{Amount of drug present} = \frac{\text{Absorbance of Sample X Concentration in } (\mu g/ml)}{\text{Absorbance of standard}}$$

Results and Discussion:

Study of Hydrotropic Agent Interference:

For determination of interference of hydrotropic agents in the spectrophotometric estimation of TDF, the absorbance's of the standard solutions of TDF were determined in D.M. water alone and in the hydrotropic blend. The absorbance's were recorded against respective reagent blanks at appropriate wavelengths

Initially, weight of a 10 ml volumetric flask filled with 5 ml of a particular blend of hydrotropic solution was determined. Then small aliquot of drug was added and flask was shaken manually so as to dissolve the drug. When drug got solubilized, further small aliquots were added and procedure was repeated till hydrotropic solution got nearly saturated. Then, weight of volumetric flask with saturated solution was determined. Finally, the difference in weight gave the approximate amount of drug dissolved in 3 ml of hydrotropic solution, from which the approximate percentage of drug solubilized was calculated.

By this technique, rough estimation of solubility enhancement by a particular hydrotropic blend was determined. Equilibrium solubility was determined only for those blends in which considerable enhancement in solubility was obtained.

From the results of above studies it was concluded that solubility of TDF was increasing with increasing concentrations of hydrotropic agents, for example solubility in 40 % urea solution was found to be much higher than solubility in 10%, 20% or 30% urea solutions.

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Table-1: Equilibrium solubility of Tenofovir disproxil fumerate in different hydrotropic blends:

S.No.	Hydrotropic Agents	Concentration (w/v)				Solubility enhancement ratio
		10%	20%	30%	40%	
1	Urea	0.067	0.094	0.131	0.191	23.875
2	Sodium Acetate	0.013	0.078	0.142	0.239	29.857
3	Sodium Benzoate	0.283	0.627	1.171	2.157	296.632
4	Sodium Citrate	0.015	0.034	0.060	0.129	16.125

Highest solubility was obtained in 40% sodium benzoate solution. Then, in order to decrease the concentration of sodium benzoate, different combinations of above mentioned 4 hydrotropic agents in different ratios were tried to determine enhancement in solubility, so that total concentration of hydrotropic agents was always 40% w/v. All possible combinations of two hydrotropic agents were taken in such a way that total concentration was always 40% with fixed ratio of 20:20.As shown in **Table-2**

Table-2: Equilibrium solubility of Tenofovir disproxil fumerate in different combination of hydrotropic blends:

S.No.	Combination	Total Conc.(%w/v)	Individual conc. (%w/v)	Solubility (%w/v)	Solubility enhancement ratio
1	U + A	40.00	20.00	0.651	81.375
2	U + B	40.00	20.00	2.909	363.625
3	U + C	40.00	20.00	0.943	117.875
4	A + B	40.00	20.00	2.148	268.516
5	A + C	40.00	20.00	0.067	8.375

Where, U= urea, A= sodium acetate, B = sodium benzoate, C = sodium citrate

Table No-3 Optimum conditions of the proposed Method

S.NO	PARAMETER	Method
1.	Drug Concentration	30µg/ml
2.	Hydrotrophic Blend	3ml

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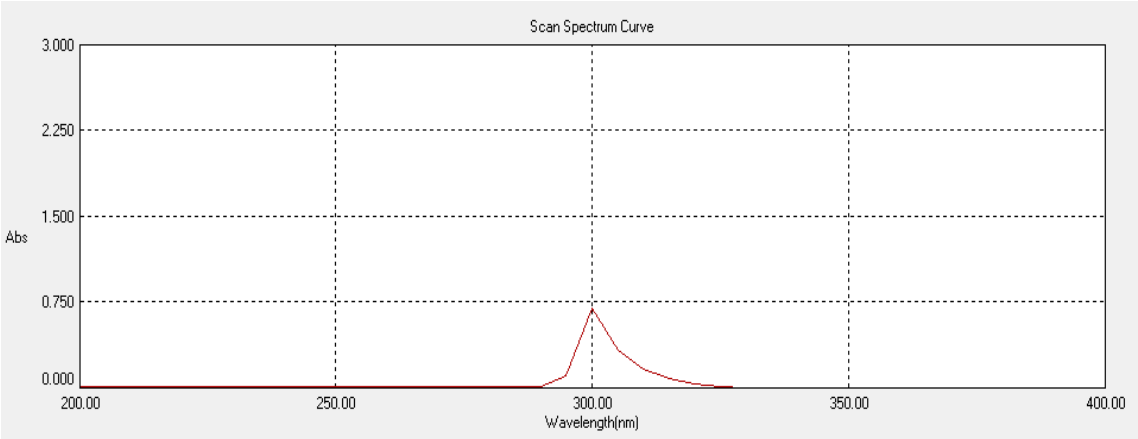
4.	Incubation	Not required
5.	Temperature	Room Temperature

Figure no: 1-Spectrum of the TDF in Hydrotrophic Blend



Linearity:

Table No-4 Beer's Plot-Verification

S.NO	Concentration(µg/ml)	Absorbance
1	10	0.059
2	20	0.138
3	30	0.198
4	40	0.268
5	50	0.344

Figure No-2: Calibration curve of Tenofovir disproxil fumerate by UV method in U+S.B solvent mixture.

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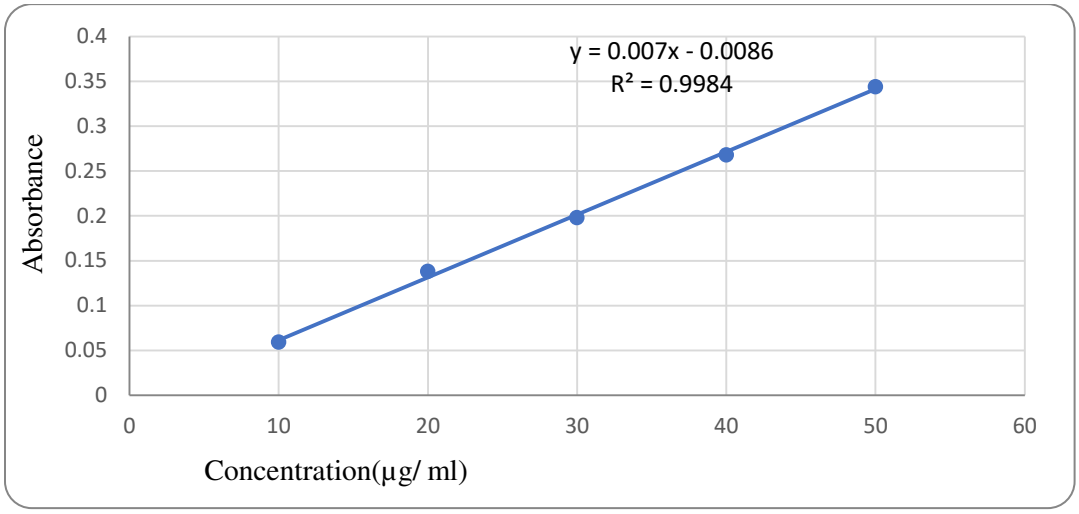

Table No-5.Study of Precision by Proposed methods

S.NO	Amount taken (mcg/ml)	Amount found*(mcg/ml)	
		Inter-day	Intra-day
1	30	29.09	29.24
2.	30	30.15	29.54
3.	30	29.84	29.54
4.	30	29.24	29.84
5.	30	29.54	29.09
6.	30	30.15	29.54
MEAN		29.66833	29.465
SD		0.413901	0.241091
%RSD		1.39	0.818

Table No-6 Study of Accuracy by the proposed methods

S. NO	Amount added(µg)	Amount recovered* (µg)	% recovered*	Average recovery (%)	LIMIT
1.	15	12.87	85.8	90.5	80-120
	15	13.93	92.8		
	15	13.93	92.8		
2.	30	30.3	101	107.3	80-120
	30	32.7	109		

	30	33.6	112		
3.	45	45.5	101	102.6	80-120
	45	47.5	105		
	45	46.0	102		

Table No-7.Determination LOD &LOQ by proposed methods

DRUG	LOD(mcg/ml)	LOQ(mcg/ml)
TNF	0.59	1.76

Table No-8 Assay by proposed methods

DRUG	Label claim (mg/tablet)	Amount estimated* (mg/tablet)	% Amount estimated*	% RSD
Tenofovir disoproxil fumerate (Tenvir) Cipla	300mg	295.6	97.8	0.85
		297.2	98.6	
		296.8	98.4	
		297.0	98.5	
		301.0	100.5	
		297.0	98.5	

Summary and Conclusion:

S. no.	Parameters	Acceptance criteria	Result
1	λ_{max}	---	300nm
2.	Beer's law limit (range)	---	10-50µg/ml
3.	Linearity	R^2=0.995to1.0	0.9984
4.	Specificity	No interference with placebo or impurity	Specific
5.	Accuracy (recovery study)	Recovery= 98.0 - 102.0%	100.5%
6.	Precision Intraday Interday	RSD NMT 2.0% RSD NMT 2.0%	0.818% 1.39%
8.	Limit of detection(LOD)	---	0.59µg/ml

9.	Limit of quantification(LOQ)	---	1.76µg/ml
10.	Intercept	---	0.008
11.	Slope	---	0.007

Conclusion:

Increasing the aqueous solubility of insoluble drugs is of major importance. Various techniques have been employed to enhance the aqueous solubility of poorly water soluble drugs, among them hydrotropic solubilization is one of them. The term Hydrotropy has been designed to increase solubility of various substances in water, due to presences of large amount of additives like Sodium benzoate, Urea, Sodium citrate, Sodium salicylates, and Sodium acetate. The solubility of drug in various hydrotropic agents (viz. sodium benzoate, sodium citrate, sodium acetate, sodium salicylate and urea) was determined by saturation solubility technique. Among various hydrotropic agents, 10%urea and 30%Sodium Benzoate was used as the solvent for spectrophotometric determination of TDF, since; it showed maximum increase in the solubility of the TDF i.e. ~15 folds.

Most of the organic solvents like ethanol, methanol, acetonitrile, hexane, cyclohexane, diethyl ether, chloroform and toluene find wide use in spectrophotometric analysis of poorly water-soluble drugs. Most of these organic solvents are toxic in nature, costlier and responsible for pollution. Inaccuracy in spectrophotometric estimation due to volatility is another drawback of organic solvents. The proposed method is new, simple, cost-effective, safe, accurate, precise and environmentally friendly. This method can be successfully employed in the routine analysis of TDF in tablet dosage form. Like this method other hydotropes can also be tried by combining them to exert synergistic effect on solubility of poorly water soluble drugs to be applied in different fields of analysis. Mixed hydrotropy may find wide use in development of aqueous formulations of poorly water soluble drugs in future.

From this study, it is obvious that there was no interference of urea solution in the estimation of TDF. Urea and sodium benzoate were cheaper than most of the organic solvents and can thus substitute expensive methanol, dimethyl formamide, chloroform and carbon tetrachloride. Drawbacks of organic solvents include toxicity, error due to volatility, pollution, and cost. Thus, hydrotropic solutions of urea to solubillized poorly water soluble drug TDF, from tablets to carry out spectrophotometric analysis precluding use of organic solvents. The tablets containing TDF were analyzed successfully.

From the present investigation, it is concluded that the proposed method is Ecofriendly, novel, simple, cost effective, accurate, safe, and precise. Thus, it can be successfully employed in the routine analysis of TDF in bulk drug and its pharmaceutical tablet dosage form.

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