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DETERMINATION OF SOLUBILITY OF TADALAFIL BY SHAKE FLASK METHOD BY EMPLOYING VALIDATED HPLC ANALYTICAL METHOD

Ojaswi Ghadge*, Mitali Samant and Anubha Khale.

H. K. College of Pharmacy, Pratiksha Nagar, Near Oshiwara, Jogeshwari (W), Mumbai-400102.

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***Correspondence for Author**
Ojaswi Ghadge
H. K. College of Pharmacy, Pratiksha Nagar, Near Oshiwara, Jogeshwari (W), Mumbai-400102.

ABSTRACT

The main aim of the work was to study the solubility behaviour of the poorly soluble drug from BCS Class IV, Tadalafil. The solubility determination was done by Shake flask method in different solvent systems (Water, Methanol, Water: Methanol, Water: SLS, in buffers of pH 1 to 7.4). The time of shaking was varied between 30 minutes and 48 hours. The time of sedimentation was varied between 1 and 24 hours. Also the study was carried out by repeating the method at different temperatures 25 ^{0}C, 37 ^{0}C, 50 ^{0}C. HPLC method was developed and validated as per ICH guidelines, for analysing samples using solvent system Methanol: Acetonitrile (50:50, t_R = 2.6min). From the Shake Flask study, it was found that the shaking time can affect the solubility of API to great extent. For Tadalafil 6 hrs shaking time found to be optimum. Since the maximum solubility of drug was found to be in Water: SLS system, it can be concluded that Water: SLS can be used as dissolution media for the formulation of Tadalafil.

KEYWORDS: Shake Flask method, Tadalafil, Solubility, HPLC.

INTRODUCTION

Solubility has been an important molecular property that influences the intestinal absorption which in turn determines bioavailability. The drug has to pass through different pH while travelling down the body which affects its solubility by the time it reaches the actual target. Solubility is a screening parameter and it is useful during lead selection and optimization, salt selection and optimization of formulation and also it is required for Biopharmaceutical Classification System (BCS),[1,2,3] Aqueous solubility is among the first physicochemical

parameter measured during the preformulation stage where it dictates many of the subsequent events and approaches in the formulation development. Later the rate of dissolution and stability of the dosage form are determined. Poor aqueous solubility is likely to give rise to increased formulation difficulties during clinical development. Thus it is of interest to accurately measure solubility of sparingly soluble compounds. The rate of dissolution is an important aspect for development of an effective formulation. For solubility determination Standard Shake flask method is widely and commonly used. [4]

Therefore, a Class IV drug (Low solubility and low permeability) was chosen for the project where studying the solubility, factors affecting solubility as well as dissolution rate and improving the solubility by appropriate way would be a challenge. The drug chosen was Tadalafil (TD) which is widely used in the treatment of erectile dysfunction,[5] It is a PDE-5 inhibitor. The inhibition of PDE5 by TD enhances erectile function by increasing the amount of CGMP. TD is a white or almost white powder and it is poorly soluble in water.[6, 7]

Since the solubility of drug is very low, HPLC method would be more accurate for its quantification. Some of the solvent systems were reviwed from literature and other parameters were referred from USP as the drug is official in USP.[8, 9, 10]

EXPERIMENTAL

Materials

Tadalafil was obtained as a gift sample from SMS Pharma. HPLC grade solvents Methanol, Acetonitrile were purchased from Merck. Deionized water was produced using a Siemens water system for all aqueous solutions. Millipore membrane filters of 0.2µm, 47mm diameter and 0.2µm, 25mm diameter were used.

Instruments

The analytical method was developed on HPLC System, Shimadzu and Prominence Model with Spinchrome Software. In addition to this Digital Balance (Contech Make, Model CB-125), Sonicator (Make Expo hi-Tech, Model CD-4820), Waterbath Shaker (Make Remi, Model ICCM1133), pH meter (Equiptronics) were used.

METHODS

Study of physicochemical properties

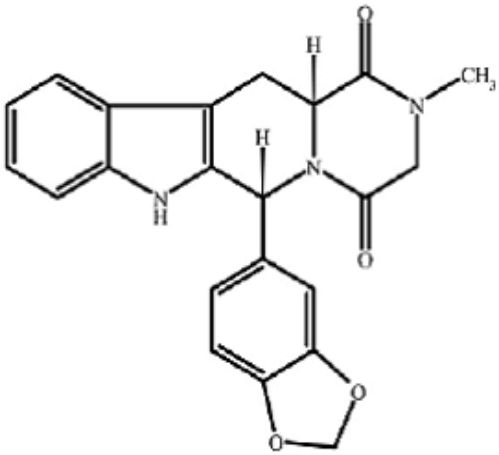


Fig. 1 Structure of Tadalafil

Tadalafil is a BCS Class IV drug with molecular formula 389.404. It is practically insoluble in water (2µg/ml). Very slightly soluble in methanol. Reported pKa of the drug were found to be 15.17 (strong acidic) and -4.2 (strong basic).[11] Particle size was studied in lab by microscopy and it was found to be 60 µm.

Solubility Determination by standard shake flask method

The solubility of Tadalafil in Water, Methanol, Water: Methanol, Water: SLS, in buffers of pH 1, 2, 3, 4.5, 6, 6.8, 7.4 was determined at 37^0c. Tadalafil (10mg) was added to 10ml of the mentioned solvents in a vial. The vials were kept on a shaker incubator maintained at 37 ± 0.5^0c for 48hrs. After shaking, the solutions were allowed to stand for 24 hrs then the supernatant solution was filtered through 0.2 µm membrane filter, diluted suitably by respective solvent and assayed by HPLC method at 285 nm. Concentrations in different solvents was then found out by extrapolation on standard plot of Tadalafil.

Study of effect of different factors on Shake-Flask method

In the shake-flask method, the achievement of equilibrium consists of two important but different parts: vigorous agitation (by stirring or shaking) and sedimentation. To discover which of these parts plays higher role in the formation of equilibrium, the time of shaking and the time of sedimentation were independently varied. The time of shaking was varied between 30 minutes and 48 hours. The time of sedimentation was varied between 1 and 24 hours.

Also the study was carried out by repeating the method at different temperatures 25 ^{0}C, 37 ^{0}C, 50 ^{0}C. In the above procedure each time 3 aliquotes were removed and analysed in HPLC.

HPLC Assay of Tadalafil

Tadalafil was assayed using High-Performance Liquid Chromatography (HPLC). The mobile phase was freshly prepared on each day of analysis, filtered through a 0.2 µ Millipore membrane filters and degassed by sonication. It consisted of a mixture of 50:50 Methanol: Acetonitrile.[12] The operating temperature was ambient, the flow rate was 1.2ml/min, and the injected volume was 20µl. The column effluent was monitored continuously using ultraviolet detection at 285nm. The HPLC system used consisted of a Shimadzu (Prominence) pump system LC-20AD, a Rheodyne Injector with a 20 µl loop, and a UV-VIS spectrophotometric detector SPD-20A. The data was recorded using Spinchrome software. A C18 Column (HiQsil HS, 4.6mm i.d X 250mm, 5 µ particle size) was used.

Preparation of Reagents and Standards

Mobile Phase

The mobile phase was prepared by mixing of methanol, acetonitrile (HPLC grade) in the ratio of 50:50 v/v. It is filtered through 0.2µm membrane filter and then sonicated for degassing.

Sample preparation

The standard stock solution of TD, of 100 µg/ml was prepared by dissolving 10 mg in 100 ml of HPLC grade Methanol. Eight different working standard solutions were prepared by for calibration by adding defined volumes of the stock standard solution and diluting with Methanol. The concentrations of TD are 2.0, 4.0, 6.0, 8.0, 10.0, 15, 20, 25µg/mL, respectively.

RESULT AND DISCUSSION

Development and Validation of HPLC method

The HPLC method for quantification of Tadalafil was developed and validated as per ICH guidelines. The t_R was found to be 2.6 min. (Fig. 2). The standard plot is given in Fig. 3. The details of validation parameters are tabulated further (Table no. 1, 3-8).

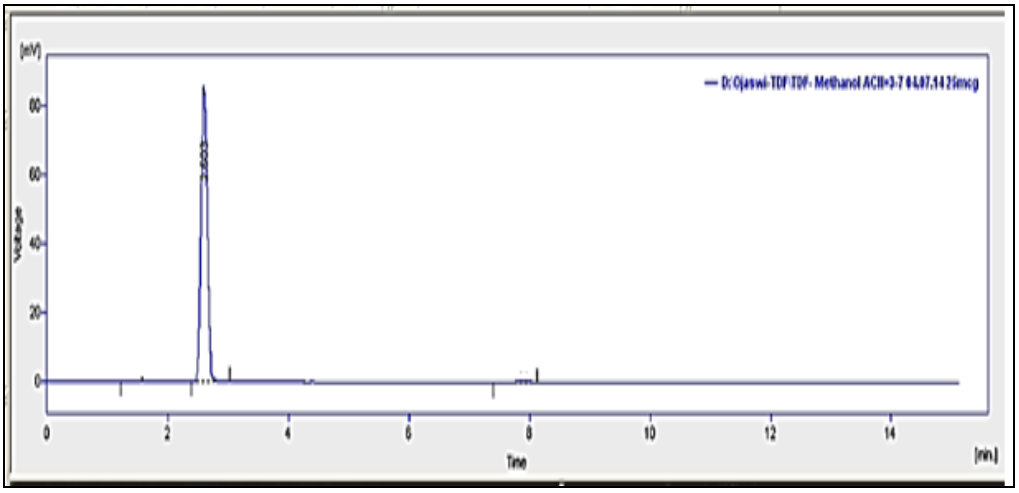


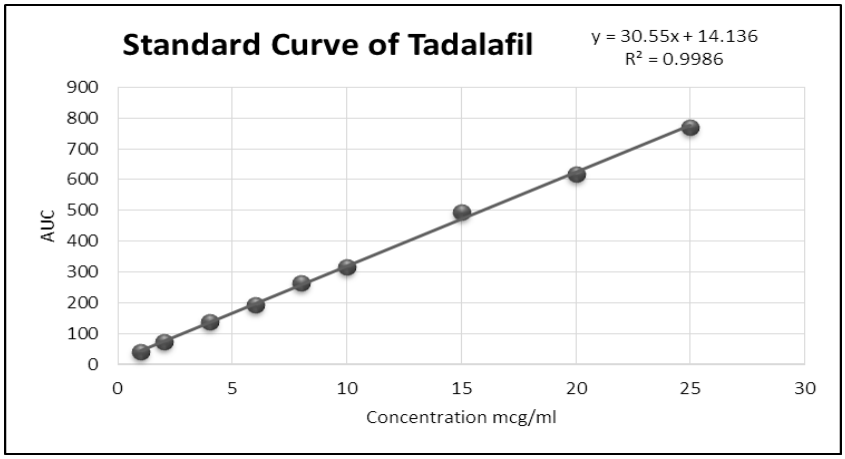
Fig. 2 Chromatogram of Tadalafil in Methanol: ACN= 50:50 as mobile phase

Calibration curve for Tadalafil

20 µl of each calibration standard solutions (1, 2, 4, 6, 8, 10, 15, 20, 25 µg/mL) were injected into the HPLC system to get the chromatograms. The average peak area and retention time were recorded. Linearity curve was constructed by plotting concentration of Tadalafil on X-axis and average peak areas of standard Tadalafil on Y-axis. The results were presented in Table 1. Results show that a phenomenal correlation exists between peak area and concentration of drug within the linearity range.

Table 1: Calibration data of the proposed HPLC method for estimation of Tadalafil

Concentrations (mcg/ml)	Area 1	Area 2	Area 3	Mean Peak Area	SD	%RSD
1	39.492	39.985	39.782	39.753	0.247776	0.623289
2	73.43	72.981	73. 501	73.2055	0.317491	0.433698
4	137.623	137.536	136.968	137.3757	0.355719	0.258939
6	193.461	192.854	193.573	193.296	0.386858	0.200138
8	264.135	264.934	265.045	264.7047	0.496458	0.187552
10	314.695	314.701	315.029	314.8083	0.191126	0.060712
15	495.063	495.318	494.5	494.9603	0.418553	0.084563
20	617.796	618.046	617.988	617.9433	0.130849	0.021175
25	770.989	771.81	770.985	771.2613	0.475163	0.061609



Graph 1 Standard Curve of Tadalafil by RP-HPLC

Precision

Intra-day precision was investigated by replicate applications and measurements of peak area for Tadalafil for six times on the same day under similar conditions. Inter-day precision was obtained from % RSD values obtained by repeating the assay six times on two different days. The percent relative standard deviation (% RSD) was calculated. The inter-day and intra -day precision results were shown in Table 2 and Table 3 respectively.

Table 2: Results of Precision Study (Inter day) for TD

Concentration (µg/ml)	Injection No.	Peak Area (mV)	% RSD
10	1	314.695	0.121
	2	314.50	
	3	314.783	
	4	314.05	
	5	313.93	
	6	314.367	

Table 3: Results of Precision Study (Intra day) for TD

Concentration (µg/ml)	Injection No.	Peak Area (mV)	% RSD
10	1	314.695	0.0482
	2	314.667	
	3	314.342	
	4	314.618	
	5	314.78	
	6	314.546	

Accuracy/Recovery

Accuracy is the degree of agreement between a measured value and the accepted reference value. The accuracy of the method was tested by triplicate samples at 3 different concentrations equivalent to 80%, 100% and 120% of the active ingredient, by adding a known amount of Tadalafil standard to a pre-determined amount of Tadalafil. The recovered amount of Tadalafil, %RSD of recovery, % recovery of each concentration is calculated to determine the accuracy. The recovery results for accuracy study of Tadalafil are presented in Table 4.

Table 4: Recovery data for TD

Sr. No	Amount of drug taken (µg/ml)	Known Amount of drug added in injection (µg/ml)	Total amount of drug (µg/ml)	Total amount of drug found (µg/ml)	% recovery	Average recovery in %	% RSD
1	8	10	18	17.77 17.84 17.88	98.72 99.11 99.33	99.05	0.311
2	10	10	20	20.04 19.91 19.75	100.2 99.55 98.75	99.5	0.729
3	12	10	22	22.13 22.68 22.74	100.59 103.09 103.36	102.34	1.492

Robustness

Robustness is the ability to provide accurate and precise results under a variety of conditions. In order to measure the extent of method robustness, the most critical parameters were interchanged while keeping the other parameters unchanged and in parallel, the chromatographic profile was observed and recorded. The studied parameters were: the composition of mobile phase, flow rate, detection wavelength. The results for robustness study in Table 5 indicated that the small change in the conditions did not significantly affect the determination of Tadalafil.

Table 5: Robustness result of TD

Sr. No	Parameter	Used	Optimized	Retention Time (min)	Remark
1	Flow Rate (±0.2ml/min)	1.0ml/min 1.2ml/min 1.4ml/min	1.2ml/min	2.81 2.63 2.21	NIL

2	Detection Wavelength	280 nm 285 nm 290 nm	285	2.58 2.61 2.70	No significant change in tR, peak area changes at 290 nm because of the typical UV spectrum of the drug
3	Mobile phase composition (Methanol: ACN)	30:70 50:50 70:30	50:50	2.59 2.63 2.55	NIL

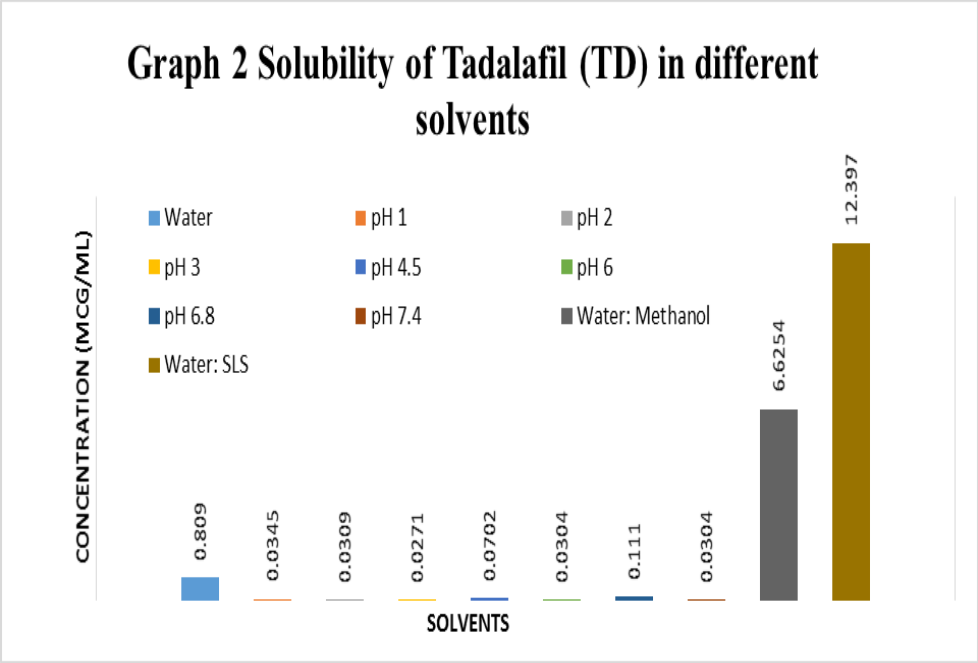
Comparison of Solubility by above HPLC method in different solvents

Solubility of TD in different solvents was analysed by HPLC. It was found that solubility of TD is maximum Water: SLS in and then in Water: Methanol solvent system and minimum in water i.e less than 1 µg/ml under the stated conditions maintained in procedure. In different pH solutions the solubility of TD is very less (less than 1µg/ml) and change in pH did not find to make any difference on solubility. Each solution was then diluted 10times of the last dilution and injected. These concentrations were found out by extrapolation on standard plot of TD.

Results are presented in Table 6 and Graph 2. It is found that out of different solvents tried, water: SLS system showed maximum solubility hence it was further studied for effect of shaking time.

Table 6 Solubility of Tadalafil in different solvents

Sr. No.	Solvent systems	AUC	Retention Time (t_R)	Concentration (µg/ml)
1	Water	38.852	2.62	0.809
2	0.1 M HCl, pH 1	15.19	2.64	0.0345
3	0.01 M HCl, pH 2	15.082	2.62	0.0309
4	Acetate buffer, pH 3	14.964	2.63	0.0271
5	Acetate buffer, pH 4.5	16.281	2.59	0.0702
6	Phosphate buffer, pH 6	15.065	2.62	0.0304
7	Phosphate buffer, pH 6.8	17.53	2.55	0.1110
8	Phosphate buffer, pH 7.4	15.065	2.63	0.0304
9	Water: Methanol	216.545	2.59	6.6254
10	Water: SLS	392.862	2.63	12.397

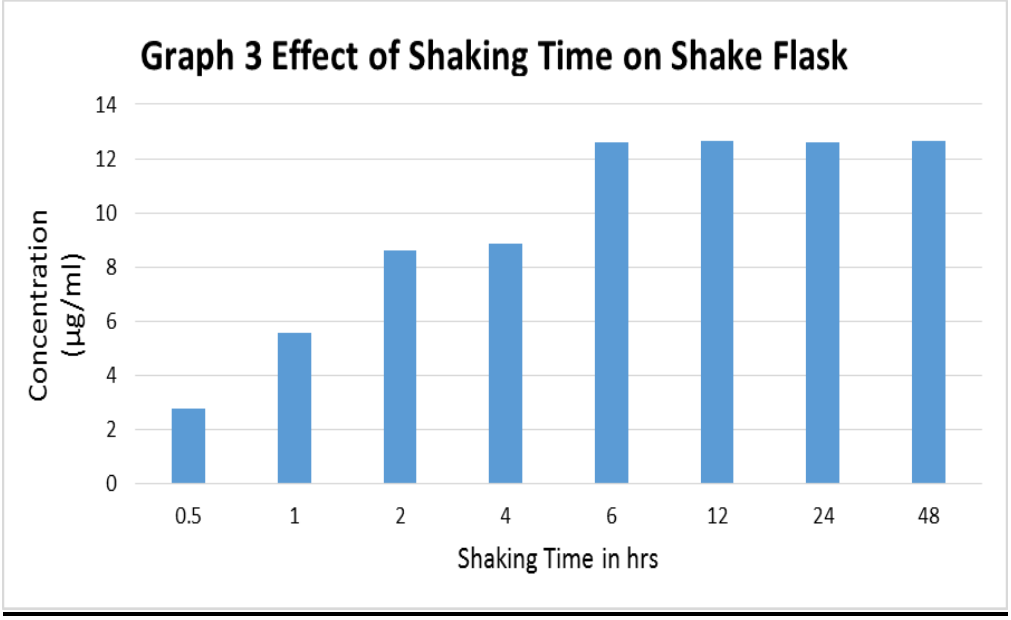


Effect of Shaking Time

The time of shaking was varied between 30 min to 48 hrs. As shown in Table 7 and Graph 3, the measured solubility of TD increases with increasing shaking time and then reaches a maximum value. There are no significant differences in the solubility results obtained after stirring for 6 hours or more. This suggests that 48 hours of stirring time is not required for the measurement of solubility of TD. The optimum shaking time is 6 hrs.

Table 7: The effect of shaking time on solubility of Tadalafil in Water SLS system

Sr. No	Shaking time (hrs)	Mean Peak area	Retention time	Concentration (µg/ml)
1	0.5	98.376	2.63	2.757
2	1	183.364	2.59	5.539
3	2	276.298	2.60	8.581
4	4	284.84	2.59	8.861
5	6	399.735	2.63	12.622
6	12	399.831	2.61	12.626
7	24	399.845	2.62	12.625
8	48	399.892	2.62	12.627

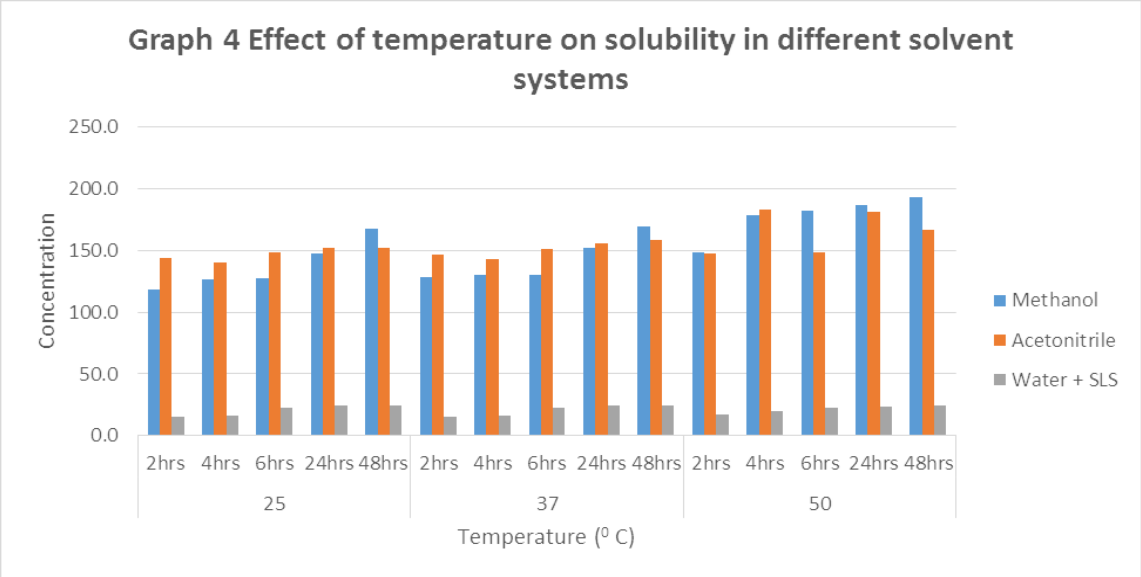


Effect of Temperature

When the drug, TD, was subjected to different conditions of temperature for each solvent system and no changes in solubility were found. All the aliquots showed almost same AUC as shown in Table 8. Graph 4 shows the comparison in three different solvent systems in which the drug was more soluble as compared to other solvents mentioned in Table 8. Thus it can be concluded that temperature does not enhance the solubility of Tadalafil in solvents mentioned in the procedure.

Table 8: Effect of temperature on solubility

Temperature	25					37					50				
SOLVENTS	2hrs	4hrs	6hrs	24hrs	48hrs	2hrs	4hrs	6hrs	24hrs	48hrs	2hrs	4hrs	6hrs	24hrs	48hrs
Methanol	118.0	126.4	127.2	147.8	167.8	128.9	130.4	130.5	152.5	169.8	148.4	178.3	182.7	187.2	193.4
Acetonitrile	144.1	140.3	148.3	151.9	151.9	146.8	143.3	151.7	155.9	159.0	147.3	183.4	148.3	181.4	166.6
Water + SLS	15.2	15.8	22.4	24.1	24.3	15.2	15.9	22.6	24.2	24.3	17.2	19.7	23.0	24.0	24.3
Water+ Methanol	12.4	13.0	14.2	15.4	15.6	12.7	12.9	14.3	15.5	15.7	21.8	19.2	15.4	15.1	14.4
Water	2.3	3.6	3.8	4.4	5.1	2.3	3.7	3.8	4.4	5.2	2.4	3.7	4.4	4.5	5.2
pH1	0.1	0.2	0.5	0.9	1.8	0.1	0.2	0.5	0.9	1.8	0.1	0.9	1.6	1.7	1.8
pH2	0.4	0.9	3.2	3.2	3.4	0.4	0.9	3.2	3.3	3.4	0.8	1.1	3.2	3.3	3.5
pH3	0.5	0.8	0.9	1.0	2.9	0.6	0.8	0.9	1.1	3.1	0.6	0.8	1.0	1.1	5.2
pH4.5	0.6	0.8	0.8	0.8	1.2	0.7	0.8	0.8	0.9	1.3	0.7	0.8	1.0	1.4	1.6
pH6	0.7	0.8	0.8	0.9	2.6	0.8	0.8	0.8	1.2	2.7	0.7	1.0	1.3	2.4	2.8
pH6.8	0.7	0.8	1.5	1.9	2.9	0.7	0.8	1.5	2.3	4.6	0.6	0.6	2.0	2.8	4.4
pH7.4	0.8	0.9	1.2	2.3	2.9	0.8	0.9	1.7	2.3	3.1	0.8	2.3	2.3	2.3	3.2



CONCLUSION

The developed RP HPLC method was validated with respect to all the parameters as per ICH guidelines. From the Shake Flask study for solubility it was found that the shaking time can affect the solubility of API to a great extent. Time of shaking can vary from drug to drug. For TD 6hrs shaking time found to be sufficient. Solubility did not change after 6 hours. Since the maximum solubility of drug was found in Water: SLS system, it can be concluded that Water: SLS can be used as dissolution media for the formulation of TD.

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