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Original Article

# Development and Validation of Discriminative Dissolution Test for Tenofovir Disoproxil Fumarate Formulation

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**ABSTRACT**

**Objectives and Introduction:** This study uses HPLC and UV spectrophotometric methods to develop and validate a discriminative dissolution test method for quality control of tenofovir disoproxil fumarate in a tablet. Tenofovir disoproxil fumarate is a drug used in combination therapy to treat HIV infection. It has antiviral, prodrug, and HIV-1 reverse transcriptase inhibitory properties. **Materials and Methods:** Phosphate buffer 6.8, water, and 0.01 N HCl were the components of the discriminative solution. The USP Apparatus II blade should be used at 75 rpm and 900 mL of 0.01 N HCl at 37º±0.5ºC for the best dissolving. Tenofovir disoproxil fumarate's *in vitro* release profiles perform well under these circumstances. This was done on Agilent ZORBAX C8 a 6x150 mm column, the temperature of the column was ambient, its flow rate was 1.0 mL/min and the detection wavelength was 260 nm. The mobile phase comprised of 70:30 v/v mixture of methanol and formic acid solution. **Results:** When the conditions were optimal, this approach demonstrated good release. Method validation was carried out as per the ICH guidelines. The drug follows zero-order release kinetics. **Conclusion:** The results obtained by the proposed method for dissolution test for Tenofovir disoproxil fumarate tablet formulation were found to be reliable, rugged, linear, accurate and precise.

**Keywords:** Discriminative dissolution, Dissolution test, HPLC, Tenofovir disoproxil fumarate.

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## INTRODUCTION

Dissolution is the process of dissolving solid drug substances in a solvent. The bioavailability and equivalence data obtained from dissolution testing can be utilized in the development of new formulation and product development processes.[1] The process of intestinal absorption, following oral administration comprises sequential events such as drug release, dissolution, solubilization, and dissolved drug permeability at the site of absorption. The development of suitable *in vitro* dissolution method is crucial for predicting *in vivo* efficacy, as the first two steps are essential to determine bioavailability.[2] Chemically Tenofovir Disoproxil Fumarate (TDF) is 2E)-but-2-enedioic acid; bis({[(propan-2yloxy) carbonyl] oxy} methyl) {[(2R)-1-(6-amino-9H-purin-9-yl) propan-2-yl] oxy} methane phosphonate.[3] It is a prodrug, antiviral, nucleotide analog reverse transcriptase inhibitor prescribed in combination with other drugs for the management of HIV infection as well as for Hepatitis B therapy.[4] A literature survey discovered that many analytical techniques have been reported for the determination of Tenofovir Disoproxil Fumarate in pharmaceutical dosage forms and pure drug, either in single or in combined forms, L. Manojkumar *et al*.,[5] Ananda Kumar Karunakaran *et al*.,[6] Ramreddy Godela *et al*.,[7] Bhavin N. Patel *et al*.,[8] but so for no methods has been reported for their dissolution analysis. The present study describes the development and validation of discriminative dissolution test method by using HPLC and UV visible spectrometry method.

## MATERIALS AND METHODS

The chemicals and solvents used for carrying out experimental work: Methanol and acetonitrile were used are of HPLC grade, Hydrochloric acid and formic acid are of GR Grade, double distilled water Tenofovir disoproxil fumarate drug sample was gifted by Sun pharma limited. HPLC system-Shimadzu-1700 double beam, UV-visible Spectroscopy- Jasco V-630, Dissolution Apparatus-Paddle, Basket, Cannula, Glass vessels, Syringe Electro lab Tablet Dissolution tester-TDP-06P, Lab India Ds1400, PH-meter-Digital pH Meter 111E, Membrane filters with 0.45-inch-thick cellulose filter paper, Weighing Balance-Shimadzu AUX 220 RADWAG PS 1500.

### Spectral study of TDF by UV spectroscopy

#### *Preparation of Working Standard solution*

A standard stock solution was prepared with a 1000 µg/mL TDF concentration in methanol and appropriately diluted to get a

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working standard solution having concentration 10 µg/mL of TDF in methanol.

## Selection of Wavelength

TDF (10 µg/mL) working standard solution was scanned in a 1.0 cm cell against water as a blank, within the 200-400 nm range.

## Selection of chromatographic method

### *Preparation of standard drug solution*

Standard stock solution (A): A precisely weighed amount of TDF was transferred and dissolved in enough methanol to create a standard stock solution with a 1000 µg/mL TDF concentration. To prepare a working standard solution with a concentration of 10 µg/mL of TDF, the solution was further diluted with mobile phase.

Standard solutions were prepared with different mobile phases varying in composition and numerous trials were taken for the selection of mobile phase, temperature, column, flow rate, wavelength, temperature and pH exhibiting well defined and resolved peak.

## Study of system suitability parameters

A study was directed to find out the suitability of the system by administering six parallel injections of a standard solution containing 10 µg/mL TDF and then examining the chromatograms for theoretical plates, RSD and tailing factor.

## Determination of solubility

To identify the medium, the drug's Physico-chemical properties, such as its solubility, should be taken into account. The medium's volume must be at least three times larger than what is needed to generate a drugs saturated solution in order for the dissolving process to ensure proper sink conditions (USP 35, 2012). TDF dissolution was assessed in a variety of physiological pH values to ascertain the sink condition in various media: water, 0.01M HCl; pH 4.5 for acetate buffer; pH 6.8 for phosphate buffer.

## Stability determination

Another essential information when selecting the dissolving media is the stability of the solution. The stability of the standard solution was tested at room temperature for 24 hr and twice every 2 hr at 37±0.5°C in 0.01M HCl. The sample solutions were also tested for 24 hr at room temperature. In order to compare absorbance at each time interval, the acceptable range for the stability of solution was established as 98.0-102.0%.

## Dissolution test condition

Dissolution test Method for TDF estimation parameters for various dissolutions were optimized utilizing the optimized chromatographic conditions and the drug solubility data to choose a set of parameters that will yield the drug maximal percent release, to get a discriminative dissolving test, various medium, volume, apparatus type, and rotating speed were assessed. Sampling aliquots of 10 mL each were withdrawn at an interval of 10 min up to 80 min and replacing it with fresh dissolution media.

The TDF drug is not soluble in Acetate buffer and the Phosphate buffer, hence the dissolution test was not performed on these buffers and were rejected.

The percentage drug release in different parameters was estimated by validated HPLC and UV spectroscopy methods at each time point.

$$Percent\ Release = \frac{\text{Volume of dissolution media} \times \text{Peak area of sample} \times \text{Concentration of std.x dilution factor} \times 100}{\text{Lable claim} \times \text{Peak arae of sample}} \quad ---1$$

## Optimization of Dissolution Parameters

### *Change in USP apparatus*

The 0.01N HCl was selected as a dissolution media as the TDF is film coated tablet. A constant media volume of 900 mL was used, and two different USP equipment types were used for the dissolution process on a trial-and-error basis and percent drug release was calculated.[9,10]

## Change in the Volume of Dissolution Medium

The dissolution medium utilized in the above investigation, in which dissolution was carried out using a USP Type II apparatus and media volumes ranging from 900 mL to 500 mL and 1000 mL. The percent drug release was calculated.

## Change in the speed of rotation (rpm)

The 0.01 N HCl was optimized dissolution media with media volume of 900 mL. The speed of rotation (rpm) is varied from 50, 75 and a 100 rate and percent drug release were calculated.

## Preparation of test solution

One tablet was dropped into each of six dissolution vessels containing 0.01 N HCl for the respective analyte drug. Aliquot part of 10.0 mL was taken at specific time intervals, used as a sample, and replaced with the same volume of fresh medium to keep a constant total volume. At the end of each time point, aliquot part was filtered, diluted and chromatographed. Also, the absorbance of the diluted sample was noted using UV spectrophotometer against blank.

## Method validation

### *Linearity and range*

By using the label claim of TDF as 100% target concentration [10 µg/mL of TDF] and creating the solutions in the mobile phase with concentrations ranging from around 5-30% of target concentration, the linearity for TDF to concentration was established.[11,12]

## Accuracy

The "spiking" technique, which adds a known amount of a standard drug (TDF) to the target dissolution concentration (10 µg/mL) of TDF (as 100% accuracy level) in accordance with the label claim of 300 mg TDF for mutation, was used to evaluate the accuracy of the suggested method. In addition to a 300 mg tablet, 150 mg, 300 mg, and 750 mg of the standard drug were introduced in dissolution vessel. The drug dissolution study was performed.

At spiking concentration levels of 50%, 100%, and 150%, respectively, 10 mL aliquots were taken, filtered through Whatman filter paper, and then subjected to chromatographic analysis. In triplicate, each concentration was examined. After giving the system 30 min to equilibrate, mobile phase was introduced into the chromatographic column at a flow rate of 1.0 mL/min.

Following equilibration, 20 µL injections in triplicate of test solutions at various concentrations were made. Total amount of drug estimated and recovered drug was calculated using following formula (2), (3) and (4).

$$Amount\ estimated = \frac{\text{At x Cs x dilution factor x volume of stock}}{\text{As}} \text{ ----2}$$

$$Percent\ Amount\ recovered = Total\ amount\ estimated - Label\ claim - - - 3$$

$$Percent\ Recovery = \frac{Amount\ Recovered}{Amolunt\ of\ standard\ drug\ added}\ X\ 100 - - - 4$$

L. C.=Label Claim in mg Where, $A_t$=Peak area/Absorbance of test sample,

$A_S$=Peak area/Absorbance of standard sample, $C_S$=Concentration of standard.

## Precision

A homogenous sample of measurements under specified conditions is expressed as closeness of agreement (degree of scatter) using an analytical process called precision. Precision studies should be conducted with true, uniform samples. A drug test sample in the vessel and a placebo in a dissolving vessel might be tested, if a homogeneous sample could not be produced.

## Repeatability study

A homogenous sample of measurements under specified conditions is expressed as closeness of agreement (degree of scatter) using an analytical process called precision. Precisions. This describes the accuracy over a brief period of time on the same day, with the same operating conditions. Studying repeatability is sometimes referred to as intra assay precision.

After passing the mobile phase through the chromatographic column at a rate of 1 mL/min, the system was given 30 min to equilibrate. After equilibration, chromatograms were recorded by five test solutions were injected in a volume of 20 µL. The peak area is calculated from the drug dissolution percentage.

## Intra-day precision

On the same day, the study was conducted. The test sample of TDF in 0.01 N HCl and Water media injected at interval of 1 hr up to 3 hr and the chromatograms were recorded. The variation of results within the same day and measured the absorbance after 1, 2 and 3 hr time intervals.

## Inter-day precision

The study was performed during three consecutive days in 0.01N HCl and water media of same working test sample. The test sample of TDF injected at interval of 1st, 2nd, and 3rd day and the chromatograms were recorded.

## Ruggedness

Two independent analysts examined the sample preparation in order to assess the ruggedness of the procedure. Sample examined on a separate day by a different analyst using different C18 columns. The parameters were followed when setting the dissolution apparatus. Each dissolving tank held 1 tablet, which was then subjected to dissolution study. The sample was then filtered through a 0.45 µm membrane filter, and the TDF tablet preparation sample in water and 0.01 N HCl was chromatographed on a different day by a different analyst using a different column and a UV Spectrophotometer set to 260 nm was used to measure the test sample's absorbance. Formula (1) was used to compute the percentage release.

## Robustness

The robustness of an analytical method is determined by its ability to endure minor but deliberate modifications in parameters and indicates its level of applicability under normal usage.

The robustness study for proposed HPLC method was carried out for following parameters:

- Change in flow rate.
- Change in wavelength.
- Change in mobile phase composition.

The purpose of the study was to ascertain the impact of flow rate fluctuation. In order to observe the impact of deliberate changes, flow rate within a range of ±0.2 mL/min, the flow rate was deliberately varied. Wavelength was varied for ±2 nm and ±5% mobile phase organic composition and analyzed by proposed HPLC method.

## Methods used to compare dissolution profiles

ANOVA models were used to compare the dissolution data, with comparisons made between model-dependent (zero-order and first-order) and model independent factors (f1 and f2 factors) methods.[13-19]

# RESULTS

## Finalizations of Chromatographic conditions

The final chromatographic conditions selected were maintained throughout the experimentation are mentioned in Table 1.

System suitability analysis was carried out to check the suitability of the instrument in order to perform the desired analysis. From the ascertained chromatograms, the peak area unit was noted and results are tabulated in Table 2.

## Finalized dissolution parameter

### *Change in USP apparatus*

In 0.01 N HCl and water the highest drug release was observed, but in drug release, no significant difference was observed in either medium. From the results, it was found that USP I released drugs more slowly than USP II hence further the experiment, USP II was chosen as one of the optimal dissolution parameters (Figure 1a).

## Change in the Volume of Dissolution Medium

According to the results, the percentage of drug released in a media capacity of 1000 mL was lower than that of 900 mL and faster in 900 mL than in 500 mL. As a result, 900 mL of media was chosen as one of the optimal dissolving parameters and used during the experiment (Figure 1b).

## Change in the speed of rotation (rpm)

From the results, it was determined that the percent of drug in 50 and a 100 rpm is lower in amount as compared to 75 rpm. Thus, 75 rpm was optimized one of the dissolution parameters and was additional utilized in the experimentation (Figure 1c).

The test dissolution was performed using the finalized dissolution parameter as shown in Table 3. The discriminative dissolution was performed in 0.01N HCl and Water as dissolution media.

**Table 1: Final Optimized Chromatographic Parameter.**

| Parameters | Condition |
|---|---|
| System | Shimadzu HPLC |
| Column (Stationary Phase) | Agilent Zorbax C8 (4.6x150 nm) |
| Mobile Phase | 0.01 N Formic acid: Methanol (50:50) v/v |
| Detection wavelength | 260 nm |
| Flow rate | 1.0 mL/min |
| Injection volume | 20 µL |
| Run time | 10 min |

## Methodology

The 10 mL aliquots were filtered, diluted and injected into the HPLC system under finalized chromatographic conditions. Figures 2a-2c displays the chromatograms of the formulation under study's dissolution analysis at chosen intervals recorded under optimal chromatographic conditions. The dissolution test method described earlier was repeated and samples were analyzed at appropriate time interval by UV-Spectrophotometric method at 260 nm.

The result of percent dissolution was calculated using formula (1) mentioned earlier the results calculated are shown in Tables 4 and 5 respectively.

## Method validation

**Linearity and Range:** Linearity of the test response was constructed by plotting a graph of peak area versus concentration of drug, similarly absorbance versus concentration of drug in µg/mL is shown in Figures 3 and 4 and determining the correlation coefficient. From the observations it was found that the correlation coefficient for was found to be TDF to be 0.9972 and 0.9954 for HPLC and UV method respectively.

## Accuracy

From the Table 6 mean recovery of TDF at each spiked level was determined, mean percent recoveries was found to be in range 99.33-99.93% by UV method and 99.275-100.86% by HPLC while percent RSD was found to be below 2%. to be 100.86 and %RSD is found to be 0.635.

## Precision

The results of various parameters under precision were evaluated from the chromatograms recorded viz. repeatability of measurement, intra and inter-day variation indicates that solution was stable in solution form up to 72 hr, and percent RSD

**Table 2: Results of System Suitability Parameters.**

| Sl. No. | Wt. of Std. drug Taken (mg) | Area (mV) |
|---|---|---|
| 1. | ~10.0 mg | 592358 |
| 2. | | 593799 |
| 3. | | 593586 |
| 4. | | 593895 |
| 5. | | 593325 |
| 6. | | 593844 |
| Mean | | 593467 |
| ±SD | | 583.0503 |
| %RSD | | 0.0098 |
| Retention time | | 7.102 |
| Tailing factor (Asymmetry) | | 1.929 |
| Theoretical Plate | | 3973 |

**Table 3: Finalized Dissolution Parameter.**

| Drug | Dissolution media | Media volume | USP Apparatus | RPM |
|---|---|---|---|---|
| Tenofovir Disoproxil Furamate | 0.01 N HCl/Water | 900 mL | Type-II Paddle | 75 |

**Table 4: Observation of Dissolution Study in HCl and Water using HPLC.**

| Sample | Time point | Retention time | AUC (mV) HCl | % Drug release | Retention time | AUC (mV) Water | % Drug release |
|---|---|---|---|---|---|---|---|
| Test Samples | 10 | 6.398 | 1142581 | 57.72 | 8.0123 | 1120213 | 56.59 |
| | 20 | 6.416 | 1234321 | 62.36 | 8.0213 | 1152895 | 58.24 |
| | 30 | 6.363 | 1385241 | 69.98 | 8.1250 | 1356958 | 68.55 |
| | 40 | 6.349 | 1462314 | 73.87 | 8.0140 | 1458942 | 73.70 |
| | 50 | 6.392 | 1591024 | 80.38 | 8.1235 | 1589652 | 80.31 |
| | 60 | 6.452 | 1828510 | 92.38 | 8.1453 | 1785962 | 90.23 |
| | 70 | 6.475 | 1914121 | 96.70 | 8.0446 | 1898562 | 95.91 |
| | 80 | 6.454 | 1961455 | 99.09 | 8.1140 | 1958942 | 98.97 |

**Table 5: Observation of Dissolution Study in HCl and Water using UV method.**

| Sample | Time point | Absorbance HCl | % Drug release | Absorbance Water | % Drug release |
|---|---|---|---|---|---|
| Test Samples | 10 | 0.3581 | 37.38 | 0.3321 | 34.65 |
| | 20 | 0.4721 | 49.26 | 0.4421 | 46.13 |
| | 30 | 0.5821 | 60.74 | 0.5312 | 55.42 |
| | 40 | 0.6733 | 70.25 | 0.5521 | 68.04 |
| | 50 | 0.7621 | 79.25 | 0.7412 | 77.34 |
| | 60 | 0.8721 | 91.00 | 0.8411 | 87.76 |
| | 70 | 0.9210 | 96.10 | 0.9011 | 94.02 |
| | 80 | 0.9455 | 98.66 | 0.9256 | 96.58 |

**Table 6: Results of Method validation.**

| Characteristics | Acceptance Criteria | UV Method | | HPLC Method | |
|---|---|---|---|---|---|
| Accuracy/Trueness | Recovery 98-102% (individual) with 80,100,120% spiked | HCl | Water | HCl | Water |
| | | 99.93% | 99.33% | 100.86% | 99.275% |
| Precision * | RSD<2% | HCl | Water | HCl | Water |
| | | 0.04912 | 0.0576 | 0.05521 | 0.09520 |
| Repeatability * | RSD<2% | HCl | | Water | |
| | | 0.0950% | | 0.0490% | |
| Ruggedness* | RSD<2% | HCl | Water | HCl | Water |
| | | 0.0285 | 0.02655 | 0.0377 | 0.0595 |
| Robustness | Overall RSD<2% | Passes | | Passes | |
| Specificity/Selectivity | No interference | No interference | | | |
| Detection limit | S/N 2 or 3 | 0.46864 | | | |
| Quantification limit | S/N 2>10 | 1.420117 | | | |
| Linearity | Correlation coefficient r>0.999 | 0.9954 | | 0.9972 | |

*Each mean is the result of three replicates.

values were found to be within limits in both media evaluated by proposed methods. The results of the precision and repeatability study of the drug by the proposed methods are summarized in Table 6.

### Ruggedness

Results of ruggedness study by different analysts on different days using the proposed methods revealed that the methods are rugged. Results are shown in Table 6.

## ROBUSTNESS

Results of the robustness study of HPLC method for deliberate variations done in the method parameters revealed that the method was found to be robust study. Overall percent RSD values were found to be within limits Table 6.

### Release kinetics

Several mathematical models were investigated (zero order, first order, Higuchi model, Korsemeyer-Peppas model.) to identify the drug-release kinetics The graphs of various models were constructed based on the release data obtained during the study to obtain the best release profile. The graphs are shown in Figures 5a-5d and results of correlation coefficient are shown in Table 7.

## DISCUSSION

Mobile phase composition containing equal amounts of ACN and Methanol was tried based on literature but negative co-eluting peaks were observed. The composition was changed to make the mobile phase more polar to resolve the coeluting peaks. Formic was introduced, 0.01N Formic acid: Methanol (50:50 v/v) was employed, giving well-defined and resolved peak. System suitability analysis showed the tailing factor (less than 2) and number of theoretical plates (more than 2000) found to be satisfactory as per ICH guidelines. The dissolution parameters like rotation speed, dissolution media volume, and dissolution media were finalized and the dissolution test method was performed for the dissolution analysis. In the present study, various dissolution media, medium volumes, and paddle stirring speeds were

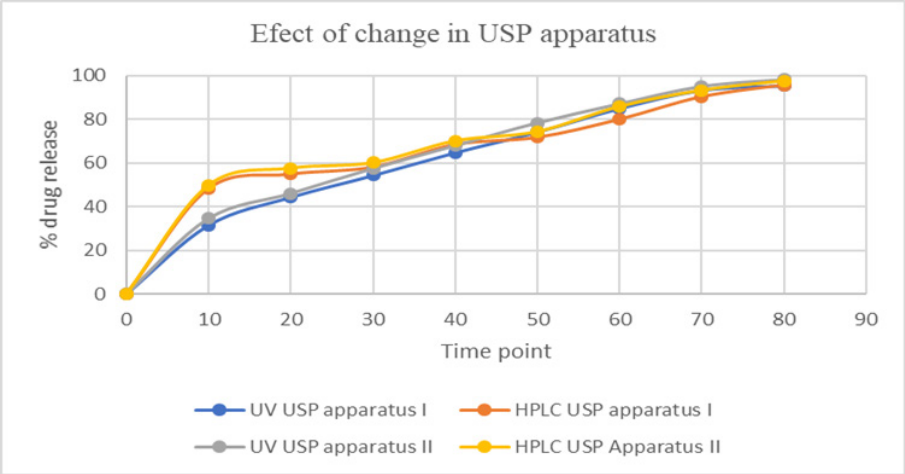


**Figure 1a:** Effect of change in USP apparatus.

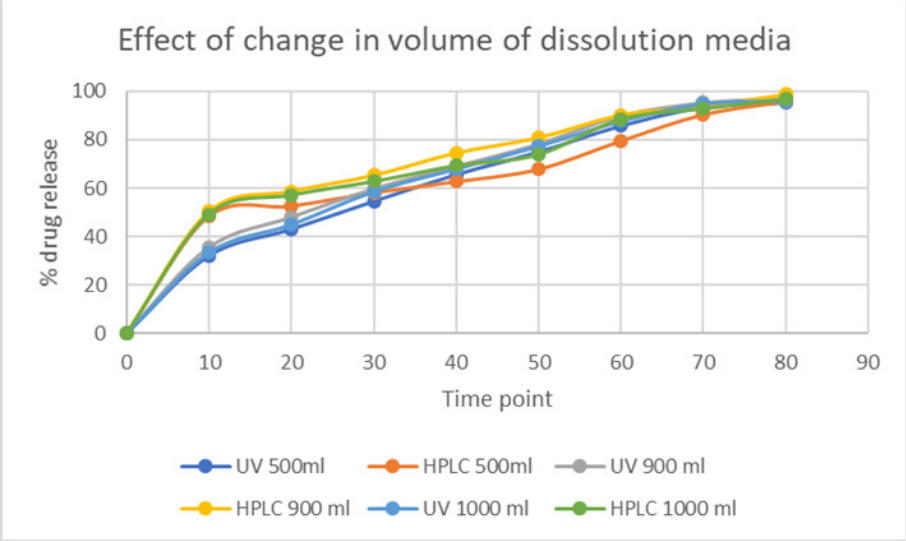


**Figure 1b:** Effect of change in volume of dissolution media.

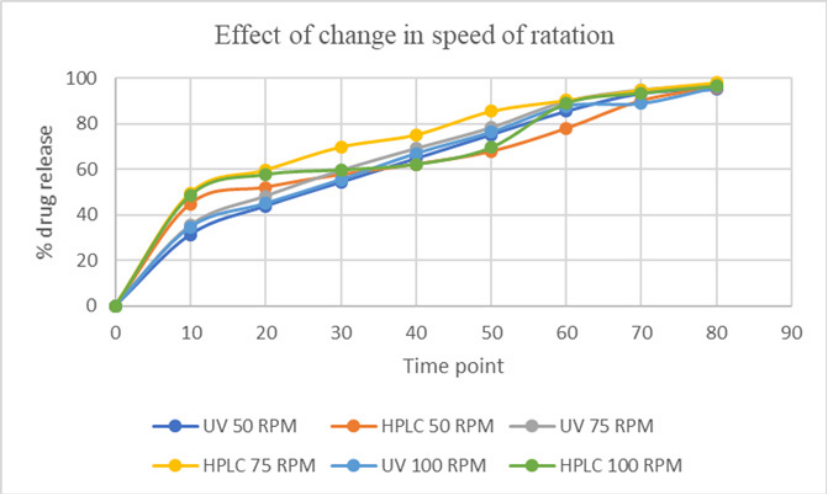


**Figure 1c:** Effect of change in speed of rotation.

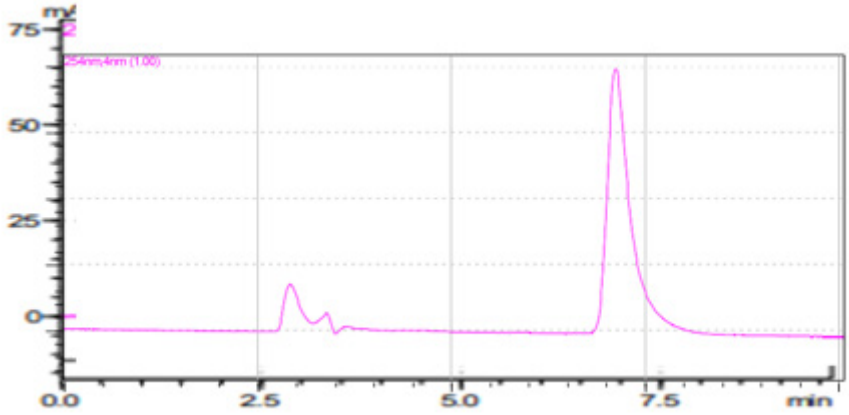


**Figure 2 (a):** Chromatogram of standard recorded using 0.01N HCl.

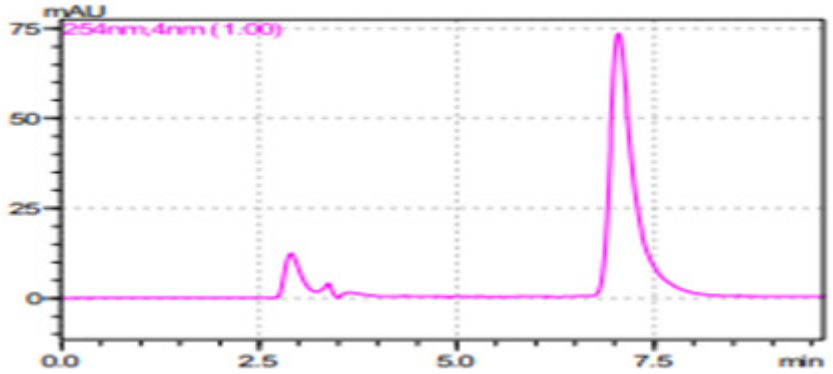


**Figure 2(b):** Chromatogram of sample recorded using 0.01N HCl.

**Table 7: Release Kinetic Model (Best fit Model).**

| Models | Correlation coefficient $R^2$ | |
|---|---|---|
| | HCl | Water |
| Zero order | 0.9819 | 0.9854 |
| First order | 0.8696 | 0.8521 |
| Higuchi Model | 0.9594 | 0.621 |
| Korsmeyer-peppas model | 0.9298 | 0.9221 |

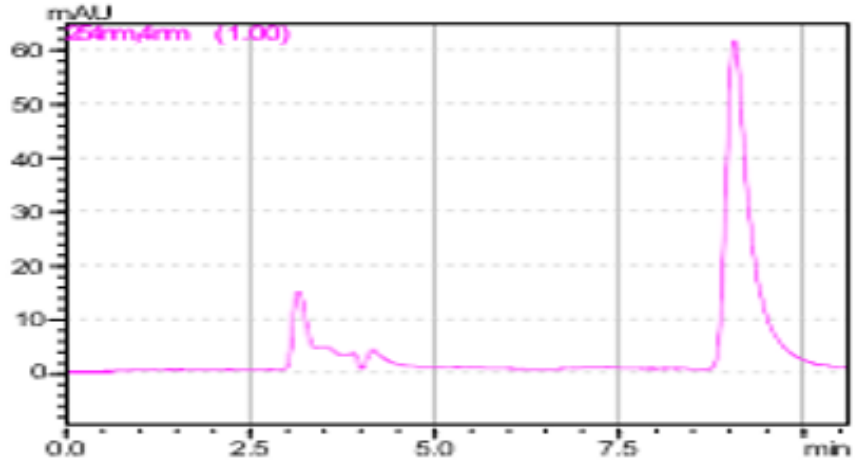


**Figure 2 (c):** Chromatogram of standard recorded using water

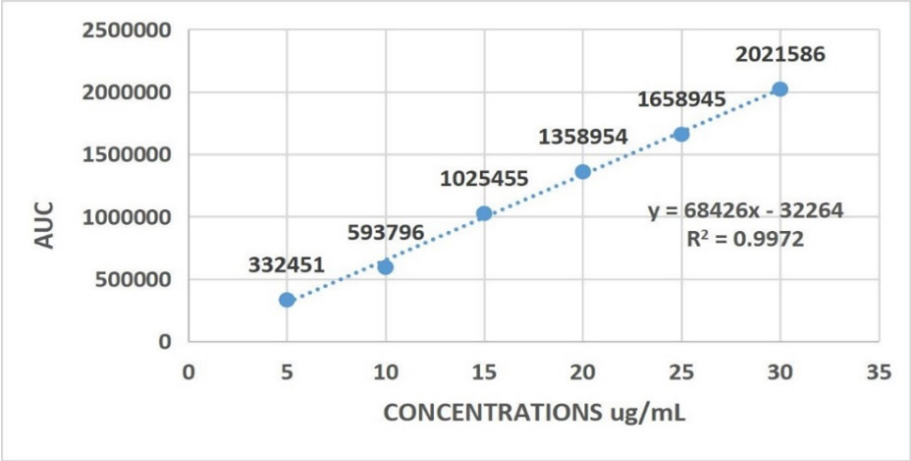


**Figure 3:** Graph showing linearity of TDF by HPLC method.

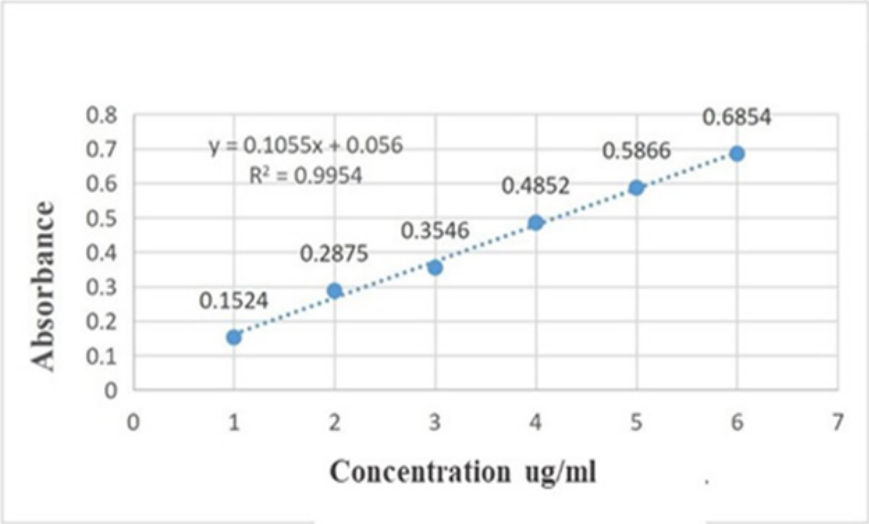


**Figure 4:** Graph showing linearity of TDF by UV method.

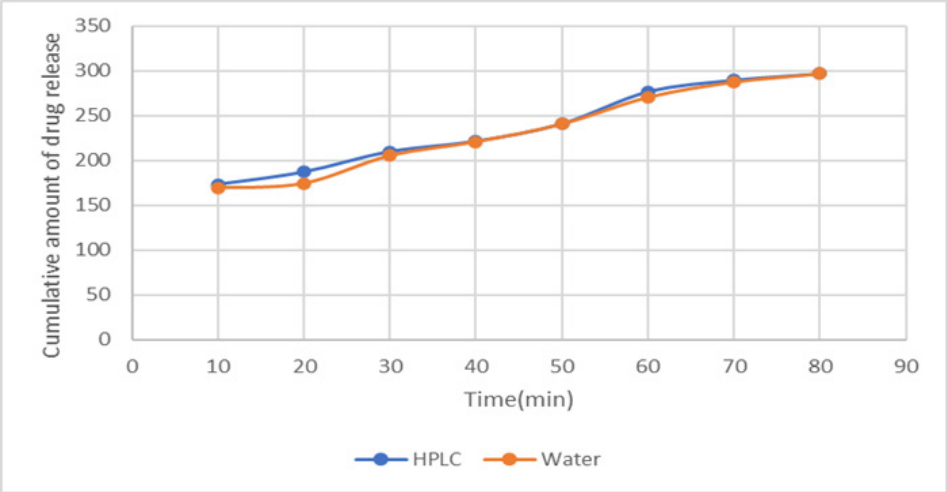


**Figure 5a:** Zero order model.

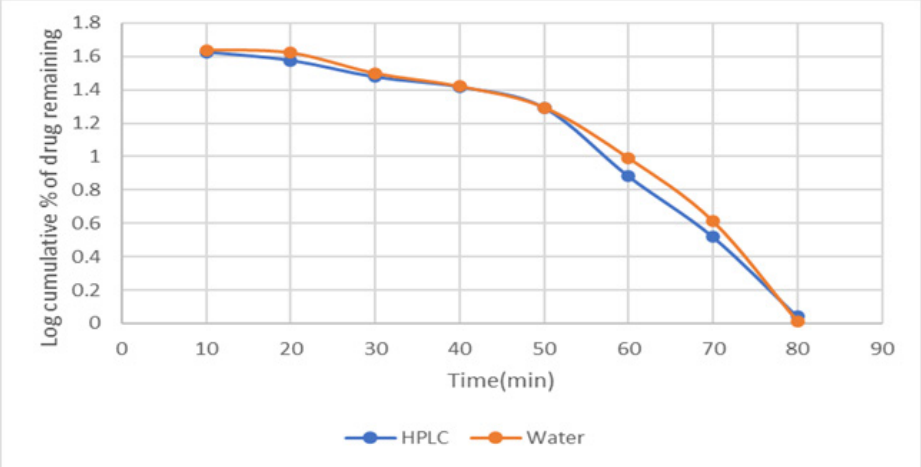


**Figure 5b:** First order model.

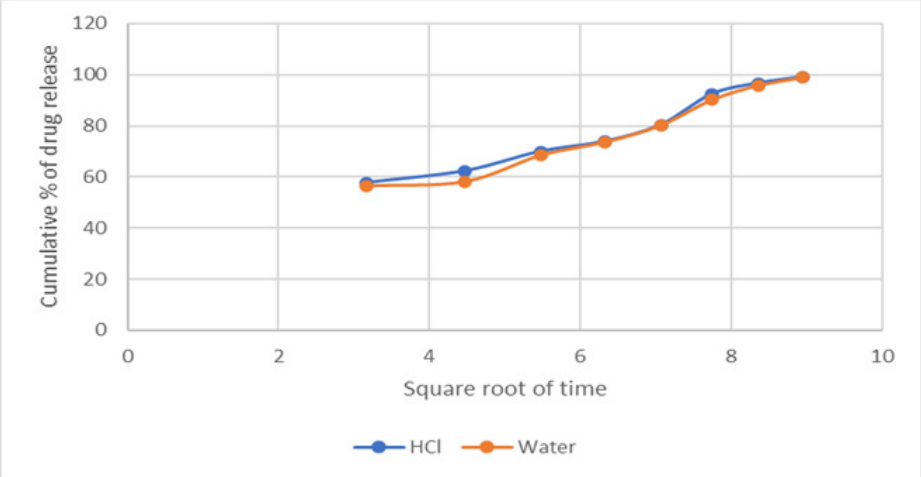


**Figure 5c:** Higuchi model.

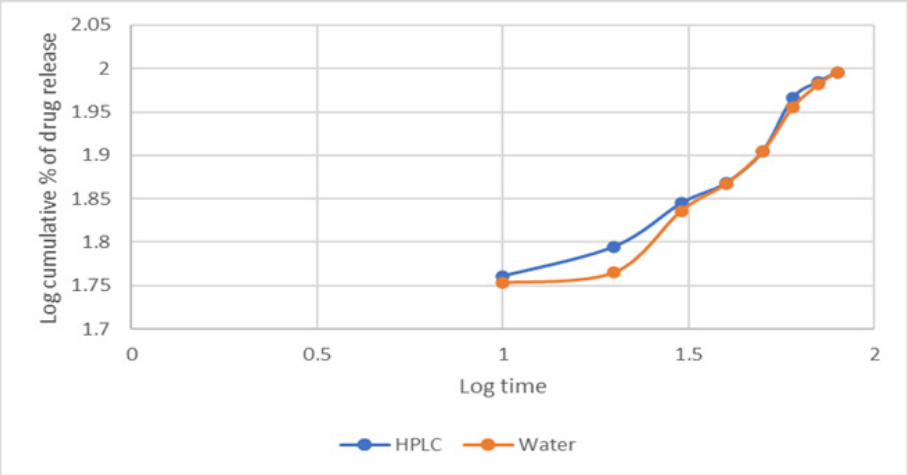


**Figure 5d:** Korsmeyer-peppas model.

assessed the sample formulation dissolution profiles. Results were satisfactory when using 0.01N HCl at 37±0.5º, 900 mL, and 75 rpm. From Table 6, various method validation parameters were found to be satisfactory and pass all the acceptance criteria as per the ICH guidelines.The goodness of fit for several models examined for products ranks in the order of zero-order>Higuchi>Korsmeyer-Peppas>First order in 0.01 N HCl while in water it follows zero-order>Korsmeyer-Peppas>First order>Higuchi. In both dissolution media, the zero-order release model had the best fit for all dissolution data and had the highest coefficient of determination (R2). Fickian diffusion-based drug release provides zero-order release kinetics, with the release rate independent of the concentration of the drug in each tablet.

## CONCLUSION

In summary, a reliable and distinct dissolution technique was developed for Tenefovir disoproxil tablets. To aid, in formulation development, a novel discriminating dissolving test method was developed and approved in compliance with the most recent ICH and FDA requirements. The sample formulation's drug release most closely fits the Zero order model, which offers a useful comparison between uniformity in profile shape and level (location).

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**TDF:** Tenofovir Disoproxil Fumarate; **HPLC:** Hogh Performance Liquid Chromatography; **UV:** Ultraviolet Spectroscopy; **USP:** United Staes Pharmacopoeia; **ACN:** Acetonitrile; **RPM:** Rotation per minute; **AUC:** Area under Curve; **HCl:** Hydrochloric Acid; **RSD:** Relative standard Deviation; **ICH:** International community on Harmonization; **FDA:** Food and Drug Administration.

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