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QbD Approach to Analytical RP-HPLC Method Development and Validation of Tenofovir Disoproxil Fumarate in Dosage Form

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Abstract:
A simple, sensitive and reproducible spectrophotometric method is developed for determining the Tenofovir Disoproxil Fumarate content in bulk and in tablet dosage form using an experimental design approach. Quality by design (QbD) refers to the achievement of certain predictable quality with desired and predetermined specifications. A very useful component of the QbD is the understanding of factors and their interaction effects by a desired set of experiments. The present study describes the development of a comprehensive science and risk based RP-HPLC method and subsequent validation for the analysis of Tenofovir Disoproxil Fumarate active pharmaceutical ingredient (API) and Tablet using a quality by design approach. An efficient experimental design based on systematic scouting of key components of the RP-HPLC method (column and mobile phase) is presented. The described method was linear. (r2=0.99). The precision, ruggedness and robustness values were also within the prescribed limits (<1% for system precision and <2% for other parameters). The proposed method can be used for routine analysis of Tenofovir Disoproxil Fumarate in quality control laboratories.

Keywords: Design approach, HPLC, Quality by Design, Tenofovir Disoproxil Fumarate.

1. INTRODUCTION:

Tenofovir disoproxil fumarate is a fumaric acid salt of the bis isopropoxycarbonyl oxymethyl ester derivative of tenofovir. The chemical name of tenofovir disoproxil fumarate is 9 [(R) 2 [[bis [[(isopropoxycarbonyl) oxy] methoxy] phosphinyl] methoxy] proyl] adenine fumarate (1:1). It has a molecular formula of $C_{19}H_{30}N_5O_{10}P \bullet C_4H_4O_4$ and a molecular weight of 635.52. Tenofovir is a nucleotide reverse transcriptase inhibitor3 used in combination with other antiretrovirals for the treatment of HIV infections. Literature survey reveals that there are several reports describing the determination of Tenofovir in plasma using HPLC coupled with fluorescence and UV detection.

Quality by Design (QbD) is a concept first outlined by well-known quality expert Joseph M. Juran in various publications, most notably Juran on Quality by Design. While Quality by Design principles have been used to advance product and process quality in every industry and particularly the automotive industry, they have most recently been adopted by the U.S. Food and Drug Administration (FDA) as a vehicle for the transformation of how drugs are discovered, developed and commercially manufactured. Since first initiated by the U.S. Food and Drug Administration (FDA) in its "Pharmaceutical cGMPs for the twenty-first century". Quality by Design (QbD) has become an important concept for the pharmaceutical industry that is further defined in the International Conference on Harmonisation (ICH) guidance on pharmaceutical development as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management". The scientific understanding gained during the method development process can be used to devise method control elements and to manage the risks identified. High-performance liquid chromatography (HPLC), particularly Reversed Phase HPLC (RP-HPLC) is the most popular analytical technique in the pharmaceutical industry. The quality of HPLC methods has become increasingly important in a QbD environment. For the purpose of QbD for HPLC methods, robustness and ruggedness should be verified early in the method development stage to ensure method performance over the lifetime of the product.

The aim of the analytical method is to separate and quantify the main compound while meeting the method performance criteria based on regulatory requirements, such as specificity, linearity, accuracy, precision, sensitivity, robustness, and ruggedness.

The primary objective of this study was to implement Qbd approach to develop and validate an RP-HPLC method that could separate drug from its potential related substances and to establish an in depth understanding of the method and build in the quality during the method development to ensure optimum method performance over the lifetime of the product.

The objectives of this work are as follows:

a. To develop simple, rapid and sensitive method for identification of critical attributes by QbD approach of this antiretroviral drug by RP-HPLC.
b. To establish a validated test method as per ICH guidelines for the determination of assay of this antiretroviral drug by RP-HPLC.

2. MATERIALS AND METHODS:

A TDF sample was obtained from Lupin Pharmaceutical Ltd., Aurangabad, India. HPLC grade Distilled Water were purchased from Modern Industries, C-74, MIDC Malegaon-Sinnar, Nashik, India. Acetonitrile 99.9% were purchased from LAB FINE CHEM Industries, Mumbai, India. A HPLC of Agilent Technologies, Model No. 1220 Infinity Series with a Column of Primesil C18, particle size of about 5µm with dimension of 4.6×250 mm, Wesley Technologies Inc, USA and Design Expert® Software (Version 7.0, Stat-Ease Inc., Minneapolis, MN) was used for measurements.

Reagents and solution

i. TDF Std. (Stock) Solution

An accurately weighed quantity of about 20 mg of TDF was dissolved in 50 ml of HPLC grade Distilled Water. From prepared 200 ppm stock solution 1 ml was taken out and diluted it up to 10 ml distilled water. The solution was freshly prepared and protected from light.

ii. TDF Test Solution

An accurately weighed quantity of about 31.5 mg of TDF tablet was dissolved in 100 ml of HPLC grade Distilled Water. From prepared test solution 1ml was taken out and diluted it up to 10 ml distilled water.

iii. Mobile Phase

A binary mobile phase consisting of Acetonitrile and Distilled Water in the proportion of 67.1:32.9, v/v was used for HPLC analysis. Mobile phase was used as diluent for HPLC analysis.

Selection of detection wavelength

The detection was carried out in the UV region and wavelength selected for detection was 260 nm in Distilled water. Solution was prepared in Distilled water and scanned in the range of 200-400 nm.

Method development by qbd approach

i. Define method intent

The goals of HPLC method development have to be clearly defined, as pharmaceutical QbD is a systemic, scientific, risk based, holistic and proactive approach that begins with predefined objectives and emphasizes product and process understanding and control.

ii. Perform experimental design

A systematic experimental design is needed to assist with obtaining in- depth method understanding and performing optimization. Here an efficient and comprehensive experimental design based on systematic scouting of two key components of the RP-HPLC method (mobile phase and pH) is presented. It forms a chromatographic database that will assist with method understanding, optimization and selection. In addition, it can be used to evaluate and implement change of the method, should it be needed in the future, for example should the chromatographic column used no longer be commercially available, or an impurity is no longer relevant.

Factorial Design

Central composite statistical screening design was used to optimize and evaluate main effects, interaction effects and quadratic effects of the formulation ingredients on the *in-vitro* release of the drug. A 2-factor, 3-level design used is suitable for exploring quadratic response surfaces and constructing second order polynomial models with Design Expert® (Version 10.0, Stat-Ease Inc., and Minneapolis, MN).

$$Y = \beta0 + \beta1A + \beta2B + \beta12AB + \beta11A2 + \beta22B2$$

Where Y is the measured response associated with each factor level combination; β0 is an intercept; β1 to β22 are regression coefficients computed from the observed experimental values of Y from experimental runs; and A and B are the coded levels of independent variables. The terms AB, A2 and B2 represent the interaction and quadratic terms, respectively. The factors were selected based on preliminary study. Flow rate (A) and Mobile phase composition (B) were selected as independent variables. The Retention time, peak area and peak asymmetry were selected as dependent variables.

Table 1 Coded values for independent variables

Name of the Factor	Coded values	Level		
		-1	0	1
Flow Rate (ml/min)	A	0.5	0.75	1
Mobile Phase Composition (% v/v)	B	60	50	80

iii. Evaluate experimental results and select final method conditions

These method conditions were evaluated using the three tiered approach. At the first level, the conditions were evaluated for peaks symmetry, retention time and peaks tailing. This resulted in different chromatographic conditions for API. The best suited experimental conditions shall be optimized using design expert software.

iv. Perform risk assessment with robustness and ruggedness evaluation

As the final method is selected against method attributes, it is highly likely that the selected method is reliable and will remain operational over the lifetime of product. Therefore, the evaluation of method robustness and ruggedness to be carried out as the final step of method development is mainly for the method verification and finalization. A risk-based approach based on the QbD principles set out in ICH Q8 and Q9 was applied to the evaluation of method robustness and ruggedness. Structured methodologies for risk assessment, such as Fishbone diagram can be implemented to identify the potential risk of the method due to a small change of method parameters or under a variety of conditions such as different laboratories, analysts, instruments, reagents, days etc.

v. define analytical method performance control strategy

As a result of robustness and ruggedness studies, the overall method understanding of method performance under various conditions can be improved and an analytical method performance control strategy along with appropriate system suitability criteria can be defined to manage risk back to the database described in experimental design to find a more appropriate method and to go through the procedure as described to ensure method robustness and ruggedness.

Analytical method validation

Validation is documented evidence, which provide a high degree of assurance for specific method. Validation is

analytical process by which it is established by laboratory studies that the performance characteristics of the procedure meet the requirement for intended analytical application.

i. Linearity

The linearity of TDF was assessed in the range of (5-35 µg/ml) in terms of slope, intercept and correlation co-efficient values.

ii. Precision

a. repeatability

Measure area of standard mixed solutions containing TDF 20µg/ml at 260nm. The area of solution was measured 6 times and % RSD was calculated.

b. intra-day precision

Intra-day precision was determined by analyzing TDF 20µg/ml concentrations were determined 6 times a day and %RSD was calculated.

% RSD should be less than 2.

c. inter-day precision

Inter-day precision was determined by using same solution of Intra-Day Precision by analysing TDF 20µg/ml concentrations were determined 6 times a day and %RSD was calculated.

% RSD should be less 2%.

iii. Accuracy

Accuracy of the method was confirmed by recovery study from tablet formulation at three level of standard addition. Percentage Recovery of TDF was found out. Recovery between 98-100% justifies the accuracy method.

iv. LOD and LOQ

LOD was calculated out by using following Formula:

$LOD = 3.3\sigma/S$

σ = Standard Deviation of the Response

S = Slope

LOQ was calculated out by using following Formula:

$LOQ = 10\sigma/S$

σ = Standard Deviation of the Response

S = Slope

v. Robustness

Robustness of the method was determined by subjecting the method to slight change in the method condition, individually the: Flow rate and Mobile Phase ratio.

% RSD was calculated.

vi. Ruggedness

The ruggedness of analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under conditions of different days and different analysts.

vii. System suitability studies

The system suitability was evaluated by five replicate analyses of TDF. The column efficiency and peak asymmetry, Theoretical Plates were calculated for standard solutions.

viii. Assay

Twenty tablets of TDF were weighed and finely powdered. The tablet powder equivalent to 300 mg of TDF was accurately weighed and transferred to a 100 ml volumetric flask, about 100 ml of Distilled Water was added and the flask was sonicated for 15 min. Further pipette out 1 ml and transfer into 10 ml volumetric flask and dilute up to mark with distilled water. The 20 µg/ml solution was prepared and 20 µl was injected for HPLC analysis.

3. RESULT AND DISCUSSION

Optimization of mobile phase

The mobile phase was successfully obtained after the many trials shown in the table**.**

Table 2 Optimization of Mobile Phase

Sr. No.	Mobile phase	Ratio (v/v)	Remark
1	Acetonitrile: water	50:50	Peak was not proper.
2	Acetonitrile: water	60:40	Peak tailing was observed.
3	Acetonitrile: water	70:30	Peak was not resolved.
4	Acetonitrile: water	62.9:37.0	Peak was not proper.
5	Acetonitrile: water	67.1:32.9	Peak was observed.

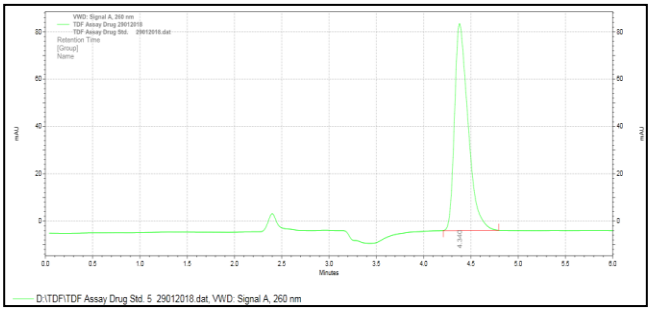

Figure 1 Chromatogram of Trial 5 – Acetonitrile: Water (67.1:32.9)

Optimization of various parameters for analysis of TDF using HPLC (by using central composite design)

Table 3 Design Summary for optimization

Study Type	Response Surface
Design Type	Central Composite Design
Design Model	Quadratic
Runs	13

Optimized condition obtained

It was obtained by studying all responses in different experimental condition using Design expert 7.0 software.

Table 4 Obtained solution for optimized formulation

Factor	Name	Units	Type	Subtype	Minimum	Maximum
Code					0	1
A	Flow Rate	ml/min	Numeric	Continuous	0.5	1
B	Mobile Phase	% v/v	Numeric	Continuous	60	80

Code	Flow Rate	Mobile Phase	Retention Time	Area	Peak Asymmetry	Desirability
16	0.66 ml/min	67.1:32.1 % v/v	4.34	14206812		

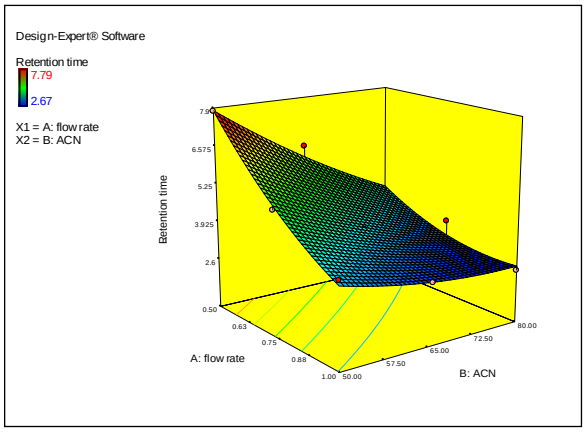

Figure 2 3D surface plot of desirability for obtaining optimized formulation

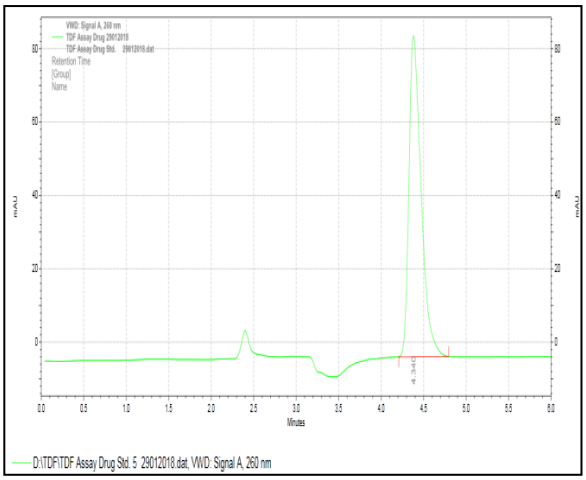

Figure 3 Chromatogram obtained from the Optimized Condition

Table 5 Final Optimized Method Condition

Sr. No.	Parameters	Results
1.	Column	C-18
2.	Flow Rate	0.66 ml/min
3.	Mobile Phase	Acetonitrile: Water (67.1:32.1 % v/v)
4.	Injection Volume	20 µl
5.	Detection Wavelength	260nm
6.	Run Time	6 min

Method validation

i. System Suitability

Table 6 System suitability test for TDF

Acceptance criteria	Result
The %RSD for five replicate injections of Standard preparation for TDF should be NMT 2.0.	
The Tailing factor for the TDF from standard preparation should be NMT 2.0	
Theoretical plates for TDF peak should be NLT 2000.	

ii. Linearity

Table 7 Linearity for TDF

Sr. No.	Conc.(µg/ml)	Peak Area
1.	5	6202211
2.	10	11377343
3.	15	19275629
4.	20	24035811
5.	25	33477919
6.	30	39999019
7.	35	49939829

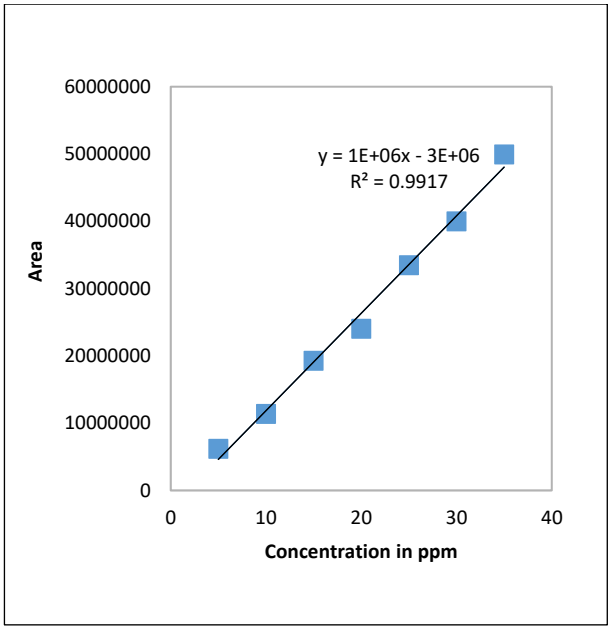

Figure 4 Calibration Curve for TDF

iii. Precision

Table 8 Data for Repeatability of TDF

Sr. No.	Conc.(µg/ml)	Area	% Assay	Mean (% Assay)	Standard Deviation	% RSD
1.	20	22287796	100.24	100.12	0.21631	0.21
2.	20	22200135	99.8			
3.	20	22270869	100.29			
4.	20	22287899	100.24			
5.	20	22222658	99.9			
6.	20	22266800	100.28			

Table 9 Data for Interday and Intraday of TDF

Sr. No.	Conc.(µg/ml)	Intraday		Interday	
		Area	% Assay	Area	% Assay
1.	20	22250778	100.07	22122710	99.5
2.	20	22374776	100.63	22337781	100.46
3.	20	22303143	100.31	22231065	99.98
4.	20	22349792	100.52	22312780	100.35
5.	20	22314046	100.36	22136775	99.56
6.	20	22205209	99.87	22335683	100.45
	Mean		100.29		100.05
	Standard Deviation		0.282324		0.439454
	% RSD		0.28		0.43

iv. Accuracy

Table 10 Accuracy/Recovery of TDF

Level	Sr. No.	Area	Amount Found	Amount Added	% Recovery	Total % Recovery (Mean)	Standard Deviation	% RSD
80%	1.	42623197	38.33	38	100.88			
	2.	42366047	38.10	38	100.27	100.49	0.336204	0.33%
	3.	42389082	38.12	38	100.33			
100%	1.	44062993	39.63	40	99.08			
	2.	44857620	40.34	40	100.86	100.26	1.021959	1.01%
	3.	44845574	40.33	40	100.84			
120%	1.	46536917	41.85	42	99.66			
	2.	46814150	42.10	42	100.25	100.01	0.313741	0.31%
	3.	46763924	42.06	42	100.14			

v. Robustness

Table 11 Robustness for TDF

Parameter	Sr. No.		Total % Recovery (Mean)	Standard Deviation	% RSD
Change in Flow Rate	1.	0.65ml/min	100.38	0.318476	0.31
	2.	0.66ml/min	100.18	0.523667	0.52
	3.	0.67ml/min	99.64	0.509094	0.51
Change in Composition	1.	62.1 % v/v	99.72	0.604406	0.64
	2.	67.1 % v/v	99.66	0.539126	0.54
	3.	72.1 % v/v	99.83	0.502888	0.53

vi. Ruggedness

Table 12 Ruggedness for TDF

Parameter	Total % Recovery (Mean)	Standard Deviation	% RSD
Analyst I	100.13	0.678351	0.67
Analyst II	100.13	0.674497	0.67
Day I	99.76	0.447724	0.44
Day II	99.82	0.535711	0.53

vii. LOD and LOQ

Table 13 LOD and LOQ of TDF

Parameters	Results
LOD (µg/ml)	0.93
LOQ (µg/ml)	2.83

viii. Assay

Table 14 Assay of TDF

Parameters	Label claim(mg)	Peak Area of Standard	Peak Area of Test	% Assay
Result	300mg	14206812	12785208	90.00%

4. CONCLUSION:

In the present work optimization of a spectrophotometric method for determining TDF content was optimized using Design Expert® Software (Version 7.0). The proposed RP-HPLC method was found to be simple, rapid, precise, accurate and sensitive for the determination of TDF in pharmaceutical dosages forms. However, the experimental design approach was systematic, less time consuming and more cost effective compared with the conventional method. Therefore, the proposed method using an experimental design approach is better for determining the TDF content and can be used for routine quality control analysis of TDF formulations.

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