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ANALYTICAL QUALITY BY DESIGHN APPROACH FOR THE DEVELOPMENT OF VALIDATED RP-HPLC METHOD IN THE SIMULTANEOUS ESTIMATION OF LAMIVUDINE AND TENOFOVIR DISPROXIL FUMERATE IN BULK AND THEIR COMBINED DOSAGE FORMS

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ABSTRACT
Qualitybydesign(QbD)referstotheachievementofcertainpredictablequalitywithdesired 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 simultaneous analysis of Lamivudine and tenofovir disposil fumarate active pharmaceutical ingredients (API) using a quality by design approach. An efficient experimental design based on systematic scouting of all three key components of the RPHPLC method (column, pH and mobile phase) is presented. The described method was linear. ((r2) =0.9998).(linearity range =)The precision, ruggedness and robustness values were also within the prescribed limits (<1% for system precision and <2% for other parameters). Chromatographic peak purity results indicated the absence of co-eluting peaks with the main peak of Lamivudine and tenofovir disposil fumarate. The proposed method can be used for routine analysis of Lamivudine and tenofovir disposil fumarate in quality control laboratories.

KEYWORDS: Quality by design, HPLC, Lamivudine and tenofovir disposil fumarate.

INTRODUCTION
Lamivudine (LAM): chemically- (2R-cis)-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H) pyrimidinone[1], is a synthetic nucleoside analogue with potent activity against human immune deficiency (HIV) and hepatitis B viruses (HBV) through inhibition of reverse transcriptase activity. It has a molecular formula of $C_8H_{11}N_3O_3S$ and a molecular weight- 229.3 g/mol, soluble in water, sparingly soluble in methanol and practically insoluble in acetone. . It has the following structural formula as shown in fig.1a.

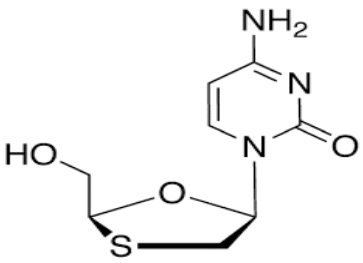


Fig 1a: Chemical structure of Lamivudine.

Tenofovir Disoproxil Fumarate: Tenofovir disoproxil fumarate is a fumaric acid salt of the bisisopropoxycarbonyloxymethyl ester derivative of tenofovir. The chemical name of TDF is 9-[(R)-2[[bis[[(isopropoxycarbonyl)oxy]methoxy]phosphinyl]methoxy]propyl]adeninefumarate. Tenofovir disoproxyl fumarate belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (NRTIs). It has a molecular formula $C_{19}H_{30}N_5O_{10}P \cdot C_4H_4O_4$ and a molecular weight 635.51. It has the following structural formula as shown in fig.1 b. TDF is a white to off-white crystalline powder with a solubility of 13.4 mg per mL in water at 25 °C.[2]

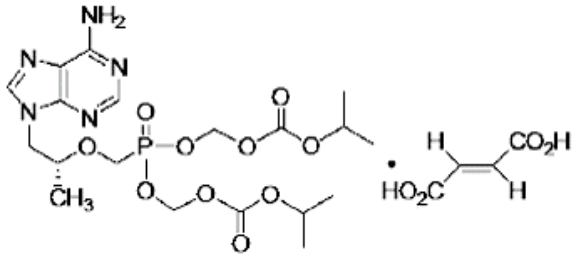


Fig 1b: Chemical structure of Tenofovir disoproxil fumarate.

Quality by Design (QbD): Quality by Design (QbD) (1-3) 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(4-7) (FDA) as a vehicle for the transformation of how drugs are discovered, developed, and commercially manufactured. Since first initiated by 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. The U.S. Food and Drug Administration (FDA) in its "Pharmaceutical.

Table: 1. The difference between Current approach and QbD approach.

S.no	QbD approach	1Current approach
1	Quality is built into product & process by design and based on scientific understanding.	Quality is assured by testing and inspection.
2	It includes Knowledge rich submission which shows product knowledge & process understanding	It includes only data intensive submission which includes disjointed information without “big picture”.
3	Here, any specifications based on product performance requirements.	Here, any specifications are based on batch history.
4	Here there is Flexible process within design space which allows continuous improvement	Here there is “Frozen process,” which always discourages changes
5	It focuses on robustness which understands and control variation	it focus on reproducibility which often avoids or ignores variation

According to literature survey, there are quite a few publications on HPLC method development strategy but the method development approaches for RP-HPLC specifically focused on pharmaceutical development in a QbD environment have not been widely discussed. Therefore, there is an unmet need to develop a systematic HPLC method development approach for pharmaceutical development using QbD principles to ensure the quality of the method throughout the product lifecycle.

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 Qb d 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

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

EXPERIMENTAL

Chemicals: Materials and reagents Lamivudine (99.5%), tenofovir (99.75%)were obtained as gift samples from Hetero laboratories, Hyderabad, commercially obtained viropovir.was India. HPLC grade Acetonitrile was purchased from Merck (Mumbai, India), HPLC grade Water (Milli Q or equivalent) all chemicals (AR Grade) were used for entire study.

Instrumentation

All HPLC experiments were carried out on a Waters Alliance 2695 separation module, with waters 2996 photodiode array detector in gradient mode using Auto sampler. Data collection and processing was done using EMPOWER PDA 2 software. The analytical column

used for the separation was Luna CN, 250mm x 4.6mm, 5µm Column, Other equipments used were ultra-sonicator (model 3210), Analytical balance (contech balance).P^H meter elico.

Preparation of solvents and solutions
Preparation of Buffer
1. Mix 1ml Ortho Phosphoric acid in 1litre water.

Preparation of Mobile Phase
Mix Acetonitirile and Buffer in the ratio of 40+60. Filter through 0.45µ membrane filter paper.

Diluent preparation
Use Mobile Phase as a diluent.

Preparation of standard solution
Weigh accurately about 12.5 mg of Lamivudine working standard and 12.5 mg of Tenofovir working standard into a 10 mL volumetric flask. Add 7 mL of diluent, sonicate to dissolve and dilute to volume with diluent. Further dilute 1mL of above solution to 10 mL with the diluent.

Preparation of sample solution
Weigh 20 tablet calculate average weight and then take one tablet equivalent weight of 49.7 mg of sample into a 10 mL volumetric flask. Add 7 mL of diluent, sonicate to dissolve and dilute to volume with diluent. Filter through 0.45µ Nylon syringe filter. Further dilute 1mL of above solution to 10 mL with the diluent.

Procedure
Inject 10µL of Standard preparation five times and Sample preparation in the Chromatograph. Record the chromatograms (Fig 2) and measure the peak responses for Lamivudine and Tenofovir. The System suitability parameters should be met. From the peak responses, calculate the content of Lamivudine + Tenofovir in the sample (Table 2).

Table 2: Estimation of marketed formulation

Brand name	Drug name	Labelled claim(mg)	Test concentration (µg/mL)	Amount found(µg)	% Recovery (n=6)
VIROFOVIR	LAM	300	30	30.1	100.3
	TDF	300	30	30.3	101.0

Determination of detection wavelength for Lamivudine and Tenofovir
Appropriate dilutions of the standard drug solutions were prepared for 10 ppm of lamivudine and tenofovir API sepatately. 10ppm solution of lamivudine and tenofovir were prepared in methanol as adiluent. Solution was scanned using double beam UV VIS spectrophotometer between the range of 200 to 400 nm. λmax of 273 nm and 245nm of lamivudine and tenofovir were considered for experimental work.

Method development by QbD approach
Step 1: Define method intent
The goals of HPLC method development have to be clearly defined, as pharmaceutical QbD is a systematic, scientific, risk based, holistic and proactive approach that begins with predefined objectives and emphasizes product and process understanding and control. The ultimate goal of the analytical method is to separate and quantify the main compound.

Step 2: Perform experimental design
A systematic experimental design is needed to assist with obtaining indepth method understanding and performing optimization. Here an efficient and comprehensive experimental design based on systematic scouting of all three key components of the RP-HPLC method (column, pH and mobile phase) 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. The scoutings of three parameters like column, pH and mobile phas are shown in table 3a, 3b, and 3c and fig 3, 4 and 5 respectively. An experimental design comprised of a standard set of 3 columns, 3 pH values and 3 mobile phases was developed. This led to a total of 27 (3 columns x 3 pH x 3 mobile phases) chromatographic conditions. For each column/pH/Organic modifier combination, a 10 run time was there. In addition, it enabled the creation of a database that describes the relationship of the compound retention and possible RP-HPLC conditions.

Chromatographic conditions: The determination was carried out on Waters HPLC 2690 equipped with PDA 996 as detector using data handling system – waters empower 2.0 software. The column used in the development for the determination is Luna Phenyl Hexyl, 250mm x 4.6mm, 5µm. The detection wavelength at 260 nm with a flow rate of 1ml/min was used for the determination of Lamivudine, and tenofovir. The 10µL sample was injected into HPLC system. The corresponding peak and retention times were recorded for each drug. From the chromatogram retention times for Lamivudine, and tenofovir were found to be 2.613, 5.114mins respectively (Table-2).

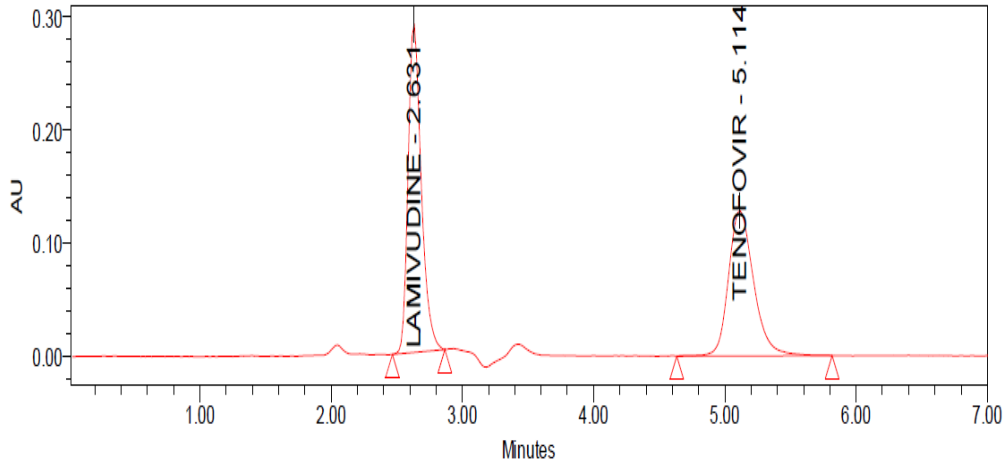


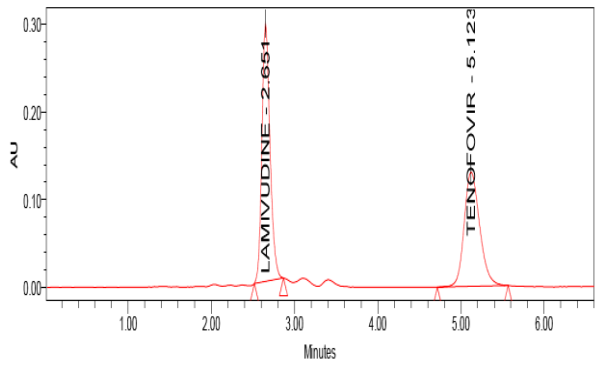
Fig 2: Standard chromatogram of Lamivudine and Tenofovir.

Table.2 Chromatographic conditions for proposed method.

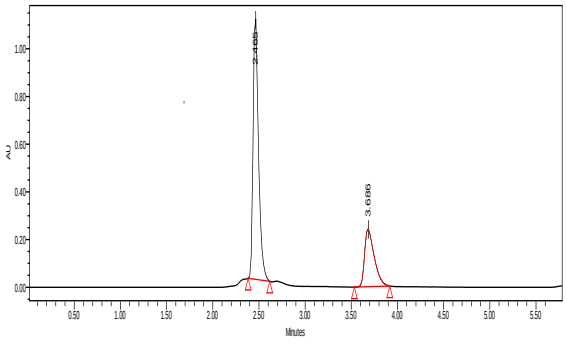
Column	**Luna Phenyl Hexyl, 250mm x 4.6mm, 5µm.**
Wavelength	260 nm
Injection Volume	10µL
Column Temperature	Ambient
Flow rate	1.0ml/min
Retention time of Lamivudine	2.613 min.
Retention time of Lamivudine	5.114 min

Table 3a: Column Variation.

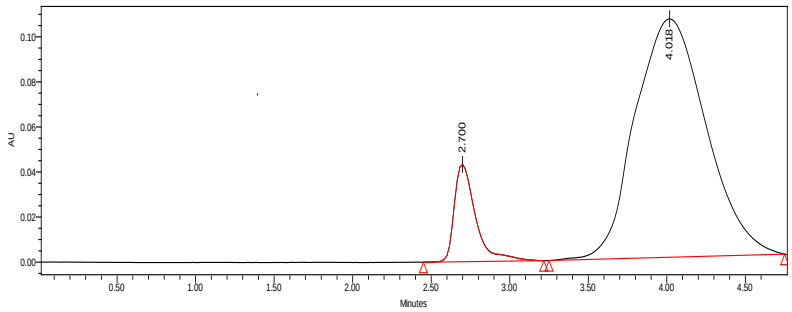
Column variations	**mobile phase**	**Diluent**	**Flow Rate**	**Wave Length**	**Injection Volume`**
Luna Phenyl Hexyl, 250x4.6mm,5µ	0.1% OPA+ACN (60+40)	Mobile Phase	1.00 ml/min	260 nm	10 µl
Luna C18, 250x4.6mm,5µ	0.1% OPA+ACN (60+40)	Mobile Phase	1.00 ml/min	260 nm	10 µl
Luna C8, 250x4.6mm,5µ	0.1% OPA+ACN (60+40)	Mobile Phase	1.00 ml/min	260 nm	10 µl



Luna Phenyl Hexyl, 250x4.6mm,5µ.



Luna C18, 250x4.6mm,5µ.

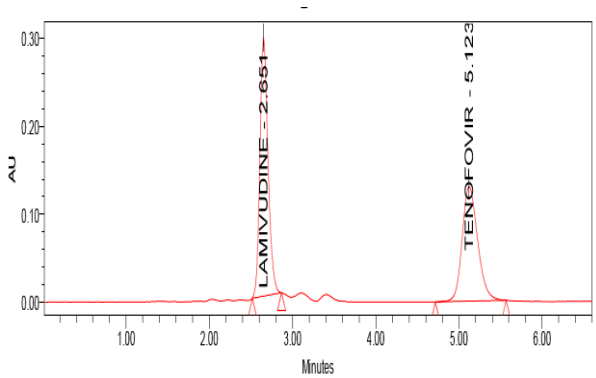


Luna C8, 250x4.6mm,5µ.

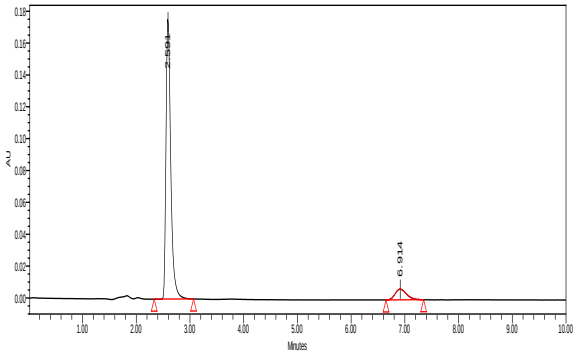
Fig 3b: Chromatogram of Column Variation of Lamivudine and Tenofovir.

Table 2b: P^H Variation.

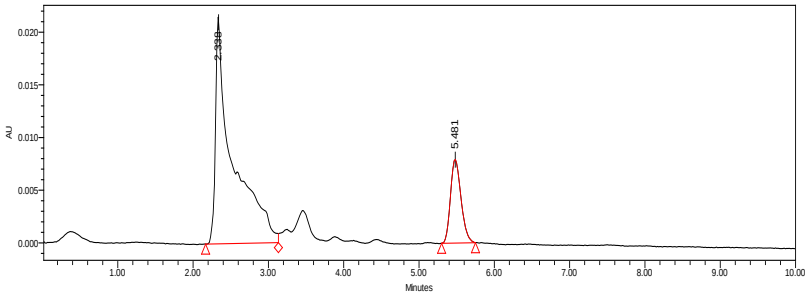
P^H Variation of mobile phase	Column	Diluent	Flow Rate	Wave Length	Injection Volume`
Water pH adjust to 2.3 (0.1% OPA) +ACN (60+40)	Luna Phenyl Hexyl, 250x4.6mm,5µ	Mobile Phase	1.00 ml/min	260 nm	10 µl
Water observed pH-7.0 +ACN (60+40)	Luna Phenyl Hexyl, 250x4.6mm,5µ	Mobile Phase	1.00 ml/min	260 nm	10 µl
Water pH adjust to 8.0 with TEA +ACN (60+40)	Luna Phenyl Hexyl, 250x4.6mm,5µ	Mobile Phase	1.00 ml/min	260 nm	10 µl



Water pH adjust to 2.3 (0.1% OPA) +ACN (60+40)



Water observed pH-7.0 +CAN (60+40)

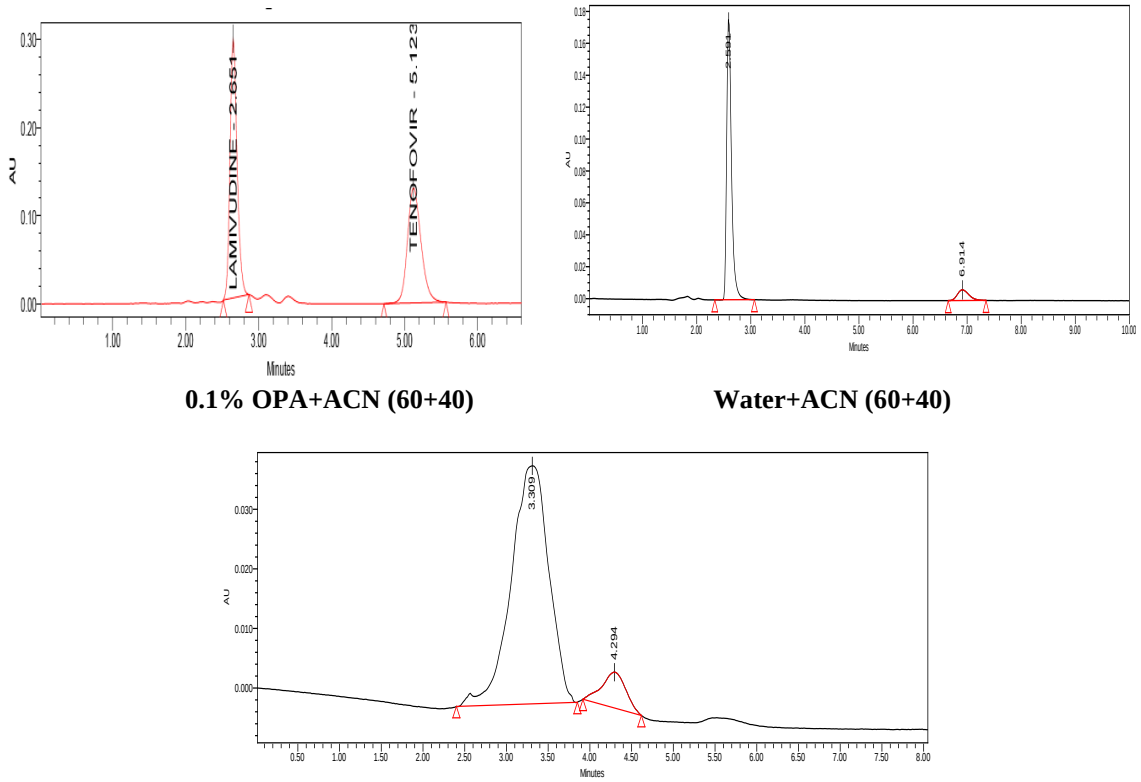


Water pH adjust to 8.0 with TEA +ACN (60+40)

Fig 4: Chromatogram of P^H Variation of Lamivudine and Tenofovir.

Table 3c: Mobile Phase Variation.

Variation of mobile phase	Column	Diluent	Flow Rate	Wave Length	Injection Volume`
0.1% OPA+ACN (60+40)	Luna Phenyl Hexyl, 250x4.6mm,5µ	Mobile Phase	1.00 ml/min	260 nm	10 µl
Water+ACN (60+40)	Luna Phenyl Hexyl, 250x4.6mm,5µ	Mobile Phase	1.00 ml/min	260 nm	10 µl
1ml TEA in 1lt water adjusted pH-2.5 with OPA+ACN (60+40)	Luna Phenyl Hexyl, 250x4.6mm,5µ	Mobile Phase	1.00 ml/min	260 nm	10 µl



0.1% OPA+ACN (60+40) **Water+ACN (60+40)**

1ml TEA in 1lt water adjusted pH-2.5 with OPA+ACN (60+40)

Fig 5: Chromatogram of mobile phase Variation of Lamivudine and Tenofovir.

Step 3

Evaluate experimental results and select final method Conditions

The method conditions were evaluated using the three tiered approach. At the first level, the conditions were evaluated for peaks symmetry, peaks fronting and peaks tailing. This resulted in chromatographic conditions for API. At the second level conditions were further evaluated by using more stringent criteria, such as tailing factor should be less than 1.5, etc.

Step 4:

Perform risk assessment with robustness and rugged ness 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 fourth 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.

A) Robustness

To establish the robustness of test method and to demonstrate its reliability for minor changes in chromatographic condition.

Table 4: Result of Robustness.

Method parameters	conditions	Retention Time		Area	
		Lam	Teno	Lam	Teno
Flow +	1.2	2.178	4.263	1732611	1446243
Flow –	0.8	3.288	6.436	1874004	2361031
Organic+	45:55	2.492	4.560	1555422	1955059
Organic-	35: 65	2.832	6.088	1503461	1856391
Wavelength+	+5nm	2.623	5.131	1815433	8872221
Wavelength-	-5nm	2.623	5.131	4562668	2450366

b) Ruggedness

The ruggedness of analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions such as different laboratories, different instruments, different lots of reagents, different assay, temperatures, different days, different analysts, etc.

Table: 6 Results of Ruggedness.

Parameters (Sample Name Time	Lamivudine		Tenofovir	
	Retention time	Area	Retention time	Area
Intermediate Prec-1	2.613	2047983	5.116	1712186
Intermediate Prec-2	2.613	2079216	5.113	1740695
Intermediate Prec-3	2.613	2072895	5.120	1727871
Intermediate Prec-4	2.614	2061563	5.122	1707694
Intermediate Prec-5	2.611	2092556	5.118	1747577
Intermediate Prec-6	2.623	2091977	5.131	1742789
Mean	2074365		1729802	
Std. Dev.	17473.8		16769.4	
% RSD	0.842		0.969	

Step 5:

Define analytical method performance control strateg y

As a result of robustness and ruggedness studies, the ove rall method understanding of method performance under various conditions can be improved and an analytical me thod performance control strategy along with appropriate system suitability criteria can be defined to manage risk and ensure the method delivers the desirable method attributes. If the risk is high and is hard to manage, it is an opportunity for the analyst to go back to the data Base described in step 2 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.

Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in sample within a given range.

Linearity solutions preparations:

1) Level - 50 %
2) Level - 100%
3) Level - 150%

Table 7: Linearity studies of proposed method.

S.No	Parameters	Lamivudine	Tenofovir
1	Linearity range (µg/ml)	12.50-187.50	12.50-187.50
2	Regression equation	y = 15621x + 17418	y = 13419x - 409.54
3	Slope	15621	13419
4	Intercept	17418	409.54
5	Correlation coefficient (r)	0.999	0.999
6	Accuracy (%Recovery)	100.3	100.2
7	System Precision (RSD)	1.5	0.92
8	Method precision(RSD)	0.25	1.017
9	LOD (µg/ml)	3.12	3.12
10	LOQ (µg/ml)	6.25	6.25

Accuracy: The accuracy of the method for assay determination was achieved at three concentration levels of 50%, 100%, and 150% for lamivudine and tenofovir known amount of standard drug concentration was added to the sample and peak area was determined. The mean percentage recovery values are shown in Table 8.

Table 8: Recovery studies of proposed method.

S. No	% Recovery Level	Amount added		Amount found		% Recovery	
		Lamivudine	Tenofovir	Lamivudine	Tenofovir	Lamivudine	Tenofovir
1	50%	6	6	5.80	6.10	96.6	101
2	100%	12	12	11.65	11.8	97	98.3
3	150%	18	18	17.89	18.03	99.3	100.1

Precision

The precision is measure of either the degree of reproducibility or repeatability of analytical method. It provides an indication of random error. The precision of an analytical method is usually expressed as the standard deviation, Relative standard deviation or coefficient of variance of a series of measurements.

Method precision (Repeatability)

Procedure: Method precision was established by determining six sample preparations under same conditions. Six replicates of sample were prepared at sample concentration by one analyst and analyzed on same day.

Table 9a: Method Precision.

S. No	Injection name	Peak area	
		Lamivudine	Tenofovir
1	Method Precision-1	2087524	1744285
2	Method Precision-2	2060175	1703863
3	Method Precision-3	2063009	1710343
4	Method Precision-4	2057159	1703491
5	Method Precision-5	2014478	1705470
	Mean	2056469	1713490
	SD	26384.84	17429.97
	%RSD	1.283	1.017

Table 9b: System Precision.

S. No	Injection name	Peak area		USP Tailing		USP Plate Count	
		LAM	TNF	LAM	TNF	LAM	TNF
1	sys pres-1	2087524	1744285	1.24	1.28	3313	3392
2	sys pres-2	2060175	1703863	1.19	1.28	3410	3143
3	sys pres-3	2063009	1710343	1.21	1.31	3478	3100
4	sys pres-4	2057159	1703491	1.22	1.27	3199	3465
5	sys pres-5	2014478	1705470	1.22	1.29	3307	3377
6	sys pres-6	2008288	1719336	1.19	1.29	3181	3245
	Mean	2048439	1714465				
	SD	30721.81	15771.44				
	%RSD	1.5	0.92				

LOD and LOQ

The limit of quantification (LOQ) is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions; it is expressed as the concentration of analyte (e.g. Percentage, parts per billion) in the sample. S/N ratio not less than 10.

System suitability

System suitability was performed and calculated at the start of study of each validation parameter. The values of system suitability results obtained during the entire study were recorded.

Table 10: System suitability parameter

Parameters	Values obtained (n=6)	
	Lamivudine	Tenofovir
Plate count	2074343	1729825
Tailing Factor	1.22	1.19
Rerention time (min)	2.613	5.114

Acceptance criteria

- Asymmetry of both the analytes peak in standard should not be more than 1.0.
- Theoretical plates of both the analytes peak in standard should not be less than 2000.
- Relative Standard Deviation for five replicates

injections of both the standard preparation should not be more than 2.0%.

RESULTS AND DISCUSSION

The result is shown in table 7.

Linearity

From the study of concentration range, the linear response for the analyte exist can be established.

Precision

The RSD of six replicate injection of zidovudine is 0.79 % and thus it is within the accepted criteria. (RSD NMT 1.0%).

LOD and LOQ

They are mentioned in table 7.

CONCLUSION

The results obtained were within the acceptance criteria.
System suitability parameters.
The mean values of system suitability parameters are sho wn in table 10.

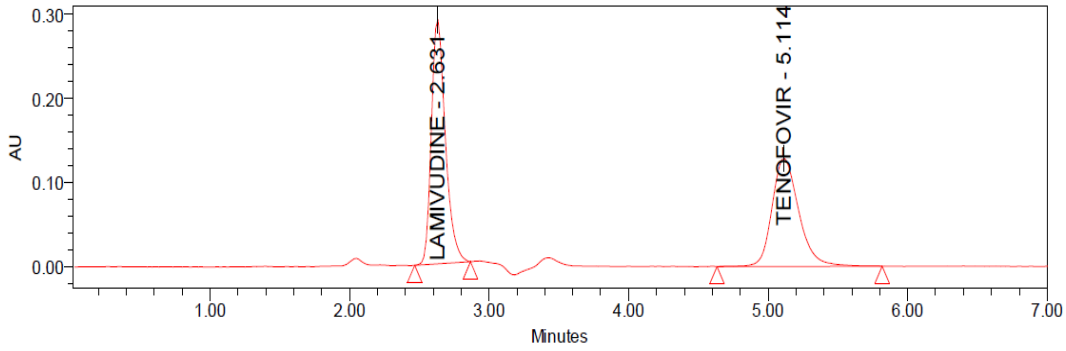


Fig 2: Standard chromatogram of Lamivudine and Tenofovir.

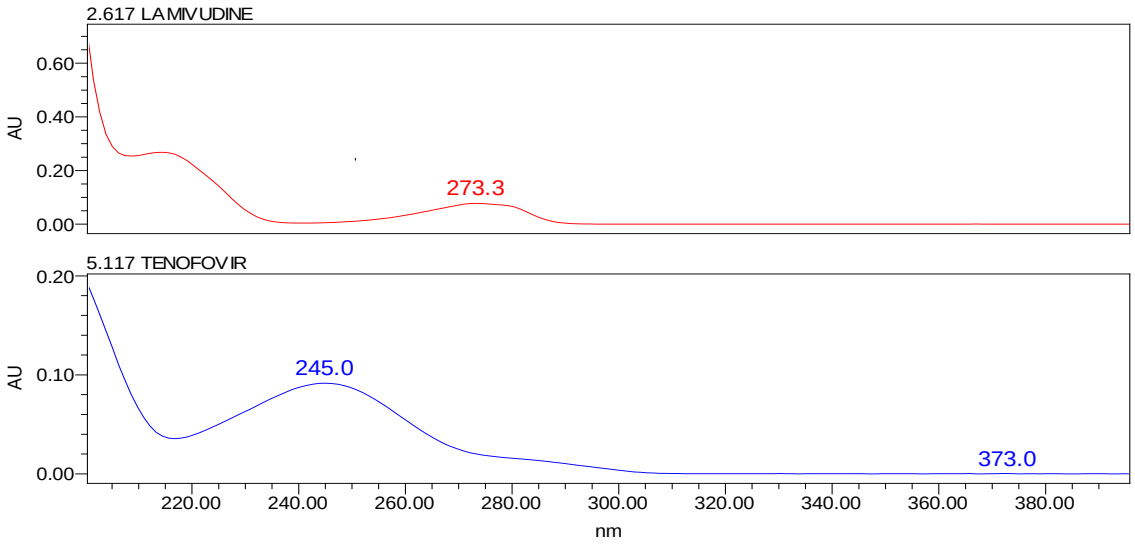
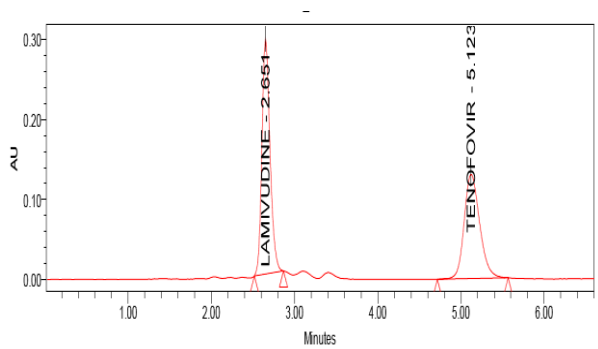
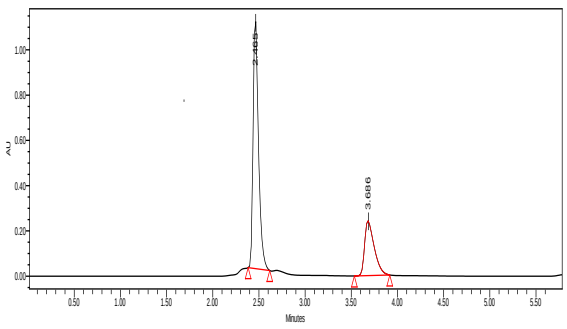


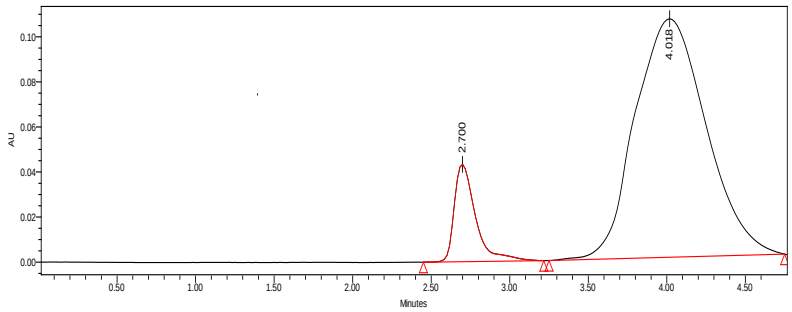
Fig.3 the UV spectrum of lamivudine and tenofovir.



Luna Phenyl Hexyl, 250x4.6mm,5µ



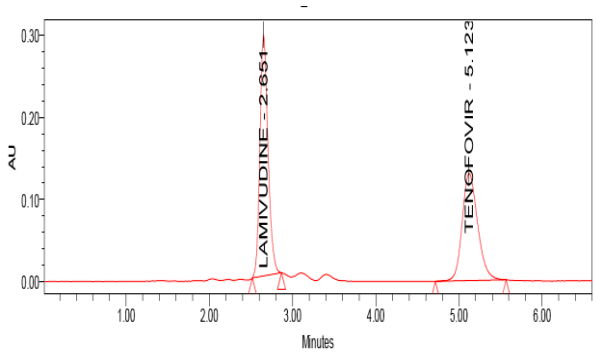
Luna C18, 250x4.6mm,5µ



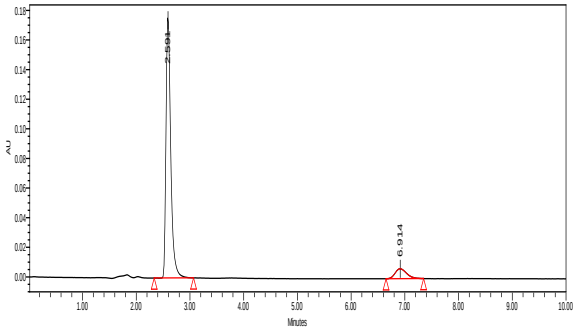
Luna C8, 250x4.6mm,5µ

Table 2b: P^H Variation

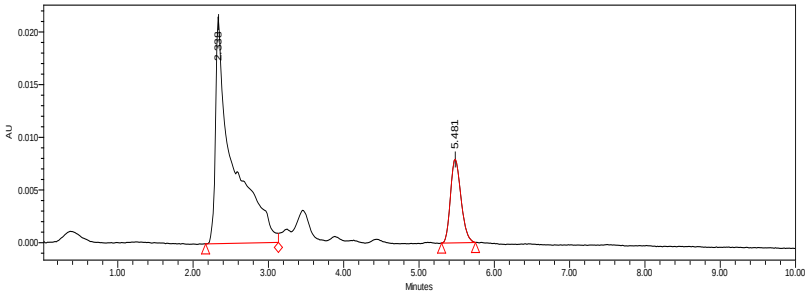
P^H Variation of mobile phase	Column	Diluent	Flow Rate	Wave Length	Injection Volume`
Water pH adjust to 2.3 (0.1% OPA) +ACN (60+40)	Luna Phenyl Hexyl, 250x4.6mm,5µ	Mobile Phase	1.00 ml/min	260 nm	10 µl
Water observed pH-7.0 +ACN (60+40)	Luna Phenyl Hexyl, 250x4.6mm,5µ	Mobile Phase	1.00 ml/min	260 nm	10 µl
Water pH adjust to 8.0 with TEA +ACN (60+40)	Luna Phenyl Hexyl, 250x4.6mm,5µ	Mobile Phase	1.00 ml/min	260 nm	10 µl



Water pH adjust to 2.3 (0.1% OPA) +ACN (60+40)



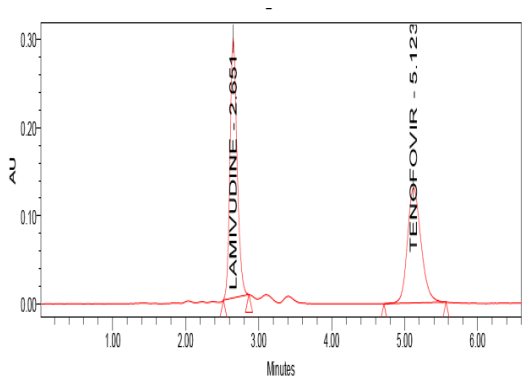
Water observed pH-7.0 +ACN (60+40)



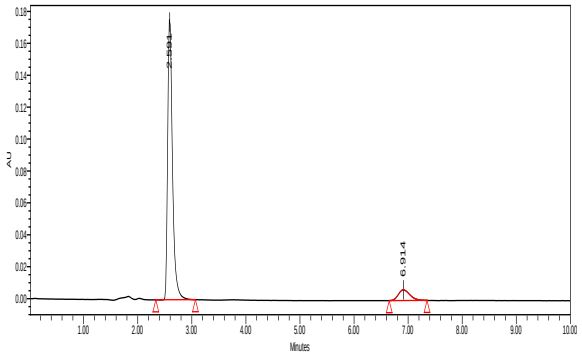
Water pH adjust to 8.0 with TEA +ACN (60+40*)*

Table 2c: Mobile Phase Variation.

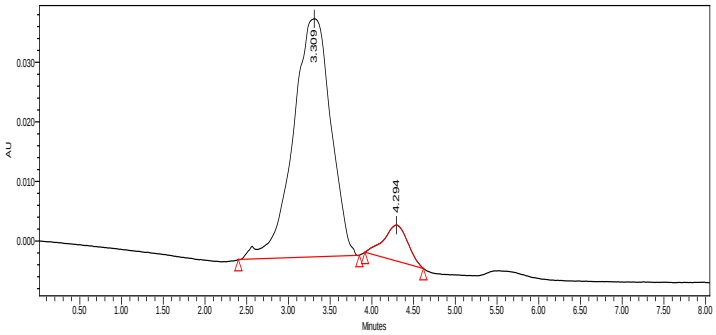
Variation of mobile phase	Column	Diluent	Flow Rate	Wave Length	Injection Volume`
0.1% OPA+ACN (60+40)	Luna Phenyl Hexyl, 250x4.6mm,5µ	Mobile Phase	1.00 ml/min	260 nm	10 µl
Water+ACN (60+40)	Luna Phenyl Hexyl, 250x4.6mm,5µ	Mobile Phase	1.00 ml/min	260 nm	10 µl
1ml TEA in 1lt water adjusted pH-2.5 with OPA+ACN (60+40)	Luna Phenyl Hexyl, 250x4.6mm,5µ	Mobile Phase	1.00 ml/min	260 nm	10 µl



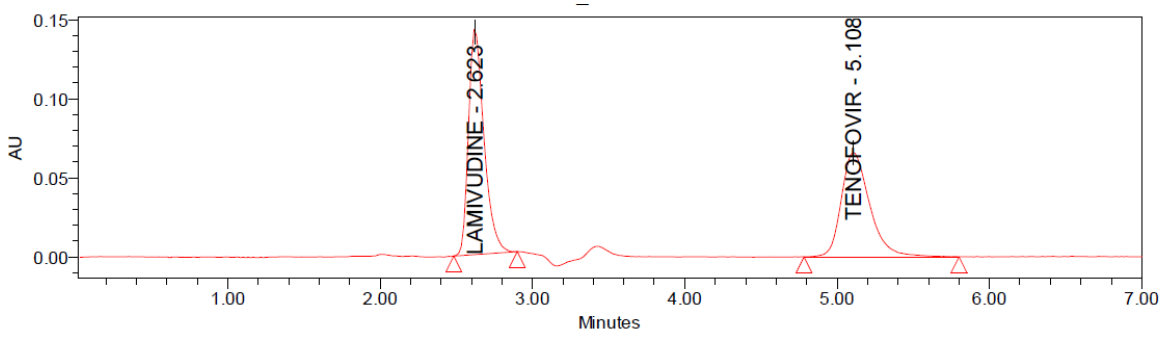
0.1% OPA+ACN (60+40)



Water+ACN (60+40)



1ml TEA in 1lt water adjusted pH-2.5 with OPA+ACN (60+40)



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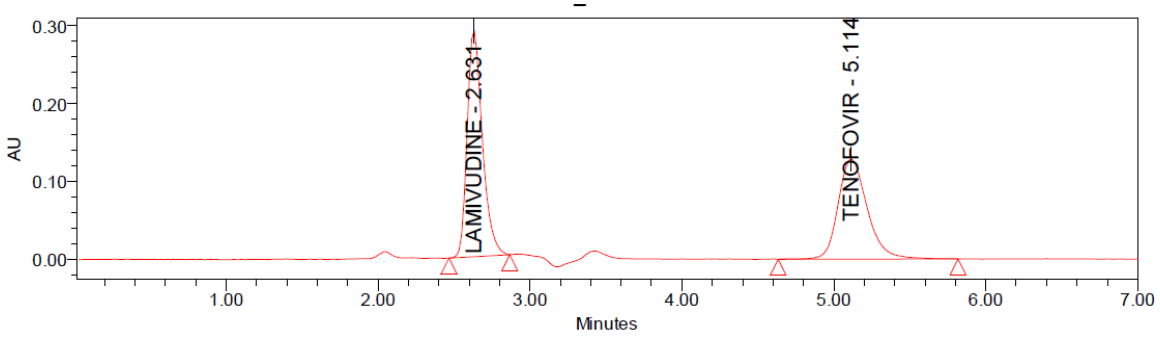

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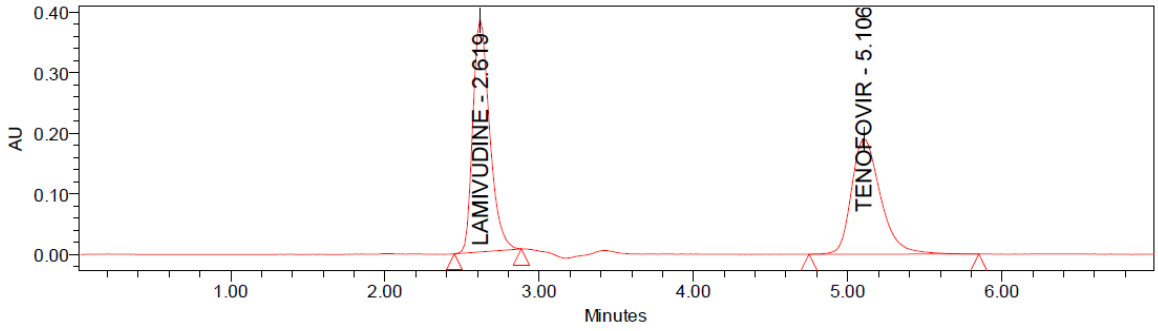

150%

Table 2a: Column Variation.

Column variations	mobile phase	Diluent	Flow Rate	Wave Length	Injection Volume`
Luna Phenyl Hexyl, 250x4.6mm,5µ	0.1% OPA+ACN (60+40)	Mobile Phase	1.00 ml/min	260 nm	10 µl
Luna C18, 250x4.6mm,5µ	0.1% OPA+ACN (60+40)	Mobile Phase	1.00 ml/min	260 nm	10 µl
Luna C8, 250x4.6mm,5µ	0.1% OPA+ACN (60+40)	Mobile Phase	1.00 ml/min	260 nm	10 µl

Table: 3 Linearity studies of proposed method

S.No	Parameters	Lamivudine	Tenofovir
1	Linearity range (µg/ml)	12.50-187.50	12.50-187.50
2	Regression equation	y = 15621x + 17418	y = 13419x - 409.54
3	Slope	15621	13419
4	Intercept	17418	409.54
5	Correlation coefficient (r)	0.999	0.999
6	Accuracy (%Recovery)	100.3	100.2
7	System Precision (RSD)	1.5	0.92
8	Method precision(RSD)	0.25	1.017
9	LOD (µg/ml)	**3.12**	**3.12**
10	LOQ (µg/ml)	**6.25**	**6.25**

Table 4: Result of Robustness.

Method parameters	Conditions	Retension Time		Area		%Recovery	
		Lam	teno	lam	teno	lam	teno
Flow +		2.178	4.263	1732611	1446243		
Flow –		3.288	6.436	1874004	2361031		
Organic+		2.492	4.560	1555422	1955059		
Organic-		2.832	6.088	1503461	1856391		
Wavelength+		2.623	5.131	1815433	8872221		
Wavelength-		2.623	5.131	4562668	2450366		

Table 5: Results of Ruggedness.

Parameters (Sample Name Time	Lamivudine		Tenofovir	
	Retention time	Area	Retention time	Area
Intermediate Prec-1	2.613	2047983	5.116	1712186
Intermediate Prec-2	2.613	2079216	5.113	1740695
Intermediate Prec-3	2.613	2072895	5.120	1727871
Intermediate Prec-4	2.614	2061563	5.122	1707694
Intermediate Prec-5	2.611	2092556	5.118	1747577
Intermediate Prec-6	2.623	2091977	5.131	1742789
Mean	2074365		1729802	
Std. Dev.	17473.8		16769.4	
% RSD	0.842		0.969	

Table 6: System suitability parameter.

Parametrs	Values obtained (n=6)	
	Lamivudine	Tenofovir
Plate count	2074343	1729825
Tailing Factor	1.22	1.19
R_t (min)	2.613	5.114

CONCLUSION

A reversed phase HPLC method development approach using QbD principles has been described. First, the method goals are clarified based on the process understanding. The experimental design describes the scouting of the key HPLC method components including column, pH, and mobile phase. Their interrelationships are studied and the preliminary optimized conditions are obtained for each combination of column, pH and mobile phase. Here a better understanding of the factors influencing chromatographic Separation and greater confidence in the ability of the methods to meet their intended purposes is done. Moreover, this approach provides an in-depth knowledge and enables the creation of a chromatographic database that can be utilized to provide alternative method conditions at a future time should changes to the method be required. Furthermore, the method development is not considered finished until a thorough risk assessment and all the necessary robustness and ruggedness studies are carried out.

All the validated parameters were found within acceptance criteria. The validated method is specific, linear, precise, accurate, robust and rugged for determination based on the knowledge of method obtained through the method development and the results of risk assessment along with robustness and ruggedness studies, detailed analytical method performance control strategy can be defined to manage the risk. This approach has been successfully used in the laboratory to develop HPLC method for Lamivudine and tenofovir disposil fumarate.

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