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Research

Design of Experimental approach for the quantification of Bictegravir, content by dissolution method in Tablets formulation using RP-HPLC method

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Abstract

In the current study the effort has been undertaken to improve most simple, economical, and sensitive and correct analytical HPLC method for the immediate valuation of these drugs without their prior separation. The method gives resolution with a short analysis time (< 25 min). The method parameter was validated and establishes to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. All the three ingredients were effectively resolved and quantified. The developed method reported herein was validated by parameters agreeing to ICH-Q2B guidelines. The offered method provided good system suitability, resolution, precision, accuracy, LOD and LOQ, linearity and robustness and requires less expensive reagents. The procedure designated now is simple, rapid, sensitive, selective and cost effective. It is apparent from the results that the suggested procedure is well suited for dissolution and assay and evaluation of drug, in dosage forms.

Keywords: Bictegravir, Dissolution, formulation, RL HPLC method

INTRODUCTION

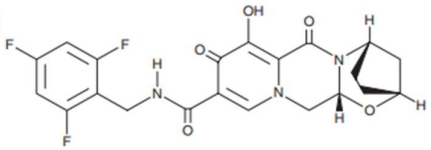
The present scope is to Design of Experimental approach for the quantification of Bictegravir, content by dissolution method in Tablets formulation using RL HPLC method Development and Validation of HPLC method for the estimation of dissolution Bictegravir, Emtricitabine and Tenofovir Alfenamide in Bictegravir, Emtricitabine and Tenofovir Alfenamide Tablets USP 50mg/200mg/25 mg The proposed method shall be used for the quantification of active material Bictegravir, Emtricitabine and Tenofovir Alfenamide. The proposed method shall be validated for Specificity, System suitability, Linearity, Accuracy, Range, Precision, and Repeatability and Robustness as per ICH guideline.
Reason for validation: Non-Pharmacopeial method.

Bictegravir/Emtricitabine/Tenofovir Alfenamide (B/FTC/TAF, BIKTARVY) is an oral tablet regimen (STR) used for the treatment of HIV type 1 (HIV-1) infection in adults with no known substitution associated with

resistance to the individual components of B/FTC/TAF.Study of literature has exposed many analytical techniques for method development and validation for immediate estimation of Tenofovir and Emtricitabine a grouping drug [6-9]. On the opposing, to the best of our insight there is no method revealing the concurrent approximation of BTG, ETC, TFRA in medicinal formulations.. The new method is effective in separating all three active ingredients. Validation of the method is performed according to ICH guidelines.

Bictegravir
Item No. 26532

CAS Registry No.:	1611493-60-7
Formal Name:	(2R,5S,13aR)-2,3,4,5,7,9,13,13a-octahydro-8-hydroxy-7,9-dioxo-N-[(2,4,6-trifluorophenyl)methyl]-2,5-methanopyrido[1',2':4,5]pyrazino[2,1-b][1,3]oxazepine-10-carboxamide
Synonym:	GS-9883
MF:	$C_{21}H_{18}F_3N_3O_5$
FW:	449.4
Purity:	≥98%
UV/Vis.:	λ_{max}: 248, 260, 322 nm
Supplied as:	A crystalline solid
Storage:	-20°C
Stability:	≥4 years



Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.

MATERIALS AND METHODS

Water, Formic acid, Methanol, Trisofium, citrate, Bictegravir, Emtricitabine Alfenamide Placebo Citric acid anhydrous HPLC Glassware Analytical Balance pH Meter Column Detector 0.45 nylon membrane filter Dissolution Apparatus.Design of Experiment –DOE by different trails by Reverse Phase -HPLC Method.

Selection of Chromatographic System:

Degradation studies were carried out on a system consisted of 1200 series HPLC (Agilent Technologies) comprising of an on-line degasser (G1222A), binary pump (G1212A), auto injector (G1267C), column oven (G1210B), DAD detector (G1215C) and Empower (software).

The published methods of analysis for determination and separation of Bictegravir, Emtricitabine and Tenofovir Alfenamide in their formulation were not evaluated for specificity and degradation study. Therefore, method having specificity for degradation products and formulation excipients is considered as a prime requirement. Degraded samples, prepared by systematic forced degradation study, were used for method development trials to optimize the method as a stability indicating method for determination of Bictegravir, Emtricitabine and Tenofovir Alfenamide.

Selection of Buffer in Mobile Phase:

Dilute citric acid was used to optimize the peak shape retention time and to proper separation of impurities peaks from main drugs peaks. The ratio of (Buffer: Methanol) was selected on the basis of resolution between the major degradation peaks and main peaks, and it was finalized as (45:55) %v/v after analyzing all the degraded samples and evaluating the peak purity, resolution, specificity and stability indicating nature of the method.

Selection of Mobile Phase:

Different ratios of Acetonitrile and Buffer were used to optimize the retention time from main drugs peaks. The ratio of (Buffer: Methanol) was selected on the basis of resolution between the major degradation peaks and main peaks, and it was finalized as (Buffer: Methanol) (45:55) %v/v after analyzing all the degraded samples and evaluating the peak purity, resolution, specificity and stability indicating nature of the method.

Selection of HPLC Column:

For HPLC, various columns are available, but as the main aim of the method to resolve the compound in the presence of polar and non-polar degradation products and impurities, a C_{18} column was preferred over other columns Zorbax SB pheny , 4.6 X250 mm, 5µm or equivalent column was chosen to give good peak shape, good lifetime and high resolution on compared to other C_{18} columns.

Selection of Diluent / Solvent for extraction

Different solvents were tried including single solvent and combination of solvents like ACN: Water, Buffer in different concentrations, But Bictegravir, Emtricitabine and Tenofovir Alfenamide. Tablet gets dissolved in methanol. Hence first stock was prepared in methanol and followed by second dilution done in diluents as (Buffer: Methanol) (45: 55) %v/v same as that of mobile phase to reduce the peak shape related problems. The results of all validation parameters are given in following tables and all lie well within the limit of acceptance criteria.

Equipment - Instrument –Glassware-Standard –Solvent-Chemicals Requirement:

Equipment and Instrument: All equipment and instrument used during method validation shall be qualify, validate and within calibration and preventive maintenances validity.

Glassware: All Class A types glassware shall be used. Before used it shall be cleaned and dried as per validated and approved procedure.

METHODOLOGY

Chemical and Reagent: Water, Bictegravir, Methanol, Trisofium citrate, Citric acid anhydrous Formic acid.

Instruments, Equipment's and Apparatus: HPLC, Dissolution Apparatus, Analytical Balance
pH Meter, Column, Detector, 0.45 nylon membrane filter, Glassware

Preparation of Citric acid solution: Dilute 10 gm of Citric acid to 100 ml with water and mix.

Preparation of Buffer solution: Preparation of pH buffer 5.5: Weight and transfer about 20.16gm of citric acid and 10.180 gm of tri sodium citrate dehydrate into 10 liter of water and sonicate and dissolved. If necessary, adjust the pH of the solution to 5.5± 0.05 with citric acid .

Preparation of pH buffer 3.0: Accurately transfer 2ml of triethlylamine into 1000ml of water and mix well. Adjust pH of the solution to 3.0 with formic acid solution. Filter the solution through 0.45 µm membrane filter paper

Preparation of mobile phase: Thoroughly mix pH buffer 3.0 and methanol solution in the ratio of 45: 55 % v/v

Dissolution

Medium	:	**pH buffer 5.5**
Volume	:	900ml
Apparatus	:	USP type ll
Speed	:	75 rpm
Temperature	:	37±0.5°C
Sampling point	:	30 minutes

Chromatographic conditions

Column : Zorbax SB pheny 4.6 X250 mm, 5µm or Equivalent
Wavelength: UV 260 nm, Flow rate Injection: 1.0ml/minute
Volume : 10µL Column oven Temperature: 30°C
Run time : 20 minute

Preparation of the standard solution : Accurately weigh and transfer about 58 mg of Bictegravir standard 220mg of Emtricitabine standard and 31mg of Tenofovir Alfenamide standard into a 100 ml volumetric flask add about 80ml of methanol and sonicate to dissolve and dilute to volume with diluent. Transfer 5.0 ml of this solution to 100 ml volumetric flask and dilute the volume with dissolution media and mix.

Preparation of Test Sample solution: Set the parameters of dissolution apparatus as mentioned above. Place one tablet into each of the dissolution jar.At the end of the specified time point withdraw 10ml of the sample solution through 10 µm full flow filters from each dissolution vessel .Filter the solution through 0.45 membrane filter by discarding first 5ml of filtrate

Procedure:

- Equilibrate the column for not less than 30 minute with mobile phase at flow rate of 1.0ml/minute.
- Inject 20 µL of blank solution into the Chromatographic system, record the Chromatogram.

- Program the data processor to inhibit the integration of peaks due to blank.
- Inject 20 µL of Reference solution into the Chromatographic system, record the Chromatogram and measure the peak response.
- Inject 20 µL of test sample ample solution into the Chromatographic system, record the Chromatogram and measure the peak response.
- Inject 20 µL of Reference solution into the Chromatographic system, record the Chromatogram and measure the peak response. (Bracketing standard).
- Inject Bracketing standard after every 4 samples analysis and/or at the end of the sequence.

Specificity and system suitability

- **Specificity** is the ability of the analytical method to distinguish between the analyte(s) and the other components in the sample matrix [13]. In case of an HPLC method, it is assured by complete separation of peak(s) of analyte(s) from other peaks originated from the sample matrix
- **The System Suitability Testing (SST)** is used to verify that an analytical method was suitable for its intended purpose the day the analysis was done. It is an essential parameter to ensure the quality of the method for correct measurements.

Sr.No	Validation Parameter	Results	Acceptance Criteria
Method and Procedure			
1.	**Identification**	Prepared standard and sample solution as per the test method and inject into the chromatographic system	
2.	**Blank and Placebo Interference**	Prepared blank and placebo solution as per the test method and inject into the chromatographic system	
3.	**System Suitability**	Prepared standard as per the test method and inject five times into the chromatographic system	
4.	**System Suitability Acceptance Criteria**		
5.	**Identification and RT Confirmation** • The retention time of standard solution and sample solution should be comparable with respect to retention time • The retention time of analyte peak obtained from sample solution should be within ±0.5 minutes of the retention time of analytic peak obtained from the standard solution **Blank and Placebo Interference :** There should not be any interfering peak in the chromatogram obtained from blank solution and placebo solution at the retention time of analyte peak in the chromatogram obtained with the standard **System Suitability** - The column efficiency as determined for the Bictegravir, Emtricitabine and Tenofovir Alfenamide from standard solution ia not less than 2000 theoretical plates. - Tailing factor for the same peak is not more than 2 - The relative standard deviation for Bictegravir, Emtricitabine and area obtained from five replicate injections of standard solution is not more Tenofovir Alfenamide .		
Observed Value			
6.	Identification RT Confirm-tion	Name / Retention time in minute: Individual / Standard / Sample Bictegravir: 16.125 / 16.295 / 16.218 Emtricitabine: 2.515 / 2.43 / 2.644 Tenofovir Alfenamide: 9.281 / 9.284 / 9.235	The retention time of standard solution and sample solution should be comparable with respect to retention time
Conclusion: RT of Bictegravir, Emtricitabine and Tenofovir Alfenamide with standard and test sample comparable. Hence method is specific			
7.	**Blank and Placebo Interference**	There are no interference peak observed due to place to at the retention time of Bictegravir, Emtricitabine and Tenofovir Alfenamide peak. Hence method is specific.	There should not be any interfering peak in the chromatogram obtained from blank solution and placebo solution at the retention time of analyte peak in the chromatogram obtained with the standard
8.	System Suitability	Component / Theoretical plate Bictegravir / 12365	The column efficiency as determined for the

Sr.No	Validation Parameter	Results		Acceptance Criteria
		Emtricitabine	6538	Bictegravir, Emtricitabine and Tenofovir Alfenamide from standard solution ia not less than 2000 theoretical plates
		Tenofovir Alfenamide	45635	
		Component	Tailing Factor	Tailing factor for the same peak is not more than 2.
		Bictegravir	1.03	
		Emtricitabine	1.04	
		Tenofovir Alfenamide	1.03	
		Component	**% RSD**	The relative standard deviation for Bictegravir, Emtricitabine and Tenofovir Alfenamide peak area obtained from five replicate injections of standard solution is not more 2.0
		Bictegravir	0.27	
		Emtricitabine	0.16	
		Tenofovir Alfenamide	0.40	

Linearity

Linearity of a method is its ability to obtain test results that are directly proportional to the sample concentration over a given range. For HPLC methods, the linear relationship between detector response (peak area and height) and sample concentration is determined. The relationship can be demonstrated directly on drug substance by dilution of standard stock or by separate weighing of the sample components, using the proposed procedures.

Linerity level	Concentraion in ppm	Y –Practical responses i.e mean peak areas are obtaines	Therotical repponse	Residaul	Residual Squares
Level-1	5.509	126785	126255	604	384591
Level-2	16.524	385462	126542	23	458
Level-3	23.045	512622	514469	-1785	345102
Level-4	27.542	644561	653860	614	376885
Level5	33.052	765321	773247	608	368456
		Reqsidual sum of square			4589632

- Trend line equation,: Y=MX + (C)Y= ResponseX= Concentration, M(Slope)=37215.08C (Y-Intercept) = -743.08Response of 1000% Concentration = 207057, 5 % of 100% Concentration= 10353

Precision

Precision of an analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Sr.No	Validation Parameter	Results	Acceptance Criteria
A. System Suitability : Method and Procedure			
1.	**System Suitabil**	Prepared standard solution as per the test methods and inject six times into the chromatographic system	
	Acceptance crit	• The column efficiency as determined for the Bictegravir e from standard solution ia not less than 3200 theoretical plates. • Tailing factor for the same peak is not more than 2.0 • The relative standard deviation for Bictegravir peak area obtained from five replicate injections of standard solution is not more 2.0	
2. Observed Values			
System Precision		Theoretical Plates 5794	The column efficiency as determined for the Bictegravir from standard solution is not less than 2000 theoretical plates.

Sr.No	Validation Parameter	Results		Acceptance Criteria
		Tailing Factors	1.05	Tailing factor for the same peak is not more than 2.0
		% RSD	0.32	The % RSD of % assay from Five samples should be more than 2.0

3. **Results :**

System Suitability and System Precision	Sr.No	Peak Area	Theoretical fa	Tailing Factor
	1	652989	5494	1.04
	2	605380	5583	1.06
	3	653175	5475	1.05
	4	652694	5475	1.04
	5	651622	5647	1.05
	6	651722	5826	1.04
	Mean	652438		
	SD	635.48		
	% RSD	0.31		

Observed Results:

- The observed theoretical plates obtained for the Bictegravi from standard solution is more than 3 theoretical plates.
- The Observed Tailing factor obtained for the Bictegravi peak from the standard solution is less than 2.0
- The % RSD of the peak area of Bictegravi obtained from five replica injections of the standard solutio

Conclusion :

The above data shows that the system is precise.

B. **Method Precision : Method and Procedure**

1.	**Methods Precis**	Prepared six sample solution of Bictegravi as per the test methods and inject into the chromatographic system
	Acceptance crit	The % RSD of % assay from six samples should be more than 2.0

2. Observed Value

Method Precision			
	% RSD	1.77	The % RSD of % assay from six samples should be more than 2.0

3. **Results:**

S.No	Dissolution % labeled amount
Injection-1	100.3
Injection-2	100.2
Injection-3	101.5
Injection-4	100.4
Injection-5	100.3
Injection-6	101.5
Mean	**100.98**
SD	**0.55**
% RSD	**0.60**
95% confidence interval of mean	**100.3 to 101.4**

Conclusion :

The above results show that the methods is precise

Accuracy

The accuracy of an analytical method expresses the closeness of agreement between the value accepted either as a conventional true value or an accepted reference value and the value obtained.

Sr.No	Validation Parameter	Results	Acceptance Criteria
Method and Procedure			
1.	Accuracy was performed by spiking the Bictegravi drugs substance to the placebo at 20% to 120 % of target concentration of Bictegravi in triplicate at each level and analyzed as per the test method		
	Acceptance Criteria	The % recovery of accuracy levels should be not less than 95.0 and not more than 105% Report the 95 % confidence interval of mean	
2. Observed Values			

Sr.No	Validation Parameter	Results	Acceptance Criteria
Accuracy		Mean recovery % 99.5	The % recovery of accuracy levels should be not less than 95.0 and not more than 105.0

3. Results

Accuracy level		Amount added in mg	Amount found in mg	% Recovery	Statistical Analysis		
					Mean	SD	%RSD
Level 1	Sample -1	1.005	0.999	99.8	99.3	0.13	0.13
	Sample -2	1.005	1.002	99.4			
	Sample -3	1.005	0.998	99.2			
Level 2	Sample -1	2.503	2.488	99.3	98.6	0.25	0.24
	Sample -2	2.503	2.468	98.5			
	Sample -3	2.505	2.468	98.5			
Level 3	Sample -1	5.013	4.954	98.6	98.7	0.15	`0.15
	Sample -2	5.013	4.941	98.5			
	Sample -3	5.013	4.949	98.6			
Level 4	Sample -1	6.012	6.013	99.4	99.5	0.13	0.16
	Sample -2	6..014	5.996	99.1			
	Sample -3	6.012	6.006	10.02			
Overall Statistical Analysis							
Mean		99.8	**SD**	0.53	**% RSD**	0.52	

Conclusion : The Form the above results , it is concluded that the test method is accurate from 20 % to 120% of test stock concentration

Range:

Range of an analytical method is the interval between the upper and lower concentration of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity. The range is normally derived from the linearity studies and depends on the intended application of the procedure

Sr.No	Validation Param	Results	Acceptance Criter
Method and Procedure			
1.	Range of analytical method can be obtained from linearity, Precision and accuracy data. Report range in % with respect to sample concentration.		
Observed Values			
2.	Range	The analytical method is linear , Precise and accurate from to 120% of target concentration	----

Conclusion :

It was concluded from the linearity, Precision and accuracy data that the analytical method is is linear , Precise and accurate from 20% to 120% of target concentration

Solution Stability

Stability of the analytical solution and extraction time are other parameters which are also evaluated as additional parameters during robustness study. Stability of analytical solution is determined by assessing the results obtained by subjecting the analytical solution to the method parameters for longer period of time e.g. 4 hrs, 24 hrs, 48 hrs etc.

Sr.No	Validation Parame	Results	Acceptance Criteria
Method and Procedure			
1.	Standard Solution and Sample Solution was prepared as per test methods and stored at refrigerator condition. Solution Stability was evaluated at initial , , 24 hours and 48 hours.		
A.	**Acceptance Criteri**	- The overall % RSA from initial replicate standard peaks and bracketing standards peak should be more than 2.0. - The % assay difference from initial and corresponding time intervals should be more than 2	
2. Observed Values			

Sr.No	Validation Parame	Results		Acceptance Criteria
	Standard Solution	Standard Solution is stable up to 48 h at refrigerator condition		The 5 assay difference from initial and at regular interval more than 2.0
	Sample Solution	Sample solution is table up to 48 hours at refrigerator condition		The % dissolution difference from initial and corresponding time intervals should be more than 2.0
3.	**Results :**			
	Standard Solution :: Over % RSD	Time Interval	% RSD	Difference
		Initial	100.6	---
		24 hours	100.5	0.2
		48 Hours	99.5	0.9
	Sample Solution :: Over % RSD	**Time Interval**	**% Dissolution**	**Difference of %Dissolution**
		Initial	102.5	--
		24 hours	101.2	1.2
		48 Hours	101.02	1.2
Conclusion : From the above results it is concluded that standard and sample solutions are stable up to 48 Hrs at Refrigerator				

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is partially evaluated during method development stages.

Sr.No	Validation Parame	Results		Acceptance Criteria
Method and Procedure				
Change in Dissolution condition: Prepare six sample solutions by varying the dissolution conditions and determine the % dissolution of samples				
Acceptance Criteria : • Dissolution results should be within the specified acceptance criteria as per the test method • The difference of Mean % dissolution from method precision study and Robustness study should not be more than 5.0.				
Samples Change in the dissolution parameters	Parameter	The difference of mean % dissolution		criteria
	Change in volume of dissolution medium 900ml	882ml	3.8	The difference of mean % dissolution between method precision study and Robustness study should not be more than 5.0%
		918ml	1.8	
	Change in rotation speed of dissolution apparatus 50 RPM	45 RPM	1.6	
		55 RPM	2.2	
	Change in temperature of dissolution 37.0ºC	35.0ºC	1.3	
		39.0ºC	0.5	
	Change in Sampling time 30 minute	28 minutes	1.7	
		32 minutes	0.3	
Conclusion: From the above data it concluded that the method is robust respect to flow rate variation, column oven temperature variation, variation of in pH of buffer, variation of mobile phase combination, variation in the speed of dissolution, variation in temperature in temperature and variation of the sampling time				
Filter variability				

Sr.No	Validation Parame	Results	Acceptance Criteria
1.	**Filter variability**	Prepared three samples solutions as per the test method. One portion of the solution was centrifuged and the other portion of sample solution was filtered through two types of filters Nylon and PVDF and calculated the difference of % assay	
	Acceptance Criteria	The results compared to unfiltered versus filter samples, the difference of % dissolution from unfiltered to the filter samples should be more than 5.0	
2. Observed Values			
Filter variability		Maximum difference 0..2 (Centrifuge Vs Nylon)	The difference of % dissolution compared from centrifuge to the filtered samples should not be more than 2.0
		Maximum difference 0.8 (Centrifuge Vs PVDF)	

3. Results

Sr.No	% Assay			Difference	
	Unfiltered	PVDF	Nylon	Unfiltered Vs PVDF	Unfiltered Vs Nylon
1	102.8	102.0	100.5	0.8	0.3
2	100.8	100.2	100.5	0.6	0.2
3	100.8	100.2	100.5	0.6	0.3

Conclusion :

The Maximum difference Unfiltered Vs Nylon and PVDF membrane filter is 0.7 and 2.2 .Hence it is concluded that both Nylon and PVDF filters are suitable for the filtration of the sample solutions.

CONCLUSION

In the current study the effort has been undertaken to improve most simple, economical, sensitive and correct analytical HPLC method for the immediate valuation of these drugs without their prior separation. The method gives resolution with a short analysis time (< 10 min). The method parameter was validated and establishes to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. All the three ingredients were effectively resolved and quantified. The developed method reported herein was validated by parameters agreeing to ICH-Q2B guidelines. The offered method provided good system suitability, resolution, precision, accuracy, LOD and LOQ, linearity and robustness and requires less expensive reagents. The procedure designated now is simple, rapid, sensitive, selective and cost effective. It is apparent from the results that the suggested procedure is well suited for dissolution and assay and evaluation of drug, in dosage forms.

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