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A NEW RP-HPLC METHOD FOR THE DETERMINATION OF TEZACAFTOR AND IVACAFTOR IN BULK FORM AND MARKETED PHARMACEUTICAL DOSAGE FORM

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Abstract:

A rapid, precise, accurate, specific and simple RP-HPLC method was developed for the simultaneous estimation of Tezacaftor and Ivacaftor in bulk and its combined pharmaceutical dosage form. A High-performance liquid chromatography Waters, software: Empower 2, 2695 separation module, 996 PDA detector, using Symmetry C18 (4.6×150mm, 5µ) column, with mobile phase composition of methanol: TEA buffer in proportion 40:60 v/v was used. The flow rate of 1.0 mL min^{-1} and effluent was detected at 260 nm. The retention time of Ivacaftor and Tezacaftor was found to be 2.781 minutes and 4.048 minutes respectively. Linearity was observed over concentration range of 5-25µg ml^{-1} for Ivacaftor and 25-125µg ml^{-1} for Tezacaftor respectively. The accuracy of the proposed method was determined by recovery studies and the Ivacaftor was found to be 99.8% and Tezacaftor was found to be 99.4% respectively. The proposed method is applicable to routine analysis of Ivacaftor and Tezacaftor in bulk and pharmaceutical formulations. The proposed method was validated for various ICH parameters like linearity, limit of detection, limits of quantification, accuracy, precision, range and specificity.

Key Words: *Ivacaftor, Tezacaftor, RP-HPLC, Robustness and ICH Guidelines.*

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INTRODUCTION:

Strategy of method development:

Method development ought to be supported many issues. It's desirable to possess most sample data to form development quick and desired for meant analytical technique application, physical and chemical properties area unit most desirable as primary data. Moreover, separation goal has to outline at starting so; acceptable technique is developed for the aim. AN LC technique development is extremely vast space for even prescribed drugs with restrictive demand of international standards. So, before technique validation and usage at internal control several aspects have to be compelled to focus as per ICH tips. Method development is supported a sample and goals moreover as offered resources for action however few basic steps for technique development area unit is mentioned as given below.

Steps in technique development

1. Sample data ,define separation goals
2. Sample pre-treatment, want of special HPLC procedure
3. choice of detector and detector settings
4. choice of LC method; preliminary run; estimate best separation conditions
5. Optimize separation conditions
6. Check for issues or demand for special procedure
7. technique validation

Sample information:

1. variety of compounds gift
2. Chemical structure of compounds
3. Chemical nature
4. relative molecular mass of compounds
5. pKa Value(s) of compounds
6. Sample solubility
7. Sample stability and storage
8. Concentration vary of compounds in sample
9. Ultraviolet illumination spectra of compounds or properties for detection of compounds

RP-HPLC continues to be comparatively new technique, and literature isn't invariably offered on operative conditions for a selected application. The primary step in developing AN RP-HPLC analysis, or the other variety of natural process analysis, is to outline the matter and state the aim of study. So as to outline the matter, the subsequent question ought to be asked:

1. Is that the analysis aiming to be used habitually for an oversized variety of samples? Is case of operation and ease of nice importance?
2. May be a qualitative and / or qualitative analysis required?
3. Is it necessary to separate all the constituents within the sample or solely a tiny low cluster of constituents?
4. Area unit the constituents similar in structure or wide diverse?
5. Area unit the constituents gift in similar concentrations, or is one constituent presenting an oversized quantity and alternative solely in trace amounts?
6. Will sample be simply ready for RP-HPLC analysis?
7. Area unit there compounds gift that will interfere with the analysis of constituents of interest?
8. Will peaks within the recording be promptly identified?

The next step may be a literature search to find-out if these compounds are separated mistreatment alternative natural process techniques.

For example: The conditions utilized in thin-layer action (TLC) or open chromatography usually are adopted for HPLC; this is a place to begin and saves a valuable time.

A total RP-HPLC technique involves the subsequent steps:

1. Sample assortment
2. Sample preparation
3. Chromatography
4. Peak identification
5. Quantification
6. Information analysis and interpretation of results (Validation)

Sample assortment:

The primary step within the analysis of biological and a few alternative samples sometimes needs sample filtration. Since RP-HPLC columns use 3-5 um packing materials, the column water is sometimes protected with a 2um frit or screen. Sample filtration is performed mistreatment membrane kind filters with zero.2 - 0.5 um pore sizes.

Several ways of macromolecule removal is used: immoderate filtration, precipitation of proteins with robust acid or organic solvents, ammonia sulphate precipitation, denaturation by heat etc.

Sample preparation:

Usually within the analysis of complicated samples, solely variety of compounds area unit of interest. Therefore, it's not necessary to realize separation of all sample constituents, however rather to optimize the conditions for speedy analysis of many elite compounds. In these cases, it's advantageous to polarity and solubility of the solutes.

Most typically used extraction procedures area unit solid-phase extraction, solid-phase small extraction, liquid-liquid extraction, liquid-phase small extraction, membrane based mostly extraction and critical fluid extraction.

Just in case of pure samples or bulk samples, merely dissolve within the mobile part consistent with their solubility and polarity.

Chromatography:
The bulk of study will currently be carried mistreatment RP-HPLC; so RP-HPLC is that the technique of selection unless the required separations can't be achieved, or unless another mode, like gel permeation, is clearly indicated. at the present industrial convenience of RP-HPLC column over ninetieth of all RP-HPLC separation is being dispensed mistreatment C18 as a bond part on 3-5 um oxide particles. In RP-HPLC, or in any separation, there area unit several parameters that may influence each resolution of compounds in mixture and also the potency of separation.

When the mobile part and stationary part area unit chosen, the optimum flows ridge and extraction mode should then be determined.

Stationary Phase:
The four necessary parameters concerned within the stationary part that may be varied in RP-HPLC separation area unit as follows:
1. Column length & internal diameter
2. Partical size
3. Variety of guaranteed phases
4. Surface coverage

Solely when exhausting the various potentialities of mobile part or mobile part mixtures ought to differ sorts of columns tried. However, in cases wherever stationary separations can't be obtained with these columns, stationary part parameters are modified individually or together. For example: Shorter columns (3-5 µm) gave higher resolution and shorter retention times for the determination of some compounds in humor. So in RP-HPLC, numerous stationary part moreover because the mobile part is altered to cive the required separation.

Mobile Phase:
One in all the nice benefits of HPLC is that the skillfulness afforded by a liquid mobile part. Currently solely will completely different parameters be varied once the mobile part is liquid, however the matter can even move with the mobile part should be ensured so as to stop precipitation.

For the mobile part, the variable to be set is whether or not AN organic or binary compound eluent ought to be used. With RP-HPLC analysis, either a binary compound eluent or sort of organic solvent like methyl alcohol or Acetonitrile is tried 1st. If the k1 values area unit overlarge with a binary compound solvent, then the separation ought to be tried by employing a mixture, the 2 in numerous proportions. Several straightforward Analyses is dispensed with isocratic extraction mistreatment AN binary compound eluent to that an organic modifier is extra. If sample to be analyzed contains a awfully complicated mixtures or mixture of compounds of numerous structure and retention behavior, then either a ternary mixture of solvents is used isocratically or gradient extraction could also be necessary.

Mode of elution & flow rate:
Whenever potential, isocratic extraction ought to be used as a result of it eliminates "turnaround time" on the column and so shortens overall analysis time. Also, retention duplicability is additional sure with isocratic extraction as a result of re-equilibration of the column when gradient extraction should be rigorously controlled. However, once adequate resolution can't be achieved at intervals cheap length of your time, thanks to the range of compounds during a mixture or once there's general extraction drawback, gradient extraction is judicious. Gradients are stepwise or continuous.

Optimization of RP-HPLC:
Optimization of latest technique development is extremely necessary. For these some equations area unit offered, however those area unit very little facilitate for unknown samples.

To optimize the retention time, several operative parameters ought to be thought of composition of eluent; extraction mode and rate if gradient extraction is being employed. To decrease k1 values, the strength of the initial or final eluent or the slope of the gradient ought to be redoubled. If the isocratic doesn't give the required resolution, the foremost obvious thanks to

improve resolution are either by isocratic extraction with mixed solvents or by gradient extraction.

Peak Identification:
The measuring of peak height or peak space is an element of the quantitative measuring. Many ways area unit offered to the analyst for the aim of confirmation of peak identity, as well as recurrent analysis on a column of a unique kind, various chemical treatment of the sample, and eventually the employment of multiple detection techniques so as to characterize any the solutes as they rinse from the column.

The main detectors in use nowadays for HPLC area unit the ratio (RI), ultraviolet illumination absorption, light detectors, and chemical science detector. The ultraviolet illumination absorption detector is out and away the foremost common detector in use in HPLC detection nowadays. Light-weight is directed through the sample stream eluting from finish of the column and also the quantity of sunshine absorbed by matter within the eluent is monitored. This light-weight is either a hard and fast wavelength or broadband wavelength. Not all analyte species absorb ultraviolet illumination radiation and thus the ultraviolet illumination detector isn't nearly as universal because the Ocean State detector.

Quantification:
Qualitative analysis mistreatment action is predicated on activity curves obtained from every of the substances analyzed. Activity is required altogether cases during which a sign associated with mass or concentration of a element in mixture, is obtained. Natural process take a look at technique use either external or internal standards for quantification.

External standard method:
AN external commonplace technique is employed once the quality is analyzed on a separate recording from the sample. Quantification is predicated on a comparison of the height space or height (HPLC / GC) or spot intensity (TLC) of the sample thereto of a reference commonplace of the analyte of interest.

Internal standard method:
With an indoor commonplace technique, compound of familiar purity that doesn't cause interference within the analysis is extra to the sample mixture. Quantification is predicated on the response quantitative relation of compound of interest to the inner commonplace vs. the response quantitative relation of an identical preparation of the reference commonplace (HPLC / GC). {This technique this system|this technique} is never used for TLC method.

Standard addition method [16]:
Once matrix interactions area unit found to be necessary, a regular addition technique might prove helpful. During this technique, a familiar amount of normal is extra to unknown compound. However it's not a lot of correct.

Though CDER doesn't specify whether or not the strategy use an indoor or external commonplace for quantification, it's usually discovered that HPLC technique for unleash and stability.

MATERIALS AND METHODS:

Ivacaftor from Sura labs, Tezacaftor from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck,

Hplc method development:
Trails:
Preparation of standard solution:
Accurately weigh and transfer 10 mg of Tezacaftor and Ivacaftor working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.75 ml of Tezacaftor and 1.125 ml of Ivacaftor from the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluents.

Procedure:
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:
Initially the mobile phase tried was methanol: water with varying proportions. Finally, the mobile phase was optimized to methanol: TEA Buffer in proportion 40:60 v/v respectively.

Optimization of Column:
The method was performed with various columns like C18 column, Symmetry and X-Bridge. Symmetry C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1 ml/min flow.

Optimized chromatographic conditions:
Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.

Temperature : 40ºC
Column : Symmetry C18 (4.6×150mm, 5µ)
pH : 4.2
Mobile phase :Methanol: TEA buffer pH 4.2 (40:60v/v)
Flow rate : 1ml/min
Wavelength :260 nm
Injection volume : 10 µl
Run time : 6 min

Validation

Preparation of buffer and mobile phase:

Preparation of Triethylamine (TEA) buffer (pH-4.2):

Dissolve 1.5ml of Ttiethyl amine in 250 ml HPLC water and adjust the pH 4.5. Filter and sonicate the solution by vaccum filtration and ultrasonication.

Preparation of mobile phase:

Accurately measured 650 ml (65%) of Methanol and 350 ml of TEA buffer (35%) a were mixed

Diluent Preparation:

The Mobile phase was used as the diluent

RESULTS AND DISCUSSION:

Optimized Chromatogram (Standard)

Mobile phase : Methanol: TEA buffer pH 4.2 (40:60)
Column : Symmetry C18 (4.6×150mm, 5.0 µm)
Flow rate : 1 ml/min
Wavelength : 260 nm
Column temp : 40ºC
Injection Volume : 10 µl
Run time : 6 minutes

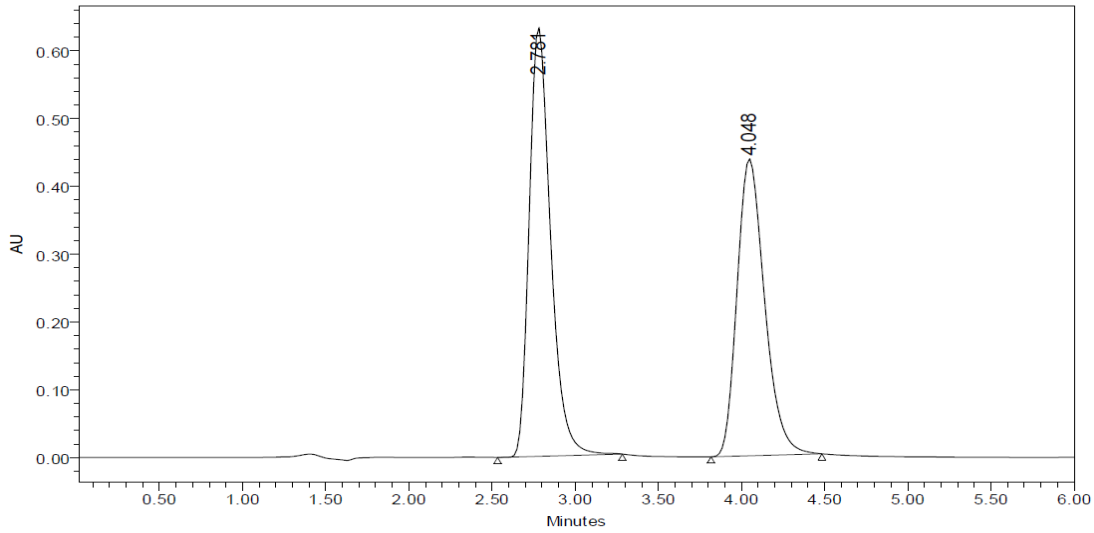

Fig-: Results of Optimized Chromatogram

Table-: Peak Results for Optimized Condition

S. No.	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Ivacaftor	2.781	2774027	299752		1.2	6314
2	Tezacaftor	4.048	2533532	210321	4.6	1.3	5521

Observation: From the above chromatogram it was observed that the Ivacaftor and Tezacaftor peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

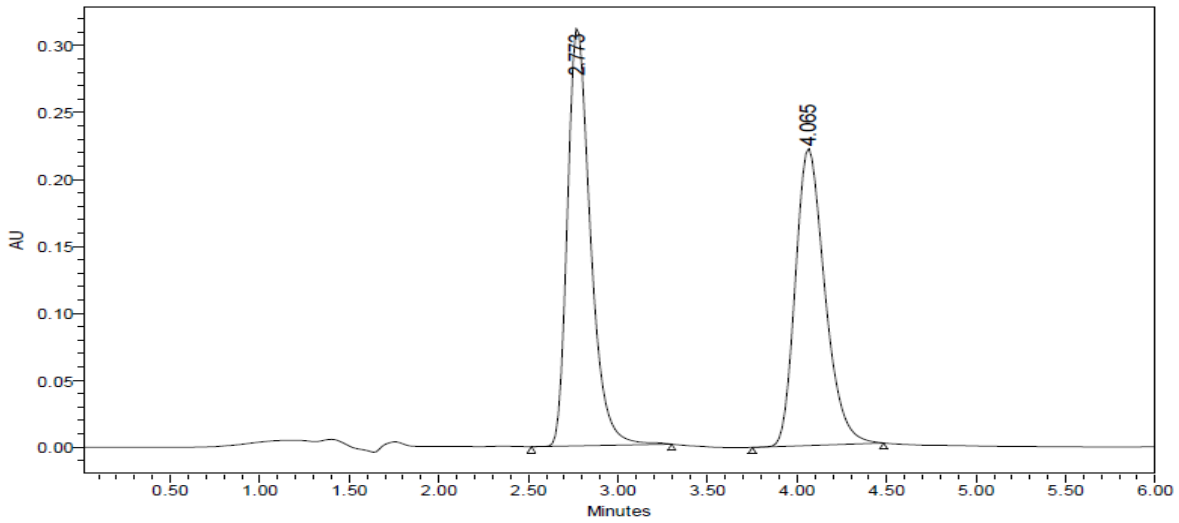

Figure-: Optimized Chromatogram (Sample)

Table-: Optimized Chromatogram (Sample)

S. No.	Peak Name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Ivacaftor	2.773	2770123	282157		1.6	5011
2	Tezacaftor	4.065	2522041	251068	3.3	1.5	5947

Acceptance criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 0.9 and not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

Table-: Peak results for assay standard of Ivacaftor

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Tailing	USP Plate Count
1	Ivacaftor	2.767	2762937	357421	1.3	6344.7
2	Ivacaftor	2.795	2774613	388745	1.3	6344.2
3	Ivacaftor	2.768	2776429	364121	1.3	6344.2
Mean			2771306			
Std. Dev.			7321.9			

% RSD			0.26			

Table-: Peak results for assay standard of Tezacaftor

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Resolution	USP Tailing	USP Plate Count
1	Tezacaftor	4.029	2534375	210326	4.6	1.3	5937.7
2	Tezacaftor	4.067	2526189	226741	4.7	1.3	5008.8
3	Tezacaftor	4.030	2546248	231494	4.7	1.3	5990.7
Mean			2535604				
Std. Dev.			10085.82				
% RSD			0.397768				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Assay (Sample):

Table-: Peak results for Assay sample

S.No.	Name	RT	Area	Height	USP Resolution	USP Tailing	USP Plate Count	Injection
1	Ivacaftor	2.764	2732203	294531		1.3	6314	1
2	Tezacaftor	4.012	2507543	216321	4.6	1.3	5954	1
3	Ivacaftor	2.767	2751843	286473		1.3	6369	2
4	Tezacaftor	4.016	2509101	216354	4.6	1.3	5944	2
5	Ivacaftor	2.764	2744776	312684		1.3	6329	3
6	Tezacaftor	4.013	2515628	206571	4.6	1.3	5990	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Ivacaftor, Tezacaftor in pharmaceutical dosage form was found to be 100. 9%, 100. 7%.

LINEARITY
CHROMATOGRAPHIC DATA FOR LINEARITY STUDY:
Ivacaftor:

Concentration µg/ml	Average Peak Area
37.5	892464
75	1866364
112.5	2777423
150	3709213
187.5	4601317

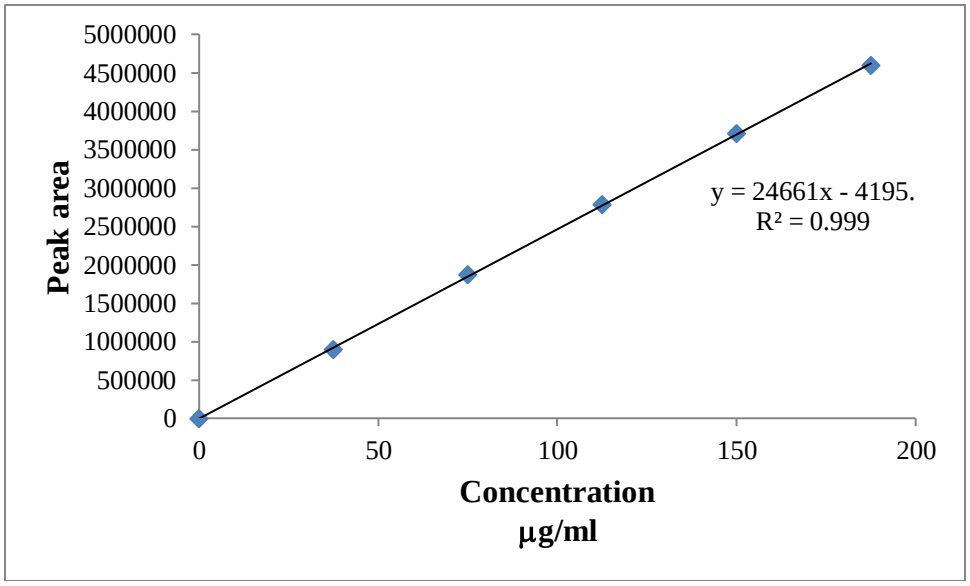

Figure calibration graph for Ivacaftor

Table : Chromatographic Data for Linearity Study Tezacaftor

Concentration µg/ml	Average Peak Area
25	920032
50	1752782
75	2521426
100	3326009
125	4217393

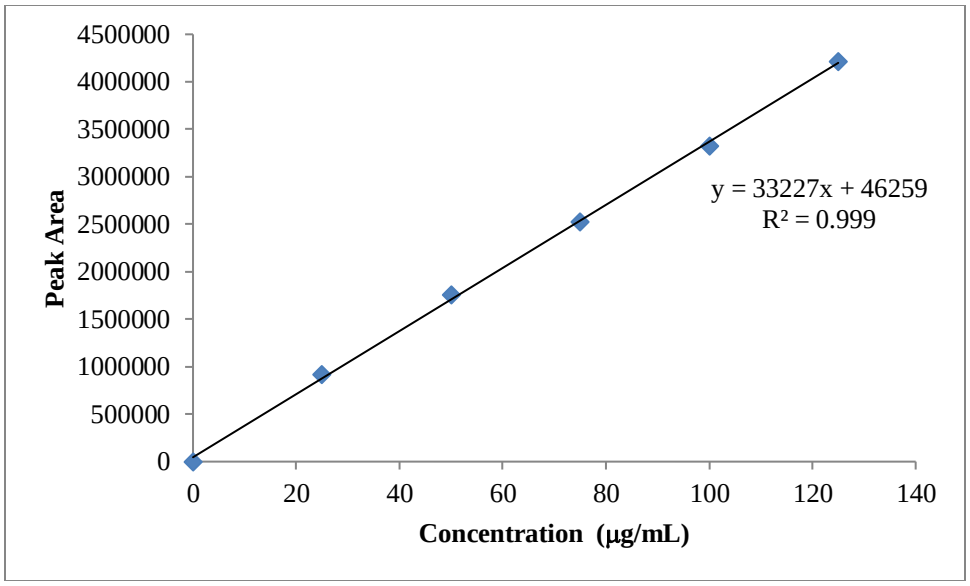

Figure calibration graph for Tezacaftor

Repeatability:

Table-: Results of repeatability for Ivacaftor:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ivacaftor	2.766	2766870	294578	6684	1.3
2	Ivacaftor	2.774	2771971	286541	6347	1.3
3	Ivacaftor	2.770	2771958	302657	6674	1.3
4	Ivacaftor	2.772	2780299	293412	6451	1.3
5	Ivacaftor	2.771	2789695	283154	6678	1.3
Mean			2776159			
Std. Dev			8969.6			
% RSD			0.32			

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table-: Results of method precision for Tezacaftor:

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Tezacaftor	4.025	2534539	193240	5761	1.3	4.7
2	Tezacaftor	4.040	2539247	201647	5489	1.3	4.6
3	Tezacaftor	4.032	2544661	193472	5367	1.3	4.6
4	Tezacaftor	4.041	2548839	196475	5845	1.3	4.6
5	Tezacaftor	4.036	2558822	201394	5347	1.3	4.7
Mean			2545221				
Std. Dev			9330.0				
% RSD			0.37				

Acceptance criteria:

- %RSD for sample should be NMT 2

- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision:

Table-: Results of Intermediate precision Day 1 for Ivacaftor

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ivacaftor	2.781	2715421	294651	6647	1.3
2	Ivacaftor	2.780	2778540	284123	6781	1.3
3	Ivacaftor	2.782	2754247	274561	6984	1.3
4	Ivacaftor	2.780	2780545	281241	6475	1.3
5	Ivacaftor	2.782	2777021	286471	6647	1.3
6	Ivacaftor	2.774	2780254	294512	6489	1.3
Mean			2764338			
Std. Dev			25974			
% RSD			0.9			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.

Table-: Results of Intermediate precision Day 1 for Tezacaftor

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Tezacaftor	4.048	2506927	211541	5495	1.4	4.6
2	Tezacaftor	4.050	2504522	206141	5694	1.4	4.6
3	Tezacaftor	4.049	2541270	198641	5785	1.4	4.7
4	Tezacaftor	4.050	2507885	206741	5947	1.4	4.6
5	Tezacaftor	4.049	2504587	209487	5742	1.4	4.6
6	Tezacaftor	4.040	2504780	193481	5914	1.4	4.6
Mean			2511662				
Std. Dev			14572.01				
% RSD			0.5				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

Table-: Results of Intermediate precision Day 2 for Ivacaftor

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ivacaftor	2.764	2781856	294651	6647	1.3
2	Ivacaftor	2.759	2761510	284123	6781	1.3
3	Ivacaftor	3.015	2748811	274561	6984	1.3

4	Ivacaftor	2.773	2790831	281241	6475	1.3
5	Ivacaftor	2.765	2785112	286471	6647	1.3
6	Ivacaftor	2.764	2781932	294512	6489	1.3
Mean			2775009			
Std. Dev			16222.05			
% RSD			0.5			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.

Table-: Results of Intermediate precision day 2 for Tezacaftor

S.No.	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Tezacaftor	4.015	2536301	211541	5495	1.4	4.6
2	Tezacaftor	4.007	2541972	206141	5694	1.4	4.6
3	Tezacaftor	4.323	2521259	198641	5785	1.4	4.7
4	Tezacaftor	4.065	2537081	206741	5947	1.4	4.6
5	Tezacaftor	4.020	2549869	209487	5742	1.4	4.6
6	Tezacaftor	4.015	2536301	193481	5914	1.4	4.6
Mean			2537131				
Std. Dev			9370.087				
% RSD			0.36				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

Accuracy:

Table 14: The accuracy results for Glipizide

%Concentration (at specification Level)	**Area**	**Amount Added (µg/ml)**	**Amount Found (µg/ml)**	**% Recovery**	**Mean Recovery**
50%	124675.7	15	15.1	101%	100.4%
100%	242006.3	30	30.1	100.5%	
150%	357449	45	44.9	99.7%	

Table 15: The accuracy results for Metformin

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
50%	1696259	18.75	18.71	99.8%	99.2%
100%	3351661	37.5	37.2	99.4%	
150%	4975094	56.25	55.47	98.6%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table-: Results for Robustness

Ivacaftor:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2774027	2.781	6314	1.2
Less Flow rate of 0.9 mL/min	2884521	3.327	6199	1.4
More Flow rate of 1.1 mL/min	2542012	2.516	6234	1.4
Less organic phase	2888515	3.326	6298	1.4
More organic phase	2541550	2.416	6287	1.2

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Tezacaftor:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	2533532	4.048	5521	1.3
Less Flow rate of 0.9 mL/min	2750214	5.319	5643	1.6
More Flow rate of 1.1 mL/min	2254107	3.649	5782	1.5
Less organic phase	2754017	5.318	5309	1.4
More organic phase	2215870	3.233	5580	1.51

Acceptance criteria:
The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION:

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Tezacaftor and Ivacaftor in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Methanol: TEA pH 4.2 (40:60) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectro photometric methods.

This method can be used for the routine determination of Tezacaftor and Ivacaftor in bulk drug and in Pharmaceutical dosage forms.

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