Research Article

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Method Development and Validation of Simultaneous Estimation of Tezacaftor and Ivacaftor in Api by Rp-Hplc

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ABSTRACT

An economic, uncomplicated, selective, detailed, and accurate RP-HPLC procedure for simultaneous quantitative determination of tezacaftor and ivacaftor in combined dosage forms, was formulated and validated according to ICH guidelines. The method was developed using Agilent 1200 series HPLC and Hypersil BDS (150x4.6mm ID) 5.0µm column in isocratic mode, with mobile phase comprising of mixed phosphate buffer: acetonitrile (70:30) the flow rate was 1.0 ml/min and the detection was carried at a wavelength of 274nm. The retention time and percentage assay of purity for tezacaftor and ivacaftor was found to be 3.386 min and 4.433 min, 99.5% and 99.9% respectively. The method was successfully validated for accuracy, precision, ruggedness, linearity and range, specificity and robustness in accordance with ICH guidelines. The proposed method was found to be within the acceptance limits indicating that the method is accurate, specific and economical.

Keywords: Tezacaftor, Ivacaftor, precision, accuracy, linearity, HPLC

INTRODUCTION

Tezacaftor 100 mg/Ivacaftor 150 mg (SYMDEKO) is indicated for the treatment of patients with cystic fibrosis (CF) aged 12 years and older who are homozygous for the F508del mutation or who have at least one mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene that is responsive to tezacaftor/ivacaftor based on in vitro data and/or clinical evidence.[1]Tezacaftor, also known as VX-661, is a drug approved by the FDA to treat some cases of cystic fibrosis. Tezacaftor helps move the CFTR protein to the correct position on the cell surface, and is designed to treat people with the F508del mutation.[2-7]Tezacaftor is a white to off-white powder that is practically insoluble in water (<5 microgram/mL).Tezacaftor facilitates the cellular processing and trafficking of normal and select mutant forms of CFTR (including F508del-CFTR) to increase the amount of mature CFTR protein delivered to the cell surface.

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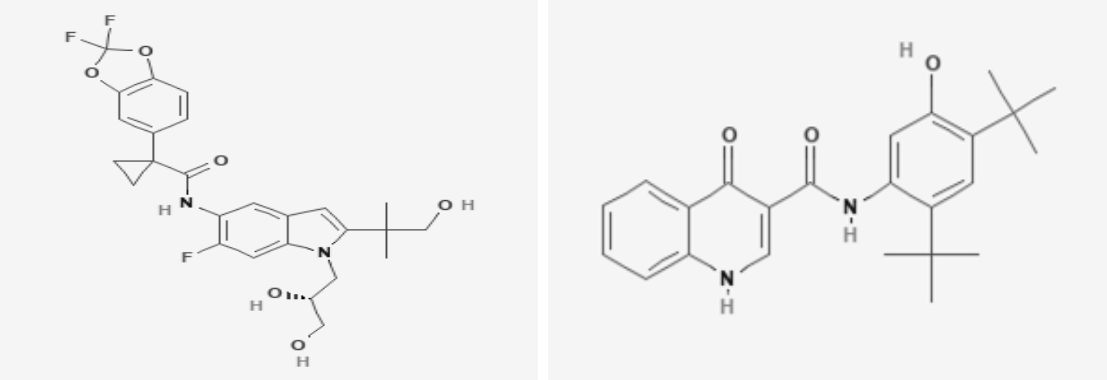

Fig 1: Structure of tezacaftor and ivacaftor

Ivacaftoris a drug used to treat cystic fibrosis in people with certain mutations in the cystic fibrosis transmembrane conductance regulator (CFTR). Ivacaftor was developed by Vertex Pharmaceuticals in conjunction with the Cystic Fibrosis Foundation and is the first drug that treats the underlying cause rather than the symptoms of the disease. Cystic fibrosis is caused by any one of several defects in the CFTR protein, which regulates fluid flow within cells and affects the components of sweat, digestive fluids, and mucus. Ivacaftor, a CFTR potentiator, improves the transport of chloride through the ion channel by binding to the channels directly to induce a non-conventional mode of gating which in turn increases the probability that the channel is open. Ivacaftor is approximately 99% bound to plasma proteins.[8-15]Ivacaftor is a white to off-white powder that is practically insoluble in water (<0.05 microgram/mL).Ivacaftor is a CFTR potentiator that facilitates increased chloride transport by potentiating the channel-open probability (or gating) of the CFTR protein at the cell surface. For ivacaftor to function CFTR protein must be present at the cell surface. Ivacaftor can potentiate the CFTR protein delivered to the cell surface by tezacaftor, leading to a further enhancement of chloride transport than either agent alone. The combined effect of tezacaftor and ivacaftor is increased quantity and function of CFTR at the cell surface, resulting in increases in chloride transport.

MATERIALS AND METHOD

Tezacaftor and Ivacaftor gift samples obtained from pharma industry were used for the study. All the solvents and reagents used were of HPLC grade.

Equipment

Agilent 1200 series HPLC system was provided. The chromatographic analysis was performed using Hypersil BDS (150x4.6mm ID) 5.0µm column as a stationary phase.

Chromatographic Conditions

Mobile phase was pumped at a flow rate of 1 mL/min using a binary mixture of mixed phosphate buffer: acetonitrile (70:30v/v) in isocratic mode. The injection volume of 20 µL was given and the detection wavelength for tezacaftor and ivacaftor was set at 274 nm and the separation was achieved at room temperature.

Preparation of mixed standard solutions

150 mg of Ivacaftor and 100mg of Tezacaftor are weighed each in a volumetric flask of 100ml dissolved it in mobile phase and then volume is make up with the mobile phase this gives 1500µg/ml of Ivacaftor and 1000µg/ml Tezacaftor. From the above solution 1ml is pipetted out in volumetric flask of 10 ml each Ivacaftor and Tezacaftor and then volume is make up with mobile phase this gives 150µg/ml of Ivacaftor and 100µg/ml Tezacaftor. For recording the chromatogram, this solution is used.

Preparation of sample solution

About 150mg of equivalent powder of Ivacaftor and 100mg of Tezacaftorin is weighed and taken in volumetric flask of 100 mL and to this mobile phase is added about 70mL and sonicated. 5 mL of the solution is pipetted out in volumetric flask of 50 mL and volume is make up by using mobile phase.

RESULTS AND DISCUSSION

Table 1: Optimized chromatographic conditions

Mobile phase	Mixed phosphate buffer: Acetonitrile (70:30)
Column	Hypersil BDS (150x4.6mm ID) 5.0µm
Flow rate	1.0mL/min
Column temperature	Room Temperature (20-25˚C)
Sample temperature	Room Temperature (20-25˚C)
Wavelength	274 nm
Injection volume	20µL
Run time	10 min
Retention time	About 3.386min fortezacaftor and 4.433min for ivacaftor

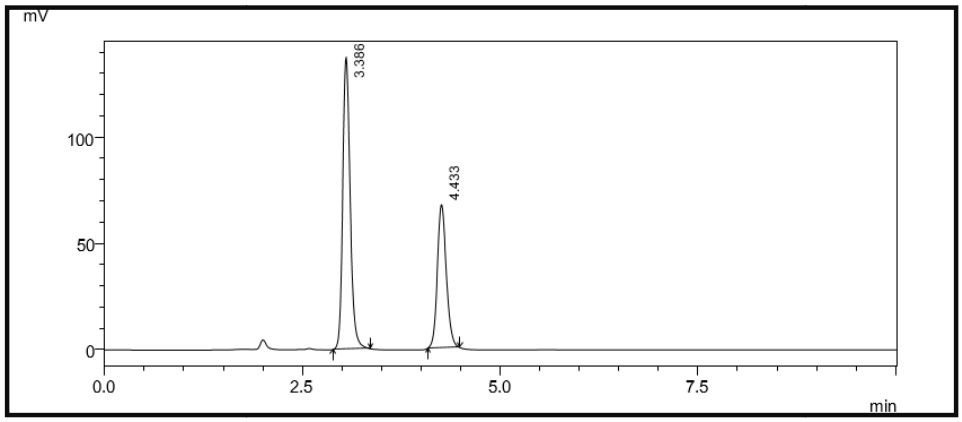

Fig. 1: Typical Chromatogram of tezacaftor and ivacaftor

Assay:

Table 2: Assay Results

Ivacaftor			Tezacaftor	
Standard Area	1	920431		546931
	2	918756		566243
	3	930543		565532
	4	908056		547057
	5	914945		553448
	6	913816		549806
	Average	917758	Average	554836
Sample area	1	902190		540819
	2	921919		558410
	3	909278		554898
	4	915997		540770
	5	923670		544623
	6	911877		539649
	Average	918546	Average	546528
Tablet average weight (mg)		190.2		190.2
Standard weight (mg)		100.2		5.1
Sample weight (mg)		190.5		190.5
Label amount (mg)		100		5
Standard purity %		99.8		99.6

Cal.: (mg)	**99.5**	**5.00**

Validation of the HPLC Method

The proposed method was validated as per ICH guidelines[16].

Linearity and range

Linearity of detector response of assay method was found by injecting standard solutions with concentration ranging from 50 % to 150 % of the test concentration Peak area is measured and each level injected into the chromatographic system and the. A graph plotted of peak area versus concentration the correlation coefficient calculated. The results were shown in Table 3, 4 and Fig 2, 3.

Table 3: Linearity of Ivacaftor

S.No.	Conc.(µg/ml)	Area
1	75	454530
2	120	733496
3	150	883374
4	180	1200148
5	225	1388503

Table 4: Linearity of Tezacaftor

S.No.	Conc.(µg/ml)	Area
1	50	264727
2	80	443366
3	100	524516
4	120	717888
5	150	842245

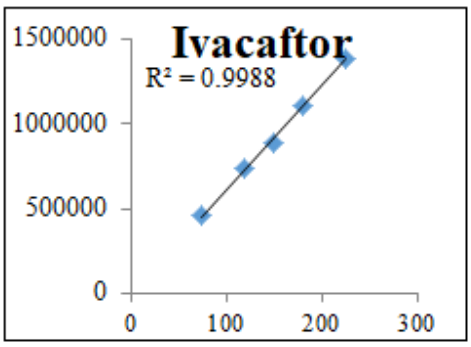

Fig. 2: Linearity graph of Ivacaftor

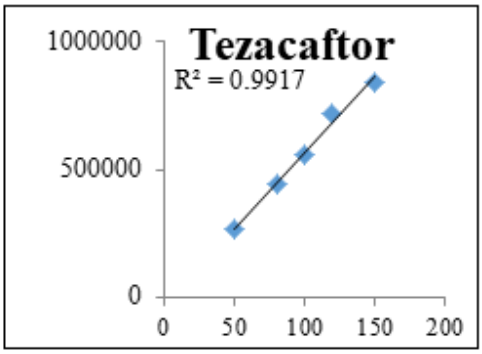

Fig. 3: Linearity graph of Tezacaftor

Accuracy

Recovery studies and accuracy of the method was determined. To the formulation which is a pre analyzed sample, at the level of 50%, 100%, 150% the reference standards of the drugs were added and the recovery studies were carried out three times. The results were shown in Table 5, 6.

Table 5: Recovery results for Ivacaftor

Accuracy of Ivacaftor						
% Recovery	**Standard Weight in mg**	**Area**	**Concentration Added**	**Concentration Recovered**	**%Recovery**	**Average**
50% -1	75	461018	75	75.08	100.2	99.3

50% -2	75	456169	75	74.55	99.1
50% -3	75	454159	75	74.33	98.7
100% -1	150	907152	150	148.54	98.5
100% -2	150	909748	150	148.82	98.8
100% -3	150	899426	150	147.70	97.7
150% -1	225	1398530	225	226.91	101.3
150% -2	225	1348013	225	222.42	97.6
150% -3	225	1400913	225	227.17	101.4

Table 6: Recovery results for Tezacaftor

Accuracy of Tezacaftor						
% Recovery	**Standard Weight in mg**	**Area**	**Concentration Added**	**Concentration Recovered**	**%Recovery**	**Average**
50% -1	50	277733	50	48.43	97.1	
50% -2	50	271508	50	46.37	94.9	
50% -3	50	261903	50	44.29	91.6	
100% -1	100	60846	100	100.53	100.6	
100% -2	100	554904	100	98.85	97.0	99.6
100% -3	100	899426	100	102.86	117.2	
150% -1	150	855433	150	149.48	99.7	
150% -2	150	857891	150	150.00	100.0	
150% -3	150	847447	150	148.41	98.8	

Precision

Sample preparations of ivacaftor and tezacaftor were prepared as per the method and injected 6 times into the column. And the relative standard deviation of assay results was calculated. The results were shown in Table 7.

Table 7: Results for Method precision of Ivacaftor and Tezacaftor

Precision					
Ivacaftor			**Tezacaftor**		
S.No.	**Retention time**	**Area**	**S.No.**	**Retention time**	**Area**
1	3.379	909854	**1**	4.439	552029
2	3.399	897813	**2**	4.426	555619
3	3.383	903496	**3**	4.465	546873
4	3.378	900330	**4**	4.439	544088
5	3.341	894921	**5**	4.462	541606
6	3.326	867809	**6**	4.483	525570
Average	3.367	895704	**Average**	4.452	544298
Standard deviation	0.009	14598	**Standard deviation**	0.036	10513
%RSD	0.3	1.6	**%RSD**	0.8	1.8

Intermediate Precision/Ruggedness

Precision was performed by different analysts by using different columns of same dimensions and method evaluated. The area was measured in HPLC for the five times injected standard solution. The results were shown in Table 8.

Table 8: Results for Ruggedness

Ivacaftor	**%Assay**	**Tezacaftor**	**%Assay**
Analyst 01	100.15	**Analyst 01**	100.96
Analyst 02	99.89	**Analyst 02**	99.14
%RSD	0.21	%RSD	1.45

Robustness

Chromatographic conditions variation

The prepared solution is injected at different variable conditions like Temperature and wavelength as per test method. System suitability parameters were compared with that of method precision. The results were shown in Table 9

Table 9: Result of Robustness study

Chromatographic Changes		Theoretical Plates		Tailing factor		Resolution
		Ivacaftor	Tezacaftor	Ivacaftor	Tezacaftor	
Flow rate(mL/min)	0.8	34208	47021	1.238	1.198	7.055
	1.2	27281	37074	1.254	1.166	6.205
Wave length (nm)	272	31000	41822	1.247	1.168	6.486
	276	30697	41839	1.248	1.205	6.370

Limit of Detection

The limit of detection of both drugs were determined by calculating the signal-to-noise(S/N) ratio of 3:1 respectively according to guidelines.

LOD = 3.3 α / S
= (3.3) * (2283.05)/9315
= 0.80µg/ml (Ivacaftor)
= (3.3) * (3547.5)/11988
= 0.97µg/ml (Tezacaftor)
Where,
σ = the standard deviation of the response
S = the slope of the calibration curve
The slope S may be estimated from the calibration curve of the analyte.

Limit of Quantification

The limit of quantification of Ivacaftor and Tezacaftor were determined by calculating the signal-to-noise(S/N) ratio of 10:1 respectively according to International Conference on Harmonization guidelines.

LOQ = 10 α / S
= (10) * (2283.05)/9315
= 2.45µg/ml (Ivacaftor)
LOQ = 10 α / S
= (10)* (3547.5)/11988
= 2.95µg/ml (Tezacaftor)
Where,
σ = the response standard deviation
S = the calibration curve slope
The slope S may be Obtained from the calibration curve.

CONCLUSION

From the above it can be concluded that all validation parameters such as precision, accuracy, linearity and Ruggedness met the predetermined acceptance criteria as mentioned in ICH guidelines. The developed RP-HPLC method is simple, rapid, accurate, and precise and can be applied for routine analysis of tezacaftor and ivacaftor in bulk and its dosage forms.

REFERENCES

1. Tezacaftor/ivacaftor for cystic fibrosis. *AustPrescr*. 2019;42(5):174-175.
2. National Center for Biotechnology Information. PubChem Database. Tezacaftor, CID=46199646, https://pubchem.ncbi.nlm.nih.gov/compound/Tezacaftor
3. Donaldson SH, Pilewski JM, Griese M, Cooke J, Viswanathan L, Tullis E, Davies JC, Lekstrom-Himes JA, Wang LT: Tezacaftor/Ivacaftor in Subjects with Cystic Fibrosis and F508del/F508del-CFTR or F508del/G551D-CFTR. Am J RespirCrit Care Med. 2018 Jan 15;197(2):214-224.
4. Taylor-Cousar JL, Munck A, McKone EF, van der Ent CK, Moeller A, Simard C, Wang LT, Ingenito EP, McKee C, Lu Y, Lekstrom-Himes J, Elborn JS: Tezacaftor-Ivacaftor in Patients with Cystic Fibrosis Homozygous for Phe508del. N Engl J Med. 2017 Nov 23;377(21):2013-2023

5. Rowe SM, Daines C, Ringshausen FC, Kerem E, Wilson J, Tullis E, Nair N, Simard C, Han L, Ingenito EP, McKee C, Lekstrom-Himes J, Davies JC: Tezacaftor-Ivacaftor in Residual-Function Heterozygotes with Cystic Fibrosis. N Engl J Med. 2017 Nov 23;377(21):2024-2035.
6. Garg V, Shen J, Li C, Agarwal S, Gebre A, Robertson S, Huang J, Han L, Jiang L, Stephan K, Wang LT, Lekstrom-Himes J: Pharmacokinetic and Drug-Drug Interaction Profiles of the Combination of Tezacaftor/Ivacaftor. ClinTransl Sci. 2019 May;12(3):267-275
7. Saint-Criq V, Gray MA: Role of CFTR in epithelial physiology. Cell Mol Life Sci. 2017 Jan;74(1):93-115. doi: 10.1007/s00018-016-2391-y. Epub 2016 Oct 6.
8. Saint-Criq V, Gray MA: Role of CFTR in epithelial physiology. Cell Mol Life Sci. 2017 Jan;74(1):93-115.
9. Kunzelmann K, Mall M: Pharmacotherapy of the ion transport defect in cystic fibrosis: role of purinergic receptor agonists and other potential therapeutics. Am J Respir Med. 2003;2(4):299-309.
10. MacDonald KD, McKenzie KR, Zeitlin PL: Cystic fibrosis transmembrane regulator protein mutations: 'class' opportunity for novel drug innovation. Paediatr Drugs. 2007;9(1):1-10.
11. Fraser-Pitt D, O'Neil D: Cystic fibrosis - a multiorgan protein misfolding disease. Future Sci OA. 2015 Sep 1;1(2):FSO57.
12. Yu H, Burton B, Huang CJ, Worley J, Cao D, Johnson JP Jr, Urrutia A, Joubran J, Seepersaud S, Sussky K, Hoffman BJ, Van Goor F: Ivacaftor potentiation of multiple CFTR channels with gating mutations. J Cyst Fibros. 2012 May;11(3):237-45.
13. Flume PA, Liou TG, Borowitz DS, Li H, Yen K, Ordonez CL, Geller DE: Ivacaftor in subjects with cystic fibrosis who are homozygous for the F508del-CFTR mutation. Chest. 2012 Sep;142(3):718-724.
14. Mayer M: Lumacaftor-ivacaftor (Orkambi) for cystic fibrosis: behind the 'breakthrough'. Evid Based Med. 2016 Jun;21(3):83-6.
15. Fohner AE, McDonagh EM, Clancy JP, Whirl Carrillo M, Altman RB, Klein TE: PharmGKB summary: ivacaftor pathway, pharmacokinetics/pharmacodynamics. Pharmacogenet Genomics. 2017 Jan;27(1):39-42.
16. ICH Guideline "Validation of analytical procedures: text and methodology Q2 (R1)" International conference on harmonization, Geneva, Switzerland, vol. 4, 2005, pp. 1-13