ISSN- 2230-7346
Journal of Global Trends in Pharmaceutical Sciences

ANALYTICAL METHOD DEVELOPMENT AND METHOD VALIDATION OF STABILITY INDICATING RELATED SUBSTANCES METHOD BY RP-HPLC FOR LUMACAFTOR AND IVACAFTOR TABLETS

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ARTICLE INFO

Key words:

Lumacaftor and Ivacaftor
RP-HPLC

Access this article online
Website:
https://www.jgtps.com/
Quick Response Code:

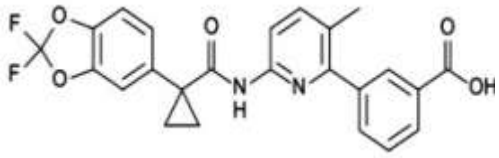
ABSTRACT

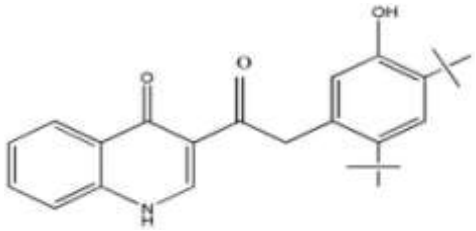
The RP-HPLC technique was developed by utilizing X Terra RP C_8, (4.6x150mm, 5µm) column with mobile phase A of Buffer which is mix of potassium dihydrogen phosphate adjusting pH to 2.6±0.05 using dilute ortho phosphoric acid and mobile phase B was made up of acetonitrile and methanol at ratio of 80:20 % v/v. The diluents-1 taken was acetonitrile and methanol at proportion of 60:40%v/v and diluents-2 taken was acetonitrile: methanol : buffer at proportion of 45:30:25%v/v. Flow rate was 1.0mL/min, UV detection at 225nm with PDA detector and the injection volume was 10µL and the run time was 112mins. The system suitability parameters also explain about the values whether in the specified limit for the proposed strategy. The theoretical plates for Lumacaftor and Ivacaftor were obtained to be not less than 5000 and the tailing factor was obtained to be not more than 2.0. The retention time of the Lumacaftor and impurities peaks take place obtained to be 72 minutes, 14.19 minutes 15.99 minutes 32.20 minutes 38.34 minutes 53.64 minutes 88.433 minutes and 98.61 minutes respectively. The retention time of the Ivacaftor and impurities peaks were respectively found to be 68 minutes, 19.24minutes, 40.49 minutes and 94.7minutes.The solution stability of the sample and standard solutions was found to be stable up to 48hrs on both benches.

INTRODUCTION

Analytical chemistry is a scientific approach that is used to study the chemical composition, structure and behaviour of matter. The purpose of chemical analysis is to gather and interpret chemical information that will be of value of society in a wide range of contexts. Analytical chemistry involves the application of range of techniques and methodologies to obtain an access qualitative, quantitative and structural information on the nature of matter[1].Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e., with the raw material on which degree of purity and quality of medicament depends. The quality of a drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulation[2-3].

LUMACAFTOR





IVACAFTOR

Impurities with IUPAC name and Structure

2	Amine Acid	3-(6-Amino-3-methylpyridin 2-yl)benzoic acid	
3	TBA dimer	[1,1'-Biphenyl]-3,3'-dicarboxylic acid	
4	DCA	1-(2,2-Difluorobenzo[d] [1,3]dioxol-5-yl) cyclopropane-1-carboxylic acid	

MATERIALS AND METHODS

Ivacaftor and lumacaftor were obtained as a gift sample from Ananth pharmaceuticals Pvt Ltd Maharashtra

Reagents & Chemicals:

S. No	Name	Grade	Manufacturer
1	Water	HPLC	Milli-Q
2	Methanol	HPLC	Rankem™ chemicals products
3	Acetonitrile	HPLC	Rankem™ chemicals products
4	Potassium di hydrogen phosphate	HPLC	Analytical Reagent grade™ chemicals products
5	Ortho Phosphoric Acid	HPLC	Analytical Reagent grade™ chemicals products

High performance liquid chromatography:

A Waters Alliance HPLC system (Waters, USA) equipped with binary gradient pump, auto sampler, column oven and for analysis PDA Detector was employed. Chromatographic statistics was obtained using Empower 3 software. Based on the available literature, Lumacaftor and Ivacaftor were found to be freely soluble in pH ranging from pH 1.2 to pH 6.8. Based on trails, pH 2.60 Buffer was finalized for preparation of Mobile phase. For better chromatographic conditions, the same buffer was considered for diluents preparation (as solubility found satisfactory from pH 2.6 buffers 100%.

Diluent-1: Acetonitrile: methanol(60:40% v/v).

Diluent-2: Mix acetonitrile: methanol: Buffer (45:30:25% v/v/v).

Diluent -3: Methanol.

Diluents-4: Methanol: acetonitrile: water (40:50:10% v/v/v).

Selection of Standard and Test concentrations and Injection volume:

Standard and Test solution concentrations and injection volume were finalized based on the Limit of Quantification of Lumacaftor and Ivacaftor peak and its respective impurities and also by considering specification level referred by ICH guidelines which is further based on Minimum daily dose.

Standard solution**:** Weigh 38mg of Lumacaftor and 24mg of Ivacaftor working standard into 100ml volumetric flask and add 75ml of diluent-1sonicated to dissolve and volume made up to mark with diluent-1.Transfer 5ml of above solution into a 50ml volumetric flask, dilute to volume with diluents-2 and mix Transfer 5ml of above solution into a 50ml volumetric flask, dilute to volume with diluents-2 and mix

Sensitivity Solution: Transfer 5ml of above solution into 50ml volumetric flask and diluted to volume with diluent-2 and mixed.

Sample solution: Weigh 216.18gm drug & add 75ml of diluent-1 and sonicated for not less than 45min with intermediate shaking& diluted with buffer. Centrifuge the solution at

5000rpm for10 min and collect the supernatant solution.

Drug product spiked sample preparation:

Preparation of TBA Acid , Amine acid, TBA dimer Impurity, DCA impurity, PBC des bromo, methyl ester impurity, TDB impurity solution: 2.169mg, 2.017mg, 2.248mg, 2.95mg, 1.930mg, 2.189mg, 2.26mg dissolved in 15ml of diluent-3. Then volume made up to 25ml with diluent-3.

Preparation of 0.2% standard solution:

Transfer 1ml of standard solution (diluents-1) into 50ml volumetric flask and volume made up with diluent-2

Preparation of 0.8% standard solution:

Transfer 4ml of standard solution (diluents-1) into 50ml volumetric flask and volume made up with diluent-2

Optimized method:

Chromatographic conditions:

Mobile phase: KH_2PO_4 pH 2.6 with OPA (20mM) and Acetonitrile: Methanol (80:20)
.

Flow rate **:** 1ml/min

Column: X terra RP C_8 (4.6 x 150mm, 5µm)

Detector wave length: 225.nm UV detector (PDA 200-400nm)

Column temperature **:** 30°C

Injection volume **:** 10µL

Run time **:** 112 min

Diluent **:** Acetonitrile: Methanol (60:40)

Results: In this programme peak shape of all impurity and both main peaks found to be satisfactory

All impurity and both main peaks are well separated from each other. In this programme gradient hump was not observed. The chromatogram for trial 5 is shown in Figure no.20

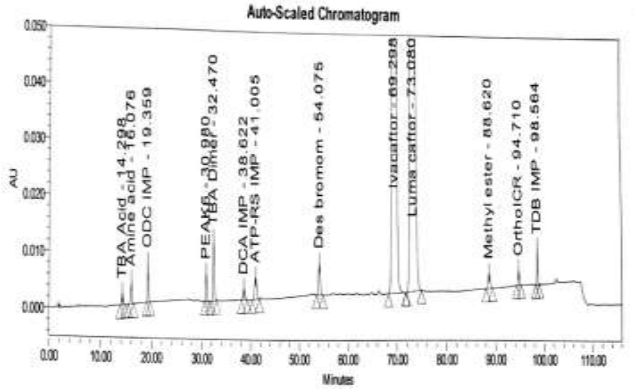


Figure No.:20 Optimized chromatogram for Drug Product

RESULTS AND DISCUSSION

Analytical Method Development for Related substance of simultaneous estimation of Lumacaftor and Ivacaftor by RP-HPLC.

Method development: Method development was done by changing various, mobile phase ratios, buffers etc.

Trial 1:

Chromatographic conditions:

Mobile phase **:** 0.1%OPA and Acetonitrile: Methanol (80:20)

Flow rate **:** 1ml/min

Column **:** X terra RP C_8 (4.6 x 150mm, 5µm)

Detector wave length **:** 225.nm UV detector (PDA 200-400nm)

Column temperature **:** 30°C

Injection volume **:** 10µL

Run time **:** 97min

Diluent **:** Acetonitrile and Methanol in the ratio (60:40)

Results **:** Amine acid and DCA impurity are not eluated within run time Base line disturbance was observed. The chromatogram for trial 1 is shown in Figure.no.16

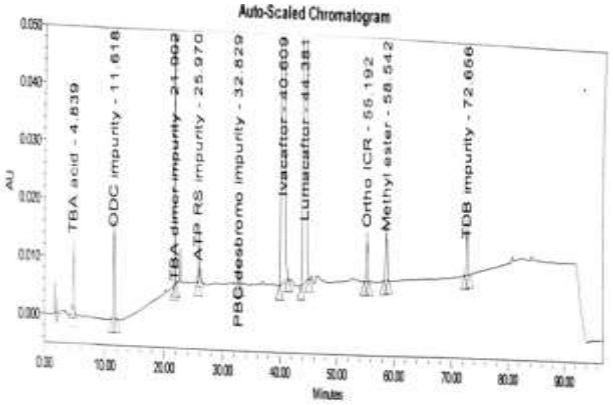


Figure No.:16 Trial-1 Chromatogram for Drug product

RRF Establishment:

Chromatographic conditions:

Mobile phase **:** KH_2PO_4 pH 2.6 with OPA (20mM) and Acetonitrile: Methanol (80:20)

Flow rate **:** 1ml/min

Column **:** X terra RP C_8 (4.6 x 150mm, 5µm)

Detector wave length **:** 225.nm UV detector (PDA 200-400nm)

Column temperature **:** 30°C

Injection volume **:** 10µL

Run time **:** 112 min

Diluent **:** Acetonitrile: Methanol (60:40)

Results **:** All impurity are separated for main peak in spiked sample solution.

The chromatogram for RRF establishment is shown in Figure no.21

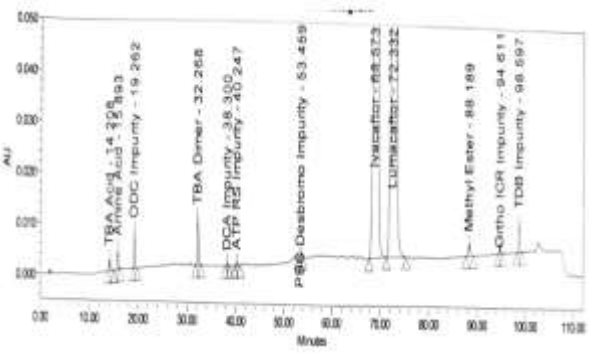


Figure No.:21- Chromatogram for RRF Establishment

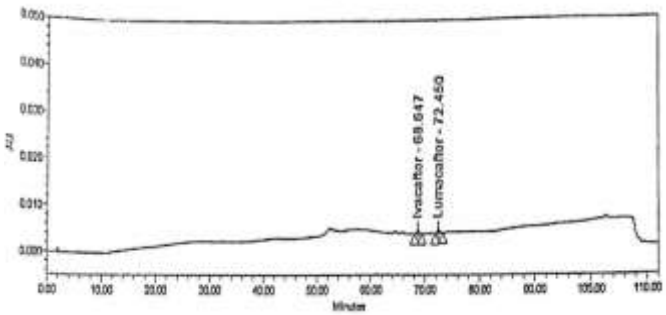


Figure No.:22 System suitability Chromatogram for Unspiked

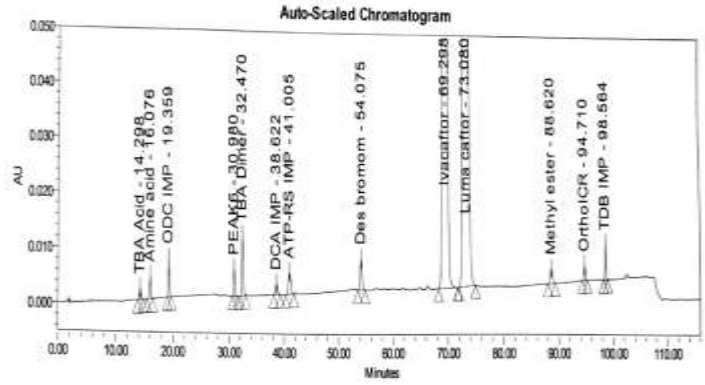


Figure No.:23 System suitability Chromatogram for spiked

Method Validation: The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. According to ICH Q2B guidelines, typical analytical performance characteristics that should be considered in the validation of the types of methods.

Specificity:

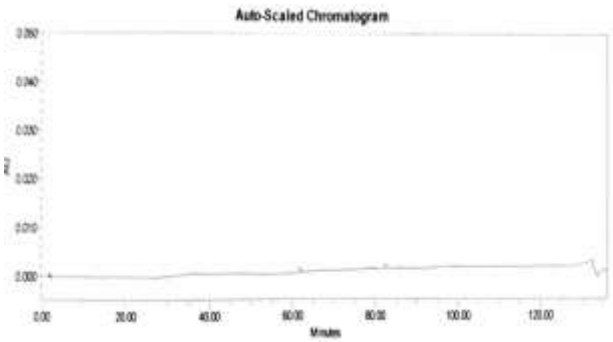


Figure No.:24 Chromatogram of blank.

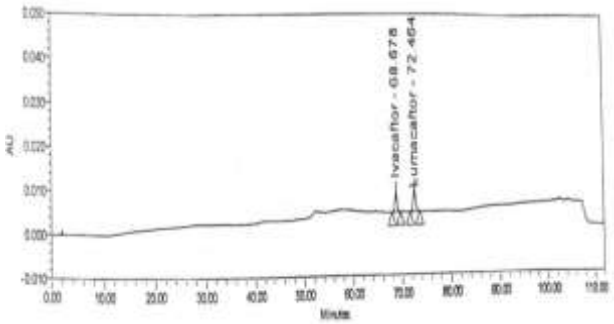


Figure No.:26 Chromatogram of Standard solution

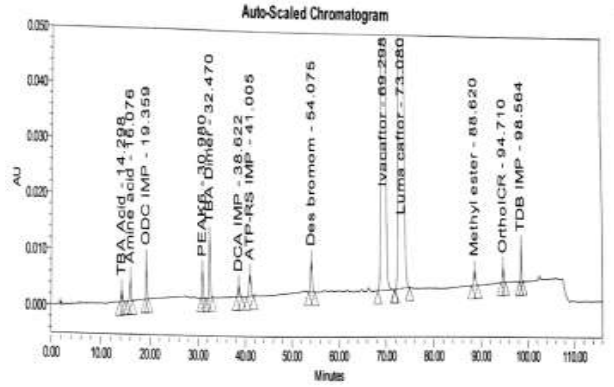


Figure No.:27 Typical Chromatogram of spiked samples

Discussion: Retention times of Lumacaftor and Ivacaftor were 73.080min and 69.298min respectively. No interfering peaks were found in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

2. Precision:

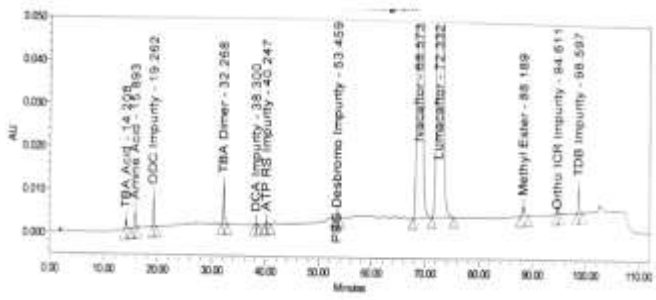


Figure No.:28 Precision chromatogram spiked

Discussion: From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated for two drugs. % RSD obtained as 0.682%and 0.775% respectively for Ivacaftor and Lumacaftor. As the limit of Precision was less than "2" the precision was passed in this method.

Solution stability: The spiked sample solution was placed on bench top and refrigerator for 12hrs, 18hrs, 24hrs, 32hrs and 48hrs and run the sample in same optimized condition

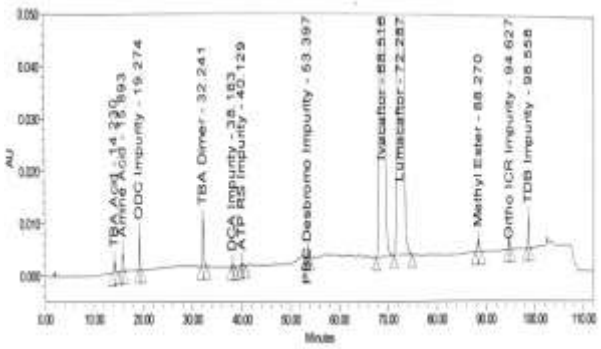


Figure No.:34 Chromatogram of Drug product spiked solution stability(48hr)- Bench top

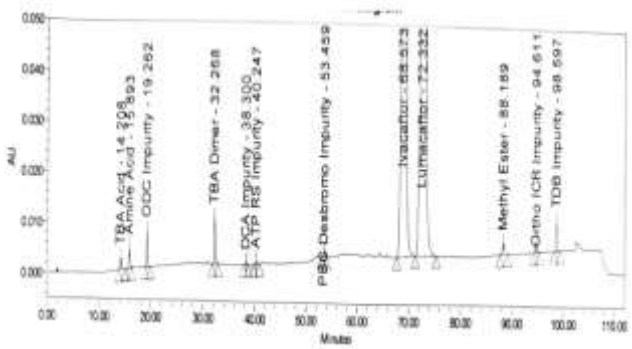


Figure No.:35 Chromatogram of Drug product spiked solution stability(48hr)- Refrigerator

Discussion: Retention times of Lumacaftor and Ivacaftor were 73.080min and 69.298min respectively. The solution stability of the sample and standard solutions was found to be stable up to 48hrs on both bench top and refrigerator conditions.

Accuracy:

S.No	NAME	% Level	Amount Spiked (mg/mL)	Amount recovered (mg/mL)	% Recovery	Mean %Recovery
1	TBA acid	50%	0.098	0.095	103.2	104.4
			0.097	0.095	102.1	
			0.097	0.095	102.1	
		100%	0.198	0.191	103.7	
			0.195	0.191	102.1	
			0.206	0.191	107.9	
		150%	0.315	0.299	105.4	
			0.329	0.299	108	
			0.314	0.299	108	
2	Amine acid	50%	0.107	0.105	101.9	101.7
			0.106	0.105	101	
			0.111	0.105	105.7	
		100%	0.210	0.210	100	
			0.210	0.21	100	
			0.211	0.21	100.5	
		150%	0.322	0.316	101.9	
			0.322	0.316	101.9	
			0.324	0.316	101.9	
3	TBA dimer	50%	0.105	0.105	100	99.8
			0.105	0.105	100	
			0.108	0.105	102.9	
		100%	0.208	0.211	98.6	
			0.208	0.211	98.6	
			0.212	0.211	100.5	
		150%	0.317	0.316	100.3	
			0.313	0.316	99.1	
			0.310	0.316	98.1	
4	DCA	50%	0.097	0.098	99	105
			0.102	0.098	104.1	
			0.093	0.098	94.9	
		100%	0.201	0.196	102.6	
			0.207	0.196	105.6	
			0.207	0.196	105.6	
		150%	0.329	0.293	112.3	
			0.330	0.293	112.6	
			0.318	0.293	108.5	
5	PBC des bromo	50%	0.089	0.090	98.9	99
			0.077	0.090	87.3	
			0.085	0.090	94.4	
		100%	0.180	0.180	100	
			0.179	0.180	99.4	
			0.177	0.180	98.3	
		150%	0.285	0.271	105.2	
			0.270	0.271	99.6	

			0.292	0.271	107.7	
6	Methyl ester	50%	0.107	0.103	103.9	103.3
			0.105	0.103	01.9	
			0.104	0.103	101	
		100%	0.217	0.206	105.3	
			0.215	0.206	104.4	
			0.213	0.206	103.4	
		150%	0.314	0.309	101.6	
			0.321	0.309	103.9	
			0.322	0.309	104.2	
7	TDB	50%	0.117	0.107	109.3	103.4
			0.116	0.107	108.4	
			0.119	0.107	111.2	
		100%	0.211	0.214	98.6	
			0.212	0.214	99.1	
			0.217	0.214	101.4	
		150%	0.325	0.321	101.2	
			0.322	0.321	100.3	
			0.324	0.321	100.3	
8	ODC	50%	0.104	0.109	95.4	97.1
			0.104	0.109	95.4	
			0.107	0.109	98.2	
		100%	0.207	0.217	95.4	
			0.207	0.217	95.4	
			0.212	0.217	97.7	
		150%	0.324	0.326	99.4	
			0.319	0.326	97.3	
			0.322	0.326	98.8	
9	ATP RS	50%	0.087	0.09	96.7	102.3
			0.076	0.09	86.7	
			0.091	0.09	101.1	
		100%	0.130	0.181	105	
			0.82	0.181	100.6	
			0.180	0.181	99.4	
		150%	0.308	0.274	112.4	
			0.285	0.274	107.7	
			0.305	0.274	111.3	
10	Ortho ICR	50%	0.102	0.097	105.2	99.7
			0.096	0.097	99	
			0.094	0.097	96.9	
		100%	0.184	0.193	95.3	
			0.179	0.193	92.7	
			0.179	0.193	92.7	
		150%	0.306	0.290	105.5	
			0.301	0.290	103.8	
			0.309	0.290	106.6	

CONCLUSION:

Extremely easy, rapid, exact, perfect and reproducible RP-HPLC technique was flourished for the stability indicating study of related substances of Lumacaftor and Ivacaftor. The mobile phase used was simple and the improved strategy can be employed for the routine analysis of related substances of Lumacaftor and Ivacaftor.

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