IJBPAS, November, 2024, 13(11): 5457-5466

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

A COMPREHENSIVE REVIEW OF ANALYTICAL TECHNIQUES FOR LUMACAFTOR AND IVACAFTOR IN PHARMACEUTICAL DOSAGE FORMS

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Received 24th Sept. 2023; Revised 25th Oct. 2023; Accepted 12th Jan. 2024; Available online 1st Nov. 2024

https://doi.org/10.31032/IJBPAS/2024/13.11.8391

ABSTRACT

The current work enumerates all developed analytical methods for the estimation of the combination of Lumacaftor and Ivacaftor in pharmaceutical dosage forms, used for the treatment of cystic fibrosis. Cystic Fibrosis is a multi-organ disorder caused by dysfunctional CFTR gene mutations. It mostly arises in the lungs but also impacts some body parts such as the colon, liver, kidneys, and pancreas. The CFTR protein mutation leads to impaired transport of fluid, Bicarbonate, Na^+ & Cl^- ions, leading to the thickening of mucus on the cell membrane and cellular damage. Most researchers have done their work on Lumacaftor and Ivacaftor regarding Pharmacology, Mode of Action, and Synthesis but in the present work, we have focused on different types of analytical techniques used to determine the Lumacaftor and Ivacaftor in pharmaceutical dosage form. We have reviewed various analytical methods, such as HPLC, RP-HPLC, UP-HPLC, and LC-MS techniques that have been developed for the combination of Lumacaftor and Ivacaftor. According to the literature, most of the elution was carried out by the Isocratic method using the mobile phase which includes Acetonitrile, water, and buffer. The HPLC method has been the most widely used analytical technique for the past decade.

Keywords: Cystic Fibrosis, HPLC, Ivacaftor, LC-MS, Lumacaftor

INTRODUCTION:

The current study concentrates on the various methods used to analyze the combination of Lumacaftor and Ivacaftor in different dosage forms were developed. The combination of Lumacaftor and Ivacaftor is an oral medication used to treat cystic fibrosis by modifying the CFTR protein in which Lumacaftor enhances the number of CFTR proteins that reach to cell's outer membrane including Ivacaftor enables dysfunctional chloride channels to open **[1, 2]**. The medication is available in the market by the name of Orkambi with different types of dosage forms, like tablets and granules **[2, 3]**. Orkambi, the formulation for individuals with cystic fibrosis who have two copies of the F508del mutation, was approved on 2 July 2015 for those over 12 years old. FDA amended this on 28 September 2016 to include children aged 6-11. Approval was extended to children aged 2-5 on 7 Aug. 2018 and to infants aged 12-24 months on 2 Sep. 2022 **[4]**. Patients suffering from cystic fibrosis carrying the F508del alteration in the CFTR gene may benefit from taking this medication **[5]**.

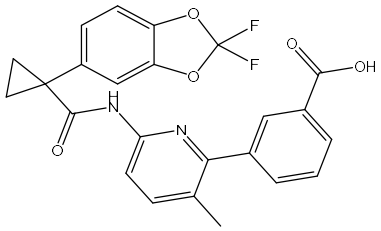
Cystic fibrosis:

Cystic fibrosis is a genetic disorder caused by mutations in the CFTR gene, resulting in impaired ion transport across cell membranes. It is a multiorgan disease, that mainly targets the respiratory organ and also can impact the pancreas, liver, kidneys, and intestine **[6]**. This is mainly caused by F508del, G551D CFTR protein mutation **[7]**. The CFTR proteins are located in the smooth muscles and epithelial cell membrane which is essential for the transport of anions, mainly chloride, and bicarbonate, across the epithelial cells. The mutation of the CFTR gene renders the malfunctioning of epithelial fluid, Na^+, and Cl^- in the lung, pancreas, and other organs causing cystic fibrosis **[8]**. The abnormalities in CFTR proteins might affect the mucus components and cause aberrant transport of fluids, bicarbonate, and H_2O. This results in forming a film of thick and sticky mucus that cilia can't remove easily, eventually leading to impaired mucociliary function and persistent infections of the lungs, intestine, and pancreas **[9, 10]**.

Physiochemical Properties:

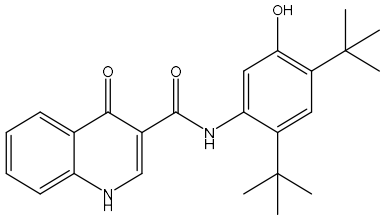
Lumacaftor is a non-hygroscopic white to off-white crystalline solid that melts at 200-205^{0}C and boils at 653.0 ± 55.0°C **[11, 12]**. Lumacaftor is chemically 3-[6-[[1-(2,2-difluoro-1,3benzodioxol-5yl) cyclopropane carbonyl] amino]-3methylpyridin-2-yl] benzoic acid which is sparingly soluble in n-butanol, practically insoluble in water, and freely soluble in 2-methyltetrahydrofuran and formic acid. It is also soluble in hydrogen ion regulators at pH 1-8 and gut juice **[11, 13]**. The molecular formula and molecular weight of the Lumacaftor are

C24H18F2N2O5 and 452.4g/mol, respectively **[13]**. It has a half-life of approximately 26 hours and is predominantly excreted unchanged in feces **[14]**.



Lumacaftor

Ivacaftor is a slightly hygroscopic white to off-white crystalline solid that melts at 212-215^{0}C and boils at 550.4^{0}C **[11, 15]**. It is also known as N-(2,4-ditert-butyl-5hydroxyphenyl)-4-oxo-1Hquinoline-3-carboxamide which is practically insoluble in water and buffers with pH between 1.0 and 7.0, but partially soluble in ethanol, methanol, and acetone. It is soluble in 2-methyl tetrahydrofuran **[11, 15]**. The molecular formula and molecular weight for Ivacaftor are $C_{24}H_{28}N_2O_3$ and 392.5g/mol respectively **[15]**. It has a half-life of approximately 12 hours and is metabolized in the liver and mostly excreted in feces **[14]**.



Ivacaftor

ANALYTICAL FINDINGS:

According to the analytical findings, we have found some Lumacaftor and ivacaftor estimation methods in pharmaceutical dosage forms, as listed in the table **(Table A)**. These techniques include HPLC, RP-HPLC, UP-HPLC, and LC-MS which are used for precise and accurate determination and validation studies to meet the quality attributes of the dosage forms.

Table 1: The Reported Analytical Methods of Lumacaftor and Ivacaftor.

Sr. No.	Drugs	Methods	Description	Ref. No.
1	Lumacaftor and Ivacaftor	RP-HPLC	Mobile Phase: 0.1% OPA: Acetonitrile (40: 60) v/v Flow Rate: 1 ml/min Stationary Phase: Agilent Eclipse column (4.6 x 150mm, 5µm) Retention Time: Lumacaftor = 1.857 min, Ivacaftor = 2.681 min Wavelength: 290 nm	[5]
2	Lumacaftor and Ivacaftor	RP-HPLC	Mobile Phase: ACN, Methanol,1 N of OPA (30:10:60) v/v Flow Rate: 1ml/min Stationary Phase: Ineertsil ODS (4.6×250 mm) 5µm Retention Time: Lumacaftor = 3.101 min, Ivacaftor = 4.205 min Wavelength: 254 nm	[2]
3	Ivacaftor and Tezacaftor	RP-HPLC	Mobile Phase: methanol and 0.05% formic acid (95:5) v/v Flow Rate: 1 mL/min Stationary Phase: Inspire C18, (4.6 × 250 mm, 5µm) Retention Time: Ivacaftor = 3.009 min, Tezacaftor = 3.806 min Wavelength: 235 nm	[16]
4	Ivacaftor	RP-HPLC	Mobile Phase: Buffer-acetonitrile (30:70) v/v Flow Rate: 1ml/min Stationary Phase: C8 Retention Time: 3.685 min Wavelength: 225 nm	[17]
5	Lumacaftor and Ivacaftor	RP-UPLC	Mobile Phase: Phosphate Buffer (pH 3.5) + Acetonitrile (75:25) v/v Flow Rate: 0.5ml/min Stationary Phase: Waters AcquityC18(50mm x2.1 mm ID) 1.8µm Retention Time: Ivacaftor = 1.827 min, Lumacaftor = 3.577 min Wavelength: 265 nm	[18]

6	**Lumacaftor and Ivacaftor**	**RP-HPLC**	**Mobile Phase: Phosphate buffer + Acetonitrile (55:45) v/v Flow Rate: 1ml/min Stationary Phase: Inertsil ODS 3V, C18(150x4.6 ID) 5µm Retention Time: Ivacaftor = 4.003min, Lumacaftor = 2.927 min Wavelength: 225 nm**	**[19]**
7	**Lumacaftor and Ivacaftor**	**RP-HPLC**	**Mobile Phase: Methanol+ Water (65:35) v/v Flow Rate: 1ml/min Stationary Phase: C18 (4.6×150mm, 5µ) Retention Times: Lumacaftor 2.460 min, Ivacaftor = 4.312 min Wavelength: 270 nm**	**[7]**
8	**Lumacaftor and Ivacaftor**	**RP-HPLC**	**Mobile Phase: 0.1%Formic acid: Acetonitrile (50:50) v/v Flow Rate: 1 ml/min Stationary Phase: Symmetry C18 column (4.6 x 150mm, 5µm) Retention Time: Lumacaftor = 2.186 min, Ivacaftor = 2.807 min Wavelength: 285 nm**	**[20]**
9	**Tezacaftor and Ivacaftor**	**RP-UPLC**	**Mobile Phase: 0.1 % orthophosphoric acid and acetonitrile in the proportion of 50:50 v/v Flow Rate: 0.3 ml/min Stationary Phase: HSS C18 (100×2.1 mm), 1.7 µ Retention Time: Ivacaftor = 0.531 min, Tezacaftor = 1.072 min Wavelength: 292 nm**	**[21]**
10	**Lumacaftor and Ivacaftor**	**RP-HPLC**	**Mobile Phase: Acetonitrile: OPA (45:55) v/v Flow Rate: 1 ml/min Stationary Phase: Xterra C18 column (4.6 x 250mm, 5µm) Retention Time: Lumacaftor = 2.323 min, Ivacaftor = 4.325 min Wavelength: 255 nm**	**[22]**
11	**Tezacaftor and Ivacaftor**	**RP-HPLC**	**Mobile Phase: buffer 0.01N potassium dihydrogen ortho-phosphate: acetonitrile (50:50) v/v Flow Rate: 1ml/min Stationary Phase: Discovery 250 x 4.6 mm, 5µ Retention Time: Ivacaftor = 2.373 min, Tezacaftor = 2.967 min Wavelength: 290 nm**	**[23]**
12	**Lumacaftor and Ivacaftor with its metabolites**	**HPLC LC-MS/MS**	**Mobile Phase: ACN/H2O:0.1% formic acid (60:40) v/v Flow Rate: 1 mL/min Stationary Phase: Waters C8 (3.9 × 150 mm id, 5µm) Retention Time: Ivacaftor = 6.2 min, Hydroxymethyl-Ivacaftor (M1) = 2.4 min Ivacaftor carboxylate (M6) = 2.1 min Lumacaftor = 5.4 min Wavelength: Ivacaftor = 309 nm Lumacaftor = 295 nm Ion spray Voltage: 4.5 kV Collision Energy: 295.9 V Collision Gas: Argon Nebulizing Gas: nitrogen at 3 L/min Drying gas Temperature: 20 L/min Desolvation Temperature: 250ºC with a heat block temperature of 400ºC. Lens Voltage Q3: −22 V Ion Transitions (m/z): Ivacaftor = 392.49–393, Hydroxymethyl-Ivacaftor (M1) = 408.49–409, Ivacaftor carboxylate (M6) = 422.47–423 and Lumacaftor = 452.40–453.**	**[24]**
13	**Lumacaftor and Ivacaftor**	**RP-HPLC**	**Mobile Phase: 80% buffer (3.4g of KH_2PO_4 + 1000ml HPLC water, (P^H was adjusted with OPA up to 3.0) 20% Acetonitrile Flow Rate: 1ml/min Stationary Phase: Inertsil ODS (4.6 x 250mm, 5µm) Retention Time: Lumacaftor = 3.607 min, Ivacaftor = 5.141min Wavelength: 259 nm**	**[25]**
14	**Ivacaftor and Tezacaftor**	**RP-UPLC**	**Mobile Phase: Buffer and Acetonitrile (50:50A V/V) Flow Rate: 0.3ml/min Stationary Phase: CHS C18 (100 x 2.1 mm, 1.7µ) Retention Time: Ivacaftor = 0.620 min, Tezacaftor = 1.155 min Wavelength: 292 nm**	**[26]**
15	**Lumacaftor in the presence of Ivacaftor**	**HPLC**	**Mobile Phase: Mobile phase A: 0.1% (v/v) formic acid in the water, mobile phase B: 0.1% (v/v) formic acid in acetonitrile Flow Rate: 1 ml/min Stationary Phase: Supelco Ascentis Express F5 reversed-phase (2.7µm, 4.6 mm id × 100 mm) Retention Time: 5.31 min Wavelength: 216 nm Nebulizing Gas Flow: 1.5 L/min, Drying Gas temperature: 200 KPa,**	**[27]**

		LCMS-IT-TOF	**Desolvation Temperature: 200◦C (same as heat block temperature). Detector Voltage of TOF: 1.6 kV Ion Transitions (m/z): 453.1235–453.1257**	
16	**Ivacaftor and Tezacaftor**	**RP-HPLC**	**Mobile Phase: Phosphate buffer and Acetonitrile (40:60) v/v Flow Rate: 1ml/min Stationary Phase: Inertsil ODS C18 (250 x 4.6 mm, 5µ) Retention Time: Ivacaftor = 3.285 min, Tezacaftor = 4.635 min Wavelength: 259 nm**	**[28]**
17	**Ivacaftor, Hydroxymethyl Ivacaftor, Ivacaftor carboxylate, Lumacaftor, Tezacaftor**	**LC-MS/MS**	**Mobile phase: A: 0.1% v/v formic acid and 0.05% v/v ammonium formate in ultrapure water, B: 0.1% v/v formic acid and 0.05% v/v ammonium formate in ACN Flow Rate: 0.5 ml/min. Stationary Phase: C18 HyPURITY (50 × 2.1 mm, 1.9 µm) Retention Time: Ivacaftor = 2.64 min, Hydroxymethyl Ivacaftor = 1.54 min, Ivacaftor carboxylate = 1.42 min, Lumacaftor = 2.56 min, and Tezacaftor = 1.88 min Ion Spray Voltage: 5500 V Collision Energy: Ivacaftor and its metabolites = 19 V, Lumacaftor = 49 V, Tezacaftor = 29 V Ion Transitions (m/z): For Fragment: Ivacaftor = 337.10, Hydroxymethyl Ivacaftor = 353.20, Ivacaftor carboxylate = 367.10, Lumacaftor = 131.10, and Tezacaftor = 449.10 For Precursor: Ivacaftor = 393.20, Hydroxymethyl Ivacaftor = 409.15, Ivacaftor carboxylate = 423.15, Lumacaftor = 453.05, and Tezacaftor = 521.10**	**[29]**
18	**Tezacaftor and Ivacaftor**	**RP-UPLC**	**Mobile Phase: sonicated 0.1 percent Orthophosphoric acid buffer: Acetonitrile (60:40) v/v Flow Rate: 0.3ml/min Stationary Phase: CHS C18 (50 X 2.1 mm, 1.8 µm) Retention Time: Tezacaftor = 0.508 min, Ivacaftor = 0.876 min Wavelength: 292 nm**	**[30]**
19	**Tezacaftor and Ivacaftor**	**RP-HPLC**	**Mobile Phase: Buffer, Methanol and Acetonitrile (42:27:31) v/v/v Flow Rate: 1.2 ml/min Stationary Phase: Symmetry Shield RP18 (4.6 mm × 250 mm, 5µm) Retention Time: Tezacaftor = 3.75 min, Ivacaftor = 6.04 min Wavelength: 257 nm**	**[31]**
20	**Tezacaftor, Ivacaftor and Elexacaftor**	**RP-HPLC**	**Mobile Phase: 0.1% orthophosphoric acid: acetonitrile (60:40) v/v Flow Rate: 1.0ml/min Stationary Phase: Ascentis C18, 150 × 4.6 mm, 5µ. Retention Time: Tezacaftor = 2.336 min, Ivacaftor = 2.901 min and Elexacaftor = 3.517 min Wavelength: 260.0 nm**	**[32]**
21	**Colistin, Ivacaftor and Ciprofloxacin**	**LC-MS/MS**	**Mobile Phase: 0.1% (V/V) aqueous formic acid (solvent A) and 0.1% (V/V) formic acid in acetonitrile (solvent B) Flow Rate: 0.4 mL/min Stationary Phase: Kinetex C18 (2.6 mm, 100 Å, 50 mm × 3 mm) Retention Time: Colistin A = 0.630 min, Colistin B = 0.627 min, Ivacaftor = 4.728 min, and Ciprofloxacin = 3.357 min Collision Energy: Colistin AandB = 20 V, Ivacaftor =8 V, and Ciprofloxacin = 17 V Capillary Voltage: 4000 V Nozzle Voltage: 1000 V Ion Transitions (m/z): Quantitative transition: Colistin A = 585.5–101.1, Colistin B = 578.5–101.1, Ivacaftor = 393.2–337.2, and Ciprofloxacin = 332.2–314.2. Qualitative transition: Colistin A = 585.5–241.1, Colistin B = 578.5–227.2, Ivacaftor = 393.2–172.1, and Ciprofloxacin = 332.2–288.2**	**[33]**
22	**Ivacaftor and Tezacaftor**	**RP-HPLC**	**Mobile Phase: water: acetonitrile (50:50) v/v Flow Rate: 1.0 ml/min Stationary Phase: Kromisil C18 (150 x 4.6 mm, 5 µm) Retention Time: Ivacaftor = 2.089 min, Tezacaftor = 2.434 min Wavelength: 292 nm**	**[34]**
23	**Ivacaftor, Tezacaftor, and Elexacaftor**	**LC-MS/MS**	**Mobile Phase: A: 0.1% formic acid (FA) in water and B: 0.1% formic acid in acetonitrile. Flow Rate: 1ml/min Stationary Phase: Accucore Polar Premium (50 mm × 2.1 mm, i.d. 2.6 µm, Thermo Scientific, Milan, Italy) Retention Time: Ivacaftor = 2.82 min, Tezacaftor = 2.44 min and Elexacaftor = 2.77 min Ion Spray Voltage: 3500 V Collision Gas Pressure: 1.5 m Torr Collision Gas: Argon Nebulising Gas: Nitrogen**	**[35]**

			Vaporizer and Capillary Temperature: 350^{0}C Ion Transitions: Multiple Reaction Monitoring: Ivacaftor = 393.3–144.1; 319.1, Tezacaftor = 521.3–387.0; 503.2, Elexacaftor = 598.2–328.9; 423.3. Deuterated IS: Ivacaftor d-9 = 402.5–328.1, Tezacaftor d-4 = 525.2–391.1, Elexacaftor d-3 = 601.3–502.2	
24	Ivacaftor and Tezacaftor	HPLC	Mobile Phase: Ammonium Acetate Buffer (ph.- 5.0) and Acetonitrile (60:40) v/v Flow Rate: 1ml/min Stationary Phase: Sunfire C 18 (150×4.6mm,3.5µ id) Retention Time: Ivacaftor = 1.8 min, Tezacaftor = 3.5 min Wavelength: 260 nm	[36]
25	Lumacaftor and Ivacaftor	RP-HPLC	Mobile Phase: Phosphate buffer (pH was adjusted to 4.5 with O-phosphoric acid) and Acetonitrile (40:60) v/v Flow Rate: 1.0 ml/min Stationary Phase: C18 Phenomenex (250 × 4.6 mm, 5 µ) Retention Time: Lumacaftor = 2.857min, Ivacaftor = 6.329 min Wavelength: 255 nm	[37]
26	Lumacaftor and Ivacaftor	RP-HPLC	Mobile Phase: 0.1% O.P.A and methanol (30:70) v/v Flow Rate: 1ml/min Stationary Phase: Agilent Eclipse XDB-C8 (4.6×150mm,5µm) Retention Time: Lumacaftor = 1.8 min, Ivacaftor = 2.6 min Wavelength: 280 nm	[38]

CONCLUSION:

This review reveals various analytical techniques that have been developed and validated for the determination of Lumacaftor and ivacaftor in various dosage forms, alone or in combination with other drugs. These methods include HPLC, RP-HPLC, UP-HPLC, and LC-MS. The most employed technique was found to be RP-HPLC, However, the LC-MS method was also significantly utilized to analyze the Ivacaftor metabolites alongside other medications. This review included an overview of the most recent, cutting-edge analytical techniques for the determination of Lumacaftor and Ivacaftor, which will be helpful for future studies on this combination or with other drugs. Comprehending the important dissolving and carrier fluids along with accessible tools in the analytical laboratory would also be helpful for future researchers and readers.

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