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COMPREHENSIVE REVIEW OF ANALYTICAL TECHNIQUES FOR THE ESTIMATION OF IVACAFTOR IN PHARMACEUTICALDOSAGE FORMS

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ABSTRACT

Ivacaftor, a cystic fibrosis transmembrane conductance regulator (CFTR) potentiator, has garnered significant attention due to its therapeutic efficacy in treating cystic fibrosis. Accurate quantification of Ivacaftor in pharmaceutical formulations is essential for quality control and regulatory compliance. This review provides a comprehensive overview of the various analytical methods developed for the estimation of Ivacaftor, with a particular focus on UV spectrophotometry, high-performance liquid chromatography (HPLC), and liquid chromatography–mass spectrometry (LC-MS). UV spectrophotometric methods are highlighted for their simplicity, cost-effectiveness, and suitability for routine analysis, although limited by lower sensitivity and specificity. HPLC methods, particularly reverse-phase HPLC (RP-HPLC), offer greater accuracy, reproducibility, and precision, making them ideal for stability-indicating and validation studies. LC-MS methods are recognized for their high sensitivity, selectivity, and capability in bioanalytical applications, especially for pharmacokinetic and metabolic studies. The review discusses method development strategies, validation parameters, and application areas of each technique, offering insights into their strengths and limitations. Collectively, these analytical methods play a pivotal role in the effective monitoring of Ivacaftor in both bulk and dosage forms, ensuring therapeutic efficacy and safety.

1. INTRODUCTION

The Respiratory, gastrointestinal, reproductive, and endocrine systems are all impacted by the genetic illness known as cystic fibrosis (CF). It results in the accumulation of unusually thick mucus, which obstructs the flow. Any one of a number of abnormalities in the cystic fibrosis transmembrane conductance regulator (CFTR) protein, including the F508del mutation and the G551D mutation, can result in cystic fibrosis (CF). One Several daily drugs are necessary for this life-limiting illness in order to prolong life and improve quality of life. CF has been treated with a variety of traditional regimens, such as multivitamins, mucolytics, antibiotics, bronchodilators, anti-inflammatory drugs, and supplements containing pancreatic enzymes. Ivacaftor (Potentiator) are new drug used for the treatment of cystic fibrosis (1). Ivacaftor (ICF) is an aromatic amide; its molecular formula is $C_{24}H_{28}N_2O_3$, and its molecular weight is 392.499. It is chemically known as N-(2,4-di tert-butyl-5-hydroxyphenyl)-4-oxo-1H-quinoline-3-carboxamide (Figure 2). The white to offwhite powder known as Ivacaftor is essentially insoluble in water (<0.05 mg/mL). Ivacaftor is the first medication to address the underlying cause of the illness instead of its symptoms. Ivacaftor promotes chloride transport by amplifying the channel-open probability (also known as gating) of the G551D-CFTR protein, which is a potentiator of the CFTR protein, a chloride channel found at the surface of epithelial cells in several organs (2-3).

Drug Profile:

Ivacaftor (also known as Kalydeco or VX-770) is a drug used for the management of Cystic Fibrosis (CF). It is manufactured and distributed by Vertex Pharmaceuticals. It was approved by the Food and Drug Administration on January 31, 2012 (4), and by Health Canada in late 2012 (6). Ivacaftor is administered as a monotherapy and also administered in combination with other drugs for the management of CF (5,7).

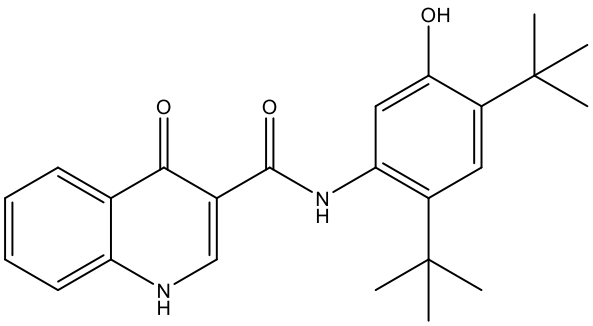

Fig-1: Structure of Ivacaftor.

Table -1: Physical and Chemical Properties of Ivacaftor.

Molecular formula	**$C_{24}H_{28}N_2O_3$**
IUPAC Name	N- (2,4ditert-butyl-5-hydroxyphenyl)-4-oxo-1Hquinoline-3-carboxamide.
Molecular weight	392.499 g/mol
Melting point	212-215 °C
Boiling point	550.5±50.0 °C at 760 mmHg
Density	1.2±0.1 g/cm3
Flash Point	286.7±30.1 °C
Solubility	Freely Soluble in Methanol, Ethanol, Acetonitrile, DMSO Insoluble in water
Physical Description	white to off-white powder Amorphous
Category	cystic Fibrosis Transmembrane conductance Regulator (CFTR) potentiator.
PKa	9.40 and 11.60
Log P	6.34
Dose	75 mg 150 mg
Dosage form	Tabletsa and Capsules
Vapour pressure	0.0±1.5 mmHg at 25°C
Index of Refraction	1.606
Storage condition	2~8°C

Mechanism of action :(8)

A wide variety of CFTR mutations correlate to the Cystic Fibrosis phenotype and are associated with differing levels of disease severity. The most common mutation, affecting approximately 70% of patients with CF worldwide, is known as F508del-CFTR or delta-F508 (ΔF508), in which a deletion in the amino acid phenylalanine at position 508 results in impaired production of the CFTR protein, thereby causing a significant reduction in the amount of ion transporter present on cell membranes

Pharmacokinetics:

Absorption : In the digestive system, ivacaftor is efficiently absorbed(9). Peak plasma concentrations were attained 4 hours after ivacaftor was administered with fat-containing meals, with a maximum concentration (Cmax) of 768 ng/mL and an AUC of 10600 ng hr/mL. Because fat-containing foods boost absorption by around 2.5 to 4 times, it is advised to take Ivacaftor with them (10).

Volume of Distribution: In healthy individuals in a fed condition, the mean (±SD) for apparent volume of distribution was 353 L following oral administration of 150 mg every 12 hours for 7 days (10).

Protein Binding: Ivacaftor, a cystic fibrosis transmembrane conductance regulator (CFTR) potentiator, exhibits high plasma protein binding (99%), primarily to albumin and alpha-1 acid glycoprotein (AAG). This extensive binding limits its free, active concentration in the bloodstream, influencing its pharmacokinetics and drug interactions. Since only the unbound fraction is pharmacologically active, changes in protein levels (e.g., AAG fluctuations during inflammation) may alter ivacaftor's efficacy and safety. This binding also contributes to its long half-life (~12 hours) and potential interactions with other highly protein-bound drugs. (9).

Metabolism : In humans, ivacaftor undergoes substantial metabolism. Clinical and in vitro research shows that CYP3A is the primary metabolizer of Ivacaftor. The two main metabolites produced by this metabolism are M1 and M6. Despite having only around one-sixth the action of the parent molecule ivacaftor, M1 is nevertheless regarded as pharmacologically active. However, because M6 only has a fraction of the original compound's impact, it is not regarded as pharmacologically active (9,10).

Route of Elimination : Ivacaftor is primarily excreted in the feces following oral treatment due to metabolic conversion. This excretion accounts for 87.8% of the dose. The majority of the eliminated dose is accounted for by the metabolites M1 and M6, which make up 22% and 43% of the total eliminated dose, respectively. Ivacaftor exhibits very little urine excretion when taken as is oral (9,10).

Half life: After a single dose of Ivacaftor, the apparent terminal half-life in a clinical investigation was roughly 12 hours. 18 According to one report, the half-life is between 12 and 14 hours (9).

Clearance: The apparent clearance (CL/F) of ivacaftor after a 150 mg dose in healthy subjects was 17.3 ± 8.4 L/hr, indicating moderate variability in drug elimination. Since CL/F reflects both systemic clearance (CL) and oral bioavailability (F), this value accounts for factors like first-pass metabolism and intestinal absorption. The relatively high standard deviation (±8.4) suggests interindividual variability, possibly due to differences in CYP3A4 metabolism (the primary enzyme responsible for ivacaftor breakdown) or protein binding effects**. This parameter is crucial for determining appropriate dosing regimens in different patient populations. (10)

Toxicity : LD50 information is not readily available. There have been no reports of overdose with ivacaftor, but when given with tezacaftor, the highest clinical dose lead to diarrhea and dizziness. Provide supportive measures in cases of a suspected overdose. No antidote is available at this time (5).

Pharmacodynamics : (11)

Ivacaftor is a "potentiator" of CFTR, meaning it increases the probability that the defective channel will be open and allow chloride ions pass through the channel pore

Chemical Classification : (12)

Description : This substance is a member of the aromatic anilide class of organic chemicals. These are aromatic compounds having an anilide group that has an aromatic group in place of the carboxamide group. Their general structure is RNC(=O)R', where R stands for aryl group and R for benzene.

Kingdom :Organic compounds

Super Class :Benzenoids

Class :Benzene and substituted derivatives

Sub Class :Anilides

Direct Parent : Aromatic anilides

Alternative Parents: Quinoline-3-carboxamides / Hydroquinolones / Hydroquinolines / Pyridinecarboxylic acids and derivatives / Phenylpropanes / 1-hydroxy-2-unsubstituted benzenoids / Vinylogous amides / Heteroaromatic compounds / Secondary carboxylic acid amides / Azacyclic compounds

Substituents :1-hydroxy-2-unsubstituted benzenoid / Aromatic anilide / Aromatic heteropolycyclic compound / Azacycle / Carboxamide group / Carboxylic acid derivative / Dihydroquinoline / Dihydroquinolone / Heteroaromatic compound / Hydrocarbon derivative show 15 more

Molecular Framework: Aromatic heteropolycyclic compounds

External Descriptors: Monocarboxylic acid amide, phenols, aromatic amide, quinolone

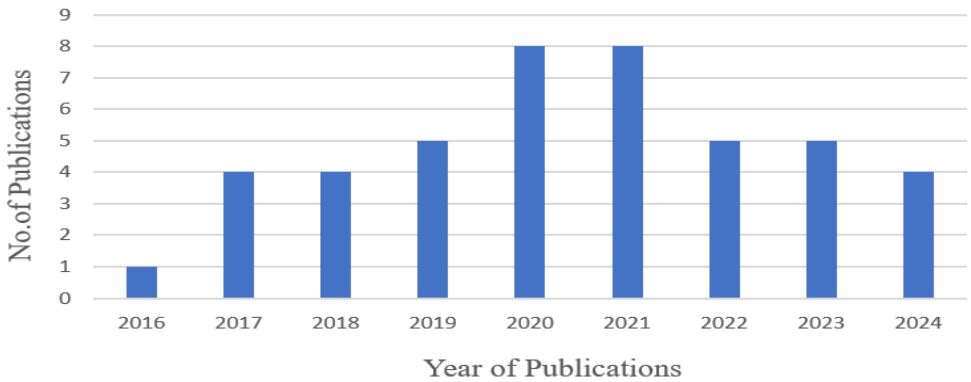

Fig-2: Reported Ivacaftor Publications from 2023-2025.

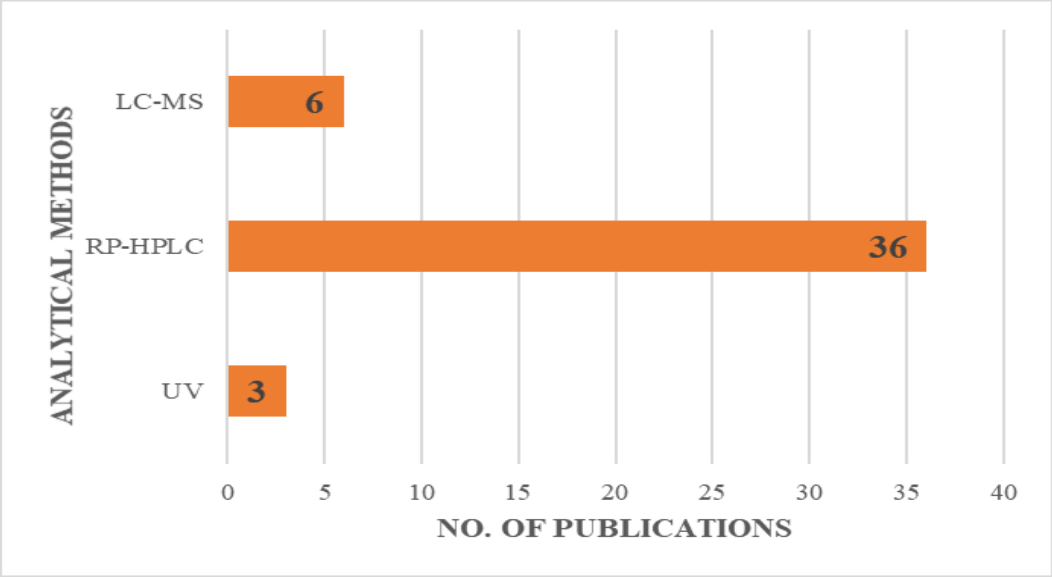

Fig-3: Reported Analytical methods on Ivacaftor.

Spectroscopic Estimation

Quantitative analysis of pharmaceutical compounds such as Ivacaftor, a medication used in the treatment of cystic fibrosis, significantly relies on the application of UV spectroscopy. This analytical technique has proven to be an essential tool for researchers aiming to evaluate these drugs both in their pure (bulk) form and in various pharmaceutical dosage forms. UV spectroscopy enables the detailed examination of a drug's optical properties, including absorbance, transmittance, and reflectance, across a range of concentrations. By systematically studying these properties, scientists are able to assess the linearity of the drug's response and identify its specific spectral characteristics. As a result, UV spectroscopy not only aids in the accurate quantification of Ivacaftor but also supports the overall development and validation of analytical methods used in quality control and pharmaceutical research.(13)

Table -2: Spectrophotometric Estimation of Ivacaftor.

S. No	Type of method	Solvent to prepare drug solution	Wavelength (nm)	Reaction condition	Linearity (ug/ml)	Co-efficient constant	LOD & LOQ	Applications	References
1.	UV	Ethanol	202	Weigh 10 mg ivacaftor, dissolve in	1-5	0.99973	0.016, 0.16	Tablets	14

				methanol in 100 mL flask (100 µg/mL). Dilute 1 mL to 10 mL for working solution (10 µg/mL).scanned from 200-400 nm to find the λmax					
2.	UV (Simultaneous Equations method) vierodt's method.	Ethanol	351	10 mg of ivacaftor was dissolved in ethanol and diluted to 100 ml to prepare a 100 µg/ml stock solution. A 10 µg/ml working solution was scanned from 200–400 nm to find the λmax.	5-30	1		Tablets	15
3.	UV (absorbance ratio method	Ethanol	274	10 mg of ivacaftor was dissolved in ethanol and diluted to 100 ml to get a 100 µg/ml stock solution. A 10 µg/ml	5-30	0.998		Tablets	15

				solution was scanned from 200–400 nm to find the λmax.					
4	UV	Ethanol	310	10 mg of Ivacaftor was dissolved in ethanol, sonicated for 5 minutes, and diluted to 10 ml to get a 1 mg/ml stock solution. Then, 1 ml of this stock was diluted to 10 ml to prepare a 10 µg/ml solution, which was scanned from 200–400 nm in the UV range.	5-25	0.9989	0.374 & 1.135	Tablets	16

Pharmaceutical Applications of Spectrophotometric Methods

Spectrophotometric techniques are frequently used in pharmaceutical applications because of their adaptability and efficiency in examining a wide range of medicinal ingredients and formulations.(17-21)

Drug assay in bulk and formulations

Applications: Spectrophotometric methods are extensively used for the analysis of Active Pharmaceutical Ingredients (APIs) in both bulk and prepared dosage forms such tablets, capsules, and injections.

Example: UV-visible spectrophotometry is frequently used to test medications such as aspirin, ibuprofen, and paracetamol in order to ascertain their concentration and guarantee that formulations contain the proper amount.

Dissolution studies:

Application: Drugs in solid dosage forms, like tablets, can have their rate of disintegration tracked using spectrophotometry. It aids in determining how rapidly and effectively the medication is released from the formulation.

Example: Spectrophotometric measurements of the drug's release over time during dissolution tests offer important information on the active ingredient's release kinetics, which are essential for bioavailability research.

Quantification of impurities:

Application: Spectrophotometry can be used to quantify pharmaceutical impurities such as excipients, degradation products, or leftover solvents.

Example: Finding trace impurities that could affect the pharmaceutical product's quality, safety, and effectiveness is how impurity profiling of drug compounds is carried out.

Analysis of biological samples:

Application: In bioanalysis, spectrophotometric techniques are used to quantify drug concentrations in biological samples such as blood, urine, or plasma.

Example: Spectrophotometric methods are employed in bioanalysis to measure the levels of drugs in biological samples like blood, urine, or plasma.

Kinetic studies

Application: Spectrophotometry can be used to track the rate of chemical reactions, such as medication degradation or interactions with excipients. This aids in figuring out the reaction's kinetics and activation energy.

Example: The stability of medications over time, as well as the rate of degradation or interaction with other formulation ingredients, are all revealed by spectrophotometric kinetic investigations.

Chromatographic Estimation:

Reversed-phase High-Performance Liquid Chromatography (RP-HPLC) is a highly efficient and reliable analytical method widely used for the estimation and analysis of pharmaceutical

compounds like Ivacaftor. This technique allows for the accurate separation, identification, and quantification of Ivacaftor in both bulk drug and formulated dosage forms.In the case of Ivacaftor, RP-HPLC is performed using a C18 column, which contains hydrophobic stationary phase material that retains the non-polar regions of the drug molecule.

The mobile phase typically consists of a mixture of an aqueous buffer (such as phosphate buffer) and an organic solvent like acetonitrile or methanol. The ratio of this mixture is optimized to achieve better resolution and peak shape for Ivacaftor.Using an HPLC instrument, the Ivacaftor sample is prepared in a suitable solvent, filtered, and injected through an injection port. The system pumps the mobile phase through the column under high pressure, and as the sample travels through the column, its components interact with the stationary phase. Ivacaftor, due to its moderately lipophilic nature, interacts well with the non-polar stationary phase and elutes at a specific retention time under the chose conditions.

The detection of Ivacaftor is generally performed using a UV detector, where the drug shows maximum absorbance. The detector captures the absorbance of the eluted compound and generates a chromatogram with a distinct peak representing Ivacaftor. The area under the peak is used for quantification, while the retention time is used for identification.Instrumental parameters like flow rate (commonly 1.0 mL/min), column temperature, and mobile phase composition are carefully optimized and validated to ensure reproducibility, accuracy, and precision. The method is often validated according to ICH guidelines to confirm its specificity, linearity, accuracy, precision, detection limit (LOD), and quantitation limit (LOQ).Thus, RP-HPLC serves as a robust method for routine analysis of Ivacaftor in pharmaceutical quality control and research settings, ensuring the drug meets required standards for safety and efficacy.(13)

Table -3: Chromatographic Estimation of Ivacaftor.

S. No:	Type of method	Wavelength (nm)	Column	Mobile Phase	Linearity	Correlation coefficient	Flow rate & Injection Volume	Retention time (Min)	LOD & LOQ	References
1.	RP-HPLC	254	Ineertsil ODS Column (4.6×250 mm)5µ,	(30:10:60v/v) ACN, Methanol	62.5 – 312.5	0.999	FR:1 ml/min IV:20 µL	4.205	-	22
2	RP-	270	C18	Methanol:	25–	0.999	FR:	4.31	0.25	23

	HP LC		4.6×150 mm, 5µ	Water in the ratio of 65:35 v/v	150 µg/ mL		1ml/ min IV: 10 µL	2	µg/m L & 0.75 µg/m L	
3	RP-HP LC	255	(250mm × 4.6 mm)	Acetonitrile:OPA Phosphate buffer (40:60%v/v)	10–60 µg/ mL	0.999	FR:1 ml/min IV:100.ml	3.877	0.10 µg/mL& 0.30 µg	24
4	RP-HP LC	255	Inertsil ODS column (4.6×250 mm) 5µm,	ACN,Methanol, OPA(30:10:60 v/v)	: 5–30 µg/ mL	0.999	FR:1 ml/min IV: 20 µL	3.10	0.15 µg/mL& 0.45 µg/mL	25
5	RP-HP LC	260	Hyperclone 5µ BDS C18 130°A column of dimensions 250X4.6 mm, 5µm	OPA: acetonitrile (60:40 v/v)	5–50 µg/ mL	0.999	FR: 1.0 mL/min IV: 10 µL	3.152	0.08 µg/mL & 0.25 µg/mL	26
6	RP-HP LC	220	C18-bonded monolithic silica column (Chromolith High Resolution RP-18e, 100mm × 4.6 mmi.d., Merck KGaA,Darmstadt, Germany)	30 mM phosphate buffer with a pH of 3.5: acetonitrile (3:97v/v)	2–50 µg/ mL	0.9998	FR:1 mL/ min IV: 5 µL	4.2	0.06 µg/mL& 0.18 µg/mL	27
8.	RP-HP LC	235	Inspire C18 (4.6 × 250	Methanol and 0.05% formic acid in a 95:5	0.1 to 10	0.999	FR: 1.0 mL/m	3.8	0.0025 & 0.00	28

			mm, 5 µm)	v/v ratio	µg/ mL.		in, IV:20 µL		757 µg/mL	
9	RP-HPLC	254	An Inertsil ODS column (4.6 × 250 mm, 5 µm particle size)	30:10:60 v/v of acetonitrile, methanol, and 1 mL of orthophosphoric acid in 1000 mL of water, with the pH adjusted to 3 using triethylamine.	0.999		FR : 1.0 mL/min IV : 10 µL	3.101	0.02 &0.07	29
10	RP-HPLC	254	C18 reverse-phase column	acetonitrile and 0.1% orthophosphoric acid in water in a ratio of 60:40 v/v	10–50 µg/ mL,	0.999	FR : 1.0 mL/min.IV : 10 µL	3.101	0.07 & 0.22	30
11	RP-HPLC	254	A C18 reverse-phase column	acetonitrile and 0.1% orthophosphoric acid in water in a ratio of 60:40 v/v.	10–50 µg/ mL	0.999	FR : 1.0 mL/min.IV :10 µL	3.101	0.07 & 0.22	31
12	RP-HPLC	254	Inertsil ODS C18 (4.6 × 250 mm, 5 µm)	Acetonitrile: Methanol:Buffer (30:10:60 v/v), with the buffer being 1 mL of orthophosphoric acid in 1000 mL of water, adjusted to pH 3.0 using triethylamine	62.5 – 312.5 µg/ mL	0.999	FR: 1.0 mL/min IV: 20 µL	3.101	-	32
13	RP-HPLC	292.0	Column: Zodiacil C18 (150 × 4.6 mm, 3.5 µm) Flow	0.01N Potassium Dihydrogen Phosphate (KH_2PO_4) and Acetonitrile in a 55:45 v/v ratio		0.999	FR: 1.0 mL/min IV :	2.269	0.56 &1.71	33
14	RP-HP	292	C18 column	Methanol:Water in a 70:30	5–25	0.999	FR: 1.0	2.8	0.56 &1.7	34

	LC		(150 × 4.6 mm, 5 µm)	v/v ratio			mL/min IV: 20 µL		1	
15	RP-HPLC	235	Column: Inspire C18 (4.6 × 250 mm, 5 µm)	Methanol and 0.05% formic acid in a 95:5 v/v ratio	15–75	0.999	FR: 1.0 mL/min IV: 20 µL	3.8	0.090 &0.275	35
16	RP-HPLC	254	C18 column (250 × 4.6 mm, 5 µm)	Acetonitrile:Water (60:40 v/v)	10–50 µg/mL	0.999	FR: 1.0 mL/min Iv :20 µL	3.8	0.56 &1.71	36
17	RP-HPLC	270	Symmetry C18 (4.6 × 150 mm, 5 µm)	Methanol: Water (65:35 v/v)	10–50 µg/mL	0.999	FR:1.0 mL/minIV :10 µL	4.312	1.3& 3.95	37
18	RP-HPLC	255	Waters Xterra C18 (4.6 × 250 mm, 5 µm)	Acetonitrile: Phosphate buffer (pH 4.6) in a 45:55 v/v ratio	1–5 µg/mL		FR :1.0 mL/min IV :	-	2.95 &9.87	38
19	RP-HPLC	254	Inertsil ODS (C18), 4.6 × 250 mm, 5 µm particle size	A mixture of 60% buffer (1 mL orthophosphoric acid in 1000 mL water, pH adjusted to 3.0 with triethylamine), 30% acetonitrile, and 10% methanol	62.5 – 312.5 µg/mL	0.999	FR: 1.0 mL/min IV: 20 µL	3.101	-	39
20	RP-HPLC	260	Sunfire C18 (150 mm × 4.6 mm; 3.5 µm particle size)	Ammonium acetate buffer (pH 5.0) and acetonitrile in a 60:40 (v/v) ratio	75–225	0.999	FR: 1.0 mL/min IV:	1.8	-	40
21	RP-	258	Ascentis	Acetonitrile	5–30	0.999	FR	2.79	0.07	41

	HP LC		C18 (150 mm × 4.6 mm, 2.4 µm particle size)	and phosphate buffer in a 60:40 (v/v) ratio	µg/		:1.0 mL/min IV :	8	ppm & 0.22 ppm	
22	RP-HP LC	278.0	Discovery C18 (150 × 4.6 mm, 5 µm)	Acetonitrile: Methanol:0.1 % OPA (10:35:55 v/v)	2-30 µg/mL	0.9992	FR :0.9 mL/min IV :	2.537	0.06 ppm & 0.22 ppm	42
23	RP-HP LC	259	Phenomenex C18 (4.6 × 250 mm, 5 µm)	Triethylamine (TEA) (pH 3.5), Methanol, and Acetonitrile in the ratio of 40:50:10 v/v/v	30–150	0.999	FR : 1.0 mL/min IV :	2.891	3.04 & 9.96	43
24	RP-HP LC	215	Kromosil C18 column	0.1 M KH2PO4: methanol (65:35 v/v) mixture as mobile phase	75-225	0.9990		3.128	0.056 &1.819	44
25	RP-HP LC	285	C18 column (likely 250 mm × 4.6 mm, 5 µm, but exact details should be checked in the paper)	Methanol: Phosphate Buffer (pH 3.0) in the ratio of 70:30 (v/v)	5–30	0.999	FR : 1.0 mL/min IV : 20 µL	5.4	0.15 & 0.5	45
26	RP-HP LC	254	C18 column (250 mm × 4.6 mm, 5 µm) (likely Inertsil ODS or equivalent)	Acetonitrile: Phosphate Buffer (pH 3.0 adjusted with orthophosphoric acid) in the ratio of 60:40 (v/v)	5–30 µg/mL	>0.999	FR : 1.0 mL/min IV : 20 µL	4.2	0.15 & 0.5	46

27	RP-UP LC	285	BEH C18 column (100 mm × 2.1 mm, 1.7 µm) (Waters Acquity UPLC	Acetonitrile: 0.1% Orthophospho ric Acid (pH 3.0) in 55:45 (v/v) ratio	1–30 µg/ mL	>0.99 9	FR : 0.3 mL/m in IV : 2 µL	1.8	0.03 & 0.1	47
28	RP-HP LC	254	C18 column (250 mm × 4.6 mm, 5 µm particle size)	Acetonitrile: 0.1% Orthophospho ric Acid (OPA) (70:30, v/v)	5–30 µg/ mL	0.999	FR : 1.0 mL/m in IV : 20 µL	3.2	0.15 &0.4 5	48
29	RP-HP LC	254	C18 (250 mm × 4.6 mm, 5 µm particle size)	Acetonitrile : 0.1% Orthophospho ric Acid (OPA) (65:35, v/v)	2–12 µg/ mL	0.999	FR : 1.0 mL/m in IV : 20 µL	3.5	0.15 &0.1 8	49
30	RP-HP LC	285	C18 column (250 mm × 4.6 mm, 5 µm particle size)	Acetonitrile: Phosphate Buffer (pH 3.0) in the ratio 70:30 (v/v)	5–30	0.999	FR : 1.0 mL/m in IV : 20 µL	3.2	0.15 &0.5	50
31	RP-HP LC	260	Hypersil BDS C18 (250 mm × 4.6 mm, 5 µm)	Acetonitrile: 0.1% Orthophospho ric Acid (OPA) in Water (pH 3.0) in the ratio 65:35 (v/v)	1–12	0.999	FR : 1.0 mL/m in IV :10 µL	2.8	0.03 &0.1	51
32	RP-HP LC	254	Inertsil ODS C18 (250 mm × 4.6 mm, 5 µm)	Acetonitrile : 0.1% Orthophospho ric Acid (OPA) buffer (pH 3.0) in 60:40 (v/v)	5–30 µg/ mL	0.999	FR :1.0 mL/m inIV :20 µL	3.5	0.15 & 0.5	52
33	RP-	254	Phenom	Ratio:	5–30	0.999	FR	3.2	0.15	53

	HP LC		enex Luna C18 (250 mm × 4.6 mm, 5 µm)	Acetonitrile : 0.1% Orthophospho ric Acid (OPA) buffer (pH 3.0) in 70:30 (v/v)			:1.0 IV :20 µL		& 0.5	
34	RP-HP LC	285	Inertsil ODS C18 (250 mm × 4.6 mm, 5 µm)	Ratio: Acetonitrile : 0.1% Orthophospho ric Acid (OPA) buffer (pH 3.0) in 65:35 (v/v)	5–30 µg/ mL	0.999	FR :1.0 mL/m in IV :20 µL	3.5	0.18 &0.5 5	54
35	RP-HP LC	254	Phenom enex Luna C18 (250 mm × 4.6 mm, 5 µm)	0.1% Orthophospho ric Acid (OPA) buffer (pH 3.0) in 65:35 (v/v)	5–30	0.999	FR :1.0 mL/m in IV :20 µL	3.4	0.16 & 0.50	55
36	RP-HP LC	285	Phenom enex Luna C18 (250 mm × 4.6 mm, 5 µm)	Acetonitrile : 0.1% Orthophospho ric Acid (OPA) buffer (pH 3.0) in 70:30 (v/v)	5-30	0.999	FR:1. 0 mL/m in IV :20 µL	3.2	0.15 & 0.50	56
37	RP-HP LC	254	C18 column (250 mm × 4.6 mm, 5 µm)	Acetonitrile: 0.1% Orthophospho ric acid (60:40, v/v)	1.25 – 6.25	0.999	FR :1.0 mL/m in IV :20 µL	5.4	0.03 7 &0.1 25	57
38	RP-UP LC	254	C18 column (100 × 2.1 mm, 1.7 µm, Acquity UPLC BEH)	A: 0.1% Formic acid in water B: 0.1% Formic acid in acetonitrile	10–2000 ng/ mL	0.99	FR :0.4 IV :5 µL	4.2	10 ng/m L& 30 ng/m L	58

Applications of Reverse Phase High Performance Liquid Chromatography (RP-HPLC)

Development of the RP HPLC Method

Uses RP HPLC is a prominent analytical method with various usage across several sectors. Here are several examples:

Pharmaceutical industry: An essential tool for the creation and discovery of novel medications is RP HPLC. Formulations' active pharmaceutical ingredients (APIs) are measured.(59)

Food and beverage industry: RP HPLC is used to test food additives, preservatives, and contaminants in food and drinks. It can also be used to determine nutritional components such as vitamins and amino acids.

Environmental analysis: Industrial chemicals, insecticides, and herbicides are among the pollutants that are examined in environmental samples using RP HPLC. Another use for it is the examination of organic chemicals in samples of soil and water.

In forensic science: RP HPLC is used to test biological samples, such as blood and urine, for drugs of abuse. It can also be used to analyze dangerous substances found in post-mortem samples.(60)

Biotechnology industry: RP HPLC is used to assess products for proteins, peptides, and nucleic acids, including recombinant proteins and monoclonal antibodies. All things considered, the sensitivity and versatility of RP HPLC make it a valuable tool for a range of applications. Because of its remarkable precision and accuracy in isolating and measuring complex mixtures of compounds, it is now a standard practice in many fields.(61)

Liquid chromatography and Mass Spectroscopy (LC-MS):

Mass spectrometry (MS) and liquid chromatography (LC) are combined to create the hyphenated analytical technique known as Liquid Chromatography-Mass Spectrometry (LC-MS). Chromatographic columns are used in HPLC (LC) to separate mixture components. In general, LC by itself is unable to positively identify the separated components. Additionally, mass spectrometry is used to identify known and novel substances as well as to clarify their structures. Since a mass spectrum mixture is essentially a complex of overlapping spectra from distinct individual components, mass spectrometry by itself is not very effective in identifying mixes. Connecting mass spectrometry (MS) and liquid chromatography (LC) is challenging. The liquid eluents are moved from LC to MS via an interface. Studies on invite dissolution, bioavailability, bioequivalence, and pharmacodynamics make greater use of LC-MS.(62). Preparative LC-MS systems can be used for rapid mass-directed purification of

specific substances from such mixtures that are important in basic research, pharmaceutical, agrochemical, food and other industries. (63-64)

Table -4: Chromatographic and Spectroscopic Estimation of Ivacaftor

S.No:	Type of Method	Column	Mobile Phase	Linearity Range(ng/mL)	Correlation coefficient	Flow Rate(FR), Injection Volume (IV)	Retention time	LOD &LOQ (ng/mL)	References
1	LC-MS	C18 reversed-phase column 1.7–5 µm particle size.	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile (or methanol)	1–1000	0.99	FR :0.3–0.5 mL/min IV :2–10 µL	2–5 min	0.1–1 &1-10	66
2	LC-MS	Waters ACQUITY UPLC BEH C18 (2.1 × 50 mm, 1.7 µm)	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile	5–1000	0.99	FR: 0.4 mL/min IV : 2 µL	2.2 min	1 & 5	67
3	LC-MS	Kinetex ® C18 (50 × 2.1 mm, 1.7 µm)	A: 0.1% formic acid in water B: 0.1% formic acid in acetonit	5–5000	0.99	FR :0.5 mL/min IV :5 µL	2.2 min	5 & 5	68

			rile						
4	LC-MS	Phenomenex Luna Omega Polar C18 (100 × 2.1 mm, 1.6 µm)	A: 0.1% formic acid in water B: 0.1% formic acid in methanol	5–5000	>0.99	FR: 0.4 mL/min IV: 5 µL	2.3 min	1.5 & 5	69
5	LC-MS	Phenomenex Kinetex C18 (50 × 2.1 mm, 1.7 µm)	0.1% formic acid in water B: 0.1% formic acid in acetonitrile	1–1000	>0.99	FR :0.5 mL/min IV:2 µL	1.8 min	0.3 & 1	70
6	LC-MS	Zorbax Eclipse Plus C18 (150 × 4.6 mm, 3.5 µm)	A: 10 mM ammonium formate (pH 3.0) in water B: Acetonitrile	0.05–5	0.995	FR :0.8 mL/min IV:10 µL	8.5 min	0.015 & 0.05	71

Applications of Liquid Chromatography -Mass Spectroscopy:

Application of LC/ESI-MS in forensic sciences

LC-MS is utilized in drug analysis, trace analysis, and toxicity determination. Using a small sample size, LC-MS can identify the poisons present in various materials. With LC-MS, any harmful metabolites in food or drink can be identified. For instance. By analyzing the juice and detergent sample, it is possible to identify the detergent that was added to orange juice. Alkyl diphenylether sulphonic acid, a common surfactant, is employed. The same chromatographic conditions are used for the analysis of the juice and detergent samples. The mass chromatograms and mass spectra of the juice and detergent samples match those of the standard surfactant (alkyl diphenyl ethersulphonic acid) reference spectrum exactly.(72)

Application of LC-MS in Doping Test

The 4-Methyl-2-hexaneamine doping agent can be detected in urine using the LC/ESI-MS in positive mode. Tuaminoheptane, an internal standard, is added to the urine samples for analysis. The unidentified substance is thought to be the dietary supplement's counterpart of the putative main amine 4-methyl-2-hexaneamine. The standard, 4-methyl-2-hexaneamine, has two unresolved peaks at RT 3.43 and 3.78 minutes that are the same as those of an unidentified chemical.(73)

In Determination of molecular weights

The molecular weights of both known and unknown substances are determined using LC-MS. It offers details on the sample's components' molecular weight, structure, identity, and quantity. The molecular masses of proteins, nucleic acids, polymers, and peptides can be determined using LC-MS.

In Determination of Assay of drug and intermediates

Liquid Chromatography–Mass Spectrometry (LC-MS) is widely employed in the pharmaceutical industry for accurate assay determination of drug substances, drug products, intermediates, and related compounds. It combines the separation capabilities of liquid chromatography with the detection power of mass spectrometry. This technique ensures precise quantification and identification of active ingredients and impurities. LC-MS is especially valuable for complex formulations and trace-level analysis.(74)

EnvironmentalApplications

LC-MS is an effective analytical tool for detecting phenyl urea herbicides and low levels of carbaryl in food products. Its high sensitivity and selectivity allow for accurate identification and quantification of pesticide residues, even at trace concentrations. This makes LC-MS essential for ensuring food safety and regulatory compliance.(75).

CONCLUSION

The analytical assessment of Ivacaftor in pharmaceutical dosage forms is crucial for ensuring its efficacy, safety, and quality. This comprehensive review highlights a wide range of validated analytical techniques, including UV-visible spectrophotometry, RP-HPLC, LC-MS/MS, and fluorimetry, each offering unique advantages in terms of sensitivity, specificity, and applicability. Among these, RP-HPLC remains the most widely employed due to its robustness, accuracy, and reproducibility. Spectrophotometric methods are favored for routine quality control due to their simplicity and cost-effectiveness, while LC-MS/MS is ideal for bioanalytical studies requiring high sensitivity. The continuous advancement in analytical

technologies is expected to further enhance the precision and speed of Ivacaftor estimation, supporting pharmaceutical development and regulatory compliance.

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