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Stability Indicating Method Development and Validation for Simultaneous Estimation of Tezacaftor and Ivacaftor and Elexacaftor in Bulk and Pharmaceutical Dosage Form by HPLC

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ABSTRACT

A simple, accurate, precise method was developed for the simultaneous estimation of the Ivacaftor, Elexacaftor and Tezacaftor in solid dosage form. Chromatogram was run through Zorbax C18 150x4.6 mm, 5µ. Mobile phase containing 0.01N KH_2PO_4 and acetonitrile in the ratio of 60:40 v/v was pumped through column at a flow rate of 1 ml/min. Temperature was maintained at 30°C. Optimized wavelength for Ivacaftor, Elexacaftor and Tezacaftor was 260 nm. Retention time of Ivacaftor, Elexacaftor and Tezacaftor were found to be 2.322 min, 2.847 min and 3.457 min %RSD of system precision for Ivacaftor, Elexacaftor and Tezacaftor were and found to be 0.6, 1.4 and 1.0 respectively. %RSD of method precision for Ivacaftor, Elexacaftor and Tezacaftor were and found to be 0.4, 1.2, and 1.0 respectively. % recovery was obtained as 100.04%, 100.15% and 99.44% for Ivacaftor, Elexacaftor and Tezacaftor respectively. LOD, LOQ values are obtained from regression equations of Ivacaftor, Elexacaftor and Tezacaftor were 0.12 ppm, 0.36 ppm, 0.06 ppm and 0.18 ppm, 0.03 ppm, 0.08 ppm respectively. Regression equation of Tezacaftor was y = 26227x + 863.8, Ivacaftor was y = 47191x + 7683 and of Elexacaftor was y = 84526x + 21311.

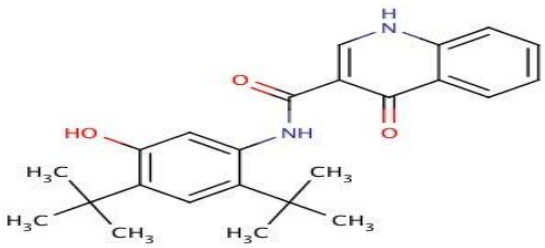
INTRODUCTION

DRUG PROFILE

Ivacaftor

Description: Ivacaftor (also known as Kalydeco or VX-770) is a drug used for the management of Cystic Fibrosis (CF) in patients aged 2 years and older. Cystic Fibrosis is an autosomal recessive disorder caused by one of several different mutations in the gene for the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) protein, an ion channel involved in the transport of chloride and sodium ions across cell membranes. CFTR is active in epithelial cells of organs such as of the lungs, pancreas, liver, digestive system, and reproductive tract. Alterations in the CFTR gene result in altered production, misfolding, or function of the protein and consequently abnormal fluid and ion transport across cell membranes [5, 6]. As a result, CF patients produce a thick, sticky mucus that clogs the ducts of organs where it is produced making patients more susceptible to complications such as infections, lung damage, pancreatic insufficiency, and malnutrition [8].

STRUCTURE



Application: A CFTR activator

CAS Number: 873054-44-5

Purity: ≥98%

Molecular Weight: 392.49

Molecular Formula: $C_{24}H_{28}N_2O_3$

IUPAC Name: N-(2,4-di-tert-butyl-5-hydroxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide

Appearance: Crystalline powder

Physical State: Solid

Solubility: Water Solubility (0.002 mg/mL) Soluble in DMSO (78 mg/ml at 25°C), water (<1 mg/ml at 25°C), and ethanol (<1 mg/ml at 25°C).

Storage: Store at room temperature

Melting Point: 253.58°C (Predicted)

Boiling Point: ~550.5°C at 760 mmHg (Predicted)

Density: ~1.2 g/cm^3 (Predicted)

pK Values: 6.57

Indication: When used as monotherapy as the product Kalydeco, ivacaftor is indicated for the management of CF in patients age 2 years and older who have a mutation in the CFTR gene that is responsive to ivacaftor potentiation. When used in combination with the drug Lumacaftor as the product Orkambi, ivacaftor is indicated for the management of CF patient's age 6 years and older who are homozygous for the F508del mutation in the CFTR gene.

Mechanism of action: Ivacaftor exerts its effect by acting as a potentiator of the CFTR protein, an ion channel involved in the transport of chloride and sodium ions across cell membranes of the lungs, pancreas, and other organs. Alterations in the CFTR gene result in altered production, misfolding, or function of the protein and consequently abnormal fluid and ion transport across cell membranes [5, 6]. Ivacaftor improves CF symptoms and underlying disease pathology by potentiating the channel open probability (or gating) of CFTR protein in patients with impaired CFTR gating mechanisms. The overall level of ivacaftor-mediated CFTR chloride transport is dependent on the amount of CFTR protein at the cell surface and how responsive a particular mutant CFTR protein is to ivacaftor potentiation [Label].

Absorption: Following administration of ivacaftor with fat containing foods, peak plasma concentrations were reached at 4 hours (Tmax) with a maximum concentration (Cmax) of 768 ng/mL and AUC of 10600 ng * hr/mL. It's recommended that ivacaftor should be taken with fat-containing foods as they increase absorption by approximately 2.5- to 4-fold.

Volume of distribution: After oral administration of 150 mg every 12 hours for 7 days to healthy volunteers in a fed state, the mean (±SD) for apparent volume of distribution was 353 (122).

Protein binding: Ivacaftor is approximately 99% bound to plasma proteins, primarily to alpha 1-acid glycoprotein and albumin. Ivacaftor does not bind to human red blood cells.

Metabolism: Ivacaftor is extensively metabolized in humans. In vitro and clinical studies indicate that ivacaftor is primarily metabolized by CYP3A. M1 and M6 are the two major metabolites of ivacaftor in humans. M1 has approximately one-sixth the potency of ivacaftor and is considered pharmacologically active. M6 has less than one-fiftieth the potency of ivacaftor and is not considered pharmacologically active.

Route of elimination: Following oral administration, the majority of ivacaftor (87.8%) is eliminated in the feces after metabolic conversion. The major metabolites M1 and M6 accounted for approximately 65% of the total dose eliminated with 22% as M1 and 43% as M6. There was negligible urinary excretion of ivacaftor as unchanged parent.

Half-life: The apparent terminal half-life was approximately 12 hours following a single dose.

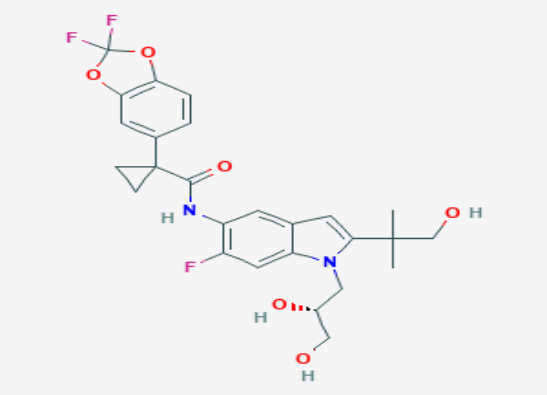
Uses: This medication is used to treat cystic fibrosis in certain people (those with an abnormal "CFTR" gene). It may help to improve breathing, reduce the risk of lung infections, and improve weight gain.

Side Effects: Dizziness, headache, and nausea may occur. If any of these effects persist or worsen, tell your doctor or pharmacist promptly.

Name: Tezacaftor

Description: Tezacaftor is a small molecule that can be used as a corrector of the cystic fibrosis transmembrane conductance regulator (CFTR) gene function [4]. It was developed by Vertex Pharmaceuticals and FDA approved in combination with ivacaftor; a CFTR potentiator that allows the proteins at the cell surface to open longer and improve nutrient transport.[5] The approval was done on February 12, 2018, to be used under prescription.

Structure:



CAS number: 1152311-62-0

Molecular Weight: 520.505

Chemical Formula: $C_{26}H_{27}F_3N_2O_6$

IUPAC Name: 1-(2,2-difluoro-2H-1,3-benzodioxol-5-yl)-N-{1-[(2R)-2,3-dihydroxypropyl]-6-fluoro-2-(1-hydroxy-2-methylpropan-2-yl)-1H-indol-5-yl}cyclopropane-1-carboxamide

Indication: Tezacaftor, in combination with ivacaftor, is indicated for the treatment of cystic fibrosis in people aged 12 years or older who have two copies of the F508del mutation or at least one mutation in the CFTR gene that is responsive to this treatment based on clinical evidence.

Cystic fibrosis is a rare, life-shortening disease caused by a defective or missing CFTR protein. This modification in the protein is caused by a mutation in the CFTR gene. The affected people carry two inherited defective copies of the CFTR gene. There are approximately 2000 known mutations in the CFTR gene. The presence of a defective or missing protein results in poor flow of salt and water into and out of the cell. This condition will cause the buildup of abnormally thick, sticky mucus in the lungs that will later potentially cause chronic lung infections and lung damage. In the patients with the F508del mutation, the CFTR protein is not processed or folded normally within the cell and thus, it does not reach the cell surface.

Pharmacodynamics: Some clinical studies have shown a significant decrease in sweat chloride and an increase in the forced expiratory volume.[1] The phase 3 clinical studies have shown that a significant increase in forced expiratory volume was attained at 4 and 8 weeks

after the beginning of the treatment. Tezacaftor significantly improved the respiratory domain as seen by the cystic fibrosis questionnaire-revised.[4] Studies have also reported that tezacaftor does not provoke QT prolongation to any clinically relevant extent.[2]

Mechanism of action: Normally, the transport of charged ions across cell membranes is achieved through the cystic fibrosis transmembrane regulator protein. This protein serves as a channel and allows passage of charged ions such as chlorine or sodium. This process is also important for the movement of water in the tissues and for the creation of a thin mucus that can lubricate some of the organs and body tissues, including the lungs. In the F508del mutation of CFTR, one amino acid is deleted in position 508 and thus the channel function is compromised and thick mucus is produced.

Absorption: After administration of tezacaftor and ivacaftor, the plasma concentration of both compounds reached steady-state within 8 days for tezacaftor and 2-5 days for ivacaftor. When reached steady state, the accumulation of both will be 1.5 for tezacaftor and 2.2 for ivacaftor. The Cmax, tmax and AUC of tezacaftor, when administered with ivacaftor, is 5.95 mcg/ml, 2-6 h and 84.5 mcg.h/ml respectively.[3] Exposure of tezacaftor/ivacaftor is 3 times higher exposure when administered with high-fat containing food.

Volume of distribution: The apparent volume of distribution of tezacaftor is 271 L.

Protein binding: Tezacaftor is approximately bound in a proportion of 99% of the administered dose. From the bound state, most of the dose is bound to albumin.

Metabolism: Tezacaftor is metabolized extensively in humans by the action of CYP3A4 and CYP3A5. There are three main circulating metabolites named M1, M2 and M5. From the metabolites, M1 is an active metabolite with a similar potency than tezacaftor, M2 is less pharmacologically active and M5 is an inactive metabolite. There is an additional circulating metabolite, named M3 that corresponds to the glucuronide form of tezacaftor. [Label]

Route of elimination: Following oral administration, the majority of tezacaftor dose reaching to even 72% of the administered dose is excreted by the feces either unchanged or as the metabolite M2 and about 14% of the administered dose is recovered in urine as the metabolite M2. These percentages correspond to 86% of the recovered dose after 21 days of administration. It was noted that less than 1% of the administered dose is excreted unchanged in the urine and thus, renal excretion is not the major elimination pathway.[Label]

Half-life: The apparent half-life of tezacaftor is approximately 15 hours.

Solubility: Water solubility 0.0124 mg/mL

pKa Value:11.54

Uses: This product contains 2 different tablets. One tablet contains 2 medications ("multi-drug"): tezacaftor and ivacaftor. The other tablet contains only ivacaftor. This product is used to treat cystic fibrosis in certain people (those with an abnormal "CFTR" gene). It may help to improve breathing, reduce the risk of lung infections, and improve weight gain.

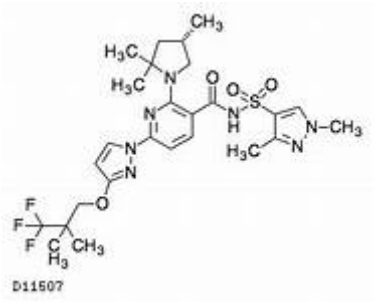
Side Effects: Nausea, headache, or dizziness may occur. If any of these effects last or get worse, tell your doctor or pharmacist promptly. Remember that your doctor has prescribed this product because he or she has judged that the benefit to you is greater than the risk of side effects. Many people using this product do not have serious side effects.

Name: Elexacaftor

Description

Elexacaftor (previously VX-445) is a small molecule, next-generation corrector of the cystic fibrosis transmembrane conductance regulator (CFTR) protein.3 It received FDA approval in October 2019 in combination with tezacaftor and ivacaftor as the combination product TrikaftaTM.6 Elexacaftor is considered a next-generation CFTR corrector as it possesses both a different structure and mechanism as compared to first generation correctors like tezacaftor.3 While dual corrector/potentiator combination therapy has proven useful in the treatment of a subset of CF patients,2 their use is typically limited to patients who are homozygous for the F508del-CFTR gene.3 Elexacaftor, along with VX-659, was designed to fill the need for an efficacious CF therapy for patients who are heterozygous for F508del-CFTR and a gene that does not produce protein or produces proteins unresponsive to ivacaftor or tezacaftor.3 The triple combination product TrikaftaTM, manufactured by Vertex Pharmaceuticals, is the first product approved for the treatment of CF in individuals who are either homo- or heterozygous for the F508del-CFTR gene - this represents approximately 704-90%3,1 of all CF patients.

Structure:



Properties:

CAS number: 2216712-66-0

Weight: Average: 597.66; Monoisotopic: 597.234508266

Chemical Formula: $C_{26}H_{34}F_3N_7O_4S$

IUPAC Name: N-[(1,3-dimethyl-1H-pyrazol-4-yl)sulfonyl]-6-[3-(3,3,3-trifluoro-2,2-dimethylpropoxy)-1H-pyrazol-1-yl]-2-[(4S)-2,2,4-trimethylpyrrolidin-1-yl]pyridine-3-carboxamide.

pKa (Strongest Acidic): 4.1

pKa (Strongest Basic): 2.09

Water Solubility: 0.0192 mg/mL

Indication:

Elexacaftor, in combination with ivacaftor and tezacaftor as the combination product TrikaftaTM, is indicated for the treatment of cystic fibrosis (CF) in patients 12 years of age and older who have at least one F508del mutation in the CTFR gene.6

Pharmacodynamics:

As a CFTR corrector, elexacaftor works to increase the amount of mature CFTR proteins present on the surface of cells. 6 When used in combination with CFTR potentiators, which enhance the function of cell-surface CFTR proteins, drugs like elexacaftor help to improve a variety of multi-organ cystic fibrosis symptoms, including lung function, nutritional status, and overall quality of life.3 TrikaftaTM, the triple combination product containing elexacaftor, may cause elevations in liver transaminases. Liver function testing should be

conducted prior to beginning Trikafta, every 3 months for the first year of treatment, and annually thereafter.6

Mechanism of action:

Cystic fibrosis (CF) is the result of a mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene.4 The CFTR proteins produced by this gene are transmembrane ion channels that move sodium and chloride across cell membranes - water follows the flow of chloride ions to the cell surface, which consequently helps to hydrate the surface of the cell and thin the secretions (i.e. mucous) around the cell.4 Mutations in the CFTR gene produce CFTR proteins of insufficient quantity and/or function, leading to defective ion transport and a build-up of thick mucus throughout the body that causes multi-organ disease involving the pulmonary, gastrointestinal, and pancreatic systems (amongst others). The most common CFTR mutation, the F508del mutation, is estimated to account for 704 to 90%3,1 of all CFTR mutations and results in severe processing and trafficking defects of the CFTR protein.2

Elexacaftor is a CFTR corrector that modulates CFTR proteins to facilitate trafficking to the cell surface for incorporation into the cell membrane.6 The end result is an increase in the number of mature CFTR proteins present at the cell surface and, therefore, improved ion transport and CF symptomatology. Elexacaftor is used in combination with tezacaftor, another CFTR corrector with a different mechanism of action, and ivacaftor, a CFTR potentiator that improves the function of CFTR proteins on the cell surface - this multi-faceted, triple-drug approach confers a synergistic effect beyond that seen in typical corrector/potentiator dual therapy regimens.6,3.

Absorption:

The absolute oral bioavailability of elexacaftor is approximately 80%. The steady-state AUC0-24h and Cmax following once daily dosing with elexacaftor 200 mg are 162 mcg·h/mL and 8.7 mcg/mL, respectively, and the median Tmax is 6 hours.6 The AUC of elexacaftor is increased 1.9-2.5-fold following a moderate-fat meal - for this reason, it is recommended to give Trikafta TM with fat-containing food.6

Volume of distribution:

The apparent volume of distribution of elexacaftor is 53.7 L.6

Protein binding:

Elexacaftor is >99% protein bound in plasma, primarily to albumin.6

Metabolism:

The metabolism of elexacaftor is extensive and primarily catalyzed via CYP3A4/5.6 Its main active metabolite, M23-ELX, carries a similar potency as the parent drug.6 The precise metabolic pathway of elexacaftor has not yet been elucidated in published research.

Route of elimination:

Approximately 87.3% of an administered radio-labeled dose of elexacaftor was found in the feces, mostly as metabolites, while only 0.23% of that same dose was found excreted in the urine.6

Half-life:

The mean terminal half-life of elexacaftor is approximately 24.7 hours.6.

Uses:

This product has 2 different tablets. One tablet contains 3 medications (it is a "multi-drug"): elexacaftor, tezacaftor, and ivacaftor. The other tablet contains only ivacaftor.

This product is used to treat cystic fibrosis in certain people (those with an abnormal "CFTR" gene). It may help to improve breathing, reduce the risk of lung infections, and improve weight gain.

Side Effects:

Headache, diarrhea, or dizziness may occur. If any of these effects last or get worse, tell your doctor or pharmacist promptly.

Remember that your doctor has prescribed this product because he or she has judged that the benefit to you is greater than the risk of side effects. Many people using this product do not have serious side effects.

MATERIALS AND METHODS

Materials and Instruments: The following materials used were either AR/LR grade or the best possible Pharma grade available as supplied by the manufacturer or supplier without further purification or investigation.

Drug Samples

Were obtained from

- Spectrum Pharma Research Solutions Pvt. Ltd

Chemicals and Solvents Used

- Water – HPLC grade
- Acetonitrile - HPLC grade
- Triethylamine – ARgrade
- Potassium dihydrogen orthophosphate – AR grade
- Orthophosphoric acid – Argrade
- All the above chemicals and solvents are from Ranchem.

Instruments

- Electronics Balance - Denver
- BVK enterprises, India, pH meter
- Waters HPLC 2695 series with quaternary pumps, Photo Diode array detector and autosampler integrated with empower software.
- BVK enterprises, Ultrasonicator.
- Labindia UV double beam spectrophotometer with UVwin5.

Sample Processing

Diluents: Based upon the solubility of the drug diluents was selected Water: Acetonitrile (50:50 v/v).

Preparation of Standard stock solutions: Accurately weighed 5 mg of Tezacaftor, 7.5 mg of Ivacaftor and 10 g of Elexacaftor and transferred to three 50 ml volumetric flasks separately. 10 ml of diluent was added to flasks and sonicated for 20mins. Flasks were made up Diluent and labeled as Standard stock solution 1, 2 and 3. (100 µg/ml of Tezacaftor, 150 µg/ml of Ivacaftor and 1000 µg/ml of Elexacaftor).

Preparation of Standard working solutions (100% solution): 1 ml from each stock solution was pipette out and taken into a 10 ml volumetric flask and made up with Diluent. (10 µg/ml of Tezacaftor, 15 µg/ml of Ivacaftor and 100 µg/ml of Elexacaftor).

Preparation of Sample stock solutions: 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, 25 mL of diluent added and sonicated for 50 min, further the volume made up with diluent and filtered. (500 µg/ml of Tezacaftor, 750 µg/ml of Ivacaftor and 1000 µg/ml of Elexacaftor)

Preparation of Sample working solutions (100% solution): From the filtered solution 0.2 ml was pipette out into a 10 ml volumetric flask and made upto 10 ml with diluents. (10 µg/ml of Tezacaftor, 15 µg/ml of Ivacaftor and 100 µg/ml of Elexacaftor)

Validation

System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of Tezacaftor, Ivacaftor and Elexacaftor and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be more than 2%.

Specificity: Checking of the interference in the optimized method. We should not found interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision:

Preparation of Standard stock solutions: Accurately weighed 5 mg of Tezacaftor, 7.5 mg of Ivacaftor and 10 g of Elexacaftor and transferred to three 50 ml volumetric flasks separately. 10 ml of diluent was added to flasks and sonicated for 20 mins. Flasks were made

up Diluent and labeled as Standard stock solution 1, 2 and 3. (100 µg/ml of Tezacaftor, 150 µg/ml of Ivacaftor and 1000 µg/ml of Elexacaftor)

Preparation of Standard working solutions (100% solution): 1 ml from each stock solution was pipette out and taken into a 10 ml volumetric flask and made up with Diluent. (10 µg/ml of Tezacaftor, 15 µg/ml of Ivacaftor and 100 µg/ml of Elexacaftor)

Preparation of Sample stock solutions: 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was transferred into a 100 mL volumetric flask, 25 mL of diluent added and sonicated for 50 min, further the volume made up with diluent and filtered. (500 µg/ml of Tezacaftor, 750 µg/ml of Ivacaftor and 1000 µg/ml of Elexacaftor).

Preparation of Sample working solutions (100% solution): From the filtered solution 0.2 ml was pipette out into a 10 ml volumetric flask and made upto 10 ml with diluents. (10 µg/ml of Tezacaftor, 15 µg/ml of Ivacaftor and 100 µg/ml of Elexacaftor)

Linearity:

Preparation of Standard stock solutions: Accurately weighed 5 mg of Tezacaftor, 7.5 mg of Ivacaftor and 10 g of Elexacaftor and transferred to three 50 ml volumetric flasks separately. 10 ml of diluent was added to flasks and sonicated for 20 mins. Flasks were made up Diluent and labeled as Standard stock solution 1, 2 and 3. (100 µg/ml of Tezacaftor, 150 µg/ml of Ivacaftor and 1000 µg/ml of Elexacaftor)

25% Standard solution: 0.25 ml each from three standard stock solutions was pipette out and made up to 10 ml.

50% Standard solution: 0.5 ml each from three standard stock solutions was pipette out and made up to 10 ml.

75% Standard solution: 0.75 ml each from three standard stock solutions was pipette out and made up to 10 ml.

100% Standard solution: 1.0 ml each from three standard stock solutions was pipette out and made up to 10 ml.

125% Standard solution: 1.25 ml each from three standard stock solutions was pipette out and made up to 10 ml.

150% Standard solution: 1.5 ml each from three standard stock solutions was pipette out and made up to 10 ml.

Accuracy:

Preparation of Standard stock solutions: Accurately weighed 5mg of Tezacaftor, 7.5 mg of Ivacaftor and 10 g of Elexacaftor and transferred to three 50 ml volumetric flasks separately. 10 ml of diluent was added to flasks and sonicated for 20 mins. Flasks were made up Diluent and labeled as Standard stock solution 1, 2 and 3. (100 µg/ml of Tezacaftor, 150 µg/ml of Ivacaftor and 1000 µg/ml of Elexacaftor)

Preparation of 50% Spiked Solution: 0.1 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 0.2 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 0.3 ml of sample stock solution was taken into a 10 ml volumetric flask, to that 1.0 ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guidelines.

Robustness conditions like Flow minus (0.9 ml/min), Flow plus (1.1 ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much effected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25 ml each from three standard stock solutions was pipette out and transferred to 3 separate 10 ml volumetric flask and made up with diluents from the

above solutions 0.1 ml, 0.1 ml and 0.1 ml of Tezacaftor, Ivacaftor and Elexacaftor solutions respectively were transferred to 10 ml volumetric flasks and made up with the same diluents.

LOQ sample Preparation: 0.25 ml each from three standard stock solutions was pipette out and transferred to 3 separate 10 ml volumetric flask and made up with diluents from the above solutions 0.3 ml, 0.3 ml and 0.3 ml of Tezacaftor, Ivacaftor and Elexacaftor solutions respectively were transferred to 10 ml volumetric flasks and made up with the same diluents.

Degradation studies:

- **Oxidation**:

To 1 ml of stock solutions of Tezacaftor, Ivacaftor and Elexacaftor. 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0C. For HPLC study, the resultant solution was diluted to obtain 10µg/ml, 15 µg/ml and 20 µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

- **Acid Degradation Studies**:

To 1 ml of stock s solution Tezacaftor, Ivacaftor and Elexacaftor, 1 ml of 2 N Hydrochloric acid was added and refluxed for 30 mins at 60^0C. The resultant solution was diluted to obtain 10 µg/ml, 15 µg/ml and 20 µg/ml of all components and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

- **Alkali Degradation Studies**:

To 1 ml of stock solution Tezacaftor, Ivacaftor and Elexacaftor, 1 ml of 2 N sodium hydroxide was added and refluxed for 30 mins at 60^0C. The resultant solution was diluted to obtain 10 µg/ml, 15 µg/ml and 20 µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

- **Dry Heat Degradation Studies**:

The standard drug solution was placed in oven at 105^0C for 1 h to study dry heat degradation. For HPLC study, the resultant solution was diluted obtain 10µg/ml, 15 µg/ml and 20 µg/ml of all components and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

- **Photo Stability studies**:

The photochemical stability of the drug was also studied by exposing the 100 µg/ml, 150 µg/ml and 200 µg/ml solution to UV Light by keeping the beaker in UV Chamber for 1 day or 200 Watt hours/m^2 in photostability chamber. For HPLC study, the resultant solution was diluted to obtain 10 µg/ml, 15 µg/ml and 20 µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

- **Neutral Degradation Studies**:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to obtain 10 µg/ml, 15 µg/ml and 20 µg/ml of all components and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample.

RESULTS AND DISCUSSION

Method Validation: The validation of the Process carried out was validated as per ICH guidelines and the following parameters were reported as follows:

System suitability: All the system suitability parameters were within the range and satisfactory as per ICH guidelines.

Plate count of the Tezacaftor was 7547, Ivacaftor was 6322, and of Elexacaftor was 8262, tailing factor of Tezacaftor was 1.05, Ivacaftor was 1.11, and of Elexacaftor was 1.01, resolution between Elexacaftor and Ivacaftor was 4.2 and resolution between Tezacaftor and Elexacaftor was 4.2. According to ICH guidelines, plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits.

Table No. 1: System suitability parameters for Tezacaftor, Ivacaftor, and Elexacaftor

S no	Tezacaftor.			Ivacaftor			Elexacaftor		
Inj	RT (min)	TP	Tailing	RT (min)	TP	Tailing	RT (min)	TP	Tailing
1	2.84	7723	1.05	4.2	4.3	4.4	3.45	8806	1.00
2	2.84	7825	1.05	4.3	4.2	4.3	3.45	9059	1.00
3	2.84	7876	1.03	4.2	4.3	4.4	3.45	8518	0.99
4	2.85	7628	1.05	4.3	4.2	4.3	3.45	8647	1.00
5	2.85	7873	1.03	4.2	4.3	4.4	3.46	8700	1.01
6	2.85	7547	1.05	4.3	4.2	4.3	3.46	8262	1.01

Accuracy:

Discussion: Three levels of Accuracy sample were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 100.02%, 99.69% and 100.63% for Tezacaftor, Ivacaftor and Elexacaftor. Respectively.

Table No. 2: Accuracy table of Tezacaftor

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	5	4.988	99.75	100.02%
	5	5.008	100.17	
	5	4.949	98.98	
100%	10	10.019	100.19	
	10	10.044	100.44	
	10	9.885	98.85	
150%	15	15.220	101.46	
	15	14.890	99.27	
	15	15.158	101.05	

Table No. 3: Accuracy table of Ivacaftor

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	7.5	7.500	100.01	99.69%
	7.5	7.518	100.24	
	7.5	7.548	100.64	
100%	15	14.877	99.18	
	15	14.768	98.45	
	15	14.943	99.62	
150%	22.5	22.363	99.39	
	22.5	22.456	99.80	
	22.5	22.466	99.85	

Table No. 4: Accuracy table of Elexacaftor

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	10	10.12	101.21	100.63%
	10	10.02	100.18	
	10	10.00	99.98	
100%	20	19.88	99.39	
	20	20.27	101.34	
	20	19.94	99.71	
150%	30	30.11	100.38	
	30	30.50	101.68	
	30	30.55	101.83	

LOD and LOQ

The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyze that give the measurable response. The LOD for

Tezacaftor, Ivacaftor and Elexacaftor was found to be 0.03 µG/ml, 0.21 µg/ml and 0.06 µg/ml respectively.

LOQ is the smallest concentration of the analyze, which gives response that can be accurately quantified signal to noise ratio of 10. The LOQ was0.08 µg/ml, 0.36 µg/ml and0.18 µg/ml for Tezacaftor, Ivacaftor and Elexacaftor.

Linearity:

Six linear concentrations of Tezacaftor (.25-15.0 µg/ml), Ivacaftor (3.75-22.50 µg/ml) and Elexacaftor (5-30 µg/ml) were injected in a triplicate maner. Average areas were mentioned above and linearity equations obtained for Tezacaftor was y = 26227x + 863.8. Ivacaftore was y = 47191x + 7683 and of Elexacaftor was y =84526x +21311. Correlation coefficient obtained was 0.999 for all three drugs.

Table No. 5: Linearity table for Tezacaftor, Ivacaftor and Elexacaftor

Tezacaftor		Ivacaftor		Elexacaftor	
Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area	Conc (µg/mL)	Peak area
2.5	71432	3.75	203034	5	464556
5	128943	7.5	350727	10	842060
7.5	196609	11.25	543909	15	1327190
10	259676	15	701470	20	1710770
12.5	330862	18.75	902412	25	2145538
15	395424	22.5	1068518	30	2534342

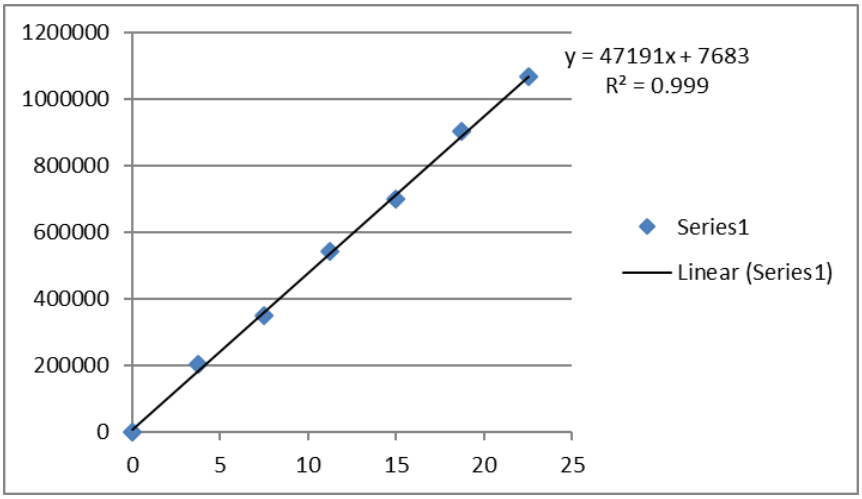


Figure No. 4: calibration curve of Ivacaftor

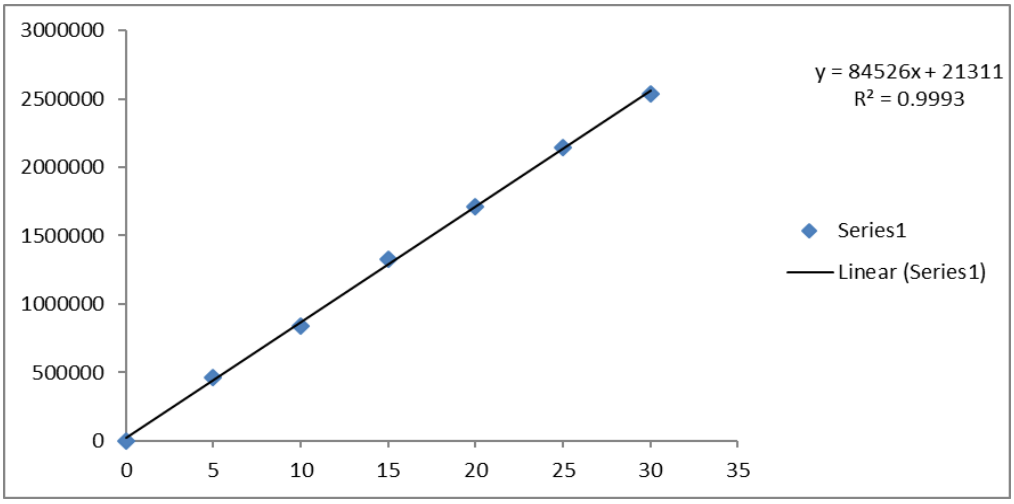


Figure No. 5: calibration curve of Elexacaftor

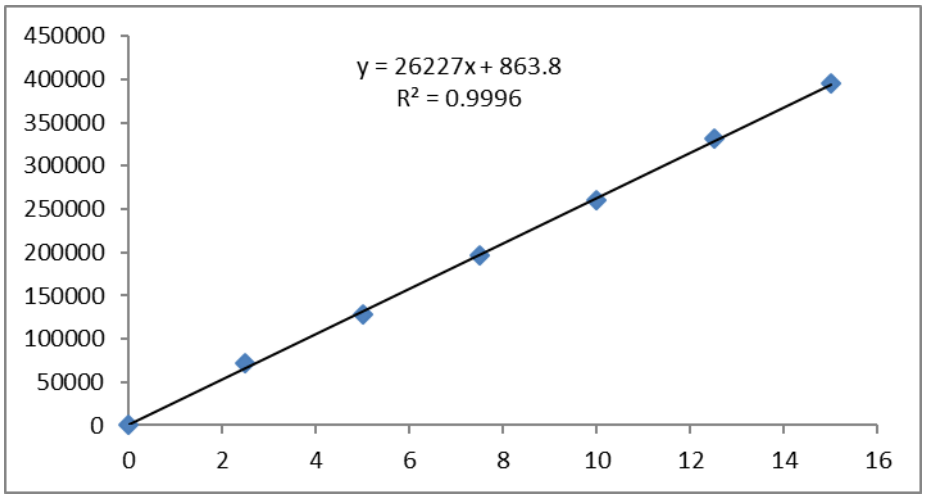


Figure No. 6: Calibration curve of Tezacaftor

Precision:

System Precision: 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 three drugs and obtained as 1.0%, 0.6% and 1.4% respectively for Tezacaftor, Ivacaftore and Elexacaftor. As the limit of Precision was less than "2" the system precision was passed in this method.

Table No. 6: System precision table of Tezacaftor, Ivacaftor and Elexacaftar

S. No.	**Area of Tezacaftor**	**Area of Ivacaftor**	**Area of Elexacaftor**
1.	241552	696496	1649897
2.	238037	695730	1662332
3.	243849	693416	1666767
4.	241828	685418	1716345
5.	243505	694133	1687564
6.	244992	689646	1687753
Mean	242294	692473	1678443
S.D	2451.7	4200.1	23736.9
%RSD	1.0	0.6	1.4

Robustness: Robustness conditions like Flow minus (0.9 ml/min), Flow plus (1.1 ml/min), mobile phase minus (65W:35M), mobile phase plus (55W:45M), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

Table No. 7: Robustness data for Tezacaftor, Ivacaftor and Elexacaftor

S.no	**Condition**	**%RSD of Ivacaftor**	**%RSD of Elexacaftor**	**%RSD of Tezacaftor**
1	**Flow rate (-) 0.9ml/min**	0.7	1.0	1.5
2	**Flow rate (+) 1.1ml/min**	1.3	1.7	0.7
3	**Mobile phase (-) 65W:35M**	0.4	0.2	0.2
4	**Mobile phase (+) 55W:45M**	0.8	1.1	0.8
5	**Temperature (-) 25°C**	0.7	0.1	0.14
6	**Temperature (+) 35°C**	0.8	0.9	0.13

Assay:

The label claim Ivacaftor 50mg, Tezacaftor 75 mg, Elexacaftor 100 mg per unit formulation Assay was performed with the above formulation. Average % Assay for Tezacaftor, Ivacaftor and Elexacaftor. Obtained was 99.45%, 99.06% and 99.71% respectively.

Table No. 8: Assay Data of Tezacaftor

S.no	Standard Area	Sample area	% Assay
1	241552	243899	100.40
2	238037	240258	98.90
3	243849	239092	98.42
4	241828	239856	98.74
5	243505	245050	100.87
6	244992	241265	99.32
Avg	242442	241570	99.44
Stdev	2451.7	2384.4	0.982
%RSD	1.0	1.0	1.0

Table No. 9: Assay Data of Ivacaftor

S.no	Standard Area	Sample area	% Assay
1	695730	694009	100.14
2	693416	692897	99.98
3	685418	689369	99.47
4	694133	691419	99.76
5	689646	696462	100.49
6	691669	695636	100.37
Avg	4146.8	693299	100.04
Stdev	0.6	2649.9	0.38
%RSD	695730	0.4	0.4

Table No. 10: Assay Data of Elexacaftor

S.no	Standard Area	Sample area	% Assay
1	1649897	1710706	101.82
2	1662332	1666432	99.19
3	1666767	1655676	98.54
4	1716345	1695007	100.89
5	1687564	1684358	100.25
6	1687753	1684057	100.23
Avg	1678443	1682706	100.15
Stdev	23736.9	19674.0	1.171
%RSD	1.4	1.2	1.2

DEGRADATION

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation.

Table No. 11: Degradation Data of Tezacaftor

S.NO	Degradation Condition	Area	% Area Recovery	% Drug Degraded
1	Acid	231994	95.56	4.44
2	Alkali	230518	94.95	5.05
3	Oxidation	231055	95.17	4.83
4	Thermal	234276	96.50	3.50
5	UV	238075	98.06	1.94
6	Water	241204	99.35	0.65

Table No. 12: Degradation Data of Ivacaftor

S.NO	Degradation Condition	Area	% Area Recovery	% Drug Degraded
1	Acid	679644	97.95	2.05
2	Alkali	675568	97.36	2.64
3	Oxidation	667770	96.24	3.76
4	Thermal	662999	95.55	4.45
5	UV	677528	97.65	2.35
6	Water	688208	99.19	0.81

Table No. 13: Degradation Data of Elexacaftor

S.NO	Degradation Condition	Area	% Area Recovery	% Drug Degraded
1	Acid	1641326	97.69	2.31
2	Alkali	1563353	93.05	6.95
3	Oxidation	1650964	98.26	1.74
4	Thermal	1660847	98.85	1.15
5	UV	1661622	98.90	1.10
6	Water	1676538	99.79	0.21

CONCLUSION

A simple, accurate, precise method was developed for the simultaneous estimation of the Ivacaftor, Elexacaftor and Tezacaftor in Tablet dosage form. Retention time of Ivacaftor, Elexacaftor and Tezacaftor were found to be 2.322 min, 2.847 min and 3.457 min %RSD of system precision for Ivacaftor, Elexacaftor and Tezacaftor were and found to be 0.6, 1.4 and 1.0 respectively. %RSD of method precision for Ivacaftor, Elexacaftor and Tezacaftor were and found to be 0.4, 1.2, and 1.0 respectively. % recovery was obtained as 100.04%, 100.15% and 99.44% for Ivacaftor, Elexacaftor and Tezacaftor respectively. LOD, LOQ values are obtained from regression equations of Ivacaftor, Elexacaftor and Tezacaftor were 0.12 ppm, 0.36 ppm, 0.06 ppm and 0.18 ppm, 0.03 ppm, 0.08 ppm respectively. Regression equation of Tezacaftor was y = 26227x + 863.8, Ivacaftor was y = 47191x + 7683 and of Elexacaftor was y = 84526x + 21311. Retention times are decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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