METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF LUMACAFTOR AND IVACAFTOR IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

Gangi.Sireesha*, Dr.D.Narendra, Lakshmi Prasanna, Laxmi Priya

Department of Pharmaceutical Analysis, VJ’S College of Pharmacy, Rajahmundry.

Abstract: A simple, Accurate, precise method was developed for the simultaneous estimation of the Ivacaftor and Lumacaftor in Tablet dosage form. Chromatogram was run through Phenominex (4.6 x 250mm, 5μm), Mobile phase containing Buffer 40% buffer 60% Acetonitrile taken in the ratio 40:60 was pumped through column at a flow rate of 1 ml/min. Buffer used in this method was 0.1N H3PO4. Temperature was maintained at 30°C. Optimized wavelength selected was 265 nm. Retention time of Ivacaftor and Lumacaftor were found to be 6.329 min and 2.857 min. %RSD of the Ivacaftor and Lumacaftor were 0.8 and 0.8 respectively. %Recovery was obtained as 100.13% and 100.53% for Ivacaftor and Lumacaftor respectively. LOD, LOQ values obtained from regression equations of Ivacaftor and Lumacaftor were 3.00, 10.00 and 3.02, 9.98 respectively. Regression equation of Ivacaftor is \( y = 27194x + 45038 \) and \( y = 332.76x + 31993 \) of Lumacaftor. Retention times were decreased and run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

INTRODUCTION

Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials. Chemical composition is the entire picture (composition) of the material at the chemical scale and includes geometric features such as molecular morphologies and distributions of species within a sample as well as single dimensional features such as percent composition and species identity.

To be effective and efficient, analyzing samples requires expertise in

1. The chemistry that can occur in a sample.
2. Analysis and sample handling methods for a wide variety of problems (the tools-of-the-trade).
3. Accuracy and precision of the method.
4. Proper data analysis and record keeping.

The major stages of an analytical process are described as follows:

![Figure 1(a): Steps in analytical cycle](image)

The pharmaceutical analysis comprises the procedures necessary to determine the “identity, strength, quality and purity” of such compounds. It also includes the analysis of raw material and intermediates during manufacturing process of drugs.
CHROMATOGRAPHY

Chromatographic separation involves the placing of a sample onto a liquid or solid stationary phase and passing a liquid or gaseous mobile phase through or over it, a process known as elution. Sample components, or solutes, whose distribution ratios between the two phases differ will migrate (be eluted) at different rates, and this differential rate of migration will lead to their separation over a period of time and distance. Chromatographic techniques can be classified according to whether the separation takes place on a planar surface or in a column. They can be further subdivided into gas and liquid chromatography, and by the physical form, solid or liquid, of the stationary phase and the nature of the interactions of solutes with it, known as sorption mechanisms.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

High performance liquid chromatography is a very sensitive analytical technique most widely used for quantitative and qualitative analysis of pharmaceuticals. The principle advantage of HPLC compared to classical column chromatography is improved resolution of the separated substance, faster separation times and the increased accuracy, precision and sensitivity.

Principle of Separation and its type

There are four types of chromatography in which the mobile phase is a liquid. The mobile phase is pumped through the packed column, under high pressure.

a. Partition chromatography
   i. Normal phase chromatography
   ii. Reverse phase chromatography
b. Adsorption or liquid solid chromatography
c. Ion exchange chromatography
d. Size exclusion or gel permeation chromatography

NORMAL PHASE CHROMATOGRAPHY

In normal phase mode, the stationary phase (e.g. silica gel) is polar in nature and the mobile phase is non-polar in this technique, non-polar compounds travel faster and are eluted first. This is because less affinity between solute and stationary phase. Polar compounds are retained for longer time in the column because more affinity towards stationary phase and takes more time to be eluted from the column. This is not advantageous in pharmaceutical applications since most of the drug molecules polar in nature and takes longer time to be eluted and detected. Hence this technique is not widely used in pharmacy.

SOLUTE POLARITY:

Adsorption strengths increased with increasing solute polarity and this is favorable interaction between the polar solutes and the polar stationary phase increases the elution time (note: the interaction strength not only depends on the functional groups in the enlight molecule, but also stearic factors).

Reverse phase chromatography

In RP-HPLC the stationary phase is non-polar often a hydrocarbon and the mobile phase is relatively polar such as water, methanol or Acetonitrile. In RPC the solutes are eluted in the order of their decreasing polarities. These are prepared by treating the surface of silanol group with an organochlorosilane reagent.

Reverse phase mode

Non-polar stationary phase and polar mobile phase is used here.

Mechanism

Retention by interaction of the stationary phase non-polar hydrocarbon chain with non-polar parts and sample molecules.

ADSORPTION OR LIQUID SOLID CHROMATOGRAPHY

The principle of separation is adsorption, separation of components takes place because of difference in affinity of compounds towards stationary phase. This principle is seen in normal phase as well as reverse phase mode, where adsorption will takes place.
ION-EXCHANGE CHROMATOGRAPHY

In ion exchange chromatography, retention is based on the attraction between solute ions and charged sites bound to stationary phase. Ions of the same charge are excluded. Some types of ion Exchangers include: (1) polystyrene resins-allows cross linkage which increases the stability of the chain. Higher cross linkage reduces swerving, which increases the equilibration time and ultimately improves selectivity. (2)Cellulose and dextran ion-exchangers (gels) these possess larger pore sizes and low charge densities making them suitable for protein separation. (3) controlled-pore glass or porous silica

In general, ion-exchangers favor the binding of ions of higher charge and smaller radius. An increase in counter ion (with respect to the functional groups in resins) concentration reduces the retention time. An increase in pH reduces the retention time in cation exchange while a decrease in pH reduces the retention time in anion exchange.

SIZE EXCLUSION CHROMATOGRAPHY

Size exclusion chromatography (SEC) also known as gel permeation chromatography or gel filtration chromatography, separates particles on the basics of size. It is generally a low resolution chromatography and thus it is often reserved for the final, “polishing” step of purification. It is also useful for determining the tertiary structure and quaternary structure of purified proteins, and is the primary technique for determining the average molecular weight of natural and synthetic polymers.

METHODS OF QUANTITATIVE ANALYSIS:

The sample or solute is analyzed quantitatively in HPLC by either peak height or peak area measurements. Peak areas are proportional to the amount of the material eluting from the column as long as the solvent flows at constant rate. Peak heights are proportional to the amount of the material only when peak widths are constant and are strongly affected by the sample injection techniques. There are five principles evaluation methods for quantifying the solute:

(a) Calibration by Standards:

Calibration curves for each component are prepared from pure standards, using identical injection volumes of operating conditions for standards and samples. The concentration of solute is read from its curve if the curve is linear

\[ X = K \times \text{Area} \]

Where, \( x = \) concentration.

\[ K = \text{proportionality constant (slope of the curve).} \]

In this evaluation method, only the area of the peaks of interest is measured. Relative response factors must be considered when converting areas to volume and when the response of the given detector differs for each molecular type of compounds.

(b) Internal Standard Method:

In this technique, a non quantity of internal standard is chromatographed and area Vs concentration is ascertained. Then a quantity of internal standard is added to the raw sample prior to any sample pretreatment or separation operations. The peak area of the standard in the sample run is compared with the peak area when the standard is run separately. This ratio serves as correction factor for variation in sample size, for losses in any preliminary pretreatment operations, or for incomplete elution of the sample.

The material selected for the internal standard must be completely resolved from adjacent sample components and should not interfere with the sample components and never be present in samples.

\[ \frac{\text{Area of sample}}{\text{Area ratio}} = \frac{\text{Area of internal standard}}{\text{Area of sample}} \]

\[ \text{Sample concentration} = \frac{\text{Area of sample}}{\text{Area of internal standard}} \times \text{concentration of standard} \]
(c) Area normalization

This technique is often used for the sample having identical components. It is used to evaluate the absolute purity of the sample. The procedure is to total up the areas under all peaks and then calculates the percentage of the total area that is contributed by the compound of interest. For this method the entire sample must be eluted, all components must be separated and each peak must be completely resolved.

(d) Standard addition method

If only few samples are to be chromatographed, it is possible to employ the method of standard addition (s). The chromatogram of the unknown is recorded, then a known amount of analyte (s) is added and the chromatogram is repeated using same reagents, instruments and other conditions. From the increase in the peak area (or peak height), the original concentration can be computed by interpolation.

If an instrumental reading (area/height) ‘Rx’ is obtained, from a sample of unknown ‘x’ and a reading ‘Rt’ is obtained from the sample to which a known concentration ‘a’ of analyte has been added, then ‘x’ can be calculated from

\[ \frac{x}{x+a} = \frac{Rx}{Rt} \]

A correction for dilution must be made if the amount of standard added, changes the total sample volume significantly. It is always advisable to check the result by adding at least one other standard.

Principle of separation

HPLC is based on the mechanism of adsorption, partition, ion exchange or size exclusion, depending on the type of stationary phase used. HPLC involves a solid stationary phase, normally packed inside a stainless-steel column, and a liquid mobile phase. Separation of the components of a solution results from difference in the relative distribution ratios of the solutes between the two phases.

The majority of the HPLC separation are done with Reversed phase separation, probably over 90%. In reversed phase separation organic molecules are separated based on the degree of hydrophobicity. There is a correlation between the degree of lipophylicity and retention in the column. This is the list of mobile phase parameters effecting retention and separation in reversed phase. Elution order in normal phase HPLC shows that the polar solutes elute later then non polar lyophilic ones.

![Figure 1(c): principles of separation in HPLC](image-url)
METHOD DEVELOPMENT IN RP-HPLC

Retention in RP-HPLC

The Reverse phase chromatography retention of a compound is determined by its polarity and experimental conditions, mobile phase, column and temperature.

1. Mobile phase effects

Retention (compound K value) can be preferably adjusted by changing mobile phase composition or solvent strength in RPC. Retention is less for stronger, less polar mobile phases. Solvent strength depends on

i) Choice of organic solvent or choice of % B.

ii) Concentration of the organic solvent in the mobile phase A: % B where A is water, B is the organic phase and % is volume % v/v.

iii) A retention range of 0.5< K<20 are allowable for sample to be separated using isocratic condition but 1< K<10 is generally preferred.

a) Choice of organic phase

A mobile phase of 100% Acetonitrile is a stronger polar solvent, which might result in (K<0.2), so weaker mobile phase is required to retain the compound. By decreasing the percentage of Acetonitrile, retention time will increase. If organic phase is decreased by 10%, the K value increases 3 times approximately. Systematic decrease of % B to investigate sample retention is a simple and convenient way to determine the best mobile phase composition for a given sample.

b) Mobile-Phase strength

Mobile phase strength in RPC depends upon both % B and the type of organic solvent. RPC solvent strength varies as water (weakest) < methanol < Acetonitrile < ethanol < tetrahydrofuran < propanol < methylene chloride (strongest). Solvent strength increases as solvent polarity decreases. Any of the above solvents can be used with water for Reverse phase chromatography, except methylene chloride since it is not water miscible. Acetonitrile is the best initial choice of organic solvent for the mobile phase. The next best organic solvent is methanol followed by tetrahydrofuran.

2. Selectivity in RP-HPLC

Three main variables can be used in RPC to change selectivity (α) for neutral samples like mobile phase composition, column type and temperature. Overall sample retention acceptable is (0.5< K<20).

a) Solvent-strength selectivity

The best sample resolution will occur for a % B value, where both pairs have the same resolution peak spacing can be
explored while % B is varied for optimum sample retention (0.5< K< 20).

b) Solvent type selectivity

A change in organic solvent type is often used to change peak spacing and improve resolution. The selection of RPC solvents for this purpose is guided by solvent properties that are believed to affect selectivity, acidity, basicity and dipolarity. Only a slight increase (2 to 5 %) in the selectivity (α value) for a critical band pair may be necessary to achieve acceptable resolution. Changing solvent type in RPC is usually the most effective procedure to alter selectivity and achieve the separation of multicomponent neutral samples.

c) Column type selectivity

A change in column type can produce useful changes in selectivity and overall sample retention. Retention is greater (run time longer) on the stronger (C₈ and phenyl column) vs. the weaker cyano column. A change of the column is usually less useful than a change in mobile phase type hence this should be tried only after the use of solvent strength or solvent type selectivity has failed. Usually a C₈ or C₁₈ column should be tried first followed by a cyano, then by a phenyl column. Column pads/bonded with cyclodextrin (CD) are useful in separation of enantiomeric isomers.

d) Temperature selectivity

Values of K decreases at higher temperature for the RPC separation of neutral compounds. This is less effective for non-ionic compounds as a mean of altering selectivity for improved separation. As the temperature is increased, the relative retention of the polar compounds decreases more rapidly than for the non-polar compounds.

Equipment for HPLC:
The essential features of modern HPLC are illustrated in the block diagram and compromise of components

1. Pumping system
2. A injector
3. Chromatographic column
4. Detector
5. Data collection device (computer, integrator, or recorder)

![Figure 1(E): BLOCK DIAGRAM FOR DIFFERENT PARTS OF HPLC](image)

**Pumping system:**

HPLC pumping systems are required to deliver metered amounts of mobile phase at a constant flow rate. Pumping systems that deliver solvent from one or more reservoirs are available. Pressure fluctuations should be minimized, e.g. by passing the pressurized solvent through a pulse dampening device. Tubing and connections should be capable of withstanding the pressures developed by the pumping systems. Many HPLC pumps are fitted with a facility for “bleeding” the system of entrapped air bubbles.
Modern computer- or microprocessor- controlled pumping systems are capable of delivering a mobile phase of either constant (isocratic elution) or varying (gradient elution) composition, according to a defined programme. In the case of gradient elution, solvent mixing can be achieved on either the low or high-pressure side of the pump(s) depending on the flow rate and composition of mobile phase, operating pressure of up to 42000 kpa (6000 psi) can be generated during routine analysis.

An injector:

The sample solution is usually introduced into the flowing mobile phase at or near the head of the column using an injection system based on an injection valve design which can operate at high pressure. Such an injection system has a fixed or a variable column device which can be operated manually or by an auto sampler. Partially filling of tuber may lead to poorer injection volume precision.

Chromatographic column:

Columns are usually made of polished stainless steel, or between 50 and 300 mm long, and have an internal diameter of between 2 to 5 mm. They are commonly filled with a stationary phase with a particle size of 5 - 10 µm. Columns with internal diameters of less than 2 mm are often referred to as micro bore columns. Ideally, the temperature of the mobile phase and the column should be kept to constant during an analysis. Most separations are performed at an ambient temperature, but columns may be heated to give better efficiency normally, columns should not be heated above 60°C because of the potential for stationary phase degradation or changes occurring to the composition of the mobile phase.

Detector:

Ultraviolet/visible (UV/vis) absorption spectrometer are the most commonly used detectors for pharmaceutical analysis. In specific cases fluorescence spectrophotometers, differential refracting meters, electro chemical detectors, light scattering detectors, mass spectrometers, or other special detectors may be used. Here an analyte possesses a chromophoric group that absorbs UV/vis radiation, the UV/vis detector is the most appropriate because of its sensitivity and stability. Such a detector is not suitable for detecting analytes with very weak chromophores.

A variant on the UV/vis type of detector, which is becoming increasingly popular because of its ability to furnish detailed spectral information, is the diode array detector. This type of detector acquires absorbance data over a certain UV/vis range and can provide chromatograms at multiple, selectable wave lengths, together with spectra for the eluted peaks. In addition, the detector and accompanying computer programs can be used to assess the spectral homogeneity of peaks, which may provide information on the chromatographic purity of the peaks. This can be especially useful in method development and validation.

Enhanced sensitivity may be achieved in certain cases by using pre-column or post-column derivatization techniques. (These techniques are to be avoided for control of impurities.)

Data collection devices:

Signals from detector may be collected on chart recorders or electronic integrators that vary in complexity and in their ability to process, store and reprocess chromatographic data. The data storage capacity of these devices is usually limited.

Modern data stations are computer based and have a large storage capacity to collect, process, and store data for possible subsequent reprocessing. Analytical reports can be customized to the needs of the analyst.

Integration of peak areas and the setting of threshold levels are not normally problematic in an assay since the peak of the substance to be analyzed should be free of interference. However, in a test for impurities, the selection of the peak area integrator parameters become very important, particularly when baseline separations are not always attainable. If baseline separations cannot be obtained, valley-to-valley integration should be employed.

HPLC allows limits to be set for individual impurities and for the sum of impurities, but there is a level at which peaks should not be integrated. This “disregard level” is set in relation to the area of the peak in the chromatogram of the prescribed reference solution and is usually equivalent to 0.05% or the substance being examined.

DRUG PROFILES

DRUG PROFILE OF IVACAFTOR

Chemical name: Ivacaftor; VX-770; 873054-44-5; Kalydeco; Ivacaftor (VX-770); VX770

Synonym : kalydeco, vx770
Structural formula:

Molecular weight : 392.49072 g/mol

Molecular formula: C24H2,N2O3

Description: Ivacaftor is a drug used to treat cystic fibrosis in people with certain mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, who account for 4–5% cases of cystic fibrosis.

Solubility: Solubility: 0.002mg/ml in Water; practically insoluble in water and buffers at pH 7
Freely soluble in methyl ethyl ketone and water mixture
Soluble in 2 methyl tetra hydro furan and PEG 400
Slightly soluble in ethanol, methanol and acetone.

Category : cystic fibrosis transductase inhibitors

Bioavailability: 1hour
log D value : 5.68
pka value : 9.40-11.60
u v /vi s : 213, 247, 254, 310nm

Pharmacology:

Mechanism of action: Cystic fibrosis is caused by any one of several defects in a protein, cystic fibrosis transmembrane conductance regulator, which regulates fluid flow within cells and affects the components of sweat, digestive fluids, and mucus. The defect, which is caused by a mutation in the individual's DNA, can be in any of several locations along the protein, each of which interferes with a different function of the protein. One mutation, G551D, lets the CFTR protein reach the epithelial cell surface, but doesn't let it transport chloride through the ion channel. Ivacaftor is a potentiator of the CFTR protein. The CFTR protein is a chloride channel present at the surface of epithelial cells in multiple organs. Ivacaftor facilitates increased chloride transport by potentiating the channel-open probability (or gating) of the G551D-CFTR protein.

DRUG PROFILE OF LUMACAFTOR

Chemical name: 3-(6-(1-(2,2-di fluoro-1-3-benzodioxol-5-yl)cyclopropyl)carbonyl)amino)-3-methylpyridin-2-yl)benzoic acid.

Structural formula:

Molecular weight: 452.41
Molecular formula: C24H18F2N2O5

Description: Lumacaftor/ivacaftor (brand name Orkambi) is a combination drug available as a single pill that is used for the treatment of cystic fibrosis in people who have the F508del mutation in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) protein. It is a combination drug that consists of lumacaftor and ivacaftor. Ivacaftor increases the activity of the CFTR protein at the surface of epithelial cell, while lumacaftor acts as a chaperone during protein folding and increases the number of CFTR proteins that are trafficked to the cell surface. It was approved by the US FDA in July 2015.

Solubility: it is soluble in water and buffers at pH 1-8 sparingly soluble in butanol freely soluble in formic acid and 2-methyl tetrahydrofuran.

PKa: strong acidic (9.16)

Category: cystic fibrosis transmembrane conductance regulator.

Pharmacology:

Mechanism of action: Orkambi is a combination of lumacaftor and ivacaftor, both of which are oral cystic fibrosis transmembrane conductance regulator (CFTR) modulators. The CFTR protein is a chloride channel present at the surface of epithelial cells in multiple.

EXPERIMENTAL METHODOLOGY

INSTRUMENTS USED

<table>
<thead>
<tr>
<th>SL. No</th>
<th>Instrument</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HPLC</td>
<td>WATERS, software: Empower, 2695 separation module, UV detector.</td>
</tr>
<tr>
<td>2</td>
<td>UV/VIS spectrophotometer</td>
<td>LABINDIA UV 3000+</td>
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<tr>
<td>3</td>
<td>pH meter</td>
<td>Adwa – AD 1020</td>
</tr>
<tr>
<td>4</td>
<td>Weighing machine</td>
<td>Afcoset ER-200A</td>
</tr>
<tr>
<td>5</td>
<td>Pipettes and Burettes</td>
<td>Borosil</td>
</tr>
<tr>
<td>6</td>
<td>Beakers</td>
<td>Borosil</td>
</tr>
</tbody>
</table>

CHEMICALS USED:

<table>
<thead>
<tr>
<th>SL. No</th>
<th>Chemical</th>
<th>Company Name</th>
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</thead>
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<tr>
<td>1</td>
<td>Lumacaftor</td>
<td>PHARMATRAIN</td>
</tr>
<tr>
<td>2</td>
<td>Ivacaftor</td>
<td>PHARMATRAIN</td>
</tr>
<tr>
<td>3</td>
<td>Water and Methanol for HPLC</td>
<td>LICHROSOLV (MERCK)</td>
</tr>
<tr>
<td>4</td>
<td>Acetonitrile for HPLC</td>
<td>MOLYCHEM</td>
</tr>
<tr>
<td>5</td>
<td>Ortho phosphoric Acid</td>
<td>MERCK</td>
</tr>
</tbody>
</table>

HPLC METHOD DEVELOPMENT:

Mobile Phase Optimization:

Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to orthophosphoric acid with buffer (pH 4.5), Acetonitrile in proportion 40: 60 v/v respectively.

Wave length selection:

UV spectrum of 10 µg/ml Lumacaftor and Ivacaftor in diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 265nm. At this wavelength both the drugs show good absorbance.
Optimization of Column:

The method was performed with various columns like C18 column, hypersil column, lichrosorb, and inertsil ODS column. Inertsil ODS (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1.0 ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

Instrument used : Waters HPLC with aut sampler and 2487 UV detector.

Temperature : Ambient

Column : Phenominex (4.6 x 250mm, 5µm)

Buffer : 1ml of orthophosphoric acid in 1000ml water, pH adjusted with NaOH.

pH : 4.5

Mobile phase : 40% buffer 60% Acetonitrile

Flow rate : 1 ml per min

Wavelength : 265 nm

Injection volume : 10 µl

Run time : 10 min.

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of 0.1% OPA buffer:

Pipette out 1ml of Ortho Phosphoric Acid was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 4.5 with NaOH

Preparation of mobile phase:

Accurately measured 400 ml (40%) of above buffer and 600 ml of Acetonitrile HPLC (60%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.
PREPARATION OF THE LUMACAFTOR & IVACAFTOR STANDARD & SAMPLE SOLUTION:

**Standard Solution Preparation:**

Accurately weigh and transfer 20 mg of Lumacaftor and 12.5 mg of Ivacaftor working standard into a 10 ml clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 1.5 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

**Sample Solution Preparation:**

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 20 mg of Lumacaftor and 12.5mg Ivacaftor sample into a 10mL clean dry volumetric flask add about 7 mL of Diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is Filtered through 0.44 micron Injection filter. (Stock solution)

Further pipette 1.5 ml of Lumacaftor and Ivacaftor from the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Procedure:**

Inject 10 μL of the standard, sample into the chromatographic system and measure the areas for Lumacaftor and Ivacaftor peaks and calculate the %Assay by using the formulae.

**SYSTEM SUITABILITY:**

Tailing factor for the peaks due to Lumacaftor and Ivacaftor in Standard solution should not be more than 2.0

Theoretical plates for the Lumacaftor and Ivacaftor peaks in Standard solution should not be less than 2000.

Resolution for the Lumacaftore and Ivacaftor peaks in standard solution should not be less than 2.

**LINEARITY:**

Preparation of stock solution:

Accurately weigh and transfer 20 mg of Lumacaftor and 12.5 mg of Ivacaftor working standard into a 10 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Preparation of Level – I:

0.5 ml of above stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – II:

1 ml of above stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – III:

1.5 ml of above stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – IV:

2.0 ml of above stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent

Preparation of Level – V: 2.5 ml of above stock solutions has taken in different 10ml of volumetric flasks, dilute up to the mark with diluent

**Procedure:**

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.
ACCURACY:

Preparation of Standard stock solution:

Accurately weigh and transfer 20 mg of Lumacaftor and 12.5 mg of Ivacaftor working standard into a 10 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1.5 ml of Lumacaftor & Ivacaftor of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration):

Accurately weigh and transfer 10 mg of Lumacaftor and 6.25 mg of Ivacaftor working standard into a 10 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1.5 ml of Lumacaftor & Ivacaftor of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 20 mg of Lumacaftor and 12.5 mg of Ivacaftor working standard into a 10 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1.5 ml of Lumacaftor & Ivacaftor of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 150% solution (With respect to target Assay concentration):

Accurately weigh and transfer 30 mg of Lumacaftor and 18.75 mg of Ivacaftor working standard into a 10 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1.5 ml of Lumacaftor & Ivacaftor of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions.

Calculate the Amount found and Amount added for Lumacaftor & Ivacaftor and calculate the individual recovery and mean recovery values.

PRECISION:

Preparation of stock solution:

Accurately weigh and transfer 20 mg of Lumacaftor and 12.5 mg of Ivacaftor working standard into a 10 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1.5 ml of Lumacaftor & Ivacaftor of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

The standard solution was injected for six times and measured the area for all six. Injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

INTERMEDIATE PRECISION/RUGGEDNESS:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day.
Preparation of stock solution:

Accurately weigh and transfer 20 mg of Lumacaftor and 12.5 mg of Ivacaftor working standard into a 10 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1.5 ml of Lumacaftor & Ivacaftor of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

The standard solutions prepared in the precision was injected on the other day, for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

DETECTION LIMIT

LIMIT OF DETECTION: (for Lumacaftor)

Preparation of 300µg/ml solution:

Accurately weigh and transfer 20 mg of Lumacaftor working standard into a 10 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1.5 ml of Lumacaftor of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 2.93 µg/ml solution:

Further pipette 0.65ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Further pipette 1.5ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

LIMIT OF QUANTIFICATION:

Preparation of 300 µg/ml solution:

Accurately weigh and transfer 20 mg of Lumacaftor working standard into a 10 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1.5 ml of Lumacaftor of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 9.53µg/ml solution:

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

LIMIT OF DETECTION: (for Ivacaftor)

Preparation of 187.5 µg/ml solution:

Accurately weigh and transfer 12.5 mg of Ivacaftor working standard into a 10 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1.5 ml of Ivacaftor of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 3.11 µg/ml solution:

Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

LIMIT OF QUANTIFICATION:

Preparation of 187.5µg/ml solution:

Accurately weigh and transfer 12.5 mg of Ivacaftor working standard into a 10 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 1.5 ml of Ivacaftor of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.
Preparation of 10.29 µg/ml solution:
Further pipette 3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.
Further pipette 1.83ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluents

Procedure for LOD and LOQ:
The LOD and LOQ solutions was prepared injected, for three times and measured the area for all three injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits

ROBUSTNESS:
As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method.

A. The flow rate was varied at 0.9 ml/min to 1.1ml/min. Standard solution 300 ppm of Lumacaftor & 187.5 ppm of Ivacaftor was prepared and analysed using the varied flow rates along with method flow rate.
On evaluation of the above results, it can be concluded that the variation in flow rate affected the method significantly. Hence it indicates that the method is robust even by change in the flow rate ±10%.

B. The Organic composition in the Mobile phase was varied from 50% to 50%.
Standard solution 300 ppm of Lumacaftor & 187.5 ppm of Ivacaftor was prepared and analysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.
On evaluation of the above results, it can be concluded that the variation in 10%. Organic composition in the mobile phase affected the method significantly. Hence it indicates that the method is robust even by chan

RESULTS AND DISCUSSION

HPLC METHOD

TRAIL NO: 1
Mobile phase - Methanol: Phosphate buffer 4.5 (50:50)
Column: Xterra(250 mm X 4.6 mm)
Flow rate: 1mL/min
Run time: 10 min
Injection volume: 10µL
Wave length: 265 nm

Figure: Chromatogram for Trail-1
TRAIL NO: 2

Mobile phase- Methanol: Triethylamine (50:50)

Column: Xterra (250 mm X 4.6 mm)

Flow rate: 1mL/min

Run time: 10 min

Injection volume: 10µL

Wave length: 265 nm

Figure: Chromatogram for Trail-2

TRAIL NO: 3

Mobile phase- Methanol: phosphate buffer 3 (50:50)

Column: Phenomenex (250 mm X 4.6 mm)

Flow rate: 1mL/min

Run time: 10 min

Injection volume: 10µL
Wave length: 265 nm

Figure: Chromatogram for Trail-3

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Retention time</th>
<th>Area</th>
<th>Height</th>
<th>Resolution</th>
<th>USP Plate count</th>
<th>USP Tailing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.335</td>
<td>26171</td>
<td>2298</td>
<td>1035.46</td>
<td>1.32</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8.844</td>
<td>13652</td>
<td>1336</td>
<td>2.96</td>
<td>2932.57</td>
<td>1.60</td>
</tr>
</tbody>
</table>

TRAIL NO: 4

Mobile phase- Methanol: Orthophosphoric acid(50:50)

Column: Phenomenex (250 mm X 4.6 mm)

Flow rate: 1mL/min

Run time: 10 min

Injection volume: 10µL

Wave length: 265 nm

Figure: Chromatogram for Trail-4

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Retention time</th>
<th>Area</th>
<th>Height</th>
<th>Resolution</th>
<th>USP Plate count</th>
<th>USP Tailing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4330</td>
<td>21863</td>
<td>20402</td>
<td>748.20</td>
<td>1.60</td>
<td></td>
</tr>
</tbody>
</table>
TRAIL NO: 5

Mobile phase: Acetonitrile: Phosphate buffer (50:50)
Column: Phenomenex (250 mm X 4.6 mm)
Flow rate: 1mL/min
Run time: 10 min
Injection volume: 10µL
Wave length: 265 nm

Figure: Chromatogram for Trail-5

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Retention time</th>
<th>Area</th>
<th>Height</th>
<th>Resolution</th>
<th>USP Plate count</th>
<th>USP Tailing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak 1</td>
<td>3.231</td>
<td>25437</td>
<td>23383</td>
<td>1390.88</td>
<td>1.02</td>
<td></td>
</tr>
<tr>
<td>Peak 2</td>
<td>4.900</td>
<td>15383</td>
<td>13827</td>
<td>4.92</td>
<td>2836.30</td>
<td>1.38</td>
</tr>
</tbody>
</table>

TRAIL NO: 6

Temperature : Ambient
Column : Phenominex (4.6 x 250mm, 5µm)
Buffer : 0.1% orthophosphoric acid
pH : 4.5
Mobile phase : 40% buffer 60% Acetonitrile
Flow rate : 1 ml per min
Wavelength : 265 nm
Injection volume : 10 µl
Run time : 10 min.

Figure: Chromatogram for Trail-6
Table 3: Results for Lumacaftor and Ivacaftor in Trail-6 (Optimized Trail)

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Retention time</th>
<th>Area</th>
<th>Height</th>
<th>Resolution</th>
<th>USP Plate count</th>
<th>USP Tailing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumacaftor</td>
<td>2.857</td>
<td>135283</td>
<td>20824</td>
<td>4535</td>
<td>1.30</td>
<td></td>
</tr>
<tr>
<td>Ivacaftor</td>
<td>6.329</td>
<td>120553</td>
<td>12053</td>
<td>16.18</td>
<td>9354</td>
<td>1.16</td>
</tr>
</tbody>
</table>

**OPTIMIZED CHROMATOGRAPHIC CONDITIONS:**

- **Instrument used**: Waters HPLC with auto sampler and 2487 UV detector.
- **Temperature**: Ambient
- **Column**: Phenominex (4.6 x 250mm, 5µm)
- **Buffer**: 1ml of orthophosphoric acid in 1000ml water, pH adjusted with NaOH.
- **pH**: 4.5
- **Mobile phase**: 40% buffer 60% Acetonitrile
- **Flow rate**: 1 ml per min
- **Wavelength**: 265 nm
- **Injection volume**: 10 µl
- **Run time**: 10 min.

Specificity
Fig 7-Chromatogram of blank

Fig 8-Chromatogram of Lumacaftor standard
Fig 9-Chromatogram of Ivacaftor standard

Fig 10-Chromatogram of sample
Fig 10-Chromatogram of standard

Table 8: System suitability parameters for Lumacaftor and Ivacaftor

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>Retention time</th>
<th>Area</th>
<th>Height</th>
<th>Resolution</th>
<th>USP Plate count</th>
<th>USP Tailing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumacaftor</td>
<td>2.857</td>
<td>135283</td>
<td>20824</td>
<td>4535</td>
<td>1.30</td>
<td></td>
</tr>
<tr>
<td>Ivacaftor</td>
<td>6.329</td>
<td>120553</td>
<td>12053</td>
<td>16.18</td>
<td>9354</td>
<td>1.16</td>
</tr>
</tbody>
</table>

Stanadard chromatogram for Lumacaftor and Ivacaftor:
Figure: Chromatogram For Standard
Sample Chromatogram For Lumacaftor and Ivacaftor:

**Figure: Chromatogram for Sample**

**Assay Results: (Lumacaftor)**

\[
\begin{array}{cccccc}
135989 & 20 & 1.5 & 10 & 10 & 473.7 & 99.8 \\
\hline
- & - & - & - & - & - & 100 \% = 100.39 \%
\end{array}
\]

\[
\begin{array}{cccccc}
135383.3 & 10 & 10 & 47.3 & 1.5 & 200 & 100 \\
\end{array}
\]

**Assay Results: (Ivacaftor)**

\[
\begin{array}{cccccc}
121004 & 12.5 & 1.5 & 10 & 10 & 473 & 99.8 \\
\hline
- & - & - & - & - & - & 100 \% = 100.17 \%
\end{array}
\]

\[
\begin{array}{cccccc}
120553 & 10 & 10 & 47.3 & 1.5 & 125 & 100 \\
\end{array}
\]
6.2. ANALYTICAL METHOD VALIDATION

The method was validated for its linearity range, accuracy, precision, sensitivity and specificity. Method validation is carried out as per ICH guidelines.

Linearity:

Figure 11 - Chromatogram of Lumacaftor and Ivacaftor (100 & 62.5 µg/ml)

Figure 12 - Chromatogram of Lumacaftor and Ivacaftor (200 & 125 µg/ml)
Figure 13 - Chromatogram of Lumacaftor and Ivacaftor (300 & 185.5 µg/ml)

Figure 14 - Chromatogram of Lumacaftor and Ivacaftor (400 & 250 µg/ml)
Figure 15 - Chromatogram of Lumacaftor and Ivacaftor (500 & 312.5 µg/ml)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Linearity Level</th>
<th>Concentration (µg/ml)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>100</td>
<td>65792</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>200</td>
<td>98696</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>300</td>
<td>131638</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>400</td>
<td>162911</td>
</tr>
<tr>
<td>5</td>
<td>V</td>
<td>500</td>
<td>200063</td>
</tr>
</tbody>
</table>

Correlation Coefficient: 0.999

10(a): Linearity results of Lumacaftor

<table>
<thead>
<tr>
<th>S. No</th>
<th>Linearity Level</th>
<th>Concentration (µg/ml)</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>62.5</td>
<td>71267</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>125</td>
<td>99725</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>187.5</td>
<td>127369</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>250</td>
<td>155275</td>
</tr>
<tr>
<td>V</td>
<td>312.5</td>
<td>179461</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.999</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10(b): Linearity results of Ivacaftor

Linearity of Lumacaftor and Ivacaftor

**Lumacaftor**

\[ y = 332.76x + 31993 \]

\[ R^2 = 0.999 \]

**Ivacaftor**

\[ y = 27194x + 45038 \]

\[ R^2 = 0.999 \]

**Figure 16- Curve calibration for Lumacaftor and Ivacaftor**

Accuracy 50% results of Lumacaftor and Ivacaftor:
Accuracy 100% results of Lumacaftor and Ivacaftor:
Accuracy 150% results of Lumacaftor and Ivacaftor:
Table Accuracy results for Lumacaftor:

<table>
<thead>
<tr>
<th>% Concentration (at specification Level)</th>
<th>Area</th>
<th>Amount Added (mg)</th>
<th>Amount Found (mg)</th>
<th>% Recovery</th>
<th>Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>67838.3</td>
<td>10</td>
<td>10</td>
<td>100.2%</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>136569</td>
<td>20</td>
<td>20.13</td>
<td>100.07</td>
<td>100.53%</td>
</tr>
<tr>
<td>150%</td>
<td>205309.3</td>
<td>30</td>
<td>30.27</td>
<td>100.90</td>
<td></td>
</tr>
</tbody>
</table>

Table 11(b): Accuracy results of Ivacaftor

<table>
<thead>
<tr>
<th>% Concentration (at specification Level)</th>
<th>Area</th>
<th>Amount Added (mg)</th>
<th>Amount Found (mg)</th>
<th>% Recovery</th>
<th>Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>60620.7</td>
<td>6.25</td>
<td>6.27</td>
<td>100.37%</td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>121845</td>
<td>12.5</td>
<td>12.61</td>
<td>100.89%</td>
<td>100.22%</td>
</tr>
<tr>
<td>150%</td>
<td>179676</td>
<td>18.75</td>
<td>18.59</td>
<td>99.16</td>
<td></td>
</tr>
</tbody>
</table>
Precision:
Table 12: Precision results for Lumacaftor

<table>
<thead>
<tr>
<th>Injection</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection-1</td>
<td>141368</td>
</tr>
<tr>
<td>Injection-2</td>
<td>140717</td>
</tr>
<tr>
<td>Injection-3</td>
<td>142655</td>
</tr>
<tr>
<td>Injection-4</td>
<td>143939</td>
</tr>
<tr>
<td>Injection-5</td>
<td>143013</td>
</tr>
<tr>
<td>Injection</td>
<td>Area</td>
</tr>
<tr>
<td>------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Injection-1</td>
<td>128876</td>
</tr>
<tr>
<td>Injection-2</td>
<td>127224</td>
</tr>
<tr>
<td>Injection-3</td>
<td>129055</td>
</tr>
<tr>
<td>Injection-4</td>
<td>128739</td>
</tr>
<tr>
<td>Injection-5</td>
<td>126699</td>
</tr>
<tr>
<td>Injection-6</td>
<td>129220</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>128302.2</strong></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td><strong>1064.1</strong></td>
</tr>
<tr>
<td><strong>% RSD</strong></td>
<td><strong>0.8</strong></td>
</tr>
</tbody>
</table>

**Table 12: Precision results for Ivacaftor:**

<table>
<thead>
<tr>
<th>Injection</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection-6</td>
<td>142282</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>142329.0</strong></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td><strong>1156.8</strong></td>
</tr>
<tr>
<td><strong>% RSD</strong></td>
<td><strong>0.8</strong></td>
</tr>
</tbody>
</table>

**ID Precision:**
**Table:** The ID Precision results are summarized Lumacaftor

<table>
<thead>
<tr>
<th>Injection</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection-1</td>
<td>139453</td>
</tr>
<tr>
<td>Injection-2</td>
<td>137162</td>
</tr>
<tr>
<td>Injection-3</td>
<td>139458</td>
</tr>
<tr>
<td>Injection-4</td>
<td>138377</td>
</tr>
<tr>
<td>Injection-5</td>
<td>138482</td>
</tr>
<tr>
<td>Injection-6</td>
<td>139771</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>138783.8</strong></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td><strong>976.1</strong></td>
</tr>
<tr>
<td><strong>%RSD</strong></td>
<td><strong>0.7</strong></td>
</tr>
</tbody>
</table>

**Table:** ID Precision The results are summarized Ivacaftor

<table>
<thead>
<tr>
<th>Injection</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection-1</td>
<td>122535</td>
</tr>
<tr>
<td>Injection-2</td>
<td>121224</td>
</tr>
<tr>
<td>Injection-3</td>
<td>122915</td>
</tr>
<tr>
<td>Injection-4</td>
<td>123391</td>
</tr>
<tr>
<td>Injection-5</td>
<td>123108</td>
</tr>
<tr>
<td>Injection-6</td>
<td>122959</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>122688.7</strong></td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td><strong>769.7</strong></td>
</tr>
<tr>
<td><strong>%RSD</strong></td>
<td><strong>0.6</strong></td>
</tr>
</tbody>
</table>

LOD:
Lumacaftor:

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 66 µV

Signal Obtained from LOD solution : 198 µV

S/N = 198/66 = 3.00

Acceptance Criteria:

S/N Ratio value shall be 3 for LOD solution.

Ivacaftor:

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 66 µV

Signal Obtained from LOD solution : 199 µV

S/N = 199/66 = 3.02

Acceptance Criteria:

S/N Ratio value shall be 3 for LOD solution.

LOQ:
LUMACAFTOR

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 66 µV
Signal Obtained from LOQ solution : 659µV

S/N = 659/66 = 9.98

Acceptance Criteria:
S/N Ratio value shall be 10 for LOQ solution.

IVACAFTOR:

Calculation of S/N Ratio:

Average Baseline Noise obtained from Blank : 66 µV
Signal Obtained from LOQ solution : 660µV

S/N = 660/66 = 10.00

Acceptance Criteria:
S/N Ratio value shall be 10 for LOQ solution.

Robustness:
Less Flow:
More Flow:

![Graph showing peak areas for Lumacaftor and Ivacaftor](image)

Table 13: Robustness studies for Lumacaftor and Ivacaftor

System suitability results for Lumacaftor:

<table>
<thead>
<tr>
<th>S. No</th>
<th>Flow Rate (ml/min)</th>
<th>System Suitability Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>USP Plate Count</td>
</tr>
<tr>
<td>1</td>
<td>0.9</td>
<td>4685.09</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>4509.7</td>
</tr>
<tr>
<td>3</td>
<td>1.1</td>
<td>4065.51</td>
</tr>
</tbody>
</table>
System suitability results for Ivacaftor:

<table>
<thead>
<tr>
<th>S. No</th>
<th>Flow Rate (ml/min)</th>
<th>System Suitability Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>USP Plate Count</td>
</tr>
<tr>
<td>1</td>
<td>0.9</td>
<td>4731.46</td>
</tr>
<tr>
<td>2</td>
<td>1.0</td>
<td>4509.7</td>
</tr>
<tr>
<td>3</td>
<td>1.1</td>
<td>4549.3</td>
</tr>
</tbody>
</table>

Less Organic:

More organic:
System suitability results for Lumacaftor:

<table>
<thead>
<tr>
<th>S. No</th>
<th>Change in Organic Composition in the Mobile Phase</th>
<th>System Suitability Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>USP Plate Count</td>
</tr>
<tr>
<td>1</td>
<td>10% less</td>
<td>4382.7</td>
</tr>
<tr>
<td>2</td>
<td>*Actual</td>
<td>4509.7</td>
</tr>
<tr>
<td>3</td>
<td>10% more</td>
<td>4982.7</td>
</tr>
</tbody>
</table>

System suitability results for Ivacaftor:

<table>
<thead>
<tr>
<th>S. No</th>
<th>Change in Organic Composition in the Mobile Phase</th>
<th>System Suitability Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>USP Plate Count</td>
</tr>
<tr>
<td>1</td>
<td>10% less</td>
<td>4643.64</td>
</tr>
<tr>
<td>2</td>
<td>*Actual</td>
<td>4509.7</td>
</tr>
<tr>
<td>3</td>
<td>10% more</td>
<td>4987.28</td>
</tr>
</tbody>
</table>

* Results for actual Mobile phase composition (40:60 Buffer: ACN) have been considered from Accuracy standard.

SUMMARY AND CONCLUSION

The estimation of Lumacaftor and Ivacaftor was done by RP-HPLC.

The assay of Lumacaftor and Ivacaftor was performed with tablets and the % assay was found to be 100.39 and 100.17 which shows that the method is useful for routine analysis.

The linearity of Lumacaftor and Ivacaftor was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity.

The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.8 and 0.8 for Lumacaftor and Ivacaftor which shows that the method is precise.
The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.7 and 0.6 for Lumacaftor and Ivacaftor which shows that the method is repeatable when performed in different days also.

The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.53% and 100.13% for Lumacaftor and Ivacaftor. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility.

The acceptance criteria for LOD and LOQ is 3 and 10. The LOD and LOQ for Lumacaftor was found to be 3.02 and 9.98 and LOD and LOQ for Ivacaftor was found to be 3.00 and 10.00.

The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

BIBLIOGRAPHY

[17] From drug bank.com