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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF IVACAFTOR AND LUMACAFTOR BY RP-HPLC METHOD

B. Sravanthi*, M. Divya

Department of Pharmaceutical Analysis & Quality Assurance, Assistant professor,
Gyana Jyothi College of Pharmacy, JNTUH, Boduppal, Hyderabad, Telangana-500039

Abstract:

A simple, selective, linear, precise, and accurate RP-HPLC method was developed and validated for the simultaneous estimation of Ivacaftor and Lumacaftor from bulk formulations. Chromatographic separation was achieved isocratically on a Waters Xterra C18 column (4.6 x 250mm, 5µm) using a mobile phase, Acetonitrile: Phosphate buffer pH adjusted to 4.6 with ortho phosphoric acid in the ratio of 45:55% v/v. The flow rate was 1 ml/min and effluent was detected at 255nm. The linearity was observed in the concentration range of 100-500µg/ml of Lumacaftor and 1-5µg/ml of Ivacaftor. Linear regression coefficient was not more than 0.999. The values of RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Ivacaftor and Lumacaftor. LOD was 2.95 and 3.04 and LOQ was 9.87 and 10 for Ivacaftor and Lumacaftor respectively. The results obtained on validation parameters met ICH and USP requirements. It inferred that the method found to be simple, accurate, precise and linear. The method was found to be having suitable applications in routine laboratory analysis with high degree of accuracy and precision.

Key Words: *Ivacaftor, Lumacaftor, Validation, Isocratically.*

Corresponding author:

B.Sravanthi,

Assistant professor,
Department of Pharmaceutical Analysis & Quality Assurance,
Gyana Jyothi College of Pharmacy, JNTUH,
Boduppal, Hyderabad, Telangana-500039
Email id: divya.pepper34@gmail.com

QR code

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INTRODUCTION:

Ivacaftor

Ivacaftor chemically N-(2, 4-Di-tert-butyl-5-hydroxyphenyl)-4-oxo-1, 4-dihydroquinoline-3-carboxamide with molecular formula $C_{24}H_{28}N_2O_3$ and brand name is kalydeco. Ivacaftor category is cystic fibrosis and 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. The structure of Ivacaftor is shown in Figure1.

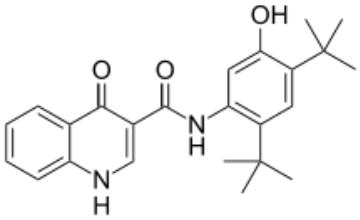

Fig 1: Chemical structure of Ivacaftor

Lumacaftor

It is chemically 3-{6-{[1-(2,2-Difluoro-1,3-benzodioxol-5-yl)cyclopropanecarbonyl]amino}-3-methylpyridin-2-yl}benzoic acid. Practically insoluble in water and molecular formula is $C_{24}H_{18}F_2N_2O_5$ of category cystic fibrosis with brand name Afatinib. The structure of Lumacaftor is shown in Figure2.

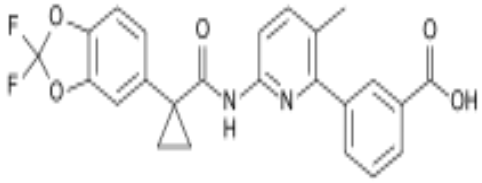

Fig 2: Chemical structure of Lumacaftor

Literature survey reveals that some analytical methods and few spectrophotometric methods have been reported for the estimation of Ivacaftor and Lumacaftor. The present work reports simple, sensitive, accurate, precise and economical methods for determination of Ivacaftor and Lumacaftor by RP-HPLC method in pure and its tablet formulation. The method was validated by parameters such as linearity, precision, accuracy, LOD & LOQ, robustness, stability and system suitability as per ICH guidelines and USP requirements.

MATERIALS AND METHODS:

The list of instruments used shown in table 1.

Table 1: List of Instruments

S.No.	Instrument	Model No.	Software	Manufacturer's name
1	HPLC Alliance PDA Detector	Waters2695 Waters996	Empower	Waters
2	UV double beam spectrophotometer	UV3000	UVWin5	Lab India
3	Digital weighing balance	BSA224SCW	-	Satorius
4	pH meter	AD102U	-	Lab India
5	Ultra sonicator	SE60US	-	-
6	Suction pump	VE115N	-	-

The list of chemicals used shown in table 2.

Table2: List of Chemicals

S.No.	Chemicals	Manufacturer	Grade
1	Water	Merck	HPLC Grade
2	Methanol	Merck	HPLC Grade
3	Acetonitrile	Merck	HPLC Grade
4	Potassium di hydrogen phosphate	Merck	A.R
5	Ivacaftor and Lumacaftor	-	-

Preparation of Sample Solution:
Accurately 10 tablets are weighed and crushed in mortar and pestle and weight equivalent to10mg of Lumacaftor and Ivacaftor (marketed formulation) sample added into a10mL clean dry volumetric flask and about 7mL of Diluents is added and sonicated to dissolve it completely and made volume up to the mark with the same solvent(Stock solution). Further 3ml of above stock solution was pipette into a10ml volumetric flask and diluted up to the mark with diluent.

Procedure:
20µL of the standard, sample are injected into the chromatographic system and the areas for Lumacaftor and Ivacaftor peaks are measured and the %Assay are calculated by using the formulae.

RESULTS AND DISCUSSION:

The present research work was designed at developing a rapid, sensitive, precise and accurate HPLC method for the simultaneous estimation of Lumacaftor and Ivacaftor in pharmaceutical dosage forms. High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Lumacaftor and Ivacaftor was done by RP-HPLC. The Phosphate buffer of p^H 4.6 and the mobile phase was optimized which consists of Acetonitrile: Phosphate buffer mixed in the ratio of 45:55% v/v. Xterra C18 column (4.6 x 250mm, 5µm) was used as stationary phase. Since the chromatographic peaks obtained were better defined and resolved and free from tailing.

After various trials, the following chromatographic conditions were finally optimized for the simultaneous estimation of Lumacaftor and Ivacaftor in a tablet dosage form. Mobile phase constitutes of ACN: Phosphate buffer pH adjusted to 4.6 with orthophosphoric acid in the ratio of 45:55% v/v.

The detection was carried out using UV detector at 255nm.The solutions were chromatographed at a constant flow rate of 1 ml/min. The linearity range of Lumacaftor and Ivacaftor were found to be from 100-500 µg/ml of Lumacaftor and 1-5µg/ml of Ivacaftor. Linear regression coefficient was found to be 0.999.

The typical chromatograms of the standard solutions were recorded for the repeatability and the respective chromatogram was given in Figure 3.

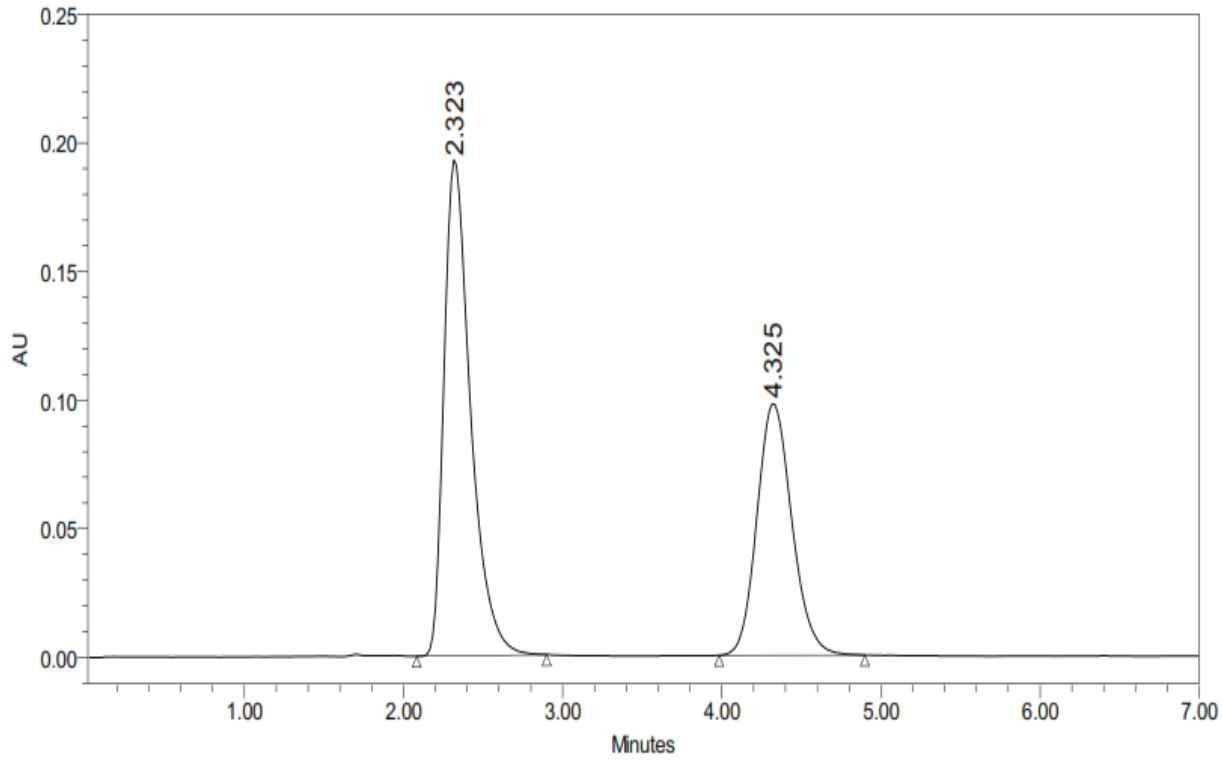

Fig 3: Typical chromatogram of Lumacaftor and Ivacaftor with detection at 255 nm.

Method Validation

After development of method, validation of the method for simultaneous estimation of Ivacaftor and Lumacaftor was performed in accordance with ICH guidelines (International Conference on Harmonization (ICH) 2000) which include System suitability, Linearity, Accuracy, Precision, LOD and LOQ, Specificity and Robustness.

Linearity:

Calibration graphs were constructed by plotting peak area vs. concentration of Ivacaftor and Lumacaftor and the regression equations were calculated. The calibration graphs were plotted over 5 different linear concentrations for all the drugs. Linearity curves of Ivacaftor and Lumacaftor were shown in figure 4 & 5 respectively.

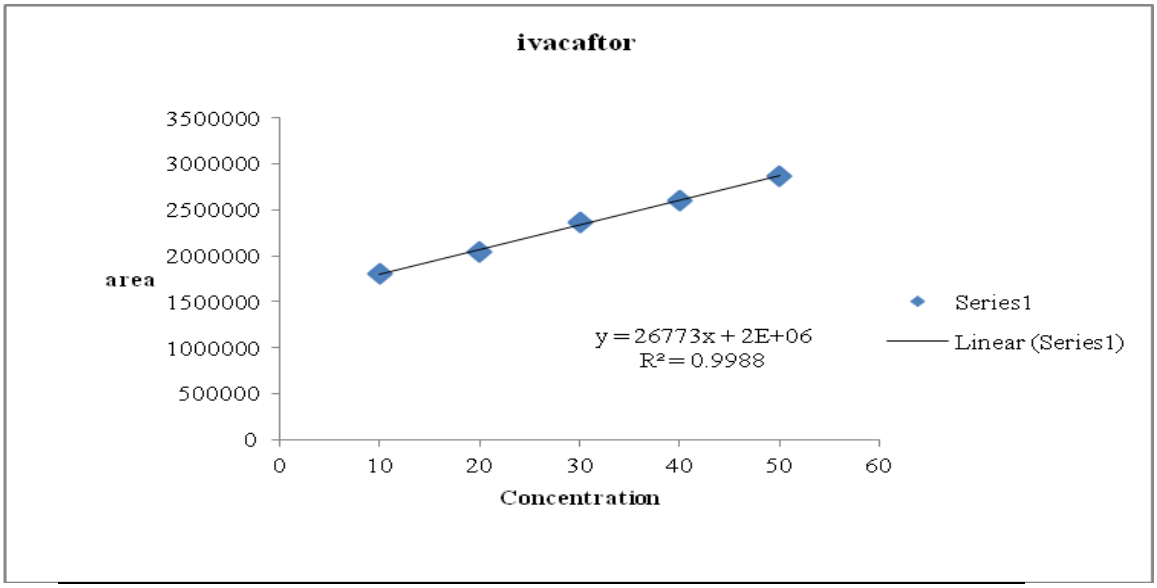

Fig 4: Calibration graph for Ivacaftor

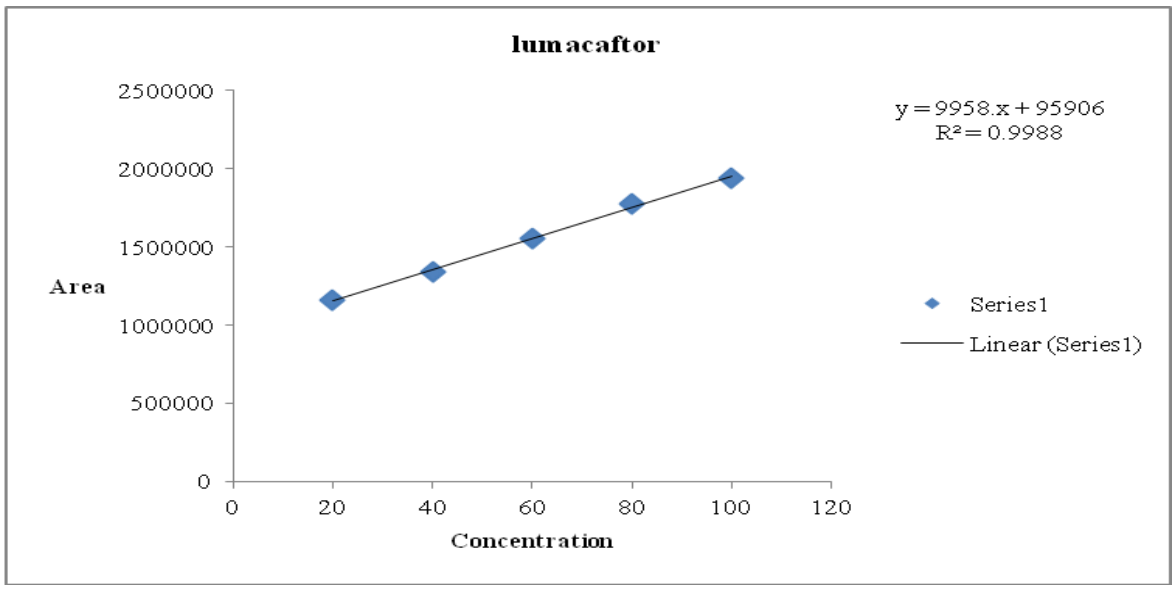

Fig 5: Calibration graph for Lumacaftor

Accuracy

The accuracy of the method was established by recovery studies i.e., external standard addition method. The known amount of standard was added at three different levels to pre analyzed sample. Each determination was performed in triplicate.

Precision

The intraday and inter day precision of the proposed method was determined by analyzing mixed standard solution of Ivacaftor and Lumacaftor for 3 times on the same day and on 3 different days. The results are reported in terms of relative standard deviation. The results of precision were tabulated in table 2.

Limit of detection (LOD) and Limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) of Ivacaftor and Lumacaftor were determined by calculating the signal to noise (S/N) ratio according to International Conference on Harmonization guidelines.

Table 3: Summary of validation parameters

S.No	Validation Parameters	Results	
		Ivacaftor	Lumacaftor
1	Accuracy (% Recovery)	100.0	100.5
2	Precession (% RSD)	0.3	0.39
3	Inter day precession (%RSD)	0.11	0.16
4	LOD	2.95	3.04
5	LOQ	9.87	10.0

Robustness:

The standard and samples of Ivacaftor and Lumacaftor were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

The system suitability parameters for Ivacaftor and Lumacaftor such as theoretical plates and tailing factor were found to be 5117.5, 1.3 and 3877.3, 1.4. Resolution was 8.1.The %purity of Ivacaftor and Lumacaftor in pharmaceutical dosage form was found to be 100.7% and101.4% respectively.

CONCLUSION:

The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Ivacaftor and Lumacaftor. LOD and LOQ were found to be within limit.

The results obtained on the validation parameters met ICH and USP requirements. It inferred that the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precise.

REFERENCES:

1. D.A.Skoog, F.J.Holler, S.R.Crouch, Textbook of Instrumental Analysis, Brooks/Cole, Cengage Learning India Private Limited, 2007p.900-906.

2. G.R.Chatwal,S.K.Anand,Text book of Instrumental Methods of Chemical Analysis, Himalaya Publishing House,5th Ed, 2002, p.2.566-2.570.

3. G.W.Ewing, Text book of Instrumental Methods of Chemical Analysis, McGraw-Hill Book Company, 5th Ed, p.375-385.

4. H. H Willard, L. L Merritt, J. A Dean, and F. A Settle, Textbook of Instrumental Methods of Analysis, CBS publishers and distributors, New Delhi, 7th Ed,1986, p.592-596.

5. ICH: Q2B, Analytical Validation – Methodology (1996).

6. Pawanjeet. J. Chhabda, m. Balaji, srinivasarao .v , development and validation of a new and stability indicating rp-hplc method for the determination of ivacaftor in presence of degradant products, international journal of pharmacy and pharmaceutical sciences issn- 0975-1491 vol 5, suppl 4, 2013.