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Analytical Method Development and Validation for the Simultaneous Estimation of Ivacaftor and Lumacaftor in its Bulk and Pharmaceutical Dosage Forms

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A B S T R A C T

A new method was established for simultaneous estimation of Ivacaftor and Lumacaftor by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Ivacaftor and Lumacaftor by using Xterra C185µm (4.6*250mm)column, flow rate was1ml/min, mobile phase ratio was Phosphate buffer(0.05M) pH4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, PDA Detector 996, Empower software version-2. The retention times were found to be 2.399mins and3.907mins. The % purity of Ivacaftor and Lumacaftor was found to be 100.7% and 101.4%respectively.ThesystemsuitabilityparametersforIvacaftor and Lumacaftor such as the oretical plates and tailing factor were found to be 1.3, 5117.5 and 1.4,3877.3 the resolution was found to be 8.0. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study for Ivacaftor and Lumacaftor was found in concentration range of 1µg-5µg and 100µg-500µg and correlation coefficient (r2) was found to be 0.999and 0.999% mean recovery was found to be 100% and 100.5%, % RSD for repeatability was 0.2 and 0.4, %RSD for intermediate precision was 0.5and 0.1 respectively. The precision study was precise, robust and repeatable. LOD value was 2.95 and 3.04 and LOQ value was 9.87and10 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Ivacaftor and Lumacaftor in API and Pharmaceutical dosage form.

Keywords: Ivacaftor, Lumacaftor, RP-HPLC

A R T I C L E I N F O

CONTENTS

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1. Introduction

High Performance Liquid Chromatography (HPLC)

A variety of methods are available for analyzing pharmaceutical compounds. High Performance/Pressure Liquid Chromatography (HPLC) is one of the best methods of choice for analyzing a variety of natural and synthetic compounds. It is because it offers high performance over ambient pressure. [3] It allows separations of a large variety of compounds by offering some major improvements over the classical column chromatography, TLC, GC; and it presents some significant advantages over more recent techniques such as supercritical fluid chromatography (SFC), capillary electrophoresis (CE), and electro kinetic chromatography. Effective and fast method development is of paramount importance throughout this drug development life cycle. This requires a thorough understanding of HPLC principles and theory which lay a solid foundation for appreciating the many variables that are optimized during fast and effective HPLC method development. Chromatographic separations are based on a forced transport of the liquid (mobile phase) carrying the analyte mixture through the porous media and the differences in the interactions at analytes with the surface of this porous media resulting in different migration times for a mixture components. The four main types of HPLC techniques are

- Normal-Phase Chromatography.
- Reversed-Phase Chromatography.
- Ion-Exchange Chromatography.
- Size-Exclusion Chromatography.

Ivacaftor (trade name Kalydeco, developed as VX-770) 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, [1][2] and is included in a combination drug, lumacaftor / ivacaftor, which is used to treat people with cystic fibrosis who have the F508del mutation in CFTR.

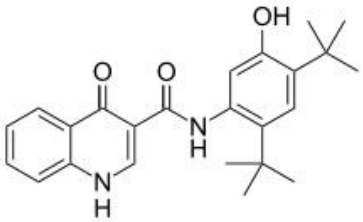

Figure 1: Ivacaftor

Lumacaftor (VX-809) is a pharmaceutical drug that acts as a chaperone during protein folding and increases the number of CFTR proteins that are trafficked to the cell surface.[1] It is available in a single pill with ivacaftor; the combination, lumacaftor /ivacaftor (brand name Orkambi), is used to treat people with cystic fibrosis who have the F508del mutation in the cystic fibrosis transmembrane conductance regulator (CFTR), the defective protein that causes the disease.[2]

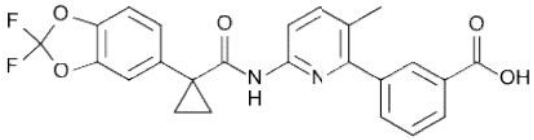

Figure 2: Lumacaftor

2. Materials and Methods

List of Standard and Sample details

S.NO.	Name	Manufaturer
1.	Ivacaftor working standard	KP labs pvt. Ltd
2.	Lumacaftor working standard	KP labs pvt. Ltd

List of Equipment/Instrument details

S.N	Instrument name	Model
1.	HPLC system	WATERS, software: Empower, 2695 separation module. 2996 PDA detector.
2.	Semi micro balance	Sartorius ME235P
3.	P^H Meter	Lab India ph. Meter
4.	Sonicator	Ultrasonic cleaner power sonic 420
5.	UV/VIS spectrophotometer	LABINDIA UV
6.	Constant temperature water bath	Thermo lab GMP

Chemicals and Reagents list of Chemicals and Reagents

S.N	Name	Manufacturer
1.	Potassium dihydrogenorthophosphate	Merck
2.	Sodium perchlorate	Merck
3.	Perchloric acid	Merck
4.	Ortho phosphoric acid	Merck
5.	Methanol	Merck
6.	Acetontrile	Merck
7.	Water	Milli-pore
8.	0.45 µm Nylon filter	Axivia
9.	0.45µm PVDF filter	Rankem

Analytical Method Development

A. Selection of wavelength [7, 8, 9]

A solution of 10 µg/ml of Ivacaftor and Lumacaftor were prepared in milliQ water. The resulting solutions were scanned individually on HPLC PDA detector from 200 to 400 nm and also in UV-Visible spectrophotometer. The

optimal response for three of them was obtained at 254 nm. Hence the complete method was processed at the wavelength of 254nm.

B. Selection of chromatographic condition

Proper selection of the method depends up on the nature of the sample (ionic/ ionisable/neutral molecule), its molecular weight and solubility. The drugs selected in the present study, were polar in nature. Thus reverse phase HPLC was selected for the initial separation because of its simplicity, suitability, ruggedness and its wider usage.

C. Initial separation condition

The mobile phase selected to elute the drug from the stationary phase was milliQ water and HPLC methanol, because of its favorable UV transmittance, low viscosity and low back pressure.

Preparation of standard solution: 10 mg of Ivacaftor and 10mg of Lumacaftor were accurately weighed and transferred into a 10 ml clean dry volumetric flask, about 7 ml of diluent was added, sonicate to dissolve it completely and the volume was made up to the mark with the same solvent to give a concentration of 1000 µg/ml. (Stock solution) Further 0.2 and 0.1 ml were pipette out fromthe above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent to give a concentration of 20 µg/ml and 10µg/ml respectively.

Preparation of sample solution:[10,11, 12]

Tablets of contents were weighed and triturated in glass mortar. The quantity of powder equivalent to 10 mg of active ingredient present in Ivacaftor and Lumacaftor was transferred into a 10 ml clean dry volumetric flask, 7 ml of diluent was added to it and was shaken by mechanical stirrer and sonicate for about 30 minutes by shaking at intervals of five minutes each and was diluted up to the mark with diluent to give a concentration of 1000 µg/ml and allowed to stand until the residue settles before taking an aliquot for further dilution (stock solution). 0.2 ml of upper clear solution was transferred to a 10 ml volumetric flask and diluted with diluent up to the mark to give the respective concentrations as par with standard solution. The solution was filtered through 0.45 µm filter before injecting into HPLC system.

Preparation of Placebo:

The amount of powdered inactive ingredient supposed to be present in 10 tablets were accurately weighed and transferred in to 10 ml volumetric flask, 7 ml of diluent was added and shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes and was diluted up to the mark with diluent and allowed to stand until the residue settles before taking an aliquot for dilution. 0.1 ml of upper clear solution was transferred to a 10 ml volumetric flask and diluted with diluent up to the mark and the solution was filtered through 0.45 µm filter before injecting into HPLC system.

Trials

Method development for the drugs was initiated based on the individual chemical characteristics' and their methods given in individual journals.

Mobile phase: Methanol: Phosphate buffer P^H3 (70:30)

Diluent: Methanol

Chromatographic conditions:

Flow rate : 1ml/min

Column : Symmetry C_{18} (4.6 x 150mm, 5µm)

Detector wavelength : 260 nm

Column oven : Ambient

Injection volume : 10µl

Observation:

Optimized Method

Preparation of mobile phase:

Take 6.8 gm of KH_2PO_4 into 1000ml volumetric flask dissolved in Hplc grated water and adjust Phupto 3 with ortho phosphoric acid. From the above prepared buffer take 300 ml(30%)and 700ml Methanol(70%) HPLC were mixed and degassed in ultrasonic water bath for 5 minutes and was filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: Mobile phase was used as Diluent.

Chromatographic conditions:

Flow rate : 0.8 ml per min

Column : symmetry C_{18} (4.6 x 250mm, 5µm)

Detector wavelength : 260nm

Column oven : Ambient

Injection volume : 10µl

Run time : 10 min

Test Procedure:

20 µl of the Standard, Sample and Blank preparations in duplicate were injected separately into HPLC system and the peak responses for Ivacaftor and Lumacaftor were measured. The quantities from the peak area in mg of Ivacaftor and Lumacaftor were calculated per tablet taken.

The developed RP-HPLC method for the simultaneous estimation of Ivacaftor and Lumacaftor were carried out on symmetry C_{18} (4.6 x 250mm), 5µm column in gradient mode using mobile phase composition of Methanol: Phosphate buffer P^H 3 [70: 30, v/v] with flow rate of 0.8 ml / min at 254nm.

Observation:

Resolution between two analytes was good. No peak asymmetry was observed. No other impurity interference was seen. All the results were found to be within the acceptance criteria. Hence the method was considered to be optimized.

Calculation: The amount of drug present was calculated by using the following formula:

3. Results and Discussions

Extraction of Lycopene by Acetone-Petroleum Ether Method: A simple liquid-liquid extraction method was employed to extract lycopene in minimum organic solvent. The yield of lycopene from papaya is extracted by acetone-petroleum ether method (**Figure 3).**

Figure 3: Extraction - Acetone-Petroleum ether method

Antimicrobial Activity

The agar well diffusion method was used to evaluate the antibacterial activity by measuring the zone of inhibition against the test microorganisms. The Petroleum Ether: Acetone (9:1) extract exhibited the prominent antibacterial activity against, *Pseudomonas spp.* and *Staphylococcus spp.* But no zone of inhibition was found against *Bacillus spp* **(Table 1 & Figure 4).**

Table 1: Zone of Inhibition

S.No	Test of organism	Zone of inhibition
1	*Staphylococcus* spp.	1.5mm
2	*Pseudomonas* spp.	1mm
3	*Bacillus* spp.	0mm

Figure 4: Anti- bacterial activity against (A) *Staphylococcus* spp. (B) *Bacillus* spp (C) Pseudomonas spp.

Food Colourant

Extracted Lycopene and standard orange colour was made to spread on sugar cubes. After 24hrs, the results were observed **(Figure 5)**.

Figure 5: Lycopene colour spreaded on sugar cubes

4. Conclusion

Lycopene sample was extracted by acetone – petroleum ether method. The antibacterial activity of lycopene was studied against *Bacillus spp. Staphylococcus spp. and Pseudomonas spp*. Due to its deep red color, lycopene can be used as a promising food colorant. Further work can be carried out to investigate anticancer activity in various cell lines. A modern life style keeps people away from healthy diet. For healthy dietary habits one should increase the consumption of food products which are helpful in the prevention of illness. Fruits and vegetables are main source of natural antioxidant and antimicrobial components. Antioxidants and Antimicrobial virtues give protection against harmful free radicals and reduce rate of cancer and heart disease and the most efficient carotenoid antioxidant is Lycopene. Thus the present study unravels two major applications of Lycopene extracted from Papaya and many more such applications by future research are yet to be unraveled.

5. References

[1] Joseph Levy and Yoav Sharoni (2004): The Functions of Tomato Lycopene and Its Role in Human Health, Herbal Gram. 2004; 62:4956 American Botanical Council, *The Journal of the American Botanical Council*, Issue: 62 Page: 4956

[2] M. Basuny, A.M. Gaffar and S.M. Arafat (2009): Tomato lycopene is a natural antioxidant and can alleviate hypercholesterolemia, *African Journal of Biotechnology*,8(23), 6627-6633

[3] Naviglio D., Pizzolongo F., Ferrara L., Naviglio B., Aragon A. and Santini (2008): A Extraction of pure lycopene from industrial tomato waste in water using the extractor Naviglio, *African J. Food. Sci.*, 2(2), 37-44

[4] Neelu Malviya (2014): Isolation and Quantification of Lycopene from Watermelon, Tomato and Papaya, Department of Chemistry, Govt. M.L.B. Girls P.G. College, Fort, Indore MP, INDIA, *Research Journal of Recent Sciences*, Vol. 3(IVC-2014), 68-70

[5] P.D. Fraser and P.M., Bramley (2004): The Biosynthesis and Nutritional Uses of Carotenoids, Progress in Lipid Research, 43(3), 228-265

[6] R. Bunghez (2011): Lycopene Determination In Tomatoes By Different Spectral Techniques (Uv-Vis, Ftir and Hplc) *Journal Of Nanomaterials And Biostructures* Vol. 6, No 3, P. 1349-1356.

[7] Sanjay Metkar., Supriya Saptarshi., Aditi Kadam (2014): Studies on extraction, isolation and applications of lycopene, Department of Biotechnology, MGM's Inst.of Biosciences and Technology, Aurangabad (MS), *India, Indo American Journal of Pharmaceutical Research*, ISSD NO: 2231-6876

[8] Simran Lilwani and Vrinda Nair (2015):Extraction and Isolation of Lycopene Form Various Natural Sources,D Y Patil University, School Of Biotechnology And Bioinformatics,IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB), Volume 1, Issue 5 (Jul. – Aug. 2015), PP 49-51

[9] Suraj Ashok Bhagat, Aditya Vikas Sakhare and Sohan Sunil Dhanawade (2012): Isolation of Lycopene from Papaya and Study of its Antimicrobial Activity, *International Journal of Science and Research (IJSR)* ISSN (Online): 2319-7064

[10] Wayne W. Fish, Penelope Perkins-Veazie, and Julie K. Collins (2002): A quantitative assay for lycopene that utilizes reduced volumes of organic solvents, United States Department of Agriculture, Agricultural Research Service, South Central Agricultural Research Laboratory, P.O. Box 159, Lane, OK 74555, U.S.A., Journal of Food Composition and Analysis (2002) 15, 309–317

[11] Wee Sim Choo and Wai Yen Sin (2012): Ascorbic Acid, Lycopene and Antioxidant Activities of Red-fleshed and Yellow-fleshed Watermelons. *Advances in Applied Science Research*, 3 (5):2779-2784.

[12] Zhao Yapinga (2002): Antioxidant activity of lycopene extracted from tomato paste towards trichloromethyl peroxyl radical CCl3O2. *Food Chemistry* 77, 209–212.