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IDENTIFICATION OF CRYSTALLINE ACTIVE PHARMACEUTICAL INGREDIENT IN LOPINAVIR RITONAVIR CAPSULE BY HORIZONTAL ATTENUATED TOTAL REFLECTANCE - FOURIER TRANSFORM INFRARED SPECTROSCOPY

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ABSTRACT

The combination of Lopinavir and Ritonavir is used with other medicines to treat Human Immuno-Deficiency Virus (HIV) infection. Lopinavir and Ritonavir exist in different crystallographic forms known as polymorphs. Since the polymorphic forms differ in their solid-state structure, they can have different aqueous solubilities and dissolution rates. According to literature survey, none of the currently available methods is helpful in the identification of crystalline forms of Lopinavir and Ritonavir. Hence, a cost-sensitive, rugged, non-destructive and environment friendly Horizontal Attenuated Total Reflectance - Fourier Transform Infrared (HATR-FTIR) Spectroscopic method has been developed and validated which can be used for routine quality control and stability analysis.

Keywords – Polymorphs, Horizontal Attenuated Total Reflectance - Fourier Transform Infrared (HATR-FTIR), Spectroscopic method, Lopinavir, Ritonavir.

1. INTRODUCTION

Viruses are the ultimate expression of parasitism. They not only take nutrition from the host cell but also direct its metabolic machinery to synthesize new virus particles. Viral chemotherapy requires interference with the cellular metabolism in the host. Drugs used in viral chemotherapy target virus specific steps like cell penetration, uncoating, reverse transcription, virus assembly or maturation.

Anti-retroviral drugs are active against Human Immuno-Deficiency Virus (HIV) which is a retrovirus. An aspartic protease enzyme encoded by HIV is involved in the production of structural proteins and enzymes of the virus. The large viral polyprotein is broken into various functional components by this enzyme. The protease acts at a late step in HIV replication, i.e. maturation of the new virus particles when the RNA genome acquires the core proteins and enzymes. The protease inhibitors (PIs) bind to the protease molecule, interfere with its cleaving function and act as effective viral inhibitors[1].

HIV PIs, including lopinavir and ritonavir, prevent cleavage of gag and gag-pol protein precursors in acutely and chronically infected cells, arresting maturation and thereby blocking the infectivity of nascent virions. The main antiviral action of HIV PIs is, thus, to prevent subsequent infection of susceptible cells. These PIs have no effect on cells already harbouring integrated proviral DNA [2, 3].

Lopinavir and Ritonavir are used in combination as an antiretroviral drug which inhibits the HIV viral protease enzyme. Lopinavir prevents cleavage of the gag-pol polyprotein due to which the viral assembly is hampered. Ritonavir inhibits the CYP3A-mediated metabolism of Lopinavir, thereby providing increased plasma levels of Lopinavir [4-7].The combination of Lopinavir and Ritonavir is used with other medicines to treat human immunodeficiency virus infection.

Lopinavir is chemically designated as [1S-[1R*, (R*), 3R*, 4R*]]-N-[4-[[(2, 6 dimethylphenoxy) acetyl] amino]-3-hydroxy-5-phenyl-1-(phenylmethyl) pentyl] tetrahydro-alpha-(1-methylethyl)-2-oxo-1(2*H*)-pyrimidineacetamide. Its molecular formula is $C_{37}H_{48}N_4O_5$, and its molecular weight is 628.80.

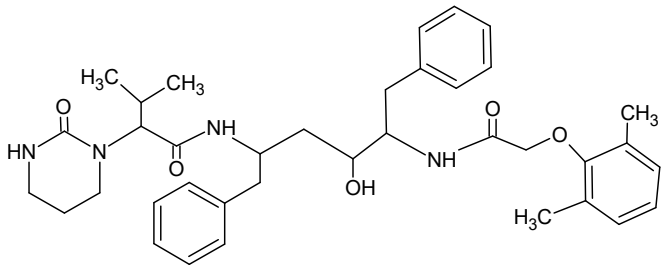

Fig. 1. Chemical Structure of Lopinavir

Ritonavir is chemically designated as 10-hydroxy-2-methyl-5-(1-methylethyl)-1-[2-(1-methylethyl)-4-thiazolyl]-3,6-dioxo-8,11-bis(phenylmethyl)-2,4,7,12-tetraazatridecan-13-oic acid, 5-thiazolylmethyl ester, [5*S*-(5*R**,8*R**,10*R**,11*R**)]. Its molecular formula is C37H48N6O5S2, and its molecular weight is 720.95.

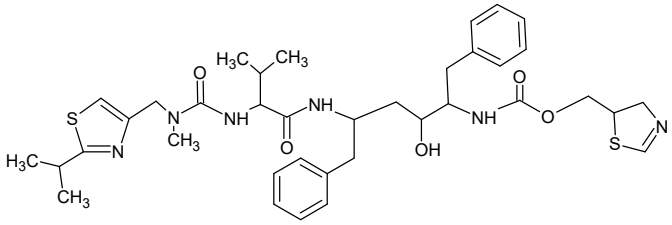

Fig. 2. Chemical Structure of Ritonavir

Lopinavir and Ritonavir exist in different crystallographic forms. Since the polymorphic forms differ in their crystal structure, they can have different aqueous solubilities and dissolution rates. The physico-chemical properties of an Active Pharmaceutical Ingredient (API) can therefore have a significant influence on the stability and bioavailability of the drug product[8-10] .The investigation of polymorphs is a key step in drug product development. In the present study, Lopinavir-Ritonavir capsules are prepared by Hot Melt Extrusion (HME) process using crystalline Lopinavir and Ritonavir API which are converted to their amorphous forms, having more bioavailability. The amorphous forms being less stable have a tendency to convert to the crystalline form during storage or stability testing. This has an impact on the bioavailability of the drug [11-13]. Hence, it is more meaningful to identify the conversion of the amorphous form to crystalline form of both APIs in the formulation.

According to literature survey, none of the currently available methods is helpful in the identification of crystalline forms of Lopinavir and Ritonavir. Hence, it is necessary to develop a method which can be used for the identification of polymorphic forms of Lopinavir and Ritonavir in formulation[14-16].

Fourier transform infrared (FTIR) spectroscopy is a technique which is used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. Many substances can be characterized, identified and also quantified by IR spectroscopy. One of the strengths of IR spectroscopy is its ability as an analytical technique to obtain spectra from a wide range of solids, liquids and gases. However, in many cases sample preparation is required in order to obtain a good quality spectrum. The technique of Attenuated Total Reflectance (ATR) has, in recent years,

revolutionized solid and liquid sample analysis as it combats the most challenging aspects of infrared analysis, namely sample preparation and spectral reproducibility.

In the present study, identification of polymorphic forms was done using non-destructive Horizontal Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy (HATR-FTIR) technique. HATR-FTIR is a reflectance method with the incident infrared radiation, reflecting off the attachment's crystal, penetrating the sample and then reflecting back to the crystal. With the FTIR spectroscopy, the spectrum was recorded without any appreciable pre-treatment. The Horizontal Attenuated Total Reflectance (HATR) accessory attached to FTIR, allowed direct measurement of the sample with minimal preparation. Thus a simple, rapid, cost effective and environment friendly analytical method for the determination of crystalline Lopinavir-Ritonavir in capsule (40/10 mg) based on HATR FT-IR spectroscopy without using any solvent was developed and validated[17-19].

2. MATERIALS AND METHODS

2.1 Materials and Reagents

Active Pharmaceutical Ingredients (APIs) of Lopinavir and Ritonavir (Form-I), Lopinavir-Ritonavir (40/10mg) capsules and placebo.

2.2 Instrumentation

FTIR spectrophotometer (Thermo scientific –Model-iS10 Nicolet HATR accessory), Analytical weighing balance (Sartorius).

2.3 Spectroscopy Measurements

Spectra were acquired using a Thermo Scientific Nicolet™ iS10. FTIR Spectrometer with a smart multi-bounce HATR Smart iTR™ accessory which has a 1.5 mm active sample area, 2 µm penetration at 1000 cm^{-1} and Zinc Selenide (ZnSe) focusing optics. The resolution was approximately 4 cm^{-1} and 32 scans were recorded. The IR spectra were measured ranging from 4000 cm^{-1} to 650cm^{-1}.

2.4 Preparation of Sample

Identification of Crystalline API: Pellets of one capsule were collected (around 220mg) and ground to make a homogenous powder. The entire sample quantity was placed the on the sample holder and the spectrum was recorded.

2.5 Method Validation

Method validation was performed following ICH guidelines[20] for specificity, linearity, precision and detection limit.

2.5.1 Specificity

Spectra of Lopinavir-Ritonavir Capsule pellets (as such sample), Crystalline Ritonavir API (Form-I), crystalline Lopinavir API were recorded.

2.5.2 Linearity

Linearity was evaluated by regression analysis of five different Lopinavir Ritonavir concentrations in the range of 3%-20% w/w. Appropriate quantities of Crystalline Lopinavir and Ritonavir APIs were mixed with the capsule blend to get around 220mg of sample and triturated to form a homogeneous powder. Correlation coefficient was analyzed and presented.

2.5.3 Limit Of Detection (LOD)

For Limit of Detection (LOD) of the method, the characteristic peak was measured at a lower concentration of sample until the related characteristic peak was barely detected.

2.5.4 Precision

Precision was evaluated with respect to both repeatability and intermediate precision. For repeatability, six individual samples of Lopinavir-Ritonavir capsule pellets (As such Samples) were analysed and spectra were recorded. Later, crystalline API was spiked in capsule pellets and the spectra were recorded. For intermediate precision, Lopinavir-Ritonavir capsule pellets with spiking of crystalline Ritonavir and Lopinavir API were analysed.

3. RESULTS AND DISCUSSION

FTIR spectra of pellets of Ritonavir and Lopinavir API were recorded in wavelengths ranging from 4000cm^{-1} to 650cm^{-1}. From the data, it was identified that the peak at 1525cm^{-1} was absent in capsule pellets, whereas the peak was present in both Lopinavir and Ritonavir API. Hence, the purpose of identification, 1525cm^{-1} peak was selected to identify crystalline API in capsule pellet.

3.1 Specificity

The FTIR spectra of crystalline Lopinavir API and Ritonavir API show a peak at wavenumber 1525±5cm^{-1}; whereas, a peak at the same wavenumber was not observed in case of the capsule pellets. This shows method is specific.

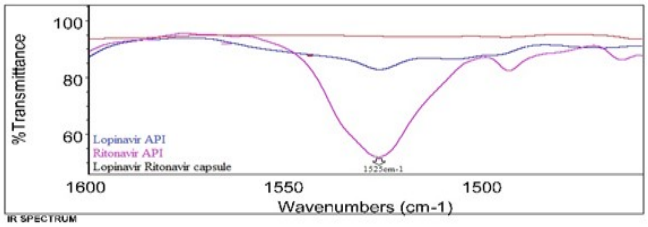

Fig. 3: Spectra of specificity

3.2 Linearity

The linearity was performed at levels 3%, 5%, 8%, 10% and 20% and the spectra were collected. The regression coefficient was calculated. The method was found to be linear.

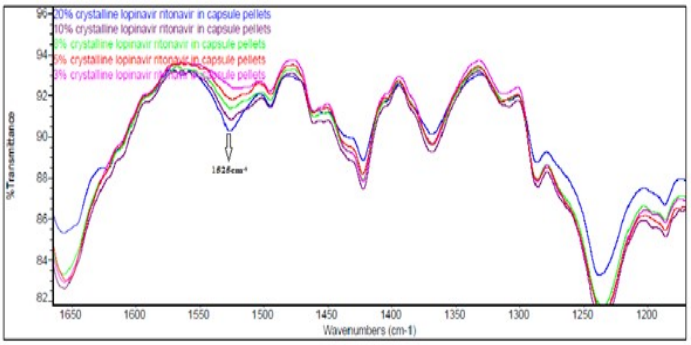

Fig. 4: Spectra of linearity

3.3 Limit of Detection

Limit of detection was examined by preparing different level (20%, 10%, 8%, 5% & 3%) and spectra were collected. It was observed that 3% LOD level had a low intensity peak at 1525±5cm^{-1}. Hence LOD was considered as 5%.

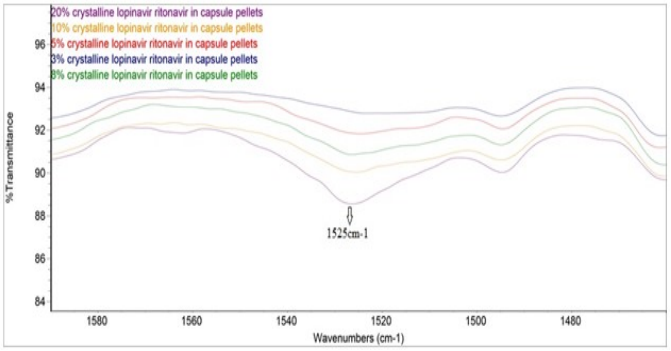

Fig. 5: LOD Spectra

3.4 Precision

In spiked and unspiked samples, results were within the acceptance criteria. In the spiked sample, a peak was observed in the range from 1526.46 cm^{-1} to 1526.98cm^{-1} and within the criteria of 1525±5cm^{-1}, indicating that the analytical method is precise.

4. CONCLUSION

An economical, rugged, non-destructive and environment friendly (Green) FTIR-HATR method was successfully developed and validated for the identification of crystalline forms of API Lopinavir and Ritonavir in capsule form. This is the only method available for identification of crystalline APIs using FTIR-HATR technique and can be used for routine quality control and stability analysis.

5. ACKNOWLEDGEMENTS

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6. CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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