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Method development and validation of Sofosbuvir and iedipasvirin by HPLC: A Review

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ABSTRACT

The aim of this review article is emphasizing on development and validation protocol which will used to develop and validate accurate, simple, selective and specific spectrophotometric methods for the determination of Sofosbuvir (SOF) and Ledipasvir (LDV) in pure and in their dosage forms. Thus it is necessary to develop methods for analysis with the help of number of analytical techniques which are available for the estimation of the drugs in combination. The analyst were determine the Specific, accurate, simple, selective and stability-indicating RP-HPLC method is developed and validated for simultaneous determination of sofosbuvir and ledipasvir in tablet dosage form.

Keywords: Sofosbuvir, Ledipasvir, RP-HPLC, Method development, Method validation.

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1. INTRODUCTION

Sofosbuvir Fig.1.(propan-2-yl (2S)-2-{[(S)-{[(3R,4R,5R) -5-(2,4-dioxo-1,2,3,4-tetrahydro pyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyloxolan-2-yl]methoxyphenoxy)phosphoryl]amino} propanoate) is a prodrug nucleotide analog used as part of combination therapy to treat hepatitis C virus (HCV) infection or to treat co-infection of HIV and HCV. It has a molecular formula of $C_{22}H_{29}FN_3O_9P$ and a molecular weight of 529.45.After metabolism to the active antiviral agent 2'-deoxy-2'-α-fluoro-β-C-methyluridine-5'-triphosphate (also known as GS-461203), the triphosphate serves as a defective substrate for the NS5B protein, an RNA dependent RNA polymerase required for replication of viral RNA.[1]

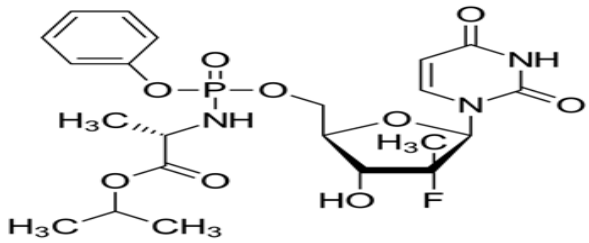

Figure 1: chemical structure of Sofosbuvir

Ledipasvir Fig.2. ((2S)-1-[(6S)-6-[5-(9,9-difluoro -7-{2-[(1R, 3S, 4S)-2-[(2S)-2-{[hydroxyl (methoxy) methylidene] amino}-3-methyl butanoyl]-2-azabicyclo [2.2.1] heptan-3-yl] -1H-1,3 benzodiazol- 6-yl}-9H-fluoren-2-yl) -1H-imidazol-2-yl]-5-azaspiro[2.4] heptan-5-yl]-2-{[hydroxyl (methoxy) methylidene] amino}-3-methyl butan-1-one) is a Hepatitis C Virus NS5A Inhibitor with potential activity against HCV. In combination with sofosbuvir for treatment in chronic hepatitis C genotype 1 patients. Upon oral administration and after intracellular uptake, ledipasvir binds to and blocks the activity of the NS5A protein. This results in the disruption of the viral RNA replication complex, blockage of HCV RNA production, and inhibition of viral replication. NS5A, a zinc-binding and proline rich hydrophilic phosphoprotein, plays a crucial role in HCV RNA replication. [2]

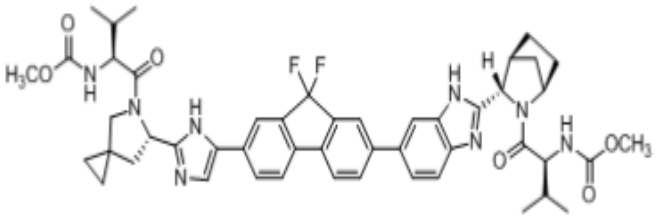

Figure 2: chemical structure of Ledipasvir

Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. There are two important reasons for validating assays in the pharmaceutical industry. The first, and by for the most important, is that assay validation is an integral part of the quality control system. The second is that current good manufacturing practice regulation requires assay validation.

Globally, 130-150 millions of people have chronic hepatitis C infection. A significant number of those who are chronically infected will develop liver cirrhosis or liver cancer. Gilead Sciences overcome most common related liver diseases by its Great invention (Harvoni). Harvoni (90 mg ledipasvir/400 mg sofosbuvir) approved by United States FDA. It is indicated for the treatment of chronic HCV genotypes 1, 4, 5, and 6 in adults and also indicated for the treatment of chronic HCV in patients co-infected with HIV [3]

The ability to provide timely, accurate, and reliable data is central to the discovery, development, and manufacture of pharmaceuticals. Analytical data are used to screen potential drug candidates, aid in the development of drug synthesis, support formulation studies, monitor the stability of bulk pharmaceuticals and formulated products, and test final products for release. The quality of analytical data is a key factor in the success of a drug development program.

Table 1: Various Instrumental Methods of Analysis

Instrumental Method	Property Measured	Application
UV- Visible Spectrophotometry	Absorption of radiation	Identification of the functional group and quantitation of unsaturated compounds.
FTIR Spectroscopy	Absorption of radiation	Quantitative analysis of organic compound at high conc. Level.
Atomic Absorption Spectroscopy	Absorption of radiation	Quantitation of metals or metalloids
Flame Photometry	Emission of radiation	Quantitation of alkali metals or alkaline earth metals.
X-Ray Diffraction	Diffraction of radiation	Identification of crystal lattice structure, determination of percent of crystallinity in polymers.
NMR, LC-NMR	Nuclear spin energy level of a mol in an applied magnetic field.	Identifies type of hydrogen and carbon in organic molecules. Analysis of trace impurity and degradants.
Thermal analysis (DTA \ DSC)	Difference in Temperature \ heat energy	Determination of melting point, Polymorphism, Drug – excipients compatibility
Mass spectrometry LC-MS \ GC-Ms	Mass to charge ratio (m\e)	Mol. Wt. determination Quantification of the analyte in liquid or gas sample. Analysis of the biological sample. Analysis of trace impurity and degradants.

2. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

High Performance Liquid Chromatography is the most widely used analytical separation technique. HPLC has been rapidly developed with the introduction of new pumping methods, more reliable columns and a variety of detectors. Most of the drugs in multicomponent dosage forms can be analyzed by HPLC methods because of the several advantages like rapidity, specificity, accuracy, precision and ease of automation in this method. HPLC method eliminates tedious extraction and isolation procedures.

In HPLC, the analyst has a wide choice of chromatographic separation methodologies from normal to reverse phase and a whole range of mobile phases using isocratic (or) gradient elution techniques. [4]

2.1 Reverse Phase Chromatography:

Reversed phase mode is the most popular mode for analytical and preparative separation of compounds of interest in chemical, biological, pharmaceutical, food and biomedical sciences. In this mode, the stationary phase is nonpolar hydrophobic packing with octyl or octa decyl functional group bonded to silica gel and the mobile phase is polar solvent. An aqueous mobile phase allows the use of secondary solute chemical equilibrium (such as ionization control, ion suppression, ion pairing and complexation) to control retention and selectivity. The polar compounds get eluted first in this mode and nonpolar compounds are retained for longer time. As most of the drugs and pharmaceuticals are polar in nature, they are not retained for longer time and hence elute faster. The different columns used are octa decyl silane (ODS) or C-18, C-8, C-4, etc., in the order of increasing polarity of the stationary phase

2.2 Partition Chromatography:

It is the most widely used liquid chromatographic procedure to separate most kinds of organic molecules. Here the components present in the analyte mixture distribute themselves between the mobile phase and stationary phase as the mobile phase moves through the column. The stationary phase actually consists of a thin liquid film either adsorbed or chemically bonded to the surface of finely divided solid particles. [5]

3. METHOD DEVELOPMENT PROTOCOL

3.1 Selection of Chromatographic Mode

Proper selection of the method depends upon the nature of the sample (ionic/ ionizable/ neutral molecule), its molecular weight and solubility. The drugs selected in present study are neutral in nature. Both the drugs are freely soluble in organic solvents. Hence, reversed phase HPLC was selected for the initial separations because of its simplicity and suitability.

3.2 Selection of Stationary Phase

On the basis of reversed phase HPLC mode and number of carbon present in the molecule (analyte) RP-Purosnosphere C18 (Grace) column (5µm, 4.6 mm × 250 mm), of following configuration was selected for further study.

3.3 Selection of Mobile Phase

Ledipasvir and Sofosbuvir are soluble in HPLC grade water and methanol on ultrasonication, insoluble in hexane and are freely soluble in methanol and water, DMSO. [6]

3.4 Selection of Detector and Detection Wavelength

UV-Visible detector was selected, as it is reliable and easy to set at the correct wavelength.

3.5 Standard Sample Preparation Ledipasvir and Sofosbuvir

An accurately weighed quantity of Ledipasvir 10 mg and 40 mg Sofosbuvir was taken in 25 ml volumetric flask and dissolved in 10 ml Methanol, with the help of ultrasonication for about 10 min. Then the volume was made up to the mark using methanol to get standard stock solution.

3.6 Optimization of HPLC Parameters

Optimizations of HPLC process is to find a set of conditions that adequately separate and enable the quantification of the analytes from the endogenous material with acceptable accuracy, precision, cost, ease and speed.

3.7 Optimization of Mobile Phase Strength

Initially methanol and water in different ratios were tried, and then methanol and water at different pH will try. [7]

3.8 Optimization of Detection Wavelength

On the basis of overlay spectra different wavelengths were selected. A fixed concentration of analyte mixture was analyzed at selected wavelengths.

4. METHOD VALIDATION PROTOCOL

The method was validated, in accordance with ICH guidelines (ICH Q2R1), for system suitability, linearity, accuracy, precision, repeatability, ruggedness, robustness, LOD and LOQ. [8]

4.1 Linearity:

The linearity range of Ledipasvir and Sofosbuvir was evaluated by varying concentrations of standard solutions were injected into HPLC system. The linear range of detectability that obeys Beer's law is dependent on the compound analyzed and the detector used. The working sample concentration and samples tested for accuracy should be in the linear range. The claim that the method is linear is to be justified with additional mention of zero intercept by processing data by linear least square regression. Data is processed by linear least square regression declaring the regression co-efficient and b of the linear equation y= ax + b together with the correlation coefficient of determination r2. For the method to be linear, the r^2 value should be close to1

4.2 Precision:

Precision is a measure of the degree of reproducibility or of the repeatability of the analytical method under normal operating circumstances. Repeatability involves analysis of replicates by the analyst using the same equipment, method and conducting the precision study over short period of time while reproducibility involves precision study at different Occasions. [9]

- Different Laboratories
- Different Batch of Reagent
- Different Analysts
- Different Equipments

4.3 Repeatability:

It is normally expected that at least six replicates should be carried out and a table showing each individual result provided from which the mean, standard deviation and co-efficient of variation should be calculated for set of n value. The RSD values are important for showing degree of variation expected when the analytical procedure is repeated several time in a standard situation. It should be below 2% for bulk drugs and below 2% for assay in finished product. [10]

4.4 Limit of Detection:

The determination of the limit of detection of instrumental procedures is carried out by determining the signal-to-noise ratio by comparing test results from the samples with known concentration of analyte with those of blank samples and establishing the minimum level at which the analyte can be reliably detected. A signal-to-noise ratio of 2:1 or 3:1 is generally accepted. The signal-to-noise ratio is determined by dividing the base peak by the standard deviation of all data points below a set threshold. Limit of detection is calculated by taking the concentration of the peak of interest divided by three times the signal-to-noise ratio.

For spectroscopic techniques or other methods that rely upon a calibration curve for quantitative measurements, the IUPAC approach employs the standard deviation of the intercept (Sa) which may be related to LOD and the slope of the calibration curve, b, by: LOD = 3.3 Sa / b. [11]

4.5 Limit of Quantitation

Where the final assessment is based on an instrumental reading, the magnitude of background response by analyzing a number of blank samples and calculating the standard deviation of this response. The standard deviation multiplied by a factor (usually 10) provides an estimate of the limit of quantitation. In many cases, the LOQ is approximately twice the limit of detection. SD is the standard deviation of the intercept which may be related to LOQ and the slope of the calibration curve, b, by:

$$LOQ = 10\ SD / slope$$

4.6 Selectivity and Specificity

Selectivity may be expressed in terms of the bias of the assay results obtained when the procedure is applied to the analyte in the presence of expected levels of other components, compared to the results obtained on the same analyte without a added substances. [12]

4.7 Determination of Reproducibility:

Reproducibility means the precision of the procedure when it is carried out under different conditions, usually in different laboratories on separate, putatively identical samples taken from the same homogeneous batch of material. Comparisons of results obtained by different analysts, by the use of different equipments or by carrying out the analysis at different times can also provide valuable information. [13]

4.8 Ruggedness:

The testing of ruggedness is normally suggested when the method is to be used in more than one laboratory. Ruggedness is normally expressed as the lack of the influence on the test results of operational and environmental variables of the analytical method.

4.9 System Suitability:

These parameters are measured during the analysis. Similar to the analytical method development, the system suitability test strategy should be revised as the analysts develop more experience with the assay. [14]

5. CONCLUSION

The presented article will give information about protocol considered during method development and validation. The method should be precise, sensitive and accurate. This method has simple sample preparation. The good recoveries and low coefficient of variation confirmed the suitability of proposed method for the routine analysis of LPS and SOFO in pharmaceuticals.

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