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Review on: Analytical Method Development and Validation of Lamivudine and Dolutegravir Sodium in Tablet Dosage Form

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ABSTRACT:

A simple, precise, accurate, efficient and reproducible, isocratic Reverse Phase- High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the simultaneous estimation of Lamivudine and Dolutegravir in bulk and tablet dosage form.

A fresh selective, rapid, accurate, precise and RP-HPLC stability-indicating method was developed and validated for the quantitative simultaneous determination of dolutegravir and lamivudine in the bulk as well as pharmaceutical dosage form.

The drugs, dolutegravir and lamivudine, were subjected to varied conditions like base hydrolysis, acid hydrolysis, oxidation, thermal, photochemical and UV. The suggested method was analysed statistically and validated to fulfil requirements of International Conference on Harmonisation (ICH) and the validation covered accuracy, precision, linearity, limit of detection (LOD), limit of quantification (LOQ), robustness, ruggedness and specificity.

KEYWORDS: Lamivudine and Dolutegravir, HPLC, Method Development and Validation.

INTRODUCTION:

Chromatography, a separation technique, is mostly used in chemical analysis. High-performance liquid chromatography (HPLC) is an extremely versatile technique. Where analytes are separated by passage through a column packed with micrometer-sized particles. The reversed-phase chromatography is commonly used separation technique in HPLC.

Lamivudine

Lamivudine (LAM) is (3TC, Epivir) is an antiviral drug that reduces the amount of HIV in the body. Anti-HIV drugs such as lamivudine slow down or prevent damage to the immune system, and reduce the risk of developing AIDS-related illnesses. Lamivudine is also active against hepatitis B virus (HBV). Lamivudine (Epivir-HBV) is used to treat hepatitis B infection. Lamivudine is in a class of medications called nucleoside reverse transcriptase inhibitors (NRTIs). It works by decreasing the amount of HIV and hepatitis B in the blood.

IUPAC name have 4-amino-1- [(2R, 5S)-2- (hydroxymethyl) - 1, 3-oxathiolan-5-yl]-1, 2- dihydropyrimidin-2-one. The molecular formula of C₈H₁₁N₃O₃S and molecular weight is 229.26gm/mol. Generally soluble in Water, ethanol, methanol, dimethylformamide (DMF), Acetonitrile. The chemical structure shown in Fig.1

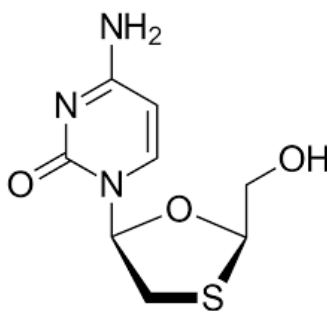


Fig. 1. Structure of Lamivudine

2. Dolutegravir Dolutegravir is a human immunodeficiency virus (HIV) integrase inhibitor, the third in this class of agents that target the viral integrase. Dolutegravir is used only in combination with other antiretroviral agents in the treatment of HIV infection, and it has had limited use. Dolutegravir is associated with a low rate of serum aminotransferase elevations during therapy, but has not been linked to instances of acute, clinically apparent liver injury.

IUPAC Name • 2H-Pyrido(1',2':4,5)pyrazino(2,1-b)(1,3)oxazine-9 carboxamide, N-((2,4-difluorophenyl)methyl) 3,4,6,8,12a-hexahydro-7-hydroxy-4-methyl-6,8-dioxo, sodium salt, hydrate (1:1:1), (4R,12aS)- Chemical Formula $C_{20}H_{19}F_2N_5O_5$. Molecular Weight 459.4 g/mol. Slightly soluble in water R, and very slightly soluble in methanol.

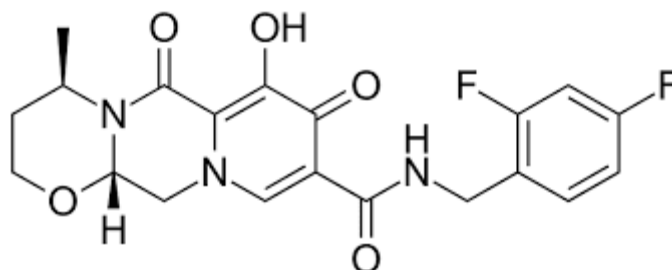


Fig. 2. Structure of Dolutegravir

ANALYTICAL METHOD DEVELOPMENT:

Analytical method development is the process by which a specific analytical method is to be developed for drug products from the stage of in process to finished product and minivaldation to be done before starting the analyses of routine samples, investigation samples and stability samples. Analytical method development and finalizing the method consists of

Standardizing the working standard from

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- Standardizing the working standard from reference standard.
- Optimizing the chromatographic condition,
- Optimizing the chromatographic condition, concentration of standard and sample solution and extraction procedure of the sample.
- Analytical method verification or mini
- Analytical method verification or mini validation to be done before analyzing (routine samples) tests like assay, dissolution and related substance in development laboratories etc.
- Prior starting the validation the
- Prior starting the validation the satisfactory result should be found in mini validation and formulation should be finalized.

Steps involved in HPLC Method development are.

- Understanding the Physicochemical properties of drug molecule.
- Selection of chromatographic conditions.
- Developing the approach of analysis.

- Sample preparation
- Method optimization
- Method validation (figure-3)

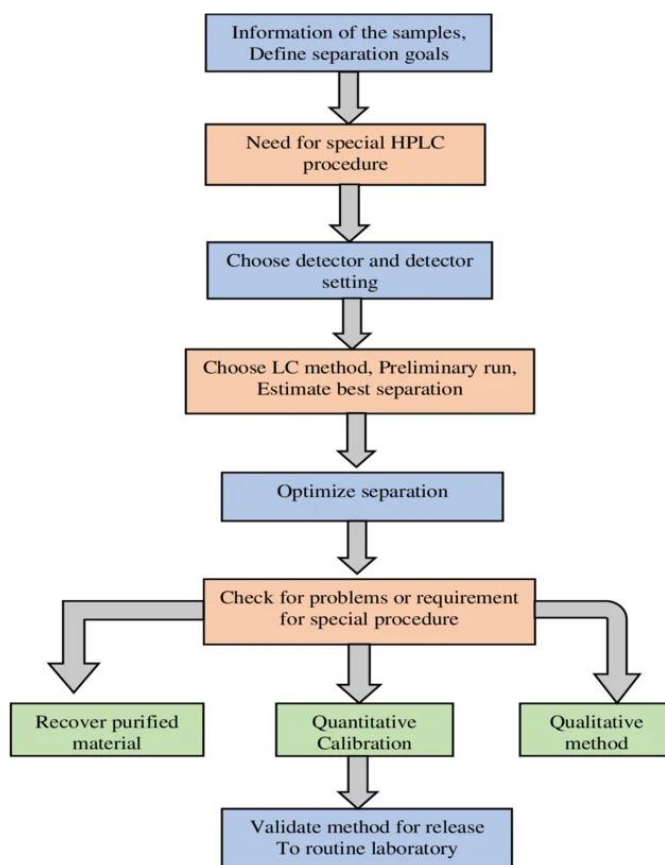


Fig.(3): Steps involved in HPLC Method development

VALIDATION PARAMETER:

The Development method should be validated as per ICH Guideline by using the various validation parameters such as,

- 1 Accuracy
- 2 Specificity
- 3 Limit of Detection (LOD)
- 4 Limit of Quantification
- 5 Linearity and Range
- 6 Ruggedness
- 7 Robustness
8. Precision
9. Sensitivity

1 Accuracy:

This type of analysis demonstrates the statistical variability and consistency of consistency between measurements obtained from repeated samples of the same sample under predetermined conditions. Accuracy is frequently referred to as average relative deviation. Multiplication, average accuracy, and multiplication are the three types of accuracy that can be identified.

2. Specificity:

In the presence of elements that can be expected to occur, testing without analytes is required. This typically entails matrix, dirt, and other detritus. This might be carried out to guarantee the analyst's identification. Prior to the analysis of individual analyses, hygiene tests are used to confirm the accurate contamination of the contaminant content, such as testing for composite materials, heavy metals, residual solvent content, etc.

3. Limit of Detection:

The definition of the acquisition limit for each analysis technique is the lowest value of the analyst in the sample that can be reached, but which is not really the measurement, as a specific value under precise test conditions.

$$DL = \frac{3.3\sigma}{S}$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

4. Limit of Quantification:

Under the required test conditions, the quantitative limit or parameter test for low level analysts in the sample can be performed, and it is generally used to identify pollutants and/or corrosive compounds.

Quantitation limit based on the slope and the response's standard deviation. It can be stated as follows:

$$QL = [10\sigma/S]$$

σ = standard deviation of the response

S = slope of the calibration curve (of the analyte)

5. Linearity and range:

Linearity (within a certain range) is the ability of an analytical method to give test results that are directly proportional to the concentration of analyte in the sample. A linear relationship must be evaluated throughout the entire analytical process. It is directly proven on the drug substance by diluting a standard stock solution of the drug product's component parts in accordance with the specified process. In most cases, linearity is expressed as the confidence interval around the regression line's slope. According to the ICH requirement, linearity should be established using a minimum of five concentrations. The range of the method is the distance between the higher and lower levels that may be calculated using an analytical method with confirmed precision, accuracy, and linearity.

6. Ruggedness:

The degree of test result reproducibility, analysis of many environmental factors, including various laboratories, analyses conducted with various tools, and analysis conducted on various days, are all indicators of how robust an analytical process is. Different reagent sources, assay times that have passed, and assay temperature conditions. The measure of test result reproducibility under the range of conditions that are typically anticipated from analyst to analyst is ruggedness. The RSD should not exceed 2% as the toughness criterion.

7. Robustness:

If a method can resist slight variations in variables like temperature, the pH of the mobile phase, the concentration of the buffer, and injection volume, it is considered to be robust. A robustness requirement should prevent the RSD from going above 2%.

CONCLUSION

The developed RP-HPLC chromatographic method was simple, accurate, specific, and selective, as well as stable, indicating that it could be used to estimate Lamivudine and Dolutegravir in bulk and pharmaceutical dose form at the same time.

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