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DEVELOPMENT OF FORCED DEGRADATION STUDY

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INTRODUCTION

Lamivudine,(2R-cis)-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone,[1] is a synthetic nucleoside analogue with activity against the human immunodeficiency virus (HIV) and hepatitis B virus (HBV).[2] The molecule has two chiral centers and is manufactured as the pure 2R, cis(−)-enantiomer. The racemic mixture from which lamivudine originates has antiretroviral activity but is less potent and substantially more toxic than the pure (−)-enantiomer. Compared with the (+)-enantiomer, the phosphorylated (−)-enantiomer is more resistant to cleavage from nascent RNA/DNA duplexes by cellular 3'-5' exonucleases, which may contribute to its greater potency. Lamivudine is either formulated alone as a tablet/oral formulation or in combination with zidovudine. The spectroscopic method for assay of lamivudine is not official in any pharmacopoeia .A few high-performance thin-layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC) techniques have been suggested for analysis of the formulation. HPLC is the most widely used technique for the estimation of lamivudine in human plasma, saliva, cerebrospinal fluid, and human blood cells, as well as for studying the drug metabolites in the urine.[8] The suggested HPTLC and HPLC methods for assay of lamivudine are expensive and need complex and sophisticated instrumentation. Lamivudine can also be determined by Reverse Phase-HPLC method with lesser runtime, but the aforementioned drawback still persists. One of the first methods for visible spectrophotometric determination of lamivudine was based on the colored condensation products of aromatic aldehydes; this method suffers from a drawback as the interference from the excipients is more since the determination is carried out at much shorter wavelengths. It is also reported that lamivudine can also be assayed by titrimetric methods based on diazo coupling, redox reaction using Folin- Ciocalteu reagent, and redox-complexation reaction using ferric chloride-ortho phenanthroline .However, the above mentioned titrimetric methods are reported to suffer from disadvantages like unstability of the reagents, high cost of the chemicals, reduced sensitivity, etc. The present research work describes a UV spectrophotometric method for estimation of lamivudine in API and its pharmaceutical preparation.

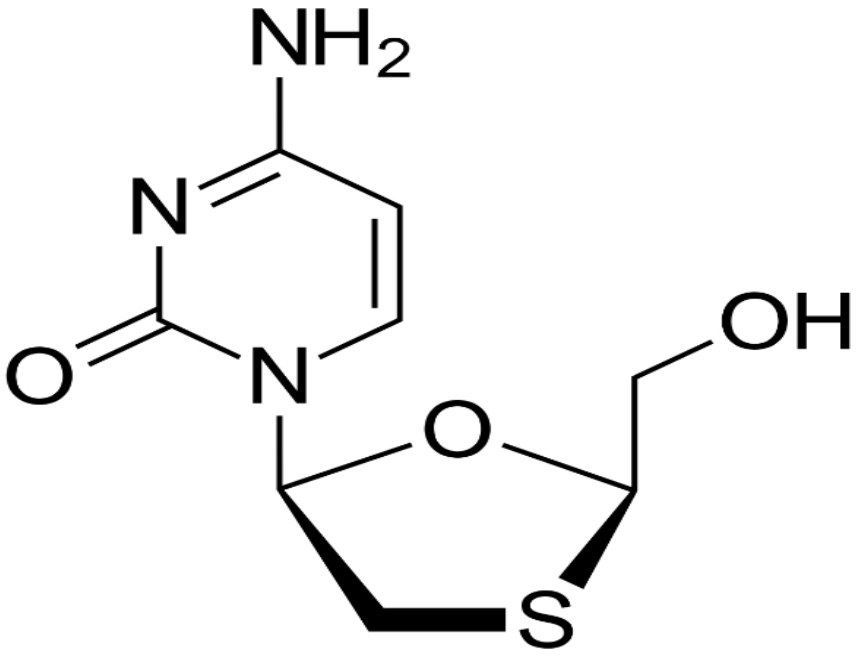


Fig:- Structure of lamivudine

Lamivudine is a monothioacetal that consists of cytosine having a (2R,5S)-2-(hydroxymethyl)-1,3-oxathiolan-5-yl moiety attached at position 1. An inhibitor of HIV-1 reverse transcriptase, it is used as an antiretroviral in the treatment of AIDS and hepatitis B.

Forced degradation studies provide the approach to analyses the stability of drug samples in pharmaceutical industries. Drug product safety and efficacy is affected by the chemical stability of the molecule. Stability of molecule information provides the data for selecting proper formulation, package, proper storage conditions and shelf life. These data also play a significant role which is required in the regulatory documentation. Before filling registration dossier, it is obligatory to execute stability studies of new drug molecules.

A forced degradation study investigates the stability of a chemical or pharmaceutical product under stressful conditions (forced degradation research is also known as stress testing). “Stress” in this case means any physical or environmental conditions that a product will encounter that could cause a chemical change. Key learning outcomes of forced degradation testing include:

- Establishing likely degradation pathways and products.
- Evaluating the stability of the molecule.
- Validating the analytical methods used to assess chemical stability.
- Gaining insight into drug packaging and storage.

Forced degradation studies can be performed for any chemical, but it is essential in pharmaceutical development. Active pharmaceutical ingredients (APIs) are carefully designed and dosed to deliver a specific pharmaceutical effect. Chemical changes can compromise the drug’s efficacy or cause harmful side effects.

Objectives for forced degradation :-

- To develop a degradation pathways of drug substances and drug products.
- To recognize the chemical properties of drug molecules.
- To resolve stability related problems.
- To establish the intrinsic stability of a drug substance in the formulation.
- To reveal the degradation mechanism of drug substance and drug products.
- To generate stability indicating nature of a developed method.
- To produce more stable formulations. It also helps in determining the expiry date of particular formulation.

- To generate a degradation profile similar to that of what would be observed in a formal stability study under ICH conditions.
- To distinguish degradation products that is related to the drug products from those that are generated from non-drug product in a formulation.

METHODS AND MATERIALS :-

Chemicals:

Lamivudine drug was obtained from lamivudine tablets. These tablets of 100 mg were purchased from local markers. The purity of lamivudine was confirmed by uv-visible spectroscopy analysis.

Apparatus:

UV visible wavelength detector, UV spectrophotometer (V-730ST), conical flask, RBF, cuvettes, condenser, measuring cylinder, burner, Bunsen burner stand.

Standard and Sample Preparation:-

Standard Preparation:

A stock standard solution of lamivudine was prepared by dissolving 100 mg of lamivudine drug dissolving in 100 ml of volumetric flask containing 25 ml methanol. It was sonicated for about 5 min to dissolve drug. Then resultant solution diluted to 100 ml methanol. 10 ml from this , transfer in another volumetric flask and diluted with 100 ml methanol. The concentration use to asses lamivudine content is 10 mg/ml.

Sample Preparation:

To prepare the stock sample solution 10 tablets of lamivudine were accurately weighted pulverized and mixed using mortar and pestle. An amount of tablets power equivalent to 100 mg of lamivudine was weighted and transfer into 100 ml of volumetric flask containing 25 ml of methanol. It was sonicated for about 5 min to increase dissolution. 10 ml of supernatant solution taken and diluted to 100 ml with methanol.

FORCED DEGRADATION STUDY:-

1] Acidic degradation:-

The procedure of degradation studies was conducted in agreement with ich guide to stability studies. The sample solution were prepared at concentration of 10mg/ml in methanol, 0.5 M HCL and 0.5 M NaOH at 80c for 24 hours. At this condition lamivudine drug was found to be stable.

While two degradation product was found to be from 2 M HCL at 80c for 72 hours. In this degradation study result was found to be bad smell and yellow color.

In 2 M NaOH at neutral condition lamivudine drug was found to be stable.

Fig. Before acidic degradation

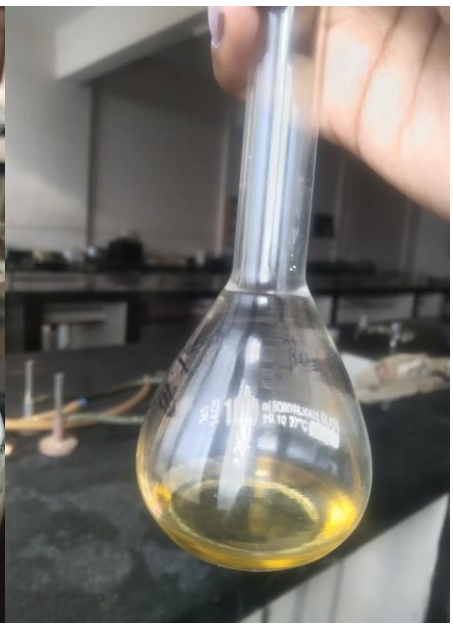


Fig. After acidic degradation

2] Thermal degradation:-

Thermal behaviour and decomposition kinetics studied by thermal analysis techniques by Differential Scanning Calorimetry (DSC) and Thermo Gravimetric Analyse (TGA)

Different heating rates were applied to study the DSC behaviour of drug sample in order to compute their thermo kinetic and thermodynamic parameter by non-isothermal kinetic methods.

DSC In this method heat is applied to sample and observed physical and chemical change whether heat is loss or gain, DSC curves used for thermo kinetic parameter.

TGA In this method accurate weight of lamivudine drug are taken and applied the heat then observe change in mass. Decomposition at several heating rates was observed.

3] Photolytic degradation:-

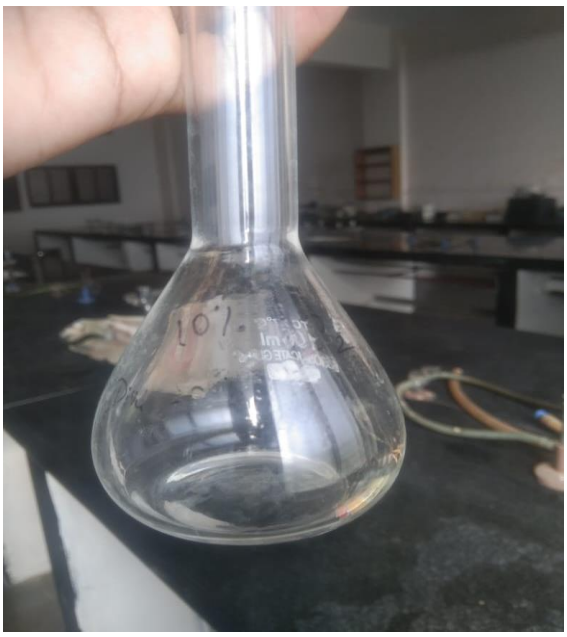
It consists of photo stability chamber which include 2 UV lamp sand 4 fluorescence lamp. The photolytic degradation of lamivudine drug detected in pure water, natural water and fresh water.

4] Hydrolytic degradation:-

Identification and characterization of lamivudine drug degradation was studied by using liquid chromatography-mass spectroscopy (LC-MS) in combination with high resolution mass spectroscopy useful in development of stable formulation.

5] Oxidative degradation: -

Stock solution of lamivudine drug diluted with 10% H202 and kept at room temp for 10 hrs. for oxidative degradation. In this condition lamivudine drug was found to be stable.



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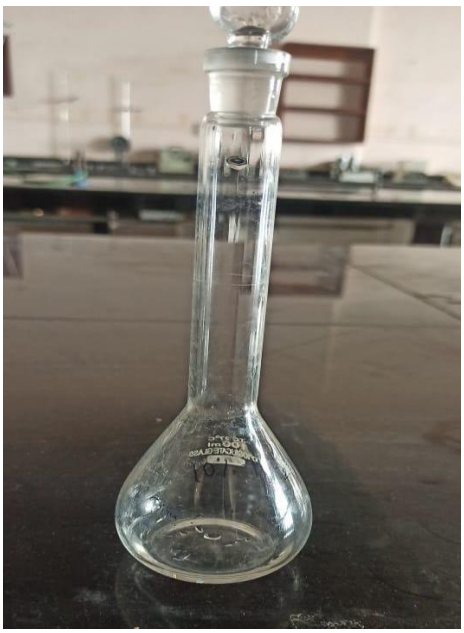

(2)

Fig. Oxidative degradation

CONCLUSION:-

A stability indicating HPLC related compounds method was developed for the quantification of Lamivudine and its potential impurities in active pharmaceutical ingredients and its dosage forms. The developed method is specific, precise, accurate, linear and robust for Lamivudine and its impurities. Degradation products formed during forced decomposition studies were very well separated from analyte peak, which demonstrates that the developed method was specific and stability indicating .This method can be used to carry out the analysis of Lamivudine drug product in regular quality check and stability samples.

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