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Analytical Method Development

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Analysis of active pharmaceutical component is an integral part of pre formulation and formulation development. It is essential to have a validated specific method of analysis of drug. The pharmaceutical analysis is a branch of chemistry which involves series of process for the identification , determination , quantification and purification. It is use for separation of components from the mixture and for the determination of the structure of compounds.

Anti retroviral drugs are nucleoside reverse transcriptase inhibitors . A type of anti retroviral drug used for treatment of HIV and AIDS . It is an analogue of thymidine . Thymidine inhibits the activity of HIV 1 reverse transcriptase (RT) by competing with natural substrate d GTP and by its incorporation into viral DNA . For the estimation of multicomponent formulation the instrumental techniques which are commonly employed are spectrophotometry , GLC , HPTLC , HPLC , etc. These methods are based upon measurement of specific and non specific physical properties of substance .

Introduction of Analytical In Instrument

HPLC (High Performance Liquid Chromatography) :-

A new method using HPLC coupled with ultra violet detection (HPLC – UV) was developed and validate for the simultaneous quantification of Anti retroviral drug in human plasma . The solid phase extraction and HPLC – UV method enable a specific , sensitive and reliable simultaneous determination of nine Anti retroviral agent in plasma.

Chemicals and reagents used in HPLC , the most common solvents used

for HPLC are water , methanol and acetonitrile.

HPLC Components :

1) Infusion System
2) Degassing Device
3) Gradient Elution Device
4) Sampling System
5) Separation System
6) Detector
7) Data Processing System

Columns Of HPLC :-

The physical properties of the target molecules (analytes) determine the most suitabe HPLC column for a given separation. The molecular characteristics that impact HPLC column selection include hydrophobicity or hydrophilicity, intermolecular courses(particularly dipole-dipole), intramolecular forces (ionic), and size. HPLC column separation can often exploit multiple differences in the molecular properties of the target molecules. Generally, the structure and chemistry of the HPLC column packing(stationary phase) determines the analyte elution profile.

HPLC column sizes range from capillary to process scale. The internal diameter and volume of column determine both how much sample can be loaded on to a column and the sensitivity of separation. The column ID can affect the separation profile, particularly when using gradient elution, with smaller IDs yielding increased separation and detection sensitivity. Therefore, for analytical separation there is typically a trade-off between sensitivity and the sample volume loaded on to a column.

HPLC column types:-

1) Ion exchange HPLC column
2) Reversed phase HPLC column
3) Normal phase HPLC column

4) Size exclusion HPLC column

Methods For Degradation Study

1) Thermal degradation :-

Thermal behavior and decompositionkinetics studied by thermal analysis techniques by Differential Scanning Calorimetry (DSC) and Thermo Gravimetric Analysis (TGA)

Different heating rates were applied to study the DSC behavior ofdrug sample in order to compute their thermokinetic and thermodynamic parameter by non isothermal kinetic methods .

- DSC :- In this method heat is applied to sample and observed physical and chemical change i.e. heat is loss or gain . DSC curves used for thermokinetic parameter .
- TGA :- In this method crystal /mass /weight are taken and applied the heat then observe change in mass . Check decomposition at several heating rates .

2) Photolytic degradation :-

It consist of photo stability chamber which include 2 UV lampsand 4 fluorescence lamp. Anti retroviral drugs were among In most hazardous therapeutic pharmaceuticals with regard to their toxicity toward algae , daphnids, fish. Thus necessary to know transformation pathway (photolysis) of anti retroviral drugs in aquatic environment. They detected in pure water, natural water , fresh water , sea water and waste water treatment plants (WWTPs).

3) Hydrolytic degradation :-

Identification and characterization of degradation of productusing liquid chromatography – mass spectroscopy (LC – MS) in combination with high resolution mass spectroscopy useful in development of stable formulation .

4) Acidic degradtion

Anti retroviral drug was found to be stable when refluxed in 0.5 M HCL and 0.5 m NaOH at 80c for 24hrs. While 2 degradation product form in 2m HCL at 80c for 72 hrs . In 2m NaOH at neutral condition stable .

5) oxidative degradation

Stock solution of antiretroviral drug diluted with 10 % H2O2 andkept at room temp for 10 hrs for oxidative degradation . Acidic , basic, neutral, hydrolytic , degradation studies carried out by refluxing in 2 m HCL , 2 m NaOH and water at 80 c for 72 hrs respectively.

Method validation

1) Accuracy :-

The accuracy of the method was determined by calculating recoveries of antiretroviral drug by standard addition method. A known amountof standard solution of solvent were added to pre quantified sample solution oftablet. The amount of drug was determined by applying this value to the regression equation of the calibration curve.

2) Precision:-

The precision was checked by repeatedly solution of solvent forthe RP – HPLC method. The accuracy of the method was evaluated by determination of the recovery of solvent on two days at 6 levels concentration.

Tablets and capsules sample solution were spiked with solvent standard solution. Corresponding to 125% of the nominal analytical concentration. The results show good recoveries ranging from 98.77-101.45%.

3) Linearity:

Linearity was demonstrated by analyzing six different concentration of active compound. Accurately measured standard working solution of drug 0.1,0.2,0.3,0.4,0.5,0.6 ug/ml were prepared in 10ml volumetric flask and diluted to the mark of mobile phase. Aliquotes of each solution were injected under the operating chromatographic condition . Peak areas were recorded for all peaks and calibration plot was constructed by plotting peak area vs concentration of drug

4) Limit of detection:-

The lowest possible concentration at which the method can detect (but not quantity) the analyte within the matrix .The sample was dissolvedby using mobile phase and injected until peak was diapered. After 0.053 ug/ml dilution peak was not clearly observed . So it confirms that 0.062 ug/ml is limit of detection.

5) Specificity and Selectivity :-

The chromatographic interference fron endogeous compound was assessed by compairing chromatograms with that of drug tabletsubstance .

6) Sensitivity :-

The lowest limit of detection is defined as the amount that couldbe detected with a signal to noise ratio 4. The lowest limit of quantification was determine as minimum concentration that could be accurately quantified with standard deviation +/- 10% (0.025 ug/ml).

7) Robustness :-

As part of robustness delibrate change in the flow rate, mobilephase composition, temprature variation was made to evaluate the impact of method . The flow rate was varied from 0.7ml /min to 0.9 ml/min . The organic composition of mobile phase was varied about 10%

onclusion

All above studies led to the conclusion that the method developed is accurate, precise, specific, cost effective, robust, rugged, simple and sensitive. This method can be applied for the estimation of antiretroviral drug in bulk and in pharmaceutical formulation. The method is also applicable for the stability testing of antiretroviral drug in bulk as well as in pharmaceutical dosage form.

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