1. INTRODUCTION.

1.1 ANTI-RETRO VIRAL DRUGS.

Anti-retro viral Drugs\(^1,2\), These are the drugs active against human immuno-deficiency virus (HIV).

The first Anti-retro viral drug Zidovudine was developed in 1987. There are number of new drug molecules that have been developed for the effective treatment of HIV infection or other viral infections. As recently the mankind has encountered some viral infections which are caused by newer viral strains like H1N1, Ebola virus, Hanta virus, Marburg virus etc, which has caused havoc in both developed & developing countries posing newer threats & challenges to the mankind. These newer virus strains which have been recently identified are the main cause for the increased mortality rate in both humans and animals. All these infections require some new drugs so that these infections can be treated & prevented. So there is a need for development of newer antiretroviral drugs and formulations which in turn makes it obligatory for their quantifications either in APIs or in the formulations.

The Mechanism of drugs acts like Nucleoside Reverse Transcriptase Inhibitors (NRTI), Non Nucleoside reverse transcriptase Inhibitors (NNRTIS), and Protease inhibitors\(^3\).

1.2 DIABETIC MELLITUS DRUGS.

Now a days due to change in lifestyle and food habits it has become a major problem causes diseases and disorders in human begins.

Since many of drugs has been came existences for human survival.

Diabetic mellitus (DM) is an metabolic disorder which result causes increase glucose level in body, does not produce enough insulin or does not responding to insulin.

This shows symptoms like polyuria, polydipsia and polyphasia\(^4\).

However with proper management like controlled diet, drugs and physical exercise we can maintain proper levels of insulin.

Further Diabetic mellitus is classified into

Type-1 DM –This is insulin dependent (IDDM) it is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas leading to insulin deficiency.
Type-2 DM – This is not insulin dependent (NIDDM) is characterized by reduced insulin secretion. Type 2 diabetes is the most common type.

Type-3 DM- This occurs due to hormonal disorder such as Acromegaly and drugs such as glucocorticoids.

Type-4 DM – It is known as Gestational diabetes mellitus (GDM) it occurs in about 2%–5% of during pregnancies and it disappear after delivery. Gestational diabetes is fully treatable but requires careful medical supervision throughout the pregnancy.

There are various pharmacological classes of drug which can use in the treatment of diabetes mellitus. These classes are as follows:

- Inhibitors of hepatic gluconeogenesis- Biguanides ,
- Stimulators of insulin release by beta cells- Meglitinides, Sulfonylurea,
- Inhibitors of intestinal Alfa glucosidases- acarbose, miglitol,
- Drugs which reduces insulin resistance-glitazones,
- Inhibitors of dipeptidyl dipeptidase-4 (DDP-4)-sitagliptin.

Due to increase in the diseases and disorder researcher facing much more problems in challenging to discoveries of new drug or formulation.

1.3 NON STEROIDAL ANTI INFLAMMATORY DRUGS (NSAIDS).

Now a days it has became a major problem for all the age peoples, due various factors like excess of strains, without any physical exercise which result increase in the body weight and also by change in food habits etc.

Many of the new chemical entities molecules were introducing into market for survival of humans, but still research is required for some drugs to have new analytical method development. They mainly acts by COX-1, COX-2 inhibitors.

1.4 DEVELOPMENT OF ANALYTICAL METHOD.

To meet quality, safety and efficacy of medicines are manufactured as per ICH and WHO regulations.

Evaluation of safety & efficacy dependent upon the quality control of the product.

Number of the drugs introduced into market and some drugs or drugs in combination the standard analysis procedure may not be available in any pharmacopoeias.
In this proposal, it is planned to develop and subsequently validate chromatographic methods for sample preparation of drugs from varying therapeutic categories, in single or in combinations. To develop a new existing analytical procedures for the Solvents, extraction and separation procedure are to be less expensive.

Analytical methods for a drug in combination with other drugs may not be available. HPLC is used for characterization of drugs, impurities, metabolites and related substance, the used of small column (3-10cm) in routine analysis of drugs which is about hundred times faster than the other conventional liquid chromatography. An advantage of HPLC over other liquid chromatography is accuracy, precision faster separation and sensitivity.

HPLC is more efficient which uses smaller particles make more rapid equilibrium approach to column which show narrow peaks with high efficient theoretical plates of 5000-10000 with adequate resolution faster separation, even the small particle when passed through closed packed column which also reduces the flow rate, in order to achieve normal flow it need to apply high pressure for function 6.

HPLC is mainly used in pharmaceutical industries for

- Identify the metabolites
- Assay active ingredients, impurities, degradation products.
- Used to identify & purify synthetic or natural product.
- In pharmacodynamic and pharmacokinetic studies.

Chromatography is an qualitative analysis and quantitative analysis.

Qualitative analysis is an important step in spectroscopic analysis used to determine the presence or absence of components of mixture.

Quantitative analysis is mostly used tool for separation, is used comparison either on height or area of the analytical peak with the one or more standard.

It also used to determine by using analysis based on peak height, peak areas, calibration internal standard method and area normalization method7.

1.5 VALIDATION OF ANALYTICAL METHODS8.

Validation provides the documented evidence whether the method is fit for intended purpose.

Validation is done for new process, new equipment, the process validation includes, Analytical test procedures, Instrument calibration, Critical support systems, Raw materials, Equipment, Effect of Stability.
The validation parameters are

- Accuracy,
- Precision, (repeatability and reproducibility)
- Linearity and range,
- Limit of detection (LOD), Limit of quantitation (LOQ)
- System suitability studies,
- Ruggedness,
- Robustness,
- Specificity, Stability studies.

RP-HPLC method were developed for some selected drugs and their formulation are Abacavir, Dolutegravir and Lamivudine, Indinavir sulphate, Saquinavir, Darunavir, Cobicistat are the Anti-retroviral Drugs. Saroglitazar is an Type 2 diabetes drug. Aceclofenac, Serratiopeptidase, Lornoxicam, Thiocolchicoside are NSAID.