4. Methodology and Work Plan:
Pharmaceutical analytical methods are categorized into five general types:

- Identification tests
- Potency assays
- Impurity tests: quantitative
- Impurity tests: limit
- Specific tests.

**Method will be developed for following drugs.**

a) Acyclovir:

b) Valacyclovir:

HPLC method development will be carried by clubbing the two molecules simultaneously in single method.

**Analytical Method development process:**
The steps of methods development and method optimization depend upon the type of method being developed, however the following steps are widely and commonly used:

- Method development plan definition
- Background information gathering
- Laboratory method development
- Generation of test procedure
- Methods optimization protocol definition
- Laboratory methods optimization
- Validated test method generation
- Method validation report

**Steps will involve in new analytical method development by HPLC as below:**

**Step 1 – Literature survey**
Collect the following information about Physico chemical property of drugs from different books, net etc. Make compilation and design the plan for develop the method.

**Knowledge of the samples of drugs:**

- Molecular weight range
- Nature of sample components
- Structure of sample components
• Number of compounds present
• Sample matrix
• pKa values of sample components
• Concentration range
• Solubility
• Other pertinent data

**Step 2 – Method development:**

**I. Selection of initial conditions.**
This step determines the optimum conditions to adequately retain all analytes; that is ensures analyte has a better capacity factor (excessive retention leads to long analysis time and broad peaks with poor detectability).

**II. Selection of Mobile phase solvent strength**
The solvent strength is a measure of its ability to pull analytes from the column. It is generally controlled by the concentration of the solvent with the highest strength; for example, in reverse phase HPLC with aqueous mobile phases, the strong solvent would be the organic modifier; in normal phase HPLC, it would be the most polar one.

**III. Selection of stationary phase (HPLC column) :**
Selection of column is important factor in HPLC method development and on base of physico chemical property of drugs we can achieve the proper selection of HPLC column. There are large nos of column available for analysis, having stationary phase Octadecyl silane, Octyl silane, Cyano, Amino, Phenyl base. Selection of column base upon nature of molecules like hydrophilic/ hydrophobic, Acid/Base, Functional groups etc.

**IV. Selection of wavelength**
Each molecule will be scan by UV region to identify the maximum wavelength absorbance. It will help in select the single wavelength for more molecules.

**V. Selectivity optimization**
The aim of this step is to achieve adequate selectivity (peak spacing). The mobile phase and stationary phase compositions need to be taken into account. To minimize the number of trial chromatograms involved, only the parameters that are likely to have a significant effect on selectivity in the optimization must be examined. To select these, the nature of the analytes must be considered.
VI. System parameter optimization.
This is used to find the desired balance between resolution and analysis time after satisfactory selectivity has been achieved. The parameters involved include column dimensions, column-packing particle size and flow rate. These parameters may be changed without affecting capacity factors or selectivity.

VII. Method Optimization.
Proper validation of analytical methods is important for pharmaceutical analysis when assurance of the continuing efficacy and safety of each batch manufactured relies solely on the determination of quality. The ability to control this quality is dependent upon the ability of the analytical methods, as applied under well-defined conditions and at an established level of sensitivity, to give a reliable demonstration of all deviation from target criteria.

Step 3 - Method Validation
The HPLC or UV-Spectrophotometer or Potentiometer instruments will use for analytical development on base of selection of drug substance (Molecule).

Each of these validation characteristics is defined as per ICH as below:

Specificity: Ability to measure desired analyte in a complex mixture. Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.

Linearity: Proportionality of measured value to concentration
The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Accuracy: Agreement between measured and real value
The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

Precision: Agreement between a series of measurements
The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.
Repeatability
Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Intermediate precision
Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.

Robustness
The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

The following characteristic will be performed to check the purity. Theses study indicates stability indicating method.

Forced degradation
This study will be carried out to check the interference of degradents with main peak of drug molecules. On base of “Peak Purity” parameters of HPLC, it will be confirm that the interference of degradents with main peak of drug molecule. Peak purity pass indicates, the principle peak of molecule is homogeneous and no interference from others.

The following condition will be applied for carry out the study.

a) Acid hydrolysis
b) Alkali hydrolysis
c) Oxidation degradation
d) Photo stability degradation
e) Thermal degradation
f) Hydrolysis degradation
h) Reduction degradation
5. WORK PLAN:
The present study might be the best way to evaluate the quality and stability of the pharmaceutical formulations. The analytical method development for the detection, identification, quantitative determination of impurities using various analytical techniques may offer various advantages such as improving drug product quality and manufacturing efficiency of the drug product. Estimation of impurity level and validation study of drug product has received considerable attention in the recent past due to growing interest in the cost effective and quality drug product. Considering the great opportunities which exist for the discovery and development of new formulation, ‘Quality’ would be today’s basic need.

So planning of work is:

- Literature survey of API and Finish product regarding their physical, chemical properties.
- To develop simple, precise, economical method in combination dosage form for a routine quality control analysis.
- Estimation of both drugs from tablet dosage form.
- Forced degradation studies.
- Validation of developed method according to ICH guidelines.