4. **Methodology:**
Pharmaceutical analytical methods are categorized into five general types:
- Identification tests
- Potency assays
- Impurity tests: quantitative
- Impurity tests: limit
- Specific tests.

From above tests we will be developed a method for “**Potency-assays**” on base of modern instrument like “High Performance Liquid Chromatography Analytical method will be developing for simultaneous determination of Hypertensive drugs. The method will be applicable in API industries and pharmaceutical industries to determine the assay of drugs.

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

a) **Diuretics (Water pills) drugs:**
   - Chlorthalidone, Hydrochlorothiazide drugs

b) **Calcium Chanel Blockers drugs:**
   - Amlodipine Besylate

c) **Angiotensin II receptor blocker:**
   - Candesartan, Irbesartan, Losartan, Telmisartan, Valsartan, Olmesartan
   - Medoxomil

d) **Statin drugs**
   - Atorvastatin Calcium

Hence assay method will be develop on 10 drug molecules and try to set the assay method by taking at least 4 molecules in single method. Also developed method will be applied for some commercial available pharmaceutical products in market to check the accuracy. The HPLC method will be classified on base molecules as below.

a) **Method I**
HPLC method development will be carried by clubbing the five molecules simultaneously by single method like Hydrochlorothiazide, Chlorthalidone, Amlodipine Besylate, Valsartan and Telmisartan

b) **Method II**
HPLC method development will be carried by clubbing the five molecules simultaneously by single method like Hydrochlorothiazide, Chlorthalidone, Olmesartan Medoxomil, Candesartan and Atorvastatin calcium

c) **Method III**
HPLC method development will be carried by clubbing the five molecules simultaneously by single method like Hydrochlorothiazide, Chlorthalidone, Losartan and Irbesartan

**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 the analyte has a better capacity factor (excessive retention leads to long analysis time and broad peaks with poor delectability).

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. The aim is to find the correct concentration of the strong solvent. With many samples, there will be a range of solvent strengths that can be used within the aforementioned capacity limits. Other factors (such as pH and the presence of ion pairing reagents) may also affect the overall retention of analytes.

Different type of buffer reagents available for mobile phase preparation like sodium orthophosphate buffer, potassium ortho phosphate buffer monobasic or dibasic, citrate buffer and orthophosphoric etc. The selection of buffer in mobile phase depend pH and solubility criteria of mobile phase. Different type of organic solvents available like methanol, acetonitrile and
tetrahydrofuran can be used for method development and it depends upon resolution and tailing of molecules on chromatographic condition.

Gradient HPLC with samples containing a more than single drug of analytes will be used to better separation and avoid excessive retention of analyte.

III. Selection of stationary phase (HPLC column)
Selection of column is an important factor in HPLC method development and on base of physicochemical 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 scanned by UV region to identify the maximum wavelength absorbance. It will help in selecting 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.

Once the analyte types are identified, the relevant optimization parameters may be selected. Note that the optimization of mobile phase parameters is always considered first as this is much easier and convenient than stationary phase optimization.

Initially gradient conditions or Isocratic condition should be optimized using a binary system based on organic solvent or aqueous buffer. If there is a serious lack of selectivity, a different organic modifier should 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). After develop the method, method will be validated on base of following validation characteristics as per “ICH Harmonised Tripartite Guideline”

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.

Assay: This definition has the implications to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

**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.
Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

**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.

**System suitability testing**
System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. See Pharmacopoeias for additional information.

**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

From above validation characteristic results, we will finalize the analytical methods for hypertensive drug analysis.

**Step 4 - Application**  
After finalize the method, it will be apply for available commercial pharmaceutical drug products and calculate the % assay. It will be help to determine the assay from drug products with accuracy. We will collect the market samples (Single dosage form or combined dosage form) from medical store and perform the analysis and calculate the potency of drugs in the percentage. Results will be recorded and reported.

**5. Work plan:**  
Work will be plan accordingly following schedule:

<table>
<thead>
<tr>
<th>Sr. Nos</th>
<th>Experiments</th>
<th>Schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Solubility and other study initiation for selected drug molecules</td>
<td>January 2010</td>
</tr>
<tr>
<td>2.</td>
<td>Determination UV absorbances and collect the information of physico-chemical properties of drugs</td>
<td>March 2010</td>
</tr>
<tr>
<td>3.</td>
<td>Method I development initiation for simultaneously determination of Hydrochlorothiazide, Chlorthalidone, Amlodipine Besylate, Valsartan and Telmisartan by HPLC</td>
<td>April 2010</td>
</tr>
<tr>
<td>4.</td>
<td>Method I development and finalization on base of optimization</td>
<td>June 2010</td>
</tr>
<tr>
<td></td>
<td>Method II development initiation for simultaneously determination of Hydrochlorothiazide, Chlorthalidone, Olmesartan Medoxomil, Candesartan and Atorvastatin calcium</td>
<td>September 2010</td>
</tr>
<tr>
<td>---</td>
<td>---------------------------------------------------------------------------------------------------------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>6.</td>
<td>Method II development and finalization on base of optimization</td>
<td>December 2010</td>
</tr>
<tr>
<td>7.</td>
<td>Method III development initiation for Simultaneously determination of Hydrochlorothiazide, Chlorthalidone, Losartan and Irbesartan</td>
<td>March 2011</td>
</tr>
<tr>
<td>8.</td>
<td>Method III development and finalization on base of optimization</td>
<td>May 2011</td>
</tr>
<tr>
<td>10.</td>
<td>Analytical Method validation of method II, calculation and report compilation</td>
<td>September 2011</td>
</tr>
<tr>
<td></td>
<td>Paper publication of Method I</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>Analytical Method validation of method III, calculation and report compilation</td>
<td>November 2011</td>
</tr>
<tr>
<td></td>
<td>Paper publication of Method II</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>Paper publication of Method III</td>
<td>January 2012</td>
</tr>
</tbody>
</table>