WORK PLAN AND METHODOLOGY

The work plan and methodologies are mentioned below are followed for each drug selected for the research study.

1. Exhaustive Literature survey:
   Literature review shall be performed by referring to, e books, books in organizational library and literatures available through different scientific databases like Elsevier, springerlink etc.

2. Selection and identification of drugs.
   a) The selection of drug shall be based on its category and severity for the need of bioanalytical method.
   b) Identification shall be performed by mass spectrometer. The available certificate of analysis shall be referred for the further identification.

3. Selection of Internal standard.
   Internal standard suitable for the drug of choice shall be selected on either a similar structural analogue or its deuterated standard.

   Drug and Internal standard shall be procured from the available resources.

5. Instrumental optimization:
   The mass and liquid chromatographic parameter for the analyte shall be optimized depending on their molecular weight, pKa and polarity characteristics.
   a) Tuning of the analyte and internal standard shall be performed using mass spectrometer to optimize the MS/MS parameters.
   b) UPLC parameter optimization shall be done for suitable LC column, mobile phase composition and injector parameters.

6. Optimization of sample preparation method:
   The extraction of analyte and sample clean-up shall be done by any of the following methods or a combination of any mentioned below by trial and error method.
   - Protein precipitation technique using aprotic solvents
   - Protein precipitation using cartridge
Synopsis

- Liquid liquid extraction
- Solid Phase extraction

7. Validation of the developed method:

The method validation experiments shall be performed as per US FDA guidelines. Following parameters shall be studied to ensure the acceptability of the performance and the reliability of analytical results:

- Selectivity
- Precision and accuracy
- Linearity
- Sensitivity (Limit of Quantification)
- Matrix effect
- Recovery
- Stability (freeze thaw, bench top, auto sampler, long term storage).