4. Methodology and Work Plan:

Methodology:

- Collection of standard & sample from reliable source in pure form.
- Solubility Determination for API and Finish product in appropriate solvent or their mixture of solvents.
- Determination of the $\lambda$ max for drug substance by U.V. Spectroscopy.
- Preparation of same concentration solution for both standard & sample by help of their label claim mentioned.
- Method development for the assay and Related substances by HPLC method.
- On the basis of solubility studies, the diluents & mobile phase composition can be decided for further research work.
- Selection of column on the basis of previous work done on individual drugs, mainly C8 or C18 column.
- Determination of isocratic or gradient mode for analysis on the basis of previous done as well as primary run on HPLC system.
- Determination of Solution stability by run on HPLC.
- Optimization will be done by changing the proportion of mobile phase, as well as doing trail on different grade column.
- According to ICH guideline, validate the above new method.

Various parameters studied for Method Validation:

For validation the developed method is subjected to following studies:

- **Accuracy**: It is the concordance between it and the true or most probable value.
- **Precision**: It is the concordance of a series of measurements of the same quantity.
- **Linearity**: The linearity of an analytical procedure is its ability within a given range to obtain test results that are directly proportional to the concentration of analyte in the sample.
- **Specificity / Selectivity**: It is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present.
- **Limit of detection**: It is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value.
● **Limit of quantitation**: The quantitation limit of an analytical procedure is the lowest amount of analyte in a sample that can be determined quantitatively with suitable precision and accuracy.

● **Robustness / Ruggedness**: 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 use.

From above validation characteristic results, we will finalize the analytical methods for Parkinson's disease.

5. **Plan of work**: (Time scheduled of the work)

Total expected duration of work is approx. 2 year. Back up of work detail are given bellow.

**First six month**: -

**Second six month**: -
Development and optimise Pramipexole standard and sample preparations for LC/UV method. Perform trials on different method parameters and validation chapters.

**Third six month**: -
Method development will be finalized and performed method validation on basis of method development. Developed methods were validated on following ground.

1) Specificity & selectivity
2) Linearity and range
3) Limit of Detection/Limit of Quantitation
4) Recovery (Accuracy)
5) Repeatability (Precision)
6) Reproducibility
7) Ruggedness
8) Solution Stability

**Four six month**: - Based on the method validation data summery report to be prepared. Thesis writing will be completed and paper publication to be done.