3. **Aim and objectives of the Present Research work**

Bioanalytical methods employed for the quantitative and qualitative determination of drugs and their metabolites in biological samples. The developed method must generate reproducible and reliable data in order to permit valid interpretation of the studies they support. It is essential to employ well-characterized and fully validated bioanalytical methods to yield reliable results that can be satisfactorily interpreted. It is recognized that bioanalytical methods and techniques are constantly undergoing changes and improvements and in many instances, they are at the cutting edge of the technology. It is also important to emphasize that each bioanalytical technique has its own characteristics, which will vary from analyte to analyte. In these instances, specific validation criteria may need to be developed for each analyte. Moreover, the appropriateness of the technique may also be influenced by the ultimate objective of the study.

Method validation employed for the quantitative determination of drugs and their metabolites in biological fluids plays a significant role in the evaluation and interpretation of bioavailability, bioequivalence, pharmacokinetic and toxicokinetic study data. These studies generally support regulatory filings. The quality of these studies is directly related to the quality of the underlying analytical data. It is therefore important
Chapter 3  
Aim & Objectives of the Present investigation

that guiding principles for the validation of these analytical methods be established and disseminated to the pharmaceutical community.

**LIST OF DRUGS SELECTED FOR PRESENT RESEARCH WORK**

<table>
<thead>
<tr>
<th>S. NO</th>
<th>Name of the Drug Substance</th>
<th>Chemical Structure</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Febuxostat</td>
<td><img src="image1" alt="Febuxostat Chemical Structure" /></td>
<td>Management of hyperuricaemia</td>
</tr>
<tr>
<td>2.</td>
<td>Milnacipran</td>
<td><img src="image2" alt="Milnacipran Chemical Structure" /></td>
<td>Treatment of depression</td>
</tr>
<tr>
<td>3.</td>
<td>Mesalamine</td>
<td><img src="image3" alt="Mesalamine Chemical Structure" /></td>
<td>Treatment of digestive tract disease (Crohn’s disease)</td>
</tr>
</tbody>
</table>
Aim is to conduct method development and method validation of the selected drugs namely Febuxostat, Milnacipran, Mesalamine and Acamprosate in human plasma by using high performance liquid chromatography coupled with mass spectrometry.

In **method development**, extensive literature survey, optimization of mass spectrometry, chromatographic parameters, extraction methods optimization, linearity range, regression model selection, sensitivity and recovery to be carried out.

In **method validation**, selectivity & sensitivity, intra-inter assay precision and accuracy, Matrix effect, Dilution integrity, ruggedness, recovery, Stability of the drug in plasma(stability parameters like short term stability, long term stability, auto sampler stability, bench top stability, freeze-thaw stability, and reinjection stability), stability of the drug in stock solution and intermediate spiking solution to be proved. After method development and method validation of selected drugs there
is a need to be prove its application of Pharmaceutical formulations in biological matrices.

For this purpose Pharmacokinetic, Bioavailability of the selected formulations to be prove based on the validated method. To compare the bioequivalence of test and reference formulations Pre-Clinical/Clinical study to be done.

The **bioequivalence study** protocol is to be designed for each selected drug formulation as per Institutional Ethics Committee (IEC) and Drug Control General of India (DCGI) guidelines. Based on the randomization schedule, test and reference formulations to be applied into screened healthy human volunteers. Blood samples from each volunteer (subject) will be collected at specified time intervals and analyzed at each sample of test and reference by LC-MS/MS. Concentration data from each subject (test, reference) will be applied to pharmacokinetic study.

**Pharmacokinetic parameters** from human plasma concentration data for each subject will be calculated by a non-compartmental statistics model using WinNon-Lin 5.0 software. In case of Preclinical study for selected drug, bioavailability to be calculated at specified time points and pharmacokinetic parameters to be evaluated.