8.0 SUMMARY AND CONCLUSION

The present work aimed to assess the applicability of Liquid chromatography tandem mass spectrometry (LC-MS/MS) for Bio analysis of different class of drugs in human subjects. The manuscript described the research work was composed of 7 chapters.

In Chapter 1, general introduction and background on the current research was given. HPLC has been suggested as an alternative but the lack of selective detection has limited its capabilities for a long time. Today this has changed with the introduction of LC-MS. The tremendous evolution in interface and instrument design over the last decade has resulted in the creation of state-of-the-art instrumentation for target analysis in complex mixtures. In recent years, LC-MS/MS has been applied in numerous scientific fields, including Toxicology. Evaluating the application of LC-MS/MS for bioanalysis of selected drugs offered an interesting research challenge and was the basis for the present work. Simultaneously, we have discussed about biopharmaceutical analysis, different extraction procedures, method development and method validation parameters, bioavailability and bioequivalence studies.

In Chapter 2, Review of literature for the selected drugs namely Febuxostat, Acamprosate, Mesalamine and its metabolite N-Acetyl mesalamine and Milnacipran was elaborated.
In **Chapter 3**, discussed the Aim & objectives of the present research study for the selected drugs namely Febuxostat, Acamprosate, Mesalamine and its metabolite N-Acetyl mesalamine in human plasma and Milnacipran in rat plasma. LC-MS/MS was used for detection and simultaneously we discussed the validation parameters like selectivity, sensitivity, intra & inter assay precision and accuracy, recovery, stability of the drug in plasma and solution. This was followed by bioequivalence study for Febuxostat, Acamprosate, Mesalamine and its metabolite N-Acetyl mesalamine in human plasma. For Milnacipran, Pharmacokinetic study was proved in rat plasma. Pharmacokinetic concentration data was calculated by non-compartmental statistics model using WinNon-Lin 5.0 software.

In **Chapter 4**, developed and validated the simple, sensitive, reproducible, specific method for febuxostat within the concentration range of 1.0 – 8000.0 ng/mL using a simple LLE technique for extraction of drug and internal standard within 2.5 minutes of analysis time. Deuterated compound febuxostat–D7 was used as an internal standard. Simultaneously it was successfully employed in the bioequivalence study of febuxostat (80 mg) in Test and Reference products in 14 healthy human subjects.

In **Chapter 5**, developed and validated the simple, sensitive, reproducible, specific method for milnacipran within the concentration range of 1.0 – 400.0 ng/mL using a simple LLE technique for extraction of drug and internal standard within 3.0 minutes of analysis time. Deuterated
compound milnacipran-D10 was used as an internal standard. Simultaneously it was successfully employed in the pharmacokinetic study of milnacipran in 6 healthy Male Sprague-Dawley rats.

In **Chapter 6**, developed and validated highly sensitive, selective and reproducible, economical analytical method for the determination of Mesalamine, and its metabolite N-Acetyl Mesalamine in plasma samples utilizing LC-MS/MS. Deuterated compound N-Acetyl Mesalamine –D3 was used as internal standard. The method was developed and validated as per FDA guidelines over a concentration range of 2.00-1500.00 ng/mL for Mesalamine and 10.00-2000.00 ng/mL for N-Acetyl Mesalamine. Liquid-liquid extraction technique was used for extraction of drug and internal standard. This method was fully validated as per FDA guidelines and was successfully employed in 34 healthy human subjects following oral administration of mesalamine tablets (400 mg).

In **Chapter 7**, developed and validated the simple, sensitive, reproducible, bio analytical method for Acamprosate within the concentration range of 1.0 – 250.0 ng/mL using a simple SPE technique for extraction of drug and internal standard within 3 minutes of analysis time. Deuterated compound Acamprosate –D12 was used as an internal standard. Simultaneously it was successfully employed in the bioequivalence study of Acamprosate (333 mg) in Test and Reference products in 14 healthy human subjects.
The above validated methods were successfully employed in Bioanalysis, followed by pharmacokinetic study by non-compartmental statistics model using WinNon-Lin 5.0 software. The $C_{max}$, $T_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ were within the acceptance criteria for selected drugs. In addition, the mean ratio of $AUC_{0-t}/AUC_{0-\infty}$ was higher than 90%. The ratio of test/reference (T/R) and 90% confidence intervals (90 CIs) for overall analysis were within the range of 80–125%.

Therefore, it can be concluded that the two formulations (test and reference) for Febuxostat, Acamprosate and Mesalamine analyzed were bioequivalent in terms of rate and extent of absorption.

For Milnacipran pharmacokinetic study was proved in rats by i.v administration of the milnacipran formulation. The validated method was applied to find of pharmacokinetics of drug.

In conclusion, the present work complied with our initial research objectives and successfully demonstrated the applicability of LC-MS/MS for bioanalysis of different class of drugs namely Febuxostat, Acamprosate and Mesalamine and its metabolite N-Acetyl- Mesalamine in human plasma and Milnacipran in rat plasma.

This research has contributions in 3 important scientific fields. From an bioanalytical point of view, the extensive study of this novel instrumentation has resulted in innovative methodology for selected drugs in human plasma and rat plasma.
From a *clinical and bioequivalence point of view*, application of the new LC-MS/MS procedures widened our knowledge about concentration-time profiles in human plasma.

From a *pharmacokinetic point of view* application of the concentration-time profiles by non-compartmental statistics model using WinNon-Lin 5.0 software for selected drugs broadened our knowledge in *in vivo* studies calculations.

The developed and validated methods for selected drugs have greater advantage than the reported methods. Hence, it is evident and will unquestionably expand future research capabilities.