8.1 SUMMARY

The present work aimed to assess the applicability of High Performance Liquid chromatography with mass spectrometry (HPLC-MS) for analysis of different class of drugs in rabbit and mice plasma. The Dissertation described the research work is composed of 8 chapters.

In Chapter 1, a general introduction and background on the current research is given. HPLC has been suggested as an alternative but the lack of selective detection has limited its capabilities for a long time.

This has been changed with the introduction of Liquid Chromatography with tandem mass spectrometry (LC-MS/MS). In recent years, LC-MS/MS has been applied in numerous scientific fields, including Toxicology. Evaluating the application of LC-MS/MS for analysis of selected drugs offered an interesting research challenges and was the basis for the present work. Simultaneously we have discussed about pharmaceutical analysis, different extraction procedures, method development, method validation parameters and pharmacokinetic studies.

In Chapter 2 we have discussed about the review of literature for the selected drugs namely Tolvaptan, Eszopiclone, Frovatriptan and Aliskiren.
In **Chapter 3** we have discussed about the aim and objective of the present research work, for the selected drugs.

In **Chapter 4** we have developed and validated the simple, highly sensitive, selective, rugged and reproducible bioanalytical method for Tolvaptan within the concentration range of 0.1 – 1000.0 ng/mL using a simple LLE extraction technique for drug and internal standard within 1.5 minutes of analysis time in biological fluids. Tolvaptan-d7 was used as an internal standard. The validated method was successfully employed in the quantification of Tolvaptan in rabbit plasma samples by oral administration of Tolvaptan (2.77 mg dose /1.8kg body weight of rabbit).

In **Chapter 5** we have developed simple, sensitive, rapid, good, linear, reproducible bio-analytical method for Eszopiclone and validated over a concentration range of 0.05 – 210.00 ng/mL using a Liquid-Liquid Extraction technique. Eszopiclone-d8 was used as an internal standard. The validated method was successfully employed in the quantification of Eszopiclone rabbit plasma samples by oral administration of Eszopiclone (277.5 µg dose/1.8kg body weight of rabbit).
In **Chapter 6** we have developed simple, sensitive, rapid, good, linear, reproducible bio-analytical method for Frovatriptan and validated over a concentration range of 5.0 – 12000.00 pg/mL using a Liquid-Liquid Extraction technique. Frovatriptan-d3 was used as an internal standard. The validated method was successfully employed in the quantification of Frovatriptan in rabbit plasma samples by oral administration of Frovatriptan (0.23 mg dose/1.8 kg body weight of rabbit).

In **Chapter 7** we have developed and validated simple, sensitive method for Aliskiren over a concentration range of 0.1 - 1600.0 ng/mL by a simple LLE extraction technique for drug and internal standard. Aliskiren-d8 was used as an internal standard. Simultaneously it was successfully employed in the analysis of mice plasma samples by i.v administration of Aliskiren (20 µg dose /200g body weight of mice).

Simultaneously for all above selected drugs, we proved the validation parameters like Selectivity, Sensitivity, Intra & Inter Assay Precision and Accuracy, Recovery, Stock solution stability and Plasma stabilities like Short time stability, Long time stability, Auto sampler stability, Bench Top Stability and Freeze-thaw stability.
The above validated methods were successfully employed in analysis, followed by pharmacokinetic study by non-compartmental statistics model using Win-Non-Lin 5.0 software. The $C_{\text{max}}$, $T_{\text{max}}$, $\text{AUC}_{0-t}$ and $\text{AUC}_{0-\infty}$ were within the acceptance criteria for selected drugs.

The overall pharmacokinetic parameters calculated for selected drugs are not deviating when compared with literature survey. Therefore, it can be concluded that, the present study provides solid evidence to support in clinical pharmacokinetic studies for further Research of selected drugs.
8.2 CONCLUSION

The present work compiled with our initial research objectives and successfully demonstrated the applicability of LC-MS/MS for biopharmaceutical analysis of different class of drugs namely Tolvaptan, Eszopiclone, Frovatriptan in rabbit plasma and Aliskiren in mice plasma.

The developed and validated methods shown high degree of sensitivity, selectivity, reproducibility and high recovery, stability with less matrix effects when compared with previously reported methods.

This research has contributions in 2 important scientific fields. From an analytical point of view, the extensive study of this novel instrumentation has resulted in innovative methodology for selected drugs in rabbit plasma and mice plasma.

From a pharmacokinetic point of view, application of the new HPLC-MS/MS procedures and usage of Non-compartmental statistics model using WinNon-Lin 5.0 software broadened our knowledge, concentration-time profiles and in-vivo studies calculations in rabbit plasma and mice plasma.

The tremendous potential use of LC-MS/MS from clinical samples is evident and will unquestionably expand future research capabilities in terms of shorter runtimes, high rugged and reproducible methods with less precision and high accuracy.