8. SUMMARY AND CONCLUSION

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 rat, rabbit and human 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.

Today this has been changed with the introduction of High Performance Liquid Chromatography with mass spectrometry (HPLC-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, HPLC-MS/MS has been applied in numerous scientific fields, including Toxicology. Evaluating the application of HPLC-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 Guanfacin, Metaxalone, Atovaquone and Leflunomide metabolite-Teriflunomide.

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 Guanfacin within the concentration range of 50.0 – 10000.0 pg/mL using a simple LLE extraction technique for drug and internal standard within 3.0 minutes of analysis time in biological fluids. Guanfacin \(^{15}\text{N}_3^{13}\text{C}_1\) was used as an internal standard. The validated method was successfully employed in the quantification of Guanfacin in rat plasma samples by i.v administration of Guanfacin (72 µg/200g rat).

In **Chapter 5** we have developed simple, sensitive, rapid, good, linear, reproducible bio-analytical method for Metaxalone and validated over a concentration range of 10.0 – 6000.00 ng/mL using a Liquid-Liquid Extraction technique. Metaxalone N-methyl analog was used as an internal standard. The validated method was successfully employed in the quantification of Metaxalone in human plasma samples by oral administration of Metaxalone (2x400 mg/60kg).
In Chapter 6 we have developed simple, sensitive, rapid, good, linear, reproducible bio-analytical method for Atovaquone and validated over a concentration range of 50.0 – 20000.00 ng/mL using a Liquid-Liquid Extraction technique. Buparvaquone was used as an internal standard. The validated method was successfully employed in the quantification of Atovaquone in rat plasma samples by i.v. administration of Atovaquone (6 mg/200g rat).

In Chapter 7 we have developed and validated simple, sensitive method for Leflunomide metabolite-Teriflunomide over a concentration range of 10.0 - 5000.0 ng/mL by a simple PPT extraction technique for drug and internal standard. Leflunomide metabolite-ethylanalog was used as an internal standard. Simultaneously it was successfully employed in the analysis of rabbit plasma samples by oral administration of Leflunomide (1.85 mg/1.8kg rabbit) in rabbits.

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 $C_{\text{max}}$, $T_{\text{max}}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ were within the acceptance criteria for selected drugs.

The overall pharmacokinetic parameters were within the range of 80-125% therefore it can be concluded that, the present study provides firm evidence to support in clinical pharmacokinetic studies for further Research of selected drugs.
CONCLUSION

The present work compiled with our initial research objectives and successfully demonstrated the applicability of HPLC-MS/MS for biopharmaceutical analysis of different class of drugs namely Guanfacin, Atovaquone in rat plasma and Metaxalone in human plasma, Leflunomide metabolite-Teriflunomide in rabbit 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 rat 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 rat plasma.

The tremendous potential use of HPLC-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.