CHAPTER 5

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1 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 biological matrices. The Dissertation described the research work is composed of 6 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, the extensive literature survey is discussed in this chapter.

In Chapter 3 I have discussed about the aim and objective of the present research work, for the selected drugs namely Bosentan, Flecainide in Rat Plasma, Betahistine in human plasma and Budesonide in rabbit plasma by using HPLC-MS detection.

In Chapter 4 I have developed and validated the simple, highly sensitive, selective, rugged and reproducible bioanalytical method for Bosentan within the concentration range of 5.0 – 5000.00 ng/mL using a simple Liquid-liquid extraction technique for drug and internal standard within 3.0minutes of analysis time in biological fluids. Bosentan - d4 was used as an internal standard. The validated method was successfully employed in the quantification of Pregabaline in rat plasma samples by i.v administration of Bosentan (23 mg / 200g rat).
I have developed simple, sensitive, rapid, good, linear, reproducible bioanalytical method for Flecainide and validated over a concentration range of 1.01–506.04 ng/mL using a Liquid-Liquid Extraction technique. Deuterated compound Flecainide impurity A was used as an internal standard. The validated method was successfully employed in the quantification of Flecainide in rat plasma samples by i.v administration of Flecainide (23 mg / 200g rat).

I have developed simple, sensitive, rapid, good, linear, reproducible bioanalytical method for Betahistine and validated over a concentration range of 10.20–501.60 ng/mL using a Liquid-Liquid Extraction technique. Deuterated compound Betahistine d4 was used as an internal standard. The validated method was successfully employed in the quantification of Betahistine in human plasma samples.

I have developed and validated simple, sensitive method for Budesonide over a concentration range of 10.00–2000.0 pg/mL by a simple SPE extraction technique for drug and internal standard. Deuterated compound Budesonide –d7 was used as an internal standard. Simultaneously it was successfully employed in the analysis of rabbit plasma samples.

Simultaneously for all above selected drugs, validation parameters like Selectivity, Sensitivity, Intra & Inter Assay Precision and Accuracy, Recovery, Stock solution stability and Plasma stabilities like reinjection stability, Short time stability, Long time stability, Auto sampler stability, Bench Top Stability and Freeze-thaw stabilities were proved as per standard guidelines.

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_{max}, T_{max}, AUC_{0-t} and AUC_{0-∞} were reported for selected drugs.

Therefore it can be concluded that, the present study provides firm evidence to support in clinical pharmacokinetic studies for further research of selected drugs.

5.2 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 Bosentan, Flecainide in Rat Plasma,
Betahistine in human plasma and Budesonide in rabbit plasma by using LC-MS detection.

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. Moreover it was proved by publishing the methods in reputed journals.

This research has contributions in two 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 rat plasma.

From a pharmacokinetic point of view, application of the new LC-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.

5.3 Recommendations:

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 Bosentan, Flecainide in Rat Plasma, Betahistine in human plasma and Budesonide in rabbit plasma by using HPLC-MS detection.

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. Moreover it was proved by publishing the methods in reputed journals and National conferences.

This research has contributions in two 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 rat, rabbit, Human 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 biological samples is evident and will unquestionably expand future research capabilities in terms of shorter
runtimes, rugged and reproducible methods with less precision and high accuracy. The Research work Recommend that, The Developed methods can be use in commercial purpose in

• Clinical Research, Drug metabolism and Pharmacokinetics, BioAnalytical Research, Novel Drug Delivery System Research