7. SUMMARY AND CONCLUSIONS

This thesis deals with the studies carried out by the writer in the laboratory for the past four years on the “Development and validation of analytical methods for the simultaneous estimation of the selected herbal constituents in plant extracts and their commercial formulations by HPLC and LC-MS techniques”.

The thesis begins with a brief introduction of the reasons for analyzing herbal constituents and their products and introduction to the analytical methods used like HPLC and LC-MS. The methods used for the quantitative analysis of herbal constituents and the steps involved are presented. The steps involved in the validation of the analytical methods as per ICH guidelines are also presented. An introduction to the herbal constituents separation and the different methods of analysis used to simultaneous estimation are given.

The second chapter of the thesis deals with the aim and objective of the present investigation. The reasons for analyzing the herbal constituents and need for newer analytical methods for the simultaneous estimation of plant constituents and their commercial formulations are briefly discussed.

The third chapter of the thesis deals with the review of literature on the analytical methods available for the estimation of the selected flavonoid constituents in the selected herbal plants.

The fourth chapter of the thesis deals with the scope and plan of work of the present study. Herbal constituents such as apigenin, luteolin, quercetin and rutin and the commercial formulations of the selected plant and the detailed plan of work are discussed.

The fifth chapter of the thesis deals with the materials and instruments used in the experimental procedures adopted. It describes in detail about the procedure adopted for the optimization of the chromatographic HPLC and LC-MS methods for the simultaneous estimation of the selected plant constituents present in the plant extracts and their products and validation of the developed methods.

The results obtained in the present investigations are reported in chapter six of the thesis. Standard and sample HPLC and LC-MS chromatograms, calibration
curves for the selected analytes are also presented. The results of the experiments carried out to check the accuracy, reproducibility of the methods carried out are presented and discussed in detail. System suitability studies and forced degradation studies carried out for various methods developed are also presented and discussed.

The following are some of the salient features and conclusions made for the present study.

- Two herbal plants for which there were no HPLC and LC-MS methods reported for the simultaneous estimation of flavonoid contents and their formulations were selected for the present study after through literature survey.
- Four flavonoids such as apigenin, luteolin, quercetin and rutin were selected for the simultaneous estimation by HPLC and LC-MS methods.
- The chromatographic conditions like detection wavelength/mass range, nature and composition of mobile phase, nature of stationary phase, selection of internal standard, peak modifiers etc. were optimized for the best possible separation and quantification of the herbal constituents.
- The herbal extracts were estimated for the content of the constituents by the developed and optimized chromatographic methods and the statistical treatment of the data were carried out.
- The developed HPLC and LC-MS methods were validated for their transferability to other laboratories, in terms of specificity, selectivity, accuracy, precision, linearity and range, detection and quantification limits, ruggedness and robustness and system suitability. The validation carried out revealed that the developed methods satisfy the ideal characteristics of the analytical methods.
- The methods developed have linearity over a wide range of concentration and avoids cumbersome some sample preparation procedures.
- The selected plant extracts were analyzed for the presence of selected flavonoids by the developed and optimized chromatographic methods.
- The RP-HPLC and LC-MS chromatographic methods developed in the present study for the simultaneous estimation of herbal constituents and
their formulations were found to be simple, rapid, accurate, precise, specific, linear, sensitive and robust. They are thus suitable for the simultaneous estimation of herbal constituents and herbal extracts from plant materials. The newly developed HPLC and LC-MS methods can be useful in the following fields,

- Research institutions,
- Academic institutes,
- Quality control department in herbal industries,
- Approved testing laboratories,
- Bio pharmaceutics & bioequivalence studies and
- Clinical & pharmacokinetic studies after suitable modification.

The developed methods may also be used as control procedures during processing of herbal products in order to assure the quality, biological values and safety of the herbal products which a manufacturer should notify in the compendia. Developed analytical methodologies may help to authenticate herbal products. Data on herbal components can be used in nutritional analysis, disease prevention, consumer protection and regulatory requirements.

In conclusion, the developed methods for the simultaneous estimation and stress degradation studies of herbal plant extracts and herbal formulations are accurate, precise and linear and therefore, can be employed for stability studies and for quality control analysis during manufacturing, packaging and storage conditions.