CHAPTER - 8
SUMMARY AND CONCLUSION

SUMMARY

It was concluded that there were few method reported for the simultaneous estimation of the selected multi component dosage forms, which promote to pursue the present work. The present work aimed to assess the applicability of High performance liquid chromatography coupled with UV detector (RP-HPLC with UV) for analysis of different classes of drugs in pharmaceutical formulations. The dissertation described the research work was composed of **7 chapters.**

In **Chapter 1** a general introduction and research on analysis of drugs using chromatographic techniques.

In **Chapter 2** discussed about the objectives and plan of the research work for the selected drugs, namely simultaneous estimation of sofosbuvir and daclatasvir, ombitasvir, paritaprevir, ritonavir and abacavir, dolutegravir, lamivudine and bictegravir emtricitabine, tenofovir alafenamide in APIs by using RP-HPLC-UV detection to explore the applicability of HPLC.

In **Chapter 3** discussed are about the review of literature related to research work.

In **Chapter 4** discussed about the validated method for simultaneous estimation of A simple, rapid reversed-phase high performance liquid chromatographic method had been developed and validated for estimation of daclatasvir and sofosbuvir in tablet dosage form. The estimation was carried out on Inertsil ODS-C_{18} column (250 x 4.6 mm, 5µ) column with a mixture of acetonitrile: methanol: 0.1% triethylamine buffer (pH-3.0) 25:35:40 (v/v/v) as mobile phase. UV detection was performed at 250 nm. The method was validated for linearity, accuracy, precision, specificity and sensitivity as per ICH norms. The developed and validated method was successfully used for the quantitative analysis

of commercially available dosage form. The retention time was 2.09 and 3.50 min for daclatasvir and sofosbuvir respectively and total run time was 6.0min at a flow rate of 1.0 mL/ min. The calibration curve was linear over the concentration range of 5.0-25.0 µg/ mL for Daclatasvir and 2.0-10.0 µg/ mL for Sofosbuvir. The LOD and LOQ values were found to be 0.313 and 0.948 µg/ mL for daclatasvir and 0.021 and 0.065 µg/mL for sofosbuvir, respectively. The high percentage of recovery and low percentage coefficient of variance confirm the suitability of the method for the simultaneous estimation of daclatasvir and Sofosbuvir in tablet dosage form.

In Chapter 5 discussed about the validated method for simultaneous estimation of The purpose of this work is to develop an accurate and precise HPLC method for the determination of ombitasvir, paritaprevir, ritonavir in tablet. Separation of drug was achieved on an Inertsil ODS-C18; 5µm (4.6 X 250mm) column using a mobile phase consisting of 0.02M phosphate buffer (pH-4.5): acetonitrile: methanol, (50:30:20) (v/v). The retention time was 2.81 and 7.42 min for ombitasvir, ritonavir, paritaprevir, RP-HPLC, ICH guide lines respectively and total run time was 10 min. at a flow rate of 1.0 mL/ min and the detection wavelength was 262 nm. The linearity was observed in the range of 15-45 ppm for ombitasvir, paritaprevir, ritonavir with a correlation coefficient of 0.9903, 0.9996 and 0.9998 respectively. The proposed method was validated for its linearity, accuracy, precision and robustness. This method can be employed for routine quality control analysis of ombitasvir, paritaprevir and ritonavir in tablet. The LOD and LOQ values were found to be 1.8, 0.29 and 0.69 µg/ mL and 5.7, 0.90 and 2.10 µg/ mL for ombitasvir, paritaprevir and ritonavir, respectively. The high percentage of recovery and low percentage coefficient of variance confirm the suitability of the method for the simultaneous estimation of ombitasvir, paritaprevir and ritonavir by RP-HPLC as per ICH guidelines in tablet dosage form.

In Chapter 6 discussed about the validated method for simultaneous estimation of Selective and novel method has been optimized for evaluation of abacavir, dolutegravir and lamivudine in bulk and formulation by HPLC. The principle analytes were eluted with the conditions of mobile phase having the ethanol: ethyl acetate (80:20, % v/v) using the Lichrosphere RP C8 column (Phenomenex, USA (250 x 4.6 mm, 5µ) analytical column with the 1.0 ml/min flow rate and 10 µl sample volume at 260 nm in UV detector. The retention times of abacavir, dolutegravir and lamivudine were 2.31 min, 3.120 min and 4.59 min with the total run time of 6 min. The curve indicates correlation coefficient (r^2) was superior by having the value nearer to 1.000 with linear range of 40 µ.g/m.l-130.0 µ.g/m.l for abacavir, dolutegravir and lamivudine. The correlation coefficient (r^2) 0.9971 for abacavir, 0.9979 for dolutegravir and 0.9947 for lamivudine were found. The LOD and LOQ for the abacavir, dolutegravir and lamivudine were found 1.40 µ.g/m.l, 3.01 µ.g/m.l, 5.84 µ.g/m.l and 4.25 µ.g/m.l, 9.12 µ.g/m.l and 17.71 µ.g/m.l. The developed method was applied for the bulk and formulation.

In Chapter 7 discussed about the validated method for simultaneous estimation of Selective and novel method has been optimized for evaluation of Bictegravir, emtricitabine and tenofovir alafenamide in bulk and formulation by HPLC. The principle analytes were eluted with the conditions of mobile phase having the acetonitrile and 0.1% Ortho phosphoric acid in the ratio of 50:50 (v/v/) using the Phenomenex, ODS 150 x 4.6 mm 5µm analytical column with the 1.0 ml/min flow rate and 20 µl sample volume at 270 nm in UV detector. The retention times of bictegravir, emtricitabine and tenofovir alafenamide were 2.56 min, 3.57 min and 3.503 min with the total run time of 5 min. The curve indicates correlation coefficient (r^2) was superior by having the value nearer to 1.000 with linear range of 40 µ.g/m.l-130.0 µ.g/m.l for bictegravir, emtricitabine and tenofovir alafenamide. The correlation coefficient (r^2) 0.9992 for bictegravir, 0.9998 for emtricitabine and 0.9983

for tenofovir alafenamide were found. The LOD and LOQ for the bictegravir, emtricitabine and tenofovir alafenamide were found 0.89 µ.g/m.l, 1.32 µ.g/m.l, 1.03 µ.g/m.l and 2.72 µ.g/m.l, 4.00 µ.g/m.l and 3.13 µ.g/m.l. The developed method was applied for the bulk and formulation.

Hence, the developed chromatographic methods free from matrix interference and useful in the analysis of drugs in pharmaceutical formulation.

Precision of the developed methods was studied under intra and inter day precision. The % RSD values for precision was found to be within the acceptable limit, which revealed that the developed method was precise. The developed method was found to be robust. The % RSD values for percentage recovery was found to be within the acceptable criteria. The result indicates satisfactory accuracy of method for simultaneous estimation of the selected drugs.

CONCLUSION

The present work compiled with initial research objectives and successfully demonstrated the applicability of RP-HPLC for pharmaceutical analysis of different classes of drugs namely sofosbuvir and daclatasvir, ombitasvir, paritaprevir, ritonavir and abacavir, dolutegravir, lamivudine and bictegravir emtricitabine, tenofovir alafenamide by RP-HPLC with UV detection. The developed and validated methods shown high degree of sensitivity, selectivity, reproducibility and good recovery, stability with negligible matrix effects when compared with previously reported method.

This research work has contributions in **four** important scientific fields.

From an **analytical research and development (AR&D) point of view**, useful for analytical research scientists, particularly developing new analytical methods for these selected drugs in different biological samples are useful.

From a **formulation research and development (FR&D) point of view**, useful for formulation research scientists, particularly working on these selected drugs in developing new formulations and pharmacokinetic parameter calculations in different biological samples.

From a **drug regulatory point of view**, generated data meeting regulatory standards (bioanalytical and pharmacokinetic data) and it is acceptable for regulatory submission.

From **GLP (Good laboratory practices) point of view**, all bioanalytical lab instruments and methods were calibrated and validated before performing bioanalysis for acquiring of precise and accurate results.

The tremendous potential of RP-HPLC for pharmaceutical analysis is evident and will unquestionably expand future research capabilities in terms of shorter runtime, high rugged and reproducible analytical methods with high precision and accuracy.