CHAPTER-7

SUMMARY, CONCLUSION AND FUTURE SCOPE

7.1 Summary:

The present experimental investigation reported in this thesis is to improve a new analytical method development and validation as per the ICH guidelines which are recommended for the analytical method validation. Four new analytical method developments were carried out for the simultaneous estimation of active pharmaceutical ingredients and its combined marketed dosage form. Sofosbuvir and Daclatasvir was the selected combination, marketed in the brand name **Sovaldi** (400mg of sofosbuvir and 60mg of daclatasvir) tablet; Lamivudine and dolutegravir was the selected combination, marketed in the brand name **Triumeq** (300mg of Lamivudine, 50mg of dolutegravir and 600mg of abacavir); Emtricitabine and Tenofovir alafenamide was the selected combination, marketed in the brand name **Truvada** (200mg emtricitabine and 300mg tenofovir alafenamide) and Sacubitril and Valsartan was the selected combination, marketed in the brand name **Entresto** (97mg of sacubitril and 103mg of valsartan). The selected drug and API were used for the simultaneous analysis and validation by reverse phase liquid chromatography.

A new improved analytical method development and validation was performed for the simultaneous estimation of Sofosbuvir and Daclatasvir, was selected at the isobestic point at which the drugs can be detected using UV detectors. The selected wavelength was 275nm and separation was achieved on C18 column (X-Terra RP18 150*4.6, 5µm) at ambient temperature by a mobile phase consists of 0.1% v/v Trifluoro acetic acid in water: Acetonitrile (60:40 %v/v). The flow rate was 1.0 ml/ min. The average retention time for Sofosbuvir and Daclatasvir found to be 2.09 and 3.50 min.

The simultaneous estimation of lamivudine and dolutegravir, was selected at the isobestic point at which the drugs can be detected using UV detector, the selected wavelength was 260nm and the chromatographic separation was achieved on C18 column (Inertsil ODS 3V 250*4.6mm) at ambient temperature by employing a mobile

phase consists of 0.1%v/v TFA in water: ACN (30:70%v/v). The flow rate was 0.8ml/ min. and ultra violet detector at 260nm. The average retention time for Lamivudine and Dolutegravir found to be 2.373min and 4.558min.

The simultaneous estimation of Emtricitabine and Tenofovir Alafenamide, was selected at the isobestic point at 260nm, the simultaneous estimation of sacubitril and valsartan, was selected wavelength was 267nm and The chromatographic separation was achieved on C18 column (Intersil ODS 3V, 150*4.6mm, 5µm) at ambient temperature .The separation achieved employing a mobile phase consists of Formic acid in water: Methanol (45:55%v/v). The flow rate was 1.0ml/ min. The average retention time for Emtricitabine and Tenofovir Alafenamide found to be 1.919min and 4.745min.

The simultaneous estimation of Valsartan and Sacubitril in drug product was done by liquid chromatography and the chromatographic separation was achieved on C18 column (X-Terra RP-18 150*4.6mm) column at ambient temperature. The separation achieved employing a mobile phase consists of 0.1%v/v Formic acid in water: Methanol (25:75%v/v). The flow rate was 1.0ml/ min. and ultra violet detector at 267nm. The average retention time for Valsartan and Sacubitril found to be 2.66 min and 3.154 min.

7.2 Conclusion:

The conclusion drawn from the results of various investigations performed are as follows.

Four combined dosage forms were selected for the present investigation namely Sofosbuvir and Daclatasvir, Lamivudine and Dolutegravir, Emtricitabine and Tenofovir Alafenamide and Sacubitril and Valsartan. From the literature survey it a was found that there are very few analytical methods available for the selected combination of drugs and these methods required tedious procedure for the chromatographic separation of these compounds and hence new analytical methods were developed for the simultaneous estimation of above said drugs by isocratic RP-HPLC method.

Table 7.1: Summary of all the methods used for the drugs

S.No.	Drugs used in the simultaneous estimation	Mobile Phase Used (%v/v)	λ (nm)	Flow rate (ml/min)
1	Sofosbuvir and Daclatasvir	TFA: ACN (60:40%v/v)	275	1.0
2	Lamivudine and Dolutegravir	TFA: ACN (30:70%v/v)	260	0.8
3	Emtricitabine and Tenofovir Alafenamide	Formic acid: Methanol (45:55%v/v)	260	1.0
4	Sacubitril and Valsartan	Formic acid: Methanol (25:75%v/v)	267	1.0

Table 7.2: SUMMARY OF ALL DRUGS

S.No.	Parameter	Drug- 1	Drug- 2	Drug- 3	Drug- 4	Drug- 5	Drug- 6	Drug- 7	Drug- 8	ACCEPTENCE CRITERIA
1	**System Suitability**									
	a)Theoretical Plates	6613	3226	4728	9459	3033	4286	4088	3583	Not less than 3000
	b)Asymmetry									
	c) RT (Min)	1.11	1.15	1.17	1.18	1.17	0.98	1.07	1.07	Not more than 1.5
	d) %RSD	3.502	2.089	2.372	4.560	1.92	4.75	3.186	2.663	-
		0.08	0.27	1.35	1.51	0.29	0.41	0.28	0.22	Not more than 2.0
2	**Specificity**	Specific	Specific	Specific	Specific	Specific	Specific	Specific	Specific	Specific
3	**Method precision (%RSD)**	0.62	0.17	0.54	0.35	0.26	0.31	0.40	0.46	Not more than 2.0%
4	**Linearity (µg/ml)**	50-150	50-150	50-150	50-150	50-150	50-150	50-150	50-150	-
5	**Correlation co-efficient (r^2)**	0.9998	0.9999	0.9991	0.9992	0.9993	0.9991	0.9997	0.9997	Not less than 0.999
6	**Accuracy**									
	a) 50%	99.4	99.8	99.4	99.2	99.7	99.4	99.8	99.4	97 - 103%
	b) 100%	100.0	100.1	99.7	99.7	99.05	99.52	99.3	99.4	
	c) 150%	99.6	99.0	99.8	100.0	99.1	99.6	99.2	99.1	
7	**Robustness**	All the system suitability parameters are with in the limits.								

Drug-1: Sofosbuvir Drug-3: Lamivudine Drug-5: Emtricitabine Drug-7: Sacubitril

Drug-2: Daclatasvir Drug-4: Dolutegravir Drug-6: Tenofovir- Alafenamide Drug-8:Valsartan

Validation parameters were performed as per ICH guidelines and for the linearity, precision, accuracy, LOD, LOQ, robustness and system suitability parameters for the developed methods. All the validation parameters were within the specified criteria.

7.3 Future scope of the present work:

The proposed RP-HPLC methods were found to be simple, specific, precise, accurate, rapid and economical for the simultaneous estimation of Sofosbuvir and daclatasvir, Lamivudine and Dolutegravir, Emtricitabine and Tenofovir Alafenamide and Sacubitril and Valsartan in combined dosage forms and active pharmaceutical ingredients. The methods were validated as per ICH guidelines. The sample recovery in the formulation was in good agreement with their respective label claims and they suggested non- interference of formulation excipients in the estimation.

Hence, these methods can be easily and conveniently adopted for routine analysis of Sofosbuvir and Daclatasvir, Lamivudine and Dolutegravir, Emtricitabine and Tenofovir Alafenamide and Sacubitril and Valsartan in combined dosage forms.