CHAPTER-7

SUMMARY, CONCLUSION AND RECOMMENDATIONS.

7.1 Summary

The present investigation was carried out to develop analytical method for simultaneous estimation of selected drug combinations by RP-HPLC technique. The brand names of the drug combinations selected were RMT (FLUMADINE), CDF (VISTIDE), PLK (ONON), SOF and VEL (SOFOSVEL), MON,ACE and DES (ACMON-DM) and PAN,CHL and DIC (ALLDEX-DT PLUS). RP-HPLC is the one of the rapidly emerging analytical tool for quantitative analysis. New RP-HPLC methods were developed for the simultaneous estimation of above drug combinations and validated according to the ICH guidelines.

7.1.1 Quantitative estimation of RMT tablets by RP- HPLC.

A new RP- HPLC method was developed for analysis of RMT tablets by conducting pre-column derivatization of RMT hydrochloride with AQSC. The isocratic mobile phase for the C-18 column consisted of Acetonitrile and 0.005 M 1-Octane sulfonic acid sodium salt monohydrate buffer (pH 6.7) in 60:40 v/v ratio .The UV detection wavelength for RMT derivative was 259 nm. The RT was 6.79 minutes. Linearity was satisfied over a range of 50 µg/mL to 250µg/mL with R^2 value 0.999. Percentage RSD for precision, accuracy and robustness were less than 2. The LOD and LOQ were 1.32µg/mL and 4.0µg/mL respectively. Validation was done as per ICH guidelines and all the results were with the limits. The RT values were minimized when compared to previously reported values.

7.1.2 Quantitative estimation of CDF injection by RP-HPLC.

A novel RP-HPLC method has been developed for analysis of CDF in its available medicinal form. Stationary phase was a sophisticated C-18 RP Column (250mm × 4.6mm). Mobile phase consist of Acetonitrile and 0.005M Citric acid buffer (pH 5.5) in 60:40v/v ratio. Mobile phase flow rate was 1.0 mL per minute and Isocratic. UV detection wavelength was 260nm. Validation was carried according to ICH guidelines. The RT of both API and the medicine were 3.88 minutes. Linearity was satisfied over the concentration range of 2µg/mL to 10µg/ml and R^2 value was 0.999. LOD and LOQ values were 0.067µg/mL and 0.205µg/ml respectively. Reported method was novel and all validation parameters met the criteria.

7.1.3 Quantitative estimation of PLK capsules by RP-HPLC.

A RP-HPLC method was developed and validated as per ICH guidelines for the analysis of PLK in its capsule form. The analysis was performed on a C-18 column. Separation was completed isocratically by using a mobile phase of Phosphate buffer and Acetonitrile in 60:40 v/v ratio at 30ºC and maintained at a flow rate of 1milliliter per minute. Ultraviolet absorption was maximum at 238 nm wavelength. Linearity was satisfied in the range 28.125 µg/mL to 168.75 µg/mL with R^2 value of 0.999.

7.1.4 Simultaneous estimation of SOF and VEL Tablets by RP-HPLC.

A new simple and precise simultaneous RP-HPLC method was developed and validated for the identification and analysis of fixed dose combination of SOF and VEL in their combined tablet dosage form. The mobile phase was a combination of Methanol and Phosphate buffer. The RT of SOF was 3.049 minutes and VEL was 4.316 minutes respectively. The LOD were 0.475µg/mL and 0.65µg/mL and the LOQ were 1.44µg/mL and 1.98µg/mL for SOF and VEL respectively. The drug content assay in the tablet was closer to 100%. All the validated parameters met the acceptance criteria. RT, LOD and LOQ values were much better when compared to previously reported values.

7.1.5 Simultaneous estimation MON, ACE, and DES Tablets by RP-HPLC

A simple, fast, accurate and specific RP-HPLC method has been developed for the simultaneous quantification of MON, ACE and DES in bulk and tablet dosage form. The chromatographic separation was performed on a reverse phase BDS C8 Column (150×4.6mm, 5µm particle size) consisting a mobile phase of Potassium hydrogen orthophosphate buffer and Acetonitrile (40:60 v/v), with a flow rate of 1mL/min, temperature 30˚C and UV detection wavelength 280nm. The RT of MON, ACE and DES were observed as 2.04min, 2.68min and 3.77 min respectively. The linearity of the drugs were obtained in the range of 5-30 µg/mL for MON, 100-600µg/mL for ACE and 2.5-15 µg/mL for DES. The %RSD from precision studies were 0.4, 0.1 and 1.0, mean percentage recovery from accuracy studies were found to be 98.47%, 98.71% and 100.11% for MON, ACE and DES respectively.

7.1.6 Simultaneous estimation of PAN, CHL and DIC capsules by RP-HPLC

A simple, fast, accurate and specific RP-HPLC method has been developed for the simultaneous quantification of PAN, CHL and DIC in bulk and capsule dosage form.

The chromatographic separation was performed on a reverse phase Zodiac C18 column (150mm × 4.6mm, 5µm) consisting of mobile phase of 0.1% Orthophosphoric acid buffer and Acetonitrile (40:60, v/v), with a flow rate 1mL/min, temperature 30˚C and UV detection wavelength 210 nm. The RT of PAN, CHL and DIC were observed as 2.17min, 2.83min and 4.40 min respectively. The linearity of the drugs were obtained in the range of 5-30 µg/mL for PAN, 125-750 µg/mL for CHL and 12.5-75 µg/mL for DIC. The percent RSD from precision studies were 0.4, 0.2 and 0.7, mean percentage recovery from accuracy studies were found to be 98.17%, 98.64% and 99.82% for PAN, CHL, and DIC respectively.

The procedure for RP-HPLC method development and its validation for all the selected drugs and their pharmaceutical dosage forms were carried as per the ICH guidelines. All the drugs which were carried out in the present investigation got good agreement with respect to all validation parameters when compared to previously reported methods. The authors selected the different kinds of drug formulations namely tablet dosage, injection and capsules type dosage forms for the experimental investigation. The reagents and the chemicals which were used in the present investigation were easily available.

Table 7.1 Table of optimized Chromatographic Conditions of all the Selected Single and Combination Drugs.

Study Drug	Diluent	λ (nm)	column	Mobile phase (v/v)	Buffer pH	Flow rate (mL/ min)	Injection volume (µL)	Column Temp (ºC)	Elution	Run Time(min)	RT (min)
RMT	Acetonitrile	260	C 18	Buffer OSA : Acetonitrile (40:60)	6.7	1.0	20	30	Isocratic	10	6.79
CDF	Acetonitrile	260	C 18	Citric Acid Buffer: Acetonitrile (40:60)	5.5	1.0	20	30	Isocratic	10	3.88
PLK	Methanol : water (50:50)	238	C 18	Phosphate Buffer : Acetonitrile 60 : 40	3.0	1.0	10	30	Isocratic	5	2.29
SOF/VEL	Methanol : Buffer (60:40)	254	C 18	Methanol : phosphate buffer (60:40)	3.2	1.0	20	25	Isocratic	8	3.049 4.317
MON/ ACE/ DES	Water:Acetonitrile (50:50)	280	BDS C 8	Phosphate buffer: Acetonitrile 40 : 60	3.0	1.0	10	30	Isocratic	7	2.04 2.68 3.77
PAN/ CHL/ DIC	Water : Acetonitrile 50 : 50	210	C 18	Phosphate buffer : Acetonitrile (40 : 60)	4.8	1.0	10	30	Isocratic	8	2.179 2.832 4.409

TABLE 7.2 TABLE OF METHOD VALIDATION PARAMETERS OF ALL THE SELECTED DRUGS

S.No	Parameter	RMT	CDF	PLK	SOF	VEL	MON	ACE	DES	PAN	CHL	DIC
1	N	7720	6694.2	3792	11036.8	8372	2031	2393	2504	5045	4254	13226
2	T	1.048	0.944	1.36	1.18	1.28	1.2	1.1	1.0	1.25	1.69	1.16
3	RT (min)	6.79	3.88	2.29	3.049	4.317	2.04	2.68	3.77	2.179	2.83	4.40
4	R	12.956	10.421	9.56	-	4.34	-	3.1	4.2	-	4.3	9.4
5	% RSD (Peak area)	0.9745	0.9698	1.4	0.27	0.289	0.4	0.1	1.0	0.4	0.2	0.7
6	Linearity range (µg/mL)	50 – 250	2 – 10	25 – 150	2.0-8.0	0.5 – 2.0	5-30	100-600	2.5 – 15	5 – 30	125 – 750	12.5 – 75
7	R^2	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999
8	LOD (µg/mL)	1.32	0.067	0.07	0.475	0.65	0.01	0.32	0.01	0.05	0.084	0.215
9	LOQ (µg/mL)	4.0	0.205	0.2126	1.44	1.98	0.03	0.98	0.03	0.15	0.26	0.65
10	% Recovery 50%	101.68	101.04	99.92	99.78	99.92	98.46	98.97	100.39	100.33	99.94	100.95
	100%	101.87	100.33	99.74	99.97	99.94	98.10	98.52	99.89	99.79	99.22	99.75
	150%	98.45	99.16	100.15	100.02	99.97	99.93	98.19	98.6	100.20	99.52	100.83

11	Method precision (% RSD)	0.974	0.9698	1.08	0.412	0.875	0.7	0.15	1.4	0.1	0.06	1.4
12	% Assay	100.76	101.1	100.28	99.56	99.87	99.02	98.96	99.85	99.17	99.64	99.82
13	Specificity	Specific	Specific	Specific	Specific	Specific	Specific	Specific	Specific	Specific	Specific	Specific
14	Robustness	Robust	Robust	Robust	Robust	Robust	Robust	Robust	Robust	Robust	Robust	Robust

7.2 Conclusion

The author concludes that the proposed methods were found to be specific for simultaneous estimation of selected fixed dose drugs in both pure and medicinal dosage form. The methods were validated as per ICH Q2 (R1) guidelines. The statistical data proved that the methods were reliable and reproducible. The methods were new, simple, specific, rapid, robust, accurate, and precise for estimation of selected drug combinations by RP-HPLC. The developed methods can be used for the assay of the drugs selected in this research work.

7.3 Recommendations

The current investigation was carried out according to the specifications of drug regulating bodies like USFDA and ICH. Based on the above results and discussion, it is recommended that the proposed methods were consistent and reproducible for the simultaneous estimation of selected predetermined dosage combinations by RP-HPLC for the release of safe drugs in to the market.

7.4 Future Scope

The research work carried out was to develop simple, specific and rapid RP-HPLC methods for the simultaneous quantification of selected drug formulations. It is necessary to carry out the forced degradation studies, impurity profiling and long term stability studies of the selected dosage forms by using more sophisticated equipments like LC/MS (Liquid chromatography/Mass spectrophotometry) in order to find out powerful genotoxics and protect the patients from long term toxic effects.