ABSTRACT

High performance liquid chromatography and UV-Spectrophotometry are most and powerful techniques for the analysis of drugs. Present investigation is focussed on the development of novel RP-HPLC analytical techniques for the development of drug contents in solid oral dosage forms and their validation. Method development is highly significant in therapeutic drug monitoring and pharmaceutical industries. It is always desirable to select and develop simple, accurate, precise and economical method for the determination of drugs in pharmaceutical dosage forms and biological fluid samples. Analytical method development and validation play an important role in the discovery, development and manufacturing of drugs and pharmaceuticals.

For the present study, four dosage forms are selected, where each dosage form consists of two drugs. Dosage forms selected are **Sovodak,** manufactured by Beacon Pharmaceuticals Limited, consists of Sofosbuvir and Daclatasvir, used as antiviral drug for the treatment of HCV type-A, B. **Triumeq,** manufactured by ViiV Healthcare company, consists of Abacavir, Lamivudine and Dolutegravir, used as anti-retroviral drug for the treatment of HIV. **Truvada,** a combination drug of Emtricitabine and Tenofovir alafenamide, used for the treatment and prevention of HIV/AIDS, manufactured by Gilead Sciences, Inc. **Entresto,** a combination drug, manufactured by Novartis Pharmaceuticals Corporation, consists of Sacubitril and Valsartan, used for the treatment of patients with chronic heart failure.

A RP-HPLC method was developed for the development and validation of Sofosbuvir and Daclatasvir in the combined dosage form. The method was an isocratic system with X-Terra RP-18 (150mm*4.6mm, 5µm) column, using a mobile phase consisting of 0.1%v/v Trifluoro acetic acid in water and Acetonitrile in the ratio of 60:40% v/v, monitored at a wavelength of 275nm using a UV-detector at the flow rate of 1.0ml/min. The retention time for Sofosbuvir and Daclatasvir was 2.089min and 3.502min, with % recovery of 99.4-100.6% for sofosbuvir and 99.0 -100.1% for daclatasvir.

A new method developed for the simultaneous estimation of Lamivudine and Dolutegravir, performed on Intersil ODS 3V (150mm*4.6mm, 5µm) column, using a mobile phase of 0.1% Formic acid in water and Acetonitrile in the ratio of 30:70% v/v, at a flow rate of 0.8ml/min at an ambient temperature. Peaks were measured using a UV-detector at 260nm. The retention time for Lamivudine and Dolutegravir was 2.372 and 4.560 min respectively. The percentage recovery for Lamivudine and Dolutegravir was 99.0-100%.

Emtricitabine and Tenofovir Alafenamide determined and validated using Intersil ODS 3V (150mm*4.6mm, 5µm) using a mobile phase consisting of formic acid in water and methanol (45:55%v/v) with a flow rate of 1.0ml/min at an ambient temperature and a wavelength of 260nm. The retention times of Emtricitabine and Tenofovir Alafenamide were 1.92 and 4.75min with percentage recovery of 99-100% respectively.

The development and validation for the simultaneous estimation of Sacubitril and Valsartan achieved by X-Terra RP-18 (150mm*4.6mm, 5µm) column, with a mobile phase of 0.1% formic acid in water and Methanol (25:75% v/v) at a flow rate of 1.0ml/min at ambient temperature. The peaks were observed by a UV-detector at 267nm and the retention time for both the drugs was 3.156 and 2.663min. The percentage recovery was 99-100% for both the drugs.

Method validation was carried out for all above new methods developed. Linearity parameter for the method was analysed by preparing solution of five levels of concentrations. Calibration curve for concentration versus peak areas was found linear. Precision was studied by preparing solutions in six replicates and analyzed, %RSD was calculated for the peak areas of each drug. Accuracy for new methods developed, evaluated by using the standard solutions of three different concentrations (50%, 100% and 150%) into sample solution and their percentage recovery at each level was determined. Robustness for above methods was carried out at different flow rates and at different organic composition, standard and sample solutions were prepared and analyzed at $\pm$ 0.1ml/min and at $\pm$ 10% and developed method found to be stable on deliberate variation. Parameters evaluated for validating novel RP-HPLC

methods for simultaneous determination of above four dosage forms meet the requirements of ICH guidelines.

The developed methods are simple, precise, cost effective and rapid. These newly developed methods can be applied for the quantitative determination in the QC laboratories for the regular release.