ABSTRACT

A simple, specific, accurate and precise reverse phase High Performance Liquid Chromatography (RP-HPLC) and High Performance Thin Layer Chromatography (HPTLC) methods have been developed which can separate and quantitatively estimate formulation of 1) **Metformin Hydrochloride and Pioglitazone** 2) **Atorvastatin, Glimepiride and Metformin Hydrochloride** 3) **Atorvastatin, Ezetimibe and Fenofibrate** 4) **Atorvastatin, Losartan Potassium, Atenolol and Aspirin**.

The chromatographic separation for Metformin Hydrochloride and Pioglitazone in HPTLC method separation was achieved with the mobile phase consisting of Toluene: Methanol: Triethylamine (8: 2: 0.1 % v/v/v). The detection of spot was carried out at 230 nm and the Rf values Metformin Hydrochloride and Pioglitazone were found to be 0.25±0.03 and 0.47±0.04. The RP-HPLC, method separation was achieved with 25 mM sodium dihydrogen Phosphate (pH 3.65 adjusted with orthophosphoric acid): Acetonitrile (60:40, v/v) as mobile phase, Phenomenax C18 column (250x4.6) mm i.d., 5µm, particle size at room temperature and UV detection at 230 nm. The compounds were eluted isocratically at flow rate of 1.0 ml/min. The average retention times of Metformin Hydrochloride and Pioglitazone were found to be 2.17 and 6.21 min, respectively.

The chromatographic separation for Atorvastatin, Glimepiride and Metformin Hydrochloride in HPTLC method separation was achieved with the mobile phase consisting of Water: Methanol: Ammonium sulphate (3.5: 3.5: 12.6 % v/v/v). The detection of spot was carried out at 245 nm and the Rf values Atorvastatin, Glimepiride and Metformin Hydrochloride were found to be 0.50±0.01, 0.65±0.01 and 0.33±0.01.
In RP-HPLC method, separation was achieved with 20 mM Potassium dihydrogen Phosphate and Acetonitrile (65: 35%v/v) as mobile phase, Phenomenax C$_{18}$ column (250x4.6) mm i.d., 5µm, and particle size at room temperature and UV detection at 245 nm. The compounds were eluted isocratically at flow rate of 1.0 ml/min. The average retention times of Atorvastatin, Glimepiride and Metformin Hydrochloride were found to be 8.51±0.51 min, 12.18 ± 0.62 min and 3.918 ± 0.70 min, respectively.

The chromatographic separation for Atorvastatin, Ezetimibe and Fenofibrate in HPTLC method separation was achieved with the mobile phase consisting of Chloroform: Toluene: Methanol: Glacial Acetic Acid (5:4:1:0.1% v/v/v/v). The detection of spot was carried out at 254 nm and the R$_f$ values Atorvastatin, Ezetimibe and Fenofibrate were found to be 0.36±0.08, 0.51±0.06 and 0.87±0.04. In RP-HPLC method, separation was achieved with 20 mM Potassium dihydrogen phosphate: Methanol: Acetonitrile (10: 60: 30 % v/v/v) as mobile phase, RP18e, Hibar$^\text{®}$ RT column (250x4.6 mm) kept at room temperature and UV detection was 254 nm. The compounds were eluted isocratically at flow rate of 1.0 ml/min. The average retention times of Atorvastatin, Ezetimibe and Fenofibrate were found to be 2.67 ± 0.23 min, 4.32 ± 0.43 min and 11.48 ± 0.56 min, respectively.

The chromatographic separation for Atorvastatin, Losartan Potassium, Atenolol and Aspirin in HPTLC method separation was achieved with the mobile phase consisting of Methanol: Hexane: Ethyl Acetate: Chloroform: GAA (4.5: 1.5: 4: 1: % v/v/v/v/v). The detection of spot was carried out at 220 nm and the R$_f$ values of Atorvastatin, Losartan Potassium, Atenolol and Aspirin were found to be 0.63±0.05, 0.83±0.03, 0.21±0.02 and 0.41±0.01. In RP-HPLC method, separation was achieved with 10 mM Potassium dihydrogen Phosphate and Acetonitrile (65: 35%v/v) as mobile phase, Phenomenax C$_{18}$ column (250x4.6) mm i.d., 5µm, and particle size at room temperature and UV detection at 245 nm. The compounds were eluted isocratically at flow rate of 1.0 ml/min. The average retention times of Atorvastatin, Glimepiride and Metformin Hydrochloride were found to be 8.51±0.51 min, 12.18 ± 0.62 min and 3.918 ± 0.70 min, respectively.

The chromatographic separation for Atorvastatin, Ezetimibe and Fenofibrate in HPTLC method separation was achieved with the mobile phase consisting of Chloroform: Toluene: Methanol: Glacial Acetic Acid (5:4:1:0.1% v/v/v/v). The detection of spot was carried out at 254 nm and the R$_f$ values Atorvastatin, Ezetimibe and Fenofibrate were found to be 0.36±0.08, 0.51±0.06 and 0.87±0.04. In RP-HPLC method, separation was achieved with 20 mM Potassium dihydrogen phosphate: Methanol: Acetonitrile (10: 60: 30 % v/v/v) as mobile phase, RP18e, Hibar$^\text{®}$ RT column (250x4.6 mm) kept at room temperature and UV detection was 254 nm. The compounds were eluted isocratically at flow rate of 1.0 ml/min. The average retention times of Atorvastatin, Ezetimibe and Fenofibrate were found to be 2.67 ± 0.23 min, 4.32 ± 0.43 min and 11.48 ± 0.56 min, respectively.

The chromatographic separation for Atorvastatin, Losartan Potassium, Atenolol and Aspirin in HPTLC method separation was achieved with the mobile phase consisting of Methanol: Hexane: Ethyl Acetate: Chloroform: GAA (4.5: 1.5: 4: 1: % v/v/v/v/v). The detection of spot was carried out at 220 nm and the R$_f$ values of Atorvastatin, Losartan Potassium, Atenolol and Aspirin were found to be 0.63±0.05, 0.83±0.03, 0.21±0.02 and 0.41±0.01. In RP-HPLC method, separation was achieved with 10 mM Potassium dihydrogen Phosphate and Acetonitrile (65: 35%v/v) as mobile phase, Phenomenax C$_{18}$ column (250x4.6) mm i.d., 5µm, and particle size at room temperature and UV detection at 245 nm. The compounds were eluted isocratically at flow rate of 1.0 ml/min. The average retention times of Atorvastatin, Glimepiride and Metformin Hydrochloride were found to be 8.51±0.51 min, 12.18 ± 0.62 min and 3.918 ± 0.70 min, respectively.
dihydrogen Phosphate (pH 2.5 with Orthophosphoric acid): Methanol (20: 80 % v/v) as mobile phase, RP18e, Hibar® RT column (250x4.6 mm) at room temperature and UV detection at 220 nm. The compounds were eluted isocratically at flow rate of 1.0 ml/min. The average retention times of Atorvastatin, Losartan Potassium, Atenolol and Aspirin were found to be 2.363 ± 0.012 min, 3.220 ± 0.019 min, 3.890 ± 0.023 min and 5.203 ± 0.054 min, respectively.

All the above methods were validated in terms of linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) etc. in accordance with ICH guidelines. The recoveries for all four combinations were above 96%. The developed methods are free from interference due to excipients present in tablets. These methods are rapid and simple, do not require any sample preparation and is suitable for routine quality control analysis.

Keywords: HPTLC; Reverse HPTLC; Method validation; Quantitative analysis