ABSTRACT (PART-A)

- Seven simple RP-HPLC methods were developed and validated for the quantitative estimation of Pioglitazone, Rosiglitazone, Metformin, Glimepiride and Repaglinide from bulk and its formulation.
- Here these four combinations were selected for method development and validated for the quantitative simultaneous estimation of ROS and MET, ROS and GLP, PIO and MET and PIO and GLP respectively.
- The proposed methods was validated according to various ICH parameters like linearity, accuracy, precision, specificity, limits of detection, limits of quantification, range, solution stability and system.

**Method I. Simultaneous estimation of ROS and MET**

Chromatographic analysis was performed with a mixture of Ammonium dihydrogen Phosphate buffer (pH 4.5): Acetonitrile in the ratio (65:35; v/v) as mobile phase, at a flow rate of 1.0 mL min\(^{-1}\). UV detection was performed at 230 nm. The retention times of ROS and MET were 7.19±0.044 and 5.57±0.038 min respectively. Calibration plots were linear over the concentration ranges 12–32 μg mL\(^{-1}\) and 20–70 μg mL\(^{-1}\) for ROS and MET respectively. The Limit of detection was found to be 1.100 and 0.712 μg/ml and the quantification limit was 3.66 μg/ml and 2.41 μg/ml for MET and ROS respectively. The accuracy of the proposed method was determined by recovery studies and found to be 97.72% to 100.46%.

**Method II. Simultaneous estimation of ROS and GLP**

Chromatographic analysis was performed with a mixture of Ammonium dihydrogen Phosphate buffer (pH 4.5): Acetonitrile in the ratio (72:28; v/v) as mobile phase, at a flow rate of 1.0 mL min\(^{-1}\). UV detection was performed at 230 nm. The retention times of ROS and GLP were 9.51 ± 0.091 and 14.42 ± 0.180 min respectively. Calibration plots were linear over the concentration ranges 30–80 μg mL\(^{-1}\) and 20–45 μg mL\(^{-1}\) for ROS and GLP respectively. The Limit of detection was found to be 0.514 and 0.447 μg/ml and the quantification limit was 1.716 μg/ml and 1.493 μg/ml for ROS and GLP respectively.
The accuracy of the proposed method was determined by recovery studies and found to be 99.46% to 101.86%.

**Method III. Simultaneous estimation of PIO and MET**

Chromatographic analysis was performed with a mixture of Methanol: Phosphate buffer (pH 4.0) in the ratio (68:32; v/v) as mobile phase, at a flow rate of 1.0 mL min\(^{-1}\). UV detection was performed at 260 nm. The retention times of PIO and MET were 7.24±0.051 and 2.54±0.038 min respectively. Calibration plots were linear over the concentration ranges 10–35 μg mL\(^{-1}\) and 15–40 μg mL\(^{-1}\) for PIO and MET respectively. The Limit of detection was found to be 0.382 and 0.131 μg/ml and the quantification limit was 1.27 μg/ml and 0.436 μg/ml for MET and PIO respectively. The accuracy of the proposed method was determined by recovery studies and found to be 98.65% to 98.90%.

**Method IV. Simultaneous estimation of PIO and GLP**

Chromatographic analysis was performed with a mixture of Methanol: Phosphate buffer (pH 4.0) in the ratio (70:30; v/v) as mobile phase, at a flow rate of 1.0 mL min\(^{-1}\). UV detection was performed at 260 nm. The retention times of PIO and GLP were 6.66±0.0366 and 9.27±0.062 min respectively. Calibration plots were linear over the concentration ranges 30–80 μg mL\(^{-1}\) and 15–40 μg mL\(^{-1}\) for PIO and GLP respectively. The Limit of detection was found to be 0.365 and 0.0702 μg/ml and the quantification limit was 1.218 μg/ml and 0.234 μg/ml for PIO and GLP respectively. The accuracy of the proposed method was determined by recovery studies and found to be 100% to 102.59%.

**Method V. Estimation of ROS**

Chromatographic analysis was performed with a mixture of Ammonium dihydrogen phosphate buffer (pH 4.5): Acetonitrile in the ratio (65:35; v/v) as mobile phase, at a flow rate of 1.0 mL min\(^{-1}\). UV detection was performed at 230 nm. The retention times of ROS was found to be 7.19±0.145 respectively. Linearity was observed in concentration ranges of 12–70 μg mL\(^{-1}\). The limit of detection was found to be 0.725 and the
quantification limit was 2.41 μg/ml. The accuracy of the proposed method was determined by recovery studies and found to be 98.26% to 101.37%.

**Method VI. Estimation of PIO**

Chromatographic analysis was performed with a mixture of Methanol: Phosphate buffer (pH 4.0) in the ratio (70:30; v/v) as mobile phase, at a flow rate of 1.0 mL min⁻¹. UV detection was performed at 260 nm. The retention times of PIO was found to be 6.66±0.0366 min respectively. Calibration plots were linear over the concentration ranges 30–80 μg mL⁻¹. The Limit of detection was found to be 0.365 and the quantification limit was 1.218 μg/ml. The accuracy of the proposed method was determined by recovery studies and found to be 100% to 102.52%.

**Method VII. Estimation of REP**

Chromatographic analysis was performed with a mixture of Acetonitrile: Ammonium formate in the ratio (65:35; v/v) as mobile phase, at a flow rate of 1.0 mL min⁻¹. UV detection was performed at 245 nm. The retention times of REP was found to be 4.22±0.0123 min respectively. Calibration plots were linear over the concentration ranges 1–6 μg mL⁻¹ for REP with correlation coefficient \( (r^2) \) 0.9996 respectively. The Limit of detection was 0.057 μg/ml and the quantification limit 0.192 μg/ml for REP respectively. The accuracy of the proposed method was determined by recovery studies and found to be 99.72% to 100.33%.

**KEYWORDS:** Rosiglitazone, Metformin, Pioglitazone, Glimepiride, Repaglinide, RP-HPLC, ICH guidelines.
ABSTRACT (PART- B)

- Four simple HPTLC methods were developed and validated for the quantitative estimation of Pioglitazone, Rosiglitazone, Repaglinide and simultaneous estimation of pioglitazone and glimepiride from bulk and its formulation.
- The analysis of PIO, ROS, REP and simultaneous estimation of PIO and GLP was carried out on TLC aluminum plates precoated with silica gel 60 F 254 as the stationary phase.
- The proposed methods was validated according to various ICH parameters like linearity, accuracy, precision, specificity, limits of detection, limits of quantification, range and solution stability.

Method I. Estimation of ROS

The mobile phase used was a mixture of Chloroform: Methanol (7:2; v/v). The detection of spot was carried out at 254 nm. The calibration curve was found to be linear between 200 to 2000 ng mL$^{-1}$ with regression coefficient of 0.9992 and Rf value 0.53±0.01. The proposed method can be successfully used to determine the drug content of marketed formulation. The accuracy of the proposed method was determined by recovery studies and found to be 98.67 to 99.11 %. The Limit of detection was 46.57 ng/ml and the quantification limit was 155.23ng/ml for raw material and formulations which represents that sensitivity of the method is high.

Method II. Estimation of PIO

The mobile phase used was a mixture of Toulene: Ethyl acetate: Formic Acid 10:3:1 v/v. The detection of spot was carried out at 254 nm. The calibration curve was found to be linear between 100 to 3000 ng mL$^{-1}$ with regression coefficient of 0.9992 and Rf value 0.53±0.023. The proposed method can be successfully used to determine the drug content of marketed formulation. The accuracy of the proposed method was determined by recovery studies and found to be 98.34 to 99.40 %. The Limit of detection was 7.27 ng/ml and the quantification limit was 24.54 ng/ml for raw material and formulations which represents that sensitivity of the method is high.
Method III. Estimation of REP

The mobile phase used was a mixture of Chloroform: Methanol (9:1) v/v. The detection of spot was carried out at 254 nm. The calibration curve was found to be linear between 300 to 3000 ng mL\(^{-1}\) Rf value 0.41±0.018 with regression coefficient of 0.9991. The proposed method can be successfully used to determine the drug content of marketed formulation. The accuracy of the proposed method was determined by recovery studies and found to be 97.98 to 98.89 %. The Limit of detection was 52.91 ng/ml and the quantification limit was 176.39 ng/ml for raw material and formulations which represents that sensitivity of the method is high.

Method IV. Simultaneous estimation of PIO and GLP

The mobile phase used was a mixture of Benzene: Ethyl acetate: Diethyl ether 6:3:1 v/v. The detection of spot was carried out at 254 nm. The calibration curve of PIO was found to be linear between 600 ng/ml to 3600 ng/ml with regression coefficient of 0.9984 and calibration curve of GLP was found to be linear between 200-1200 ng/ml for with regression coefficient of 0.9991 respectively. The limit of detection was found to be 57.22 ng/ml and 16.67 ng/ml and the quantification limit was 190.73 ng/ml and 55.58 ng/ml for PIO and GLP respectively. The accuracy of the proposed method was determined by recovery studies and found to be 97.84 to 99.07 %.

KEYWORDS: Pioglitazone, Rosiglitazone, Glimepiride, Repaglinide, HPTLC, ICH guidelines