6. ANALYTICAL AND BIOANALYTICAL STUDIES

6.1.1 Simultaneous determination of glimepiride and atorvastatin calcium by HPLC

6.1.2 Simultaneous determination of pioglitazone hydrochloride and simvastatin by HPLC

6.1.3 Simultaneous determination of glibenclamide and atorvastatin calcium by HPLC
6.1 Analytical and bioanalytical studies

6.1.1 Simultaneous determination of glimepiride and atorvastatin calcium by high performance liquid chromatography (HPLC)

Analytical method

The HPLC method used for simultaneous determination of glimepiride and atorvastatin calcium, showed the drugs retention time at 9.63±0.19 min for glimepiride and 6.61±0.22 min for atorvastatin calcium respectively. A linear relationship was observed in the concentration range of 1 - 25 μg/ml ($r^2 = 0.998$; $n = 6$) for glimepiride and 10 - 60 μg/ml ($r^2 = 0.998$; $n = 6$) for atorvastatin calcium. The developed method was specific as there was no interference by placebo solution at the retention time of the analytes and the LOQ i.e., the limit of quantification, with acceptable precision and accuracy was of 0.22 μg/ml for glimepiride and 0.24 μg/ml for atorvastatin calcium. The accuracy of glimepiride at the low (8 μg/ml), moderate (10 μg/ml) and higher (12 μg/ml) concentrations ranged from 98.1 to 102.09 % and 94.5 to 103.7 % for intra-day ($n = 6$) and inter-day ($n = 3$) estimations respectively. Similarly the accuracy of atorvastatin calcium at the low (16 μg/ml), moderate (20 μg/ml) and higher (24 μg/ml) concentrations ranged from 97.4 to 101.04 % and 94.5 to 102.8 % for intra-day ($n = 6$) and inter-day ($n = 3$) estimations respectively. The precision of the aforesaid low, moderate and higher concentration expressed as % coefficient of variation (C.V) was found to be less than 4% for intra-day assay and less than 6% for inter-day assay for glimepiride and less than 6% for intra-day assay and less than 8% for inter-day assay for atorvastatin calcium indicating that % C.V was within the limits (% C.V should be within 20% for the lowest concentration and 15% for the upper levels). The calibration curve was established by
plotting the concentrations of glimepiride and atorvastatin calcium samples versus their peak areas are shown in below Figure 13 and Figure 14.

**Figure 13. Standard calibration curve for glimepiride by RP-HPLC**

**Figure 14. Standard calibration curve for atorvastatin calcium by RP-HPLC**
Bio-analytical method

The HPLC bio-analytical method was used for simultaneous determination of glimepiride and atorvastatin calcium, showed the drugs retention time at 9.81±0.28 min for glimepiride, 8.12±0.31 min for pioglitazone hydrochloride an internal standard, and 6.58±0.24 min atorvastatin calcium respectively. A linear relationship was observed in the concentration range of 25 - 1000 ng/ml ($r^2 = 0.997; n = 6$) for glimepiride and 25 - 1500 ng/ml ($r^2 = 0.997; n = 6$) for atorvastatin calcium. The developed method was specific as there was no interference by placebo solution at the retention time of the analytes and the LOQ i.e., the limit of quantification with acceptable precision and accuracy was of 30.5 ng/ml for glimepiride and 5.75 ng/ml for atorvastatin calcium. The accuracy of glimepiride at the low (25 ng/ml), moderate (50 ng/ml) and higher (100 ng/ml) concentrations ranged from 97.4 to 101.12 % and 95.1 to 103.9 % for intra-day (n = 6) and inter-day (n = 3) estimations respectively. Similarly the accuracy of atorvastatin calcium at the low (25 ng/ml), moderate (50 ng/ml) and higher (100 ng/ml) concentrations ranged from 98.1 to 101.15 % and 94.8 to 103.2 % for intra-day (n = 6) and inter-day (n = 3) estimations respectively. The precision of the aforesaid low, moderate and higher concentration expressed as % coefficient of variation (C.V) was found to be less than 6% for intra-day assay and less than 9% for inter-day assay for glimepiride and less than 6% for intra-day assay and less than 10% for inter-day assay for atorvastatin calcium indicating that % C.V was within the limits (% C.V should be within 20% for the lowest concentration and 15% for the upper levels). The calibration curve was established by plotting the concentrations of glimepiride versus their retention factor and atorvastatin calcium versus their retention factor versus their retention factor are
shown in below Figure 15 and Figure 16. Representative chromatograms of plasma spiked with glimepiride, atorvastatin calcium and pioglitazone HCl is depicted in Figure 17, respectively.

Figure 15. Standard calibration curve of glimepiride and internal standard in rat plasma by RP-HPLC
Results and Discussion

Figure 16. Standard calibration curve of atorvastatin calcium and internal standard in rat plasma by RP-HPLC

\[ R^2 = 0.997 \]

Figure 17. RP-HPLC chromatogram of glimepiride, atorvastatin calcium and internal standard (pioglitazone HCl)
6.1.2 Simultaneous determination of pioglitazone hydrochloride and simvastatin by high performance liquid chromatography (HPLC)

Analytical methods

The HPLC method used for simultaneous determination of pioglitazone HCl and simvastatin, showed the drugs retention time at 2.49±0.20 min for pioglitazone HCl and 9.01±0.16 min for simvastatin respectively. A linear relationship was observed in the concentration range of 5 - 50 μg/ml (r² = 0.997; n = 6) for pioglitazone HCl and 5 - 50 μg/ml (r² = 0.998; n = 6) for simvastatin. The developed method was specific as there was no interference by placebo solution at the retention time of the analytes and the LOQ i.e., the limit of quantification with acceptable precision and accuracy was of 0.36 μg/ml for pioglitazone HCl and 0.28 μg/ml for simvastatin. The accuracy of pioglitazone HCl at the low (5 μg/ml), moderate (10 μg/ml) and higher (15 μg/ml) concentrations ranged from 98.4 to 101.02 % and 95.4 to 102.3 % for intra-day (n = 6) and inter-day (n = 3) estimations respectively. Similarly the accuracy of simvastatin at the low (5 μg/ml), moderate (10 μg/ml) and higher (15μg/ml) concentrations ranged from 97.0 to 101.05 % and 95.1 to 102.1 % for intra-day (n = 6) and inter-day (n = 3) estimations respectively. The precision of the aforesaid low, moderate and higher concentration expressed as % coefficient of variation (C.V) was found to be less than 8% for intra-day assay and less than 11% for inter-day assay for pioglitazone HCl and less than 7% for intra-day assay and less than 10% for inter-day assay for simvastatin indicating that % C.V was within the limits (% C.V should be within 20 % for the lowest concentration and 15 % for the upper levels). The calibration curves were established by plotting the concentrations of pioglitazone HCl versus their peak areas and simvastatin samples versus their peak areas
are shown in below Figure 18 and Figure 19. Representative chromatograms of pioglitazone HCl and simvastatin is depicted in Figure 20 respectively.

Figure 18. Standard calibration curve for pioglitazone HCl by RP-HPLC
Results and Discussion

Figure 19. Standard calibration curve for simvastatin by RP-HPLC

Figure 20. RP-HPLC chromatogram of pioglitazone HCl and simvastatin

6.1.3 Simultaneous determination of glibenclamide and atorvastatin calcium by high performance liquid chromatography (HPLC)

Analytical methods

The HPLC method used for simultaneous determination of glibenclamide and atorvastatin calcium, showed the drugs retention time at 8.32±0.26 min for glibenclamide.
and 5.23±0.28 min for atorvastatin calcium respectively. A linear relationship was observed in the concentration range of 5 - 30 μg/ml ($r^2 = 0.998$; $n = 6$) for glibenclamide and 5 - 30 μg/ml ($r^2 = 0.998$; $n = 6$) for atorvastatin calcium. The developed method was specific as there was no interference by placebo solution at the retention time of the analytes and the LOQ i.e., the limit of quantification with acceptable precision and accuracy was of 0.26 μg/ml for glimepiride and 0.22 μg/ml for atorvastatin calcium. The accuracy of glibenclamide at the low (5 μg/ml), moderate (10 μg/ml) and higher (15 μg/ml) concentrations ranged from 97.7 to 101.3 % and 95.2 to 102.6 % for intra-day ($n = 6$) and inter-day ($n = 3$) estimations respectively. Similarly the accuracy of atorvastatin calcium at the low (5 μg/ml), moderate (10 μg/ml) and higher (15 μg/ml) concentrations ranged from 98.1 to 101.7 % and 94.2 to 102.4 % for intra-day ($n = 6$) and inter-day ($n = 3$) estimations respectively. The precision of the aforesaid low, moderate and higher concentration expressed as % coefficient of variation (C.V) was found to be less than 6% for intra-day assay and less than 13% for inter-day assay for glibenclamide and less than 7% for intra-day assay and less than 9% for inter-day assay for atorvastatin calcium indicating that % C.V was within the limits (% C.V should be within 20 % for the lowest concentration and 15 % for the upper levels). The calibration curve was established by plotting the concentrations of glibenclamide and atorvastatin calcium samples versus their peak areas are shown in below Figure 21 and Figure 22.
Results and Discussion

Figure 21. Standard calibration curve for glibenclamide by RP-HPLC

![Glibenclamide Calibration Curve](image)

R² = 0.9982

Figure 22. Standard calibration curve for atorvastatin calcium by RP-HPLC

![Atorvastatin Calcium Calibration Curve](image)

R² = 0.998
Results and Discussion

Bio-analytical method

The HPLC bio-analytical method was used for simultaneous determination of glibenclamide and atorvastatin calcium, showed the drugs retention time at 8.45±0.16 min for glibenclamide, 6.82±0.27 min for spironolactone an internal standard, and 5.34±0.21 min atorvastatin calcium respectively. A linear relationship was observed in the concentration range of 500 - 30000 ng/ml (r² = 0.996; n = 6) for glibenclamide and 25 - 1500 ng/ml (r² = 0.996; n = 6) for atorvastatin calcium. The developed method was specific as there was no interference by placebo solution at the retention time of the analytes and the LOQ i.e., the limit of quantification with acceptable precision and accuracy was of 0.0036 ng/ml for glibenclamide and 20 ng/ml for atorvastatin calcium. The accuracy of glibenclamide at the low (500 ng/ml), moderate (1000 ng/ml) and higher (1500 ng/ml) concentrations ranged from 98.0 to 101.3 % and 94.8 to 103.4 % for intra-day (n = 6) and inter-day (n = 3) estimations respectively, similarly the accuracy of atorvastatin calcium at the low (30 ng/ml), moderate (60 ng/ml) and higher (100 ng/ml) concentrations ranged from 97.5 to 101.6 % and 94.3 to 102.8 % for intra-day (n = 6) and inter-day (n = 3) estimations respectively. The precision of the aforesaid low, moderate and higher concentration expressed as % coefficient of variation (C.V) was found to be less than 9% for intra-day assay and less than 12% for inter-day assay for glibenclamide and less than 7% for intra-day assay and less than 10% for inter-day assay for atorvastatin calcium indicating that % C.V was within the limits (% C.V should be within 20 % for the lowest concentration and 15 % for the upper levels). The calibration curve was established by plotting the concentrations of glibenclamide versus their retention factor and atorvastatin calcium concentrations versus their retention factor as shown in below
Figure 23 and Figure 24. Representative chromatograms of plasma spiked with glibenclamide, atorvastatin calcium and spironolactone is depicted in Figure 25 respectively.

![Standard calibration curve of glibenclamide and internal standard in rat plasma by RP-HPLC](image)

Figure 23. Standard calibration curve of glibenclamide and internal standard in rat plasma by RP-HPLC
Figure 24. Standard calibration curve of atorvastatin calcium and internal standard in rat plasma by RP-HPLC

Figure 25. RP-HPLC chromatogram of glibenclamide, atorvastatin calcium and internal standard (spironolactone)