DISCUSSION

ANALYSIS OF METFORMIN HYDROCHLORIDE AND PIOGLITAZONE BY HPTLC AND HPLC METHODS

HPTLC METHOD

The present investigation was aimed to develop a HPTLC method for the estimation of Metformin Hydrochloride and Pioglitazone in combined dosage form.

Different mobile phase systems in different proportions were tried. A mixture of Toluene: Methanol: Triethylamine (8:2:0.1 % v/v/v) show describable levels of separation with minimum tailing. The UV detection was carried out densitometrically at 230 nm as Metformin Hydrochloride and Pioglitazone showed good response at the selected wavelength. The Rf values of Metformin Hydrochloride and Pioglitazone were found to be 0.25 ± 0.03 and 0.47 ± 0.04, respectively.

The method was validated as per ICH guidelines. Linearity experiments were performed for both analytes based on peak area response factor. The response was found to be linear in the range of 50 to 300 ng/spot for Metformin Hydrochloride and 1.5 to 9 ng/spot for Pioglitazone, respectively. The correlation coefficient values of Metformin Hydrochloride and Pioglitazone were found to be 0.994 and 0.999, respectively, which proves good correlation between peak area and concentration of each drug. Precision studies were also performed. Low % RSD value indicates that the proposed method has good precision. LOD for Metformin Hydrochloride and Pioglitazone were found to be 10 and 0.5 ng/spot and their LOQ values were found to be 50 and 1.5 ng/spot, respectively. In this method, accuracy was determined by calculation of percentage recovery and average recovery which were calculated at
80, 100, and 120% levels. The recovery values between prescribed limit of 98-102% shows that the method is free from interference of excipients present in formulation. The method was successfully used for determination of drugs in tablets.

The above discussion proves that the proposed method is simple, rapid and precise. The good recovery values and low relative standard deviation confirm the suitability of the method.

HPLC METHOD

A wavelength of 230 nm was selected for the study. 25 mM sodium dihydrogen Phosphate (pH 3.65 adjusted with orthophosphoric acid): Acetonitrile was fixed as mobile phase in the ratio of 40:60% v/v which gives symmetry peaks with good resolution. The drugs were chromatographed under different flow rates, from which a flow rate of 1 ml/minute was selected. The retention times of Metformin Hydrochloride and Pioglitazone were found to be 2.17 and 6.21 minutes, respectively.

The developed method was validated as per ICH guidelines. Calibration graphs were plotted using standard peak areas Vs concentration of standard drug solutions. The slope, intercept and correlation coefficient values were found to be 10151.54440, 49424.2 and 0.994, respectively for Metformin Hydrochloride and 28205.6667, 6532.30 and 0.999, respectively for Pioglitazone. The results show that within the concentration range tested, there was excellent correlation between peak area and concentration. Metformin Hydrochloride and Pioglitazone were found to be linear in the range of 5 to 45 µg/ml and 0.15 to 0.9 µg/ml, respectively. The LOD of Metformin Hydrochloride and Pioglitazone were found to be 0.005 and 0.01 µg/ml.
respectively. The LOQ of Metformin Hydrochloride and Pioglitazone were found to be 0.015 and 0.03 µg/ml, respectively. Precision of the developed method was studied under Intra day precision; inter day precision and repeatability of injection. Low % RSD values show that the developed method is precise. System suitability parameters like number of theoretical plates (N), peak asymmetry factor (As) and resolution (Rs) etc. were studied and it was found that all results were within limits.

*The developed methods can be used for the routine analysis of the simultaneous estimation of Metformin Hydrochloride and Pioglitazone in formulation.*
ANALYSIS OF ATORVASTATIN, GLIMEPIRIDE AND METFORMIN HYDROCHLORIDE BY VARIOUS METHODS

HPTLC METHOD

The present investigation was aimed to develop a HPTLC method for the estimation of Atorvastatin, Glimepiride and Metformin Hydrochloride in combined dosage form.

Different mobile phase systems in different proportions were tried. A mixture of Water: Methanol: Ammonium Sulphate (3.5: 3.5: 12.6% v/v/v) gave good separation with minimum tailing. The UV detection was carried out densitometrically at 245 nm as Atorvastatin, Glimepiride and Metformin Hydrochloride showed good response at the selected wavelength. The \( R_f \) values of Atorvastatin, Glimepiride and Metformin Hydrochloride were found to be 0.50 ± 0.01, 0.65 ± 0.01 and 0.33 ± 0.01 respectively.

The method was validated as per ICH guidelines. Linearity experiments were performed for three analytes based on peak area response factor. The response was found to be linear in the range of 1 to 7 ng/spot for Atorvastatin, 0.1 to 0.7 ng/spot for Glimepiride and 50 to 350 ng/spot Metformin Hydrochloride respectively. The correlation coefficient values of Atorvastatin, Glimepiride and Metformin Hydrochloride were found to be 0.999±0.001, 0.998±0.002 and 0.999±0.001 respectively, which prove good correlation between peak area and concentration of each drug. Precision studies were also performed. Low % RSD value indicates that the proposed method has good precision. LOD for Atorvastatin, Glimepiride and Metformin Hydrochloride were found to be 0.2, 0.02 and 5 ng/spot and their LOQ...
values were found to be 1, 0.1 and 50 ng/spot respectively. In this method, accuracy was determined by calculating percentage recovery and average recovery which were calculated at 80, 100, and 120% levels. The recovery values between prescribed limit of 98-102% show that the method is free from interference of excipients present in formulation.

The above results prove that the proposed method is simple, rapid and precise. The good recovery values and low relative standard deviation confirm the suitability of the method.

Hence, these methods were successfully applied for determination of drugs in the tablets.

HPLC METHOD

A wavelength of 245 nm was selected for the study. 20mM Potassium dihydrogen phosphate: Acetonitrile was fixed as mobile phase in the ratio of 65: 35 % v/v which gives symmetry peaks with good resolution. The drugs were chromatographed under different flow rates from which a flow rate of 1 ml/minute was selected. The retention times of Atorvastatin, Glimepiride and Metformin Hydrochloride were found to be 8.51±0.51, 12.18± 0.62 and 3.918± 0.70 minutes, respectively.

The developed method was validated as per ICH guidelines. Calibration graphs were plotted using standard peak areas Vs concentration of standard drug solutions. The slope, intercept and correlation coefficient values were found to be 37754.56, 115122.6 and 0.9992, for Atorvastatin. The slope, intercept and correlation
coefficient values were found to be 288393.7120, -15315.8 and 0.996 for Glimepiride. The slope, intercept and correlation coefficient values were found to be 3934.7120, 351927.6 and 0.9914 for Metformin Hydrochloride. The results show that within the concentration range tested, there was excellent correlation between peak area and concentration. Atorvastatin, Glimepiride and Metformin Hydrochloride were found to be linear in the range of 1 to 5 µg/ml, 0.1 to 0.5µg/ml and 50 to 250µg/ml, respectively. The LOD of Atorvastatin, Glimepiride and Metformin Hydrochloride were found to be 0.01, 0.001 and 0.1µg/ml respectively. The LOQ of Atorvastatin, Glimepiride and Metformin Hydrochloride were found to be 0.3, 0.03 and 1µg/ml, respectively. Precision of the developed method was studied under Intra day precision; inter day precision and repeatability of injection. Low % RSD values show that the developed method is precise. System suitability parameters like number of theoretical plates (N), peak asymmetry factor (As) and resolution (Rs) etc. were studied and it was found that all results were within limits.

_The developed methods can be used for the routine simultaneous analysis of the simultaneous estimation of_ Atorvastatin, Glimepiride and Metformin Hydrochloride in formulation._
ANALYSIS OF ATORVASTATIN, EZETIMIBE AND FENOFIBRATE BY VARIOUS METHODS

HPTLC METHOD

The present investigation was aimed at developing a HPTLC method for the estimation of Atorvastatin, Ezetimibe and Fenofibrate in combined dosage form.

Different mobile phase systems in different proportions were tried. A mixture of Methanol: Chloroform: Toluene: Glacial Acetic Acid (1: 5: 4: 0.1 %v/v/v/v) gave good separation with minimum tailing. The UV detection was carried out densitometrically at 254 nm. Atorvastatin, Ezetimibe and Fenofibrate have showed good response at the selected wavelength. The Rf values of Atorvastatin, Ezetimibe and Fenofibrate were found to be 0.36 ± 0.08, 0.51 ± 0.06 and 0.87 ± 0.04, respectively.

The method was validated as per ICH guidelines. Linearity experiments were performed for both analytes based on peak area response factor. The response was found to be linear in the range of 10-60 ng/spot for Atorvastatin, 10-60 ng/spot for Ezetimibe and 160 to 960 ng/spot for Fenofibrate, respectively. The correlation coefficient values of Atorvastatin, Ezetimibe and Fenofibrate were found to be 0.989, 0.999 and 0.999 respectively, which proves good correlation between peak area and concentration of each drug. Precision studies were performed; low % RSD value indicates that the proposed method has good precision. LOD for Atorvastatin, Ezetimibe and Fenofibrate were found to be 1, 1.5 and 40 ng/spot and their LOQ values were found to be 10, 10 and 160 ng/spot, respectively. In this method, accuracy
was determined by calculation of percentage recovery and average recovery which were calculated at 80, 100 and 120% levels. The recovery values between prescribed limit of 98-102% shows that method is free from interference of excipients present in formulation.

The above details prove that the proposed method is simple, rapid and precise. The good recovery values and low relative standard deviation confirm the suitability of the method. Therefore method was successfully applied for determination of drugs in tablets.

**HPLC METHOD**

A wavelength of 254 nm was selected for the study. 20 mM Potassium dihydrogen Phosphate: Methanol: Acetonitrile was fixed as mobile phase in the ratio of 10: 60: 30 % v/v/v symmetry peaks and good resolution was obtained. Next, the drugs were chromatographed under different flow rates from which a flow rate of 1 ml/minute was selected. The retention times of Atorvastatin, Ezetimibe and Fenofibrate were found to be 2.67 ± 0.23, 4.32 ± 0.43 and 11.48 ± 0.56 minutes, respectively.

The developed method was validated as per ICH guidelines. Calibration graphs were plotted using standard peak areas Vs concentration of standard drug solutions. The slope, intercept and correlation coefficient values were found to be 287277.9, 6381.7 and 0.999 respectively for Atorvastatin. The slope, intercept and correlation coefficient values were found to be 292671.3, 50325.9 and 0.999 respectively for Ezetimibe. The slope, intercept and correlation coefficient values were found to be 64386.5, 216857.6 and 0.997 respectively for Fenofibrate.
Atorvastatin, Ezetimibe and Fenofibrate were found to be linear in the range of 1-5 µg/ml, 1-5 µg/ml and 16-80 µg/ml, respectively. The LOD of Atorvastatin, Ezetimibe and Fenofibrate were found to be 0.5, 0.2 and 2 µg/ml, respectively. The LOQ of Atorvastatin, Ezetimibe and Fenofibrate were found to be 1, 1 and 16 µg/ml, respectively. Precision of the developed method was studied under Intra day precision; inter day precision and repeatability of injection. Low % RSD values show that the developed method is precise. System suitability parameters like number of theoretical plates (N), peak asymmetry factor (As) and resolution (Rs) etc. were studied and it was found that all results were within limits.

_The developed methods can be used for the routine simultaneous analysis of the simultaneous estimation of Atorvastatin, Ezetimibe and Fenofibrate in formulation._
HPTLC METHOD

The present investigation was aimed at developing a HPTLC method for the estimation of Atorvastatin, Losartan Potassium, Atenolol and Aspirin in combined dosage form.

Different mobile phase systems in different proportions were tried. A mixture of Methanol: Hexane: Ethyl acetate: Chloroform: Glacial Acetic Acid (4.5: 1.5: 4: 1: 0.1 % v/v/v/v/v) gave good separation with minimum tailing. The UV detection was carried out densitometrically at 220 nm as Atorvastatin, Losartan Potassium, Atenolol and Aspirin showed good response at the selected wavelength. The R_f values of Atorvastatin, Losartan Potassium, Atenolol and Aspirin were found to be 0.63 ± 0.05, 0.85 ± 0.03, 0.21 ± 0.02 and 0.41 ± 0.04, respectively.

The method was validated as per ICH guidelines. Linearity experiments were performed for both analytes based on peak area response factor. The response was found to be linear in the range of 30-70 ng/spot, 150-350 ng/spot, 150-350 ng/spot and 225-525 ng/spot for Atorvastatin, Losartan Potassium, Atenolol and Aspirin, respectively. The correlation coefficient values of Atorvastatin, Losartan Potassium, Atenolol and Aspirin were found to be 0.996, 0.999, 0.993 and 0.999 respectively, which proves good correlation between peak area and concentration of each drug. Precision studies were also performed. Low % RSD value indicates that the proposed method has good precision. LOD for Atorvastatin, Losartan Potassium, Atenolol and
Aspirin values were found to be 0.5, 0.1, 1.0 and 1.5. And their LOQ values were found to be 10, 50, 50 and 75 ng/spot, respectively. In this method, accuracy was determined by calculation of percentage recovery and average recovery which were calculated at 80,100, and 120% levels. The recovery values between prescribed limit of 98-102% shows that the method is free from interference of excipients present in formulation.

The method was successfully used for determination of drugs in tablets. The above results prove that the proposed method is simple, rapid and precise. The good recovery values and low relative standard deviation, confirm the suitability of the method.

**HPLC METHOD**

A wavelength of 220 nm was selected for the study. 10 mM Potassium Dihydrogen Phosphate (pH 2.5 with Orthophosphoric acid): Methanol was fixed as mobile phase in the ratio of 20:80% v/v symmetry peaks and a good resolution was obtained. The drugs were chromatographed under different flow rates from which a flow rate of 1 ml/min was selected. The retention times of Atorvastatin, Losartan Potassium, Atenolol and Aspirin were found to be 2.363±0.012, 3.220±0.019, 3.890±0.043 and 5.203±0.054 minutes, respectively.

The developed method was validated as per ICH guidelines. Calibration graphs were plotted using standard peak areas Vs concentration of standard drug solutions. The slope, intercept and correlation coefficient values were found to be 30715.32, -70813.80 and 0.9989 respectively for Atorvastatin. The slope, intercept and correlation coefficient values were found to be 15807.5380, 56352.30 and 0.9999
respectively for Losartan Potassium. The slope, intercept and correlation coefficient values were found to be 15756.5175, 164234.6 and 0.99434 respectively for Atenolol.

The slope, intercept and correlation coefficient values were found to be 14752.8973, 188435.7000 and 0.998260 respectively for Aspirin. The results show that within the concentration range tested, there was excellent correlation between peak area and concentration. Atorvastatin, Losartan Potassium, Atenolol and Aspirin were found to be linear in the range of 10-50 µg/ml, 50-250 µg/ml, 50-250 µg/ml and 75-375 µg/ml, respectively. The LOD of Atorvastatin, Losartan Potassium, Atenolol and Aspirin were found to be 0.0005, 0.002, 0.0025 and 0.0045 µg/ml respectively. The LOQ of Atorvastatin, Losartan Potassium, Atenolol and Aspirin were found to be 0.001, 0.02, 0.025 and 0.01 µg/ml, respectively. Precision of the developed method was studied under intra day precision; inter day precision and repeatability of injection. Low % RSD values show that the developed method is precise. System suitability parameters like number of theoretical plates (N), peak asymmetry factor (As) and resolution (Rs) etc. were studied and it was found that all results were within limits.

The developed methods can be used for the routine simultaneous analysis of the simultaneous estimation of Atorvastatin, Losartan Potassium, Atenolol and Aspirin in formulation.