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VALIDATED UHPLC-PDA METHOD FOR SIMULTANEOUS DETERMINATION OF METFORMIN, GLIMEPIRIDE AND PIOGLITAZONE IN THEIR FDC TABLETS

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ABSTRACT
In the presented work the Ultra-High Performance Liquid Chromatographic-Photodiode Array Detection (UHPLC-PDA) method has been developed and validated for simultaneous determination of Anti-diadetic drugs such as Metformin, Glimepiride and Pioglitazone in their Fixed Dose Combination (FDC) tablets. The chromatographic separation was achieved on Acquity UPLC^{TM} BEH C_{18} (100.0 × 2.1 mm, 1.7µm) column using isocratic mobile phase consisting of acetonitrile-2mM ammonium acetate (50:50, v/v) at a flow rate of 0.25 mL/min. The detection was carried out at 250 nm by PDA detector. The elution of metformin, glimepiride and pioglitazone was occurred at 0.75 min, 1.50 min and 2.25 min, respectively. The calibration curves were linear over the concentration range of 1-1000 ng/mL for all the drugs. The developed method was validated according to ICH guidelines. The proposed method was applied for simultaneous determination of metformin, glimepiride and pioglitazone during routine quality control analysis in their combined tablets.

KEYWORDS: UHPLC-PDA, Anti-diabetic drugs, FDC, Validation, Determination.

INTRODUCTION
The UHPLC-PDA (Ultra-High Performance Liquid Chromatography-Photodiode Array Detection) technique has been widely used technique in the identification and quantitative analysis of drug products. The UHPLC column packing composed with bridged ethylsiloxane/silica hybrid (BEH) structure which provides improved efficiency, strength and wide pH range. UHPLC offers an advancement of HPLC which is based on the principal of use of stationary phase consisting of particles less than 2µm. By using smaller particles, speed of analysis, peak capacity can be extended to new limits and the sample can be analyzed in a shorter period of time. By the improved column packing material and particle size, Waters Company was given the trade name UPLC (Ultra Performance Liquid Chromatography) for UHPLC technique. UHPLC provides the fast, better chromatographic separation with shorter chromatographic run time. PDA detector provides higher sensitivity, selectivity, sharp peaks of drugs.[1-7]

Metformin hydrochloride is an orally administered biguanide widely used in the treatment of type 2 (non-insulin dependent) diabetes mellitus. It is anti-hyperglycemic drug that lowers glucose by reducing hepatic glucose production and gluconeogenesis and by enhancing peripheral glucose uptake.[8] Glimepiride lower blood glucose levels by stimulating insulin release from the pancrease. Pioglitazone hydrochloride is an oral anti-hyperglycemic agent which acts by decreasing insulin resistance in the periphery and liver resulting in increased insulin dependent glucose disposal and decreased hepatic glucose output. It is used in the treatment of type-II diabetes mellitus.[9] Fixed dose combination (FDC) tablets containing 500 mg of metformin hydrochloride, 2 mg of glimepiride and 15 mg of pioglitazone hydrochloride has been approved for the treatment of diabetes and widely available in Indian market.

The literature survey revealed that few analytical methods have been reported for determination of metformin as an individual drug in biological fluids such as UV-Spectrophotometry,[10,11] HPLC,[12,13] and LC-MS.[14-16] Determination of glimepiride as an individual drug in biological fluids has been reported by HPLC[17-19] and LC-MS.[20-22] Determination of piogliazone as an individual drug has been reported by UV-Spectrophotometry,[23] HPLC,[24-26] HPTLC[27] and LC-MS/MS.[28] Simultaneous determination of metformin, glimepiride and pioglitazone in formulations and/or biological fluids has also been reported by HPLC.[29-31] HPTLC[32] and LC-MS-MS.[33] However author was developed UPLC-QTOF-MS method for simultaneous determination of metformin, glimepiride and pioglitazone in human plasma but the developed method employed Q-TOF-MS (Time-of-flight mass

spectrometer) for detection of drugs, which was found highly costly for identification and quantitative analysis.[34] Hence in the presented work an UHPLC-PDA method is developed and validated for simultaneous determination of metformin, glimepiride and pioglitazone in their combined tablet dosage forms.

Experimental

Chemicals and Reagents

Metformin hydrochloride ($C_4H_{11}N_5$.HCl, Molecular weight 165.62 and purity 99.98%), Glimepiride ($C_{24}H_{34}N_4O_5S$, Molecular weight 490.62 and purity 99.99%) and pioglitazone hydrochloride ($C_{19}H_{21}ClN_2O_3S$, Molecular weight 392.90 and purity 99.98%) were kindly supplied as pure samples by Systopic Pharmaceuticals Ltd. (New Delhi, India). Tablets (TRIEXER, Cipla) were obtained commercially with labeled amounts of 500 mg of metformin hydrochloride, 2 mg of glimepiride and 15 mg of piogliazone hydrochloride. HPLC grade water; acetonitrile, methanol, and ammonium acetate were purchased from Fluka analytical, Sigma-Aldrich Corporation, St. Louis, MO, USA.

UHPLC-PDA Conditions

UHPLC was performed with a Waters Acquity UPLC system equipped with a binary solvent manager, an auto-sampler, column manager and PDA detector. Chromatographic separation was performed on a Waters Acquity UPLC BEH C_{18} (100.0 × 2.1 mm, 1.7µm) column. The mobile phase for UPLC analysis consisted of acetonitrile-2 mM ammonium acetate (50:50, v/v) which was filtered through 0.45 mm membrane filter and degassed by sonication. For isocratic elution, the flow rate of the mobile phase was kept at 0.25 mL/min and 10 mL of sample solution was injected in each run. The total chromatographic run time was 3.0 min. PDA detection by carried out by Waters PDA detector at wavelength of 250 nm for all the compounds.

Preparation of standard solutions

Each of the drugs was weighed accurately and transfer to 50 mL volumetric flasks separately. The powders were then dissolved with approximately 25 mL of methanol and ultrasonicated for 5 min. The final volume was made up with methanol. The solutions were further diluted with methanol: water (50:50, v/v) to give a series of standard solutions containing required concentrations for each compound.

Preparation of sample solutions

Twenty tablets were weighed accurately and powdered. Powder equivalent to 500 mg of metformin hydrochloride, 2 mg of glimepiride and 15 mg of pioglitazone hydrochloride was taken and transferred to a 50 mL volumetric flask. The powder was dissolved with approximately 25 mL of methanol and ultra-sonicated for 10 min. The final volume was made up with methanol. This solution was filtered through a 0.45 mm nylon membrane filter to remove all the excipients. The resultant filtrate was further diluted with methanol: water (50:50, v/v) to give adequate concentrations of sample solutions. 20 µL sample solution was injected in to UPLC-PDA system.

Validation of method

The developed method was validated according to ICH validation guidelines.[35] Different standard concentrations each of the compound in the range of 1-1000 ng/mL (1, 10, 50, 100, 200, 500, and 1000 ng/mL) were prepared separately in methanol: water (50:50, v/v). The solutions were filtered through 0.20 µm nylon syringe filter and injected in to the UPLC-PDA system for analysis. Linearity graph was prepared by average peak area of each concentration. Standard stock solutions were diluted appropriately to obtain concentrations for the determination of limit of detection (LOD) and the limit of quantitation (LOQ). Intraday and interday precision was evaluated by analyzing the samples for three consecutive days. The accuracy of the method was determined by standard addition technique. Three different levels (50, 100, and 150%) of standards were added to pre-analyzed tablet sample in six replicates and the mixtures were re-analyzed by the proposed method. The percentage recoveries of all the compounds at each level and each replicate were determined. The mean of percentage recoveries and the RSD (%) was calculated Specificity is the ability of the method to measure the analyte response in the presence of sample components or matrix such as excipients, potential impurities and degradation products. The samples were chromatographed to determine the extent to which mobile phase components and excipients could contribute to the interference with the analytes. Robustness is the ability of the method to remain unaffected by small and deliberate variations in the method parameters and provides an indication of its reliability for routine analysis. The robustness was determined by analyzing the sample solution containing appropriate concentrations all three drugs under a variety of conditions of the method parameters, such as flow rate, mobile phase composition, column temperature, and injection volume. The mean of percentage recoveries and the RSD (%) was calculated from six replicates.

Analysis of marketed fdc tablets

The procedure for analysis of marketed combined (FDC) tablets was similar as described in preparation of sample solution. The amount of metformin hydrochloride, glimepiride and pioglitazone hydrochloride in tablets was determined by calibration equations obtained from their respective calibration plots.

RESULTS AND DISCUSSION

The isocratic mobile phase containing acetonitrile-2mM ammonium acetate (50:50, v/v) at a flow rate of 0.25 mL/min provide peaks with short retention times. All the drugs were shown the significant absorption at 250 nm and hence it was selected for identification all the drugs. The retention time was found to be 0.75 min for

Metformin hydrochloride, 1.50 min for Glimepiride, 2.50 min for Pioglitazone hydrochloride as shown in **Figure 1.** The total chromatographic run time was 3.0 min for each compound.

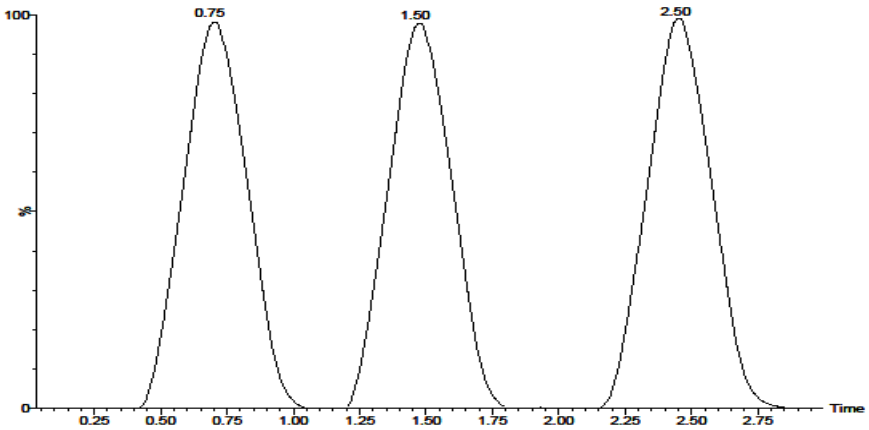

Figure 1: UPLC-PDA Chromatogram obtained from mixed standards (1 ng/mL each) of Metformin hydrochloride (R_t 0.75 min), Glimepiride (R_t 1.50 min) and Pioglitazone hydrochloride (R_t 2.50 min)

Validation of the method

Linearity and Range, LOD and LOQ

The linear calibration plot was obtained over the concentration range of 1-1000 ng/mL for all the compounds with correlation coefficient was more than 0.999. The results exhibited that an excellent correlation existed between the peak area and concentration ranges as stated for all the compounds. The results obtained from Linearity, LOD, and LOQ is presented in **Table 1**. The obtained results indicated that higher sensitivity of the method.

Table 1: Results Obtained from Linearity, LOD, and LOQ.

Parameters	Metformin Hydrochloride	Glimepiride	Pioglitazone Hydrochloride
Linear range (ng/mL)	1-1000	1-1000	1-1000
Correlation coefficient	0.9997	0.9998	0.9998
LOD (ng/mL)	0.01	0.01	0.01
LOQ (ng/mL)	1	1	1

Precision, Accuracy, Robustness and Specificity

The RSD less than 2% were obtained for all the compounds by evaluation of intraday, interday, and different analysts precision suggested that an acceptable precision of the method. The recoveries of all the compounds was found to be in the range 98-101% with RSD less than 2%, indicating that accuracy of the method was adequate. The results obtained from Recovery Studies and Precision is presented in **Table 2.** The method was found to be robust with respect to variability in chromatographic conditions. The retention times and peak area of each compound did not change significantly when mobile phase composition, flow rate, injection volume, and column temperature were deliberately modified. The comparison of the chromatograms of the blank and sample solutions indicated that no interferences were detected from mobile phase components and excipients of the formulation, suggested that method was specific.

Table 2: Results Obtained from Recovery Studies and Precision.

Drugs	Conc. Added (ng/mL)	Conc. Found (ng/mL)	Recovery (%)	RSD (%)	
				Intraday	Interday
Metformin Hydrochloride	250	248.95	99.58	1.25	0.98
	500	500.50	100.10	1.54	1.12
Glimepiride	100	99.98	99.98	1.75	1.55
	200	199.97	99.99	1.24	1.25
Pioglitazone Hydrochloride	150	150.12	100.08	1.44	1.92
	300	299.50	99.83	1.85	1.74

Analysis of marketed tablets

The validated method was applied for the determination of metformin hydrochloride, glimepiride and pioglitazone hydrochloride in commercially available fixed dose combination (FDC) tablets containing 500 mg of metformin hydrochloride, 2mg of glimepiride and 15 mg of pioglitazone hydrochloride. The content of drugs from the tablets was in between 98.00 to 99.98% with

RSD 1.75%. The low values of RSD indicated that method was suitable for routine analysis of drugs in tablets without any interference from excipients.

CONCLUSION

The simple UHPLC-PDA method was developed, validated and applied for simultaneous determination of metformin hydrochloride, glimepiride and pioglitazone hydrochloride in their combined tablet dosage forms. The obtained chromatograms of these drugs indicated that all the drugs were well separated with higher sensitivity and selectivity. The advantages of our developed method are simple, cost effective, all compounds are separated in single chromatographic run time (3 min), and high sensitivity (LOQ: 1.0 ng/mL for all the compounds). Hence, it is suggested that the method should be applied for routine quality control analysis and during stability studies of all drugs in their bulk drugs and in combined tablets.

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