Development and Validation of Liquid Chromatographic and UV Derivative Spectrophotometric Methods for the Determination of Metformin, Pioglitazone and Glimepiride in Pharmaceutical Formulations

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Abstract

A high-performance liquid chromatographic method and a UV derivative spectrophotometric method for the simultaneous determination of metformin (MFN), pioglitazone (PLZ) and glimepiride (GLM), in tablets were developed in the present work. The various parameters, such as linearity, precision, accuracy, specificity, robustness, limit of detection and limit of quantitation were studied according to International Conference on Harmonization guidelines. HPLC was carried out by using the reversed-phase technique on an phenomenex RP-18 column (150x 4.6mm, 5µ) with a mobile phase consisted of acetonitrile and phosphate buffer (pH 3) in the ratio of 65: 35. The flow rate was fixed at 0.5ml/min and the drugs were monitored at 245nm with UV dual absorbance detector and the elution time was found less than 10 min indicates shorter analysis time. The first derivative UV spectrophotometric method was performed at 260.1, 280.7 and 251.5nm for MFN, PLZ and GLM respectively. Statistical analysis was done by Student's t-test and F-test, which showed no significant difference between the results obtained by the two methods. The proposed methods are highly sensitive, precise and accurate and therefore can be used for its intended purpose.

Keywords: Anti-diabetic drugs, HPLC, UV derivative spectrophotometry, validation, pharmaceutical dosage form

Introduction

Chemically, metformin is 1,1-dimethyl biguanide hydrochloride, pioglitazone is (±)-5-[p- [2-(5-ethyl-2-pyridyl)-ethoxy] benzyl]-2,4-thiazolidinedione where as glimepiride is 1-(4-(2-(3-ethyl-4-methyl-2-oxo-2,5-dihydro-1H-pyrrole-1-carboxamido)ethyl)phenylsulfonyl)-3-(4-methyl cyclo hexyl)urea[1] (structures shown in figure 1a, 1b and 1c). Metformin improves hepatic and peripheral tissue sensitivity to insulin without the problem of serious lactic acidosis, pioglitazone has been shown to affect abnormal glucose and lipid metabolism associated with insulin

resistance by enhancing insulin action on peripheral tissues where as glimepiride is a sulfonyl urea group oral anti-diabetic drug with prolonged effect and more over it maintains a more physiological regulation of insulin secretion than glibenclamide during physical exercise, suggesting that there may be less risk of hypoglycaemia with glimepiride, and act by increasing the secretion of insulin by the functioning β-cells of the pancreas[2]. This combination can be achieved by taking each of the drugs separately or alternatively fixed formulations have been developed. A combination tablet formulation is beneficial in terms of its convenience and patient compliance.

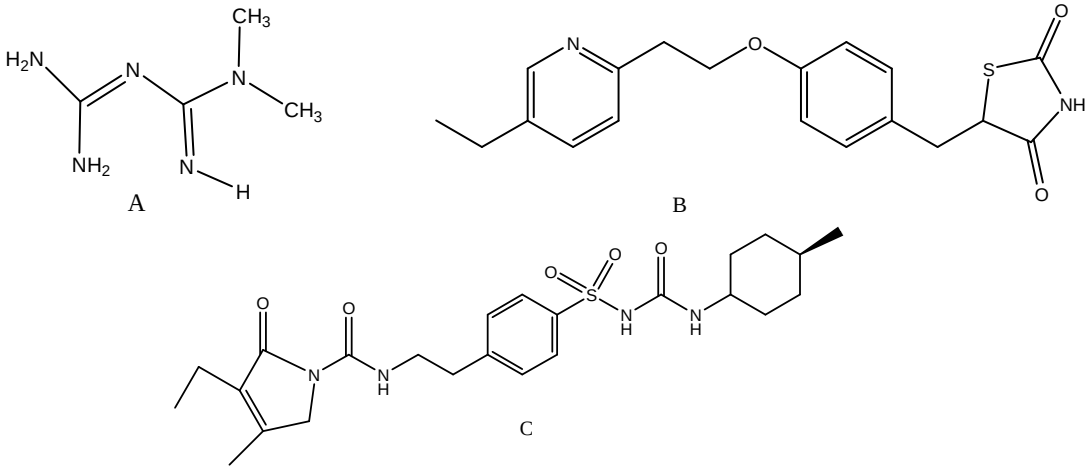

Fig. 1 Structures of anti-diabetic drugs (a) Metformin, (b) Pioglitazone and (c) Glimepiride

The review of literature reveals that there were analytical methods of all the three drugs individually in pharmaceutical dosage forms and even in biological samples [3-11] and a few methods reported for combination of either of the two drugs [12-18]. But no method was reported for these drugs as per our knowledge except a simple RP-HPLC method [19] in which there was a variation of ±0.5min for retention times of the drugs. The present paper describes both HPLC and UVDS methods for the determination of metformin, pioglitazone and glimepiride in pharmaceutical dosage forms.

Results and Discussion

HPLC method

A reversed-phase HPLC method was proposed as a suitable method for the estimation of MFN, PLZ and GLM in pharmaceutical dosage forms. The chromatographic conditions were adjusted in order to provide a good performance of the assay. The HPLC procedure was optimized with a view to develop an accurate and reproducible method so as to resolve thee drugs from each other. Various conditions such as mobile phase compositions, analyzing columns with different packing materials (C18, C8, phenyl), and configurations (10, 15, 25 cm columns) were tested so as to obtain a sharp peak and also to resolve the peak of internal standard. Mobile phase was

selected from peak parameters (symmetry, tailing), run time, easy of preparation and cost. Figure 2 shows a typical chromatogram obtained from the standard MFN, PLZ and GLM solution using the proposed method. As shown in this figure, MFN, PLZ and GLM were eluted forming symmetrical peak, well separated from each other. The retention time observed were 2.75, 4.35 and 8.75 min for MFN, PLZ and GLM respectively, and allows a rapid determination of the drugs (less than 10 min), which is important for routine analysis. From the peak of drug, the mobile phase consisting of acetonitrile and phosphate buffer (pH 3) in the ratio 65: 35 (%v/v), found to be an appropriate mobile phase on the column used at a flow rate of 0.5ml/min. In the proposed system MFN, PLZ and GLM peaks were eluted with a capacity factor (k^1) 3.75, 4.73 and 6.49, tailing factor (*T*) 1.2, 1.31 and 1.12 respectively. The calibration curves for MFN, PLZ and GLM were constructed by plotting concentration *versus* peak area ratio, and showed good linearity in the 0.25-25 µg/ml range. The representative correlation coefficient (r^2=0.9991±0.0005) for all the three drugs indicating a high sensitivity of the method (Table 1). The LOD were found to be 0.052, 0.061 and 0.058 and LOQ were 0.19, 0.21 and 0.20µg/ml, for MFN, PLZ and GLM respectively. The precision of this method was determined by repeatability (intra-day) and intermediate precision (inter-day) and was expressed as R.S.D. (%) of a series of measurement. The experimental values obtained for the determination of MFN, PLZ and GLM in samples are presented in Table 2. The result obtained shows R.S.D. of 0.26%, indicating good intra-day precision. Inter-day variability was also calculated from assays on 3 d a mean R.S.D was 0.24%. The mean recovery was found to be 98.79 for Pioz MF-G (MFN-500mg, PLZ-15mg and GLM-1mg), 98.84 for Matce-PG 2 (MFn-500mg, PLZ-15mg and GLM-2mg), and 99.05 for Glamor-PM (MFN-500mg, PLZ-15mg and GLM-2mg) (Table 3), indicating an agreement between the true value and the value found.

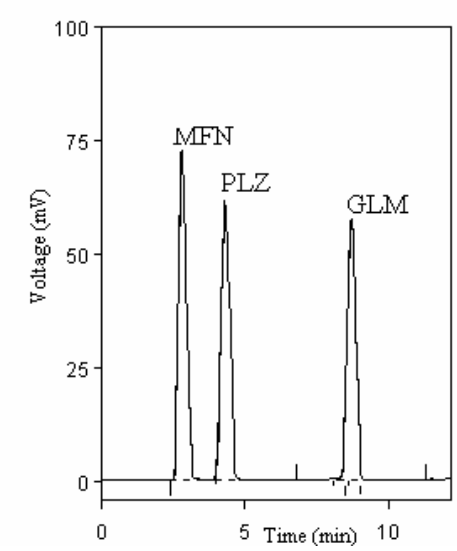

Fig 2: Typical Chromatogram showing Metformin (2.75min), Pioglitazone (4.35min) and Glimepiride (8.75min)

Table-1 Results of the analysis of the data for the quantitative determination of Metformin, Pioglitazone and Glimepiride by the proposed methods

Statistical parameter	HPLC			UVDS		
	MFN	**PLZ**	**GLM**	**MFN**	**PLZ**	**GLM**
Concentration range (µg/ml)	0.25-25	0.25-25	0.25-25	0.5-50	0.5-50	0.5-50
Correlation coefficient (r^2)	0.9992	0.9989	0.9991	0.9990	0.9986	0.9992
Standard error on estimation (S_c)	-0.02271	0.03127	0.02915	0.01571	-0.03412	-0.02777
Standard deviation on slope (S_b)	0.0008	0.0010	0.0006	0.0002	0.0005	0.0008
Standard deviation on intercept (S_a)	0.0169	0.0147	0.0171	0.0132	0.0113	0.0124
Limit of detection LOD (*µg/ml*)	0.052	0.061	0.058	0.09	0.11	0.13
Limit of quantification LOQ (*µg/ml*)	0.19	0.21	0.20	0.35	0.39	0.44

Table-2 Results of the determination of the drugs by the proposed methods (n=3)

Method		% Purity			R.S.D		
		MFN	PLZ	GLM	MFN	PLZ	GLM
HPLC	1 d	100.11	99.89	99.67	0.266	0.245	0.251
	2 d	100.08	99.67	99.55	0.219	0.240	0.216
	3 d	99.87	99.29	99.32	0.264	0.209	0.191
UVDS	1 d	100.08	100.01	99.87	0.481	0.462	0.512
	2 d	99.56	99.49	99.67	0.612	0.509	0.591
	3 d	99.23	98.97	98.56	0.681	0.587	0.556

Experimental amount was selected based on the ratio of drugs as per the label claim in formulations i.e, 500: 15: 1 for MFN, PLZ and GLM respectively

UVDS Method

The overlay spectrum of a 40 µg/ml MFN, PLZ and GLM solution in methanol (against a blank of the same) is shown in Fig. 3. One particular wavelength was selected for each drug such that the value for the other was found zero. Several assays were carried out using the first, second, third and fourth derivative of the spectra, and the best results were obtained when using the amplitude from the valley at the wavelengths of 260.1, 280.7 and 251.5nm for MFN, PLZ and GLM respectively, to the zero base line. With first derivative spectra good linearity was obtained on standard solutions of MFN, PLZ and GLM over the 0.5-50µg/ml concentration range. The linearity equations was y=-0.00174x-0.01820, y=-0.035x-0.002 and y=-0.0025x-0.00191 for MFN, PLZ and GLM respectively (r^2=0.9986±0.0007), where x is the concentration of MFN, PLZ and GLM (expressed as m g/ml) and y is the amplitude from the valley at a wavelength of 260.1, 280.7 and 251.5nm for MFN, PLZ and GLM respectively to the zero base line was chosen. Precision assessed on the standard solutions was satisfactory; R.S.D. % values of 0.46% (repeatability) and 0.68% (intermediate precision) were found for five replicates at a concentration of 500 µg/ml. The first derivative spectra of formulation sample solutions (Fig. 3) are morphologically identical to those of the standard solutions. The results obtained shows R.S.D of 0.46 indicating good intraday precision. Inter-day variability was calculated from assays on 3 d and a mean R.S.D. was found to be 0.68 (Table 2). Accuracy was calculated adding known amounts of MFN, PLZ and GLM pure substance to powdered formulations, obtaining additions of 25, 50, 75, 100, 125 µg/ml (total concentrations: 25, 50, 75, 100, 125 µg/ml). As seen from Tables 2 and 3, all assays gave satisfactory results: the mean amount found of declared was always between 97.5 and 99.26% for all formulations, while precision R.S.D.%

values were always under 1.3% and accuracy above 98.6%. The LOQ were 0.35, 0.39, 0.44 µg/ml and the LOD were 0.09, 0.11 and 0.13 µg/ml, according to ICH guidelines [20].

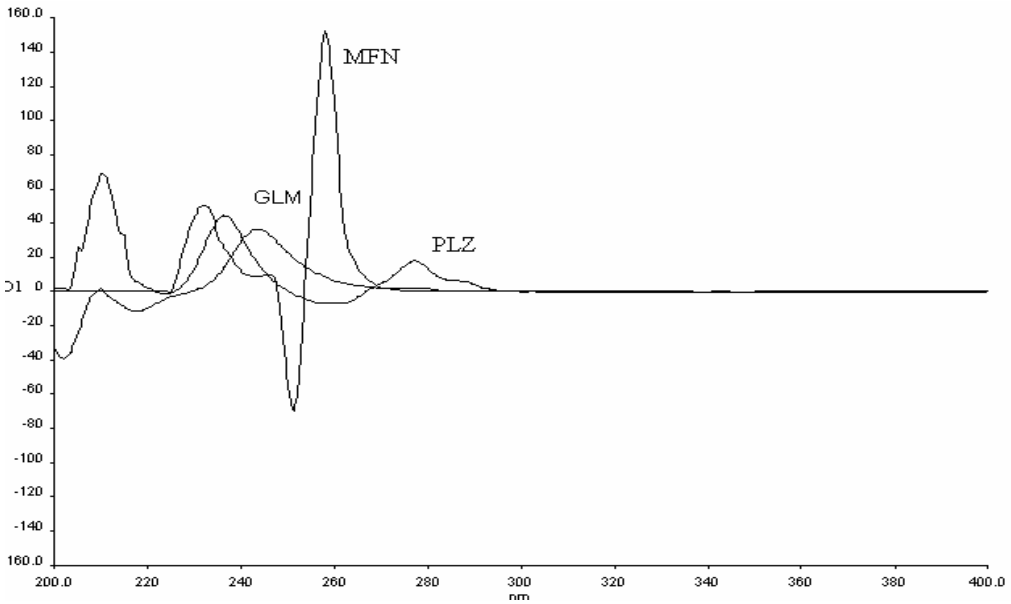

Fig 3 Overlay first derivative UV spectrum of Metformin, Pioglitazone and Glimepiride.

Table-3 Results of the determination of Metformin, Pioglitazone and Glimepiride in tablets

Formulation		amount	HPLC		UVDS	
		present (mg)	found (mg)	% recovery	found (mg)	% recovery
Pioz MF-G	MFN	500	491.23	98.25	490.87	98.17
	PLZ	15	14.87	99.13	14.85	99.00
	GLM	1	0.99	99.00	0.986	98.60
Matce-PG 2	MFN	500	495.53	99.11	492.35	98.47
	PLZ	15	14.91	99.40	14.89	99.26
	GLM	2	1.96	98.00	1.97	98.50
Glamor-PM	MFN	500	490.11	98.02	490.12	98.02
	PLZ	15	15.02	100.13	14.88	99.20
	GLM	2	1.98	99.00	1.95	97.50

Comparison between HPLC Method and UVDS Method

The Student's *t*-test was applied and does not reveal significant difference between the experimental values obtained in the sample analysis by the two methods. The calculated *t*-value and *F*-value was found to be less than the tabular values at 95% confidence limits (Table 4).

Table-4 Results obtained in the comparison of HPLC and UVDS methods

Sample	% RSD (HPLC)	% RSD (UVDS)	*F*-test[a]	*t*-test[a]
MFN	0.7912	0.8154	1.061	0.3724
PLZ	0.8187	0.8217	1.348	0.1950
GLM	0.9797	1.6241	0.366	0.4356

[a]value at 95% confidence

Materials and Methods

Chemicals and reagents

Drug samples were obtained from Orchid Chemicals and Pharmaceuticals Ltd., Chennai, India. Pharmaceutical dosage forms (Glamor-PM, Matce-PG, Pioz MF-G) containing MFN, PLZ and GLM were obtained commercially. Acetonitrile HPLC grade (Rankem, New Delhi, India) potassium dihydrogenphosphate (A.R. grade), and orthophosphoric acid (A.R. grade) were obtained from Qualigens (Mumbai, India). Ultra pure water was obtained from a Milli-Q® UF-Plus apparatus (Millipore). Methanol (Qualigens) was used to prepare all solutions for the UVDS method. All solutions were prepared daily.

Instrumentation and Analytical Conditions

The HPLC method was performed on a Shimadzu Class LC-10AT vp and LC-20AD pumps connected with SPD-10A vp UV-Visible detector. The data acquisition was performed by Spincotech software version 1.7. The method was conducted using a reversed-phase technique. Drugs were eluted isocratically with a flow rate of 0.5 ml/min using a mobile phase consisting of acetonitrile and phosphate buffer (pH 3) in the ratio of 65: 35. The wavelength of the UV detector was set to 245 nm. The mobile phase was prepared daily, filtered through a 0.45- µm membrane filter (Millipore) and sonicated before use. A phenomenex C_{18} analytical column (150mmx4.6 mm i.d., 5µm particle size) was used. The HPLC system was operated at ambient temperature. UVDS method was performed on a UV-visible Spectrophotometer (model Perkin Elmer lambda 25) at 260, 280 and 251.5nm for MFN, PLZ and GLM respectively using 1.0 cm quartz cells and UV Winlamb version 2.8.04 software was used for all absorbance measurements.

Preparation of the Standard Solutions

HPLC: Accurately weighed 25 mg of MFN, PLZ and GLM reference standards were transferred to 25 ml volumetric flask and dissolved in methanol HPLC grade (to get a final concentration of 1 mg/ml). From this solution, working standard solutions 100 µg/ml was prepared. The concentrations in the range of 0.25-25 µg/ml were made in 10 ml volumetric flasks and the volume was adjusted with mobile phase.

UVDS: Accurately weighed 25 mg of reference standards were transferred to 25 ml volumetric flask and dissolved in methanol AR grade (to get a final concentration of 1 mg/ml). From this solution, the concentrations in the range of 0.5-50 µg/ml were made in 10 ml volumetric flasks and volume was adjusted with methanol.

*Preparation of Samples from Tablets***:** About 20 tablets were weighed and thoroughly powdered. The amount of powder equivalent to labeled claim of the drugs was placed in a volumetric flask. To it around 20ml of solvent (methanol) was added and the flask was placed in an ultrasonic bath for 15 min. The solution was then cooled and diluted to volume with the same solvent. The solution was filtered though a 0.45 µm filter and then the filtrate were used to prepare sample solutions of different concentrations.

Conditions **:** HPLC: HPLC separation was carried out by a phenomenex C_{18}, 5 µm, 150 x 4.6 mm column. The mobile phase flow rate was 0.5 ml min^{-1}. The analysis was carried out at ambient temperature. The sample injection volume was 20 µl. The UV detection was carried out at 245nm for the determination of three drugs.

UVDS: For drugs solutions, the first derivative spectra were recorded in the wavelength range 200-400 nm using methanol as reference. The instrument settings were optimized to produce a spectrum with about 80% full-scale deflection and acceptable noise level. Each spectrum was recorded in triplicate. For each replicate measurement the cell was refilled with fresh solution.

Method Validation: The methods were validated according to International Conference on Harmonization guidelines for validation of analytical procedures [20]. Student's *t*-test and *F*-test were used to verify the validity of the methods.

Linearity: The calibration curve was tested with five concentrations of the standard solutions, as 0.25-25µg/ml for HPLC method and 0.5-50 µg/ml for UVDS method, respectively. The solutions were prepared in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Precision: The precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability was evaluated by assaying samples, at same concentration and during the same day. The intermediate precision was studied by comparing the assays on different days (3 d). Five sample solutions were prepared and assayed.

Robustness: The robustness of the HPLC method was determined by analysis of samples under a variety of conditions such as small changes in the pH (2.8-3.4) and in the percentage of

acetonitrile (60-70%) in mobile phase and changes in flow rate (0.3-0.8ml/min). The effect on retention time and peak parameters were studied.

Limit of Detection and Limit of Quantitation: The parameters LOD and LOQ were determined on the basis of response and slope of the regression equation.

Conclusion

The two proposed methods based on the UVDS and HPLC, are suitable for determination of MFN, PLZ and GLM in the commercial tablets. The methods are simple, reliable, fast and reproducible. The spectrophotometric method requires only wavelength scan and automatic calculation of the first derivative value, while the HPLC was less than ten minutes. Furthermore, the proposed methods are inexpensive and low polluting, because small volumes are required for preparation of samples.

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