Simultaneous Estimation of Metformin Hydrochloride, Pioglitazone Hydrochloride, and Glimepiride by RP-HPLC in Tablet Formulation

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Abstract

A simple, precise, rapid, and reproducible reversed-phase high-performance liquid chromatography method is developed for the simultaneous estimation of metformin hydrochloride (MET), pioglitazone hydrochloride (PIO), and glimepiride (GLP) present in multicomponent dosage forms. Chromatography is carried out isocratically at 25°C ± 0.5°C on an Inertsil-ODS-3 (C-18) Column (250 × 4.60 mm, 5 µm) with a mobile phase composed of methanol–phosphate buffer (pH 4.3) in the ratio of 75:25 v/v at a flow rate of 1 mL/min. Detection is carried out using a UV-PDA detector at 258 nm. Parameters such as linearity, precision, accuracy, recovery, specificity, and ruggedness are studied as reported in the International Conference on Harmonization guidelines. The retention times for MET, PIO, and GLP are 2.66 + 0.5 min, 7.12 + 0.5 min, and 10.17 + 0.5 min, respectively. The linearity range and percentage recoveries for MET, PIO, and GLP are 10–5000, 10–150, and 1–10 µg/mL and 100.4%, 100.06%, and 100.2%, respectively. The correlation coefficients for all components are close to 1. The relative standard deviations for three replicate measurements in three concentrations of samples in tablets are always less than 2%.

Introduction

Metformin hydrochloride (MET) (*N*,*N*-dimethylimidodicarbonimidic diamide hydrochloride) (Figure 1A) is an orally administered biguanide widely used in the treatment of type 2 (non-insulin dependent) diabetes mellitus. It improves hepatic and peripheral tissue sensitivity to insulin without the problem of serious lactic acidosis commonly found with its analogue, phenformin. MET is a hydrophilic drug with an oral bioavailability of 50–60% and a relatively short half-life of 1.5–4.5 h (1).

Pioglitazone hydrochloride (PIO) [(±)-5-[[4-[2-(5-ethyl-2-pyridinyl) ethoxy] phenyl] methyl]-2,4-] thiazolidine-dione monohydrochloride (Figure 1B) is an oral anti-hyperglycemic agent which acts primarily by decreasing insulin resistance. It is used in the treatment of type-II diabetes mellitus (*2*).

Glimepiride (GLP) 1-[[*p*-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)ethyl]-phenyl]-sulfonyl]-3-(*trans*-4-methylcyclohexyl) urea (Figure 1C) is a new oral anti-diabetic drug in the sulfonylurea class, with the advantage of being completely bioavailable, being effective at low doses in patients with non-

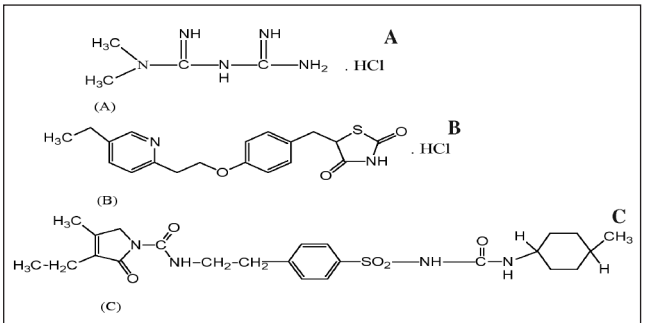

Figure 1. Chemical structures of MET HCl (A), PIO HCl (B), GLP (C).

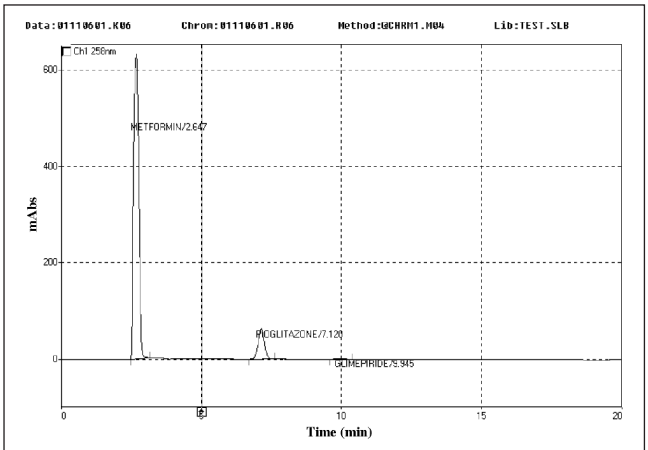

Figure 2. Representative chromatogram of MET, PIO, and GLP.

Table I. System Suitability

Serial No.	Parameters	MET	PIO	GLP
1	No. of theoretical plates	991	4599	4907
2	HETP	0.251	0.055	0.051
3	Tailing factor	1.13	1.04	0.97

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insulin-dependent diabetes mellitus, showing linear pharmacokinetics, and having a prolonged effect. As with the other sulphonylureas, glimepiride appears to lower blood glucose levels by stimulating insulin release from the pancreas (3).

Tablet dosage forms containing MET, PIO, and GLP in ratio of: 500 mg, 15 mg, and 1 mg; and 500 mg, 15 mg, and 2 mg, respectively, of various brands are available in market. MET has been reported to be determined by HPLC (4,5) from formulations and in biological fluids. PIO determination has been done by high-performance liquid chromatography (HPLC) (6,7) and liquid chromatography (LC)–mass spectrometry (MS)–MS (8) in a variety of samples, while GLP determinations have been reported by UV derivative spectrophotometry (9), HPLC (10), and LC–MS–MS (11). Simultaneous determination of GLP, PIO (12), and MET, PIO (13) in pharmaceutical dosage forms was reported by HPLC. However, there is no method available for the simultaneous determination of these three drugs. Therefore, an attempt was made to develop a new, rapid, and sensitive method for the simultaneous determination of MET, PIO, and GLP. To access the reproducibility and wide applicability of the developed method, it was validated as per ICH norm, which is also mandatory (14–15)

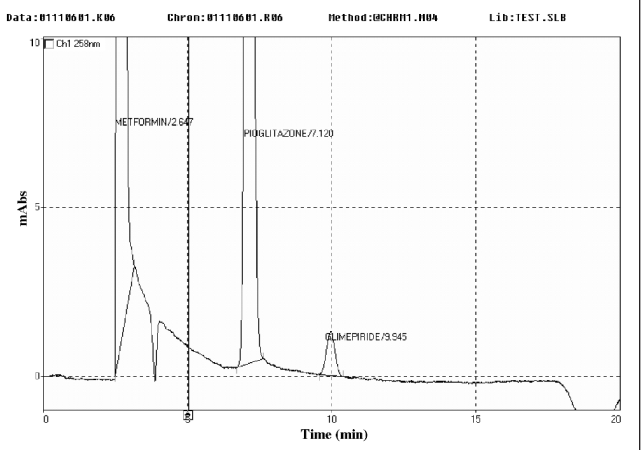

Figure 3. Representative chromatogram of Metformin, Pioglitazone, and Glimepiride (by minimizing the scale).

Table II. Results of Recovery Experiments

Serial. No.	Conc. of drug in preanalyzed samples (µg/mL)			Std. drug sol. Added (µg/mL)			Recovered amount* (µg/mL)			%Recovered		
	MET	PIO	GLP	MET	PIO	GLP	MET	PIO	GLP	MET	PIO	GLP
1	1000	30	2	1000	30	2	1003.4	30.02	2.2	100.3	100.0	100.2
2	2000	60	4	2000	60	4	2001.1	59.56	4.1	100.0	97.6	100.3
3	3000	90	6	3000	90	6	3002.5	89.92	6.3	100.0	99.9	100.2
									Mean	100.1	99.16	100.23
									S.D.	0.17	1.35	0.057
									%RSD	0.16	1.36	0.056

* Mean of three readings.

Table III. Results of Precision

Serial. no.	Validation parameter	% Mean*			S.D.			% R.S.D.		
		MET	PIO	GLP	MET	PIO	GLP	MET	PIO	GLP
1	Repeatability	100.5	99.1	97.69	0.39	0.20	0.68	0.38	0.2	0.69
2	Intermediate precision (day to day)	100.4	99.19	98.60	0.31	0.26	1.10	0.31	0.27	1.11
3	Intermediate precision (analyst to analyst)	100.6	99.33	100.6	0.30	0.52	0.99	0.29	0.52	0.98

* Mean of fifteen determinations (3 replicates at 5 concentration levels).

Experimental

Instrumentation

The LC system was from Shimadzu (Kyoto, Japan) and was comprised of a manual injector, double reciprocating plunger pump LC10 ATvp for constant flow and constant pressure delivery, and a photodiode array (PDA) detector SPD-M10 Avp connected to software Class M10A for controlling the instrumentation as well as processing the data generated was used.

Reagents and chemicals

PIO and GLP were obtained as pure samples from Cadila Health Care, Ahemdabad and MET Hydrochloride was obtained from Ranbaxy Labs, Dewas as a gift sample. Acetonitrile, methanol, and glacial acetic acid were of HPLC grade and supplied by Merck Ltd., India. Triple distilled water was generated in house. Tablets, Tribet-1, and Tribet-2 of Nicholas Piramal India Limited containing MET, PIO, and GLP in ratio of: 500 mg, 15 mg, and 1 mg; and 500 mg, 15 mg, 2 mg, respectively, were purchased from a local market.

Chromatographic condition

The isocratic mobile phase consisted of methanol–phosphate buffer (pH 4.3) in the ratio of 75:25, v/v, flowing through the column at a constant flow rate of 1.0 mL/min. An Inertsil-ODS-3 (C-18) Column (250 × 4.60 mm, 5 µm) was used as the stationary phase. MET, PIO, and GLP have different λ_{max} (viz 235, 265, and 227 nm, respectively), but considering the chromatographic parameter, sensitivity, and selectivity of the method for all three drugs, 258 nm was selected as the detection wavelength for UV–PDA detector.

Standard preparation

Standard stock solution

Standard stock solutions of 10000, 1500,

1000 µg/mL of MET, PIO, and GLP were prepared in methanol respectively.

Working standard solution

Working standard solutions were prepared by taking dilutions ranging from 10–5000, 10–150, 1–10 µg/mL for MET, PIO, and GLP, respectively.

Sample preparation

Twenty tablets of Tribet-1 and Tribet-2 of Nicholas Piramal India Limited containing MET, PIO, and GLP: 500 mg, 15 mg, and 1 mg; 500 mg, 15 mg, and 2 mg, respectively, were weighed and crushed to fine powder. Powder equivalent to 500 mg MET was weighed and dissolved in 100 mL methanol, sonicated for 10 min, and filtered through Whatmann filter paper No. 42; finally, different concentrations of tablet sample were prepared by the serial dilution technique.

Results and Discussion

Chromatography

Initially, reversed-phase LC separation was tried using methanol and water (75:25) as the mobile phase, in which GLP gave tailing of 2.6, although the other two drugs responded properly, and the resolution was also poor. The organic content of mobile phase was also investigated to optimize the separation of PIO and GLP. To improve the tailing factor, the pH of mobile phase becomes an important factor. At pH 6.4, the signal-to-noise ratio for GLP was less, and the retention time was also 14 min. Thereafter, methanol–phosphate buffer of pH 4.3 in the ratio of 75:25 v/v was selected to improve the resolution, and the tailing for the three peaks were reduced considerably and brought close to 1, and the retention time of GLP was also reduced to 10 min. To analyze these three drugs, detection were tried at various wavelengths: from 233 nm to 260nm. Initially, 233 nm was selected, considering the λ_{max} of three drugs (λ_{max} of MET 235 nm, λ_{max} of PIO 227 nm and 265 nm, and λ_{max} of GLP 227).

At 233 nm, MET was found to merge with a component, which is structurally similar to MET, as it showed a similar spectra but did not absorb at 258 nm. Because of this component, base-to-base separation between MET and PIO was also not observed below 258 nm.

The spectra of MET extended from below 200 nm to 267 nm. Although the absorbance is less at 258 nm, it is considerable, and secondly, the concentration of MET in combination is also very high.

Therefore, 258 nm was found to be suitable where all the three drugs could be detected simultaneously. The sensitivity of the detector is 0.5.

The concentration of GLP is low, hence the AUC is not noticeable in comparison to MET and PIO; therefore, the peak is not clearly visible on the same scale in chromatogram (Figure 2). By minimizing the scale, the peak corresponding to GLP is clearly visible (Figure 3).

Table IV. Results of Robustness

Serial. no.	Validation parameter	% Mean*			S.D.			% R.S.D.		
		MET	PIO	GLP	MET	PIO	GLP	MET	PIO	GLP
1	Robustness	98.87	100.59	100.22	0.64	0.99	1.30	0.64	0.98	1.29

* Mean of six determinations.

Table V. Stability Data of MET, PIO, and GLP

Hours	AUC ± %RSD		
	MET (1000 µg/mL)	PIO (30 µg/mL)	GLP (2 µg/mL)
0	3858142 ± 0.31	483857 ± 0.53	15474 ± 1.25
6	3858231 ± 0.42	483745 ± 0.68	15467 ± 2.23
12	3858443 ± 0.35	483687 ± 0.93	15479 ± 2.25

System suitability

System suitability parameters, such as number of theoretical plates, HETP, and peak tailing, are determined. The results obtained are shown in Table I. The number of theoretical plates for MET, PIO, and GLP were 991, 4599, and 4907, respectively.

Linearity

MET, PIO, and GLP showed a linearity of response between 10–5000, 10–150, and 1–10 µg/mL, respectively. The linearity was represented by a linear regression equation as follows.

Y (MET) = 3706.27 conc. + 98586.40 (r^2 = 0.9998)

Y (PIO)= 16231.16conc. + 5021.31 (r^2 = 0.9981)

Y (GP) = 7647.59conc. + 41.12 (r^2 = 0.9995)

Table VI. Results of the HPLC Analysis for Tablets

Serial. no.	Parameters	TRIBET-1			TRIBET-2		
		MET	PIO	GLP	MET	PIO	GLP
1	% Mean*	100.7	99.95	98.77	100.06	98.68	98.88
2	S.D.	0.44	1.21	0.77	0.40	0.98	1.6
3	% R.S.D	0.43	1.20	0.78	0.39	0.99	1.63
4	SEσ	0.17	0.49	0.31	0.16	0.39	0.65

* Mean of fifteen determinations (3 replicates at 5 concentration levels).

Accuracy

Recovery studies were performed to validate the accuracy of the developed method. To a pre-analyzed sample solution, a definite concentration of standard drug was added, and the recovery was studied. These results are summarized in Table II.

Precision

Repeatability

Five dilutions in three replicates were analyzed in same day for repeatability, and the results were found within acceptable limits (RSD < 2), as shown in Table III.

Intermediate precision

Five dilutions in three replicates were analyzed on two different days and by two analysts for day-to-day and analyst-to-analyst variation. Although the relative standard deviation (RSD) value for GLP is higher than that of MET and PIO, this is because of its low concentration; however, all results fall within acceptable limits (RSD < 2), as shown in Table III.

Robustness

As per ICH norms, small but deliberate variations, by altering the pH or concentration of the mobile phase, were made to check the method's capacity to remain unaffected (method stability). The change was made in the ratio of mobile phase: instead of methanol–phosphate buffer (pH 4.3) (75:25 v/v), methanol–phosphate buffer (pH 4.3) (70:30 v/v) was used as the mobile phase. Results of analysis are summarized in Table IV.

Stability of sample solution

The sample solution injected after 12 h did not show any appreciable change. Results are shown in Table V.

Tablet analysis

Contents of MET, PIO, and GLP found in the tablets by the proposed method are shown in Table VI. The low RSD values indicate that the method is precise and accurate.

Conclusions

An RP-HPLC method was developed and validated for simultaneous estimation of MET, PIO, and GLP in tablet dosage form. The proposed method is fast, accurate, precise, and sensitive, as it could estimate GLP concentration, which is far less when compared to the other two components; hence, it can be employed for routine quality control of tablets containing these three drugs in industries.

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Manuscript received February 7, 2007;
revision received July 7, 2007.