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A RP-HPLC method for simultaneous estimation of metformin and pioglitazone in pharmaceutical formulation

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ABSTRACT

A simple, selective, rapid, precise and economical reverse phase HPLC method has been developed for the simultaneous estimation of metformin and pioglitazone from pharmaceutical dosage forms. The method was carried out on a phenomenex C_{18} (25 cm x 4.6 mm i.d., 5 µ) column with a mobile phase consisting of acetonitrile: phosphate buffer (adjusted to pH 5.0 using orthophosphoric acid) (50:50 v/v) at a flow rate of 1. ml/min. Detection was carried out at 258 nm. Etoricoxib was used as an internal standard. The retention time of paracetamol, aceclofenac and etoricoxib was 4.75, 6.44 and 8.83 min, respectively. The developed method was validated in terms of accuracy, precision, linearity, limit of detection, limit of quantitation and solution stability. The proposed method can be used for the estimation of these drugs in combined dosage forms.

Keywords: RP-HPLC, metformin, pioglitazone

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INTRODUCTION

Metformin hydrochloride (*N,N*-dimethylimidodicarbonimidic diamide hydrochloride}, Pioglitazone[(±)-5-[[4-[2-(5-ethyl-2-pyridinyl)ethoxy]phenyl]methyl]-2,4-]thiazolidine-dione monohydrochloride. It is used as an analgesic and antipyretic. Many methods have been described in the literature for the determination of Metformin and Pioglitazone individually and in combination with other drugs [1-11]. However, there is no HPLC method reported for the simultaneous estimation of these drugs in combined dosage forms. Fixed dose combination containing Metformin (500 mg) and Pioglitazone (15 mg) is available in the tablet form in the market. The aim of this work was to develop an RP-HPLC method with ultraviolet detection for the simultaneous determination of Metformin and Pioglitazone in pharmaceutical dosage forms. The present RP-HPLC method was validated following the ICH guidelines [12,13].

MATERIALS AND METHODS

Acetonitrile HPLC grade was procured from E.merck (India) Ltd, Mumbai. Disodium hydrogen orthophosphate and orthophosphoric acid AR grade were procured from Qualigens fine chemicals, Mumbai. Water HPLC grade was obtained from a Milli-QRO water purification system. Reference standard of metformin and pioglitazone are procured from Aristo Pharmaceuticals, Mumbai and etoricoxib was procured from Cadila Pharmaceuticals Ltd, Ahmedabad.

Chromatographic separation was performed on a Shimadzu® liquid chromatographic system equipped with a LC-10AT-vp solvent delivery system (pump), UV detector, Rheodyne 7725i injector with 50µl loop volume. . A phenomenex C_{18} column (25cm x 4.6mm i.d., 5µ) was used for the separation.

Preparation of mobile phase and standard solutions

The mobile phase prepared is a mixture of acetonitrile and phosphate buffer (pH 5.0 adjusted with orthophosphoric acid) (50:50 v/v). It was filtered through a 0.2 µ membrane filter and degassed. Standard stock solutions of 1mg/ml metformin, pioglitazone and etoricoxib were prepared separately using a mixture of water and acetonitrile in the ratio 1:1 v/v. From the standard stock solution, mixed standard solution was prepared to contain 50 µg/ml of metformin, 2 µg/ml of pioglitazone and 50 µg/ml of etoricoxib as internal standard. The mobile phase was delivered at a flow rate of 1 ml/min with detection at 258 nm. The injection volume was 50 µl; Analysis was performed at ambient temperature.

Preparation of sample solutions

Twenty tablets, each containing 500 mg of Metformin and 15 mg of Pioglitazone were weighed and finely powdered; a quantity of powder equivalent to 50 mg of Metformin and 1.5 mg of Pioglitazone was weighed and transferred to a sintered glass crucible. To this 10 ml of 50 mg/ml solution of etoricoxib was added and the drugs were extracted with three quantities, each of 20 ml of

mixture of acetonitrile and water (1:1 v/v). The combined extracts were made up to 100 ml with mobile phase and further dilutions were made to get a concentration of 50 µg/ml of Metformin, 1.5 µg/ml of Pioglitazone (theoretical value) and 50 µg/ml of etoricoxib as internal standard and this solution was used for the estimation.

Assay method

With the optimized chromatographic conditions, a steady baseline was recorded, the mixed standard solution was injected and the chromatogram was recorded. The retention time of metformin, pioglitazone and etoricoxib was found to be 4.75, 6.44 and 8.83 min, respectively. This procedure was repeated for the sample solution obtained from the formulation. The response factor (peak area ratio of standard peak area and internal standard peak area) of the standard solution and sample solution were calculated. The concentration of the drugs were calculated (Table 1) using following formula,

$$\text{Concentration of drugs} = \frac{\text{Response factor of the sample}}{\text{Response factor of the standard}} \times \text{Concentration of standard}$$

RESULTS AND DISCUSSION

Estimation of metformin and pioglitazone in dosage forms by RP-HPLC method was carried out using optimized chromatographic conditions. The standard and sample solutions were prepared. The chromatograms were recorded. Detection found at 254 nm. The overlaid UV spectrum of metformin and pioglitazone is shown in Fig 1. The typical chromatogram of sample solution is given in Fig 2**.** The peak area ratio of standard and sample solutions was calculated. The assay procedure was repeated for six times and mean peak area ratio and mean weight of standard drugs were calculated. The percentage of individual drugs found in formulation, mean, standard deviation in formulation were calculated and presented in Table 1**.** The results of analysis shows that the amount of drugs was in good agreement with the label claim of the formulation.

The method was validated as per ICH guidelines. The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in Table 1. From the data obtained, added recoveries of standard drugs were found to be accurate.

The precision of the method was demonstrated by inter day and intra day variation studies. In the intra day studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated and presented in Table 2**.** In the inter day variation studies, six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated and presented in Table 2. From the data obtained, the developed HPLC method was found to be precise.

The linearity of the method was determined at seven concentration levels ranging from 10 to 50 µg/ml for metformin and 0.3 to 1.5µg/ml for pioglitazone (Table 3)**.** The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y = 0.0072 x -0.001 (R^2=0.998) for paracetamol and y=0.0252 x +0.003 (R^2=0.996) for aceclofenac. The results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above. The calibration curves are shown in Fig 3 & 4**.**

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLC method. The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3). The LOD for metformin and pioglitazone was found to be 5 ng/ml and 10 ng/ml, respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ was 15 ng/ml and 30 ng/ml for metformin and pioglitazone, respectively (Table 4)**.**

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 5 h at room temperature. The results show that for both solutions, the retention time and peak area of metformin and pioglitazone remained almost unchanged (% R.S.D. less than 2.0) and no significant degradation within the indicated period, thus indicated that both solutions were stable for at least 5hr, which was sufficient to complete the whole analytical process.

The column efficiency, resolution and peak asymmetry were calculated for the standard solutions (Table 4)**.** The values obtained demonstrated the suitability of the system for the analysis of this drug combinations, system suitability parameters may fall within $\pm$ 3 % standard deviation range during routine performance of the method.

Thus the proposed RP-HPLC method for the simultaneous estimation of metformin and pioglitazone in combined dosage forms is accurate, precise, linear, rugged, robusted, simple and rapid. Hence the present RP-HPLC method is suitable for the quality control of the raw materials, formulations and dissolution studies.

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TABLE 1: RESULTS OF ANALYSIS OF FORMULATION AND RECOVERY STUDIES

Drug	Amount mg/ tab		% Label claim*	% Recovery*
	Labelled	Found *		
Metformin	500	499.07 ± 1.047	99.81 ± 1.023	98.89 ± 0.813
Pioglitazone	15	19.01 ± 1.132	95.05 ± 1.098	95.01 ± 0.571

* Average of 6 determinations ± standard deviation

OLFENAC-P (Olcare pharmaceuticals) each tablet containing 500 mg of Paracetamol and 20 mg of Aceclofenac

TABLE 2: INTRADAY AND INTERDAY PRECISION STUDIES

Intraday studies				Interday studies				
RF* of Paracetamol	Mean (% RSD*)	RF of Aceclofenac	Mean (% RSD)	Day	RF of Paracetamol	Mean (% RSD)	RF of Aceclofenac	Mean (% RSD)
0.3612 0.3613 0.3611 0.3610 0.3612 0.3612	0.3612 (0.0286)	0.0521 0.0522 0.0521 0.0523 0.0520 0.0521	0.0521 (0.1981)	Day 1	0.3610 0.3611 0.3612 0.3613 0.3611 0.3610	0.3611 (0.0324)	0.0522 0.0521 0.0523 0.0520 0.0522 0.0522	0.0522 (0.1980)
				Day 2	0.3609 0.3610 0.3613 0.3611 0.3610 0.3612	0.3611 (0.0418)	0.0520 0.0521 0.0522 0.0520 0.0521 0.0519	0.0521 (0.2015)
				Day 3	0.3611 0.3608 0.3611 0.3612 0.3610 0.3609	0.3610 (0.0408)	0.0521 0.0520 0.0519 0.0521 0.0522 0.0520	0.0521 (0.2016)

* RF-Response Factor, % RSD- Relative standard deviation

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TABLE 3: LINEARITY AND RANGE

Internal standard peak area (100µg/ml Etoricoxib)	Metformin			Pioglitazone		
	Concentration (µg/ml)	Peak area	Response factor	Concentration (µg/ml)	Peak area	Response factor
11979960	20	1718492	0.143	0.5	151147	0.013
	30	2577835	0.215	1.0	302534	0.025
	40	3437240	0.287	1.5	455216	0.038
	50	4315895	0.360	2.0	624543	0.052
	60	5205470	0.435	2.5	755658	0.063
	70	6114715	0.510	3.0	906413	0.076
	80	6873960	0.574	3.5	1054874	0.088

TABLE 4: VALIDATION AND SYSTEM SUITABILITY STUDIES

S. No.	Parameters	Metformin	Pioglitazone
1	Linearity range	20.0 to 80.0 µg/ml	0.5 to 3.5µg/ml
2	Regression equation Y = mx + c*	y = 0.0072x - 0.001	y = 0.0252x + 0.0003
3	Correlation coefficient	0.9998	0.9996
4	Theoretical plate/meter	25478	29784
5	Resolution factor	1.32	1.32
6	Asymmetric factor	0.91	1.02
7	LOD (ng/ml)	5	10
8	LOQ (ng/ml)	15	30

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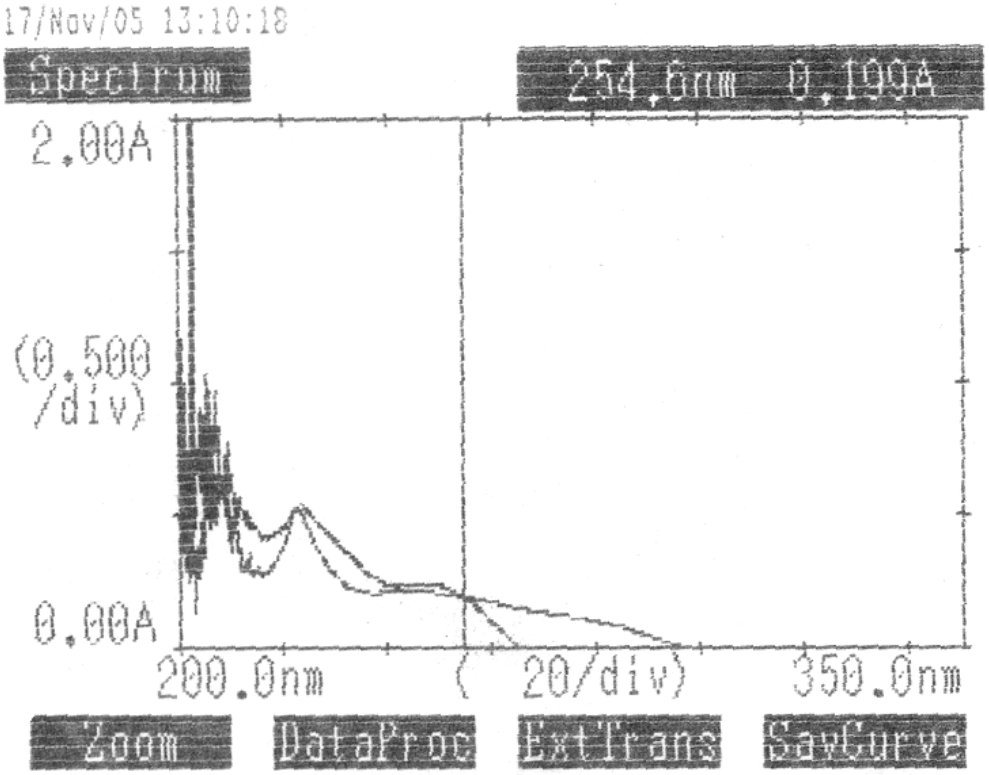

Fig.1: Overlay spectrum of Metformin and pioglitazone

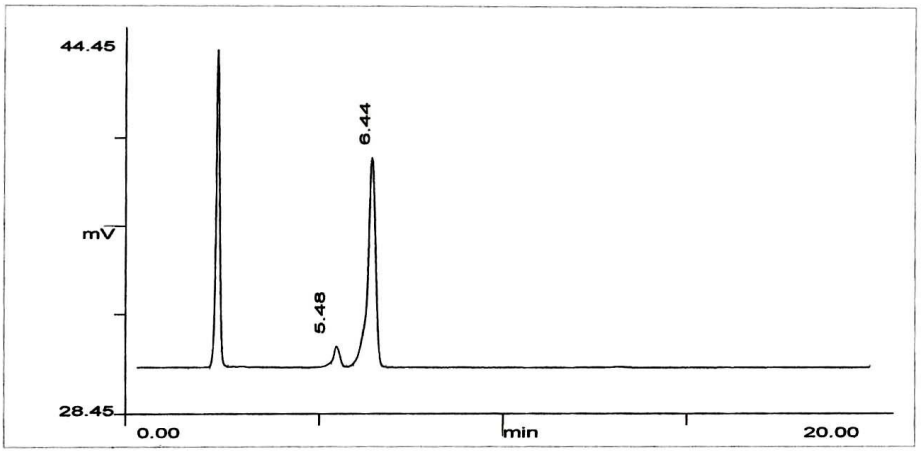

Fig.2: Chromatogram of sample solution

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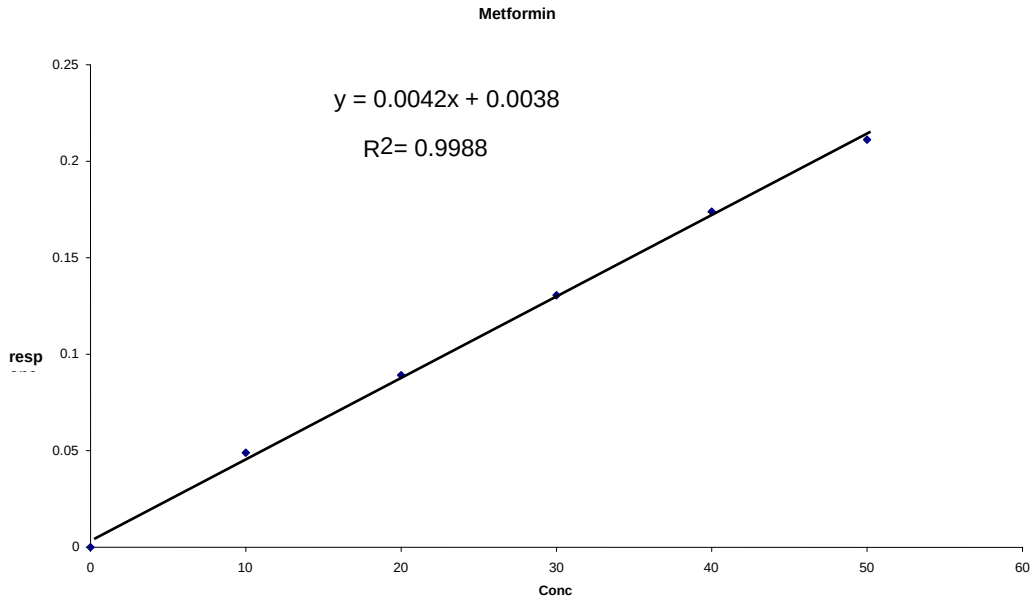

Fig. No. 3. Calibration curve of Metformin

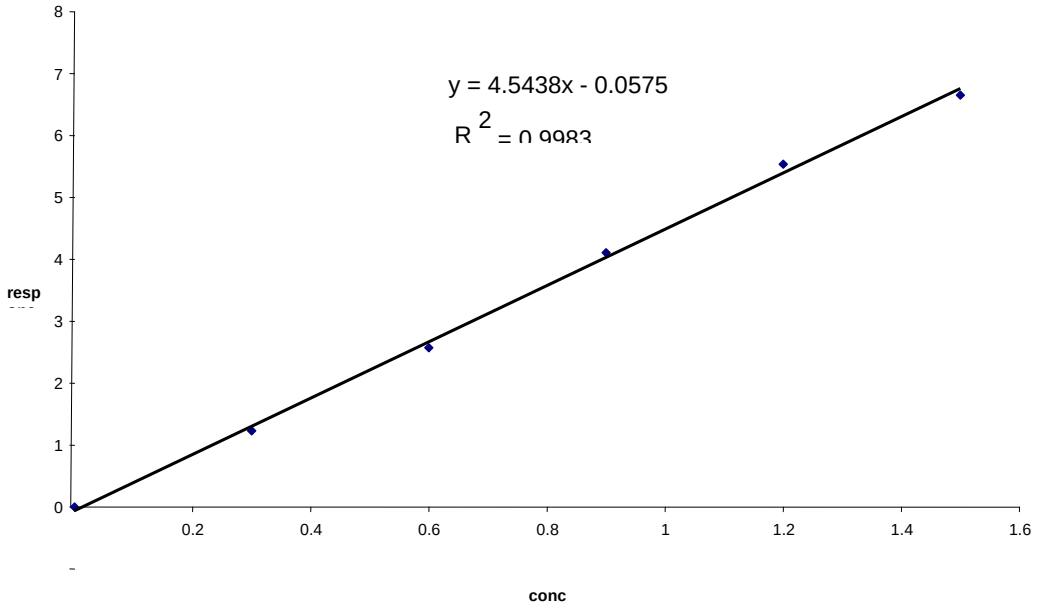

Fig. No. 4. Calibration curve of Pioglitazone

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