Kinetic and Spectrophotometric Methods for Determination of Two Hypoglycemic Drugs, Pioglitazone Hydrochloride and Glimepiride in their Pharmaceutical Formulations

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Abstract

Simple and sensitive kinetic and spectrophotometric methods have been developed and validated for the determination of two hypoglycemic drugs, pioglitazone hydrochloride and glimepiride in bulk powder and in their pharmaceutical formulations. Both methods are based on the oxidative coupling of each drug with 3-methylbenzothiazolinone hydrazone / cerric sulphate system. The formation of the colored products was monitored spectrophotometrically by measuring the absorbance at 645 nm. Factors affecting the reactions were studied and optimized. The stoichiometries of the reactions were determined using the limiting logarithmic method and the proposed reactions pathways were postulated. The fixed time (at 20 min) method was utilized for constructing the calibration graph of pioglitazone hydrochloride at room temperature. Concentrations of the drugs were calculated using the calibration equation for fixed time method for pioglitazone hydrochloride and using direct absorbance measurement of reaction product for glimepiride where good correlations were obtained in the range 2 – 16 µg ml^{-1} of both drugs with limits of detection of 0.37 and 0.06 µg ml^{-1} for pioglitazone hydrochloride and glimepiride respectively.

No interference was observed from the excipients that are commonly present in the pharmaceutical formulations. The proposed methods were successfully applied to the determination of pioglitazone hydrochloride and glimepiride in their pharmaceutical formulations. The label claim percentages were 99.9 – 100.1 ± 0.87 – 0.89 and 99.2 – 99.4 ± 0.75 – 1.02 for pioglitazone hydrochloride and glimepiride respectively. Statistical comparison of the results with those obtained by reference spectrophotometric methods showed excellent agreement between the accuracy and precision of the two methods. The proposed methods have great value in their applications to the analysis of pioglitazone hydrochloride and glimepiride in quality control laboratories.

Keywords: Kinetic Spectrophotometry, Pioglitazone hydrochloride, Glimepiride, Pharmaceutical formulations, 3-methylbenzothiazolinone hydrazone / cerric sulphate system.

Introduction

Anti-diabetics such as sulfonylurea and thiazolidinedione derivatives are commonly prescribed hypoglycemic drugs for non-insulin dependent type II diabetes mellitus. However, they can also be used as a stopper in race-horses by reducing the blood glucose level[1].

Pioglitazone hydrochloride, (±)-5-{4-[2-(5-ethyl-2-pyridyl) ethyl] benzyl}-2,4-thiazolidinedione hydrochloride salt (Fig. 1), is a member of thizolidinedione class, which exerts its glucose-lowering effect by binding to peroxisome proliferator-activated receptors gamma. (PPARγ), thus increasing the receptor sensitivity to insulin[2,3].

Glimepiride, 1-H-pyroll-1-carboxamide-3-ethyl-2, 5-dihydro-4-methyl-N-[2-[4-[[[[(4-methylsiklohexyl) amino] carbonyl] amino] sulfonyl] [phenyl] ethyl]-2-oxo-trans (Fig. 1), is a member of sulfonylurea drugs, which can increase the secretion of insulin by functioning islet β - cells. In the past few decades, several generations of sulfonylurea drugs have been developed for common use such as glimepiride. This generation of hypoglycemic drugs is much more potent and are therefore effective at much lower dosages[4,5].

Several analytical methods have been reported for the determination of pioglitazone hydrochloride in bulk form, pharmaceuticals and biological fluids either alone or in combination with other diabetic drugs. Most of the reported methods are chromatographic methods and no official methods have been reported for its determination. The reported methods include: HPLC [6-11], LC/MS/MS[12,13], micellar electrokinetic chromatography[14], TLC[15-17], HPTLC[18], CE[19], voltammetry [20] and flow-injection chemiluminescence [21]. Also potentiometric sensors [22] and ion selective membrane sensors[23] were fabricated for the determination of pioglitazone hydrochloride in some pharmaceutical formulations and plasma. Some spectrophotometric methods are available for the determination of pioglitazone hydrochloride including UV spectrophotometry[24-27] and visible spectrophotometry based on the formation of an ion associated complex with bromocresol green, bromocresol purple, bromophenol blue

and bromothymol blue [28], with diazotized sulphanilic acid in alkaline medium[24] and with tropaeolin-ooo, wool fast blue, saffranin-o and methylene blue[29].

Few analytical methods have been reported for the determination of glimepiride. These methods were based mainly on chromatographic techniques, including LC [30-32], LC/MS/MS[33,34], HPLC [35-37], HPTLC [38] and micellar electrokinetic chromatography[39], In addition, one polarographic method[40] and one method of ion selective electrodes[41] were used for the determination of glimepiride. Concerning spectrophotometry, glimepiride was assayed by UV spectrophotometry[25,42] and one visible spectrophotometric method[43] was reported for its determination based on complex formation.

The chromatographic, voltammetric and electrophoretic methods utilized delicate and /or expensive instruments that are not available in most quality control laboratories. Spectrophotometric techniques are the most widely used in pharmaceutical analysis[44,45]. The widespread of spectrophotometric methods is attributed to their inherent simplicity, economic advantages and wide availability in most quality control laboratories.

However, the few spectrophotometric methods used for determination of pioglitazone hydrochloride and glimepiride in their pharmaceutical dosage forms were associated with some major drawbacks such as decrease selectivity due to measurement in ultraviolet region and/or decreased simplicity of the assay procedure.

For these reasons, it was worthwhile to develop new simple and selective spectrophotometric methods for the determination of pioglitazone hydrochloride and glimepiride in their pharmaceutical dosage forms.

Kinetic spectrophotometric methods are becoming of great interest in the pharmaceutical analysis[46-49]. The application of these methods offers some specific advantages such as improved selectivity, avoiding the interference of the colored and /or turbidity background of the samples, possibility of avoiding the interference of the other active ingredients present in the commercial product, and reduction of the analysis time when the analytical reaction requires long time for completion.

No attempts have been reported for the kinetic spectrophotometric determination of the studied drugs. The present study describes, for the first time, the development and validation of a sensitive, selective and simple kinetic spectrophotometric method for the determination of pioglitazone hydrochloride. In case of glimepiride, the reaction with 3-methylbenzothiazolinone hydrazone/cerric sulphate system is very fast and completed after 5 min, so no kinetic study has been adopted. Both drugs were determined in their commercial pharmaceutical dosage forms depending on the oxidative coupling of pioglitazone hydrochloride and glimepiride with 3-methylbenzothiazolinone hydrazone /cerric sulphate system.

Material and Methods

Apparatus: Ultrospec 2100 pro: 80 – 2112 – 21 Ultraviolet / Visible Spectrophotometer (Biochrom, Cambridge, UK) with matched 1-cm quartz cells was used for all the spectrophotometric measurements.

Materials, chemicals and dosage forms: Pioglitazone hydrochloride was a kind gift from Saudi Pharmaceutical Industries and Medical Appliances Corporation /Al-Qassim Pharmaceutical Plant (SPIMACO), KSA. Glimepiride was obtained from Chargen-Zert Brand Co., Germany.

3-Methyl benzothiazoline-2-one hydrazone (Wako) was 2 x 10^{-2} M, freshly prepared in distilled water. Ammonium cerric sulphate dihydrate (Fluka) was 1.6x10^{-2} M, prepared in 0.8 M sulphuric acid (BDH, UK). Methanol (BDH, UK) was used. All solvents and other chemicals used through this study were of analytical grade. Pharmaceutical formulations, Amaryl® tablets (Aventis, Germany) are labeled to contain 1.0, 2.0 and 3.0 mg glimepiride per tablet, Actos® tablets (Takeda Chemical Industries, Ltd Osaka, Japan) are labeled to contain 15 and 30 mg pioglitazone hydrochloride per tablet.

Preparation of stock standard solutions: Into a 100-ml calibrated flask, an accurately weighed amount (0.01 g) of the standard drug (pioglitazone hydrochloride and glimepiride) was dissolved in 50 ml methanol. The resulting solution was completed to volume with the same solvent. This stock solution (100 µg ml^{-1}) was diluted with water to obtain working concentrations in the range of 2 – 16 µg ml^{-1} for both drugs.

Preparation of dosage: Twenty tablets were weighed and finely powdered. A quantity of the mixed powder equivalent to 10 mg of pioglitazone hydrochloride and glimepiride was transferred into a 50- ml conical flask, dissolved in 25 ml of methanol, swirled and sonicated for 30 min, shaken well and filtered in a 100- ml calibrated flask. The filtrate was completed to volume with methanol to obtain a 100 µg ml^{-1} stock solution. The working solutions were prepared by appropriate dilutions of the stock solution.

General analytical procedures and data treatment

Procedure for pioglitazone hydrochloride: Aliquots of the standard or sample solution equivalent to 2 – 16 µgml^{-1} of pioglitazone hydrochloride were transferred into a series of 25- ml calibrated flasks, 6 ml of 1.6 x 10^{-2} M cerium (IV) solution were added, followed by 2 ml of 2 x 10^{-2} M MBTH solution. The reaction mixture was mixed and completed to volume with water. After 20 min, the reaction mixture was transferred into a spectrophotometric cell and the absorbance was recorded (at 645 nm) at the

ambient temperature against a reagent blank treated similarly. The kinetic data was recorded for curve fitting, regression analysis and statistical calculations. Pioglitazone hydrochloride concentration was computed from the appropriate equation of the calibration graph for the fixed time method.

Procedure for glimepiride: In a series of 25-ml calibrated flasks, 6 ml of 1.6 x 10^{-2} M cerium (IV) solution were transferred followed by 2 ml of 2 x 10^{-2} M MBTH solution. Aliquots of the standard or sample solution equivalent to 2 – 6 µg ml^{-1} of glimepiride were added, the reaction mixture was mixed and completed to volume with water and the absorbance of the green colored product was recorded (at 645 nm) at the ambient temperature against a reagent blank treated similarly.

Determination of molar ratio of the reactions: The limiting logarithmic method[50] was employed. Two sets of experiments were carried out employing the general recommended procedures described above. The first set of experiments was carried using increasing MBTH concentrations (1.0 x 10^{-3} – 1.75 x 10^{-3} M) at a fixed concentration of pioglitazone hydrochloride and glimepiride. The second set of experiments was carried using increasing drug concentrations (4.0 x 10^{-6} – 2.5 x 10^{-5} M) at a fixed MBTH concentration. 6 ml of 1.6 x 10^{-2} M cerium (IV) were added to each solution in both sets and the logarithms of the obtained absorbances were plotted as function of the logarithms of the MBTH and drug concentrations in the first and second sets of experiments, respectively. The slopes of the fitting lines in both sets of experiments were calculated.

Results and Discussion

Involved reaction and absorption spectra: The reactions involved in the present study were based on the formation of colored oxidative coupling of pioglitazone hydrochloride and glimepiride by their reaction with 3-methylbenzothiazolinone hydrazone /cerric sulphate system. 3-methylbenzothiazolinone hydrazone (MBTH) has been frequently used for the spectrophotometric determination of several pharmaceutical compounds, as quinolone antibacterials[51], ritodsine and amoxicillin[52] and josamycin[53].

The formation of the colored products is monitored spectrophotometrically at their maximum absorption peaks (645 nm). The absorption spectrum for MBTH, glimepiride (as an example) and their reaction product is given in fig. 2.

Optimization of reactions conditions: The factors affecting reactions conditions (concentrations of MBTH and cerric sulphate, acidity, temperature and the diluting solvents) were studied by altering each variable in turn while keeping the others constant. The intensity of the developed color was recorded as a function of the concentrations of MBTH and ceric sulphate reagents. It was found that the color intensity was dependent on the concentration of both reagents. The highest color intensity was attained when the concentration of MBTH was 1.6 x 10^{-3} M in the final reaction solution (2 ml of 2 x 10^{-2} M of the working MBTH aqueous solution) either for pioglitazone hydrochloride or glimepiride and this condition was employed in all the subsequent experiments.

Different oxidizing agents (ceric sulphate, potassium permanganate, potassium dichromate and ferric chloride, all used as 3.8 x 10^{-3} M in the final reaction solutions) were tested for oxidation of MBTH reagent. Best results were obtained when ammonium ceric sulphate was used. In separate experiments, the effect of ceric sulphate concentration was investigated by carrying out the reaction in different concentrations of ceric sulphate solution. It was found that the color intensity increased as the concentration of cerium (IV) ions in the final reaction solution of either pioglitazone hydrochloride or glimepiride was 3.84 x 10^{-3} M after which a decreased intensity was observed. The subsequent experiments were carried out using 3.84 x 10^{-3} M cerium (IV) in the final reaction solution (6 ml of 1.6 x 10^{-2} M working ceric sulphate solution).

The effect of acidity was also investigated, although the acidity had no effect on the color development yet it was found to be important to dissolve and stabilize cerium (IV) ions and it was optimized by preparing ceric sulphate in different concentrations of sulphuric acid in the range 0.05 – 1.5 M. It was found that 0.8 M is the least concentration of sulphuric acid required to dissolve and stabilize the acidity of the reaction medium for both pioglitazone hydrochloride and glimepiride.

The effect of temperature was also investigated. The reaction was carried out at room temperature (25 ± 5^{o}C) and at elevated temperatures using a thermostatically controlled water bath. It was found that the color intensity decreased significantly when the reaction temperature increased. This was probably attributed to the instability of the colored product and a formation of a precipitate at elevated temperatures. Therefore, the further experiments were carried out at ambient temperature.

In order to select the most appropriate solvent for dilution, various experiments were performed to study the effect of diluting solvent. The reaction was firstly tried in aqueous medium then different solvents were tried as diluents namely methanol, ethanol, acetone, acetonitrile and isopropanol. The highest color intensity was attained when water was used as a diluting solvent, therefore it was selected for the further investigations.

It was noteworthy to mention that the sequence of the addition of reactants had some effect on color development and intensity of the reaction product. For

pioglitazone hydrochloride, the addition of the drug followed by the addition of cerium (IV) solution then MBTH solution gave the highest color intensity. In case of glimepiride, the ideal sequence of addition was started with cerium (IV) solution followed by the addition of MBTH solution and finally the addition of drug solution which gave the highest intensity.

Stoichiometry and mechanism of the reaction: The stoichiometry of the reaction of MBTH with each glimepiride and pioglitazone hydrochloride was investigated by limiting logarithmic method [50]. A plot of log absorbance versus log [drug] at a constant concentration of MBTH and cerium (IV) gave straight lines with slopes of 1.09 and 0.95 for glimepiride and pioglitazone hydrochloride respectively (Fig. 3a). A plot of log absorbance versus log [MBTH] at a constant concentration of drug gave straight lines with slopes of 1.13 and 1.3 for glimepiride and pioglitazone hydrochloride respectively (Fig. 3b). Thus, the molar ratio of the reaction is 1.09:1.13 and 0.95:1.3 which is almost 1:1 (drug:MBTH).

The reaction pathways are assumed to proceed according to the previously reported methods[51-53], the reaction of MBTH with each glimepiride and pioglitazone hydrochloride in presence of an oxidant proceeds via oxidation coupling. MBTH when oxidized with cerium (IV) ions, forms an electrophilic intermediate (E^+) which is the active coupling species. The later would be expected to attach carbon atom with maximum electron density according to the pathway shown for glimepiride in fig. 4.

Kinetics of the reaction of pioglitazone hydrochloride with MBTH: Under the optimum conditions, the absorbance-time curves for the reaction of varying concentrations of pioglitazone hydrochloride ($0.50x10^{-5}$-$4.07x10^{-5}$M) with a fixed concentration of MBTH (in excess) in presence of cerium (IV) solution were generated (Fig. 5). The initial reaction rates (υ) were determined from the slopes of these curves. The logarithms of the reaction rate (log υ) were plotted as a function of logarithms of pioglitazone hydrochloride concentration (log C) (Fig. 6). The regression analysis for the values was performed by fitting the data to the following equation:

$$\log \upsilon = \log K + n \log C$$

where υ is the reaction rate, K is the rate constant, C is the molar concentration of pioglitazone hydrochloride and n (slope of the regression line) is the order of the reaction. A straight line with slope value of 0.9094 (≈1) was obtained confirming that the reaction was first order. However, under the optimized reaction conditions, the concentrations of MBTH and cerium (IV) were in much more excess than that of pioglitazone hydrochloride in the reaction solution. Therefore, the reaction was regarded as a pseudo-first order reaction.

Evaluation of kinetic method and quantitation: The analysis of pioglitazone hydrochloride under the above mentioned optimum conditions where [MBTH] is about 20 times the final concentration of pioglitazone hydrochloride, would result in a pseudo-zero-order reaction with respect to [MBTH]. However, the rate will be directly proportional to pioglitazone hydrochloride concentration in a pseudo-first order reaction as follows:

Rate = K [pioglitazone hydrochloride].

where K is the pseudo first order constant. This equation was the basis for several experiments that were run to obtain pioglitazone hydrochloride concentrations using the rate data. Initial rate, fixed concentration and fixed time methods were tried and the most suitable analytical method was selected by taking into account the applicability, sensitivity, correlation coefficient (r) and intercept of the regression equations.

Initial rate method: The initial rate of the reaction for pioglitazone hydrochloride followed a pseudo-first order. Regression analysis, using the method of least squares was performed for the data of initial rates (υ) and initial concentrations of the drug (C). The initial rate of the reaction at different concentrations was obtained from the slope of the tangent to the absorbance-time curve and the calibration curve was constructed by plotting the logarithm of the initial rate of reaction versus logarithm of the concentration of pioglitazone hydrochloride. The relation appeared to be linear in the range 5.00×10^{-6} - 4.07×10^{-5} M (2.0 – 16.0 μg ml^{-1}) and gave the following equation:

$$\upsilon = 4.0 \times 10^{-5} + 20.325\ C \qquad r = 0.9985$$

The value of correlation coefficient (r = 0.9985) indicates poor linearity, thus this method was abandoned.

Fixed concentration method: Reaction rates were recorded for different concentrations of pioglitazone hydrochloride in the range of 8.0 – 14.0 μg ml^{-1}. A pre-selected value of absorbance (0.35) was fixed and the time was measured in seconds. The reciprocal of time against the initial concentration of pioglitazone hydrochloride was plotted and the following equation of the calibration graph was obtained:

$$1/t = -0.0034 + 0.0009\ C \qquad (r = 0.9940)$$

where C is the concentration of pioglitazone hydrochloride in μg ml^{-1} and t is the time in seconds. The range of concentration of pioglitazone hydrochloride giving the most satisfactory calibration graph with the above equation was limited and the linearity was also poor.

Fixed time method: In this method, the absorbance of the reaction solution containing varying amounts of pioglitazone hydrochloride was measured at a pre-selected

fixed time. Calibration plots of absorbance versus the concentrations of pioglitazone hydrochloride were established at fixed periods of time for the reaction. The regression equation coefficients of correlation, and limits of detection (LOD) and quatitation (LOQ) are given in Table 1. It was clear that the slopes increased with time and the correlation coefficient at 20 min was the highest and the standard deviation of slope (S_b), standard deviation of intercept (S_a), limit of detection (LOD) and limit of quantitation (LOQ) were the lowest, therefore, the fixed time of 20 min was recommended as the most suitable time for analytical procedure.

Quantitation of pioglitazone hydrochloride: Under the described optimum conditions, standard calibration curve for pioglitazone hydrochloride by the proposed kinetic spectrophotometric method was constructed. The absorbance was linearly related to pioglitazone hydrochloride concentration over the range 2 – 16 µg ml^{-1} with a limit of detection of 0.37 µg ml^{-1} and a limit of quantitation of 1.12 µg ml^{-1} (Table 2). The concentration of pioglitazone hydrochloride was calculated using the corresponding calibration equation presented in Table 1 at a fixed time of 20 min.

Quantitation of glimepiride: Under the optimized experimental conditions described earlier for glimepiride, the standard calibration curve was constructed. The absorbance was linearly related to glimepiride concentration over the range 2 – 16 µg ml^{-1} with a limit of detection of 0.06µg ml^{-1} and a limit of quantitation of 0.2 µg ml^{-1}. The concentration of glimepiride was calculated using the calibration equation cited in table 2.

Statistical evaluation of regression lines of calibration equations for both pioglitazone hydrochloride and glimepiride gave low values of standard deviations of slopes (S_b) and intercepts (S_a) as shown in table 2. The low values of LOD confirmed the high sensitivity of the methods and consequently their capability to determine low amounts of the investigated drugs.

Validation of the proposed methods

Precision: The precision (intra- and inter-assay) of the proposed spectrophotometric method for glimepiride and kinetic spectrophotometric method for pioglitazone hydrochloride was determined at three concentration levels (Table 3). The intra-assay precision was assessed by analyzing 3 replicates of each sample as a batch in a single assay run, and the inter-assay precision was assessed by analyzing the same sample, as triplicate, in a separate assay run on three days within two weeks. The relative standard deviations (%RSD) for the results did not exceed 2% (Table 3), proving the high reproducibility of the results and the precision of the methods. This good level of precision was suitable for quality control analysis of both pioglitazone hydrochloride and glimepiride in their pharmaceutical dosage forms.

Analytical recovery studies and selectivity: The analytical recovery of the proposed methods was also checked. The obtained mean recoveries and relative standard deviations were in the range 99.2 – 100.1 ± 0.25 – 0.76. The accuracy was also studied as percent relative error (% Error). It was found that % Error was in the range 0.14 – 0.44 (Table 3). These results prove the accuracy of the proposed methods. It is worth noting that all the proposed kinetic and spectrophotometric methods for pioglitazone hydrochloride and glimepiride determination were performed in the visible region away from the UV-absorption region of the UV-absorbing interfering excipient materials that might be co-extracted from the dosage forms.

Application of the proposed methods: It is evident from the results obtained previously that the proposed kinetic and spectrophotometric methods gave satisfactory results for determination of pioglitazone hydrochloride and glimepiride in bulk drugs. The proposed methods have been applied in the analysis of the commercial pharmaceutical dosage forms. The concentrations of the studied drugs were computed from their corresponding regression equations. The results of the proposed methods (fixed time or the spectrophotometric assay) were statistically compared with those of the reference methods[24,42] with respect to the accuracy and precision. The obtained mean recovery of the labeled amounts is 99.2 – 100.1 ± 0.67 – 1.02 (Table 4). In the t- and F- tests, no significant difference were found between the calculated and theoretical values of both the proposed and the reported methods at 95% confidence level. This indicated the similarities between the precision and accuracy of the proposed and the reference methods for the determination of both pioglitazone hydrochloride and glimepiride in their dosage forms.

Advantages of the proposed methods over the reported spectrophotometric methods: The proposed methods, because they involve measurements in visible region, are more selective than the previously reported spectrophotometric methods that involved measurements in the ultraviolet region[24-27]. As well, the proposed methods are superior to the few reported visible spectrophotometric methods that were based on the ion-pair associated or formation of charge-transfer complexes[28,29,43] as the proposed methods do not require elaborate treatment of the samples, careful adjustment of the critical optimum pH of the reaction medium and /or tedious liquid-liquid extraction for the chromophores. Also, they are superior to the other visible methods in terms of health and environmental safety, cost and procedure simplicity. The high sensitivity that has been achieved in the proposed methods confers the ease in preparation of the sample for analysis.

These advantages encourage the application of the proposed methods in routine analysis of pioglitazone hydrochloride and glimepiride in quality control laboratories, as alternatives for the existing methods.

Conclusion

The present study described for the first time, a simple, selective, sensitive and high reproducible kinetic spectrophotometric method for the determination of pioglitazone hydrochloride and a simple and rapid spectrophotometric method for the determination of glimepiride in pure form and in their dosage forms. The proposed methods can be easily applied as they do not require elaborate treatment of the samples and /or tedious procedures for extraction of the chromophores. As well, both methods were sensitive enough for analysis of lower amounts of the drugs. Furthermore, the proposed methods do not require expensive or sophisticated instrumentation and /or critical analytical reagents. These advantages give the proposed methods a great value and encourage their application to the analysis of pioglitazone hydrochloride and glimepiride in quality control laboratories.

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Table 1
Analytical parameters for the proposed fixed time spectrophotometric method for determination of pioglitazone hydrochloride

Reaction time (min)	Linear range (µg ml^{-1})	Intercept	Standard deviation of intercept (S_a)	Slope	Standard deviation of intercept (S_b)	Correlation coefficient (r)	LOD (µg ml^{-1})	LOQ (µg ml^{-1})
5	2.0-16.0	-0.0066	$7.60x10^{-3}$	0.0186	$3.61x10^{-3}$	0.9970	1.35	4.0
10	2.0-16.0	-0.0021	$5.35x10^{-3}$	0.0206	$2.00x10^{-3}$	0.9993	0.86	2.6
15	2.0-16.0	-0.0035	$5.98x10^{-3}$	0.0237	$2.83x10^{-3}$	0.9990	0.83	2.5
20	2.0-16.0	-0.0046	$2.96x10^{-3}$	0.0265	$1.41x10^{-3}$	0.9999	0.37	1.12
25	2.0-16.0	0.0021	$3.50x10^{-3}$	0.0298	$1.85x10^{-3}$	0.9997	0.39	1.17
30	2.0-16.0	0.0053	$5.60x10^{-3}$	0.0343	$2.53x10^{-3}$	0.9992	0.54	1.63

Table 2
Analytical parameters for the spectrophotometric method for determination of glimepiride and the fixed time method for determination of pioglitazone hydrochloride

Drug	Linear range (µgml^{-1})	Least square equation*		Correlation coefficient (r)	LOD (µg ml^{-1})	LOQ (µg ml^{-1})	S_a**	S_b***
		Intercept	Slope					
Glimepiride	2 - 16	-0.0368	0.0394	0.9999	0.06	0.20	$7.16x10^{-4}$	$4.20 x10^{-4}$
Pioglitazone hydrochloride	2 - 16	-0.0046	0.0265	0.9999	0.37	1.12	$2.96x10^{-3}$	$1.41 x10^{-3}$

* Least square equation for pioglitazone hydrochloride is: Log υ = log K + n log C. Where υ is the reaction rate, K is the conditional rate constant (intercept = log K), n is the order of reaction (slope = n) and C is the concentration.
**The standard deviation of intercept.
***The standard deviation of slope.

Table 3
Precision and accuracy for the proposed fixed time and spectrophotometric methods for determination of pioglitazone hydrochloride and glimepiride

Drug	Concentration (µg ml^{-1})	%Recovery (± RSD)*			
		Intra-assay,	(% Error)	Inter-assay,	(% Error)
Pioglitazone hydrochloride	6.0	99.8±0.76 ,	(0.44)	100.1±0.25 ,	(0.14)
	8.0	99.4±0.47 ,	(0.27)	99.5±0.50 ,	(0.29)
	13.0	99.3±0.35 ,	(0.20)	99.2±0.32 ,	(0.18)
Glimepiride	6.0	99.2±0.32 ,	(0.18)	99.7±0.61 ,	(0.35)
	10.0	99.8±0.25 ,	(0.14)	99.8±0.42 ,	(0.24)
	14.0	99.5±0.57,	(0.33)	99.5±0.44 ,	(0.25)

* Values are mean of three determinations.

Table 4
Results of analysis of pioglitazone hydrochloride and glimepiride and their dosage forms by the proposed and comparison methods

Drug form	Proposed method			Comparison method[a]
	Label claim (%±S.D.)	t-value	F-value	
Pioglitazone hydrochloride (pure)	99.3±0.68 (n = 7)	1.001 (2.306)[b]	2.672 (19.30)[b]	99.2±0.42 (n = 3)
Actos® tablets (15 mg/tablet)	99.9±0.89 (n = 8)	1.257 (2.228)[b]	1.178 (6.16)[b]	99.3±0.82 (n = 5)
Actos® tablets (30 mg/tablet)	100.1±0.87 (n = 8)	1.240 (2.228)[b]	1.934 (6.16)[b]	99.5±1.21 (n = 5)
Glimepiride (pure)	100.1±0.67 (n = 7)	1.274 (2.228)[b]	1.207 (6.16)[b]	99.6±0.74 (n = 5)
Amaryl® tablets (1.0 mg/tablet)	99.2±1.02 (n = 7)	0.716 (2.201)[b]	1.50 (4.95)[b]	99.7±1.25 (n = 6)
Amaryl® tablets (2.0 mg/tablet)	99.4±0.75 (n = 7)	0.142 (2.228)[b]	2.60 (6.16)[b]	99.5±1.21 (n = 5)
Amaryl® tablets (3.0 mg/tablet)	99.3±0.80 (n = 7)	0.356 (2.262)[b]	1.76 (8.94)[b]	99.1±1.o6 (n = 4)

[a]References:24 and 42 for pioglitazone hydrochloride and glimepiride, respectively. Values are mean± standard deviation.
[b]Values in parenthesis are tabulated values at 95% confidence limit.

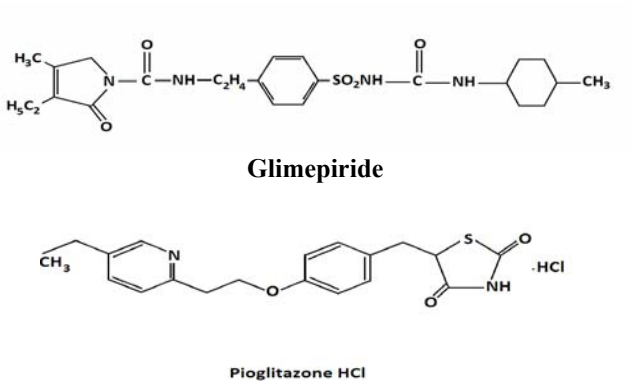


Fig. 1: Chemical structure of pioglitazone hydrochloride and glimepiride.

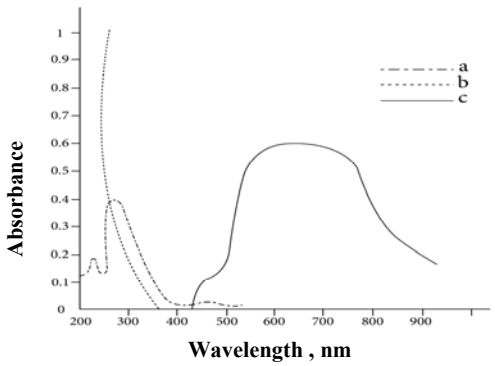
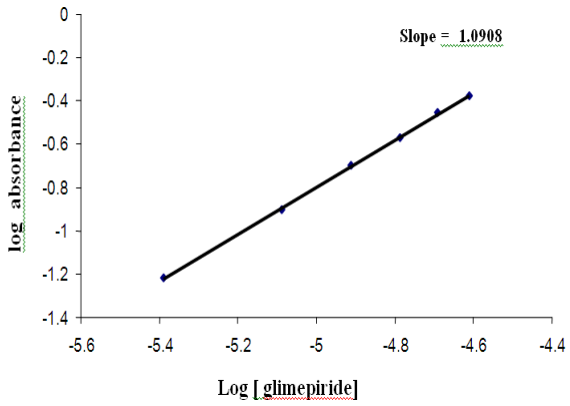
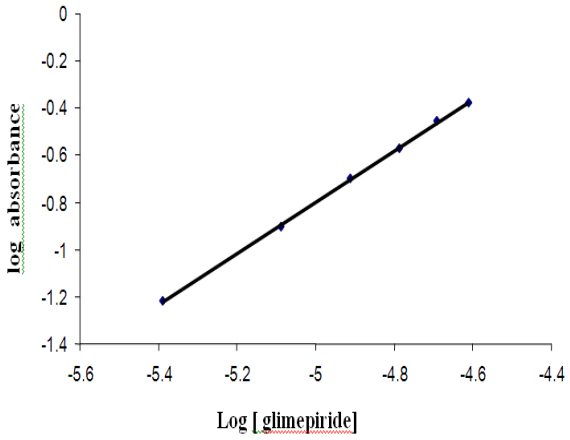
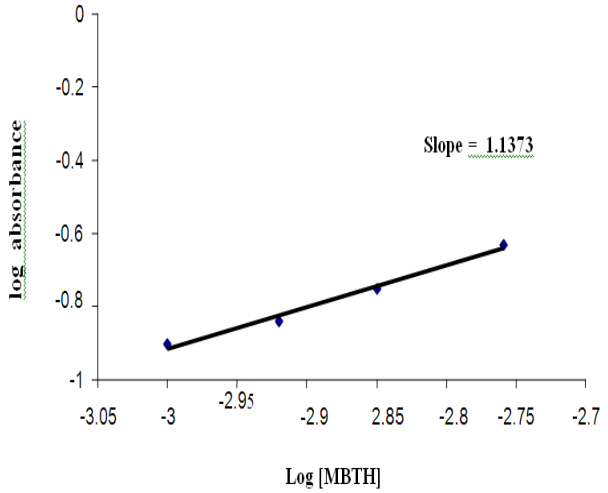


Fig. 2: Absorption spectra of : 12 µg ml^{-1} of glimepiride in methanol (a), 1.6 x 10^{-3} M of 3-methylbenzothiazolinone hydrazone in water (b) and their reaction product (c) in presence of acidic cerium (IV) ions.





(a)



(b)

Fig. 3: Limiting logarithmic plots for determination of the molar ratio for the reaction (a) log absorbance versus log [glimepiride] at fixed concentrations of MBTH and cerium (IV), (b) log absorbance versus log [MBTH] at fixed concentrations of glimepiride and cerium(IV).

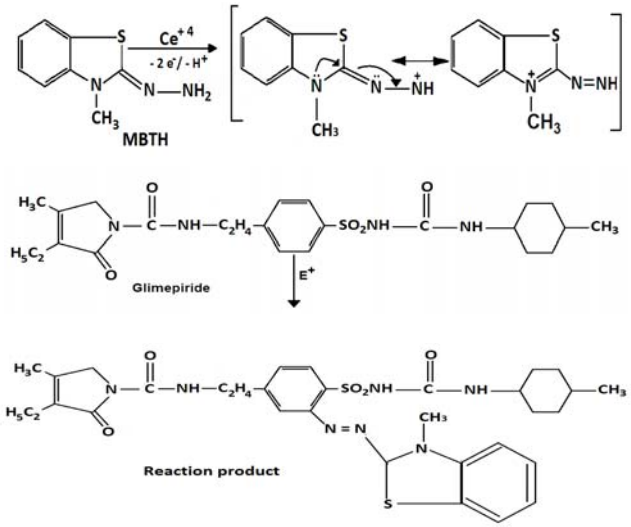


Fig. 4: The proposed reaction pathway of glimepiride with MBTH / cerium (IV) system.

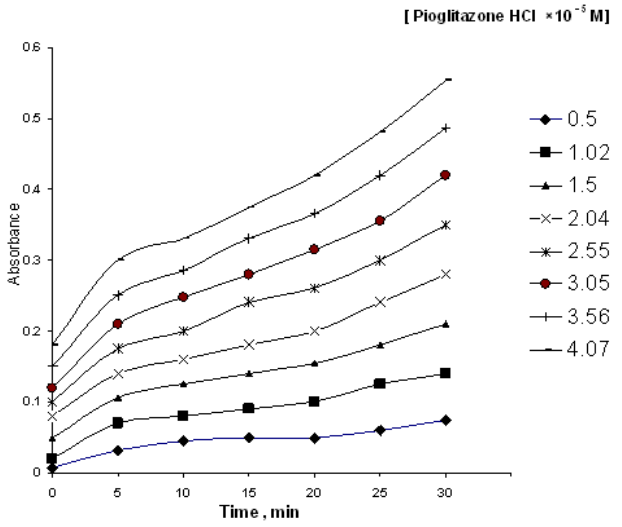


Fig. 5: Absorbance - time curves for the reaction of varying concentrations of pioglitazone hydrochloride with MBTH/Cerium (IV) system.

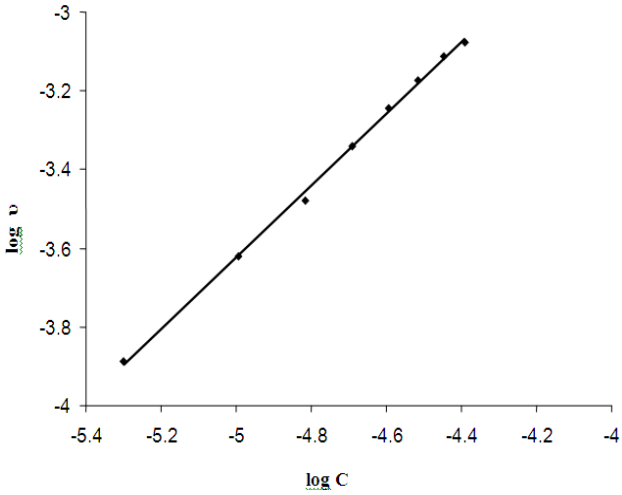


Fig. 6: Linear plot for log C versus log υ for the kinetic reaction of pioglitazone hydrochloride with MBTH/Cerium (IV) system and C is [pioglitazone HCl], (0.50 x 10^{-5} – 4.07 x 10^{-5}M); υ is the reaction rate (s^{-1})

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