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Original article

Spectrophotometric and HPLC determinations of anti-diabetic drugs, rosiglitazone maleate and metformin hydrochloride, in pure form and in pharmaceutical preparations

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ABSTRACT

In this study, three spectrophotometric methods and one HPLC method were developed for analysis of anti-diabetic drugs in tablets. The two spectrophotometric methods were based on the reaction of rosiglitazone (RSG) with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and bromocresol green (BCG). Linear relationship between the absorbance at λ_{max} and the drug concentration was found to be in the ranges 6.0–50.0 and 1.5–12 μg ml^{-1} for DDQ and BCG methods, respectively. The third spectrophotometric method consists of a zero-crossing first-derivative spectrophotometric method for simultaneous analysis of RSG and metformin (MTF) in tablets. The calibration curves were linear within the concentration ranges of 5.0–50 μg ml^{-1} for RSG and 1.0–10.0 μg ml^{-1} for MTF. The fourth method is a rapid stability-indicating HPLC method developed for the determination of RSG. A linear response was observed within the concentration range of 0.25–2.5 μg ml^{-1}. The proposed methods have been successfully applied to the tablet analysis.

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1. Introduction

Drugs belonging to class such as biguanides (e.g. metformin), sulfonylureas and thiazolidinedione (TZD) derivatives (pioglitazone, rosiglitazone) are commonly prescribed anti-diabetic drugs for the treatment of non-insulin dependent type II diabetes mellitus. For many patients with Type 2 diabetes, monotherapy with an oral anti-diabetic agent is not sufficient to reach target glycaemic goals and multiple drugs may be necessary to achieve adequate control [1]. The use of combination of biguanides and TZDs is commonly observed in clinical practice. This combination can be achieved by taking each of the drugs separately or alternatively fixed formulations have been developed.

Rosiglitazone maleate (RSG), chemically [(±)-5-[4-[2-[*N*-methyl-*N*(2-pyridyl) amino] ethoxy] benzyl]-2,4-dione thiazolidine] maleate (Fig. 1A), it's a potent new oral antihyperglycemic agent that reduces insulin resistance in patients with type 2 diabetes by binding to peroxisome proliferator-activated receptors gamma [2–4]. Metformin hydrochloride (MTF) is chemically 1,1-dimethyl biguanide hydrochloride (Fig. 1B). These drugs are oral hypoglycemic agents prescribed individually and concomittantly. A combination of 500 mg of metformin hydrochloride and 2 mg of rosiglitazone is available commercially and indicated for the treatment of type 2 diabetes mellitus.

Several methods have been reported for the determination of RSG in pharmaceutical preparations such as UV-spectrophotometry [5, 6], high performance thin layer chromatography [7–9], high performance liquid chromatography (HPLC) [10, 11] and micellar electrokinetic capillary chromatography [10]. There is no visible spectrophotometric method available for determination of RSG in tablets; therefore two visible spectrophotometric analyses were developed for the analysis of RSG in tablets. These spectrophotometric methods were based on charge transfer reaction (with DDQ) and ion-pair extraction method (with BCG). The reagents used in the proposed methods are inexpensive, readily available in any analytical laboratory and procedures do not involve any critical reaction conditions or tedious sample preparation.

Several methods are available for determination of MTF either alone or in combination with various drugs in bulk and pharmaceutical preparations using spectrophotometry [12,13], potentiometry [14], LC [15,16]. On the other hand, there are three chromatographic analyses available for the simultaneous determination of RSG and MTF in pharmaceutical preparations [17–19]. In literature survey, no spectrophotometric method was encountered for simultaneous analysis of these drugs in pharmaceutical

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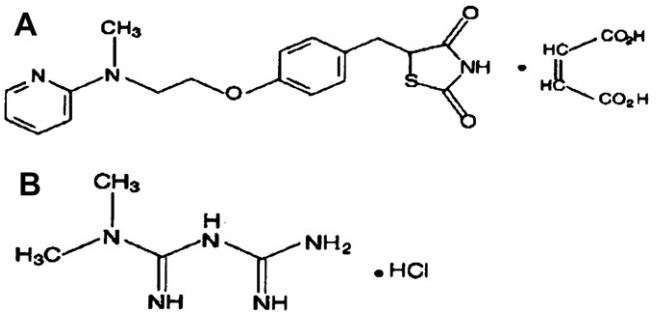

Fig. 1. Chemical structure of (A) rosiglitazone maleate and (B) metformin HCl.

preparations. Therefore, a zero-crossing first-derivative spectrophotometric method was developed for simultaneous analysis of RSG and MTF in fixed dose combination tablet preparations. The derivative UV-spectrophotometric method is very simple and requires no reagent, pH-adjustment and extraction as compared to chromatographic technique and can be used for routine analysis of RSG and MTF, simultaneously in fixed dose combination tablets in quality control laboratories.

In the last method, a simple, accurate and reproducible HPLC for determination of RSG in tablets unaffected by interferences from the excipients. Degradation profile of drug was also investigated by this HPLC method. For this purpose stock solution of RSG was subjected to stress conditions; neutral, acid and alkali hydrolysis as well as oxidation, dry heat treatment. The method proved to be selective and useful for the investigation of the stability of RSG.

2. Results and discussions

Rosiglitazone maleate, an anti-diabetic agent, indicated for the treatment of non-insulin dependent type II diabetes mellitus. Only two UV-spectrophotometric analysis of RSG in tablets are available in literature [5,6]. Literature scan revealed no visible spectrophotometric method for the determination of RSG in tablets. Therefore, two visible spectrophotometric analytical methods were developed for the determination of RSG in tablets. The first spectrophotometric method is based on the reaction of RSG as *n*-electron donor with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) as an π-acceptor to give highly colored complex species. π-acceptors are known to yield charge transfer complexes and radical anions with a variety of electron donors [20–25]. The second spectrophotometric method is based on ion-pair extraction spectrophotometry. This technique has received considerable attention for quantitative determination of many pharmaceutical compounds [26–30]. An ion-pair is formed between basic compounds and an anionic dye (such as bromophenol blue). At a specific pH, the ion-pair is extracted into an organic solvent, which is immiscible with water, and the concentration of the resulting ion-pair in the organic phase is determined spectrophotometrically.

The interaction of RSG with DDQ, in acetonitrile yielded intense orange red colored chromogen absorbing maximally at wavelengths 494 nm (Fig. 2A). The RSG reacts with BCG in an acidic buffer to give a chloroform soluble yellow colored ion-association complex which exhibits an absorption maximum at 420 nm (Fig. 2B).

The influence of different parameters on the color formation was studied to determine optimum conditions for the visible spectrophotometric methods.

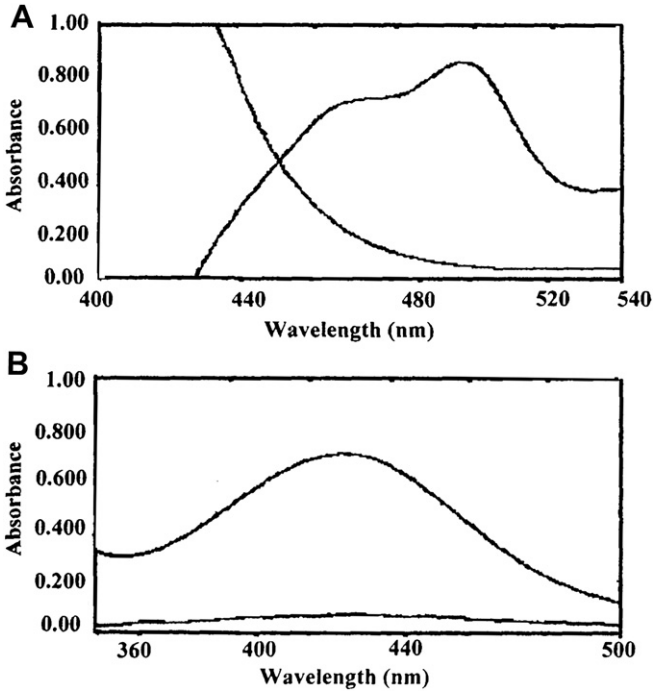

Fig. 2. (A) Absorption spectrum of charger transfer complex between RSG and DDQ reagent (50 μg ml^{-1}) against reagent blank, (B) absorption spectrum of ion-pair complex between RSG and BCG reagent (9 μg ml^{-1}) against reagent blank.

2.1. Choice of solvent

For DDQ method, different solvents including chloroform, acetonitrile, acetone, ethanol, 1,4–dioxan, methanol, and methylene chloride were investigated in order to select the suitable solvent. Acetonitrile is considered to be an ideal solvent for the color reaction as it offers solvent capacity and gives the highest yield of the radical as indicated by high ε values.

For BCG method, the effect of the extracting solvent on the ion-pair complex was examined. Chloroform, ethyl acetate, dichloromethane, ether, benzene, methyl isobutyl ketone were tested as extractive solvents for effective extraction of colored species from aqueous phase. Chloroform was selected because of its slightly higher efficiency on color intensity and its suitability for selective extraction of the drug–dye complexes from the aqueous phase.

2.2. Reagent concentration

For DDQ method, the effect of DDQ concentration (indicated as volume) on its reaction with the RSG was investigated. When various concentrations (by volume) of DDQ solution added to a fixed concentration of RSG, a 1.5 ml of DDQ solutions 0.2% (w/v) is found to be effective for the quantitative determination of RSG (Fig. 3).

For BCG method, the effect of dye concentration on the intensity of the color developed was tested using different milliliters of the reagent. It was found that 0.6 ml of BCG solution 0.3% (w/v) was adequate to obtain a stable product exhibiting maximum absorption at 420 nm (Fig. 3).

2.3. Reaction time

For DDQ method, the optimum reaction time was determined by following the color development at room temperature, 50 °C and 60 °C. Complete color development was attained after heating on a water bath at 50 °C for 25 min. The color remained stable for 7 h. For BCG method, the effect of temperature on the colored

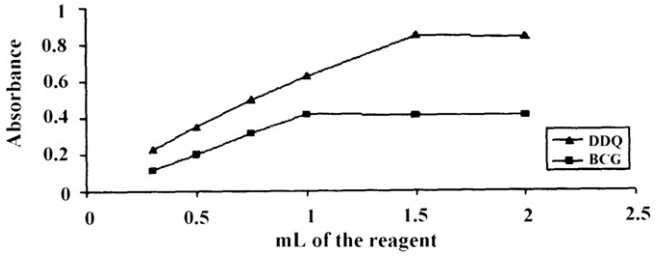

Fig. 3. Effect of volume of DDQ, (0.2%, w/v) and BCG (0.3%, w/v) on the formation of the reaction product of RSG with these reagents at (▲) DDQ and (■) BCG.

complexes was studied at room temperature. Complete color development was attained immediately. The resultant complexes with both of DDQ and BCG were stable up to 24 h at room temperature when kept in the dark.

2.4. Stoichiometry of the reaction

The molar ratio of DDQ and BCG to RSG in the reaction mixture was studied according to Job's method of continuous variation [31]. Utilizing equimolar solutions of RSG and DDQ and BCG, the reaction stoichiometry for both of DDQ–RSG and BCG–RSG reactions was found to be a good approximation 1:1 ratio (drug/reagent), confirming that one molecule of RSG reacts either with one molecule of DDQ or with one molecule of BCG.

2.5. Derivative spectrophotometric method

RSG and MTF are oral hypoglycemic agents. A fixed dose combination of 500 mg of MTF and 2 mg of RSG is available commercially and indicated for the treatment of type 2 diabetes mellitus. For the simultaneous analysis of these drugs, a zero-crossing first-derivative spectrophotometric method was developed in fixed dose combination tablet preparations.

The absorption spectra of the two compounds, RSG and MTF overlapped as shown in Fig. 4. Since spectral overlap is quite clear in this figure, simultaneous determination of these components cannot be performed. For this reason, the simultaneous determination of these drugs was not possible by direct measurements of absorbance in zero-order spectra. On the other hand, derivative spectrophotometry based on a mathematical transformation of the zero-order curve into the derivative spectra can overcome this problem [32–37]. The RSG and MTF were prepared with different solvents (acetonitrile, methanol, water) and their mixture. The best results were obtained with methanol: water (50:50). By using memory channels, the first-fourth order derivative spectra were investigated. The first order derivative spectrum was selected when compared by the zero-crossing point (ZCP). As it can be seen on Fig. 5C, the wavelength 334 nm was selected for the determination of RSG (where the derivative response for MET was zero) and 227 nm was selected for the determination of MTF (where the derivative response for RSG was zero). Characteristic wavelengths (ZCP) for RSG and MTF were verified by working with various concentrations of each drug.

2.6. HPLC method

In order to separate RSG and its degradation products formed under stress conditions, aqueous buffer–acetonitrile mixtures were used as the mobile phase. Satisfactory resolution was obtained using the mobile phase system of acetonitrile-*o*-nitric acid (pH:3) (30:70, v/v) at a flow rate of 1.0 ml min^{-1} using C_{18} column and UV

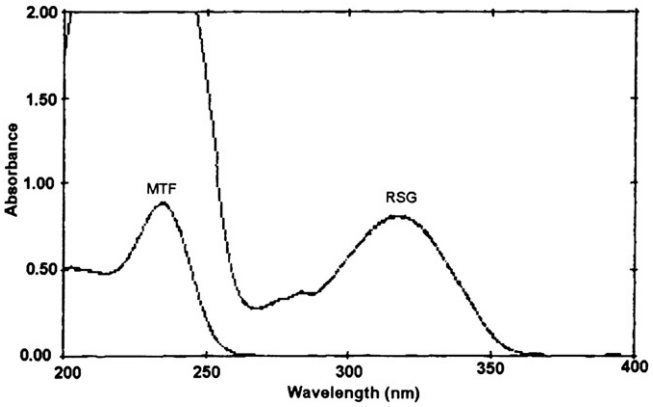

Fig. 4. Overlaid zero-order spectra of RSG (50 μg ml^{-1}) and MTF (10 μg ml^{-1}) in methanol–water (50:50).

detector was set at 243 nm. In Fig. 6, a typical chromatogram obtained under these conditions is presented. Chromatograms of stressed reaction solutions are also presented in Fig. 6. These chromatograms indicate that the developed method was successful for the separation the drug and its chromophoric degradation products.

2.7. Method validation

In the visible spectrophotometric methods, linear relationship between the absorbance at λ_{max} and the concentration of the drug was found within the range of 6.0–50.0 and 1.5–12 μg ml^{-1} for DDQ and BCG methods, respectively. Molar absorptivity value of DDQ and BCG method was found as 1.5×10^4 and 7×10^4 L/mol cm, respectively. The BCG method is the most sensitive method when compared to the DDQ method, with a high ε value. Sandell's sensitivity (S) represents the number of micrograms of the determinant per milliliter of a solution having an absorbance (A) of 0.001 for a path length (l) of 1-cm. Thus, $S = 10^{-3}/a = \mu g\ cm^{-2}$ where, a is the specific absorptivity and its value (in ml g^{-1} cm^{-1}) corresponds to the determinant in a cuvette with an optical length of 1-cm. Also, $a = (b/\text{molecular weight of rosiglitazone base}) \times 1000$, where b = molar absorptivity = A/Cl, where C is the molar concentration of the determinant and l = 1-cm path length. Sandell sensitivity was found to be 0.023 and 0.050 μg cm^{-2} for DDQ and BCG methods, respectively

In the derivative spectrophotometric method, the absorbance of the standard solutions of RSG and MTF were measured at wavelengths of 334 and 227 nm for rosiglitazone and metformin, respectively. The calibration curves were constructed by plotting the D1 values against RSG or MTF. The concentration ranges were found to be 5.0–50.0 and 1.0–10.0 μg ml^{-1} for RSG and MTF, respectively.

In the HPLC method, the calibration curve was prepared by plotting the peak area of RSG against drug concentration and it was linear within the range of 0.25–2.5 μg ml^{-1}. Peak area and concentration were subjected to least square linear regression analysis for the calculation of the calibration equation and correlation coefficients.

In the spectrophotometric methods, the limits of detection (LOD) and limits of quantitation (LOQ) were determined using the formula: LOD or LOQ = $\kappa SDa/b$, where $\kappa = 3$ for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope. In the HPLC method, the LOD was found to be 0.05 μg ml^{-1} when the signal-to-noise ratio 3:1. The limit of quantitation was 0.25 μg ml^{-1} with a coefficient of variation 1.02% ($n = 5$).

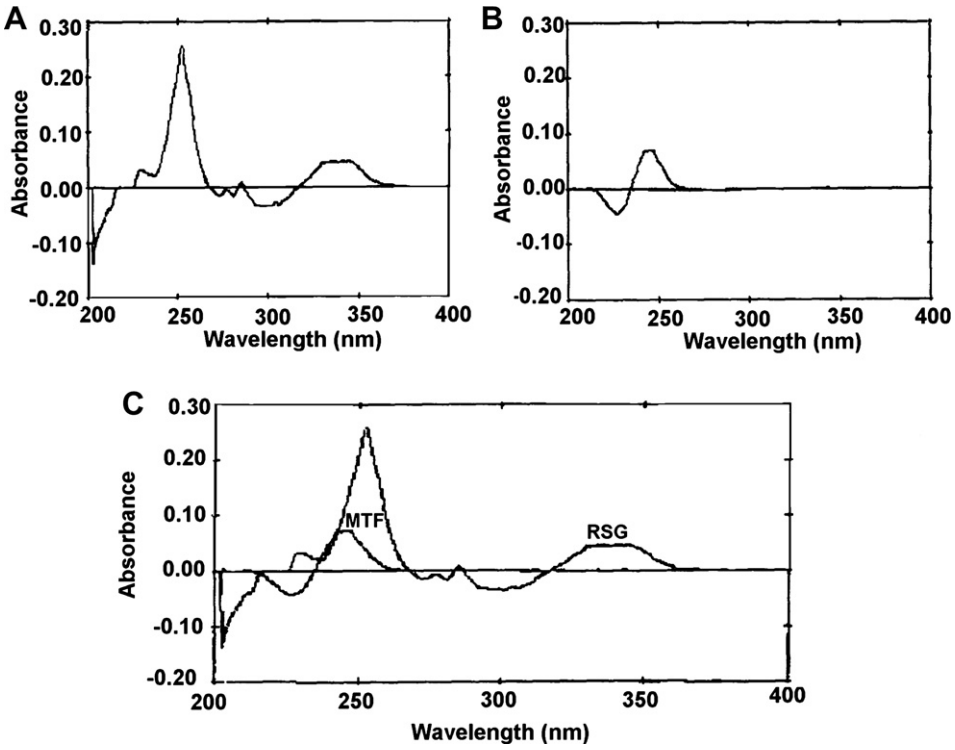

Fig. 5. (A) First-derivative spectrum of RSG in methanol–water (50:50), (B) first-derivative spectrum of MTF in methanol–water (50:50) and (C) overlaid first-derivative spectra of RSG (50 μg ml^{-1}) and MTF (10 μg ml^{-1}) in methanol–water (50:50).

The inter- and intra-day precisions were examined by analysis of drugs for the same day and seven consecutive days (each $n = 5$). The RSD values for intra-day precision was 0.43–0.89% and inter-day precision was 0.67–1.32% for all developed methods indicating good precision. The obtained results are summarized in Table 1.

To check accuracy of the proposed methods, the standard addition technique was applied. A different amount of pure sample solution was added to three different concentrations of the standard drug solution and assayed. The percent recovery of the added standard to the assay samples was calculated from:

$$\text{Recovery}\% = [(C_t - C_u)/C_a] \times 100$$

where C_t is the total concentration of the analyte found; C_u is the concentration of the analyte present in the formulation; and C_a is the concentration of the pure analyte added to the formulation. The results of analysis of the commercial dosage forms and the recovery study as shown in Table 2. The average percent recoveries obtained were quantitative (99.69–101.96%), indicating good accuracy of the methods.

Degradation behavior of drug was investigated using HPLC study mentioned above. RSG solution remained stable under thermal stress, but about 15% of the drug was decomposed with acid hydrolysis and chemical oxidation. On the other hand, after the basic and neutral hydrolysis, the peak corresponding to the parent drug substantially reduced. The proposed method can be used as a stability-indicating one because the peak of the parent drug, RSG, is not interfered by any other signal in the chromatogram (Fig. 6). The method proved to be both selective and useful for the investigation of the stability. Recovery data of the degradation tests for RSG are given in Table 3.

The applicability of the proposed method was tested by the determination of drugs in their pharmaceutical preparations. The results obtained are satisfactorily accurate and precise as indicated by the excellent % recovery and SD < 2 (Table 4). Experiments showed that there was no interference from the additions and excipients, e.g. lactose, glucose, fructose, magnesium stearate and starch.

3. Conclusion

The aim of this study was to develop simple, fast, validated and very economic methods for either analysis of RSG individually in pharmaceutical preparations or simultaneous analysis of RSG and MTF in fixed dose combination pharmaceutical preparations.

Two selective, simple and less time consuming visible spectrophotometric methods using DDQ and BCG reagents were described for analysing of RSG in tablets. BCQ method was the most sensitive compared when compared to the DDQ method, with a high ε value (7×10^4 L/mol cm). The proposed visible spectrophotometric methods are simple, accurate and highly cost-effective and suitable for routine determination of the rosiglitazone in tablets.

For the simultaneous analysis of RSG and MTF in fixed dose combination tablets, first-derivative spectrophotometric method was proposed. Derivative spectroscopy shows better resolution and enables the method to analyse each drug in presence of one another as well as in presence of other excipients without any pretreatment. The proposed first-derivative spectrophotometric method is simple, practical, inexpensive and fast with respect to analysis time when compared to chromatographic technique and the proposed method can be used for routine analysis of RSG and MTF, simultaneously in fixed dose combination tablets without any prior separation in quality control laboratories.

The proposed HPLC method provides simple, accurate and reproducible quantitative analysis for determination of RSG in tablets without any interference from the excipients and also allows analysis of RSG without any interference in the presence of its neutral, acidic, alkaline, oxidative degradation products. It is a simple analytical procedure and short retention time allows the

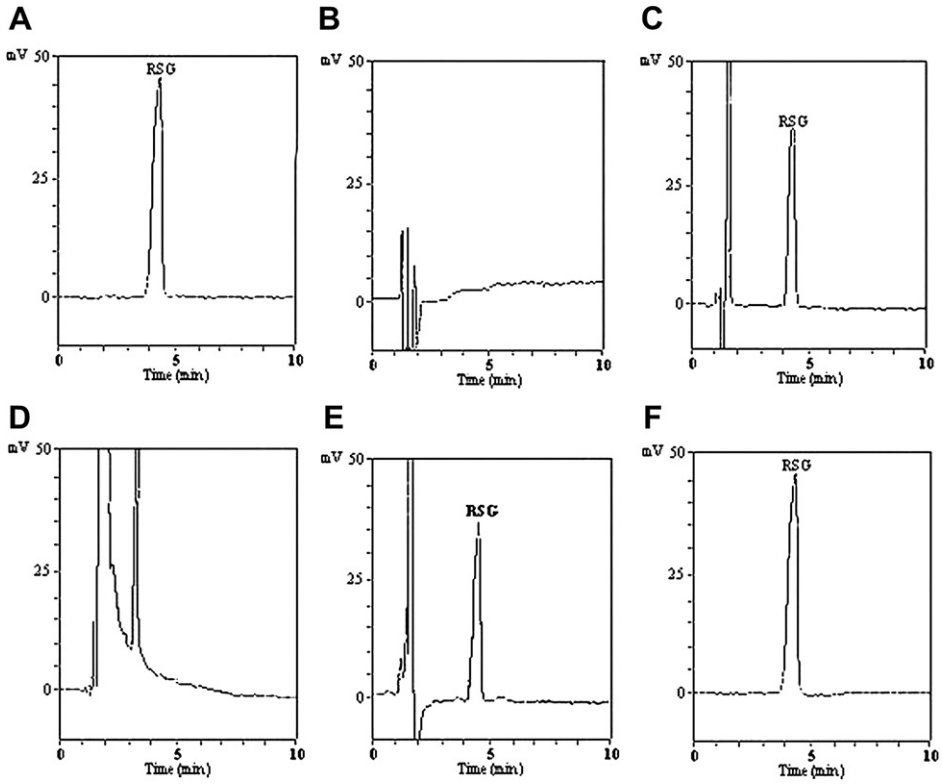

Fig. 6. Chromatograms corresponding to (A) a typical chromatogram of 2.5 μg mL^{-1} RSG standard, RSG solution subjected to (B) neutral hydrolysis (C) acid hyrolysis, (D) alkaline hydrolysis, (E) chemical oxidation and (F) thermal stress.

analysis of a large number of samples in a short period of time. The proposed HPLC method for determination of RSG has advantages over other analytical methods due to selectivity and better sensitivity.

The developed methods were validated and applied for the determination of drugs in pharmaceutical preparations. The high recovery percentage and low relative standard deviation reflect the high accuracy and precision of the proposed methods; moreover, the methods are easy, applicable to a wide range of concentration, besides being less time consuming and depending on simple and available reagents thus offering economic and acceptable methods for the routine determination of drugs in pure form and its pharmaceutical preparations.

4. Experimental protocols

4.1. Apparatus

Spectrophotometric measurements were carried out using a Shimadzu UV-160 A spectrophotometer with 1-cm glass cells.

The HPLC analyses were performed on a Shimadzu LC-20A (Kyoto, Japan) which consisted of an LC-20AT solvent delivery system equipped with a Rheodyne injection valve with a 20 L loop, DGU-20A5 vacuum degasser, CTO-10ASVP column oven and SPD-M20A photodiode array detector (PDA) set at 243 nm. The data was collected and analyzed via the automation system software. Separations were performed at room temperature on a Luna C_{18} column

Table 1
Results of validation parameters for proposed methods.

Parameter	Visible spectrophotometric methods		Derivative spectrophotometric method		HPLC method
	Using DDQ reagent	Using BCG reagent	For RSG	For MTF	For RSG
Linearity range[a] (μg ml^{-1})	6.0–50.0	1.5–12	5.0–50.0	1.0–10.0	0.25–2.5
Intra-day[b], RSD %	0.65	0.46	0.89	0.80	0.43
Inter-day[c], RSD %	0.87	0.95	1.03	1.32	0.67
Regression equation[d]					
Slope ± SD	0.0152 ± 0.00023	0.0656 ± 0.0016	0.001 ± 0.00005	0.0053 ± 0.0001	443 544 ± 3178
Intercept ± SD	−0.0041 ± 0.00031	0.022 ± 0.0007	0.0013 ± 0.0005	0.032 ± 0.00008	17820 ± 78
Correlation coefficient, *r*	0.9995	0.9986	0.9998	0.9996	0.9997
LOD (μg ml^{-1})	0.061	0.032	0.165	0.050	0.05
LOQ (μg ml^{-1})	0.205	0.106	0.55	0.168	0.25

[a] Average of six determinations.
[b] $n = 5$ Correspond to replicate analysis for each level.
[c] Results of five different days.
[d] $A = a + bC$ (where C is the concentration of drug in μg ml^{-1}, A is the absorbance at λ_{max} for spectrophotometry, peak area for HPLC).

Table 2
Results of recovery studies by standard addition method.

	Amount taken (μg ml^{-1})	Amount added (μg ml^{-1})	Total amount found[c] (μg ml^{-1}) (Mean ± SD[d])	Recovery (%)	RSD (%)
Visible spectrophotometric methods[a]					
Using DDQ reagent	10	6.0	16.043 ± 0.087	100.72	0.54
		20.0	30.132 ± 0.38	100.66	1.26
		40.0	49.876 ± 0.70	99.69	1.40
Using BCG reagent	4	1.5	5.510 ± 0.074	100.65	1.34
		5.0	9.036 ± 0.045	100.73	0.50
		8.0	12.103 ± 0.169	101.29	1.40
Derivative spectrophotometric method[b]					
For RSG	10	5.0	15.098 ± 0.073	101.96	0.48
		20.0	30.054 ± 0.35	100.27	1.16
		40.0	50.210 ± 0.43	100.53	0.86
For MTF	5	1.0	5.997 ± 0.052	99.70	0.87
		3.0	8.056 ± 0.056	101.87	0.70
		5.0	11.007 ± 0.066	101.34	0.66
HPLC method[a]					
For RSG	1	0.25	1.2504 ± 0.0065	100.16	0.52
		1.0	1.9976 ± 0.012	99.76	0.60
		1.50	2.4989 ± 0.025	99.93	1.02

[a] Avandia film tablet®, containing 4 mg of rosiglitazone per tablets.
[b] Avandamet film tablet® containing 2 mg of rosiglitazone and 500 mg metformin hydrochloride per tablets.
[c] Five independent analyses.
[d] Standard deviation.

(4.6 mm i.d. · 250 mm, 5 lm particle; Phenomenex, TX, USA), with a guard column (4 mm · 3 mm i.d.; Phenomenex) packed with the same material. The mobile phase consists of acetonitrile-*o*-nitric acid pH adjusted to 3 with 1 N NaOH (30:70), v/v at a flow rate of 1.0 ml min^{-1}. The mobile phase was degassed by an ultrasonic bath and filtered by a Millipore vacuum filter system equipped with a 0.45 μm HV filter.

4.2. Reagents and solutions

Rosiglitazone maleate and metformin hydrochloride were kindly supplied by Abdi Ibrahim Ilac (Istanbul, Turkey) and Bilim Ilac (Istanbul, Turkey), respectively. Their pharmaceutical preparation Avandia film tablet®, containing 4 mg of rosiglitazone per tablets and Avandamet film tablet® containing 2 mg of rosiglitazone and 500 mg metformin hydrochloride per tablets were obtained from local drugstore. All chemicals and reagents were of analytical-reagent grade.

For visible spectrophotometric methods, stock solutions were prepared by dissolving 33.1 mg of rosiglitazone maleate (equivalent to 25 mg of the rosiglitazone base) in water–methanol (98:2) in a 100-ml volumetric flask to give a concentration of 250 μg ml^{-1} of RSG.

For DDQ method, RSG base solution was prepared. For this purpose, 10 ml aliquot of the stock solution was transferred to stoppered tube and 1 ml 1 N NaOH solution was added. Three 5 ml portion of chloroform was added for the extraction. Combined extracts were dried using anhydrous Na_2SO_4 and diluted with 20 ml with chloroform. All of this solution was evaporated to dryness under nitrogen with mild heating. The residue was dissolved with acetonitrile (in 20 ml volumetric flask) using an ultrasonic bath. Acetonitrile was added to the mark (125 μg ml^{-1} as the base).

2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) (Merck-Schuchardt, Munich, Germany) was freshly prepared as (0.2%(w/v)) solutions in acetonitrile; the solution was stable for 1 week at 4 °C. BCG was obtained from Merck (Darmstadt, Germany). 0.3% BCG solution was prepared in water.

For derivative spectrophotometric method, portions (50 mg each) of standard RSG (calculated as a base) and MTF were weighed and transferred to 2 separate 100 ml volumetric flasks and dissolved in methanol–water (50:50) and further diluted with the same solvent mixture to obtain standard solutions of RSG and MTF having final concentrations of 100 μg ml^{-1} each.

For HPLC method, stock solutions of RSG (as bases) were prepared in methanol to give a concentration of 250 μg ml^{-1} of RSG and diluted further with the mobile phase (acetonitrile-*o*-nitric acid (30:70), v/v) to obtain standard solutions of 25 μg ml^{-1}.

Table 3
Degradation trial for RSG.

Condition	Time (h)	Recovery (%)
Acid, 1 N HCl, (80 °C)	1	87.89
Base, 1 N NaOH, (80 °C)	1	–
Neutral hydrolysis, (80 °C)	1	–
H_2O_2, 30%, (at room temperature)	1	84.59
Dry heat (100 °C)	5	99.83

Table 4
Analysis of RSG using visible spectrophotometric and HPLC methods in tablets containing 4 mg of the drug and analysis of RSG and MTF simultaneously using derivative spectrophotometric method in tablets containing 2 and 500 mg rosiglitazone and MTF, respectively ($n = 5$).

Visible-spectrophotometric methods[a]	Using DDQ reagent	Using BCG reagent
Mean[c] ± SD	3.99 ± 0.033	4.012 ± 0.047
Recovery (%)	99.75	100.30
RSD (%)	1.20	1.17
Derivative spectrophotometric method[b]	For RSG	For MTF
Mean[c] ± SD	2.015 ± 0.024	502.56 ± 2.56
Recovery (%)	100.75	100.51
RSD (%)	1.19	0.51
HPLC Method[a]	For RSG	
Mean[c] ± SD	4.032 ± 0.009	
Recovery (%)	100.80	
RSD (%)	0.37	

[a] Avandia film tablet®, containing 4 mg of rosiglitazone per tablets.
[b] Avandamet film tablet® containing 2 mg of rosiglitazone and 500 mg metformin hydrochloride per tablets.
[c] Five independent analyses.

4.3. General procedure

4.3.1. Visible spectrophotometric methods for RSG using DDQ and BCG reagents

For DDQ method, aliquots of 0.24–2.0 ml of standard or sample solution (from 125 μg ml^{-1} of base solution in acetonitrile) were pipetted into a series of 5.0 ml volumetric flask and added 1.5 ml of DDQ solution. Heat at 50 °C in a hot water bath for about 25 min; cool the mixture to the laboratory temperature and make up to volume with acetonitrile. The absorbance of the resulting solutions was measured at 496 nm against reagent blank treated similarly.

For BCG method, aliquot of 0.030–0.240 ml of stock RSG solutions was transferred to stoppered glass tubes and total volumes were brought to 0.240 ml with water. 0.6 ml of BCG solution and 1 ml of buffer (pH 3.5 phthalate buffers) solution were added in each tube. The reaction mixtures were extracted with 5 ml chloroform for 1 min with a vortex mixer. The two phases were allowed to separate and the chloroform layers were passed through anhydrous sodium sulphate. The absorbance of the yellow-colored chloroformic extracts was measured at 420 nm against corresponding reagent blank, prepared similarly except addition of drugs. All measurements were made at room temperature.

4.3.2. Derivative spectrophotometric method for RSG and MTF, simultaneously

Two different diluted standards were prepared from the stock solutions of RSG and MET, series A and B, as follows:

Series A: Different aliquots of drug solution (0.25–2.5 ml) were transferred to 5 ml volumetric flask to provide final concentration range 5.0–50.0 μg ml^{-1} and the volume was diluted to volume with methanol–water (50:50).
Series B: Different aliquots of drug solution (0.05–0.5 ml) were transferred to 5 ml volumetric flask to provide final concentration range 1.0–10.0 μg ml^{-1} and the volume was diluted to volume with methanol–water (50:50).

The absorbance of the standard solutions of RSG and MTF were measured at wavelengths of 334 and 227 nm for rosiglitazone maleate and metformin, respectively.

4.4. HPLC method

4.4.1. Selectivity

Selectivity study was performed using HPLC procedure.

Forced degradation or stress testing is undertaken to demonstrate specificity when developing stability-indicating methods, particularly when little information is available about potential degradation products

The stress conditions were as follows:

Hydrolysis: Individually, 5 mg RSG were dissolved in 5 mL methanol in a 10 mL volumetric flask and boiled for 1 h at 80 °C after adding: (a) 5 mL water for neutral hydrolysis, (b) 5 mL 1 N HCl for acid hydrolysis (c) 5 mL 1 N NaOH for basic hydrolysis.
Chemical oxidation: 5 mg RSG were dissolved in 5 mL methanol in a 10 mL volumetric flask and 100 μL 30% H_2O_2 solution (v/v) were added and mixed. The solution was left at room temperature for 1hr in the dark.
Thermal stress: Bulk drug was subjected to dry heat at 105 °C for 5 h.

To each of the stressed solutions was diluted with the mobile phase to obtain a theoretical concentration of 2.5 μg ml^{-1} for RSG. Each solution was analyzed in triplicate.

4.5. Assay procedure for tablets

For visible spectrophotometric methods, twenty tablets were individually weighed to get the average weight of the tablets. A sample of the powdered tablets, claimed to contain 40 mg of RSG base was transferred to 100 ml volumetric flask. About 75.0 ml of water: methanol (98:2) was added and then extraction was performed mechanically for 20 min and sonicated for 20 more minutes. The volume was brought to 100 ml with same solvent mixture and the content was centrifuged for 10 min at 3000 × *g*. Aliquots of filtrate were diluted further with same solvent then proceeds as described under general procedure. The nominal contents of the film tablets were calculated using either the calibration graph or the corresponding regression equation ($n = 5$).

For derivative spectrophotometric method, twenty tablets were weighed and finely powdered. Powder equivalent to 10.0 mg RSG and 2500 mg MTF was accurately weighed and transferred to a 100 ml volumetric flask. 75 ml methanol–water (50:50) was transferred to the volumetric flask and then extraction was performed mechanically for 20 min and sonicated for 20 more minutes. The volume was brought to 100 ml with same solvent mixture and the content was centrifuged for 10 min at 3000 × *g*. The further dilution was made with methanol–water (50:50) to give a solution containing 10 μg ml^{-1} RSG and 2.50 mg ml^{-1} MTF (Solution 1), which was used for the determination of RSG. From this solution, 1 ml was transferred to a 250 ml volumetric flask. The volume was diluted with methanol–water (50:50) to the mark to give a solution containing 0.04 μg ml^{-1} RSG and 10 μg ml^{-1} MTF (Solution 2), which was used for the determination of MTF. RSG and MTF are present in very different quantities in the formulation which requires their measurement in different dilution levels to avoid any need for addition of RSG as standart to the sample solution; firstly RSG is measured at lower dilution then MTF at higher dilution.

For HPLC method, powder equivalent to 1.0 mg RSG was accurately weighed and transferred to a 100 ml volumetric flask. 75 ml methanol was transferred to the volumetric flask and then extraction was performed mechanically for 20 min and sonicated for 20 more minutes. A 1 mL aliquot of the supernatant was diluted to 100 mL with the mobile phase. A 20 μL of its aliquot was injected and chromatographed ($n = 5$).

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