Research article

Drug Testing and Analysis

Received: 31 July 2010 Revised: 23 November 2010 Accepted: 7 December 2010 Published online in Wiley Online Library: 18 March 2011

(www.drugtestinganalysis.com) DOI 10.1002/dta.260

Multi-objective optimization strategy based on desirability functions used for electrophoratic separation and quantification of rosiglitazone and glimepiride in plasma and formulations

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Multiple response simultaneous optimization employing Derringer's desirability function was used for the development of a capillary electrophoresis method for the simultaneous determination of rosiglitazone (RSG) and glimepiride (GLM) in plasma and formulations. Twenty experiments, taking the two resolutions, the analysis time, and the capillary current as the responses with three important factors – buffer morality, volte and column temperature – were used to design mathematical models. The experimental responses were fitted into a second order polynomial and the six responses were simultaneously optimized to predict the optimum conditions for the effective separation of the studied compounds. The separation was carried out by using capillary zone electrophoresis (CZE) with a silica capillary column and diode array detector at 210 nm. The optimum assay conditions were 52 mmol l^{-1} phosphate buffer, pH 7, and voltage of 22 kV at 29 °C. The method showed good agreement between the experimental data and predictive value throughout the studied parameter space. The assay limit of detection was 0.02 μgml^{-1} and the effective working range at relative standard deviation (RSD) of ≤ 5% was 0.05–16 μgml^{-1} (r = 0.999) for both drugs. Analytical recoveries of the studied drugs from spiked plasma were 97.2–101.9 ± 0.31–3.0%. The precision of the assay was satisfactory; RSD was 1.07 and 1.14 for intra- and inter-assay precision, respectively. The proposed method has a great value in routine analysis of RSG and GLM for its therapeutic monitoring and pharmacokinetic studies.

Keywords: multi-objective optimization; desirability function; capillary electrophoresis; rosiglitazone and glimepiride

Introduction

Diabetes is a syndrome of disordered metabolism, usually due to a combination of hereditary and environmental causes, resulting in abnormally high blood sugar levels (hyperglycemia) due to defects in either insulin secretion (*type I*) or insulin action in the body (*type II*).[1] All forms of diabetes have been treatable since insulin became medically available in 1921, but there is no cure. The World Health Organization projects that the number of diabetics will exceed 350 million by 2030. Governments and other healthcare providers around the world are investing in health education, diagnosis, and treatments for this chronic, debilitating – but controllable – disorder. Many patients with type II diabetes require treatment with more than one anti-hyperglycemic drugs to achieve optimal glycemic control (combination products) which is also known as fixed dose combinations (FDCs). More than one-third of all the new drug products introduced worldwide during the last decade were FDC preparations. So there is a need for analytical methods capable of performing simultaneous determination of active compounds in FDCs.

In recent years, capillary electrophoresis (CE) has appeared as an appropriate technique for the analysis of complex formulations, as demonstrated in several published papers in this area, in which CE has been shown as a valuable alternative technique for their separation.[2–4] CE utilizes a wide selection of electrophoratic factors, *viz.*, the type and molarity of buffer, pH, type and concentration of organic modifier, voltage, temperature, etc. Optimization of the experimental conditions is a complicated process. However, the analysis time is usually optimized by reduced migration time without losing the resolution between the peaks originated by the analyte migration. The need to take into account different aspects of the analysis at the same time calls for the use of multi-criteria optimization. In order to carry out the latter type of optimization, experimental design is valuable tool, specifically surface response analysis[5] by using Derringer's

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desirability functions.[6–12] It is an effective tool for that purpose due to the versatility that these functions have to transform each response separately and comprising all of them in an overall desirability function to be globally optimized in a final step.

Rosiglitazone (RSG) 5-[4-[2-[N-methyl-N-(2-pyridinyl)amino] benzyl]-2-4-thiazolidinedione[13] is a novel anti-hyperglycemic drug that improves glycemic control primarily by decreasing insulin resistance by sensitizing the skeletal muscle, liver and adipose tissue to the actions of insulin.[14] Glimepiride (GLM) 1-[4-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)-ethyl]phenylsulphonyl]-3-(4- methylcyclohexyl) urea[13] is a second generation sulfonylurea widely used in type II diabetes mellitus. It is completely bioavailable from the gastrointestinal tract and achieves metabolic control with the lowest dose.[14] The effect of lowering blood glucose through a combination of RSG and GLM is significantly better than monotherapy with sulfonylurea especially in patients who are unable to achieve sufficient control on optimal dosage of sulfonylurea monotherapy and for whom metformin is inappropriate because of contraindications or intolerance.[14] As an effective treatment for type II diabetic patients, it is necessary to monitor the plasma concentrations of RSG and GLM and to study their pharmacokinetics in the human body for optimization of dose and dose regimen.

Because of the therapeutic important and clinical success of RSG and GLM, several methods have been developed for its quantitative determination in plasma samples and in pharmaceutical preparations. These methods for RSG alone include high performance liquid chromatography (HPLC),[15–19] liquid chromatography-tandem mass spectrometry (LC-MS/MS),[20] capillary electrophoresis,[21] high performance tin layer chromatography (HPTLC),[22, 23] or in combination with other anti-diabetic drugs,[24–29] and for GLM alone include derivative spectrophotometric technique,[30] HPLC,[31–33] LC-MS/MS,[34–37] or in combination with other anti-diabetic drugs.[38–41] A literature survey revealed that only one spectrophotometric estimation of RSG and GLM in tablet dosage form has been reported.[42] This method involved tedious steps for calculations, pretreatment of the samples and low accuracy and reproducibility. For these reasons, the development of new, alternative, analytical technology for simultaneous determination of RSG and GLM in plasma and formulations with adequate sensitivity, improved simplicity was seriously needed. For the first time, the present study described the development and validation of a sensitive CE method for determination of RSG and GLM at concentration as low as 20 ngml^{-1} in plasma samples.

Experimental

Electrophoretic instrumentation and conditions

The employed CE system consisted of a Beckman P/ACE MDQ instrument (Beckman Coulter, Inc., Fullerton, CA, USA) equipped with a diode array detector and a data-handling system comprising an IBM personal computer and P/ACE system MDQ software. Detection was performed at 210 nm. An undeactivated fused silica capillary was obtained from Agilent Technologies (Santa Clara, CA, USA). and had the following dimensions: 67 cm total length, 50 cm effective length, 75 µm ID, 375 µm OD. The temperature of the capillary and the samples was maintained at 29 °C. The background electrolyte solution (BGE) consisted of phosphate buffer, 52 mM pH 7.0. Samples were injected into the capillary by pressure at the anodic side at 0.5 psi for 10 s. The electrophoresis was carried out by applying high voltage to the capillary, with the cathode being at the detector end. The capillary was washed between runs with a sequence of rinses: 0.1 M sodium hydroxide (2 min), water (1 min), then equilibrated with the running buffer (4 min), to ensure reproducibility of the assay. Before sample injection, the capillary was conditioned with 0.1 M sodium hydroxide (5 min), water (5 min) and running buffer electrolyte (10 min).

Chemicals and reagents

RSG maleate and GLM reference standards were kindly supplied by GlaxoSmithKline (gsk) (Glaxo Welcome Ltd, Middlesex, UK). Combination product of RSG and GLM (Label claim: 4/1, 4/2, 4/4, mg/tablet for each drug respectively), Avandaryl™, (Smithkline Beecham Corporation, Zebulon, NC, USA). (Lot. 6ZM7410) were purchased from a local market. Isopropanol, disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium hydroxide, hydrochloric acid, and orthophosphoric acid were obtained from Merck (Darmastud, Germany). All chemicals were of analytical grade and used without further purification. Purified HPLC grade deionized water was used. Human plasma was obtained from King Khalid University Hospital (Riyadh, Saudi Arabia). All solutions were filtered through 0.2 µm PTFTE filter from Agilent Technologies (Willington, DE, USA).

Software

Experimental design, data analysis and desirability function calculations were performed by using Design-Expert® trial version 7.1.6 (Stat-Ease Inc., Minneapolis, MN, USA).

Preparation of stock and standard solutions

Stock solutions containing 1 mg ml^{-1} of GLM and RSG maleate were prepared in isopropanol on a free-base basis and corrected for salt and purity. The internal standard (IS) losartan was prepared in isopropanol to give a concentration of 1 mg ml^{-1} and was further diluted with isopropanol to get the working solution 400 µgml^{-1}. The solutions were stable for at least three weeks if kept in the refrigerator. Aliquots of the standard stock solutions of GLM and RSG were transferred into 20-ml volumetric flasks, 1.0 ml of IS solution (400 µgml^{-1}) was added to each flask, then completed to the mark with 52 mM phosphate buffer to yield final concentrations 0.05, 0.1, 0.15, 0.2, 0.25, 0.5, 1, 2, 4, 8, and 16 µgml^{-1} for RSG (maintaining the GLM concentration at a constant level 8 µgml^{-1}) and concentration of 0.05, 0.1, 0.15, 0.2, 0.25, 0.5, 1, 2, 4, 8, and 16 µgml^{-1} for GLM (maintaining the RSG concentration at a constant level 8 µgml^{-1}). Triplicate injections were made for each concentration. The peak-area ratios of each concentration to the IS against the corresponding standard concentration were plotted to obtain the calibration graphs. Alternatively, the corresponding regression equation was derived.

Analysis of authentic mixture

Aliquots of the standard stock solutions of RSG and GLM were transferred into 20-ml volumetric flasks, 1.0 ml of IS solution (400 µgml^{-1}) was added to each flask, then completed to the mark with 52 mM phosphate buffer to yield different concentrations ratios (4 : 1, 2 : 1, 1 : 1) respectively. Triplicate injections for each solution were made. The peak-area ratio of each concentration to the IS was calculated. The concentration of each drug is obtained using the calibration curve or the corresponding regression equation.

Preparation of plasma quality control samples

The quality control (QC) samples at six concentration levels, i.e. 0.2, 0.5, 3.0, 5.0, 10.0, and 12.0, $\mu g ml^{-1}$ were prepared by spiking the drug-free plasma with appropriate volumes of RSG and GLM. Before spiking, the drug-free plasma was tested to make sure that there was no endogenous interference at retention time of RSG and GLM and the internal standard. The QC samples were extracted with the calibration standards to verify the integrity of the method.

Plasma samples extraction procedure

A human plasma sample (300 µl) was placed in a 1.5 ml eppendorf tubes, and accurately measured aliquots of the standard RSG and GLM solutions were added. Then 100 µl of the internal standard solution was added to each tube and diluted with deionized water to 900 µl and mixed well to give final concentrations of 0.2, 0.5, 3.0, 5.0, 10.0, and 12.0, $\mu g ml^{-1}$ of each RSG and GLM. The mixture was vortexed vigorously for 30 s, then 400 µl of acetonitrial was added and centrifuged at 4000 rpm for 15 min. The supernatant was transferred into a 10-ml volumetric flask and completed to the volume with 52 mM phosphate buffer, filtered through 0.2 µm PTFTE filter then injected into CE. Blank human plasma samples were processed in the same manner using deionized water instead of RSG and GLM.

Preparation of tablets for assay

Twenty Avandaryl tablets were weighed, their mean weight determined, and crushed in mortar. An amount of powdered mass equivalent to half the tablets' content was transferred into each of ten 50-ml volumetric flasks containing 20 ml HPLC-grade isopropanol, mechanically shaken for 10 min, ultrasonicated for 5 min, then made up to volume with isopropanol and mixed well. Centrifuge at 3000 rpm for 10 min. A 2-ml aliquot of the solution was transferred to a 10-ml volumetric flask and made up to volume with 52 mM phosphate buffer, to yield concentrations of each of the two drugs in the range of linearity previously described. The solutions were filtered through a Millipore embrane filter (0.2 µm) from Nihon, Millipore (Yonezawa, Japan) before injection.

Selectivity

The selectivity of the assay was checked by analyzing four independent blank human plasma samples. The chromatograms of these blank plasma samples were compared with chromatograms obtained by analyzing human plasma samples spiked with the analytes.

Linearity

Calibration plots for the analytes in plasma were prepared by diluting stock solutions with pooled human plasma to yield seven concentrations over the range of 0.05–16 $\mu g ml^{-1}$ for RSG and GLM. Calibration standards at each concentration were extracted and analyzed in five replicates in three different runs. Calibration curves, based on the ratio of peak area signal of the analytes to that of the IS versus the theoretical concentration, were prepared for each run. Least squares linear regression analysis of the data gave slope, intercept, and correlation coefficient data.

Precision and accuracy

QC samples were processed in five replicates at each concentration (0.2, 0.5, 3.0, 5.0, 10.0, and 12.0 $\mu g ml^{-1}$) for three different analytical runs in order to evaluate the intra- and inter-assay accuracy and precision. The concentrations represented the entire range of the calibration curves. The recovery was determined by comparing the peak areas obtained from the quality control samples after the extraction procedure, to the peak areas obtained from the analysis of standard solutions of equivalent concentrations. Precision was reported as % relative standard deviation (% RSD) = (SD/mean) × 100. Percent accuracy was determined (using the data from the precision assessment) as the closeness of spiked samples to the nominal value of in-house standards. Percent accuracy was reported as % error = (nominal conc. − measured conc.) × 100/nominal conc.

Limit of detection and limit of quantitation

The limit of detection (LOD) was calculated using Eqns 1 and 2:

$$y - \alpha = 3.3 \times S\alpha \quad (1)$$

$$y - \alpha = b \times \mathrm{LOD} \quad (2)$$

while the limit of quantitation (LOQ) was attained using Eqns 3 and 4:

$$y - \alpha = 10 \times S\alpha \quad (3)$$

$$y - \alpha = b \times \mathrm{LOQ} \quad (4)$$

where b is the slope and $S\alpha$ is the standard deviation of the intercept of the regression line. In particular, LOD and LOQ were calculated taking under consideration data obtained from the calibration equations.[43] The results of the statistical analysis of the experimental data, such as the slopes, the intercepts, the correlation coefficients obtained by the linear squares treatment of the results along with standard deviation of the slope (S_b) and intercept (S_a) on the ordinate and the standard deviation of the residuals ($S_{y/x}$). The good linearity of the calibration graphs and the negligible scatter of experimental points are clearly evident by the values of the correlation coefficient and standard deviation.[43]

Recovery

Recovery of the proposed extraction procedure was determined by calculating the ratio of the absolute peak areas of extracted spiked plasma samples to the absolute peak areas of aqueous standard solutions containing equivalent concentrations of RSG and GLM (unextracted standards) that represent 100% recovery.

Result and discussion

Optimization design and analysis

A screening phase was carried out by demonstrating the ability of the method to accurately measure the analyte response in the presence of all potential sample components (specificity). Due to the difference in polarity and solubility between the two studied drugs, and to find out the best conditions for the separation of the two analytes and the IS, we tried to use different organic solvent and extraction mixtures to dissolve both drugs. A good result and

reasonable separation was obtained when we used isopropanol and completed the volume with 25 mM phosphate buffer.

Two buffers were evaluated (phosphate and borate) at two concentration levels (25 and 55 mM). On the other hand, two pH values were considered for each buffer at each concentration level (4 and 7.0 for phosphate, and 7.0 and 9.0 for borate). These pHs were selected according to the pK_as (6.1; 6.8 for RSG and 6.8 for GLM). The number, shape and resolution of the electrophoretic peaks were evaluated. At low pH (<5.0) GLM was not apparent; when pH increased both drugs and IS were separated from each other; at high pH (>7.0), the intensity of RSG, the migration times of three compounds decreased with increasing pH, and the current was increased. The good resolution ($Rs_1 = 32.30$, between RSG and GLM and $Rs_2 = 7.10$, between GLM and IS) were obtained in phosphate buffer pH 7.0 and 55 mM. These experiments were performed at a fixed voltage of 20 kV and 20 °C of temperature.

In order to carry out a quadratic regression on the model coefficients, each design variable has to be studied at least at three distinct levels, and consequently the central composite design is often used to provide an estimate of a second-order equation. Among the standard designs used in response surface methodology, the latter represents a good choice because of its high efficiency with respect to the number of required runs and also because it is built considering five levels of the factors being studied.

From preliminary experiments, the important variables to be tested during the optimization process are voltage, pH, buffer concentration, and temperature. Taking into account the screening experiments, pH 7.0 is a critical value that should not be varied to produce good electropherograms. Therefore buffer concentration (A), voltage (B), and temperature (C) were the variables considered in the present study. Table 1 shows the levels of each variable studied for finding out the optimum values and responses. As can be seen in this table, the ranges used were: voltage (15–25 μA), buffer concentration (25–75 mM), and temperature (15–45 C°). These ranges were selected by taking previous studies and certain instrumental limitations into consideration. As response variables, the resolution between RSG and GLM (Rs_1), the resolution between GLM and IS (Rs_2), analysis time and current were chosen.

All experimental were performed in randomized order to minimize the effects of uncontrolled variables that may introduce a bias on the measurements. Replicates (n = 6) of the central points were performed to estimate the experimental error. For an experimental design with three factors, the model including linear, quadratic, and cross terms can be expressed as:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1X_2 + \beta_{13} X_1 X3 + \beta_{23} X_2 X_3 + \beta_{11} {X_1}^2 + \beta_{22} {X_2}^2 + \beta_{33} {X_3}^2 \quad (5)$$

where Y is the response to be modelled, β is the regression coefficient, and X_1, X_2 and X_3 represent factors A, B, and C, respectively. To obtain a simple and yet a realistic model, the insignificant terms ($P > 0.05$) are eliminated from the model through 'backward elimination process'. The statistical parameters obtained from ANOVA for the reduced models are given in Table 2. Since R^2 always decreases when a regressor variable is eliminated from a regression model, in statistical modelling the adjusted R^2 which takes the number of regressor variables into account, is usually selected.[44] In the present study, the adjusted R^2 were well within the acceptable limits of $R^2 \geq 0.80$ [45] which revealed that the experimental data show a good fit with the second-order

Table 1. Central composite design used for the multiple response optimization procedure

run	Buffer conc.(mM)	Voltage (kV)	Temp. (C°)	Rs_1	Rs_2	Analysis time (min)	Current (μA)
1	25	15	15	22.7	5.56	14	100
2	75	15	15	68.9	11.4	13	110
3	25	25	15	5.5	0.88	11	160
4	75	25	15	19	3.3	12	160
5	25	15	45	5.2	1.1	10	110
6	75	15	45	24	5.9	11	190
7	25	25	45	3.3	0.74	5.7	210
8	75	25	45	9.1	1.5	5	270
9	50	20	30	7.3	2.2	8.5	180
10	50	20	30	7.5	2.3	8.4	180
11	50	20	30	7.33	2.4	8.6	183
12	50	20	30	7.45	2.2	8.3	179
13	25	20	30	12.4	2.7	12	140
14	75	20	30	5.4	8.2	14	220
15	50	20	30	9.5	1.4	12	110
16	50	25	30	5.3	1.0	7	200
17	50	20	20	32.3	7.1	13	133
18	50	20	25	24	6.4	12	140
19	50	20	30	7.4	2.1	8.8	177
20	50	20	30	7.32	2.25	8.5	180

Table 2. Reduced response models and statistical data obtained from ANOVA (after backward elimination)

Response	Reduced response models[a]	Adjus-ted R^2	Model *P* value	Adequate precision
Rs_1	$10.9 + 12.6A - 10.0\,B - 10.3\,C + 6.3\,BC + 15.6\,A^2$	0.81	0.0011	12.28
Rs_2	$2.8 + 2.3\,A - 2.3\,B - 2.2\,C - 1.2\,AB + 1.2\,BC$	0. 85	0.0005	14.12
Mt[b]	$9.4 - 1.8\,B - 2.4\,C + 2.5\,A^2$	0.82	0.0017	9.81
current	$165.7 + 23.0\,A + 38.0\,B + 36.5\,C + 32.3\,A\,C$	0.80	0.0001	15.50

[a] Only significant coefficients with $P < 0.05$ are included, factors are in coded levels.
[b] Migration time.

polynomial equations. For all reduced models, P value of < 0.05 is obtained, implying these models are significant. The adequate precision value is a measure of the 'signal (response) to the noise (deviation) ratio'. A ratio greater than 4 is desirable.[46] In this study, the ratio was found to be in the range of 10.97–20.47, which indicates an adequate signal and therefore the model is significant for the separation process.

Multi-criteria decision-making

In the present study, to optimize four responses with different targets, Derringer's desirability functions, was used.[47] Derringer's desirability function, *D*, is defined as the geometric mean, weighted, or otherwise, of the individual desirability functions.

Table 3. Criteria for the optimization of the individual responses

Response	Goal	Lower limit	Upper limit
Resolution 1	Range	6	9
Resolution 2 (1.6)	Target (1.4)	1.0	2.2
Analysis time (min)	Range	5	10
Current (μA)	minimize	150	250

The expression that defines the Derringer's desirability function is:

$$D = [d_1^{p1} \times d_2^{p2} \times d_3^{p3} \times \ldots\ldots\ldots d_n^{pn}]^{1/n} \quad (6)$$

Where *pi* is the weight of the response (*i*), *n* the number of responses (*i*) and d_i is the individual desirability function of each response (*i*) obtained from the transformation of the individual response of each experiment. The scale of the individual desirability function ranges between $d_i = 0$, for a completely undesired response, and $d_i = 1$ for a fully desired response. Weight can range from 0.1 to 10. Weights lower than 1.0 give less emphasis to the goal, whereas weights greater than 1.0 give more emphasis to the goal (in both cases, d_i varies in a non-linear way while approaching the the desired value). But with a weight of 1.0, d_i varies in a linear way. In the present study we chose weights equal to 1.0 for all four responses. A value of *D* different to zero implies that all responses are in a desirable range simultaneously and consequently, for a value of *D* close to 1.0, the combination of the different criteria is globally optimal, so as the response values are near target values.

The criteria for the optimization of each individual response are shown in Table 3. As can be seen, two responses were minimized: Rs_1 and current. These resolution corresponded to peaks with excellent separation and which can be minimized in order to shorten the analysis time; also current was minimized to decrease joule's heat effect inside the column. On the other hand, Rs_2 was adjusted to a fixed value (1.6). This was due to the fact that we could see good separation up to the separation value ≤ 1.0. Finally, the analysis time was set in the range to get reasonable resolutions for both drugs peaks and IS in a short time.

Following the conditions and restrictions previously discussed, the optimization procedure was carried out. The response surface obtained for the global desirability function is presented in Figure 1, were the best compromise is obtained at the top of the graph, *D*. The coordinates of *D* producing the maximum desirability value ($D = 0.871$), are presented in Figure 2, were buffer concentration 52 mM, voltage of 22 kV and temperature = 29 °C. The individual response values corresponding to the latter *D* are: $Rs_1 = 6.0$, $Rs_2 = 1.63$, time = 8.2 min and current = 184.0 µA. According to the fitting performed, the obtained desirability is highly acceptable, taking into account the large number of responses being simultaneously optimized. The suggested values during the optimization procedure were experimentally corroborated and the agreement between experiments and predicted responses can be seen in Table 4, where the corresponding electropherogram is shown in Figure 3B.

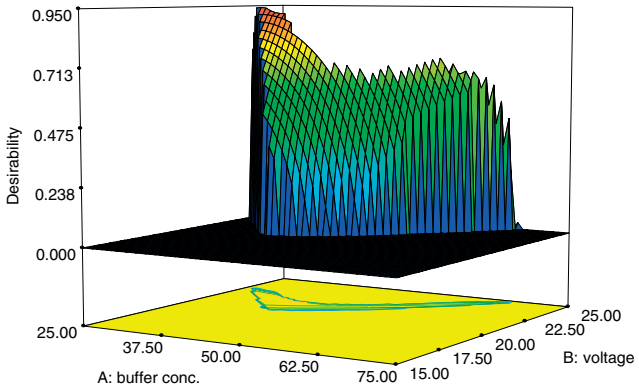

Figure 1. Graphical representation of the maximum global desirability function corresponding to formulation samples. The compromise is obtained at the top of the graph, $D = 0.870$.

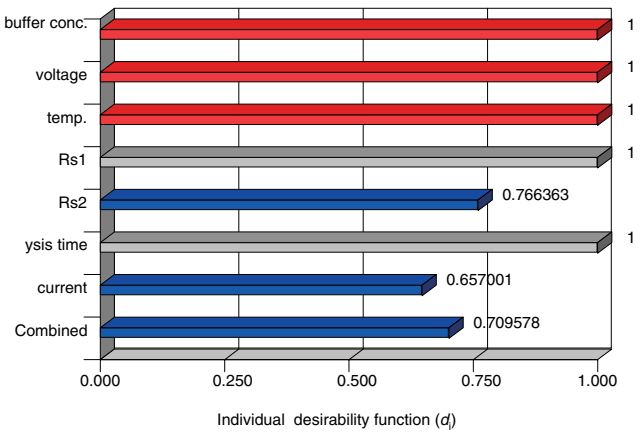

Figure 2. Bar graph showing individual desirability values (d_i) of various objective responses and their association as a geometric mean ($D = 0.870$) corresponding to formulation samples.

Validation of the assay

Linearity, limit of detection, and limit of quantitation

Under optimum analysis conditions, linearity was studied simultaneously in the concentration range of 0.05–16.0 µg ml^{-1} for RSG and GLM. The peak normalization ratios of RSG and GLM to the IS were plotted versus the nominal concentrations of the calibration standards. The linearity curves were defined by the Eqns 7 and 8 for RSG:

$$y = (0.0546 \pm 7.88 \times 10^{-4})\, x + (0.0176 \pm 5.13 \times 10^{-3}) \quad (7)$$

$$r = 0.9997 \quad (8)$$

and Eqns 9 and 10 for GLM:

$$y = (0.0535 \pm 6.84 \times 10^{-4})\, x + (0.0012 \pm 4.46 \times 10^{-3}) \quad (9)$$

$$r = 0.9998 \quad (10)$$

where *y* is the ratio of peak normalization and *x* is the concentration expressed in µg ml^{-1} ($n = 6$). The good linearity of the calibration graphs and the negligible scatter of experimental points are evident by the values of the correlation coefficient and standard deviation. The LOD were 0.02 µgml^{-1} for RSG and GLM. The LOQ of each calibration graph was 0.05 µgml^{-1} for both RSG and GLM.

Precision and accuracy

A summary of the accuracy and precision results is given in Table 6. The acceptance criteria (intra-day and inter-day %

Table 4. The comparison of experimental and predictive of different objective functions under optimal conditions

Optimum conditions	Buffer conc.(mM)	Voltage (volt)	Temp. C°	Rs_1	Rs_2	Mt	Current (μA)
Desir.value (D) = 0.870	52	22	29				
Experimental [a]				5.9	1.56	7.8	188
predictive				6	1.63	8.2	184
Average % error [b]				3.0 ± 0.032		4.8 ± 0.056	2.2 ± 0.023

[a] n = 6.
[b] Mean ± SD.

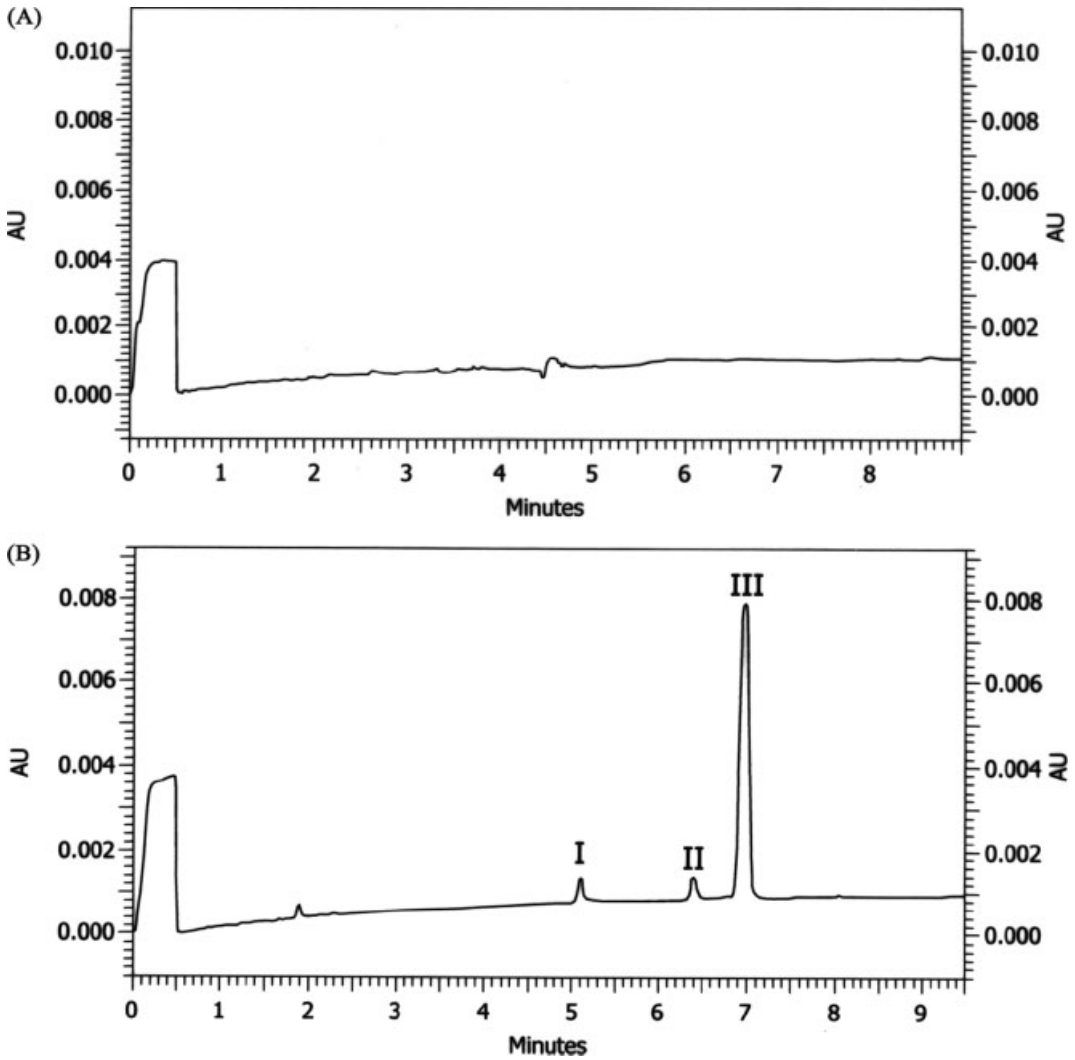

Figure 3. Electropherograms of (A) blank human plasma, (B) spiked with 0.05 μgml^{-1} of rosiglitazone (I), glimepiride (II), and 5.0 μgml^{-1} losartan (III).

RSD of < 15% and accuracy between 85 and 115%) were met in all cases. The precision and accuracy of the method were determined by using plasma samples spiked at six levels. The intra-day precision and accuracy (n = 6), as expressed by %RSD and % error were 0.29–2.43% and 0.44–2.50%, respectively, for RSG, 0.49–2.60% and 0.17–2.41% for GLM, respectively. The inter-day precision and accuracy (n = 6), expressed by % RSD and % error were 0.29–2.30% and 0.64–3.00% respectively, for RSG and 0.39–2.94 and 0.19–2.80%, respectively for GLM.

Selectivity

Selectivity is described as the ability of a method to discriminate the analytes from all potential interfering substances. Selectivity of method was investigated by both peak purity and spiking experiments with pure standard compounds. Peak purity was evaluated by use of diode array detector and the P/ACE system MDQ software. RSG and GLM were well separated under the CE conditions applied. No interference was observed in drug-free human plasma samples (Figures 3A and 3B). Otherwise, there are

Table 5. Accuracy and precision data for RSG and GLM in spiked human plasma

	Analyte	Conc. Added (μg ml^{-1})	Conc. Found (μg ml^{-1}) Mean ± SD (n=6)	Recovery %	Error (%)	RSD (%)
Intra-day	Rosiglitazone	0.5	0.504 ± 0.0049	100.8	0.81	0.97
		1.5	1.514 ± 0.0039	100.3	0.78	0.93
		3.0	2.963 ± 0.0720	98.7	1.33	2.43
		5.0	4.981 ± 0.0120	99.6	0.44	0.24
		10	10.13 ± 0.0480	101.3	1.30	0.47
		12	11.90 ± 0.0350	99.16	0.83	0.29
	Glimepiride	0.5	0.488 ± 0.0024	97.6	2.41	0.49
		1.5	1.408 ± 0.0041	98.5	1.51	2.41
		3.0	2.980 ± 0.0770	99.3	1.10	2.60
		5.0	4.99 ± 0.0249	99.8	0.21	0.50
		10	10.017 ± 0.0340	100.2	0.17	0.34
		12	11.93 ± 0.0740	99.41	0.58	0.62
Inter-day	Rosiglitazone	0.5	0.505 ± 0.0045	100.6	0.78	0.89
		1.5	1.535 ± 0.0039	99.2	1.28	1.47
		3.0	2.965 ± 0.0680	98.8	1.20	2.30
		5.0	5.032 ± 0.0135	100.6	0.64	0.29
		10	10.19 ± 0.0590	101.9	1.9	0.57
		12	11.91 ± 0.0370	99.3	0.75	0.31
	Glimepiride	0.5	0.486 ± 0.0014	97.2	2.80	0.45
		1.5	1.286 ± 0.0029	98.2	2.20	2.13
		3.0	2.958±. 08700	98.3	1.30	2.94
		5.0	4.98 ± 0.0320	99.6	0.31	0.65
		10	10.11 ± 0.0390	98.9	0.19	0.39
		12	12.07 ± 0.0840	100.6	0.62	0.70

Table 6. Intra-day and inter-day precision and accuracy results of RSG and GLM in tablet formulations ($n = 6$)

Ratio RSG/GLM	Quantity taken μgml^{-1} RSG/GLM	RSG				GLM			
		Found [a]	% Recovery	% RSD[b]	% Error [c]	Found [a]	% Recovery	% RSD[b]	% Error [c]
1 : 1	10 : 10	10.21 ± 0.036	102.12 ± 1.98	0.35	2.10	10.16 ± 0.078	101.6 ± 1.1	0.76	1.6
2 : 1	16 : 8	15.93 ± 0.031	99.52 ± 1.8	0.19	−0.43	7.98 ± 0.006	99.75 ± 0.83	0.75	−0.25
4 : 1	8 : 2	8.13 ± 0.096	100.85 ± 1.09	0.11	1.62	2.02 ± 0.003	100.83 ± 1.69	0.16	1.0

[a] Mean ± SD.
[b] [SD/Mean] ×100.
[c] [found conc. − actual conc./actual conc.] ×100.

no peaks detected at the retention time of RSG, GLM, and IS at the level of LOQ or less. Excipients commonly co-formulated with the studied drug such as magnesium stearate, cellulose, starch, calcium hydrogen phosphate, colloidal silicon dioxide, and colouring agents, did not interfere with the determination of RSG and GLM, since no excipient carries either a charge under the conditions employed or has an inherent UV absorbance at the wavelength employed, indicating the high selectivity of the proposed method (Figures 4A and 4B).

Application to plasma samples

Prior to the determination of RSG and GLM in human plasma samples using the optimized CE conditions obtained, a plasma sample pretreatment step had to be employed. A fast and simple technique of plasma deproteinization as a sample preparation method has been developed by using different solvent (acetonitrile, methanol, ethanol, and trifluoroacetic acid). Acetonitrile was selected for use since it provided the best data in terms of sample clean-up and high recoveries of both RSG and GLM. The mean recoveries using acetonitrile were 99.51 ± 1.39 for RSG, 99.14 ± 0.94 for GLM (n = 6). Results are shown in Table 5.

Application of the LC method for the analysis of pharmaceutical formulations

Evaluation of pharmaceutical formulations was performed by using the calibration curve method, since no significant differences between the slopes of the standard calibration curve to that of Avandaryl tablets were observed. Each pharmaceutical preparation was analyzed by performing six independent determinations and each series was injected three times. The same procedure was used to estimate the concentration of the drugs under study in three pharmaceutical tablet formulations containing both RSG and GLM in different strengths (Table 6). The percentage recoveries ranged from 99.52 ± 1.8 − 100.85 ± 1.09 and 99.75 ± 0.83 − 101.6 ± 1.1 for RSG and GLM respectively. The results indicated that the proposed method was reliable for

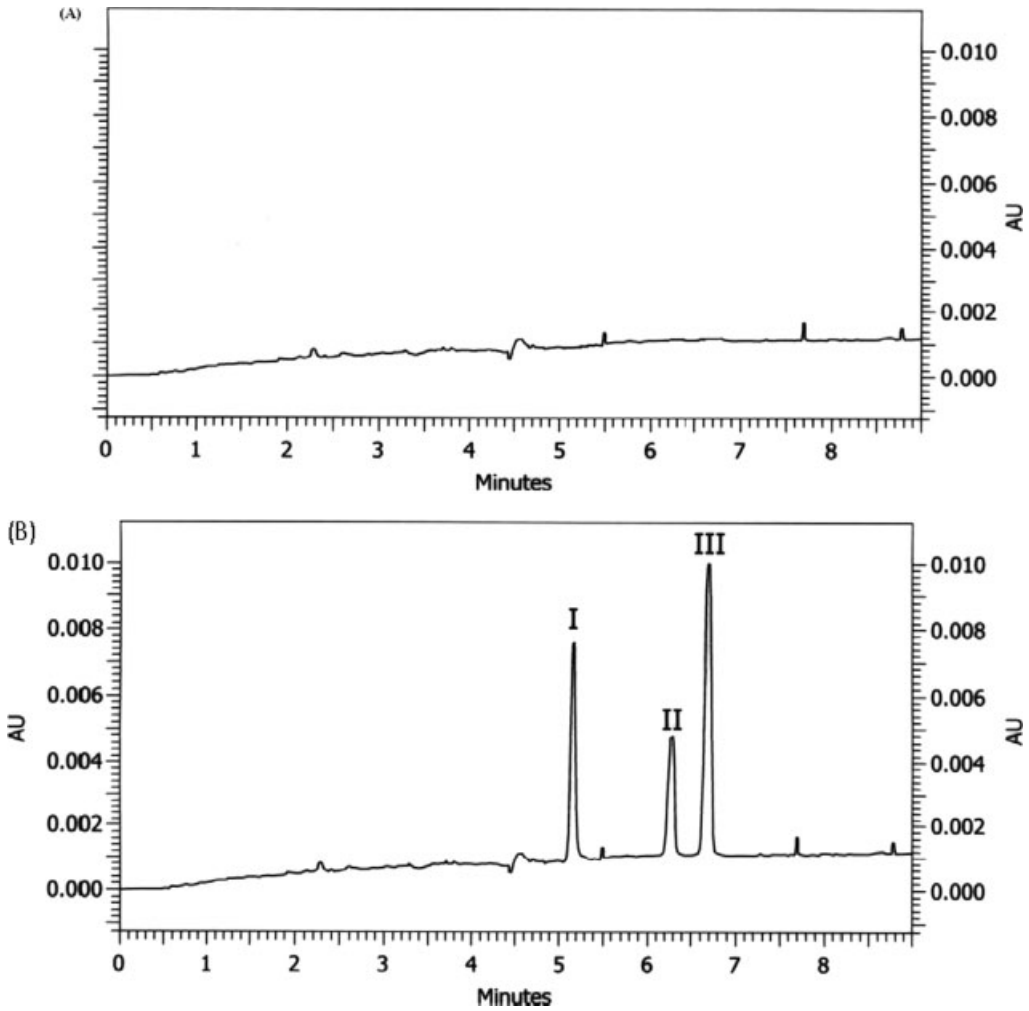

Figure 4. Electropherograms of (A) tablets blank (placebo), (B) 5.0 μgml^{-1} of rosiglitazone (I), glimepiride (II), and 5 μgml^{-1} losartan (III) recovered from Avandaryl tablets.

quantification of RSG and GLM in pharmaceutical formulations. In addition, the slope of the standard calibration curve for RSG and GLM was compared with the slope of the tablets calibration curve. It was found that there were no significant differences between the slopes which indicated that excipients did not interfere with RSG and GLM.

Specificity. The specificity of an analytical method may be defined as the ability to obviously determine the analyte in the presence of additional components such as impurities, degradation products, and matrix.[48–50] A solution of analytical placebo (containing all the tablet excipients except RSG and GLM) was prepared according to the sample preparation procedure and injected. To identify the interference by these excipients, a mixture of inactive ingredients (placebo), standard solutions, and commercial pharmaceutical preparations including RSG and GLM were analyzed by the developed method (Figures 4A and 4B). The representative chromatograms did not show any other peaks, which confirmed the specificity of the method.

Precision. The precision of the method was evaluated in terms of intermediate precision (intra-day and inter-day).[48–50] Three different concentrations of RSG and GLM were analyzed in six independent series during the same day (intra-day precision) and six consecutive days (inter-day precision); within each series every sample was injected in triplicate. The RSD values of intra- and inter-day studies for RSG and GLM showed that the precision of the method was satisfactory (Table 6).

Accuracy. The accuracy of an analytical method expresses the nearness between the reference value and found value.[48–50] Accuracy was evaluated as percentage relative error between the found mean concentrations and added concentrations for RSG and GLM. The results obtained are shown in Table 6, from which it is clear that accuracy is excellent for both active ingredients.

Conclusion

The present study described, for the first time, the development and validation of a sensitive CE method for the simultaneous analysis of RSG and GLM in plasma and pharmaceutical formulations. The studied drugs were extracted by protein precipitation procedure for sample clean-up of plasma. The method is selective where co-formulation drug excipients do not interfere. The total run time is 16 min, which allows to processing of over 90 samples per day.

The sensitivity of the developed method was found to be sufficient for accurate monitoring of the plasma concentration of RSG and GLM (103.2–550.8 μgL^{-1}) in diabetic patients. For these reasons, the proposed CE is expected to contribute to the therapeutic monitoring and pharmacokinetic studies of RSG and GLM.

Acknowledgments

The authors thank the King Abdulaziz City for Science and Technology, and the Research Center at College of Pharmacy, King Saud University for funding the work.

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