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Short communication

Determination of rosiglitazone in coated tablets by MEKC and HPLC methods

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Abstract

Micellar electrokinetic chromatographic (MEKC) and high-performance liquid chromatographic (HPLC) methods were developed and subsequently validated for the determination of rosiglitazone (RSG) in coated tablet, a potent new oral antihyperglicemic agent. The electrophoretic separation was performed in a fused-silica capillary of total length 48.0 cm (effective length 39.5 cm, 75 μm i.d.) using 10 mM sodium tetraborate buffer (pH 9.0) containing 30 mM sodium dodecyl sulfate (SDS) as the background electrolyte (BGE). The separating voltage used was of 20 kV at 25 °C and the diode array detector was set at 247 nm. The MEKC method was compared with HPLC method using a RP-18 column (125 × 4.0 mm i.d.) eluted with a mobile phase consisting of mixture of 25 mM potassium dihydrogen phosphate buffer and acetonitrile (55:45, v/v), adjusting the pH to 6.2 with dilute potassium hydroxide. Statistical analysis by Student's *t*-test showed no significant differences between the results obtained by two methods. The results indicated that MEKC can be used an alternative method to HPLC for the determination of rosiglitazone in pharmaceutical dosage form.

Keywords: Rosiglitazone; MEKC; HPLC

1. Introduction

Diabetes mellitus includes several diseases that are characterized by chronic hyperglycemia with disturbances in fat, carbohydrate and protein metabolism due to abnormal insulin secretion and/or action [1,2].

Several drugs are available for the treatment of type 2 diabetes mellitus which the rosiglitazone (RSG), chemically [(±)-5-[4-[2-[*N*-methyl-*N*(2-pyridyl)amino]ethoxy]benzyl]-2,4-dione thiozolidine] (Fig. 1), it's a potent new oral antihyperglicemic agent that reduces insulin resistance in patients with type 2 diabetes by binding to peroxisome proliferator-activated receptors gamma (PPAR-γ) [3–5].

The liquid chromatographic determination of RSG in plasma and pharmaceutical dosage form has been reported in literature [6–10]. HPLC method has some disadvantages—requires large amount of high purity organic solvents and generates high amount of waste. The new analytical separation method—capillary electrophoresis (CE), it is an alternative complementary technique to HPLC. CE has proven to be an interesting alternative for the analysis of pharmaceutical compounds because of its efficiency, flexibility, accuracy and very high resolution [11]. It offers a broad range of selectivity in combination with high separation efficiency, working with minute sample volume and short analysis time. Major drawbacks of CE are its sensitivity and

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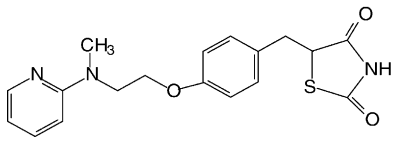

Fig. 1. The chemical structure of rosiglitazone.

reproducibility, which usually are lower than those obtained from HPLC.

A study revealed that the pioglitazone, a representative the class of thiozolidinediones, was determined using the micellar electrokinetic chromatography (MEKC) [12]. However, there are no other references concerning the analysis the other thiozolidinediones as the rosiglitazone in pharmaceutical dosage form by MEKC method. In MEKC, ionic or neutral surfactants are added to the operating buffer at a concentration above their critical micelle concentration. The micelles provide a pseudostationary phase with which analytes can partition. Although MEKC is particularly useful in the separation of neutral species, this technique may also be used for separation of charged solutes [13,14].

The aim of this work was to develop and validate the MEKC and the HPLC methods, which allowed the determination of rosiglitazone in coated tablets.

2. Experimental

2.1. Materials

Rosiglitazone maleate reference standard (74.3% of RSG free base) was obtained from GlaxoSmithKline (Rio de Janeiro, Brazil). Rosiglitazone tablets (Avandia® 8 mg) were purchased from the market. The excipients contained in pharmaceutical dosage form (hydroxypropyl methylcellulose, lactose monohydrate, magnesium stearate, microcrystalline cellulose, polyethylenoglycol 3000, sodium starch glycolate, titanium dioxide, triacetin and red iron oxide E172) were obtained from Blanver (São Paulo, Brazil). Sodium dodecyl sulfate (SDS) was purchased from Synth (São Paulo, Brazil). Acetonitrile (LiChrosolv®), potassium dihydrogen phosphate, potassium hydroxide, sodium tetraborate decahydrate, ethanol analytical grade was obtained from Merck (Darmstadt, Germany).

2.2. MEKC method

2.2.1. Instrumentation

Capillary electrophoresis experiments were performed using a ^{3D}CE system (Hewlett Packard, Waldbronn, Germany) equipped with an on-column diode-array detector, an autosampler and a power supply able to deliver up to 30 kV. A ^{3D}CE Chemstation software (rev.A.06.03, Hewlett-Packard) was used for instrumental control, data acquisition and data handling. The separations were achieved using fused-silica capillary tubes (Hewlett-Packard) with a total length of 48.0 cm (effective length 39.5 cm, 75 μm i.d.).

2.2.2. Capillary preparation and preconditioning

Before the first use, the fused-silica capillary was sequentially rinsed with 1 M sodium hydroxide for 30 min, followed, by deionized water for 15 min and background electrolyte (BGE) by 15 min. The preconditioning was consisted the washing the capillary between analyses with 0.1 M sodium hydroxide for 2 min, followed by deionized water for 2 min, then equilibrated with the BGE for 3 min.

2.2.3. Eletrophoretic separation conditions

The aqueous BGE was constituted of 10 mM sodium tetraborate (pH 9.0) containing 30 mM sodium dodecyl sulfate. The BGE was filtered through a 0.45 μm membrane filter (Millipore®, Bedford, USA) prior to use and sonicated before use. A constant voltage of 20 kV, with an initial ramping of 1 kV s^{-1}, was applied during analysis. Hydrodynamic sample injection was performed at 50 mbar for 5 s. The diode array UV detector was set at 247 nm. The capillary temperature was maintained constant at 25 °C. All experiments were carried out by applying positive mode.

2.2.4. Preparation of the standard solution

Stock standard solution of rosiglitazone (100 μg ml^{-1}) was prepared in ethanol. Aliquots of this solution were diluted in 10 mM sodium tetraborate solution to obtain the concentration range of 20–60.0 μg ml^{-1}.

2.2.5. Preparation of the sample solution

Twenty weighed tablets of Avandia® (8 mg of RSG) were ground and an amount of powder equivalent to 10 mg of active compound was diluted with ethanol. The sample solution was filtered through a filter paper and further dilution of the appropriate aliquot was made with 10 mM sodium tetraborate solution to obtain a final solution containing 40 μg ml^{-1} of RSG.

2.3. HPLC method

2.3.1. Instrumentation

The LC system consisted of a Shimadzu LC-10A with a SPD-10A variable-wavelength UV detector, a LC-10AS solvent delivery pump, a Rheodyne injection valve with a 20 μl loop and a C-R6A integrator (Shimadzu, Kyoto, Japan).

2.3.2. Chromatographic separation conditions

The mobile phase consisted of potassium dihydrogen phosphate buffer (25 mM) and acetonitrile mixture (55:45, v/v), adjusted to pH 6.2 with dilute potassium hydroxide. The mobile phase was filtered through a 0.45 μm membrane filter (Millipore®, Bedford, USA) prior to use and sonicated before use. Separation was achieved using a LiChrospher® (Merck) 100 RP-18 column (125 × 4.0 mm i.d.), the mobile phase

flow rate was 0.8 ml min^{-1} and the sample injection volume was 20 μl. The detector was set a wavelength of 247 nm. The instrument was operating at room temperature (24 ± 1 °C).

2.3.3. Preparation of the standard solution

Stock standard solution of rosiglitazone (40 μg ml^{-1}) was prepared in mobile phase. Aliquots of this solution were diluted in the same solvent to obtain the concentration range of 4–16.0 μg ml^{-1}.

2.3.4. Preparation of the sample solution

Twenty weighed tablets of Avandia® (8 mg of RSG) were ground and an amount of powder equivalent to 4 mg of active compound was diluted with mobile phase. The sample solution was filtered through a filter paper and further dilution of the appropriate aliquot was made the same solvent to obtain a final solution containing 10 μg ml^{-1} of RSG.

2.4. Method validation

The validation procedure was followed the International Conference on Harmonization guideline and United States Pharmacopoeia for the analysis of rosiglitazone by MEKC and HPLC methods [15,16]. The performance parameters evaluated these methods were: linearity, limit of detection (LOD) and limit of quantitation (LOQ), specificity, precision and accuracy.

2.4.1. Linearity

The standard curve was obtained in the range of standard solution 4–16.0 μg ml^{-1} for HPLC method and 20–60.0 μg ml^{-1} for MECK method. The linearity these methods was evaluated by linear regression analysis, which was calculated by the least square method.

2.4.2. Limit of detection and limit of quantitation

The parameters LOD and LOQ were calculated using the following equations [15]:

$$\text{LOD} = \frac{3.3s}{I} \qquad \text{LOQ} = \frac{10s}{I}$$

where s is standard deviation (S.D.) response and I is slope of regression equation.

2.4.3. Specificity

The specificity of the MEKC and HPLC methods was evaluated through the analysis the mixture of all excipients contained in coated tablet and the results was evaluated by analyzing with standard and sample solution of RSG.

2.4.4. Precision

The precision of the reported methods for the determination of RSG was studied using the parameters repeatability (intra-day) and intermediate precision (inter-day). It was expressed as relative standard deviation (R.S.D.) at series of measurements. Repeatability of these methods was verified the same day, at the same concentration and under the same experimental conditions for each one the samples evaluated. The intermediate precision, which is the inter-day variation at the same concentration level, was determined on three consecutive days. In order to evaluate the precision of the methods, six solutions were prepared (40 μg ml^{-1} for MEKC method and 10 μg ml^{-1} for HPLC method) and the amount was determined in pharmaceutical dosage form.

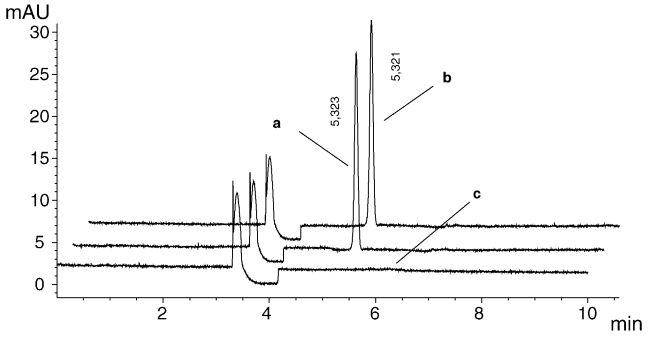

Fig. 2. Specificity test of MEKC method for solutions (40 μg ml^{-1}): RSG reference standard (a), RSG tablet (b) and excipients simulated sample (c).

2.4.5. Accuracy

The accuracy of the methods was determined through the recovery test, using the equation proposed [17]:

$$R\% = \frac{[(C_{S+STD}) - C_S]}{C_{STD}} \times 100$$

where C_{S+STD} is recovery solution (RSG tablets + RSG reference standard); C_S is concentration solution of RSG tablets and C_{STD} is concentration solution of RSG reference standard.

For analysis of RSG by MEKC method, aliquots of 2.0, 3.0 and 4.0 ml of a RSG standard solution (100 μg ml^{-1}) were added to three samples solution containing a fixed amount of RSG (20 μg) in BGE, respectively. Therefore, this recovery study was performed at a final concentration solution of 30, 35 and 40 μg ml^{-1} RSG. For HPLC method, aliquots of 1.0, 1.5 and 2.0 ml of a RSG standard solution (40 μg ml^{-1}) were added to three samples solution containing a fixed amount of RSG (8 μg) in ethanol, respectively. Therefore, this recovery study was performed at a final concentration solution of 12, 14 and 16 μg ml^{-1} RSG. All solutions were prepared in triplicate and assayed.

3. Results and discussion

3.1. MEKC method

In the development of a CE method for the determination of RSG in coated tablets different concentration buffer (sodium tetraborate) were tested using capillary zone electrophoresis (CZE). However, this mode caused a slightly

Table 1
Statistical parameters of standard curve the proposed methods for determination of RSG

Statistical parameters[a]	MEKC method	HPLC method
Concentration range ($\mu g\,ml^{-1}$)	20–60	4–16
Intercept ± standard error	3.6362 ± 0.35	−12,352 ± 0.78
Slope ± standard error	2.7443 ± 0.48	55,000 ± 0.87
Correlation coefficient (r)	0.9998	0.9998
Limit of detection ($\mu g\,ml^{-1}$)	1.41	0.23
Limit of quantitation ($\mu g\,ml^{-1}$)	4.26	0.71

[a] Data obtained from three standard curves.

peak asymmetry, and then the MEKC was selected for the analysis.

The concentration of sodium tetraborate buffer was varied from 10 to 30 mM (pH 9.0) for this study. An increase in the buffer concentration resulted in a decrease in the electroosmotic flow (EOF) due to compression double-layer and thereby an increase the current generated in capillary tubes causing the Joule heating, which may cause reduce efficiency by the zone broadening, instability of baseline and lower migration time reproducibility [14]. In this study, 10 mM buffer concentration was considered as suitable for its peak shape and run time.

An SDS concentration range from 10 to 30 mM was taken for this part of the study keeping the buffer concentration at 10 mM and pH 9.0. The results showed that an increase of SDS concentration influence the RSG retention time. A 30 mM SDS concentration was selected for further experiments since it gave high narrow peak making it easier for integration.

A potential of 20 kV, with a ramping of $1\,kV\,s^{-1}$, was the best compromise in terms of run time and current generated. As expected, on increasing the applied voltage there is an increase in EOF, leading to shorter analysis time and higher efficiencies. However, higher applied voltages exhibit higher currents and increased Joule heating [13].

To achieve high migration time reproducibility and to avoid solute adsorption on the capillary wall, the capillary preconditioning was accomplished through the rinsing between analyses improving the precision and accuracy.

The standard curves for RSG were constructed and it demonstrated to be linear in the concentration range of 20–60.0 $\mu g\,ml^{-1}$. The representative linear equation was $y = 2.7443x + 3.6362$, where x is a concentration ($\mu g\,ml^{-1}$) and y is peak area. The correlation coefficient ($r = 0.9998$) demonstrated to be highly significant for the method (Table 1). The LOD and LOQ were estimated to be 1.41 and 4.26 $\mu g\,ml^{-1}$, respectively indicating a suitable sensitivity of the method. The linearity data were validated by the analysis of variance (ANOVA), which demonstrated significative linear regression and no significative linearity deviation ($p < 0.05$).

The specificity test demonstrated that the excipients of Avandia® tablet do not cause interference in the RSG analysis. The specificity is very important, since this coated tablet is a complex matrix and contains a lot of excipients that could cause problems in determination of RSG (Fig. 2).

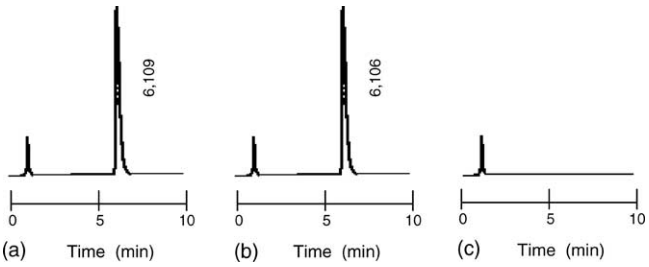

Fig. 3. Specificity test of HPLC method for solutions ($10\,\mu g\,ml^{-1}$): RSG reference standard (a), RSG tablet (b) and excipients simulated sample (c).

The precision values obtained for the determination of RSG in samples with their RSD are shown in Table 2. The RSD values varied from 0.83 to 1.24 showed that the inter-day precision of the method was satisfactory. The RSD are acceptable in CE analysis, although they are slightly higher than those obtained from HPLC method.

The accuracy of the proposed method was evaluated by recovery experiments, using the standard addition technique. Three different concentrations of RSG standard were added to Avandia® tablets diluted, as shown in Table 3. The mean recovery was found to be 100.35% indicating high accuracy of this method.

3.2. HPLC method

The best chromatographic conditions were adequately selected to develop a reversed-phase liquid chromatographic method that, working in isocratic mode, allowed the determination of RSG in coated tablets, without interference of its common excipients and in shortest time (Fig. 3). Some mobile phases were investigated for this HPLC method, however, the mixture of potassium dihydrogen phosphate buffer (25 mM) and acetonitrile (55:45, v/v), adjusting to pH 6.2 with dilute potassium hydroxide, was that allowed good separation from the solvent front and formed symmetrical peak for RSG at a flow rate of $0.8\,ml\,min^{-1}$ using C18 column.

The corresponding regression equation and other characteristic parameters for determination of RSG by HPLC are shown in Table 1. The described method was linear over a range 4–16.0 $\mu g\,ml^{-1}$ and the representative equation for standard curve was $y = 55{,}000x - 12{,}352$ ($r = 0.9998$). The low values of LOD and LOQ indicated the high sensitivity of this HPLC method. The data were validated by ANOVA, which demonstrated significant linear regression and non-significant linearity deviation ($p < 0.05$).

The RSD experimental values of intra-day and inter-day assays showed a satisfactory and acceptable variability (Table 2). The mean recovery test was found to be 99.18%, which can be observed in Table 3.

3.3. Comparison between MECK and HPLC methods

The results obtained from the MECK method were compared statiscally by the Student's t-test with the HPLC method

Table 2
Inter and intra-day assay variation of RSG by proposed methods

Method	Sample (mg/tablet)	Days	Experimental amount (mg)	Value RSG (%) ± S.D.	R.S.D. Intra-day	R.S.D. Inter-day
MEKC	8	0	7.99[a]	99.92 ± 0.42	1.03	0.99[b]
		1	8.01	100.14 ± 0.50	1.24	
		2	7.99	99.95 ± 0.34	0.83	
HPLC	8	0	8.03	100.38 ± 0.31	0.75	0.69
		1	8.06	100.81 ± 0.24	0.59	
		2	8.05	100.63 ± 0.34	0.82	

[a] Mean of six samples in triplicate ($n = 6$).
[b] $n = 18$.

Table 3
Recovery of standard solution added to commercially available samples

Method	Amount added ($\mu g\,ml^{-1}$)	Amount found ($\mu g\,ml^{-1}$)	% Recovery[a] ± RSD	% Recovery
	30	29.94	99.80 ± 1.32	
MEKC	35	35.28	100.80 ± 0.89	100.35[b]
	40	40.18	100.45 ± 1.12	
	12	11.94	99.50 ± 0.79	
HPLC	14	13.88	99.17 ± 0.58	99.18
	16	15.82	98.87 ± 0.91	

[a] Each value is a mean of nine determinations.
[b] $n = 27$.

and does not reveal significant difference between the experimental values obtained in the sample by the two methods. The calculated t-value (t_{cal} = 1.208) was found to be less than the critical t-value (t_{crit} = 2.228) at 5% significance level.

4. Conclusion

MEKC and HPLC methods were developed for determination of rosiglitazone in coated tablets. Once the optimized conditions selected, both methods were validated and showed good performances with respect to linearity, precision and accuracy. Comparing to HPLC, the developed MEKC technique was less expensive, low solvent and sample consumption. The results of this study demonstrated that both methods could be used for the routine determinations of rosiglitazone in pharmaceutical dosage form.

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