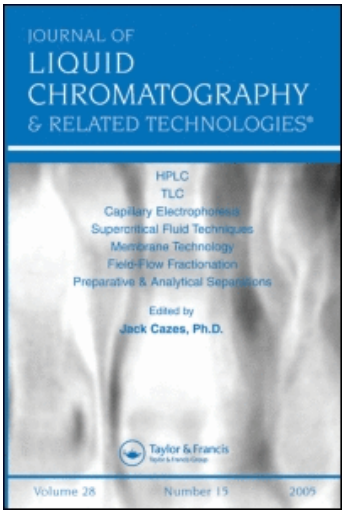

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Rapid HPTLC Determination of Rosiglitazone in Pharmaceutical Formulations

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ABSTRACT

A new, simple, rapid, and stability-indicating high-performance thin layer chromatographic (HPTLC) method has been developed and validated for the determination of rosiglitazone in tablets. Analysis was performed on silica gel $60F_{254}$ plates in horizontal chambers with chloroform–ethyl acetate–25% ammonium hydroxide (5 : 5 : 0.1, v/v) as mobile phase. Detection and quantification were performed by classical densitometry at 240 and 254 nm. The active substance was extracted from tablets with ethanol. Calibration plots were constructed in the range 0.2−1.0 μg/10 μL and were correlated with good correlation coefficients ($r_{240} = 0.9993$; $r_{254} = 0.9994$). Precision was validated by replicate analyses of standard solutions, and accuracy by analysis of fortified samples. The precision of

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the proposed chromatographic method, expressed as mean RSD was 3.58 and 2.76% for 240 nm, and 8.23 and 6.56% for 254 nm, for the lowest and the highest calibration levels, respectively. The mean recoveries from the fortified samples ranged from 89.48% to 99.38% for 240 nm, and from 89.05% to 100.89% for 254 nm. The mean recoveries from tablets were 101.95% and 103.2% for assays at 240 and 254 nm, respectively.

Key Words: Rosiglitazone; HPTLC; Densitometry; Tablets.

INTRODUCTION

Rosiglitazone maleate, (±)-5-[4-[2-[*N*-methyl-*N*-(2-pyridinyl)amino]-ethoxy]benzyl]-2,4-thiazolidinedione maleate, is a new oral antidiabetic agent, a member of the thiazolidinedione family, which appears to improve sensitivity to insulin in liver, muscle, and adipose tissue, but does not directly stimulate insulin secretion. These compounds are high affinity ligands of peroxisome proliferator activated receptor gamma (PPARγ), a member of the nuclear receptor super family, which controls the expression of genes involved in lipid and carbohydrate metabolism in target tissues.[1] They are introduced as treatments of non-insulin dependent diabetes mellitus (type 2 diabetes).

The literature concerning the quantitative determination of rosiglitazone is rather sparse. An isocratic reversed phase liquid chromatography has been reported for quantification of the drug in pharmaceuticals.[2] The HPLC methods for the determination of rosiglitazone in human and animal plasma were also elaborated.[3–6] One paper concerns HPTLC determination of rosiglitazone in its dosage forms. The authors used aluminum-backed silica gel $60F_{254}$ plates and the mobile phase ethyl acetate–toluene–methanol (45 : 55 : 1, v/v). Another drug from the thiazolidinedione family, pioglitazone was used as an internal standard.[7]

Rosiglitazone is the newest drug but it is more and more frequently used in therapy of type 2 diabetes. Therefore, rapid and simple analytical methods are continually required for determining its concentration in different preparations. This paper describes a simple, rapid, quantitative high performance thin layer chromatographic (HPTLC) method for analysis of tablets of rosiglitazone by automated instrumental application of standard and sample solutions to high performance silica gel plates, and measurement of fluorescence by scanning densitometry. Internal standards are not required because samples and standards are separated and scanned under identical conditions on the same plates.

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EXPERIMENTAL

Instrumentation and Layers

A Camag (Switzerland) HPTLC system equipped with an automatic TLC Sampler ATS3 and TLC Scanner 3 with a deuterium source with slit dimensions of 0.3 × 4.0 mm was used. Rosiglitazone spots were scanned in the reflectance/absorbance mode at 240 and 254 nm. The scanning speed was 20 mm/sec. A regression program provided by the CATS V 4.05 software controlling the densitometer was applied. The horizontal Teflon DS II chambers with mobile phase distributor from Chromdes Lublin (Poland) were used. Analysis was performed on 10 × 20 cm HPTLC silica gel $60F_{254}$ plates from Merck (Germany). A single bath number was used throughout the calibration and validation procedures. Plates were developed to a distance of 8 cm with mobile phase chloroform–ethyl acetate–25% ammonium hydroxide (5 : 5 : 0.1, v/v).

Reagents and Chemicals

Rosiglitazone maleate pure substance and 4 mg Avandia® tablets from SmithKline Beecham (England) were used. Chloroform, ethyl acetate for chromatography, ethanol and 25% ammonium hydroxide pro-analysis grade from Merck (Germany) were applied.

Procedure for Calibration

The working solution of rosiglitazone was prepared by dissolving 10 mg of pure substance in 10 mL of ethanol. It was stored at 4°C and was stable for at least 6 weeks. The HPTLC standards were prepared by dilution, covering the range 0.2–1.0 μg/10 μL. Ten microliter of each of these solutions was applied to the plate. After development, the plates were air dried, and standard zones were quantified by densitometric evaluation at 240 and 254 nm, using the scanner described above. Peaks areas were recorded for all the tracks. Following five analyses of each of five different standards, the calibration curve (dependence of peak area on the amount of rosiglitazone applied to the plate) was determined by polynomial regression.

Procedure for Assay in Fortified Samples

Three different levels of standards: 2, 4, and 6 mg of rosiglitazone (50%, 100%, and 150% of theoretical weight predicted by the label declaration) were

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added to the weighed portions of powdered tablets adequate to the mean mass of tablets (the average mass of Avandia® tablets was determined as 0.1541 g). They were transferred to 25-mL volumetric flasks containing approximately 20 mL of ethanol and vortex-mixed for 20 min. The solutions were then diluted to volume with ethanol and filtered through Whatman No. 42 filter paper. One milliliter of the filtrate was then diluted to 5 mL with ethanol. Ten microliter of each solution and working standards were applied to HPTLC plates, developed, dried, and scanned. The peak areas were recorded as was described in the calibration procedure. Each level was repeated five times and the percentage recoveries were calculated by comparing the theoretical contents predicted by the label declaration to the mean experimental contents of the sample zones.

Procedure for Assay in Tablets

Extraction of the active substance from tablets was performed with ethanol. The tablets were ground and amounts of about 0.1 g were transferred to 50-mL volumetric flasks containing approximately 30 mL of ethanol and the solution was vortex-mixed for 20 min to dissolve the active ingredient completely. The extracts were then diluted to volume with ethanol and filtered through Whatman No. 42 filter paper. The resulting solutions were used for analysis. Ten microliter of each solution and working standards were applied to HPTLC plates, developed, dried, and scanned. The peak areas were recorded as described in the calibration procedure. The procedure was repeated five times, individually weighing the tablet powder each time. The contents of drug in the sample zone were determined automatically from the standard solutions. The percentage recoveries were calculated as was described for assay in the fortified samples.

RESULTS AND DISCUSSION

The mobile phase chloroform–ethyl acetate–25% ammonium hydroxide (5 : 5 : 0.1, v/v) was selected as optimal for obtaining well shaped, symmetrical single spots. The horizontal technique and a migration distance of 8 cm were chosen as the best for chromatogram development. The R_F value of rosiglitazone was obtained as 0.52 ± 0.01 (mean $\pm$ SD; $n = 10$).

In this experiment, any additional spots and peaks were not observed. The wavelength 240 nm was selected for densitometric evaluation, because at this wavelength there was maximum of the absorption spectrum of rosiglitazone. For comparing the results, a standard wavelength 254 nm was also applied.

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Minimum detectable concentration, using a signal to noise ratio of 3 : 1 was found to be 0.05 and 0.1 μg/10μL for assay at 240 and 254 nm, respectively. The limit of quantification, using a signal to noise ratio of 10 : 1 was found to be 0.1 and 0.2 μg/10 μL, respectively.

Calibration procedures were done using five points. For each point, five measurements were made to improve the precision of an analytical procedure. The data were averaged and the mean calibration curves were calculated. The plot of the peak areas vs. concentration of rosiglitazone was constructed in the range 0.2–1.0 μg/10 μL. The calibration curves obtained were expressed as second-order polynomial functions. For mathematical verification of linearity, Mandel's fitting test was used. This test confirmed that a second-order calibration function was a better fit than a first-order function (variance homogeneity was tested by use of *F*-Snedecor test for the standard solutions of the highest and the lowest concentrations). The parameters of the polynomial equations, with their standard errors and the correlation coefficients obtained, are given in Table 1.

The accuracy of the proposed method was confirmed by recovery experiments from the fortified samples at three different levels of standard. Each level being repeated five times, and the percentage recoveries were calculated. Recoveries of rosiglitazone ranged from 89.48% to 99.38% for the lowest and the highest calibration level at 240 nm. The same values for assay at 254 nm ranged from 89.05% to 100.89%. The results of these determinations are given in Table 2.

The rosiglitazone contents in a commercial brand of tablets were analyzed according to the procedure described below. The contents were found to be 4.08 ± 0.15 mg per tablet for assay at 240 nm, and 4.13 ± 0.35 mg per tablet for assay at 254 nm. These values were calculated from the results obtained by

Table 1. Characteristics of the regression equations ($y = ax^2 + bx + c$; $n = 5$).

	Analytical wavelength (nm)	
	240	254
Parameter		
a ± SD	−2241.3 ± 122.6	−2679.6 ± 236.9
b ± SD	13448.6 ± 315.2	10575.6 ± 712.1
c ± SD	1164.1 ± 29.1	673.9 ± 36.5
Correlation coefficient	0.9993	0.9994
RSD for lowest calibration level (%)	3.58	8.23
RSD for higher calibration level (%)	2.76	6.56

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Table 2. Assay of the fortified samples.

Analytical wavelength (nm)	Amount of drug added (mg)	Mean recovery found ($n = 5$) (%)	Standard deviation	Standard error of the mean	Relative standard deviation (%)	95% Confidence interval (%)	Total mean recovery ($n = 15$) (%)
240	2	89.48	1.95	0.87	2.18	89.48 ± 2.42	95.41
	4	97.37	2.62	1.17	2.69	97.37 ± 3.25	
	6	99.38	1.75	0.78	1.76	99.38 ± 2.17	
240	2	89.05	8.20	3.67	9.21	89.05 ± 10.18	94.05
	4	100.89	6.16	2.75	6.11	100.89 ± 7.65	
	6	92.21	5.80	2.59	6.29	92.21 ± 7.20	

Table 3. Assay of Avandia® tablets ($n = 5$).

	Analytical wavelength (nm)	
	240	254
Amount claimed (mg/Tablet)	4.0	4.0
Mean amount found (mg/Tablet)	4.08	4.13
Standard deviation	0.12	0.28
Standard error of the mean	0.03	0.12
Relative standard deviation (%)	3.09	6.78
95% Confidence interval (mg)	4.08 ± 0.15	4.13 ± 0.35

performing the assay five times, each time with individual weighing. There is no interferences due to the excipients present in the brand of tablets. The results of these determination are given in Table 3.

All results were obtained at two different wavelengths, 240 and 254 nm. The results of the calibration experiments showed similar correlation, but at 240 nm, where the maximum of absorbance of rosiglitazone had been obtained, the RSD values for lowest and highest calibration levels were better. The differences in recovery data obtained for these two wavelengths were statistically compared, using *F*-Snedecor and *t*-Student tests at $P = 95\%$. The assay of rosiglitazone in the fortified samples at 240 nm was more precise, but the difference between the mean values of recoveries was not significant. The assays of the drug in the tablets at 240 and at 254 nm were equally precise and the difference between the mean values of recoveries was not significant.

In summary, a new, simple HPTLC assay was developed and validated for quantitation of a new oral hypoglycemic agent rosiglitazone in pharmaceutical formulations. This method is simple to perform, rapid, and practical, and could be an alternative method to other analytical procedures. Its accuracy and precision determined at 240 nm are satisfactory for routine use in a pharmaceutical quality control.

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