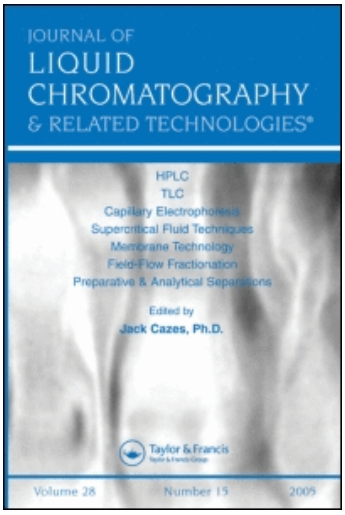

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Reversed-Phase Thin-Layer Chromatography of Three New Oral Antidiabetics and Densitometric Determination of Pioglitazone

Anna Gumieniczek[a]; Hanna Hopkała[a]; Anna Berecka[a]
[a] Department of Medicinal Chemistry, Medical University of Lublin, Lublin, Poland

Online publication date: 29 July 2004

To cite this Article Gumieniczek, Anna , Hopkała, Hanna and Berecka, Anna(2005) 'Reversed-Phase Thin-Layer Chromatography of Three New Oral Antidiabetics and Densitometric Determination of Pioglitazone', Journal of Liquid Chromatography & Related Technologies, 27: 13, 2057 — 2070
To link to this Article: DOI: 10.1081/JLC-120039418
URL: http://dx.doi.org/10.1081/JLC-120039418

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 27, No. 13, pp. 2057–2070, 2004

Reversed-Phase Thin-Layer Chromatography of Three New Oral Antidiabetics and Densitometric Determination of Pioglitazone

Anna Gumieniczek,* Hanna Hopkała, and Anna Berecka

Department of Medicinal Chemistry, Medical University of Lublin, Lublin, Poland

ABSTRACT

The thin-layer chromatographic behavior of new oral antidiabetic drugs, pioglitazone, rosiglitazone, and repaglinide has been investigated. For reversed-phase (RP) chromatography, chemically bonded cyanopropyl plates with mobile phases comprising 1,4-dioxane with phosphate buffers were used. The influence of the pH on the separation of the drugs was also examined. Then, a simple, rapid, and stability-indicating high performance thin-layer chromatographic method has been developed and validated for the quantitative determination of pioglitazone in tablets. Analysis was performed with 1,4-dioxane–phosphate buffer of pH 4.4 (5 : 5) as the mobile phase. Detection and quantification were performed

*Correspondence: Anna Gumieniczek, Department of Medicinal Chemistry, Medical University of Lublin, Chodźki 6, 20-093 Lublin, Poland; E-mail: kzchl@asklepios.am.lublin.pl.

DOI: 10.1081/JLC-120039418 1082-6076 (Print); 1520-572X (Online)

by classical densitometry at the wavelength of maximum absorption of pioglitazone, 266 nm. A calibration plot was constructed in the range of 0.4–2.4 μg/10 μL and was linear with a good correlation coefficient ($r = 0.9957$). Precision was validated by replicate analyses of standard solutions, and accuracy by analysis of fortified samples. The precision of the proposed chromatographic method expressed as mean relative standard deviation (RSD) was 4.99% and 2.57%, respectively, for the lowest and the highest calibration levels. Recovery from the fortified samples ranged from 98.09% to 103.28%. The mean (±SD) recovery from tablets was 99.79% ± 1.57%.

Key Words: Reversed-phase HPTLC; Oral antidiabetics; Separation; Pioglitazone; Densitometry; Tablets.

INTRODUCTION

Pioglitazone hydrochloride, (±)-5-[*p*-[2-(ethyl-2-pyridinyl)ethoxy]-benzyl]-2,4-thiazolidinedione hydrochloride, and rosiglitazone maleate, (±)-5-[4-[2-[*N*-methyl-*N*-(2-pyridinyl)amino]-ethoxy]benzyl]-2,4-thiazolidinedione maleate, are new oral antidiabetic agents that are members of the thiazolidinedione (TZD) family. TZDs appear to improve sensitivity to insulin in liver, muscle, and adipose tissue, but they do not directly stimulate insulin secretion. These compounds are high affinity ligands of peroxisome proliferator activated receptor gamma (PPARγ). This receptor is a member of the nuclear receptor family, which controls the expression of genes involved in lipid and carbohydrate metabolism in target tissues.[1] Repaglinide, *S*(+)-2-ethoxy-4-[*N*-[1-(2-(1-piperidinophenyl)-3-methyl-1-butyl]-aminocarbonylmethyl]benzoic acid, has been recently introduced as a rapidly absorbed and rapidly eliminated insulin releaser. This drug acts on the sulfonylurea receptor SUR1 of the pancreatic B-cell at a different site to sulfonylureas, but by closing K_{ATP} channels it depolarizes the cell and opens voltage-dependent Ca^{2+} channels. Through this pathway, repaglinide – like sulfonylureas stimulate insulin secretion.[2]

The literature on thin-layer chromatography (TLC) analysis of TZDs and repaglinide is rather sparse. In our previous paper, the chromatographic separation of seven oral antidiabetic drugs, including pioglitazone, rosiglitazone, and repaglinide, was elaborated on silica gel, alumina, and RP-18 plates. For separation of these drugs, reversed-phase (RP) chromatography was more effective than the normal-phase mode.[3] Two other papers concern the quantitative TLC 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). Pioglitazone was used as an internal

standard.[4] In the second paper, the authors used silica gel plates and the mobile phase chloroform–ethyl acetate–25% ammonium hydroxide (50 : 50 : 1).[5] Methods based on high performance liquid chromatography (HPLC) have been reported for analysis of pioglitazone in pharmaceuticals[6,7] and in human or animal plasma.[8–11] The HPLC methods were also used for quantitative determination of rosiglitazone in pharmaceuticals[12] and plasma samples.[13–18] There are also some papers concerning the HPLC analysis of repaglinide in biological material.[19,20]

These newest drugs are more and more frequently used in therapy of type 2 diabetes. Therefore, rapid and simple analytical methods are continually required for qualitative and quantitative determination of these drugs in different preparations. The aim of this presented work was to develop a simple separation of three new antidiabetic drugs by RP TLC. This paper describes and compares their chromatographic behavior on cyanopropyl-bonded layers with mobile phases comprising 1,4-dioxane with phosphate buffers of different pH values. Because no TLC or high performance TLC (HPTLC) method has yet been reported for the determination of pioglitazone, this paper also describes a simple, rapid quantitative HPTLC method for analysis of pioglitazone in tablets.

EXPERIMENTAL

Reagents and Chemicals

Pioglitazone hydrochloride and Actos® tablets (containing 30 mg of pioglitazone) from Takeda Industries Ltd. (Osaka, Japan), rosiglitazone maleate from SmithKline Beecham (Brentford, England), and repaglinide from NovoNordisk (Bagsværd, Denmark) were used. The working solutions were prepared by dissolving pioglitazone and repaglinide with methanol and rosiglitazone maleate with ethanol. The working concentrations of 1.0 mg/mL for pioglitazone, rosiglitazone, and repaglinide were prepared and stored at -4°C. They were stable for at least 6 weeks. 1,4-Dioxane, ethanol, and methanol for chromatography from E. Merck (Darmstadt, Germany) were used. Buffer solutions were prepared with 0.067 mol/L KH_2PO_4 and 0.067 mol/L Na_2HPO_4 and 85% H_3PO_4. The pH was measured in the buffer solutions and not in the final mobile phases.

Chromatography

Analysis was performed on 10×20 cm HPTLC CN F_{254} plates from E. Merck (Darmstadt, Germany). A single batch number was used throughout

the separation, calibration, and validation procedures. Horizontal Teflon DS II chambers with mobile phase distributors from Chromdes (Lublin, Poland) were used.

Plates were developed to a distance of 9 cm in unsaturated chambers, and the temperature was maintained at 20°C ± 1°C. Migration distances were measured to an accuracy of 1 mm and converted to R_F values. The examined chromatographic conditions and the obtained R_F values are given in Table 1.

A densitometer CD 60 from Desaga (Wiesloch, Germany) controlled by Desaga ProQuant® Windows software with an AS 30 HPTLC applicator were used. Pioglitazone spots were scanned in the reflectance/transmittance mode at 266 nm with slit dimensions of 0.1 mm × 2.0 mm; 360 nm was used as the reference wavelength.

Procedure for Qualitative Analysis of Pioglitazone, Rosiglitazone, and Repaglinide

The working solutions of pioglitazone, rosiglitazone, and repaglinide, in volumes of 2 μL, were applied manually to the plates, using a 25 μL microsyringe from the Hamilton Company (Bonaduz, Switzerland). All experiments were repeated three times. The locations of the spots were determined under ultraviolet (UV) light at 254 nm.

Table 1. R_F values of the investigated antidiabetic drugs.

		R_F in mobile phase: 1,4-dioxane–buffer				
Drugs	pH	(2 : 8)	(4 : 6)	(5 : 5)	(6 : 4)	(8 : 2)
Pioglitazone	2.8	0.22	0.44	0.52	0.62	0.83
	4.4	0.80	0.28	0.53	0.63	0.87
	6.4	0.70	0.26	0.41	0.58	0.83
	7.9	0.10	0.21	0.41	0.49	0.83
Rosiglitazone	2.8	0.28	0.54	0.63	0.71	0.60
	4.4	0.13	0.44	0.61	0.71	0.74
	6.4	0.17	0.46	0.57	0.59	0.70
	7.9	0.11	0.33	0.53	0.50	0.67
Repaglinide	2.8	0.17	0.38	0.30	0.61	0.78
	4.4	0.01	0.16	0.32	0.46	0.87
	6.4	0.02	0.11	0.23	0.54	0.81
	7.9	0.06	0.14	0.24	0.50	0.78

Procedure for Quantitative Analysis of Pioglitazone

Procedure for Calibration

The standard solutions of pioglitazone were prepared by dilution covering the range 0.4–2.4 μg/10 μL (per spot). A volume of 10 μL of each solution was applied to the plate using the applicator described above. After development, the standard zones were quantified by densitometric evaluation at 266 nm. Following five analyses of each standard solution, the calibration curve (dependence of peak area on the amount of pioglitazone applied to the plate) was determined by linear regression. Typical densitograms recorded for the standard solutions of pioglitazone are presented in Fig. 1.

Procedure for Assay in Tablets

Extraction of the active substance from tablets was performed with methanol. The average mass of Actos® tablets was determined as 0.1194 g. The tablets were ground, and amounts of ca. 0.2 g were transferred to 50-mL volumetric flasks containing approximately 30 mL of methanol. Solutions were vortex-mixed for 20 min to dissolve the active ingredient. The extracts were then diluted to volume with methanol and filtered through Whatman no. 42 paper. Equal 10 μL volumes of each sample solution and standard solution were applied to HPTLC plates, which were then developed, dried, and scanned. The peak areas were recorded as described in the calibration procedure. The assay was repeated 10 times, individually weighing the tablet powder. The contents of drug in the sample zones were determined automatically from the standard solutions. The percent recoveries were evaluated by comparing the theoretical contents predicted by the label declaration to the experimental contents of the sample zones. The results from this experiment are given in Table 2 and Fig. 1.

Procedure for Assay in Fortified Samples

Three different levels of standards, 15, 30, 45 mg of pioglitazone (50%, 100%, and 150% of theoretical content predicted by the label declaration), were added to the weighed portions of tablet powder, corresponding with the mean mass of tablet. The samples were transferred to 100-mL volumetric flasks containing approximately 50 mL of methanol and vortex-mixed for 20 min. The solutions were then diluted to volume with methanol and filtered. A 1.0 mL volume of each filtrate was then diluted to 10 mL with methanol. Equal 10 μL volumes of each sample solution and standard solution were applied to HPTLC plates, which were then developed, dried, and scanned. The peak areas were recorded as described in the calibration procedure.

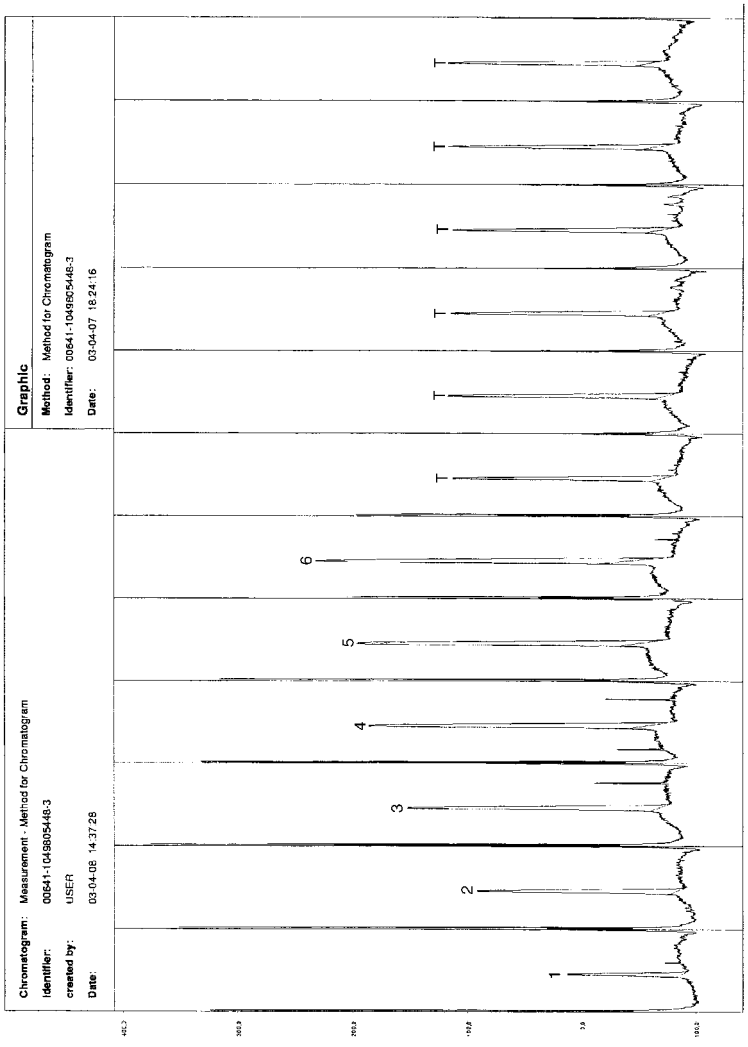

Figure 1. Densitogram recorded for the standard solutions of pioglitazone (1–6) in the calibration range 0.4–2.4 μg/10 μL, and for the samples obtained from Actos® tablets (T).

Table 2. Assay of pioglitazone in Actos® tablets ($n = 10$).

Amount claimed (mg/tablet)	Amount found (mg/tablet)	Mean amount found (mg/tablet)	Standard deviation	Standard error of the mean	Relative standard deviation (%)	95% Confidence interval (mg)
	30.16					
	30.09					
	28.65					
	29.84					
30.0	30.08	30.04	0.19	0.06	0.63	30.04 ± 0.14
	30.00					
	30.06					
	29.96					
	30.23					
	30.30					

Each level was repeated three times, being analyzed and calculated in a manner similar to that described for the assay in tablets. Results from the accuracy experiment are given in Table 3.

RESULTS AND DISCUSSION

Qualitative Analysis of Pioglitazone, Rosiglitazone, and Repaglinide

The separation efficiency by RP chromatography has been examined for three oral antidiabetic drugs, including the newest TZDs (pioglitazone, rosiglitazone) and repaglinide. Cyanopropyl-bonded layers in the RP mode were used. Optimization of retention was carried out by changing the concentration of the organic solvent 1,4-dioxane in the aqueous mobile phase. The effect of pH was also examined. The concentration of 1,4-dioxane was varied between 20% and 80%. Phosphate buffers of pH 2.8, 4.4, 6.4, and 7.9 were used. All of the drugs were detected by UV irradiation at 254 nm. The spots obtained by the elaborated chromatographic systems were well defined, symmetric, and oval. The horizontal technique and a migration distance of 9 cm were chosen as the best for chromatogram development.

The plots showing the effect of mobile phase on the retention of the target drugs on cyanopropyl-bonded plates are presented in Figs. 2–5. The pH of the buffer strongly affected the retention of pioglitazone when

Table 3. Assay of pioglitazone in the fortified samples.

Amount of drug added (mg)	Recovery (%)	Relative standard deviation ($n = 3$) (%)	Total mean recovery ($n = 9$) (%)	Total standard deviation ($n = 9$)	Standard error of the mean ($n = 9$)	95% Confidence interval ($n = 9$) (%)
15	99.04; 98.09; 99.34	0.66				
30	103.28; 101.08; 102.25	1.09	100.25	1.71	0.57	100.25 ± 1.31
45	100.78; 99.44; 98.97	0.94				

the content of organic modifier was 20%. For two other drugs and for other mobile phases, this effect was less apparent. In mobile phases containing 20% 1,4-dioxane in buffers of pH 4.4, 6.4, or 7.9, the compounds did not migrate from the origin, especially repaglinide. These weaker mobile phases drastically increased the retention and made this assay of less practical interest. In mobile phases containing more than 20% 1,4-dioxane in all examined buffers, the substances migrated from the origin with R_F values of more than 0.2, except repaglinide. When the concentration of 1,4-dioxane was 80%, the R_F values of some drugs was higher than 0.8. The best separation of the three target drugs was achieved using 50% 1,4-dioxane in each phosphate buffer, 40% 1,4-dioxane in the buffer of pH 2.8, or 60% 1,4-dioxane in the buffer of pH 4.4. However, a mobile phase containing 40% 1,4-dioxane in pH 6.4 buffer provided the best separation of the similar drugs pioglitazone and rosiglitazone. On the basis of the obtained results, it was possible to choose the optimal chromatographic system for quantitative determination of pioglitazone.

Quantitative Analysis of Pioglitazone

The mobile phase 1,4-dioxane—pH 4.4 buffer (5 : 5) was selected as optimal for obtaining well-shaped, symmetrical single spots of pioglitazone. The R_F value of the drug was 0.53 ± 0.01 (mean $\pm$ SD; $n = 10$). The wavelength 266 nm was selected for densitometric evaluation, because at this wavelength there was a maximum of the absorption spectrum of pioglitazone.

Calibration was done using six points. For each point, five measurements were made to improve the precision of the analytical procedure. The data were averaged and the mean ($\pm$SD) calibration curve was calculated. It was

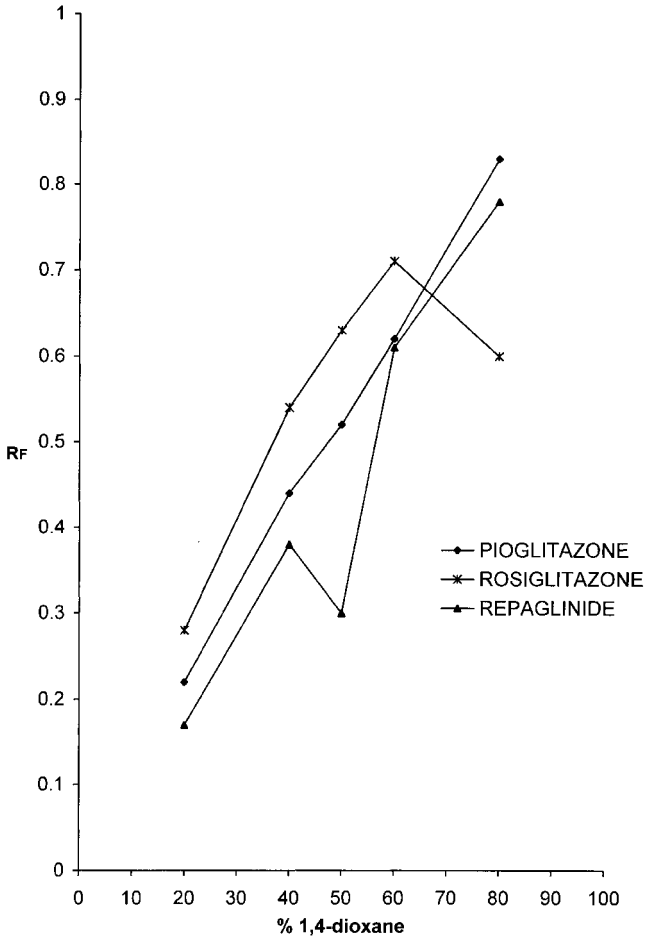

Figure 2. Dependence of R_F of pioglitazone, rosiglitazone, and repaglinide on the composition of the mobile phase containing buffer of pH 2.8.

represented by the linear regression equation $y = 581.35\ (\pm 19.44)x + 121.11\ (\pm 21.59)$; $r = 0.9957\ (\pm 0.001)$. The plot of the peak area vs. concentration of pioglitazone was found to be linear in the range 0.4–2.4 μg/10 μL. The limit of detection (LOD) and limit of quantification (LOQ) were calculated by use of the equations $\text{LOD} = 3\times N/B$ and $\text{LOQ} = 10\times N/B$, where N is the SD of the peak area of the standard ($n = 3$), taken as a measure of the noise, and B is the slope of the corresponding calibration curve. The LOD was found to be 0.06 μg/10 μL and the LOQ 0.2 μg/10 μL.

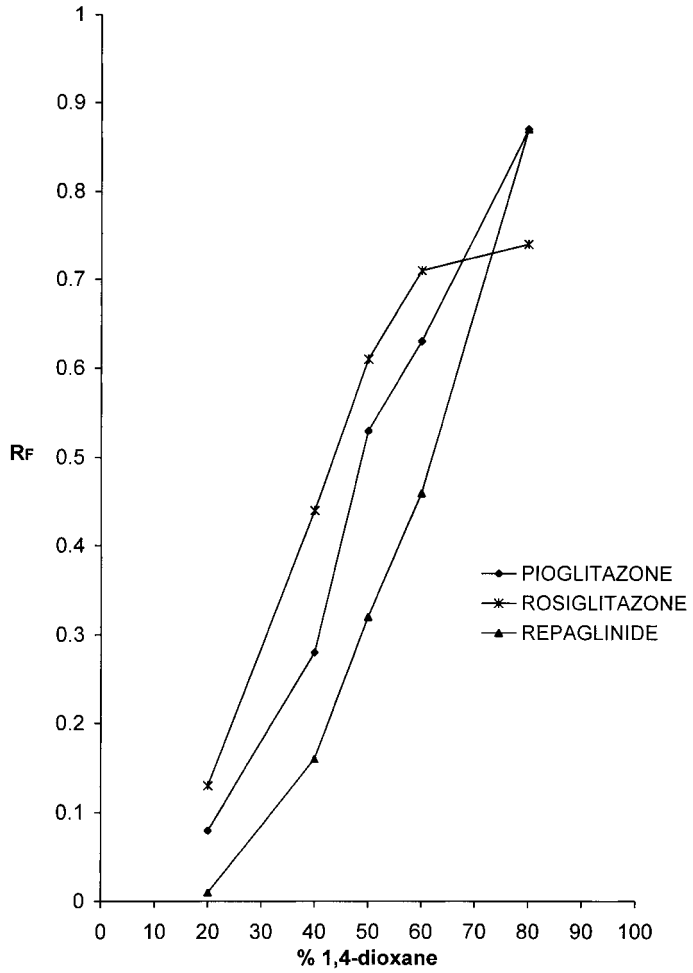

Figure 3. Dependence of R_F of pioglitazone, rosiglitazone, and repaglinide on the composition of the mobile phase containing buffer of pH 4.4.

The densitograms recorded for the standard solutions are presented in Fig. 1. Pioglitazone content in a commercial brand of tablets was analyzed according to the procedure described above. The content was found to be 30.04 ± 0.19 (mean $\pm$ SD) mg per tablet. It was calculated from the results obtained by performing the assay 10 times, each time with individual weighing. There were no interferences due to the excipients present in the brand of tablets. The results of these determinations are given in Table 2 and Fig. 1. The accuracy

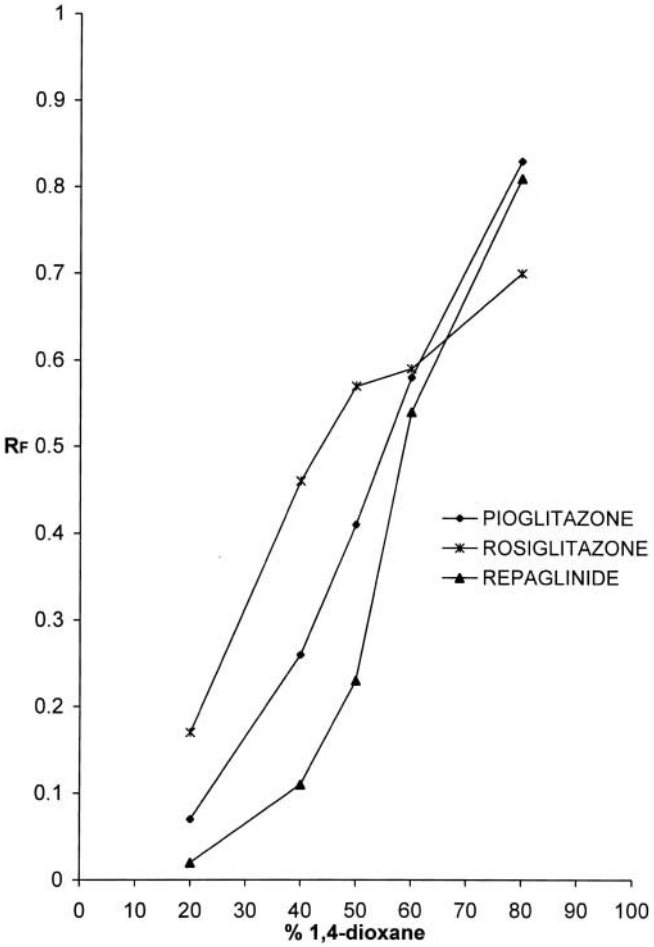

Figure 4. Dependence of R_F of pioglitazone, rosiglitazone, and repaglinide on the composition of the mobile phase containing buffer of pH 6.4.

of the proposed method was confirmed by recovery experiments from fortified samples at three different levels of standard. Each level was repeated three times, and the percent recoveries were calculated. The recoveries of pioglitazone ranged from 98.09% to 103.28%, including all three levels of addition ($n = 9$). The results of these determinations are given in Table 3.

In summary, three new oral antidiabetic drugs, pioglitazone, rosiglitazone, and repaglinide, were separated by RP TLC on cyanopropyl-bonded plates. The

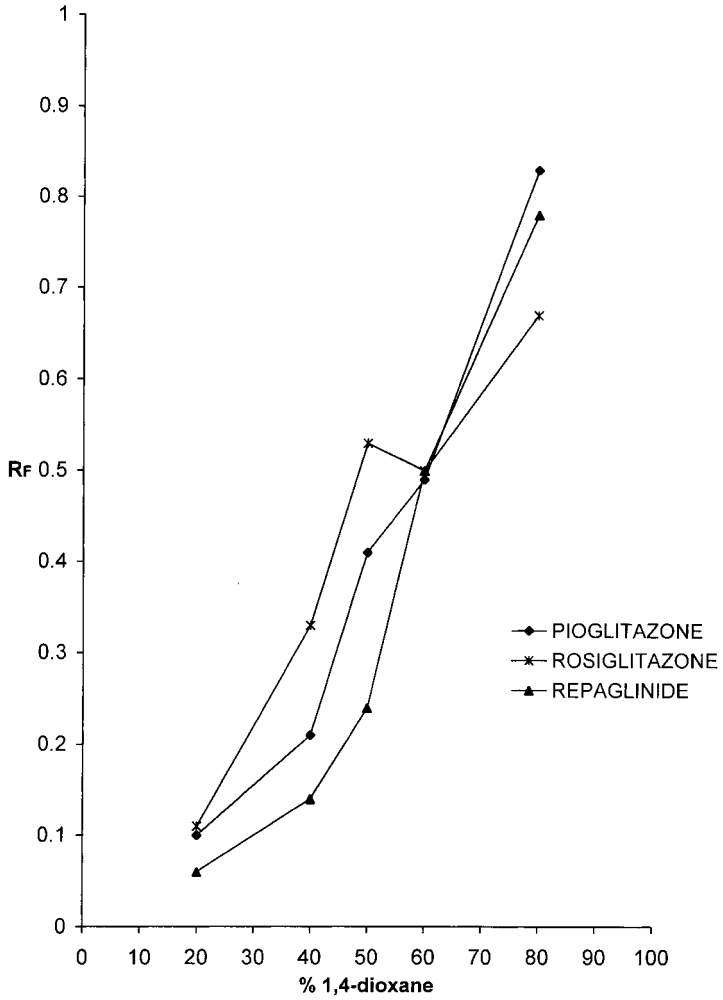

Figure 5. Dependence of R_F of pioglitazone, rosiglitazone, and repaglinide on the composition of the mobile phase containing buffer of pH 7.9.

differences in selectivity of the solvent systems were examined for separation of the closely related compounds, pioglitazone and rosiglitazone. The described investigations enabled the choice of a suitable chromatographic system for identification and quantification of pioglitazone, rosiglitazone, and repaglinide.

A simple HPTLC assay was developed and validated for quantitation of pioglitazone in pharmaceutical formulations. Because of its relatively good

cost effectiveness, rapidity, and simple technique and instrumentation, the HPTLC method can be used as a more effective alternative to other chromatographic techniques. Its accuracy and precision are satisfactory for routine use in a pharmaceutical analysis.

ACKNOWLEDGMENT

The authors wish to thank Takeda Industries Ltd. (Osaka, Japan) for supplying pioglitazone hydrochloride pure substance.

REFERENCES

1. Madhavan, G.; Chakrabarti, R.; Kumar, S.K.B.; Misra, P.; Mamidi, R.N.V.S.; Balraju, V.; Kasiram, K.; Babu, R.K.; Suresh, J.; Lohray, B.B.; Lohray, V.B.; Iqbal, J.; Rajagopalan, R. Novel phthalazinone and benzooxazinone containing thiazolidinediones as antidiabetic and hypolipidemic agents. Eur. J. Med. Chem. **2001**, *36*, 627–637.
2. Bailey, C.J. Potential new treatments for type 2 diabetes. Trends Pharmacol. Sci. **2000**, *21*, 259–265.
3. Gumieniczek, A.; Hopkala, H.; Berecka, A.; Kowalczuk, D. Normal and reversed-phase thin-layer chromatography of seven oral antidiabetic agents. J. Planar Chromatogr.-Mod. TLC **2003**, *16*, 271–275.
4. Sane, R.T.; Francis, M.; Moghe, A.; Khedkar, S.; Anerao, A. High performance thin-layer chromatographic determination of rosiglitazone in its dosage form. J. Planar Chromatogr.-Mod. TLC **2002**, *15*, 192–195.
5. Gumieniczek, A.; Berecka, A.; Hopkala, H.; Mroczek, T. Rapid HPTLC determination of rosiglitazone in pharmaceutical formulations. J. Liq. Chromatogr. Relat. Technol. **2003**, *26*, 3301–3308.
6. Radhakrishna, T.; Sreenivas, R.D.; Om, R.G. Determination of pioglitazone hydrochloride in bulk and pharmaceutical formulations by HPLC and MEKC methods. J. Pharm. Biomed. Anal. **2002**, *29*, 593–607.
7. Dai, J.; Jin, W.; Liu, Y. Study on determination of related substances of pioglitazone hydrochloride by RP-HPLC. Yaowu Fenxi Zazhi **2001**, *21*, 36–39.
8. Zhong, W.Z.; Williams, M.G. Simultaneous quantitation of pioglitazone and its metabolites in human serum by liquid chromatography and solid phase extraction. J. Pharm. Biomed. Anal. **1996**, *14*, 465–473.
9. Yamashita, K.; Murakami, H.; Okuda, T.; Motohashi, M. High performance liquid chromatographic determination of pioglitazone and its metabolites in human serum and urine. J. Chromatogr. B **1996**, *677*, 141–146.

10. Xue, Y.J.; Turner, K.C.; Meeker, J.B.; Pursley, J.; Arnold, M.; Unger, S. Quantitative determination of pioglitazone in human serum by direct-injection high performance liquid chromatography mass spectrometry and its application to a bioequivalence study. J. Chromatogr. B **2003**, *795*, 215–226.
11. Lin, Z.J.; Ji, W.; Desai-Krieger, D.; Shum, L. Simultaneous determination of pioglitazone and its two active metabolites in human plasma by LC-MS/MS. J. Pharm. Biomed. Anal. **2003**, *33*, 101–108.
12. Radhakrishna, T.; Satyanarayana, J.; Satyanarayana, A. LC determination of rosiglitazone in bulk and pharmaceutical formulation. J. Pharm. Biomed. Anal. **2002**, *29*, 873–880.
13. Wei, S.; Zhang, Z. RP-HPLC determination of rosiglitazone in plasma. Yaowu Fenxi Zazhi **2001**, *21*, 365–366.
14. Muxlow, A.M.; Fowles, S.; Russell, P. Automated high performance liquid chromatography method for the determination of rosiglitazone in human plasma. J. Chromatogr. B **2001**, *752*, 77–84.
15. Abbott, R.W.; Allen, G.D.; Rhodes, G. Chiral HPLC analysis of BRL 49653 and its enantiomers in mouse, rat, dog, and human plasma. Methodol. Surv. Bioanal. Drugs **1994**, *23*, 255–256.
16. Gaffney, M.H.; Allen, G.D.; Abbott, R.W.; Deeks, N.J.; Hollis, F.J.; Rhodes, G. Quantification of BRL 49653 in rat and dog plasma with fluorescence detection. Methodol. Surv. Bioanal. Drugs **1994**, *23*, 251–254.
17. Kolte, B.L.; Raut, B.B.; Deo, A.A.; Bagool, M.A.; Shide, D.B. Liquid chromatographic method for the determination of rosiglitazone in human plasma. J. Chromatogr. B **2003**, *788*, 37–44.
18. Mamidi, R.; Chaluvadi, M.R.; Benjamin, B.; Ramesh, M.; Katneni, K.; Babu, A.P.; Bhanduri, J.; Rao, N.; Rajagopalan, R. HPLC method for the determination of rosiglitazone in human plasma and its application in a clinical pharmacokinetic study. Arzneim.-Forsch. **2002**, *52*, 560–564.
19. Ji, H.; Xie, L.; Yan, M.; Zhang, L.; Huang, X.; Liu, X. Determination of repaglinide in plasma by HPLC and its pharmacokinetics in men. Zhongguo Yaoke Daxue Xuebao **2001**, *32*, 30–33.
20. Greischel, A.; Beschke, K.; Rapp, H.; Roth, W. Quantitation of the new hypoglycaemic agent AG-EE 388 ZW in human plasma by automated high performance liquid chromatography with electrochemical detection. J. Chromatogr. **1991**, *568*, 246–252.

Received November 10, 2003
Accepted December 16, 2003
Manuscript 6346L