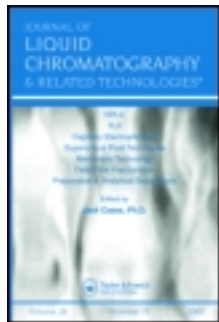


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TLC DETERMINATION OF GLIMEPIRIDE AND ITS MAIN IMPURITIES IN PHARMACEUTICALS

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Abstract

A quantitative TLC method was developed for the simultaneous separation and quantification of glimepiride and its main degradation impurities, glimepiride-sulfonamide and glimepiride-carbamate. Chromatographic analysis was performed using the commercial aluminium-backed TLC plates precoated with silica gel 60F254 as stationary phase, and toluene-ethyl acetate-methanol 8:5:1 (v/v) as mobile phase. Detection was performed at 230 nm. Regression coefficients ($r > 0.997$), recovery (94.9 to 105.1 %), determination limit of impurities (7 ng spot⁻¹ equivalent to the 0.1% impurity level), and robustness were validated and found to be satisfactory. The method is convenient for quantitative analysis and purity control of glimepiride in its dosage forms.

KEYWORDS: TLC, glimepiride, impurities, quantification, pharmaceutical dosage forms

INTRODUCTION

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Glimepiride is an orally active hypoglycemic drug belonging to the sulfonylurea group and it can be used in the non-insulin dependent diabetes mellitus cases. It is classified as a second-generation antidiabetic drug^[1] and – like the other drugs belonging to this group – it shows an increased potency, a more rapid onset, a shorter plasma half-life, and a longer duration of action.

The presence of the sulfonylurea bridge, a carboxamide linkage, a constrained lactam ring, and an α,β -unsaturated carbonyl system make glimepiride susceptible to degradation as a result of the lability of these linkages and functional groups to hydrolysis and photolysis^[2]. As a result, several degradation products are assumed to be formed in the course of the formal stability testing of the drug. The presence of impurities can have a significant impact on the quality and safety of the glimepiride dosage products.

A recently published review paper devoted to the different analytical techniques for qualitative and quantitative determination of glimepiride in biological samples and pharmaceutical formulations has covered information contained in 55 references^[3]. With respect of the pronounced lability of glimepiride, several high performance liquid chromatographic (HPLC) methods were developed for the separation of glimepiride and its impurities originating from the synthesis (as process impurities), or appearing as the degradation products, mostly from API (active pharmaceutical ingredients)^[2,4,5], or the pharmaceutical drug products^[6,7]. The drug substance monograph of glimepiride in European Pharmacopoeia (EP) lists ten impurities (A-J)^[8], while the United States Pharmacopoeia (USP) lists three impurities, glimepiride-sulfonamide, glimepiride-

urethane (carbamate), and glimepiride-3-isomer^[9]. According to USP and the requirements for dosage formulations (tablets), such degradants as glimepiride-sulfonamide, are required to be quantitatively tested. Besides glimepiride-sulfonamide, certain manufacturers require quantitative testing of glimepiride-carbamate in the final dosage form. These two degradants were suggested to be formed due to the hydrolysis of the sulfonylurea bridge. The chemical structures of glimepiride, glimepiride-sulfonamide, and glimepiride-carbamate are given in Fig. 1.

TLC has been used for the determination of the multi-component dosage formulations containing glimepiride in the presence of pioglitazone^[10,11], pioglitazone and metformin^[12], and metformin and atorvastatin^[13]. HPLC and TLC have been used for the determination of glimepiride and pioglitazone in pharmaceutical formulations^[14]. So far, no reports are available on the thin-layer chromatographic methods for the determination of glimepiride and its main degradation impurities.

In this paper, we present a simple, rapid, accurate, and precise thin-layer chromatographic method for a simultaneous determination of glimepiride and its main degradation products (glimepiride-sulfonamide and glimepiride-carbamate), which need to be monitored in the pharmaceutical dosage forms, according to certain manufacturers, or to the pharmacopoeia requirements.

EXPERIMENTAL

Materials

1-[[4-[2-(3-Ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)-ethyl]phenyl]sulphonyl]-3-*trans*-(4-methylcyclohexyl)urea (glimepiride), 3-ethyl-4-methyl-2-oxo-N – [2-(4-sulfamoylphenyl) ethyl]-2,3-dihydro-1*H*-pyrrole-1-carboxamide (glimepiride-sulfonamide), and methyl [4-[2-(3-ethyl-4-methyl-2-oxo-2,3-dihydro-1*H*-pyrrol-1-yl)carbonyl] ethyl] phenyl]sulfonyl]carbamate (glimepiride-carbamate), were kindly donated by Sanofi-Aventis, Scoppito (Italy). Amaryl[®] 3 mg tablets (Sanofi-Aventis, Scoppito, Italy), Limeral[®] 4 mg tablets (Zdravlje-Actavis Company, Leskovac, Serbia), and Dibiglim[®] 3 mg tablets (Sandoz Pharmaceuticals, Ljubljana, Slovenia) were also used in our study.

Solutions

Standard Solutions

Stock solution of glimepiride as the standard substance (0.3 mg mL^{-1}) and stock solutions of the impurities, glimepiride-sulfonamide and glimepiride-carbamate (0.1 mg mL^{-1}), were prepared in methanol.

Five glimepiride calibration solutions containing $0.120\text{--}0.180 \text{ mg mL}^{-1}$ of the test compound were prepared by diluting the stock solution. The $1\text{-}\mu\text{L}$ aliquot of each glimepiride calibration solution was applied to the chromatographic plate. Five calibration solutions containing the impurities were prepared by diluting the stock solutions to obtain the solutions containing $0.002\text{--}0.02 \text{ mg mL}^{-1}$ of glimepiride-sulfonamide and glimepiride-carbamate. The $5\text{-}\mu\text{L}$ aliquots of each glimepiride-

sulfonamide and glimepiride-carbamate calibration solution were applied to the chromatographic plates.

Sample Solutions

Ten tablets of each sample were weighed and pulverized. The quantity of the powdered tablets containing 1.5 mg glimepiride was transferred to the 10-mL volumetric flask and dissolved in 5 mL methanol, using an ultrasonic bath for 5 min. The solutions were then diluted to the volume with the same solvent and filtered through the 0.45- μ m pore size membrane filter (Millipore). For the assay of glimepiride, the 1- μ L aliquot of the filtrate was applied to the plate.

The quantity of the powdered tablets containing 3 mg glimepiride was transferred to the 5-mL volumetric flask and dissolved in 3 mL methanol, using an ultrasonic bath for 5 min. The solution was then diluted to the volume with the same solvent and filtered through the 0.45- μ m pore size membrane filter (Millipore). For the assay of the impurities, the 10- μ L aliquots of the filtrates were applied to the plate.

Chromatography

Chromatography was performed on the 20 cm \times 10 cm thin-layer chromatographic plates cut from the 20 cm \times 20 cm aluminium plates, precoated with silica gel 60 F₂₅₄ (Merck, Darmstadt, Germany). Standard and sample solutions were applied 15 mm above the lower edge of the plate, using a Camag (Muttens, Switzerland) Nanomat II application device. Ascending chromatography to a distance of 80 mm was performed in the twin-

trough TLC chamber, previously saturated for 20 min . The mobile phases was toluene-ethyl acetate–methanol, 8:5:1 (v/v). After the development, the plates were dried in ambient air and the separated zones were scanned in the linear reflectance–absorbance mode at 230 nm by means of a Camag TLC Scanner II with a computer system and Cats software (V.3.15). The peak areas were used for quantification.

RESULTS AND DISCUSSION

Optimization of working conditions for a simple, rapid and reproducible analysis usually involves selection of appropriate stationary or mobile phases. In our case, in order to obtain a satisfactory resolution and to avoid peak tailing, optimization was performed with different mobile phase. The retention behavior using single non polar (toluene, cyclohexane) and polar (methanol, acetonitrile) solvents was investigated.

In a non polar solvent (e.g., toluene), all investigated substances were retained on the start line. In the binary system (toluene-ethyl acetate, 1:1 (v/)), a diffused and largely retained zone with poor resolution was observed close to the start line. Polar solvents were apparently needed to suppress strong intermolecular interactions between the polar groups of the investigated impurities and the silanol groups of silica gel. A satisfactory resolution and separation of glimepiride and its impurities (glimepiride-sulfonamid and glimepiride-carbamate) was obtained using methanol as a modifier. The best resolution was obtained with toluene-ethyl acetate–methanol, 8:5:1 (v/v). Using the same solvent system, no better separation, or better peak response was achieved on the HPTLC, or HPTLC LiCrospher Si 60 plates, or on the modified silica reverse phase stationary phase.

Hence, the less expensive TLC plates were used for further validation of the elaborated thin-layer chromatographic method.

The migration distances of glimepiride, glimepiride-sulfonamide, and glimepiride-carbamate were 48.4 mm, 43.0 mm, and 34.5 mm, respectively. The absorption UV spectra of all three substances were recorded and the optimum wavelength for the densitometric assessment was chosen as equal to 230 nm. The relationships between the peak areas and the amounts of the substances applied were evaluated with use of the linear and the second degree polynomial regression functions. For glimepiride, linear regression was proved as performing well enough, because of the narrower range of the concentrations tested. For the two impurities, i.e., glimepiride-sulfonamide and glimepiride-carbamate, the second-degree polynomial function was employed, because a wider range of concentrations was required for quantitation of the impurities in the method of purity assessment. The regression parameters are summarized in Table 1.

An effect of larger amounts of the drug on the peak shape and the resolution of the impurities had to be determined, in order to avoid systematic errors. An accuracy of the method was therefore proved by determination of the impurities (i.e., glimepiride-sulfonamide and glimepiride-carbamate) in the presence of glimepiride. The tablet matrix (i.e., a mixture of the excipients) was spiked with 0.6 mg mL^{-1} glimepiride, and with 0.02 mg mL^{-1} , 0.005 mg mL^{-1} , and 0.002 mg mL^{-1} glimepiride-sulfonamide and glimepiride-carbamate, respectively (corresponding to 0.3-3.0%, respectively). The densitometrically scanned concentration profiles obtained from the spiked samples of glimepiride are

presented in Fig. 2. The calculated recoveries (the corresponding standards without glimepiride) were plotted against the expected values. The recoveries and the relative standard deviations (RSD) for glimepiride-sulfonamide and glimepiride-carbamate were acceptable for the method of purity assessment (Table 2). The recovery of glimepiride from a laboratory-prepared mixture of the excipients spiked with the three different concentrations (0.18 mg mL^{-1} , 0.15 mg mL^{-1} and 0.12 mg mL^{-1}) is given also in Table 2. The recovery of glimepiride ranging from 98.9 to 102.1%, and the RSD values lower than 2% confirms the accuracy of the method.

The repeatability of the method was assessed the by replicate chromatography applications ($n=6$) of glimepiride and the impurities (i.e., glimepiride-sulfonamide and glimepiride-carbamate) at the three different concentrations. The statistical data (i.e., the RSD values) obtained from these results are listed in Table 3.

The limit of detection (LOD) and the limit of quantification (LOQ) were determined by fitting the back-calculated inter-day standard deviation for each calibration standard. The y -intercept was then equal to SD_0 (the estimated standard deviation at the zero concentration). According to the defined LOD and LOQ values ($3SD_0$ and $10SD_0$, respectively), the sample amount applied to the plate at which it can be detected or quantified was established. The LOD values for the impurities (i.e., glimepiride-sulfonamide and glimepiride-carbamate) were found as equal to 2.19 and $2.30 \text{ ng spot}^{-1}$, respectively (which is equivalent to the percent level of 0.04% for both impurities). The LOQ values for the impurities (i.e., glimepiride-sulfonamide and glimepiride-carbamate)

were found as equal to 7.24 and 7.69 ng spot⁻¹ (which is equivalent to the percent level of 0.1% for both impurities).

Robustness is the measure of the capacity of the method to remain unaffected by small but deliberate variation of the method conditions, and it is an indication of the reliability of the method [15,16]. Since planar chromatography is an open chromatographic method, the environmental conditions can significantly influence its results. Thus, the temperature was chosen as one of the critical factors to be tested. Variation of the temperature between 15 and 25°C exerts no observable effect on the separation of glimepiride and its impurities. Apart from the temperature changes, we also examined the influence of the contents of all three solvents, i.e., methanol, toluene, and ethyl acetate, in mobile phase. These contents varied in the ±10% range and no observable effect on the separation and resolution among the peaks was perceived. The geometry of the twin-trough chamber and the flat-bottom chamber can influence (i.e., differentiate) the retention of the investigated compounds. The use of the twin-trough chamber was found as preferable for quantitative evaluation of glimepiride and its impurities.

The method was used to screen the commercial glimepiride tablets. The results are presented in Table 4. Compared with the label declaration, the recoveries of glimepiride from the dosage forms were very high. The RSD values obtained for the tablets (1.8 – 2.5 %) confirm the accuracy of the method. The impurity levels for glimepiride-sulfonamide (0.21 – 0.31 %) did not exceed the requirement for its limit, neither according to the different manufacturers of the generic glimepiride tablets (0.8-2%), nor according to USP

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(2.5%). The low LOQ value of the proposed thin-layer chromatographic method for both impurities enables testing of these two compounds in the glimepiride drug substances according to the pharmacopoeial requirements, not exceeded 0.4%.

CONCLUSION

The obtained results suggest that TLC is an efficient method for the separation and quantitative determination of glimepiride and its main degradation products. This simple and economical method is suitable for the separation and quantitative determination of the purity of glimepiride in its dosage forms. It can therefore be used for the routine quality control and performance of the formal stability study of glimepiride in its dosage forms.

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Table 1. Statistical Data for the Calibration Curves

Substance	n	ng spot ⁻¹	Calibration function y=a +bx			SD	R
			a	b			
Glimepiride	5	180-120	-276.276	5.2014		12.76	0.997
			Calibration function y=a +bx+cx ²				
			a	b	c		
Glimepiride-sulfonamide	5	100-10	5.534	10.442	-0.028	7.68	0.999
Glimepiride-carbamate	5	100-10	10.870	7.41	-0.000708	5.7	0.998

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Table 2. Accuracy of Method Expressed as a Recovery of the Analyte Spiked to the Placebo Mixture

Compound	Spiked ng spot ⁻¹	Found ng spot ⁻¹	Recovery (%)	RSD (%)
Glimepiride	180	183.85	102.1	1.55
	150	150.36	100.2	1.15
	120	118.75	98.9	1.21
Glimepiride-sulfonamide	100	94.88	94.9	3.85
	25	26.27	105.1	2.64
	10	10.17	101.7	3.80
Glimepiride-carbamate	100	96.74	96.7	3.50
	25	25.37	101.5	3.35
	10	10.23	102.3	5.17

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Table 3. Precision of the Method

Amount ng spot ⁻¹	Glimepiride RSD (%)	Glimepiride-sulfonamide RSD (%)	Glimepiride-carbamate RSD (%)
180	0.9		
150	1.2		
130	1.8		
100		1.7	3.8
25		2.7	2.7
10		3.9	4.2

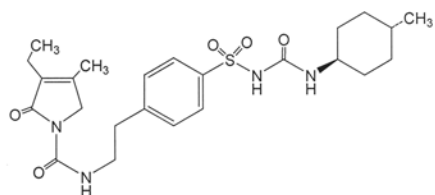
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Table 4. Results of TLC Determination of Glimepiride and Its Impurities

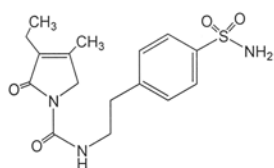
Sample	Glimepiride (mg \pm RSD)	Glimepiride- sulfonamide (% \pm RSD)	Glimepiride-carbamate (% \pm RSD)
Amaryl [®] 3 mg	2.95 \pm 1.83	0.21 \pm 5.4	-
Limeral [®] 4 mg	4.05 \pm 2.47	0.31 \pm 4.4	-
Dibiglim [®] 3 mg	2.85 \pm 2.23	0.29 \pm 4.8	-

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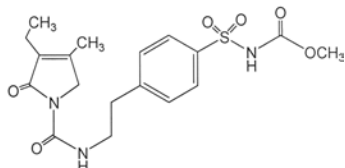
Figure 1. Chemical structures of glimepiride, glimepiride-sulfonamide, and glimepiride-carbamate.



Glimepiride



Glimepiride-sulfonamide



Glimepiride-carbamate

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Figure 2. Densitograms obtained for: (a,c) 25 ng and 10 ng mixture of each standard, respectively; glimepiride-carbamate (peak 1) and glimepiride-sulfonamide (peak 2); (b,d) samples of glimepiride (peak 3) spiked with 0.8 and 0.3%, respectively, of the impurities glimepiride-carbamate and glimepiride-sulfonamide; (e) sample of the glimepiride tablet.

