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Direct separation and quantitative determination of glimepiride isomers by high performance liquid chromatography

A simple and sensitive high-performance liquid chromatographic procedure for the determination of the *trans* isomer of glimepiride is reported. Chromatography accomplished direct separation of the *cis* and *trans* isomers of glimepiride on a Dikmonsil C18 (250 × 4.6 mm, 5 μm) column with a mobile phase consisting of methanol-acetonitrile-NH_4Ac buffer solution (1.5 mol L^{-1}, pH = 4.5) (1.1 : 1.3 : 1.0, *v/v*) at a flow rate 0.5 mL min^{-1}. The resolution (R_S) was 1.73 with a retention time of 24.885 and 23.018 min for the *cis* and the *trans* isomer, respectively. A standard linear calibration curve was established for the *trans* isomer of glimepiride over the range of 4.95–198.00 μg mL^{-1} with a correlation coefficient of 0.99997. This method has been successfully used to analyze four different kinds of glimepiride product.

Key Words: High performance liquid chromatography; Glimepiride; *cis-trans* Isomers

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1 Introduction

Glimepiride (Amaryl) is the first of a new generation of sulphonylurea antidiabetics, which can stimulate insulin release from pancreatic β-cells and may act via an extrapancreatic mechanism. This compound is suggested as first-line therapy to lower blood glucose in patients with type II diabetes whose high blood glucose can not be controlled by diet and exercise alone. Glimepiride was first introduced in Sweden in 1995. It has already been used in more than 60 countries in the world and has recently been launched in the US and UK. Several clinical studies have demonstrated that glimepiride leads to less hypoglycemia and no weight gain. It is administered once daily to patients with type II (non-insulin-dependent) diabetes and may be combined with insulin in patients with secondary sulphonylurea failure [1–5].

The chemical structure of glimepiride is *N*-[4-[2-(3-ethyl-4-methyl-2-oxo-3-pyrrolinyl-1-carboxamide)ethyl]phenylsulfonyl]-*N′-trans*-4-methylcyclohexylurea, as shown in **Figure 1**. It can be prepared by various well-known methods, for instance by reaction of the sulfonamide with *trans*-4-methyl cyclohexylisocyanate [6] or the sulfonyl carbamino acid methyl ester with *trans*-4-methylcyclohexylamine [7, 8]. In these syntheses, *cis* isomer was also present in the final products. However, there are no reports about the activity of the *cis* isomer, and what amount in final products will lower the pharmaceutical effect of glimepiride. It is therefore necessary to develop a method for determination of the *trans* isomer of glimepiride.

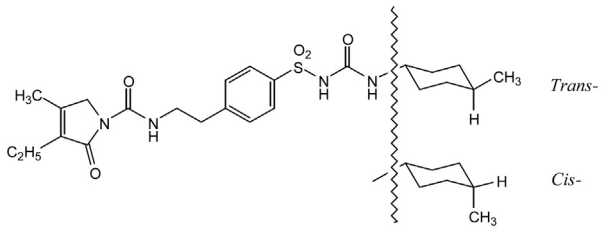

Figure 1. Chemical structures of *trans*- and *cis*-glimepiride.

Some methods have been developed to determine the content of glimepiride, such as reversed phase liquid chromatography [9] and second derivative UV spectrophotometry [10]. However, these methods can only give the total amount of *trans* and *cis* isomers, but fail to distinguish *trans*-glimepiride from the *cis* isomer.

This paper describes an improved HPLC method for separation and determination of the *trans* isomer of glimepiride.

2 Experimental

2.1 Materials

Standard reference for *trans*-glimepiride was provided by Beijing Institute for Drug Control (Beijing, China). Other glimepiride samples (mixture of *cis* and *trans* isomers,

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crude product, and refined product) were all provided by Beijing Beilu Pharmaceutical Co., Ltd. (Beijing, China). Methanol and acetonitrile were both of chromatographic grade, the former was from Tianjing Xiehe Physics Technology Company of the Chinese Academy of Medical Sciences (Tianjing, China) and the latter was purchased from Fine Chemical Plant of Nankai University (Tianjing, China). Water was redistilled before use. Ammonium dihydrophosphate, ammonium acetate, phosphoric acid, and acetic acid were all of analytical grade and were provided by Beijing Chemical Company (Beijing, China).

Mobile phases were prepared by mixing HAc-NH_4Ac (1.5 mol L^{-1}, pH 4.5) solution and organic modifier (MeOH, MeCN, or both) in different proportions. Then the mixtures were filtered using 0.45-μm filters and degassed by vacuum. Buffer molarities were expressed as concentrations in the aqueous portion of the mobile phase. The aqueous NH_4Ac buffer was pH adjusted with diluted $NH_3 \cdot H_2O$ and HAc.

2.2 Liquid chromatography

Chromatography was carried out at room temperature with standard HPLC equipment (Agilent, USA) including a G1310A liquid chromatographic pump, a G1313A autoinjection system, a G1314A UV-vis detector with the wavelength of 228 nm selected according to the UV spectrum of glimipiride, and an HPLC 2D chemstation. The stationary phase (Dikma, Beijing China) in the HPLC was Dikmonsil C18 (250 × 4.6 mm, 5 μm) column. All samples were prepared in methanol solvent at suitable concentration and 10 μL was injected.

3 Results and discussion

3.1 Effects of organic modifier in the mobile phase on separation

Being one of the components of the mobile phase, the organic modifier has a pronounced effect on the separation. In this paper, methanol, acetonitrile, and mixtures thereof were studied as organic modifier.

When only methanol was used, decreasing its content in the mobile phase from 2.5% to 1.2% (by volume) led to a corresponding increase of R_S from 1.17 to 1.36. However, the elution time (t_R) for both isomers increased from 14 min to 3 h, the baseline became noisy, and the peaks grew broad. The decrease in methanol content probably the increased polarity of the mobile phase, making elution difficult.

When only acetonitrile was used, R_S was always less than 1.0 at any ratio of modifier to buffer, although the retention time was shorter and the baseline was smoother with acetonitrile modifier than with methanol.

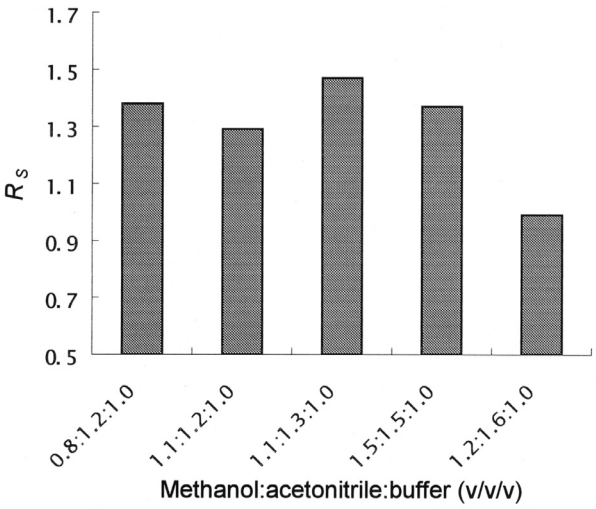

Figure 2. Effects of organic modifier in mobile phase on separation. Assay conditions: concentration of NH_4Ac 1.5 mol L^{-1} (pH = 4.5) and flow rate 1.0 mL min^{-1}. Sample concentrations were all 100 μg mL^{-1}

However, adding a mixture of methanol and acetonitrile to the HAc-NH_4Ac (1.5 mol L^{-1}, pH 4.5) solution afforded a mobile phase which gave not only high resolution but also short retention times and a smooth baseline. As shown in **Figure 2**, methanol:acetonitrile:buffer = 1.1 : 1.3 : 1.0 gave the best resolution. This mixed organic modifier apparently combined the advantages of both methanol and acetonitrile, improving the selectivity for *trans*- and *cis*-glimepiride isomers.

3.2 Flow rate of mobile phase

It is thought that the flow rate has some effect on the theoretical plate height (*H*) and theoretical plate number (*N*), which influence chromatographic resolution. Our experiments showed that the flow rate indeed affected R_S dramatically. When the flow rate was changed from 1.0 to 0.5 mL min^{-1}, R_S increased from 1.47 to 1.73 and baseline separation was accomplished. Thus 0.5 mL min^{-1} of flow rate was used for quantitative analysis. A typical chromatogram is shown in **Figure 3**.

3.3 Analytical characteristics

Under the optimum working conditions, a standard calibration curve of *trans*-glimepiride was constructed with concentrations of 4.95, 9.90, 19.80, 39.60, 49.50, 59.40, 79.20, 99.00, 118.80, 158.40, 198.00 μg mL^{-1}. The linearity was described by the regression equation: $Y = -10382 + 11716X$ (Y, peak area; X, concentration in μg mL^{-1}), with a coefficient of 0.99997. The limit of detection concentration was calculated to be 0.88 μg mL^{-1}, when a signal-to-noise ratio of 3 was used as the criterion.

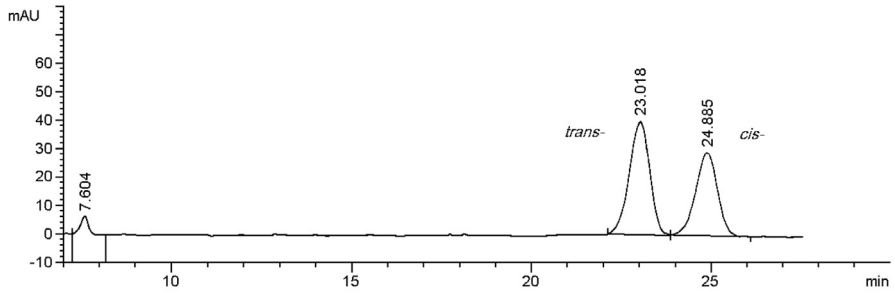

Figure 3. Typical chromatogram for the separation of mixture of *trans*- and *cis*-glimepiride. Mobile phase composed by methanol:acetonitrile : HAc-NH_4Ac (1.5 mol L^{-1}, pH 4.5) = 1.1 : 1.3 : 1.0 (*v/v*); flow rate: 0.5 mL min^{-1}. Sample concentration was 100 μg mL^{-1}.

Table 1. Determination results of glimepiride in the four samples by this method and that described in reference [9].

Sample	Found by this method (%)[a]		Found by method [9] (%)[b]
	trans	*cis*	
trans-Isomer reference	100.00	0.00	100.00
Crude product	96.77	2.30	98.60
Refined product	98.65	0.20	99.05
Mixture	53.31	42.04	98.43

UV detection at 228 nm; injection volume 10 μL; sample concentrations were all 100 μg mL^{-1}. [a] Mobile phase composed by methanol: acetonitrile:HAc-NH_4Ac (1.5 mol L^{-1}, pH 4.5) = 1.1:1.3:1.0 (*v/v*); flow rate: 0.5 mL min^{-1}. [b] Mobile phase composed by methanol:K_2HPO_4 (0.03 mol L^{-1}, pH 3.5) = 8:3 (*v/v*), flow rate 1.0 mL min^{-1}.

Six repeated experiments were done for inter-day assay at a concentration of *trans*-glimepiride reference of 10 μg mL^{-1}. The RSD was 1.8% for peak area. The intra-day assay result was 3.1% for five continuous days.

3.4 Assay of different glimepiride samples

Four different glimepiride samples were assayed by this improved method and reference [9], respectively. The results are listed in **Table 1**, which showed that different contents of *trans*-glimepiride in these four samples were found by the improved method. However, the method of Ref. [9] could only give the total amount of the *trans* and *cis* isomers, but failed to reveal the actual content of *trans*-glimepiride. This is because the two methods have different mobile phases. While the method of Ref. [9] used only methanol as modifier in the mobile phase, the improved method employed two kinds of organic modifiers, methanol and acetonitrile. Moreover, the improved method operated at much higher salt concentration, lower acidity, and lower flow rate than the previous method [9].

4 Conclusion

As discussed above, HPLC determination of *trans*-glimepiride was improved by using optimized chromatographic conditions: a mobile phase consisting of methanol-acetonitrile-NH_4Ac buffer solution (1.5 mol L^{-1}, pH = 4.5) (1.1 : 1.3 : 1.0, *v/v*) and a flow rate of 0.5 mL min^{-1}. This method possesses a wide range of linearity and a low detection limit. Repeated experiments and real sample analysis show this improved method is suitable for monitor the *trans* isomer in glimepiride preparations.

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