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Short communication

# HPLC study of glimepiride under hydrolytic stress conditions

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**Abstract**

Glimepiride is a modern hypoglycaemic agent, which belongs to the group of sulfonylurea derivates. In this paper, simple, specific and accurate RP-HPLC method was developed in order to study decomposition of glimepiride under the hydrolytic stress conditions (acid, neutral, alkaline and oxidative). The best separation of glimepiride and its degradation products was achieved on reverse phase $C_{18}$ column. The mobile phase was composed of acetonitrile–phosphate buffer (pH 3.5, 0.03 M) (48:52, v/v). Employing RP-HPLC method, five main degradation products were detected in the exposed samples. It was found that the susceptibility of glimepiride to hydrolytic decomposition increased in following manner: neutral condition < alkaline condition < acid condition < oxidative condition.


*Keywords:* Glimepiride; HPLC; Hydrolysis; Stability

## 1. Introduction

Glimepiride (1-[[p-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido) ethyl] phenyl] sulfonyl]-3-(trans-4-methylcyclohexyl) urea (Fig. 1) is a modern oral hypoglycaemic agent, which belongs to the group of sulfonylurea derivates.

These drugs are used for the treatment of non-insulin dependent diabetes mellitus (NIDDM) in order to achieve appropriate control of blood glucose level. Although glimepiride shares the principal mechanism of action with other drugs of this class (the stimulation of insulin secretion from pancreatic β-cells), it has several clinical benefits [1,2].

In order to achieve high level of safety and effectiveness of pharmacotherapy, the regulatory authorities escalate the requirements on quality of pharmaceutical products. The investigation of stability of drugs represents an important issue in drug quality evaluation. Many environmental conditions such as heat, light, moisture as well as the chemical susceptibility of substances to hydrolysis or oxidation can play extremely serious role in pharmaceutical stability [3,4].

A stress testing of drug substance can help to identify the likely degradation products and to provide important information on drug's inherent stability. Consecutively, it can be a fundamental contribution to development and validation of stability indicating analytical method used in monitoring of quality of pharmaceutical products. [5]. Independent of the final dosage form, forced drug degradation by exposure of drug solution to acid, alkaline or oxidative conditions is useful to predict the potential hydrolytic degradation products. Hydrolysis (during wide range of pH) is one of the most common degradation chemical reactions. Since water, either as a solvent or in the form of the potential moisture in the air, contacts most pharmaceutical dosage forms to some degree; the potential for this degradation pathway exists for most drugs and excipients [6].

Several analytical methods for determination of glimepiride have been reported in literature: determination of glimepiride in human plasma by liquid chromatography–electrospray ionization tandem mass spectrometry [7], HPLC analysis of glimepiride in human serum and urine after pre-column derivatization [8], liquid chromatography–mass

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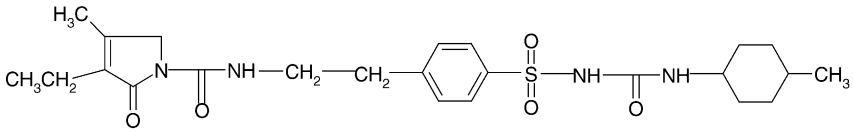

Fig. 1. Chemical structure of glimepiride.

spectrometry analysis in human plasma [9] and UV analysis of glimepiride in pharmaceutical preparation [10]. No HPLC stability indicating analytical method has been described in literature to the date. Any systematic study about the behaviour of glimepiride under the stress conditions, e.g. hydrolytic, is not available. Only short note about the influence of acid and alkali on glimepiride was published in addition to the UV analysis of this substance [10]. No data about the degradation kinetics as well as the chemical structures of potential degradation products have been found yet.

The aim of this paper was to study hydrolytic stability of glimepiride and to develop RP-HPLC analytical method for determination of glimepiride in the presence of its hydrolytic degradation products. The novelty of this work is based on the description of new analytical method, which is suitable for monitoring the purity of drug substance. Moreover, the conclusions can be helpful in effort to assure the quality, safety and effectiveness of pharmacotherapy.

## 2. Experimental

### 2.1. Chemicals and reagents

Glimepiride was kindly provided by The Research Institute for Pharmacy and Biochemistry (Czech Republic). Acetonitrile and methanol (both HPLC-grade) were obtained from Merck (Germany). Ammonium formate, formic acid, hydrogen peroxide, hydrochloric acid, sodium hydroxide, sodium phosphate dibasic, phosphoric acid were provided by Fluka Chemica (Switzerland). Ultra-pure water was obtained from Milli-Q system.

### 2.2. Instruments and chromatographic conditions

Pierce Reacting-thermo Stirring/Heating module constant temperature bath (Germany) was used for the stress testing. The HPLC chromatographic system Spectra-Physics (Germany), which consisted of a gradient pump (P 400), vacuum membrane degasser, automatic injector (AS 3000) and UV/VIS detector (SN 4000), was used for the analysis. The HPLC data were processed with PC 1000 Software. An appropriate separation was achieved on a LiChroCART column (250 × 4.6 mm i.d.) with Purospher (RP-18e, 5 μm) as a stationary phase (Merck, Germany). Detection was performed at 228 nm. The mobile phase was composed of acetonitrile–phosphate buffer (pH 3.5; 0.03 M) (48:52, v/v). The flow rate was 1 ml/min.

### 2.3. Stability study

#### 2.3.1. Standard curve

The stock solution (500 μg/ml) was prepared by dissolving an appropriate amount of solid substance of glimepiride in methanol. The calibration curve was made using five standard solutions of different concentrations (20, 50, 100, 200 and 300 μg/ml). The standard solutions were prepared by diluting an appropriate volume of stock solution with methanol. Each solution was analysed in triplicate. The peak area values were plotted against the corresponding analyte concentrations to obtain the linear calibration.

#### 2.3.2. Precision and accuracy

Six injections of one concentration of glimepiride (200 μg/ml) were analysed on the same day. The value of relative standard deviation of this assay was calculated to determine intra-day precision. This analysis was also repeated next day in order to evaluate inter-day precision. Accuracy was evaluated as a percentage of recovery obtained from analysis of samples spiked with known amount of glimepiride (30, 80 and 120 μg/ml).

#### 2.3.3. Determination of stability

The forced degradation of glimepiride was carried out under the condition of acid, neutral, alkaline and oxidative hydrolysis. Appropriate amount of glimepiride was dissolved in methanol to prepare glimepiride stock solution concentration of 400 μg/ml.

One millilitre of glimepiride stock solution was transferred into each of four glass vials. One millilitre of HCl (0.2 M) was added into first vial, 1 ml of NaOH (0.2 M) into the second vial, 1 ml of water into third vial, and finally 1 ml of the solution of 4% $H_2O_2$ into the fifth vial. All vials were tightly closed and maintained at the constant temperature (90 °C) in a heating block with simultaneous stirring. After the periods of 0, 30, 60, 180 and 360 min, 20 μl of each sample was analyzed employing HPLC. The blanks consisting of 1 ml of methanol and 1 ml of degradation medium were injected on to the column before every single analysis.

In order to determine relative rate of hydrolytic decomposition of drug, the logarithm of remaining concentration of glimepiride was plotted versus time. The linearity of this dependence was investigated employing linear regression. The presence of outliers was checked using Grubbs test. [11] The rate constants were calculated from the slope of the kinetic curves.

## 3. Results and discussion

A simple, accurate and precise HPLC method was developed for determination of glimepiride in the presence of its degradation products. Consecutively, this stability indicating analytical method was employed to gain basic information about the susceptibility of glimepiride to hydrolytic decomposition.

Table 1
Accuracy of this method

| Concentration added (μg/ml) | Concentration calculated ± S.D. (μg/ml); R.S.D% | Recovery (%) |
|---|---|---|
| 230 | 236.160 ± 0.703; 0.297 | 102.678 |
| 100 | 98.137 ± 0.263; 0.268 | 98.1378 |
| 40 | 40.412 ± 0.021; 0.053 | 101.032 |

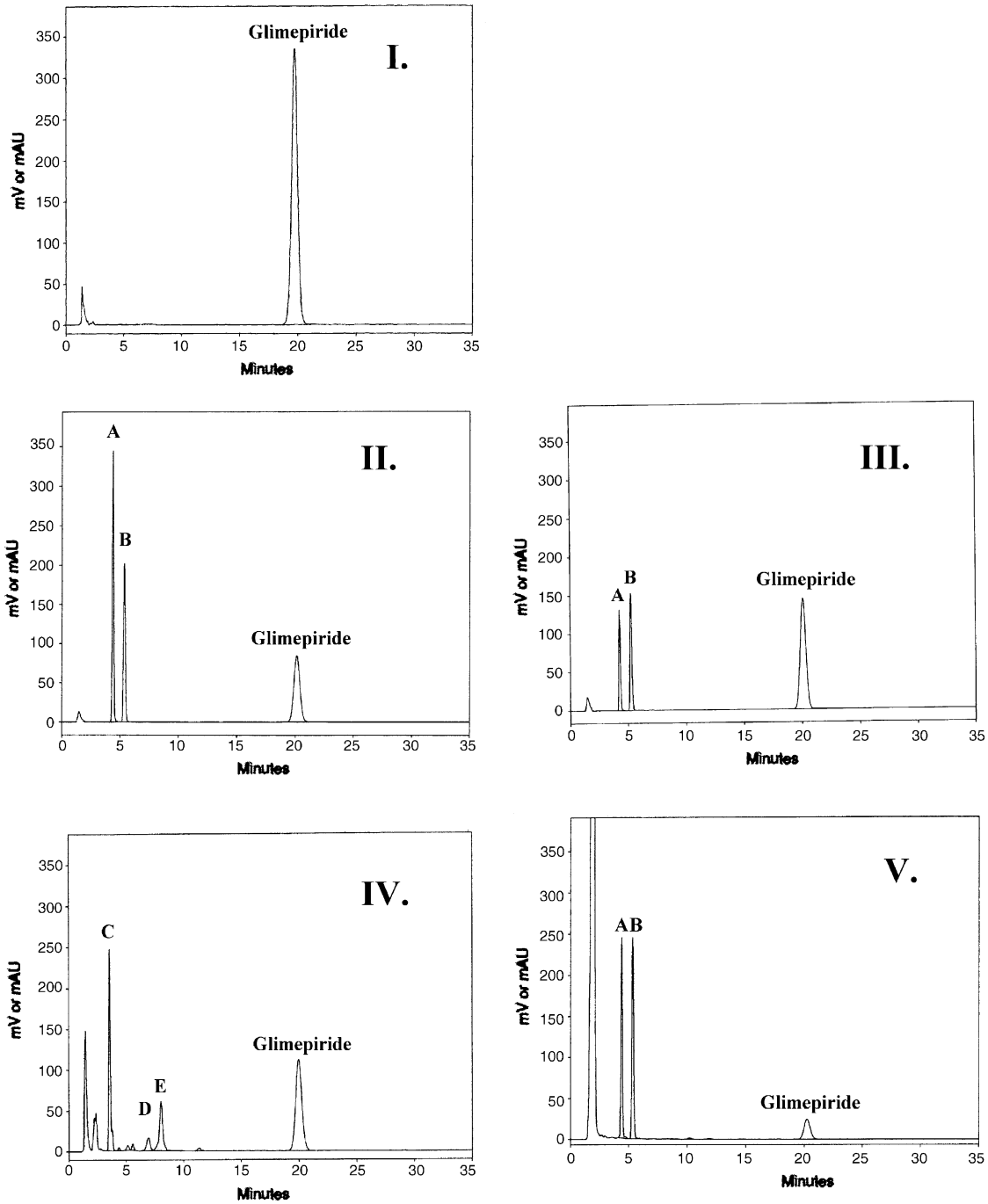


Fig. 2. Chromatograms showing decomposition of glimepiride: Keys: (I) Sample in the time of 0 min; (II) acid hydrolysis (360 min of decomposition); (III) neutral hydrolysis (360 min of decomposition); (IV) alkaline hydrolysis (360 min of decomposition); and (V) oxidative hydrolysis (360 min of decomposition).

### 3.1. Method validation

The linearity of this method was proved using linear correlation of the peak area values and appropriate concentrations of glimepiride in a range of 20–300 μg/ml. The correlation coefficient of this dependence was calculated to be 0.999. The intra- and inter-day precisions of method were determined to be 0.358 and 0.655, respectively. The results of method accuracy are presented in the Table 1.

### 3.2. Stability study

HPLC study of glimepiride hydrolytic decomposition suggested following degradation behaviour. After acid hydrolysis employing HPLC, two degradation products (labelled A and B) were detected at the retention times of 4.2 and 5.2 min. It was observed that the area values of both peaks were growing in time and this observation was accompanied with decreasing of concentration of glimepiride Fig. 2. The stability of glimepiride was also studied using water as a medium for degradation. Although both degradation products were detected on chromatogram, the ratio between the areas of peaks A and B was different in comparison with previous experiment (acid hydrolysis).

In contrast to acid hydrolysis, alkaline conditions led to decomposition of glimepiride in to three main degradation products (labelled C–E). Employing HPLC, their presence was detected at the retention times of 3.5 min (degradation product C), 6.6 min (degradation product D) and 8.0 min (degradation product E). As it is evident from Fig. 2, the peak area values of all main degradation products were growing in time. Besides the peaks of these degradation products, other small peaks were also found in the exposed samples, but none of them had the area value grater than 1% of concentration of glimepiride. Furthermore, the amount of these degradation products did not grow markedly during the time of the experiment.

The degradation of glimepiride in the solution of 2% hydrogen peroxide resulted into the formation of two major peaks. The retention times indicated the agreement of oxidative degradation products with acid ones; but however, the decline of concentration of glimepiride was much higher than concentration fall in acid hydrolysis (Fig. 2). The absence of distinct oxidative degradation product shows that the chemical structure of glimepiride is not sensitive to oxidation resulting from exposition to diluted hydrogen peroxide. Thus, in standard conditions (laboratory temperature, absence of oxidative agents etc.), the formation of oxidative impurities seems unlikely. As evident, acid, neutral and oxidative hydrolysis led to the formation of the same degradation products A and B. The possible explanation of this point can be derived from the fact, that the acid-catalyzed hydrolytic reaction could take place in all the above mentioned conditions. The Milli-Q water used in this experiment, made the medium for neutral conditions slightly acid. Diluted hydrogen peroxide solution is known to have slightly acid character.

### 3.3. Degradation behaviour

The susceptibility of glimepiride to hydrolytic decomposition was determined as a fall of concentration of drug during the time of the experiment. The kinetic slopes are shown in Fig. 3.

The straight-line behaviour was obtained for neutral, alkaline and oxidative conditions with correlation coefficients $R = 0.99977$, $R = 0.9986$ and $R = 0.9956$, respectively. This fact implies that the hydrolytic degradation followed pseudo-first-order kinetic behaviour. The correlation coefficient for acid hydrolysis was calculated to be $R = 0.927$. The rate constants were determined from the slope of kinetic curves and their values are: $3.91 \times 10^{-3}\,\mathrm{min}^{-1}$ (acid condition), $2.30 \times 10^{-3}\,\mathrm{min}^{-1}$ (neutral condition), $3.22 \times 10^{-3}\,\mathrm{min}^{-1}$ (alkaline condition) and $9.44 \times 10^{-3}\,\mathrm{min}^{-1}$ (oxidative condition). The values of rate constants determined that the susceptibility of glimepiride to hydrolytic decomposition is increasing in the following manner: neutral condition < alkaline condition < acid condition < oxidative condition.

Although no special oxidative degradation product of the drug was formed in experiment with 2% hydrogen peroxide, the fastest decomposition was observed in this

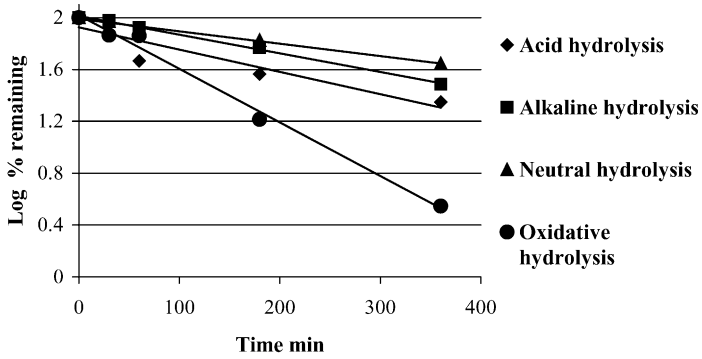


Fig. 3. Kinetic curves of glimepiride decomposition.

solution. Thus, hydrogen peroxide could probably facilitate the acid-catalyzed hydrolytic reaction. Although the glimepiride decomposition in water was determined to be the slowest one, the fall in the concentration of the drug was considerably significant. In this medium, only 44% of initial amount of glimepiride was determined at the end of our experiment (6 h). Therefore, the structure of glimepiride appears to be rather sensitive to hydrolytic decomposition. The addition of diluted acid made this degradation process only 1.7 times faster, but however, this acceleration of the reaction was quite distinct.

## 4. Conclusion

In this paper, the simple, accurate and well-defined HPLC analytical method for determination of glimepiride in the presence of its degradation products was described for the first time. The behaviour of glimepiride under the hydrolytic stress conditions in acid, neutral, alkaline and oxidative media was studied. However, further study needs to be done to determine the most likely structures of degradation products and to apply these results into advanced stability testing. The information presented herein could be very useful for quality monitoring of bulk substance as well as the pharmaceutical preparation containing this modern sulfonylurea derivate.

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## References

[1] E. Drager, Diab. Res. Clin. Pract. 28 (1995) 139–146.
[2] R. Robkamp, K. Wernicke-Panten, E. Dragaer, Diab. Res. Clin. Pract. 31 (1996) 33–42.
[3] ICH, Stability Testing of New Drugs Substances and Products: Proceeding of the International Conference on Harmonization, IFPMA, Geneva, 2000.
[4] B. Kommanaboyina, C.T. Rhodes, Drug Dev. Ind. Pharm. 25 (1999) 857–868.
[5] M. Baksi, S. Singh, J. Pharm. Biomed. Anal. 28 (2002) 1011–1040.
[6] K.C. Waterman, C.R. Adami, K.M. Alsante, A.S. Antipas, D.R. Arenson, R. Carrier, J. Hong, M.S. Landis, F. Lombardo, J.C. Shah, E. Shalaev, S.W. Smith, H. Wang, Pharm. Dev. Technol. 7 (2002) 113–146.
[7] I.I. Salem, J. Idrees, J.I. Al Tamini, J. Chromatogr. B. 779 (2004) 103–109.
[8] K.H. Lehr, P. Damm, J. Chromatogr. B. 526 (1990) 497–505.
[9] H.M. Maurer, C. Kratzsh, T. Kraemer, F.T. Peters, A.A. Weber, J. Chromatogr. B. 773 (2002) 63–73.
[10] S. Altinöz, D. Tekeli, J. Pharm. Biomed. Anal. 24 (2001) 507–515.
[11] F. Grubbs, Technometrics 11 (1997) 1–21.