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Stress degradation study of two oral antidiabetics, gliclazide and glipizide, and chemical analysis by LC and LC/MS methods

Research Article

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Abstract: Kinetic study of degradation of two oral antidiabetics, gliclazide and glipizide, was performed using new HPLC method which was validated in terms of selectivity, sensitivity, linearity, precision and accuracy. The stress degradation was performed in 0.2 M HCl, 0.2 M NaOH as well as in acetate and phosphate buffers over the pH range 3.8-8.3 at 30 and 70°C.

In strong acidic and alkaline media gliclazide was almost fully degraded while glipizide showed much higher stability. Generally, degradation processes of gliclazide and glipizide were observed as the first order reactions while the rates of decomposition for both drugs were smallest at pH 8.3. The samples of gliclazide and glipizide stressed in strong acid and alkali at 70°C were additionally analyzed using an LC/MS method and some products of decomposition were detected and identified.

It was concluded that glipizide was more resistant to very high or very low pH and would have higher stability compared to gliclazide. Such comparisons have not been performed so far for these valuable drugs. Additional LC/MS study showed that during decomposition of sulfonylureas, different degradation pathways were possible.

Keywords: *Gliclazide and glipizide • Stress degradation • Kinetics • LC and LC/MS methods*

1. Introduction

It is well known that degradation processes can cause changes of chemical, pharmacological and toxicological properties of drugs having significant impact on product quality and safety for patients. In order to gain a better understanding of an active pharmaceutical ingredient (API), forced degradation study under different conditions can be performed. Adequate stress testing of API must provide information on how the quality varies with time when the drug is influenced by a variety of environmental factors such as temperature, humidity, light or pH changes, *etc*. On the other hand, suitable analytical methods for detection, separation and, if possible, for structural characterization of drug contaminants should also be developed as a part of reliable experiments.

Gliclazide and glipizide (Fig. 1) belong to oral antidiabetic drugs, namely sulfonylureas. They bind to K_{ATP} channels on the cell membrane of pancreatic β cells. This leads to increased fusion of insulin granulae with the cell membrane, and therefore increased secretion of insulin. For that reason they are widely used in the treatment of type 2 diabetes mellitus.

Several chromatographic procedures for quantitative determination of gliclazide and glipizide are reported in the literature. The majority of them include HPLC methods for determination of gliclazide [1-5], glipizide [6-10] or both [10-14] in different pharmaceutical combinations.

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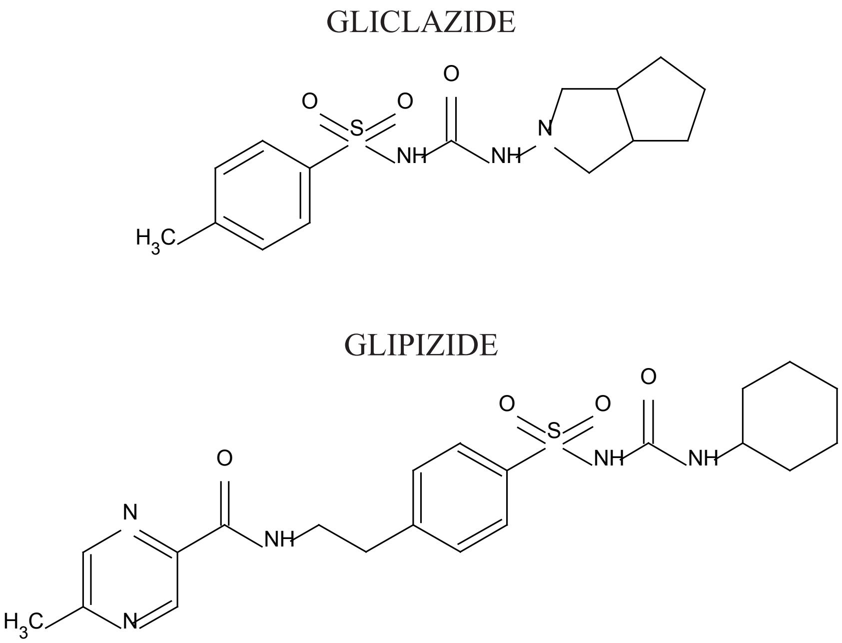
GLICLAZIDE

GLIPIZIDE

Figure 1. The chemical structures of gliclazide and glipizide.

Furthermore, some TLC [15-17], HPLC [18-22] and LC/MS [23,24] methods for determination of gliclazide or glipizide in the presence of their impurities or degradation products are presented. In the study of Krzek *et al.* [15], gliclazide and its impurities present in commercially available formulations were separated using normal phase TLC. In the next report [16], stress degradation of gliclazide and glipizide in strong acidic medium was performed and then the products were examined by the same TLC technique. For determination of cyclohexanamine which is related impurity of glipizide, a HPLC method through derivatization with *o*-phtaldialdehyde was elaborated [18]. Degradation study of gliclazide or glipizide in acidic, alkaline and oxidative media was included as a part of validation procedure in several TLC and HPLC methods which were elaborated for quantitative determinations of the drugs [17,19,20]. In these reports, susceptibility of gliclazide or glipizide on decomposition, especially in low pH and elevated temperature, was stated. In the study of Bansal *et al*. [21], the stability of gliclazide in acidic and alkaline media was examined by HPLC and finally, six impurities were detected. The same impurities were then characterized by new LC/MS method [23]. A validated stability-indicating HPLC-UV method for simultaneous determination of glipizide and its four impurities formed under hydrolytic conditions was described by Gupta and Bansal [22]. Additionally, the LC-MS study on degradation pathway of glipizide was reported in the literature by Bansal *et al.* [24]. However, the kinetic study of degradation under different stress conditions has not been performed so far for these valuable drugs. Therefore, the main goal of the present study was to examine the kinetics of degradation of gliclazide and glipizide and to compare the behavior of these two chemically related drugs under the same stress conditions and in the same time period. The first step of the present study was to develop and validate a quantitative HPLC method suitable for determination of gliclazide or glipizide in the presence of their degradation products. It was assumed that gliclazide and glipizide could be determined by the same LC method using one as an internal standard for the second. Then, degradation of gliclazide and glipizide as a function of time under extraordinary pH and temperature was investigated using the elaborated method to obtain respective equations, allowing estimation of next kinetic parameters, *i.e.*, the half-life time ($t_{0.5}$) and the time of decomposition of 10% of the drug ($t_{0.1}$). An additional goal of the study was to elucidate the possible degradation pattern of both drugs by analyzing the acid and alkali stressed samples using a suitable LC/MS method. Based on these studies, the behavior of two chemically related drugs under the same stress conditions and in the same time period were compared. The conclusions learned from such analysis could facilitate the future design of new sulfonylurea type molecules. Such comparisons have not been published so far for the sulfonylurea drugs.

2. Experimental procedure

2.1. Materials and reagents

Gliclazide from Jelfa S.A. (Poland) and glipizide from Galena (Poland) were used. In addition, gliclazide and glipizide standards from Sigma-Aldrich (Poland) were applied for preparing quality control (QC) samples. All solvents were of HPLC or LC/MS grade and were purchased from E. Merck (Germany) or Sigma-Aldrich. Other chemicals were of analytical grade and were supplied by POCh Gliwice (Poland) and by Sigma-Aldrich.

2.2. Stress degradation

The degradation of gliclazide and glipizide was performed in 0.2 M HCl, 0.2 M NaOH, acetate and phosphate buffers (pH 3.8, 4.7, 5.3, 6.3, 7.3 and 8.3) at 30 and 70°C. Acetate buffers were prepared with 0.05 M CH_3COOH and 0.05 M CH_3COONa. Phosphate buffers were prepared with 0.067 M KH_2PO_4 and 0.067 M Na_2HPO_4. The composition of the buffers was calculated from the Henderson-Hasselbalch equation while the ionic strength was attained to 0.5 M with 4.0 M NaCl. All pH measurements were performed with a pH-meter, model HI9024C from Hanna Instruments (Italy).

Samples of gliclazide and glipizide ca. 5 mg of the substance were dissolved in 0.5 mL of methanol and then 2 mL of respective medium was added. The obtained solutions were stored in tightly capped glass vials (Medlab, Poland) in a climatic chamber KBF-LQC (Binder, Germany) at the relevant temperature. Samples were collected at time intervals: 15, 30, 45, 60, 90, 120, 180, 240 and 300 min to allow sufficient reflection of the kinetics. They were cooled in a mixture of ice and water, neutralized if necessary and diluted to 10 mL. Then, 1.0 mL volumes were diluted to 10 mL with a mobile phase together with respective amounts of the internal standard and analyzed by the described quantitative LC method. The above procedure was repeated three times and the mean concentrations of the remaining drugs were calculated from the linear calibration.

2.3. Equipment

The LC system for quantitative determinations consisted of a model 306 pump and a model UV170 DAD detector controlled by OMNIC software from Gilson (USA). Separation was carried out on a LiChrospher® 100RP-18 column (125×4.0 mm, 5 μm) from E. Merck.

For LC/MS method, the samples were analyzed using an Agilent (USA) liquid chromatograph Series 1290 consisting of a binary pump G4220A, a thermostated autosampler G4226A, a thermostated column compartment G1316C and a triple quadrupole mass spectrometer G6460A with an ESI source operating in a positive ionization mode. Separation was achieved on a Poroshell 120SB-C18 column (100×3.0 mm, 2.7 μm) from Agilent.

2.4. LC procedure for quantitative determination

2.4.1. Method

The mobile phase consisted of 0.067 M phosphate buffer at pH 4.3 (diluted ten times) and acetonitrile (60:40, *v/v*). A flow rate of 1.0 mL min^{-1} was used. The separations were conducted at 22°C. Volumes of 20 μL were injected onto the column and the peaks were monitored at wavelength of 230 nm.

2.4.2. Stock solutions

Gliclazide and glipizide stock solutions were prepared by dissolving 10 mg of the compounds in methanol to obtain the concentration of 1 mg mL^{-1}. These stock solutions were stored at temperature 25°C for 3, 6, 12, 24 and 48 h in tightly capped volumetric flasks. Then, 0.5 mL volumes were diluted to 10 mL, analyzed and checked for the presence of some additive peaks. Recovery of the drugs was calculated in relation to respective standards.

2.4.3. Calibration

Series of solutions of gliclazide or glipizide were prepared by the appropriate dilution of the stock solutions with the mobile phase to reach the concentrations in range of 0.015 to 0.09 mg mL^{-1} for both drugs. Each sample contained respective concentration of the internal standard (glipizide was the internal standard for gliclazide determination and *vice versa*). Then, six injections were made onto the column for each concentration. The limit of detection and limit of quantification were calculated from the standard deviation of the blank signal and respective slopes of the calibration curves.

2.4.4. Precision and accuracy

The precision and accuracy were determined by analyzing the standard QC samples of the drugs. The solutions containing 0.02, 0.05 and 0.085 mg of gliclazide or glipizide and respective amounts of the internal standard per mL, were evaluated by injecting the solutions three times during the same day and then on three subsequent days. Finally, accuracy was established by comparing the determined amounts from the QC samples to the respective nominal values and expressed as percent recovery while precision was expressed by respective RSD values of replicate measurements.

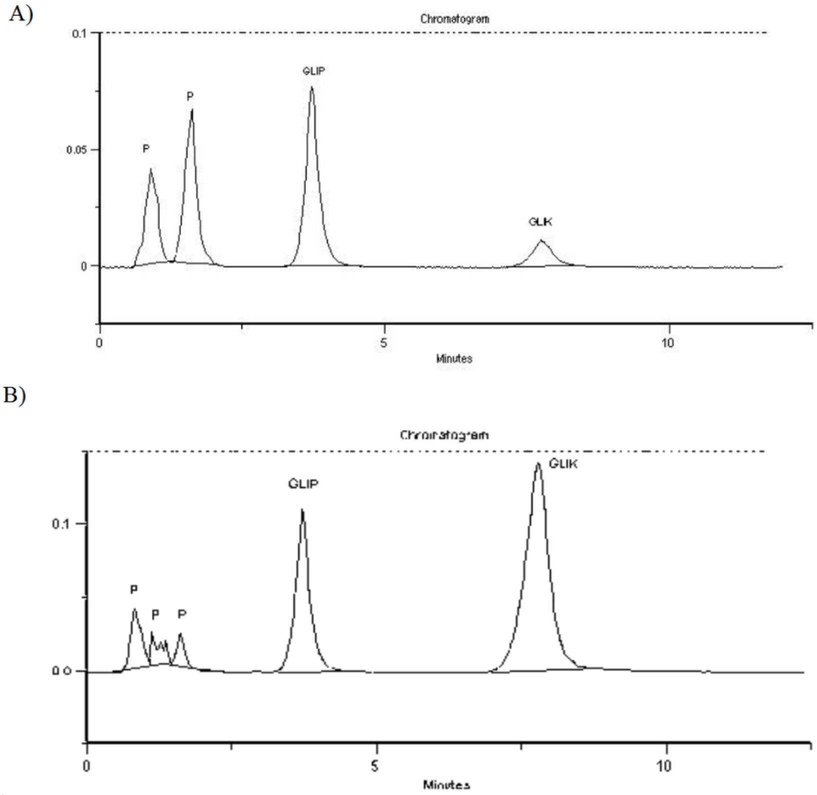

Figure 2. The LC chromatograms of the stressed samples of gliclazide (A) and glipizide (B) obtained with LiChrospher® 100RP-18 column (125×4.0 mm) at 22°C, mobile phase containing phosphate buffer at pH 4.3 and acetonitrile (60:40, *v/v*) with a flow rate of 1.0 mL min^{-1}, and UV detection at 230 nm: A) gliclazide (Glik) and its degradation products (P) after stressing in 0.2 M HCl and glipizide (Glip) added as the internal standard; B) glipizide (Glip) and its degradation products (P) after stressing in 0.2 M NaOH and gliclazide (Glik) added as the internal standard.

2.5. LC/MS procedure

A gradient elution was applied using a solvent A consisting of 0.1% CH_3COOH in H_2O and a solvent B consisting of 0.1% CH_3COOH in acetonitrile+H_2O (3+1, *v/v*). The gradient was programmed as 5 to 100% B in 20 min while the flow rate was 0.6 mL min^{-1} and the column temperature was 20°C. The working parameters of Agilent Jet Stream ESI were: drying gas temperature 300°C, drying gas flow 5 L min^{-1}, a nebulizer pressure 45 psi, sheath gas temperature 250°C, sheath gas flow 11 L min^{-1}, capillary voltage 3000 V and charging voltage 500 V. The mass spectrometer was operated in a scan mode in the range of 50 to 500 m/z with a variable fragmentor potential. The data were processed with Agilent MassHunter B.04 software.

3. Results and discussion

3.1. Optimization of quantitative LC method

It was assumed that gliclazide and glipizide could be determined by the same LC method using one as an internal standard for the second. For development of the method, the standard solutions of glipizide and gliclazide were used first. Different mobile phases containing acetonitrile in phosphate buffers were examined. Finally, the mobile phase containing phosphate buffer at pH 4.3 and acetonitrile (60:40, *v/v*) was selected as optimal for obtaining well defined and resolved peaks with retention times of ca. 7.8 and 3.7 min, for gliclazide and glipizide, respectively. Although the same mobile phase was used in one previous report for simultaneously determination of glipizide and two thiazolidinediones [19], the described chromatographic conditions have not been applied for simultaneous determination of gliclazide and glipizide. Therefore, the present HPLC procedure is practically new. It was validated and proved to be suitable for quantitative determination of gliclazide against glipizide as an internal standard and *vice versa*. In the next step, the solutions prepared from the samples of gliclazide or glipizide stressed in 0.2 M HCl and 0.2 M NaOH at 70°C were used and suitability of the elaborated method for determining the drugs in the presence of their degradation products was proved (Fig. 2).

Table 1. The results of validation procedure for quantitative LC method.

Parameter	Values	
	Gliclazide	Glipizide
Linearity range (mg mL^{-1})	0.0150-0.09	0.015-0.09
Slope ± SD (n=6×6)	44.1793 ± 0.5272*	12.4474 ± 0.1792*
t Student test for slope	$146.2954 > t_{0.05,34 =} 2.0322$	$117.8503 > t_{0.05,34 =} 2.0322$
Intercept ± SD (n=6×6)	-0.0241±0.0213	0.0194 ± 0.0073*
t Student test for intercept	$-1.3629 < t_{0.05,34 =} 2.0322$	$3.1348 > t_{0.05,34 =} 2.0322$
Correlation coefficient	0.9993	0.9990
Limit of detection (μg mL^{-1})	3.9	4.5
Limit of quantification (μg mL^{-1})	11.7	13.7
Intra-day precision (%) (n=3)	0.38 - 2.19	0.21 - 1.28
Inter-day precision (%) (n=9)	1.19	0.70
Recovery ± SD (%) (n=9)	99.38 ± 0.81	100.37 ± 1.30

**statistically significant at p=0.05*

3.2. Stability

The drugs were dissolved in methanol and stored for 48 h at the temperature 25°C. These solutions did not show any additional peaks on the chromatograms. Also, recoveries of both drugs from the stored solutions in comparison with respective standards were sufficient (data not shown).

3.3. Linearity

For calibration, six independent determinations were performed at each of six concentrations of the drugs. The relationships were constructed between the peak area ratio of the respective drug and internal standard, and the corresponding concentration (Table 1).

3.4. Precision and accuracy

The data obtained from precision and accuracy study are given in Table 1. RSD values for intra-day and for inter-day precision were in the ranges 2.19-0.38% for gliclazide and 1.28-0.21% for glipizide, confirming that the applied method was sufficiently precise. In gliclazide assay, the recovery ranged from 98.66 to 100.47% while for glipizide it ranged from 98.39 to 101.5%.

3.5. Optimization of LC/MS method

The method was developed using the same gliclazide and glipizide standards as for quantitative LC procedure. In the next step, the method was optimized using the samples of gliclazide and glipizide which were stressed in 0.2 M HCl and 0.2 M NaOH at 70°C. These samples were chosen after that the decomposition of both drugs was confirmed by the described LC method.

For the presented LC/MS analysis, methodology of Pang *et al.* [25] with an in-house modifications of Perlan Technologies Polska was used. According to the above paper, electrospray ionization (ESI) was found to be more sensitive than atmospheric pressure chemical ionization (APCI). Moreover, the response of positive ESI was found to be much better than that of negative ESI. Therefore, ESI in positive ionization mode was adopted for the presented procedure. The addition of 0.1% of acetic acid to the mobile phase (resulting in pH ca. 3.2) ensured good condition for protonation of the analytes. The gradient profile was chosen to obtain proper resolution of all chromatographic peaks.

3.6. Degradation

Gliclazide showed very high decomposition since it was fully degraded in 0.2 M HCl at 70°C after 15 min of the test. Also, ca. 90% of the substance was degraded in 0.2 NaOH and in acetate buffer at pH 3.8 during 300 min. The extent of decomposition was ca. 20% over the pH range 4.8-6.3. On the other hand, the drug was rather stable at pH 7.3-8.3 where the degradation was ca. 10% or less. The decomposition of glipizide in 0.2 M NaOH at 70°C was ca. 10%. This drug showed higher degradation in 0.2 M HCl where more than 30% of the initial concentration disappeared. Over the pH range 3.8-8.3 the extent of decomposition was lower than 10%. Generally, it was stated that the rates of decomposition for both drugs were smallest at pH 8.3 (Table 2). The results obtained in acidic and alkaline media at 70°C were not suitable for the comparisons over time since the degradation of gliclazide was practically immediate. Therefore, it was

Table 2. The results of stress degradation of gliclazide and glipizide in solutions (n=9).

Solution	Decompositon after 300 min (%)					
	Gliclazide	r^2	k (s^{-1})	Glipizide	r^2	k (s^{-1})
0.2 M HCl 70°C	100.0	-	-	31.69	-0.9906	$1.28\text{x}10^{-5}$
0.2 M HCl 30°C	92.37	-0.9929	$4.82\text{x}10^{-5}$	18.22	-0.9873	$9.00\text{x}10^{-6}$
Buffer 3.8	88.03	-0.9946	$1.87\text{x}10^{-5}$	9.15	-0.9904	$4.83\text{x}10^{-6}$
4.8	25.23	-0.9847	$1.45\text{x}10^{-5}$	8.01	-0.9937	$4.33\text{x}10^{-6}$
5.3	21.09	-0.9714	$9.50\text{x}10^{-6}$	7.57	-0.9745	$3.66\text{x}10^{-6}$
6.3	20.09	-0.9920	$6.83\text{x}10^{-6}$	6.87	-0.9861	$2.67\text{x}10^{-6}$
7.3	10.09	-0.9860	$4.33\text{x}10^{-6}$	5.37	-0.9930	$2.67\text{x}10^{-6}$
8.3	6.02	-0.9839	$2.67\text{x}10^{-6}$	4.49	-0.9936	$1.83\text{x}10^{-6}$
0.2 M NaOH 30°C	88.87	-0.9900	$4.43\text{x}10^{-5}$	10.34	-0.9845	$3.28\text{x}10^{-6}$
0.2 M NaOH 70°C	95.67	-0.9933	$8.90\text{x}10^{-5}$	10.65	-0.9904	$6.83\text{x}10^{-6}$

Table 3. The results of kinetic studies for gliclazide.

Solution	$t_{0.5}$ (h)	$t_{0.1}$ (h)
0.2 M HCl 30°C	3.99	0.61
Buffer 3.8	10.29	1.57
0.2 M NaOH 30°C	4.35	0.66
0.2 M NaOH 70°C	2.16	0.33

decided to extend the study performing them at 30°C. In these conditions gliclazide showed degradation up to 90% or higher again. It was also confirmed that glipizide was more stable since its decomposition in 0.2 M HCl did not exceed 20% (Table 2).

Although the degradation extent of gliclazide and glipizide in the established time was low in some conditions but some trends were clearly shown. The presented results could be helpful for comparing the stability and then the pharmaceutical utility of different sulfonylurea molecules. Generally, the respective decomposition processes were determined as the first order reactions. The rate constants (-*k*) were equal to the slopes of the respective plots $\ln(c)=f(t)$, where *c* was the drug concentration remaining at time intervals *t*. High correlations were obtained in these linear regressions because all obtained r^2 values were above 0.97 while the *k* values of gliclazide were higher than those of glipizide (Table 2). Because of the sufficient extent of decomposition, more kinetic results were presented for gliclazide after stressing in 0.2 M HCl, 0.2 M NaOH and in acetate buffer at pH 3.8. From the slopes of the obtained plots, the half-life time ($t_{0.5}$) and the time of decomposition of 10% of the drug ($t_{0.1}$) were obtained (Table 3). These results clearly showed that gliclazide was particularly susceptible to degradation at pH values below 3.8 and above 8.3.

In the study of Bansal *et al*. [21], gliclazide was shown to be similarly degraded in acidic as well as in neutral and alkaline media although different degradation products were detected. Also, in the study of Gupta *et al*. [22], the rate of degradation of glipizide in alkaline medium was equivalent to that in acidic medium. However, from one other report [26], the order of sensitivity of sulfonylureas was reported as the highest in acidic and lower in neutral and alkaline media that was confirmed in the present study. These discrepancies could be explained by the fact that both drugs have not been compared in one decomposition study and any such comparisons have not been published so far.

In the mentioned study of Bansal *et al*. [21], two products, namely I and III, were detected as the major degradation products of gliclazide in acid while in alkaline medium, products II, V and VI were formed. These degradation products were characterized through subsequent LC/MS study [23] as p-tolylsulfonamide (I), methyl *N*-(p-tolylsulfonyl) carbamate (II), 4,4a,5,6,7,7a-hexahydro-3*H*-cyclopenta[d]pyridazin-1yl *N*-(p-tolylsulfonyl) carbamate (V) and *N*-(4,4a,5,6,7,7a-hexahydro-3*H*-cyclopenta[d]pyridazin-1-carbonyl)-4-methyl benzene sulfonamide (VI).

In the presented +ESI MS spectra of the stressed samples of gliclazide (0.2 M HCl and 0.2 M NaOH at 70°C), at least two compounds of the main ions at *m/z* 252 (Fig. 3B) and *m/z* 141 (Fig. 3C) were visible. However, any of the obtained *m/z* values did not correspond to values proposed previously. Arguably, mass spectra of these compounds did not contain pseudo-molecular

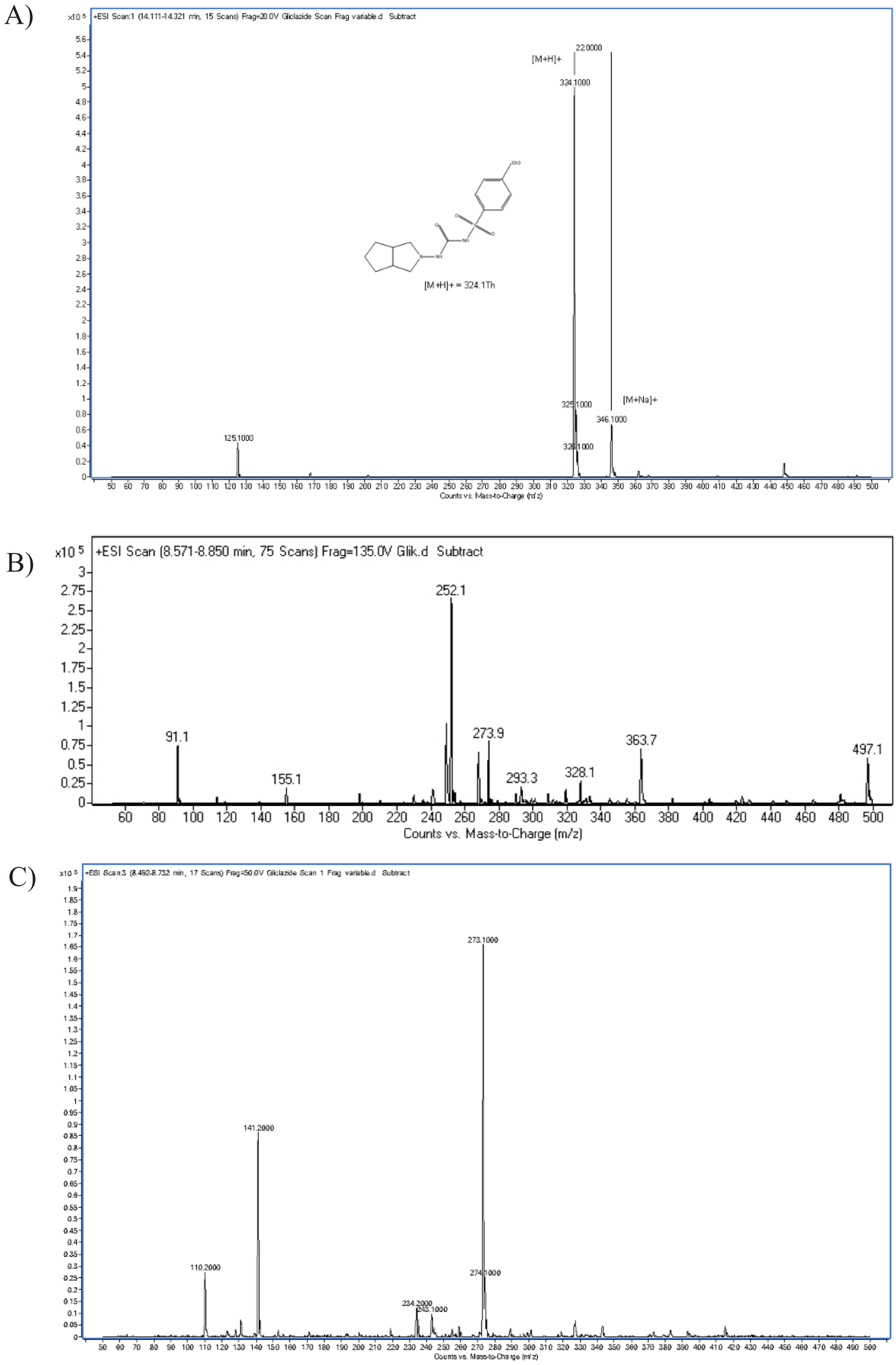

Figure 3. Typical LC/MS spectra of gliclazide: (A) gliclazide standard, (B) gliclazide after degradation in 0.2 M HCl (B) and (C) gliclazide after degradation in 0.2 M NaOH.

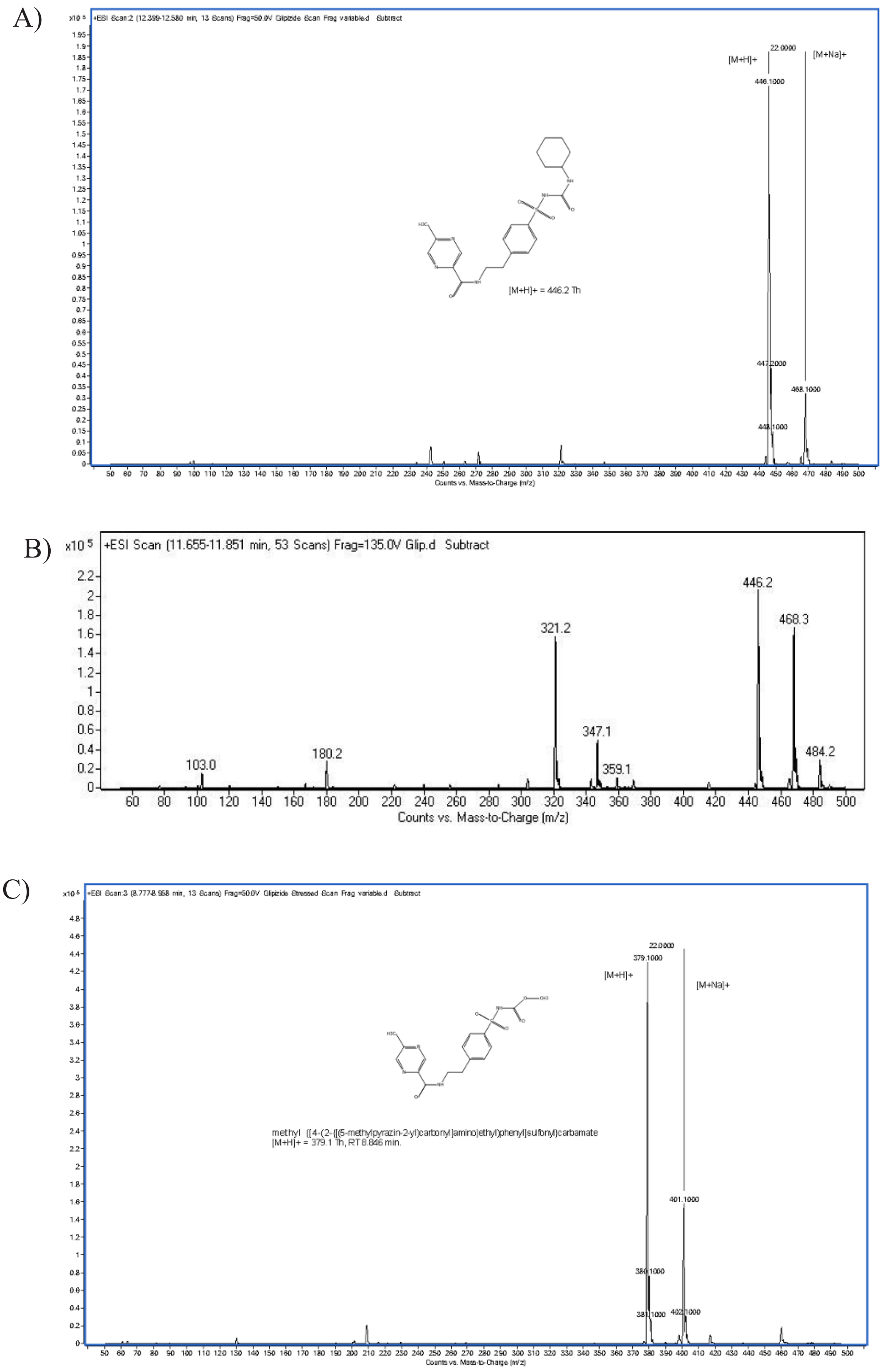

Figure 4. Typical LC/MS spectra of glipizide: (A) glipizide standard, (B) glipizide after degradation in 0.2 M NaOH and (C) glipizide after degradation in 0.2 M HCl.

ions. It was supposed that they had been fragmented in the source. Bearing in mind the limited resolution of the quadrupole spectrometer, it was difficult to infer the composition of these nuclear fragments. However, a complete degradation of the drug in acid was confirmed (Fig. 3B). After alkaline degradation, the parent drug was not detected either (Fig. 3C). In European Pharmacopoeia 7th Edition, seven impurities and related substances are listed in the monograph concerning gliclazide. However, any of them does not correspond to degradation products described by Bansal *et al*. [21]. Similarly, the degradation of glipizide was characterized through LC and then through LC/MS study [22,24]. It was shown to be degraded in 0.1 M HCl at 85°C forming two products, II (5-methyl-*N*-[2-(4-sulphamoylphenyl) ethyl]pyrazine-2-carboxamide) and III (methyl *N*-[4-[2-{(5-methyl-2-pyrazinoyl)amino}ethyl]phenyl]sulfonyl carbamate). Heating of glipizide in alkali at 85°C yielded a new product IV along with the small quantities of I and VI. After heating the drug at lower temperature of 40°C, II, III, IV and V products were detected.

In the present LC/MS study of the stressed samples of glipizide (0.2 M HCl and 0.2 M NaOH at 70°C), the product II was only detected after alkaline hydrolysis where similar fragment peak at *m/z* 321 in the +ESI MS spectrum occurred (Fig. 4B). The conversion of sulfonylureas to II was explained previously by hydrolysis to sulfonamide and an amine, with evolution of carbon dioxide [26,27]. In the present study the product III was also detected, however, after the acidic hydrolysis only (Fig. 4C). In European Pharmacopoeia 7th Edition, nine impurities and related substances are listed in the monograph concerning glipizide. The product III is listed there as the impurity G. It is probably formed only in the presence of methanol used as solubilizer because the conversion of the drug to III is postulated to occur through nucleophilic attack of methanol on the carbonyl carbon of sulfonylurea moiety, accompanied by elimination of cyclohexanamine. It is also postulated that product III is an intermediate for the formation of II [21]. However, the latter was not detected in the present study after acidic hydrolysis of glipizide (Fig. 4C).

4. Conclusions

The present LC/MS study showed that during decomposition of gliclazide and glipizide in strong acidic and alkaline media, different degradation pathways were possible. Only one of the degradation products (in the case of glipizide) was identified as the impurity listed in European Pharmacopoeia 7th edition. When the effects of pH and temperature on stability of both drugs were compared over time, it was stated that glipizide was more resistant to very high or very low pH and would therefore have higher stability in environments of differentiated pH compared to gliclazide. At the same time, the rates of decomposition for both drugs were smallest at pH 8.3. When the kinetic study was performed, degradation processes of gliclazide and glipizide were observed as the first order reactions.

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