LC and LC–MS Study on Establishment of Degradation Pathway of Glipizide Under Forced Decomposition Conditions

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Abstract

Forced degradation studies on glipizide are conducted under the conditions of hydrolysis, oxidation, photolysis, and dry heat. The solutions are subjected to liquid chromatographic (LC) investigations to establish the number of products formed in each condition. The degradation products are characterized through isolation and subsequent NMR, IR, and MS spectral analyses, or through LC–mass spectrometry (MS) fragmentation pattern study. The drug is shown to degrade in 0.1M HCl at 85°C to two products: 5-methyl-*N*-[2-(4-sulphamoylphenyl)ethyl]pyrazine-2-carboxamide (II) and methyl *N*-[4-[2-{(5-methyl-2-pyrazinoyl)amino}ethyl] phenyl]sulfonyl carbamate (III). The latter, a methyl ester, is formed only in the presence of methanol (used as a solubilizer), and does not appear on use of acetonitrile. III is shown to convert to II on continued heating in acid. The drug degrades slowly in water at the same temperature, and both II and III could be seen in the chromatograms utill the end of the study. The heating of the drug in alkali (0.1M NaOH) at 85°C yields 5-methyl-2-pyrazinecarboxylic acid (IV), along with a small quantity of 4-(2-aminoethyl) benzenesulfonamide (I). On extended heating in the same condition, a new product, 4-(2-aminoethyl)-*N*,*N*-*bis*[(cyclohexylamino)carbonyl] benzenesulfonamide (VI) is formed in small quantities. At the lower temperature of 40°C, the drug converts under each hydrolytic condition and in both the absence and presence of light to products II, III, or IV, along with a new product, 1-cyclohexyl-3-[[4-(2aminoethyl)phenyl] sulfonyl]urea (V). The light catalyzes formation of V, and it is formed utill one or two weeks, after which its level decreases. The drug remains stable in 30% H_2O_2, except that products II and III appear as small peaks due to acidic character of the peroxide solution. Also, the drug remains unaffected in solid state under thermal and photolytic stress conditions. Based on the results, a more complete picture on degradation pathway of the drug is obtained, highlighting a clear advantage of the approach suggested by International Conference on Harmonization.

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Introduction

The International Conference on Harmonization (ICH) guideline Q1A(R2) requires forced decomposition of the drugs under conditions of hydrolysis, oxidation, photolysis, and thermal stress to determine their intrinsic stability and to justify the stability-indicating nature of the analytical method employed in stability studies (1). It also emphasizes the establishment of degradation pathway of the drug. Several studies in the literature report on the protocol of stress testing (2–6), establishment of stability-indicating assays (7–14) and characterization of degradation products through isolation followed by spectral analyses and/or LC–MS fragmentation studies (15–18).

Glipizide is a second generation sulfonylurea-type oral hypoglycaemic agent (19). Chemically, it is 1-cyclohexyl-3-[[4-[2-[[(5-methyl pyrazin-2-yl)carbonyl]amino]ethyl]phenyl] sulfonyl]urea (Figure 1). The presence of amide and sulfonylurea moieties in the drug molecule makes it susceptible to hydrolytic and photolytic degradation (20–22). However, there is only one report by El Kousy (23) in the literature where the drug was decomposed under strong acidic conditions. The degradation products formed were 1-carboxy-4-methylpyrazine, 4-(2-aminoethyl)benzene sulfonamide, cyclohexanamine, and carbon dioxide. In European Pharmacopoeia 2005 (24), two impurities were listed in the monograph of the drug substance, with one of them being cyclohexanamine. A recent issue of Pharmaeuropa (25) lists nine potential impurities in total, including cyclohexanamine, but other degradation products reported by El Kousy (23) find no mention. As regards the analytical methods, several of them have been reported for the analysis of glipizide in formulations (26–29), in biological samples (30–37), and in the presence of other anti-diabetic agents (39–42).

However, to date, no systematic forced degradation studies under different stress conditions prescribed by ICH guidelines Q1A(R2) are reported on glipizide. No analytical method is known so far for the drug that is capable of studying its decom-

position behavior in different chemical environments. Hence, the present study was designed to (*i*) carry out stress studies on the drug under ICH prescribed stress conditions, (*ii*) develop an LC method for separation of various degradation products, (*iii*) characterize the products through isolation followed by spectral analysis and/or LC–MS fragmentation studies, and (*iv*) propose the degradation pathway of the drug under prescribed ICH conditions. An indirect objective was to explore if studies carried out following ICH recommendations yielded any additional information to the drug's known degradation pathway.

Experimental

Materials

Glipizide was supplied by M/S Panacea Biotec Limited (Lalru, India) and used without further purification. Sodium hydroxide, hydrochloric acid, and anhydrous sodium sulphate (all AR grade) were obtained from Ranbaxy Laboratories (SAS Nagar, India). Hydrogen peroxide was procured from S.D. Fine-Chem Ltd. (Boisar, India). Acetonitrile, methanol, and chloroform (all HPLC grade) were purchased from Ranbaxy Laboratories (SAS Nagar, India). Water used was purified using a glass triple distillation unit (Gupta Scientific Store, Ambala, India).

Instrumentation

High precision water bath and hot air oven equipped with digital temperature control (Narang Scientific Works, New Delhi, India) were used for hydrolytic and thermal stress testing, respectively. Photodegradation was carried out in a photostability chamber (KBF 240, WTB Binder, Tuttlingen, Germany) equipped with a light bank consisting of two UV (OSRAM L73) and four fluorescent (OSRAM L20) lamps and capable of controlling temperature and humidity in the range of ± 2°C and ± 5% RH, respectively. The light system complied with option 2 of the ICH guideline Q1B (43). At any given time, UV energy at the point of placement of samples was ~0.6 W/m^2 (tested with a calibrated radiometer, model 206, PRC Krochmann GmbH, Berlin, Germany) and visible illumination was ~1100 lux (tested using a calibrated lux meter, model ELM 201, Escorp, New Delhi, India). The chamber was set at accelerated condition of 40°C/75% RH during the studies. The LC system consisted of binary pump (515), dual wavelength detector (2487), Rheodyne manual injector, and Millenium 2.01 software (all from Waters Corporation, MA). The chromatographic separations were carried out on a Spherisorb C-18 (250 mm × 4.6 mm i.d., particle size 5 µm) column from Waters Corporation. LC–MS studies were carried out using negative electro spray ionization (ESI) mode on Bruker Daltonics microTOF instrument (Bruker Daltonik GmbH, Bremen, Germany), which was controlled by microTOF control software ver. 2.0. LC part of the LC–MS comprised of Agilent 1100 series LC system (Agilent Technologies Inc., Palo Alto, CA), controlled by Hystar (ver. 3.1) software. The column used for LC–MS studies was same as that for LC. IR spectra of the drug and the isolated degradation products were obtained on Spectrum One Series FTIR spectrometer (PerkinElmer, Wellesley, MA). ^{1}H-NMR spectra were recorded on Avance II 400 spectrometer (Bruker, Fallanden, Switzerland). Mass spectra of the drug and the isolated degradation products were recorded on API 3000 LC–MS–MS system (Applied Biosystems, Foster City, CA). The other equipments used were an ultrasonic bath (570H, Elma, Germany) and a precision analytical balance (AG 135, Mettler Toledo, Greifensee, Switzerland).

Stress decomposition studies

Acidic, neutral, and alkaline hydrolytic decomposition studies were carried out in HCl (0.01M and 0.1M), water, and 0.1M NaOH, respectively. Studies were performed at 85°C as well as at room temperature (r.t.) over 72 h. Oxidative study was carried out in 30% H_2O_2 at r.t. for the same period. For thermal stress testing, the drug was sealed in glass vials and placed in a hot air oven at 50°C for 31 days. Photolytic studies in solution phase were carried out by exposing the drug solution in 0.01M HCl, water, and 0.1M NaOH to the combination of UV and fluorescent light, whereas solid drug was exposed to light as a thin layer in a petri-dish for 6 weeks. The total exposure was greater than 200 Wh/m^2 of UV and about 1.2 million lux of fluorescent light. A parallel set was kept in dark under similar conditions for 6 weeks. The drug concentration in all the solution phase studies was 0.1% w/v. Solutions of the drug in 0.1M HCl, 0.01M HCl, and 30% H_2O_2 were prepared by dissolving accurately about 0.1 g of the drug in 55 mL methanol or 40 mL acetonitrile, followed by addition of sufficient concentrated HCl or H_2O_2 solution and finally making up the volume to 100 mL with water. Samples were withdrawn initially and subsequently at various prefixed time intervals. All the samples were stored in refrigerated conditions and were analyzed together. The samples were neutralized by acid or alkali, wherever necessary, and diluted ten times with the mobile phase before injection.

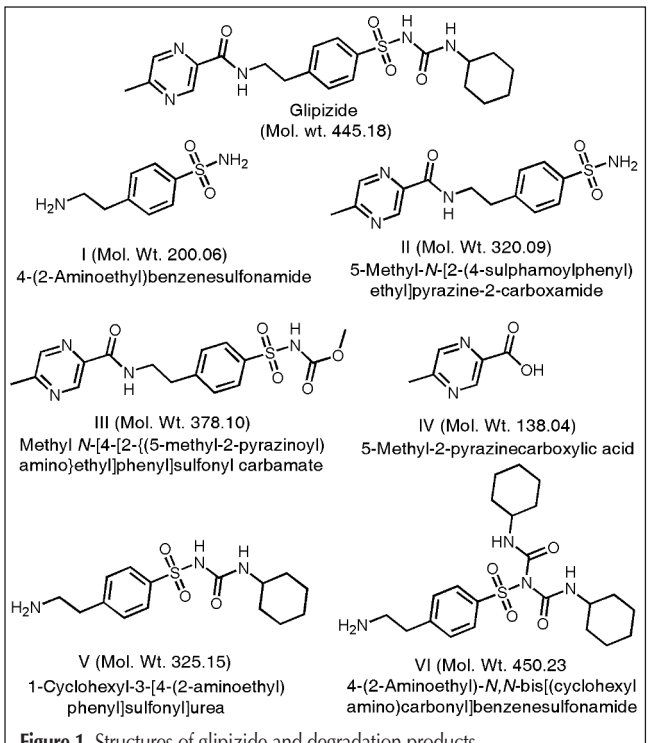

Figure 1. Structures of glipizide and degradation products.

Development and optimization of the separation method

The mobile phase in the initial studies was composed of acetonitrile and 20mM ammonium acetate. It was observed that the resolution of drug and degradation products was influenced by pH of the aqueous phase, ratio of the organic modifier and flow rate. An increase in pH of the buffer shifted all the peaks to lower t_R values and decreased the resolution. The increase in organic modifier (acetonitrile) resulted in merging of peak II with peak III, and peak IV with peak V. On the other hand, the decrease in percentage of the organic modifier improved the resolution. There was merging of peaks at a flow rate of 1.0 mL/min, but better separation was achieved on decreasing the same. Finally, acceptable separations with reasonable peak shapes were achieved by using 20mM ammonium acetate buffer (pH 3.0) and acetonitrile in the ratio of 60:40% v/v at a flow rate of 0.3 mL/min. The injection volume and detection wavelength were 20 µL and 230 nm, respectively. The diluted samples from each stress condition were initially injected individually, but subsequent investigations were carried out using a mixture of a 72 h sample generated in water at 85°C and a 6 weeks light exposed sample generated in 0.1M NaOH. The purpose was that the drug as well as all six degradation products present in a mixture could be resolved in a single run.

Table I. t_R, Rt_R, and Clog P Values of Drug and Degradation Products.

Peak	t_R (min)	Rt_R	Clog P
I	5.80	0.32	–0.414
II	6.80	0.39	0.435
III	8.13	0.49	0.801
IV	10.15	0.57	0.819
V	11.50	0.65	1.733
GLIPIZIDE	17.63	1.00	2.572
VI	27.60	1.56	3.809

Isolation and characterization of degradation products

The major degradation product II formed in acidic hydrolytic condition after 72 h at 85°C was separated as fine crystals after concentrating the reaction solution to ~30% of its original volume. The crystals were filtered, washed with water, and dried in a vacuum desiccator. The product IV formed in alkaline hydrolytic medium was isolated by extracting the stressed solution with chloroform. The organic layer was separated and dried over anhydrous sodium sulphate. The solvent was recovered under vacuum using a rotary evaporator, resulting in an off-white amorphous powder, which was finally dried in air. Purity of these isolated products was ascertained by HPLC analysis, and their structures were established by comparison of FT-IR, ^{1}H-NMR, and mass data with the drug. The identity of other degradation products was established by LC–MS analyses of the selected samples.

Results and Discussion

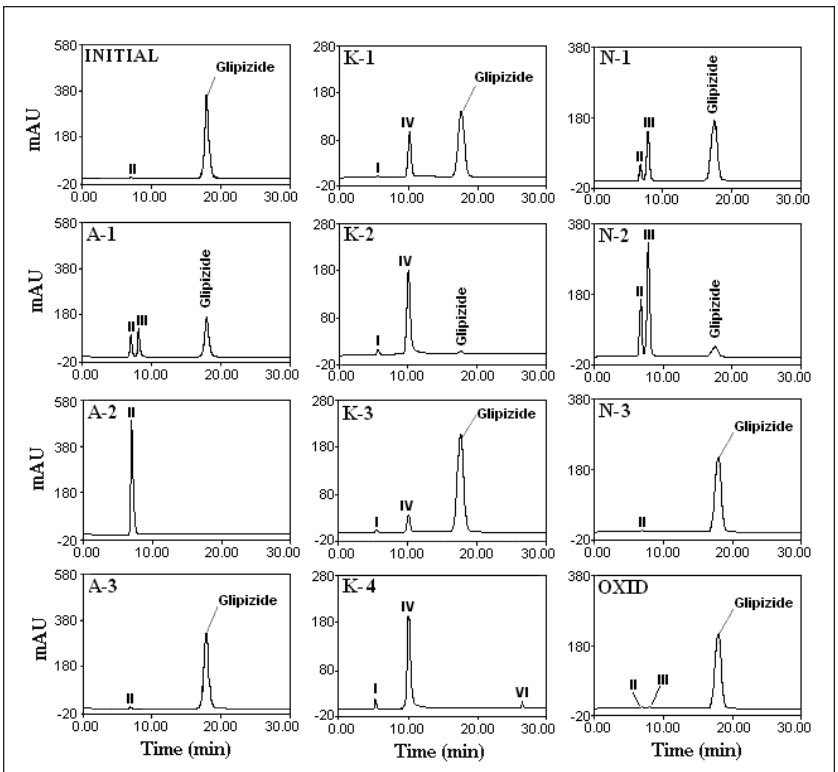

Figure 2. Typical zero time chromatogram of glipizide (INITIAL) and chromatograms showing drug degradation in acidic (A), alkaline (K), neutral (N) hydrolytic conditions (1 and 2 correspond to 8 h and 72 h samples, respectively at 85°C, whereas 3 corresponds to 72 h sample at r.t. and K-4 corresponds to refluxing in alkaline medium for 90 h) and in 30% H2O2 at r.t. after 72 h (OXID).

Degradation behavior

Six degradation products (I–VI) were detected by LC under various stress conditions. The retention times (t_R) and relative retention times (Rt_R) of the drug and the products are listed in Table I.

The drug degraded in 0.01M HCl at 85°C, forming two degradation products II and III, resolving at Rt_R of 0.39 and 0.49, respectively (Figure 2, A-1). The product III was found to increase up to 8 h, and thereafter it decreased gradually with concomitant increase in II. There was almost total conversion of the drug to II at 72 h (Figure 2, A-2). In 0.1M Hcl at the same temperature, the drug decomposed at a much faster rate to form II alone. The drug hydrolyzed at a comparatively slower rate in water at 85°C, and 10% of the drug was left after 72 h (Figure 2, N-2). Again, II and III were the main degradation products, and the level of the two increased with time (Figure 2, N-1 and N-2). Heating of drug at 85°C in 0.1M NaOH for 72 h resulted in major (IV) and minor (I) degradation products appearing at Rt_R of 0.57 and 0.32, respectively (Figure 2, K-1 and K-2). Refluxing of drug in the same alkaline medium for 90 h, which was done with an objective to generate IV in bulk, produced a new degradation product VI in small quantities at Rt_R 1.56, along with products I and IV (Figure 2; K-4).

In comparison to high temperature, the drug degraded insignificantly (~2.5%) at r.t. over 72 h in acidic and neutral media, showing only II as a minor peak (Figure 2, A-3 and N-3), which was also present in the initial sample (Figure 2, INITIAL). About 25% drug decomposition was observed in alkaline medium at r.t. over the same period with the formation of IV and I (Figure 2, K-3). The drug degraded insignificantly (< 1% with respect to initial) in oxidative medium at r.t. over 72 h (Figure 2; OXID), showing only minute peaks of II and III.

Table II. Comparative Spectral Data of Glipizide and Degradation Products II and IV

Spectral technique	Glipizide	II	IV
IR (cm^{-1})	3252, 3326 (NH) 1650 (C=O; amide) 1334, 1160 (S=O) 1444 (cyc-hexyl)	3363, 3276 (NH) 1654 (C=O; amide) 1329, 1158 (S=O) 1538 (NH bend; -SO2NH2)	3600-3100 (OH) 1709 (C=O; acid) 1262 (C-O) 1038 (OH bend)
^{1}H-NMR ($CDCl_3$, 400 MHz)	9.25 (1H, d, J = 1.2) 8.35 (1H, d, J = 1.2) 7.89 (1H, t, J = 6.0) 7.83 (2H, d, J = 8.4) 7.39 (2H, d, J = 8.0) 6.46 (1H, d, J = 8.0) 3.76 (2H, q, J = 6.8) 3.60 (1H, m) 3.04 (2H, t, J = 7.2) 2.64 (3H, s) 1.80–1.84 (2H, m) 1.56–1.68 (3H, m) 1.27–1.33 (2H, m) 1.13–1.22 (3H, m)	9.21 (1H, s) 8.29 (1H, s) 8.21 (2H, bs) 7.82 (2H, d, J = 8.0) 7.75 (1H, bs) 7.34 (2H, d, J = 8.0) 3.71 (2H, q, J = 6.8) 2.98 (2H, t, J = 6.8) 2.60 (3H, s)	9.27 (1H, s) 8.59 (1H, s) 6.80 (1H, bs) 2.67 (3H, s)
m/z (+ESI)	446	321	139

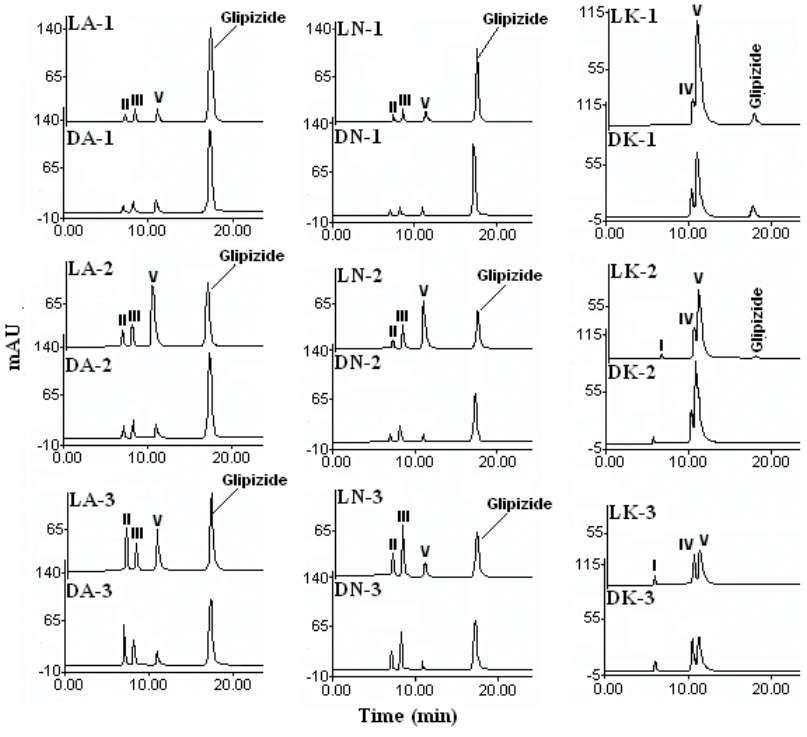

Figure 3. Overlaid LC chromatograms showing degradation of the glipizide in dark (D) and light (L) conditions in acidic (A), neutral (L), and alkaline (K) medium. 1, 2, and 3 refers to 1, 2, and 6 weeks sample, respectively.

The drug showed labiality on exposure of acid, water, and alkaline solutions to light in a photostability chamber set at 40°C. A new product V was formed both under dark and light conditions at lower temperature of 40°C (Figure 3), which was not observed during hydrolytic studies carried out at 85°C (Figure 2). The formation of product V was initially facilitated under light up to one (alkali) or two weeks (neutral and acid), and then height of its peak decreased till end of the study. The degradation behavior in acidic and neutral solutions was similar to each other, resulting in formation of products II, III, and V (Figure 3, LA versus LN and DA versus DN), while in alkali, the products formed were different [viz., I, IV, and V (Figure 3, DK and LK)]. Other than V, the behavior of formation of remaining products under light was similar to hydrolysis in acid, water, and alkali solutions at high temperatures.

Exposure of solid drug to light at 40°C did not result in any significant degradation. The observed chromatographic behavior was similar to that obtained in dark. Even the exposure of drug in solid state to dry heat at 50°C for 31 days did not result in perceptible decomposition.

Thus, it was found that decomposition of drug was temperature and pH dependent in the solution phase, while the drug was stable to light and dry heat in the solid state. The comparison of decomposition behaviour of drug in acid and neutral conditions suggested that III was an intermediate for the formation of II. As III was formed only when methanol was used as a solubilizer, and it did not form on replacement of methanol with acetonitrile, it indicated that III was probably a methyl ester of II or the drug, which was converted further to II. The formation of small quantities of products II and III in oxidative medium was also perhaps due to acidic nature of the peroxide solution.

Overall, the same degradation products were formed in acidic and neutral media, but they were different in alkaline medium. Although sulfonylureas are reported to form same degradation products at all pH values, the different degradation behaviour of glipizide in alkali could be attributed to the presence and hydrolysis of carboxamide moiety in

the molecule. The specific sensitivity of carboxamide group in alkali is reported in litearture (44). In general, the order of sensitivity of sulfonylureas is known to be acid > neutral ≤ alkali (21). In the present study, the order of the rate of degradation in acidic and neutral media was in agreement with the above, but in the alkaline medium, the rate was higher than that in neutral and equivalent to that in acidic medium (i.e., acidic ≈ alkaline > neutral). The same is again explained through the presence of alkali sensitive carboxamide moiety in the structure of glipizide.

Characterization of the Isolated degradation products

The degradation products, II and IV, which could be isolated from acidic and alkaline stress solutions, respectively (detailed in "Experimental" section), were characterized by comparison of their spectral data with that of the drug (Table II).

As shown from the data, the S=O stretching and C-H bending bands for sulphonamide and cyclohexyl groups, respectively, were absent in the IR spectrum of IV. Also, the C=O stretching band shifted from 1650 cm^{-1} to 1709 cm^{-1}, and there was the appearance of a broad band at 3600–3100 cm^{-1}. This indicated the presence of a carboxylic acid functional group. The ^{1}H-NMR spectrum indicated the absence of signals corresponding to ethyl linker, cyclohexyl, and phenyl moieties, and appearance of signals corresponding to pyrazine nucleus and the methyl group. The mass spectrum of the product in positive ESI mode showed a quasimolecular ion at *m/z* 139. The IR/^{1}H-NMR changes and the mass value suggested IV to be 5-methyl-2-pyrazinecarboxylic acid (Figure 1).

The IR spectrum of II showed retention of vibrational bands corresponding to C=O (amide) and S=O (sulphonamide) stretches and absence of C-H bending bands for cyclohexyl group. These indicated that carboxamide and sulfonyl moieties were retained, whereas the cyclohexyl group was lost in the product. Additionally, the appearance of a sharp band at 1538 cm^{-1} for N-H bending vibration (primary sulphonamide) suggested the presence of $-SO_2NH_2$ group. All the signals in ^{1}H-NMR spectrum corresponding to cyclohexyl group were absent, whereas the rest of them appeared as in glipizide. The *m/z* value of 321 was recorded for this product in positive ESI mode. These observations suggested the product to be 5-methyl-*N*-[2-(4-sulphamoylphenyl)ethyl]pyrazine-2-carboxamide (Figure 1).

LC–MS studies

The mixture of drug and degradation products was subjected to LC–MS studies in negative ESI mode. The drug was additionally subjected to LC–MS–MS analysis. Line spectra of drug and the degradation products I–VI (Figure 4) showed mass values of *m/z* 444, 199, 319, 377, 137, 324, and 449, respectively. Molecular weight of the drug corresponded to its known mass value, whereas that of the products II and IV also corresponded to the mass values determined using the positive ESI mode. Indirectly, it validated the output of the mass spectrometer used in the LC–MS study. Mass fragmentation pattern of drug and degradation products (Figure 5) was proposed on the basis of their line spectra. Structures of the degradation products, other than II and IV, could be proposed (Figure 1) based on the mass values and fragmentation pattern.

The drug on ionization yielded a major fragment of *m/z* 319, which incidentally corresponded to the molecular weight of the degradation product II. The major fragment further yielded daughter ions at *m/z* 225, 197, 182, and 170. Almost similar fragments were observed with II, thus supporting its structure. A fragment at *m/z* 319 in the line spectrum of III (*m/z* 377) indicated it to be related to structure of II. A difference of 58 atomic

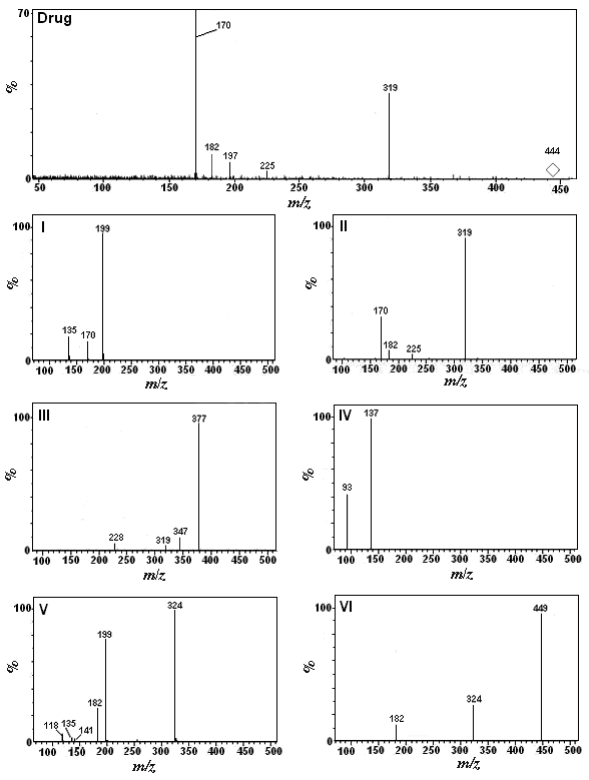

Figure 4. LC–MS–MS line spectrum of glipizide and LC–MS spectra of its degradation products (I–VI).

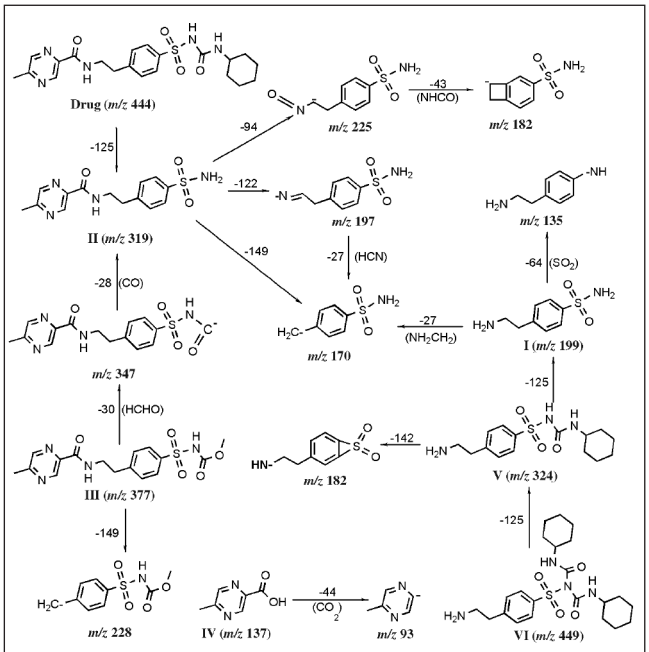

Figure 5. Fragmentation pattern for the drug and the degradation products.

mass units (amu) between mass value of 377 and *m/z* 319 of the major fragment supported it to be the methyl ester of II, as proposed earlier in the degradation behavior. III ionized to daughter ion *m/z* 347 on loss of HCHO (30 amu), and a further loss of CO (28 amu) produced the major fragment of *m/z* 319 (Figure 5). The ion with *m/z* 228 could be produced from III on cleavage of the ethylene bridge. The same cleavage was apparently responsible for production of ion with *m/z* 170 in the line spectra of II as well as the drug. This further lent support to structure of III.

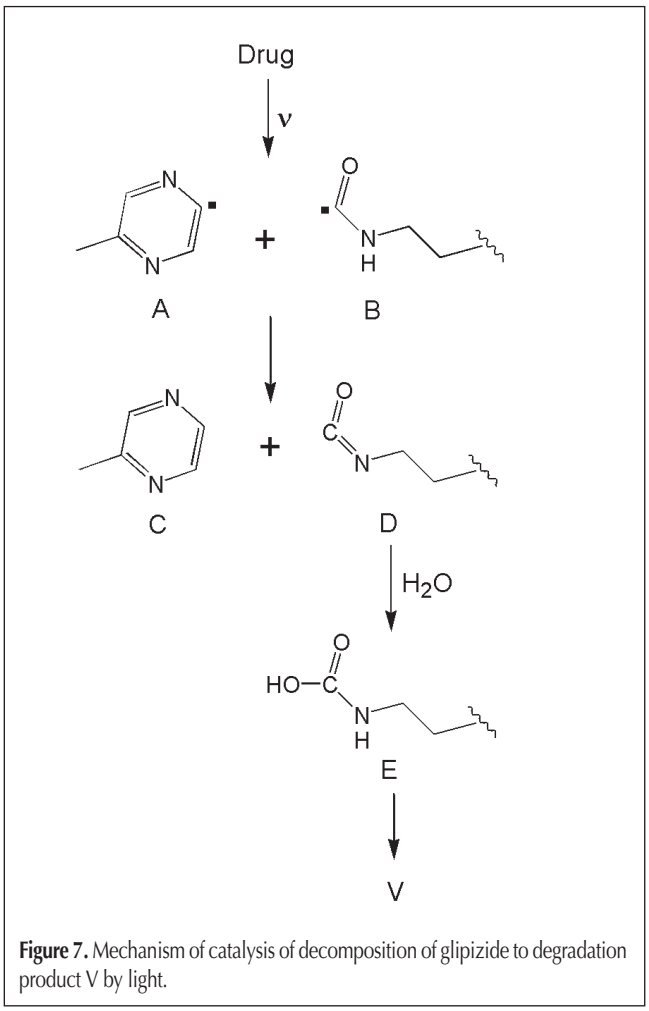

Figure 7. Mechanism of catalysis of decomposition of glipizide to degradation product V by light.

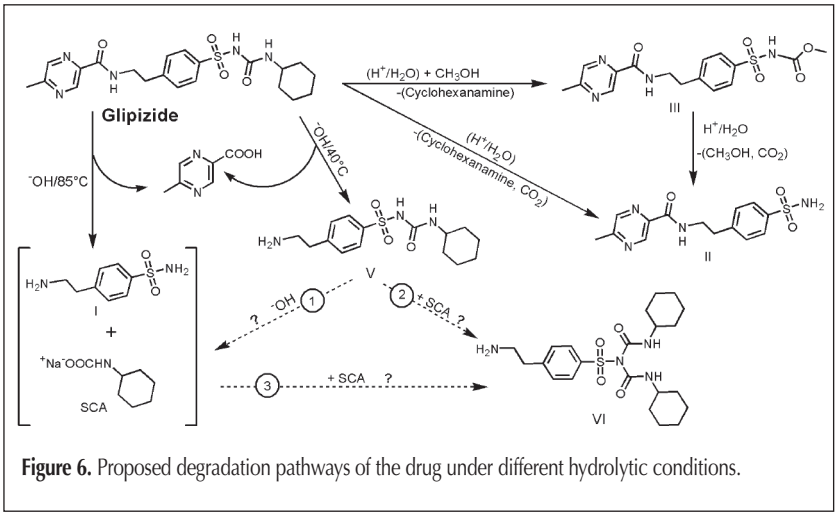

Figure 6. Proposed degradation pathways of the drug under different hydrolytic conditions.

The mass value of product I (*m/z* 199) was found to correspond to the molecular weight of a known degradation product (23). Its structure was further supported by fragments of *m/z* 170 and 135. The product I was also seen as a major fragment in the line spectrum of V (*m/z* 324), which was purportedly produced by elimination of cyclohexylisocyanate moiety (125 amu) from the molecule. Elimination of the same group in the drug produced a major fragment with *m/z* 319. The common fragment of *m/z* 135 in I and V confirmed their structural relationship, but also notable was the difference of ions with *m/z* 170 and *m/z* of 182 in the line spectrum of I and V, respectively. The sulphonamide moiety was not subject to ionization as evidenced from several other fragments with the same moiety (Figure 5). Hence, no change happened to the moiety even in the case of I and the ionization moved in the direction to produce an ion with *m/z* 170. The latter was also incidentally formed from II with an intact sulphonamide moiety. The formation of ion of *m/z* 182 in the spectrum of V was justified by elimination of cyclohexylurea moiety, present in V but not existent in I. The above contention found further support in fragmentation pathway 449 → 324 → 182 observed in the line spectrum of VI. This even justified the structure of VI itself. Compound IV, a known breakaway degradation product of glipizide (23), ionized to a single fragment of *m/z* 93 (Figure 4). This was reasonably explained by the loss of CO_2 (44 amu) (Figure 5).

Elution pattern versus lipophilicity of degradation products

To further confirm the proposed structures of the various degradation products, an attempt was made to correlate calculated lipophilicity of the each structure with its elution order on HPLC. The lipohilicity was determined for the drug and degradation products using Clog P module of ChemDraw Ultra 6.0. The elution time (t_R) and the corresponding Clog P values of the drug and products are included in Table I. It is evident that the Clog P values correlated well to the elution pattern on LC chromatogram as expected, thus supporting the propositions made earlier.

Degradation pathways

Based on the results, the pathway of degradation of glipizide under the ICH recommended stress conditions is outlined in Figure 6. The conversion of glipizide to II in acidic and neutral medium is well explained by the already known degradation pathway of sulfonylureas, according to which they are hydrolyzed to sulphonamide and an amine, with evolution of carbon dioxide (20,21). The conversion of glipizide to III in the presence of methanol is postulated to occur through nucleophilic attack of methanol on the carbonyl carbon of sulfonylurea moiety, accompanied by elimination of cyclohexanamine. Further hydrolysis of the urethane moiety in III could result in II, with loss of methanol and CO_2. However, in the absence of methanol, it is proposed that the drug is hydrolyzed directly to II through an unstable carbamic acid derivative, considered as a hypothetical carboxylic acid, existing either as a sodium

salt or ester (45).

The conversion of glipizide to products IV and I in the alkaline medium is postulated to occur by hydrolysis of both the carboxamide and sulfonylurea moieties at 85°C. On the basis of literature reports on the reactivities of sulfonylureas and carboxamides (20,21,44), it is postulated that hydrolysis of carboxamide linkage is preferred over sulfonylurea moiety under the alkaline conditions to form IV and V. The formation of product V, which does not show up at high temperatures, is supported by its presence in reaction solutions at low temperature of 40°C, under both dark and light conditions (Figure 3, DK-1 through DK-3 and LK-1 through LK-3). Most probably, V is rapidly converted in alkali at high temperature to I and sodium salt of cyclohexylcarbamic acid (SCA) (Figure 6; dotted arrow 1). But again the chromatograms (Figure 3) show absence of significant rise of I, perhaps due to relatively low extinction coefficient of this product at the detection wavelength. That is also indicated through the lack of detection of I even in acid and neutral solutions, where decrease of V was seen during the course of reaction in light conditions (Figure 3; LA-2 versus LA-3 and LN-2 versus LN-3). Alternatively, it is possible that V decreases by an alternate route in acid, neutral, and even alkaline conditions, especially in the presence of light. The product VI, generated upon prolonged boiling (90 h) in alkaline medium and having mass of 449 (even higher than the drug), can be said to be the result of adduct formation between either V and SCA (1:1 moles) or I and SCA (1:2 moles) (Figure 6; dotted arrows 2 and 3). The latter is seemingly more probable because V is not expected to remain intact at boiling temperature, which is also supported by studies at 85°C (Figure 2, K-1–K-2). Hence, formation of VI is proposed to be coupled with degradation of V. More exhaustive studies involving monitoring of kinetics at low and high temperatures and in the presence of light in the three reaction conditions, using a universal detector and having standards of VI, V, and I in hand, are needed to explain the fate of V at all the pH, and the formation of VI in alkaline medium.

The acceleration of decomposition of drug to V in acid, neutral, and alkaline conditions by light is explained in Figure 7. The photolytic cleavage of pyrazine-carbonyl carbon bond in glipizide results in free radicals A and B. The two radicals recombine to produce 2-methylpyrazine (C) and isocyanate (D). Owing to rapid hydrolysis, D gets readily converted to V via a carbamic acid derivative E. The pathway is supported by literature reports, which emphasize that amides undergo photolytic cleavage in aqueous medium via free radical mechanism to hydrocarbon and isocyanate (22). The latter gets hydrolysed further in an aqueous environment to carbamic acid or urethane, which spontaneously loses CO_2 to produce an amine (46).

Conclusion

Forced degradation studies on glipizide under the conditions of hydrolysis, oxidation, photolysis and dry heat, followed by LC–UV analyses of the degraded solutions, revealed formation of six degradation products in total. The products were characterised through isolation and subsequent NMR, IR, and MS spectral analyses, and/or through LC–MS fragmentation pattern studies. It is observed that two of the degradation products are hitherto unknown, which even do not form part of the compendial list of impurities. Based on the results, a more complete degradation pathway of the drug is proposed. It clearly means that stress studies carried out using ICH approach gives more complete information on the degradation chemistry of the drugs.

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