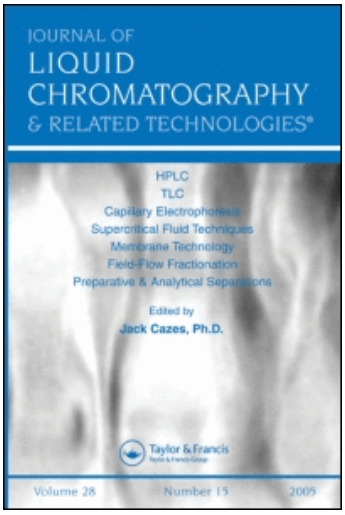

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Characterization of Mass Ionizable Degradation Products of Gliclazide by LC/ESI-MS

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Abstract: A solution containing six UV active degradation products (I-VI) of gliclazide formed under different stress conditions prescribed by International Conference on Harmonisation is analyzed by LC/ESI-MS. Of the six, four products I, II, V, and VI are detected in MS. The molecular weights of these are 171, 229, 337, and 321, respectively. The comparison of their mass fragmentation pattern with the drug has helped in establishment of their structures. These are identified as *p*-tolylsulfonamide (I), methyl *N*-(*p*-tolylsulfonyl)carbamate (II), 4,4a,5,6,7,7a-hexahydro-3H-cyclopenta[d]pyridazin-1-yl *N*-(*p*-tolylsulfonyl)carbamate (V), and *N*-(4,4a,5,6,7,7a-hexahydro-3H-cyclopenta[d]pyridazine-1-carbonyl)-4-methyl benzenesulfonamide (VI). It is indicated that product II is not a true degradation product, but a methyl ester formed in the presence of methanol, used to solubilize the drug in reaction solutions. Another product, which is not detected in LC/UV but showed up in the LC/MS chromatogram, is identified as (2-diazenylmethyl)cyclopentyl-methanol (VII). The pathways for the formation of these products are proposed.

Keywords: Degradation pathway, Degradation products, Gliclazide, LC/ESI-MS, Mass fragmentation, Stress testing

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INTRODUCTION

The guidelines issued by International Conference on Harmonisation (ICH) and other international agencies require reporting and identification of degradation products and establishment of degradation pathways of the drugs.[1–4] The identification of degradation products requires great effort, as they are generally formed only in small amounts on storage of drug substances and products, even under accelerated conditions. Therefore, to produce degradation products in sufficient amounts in order to facilitate their characterization, forced degradation studies are suggested to be carried out on active pharmaceutical ingredients under hydrolytic, oxidative, photolytic, and thermal stress conditions.[5] The degradation products formed under different conditions are separated on a LC column, isolated using preparative techniques, and characterized by spectral analyses. An alternate to this conventional approach is the use of sophisticated hyphenated techniques, like LC/MS, LC/NMR, etc. Of these, study of the mass fragmentation pattern, in particular, has proven very useful in identification of drug degradation products and prediction of their degradation pathways.[6–12]

Gliclazide is a second generation sulfonylurea type oral hypoglycaemic agent, used in treatment of type-2 diabetes mellitus.[13] Chemically, it is 1-(3-azabicyclo[3.3.0]oct-3-yl)-3-(*p*-tolylsulfonyl)urea (Figure 1). It is supposed to be a highly unstable drug, based on the reported instability of sulfonylureas[14,15] and strained heterocyclic rings[16] in acidic and alkaline media. Despite the same, there are not many reports on the degradation behaviour of this drug. El Kousy[17] has investigated degradation of gliclazide in acidic hydrolytic medium and reported *p*-toluenesulfonamide and *N*-aminocyclopentanopyrrolidine as the degradation products. British Pharmacopoeia enlists certain process related and isomeric impurities of

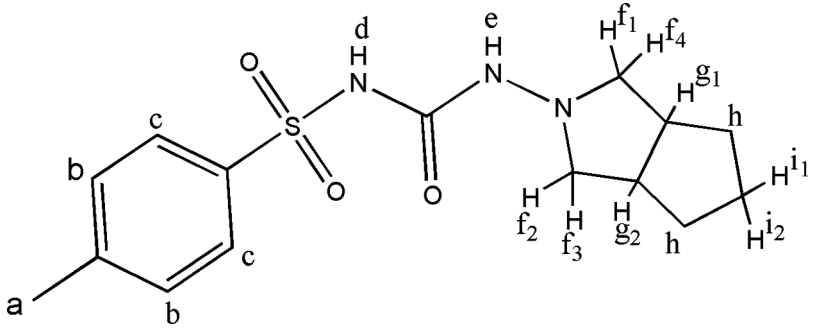

Figure 1. Structure of gliclazide.

gliclazide.[18] A systematic forced decomposition study on gliclazide under ICH recommended stress conditions has been reported by us earlier, where a total of six degradation products (I–VI) were separated using a validated LC method.[19]

The purpose of the present investigation was to establish structures of the degradation products through spectral analyses and LC/ESI-MS studies. Of the six, four products were ionized in MS, along with another that showed up in the LC/MS chromatogram, though it was not detected in LC/UV. These were characterized based on comparison of their mass fragmentation pattern with the drug. The pathway to their formation was also established.

EXPERIMENTAL

Chemicals and Reagents

Gliclazide was supplied by Panacea Biotec Ltd. (Lalru, India) as a gift sample. Acetonitrile and methanol (HPLC grade), hydrochloric acid, sodium hydroxide pellets, hydrogen peroxide solution, acetic acid glacial, and ammonium acetate (all of analytical reagent grade) were purchased from Ranbaxy fine chemicals (Gurgaon, India). *p*-Toluenesulfonamide was procured from Spectrochem Pvt. Ltd. (Mumbai, India). HPLC grade water was produced using a triple distillation glass assembly (Perfit, Ambala, India).

Instrumentation

A precision water bath (Narang Scientific Works, New Delhi, India), capable of controlling temperature within ±1°C, was used for hydrolytic stress studies. LC/UV analyses of the degradation samples were carried out on HPLC system (Waters Corporation, Milford, MA, USA) consisting of binary pumps (model 515), dual wavelength detector (model 2487), and a manual injector (Rheodyne, Rohnert, USA). The data were acquired and processed using Millenium software ver. 2.01. The LC/MS studies were carried out using positive, as well as negative, electrospray ionization (+ESI and −ESI) modes on Bruker Daltonics microTOF instrument (Bruker Daltonik GmbH, Bremen, Germany). The data were acquired and processed using micrOTOF control software ver. 2.0. LC part of the LC/MS comprised of 1100 series system (Agilent Technologies Inc., CA, USA), controlled by Hystar (ver. 3.1) software. IR spectra were recorded using KBr discs on Spectrum one series FTIR spectrophotometer (Perkin Elmer, Wellesley, USA). ^{1}H-NMR spectra were recorded in $CDCl_3$

on Avance II 400 spectrometer (Bruker, Fallanden, Switzerland). Mass spectrum of the isolated product was recorded on API 3000 LC/MS/MS system (Applied Biosystems, Foster City, USA).

Chromatographic Conditions[19]

The drug and its six degradation products (I–VI) were optimally resolved on a Waters Spherisorb® C_{18} (4.6 × 250 mm, 5 μm) column using a mobile phase composed of acetonitrile-ammonium acetate (25 mM, pH 3.0) (40:60, v/v) flowing at a rate of 0.25 mL/min. A Nucleosil® C_{18} (4.6 × 8 mm, 5 μm) guard column was placed before the main analytical column. The injection volume was 20 μL and the eluent was detected at 235 nm.

MS Conditions

The chromatographic conditions used for LC/MS and LC/MS/MS analyses were the same as that for LC/UV analyses, except that the injection volume was reduced to 10 μL. A splitter was placed before the mass detector allowing entry of only 35% of the eluent. The MS operating conditions in +ESI mode were optimized as follows: end plate offset, 5000 V; capillary, 4500 V; nebulizer, 1.2 bar; dry gas, 6.0 L/min, and dry temperature, 180°C. The operating conditions in −ESI mode were same as in +ESI, except that the dry gas flow was decreased to 4.0 L/min. The MS scans in both the modes were recorded in the range of m/z 50 to m/z 3000. The LC/MS/MS studies were conducted in the +ESI mode to generate mass spectra of both the drug and the degradation products.

Sample Preparation

The acid drug solution (0.1% w/v) was prepared by dissolving 0.1 g of the drug in 55 mL HPLC grade methanol, adding 0.85 mL of concentrated HCl, and finally making volume to 100 mL with water. The alkaline drug solution was prepared by dissolving 0.1 g of the drug in sufficient 0.1N NaOH solution to produce 100 mL. The stressed samples were generated by storing the acidic drug solution at 30°C for 12 h and alkaline drug solution at 85°C for 8 h. A mixture of both of these solutions was prepared by taking their equal volumes (2 mL each) and diluting up to five times with mobile phase, so that all six degradation products were present in the sample. The LC/UV,

LC/MS, and LC/MS/MS analyses were performed on this mixture to characterise the degradation products.

Isolation and Structure Elucidation of Acid Degradation Product

The degradation product I was generated in pure form by refluxing the drug solution (0.1% w/v) in 0.1N HCl for 72 h, using methanol (55% v/v) as a solubiliser. The product separated as fine crystals on removal of methanol by distillation. Its structure was established by comparative spectral analyses with gliclazide. Its structure was also authenticated by peak matching and spiking studies using a standard BP impurity A (*p*-tolylsulfonamide).

RESULTS AND DISCUSSION

The six products, I–VI, which were formed in different stress conditions, were resolved in the LC/UV chromatogram at relative retention times (RR_T) of 0.31, 0.40, 0.43, 0.52, 0.58, and 0.89, respectively.[19] While peaks due to products I, II, V, VI, and gliclazide were ionized in LC/MS in both +ESI and −ESI modes, the peaks due to III and IV were not detected in either of the two modes (Figure 2). Oppositely, a new product VII was observed in the LC/MS chromatogram upon −ESI detection, which was not seen in the LC/UV chromatogram, indicating it to be a non-chromophoric compound. The drug and degradation products were detected as $[M + H]^+$, $[M + Na]^+$, and $[M + K]^+$ ions in +ESI mode (Figure 3A–E), and as $[M - H]^-$ in −ESI mode (Figure 3F–K). The ESI mode was employed in the present study in agreement with various

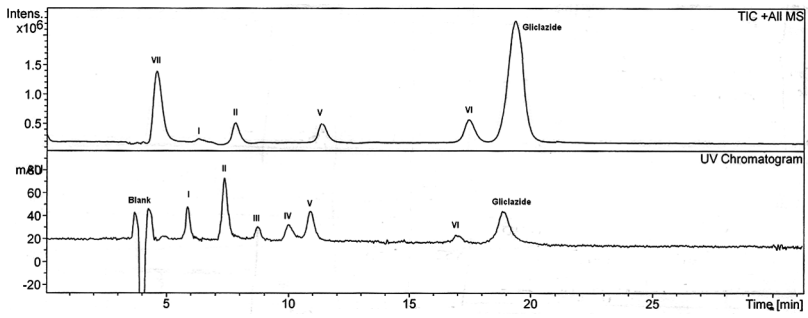

Figure 2. LC/UV and LC/MS chromatogram of mixture of stressed samples of gliclazide.

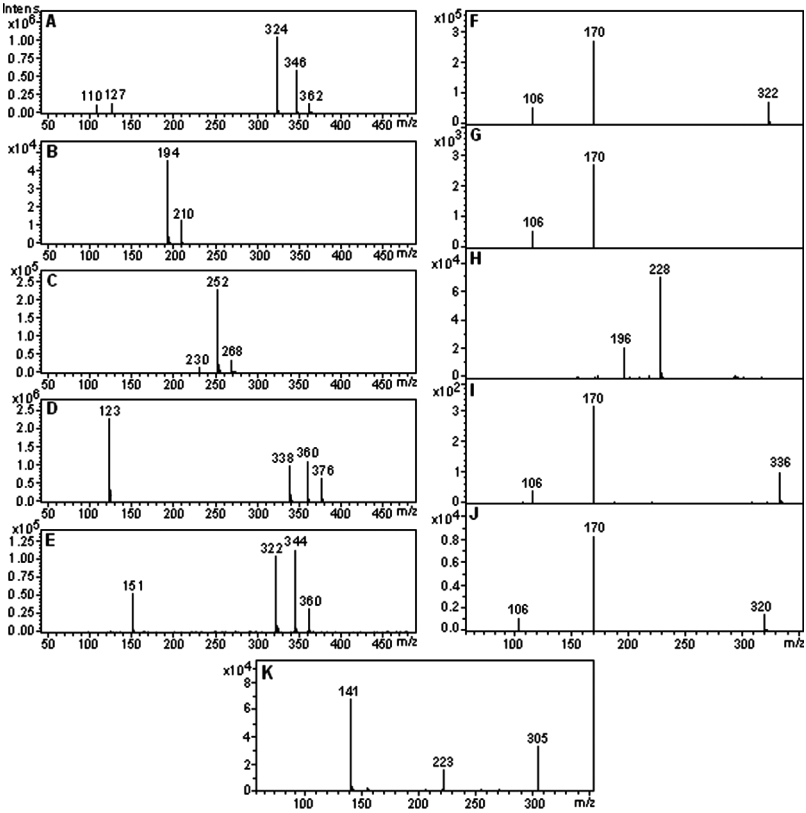

Figure 3. LC/MS scans (A–E) of gliclazide, I, II, V, and VI, respectively, in +ESI mode and (F–K) of gliclazide, I, II, V, VI, and VII, respectively, in −ESI mode.

reports on LC/MS and MS analysis of sulfonylurea based drugs.[20–26] Eventually, +ESI mode was chosen for recording MS/MS scans of gliclazide and degradation products (Figure 4), because of the greater abundance of the parent ions in this mode.

Mass Fragmentation Pattern of Gliclazide

Gliclazide was noted as peaks at m/z 324 $[M + H]^+$, 346 $[M + Na]^+$, and 362 $[M+K]^+$ in +ESI LC/MS scan (Figure 3A), similar to those reported by Liang et al.[22] Also, the peak observed at m/z 322 $[M - H]^-$ in the −ESI LC/MS scan (Figure 3F) corresponded to the molecular mass

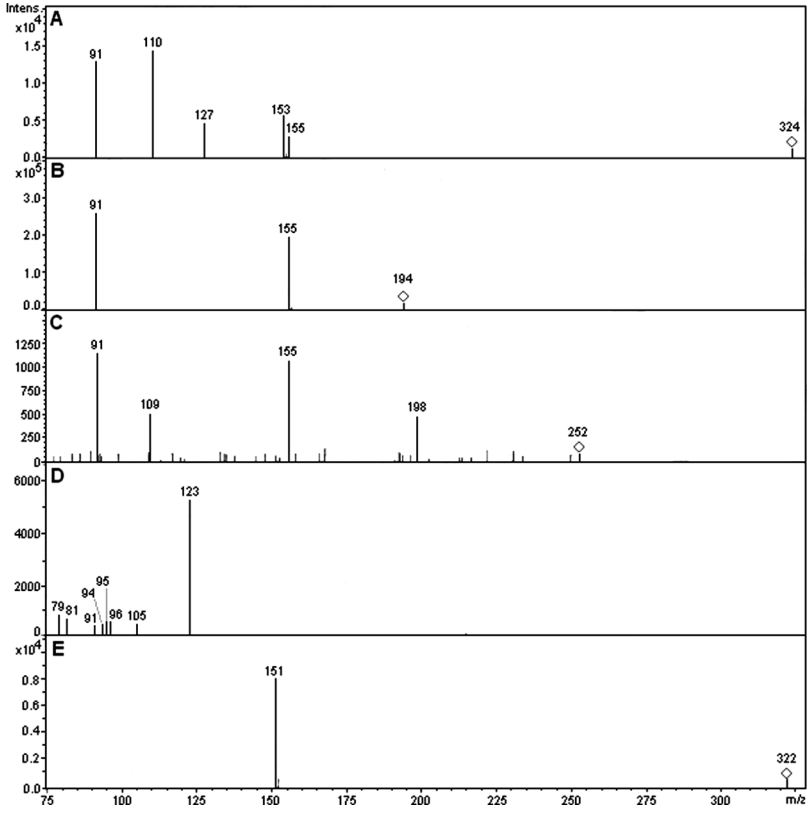

Figure 4. MS/MS scans (A–E) of gliclazide, I, II, V, and VI in +ESI mode.

(323 Da) of the drug. Even, the MS/MS scan of gliclazide (Figure 4A) showed fragments at m/z 155, 153, 127, 110, and 91, similar to those observed by Hoizey et al.[23] The fragments at m/z 110 and m/z 127, which appeared directly from gliclazide,[24,25] were assigned to cyclopentanopyrrolidine cation (F_1) and N-amino cyclopentanopyrrolidinium ion (F_2), respectively (Figure 5). Fragment F_1 was also proposed to be formed from m/z 153 (F_3), which itself was generated from the loss of *p*-tolylsulfonamide from gliclazide. The major fragment at m/z 91, corresponding to tolyl cation (F_4), was postulated to be formed directly from gliclazide, and also through the fragment of m/z 155 (F_5) by loss of SO_2.[26]

The fragment peak at m/z 170 (F_6), observed in −ESI MS scan of gliclazide (Figure 3F), could be rationalized by deprotonation at N_2 of gliclazide, followed by cleavage of N_1–C bond. Another fragment at m/z

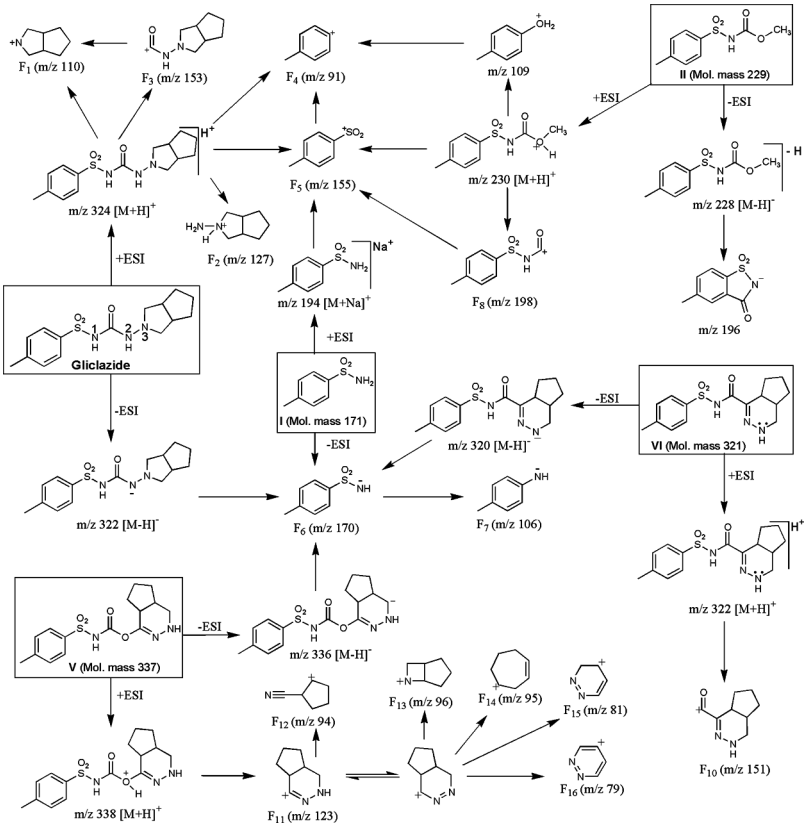

Figure 5. Proposed mass fragmentation patterns of gliclazide, I, II, V, and VI.

106 (F_7) was supposedly formed directly from the drug similar to that reported from glibenclamide.[26] However, in contrast to this, F_7 was also formed by the loss of SO_2 from F_6, as indicated by the fragment peak at m/z 106 and deprotonated molecular ion peak $[M - H]^-$ at m/z 170 in the −ESI MS scan of degradation product I (Figure 3G).

Thus, based on results of the present study and literature reports, it could be proposed that fragmentation of gliclazide was triggered by deprotonation at N_2 in −ESI mode, and by protonation at sulfonyl oxygen, carbonyl oxygen, or N_3 in +ESI mode. Also, with respect to fragmentation, the drug molecule could be visualized as divided into left and right portions, attached through a central carbonyl group (Figure 6). The molecule underwent fragmentation on the left side in −ESI mode, while the fragmentation occurred on both sides in +ESI mode.

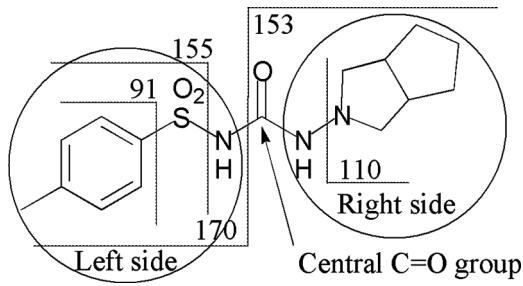

Figure 6. Representative fragmentation of gliclazide.

Characterization of Degradation Products

Apart from ascertaining the structure of the isolated degradation product I based on IR, NMR, and Mass spectral data, its structure and also those of other mass ionizable degradation products was established considering their fragmentation peaks in LC/MS (Figure 3) and MS/MS scans (Figure 4), and comparing their fragmentation pattern with gliclazide (Figure 5).

Characterization of Product I

The signals corresponding to cyclopentanopyrrolidine nucleus were absent in the NMR spectrum of I (Table 1), indicating that the right portion of gliclazide molecule was removed in this product. The IR spectrum showed retention of S=O and absence of C=O stretching bands, suggesting that the sulfonyl group was retained and the carbonyl group was lost during acid hydrolysis of gliclazide to product I. Additionally, conversion of the signal at 3274 cm^{-1} in gliclazide to a doublet at 3359 and 3262 cm^{-1} (corresponding to a primary amino group) in product I indicated that the –NH– group of gliclazide was converted to $–NH_2$. On the basis of these observations, product I was proposed to be *p*-tolylsulfonamide, which was supported by complete similarity between IR spectra of product I and *p*-tolylsulfonamide.[27] The deprotonated molecular ion peak at m/z 170 $[M - H]^-$ in the −ESI LC/MS scan (Figure 3G) and adduct ion peaks at m/z 194 $[M + Na]^+$ and m/z 210 $[M + K]^+$ in the +ESI LC/MS scan (Figure 3B) suggested the molecular mass of product I to be 171 Da, which again corresponded to *p*-tolylsulfonamide. The structure was also confirmed by fragment peaks at m/z 155 and 91 in the MS/MS scan of product I (Figure 4B); it resembles with mass spectral data of *p*-tolylsulfonamide.[28] The

Table 1. Comparative spectral data of gliclazide and product I

NMR data Proton	Gliclazide	Description	Product I	Description
d	8.77	1H, br	4.84	2H, s
e	6.02	1H, br	–	
c	7.94	2H, d, J = 8.4	7.81	2H, d, J = 8.2
b	7.30	2H, d, J = 8.0	7.32	2H, d, J = 8.0
f_1	3.28	1H, bs	–	
f_2	2.81	1H, bs	–	
a	2.43	3H, s	2.44	3H, s
f_3, f_4, g_1	2.55	3H, bs	–	
g_2, i_1	1.91–1.96	2H, m	–	
h, i_2	1.36–1.6	5H, m	–	
IR data	Wave number (cm^{-1})			
Band	Gliclazide		Product I	
N-H stretch	3274		3359, 3262	
C=O stretch	1708		Absent	
N-H bend (Primary sulfonamides)	Absent		1529	
S=O stretch	1348, 1164		1302, 1159	
Mass spectral data Ionization mode				
+ESI	m/z 362 $[M + K]^+$ m/z 346 $[M + Na]^+$ m/z 324 $[M + H]^+$ m/z 155, 153, 127, 110 and 91		m/z 210 $[M + K]^+$ m/z 194 $[M + Na]^+$ m/z 155 and 91	
−ESI	m/z 322 $[M - H]^-$ m/z 170 and 106		m/z 170 $[M - H]^-$ m/z 106	

fragments of m/z 155 and 91 in +ESI and m/z 106 in −ESI were proposed to be formed parallel with the drug (Figure 5).

Characterization of Product II

The product II was formed in acidic and neutral media. Peaks at m/z 230 $[M + H]^+$, 252 $[M + Na]^+$, 268 $[M + K]^+$ in the +ESI LC/MS scan (Figure 3C) and m/z 228 $[M - H]^-$ in the −ESI LC/MS scan (Figure 3H) established the molecular mass of product II to be 229 Da. Isotopic peaks at m/z 254 and 270 (5.7% and 5.2% of the peaks at

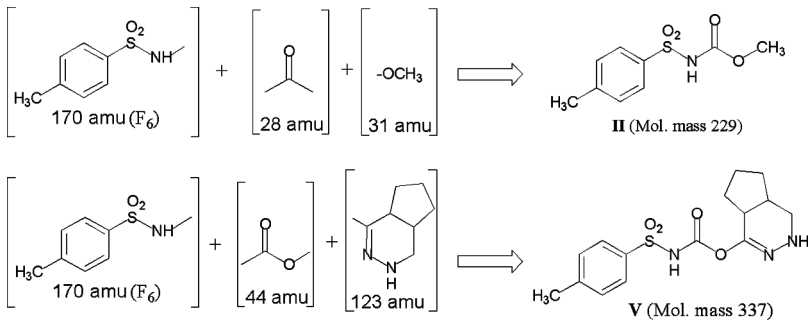

Figure 7. Assembling of fragments to generate structures of II and V.

m/z 252 and 268, respectively) in the +ESI scan and m/z 230 (5.3% of the peak at m/z 228) in the −ESI scan indicated the presence of one sulphur atom in product II. The MS/MS scan recorded by targeting m/z 252 as the precursor ion showed fragment peaks at m/z 198, 155, 109, and 91 (Figure 4C). Peaks at m/z 91 and 155 indicated the presence of F_4 and F_5 fragments in product II, similarly as in gliclazide and product I, suggesting presence of the left portion (F_6; m/z 170) in product II. However, the absence of peak at m/z 170 in the −ESI LC/MS scan suggested that the right side portion of gliclazide, responsible for triggering formation of the particular fragment, was not present in product II. A difference of 28 Da (corresponding to C=O group)[6] between F_6 and the heaviest fragment peak (m/z 198) suggested the central C=O group to be bonded to F_6. Finally, a difference of 31 Da between m/z 198 and molecular mass (229 Da) of product II was attributed to the presence of $-OCH_3$ group.[6] Hence, the three components, i.e., F_6, C=O and $-OCH_3$ could be assembled (Figure 7) to generate the structure of product II as methyl *N*-(*p*-tolylsulfonyl)carbamate, which complied even with the nitrogen rule. The mass fragmentation pattern in the +ESI mode was thus proposed to follow similar route as for gliclazide, i.e., formation of F_5 directly from product II, followed by loss of SO_2 to form F_4 (Figure 5). Formation of m/z 198 was possible by loss of CH_3OH mediated by protonation of methoxyl oxygen.[6] A fragment peak at m/z 196 in the −ESI MS scan could be attributed also to loss of CH_3OH by intramolecular rearrangement in the deprotonated parent ion. Product II was not noted in the chromatograms when acetonitrile was used as a solubilizer. This indicated that product II was not a true degradation product, but a methyl ester formed in the presence of methanol, used as a solubilizer for the drug in the reaction solutions.

Characterization of Product VI

Product VI was formed in an alkaline hydrolytic condition only. The peaks at m/z 322 $[M + H]^+$, 344 $[M + Na]^+$, and 360 $[M + K]^+$ in the +ESI LC/MS scan (Figure 3E) and m/z 320 $[M - H]^-$ in the −ESI LC/MS scan (Figure 3J) established the molecular mass to be 321 Da, suggesting it to contain an odd number of nitrogen atoms. A difference of 2 Da in the molecular masses of product VI and gliclazide suggested that the former could be formed by dehydrogenation of the latter. A single fragment peak of m/z 151 in MS/MS scan of product VI (Figure 4E) vis-à-vis m/z 153 in gliclazide (Figure 4A) indicated that dehydrogenation might have occurred on the right of the drug. Fragment peaks at m/z 170 and 106 in the −ESI MS scans of both gliclazide and product VI (Figure 3F *versus* 3J) directly suggested that the left side of gliclazide was retained in the product. On the basis of these observations, two possible dehydrogenated gliclazide derivatives, VI-A and VI-B (Figure 8), were considered possible. However, structure VI-A was ruled out because formation of a double bond at an angular position in fused bicyclic systems is not favoured.[29] On the other hand, structure VI-B was expected to produce a fragment of m/z 108, similarly as F_1 was produced from gliclazide (Figure 5). But, absence of this m/z 108 fragment in the MS/MS scan of product VI ruled out VI-B as the structure of VI. This suggested that cyclopentanopyrrolidine nucleus was absent in product VI. Further, on the basis of literature reports on nucleophile mediated ring expansion of strained aliphatic ring systems,[30] it was hypothesized that 1-aminocyclopentanopyrrolidine nucleus underwent ring expansion in alkaline medium to form a cyclopentapyridazine

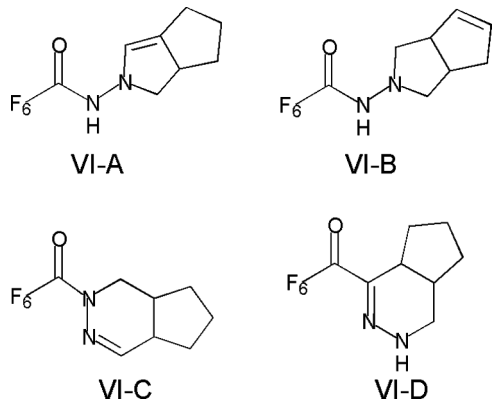

Figure 8. Probable structures of VI.

nucleus. This hypothesis facilitated VI-C and VI-D as two other possible structures of VI (Figure 8). Both VI-C and VI-D could produce a fragment of m/z 151, without the possibility of m/z 108 in the +ESI mode. However, formation of F_6 and F_7 from VI-C was not possible in the −ESI mode, due to non-availability of acidic hydrogen on the right side of the structure. On the contrary, formation of F_6 from structure VI-D was possible by deprotonation of –NH– of the pyridazine nucleus. Hence, product VI was characterized as *N*-(4,4a,5,6,7,7a-hexahydro-3H-cyclopenta[d]pyridazine-1-carbonyl)-4-methyl benzenesulfonamide.

Characterization of Product V

The peaks at m/z 338 $[M + H]^+$, 360 $[M + Na]^+$, and 376 $[M + K]^+$ in the +ESI LC/MS scan (Figure 3D) and m/z 336 $[M - H]^-$ in the −ESI LC/MS scan (Figure 3I) established molecular mass of product V to be 337 Da. The isotopic peaks at m/z 340, 362, and 378 with intensities of 5.7%–6.4% of the peaks at m/z 338, 360 and 376, respectively, in +ESI, and isotopic peak at m/z 338 with intensity of 5.2% of the peak at m/z 336 in the −ESI MS scan indicated one sulphur atom in product V. An increase in molecular mass of product V by 14 Da with respect to gliclazide suggested that the former was formed by addition of a nitrogen atom or a methylene group to the latter. However, addition of nitrogen to gliclazide to form product V meant violation of the nitrogen rule, while addition of a methylene group in gliclazide was not feasible under the given alkaline hydrolytic conditions, suggesting that product V was not formed from gliclazide directly. Increase in molecular mass of product V by 16 Da with respect to product VI suggested that the former could be produced from the latter by addition of an oxygen atom. Formation of product V from product VI was further supported by LC profiles of gliclazide in an alkaline stress condition, where V was seen only after the formation of VI.[19] The fragment peaks at m/z 170 and 106 in the −ESI LC/MS scan of V, as in gliclazide and products I and VI (Figure 3I versus Figures 3F, 3G and 3J), indicated that left side of gliclazide was retained in product V. The heaviest fragment peak at m/z 123 in MS/MS scan of product V (Figure 4D) indicated it to be contributed by cyclopentapyridazine nucleus (right side) of product VI. However, absence of any peak at m/z 151 or 153 suggested that the right side was not bonded to C=O group as in product VI and gliclazide. A difference of 44 Da between molecular mass of product V (337 Da) and sum of masses of the heaviest fragments in −ESI and +ESI MS scans, i.e., m/z 170 (F_6) and m/z 123, respectively, could be attributed to CO_2.[6] This suggested that the two heaviest fragments were linked through –C(=O)O– (carbonyloxy) linkage. Hence, structure of product

V was proposed by assembling F_6, fragment of m/z 123 and CO_2 (Figure 7) as 4,4a,5,6,7,7a-hexahydro-3H-cyclopenta[d]pyridazin-1-yl *N*-(*p*-tolylsulfonyl)carbamate, which underwent fragmentation (Figure 5) in accordance with MS/MS and −ESI LC/MS scans.

Characterization of Product VII

The product VII was detected as peak at m/z 141 $[M - H]^-$ in the −ESI LC/MS scan (Figure 3K) but it was not detected in the +ESI mode. Additional peaks at m/z 223 and m/z 305 spanned at a difference of 82 Da were attributed to adduct ions $[M - H + 2ACN]^-$ and $[M - H + 4ACN]^-$, respectively. Formation of these adduct ions was possible due to the presence of acetonitrile in the mobile phase. Further, absence of peak of product VII in the LC/UV chromatogram suggested that it was a non-chromophoric compound. Based on degradation behaviour of the drug in alkaline medium this product was proposed to be (2-diazenylmethyl)cyclopentyl methanol.

Pathways to the Formation of Degradation Products

Formation of Products I and II

The sulfonylureas are hydrolyzed to a sulfonamide and an amine with liberation of CO_2 upon cleavage of the amide linkage.[14] This cleavage is facilitated in acidic medium by protonation either at the C=O oxygen (O-protonation) or the amide nitrogen (N-protonation), followed by

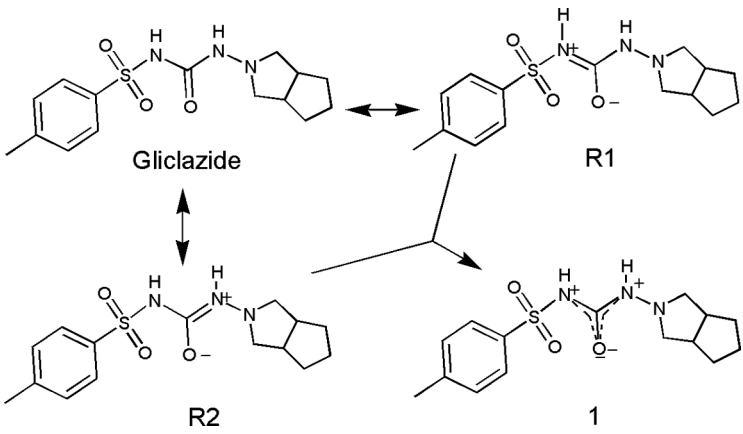

Figure 9. Resonating structures of gliclazide favoring O-protonation.

nucleophilic attack of water on the carbonyl carbon. Literature reports suggest that any factor that increases electron density at C=O oxygen favors O-protonation, and *vice versa* for N-protonation.[31,32] While sulfonylurea is reported to hydrolyze through N-protonation,[33] the presence of two –NH– groups besides C=O increases the electron density on C=O oxygen (1) due to two resonating structures (R1 and R2), and, hence, even favors O-protonation (Figure 9). Therefore, hydrolysis of amide linkage in gliclazide occurs either through O- or N-protonation routes, as postulated in Figure 10. Nucleophilic attack of water on

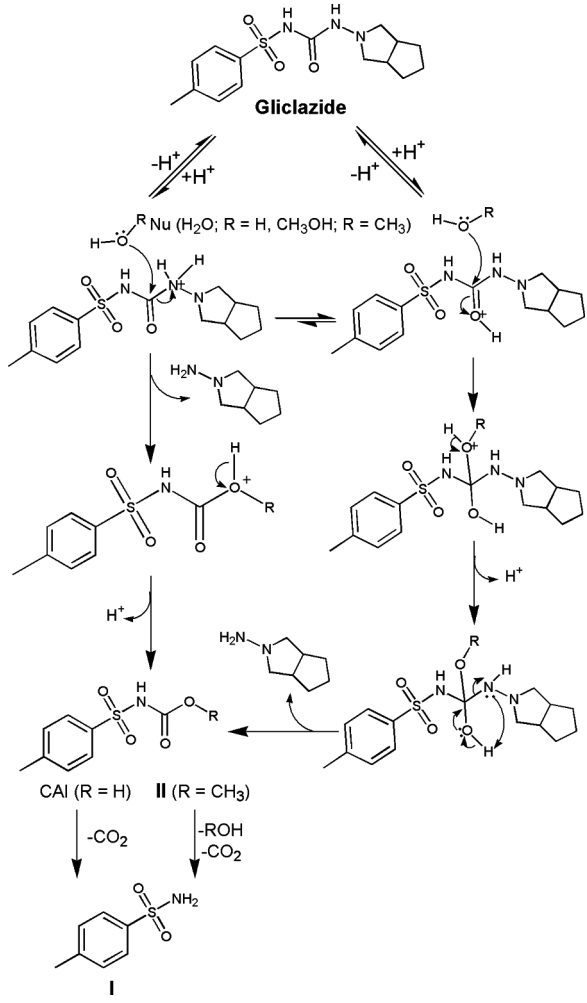

Figure 10. Proposed degradation route of gliclazide to products I and II.

C=O carbon is expected to result in cleavage of the C-N bond to produce a carbamic acid intermediate (CAI), which would decarboxylate immediately to produce sulfonamide (product I). On using methanol as solubiliser, an attack of more nucleophilic methanolic oxygen on C=O carbon generates urethane (product II), which is subsequently hydrolysed to CAI, and finally decarboxylates[34] to product I (Figure 9). This is supported by LC profiles in acidic and neutral media, wherein the decrease in product II was accompanied by an increase in product I.[19]

Formation of Product VI

The pathway of conversion of gliclazide to product VI is proposed in Figure 11. The same is explained on the basis of acidic character of α-hydrogens of aliphatic strained rings[16] and nucleophile mediated ring expansion of strained ring systems.[30] Abstraction of α-hydrogen of the fused pyrrollidine nucleus in alkaline medium generates a carbanion (i), which attacks the electrophilic carbon of the central C=O group, resulting in formation of a nitranion (ii). The latter undergoes ring expansion to product (iii), which tautomerises to stable isomer VI.

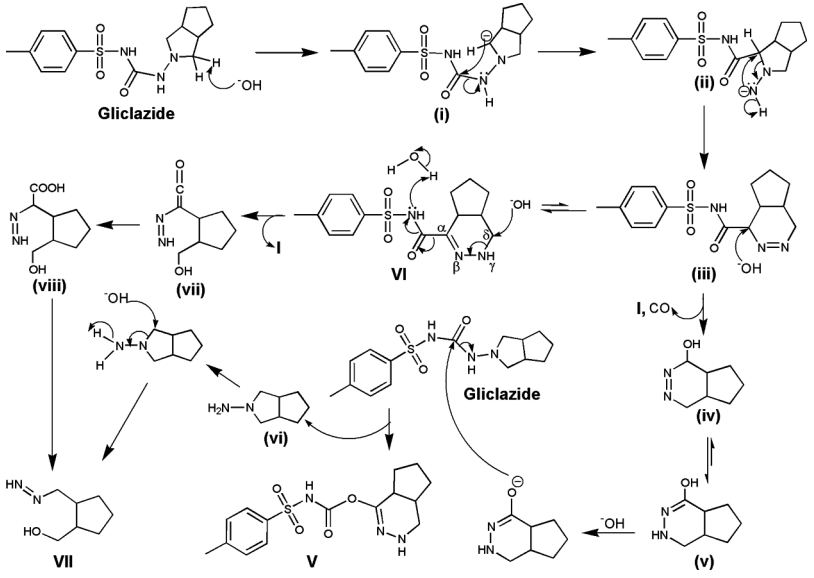

Figure 11. Degradation route of gliclazide to products I, V, VI, and VII.

Formation of Products V and VII

The study of LC chromatograms in alkali revealed that product V was formed only after appearance of product VI, and subsequently it increased continuously at the cost of the latter.[19] LC/UV chromatograms of alkaline hydrolytic samples also showed a simultaneous increase in product I,[19] despite the known stability of sulfonylureas in alkaline medium.[15] Further, a very prominent peak of product VII at m/z 141 in the LC/MS chromatogram of the sample in −ESI mode at relative retention time of 0.20 suggested the formation of a non-chromophoric or weak chromophoric product of molecular mass of 142 Da. Based on these observations, it is postulated that product V was formed from product VI in such a way that products I and VII were also formed. The proposed conversion of VI to V (Figure 11) is explained by the nucleophilic attack of OH^- on the pyridazine ring carbon flanked by carbonyl and the ring nitrogen of (iii), leading to formation of I and (iv), with a loss of CO. The intermediate (iv) is supposedly tautomerized to a more stable isomer (v) because of extension of conjugation from a lone pair of electrons of oxygen to that of nitrogen (-NH-) through the C=N bond. In alkaline medium, (v) should exist as an alkoxide ion, which attacks the carbonyl carbon of gliclazide to displace its right side as hexahydro-cyclopenta[c]pyrrol-2-ylamine (vi) to form V. Finally, it is postulated that (vi) undergoes a nucleophile mediated ring opening to give 2-(diazenylmethyl)cyclopentyl methanol (VII). Alternatively, a nucleophilic attack of OH^- on δ-carbon of the α, β-unsaturated carbonyl system of VI results in a ketene (vii), which is readily hydrolyzed to carboxylic acid (viii) and subsequently decarboxylated in alkaline medium to form VII.

CONCLUSION

LC/MS and LC/MS/MS studies on gliclazide have been carried out to characterize all the six (I–VI) degradation products formed in acidic and alkaline hydrolytic stress conditions. A mass fragmentation pattern of gliclazide has been proposed on the basis of mass spectra in +ESI and −ESI modes and known mass spectral reports to facilitate structural characterization of degradation products through comparative mass spectral data. The degradation product I is isolated and characterized through IR, NMR, and Mass spectral analyses as *p*-tolylsulfonamide, which is a known degradation product and pharmacopoeial impurity. The additional findings of the present study are that the product II is formed only when methanol is used to solubilize the drug in reaction solutions and is characterized as *N*-(*p*-tolylsulfonyl)carbamate.

The products V and VI are formed in alkaline medium and are characterized as 4,4a,5,6,7,7a-hexahydro-3H-cyclopenta[d]pyridazin-1-yl *N*-(*p*-tolylsulfonyl)carbamate and *N*-(4,4a,5,6,7,7a-hexahydro-3H-cyclopenta[d]pyridazine-1-carbonyl)-4-methyl benzenesulfonamide, respectively. The products III, IV, and VI are not ionized in both +ESI and −ESI modes and, hence, are not characterized. A new peak of product VII has been noted in the LC/MS chromatogram, which is not seen in the LC/UV chromatogram, and is characterized as 2-(diazenylmethyl)cyclopentyl methanol. The routes of degradation of gliclazide in acidic and alkaline media have been proposed based on the known reactivities of sulfonylureas and strained ring systems in different chemical environments.

REFERENCES

1. International Conference on Harmonisation, ICH Q3A(R2) Impurities in New Drug Substances 2006.
2. International Conference on Harmonisation, ICH Q3B(R2) Impurities in New Drug Products 2006.
3. International Conference on Harmonisation, ICH Q3C(R3) Impurities: Guidelines for Residual Solvents 2005.
4. Committee for Proprietary Medicinal Products, CPMP Guidelines on Control of Impurities of Pharmacopoeial Substances 2004.
5. International Conference on Harmonisation, ICH Q1A(R2) Stability Testing of New Drug Substances and Products 2003.
6. Smyth, W.F.; Brooks, P. A critical evaluation of high performance liquid chromatography-electrospray ionisation-mass spectrometry and capillary electrophoresis electrospray- mass spectrometry for the detection and determination of small molecules of significance in clinical and forensic science. Electrophoresis **2004**, *25*, 1413–1446.
7. Chen, G.; Pramanik, B.N.; Liu, Y.; Mirza, U.A. Applications of LC/MS in structure identifications of small molecules and proteins in drug discovery. J. Mass Spectrom. **2007**, *42*, 279–287.
8. Pan, C.; Liu, F.; Ji, Q.; Wang, W.; Drinkwater, D.; Vivilecchia, R. The use of LC/MS, GC/MS, and LC/NMR hyphenated techniques to identify a drug degradation product in pharmaceutical development. J. Pharm. Biomed. Anal. **2006**, *40*, 581–590.
9. Reddy, G.M.; Bhaskar, B.V.; Reddy, P.P.; Sudhakar, P.; Babu, J.M.; Vyas, K.; Reddy, P.R.; Mukkanti, K. Identification and characterization of potential impurities of rabeprazole sodium. J. Pharm. Biomed. Anal. **2007**, *43*, 1262–1269.
10. Francese, G.; Corana, F.; Meneghetti, O.; Marazza, F. LC-MS characterization of trace impurities contained in calcium folinate. J. Pharm. Biomed. Anal. **2005**, *39*, 757–763.

11. Barbarin, N.; Henion, J.D.; Wu, Y. Comparison between liquid chromatography-UV detection and liquid chromatography-mass spectrometry for the characterization of impurities and/or degradants present in trimethoprim tablets. J. Chromatogr. A **2002**, *970*, 141–154.
12. Ermer, J. The use of hyphenated LC-MS technique for characterization of impurity profiles during drug development. J. Pharm. Biomed. Anal. **1998**, *18*, 707–714.
13. da Tos, V.; Maran, A.; de Kreutzenberg, S.V.; Marchetto, S.; Tadiotto, F.; Bettio, M.; Marescotti, M.C.; Tiengo, A.; Prato, S.D. Mechanisms of acute and chronic hypoglycemic action of gliclazide. Acta Diabetol. **2000**, *37*, 201–206.
14. Kurzer, F. Sulfonylurea and sulfonylthioureas. Chem. Rev. **1952**, *50*, 1–46.
15. Sarmah, A.K.; Sabadie, J. Hydrolysis of sulfonylurea herbicides in soils and aqueous solutions: A review. J. Agric. Food. Chem. **2002**, *50*, 6253–6265.
16. Lwowski, W. Comprehensive heterocyclic chemistry. In *The Structure, Reactions, Synthesis and Uses of Heterocyclic Compounds*, Vol. 7; Lwowski, W., Ed.; Pergamon Press: Oxford, 1984; Part 5, 17 p.
17. El Kousy, N.M. Stability-indicating densitometric determination of some antidiabetic drugs in dosage forms using TLC. Mikrochimica Acta **1998**, *128*, 65–68.
18. *British Pharmacopoeia*; Published by The Stationary Office on behalf of the Medicines and Healthcare Products Regulatory Agency (MHRA): London, 2005; CD-ROM.
19. Bansal, G.; Singh, M.; Jindal, K.C. Forced degradation study on gliclazide and application of validated stability-indicating HPLC-UV method in stability testing of gliclazide tablets. Chromatographia **2007**, *66*, 751–755.
20. Khan, M.A.; Sinha, S.; Vartak, S.; Bhartiya, A.; Kumar, S. LC determination of glimepiride and its related impurities J. Pharm. Biomed. Anal. **2005**, *39*, 928–943.
21. Wang, M.; Miksa, I.R. Multi-component plasma quantitation of anti-hyperglycemic pharmaceutical compounds using liquid chromatography–tandem mass spectrometry. J. Chromatogr. B **2007**, *856*, 318–327.
22. Liang, J.; Tian, Y.; Zhang, Z.; Feng, S.; Zhao, Y.; Mao, G. High-performance liquid chromatography-electrospray ionization mass spectrometry determination of mitiglinide in human plasma and its pharmacokinetics. J. Mass Spectrom. **2007**, *42*, 171–177.
23. Hoizey, G.; Lamiable, D.; Trenque, T.; Robinet, A.; Binet, L.; Kalltenbach, M.L.; Havet, S.; Millart, H. Identification and quantification of 8 sulfonylureas with clinical toxicology interest by liquid chromatography-ion-trap tandem mass spectrometry and library searching. Clin. Chem. **2005**, *51*, 1666–1672.
24. Ho, E.N.M.; Yiu, K.C.H.; Wan, T.S.M.; Stewart, B.D.; Watkins, K.L. Detection of anti-diabetics in equine plasma and urine by liquid chromatography-tandem mass spectrometry. J. Chromatogr. B **2004**, *811*, 65–73.
25. Zhong, P.; Bi, H.; Zhou, S.; Chen, X.; Huang, M. Simultaneous determination of metformin and gliclazide in human plasma by liquid chromatography-tandem mass spectrometry: application to a bioequivalence

study of two formulations in healthy volunteers. J. Mass Spectrom. **2005**, *40*, 1462–1471.
26. Tiller, P.R.; Land, A.P.; Jardine, I.; Murphy, D.M.; Sozio, R.; Ayrton, A.; Schaefer, W.H. Application of liquid chromatography–mass spectrometry^n analyses to the characterization of novel glyburide metabolites formed in vitro. J. Chromatogr. A **1998**, *794*, 15–25.
27. Kemp, W. *Organic Molecular Spectroscopy*; Palgrave: New York, 2002; 87 pp.
28. *Eight Peak Index of Mass Spectra*, Vol. 3; Mass Spectrometry Data Center: Aldermaston, 1974; Part 2, 2475 pp.
29. Gilchrist, T.L. *Heterocyclic Chemistry*; Pearson Education (Singapore) Pte. Ltd.: Delhi, 2005; 42 pp.
30. March, J. *Advanced Organic Chemistry*; John Wiley & Sons: New York, 1992; 1077 pp.
31. Homer, R.B.; Johnson, C.D. In *The Chemistry of Amides*; Zabicky, J., Ed.; Interscience Publishers: London, 1970; 187 pp.
32. Challis, B.C.; Challis, J.A. In *The Chemistry of Amides*; Zabicky, J., Ed.; Interscience Publishers: London, 1970; 731 pp.
33. Baertschi, S.W.; Alsante, K.M. In *Pharmaceutical Stress Testing: Predicting Drug Degradation*; Baertschi, S.W., Ed.; Taylor & Francis: New York, 2005; 83–85 pp.
34. Cason, J. *Principles of Modern Organic chemistry*; Prentice-Hall: New Jersey, 1966; 336 pp.

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