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Mass spectra of gliclazide drug at various ion sources temperature

Its thermal behavior and molecular orbital calculations

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Abstract Gliclazide (GL, $C_{15}H_{21}N_3O_3S$) drug is used as non-insulin-dependant diabetes mellitus. The drug was investigated using thermal analysis (TA) measurements (TG/DTG) and electron impact mass spectral (EI–MS) fragmentation at 70 eV techniques. The mass spectra of GL at different values of ion source temperatures (400, 416, 425, and 440 K) are recorded and investigated. Semiempirical MO calculation, using PM3 procedure, has been carried out on neutral molecule and positively charged species. These calculations included bond length, bond order, bond strain, partial charge distribution, ionization energy, and heats of formation (ΔH_f). PM3 procedure provides a basis for fine distinction among sites of initial bond cleavage, which is crucial to the rationalization of subsequent fragmentation of the molecule. The primary fragmentation pathway in both TA and MS (at different values of ion source temperature) is initiated by S–N bond rupture. TA and DTG show one main weight loss at 250.38 °C and four peaks at 271.6, 360.99, 427.93 and 479.17 °C in DTA, which may be attributed to various fragments. Also, the rate constant (K') of thermal degradation has been tested isothermally at 210 and 600 °C. The calculated rate values are 9.6×10^{-3} and 0.33×10^{-3} s^{-1}, respectively, and discussed. In MS, the effect of ion source temperature on mass spectral fragmentation processes is discussed on the basis of energy considerations using quasi equilibrium theory.

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Introduction

Gliclazide (GL, Fig. 1), *N*-(4-methylbenzenesulfonyl)-*N*-(3-azabicyclo-[3.3.0] act-3yl) urea, is a second-generation of sulfonylurea commonly used in the treatment of non-insulin-dependent diabetes mellitus (NIDDM) [1–3]. GL undergoes extensive hepatic metabolic biotransformation to give seven inactive metabolites [1, 4, 5]. These metabolites are excreted in urine and feces [1, 2]. Several methods were reported in the literature for determination of GL in biological specimens using high performance liquid chromatography (HPLC), capillary electrophoresis (EC) methods [4–8] and using electrospray ionization (ESI) tandem mass spectrometry (MS–MS) [9].

Rapid advances in biological sciences have led to an increased demand for chemical and structural information from biological systems. Mass spectrometry plays a pivotal role in the structural characterization of biological molecules [10]. The technique is important because it provides a lot of structural information with little expenditure of the sample. Also, the techniques offer comparative advantages for speed and productivity for pharmaceutical analysis [11]. On the other hand, thermal analysis (TA) technique that delivers extremely sensitive measurements of heat change can be applied on a broad scale with pharmaceutical development. These methods provide unique information relating to thermodynamic data of the system studied [12]. The increasing use of the combined techniques in TA can provide more specific information, and thus facilitates more rapid interpretation of the curves obtained [12].

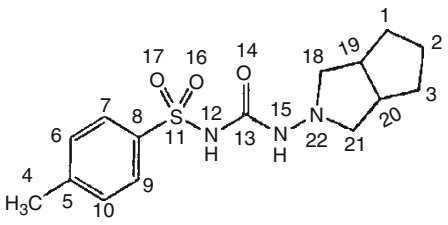


Fig. 1 Structure and numbering system of GL for C, N, O, and S skeleton

In electron ionization (EI) mass spectra, the fragmentation consists of competitive and consecutive unimolecular fragmentation [13]. The fragmentation of ionized molecule depends mainly on their internal energy [14]. The thermogravimetric TG/DTG analysis [15–17] used to provide quantitative information on weight losses due to decomposition and/or evaporation of low molecular materials as a function of time and temperature. In conjunction with mass spectrometric analysis [14], the nature of the released volatilize may be deduced, thus greatly facilitating the interpretation of thermal degradation processes. On the other hand, computational quantum chemistry can provide additional information about the atoms and bonds, which can be used successfully in an interpretation of experimental results [18]. Application of computational quantum chemistry in addition to experimental results (MS and TA) gives valuable information about the atoms and bonds which helps in the description and prediction of primary fragmentation site of cleavage and subsequent one [19–22].

The fragmentation of large organic ions produced be EI-MS has considerable similarity to the fragmentation processed associated with the thermal decomposition of organic molecules [18]. This similarity consists of large body of observations which indicate that ions undergo bond ruptures at weak bonds, loss of molecular species and molecular rearrangements, all of which are known to occur preferentially in thermal reactions [15–17]. In addition, fragmentation threshold on the ions indicate that in most instance the preferred processes are those which have the lowest energy requirement. It is noteworthy that at 100 °C the average thermal energy of such a rather small molecule is already 0.2 eV are rise more than 1 eV at 1000 °C. The typical temperature of an electron ionization source ranges from 150 to 250 °C. With increasing molecular weight the maximum of thermal energy distribution in mass spectra is shifted to even higher energies [13].

The aim of this study is focusing on further application of our previous study on GL drug [19–22]. This study includes a correlation between, mass spectral fragmentation and thermal analyses degradation of the drug and compared the experimental data with the theoretical MO calculations to identify the weakest bonds ruptured during both mass and thermal studies. Consequently the choice of the correct pathway of such fragmentation knowing this structural session of bonds can be used to decide the active sites of the drug responsible for its chemical, biological, and medical reactivities. In addition to this work, thermal energy effect (related to ion source temperature) on the breakdown fragmentation is discussed.

Experimental

Mass spectrometry (MS)

Electron ionization (EI) mass spectra of GL were obtained using Shimadzu GC–MS–Qp 1000 PX quadruple mass spectrometer with electron multiplier detector equipped with GC–MS data system. The direct probe for solid material was used in this study. The sample was put into a glass sample micro vial, by a needle (≈ 1 μg max), the vial installed on the tip of the DP containing heating cable and inserted into the evacuated ion source. The sample was ionized by electron beam emitted from the filament, the generated ions being effectively introduced into the analyzer by the focusing and extractor lenses system. The MS was continuously scanned and the spectra obtained were stored. EI mass spectra were obtained at ionizing energy value of 70 eV, ionization current of 60 μA, and vacuum is better than 10^{-6} torr.

Thermal analyses

The thermal analyses of GL drug were made using conventional thermal analyzer (Shimadzu system of DTA-50 and 30 series TGA-50). The mass losses of 5-mg sample and heat responds of the change of the sample were measured from room temperature up to 600 °C. The heating rate used was 10 °C min^{-1} in an inert argon atmosphere. These instruments were calibrated using indium metal as a thermal stable material. The reproducibility of the instrument reading was determined by repeating each experiment more than twice.

Quantum chemical calculations

The MO calculations were performed using semiempirical molecular orbital calculation. The method used in these computations is the parametric method (PM-3) described by Stewart [23]. The geometric of all stable species studied was completely optimized with respect to all geometrical variables using the Eigen vector following (EF) routine [24]. The program is run under the molecular orbital calculation package MOPAC2000 by Stewart [25] for microcomputers.

Results and discussion

It is of great interest to study the chemistry and reactivity of GL drug because of its importance in medicine. Knowledge obtained from thermal decomposition mechanisms of the neutral drug is very important to understand the chemical process that shard in biological systems. It is difficult to establish the exact major fragmentation pathway in El using conventional MS. Combining of the above two techniques and the data obtained from the MO calculation, it is possible to understand the following topics:

1. The stability of the drug under thermal degradation in solid state and mass spectral fragmentation in gas phase.
2. Prediction of the primary site of the fragmentation in both techniques, which helps to rationalize subsequent, bond cleavage.
3. The correct pathway in both techniques.
4. Consequently the understanding of what is actually happened in biodegradation of the drug or its derivatives in vivo system and its metabolites.
5. The effect of different ion source temperature on the fragmentation pattern.

Thermal analyses

The TG (Fig. 2) of GL shows one main mass loss at the temperature range 130–600 °C of practical mass loss percent = 89.57%. This means that GL drug is completely dissociated in this temperature range. This main mass loss occurs at 250.38 °C as indicated by DTG (Fig. 2). Consequently TG cannot give the detailed description of thermal gradual decomposition of the drug which may be due to the fast consecutive or parallel degradation of this compound as given by the proposed thermal decomposition Scheme 1. These detailed physical and chemical changes occurring during thermal degradation can be given by the DTA (Fig. 3).

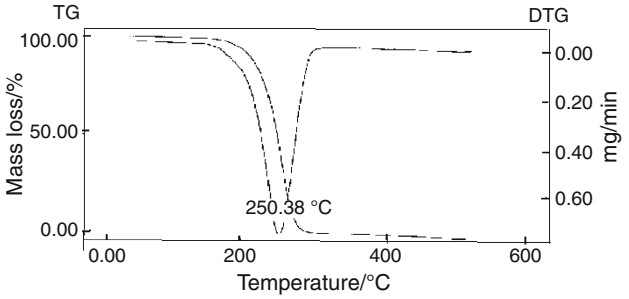


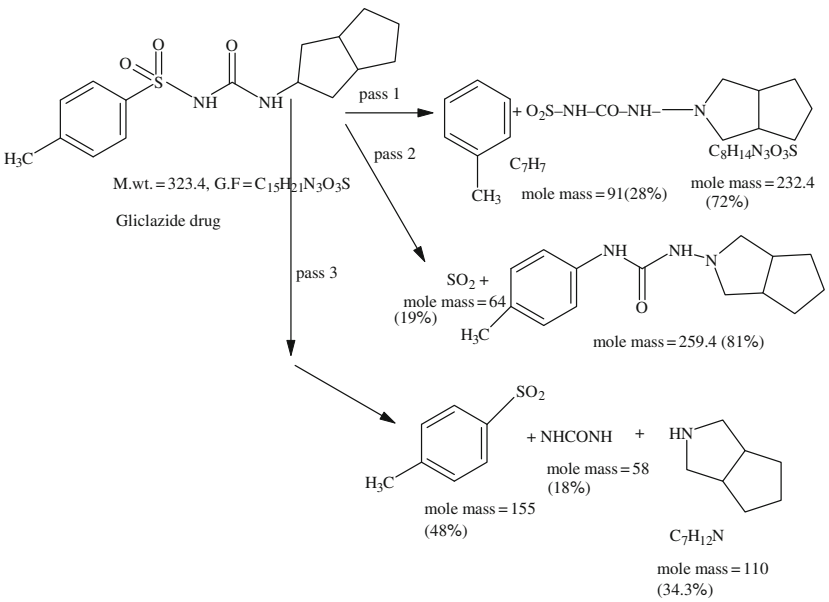
Fig. 2 The TG curve and DTG curve of GL

These data show very sharp endothermic peak at 164.24 °C, which reasonably accounts for the sharp melting of GL drug (its measured m.p. = 163–165 °C). The endothermic peaks at 271.6, 360.99, 427.93, and 479.17 °C may be attributed to the various proposed fragments given by Scheme 1.

The exothermic peak at 614.14 °C, which out of the decomposition temperature range as given by TG, may be assigned as the recombination of some fragments to form new chemical compounds such as the radical $O{=}SO^-$ changed into SO_2 gas and the change of the loosed NHCONH radical into a cyclic form. Also the formation of $CH_3C_6H_4{-}SO_2^-$ radical which may be changed into $CH_3{-}C_6H_4{=}S{=}O_2^-$ conjugate system.

The rate of thermal degradation of GL has been tested by the isothermal changes of 2.536 and 4.441 mg of the

Scheme 1 Thermal decomposition scheme of gliclazide drug



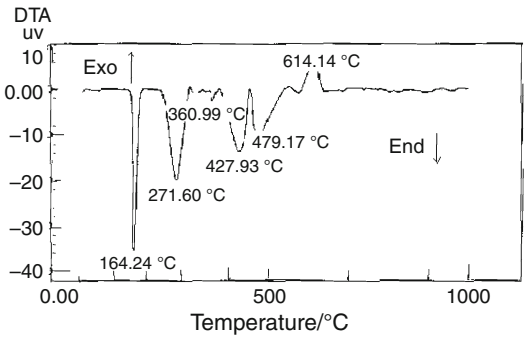


Fig. 3 The DTA curve of GL

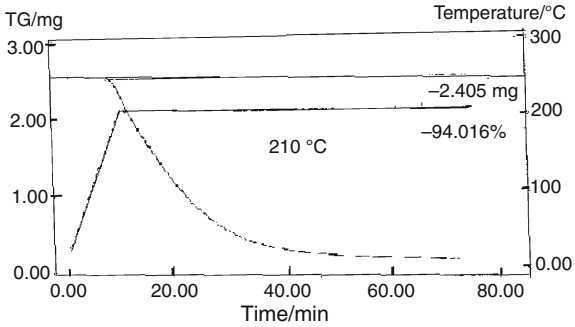


Fig. 4 Mass loss–time curve at temperature 210 °C

drug occur at two different temperatures 210 and 600 °C, respectively. The rate constant (K') values of the isothermal degradation of the drug at these two temperatures are measured by the plot of mg mass loss against time (min) and considering the process is a pseudo first-order, using the rate equation,

Rate $= K'\,[i]$ where the i is the decomposed species and $K' = 0.695/t_{0.5}$. The $t_{0.5}$ is the half-life time of the decomposed species i.

At the temperature 210 °C, the mg mass loss–time curve (Fig. 4) gives,

$$\delta w = 2.2 - 0.3 = 1.9 \text{ mg}, \; \delta t = 18.00 - 0.0 = 18 \text{ min} = 18 \times 60 \text{ seconds (s)},$$

therefore

$$t = 2.536 \times 1080/1.9 = 144 \text{ s and } t_{0.5} = 72 \text{ s and}$$

$$K' = 0.695/72 = 0.0096 \quad = 9.6 \times 10^{-3} \text{ s}^{-1}$$

At the temperature, 600 °C (Fig. 5), the mass taken of the sample was 4.441 mg at which, $\delta w = 4.00 - 3.5 = 0.5$ mg and $\delta t = 46.0 - 38.0 = 8.0$ min $\times$ 60 = 480 s and $t = 480 \times 4.441/0.5 = 71 \times 60 = 4260$ s and $t_{0.5} = 4260/2 = 2130$ s and $K' = 0.695/2130 = 0.33 \times 10^{-3}$ s^{-1}. From these data it is clear that the rate of decomposition of GL at 210 °C is more than the rate of decomposition of the stable remainder part at 600 °C which is actually logic.

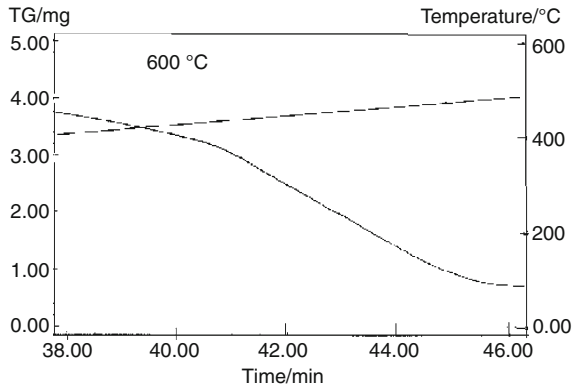


Fig. 5 Mass loss–time curve at temperature 600 °C

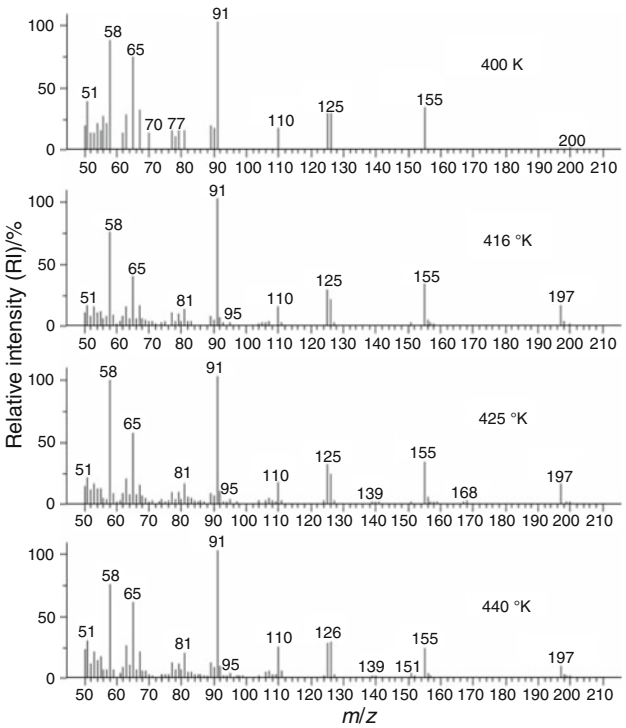


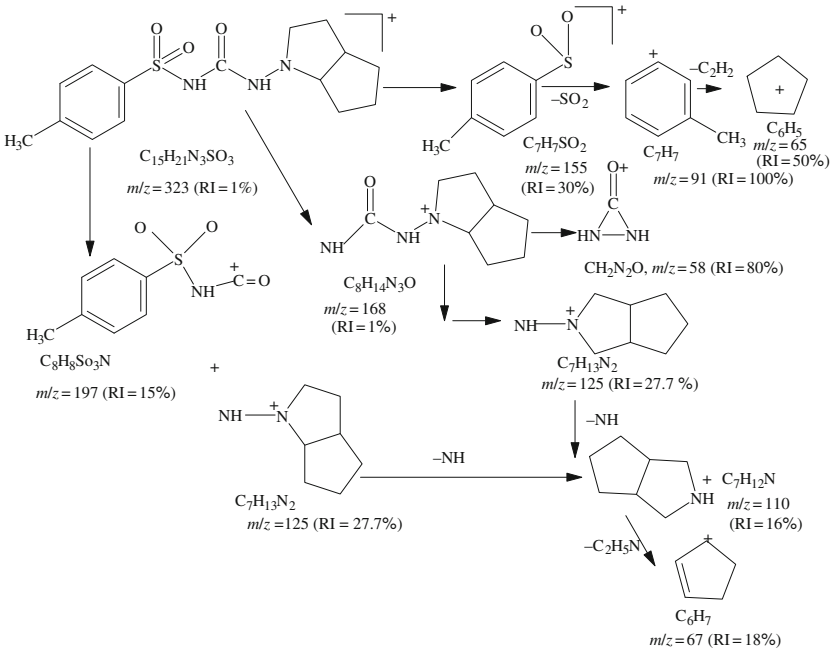
Fig. 6 70 eV mass spectrum of GL at different ion source temperature

Mass spectral (MS) fragmentation

Mass spectrometric techniques offer comparative advantages for speed and productivity for pharmaceutical analysis [11]. EI mass spectra of GL drug at 70 eV with different ion source temperature of values at 400, 416, 425, and 440 K are recorded and investigated as shown in Fig. 6.

The spectra are characterized by many competitive and consecutive fragmentation pathways forming many intense fragment ions. The proposed main fragmentation pathways following electron impact of GL was displayed in Scheme 2. On the other hand, no signal corresponding to

Scheme 2 Proposed mass spectral fragmentation of gliclazide drug



molecular ion at $m/z = 323$ $[C_{15}H_{21}N_3O_3S]^+$ and all fragment ions above $m/z = 197$ (Fig. 7).

Two important ions can be observed in MS of GL at signals $m/z = 155$ and 197. The first one is due to S–N bond cleavage and formation of methylbenzene sulphonyl $[C_7H_7SO_2]^+$, which can undergo the loss of SO_2 molecule to form the base peak (RI = 100%) at $m/z = 91$ (C_7H_7). The second one was observed at signal $m/z = 197$ which is mainly due to the rupture of CO–N bond (nearest to bicyclic rings) forming $[C_8H_8NO_3S]^+$ ion. This fragment ion is absent in the mass spectrum related to temperature at 400 K. Fragment ion observed at $m/z = 58$ (RI = 80%) (the second prominent peak in the mass spectra) is presumably formed from the cleavage of N–N and S–N bonds forming fragment of urea ion $[N_2H_2CO]^+$.

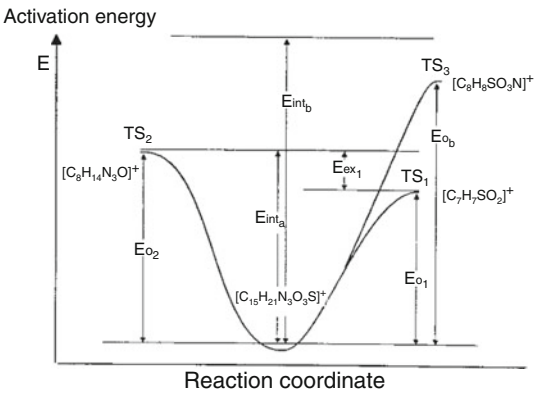


Fig. 7 Energy evaluation of competitive mass spectral fragmentation pathways

Computation

Molecular orbital (MO) calculation gives valuable information about the structure and reactivity of the molecules, which actually be used to support the experimental evidence. The much important parameters calculated using MO calculation includes bond orders, bond length, charge distribution, bond strain, and heats of formation.

In this study the calculations have been carried out on GL, neutral molecule (as in TA decomposition), and charged molecular ion (as in MS fragmentation) which are used for prediction of the weakest bond rupture to follow the fragmentation pathways in both techniques.

Figure 1 shows the numbering system of GL skeleton that helps in ordering the bond length, bond order, and bond strain and charge distribution.

Table 1 presents the values of bond length (Å), bond order, and bond strain (kJ mol^{-1}) from which one can conclude that,

1. Small differences in bond length in GL system upon ionization, indicating no appreciable change in the geometries upon ionization.
2. The lowest bond order (important for prediction of primary site of cleavage) observed at S11–N14 bond for both neutral (0.628) and positive species (0.501).
3. Upon ionization the stability of the molecule was decreased by -80.860 k Cal mol^{-1} (ΔH_f neutral $(-78.652) - \Delta H_f$ ion (2.208)).

The charge distributions on different atoms (C, N, O, and S) for neutral and charged species are summarized in Table 2. Significant change in the charge distribution with

Table 1 Comparison of computed bond length (in Å), bond order, and bond strain (kJ mol^{-1}) using PM3 method for neutral and molecular cation

Bond	Bond length/Å		Bond order		Bond strain/kJ mol^{-1}	
	Neutral	Cation	Neutral	Cation	Neutral	Cation
C1–C2	1.524	1.524	0.990	0.994	0.027	0.027
C1–C19	1.531	1.532	0.982	0.984	0.077	0.077
C3–C20	1.524	1.524	0.990	0.994	0.027	0.027
C3–C25	1.531	1.532	0.982	0.983	0.079	0.080
C4–C5	1.484	1.483	1.004	1.011	0.029	0.031
C5–C6	1.396	1.398	1.393	1.387	0.018	0.019
C5–C10	1.396	1.398	1.394	1.387	0.016	0.016
C6–C7	1.388	1.386	1.445	1.467	0.012	0.012
C7–C8	1.398	1.402	1.383	1.360	0.017	0.017
C8–C9	1.398	1.402	1.384	1.360	0.019	0.020
C8–S11	1.783	1.747	0.706	0.762	0.040	0.040
C9–C10	1.388	1.386	1.443	1.467	0.010	0.010
S11–N12	1.775	1.820	0.628	0.501	0.010	0.011
S11–O16	1.440	1.425	1.245	1.287	0.000	0.000
S11–O17	1.442	1.426	1.233	1.283	0.000	0.000
N12–C13	1.439	1.396	0.990	1.144	0.010	0.010
C13–O14	1.216	1.209	1.806	1.846	0.002	0.002
C13–N15	1.454	1.519	1.002	0.812	0.003	0.003
N15–N22	1.475	1.352	0.960	1.147	0.049	0.048
C18–C19	1.533	1.532	0.977	0.982	0.078	0.076
C18–N22	1.498	1.488	0.981	0.949	0.035	0.033
C19–C20	1.542	1.547	0.969	0.964	0.183	0.186
C20–C21	1.533	1.532	0.976	0.982	0.068	0.067
C21–N22	1.497	1.491	0.977	0.952	0.034	0.036

given system often takes place during the ionization process [26]. For neutral species the highest positive charges are that located on S11 (2.318) > C13 (0.304) atoms, while for negative charge O_{17} (−0.818) < O_{16} (−0.805) < O_8 (−0.604) < N_{12} (−0.523) < O_{14} (−0.348). For neutral species, positive charges are that located on S11 (2.365) > C13 (0.326) > N22 (0.288) and for negative charges O_{17} (−0.788) < O_{16} (−0.783) < O_8 (−0.653) < N_{12} (−0.473) < O_{14} (−0.303). The greatest change occur in the charge upon electron rupture is that occur on N22 (changed from 0.074 to 0.288) which is surely a site of electron rupture upon ionization at values, 9.14 eV. No appreciable change in bond order upon ionization except for C13–N15 and S11–N12 the bond order decreases by 0.190 and 0.127, respectively.

Correlation of TA decomposition and MO calculation for neutral molecules

Gliclazide is an essential drug used in remedy of diseases. In the literature there is no study on the thermal stability of this drug with temperature changes either in vitro or in vivo systems. As indicated previously [18], a determination of initial bond cleavage would be an important first step using these calculations in predictive manner. On the base of MO calculation the bond S11–N12 (Table 1) refers to the possible starting decomposition of neutral compound since this bond represents the lowest bond order in the system (0.628) with large bond length (1.775 Å) and bond strain = 0.010 kJ mol^{-1}. Also, the C8–S11 represents the second lowest bond order in the system (0.706) with bond length at 1.783 Å and bond strain at 0.040 kJ mol^{-1}. The inspection of partial change localized on C8 (−0.604), S11 (2.318), and N12 (−0.523) (Table 2) facilitates a rapid decomposition of these two bonds (S11–N12 and C8–S11) causing consecutive and parallel degradation as discussed before (path 1–3).

Table 2 Comparison of computed partial charge (for C, N, O, and S) on different atoms for neutral and molecular ion

Atom	Neutral	Charged
C1	−0.095	−0.099
C2	−0.097	−0.102
C3	−0.095	−0.098
C4	−0.078	−0.091
C5	−0.01	0.029
C6	−0.137	−0.133
C7	0.008	0.021
C8	−0.604	−0.653
C9	−0.013	0.021
C10	−0.137	−0.133
S11	2.318	2.365
N12	−0.523	−0.473
C13	0.304	0.326
O14	−0.348	−0.303
N15	−0.055	−0.013
O16	−0.805	−0.783
O17	−0.818	−0.788
C18	−0.076	−0.112
C19	−0.091	−0.085
C20	−0.092	−0.084
C21	−0.065	−0.121
N22	−0.074	0.288

Correlation of MS fragmentation and MO calculations for charged molecular ion, with the effect of thermal energy of the fragmentation process

The scope of the present investigation is restricted to search for prediction of the first bond rupture and subsequent one. The subsequent fragmentation in MS is determined to large extent by the initial bond rupture of molecular ion [27]. Computational quantum chemistry can provide additional

information which can be used successfully for interpretation of experimental results. These theoretical data can particularly be valuable for MS because they study in gas phase state, which can be handled much more easily by quantum chemistry than those surrounded by solvent [18]. Mass spectra of GL system show three competitive processes (Scheme 2) including principle fragmentation pathways. PM3 semiempirical molecule orbital calculation data (Fig. 1; Table 1) reveal that the S11–N12 has the smallest bond order = 0.501 of the charged system and the longest bond length = 1.820 Å with bond strain = 0.011 kJ mol^{-1}. Also, due to the force of attraction between C8 (−0.653) and S11 (2.365) (Table 2) which is larger than the force between N12 (−0.473) and S11 (2.365), consequently, one can expect that the S11–N12 is the first site for bond rupture (Scheme 1, process 1) and formation of two ring systems, i.e., methylbenzene sulphonyl (m/z = 155, $[C_7H_7SO_2]^+$) and azabicyclo urea (m/z = 168, $[C_8H_{14}N_3O]^+$) (Scheme 1 processes 1 and 2). Methylbenzene sulphonyl can undergo sulphonyl (SO_2) loss to form the most prominent peak (base peak, RI = 100%) at m/z = 91 $[C_7H_7]^+$ in the mass spectra at different ion source temperature (bond order = 0.762, bond length = 1.747 Å, bond strain = 0.040 kJ mol^{-1}) (process 1). On getting the look to another system (azabicyclo urea, m/z = 168), no signal can be observed for the presence of this fragment ion in the present mass spectrum (Fig. 7), while it represent a prominent ion using soft ionization measured by electro spray ionization–tandum mass spectrometer (ESI–MS/MS) technique [9]. The very low stability of this fragment ion using EI technique may be due to low value of activation energy.

In the present mass spectrum, identical mass spectrum for the different ion source temperatures are found except the absence of the signal at m/z = 197 (formation of $C_8H_8SO_3N$ fragment ion) at 400 K of source temperature. The interpretation can be discussed on the bases of thermodynamic consideration. Firstly, we consider the three main competitive pathways from molecular ion of GL.

1. $[C_{15}H_{21}N_3O_3S]^+ \rightarrow \underset{m/z=155}{[C_7H_7SO_2]^+} + N_1$
2. $[C_{15}H_{21}N_3O_3S]^+ \rightarrow \underset{m/z=168}{[C_8H_{14}N_3O]^+} + N_2$
3. $[C_{15}H_{21}N_3O_3S]^+ \rightarrow \underset{m/z=197}{[C_8H_8SO_3N]^+} + N_3$

In EI–MS, it is known that [28], after ionization the molecular ion accepts internal energy which can randomize overall vibration modes of molecular ion before any fragmentation occurs. As a result, mass spectra would show fragmentation. As a result, mass spectra would show an almost a statistical bond breaking through the molecular ion. The molecular ion explores many pathways up to their respective transition states and prefers the thermodynamically more favorable ones.

From the thermodynamic point of view, we consider that the hypothetical molecular ion has some internal energy and being faced to the selection of fragmentation pathway (Fig. 7).

Suppose the molecular ion $[C_{15}H_{21}N_3O_3S]^+$ has internal energy $E_{initial}$ being above the activation E_{02}, to cross the transition state TS_2 leading to the formation of $[C_7H_7SO_2]^+$, but definitely more than needed to dissociate into $[C_8H_{14}N_3O]^+$. The difference between the energy content E_{intial} of E_{01} is termed excess energy, $E_{ex} = E_{initial} - E_{01}$ of transition state TS_1. In this case both ionic products would be observed. This is the situation of source temperature = 400 K.

Increasing the ion temperature above 400 K and formation of all possible products (including m/z = 197 in the three mass spectrum), one can expect that molecular ion gain internal energy $E_{initial}$ being clearly higher than any of the three activation energies.

Conclusions

This study provides further insights into applicability of experimental TA and MS techniques and theoretical investigation using PM3 procedure on GL drug. From the application of the above techniques it is concluded that the primary fragmentation of GL in both TA and MS is due S–N rupture. Subsequent fragmentation of both techniques reveals many competitive and successive pathways with some different subsequent one. In TA decomposition the drug is completely dissociated at temperature range 130–600 °C. Also, the rate of thermal degradation was tested by isothermal degradation. In MS the effect of ion source-temperature on MS and consequently internal energy effect was recorded and investigated.

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