Investigation of diazepam drug using thermal analyses, mass spectrometry and semi-empirical MO calculation

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Abstract

In the present work diazepam (Dz) drug was investigated using thermal analyses (TA) measurements (TG/DTG) in comparison with EI mass spectral (MS) fragmentation at 70 and 20 eV. Semi-empirical MO calculations, MNDO procedure, have been carried out on diazepam both as neutral molecule and the corresponding positively charged molecular ion. These include molecular geometry, bond order, charge distribution, heats of formation and ionization energy. Thermogravimetric and kinetic analysis, reveal a high response of the drug to the temperature variation with very fast rate. It is completely decomposed in the temperature range between 204 and 340 °C with average kinetic energy (KE) at 164.69 kJ mol⁻¹. On the other hand, diazepam can easily fragmented at low energy after ionization by electron energy at 9.56 eV. The losses of CO gas molecules followed by chlorine gas from the entity of diazepam (both neutral and charged molecular ion) as the best selected pathway were observed in both mass spectra (MS) and thermal analyses (TA). MNDO calculation was applied to declare both TA and MS observations.

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1. Introduction

Diazepam is (7-chloro-1-methyl-5-phenyl-3H-1,4-benzo diazepin-2 [IH]-one) a benzodiazepine tranquilizer with anticonvulsant sedative, muscle relaxant and amnesic properties. It is used in treatment of anxiety disorders and pension states, as a sedative and premedicatinal, in the control of muscle spasm as in tetanus, and in the management of alcohol withdrawal symptoms. It is of value in patients undergoing orthopedics procedures, endoscopy and cardio version. When given by a mouth diazepam may be beneficial in the treatment of some patients with epilepsy. It is recommended when given by slow injection for the control of status epileptics.

The mass spectrometry has become a power tool for drug metabolism studies [1]. The technique is important because it provides a large amount of structural information with little expenditure of sample. In electron impact (EI) mass spectrum, the fragmentation consist of series of competitive and consecutive unimolecular fragmentation [2]. The fragmentation of ionized molecule depends mainly on their internals energy [3]. At 70 eV the spectra are very complex, it is difficult to uncover all the competing and consecutive fragmentation reactions. Lowering the energy of the ionized electron beam it is possible to make the spectra more simple and the high energy processes are thus suppressed [4].

Thermogravimetric (TG/DTG) analysis is used to provide quantiative information on weight losses due to decomposition and/or evaporation of low molecular material as a function of time and temperature. In conjunction with mass spectrometric analysis [5–7], the nature of the released volatile may be deduced, thus greatly facilitating the interpre-
tation of thermal degradation processes. On the other hand, computational quantum chemistry can provide additional data, which can be used successfully for the interpretation of experimental results [8] which may used in the description and prediction of primary fragmentation processes.

A number of methods are in use for the qualitative and quantitative analysis of diazepam and other 1,4-benzodiazepam using chromatographic methods as well as mass spectrometric analysis such as thin layer chromatography [9], column-switch high performance liquid chromatography HPLC [10,11], gas chromatography [12–14] and high performance liquid chromatography with MS [15]. Recently, the complexing behavior of the drug toward transition and rare earth elements, are investigated and discussed recently by Zayed et al. [16,17] using combined some physico-chemical methods. They concluded the high stability of the formed complexes which are of essential biological roles.

Although the literature is wealthy in information related to the biological activities of diazepam and its derivatives [9–17] it seems a lack of any correlation’s between chemical behavior and its electronic structure of this drug.

The main aim of the present work is to carry out experimental and theoretical investigation on diazepam using thermal analyses (TA) measurement including kinetic analysis of the drug and EI mass spectral (MS) fragmentation at 70 and 20 eV. Also, MO calculations are performed using MNDO procedure on the neutral molecules and charged molecular ion to investigate the geometrical parameters, heat of formation, bond orders and the atomic charge distribution. MNDO calculations are correlated with the obtained important results of both TA and MS experimental techniques about the stability of the drug and predication of the site of primary fragmentation step and subsequent ones. This study is thought to be helpful in establish a quantitative and qualitative structure—activity relationship for the drug, which is the mean objective of the present work. It is worth mentioning here that, the literature about such comparative study is scanty.

2. Experimental

2.1. Mass spectrometry (MS)

EI mass spectra were obtained using Shimadzu-GCMS-QP1000EX quadrupol mass spectrometer with electron multiplier detector equipped with a 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 values of 70 and 20 eV, ionization current of 60 mA and vacuum of 10−6 Torr.

2.2. Thermal analyses (TA)

The TA studies of diazepam was made using conventional thermal analyzer (Shimadzu system of DSC-50 and 30 series thermal analyses instrument TG-50). The mass losses (of 5 mg sample) and heat response of the changes in the sample were measured from room temperature up to 400 °C. The heating rate was 10 °C min−1 in an inert argon atmosphere. These instruments were calibrated using indium metal as thermally stable material. The reproducibility of the instrument readings was determined by repeating each experiment more than twice.

2.3. Quantum-chemical calculations (MOC) of the diazepam structural data

The calculations were performed using semi-empirical MO procedure. The program used in these computations is the modified neglect of diatomic overlap (MNDO) procedure described by Dewar and Thiel [18,19] with the standard parameters derived and extensively tested by these authors [20]. The geometries of all stable species studied were completely optimized with respect to all geometrical variables using the modified Davidson–Fletcher–Powell (DFP) [21] algorithm incorporated with the program. The program is run under the molecular orbital calculation package MOPAC 6.0 [22] for microcomputers.

3. Results and discussion

The chemistry and reactivity of diazepam drug have always been of great interest because of its importance in the remedy of various kinds of diseases. Knowledge of thermal decomposition mechanism of diazepam and other related drugs is very important in order to understand the chemical processes that the shared in biological systems. It is difficult to establish the exact major fragmentation pathway in EI using conventional MS. Combination of the experimental techniques (MS and TA) and MO calculation is very important to understand the following topics:

1. The stability of the drug as a neutral molecule and as ionized molecular ion.
2. The primary sites of fragmentation mechanism.
3. The major fragmentation pathways in both techniques.
4. The selection of the most probable decomposition pathway of diazepam by both TA and MS.

3.1. Thermal analyses (TA)

The TG/DTG curves of diazepam (Fig. 1) were displayed within the temperature range 25–400 °C. It is clear from the...
Fig. 1. Thermal analyses (TG and DTG) of diazepam (DZ).

Fig. 2. Mass spectra of DZ at 70 eV (a) and 20 eV (b).

3.2. Mass spectral behavior of diazepam (MS)

Electron ionization (EI) mass spectra of diazepam drug at 70 and 20 eV were recorded and investigated. A typical mass spectrum of the drug is shown in Fig. 2.

The main fragmentation path following electron impact of diazepam may be rationalized to elimination of the molecular ion \([M^+]\) as represented by Scheme 1. The signal appears at \(m/z = 256\) is due to the formation of molecular ion as a result of elimination of CO gas molecule from the first molecular ion.
Fig. 3. The numbering system of DZ molecule.

$(m/z = 284)$ (path 1). This fragment represents the base peak in the mass spectra at both 70 and 20 eV. The appearance of signals at $m/z = 283$ and 257 is due to elimination of hydrogen as radicals (path 2) and HCN gases (path 3) from the diazepam molecular ion $(m/z = 284)$ [23]. The formation of the fragment at $m/z = 221$ with a relative high abundance at both 70 and 20 eV indicates a moderate stability of high and low energy of ionizing electron, which may be due to the presence of chlorine atom [23]. On lowering the ionizing energy from 70 to 20 eV it is noticed that, no appreciable change in the relative intensity of the main fragmentation process (Fig. 2). Finally, the signal at $m/z = 285$ and at 286 in the same spectra are due to the isotopic effect pattern of signal of chlorine atom present in the structure of DZ [24]. Also the same effect is observed at $m/z = 258$ and 259 as a result of the same isotopic effect.

3.3. Theoretical calculations

Molecular orbital (MO) calculations give valuable information about the structure of the molecules, which actually be used to support the experimental evidence. The much important parameters calculated using MO calculation include geometries, bond orders, charge distribution and heat of formation.

In our previous work [25], malanilides compound have been studied using MS, TA and MNDO calculation. The MO calculations were performed for only neutral molecule. In the present work, the calculations have been carried out on diazepam drug-neutral molecule and charged molecular ion and have been used for predication of the weakest bond cleavage and follow the fragmentation pathway in neutral molecule (as in the TA decomposition) and charged molecular ion (as in the MS fragmentation).

Fig. 3 shows the numbering system of DZ skeleton that helps in ordering of charge distribution, heat of formation and bond orders.

Geometrical parameters (bond length and bond angle) and bond order for both neutral molecule and charged molecular ion are presented in Table 2. The calculation showed that little differences are predicted in geometries and bond order between neutral and charged species and can briefly summarized in the following main points:

\[ \Delta H_f[DZ] = 184.67 \text{ KJ mol}^{-1} \]

\[ \Delta H_f[DZ]^+ = 980.56 \text{ KJ mol}^{-1} \]

Fig. 4. Charge distribution on different atoms for DZ (a) neutral molecule, (b) charged molecular ion.
Table 2

<table>
<thead>
<tr>
<th>Bond</th>
<th>Bond length (Å)</th>
<th>Bond order</th>
<th>Bond angle (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1=C2</td>
<td>1.415</td>
<td>1.425</td>
<td>1.386</td>
</tr>
<tr>
<td>C2=C3</td>
<td>1.420</td>
<td>1.440</td>
<td>1.362</td>
</tr>
<tr>
<td>C3=C4</td>
<td>1.403</td>
<td>1.428</td>
<td>1.362</td>
</tr>
<tr>
<td>C4=C5</td>
<td>1.406</td>
<td>1.417</td>
<td>1.375</td>
</tr>
<tr>
<td>C5=C6</td>
<td>1.750</td>
<td>1.741</td>
<td>0.968</td>
</tr>
<tr>
<td>C6=C17</td>
<td>1.428</td>
<td>1.426</td>
<td>0.976</td>
</tr>
<tr>
<td>C7=N8</td>
<td>1.473</td>
<td>1.479</td>
<td>0.905</td>
</tr>
<tr>
<td>N8=C9</td>
<td>1.431</td>
<td>1.433</td>
<td>0.945</td>
</tr>
<tr>
<td>C9=C10</td>
<td>1.427</td>
<td>1.479</td>
<td>0.905</td>
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<tr>
<td>C10=C12</td>
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<td>1.542</td>
<td>0.887</td>
</tr>
<tr>
<td>C12=C14</td>
<td>1.531</td>
<td>1.542</td>
<td>0.887</td>
</tr>
<tr>
<td>C14=C15</td>
<td>1.495</td>
<td>1.471</td>
<td>0.945</td>
</tr>
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<td>C15=C16</td>
<td>1.406</td>
<td>1.406</td>
<td>1.393</td>
</tr>
<tr>
<td>C16=C17</td>
<td>1.406</td>
<td>1.406</td>
<td>1.393</td>
</tr>
<tr>
<td>C17=C18</td>
<td>1.406</td>
<td>1.406</td>
<td>1.393</td>
</tr>
<tr>
<td>C18=C19</td>
<td>1.406</td>
<td>1.406</td>
<td>1.393</td>
</tr>
<tr>
<td>C19=C20</td>
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<td>1.406</td>
<td>1.393</td>
</tr>
<tr>
<td>H-avg (average)</td>
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<td>1.113</td>
<td>0.963</td>
</tr>
<tr>
<td>H-avg (average)</td>
<td>1.119</td>
<td>1.120</td>
<td>0.955</td>
</tr>
</tbody>
</table>

1. Bond angle changes in the range ±1° except at the angle N13=C14=C15 decreased by 2° from neutral to charged species (i.e. 116–114°).
2. For neutral species the bond lengths changed in the order: C6=C7 > C10=C12 > C14=C15 > N8=C9 while in case of charged species the bond length is as follows: C6=C7 > C10=C12 > N8=C9 > C14=C15.
3. Bond order is related to bond strength. The weakest bonds, which are assumed to be cleaved preferentially and relatively low 1° bond character. For neutral DZ molecule the bond order changes in the order: C10=C12 > N8=C9 > C14=C15 and in charged molecular ion of DZ the change in bond order: N8=C9 > N8=C10 > C3=N8.

The charge distribution on different atoms (C, N, and O) and heats of formation ∆H (kJ mol⁻¹) for neutral and charged DZ species are summarized in Fig. 4. Significant changes in the electron distribution within given system during the ionization of DZ process [26]. The most negative values are that present at nitrogen and oxygen in the order: N8 (−0.4216) < O11 (−0.3194) < N13 (−0.2533) for anion forms. The most positive charges are concentrated on carbon of carbonyl group (C10) and C14 for both species. All ring carbons have a slight negative or positive charge (Fig. 4). The greatest change occurs in negative charge as a result of electron rupture (negative charge), while no appreciable change in the positive charges.

3.4. Correlation of the thermal analysis behavior and the MO calculation for neutral molecule

Diazepam is an essential drug used in remedy of diseases. In 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 [27], a determination of initial bond cleavage would be an important first step in using these calculations in a predictive manner. On the base of MO calculation the bond order of the C=C system (Table 1) refers to the possible starting decomposition of the neutral composed at C10=C12 (lowest band order = 0.887) followed by rupture of N8=C9 bond (bond order = 0.905). This means that the carbon monoxide (wt. loss (%) = 10), the rate constant (m⁻¹) of this decomposition as calculated from OZAWA method = 1.46 × 10¹¹ and the energy required for this processes is 191.71 kJ mol⁻¹ is the first volatile gas molecule evolved on heating DZ molecule starting at 204°C. This step may be followed by loss of CH3NCO (wt. loss (%) = 20.1, rate constant = 2.18 × 10¹¹ m⁻¹, energy = 178.81 kJ mol⁻¹) or CH3NCOCH2 (wt. loss (%) = 25, rate constant = 3.17 × 10¹¹ m⁻¹, energy = 161.51 kJ mol⁻¹). This also followed by the rupture of C2=C4 bond (bond order = 0.961) leading to the two molecules of chlorobenzene and cyanobenzene. Firstly, chlorobenzene starts to lose 1/2 Cl2 gas molecule (wt. loss (%) = 12, rate constant = 4.48 × 10¹¹ m⁻¹, energy = 148.94 kJ mol⁻¹). As a result of C12=N13 rupture (bond order = 0.999) leaving phenyl radical (mole mass = 77). This step may be
followed by the loss of HCN gas from cyanobenzene (wt loss (%) = 20.1, rate constant = 6.18 × 10^{11} \text{ m}^{-1} \text{ s}^{-1}, energy = 142.83 \text{ kJ mol}^{-1})

It is clear from the thermal survey that diazepam is completely decomposed in the temperature range from 204 to 340°C as shown in Fig. 1. This thermal decomposition may be tentatively given by the Scheme 2.

The calculated charge density on different atoms of diazepam (Fig. 4), the data refer to the high charge density on oxygen of CO (0.3194) and thirdly on N13 (0.2533). The charge density on N8 may be related to electron repelling power CH3 group attached to this nitrogen. The high charge density of oxygen of CO is actually related to the withdrawing power of the oxygen as a result of its electron negativity. The low charge density of N13 may be related to sharing of this nitrogen lone pair in delocalized electron cloud on cyanobenzene molecule. This is the main reason for the remaining of this part of diazepam tell high temperature (340°C) before the very low charge density on C1 (≈0.0935) of chlorobenzene part refer to the chairing of its unpaired electron is delocalize electron cloud on chlorobenzene part of diazepam leading to its loss at high temperature (>300°C).

3.5. Correlation of the mass spectral (MS) behavior and the MO calculation for neutral molecular ion

The scope of this investigation is restricted to a search for prediction and discern features of initial bond ruptures during the course of fragmentation of DZ complex molecule. Empirical observations indicate that the course of subsequent fragmentation is determined to large extent by the initial bond ruptures of the molecular ion in MS [28]. It is quite acceptable to say that the computational quantum chemical calculation can provide additional data which can be used successfully for the interpretation both TA and MS experimental results. These theoretical data can, particularly, valuable for mass spectral scientists; they study gas-phase species, which can be handled much more easily by quantum chemistry than those surrounded by solvent [27]. Mass spectra of DZ reveals three competitive process (1–3) including principle fragmentation pathways (Scheme 1). Fragment ion at different m/z ratios (Scheme 2) may be formed from the main DZ molecular ion (molecular mass = 284) by CO rupture (process 1) or by successive loss of H + HCN (process 2) leaving the corresponding molecular ions [C16H13N2Cl]+ or [C16H12NOCl]+ respectively. MNDO calculation data (Table 2) show that the C10–C12 has the smallest bond order of the charged system.

It is known that the organic molecule is more stable in neutral state than in the ionic one [23]. In DZ, the molecule

\[ \text{loss } m/z = 221 + \text{Cl} \]

It is quite acceptable to say that the computational quantum chemical calculation can provide additional data which can be used successfully for the interpretation both TA and MS experimental results.

is ionized at energy = 9.56 eV and fragmented after few electron volt above ionization energy, while in solid state phase the molecule begin its fragmentation at the temperature 304 °C. MNDO calculation of the values of heats of formation for neutral molecule (148.67 kJ mol⁻¹) and ionic (ΔΗᵣ = 980.56 kJ mol⁻¹) confirms the experimental TA and MS data.

The relative change of the calculated parameters using MNDO procedure (geometries, bond order, heats of formation and ionization energy) in the neutral molecule–molecular ion relation and furthermore, whether they could be correlated to the experimental observed primary bond cleavage. In the present work, no appreciable change in the values of geometries and bond order in both neutral and charged molecular ion of DZ. Greatest change in the values of charge distribution on different atoms especially the negative values is mainly due to the electron rupture from electron pairs on heteroatoms (N and O).

4. Conclusions

This study provides further insights into applicability of experimental TA and MS techniques and theoretical investigation using MNDO procedure on DZ drug. From the application of both practical and theoretical techniques in commitment it is concluded that: The primary fragmentation of DZ by applying both experimental techniques (TA and MS) can be explained by the CO gas loss from the initial DZ molecule. Subsequent fragmentation in mass spectrometry in five fast successive processes can be rationalized and confirmed by using MO-calculations. Di-azepam can be completely dissociated in temperature range 204–340 °C, while the molecular ion can fragmented after few electron volts above the ionization energy of DZ (9.56 eV). The theoretical MO calculation data helps the selection of the most probable fragmentation pathway in both TA and MS techniques. It worth mentioning here that a disagreement between experimental and theoretical calculation data itself does not necessarily mean unreliable theoretical results but even inspire one to think about, for example, significantly different isomerizes molecular ion structures.

References