Investigation of Naproxen Drug Using Mass Spectrometry, Thermal Analyses and Semi-Empirical Molecular Orbital Calculation

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ABSTRACT

A Naproxen (C₁₄H₁₄O₃ M.W. =230) drug is a non-steroidal anti-inflammatory agent. The drug was investigated by mass spectrometry (MS) at 70 and 12 eV of ionizing electron, and by thermal analysis (TA) measurements (TG/DTG and DTA). On the other hand, the calculations were performed on the neutral and charged species using semi-empirical molecular orbital (MO) calculation, PM3 procedure. These calculations included, bond length, bond order, bond strain, partial charge distribution, ionization energy and heats of formation (ΔHᵣ). Mass spectral fragmentation (MS) and thermal analysis (TA) decomposition were proposed and compared to each other to select the most suitable scheme representing the correct fragmentation pathway of the drug in both techniques. MO calculations provide additional information about the first rupture and subsequent one as well as the stability of neutral and ionized species.

Key Words: Naproxen / Mass spectrometry / Thermal analysis / Molecular Orbital Calculation, PM3.

INTRODUCTION

Naproxen (NAP) has an IUPAC name, 2-Naphthaleneacetic acid, 6-methoxy-α-methyl-,(s)-(+)-(s)-6-Methoxy-α-methyl-2-naphthaleneacetic acid¹,² and general formula (C₁₄H₁₄O₃). It belongs to a group of drugs called nonsteroidal anti-inflammatory drugs (NSAIDs). It works by reducing hormones that cause inflammation and pain in the body. NAP is used to treat pain or inflammation caused by conditions such as arthritis, ankylosing spondylitis, tendinitis, bursitis, or menstrual cramps³,⁴.

Mass spectrometry (MS) is one of the most powerful analytical techniques, particularly for pharmaceutical analysis, where good selectivity and high sensitivity are often needed. In the pharmaceutical industry measurements of drugs and their metabolites in plasma are essential for drug discovery and development. The more accurate and rapid measurements, is the more quickly a drug can progress towards regulatory approval. Time-of-flight mass spectrometer (TOF-MS) delivers high sensitivity, resolution, and exact mass measurements. A variety of ion source and software options makes MS a versatile choice for a range of analytical challenges⁶-¹⁰.

Thermal analytical techniques can provide important information regarding storage and stability of pharmaceuticals. Thermal analytical methods have thus become important tools for the development of modern medicines¹¹-¹⁵. These are precise and accurate techniques with low sample requirements, and can provide detailed information about new chemical entities even at the very earliest stages of discovery and development of the new compositions and drugs¹⁶-¹⁹. Thermo
gravimetric TG/DTG analysis 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 (20-23), the nature of the released volatilize may be deduced, thus great 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 (24). 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 (25-28).

The aim of the present work is focusing on further application of our previous work (25-28) to the case of NAP drug. This work includes a correlation between, mass spectral fragmentation and thermal analysis degradation of the drug and comparing these experimental data with the theoretical Molecular Orbital (MO) calculation 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 this drug responsible for its chemical, biological and medical reactivity.

**EXPERIMENTAL**

**Materials:**

All chemicals used were of the analytical reagent grade (AR), and of highest purity available. They included NAP (M.wt = 230.26 g mole), as an authentic sample which was kindly supplied by Egyptian Drug Control Authority (EDCA), Cairo (Egypt).

**Mass spectrometry (MS):**

Electron ionization (EI) mass spectrum of NAP is obtained using Thermo Finnegan TRACE DSQ quadruple mass spectrometer with electron multiplier detector equipped with GC-MS data system at Experimental Nuclear Physics - Nuclear Research Center. The direct probe (DIP) 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 obtained spectra were stored. Electron ionization mass spectra were obtained at ionizing energy value of 70 and 12 eV, ionization current of 60 μA and vacuum is better than 10^{-6} torr.

**Thermal analyses (TA):**

The thermal analyses of NAP drug were made using conventional thermal analyzer (Shimadzu system of DTA-50 and 30 series TG-50) at Cairo University micro-analytical center. The mass losses of 5 mg sample and heat reopens of the change of the sample were measured from room temperature up to 600 ºC. The heating rate, in an inert argon atmosphere, was selected as 10 ºC min−1. 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.

**Computational method:**

The MO calculations were performed using semi-empirical molecular orbital calculation. The method used in these computations is the parametric method (PM-3) described by Stewart (29). The default criteria for terminating all optimizations were increased by a factor of 100 (keyword PRECISE). All the molecular orbital calculations were carried out at the restricted Hartree-Fock level.
(RHF) for the neutral molecule of NAP while the unrestricted Hartree-Fock level (UHF) were carried out for its cation by using PM-3 method followed by full optimization of all geometrical variables (bond lengths, bond angles, and dihedral angles), without any symmetry constraint. All structures were optimized to a gradient norm 0.01-0.05, using the eigenvector following (EF) routine. All the semi empirical MO calculations were performed with the MOPAC2000 software package implemented on an Intel Pentium IV 3.0 GHz computer.

RESULTS AND DISCUSSION

It is of great interest to study the chemistry and reactivity of NAP drug because of its importance in medicine. The geometrical structure of NAP is shown in Fig. 1.

![Fig. 1: The geometrical structure of NAP.](image)

Knowledge obtained from thermal decomposition mechanisms of the neutral drug is very important to understand the chemical process that shared in biological systems. It is difficult to establish the exact major fragmentation pathway in EI using conventional MS. With combining the above two techniques and the data obtained from the MO calculation, it is possible to understand the following topics:

1. Stability of the drug under thermal degradation in solid state and mass spectral fragmentation in gas phase.
2. Prediction of the primary site of bond rupture and subsequent one.
3. The correct pathways under TA and MS techniques.
4. Understanding what actually happened in biodegradation of the drug or its derivatives in vivo system and metabolites.

1. Thermal analysis:

The TA data of NAP are illustrated in Figs.2a and 2b. It is clear from TG/DTG curves (Fig.2a) that, this compound decomposed completely within the temperature range of 80-480 °C (mass loss= 99.0%). The main mass loss of this compound occurs at 266.93 °C as shown by DTG curve (Fig.2a). From DTA curve (Fig. 2b) it is clear that thermal decomposition of NAP occurs in two main endothermic regions and one exothermic region: 150-170, 210-280 and 400-480 °C which cannot easy described by TG technique. These endothermic and exothermic mass losses required energy values of 255.41, 10.67 and 371.45 kJmol⁻¹ at 157.0, 270.0 and 452.0 °C respectively. Therefore, the proposed thermal decomposition of NAP can be carried out in three consecutive steps as shown in scheme 1.
Fig. 2a: Thermal analyses (TGA and DrTGA) of NAP drug

Fig. 2b: Thermal analyses (DTA) of NAP drug
2. Mass spectral (MS) fragmentation of NAP drug:

The electron ionization (EI) mass spectrum for NAP drug measured at 70 and 12 eV was recorded (Figs. 3a, b) and investigated. The proposed fragmentation pathway is shown in scheme 2.
Fig. 3. Mass spectrum of NAP at 70 eV (a) and 12 eV (b)

Scheme 2: Proposed mass spectral fragmentation pathway of NAP drug.

The spectrum of NAP drug at 70 eV (Fig. 3a) is characterized by many competitive and consecutive pathways, thus forming many intense fragment ions (scheme 2). The main fragmentation pathways for NAP after ionization of neutral molecule at 8.764 eV consists of three principal pathways (path1-3) as rationalized in scheme 2. The signal that appears at m/z = 230 (RI = 100%) refers to the appearance of the main molecular ion \([C_{14}H_{14}O_3]^+\). The high intensity reflects the stability of the molecular ion of NAP. The fragment ion at signal m/z=185 (scheme 2, path 2) represent the second most prominent ion \([C_3H_3O]^+\), (R.I. = 90.42 %) and is mainly due to rupture of COOH molecule from molecular ion. Also, its high stability is due to presence of the lone pairs of electrons of oxygen atom with two aromatic rings. Two important fragment ions are observed in the mass spectra at m/z = 170 (RI = 26.65%) and at m/z = 154 (RI = 12.85%). These fragment ions may be due to the
rupture of CH$_3$ and OCH$_3$ radicals, respectively. The fragment ion that observed at m/z = 139 may be due to the formation of [C$_{11}$H$_7$]$^+$. Comparison of TA (Scheme 1) and MS path-2 (Scheme 2) refers to the coincidence between TA scheme and MS of NAP drug metabolites (vitro fragments).

At the low energy value of 12 eV (Fig.3b), the base peak observed at molecular ion m/z = 230, R.I.= 100%) and it is noted that the peaks corresponding secondary process (m/z = 185) observed at RI < 10%. Comparing the data in Fig. 3a to that in Fig. 3b, refers to the fact that at 70 eV it is sufficient energy for fragmentation of NAP (mole mass 230) to give its daughter fragment ion [C$_{13}$H$_{13}$O]$^+$ (m/z = 185, R.I. = 90.42%), but at 12 eV the lower energy is insufficient to give this fragment from its parent drug molecule. This also confirms the proposed mass scheme 2, which indicates that the path 2 is the only possible way to form [C$_{13}$H$_{13}$O]$^+$ at 70 eV. This is also confirmed by the full scan MS/MS spectrum$^{(32)}$ (Fig.4) of NAP showed a molecular ion at m/z = 229.1 of very low intensity, whereas its daughter fragment ion [C$_{13}$H$_{13}$O]$^+$ (m/z = 184.9, RI = 100%) appeared as base peak.

3. Computational Molecular Orbital (MO) calculation:

Molecular orbital (MO) calculation gives valuable information about the structure and reactivity of the molecules, which actually be used to support the experimental evidence$^{(21-23,25-28)}$. The much important parameters calculated using MO calculation which includes bond orders, bond length, charge distribution, bond strain, heat of formation and ionization energy. In the present work, the calculations have been carried out on NAP neutral molecule (related to TA decomposition) and charged molecular ion (related to MS fragmentation) which is used for prediction of weakest bond rupture to follow the fragmentation pathways in both techniques.

Fig.5. shows the numbering system of NAP skeleton that helps in ordering the calculated parameters Table 1 and 2 presents the values of bond length (Å), bond order, bond strain (kcal mol$^{-1}$) heat of formation kcalmol$^{-1}$ and ionization energy (eV). One can conclude the following from Table 1 and 2.
Fig. 5: The numbering system of NAP molecule.

Table (1): Comparison between of computed bond length (in Å), bond order and bond strain (k Cal mol\(^{-1}\)) using PM3 method for neutral and molecular cation of NAP drug.

<table>
<thead>
<tr>
<th>Bond</th>
<th>Bond length (Å)</th>
<th>Bond order</th>
<th>Bond strain (kCal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neutral</td>
<td>Cation</td>
<td>Neutral</td>
</tr>
<tr>
<td>C1-C2</td>
<td>1.365</td>
<td>1.396</td>
<td>1.634</td>
</tr>
<tr>
<td>C2-C3</td>
<td>1.428</td>
<td>1.397</td>
<td>1.197</td>
</tr>
<tr>
<td>C3-C4</td>
<td>1.377</td>
<td>1.400</td>
<td>1.548</td>
</tr>
<tr>
<td>C4-C5</td>
<td>1.420</td>
<td>1.399</td>
<td>1.218</td>
</tr>
<tr>
<td>C5-C6</td>
<td>1.409</td>
<td>1.398</td>
<td>1.334</td>
</tr>
<tr>
<td>C6-C1</td>
<td>1.424</td>
<td>1.397</td>
<td>1.201</td>
</tr>
<tr>
<td>C6-C7</td>
<td>1.418</td>
<td>1.398</td>
<td>1.232</td>
</tr>
<tr>
<td>C7-C8</td>
<td>1.375</td>
<td>1.398</td>
<td>1.577</td>
</tr>
<tr>
<td>C8-C9</td>
<td>1.419</td>
<td>1.398</td>
<td>1.241</td>
</tr>
<tr>
<td>C9-C10</td>
<td>1.368</td>
<td>1.396</td>
<td>1.607</td>
</tr>
<tr>
<td>C10-C5</td>
<td>1.421</td>
<td>1.398</td>
<td>1.220</td>
</tr>
<tr>
<td>C3-O11</td>
<td>1.379</td>
<td>1.364</td>
<td>1.037</td>
</tr>
<tr>
<td>C8-C13</td>
<td>1.504</td>
<td>1.513</td>
<td>0.973</td>
</tr>
<tr>
<td>O11-C12</td>
<td>1.406</td>
<td>1.405</td>
<td>0.985</td>
</tr>
<tr>
<td>C13-C14</td>
<td>1.522</td>
<td>1.535</td>
<td>0.983</td>
</tr>
<tr>
<td>C13-C15</td>
<td>1.521</td>
<td>1.520</td>
<td>0.919</td>
</tr>
<tr>
<td>C15-O16</td>
<td>1.354</td>
<td>1.342</td>
<td>1.053</td>
</tr>
<tr>
<td>C15-O17</td>
<td>1.218</td>
<td>1.209</td>
<td>1.807</td>
</tr>
</tbody>
</table>

The order of the bond strength: C21-O22>C1-C2>C9-C10>C7-C8>C3-C4>C5-C6>C8- C9>C6-C7>C10-C5>C4-C4>C6-C1>C2-C3>C15-O16>C3-O11>O11-C12>C13-C14>C8-C15>C13-C15.

Table 2: Computed heat of formation (kcal/mol) and ionization energy (eV) for neutral and molecular cation of NAP drug using PM3.

<table>
<thead>
<tr>
<th></th>
<th>Heat of Formation (kcal/mol)</th>
<th>Ionization Energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neutral</td>
<td>Cation</td>
</tr>
<tr>
<td></td>
<td>-97.78709</td>
<td>92.74</td>
</tr>
</tbody>
</table>

1. Small differences in bond length in NAP system upon ionization, indicating that no appreciable change in the geometries upon ionization.
2. The lowest bond order (important for prediction of primary site of cleavage) observed at bond C13-C15 for both neutral (0.919) and positive species (0.932).
3. Upon ionization the stability of the molecule decreased by 180.53 kcal mol\(^{-1}\) (\(\Delta H_f (-97.79) - \Delta H_f^+ (92.74)\)).
The charge distribution on only carbon and oxygen and heats of formation; $\Delta H_f$ (kcal mol$^{-1}$) for neutral and charged NAP species are summarized in Fig. 6. Significant changes in the electron distribution with given system often takes place during the ionization.

![Figure 6](image_url)

$\Delta H_f$ [NAP] = -97.78 kcal mol$^{-1}$ of neutral species

$\Delta H_f$ [NAP]$^+ = 92.74$ kcal mol$^{-1}$ of charged species

Fig. 6: Charge distribution on different atom for NAP (a) neutral molecule and (b) charge molecular ion.

4. Correlation between thermal analysis (TA) decomposition and MO-calculation:

As indicated, the determination of initial bond rupture would be an important first step in using this calculation in predicative manner. MO calculation, PM3 procedure reveal that the C$_{13}$-C$_{15}$ bond has the lowest bond order at 0.919 and large bond length= 1.521 Å and bond strain= 0.032 kcal mol$^{-1}$. Experimental TA curves (Fig. 2) of NAP reveal that the first weight loss equal to 19.13%. This weight loss corresponding to the rupture of COOH (lowest bond order) at temperature 157 °C of range 150-170 °C, followed by rupture of C$_3$-O$_{11}$ bond (bond order= 1.037, bond length=1.364 Å, bond strain= 0.032 kcal mol$^{-1}$). This second weight loss of a value 13.65% at temperature and range 180-280°C. This weight loss is mainly due to rupture of OCH$_3$ (O+CH$_3$). This wide range of temperature may due to slow fragmentation with small half life time, since in TA decomposition the molecule are continuously energized and determined by a gas evolution and the distribution of energy can be described by energy (34).

On the other hand, the electrostatic repulsion between C$_{13}$ (-0.012) and C$_{15}$(-0.386) for neutral, facility the rupture of this bond (C$_{13}$-C$_{15}$), than the second rupture C$_3$ (-0.100) and O$_{11}$ (-0.187) atoms, indicating that the COOH loss is more easy than O-CH$_3$ loss.

5. Correlation between mass spectral (MS) fragmentation and MO calculations of charged molecule:

The scope of this investigation is restricted to a search for prediction of the first and subsequent bond ruptures during the course of fragmentation of NAP drug in MS technique. The subsequent fragmentation in MS is determined to large extent by the initial bond rupture of molecular ion (28,31). A
number of mass spectrometric techniques have utilized helping in rationalized the correct pathways of the molecules, among which are: threshold measurement and metastable abundance ratios (17). On the other hand, computational can provide important information which can be used successfully in description of primary site of cleavage. These theoretical data can particularly, valuable for MS because they study in gas phase species, which can be handled much more easily by quantum chemistry (29-31). Mass spectrum of NAP reveals (scheme2) three competitive and consecutive fragmentation pathways.

PM3 procedure reveals that the C\textsubscript{13}-C\textsubscript{15} (Table 1) bond is the first site of bond cleavage (lowest bond order=0.932, large bond length =1.520, bond strain = 0.037 kcal mol\textsuperscript{-1}). This bond cleavage accompanied by rupture of COOH molecule forming the fragment ion [C\textsubscript{13}H\textsubscript{12}O]+ at m/z =185 and relative intensity =90.42%. Further loss is a rupture of CH\textsubscript{3} molecule from this fragment at m/z= 170 [C\textsubscript{12}H\textsubscript{10}O]+ (scheme 2, path 1). On the other hand, the electrostatic repulsion between the charge localized on C\textsubscript{13}(-0.057) and C\textsubscript{15}(-0.392) atom (Fig. 5) facility the COOH molecule. The MO calculation (bond order and bond length) calculated for fragment ion at m/z =185 [C\textsubscript{13}H\textsubscript{12}O]+ are shown in Table 3. These data refer to ordering of its bond strength is given by C9-C10>C1-C2>C3-C4>C8- C13>C7-C8>C6-C7>C4-C5>C2-C3>C6-C1>C10>C5>C8>C9>C3- O11>C13-C14> O11-C12. This means that, the first ruptured bond is O11-C12 leading to formation of fragment ion m/z= 170 [C\textsubscript{12}H\textsubscript{10}O]+ via loss of CH\textsubscript{3} group , followed by the rupture of the bond C13-C14 leading to the formation of m/z = 154 (RI = 12.85%) via the loss of CH\textsubscript{3}O and finally to the rupture of the bond C3-O11 leading to the formation of fragment ion [C\textsubscript{11}H\textsubscript{12}]+ that observed at m/z = 139. This means that all fragment ions are coming from the daughter m/z = 185 not from the parent ion m/z =230. This is also is in good agreement with the proposed thermal and mass schemes (1 and 2) and MS/MS data (32).

**Table 3:** Comparison between of computed bond length (in Å) and bond order using PM3 method for neutral and molecular cation of [C\textsubscript{13}H\textsubscript{12}O]+ at m/z =185 system.

<table>
<thead>
<tr>
<th>Bond</th>
<th>Neutral Bond length (Å)</th>
<th>Cation Bond length (Å)</th>
<th>Bond order Neutral</th>
<th>Bond order Cation</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1-C2</td>
<td>1.380</td>
<td>1.360</td>
<td>1.484</td>
<td>1.679</td>
</tr>
<tr>
<td>C2-C3</td>
<td>1.422</td>
<td>1.436</td>
<td>1.222</td>
<td>1.165</td>
</tr>
<tr>
<td>C3-C4</td>
<td>1.394</td>
<td>1.400</td>
<td>1.389</td>
<td>1.364</td>
</tr>
<tr>
<td>C4-C5</td>
<td>1.413</td>
<td>1.394</td>
<td>1.252</td>
<td>1.394</td>
</tr>
<tr>
<td>C5-C6</td>
<td>1.424</td>
<td>1.435</td>
<td>1.218</td>
<td>1.167</td>
</tr>
<tr>
<td>C6-C7</td>
<td>1.421</td>
<td>1.437</td>
<td>1.206</td>
<td>1.131</td>
</tr>
<tr>
<td>C6-C7</td>
<td>1.410</td>
<td>1.381</td>
<td>1.256</td>
<td>1.489</td>
</tr>
<tr>
<td>C7-C8</td>
<td>1.412</td>
<td>1.429</td>
<td>1.257</td>
<td>1.174</td>
</tr>
<tr>
<td>C8-C9</td>
<td>1.430</td>
<td>1.448</td>
<td>1.164</td>
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<tr>
<td>C9-C10</td>
<td>1.376</td>
<td>1.355</td>
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<tr>
<td>C10-C5</td>
<td>1.424</td>
<td>1.436</td>
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<tr>
<td>C3-O11</td>
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<td>1.347</td>
<td>1.033</td>
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<tr>
<td>C8-C13</td>
<td>1.406</td>
<td>1.373</td>
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<tr>
<td>O11-C12</td>
<td>1.406</td>
<td>1.415</td>
<td>0.986</td>
<td>0.958</td>
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<tr>
<td>C13-C14</td>
<td>1.700</td>
<td>1.464</td>
<td>1.022</td>
<td>1.055</td>
</tr>
</tbody>
</table>

The order of the bond strength: C9-C10>C1-C2>C3-C4>C8-C17>C7-C8>C6-C7>C4- C5>C2-C3>C5-C6>C6-C1>C10-C5>C8-C9>C3-O11>C13-C14> O11-C12

6. Correlation between TA and MS:

It is important to make a discussion between results of TA and MS of NAP, to see the behavior of the drug in both techniques. This comparison shows the agreement between mass path-1 and TA. In both TA and MS techniques, it is proved that the C13-C15 bond is the first site of rupture. In TA,
COOH rupture is followed by OCH$_3$ (O+CH$_3$) (scheme1). In MS fragmentation it is initiated by COOH rupture and followed by CH$_3$ group rupture in path-1 (scheme 2). The obtained fragment ions in MS are m/z = 230, 185, 170, 154 and 139, that confirmed by TA can be considered as a vitro metabolites of NAP drug; which are very similar to vivo urinary metabolites of NAP obtained by liquid chromatography–electro-spray mass spectrometry$^{(35)}$.

**CONCLUSION**

The aim of this study is concerning with applicability of experimental TA and MS techniques and theoretical investigation MO calculations, using PM3 procedure on NAP drug. From correlation between MS and MO calculations, it is clear that the C13-C15 bond is the first site of bond cleavage. This refers to lowest bond order, large bond length and less bond strain. This bond cleavage accompanied by rupture of COOH molecule forming the fragment ion [C$_{13}$H$_{13}$O]$^+$ at m/z =185 and relative intensity $=90.42\%$. Further loss is a rupture of CH$_3$ molecule from this fragment at m/z= 170 [C$_{12}$H$_{10}$O]$^+$. On the other hand, the electrostatic repulsion between the charges localized on C13 and C15 atoms facility the COOH molecule. Therefore, this investigation concluded that the correlation between both practical and theoretical techniques helps in the selection of the proper pathway representing the decomposition of this drug to give its vitro metabolites. From the obtained data of both practical (TA, MS) and theoretical (MO) techniques in this investigation, it is proved that, the C13-C15 bond is the first site of rupture in TA, COOH followed by OCH$_3$ (O+CH$_3$), while in MS the rupture initiated by COOH rupture followed by CH$_3$ group. MO calculations reveal that the C13-C15 bond has the lowest bond order, large bond length and bond strain. Also, the electrostatic repulsion between C17 and C21 for neutral and cationic NAP forms facilitates the rupture of the bond (C13-C15). The charges on atoms C3 and O11 make the second bond C3-O11 rupture is less possible. This indicates that the COOH loss is easier than O-CH$_3$ loss. NAP can be complete thermally dissociated in the temperature range of 80-480 oC (mass loss $= 99.0\%$).

The obtained fragment ions in MS are m/z = 230, 185, 170, 154 and 139, that confirmed by TA can be considered as a vitro metabolites of NAP drug. These vitro metabolites are suggested and confirmed by MO calculation and are very similar to vivo urinary metabolites of NAP obtained by liquid chromatography–electro-spray mass spectrometry$^{(35)}$.

**REFERENCE**