# Studies of the Thermal Degradation of Acetaminophen Using a Conventional HPLC Approach and Electrospray Ionization–Mass Spectrometry

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## Abstract

**The thermal degradation of acetaminophen is studied via conventional accelerated aging studies by initially thermally stressing the compound at temperatures between 160°C and 190°C and measuring the rate of decomposition by reversed-phase high-performance liquid chromatography. Rates of decomposition of the compound in the dry state and the activation energy for the process are determined and compared with previously published kinetic and thermodynamic data for the degradation of acetaminophen in solution. In addition, the thermal fragmentation of acetaminophen under electrospray ionization (ESI) conditions using an interface with a heated capillary inlet is studied and the apparent activation energy for this process also is characterized. A comparison of the data shows that acetaminophen is significantly more stable in the dry state than in solution. However, the gas-phase fragmentation of acetaminophen under ESI conditions occurs more readily than either dry- or solution-state degradation. Although the resulting electrospray fragmentation mimics the breakdown product that is formed when the compound undergoes either acid or base catalyzed hydrolysis in aqueous solutions, the mechanism that produces the fragment ion appears to involve a two-step process. Initially, the parent ion forms of the analyte are produced in the spray region of the interface followed by wall-catalyzed decomposition and re-ionization in the heated inlet capillary of the spectrometer.**

## Introduction

Acetaminophen is a widely used analgesic/antipyretic that is routinely manufactured and sold throughout the world as an over-the-counter product. Since its introduction, there have been many hundreds of manuscripts published that discuss various aspects of the compound including its analytical profile (1), as well as numerous individual analytical studies and quantitative methods. An overview of this work may be found elsewhere (2–4) as well as in similar past biannual reviews in the series appearing over the last three decades. Among the body of published work are several investigations that have addressed various aspects of the thermal, hydrolytic, and photolytic stability of acetaminophen.

When maintained under dry conditions, the compound is very stable at room temperature (1). However, at elevated temperatures and in the presence of trace moisture, acetaminophen degrades more rapidly to *p*-aminophenol, which subsequently undergoes additional oxidative changes (1,5,6). Likewise, the degradation of acetaminophen in aqueous solutions is both acid and base catalyzed and degrades via first order kinetics (7–9). The activation for its degradation under these conditions has been reported to be in the 16.7 to 18 kcal/mole range, depending on solution conditions.

Because the degradation chemistry of acetaminophen is well understood under various conditions, it is a good model compound to use to study thermal fragmentation that can occur under electrospray ionization (ESI)–mass spectrometric (MS) conditions. In addition to using previously tabulated solution state degradation data, a series of conventional accelerated aging studies were carried out in order to measure the rates of oxidative decomposition of acetaminophen under dry state conditions, as well as to determine the activation energy for this process. Although this compound has been known to be very stable in the dry state for several decades, a comprehensive set of reaction rates and activation energy data could not be located in the literature. Thus, this aspect of the current study involved thermally stressing the compound at elevated temperatures in combination with subsequent analysis of the resulting thermal decomposition profiles via reversed-phase high-performance liquid chromatography (HPLC).

In addition to the previously mentioned more classical thermal aging studies, ESI–MS measurements were carried out as a function of varying inlet capillary interface temperatures while keeping all other operating conditions constant. Subsequently, the ratios of thermal fragmentation to nonfragmented and to all forms of acetaminophen were determined from the resulting mass spectra and the latter ratio used to calculate apparent fragmentation energy.

Over the last decade, ESI has become an increasingly popular

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means of coupling the effluent of liquid-based separation methodology to MS, and today it is the most widely used approach. During the electrospray operation, there are three steps that occur: nebulization, desolvation and ion formation, and transport of the gas-phase products from nearly atmospheric pressure into the vacuum region of the spectrometer. Currently, the state-of-the-art in terms of technology is such that it is relatively easy and convenient to operate most electrospray interfaces. Nevertheless, although the operational details are relatively well defined and the use of combined HPLC–MS hardware has become relatively routine, there are many mechanistic questions about ion formation and transport that are open to interpretation. Likewise, the particular interface design can have a significant influence on the resulting spectra that are obtained.

One of the important features in instrument performance is the design of the transport zone (i.e., the region where ions must migrate from approximately atmospheric pressure to the vacuum region of the spectrometer). The current work was carried out on a Finningan (San Jose, CA) instrument that is equipped with a small heated capillary through which the ions travel at relatively high linear flow velocities as they enter the vacuum region of the spectrometer. During this transport process, appreciable thermal decomposition of compounds can occur. The result of this is the production of fragments that reflect the thermal stability of the analyte being studied.

## Experimental

### Chemical and reagents

The HPLC-grade acetonitrile and methanol as well as the American Chemical Society grade reagents used for buffer preparation were from Fisher Scientific (Pittsburgh, PA). The acetaminophen was obtained from Sigma (St. Louis, MO). The deionized water was prepared in-house with a Labconco water deionization system (Kansas, MO).

### Equipment and procedures

The liquid chromatographic experiments were carried out using a Varian (Walnut Creek, CA) model 9002 isocratic pump, Spectra Physics (San Jose, CA) model Spectra Focus detector set at 254 nm, and a model 4400 Chromjet integrator. The samples were injected using a Rheodyne (Berkely, CA) model 7125 injection valve equipped with a 20-µL sample loop. The separations were carried out on a 4.6- × 250-mm i.d. Hypersil ODS 5-µm column (Supelco, Bellefonte, PA) using 7:93 (v/v) acetonitrile–10mM pH 4.0 ammonium acetate buffer. Quantitation was carried out using six-point calibration curves that were linear (i.e., $r^2 > 0.99$) over the range studied. All samples were injected at least three times for an individual assay point.

In carrying out the classical evaluated temperature studies to evaluate the accelerated aging of acetaminophen, samples were carefully weighted into amber glass ampoules (10 mg), the ampoules were heat sealed, placed into a constant temperature oven (i.e., a gas chromatography oven), and maintained at elevated temperatures for varying periods up to four days. Duplicate sampling was made at the different time points by removing two-sample ampoules from the oven, cooling them to room temperature, carefully adding a known volume of methanol, and analyzing them by HPLC. The rate of decomposition of acetaminophen at four different temperatures were studied: 160°C, 170°C, 180°C, and 190°C. This range of temperatures provided the optimum in terms of being long enough to provide reliable data and short enough to complete the measurements in a reasonable time.

The ESI–MS experiments were carried out on a Finningan model MAT TSQ 700 triple quadrupole MS equipped with a Harvard model syringe introduction pump. Sample introduction was made using a Rheodyne model 7125 injection valve equipped with a 20-µL sample loop and a carrier solution of 80:20 (v/v) methanol–water at a pumping rate of 50 µL/min. All mass spectra were acquired in the positive ion mode. The composite spectra used for each calculation was obtained from an average of 12 scans collected across the plateau region of the sample introduction ion profile. The acetaminophen samples were prepared at a concentration of 0.1 mg/mL in 80:20 (v/v) methanol–water containing 10mM ammonium acetate. Data were collected at inlet capillary interface temperatures of 130°C, 150°C, 170°C, 190°C, 210°C, and 230°C using nitrogen as the sheath gas (80 psi) and auxiliary gas (25 L/min). The electrospray voltage was set at 4.5 kV, and scans were collected over a range of 12–800 amu in 1.5 s.

## Results and Discussion

Shown in Figure 1 are plots of the decomposition profiles as a function of time for the oxidative degradation of acetaminophen obtained at 160°C, 170°C, 180°C, and 190°C. Additional data were collected beyond the time points shown for each of the temperatures studied. However, because the samples were heat sealed into ampoules, the rate of change of the oxidation of acetaminophen slowed and showed a nonlinear behavior past approximately 50% decomposition because of insufficient oxygen in the sealed containers. As such, only the linear region of the degradation curves were used to calculate the reaction rates for oxidative degradation. The slopes of the linear regression fits for the data in Figure 1 were determined (i.e., the degradation rates with $7.44 \times 10^{-3}$, $1.52 \times 10^{-2}$, $2.61 \times 10^{-2}$, $3.70 \times 10^{-2}$ $h^{-1}$ for plots A to D, respec-

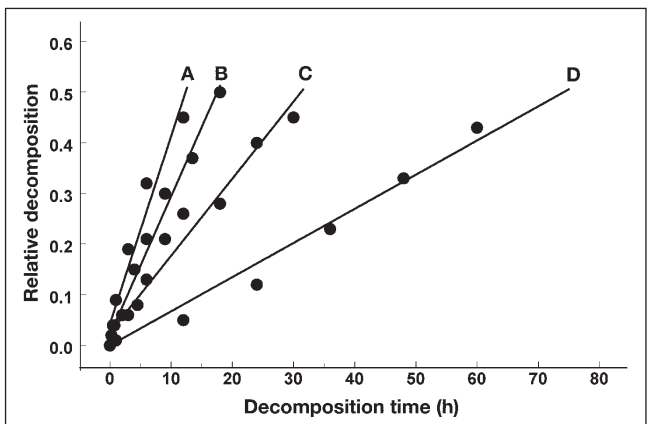


**Figure 1.** Relative decomposition of acetaminophen as a function of time for samples maintained at: (A) 190°C, (B) 180°C, (C) 170°C, and (D) 160°C.

tively) and subsequently plotted as a function of the accelerated aging temperature. These data are shown in Figure 2 as a plot of the natural logarithm of the degradation rate for the individual decomposition profiles versus the reciprocal of the temperature in K. The linear regression fit for these data was better than 0.99.

The linear regression data in Figure 2 were used to predict the degradation rate constants at temperatures between 25°C and 50°C. These constants, which are listed in Table I, are approximately two to three orders of magnitude smaller than reported solution degradation rates (7). The values in Table I were used to construct the family of predicted decomposition profiles shown in Figure 3 for a time period of 5 years. Curves A thru F correspond, respectively, to storage conditions of 25°C, 30°C, 35°C, 40°C, 45°C, and 50°C. A comparison of the dry state stability of acetaminophen (plot A) with previously published solution state degradation data (plots B–E) is shown in Figure 4 for a temperature of 35°C. Plots B, C, D, and E are for the degradation of acetaminophen at solution pH values of 6, 8, 9, and 2, respectively. These plots were generated using a combination of the kinetic and thermodynamic information appearing elsewhere (1,7–9). Likewise, appearing in Figure 5 is a comparison of the rate of change of the degradation of acetaminophen as a function of temperature for the dry state (i.e., the measurements shown in Figures 1 and 2) and the solution state using previously published stability data appearing elsewhere (1,7).

In carrying out the ESI–MS part of the current study, measurements were made over a temperature range of 100°C for the inlet capillary of electrospray interface. Shown in Figure 6 is a representative mass spectrum of acetaminophen obtained at a capillary inlet temperature of 130°C. The ions present are a single thermal decomposition fragment (*m/z* 110), the $MH^+$ ion (*m/z* 152), the

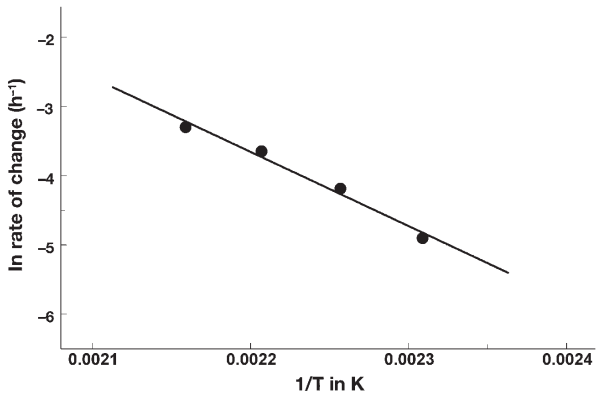


**Figure 2.** Rate of change in the decomposition rates (i.e., obtained from the plots shown in Figure 1) as a function of the reciprocal temperature in K.

**Table I. Predicted Rates for the Dry State Degradation of Acetaminophen**

| Temperature | Degradation rate ($h^{-1}$) |
|---|---|
| 25°C | $1.12 \times 10^{-7}$ |
| 30°C | $2.00 \times 10^{-7}$ |
| 35°C | $3.59 \times 10^{-7}$ |
| 40°C | $6.27 \times 10^{-7}$ |
| 45°C | $1.06 \times 10^{-6}$ |
| 50°C | $1.78 \times 10^{-6}$ |

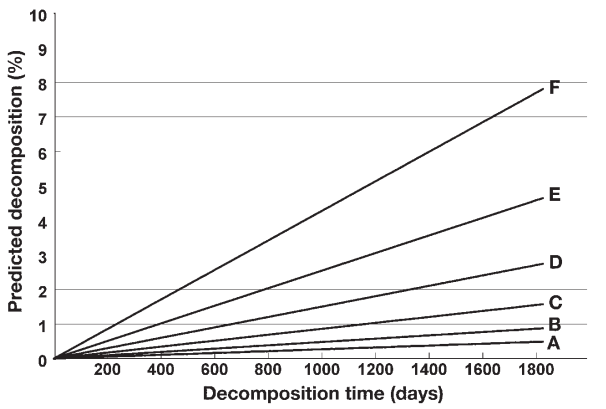


**Figure 3.** Predicted decomposition of acetaminophen over a five-year period for samples maintained at: (A) 25°C, (B) 30°C, (C) 35°C, (D) 40°C, (E) 45°C, and (F) 50°C.

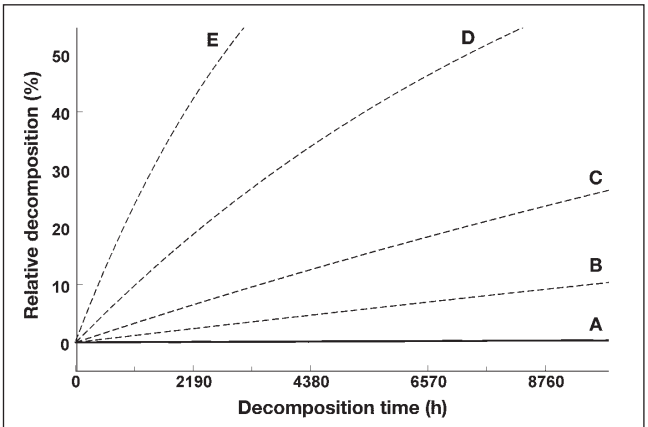


**Figure 4.** Comparison of the relative decomposition of acetaminophen at 35°C for the: (A) dry state and in solution at (B) pH 6, (C) pH 8, (D) pH 9, and (E) pH 2. Solution degradation plots are generated from the data published in references 1 and 7.

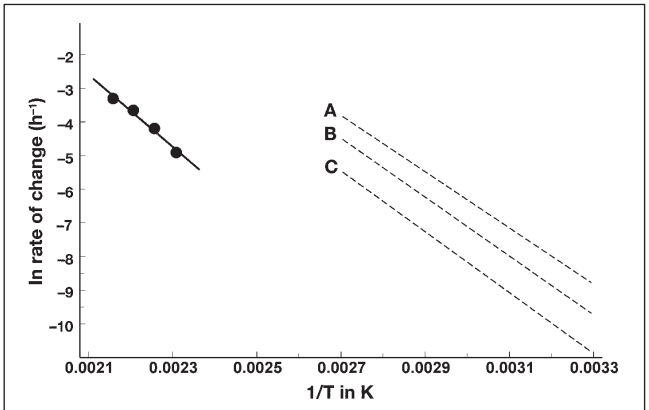


**Figure 5.** Comparison of the rate of change in $h^{-1}$ of acetaminophen versus reciprocal temperature for the dry state measurements shown in Figures 1 and 2 (i.e., the solid line with accompanying data points). Also shown as dashed lines are generated curves for the solution degradation data for acetaminophen given in references 1 and 7. Plots: (A) pH 2, (B) pH 9, and (C) pH 8.

parent-ammonium adduct, $MNH_4^+$(*m/z* 169), as well as other sodium and potassium adducts $MNa^+$(*m/z* 174) and $MK^+$(*m/z* 190), and the dimeric products $M_2H^+$(*m/z* 303), $M_2NH_4^+$(*m/z* 320), $M_2Na^+$(*m/z* 325), and $M_2K^+$ (*m/z* 341). The relative abundancies of these ions as a function of temperature are summarized in Table II for a single set of experiments. The reproducibility of these thermal changes is discussed later.

As noted from Table II, the three major ions present (i.e., over the total temperature range studied) were the thermal decomposition fragment (*m/z* 110), $MH^+$ ion (*m/z* 152), and parent-sodium adduct $MNa^+$ (*m/z* 174). Shown in Figure 7 are partial spectra of the region that contained these ions for inlet temperatures of 130°C, 150°C, 170°C, 190°C, 210°C, and 230°C. As can be seen from these data, the temperature of the inlet capillary had a significant effect on the ESI–MS spectra, consistent with changes in the spectra observed for other compounds (10,11) and similar interface designs (12–14). The *m/z* 110 fragment increased over an order of magnitude (i.e., from 6.7% to 100%) when the capillary temperature was changed from 130°C to 230°C. The *m/z* 110 fragment resulted from cleavage of the amide bond as shown in Figure 8. This fragmentation mechanism involves the thermal decarboxylation and formation of the corresponding amine and is consistent with the expected decomposition of acetaminophen obtained by more classical procedures (5–7) as well as ESI–MS data for a related analyte (15). Likewise, the sodium adduct, which is most likely attributable to the presence of a sodium impurity in the ammonium acetate delivery buffer, follows a curved relation with temperature, first increasing and then decreasing.

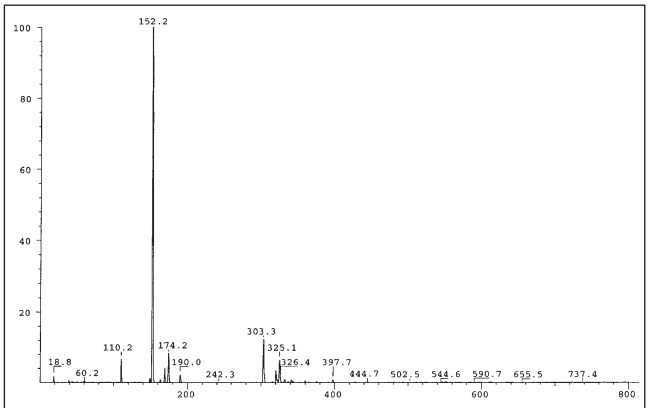


**Figure 6.** Mass spectrum of acetaminophen obtained on a Finningan spectrometer equipped with an in-line electrospray with a heated capillary inlet maintained at 130°C.

Because of the complexity of the spectral changes, simple comparisons based on variations in the relative abundance of the fragment ion with temperature did not correlate satisfactorily with the relative stability of acetaminophen in the capillary inlet transfer tube. However, when changes in the relative abundance of the fragment ion with respect to all forms of the undecomposed analyte were compared, as discussed later, an estimate of the relative stability of acetaminophen could be obtained.

The fragmentation ratios with respect to the parent forms ($FR_{PF}$) and total forms ($FR_{TF}$) were calculated using equations 1 and 2, respectively:

$$FR_{PF} = \frac{I_{fragment}}{\Sigma I_{monomeric\ forms} + 2 \times \Sigma I_{dimeric\ forms}} \quad \text{Eq. 1}$$

$$FR_{TF} = \frac{I_{fragment}}{I_{fragment} + \Sigma I_{monomeric\ forms} + 2 \times \Sigma I_{dimeric\ forms}} \quad \text{Eq. 2}$$

where $I_{fragment}$ is the relative ion abundance of the *m/z* 110 fragment for acetaminophen; $\Sigma I_{monomeric\ forms}$ is the sum of the relative ion abundance of the $MH^+$ and other analyte parent-adduct ions $MNH_4^+$, $MNa^+$, and $MK^+$; and $\Sigma I_{dimeric\ forms}$ is the sum of the relative ion abundance of the dimeric products $M_2H^+$, $M_2NH_4^+$, $M_2Na^+$, and $M_2K^+$. The relationship between various target ions and the total ion current has been used to study other gas-phase processes (16,17).

**Table II. Influence of Temperature on the Relative Ion Abundances (%Base Peak) for Acetaminophen***

| Ion (*m/z*) | 130°C | 150°C | 170°C | 190°C | 210°C | 230°C |
|---|---|---|---|---|---|---|
| Decomp. frag. (110) | 6.7 | 14.7 | 26.7 | 42.5 | 65.0 | 100.0 |
| $MH^+$ (152) | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 99.9 |
| $MNH_4^+$ (169) | 4.4 | 0.7 | 0.1 | 0.1 | 0.3 | 0.5 |
| $MNa^+$ (174) | 8.7 | 18.4 | 31.0 | 33.1 | 32.4 | 28.0 |
| $MK^+$ (190) | 1.9 | 2.3 | 2.3 | 0.7 | 0.3 | 0.6 |
| $M_2H^+$ (303) | 11.7 | 5.8 | 2.6 | 1.4 | 1.1 | 2.4 |
| $M_2NH_4^+$ (320) | 3.6 | 0.7 | 0.3 | 0.5 | 0.4 | 2.9 |
| $M_2Na^+$ (325) | 6.6 | 9.5 | 11.3 | 7.5 | 4.5 | 2.6 |
| $M_2K^+$ (341) | 1.7 | 0.7 | 0.6 | 0.5 | 1.0 | 2.0 |
| Total parent forms | 162.2 | 154.8 | 163.0 | 153.7 | 147.0 | 148.8 |
| Frag./parent forms | 0.04 | 0.09 | 0.16 | 0.29 | 0.47 | 0.67 |
| Total all forms | 169.9 | 169.5 | 189.7 | 196.2 | 212.0 | 248.8 |
| Frag./all forms | 0.04 | 0.09 | 0.14 | 0.22 | 0.31 | 0.40 |
| **Ave. frag./parent** | **0.04** | **0.10** | **0.19** | **0.31** | **0.49** | **0.75** |
| **Standard deviation** | **± 0.004** | **± 0.014** | **± 0.029** | **± 0.021** | **± 0.021** | **± 0.067** |
| **Ave. frag/all forms** | **0.04** | **0.09** | **0.16** | **0.24** | **0.33** | **0.43** |
| **Standard deviation** | **± 0.003** | **± 0.012** | **± 0.020** | **± 0.012** | **± 0.009** | **± 0.022** |

* Bolded data in last two rows represent the average of six different determinations.

Summarized in Table II are the calculated fragmentation ratios (i.e., both $FR_{PF}$ and $FR_{TF}$) for a single set of capillary inlet temperature experiments. Likewise, included are the composite data (bolded rows) for a set of six independent determinations along with the corresponding statistical variation reported as the standard deviation. These data were used to construct Figure 9, which shows the relationships of the natural logarithm of the relative fragmentation versus 1/T in K calculated using equations 1 (data shown as ●) and 2 (data shown as ▲). In the latter instance the slope of the plot yields an apparent activation energy for fragmentation of 9.3 kcal/mole, a value much smaller than that obtained in either the current solid state degradation study or for previously reported solution degradation investigations carried out under acid, basic, and neutral pH conditions (1,7). A possible mechanism for this reduced apparent activation energy may be attributable to the analyte interacting with the stainless steel sur-

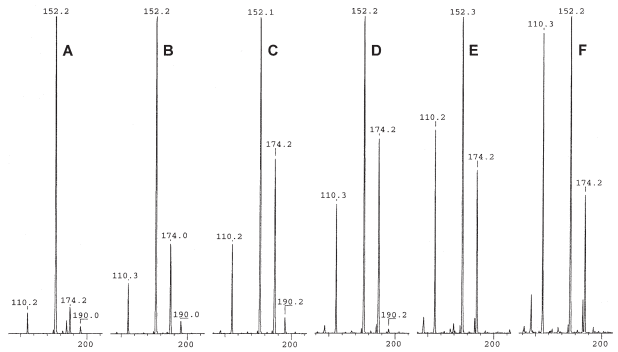


**Figure 7.** Partial mass spectrum of acetaminophen covering the mass range in which the dominant ions are present. Spectra at inlet capillary temperatures of: (A) 130°C, (B) 150°C, (C) 170°C, (D) 190°C, (E) 210°C, and (F) 230°C.

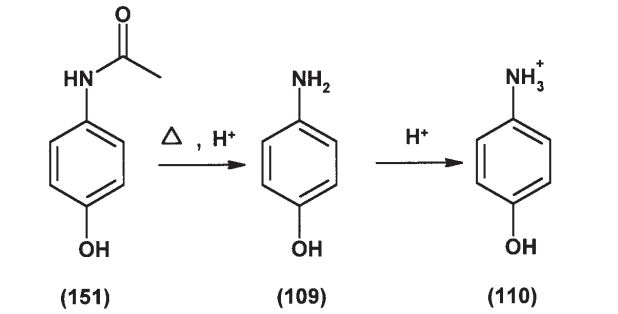


**Figure 8.** The thermal fragmentation of acetaminophen under ESI conditions and the production of the *m/z* 110 ion.

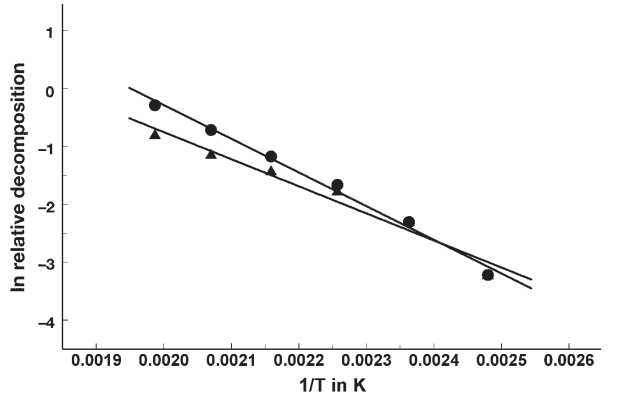


**Figure 9.** Plot of the relative decomposition of acetaminophen under ESI conditions versus the reciprocal temperature in K of the interface inlet capillary. Lines: (●) calculated using equation 1 and (▲) calculated using equation 2.

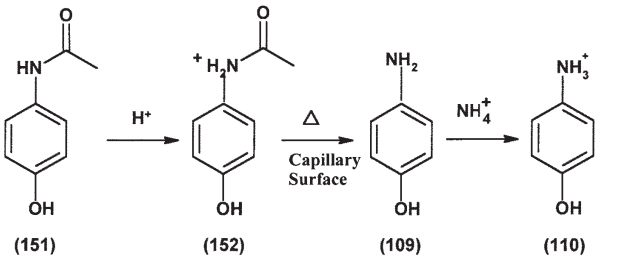


**Figure 10.** A possible two-step mechanism for the fragmentation of acetaminophen shown in Figure 8.

face of the inlet capillary and subsequent re-ionization of the neutral fragmentation/degradation product via proton transfer once it re-enters the gas phase. This two-step mechanism is shown in Figure 10. The initial production of the parent-$H^+$ ion in the electrospray region and subsequent degradation of it and the re-ionization of the product that forms (i.e., *p*-aminophenol) in the capillary region is also consistent with the absence of thermal fragment-ammonium, fragment-sodium, and fragment-potassium adduct ions that are present for the parent compound in any of the mass spectra (Table II and Figures 6 and 7).

## Conclusion

A systematic study has been carried out to compare the thermal and hydrolytic degradation of a well-understood compound, acetaminophen, with its thermal fragmentation as it undergoes ESI and transport via heated capillary inlet interface. Clearly there are many similarities between the analyte's degradation under acid- and base-catalyzed solution conditions in terms of the gas-phase fragmentation ion formed. However, the process occurs at a much lower apparent activation energy and is consistent with a mechanism that involves inlet capillary wall effects and re-ionization via gas-phase proton transfer reactions. Additional studies are in progress to examine the breakdown of other model compounds in order to develop a better understanding of ion transport in electrospray interfaces, as well as to evaluate further the proposed two-step fragmentation mechanism shown in Figure 10.

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