Fine Chemicals: Identification of Impurities in Drug Intermediates by LC/MSD Ion Trap

Application

Specialty Chemical

Authors

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Abstract

The production of fine chemicals requires high standards of purity for drug intermediates. Using the Agilent LC/MSD Ion Trap, sensitive impurity detection was demonstrated for intermediates used in the production of the drugs flurazepam, enalapril, and flunarizine. For each intermediate, LC/MS and MS/MS data were obtained in one run and the results provided sufficient information to propose structures for the corresponding impurities.

Introduction

Production of fine chemicals requires high standards of purity for drug intermediates and other critical applications. Impurities can cause unwanted and deleterious reactions and by-products. If carried through to final product, some impurities can be physiologically harmful. Others may be innocuous, having little effect on efficacy and concentration of the resulting drug. Regardless, it is necessary to determine the identity of impurities and establish a tolerance level. This level could be set as some percentage of total signal measured. Authenticated standards of the impurity may need to be prepared to confirm the identity and provide a quantitative measure. The need for extensive measures as these would wholly depend on the identification and evaluation of detected compounds.

Liquid chromatography/ion trap mass spectrometry (the Agilent LC/MSD Ion Trap) is ideally suited for the screening of intermediates for detection of impurities at low levels. Typically, the compound is dissolved at a high concentration and analyzed for impurities. The chromatographic method needs to provide sufficient separation of the material from its impurities. This article provides examples that show the unique and powerful capabilities of Agilent's LC/MSD Ion Trap. Sensitivity and the "Auto MS/MS" capability with the application of the powerful "SmartFrag" are demonstrated [1].

Experimental

The system used was an Agilent LC/MSD Ion Trap SL with an 1100 binary pump and well plate autosampler. A StableBond C_{18} column 2.1 × 50 mm with 10 mM ammonium acetate and acetonitrile as the mobile phase was used. A gradient from 10% to 90% organic produced separation and elution of all compounds present. Intermediates were prepared at concentrations of 5 to 10 mg/mL. Injections were made at 1 µL. The ion trap was operated with SmartFrag setting from 20% to 200% of the fragmentation amplitude (set at 1.0 V). The capillary exit and skimmer was set at a low energy to minimize fragmentation in the ion transport region. This was done using Smart Parameter Settings with a compound stability of 20%. With auto MS/MS turned on, only one run per intermediate was necessary to detect the protonated molecule (in positive ion mode) of impurities present and obtain MS/MS fragmentation of that precursor ion.

Results

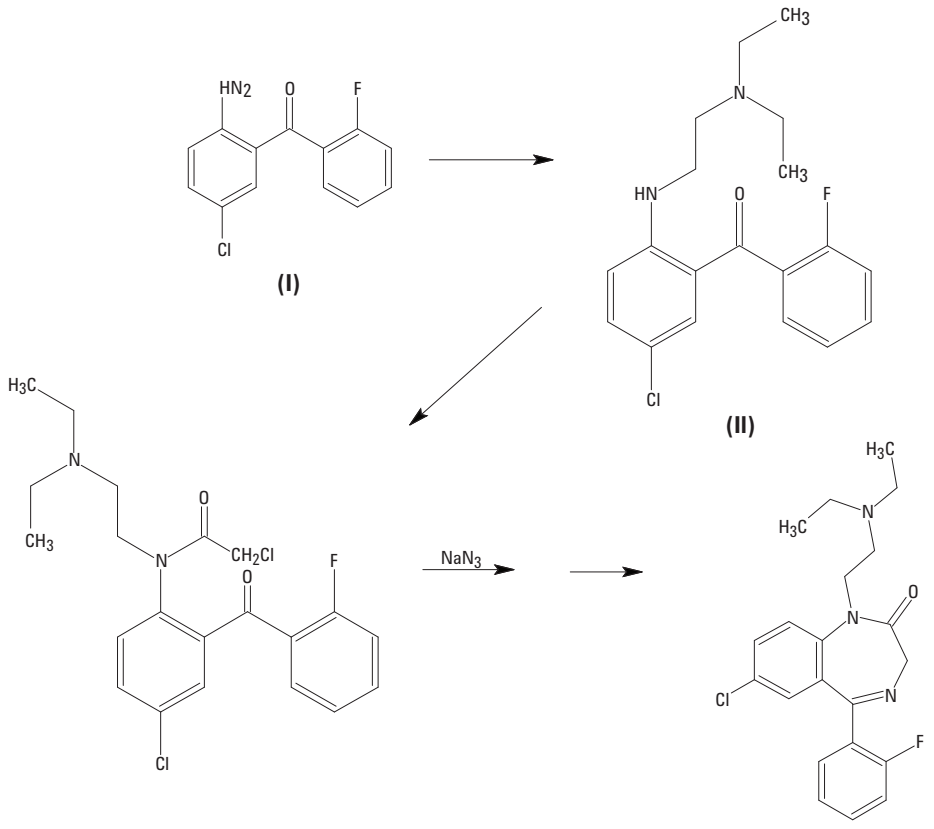
Example 1

1,4-Benzodiazepine intermediate

The compound 2-amino-5-chloro -2' -fluorobenzophenone (I) was analyzed as an intermediate to the drug flurazepam (IV). Scheme I shows the reaction of this compound to form the drug [2].

The total ion MS/MS chromatogram of the intermediate is shown in Figure 1. An impurity is observed at a low level as compared to the response of the intermediate. The LC/MSD Ion Trap detects the major ion in the impurity and performs an auto MS/MS ramping of the fragmentation amplitude from 0.2 to 2.0 V (SmartFrag). The resulting spectrum provides sufficient information to propose a structure. The single MS data shows that the $[M+H]^+$ ion is the same as the intermediate. It also

Scheme I



gives an isotope ratio consistent with one chlorine (as with the intermediate). However, the MS/MS fragmentation of the impurity shows only a major product ion at *m/z* 123. The intermediate's MS/MS produces an *m/z* 154 and a less intense *m/z* 123 product ion. This is consistent with fragmentation on both sides of the carbonyl bond. The proposed structure favors the cleavage of the amido bond and neutral loss of chloroaniline. The spectrum and the proposed impurity are shown in Figure 2. The StableBond column shows excellent stability at the low pH of the chromatographic conditions, and even with a 2.1 × 50 mm column, the needed chromatographic resolution is obtained.

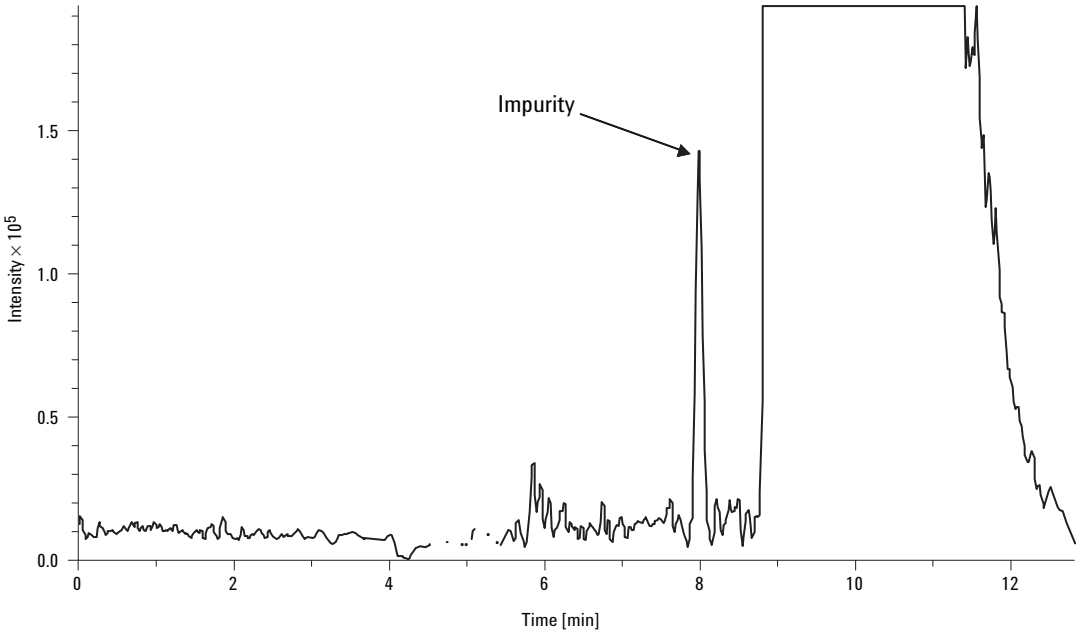


Figure 1. Total ion chromatogram (auto MS/MS) of flurazepam intermediate 2-amino-5-chloro-2'-fluorobenzophenone. Note the good separation obtained for the impurity (denoted by the arrow) using the StableBond C_{18} 2.1 × 50 mm rapid resolution column.

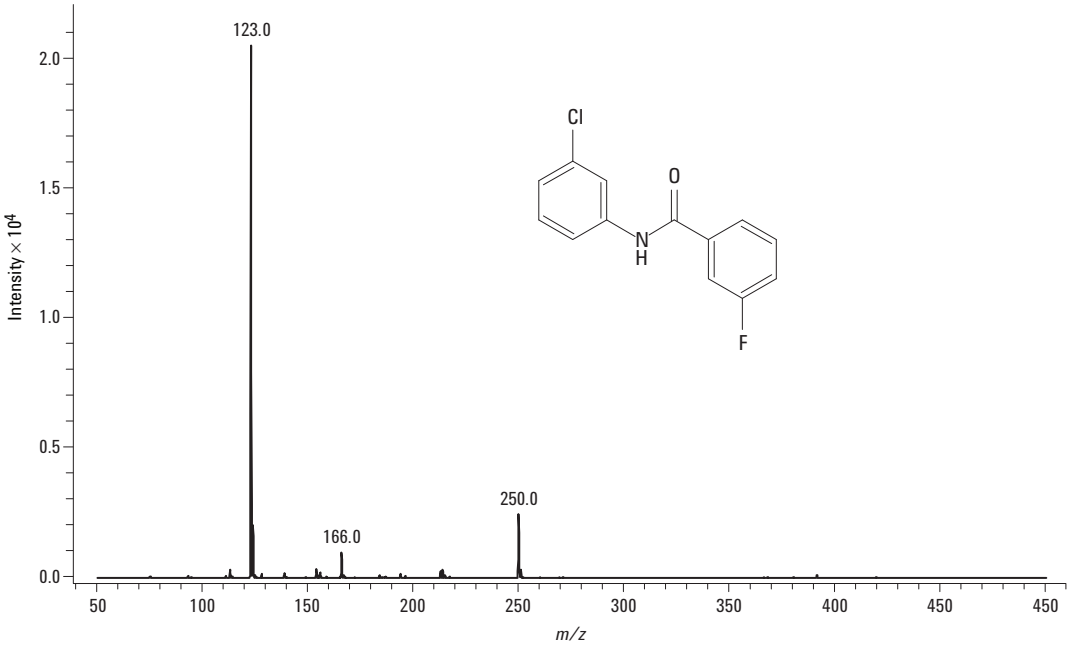
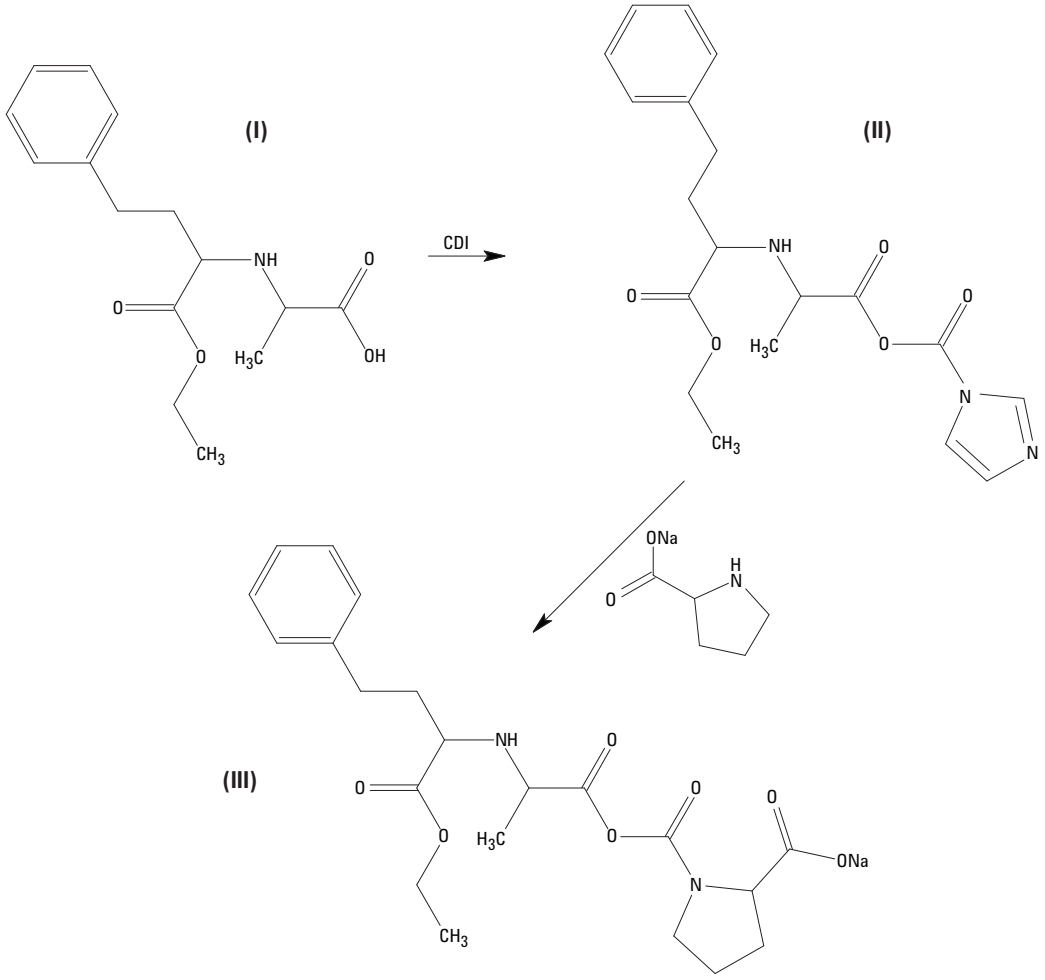


Figure 2. Auto MS/MS of impurity in 2-amino-5-chloro-2'-fluorobenzophenone with proposed identity.

Example 2

Another example involves the ACE inhibitor Enalapril (III) prescribed for chronic heart failure. A reaction pathway for the production of Enalapril is shown in Scheme II.

Scheme II



In this reaction the intermediate, N-(1-ethoxycarbonyl-3-phenyl propyl) alanine (I) is reacted with 1,1'-carbonydiimidazole (CDI) and then further reacted with sodium proline to produce Enalapril (III). A concentrated solution of the intermediate analyzed with the LC/MSD Ion Trap again, in Figure 3, shows impurities. The auto MS/MS spectrum of the early-eluting impurity and a proposed structure is given in Figure 4. This impurity displays a single MS spectrum with an ion at *m/z* 266. This is 14 amu less than the intermediate and suggests the impurity contains one less methylene. The MS/MS of the intermediate (not shown) gives prominent *m/z* 234 and 206 product ions. Loss of formaldehyde would produce the *m/z* 234. Loss of ethylformate produces the *m/z* 206. In the impurity MS/MS spectrum, the *m/z* 206 is present suggesting preservation of the phenylpropylalanine. The *m/z* 220 suggests the methylene is missing from the ester. Again the combination of good separation by LC and the power of auto MS/MS and SmartFrag demonstrates the ability to detect and identify impurities.

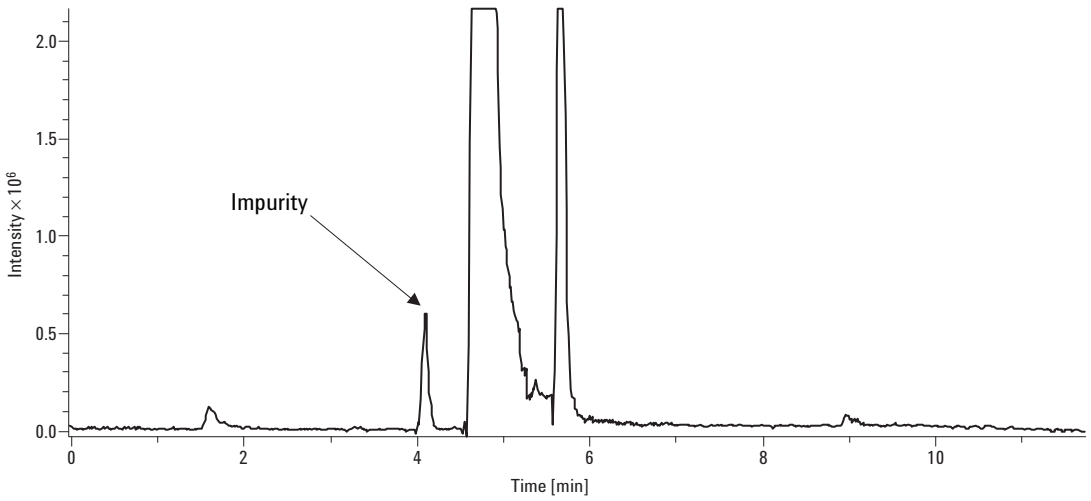


Figure 3. Total ion chromatogram of auto MS/MS showing impurity separated from intermediate of Enalapril.

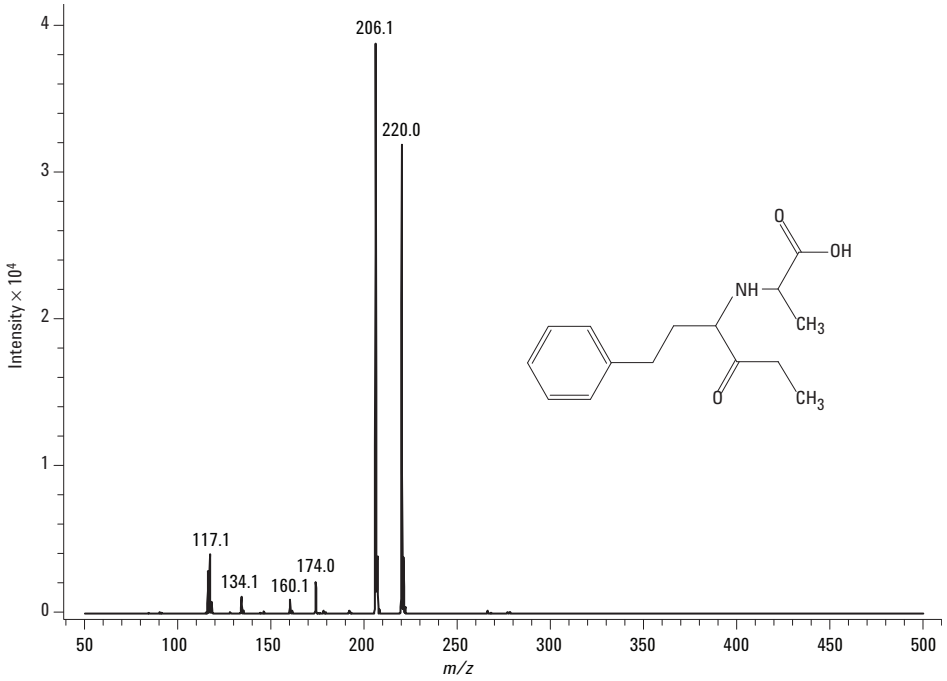
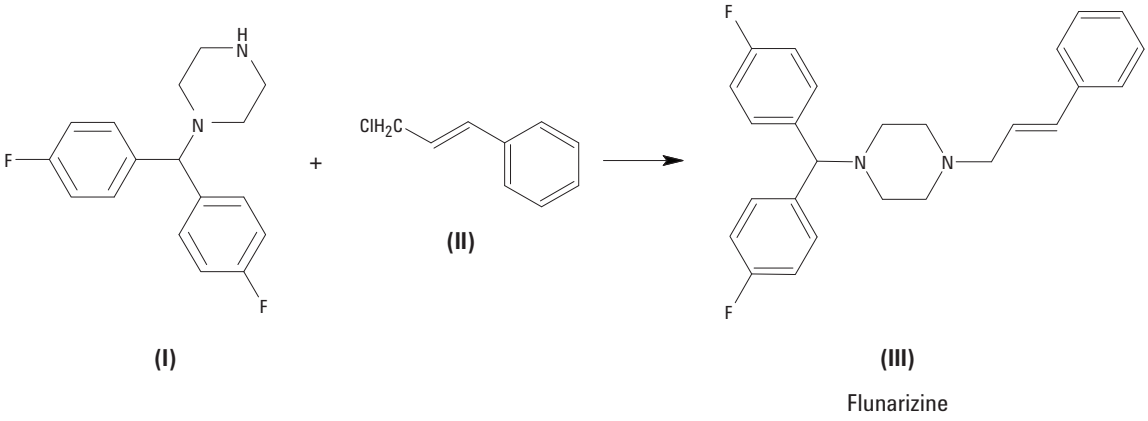


Figure 4. Auto MS/MS of impurity in N-(1-ethoxycarbonyl-3-phenyl propyl) alanine with proposed structure.

Example 3

A third example is the intermediate (I) for flunarizine (III), 1-[(4-fluorophenyl)(phenyl)methyl]piperazine (I). A synthetic pathway for flunarizine, a calcium blocker used for migraine headaches, is shown in Scheme III. In this example Auto MS/MS was not used. The total ion single MS chromatogram of the intermediate is given in Figure 5.

Scheme III



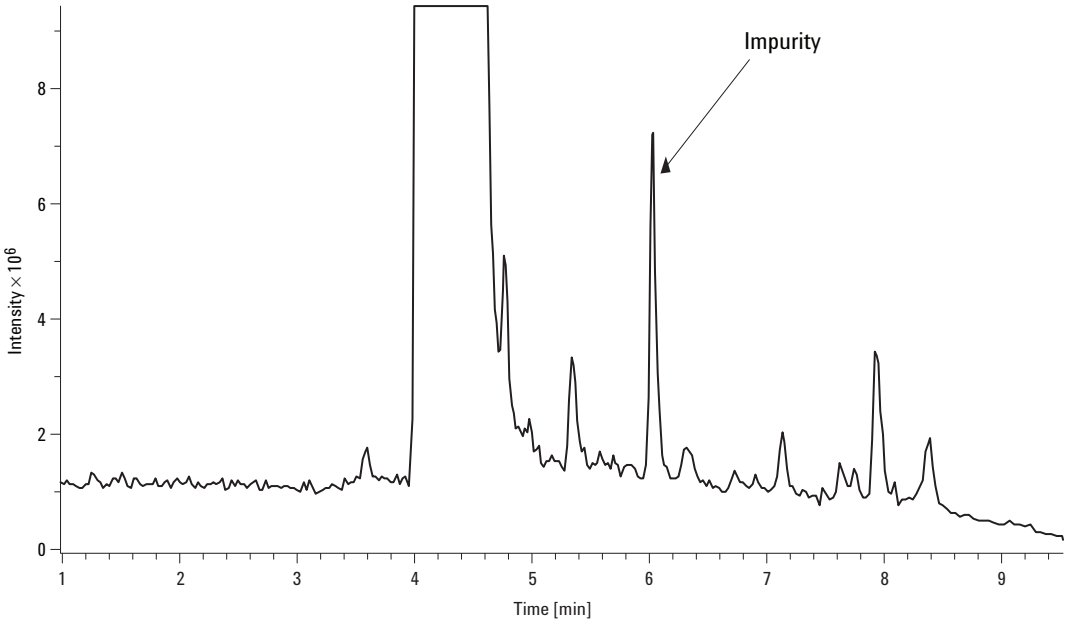


Figure 5. Total single MS ion chromatogram of flunarizine intermediate. The arrow indicates the impurity with *m/z* 317.

A second analysis was performed using MS/MS of an impurity peak with *m/z* 317, and the resulting spectrum is shown in Figure 6 with a proposed structure. Both the intermediate and the impurity show a prominent *m/z* 203, suggesting the loss of the piperazine moeity. The addition of the ethyl group would explain the data. In this analysis, a minimum of two experiments, one MS and one MS/MS, were required without auto MS/MS to attain sufficient information to propose a structure.

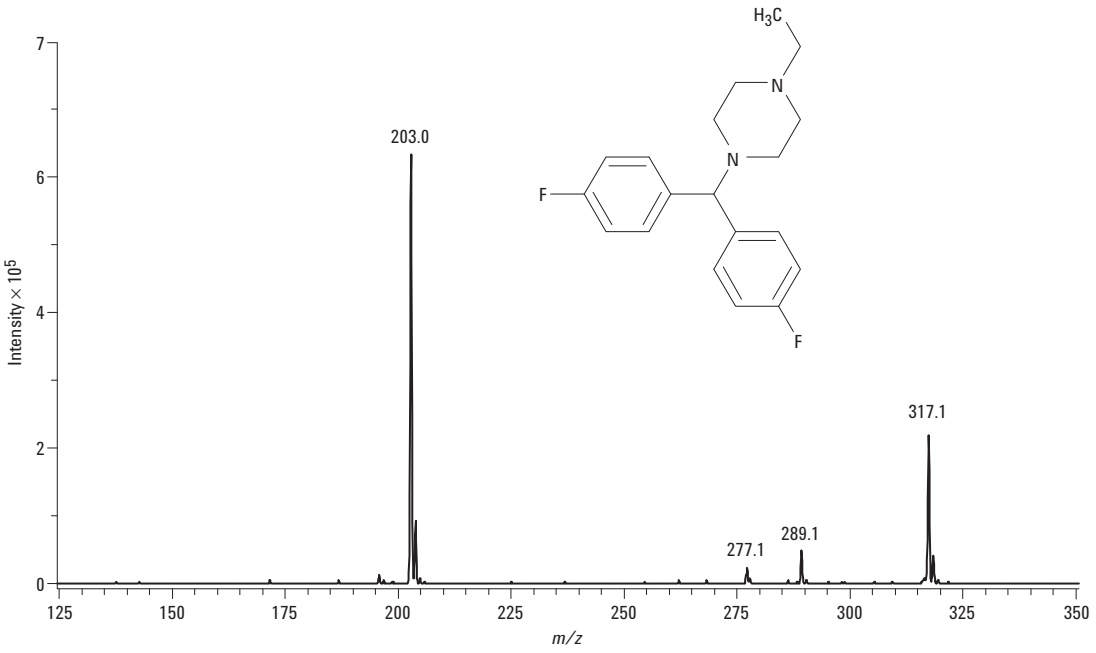


Figure 6. MS/MS of *m/z* 317 with proposed structure in the analysis of the intermediate 1-[(4-fluorophenyl)(phenyl)methyl]piperazine.

Conclusions

Three examples of fine chemicals have been used to demonstrate the power of the LC/MSD Ion Trap for identification of impurities. Agilent's SmartFrag and Auto MS/MS provided the capability to obtain precursor ion and representative fragmentation in one LC/MS and MS/MS run. The robustness of the orthogonal sprayer and Agilent StableBond column allowed overload of the subject chemical with good separation and signal of the impurities detected. The resulting data provided enough information to propose structures. It must be noted that MS/MS alone is insufficient for positive identification of an unknown. Comparison of the MS/MS spectrum with an authenticated reference is required. Information, such as matching chromatographic retention time with an authenticated standard, provides additional confirmation.

References

1. Paul Goodley, "Maximizing MS/MS Fragmentation in the Ion Trap Using CID Voltage Ramping," Agilent Technologies, publication 5988-0704EN, www.agilent.com/chem
2. Norman W. Gilman et. al., (1990) "Atropisomers of 1,4-Benzodiazepines. Synthesis and Resolution of a Diazepam-Related 1,4-Benzodiazapine," Journal of the American Chemical Society, 112, 3969-3978.

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