

Using High Resolution Mass Spectrometry to Clarify Ambiguous Benzodiazepine Ions in the NIST EI-MS Library

Edward P. Erisman*; Arun S. Moorthy; William E. Wallace; Stephen E. Stein

We have observed ambiguity in the results of NIST MS Interpreter (MS-I) analysis of low resolution (lr) GCMS data of the “triazolo” and “2-keto” subclasses of benzodiazepines. MS-I is a software tool that attempts to explain fragment peaks in a mass spectrum based on a user-provided chemical structure and a set of well-defined fragmentation rules. It can work with either high or low-resolution ms data. Ambiguity in MS-I results can come from a failure to assign a fragment peak, multiple different assignments of a single fragment peak, or an incorrect assignment of a fragment peak. High resolution (hr) ms data can clarify an incorrect fragment assignment or help choose between multiple possible assignments.

Assessing Fragments

Low-resolution spectra were from NIST2020.

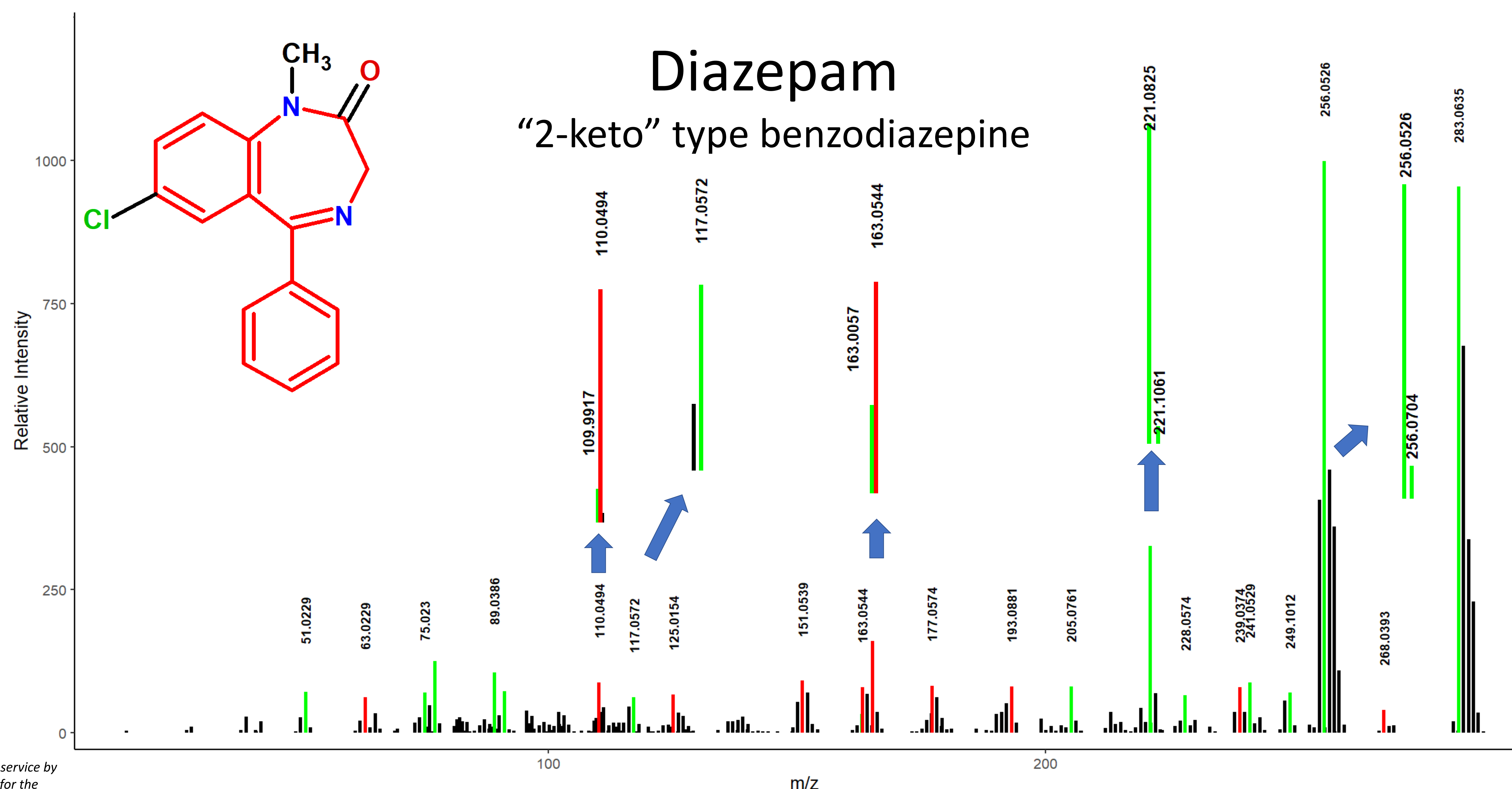
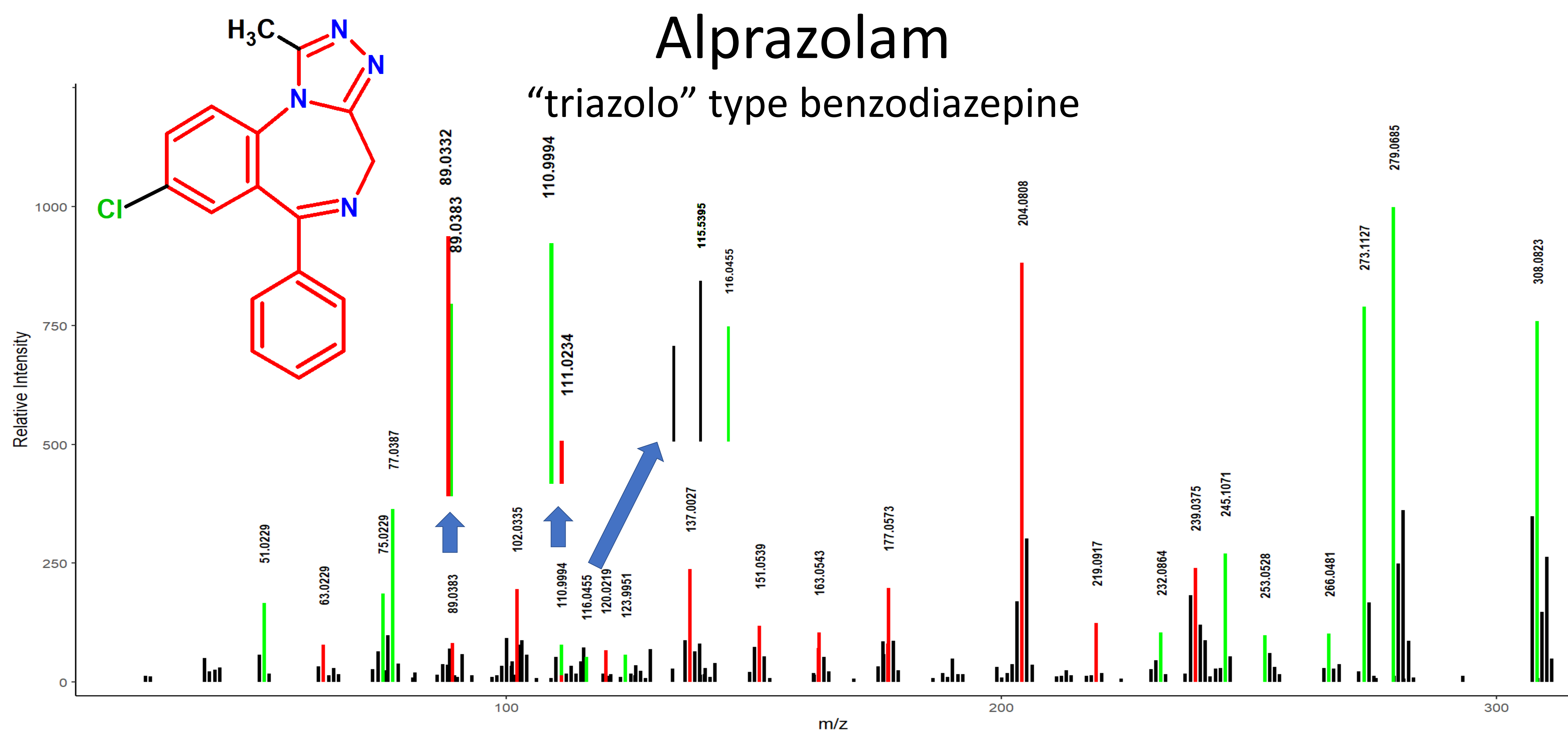
Alprazolam and Diazepam were chosen as characteristic compounds of “triazolo” or “2-keto” benzodiazepines.

High resolution 70 eV spectra were obtained with a Agilent 8890 GC mated to a Jeol GC-Alpha Time Of Flight mass spectrometer.

Raw data was centroided and drift corrected to column bleed (m/z - 207.032535) with Jeol’s msAxel.

Jeol’s msFine Analysis was used to extract deconvoluted spectra and calculate fragment formulas.

NIST MS Interpreter was used to analyze possible fragment structures from both high and low resolution data.



Evaluation of Results:

Twenty-four of the most abundant fragments in alprazolam were analyzed. In lr MS-I analysis 16 fragments are assigned and in hr analysis 14 are assigned. Two of the assigned fragments (m/z ~89 and 111) have an unassigned second peak in the hr data. The peak at m/z 89.0332 corresponds to a doubly charged $C_{13}H_8N$ fragment. Peaks at m/z ~204 and 219 have explanations in lr analysis that are not corroborated with hr data. NIST main and replicate libraries have multiple lr spectra with inconsistent m/z 115 and 116 abundances. In the hr data there are peaks at ~115.5 (double charged $C_{15}H_9N_3$) and ~116 (C_8H_6N).

Twenty-three of the most abundant fragments in diazepam were analyzed. In lr MS-I analysis 16 fragments are assigned and in hr analysis 13 are assigned. The peak at m/z ~193 had a lr assignment but no hr assignment and the calculated formula did not match the MS-I assignment. For m/z ~110, 163, and 165 the most abundant peak is unassigned, but an assignment can be made for a less abundant peak. In lr analysis the peaks m/z ~221 and 256 have two explanations each. The hr data and analysis corroborates both assignments with two peaks each for m/z ~221 and 256. There are two peaks at m/z ~117 one of which is explained and the other corresponds to doubly charged $C_{15}H_{10}N_2O$.

Many of the unexplained peaks are common between the two benzodiazepine types with equivalent calculated fragment compositions.

Using high resolution data, we are able discern if multiple assignments in lr analysis are appropriate or if any particular assignment is correct.

Future Work:

High resolution data could be used to refine and expand MS Interpreter rules to help clarify ambiguity in low resolution data and explain more fragments.

*For more information, contact: edward.erisman@nist.gov or go to <https://chemdata.nist.gov>