

Use of a Model Acid System to Interpret Results from GC/MS Analysis of Environmental Naphthenic Acids

C. Dustin Clark¹; Henry Allred²; Nicole Glines¹; Charley C. Langley²

¹Department of Biology; ²Department of Chemistry and Biochemistry; Utah State University- Uintah Basin, Vernal, UT



Overview

Purpose

In this study we use a collection of compounds that meet the standard definition of a naphthenic acid (NA) as a model. This model was used to interpret observations made in the gas chromatography-mass spectrometric (GC/MS) analysis of naphthenic acid species found in environmental samples.

Methods

- Environmental naphthenic acids from Asphalt Ridge (Vernal, Utah) oil sands ores were obtained via caustic digestion followed by organic extraction.
- Extracted naphthenic acids were derivatized with N-methyl-N-(tert-butyl-dimethyl-silyl)trifluoroacetamide (MTBSTFA) and analyzed using GC/MS.
- Model naphthenic acids were obtained, derivatized and analyzed using GC/MS, both alone and as an equimolar mixture.
- Intensity plots were generated from environmental and model naphthenic acids systems and compared to determine whether the model replicated behaviors observed for the environmental sample.

Results

The model acid system replicated band patterns observed in environmental samples. Presence of the *tert*-butyldimethylsilyl substituent appears to dominate fragmentation of model acids and minimize homologous fragment ions. If we can extrapolate to larger, less saturated systems then our results validate the assumption that we are seeing discrete, co-eluting organic acids.

Introduction

Naphthenic acids (NAs) are aliphatic carboxylic acids containing at least one ring, conforming to the general formula $C_nH_{2n+2}O_2$, where n is carbon number and z is degrees of unsaturation, and found in petrochemical resources. These acids can cause corrosion in industrial equipment and some are known to be toxic in the environment¹. Not all NAs are equally corrosive or toxic, however, and being able to differentiate between NA structures using affordable, bench-top technologies could result in savings for both business and environmental concerns.

Derivatization of NAs with *N*-methyl-*N*-(*tert*-butyldimethyl-silyl)trifluoroacetamide (MTBSTFA) replaces the acidic proton with a *tert*-butyldimethylsilyl substituent. Derivatization reduces fragmentation and increases sample volatility². For derivatized carboxylic acids the base peak is usually $M-57^+$, consistent with the loss of C_4H_9 . Gas Chromatographic Mass Spectrometric (GC/MS) analysis of NAs extracted from process water yields a chromatogram containing a profusion of unresolved peaks. A mass spectrum from one of these peaks often contains more than one possible derivatized NA species, as identified by the presence of an $M-57^+$ mass peak. In this study we use a model system composed of ten commercially available compounds conforming to the definition of a NA to replicate chromatographic and mass spectrometric structures observed in a more complex system.

Methods

Environmental Naphthenic Acids

- Obtained from Asphalt Ridge oil sands ore using hot water caustic extraction methods adapted from the work of Holowenko et al.³
- Extracted organic material was diluted with dichloromethane (DCM) to a concentration of 0.32 mg/mL and reacted with MTBSTFA for 1 hr. at 60°C prior to GC/MS analysis.

Model Naphthenic Acids

- Obtained from commercial sources.
- Diluted with DCM to 3.2×10^{-4} M for individual analysis or 3.2×10^{-5} M for analysis as ten acid mixture and reacted with MTBSTFA for 1 hr. at 60°C prior to GC/MS analysis.

GC/MS Analysis

- Shimadzu QP2010 GC/MS with an AOC-20i auto-injector, running GC/MS Solution version 2.50 SU1.
- GC Column: Shimadzu SHR5XLB with 30m length and a 0.25mm ID.
- The column temperature profile for environmental acid analysis was: initial column temperature of 40°C was held for one minute, increased at a rate of 12°C/minute to 150°C, increased at a rate of 6°C/min. to 260°C, then increased at a rate of 15°C/min. to a final temperature of 320°C and held for 4 min.
- A similar temperature profile was used for model systems, but terminated at a final temperature of 239°C.
- Mass spectral scanning began seven minutes after injections.
- A range of m/z 35-1000 at a rate of 2 scans/sec. was used for environmental NA samples.
- A range of m/z 35-400 at a rate of 5 scans/sec. was used for model NA samples.
- Electron impact ionization at 70 eV.

Model NAs used were:

- cyclohexanecarboxylic acid (CHCA), Alfa Aesar
- 1-methylcyclohexanecarboxylic acid (1MCHCA), Alfa Aesar
- 2-methylcyclohexanecarboxylic acid (2MCHCA), Aldrich
- 3-methylcyclohexanecarboxylic acid (3MCHCA), Aldrich
- 4-methylcyclohexanecarboxylic acid (4MCHCA), Aldrich
- cyclohexylacetic acid (CHAA), Aldrich
- cyclohexylpropionic acid (CHPPA), Alfa Aesar
- cyclohexanbutanoic acid (CHBA), Acros
- cyclohexanepentanoic acid (CHPTA), Aldrich
- trans-4-ethylcyclohexanecarboxylic acid (T4ECHCA), Alfa Aesar

Data Analysis

- Mass spectra were generated using GC/MS Solution Postrun Analysis.
- Igor Pro version 6.03 was used to generate intensity plots from exported data.
- Molecular Fragment Calculator version 1.0 was used to generate possible molecular formulas for mass spectral peaks.
- Isotopic distribution for selected mass spectral peaks was calculated using Molecular Weight Calculator version 6.46 (build 234), then compared with experimentally derived data.

Results

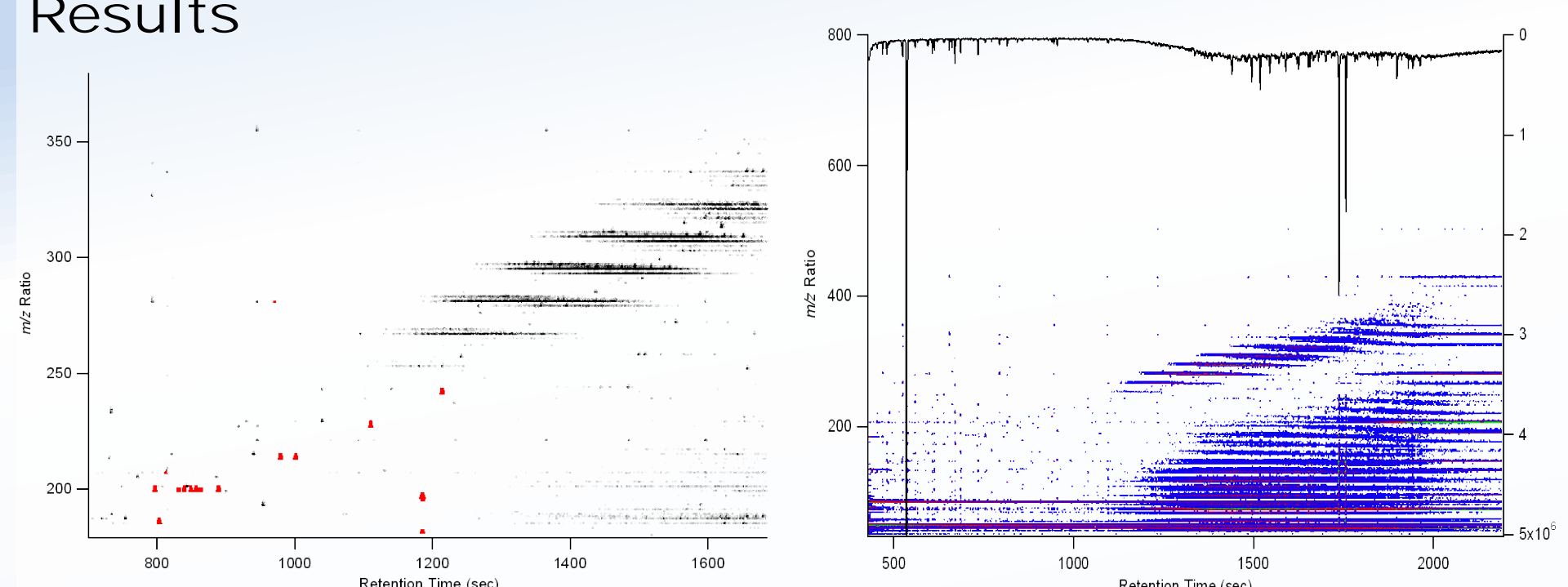


Figure 1. Above left: Intensity plot of model NA system (red) superposed upon environmental NAs (black). Note that the model NA $M-57^+$ ion bands follow the same trend as observed for the environmental NAs. Note also that there is little or no fragmentation observed in the region immediately below the $M-57^+$ ion bands. Above right: Intensity plot of environmental NAs showing the series of co-eluting bands observed. At top is the TIC for the same sample run. The TIC has been inverted to allow juxtaposition without obscuration.

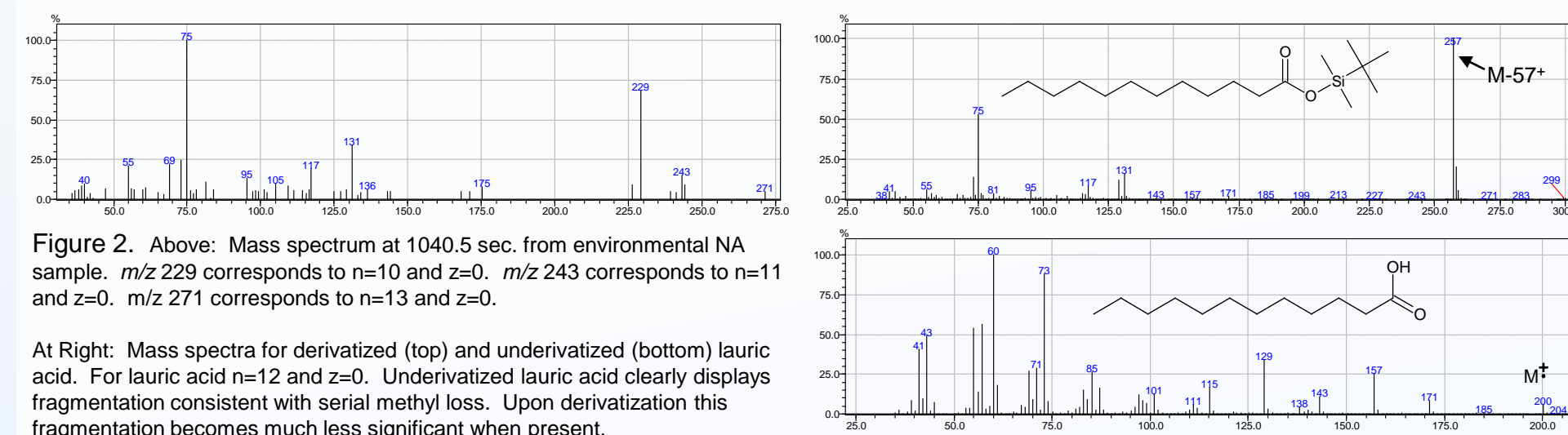


Figure 2. Above: Mass spectrum at 1040.5 sec. from environmental NA sample. m/z 229 corresponds to $n=10$ and $z=0$. m/z 243 corresponds to $n=11$ and $z=0$. m/z 271 corresponds to $n=13$ and $z=0$. At Right: Mass spectra for derivatized (top) and underivatized (bottom) lauric acid. For lauric acid $n=12$ and $z=0$. Underivatized lauric acid clearly displays fragmentation consistent with serial methyl loss. Upon derivatization this fragmentation becomes much less significant when present.

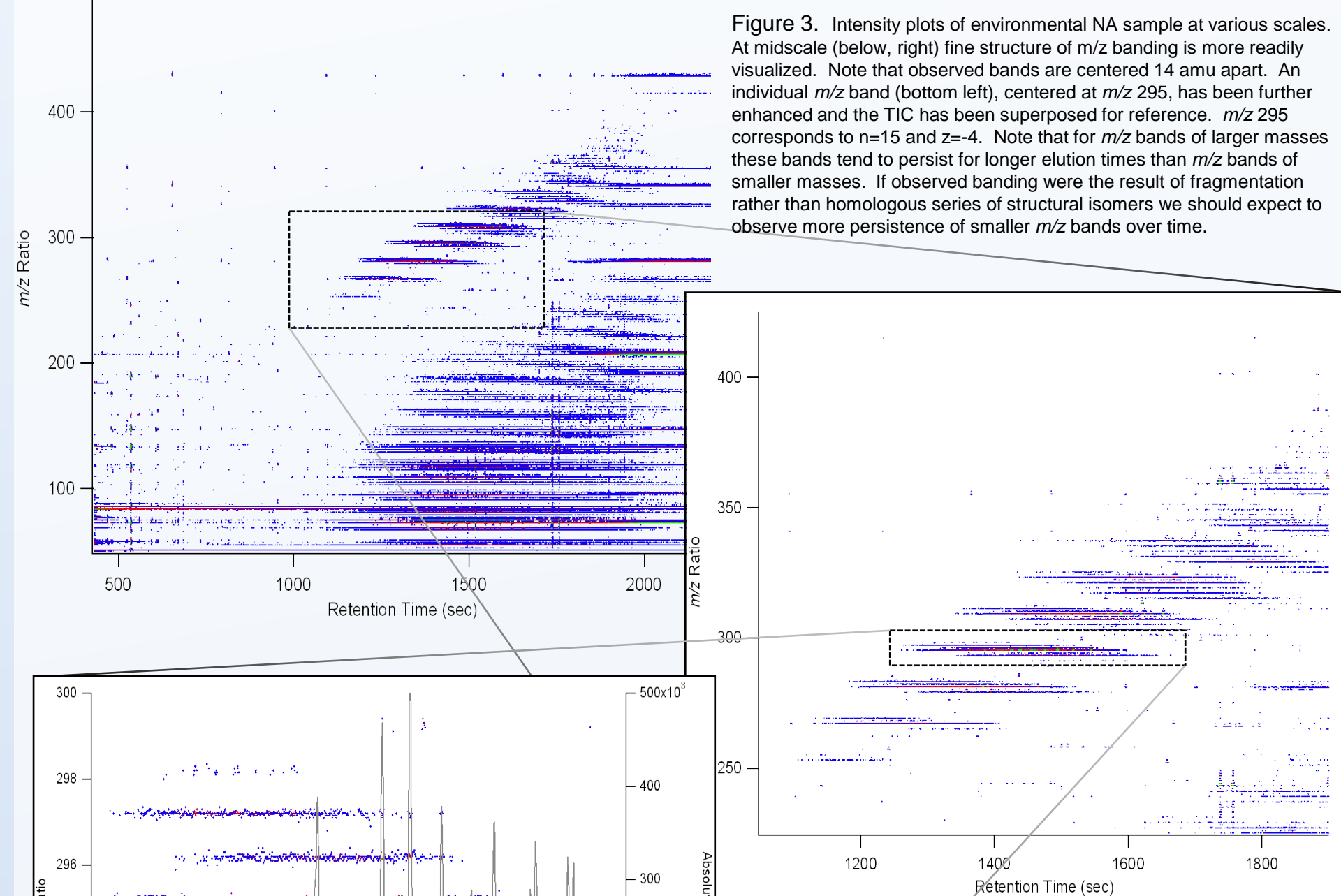
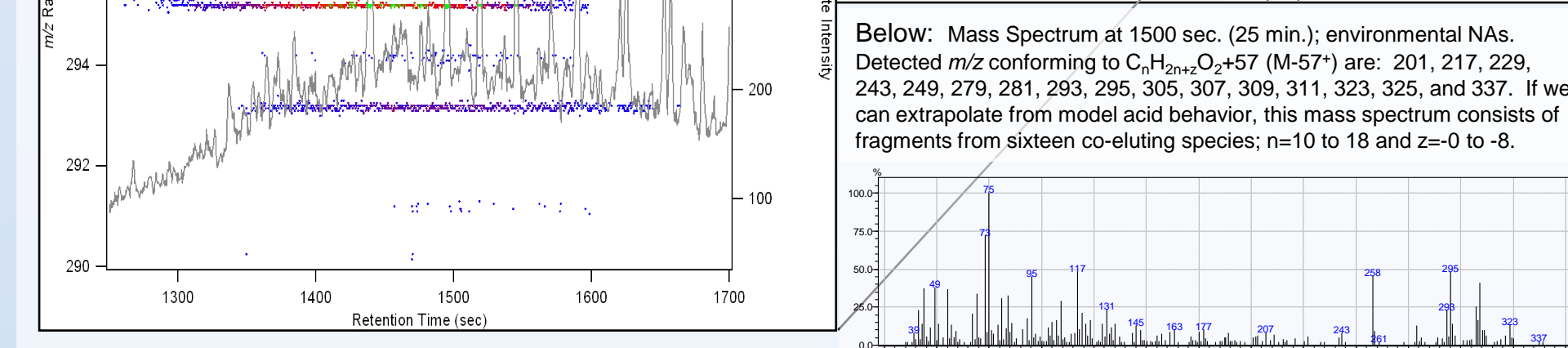


Figure 3. Intensity plots of environmental NA sample at various scales. At midscale (below, right) fine structure of m/z banding is more readily visualized. Note that observed bands are centered 14 amu apart. An individual m/z band (bottom left), centered at m/z 295, has been further enhanced and the TIC has been superposed for reference. m/z 295 corresponds to $n=15$ and $z=4$. Note that for m/z bands of larger masses these bands tend to persist for longer elution times than m/z bands of smaller masses. If observed banding were the result of fragmentation rather than homologous series of structural isomers we should expect to observe more persistence of smaller m/z bands over time.



Below: Mass Spectrum at 1500 sec. (25 min.); environmental NAs. Detected m/z conforming to $C_nH_{2n+2}O_2+57$ ($M-57^+$) are: 201, 217, 229, 243, 249, 279, 281, 293, 295, 305, 307, 309, 311, 323, 325, and 337. If we can extrapolate from model acid behavior, this mass spectrum consists of fragments from sixteen co-eluting species; $n=10$ to 18 and $z=0$ to -8.

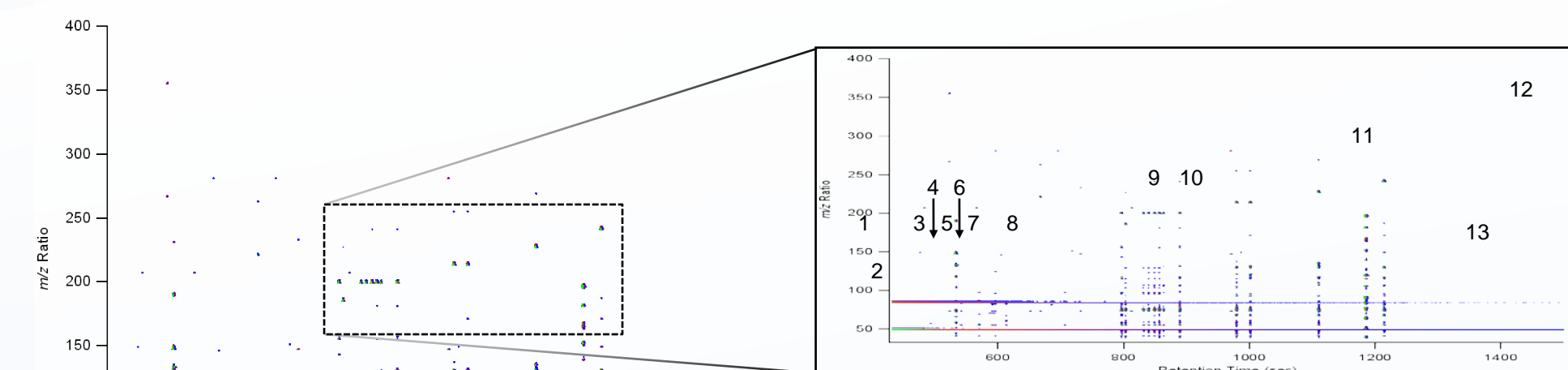


Figure 4. At Right: Intensity plot of model NA system. Bands at 84 and 49 amu are due to solvent elution. Above: Detail of model NA intensity plot. System components have been numbered. 1) 1MCHCA, 2) CHCA, 3) 2MCHCA and 3MCHCA co-eluting, 4) 4MCHCA, 5) 3MCHCA, 6) 2MCHCA, 7) 4MCHCA, 8) CHAA, 9) T4ECHCA, 10) CHPPA, 11) CHBA, 12) CHPTA, 13) 4-Methyl-benzophenone (4MBPhen). 4MBPhen is not a model NA, but was added as an internal calibrant.

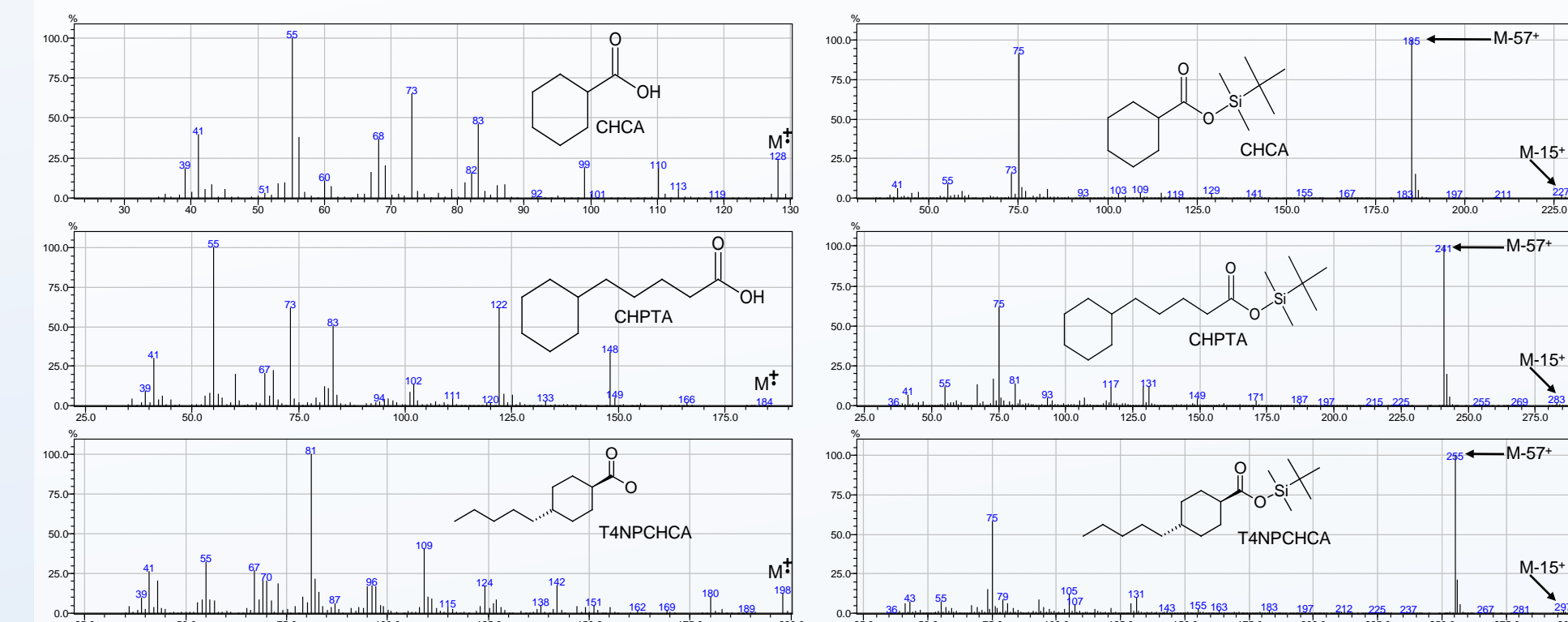


Figure 5. Selected model NAs are shown above, representing structural extremes among commercially available NAs. Derivatized acids are shown to the right and corresponding mass spectra for the underivatized acids appear immediately to their left. In every case we see that fragmentation of the derivatized acid appears to be dominated by the structure of the silyl ester, taking precedence over structural features of the carbon skeleton to which it is affixed. Trans-4-ethylcyclohexanecarboxylic acid (T4NPCHCA) is not represented in our model acid system but is shown here because it features a longer alkyl substituent than T4ECHCA.

Mass spectra for the remaining eight model NAs are shown below. Note that fragmentation of the silyl substituent is a dominant feature in every case. The $M-15^+$ ion has been labeled for each derivatized acid. The base peaks for all spectra correspond to $M-57^+$.

- $M-15^+$ appears in the mass spectra for all model acids at approximately the same relative intensity, even in cases where there is not a free methyl group on the NA skeleton, supporting loss of a methyl from the *tert*-butyldimethylsilyl substituent.
- m/z 131 and m/z 117 are fragments common to model acids in which the carboxylic functional group is not a substituent of the ring. Isotope distribution is examined in Figure 6.

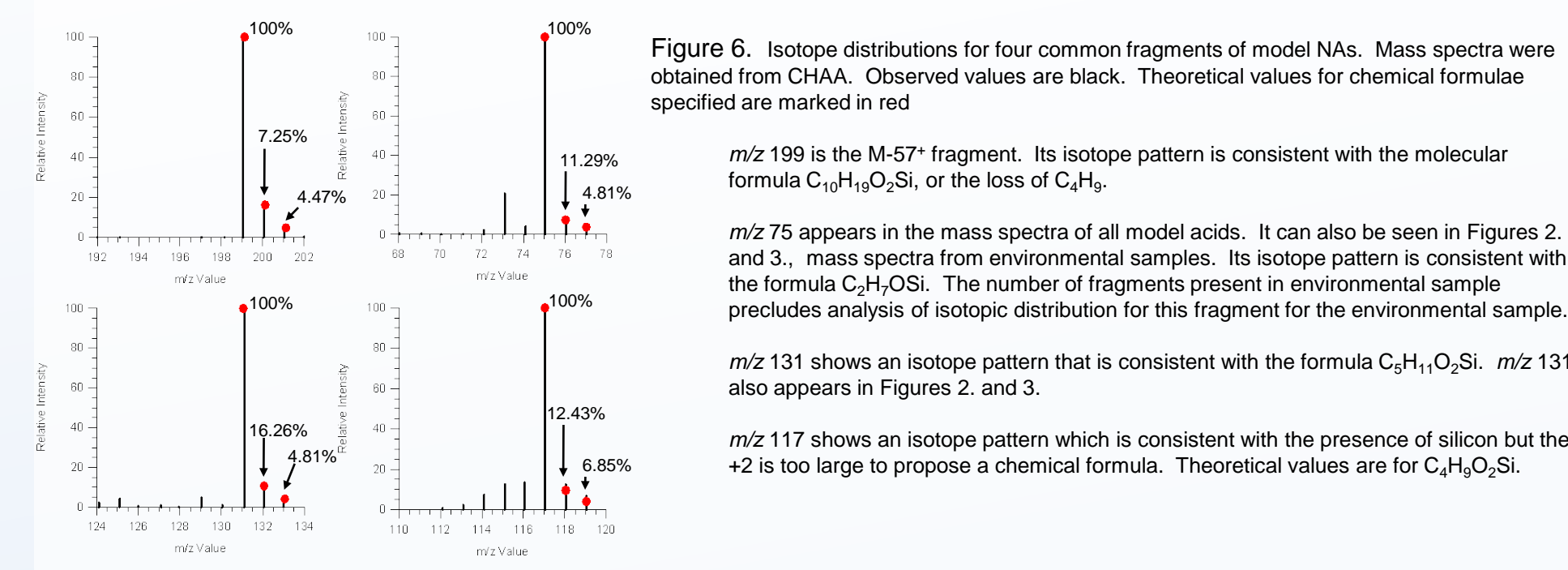
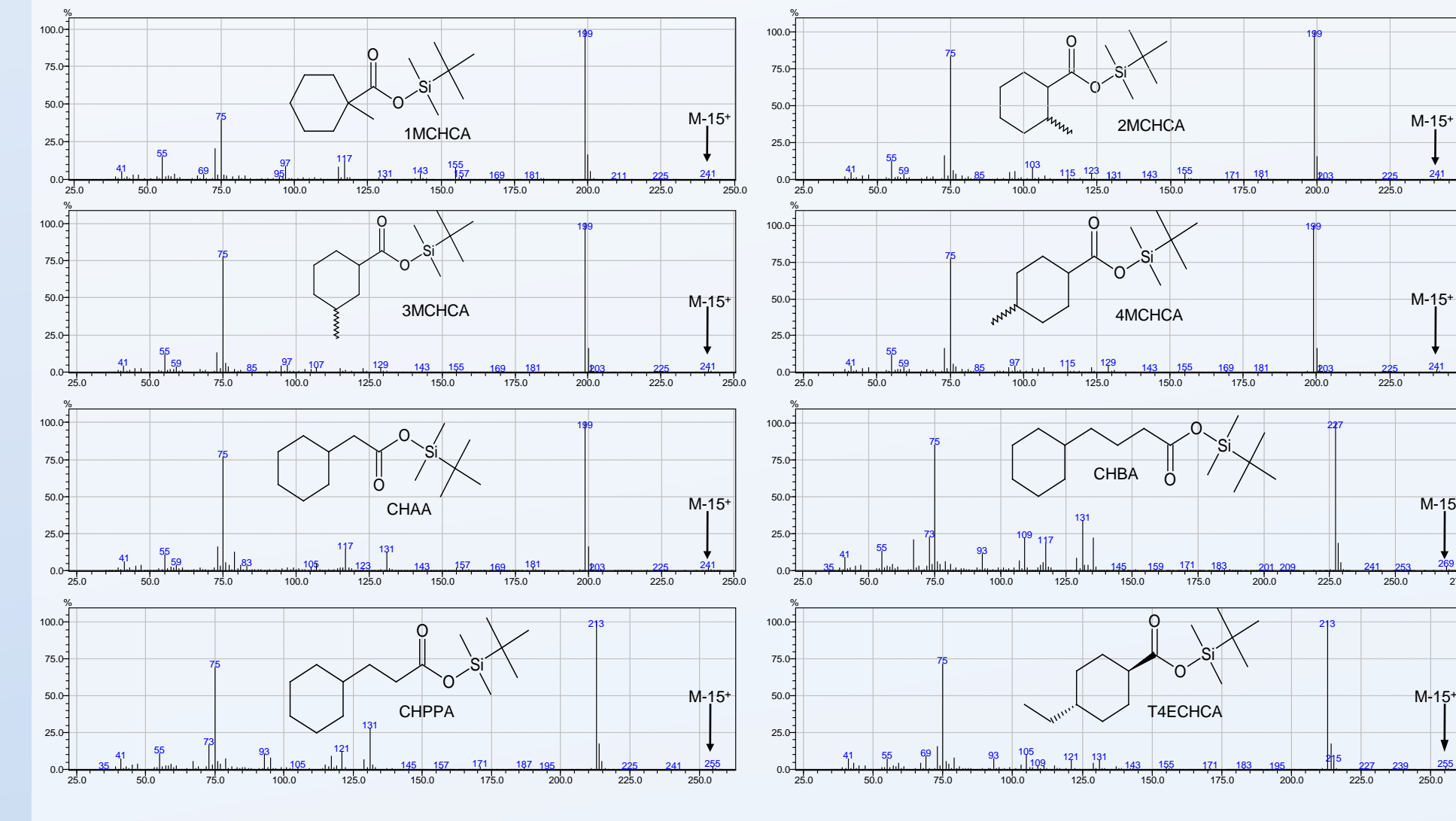


Figure 6. Isotope distributions for four common fragments of model NAs. Mass spectra were obtained from CHAA. Observed values are black. Theoretical values for chemical formulae are marked in red.

m/z 199 is the $M-57^+$ fragment. Its isotope pattern is consistent with the molecular formula $C_{11}H_{18}O_2Si$, or the loss of C_4H_9 .

m/z 75 appears in the mass spectra of all model acids. It can also be seen in Figures 2. and 3., mass spectra from environmental samples. Its isotope pattern is consistent with the formula $C_7H_{10}OSi$. The number of fragments present in environmental sample precludes analysis of isotopic distribution for this fragment for the environmental sample.

m/z 131 shows an isotope pattern that is consistent with the formula $C_9H_{14}O_2Si$. m/z 131 also appears in Figures 2. and 3.

m/z 117 shows an isotope pattern which is consistent with the presence of silicon but the +2 is too large to propose a chemical formula. Theoretical values are for $C_7H_{10}OSi$.

Conclusions

Model acid behavior

- Consistent with trends observed for environmental intensity plot (Figure 1.).
- Intensity of fragmentation attributable to carbon skeleton structure is negligible relative to fragmentation due to the *tert*-butyldimethylsilyl ester (Figures 2. and 5.)
- Isotopic distribution of stereotypical model NA fragments is consistent with dominant silyl ester fragmentation (Figure 6.).
- Region of reduced fragment intensity between $M-57^+$ and m/z 131 fragments supports the assumption that formation of a homologous fragment series does not occur (Figures 2. and 5.).

Implications for environmental NA analysis

- Extrapolating from the model system, detected $M-57^+$ masses in homologous series within mass spectra of derivatized NAs most likely represent co-eluting species.
- Except in cases of perfect TIC peak overlap, it may be possible to extract qualitative information from low resolution GC/MS analysis of these complex samples by treating co-eluting species as such.

Model NA limitations

- NAs selected for model system conform to the formula $C_nH_{2n+2}O_2$, where $z = -2$ and $n = 7$ thru $n = 11$. The mass spectrum in Figure 3. alone contains $M-57^+$ peaks consistent with values n up to 18 and values z to -8.
- As the model system falls outside the n and z range for environmental NAs, conclusions from this data are extrapolative rather than interpolative (see Figures 1. and 3.)
- Model system contains ten components compared to the very large system we're attempting to model.
- A more complex model system, if shown to replicate environmental NA behavior, would be more relevant for interpreting environmental NA systems.

Expanded model

- Would allow interpolation from model behavior.
- Expansion to include model NAs for which $n \geq 12$ and $z \leq -2$ would require custom synthesis as molecules conforming to these values are not commercially available.
- While chemical stability decreases with molecular weight, increased unsaturation, as with greater incidence of rings, tends to increase chemical stability and decrease fragmentation⁴. An expanded model system would allow experimental determination of whether larger molecules with z values of greater magnitude will behave in the same way our smaller model acids were observed to behave.

References

- Clemente, J.S.; Fedorak, P.M. *Chemosphere* **2005** 60 585-600
- St. John, W.P., Rughani, J., Green, S.A., McGinnis, G.D. *J. Chromatogr. A* **1998** 807, 241-251
- Holowenko, F.M.; MacKinnon, M.D.; Fedorak, P.M. *Water Research* **2002** 36 2843-2855
- McLafferty, F.W.; Tureček, F. *Interpretation of Mass Spectra*, 4th ed.; University Science Books: Mill Valley, California p.48

Acknowledgements

Kevin Woodward
Chad Mangum, Undergraduate Researcher, Utah State University- Uintah Basin
Anonymous Donor, whose generous contribution made possible the acquisition of our GC/MS