

Supplementary materials

Comparison of Three Untargeted Data Processing Workflows for Evaluating LC-HRMS Metabolomics Data

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Table S1. Peak picking and alignment parameters used for preprocessing using R and XCMS Online.

| Column | Polarity | Peakwidth, min | Peakwidth, max | pp m | Snthresh | Mzdiff | Prefilte r 1 | Prefilter 2 | bw |
|--------|----------|-------------------|-------------------|---------|----------|--------|-----------------|----------------|----------|
| HI | pos | 10 | 22 | 1.0 | 39 | 0.07 | 7 | 900 | 0.1 (1*) |
| HI | neg | 10 | 12 | 1.9 | 69 | 0.016 | 5 | 6100 | 0.1 (1*) |
| PH | pos | 9.3 | 12 | 1.2 | 83 | 0.02 | 10 | 5400 | 0.1 (1*) |
| PH | neg | 9.3 | 12 | 1.0 | 96 | 0.068 | 6 | 8200 | 0.5 (1*) |

HI = HILIC, PH = PhenylHexyl, pos = positive, neg = negative, ppm = allowed ppm deviation of mass traces for peak picking, snthresh = signal to noise threshold, mzdiff = minimum difference in m/z for two peaks to be considered as separate, prefilter 1 = minimum of scan points, prefilter 2 = minimum abundance, bw = bandwidth for grouping of peaks across separate chromatograms, * value used for XCMS Online.

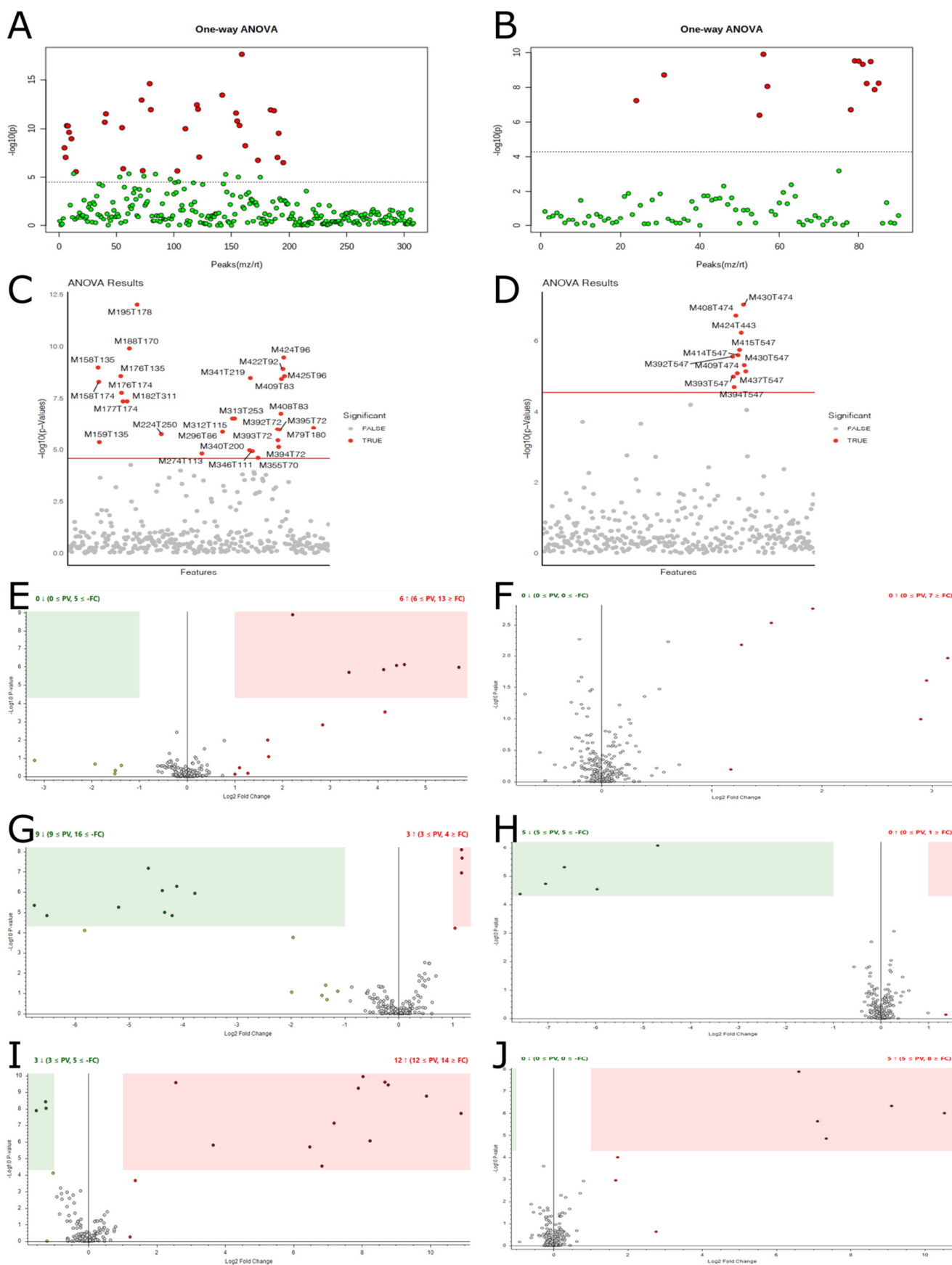


Figure S1. Results of one-way ANOVA for A-CHMINACA incubations analyzed in positive ionization mode. **A** = XCMS Online/MetaboAnalyst, HILIC column; **B** = XCMS Online/MetaboAnalyst, PhenylHexyl column; **C** = R, HILIC column; **D** = R, PhenylHexyl column; **E** = Compound Discoverer, Low and Blank, HILIC column; **F** = Compound Discoverer, Low and Blank,

PhenylHexyl column; **G** = Compound Discoverer, Low and High, HILIC column; **H** = Compound Discoverer, Low and High, PhenylHexyl column; **I** = Compound Discoverer, High and Blank, HILIC column; **J** = Compound Discoverer, High and Blank, PhenylHexyl column.

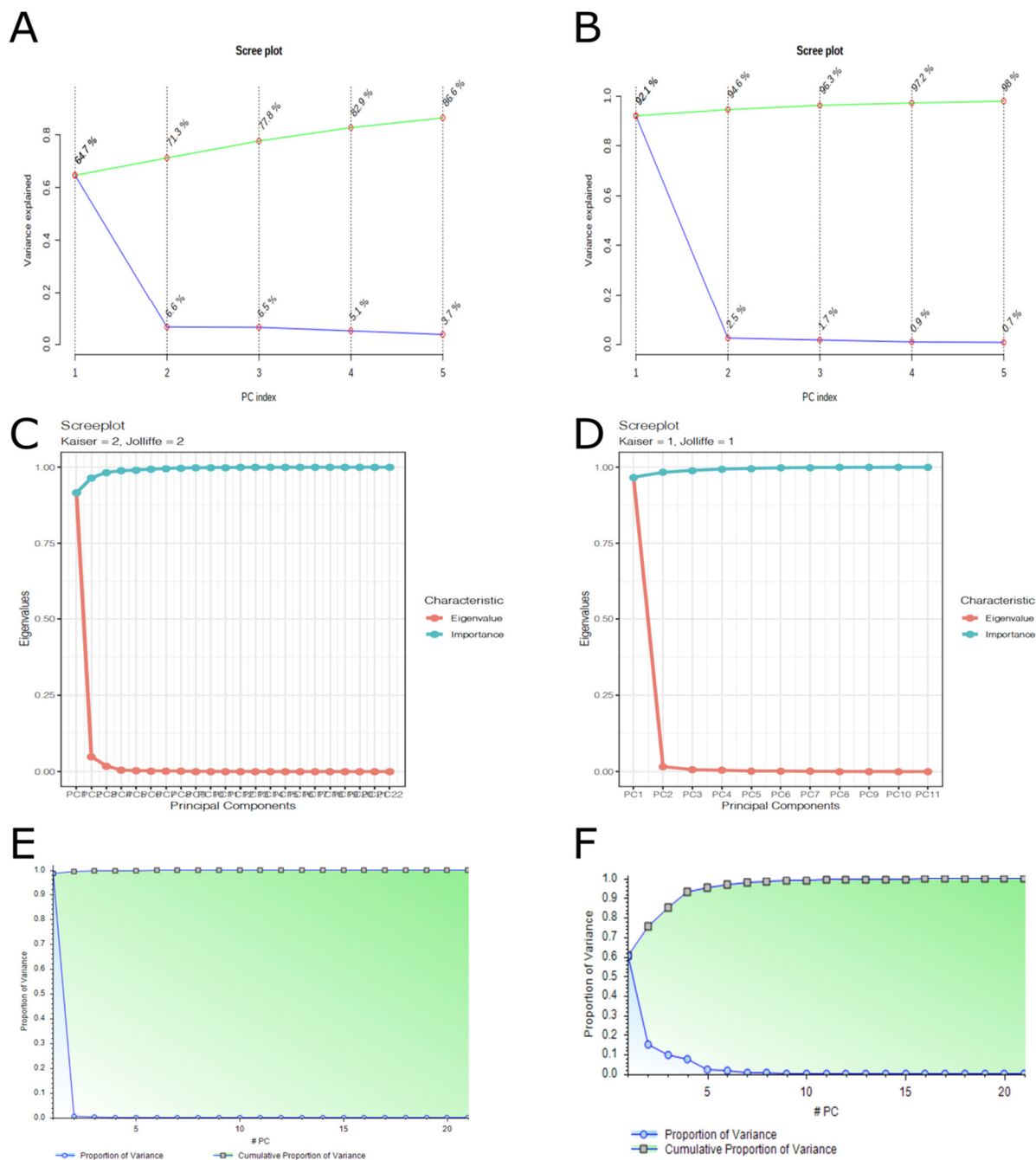


Figure S2. Results of scree plots for A-CHMINACA incubations analyzed in positive ionization mode. **A** = XCMS Online/MetaboAnalyst, HILIC column; **B** = XCMS Online/MetaboAnalyst, PhenylHexyl column; **C** = R, HILIC column; **D** = R, PhenylHexyl column; **E** = Compound Discoverer, HILIC column; **F** = Compound Discoverer, PhenylHexyl column.

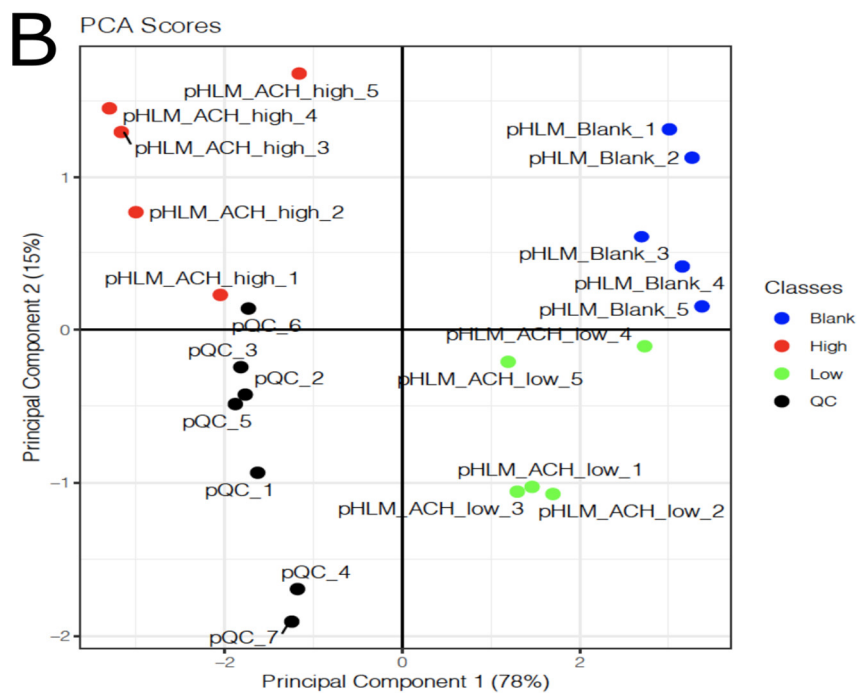
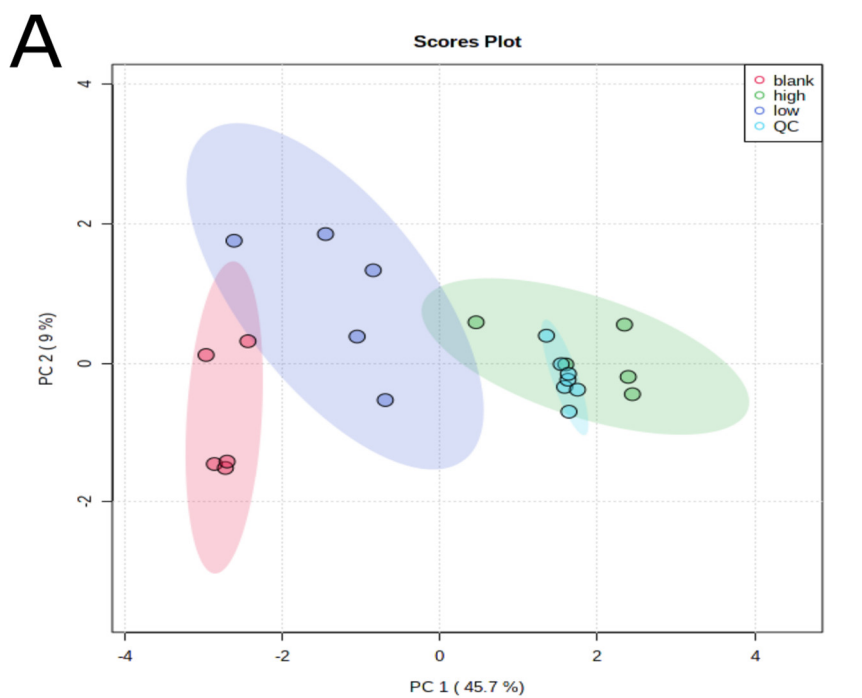
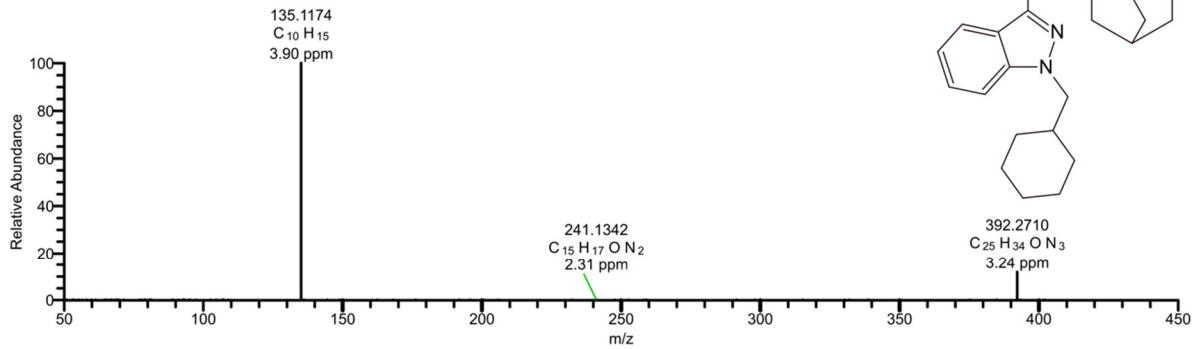
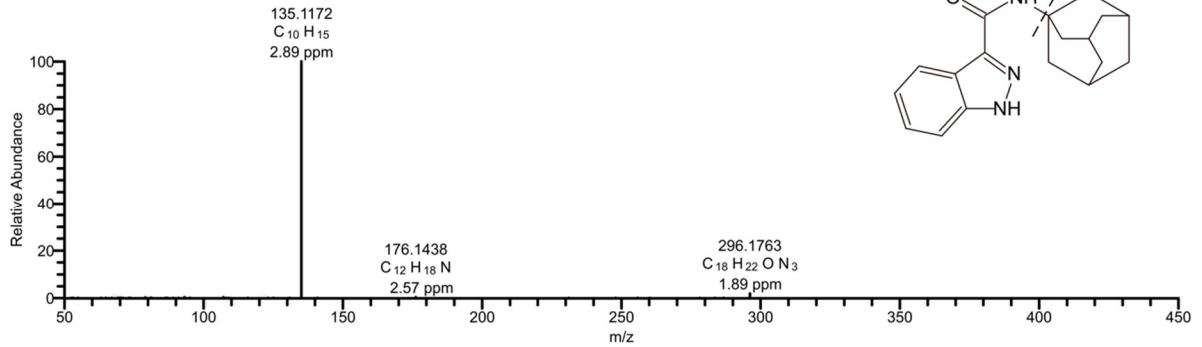


Figure S3. Results of scores of principal component analysis for A-CHMINACA incubations analyzed in positive ionization mode without isotopes and adducts. **A** = XCMS Online/MetaboAnalyst, PhenylHexyl column; **B** = R, PhenylHexyl column.

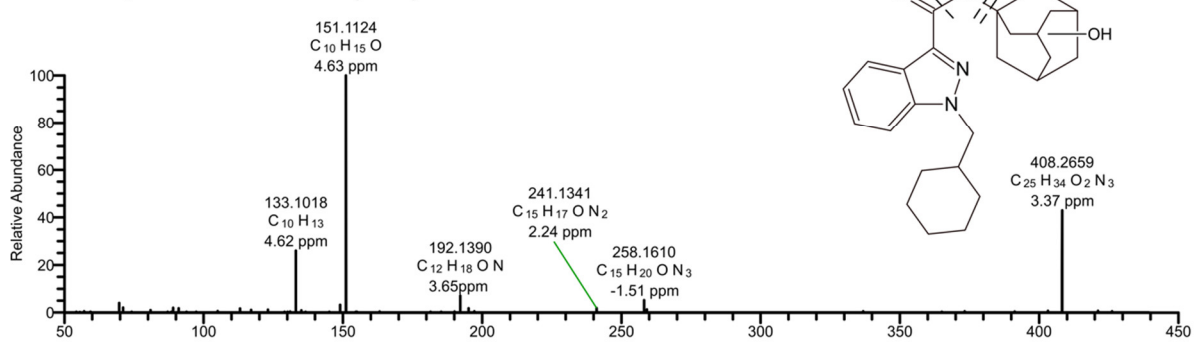
M392T72, A-CHMINACA



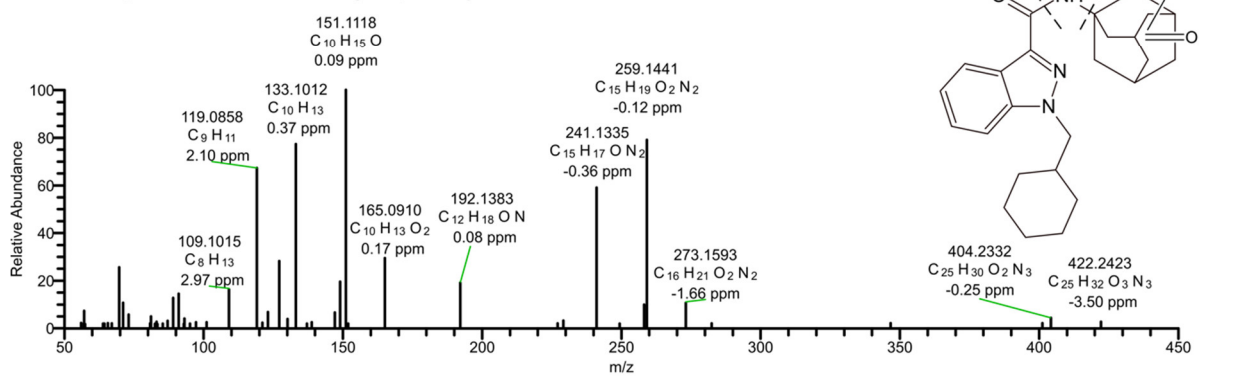
M296T86, A-CHMINACA (N-dealkyl-)



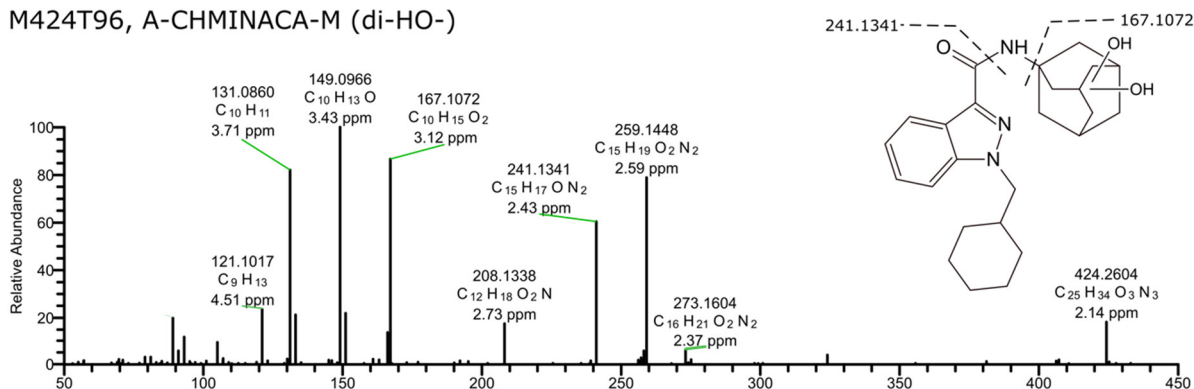
M408T83, A-CHMINACA-M (HO-)



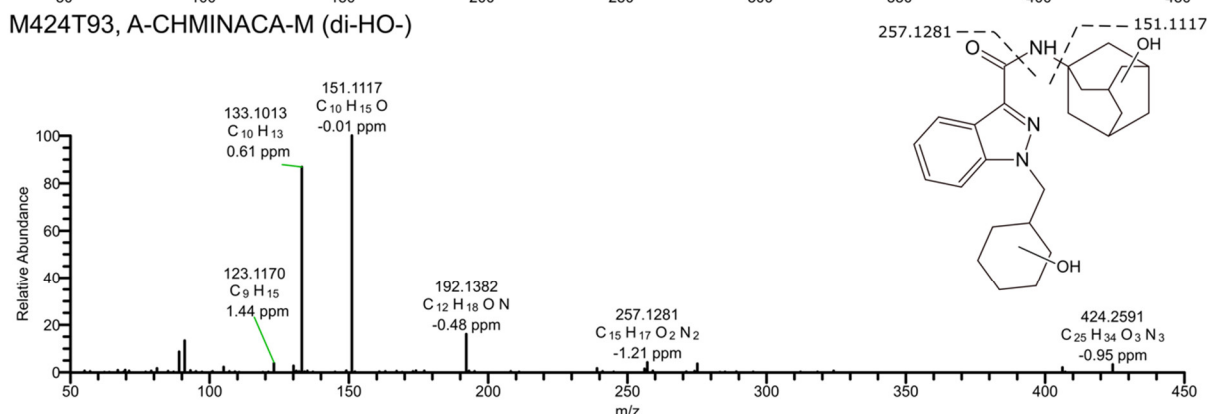
M422T92, A-CHMINACA-M (HO, Oxo)



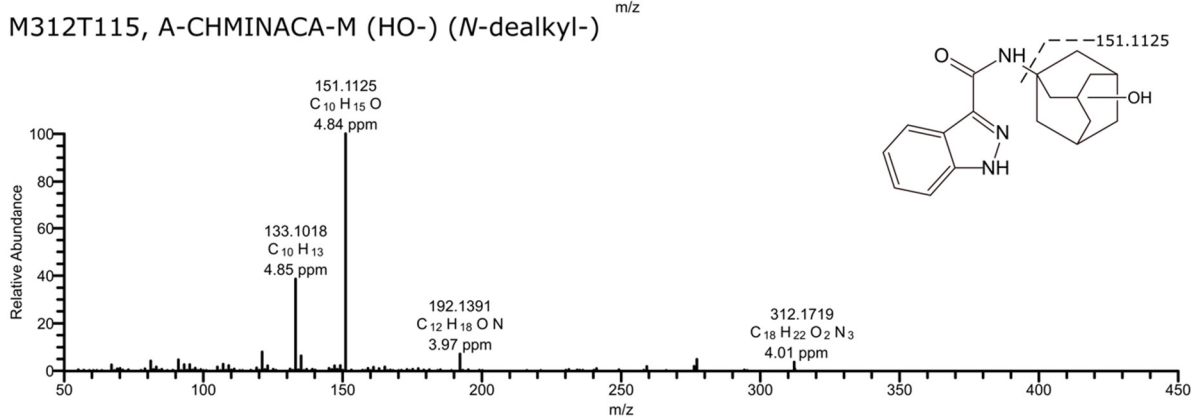
M424T96, A-CHMINACA-M (di-HO-)



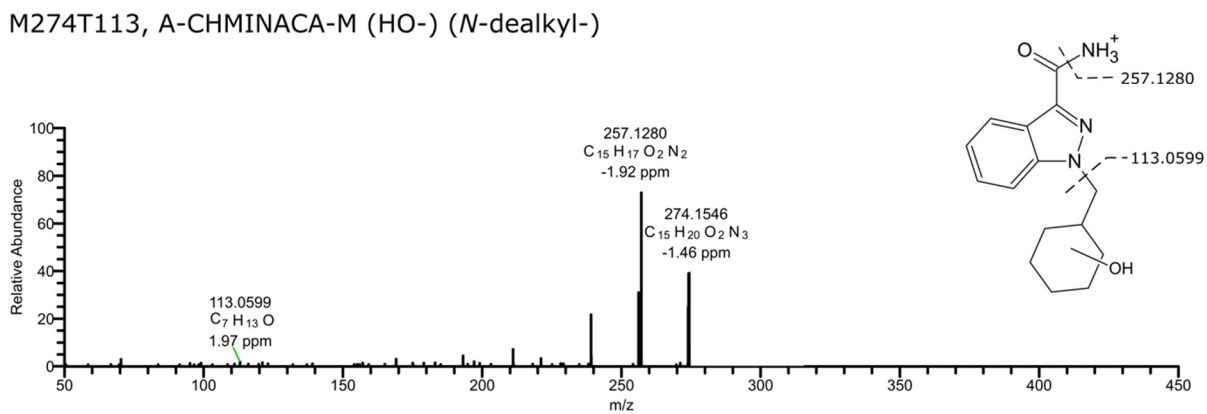
M424T93, A-CHMINACA-M (di-HO-)



M312T115, A-CHMINACA-M (HO-) (N-dealkyl-)



M274T113, A-CHMINACA-M (HO-) (N-dealkyl-)



M440T117/M440T122 A-CHMINACA-M (tri-HO)

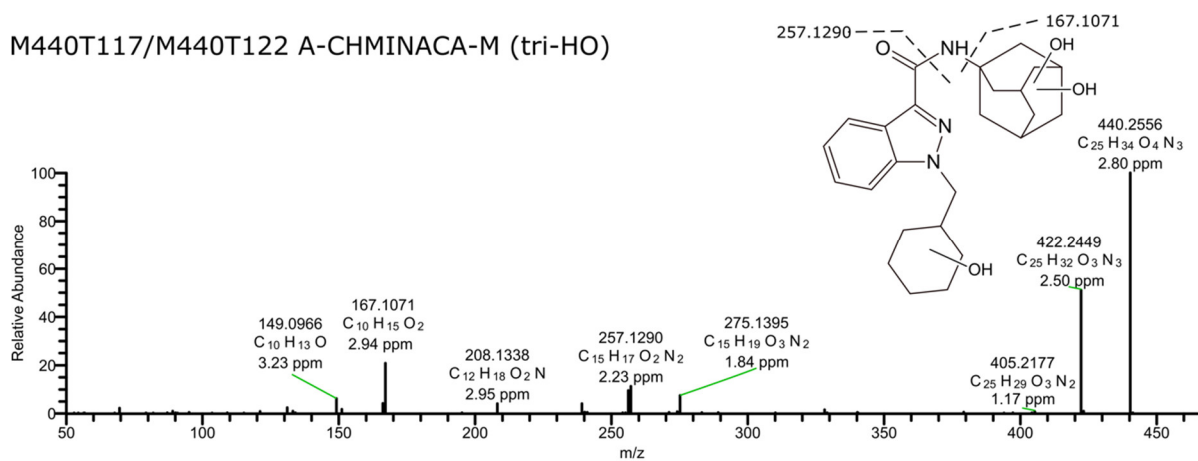


Figure S4. LC-HRMS/MS spectra of significant features in A-CHMINACA incubations analyzed with a HILIC column in positive ionization mode. Fragments with accurate mass, calculated elemental formula, and mass error value in parts per million (ppm).