SPECTRUM ACQUISITION AND EVALUATION FOR BUILDING THE 2020 NIST TANDEM MS LIBRARY

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ASMS 2020

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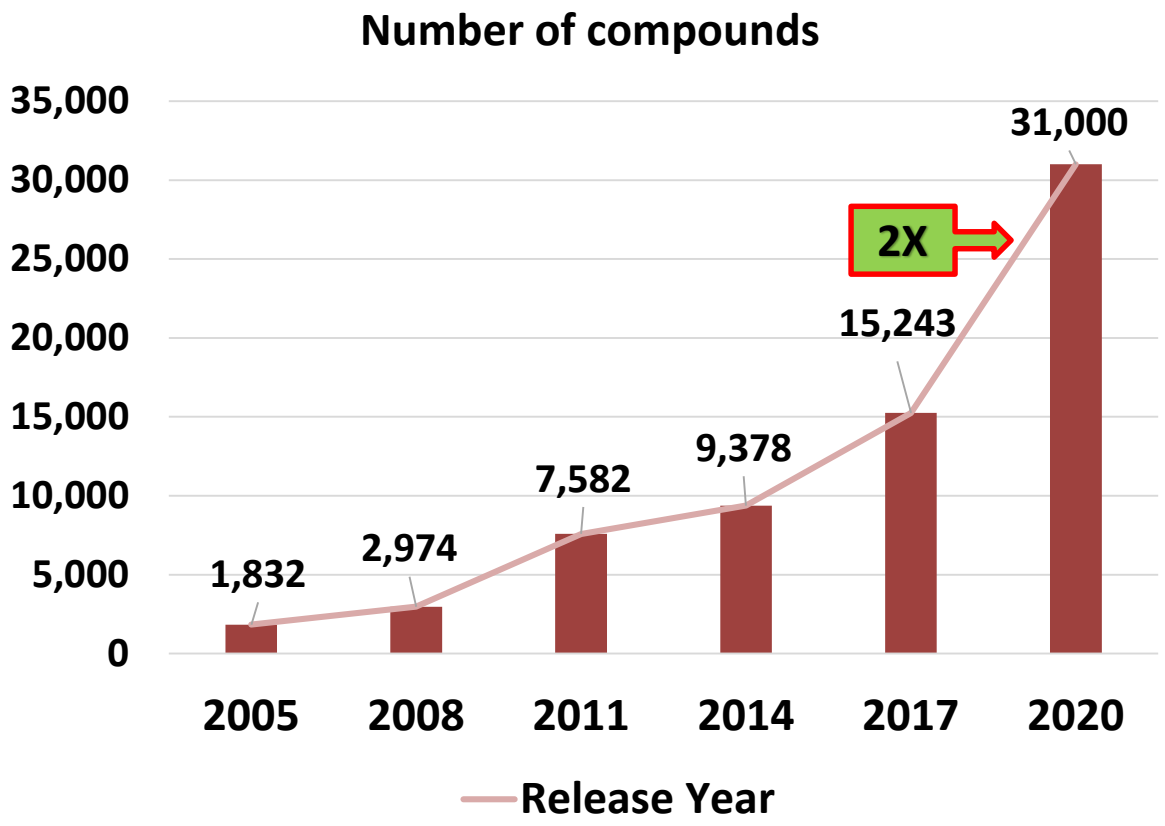
U.S. Department of Commerce

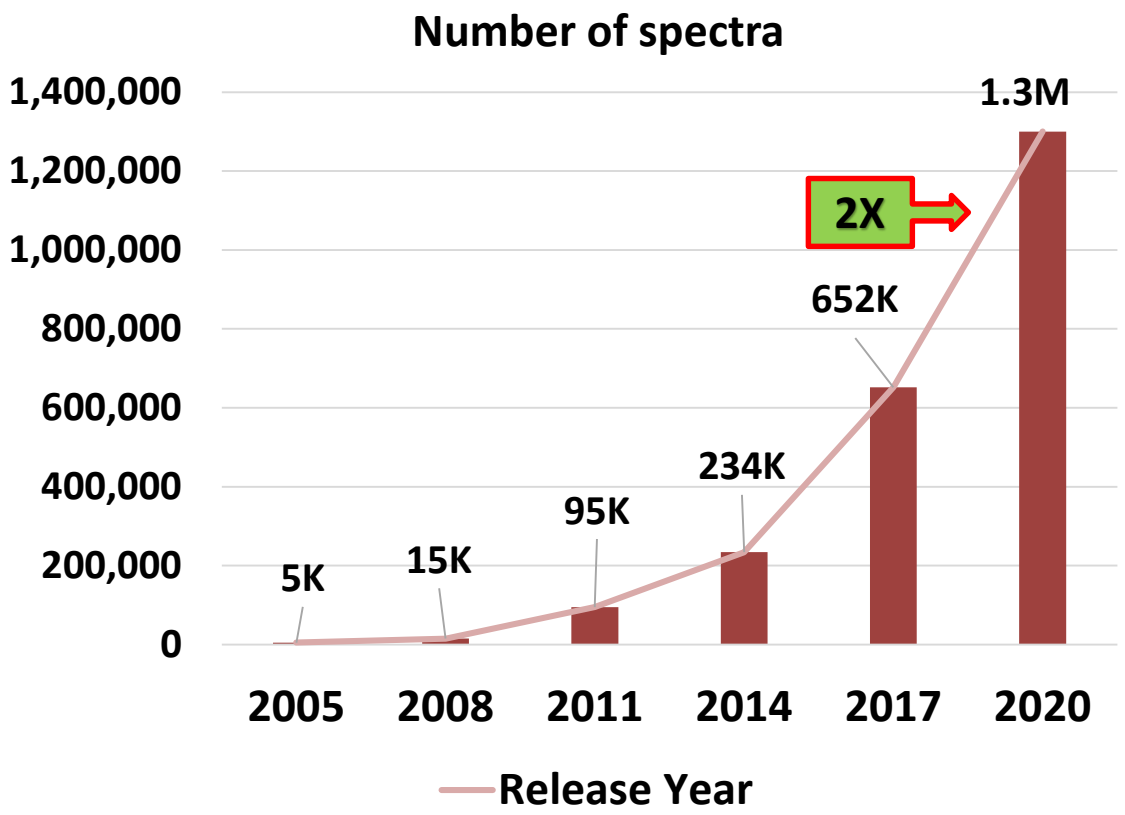
Mass Spectrometry Data Center

INTRODUCTION

- The 2020 NIST Tandem Mass Spectral Library will be released on June 2, 2020.
- The NIST tandem MS library provides reference mass spectral data for the identification of compounds, such as metabolites, drugs, pesticides, peptides, lipids, sugars, glycan, dyes and environmental contaminants.
- The 2020 release includes 1.3 M tandem mass spectra of 186 K precursor ions from 31 K compounds. Most of the MS/MS spectra have been measured on ion trap and collision cell (qTOF, QQQ, and HCD) instruments using electrospray ionization (ESI). Some of tandem spectra were acquired on qTOF instrument by atmospheric pressure chemical ionization (APCI).

The new MS library includes more than double compounds and spectra compared with the previous version (2017)





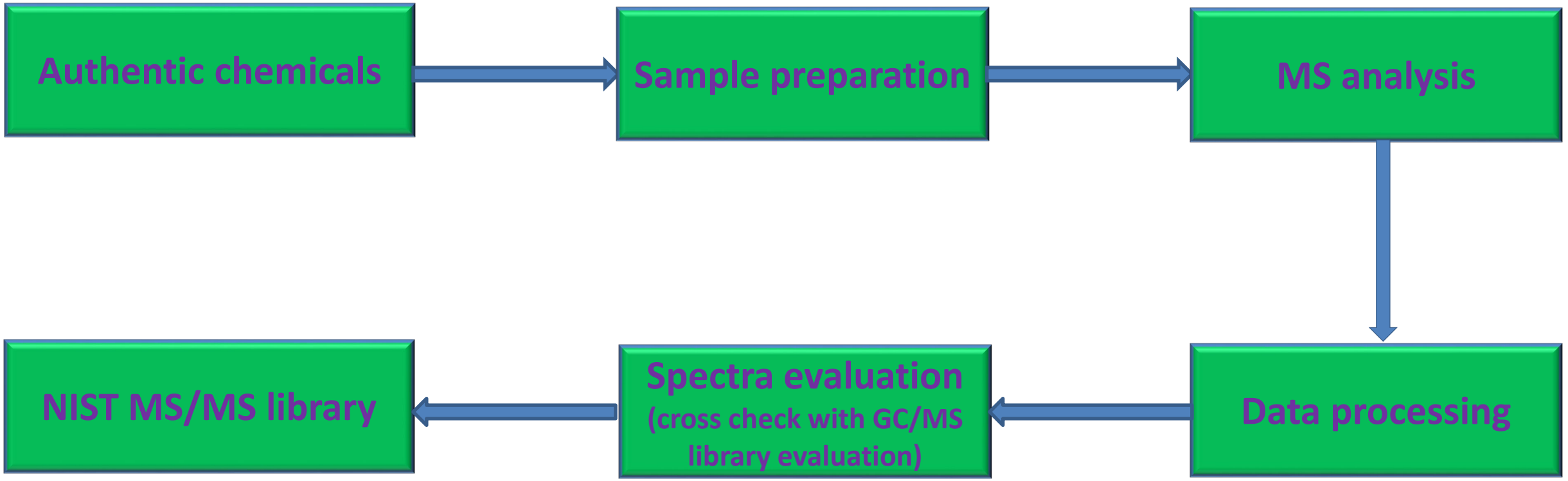
- In addition to the protonated and deprotonated precursors, spectra were collected for metal ion adducts and abundant fragment ions formed by in-source reactions of precursor ions. For example: $[M+Na]^+$, $[M-H_2O]^+$, $[M-NH_3]^+$, etc.

- The spectra of about 90% compounds in the 2020 version are high resolution and high accuracy spectra (less than 5 ppm), including MSn. The library was divided into two sub-libraries, hr_msms_nist and lr_msms_nist.

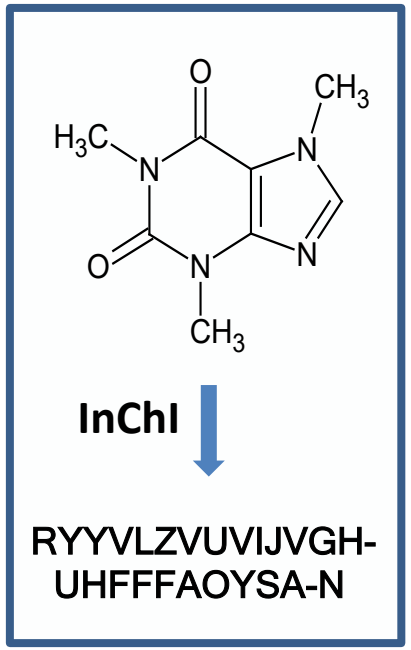
- ***Thermo Fisher and AB Sciex are our newest distributors !***

EXPERIMENTAL

Work flow for building library:

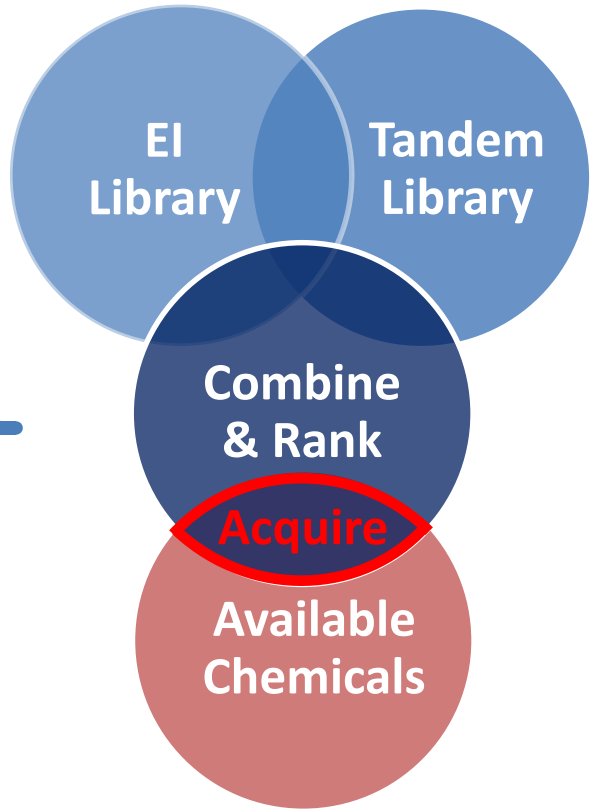


New Compound Selection Process



30+ Collections

- Wikipedia
- HMDB
- KEGG
- FoodDB
- EPA/FDA Lists
- Drug Bank
- ⋮



Sample preparation and Instrument conditions

- Samples were dissolved in ACN/H_2O/FA (50/50/0.1) at a concentration of about 0.1 mg/mL.

- **Nano LC conditions:**

a) Gradient: ACN/H_2O/FA (50/50/0.1).

b) Flow rate: 350 nl/min.

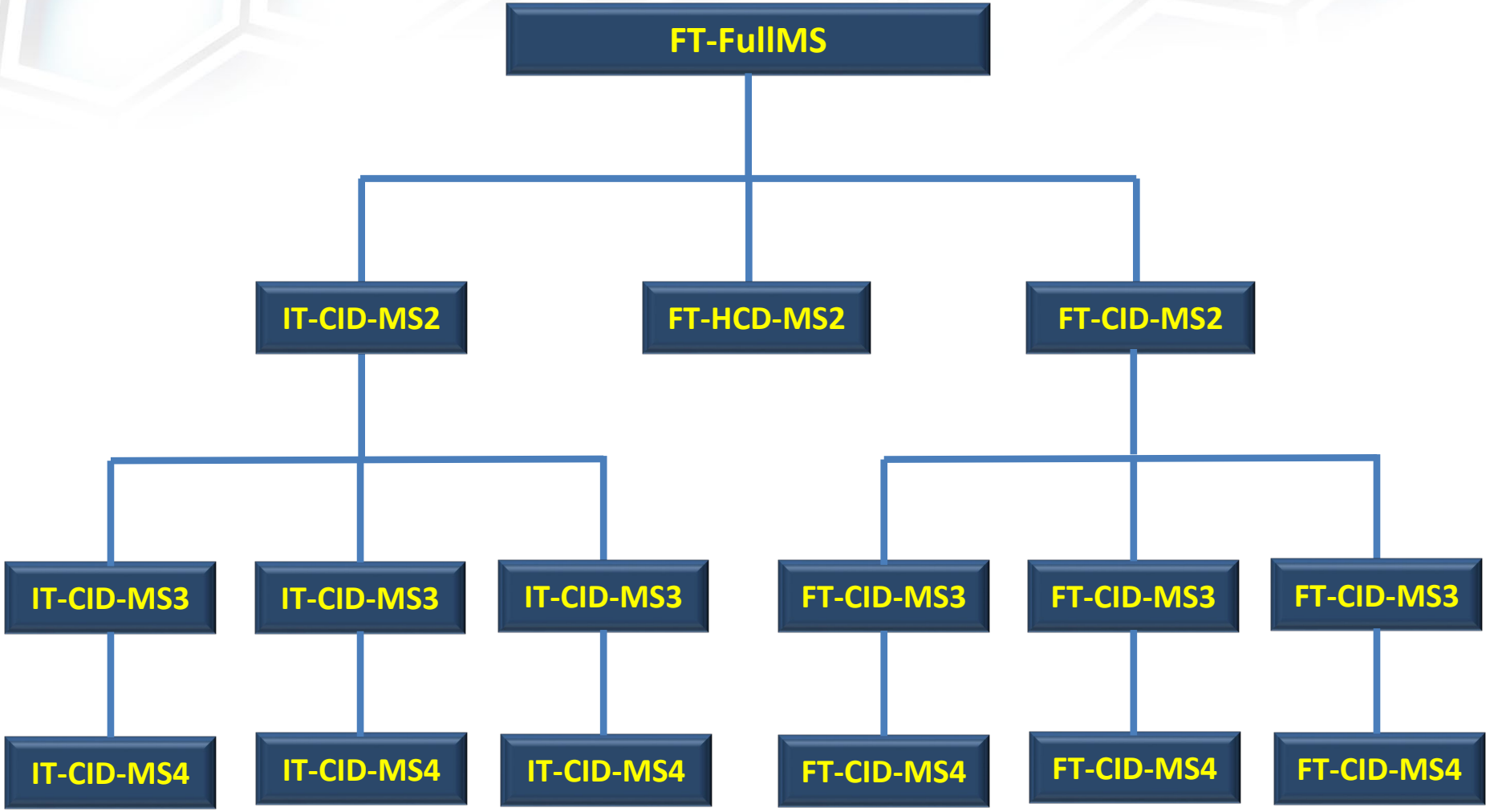
- **MS conditions:**

a) Instruments were operated in a data-dependent mode for the MS2, MS3 and MS4, the most intense ions were selected for further analysis.

b) Dynamic exclusion was set at 90 s to allow sampling of other precursors. The resolution of MS1 and MSn were set as 60,000 and 30,000, respectively.

c) For MS2 analyses, precursors were fragmented by higher-energy collision induced dissociation (HCD) followed by FT analysis (FT-HCD). FTMS2 precursors were fragmented with a wide range of Normalized Collision Energies (NCE) (…,14, 20, 30, 40, 50, 65,…). Ion trap spectra MSn were acquired at a normalized collision energy of 35 %.

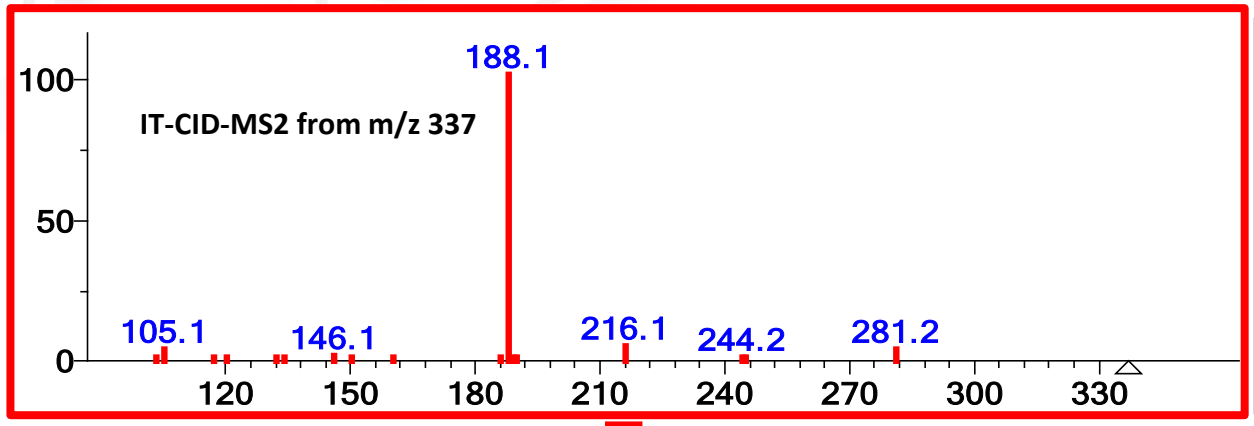
Workflow for acquiring spectra on MS instruments

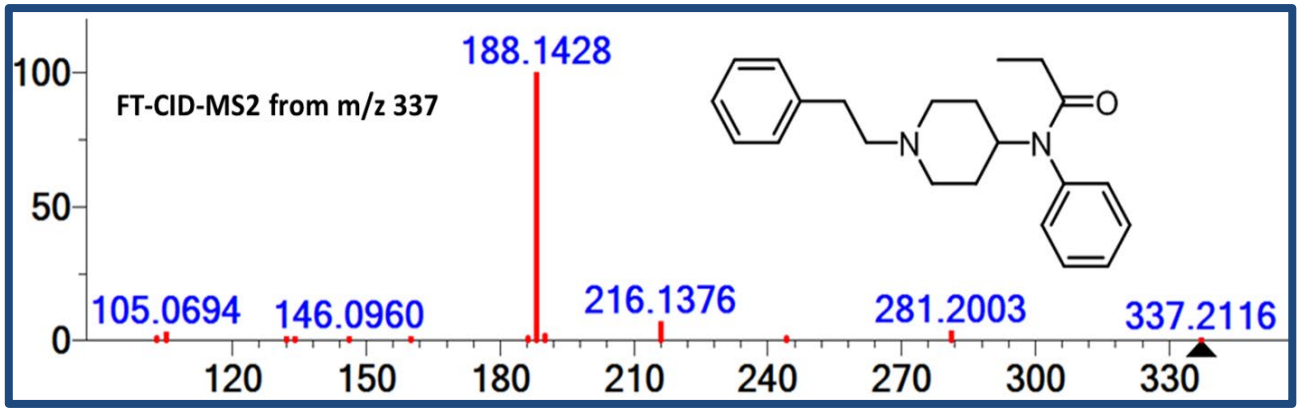


29 spectra per precursor !

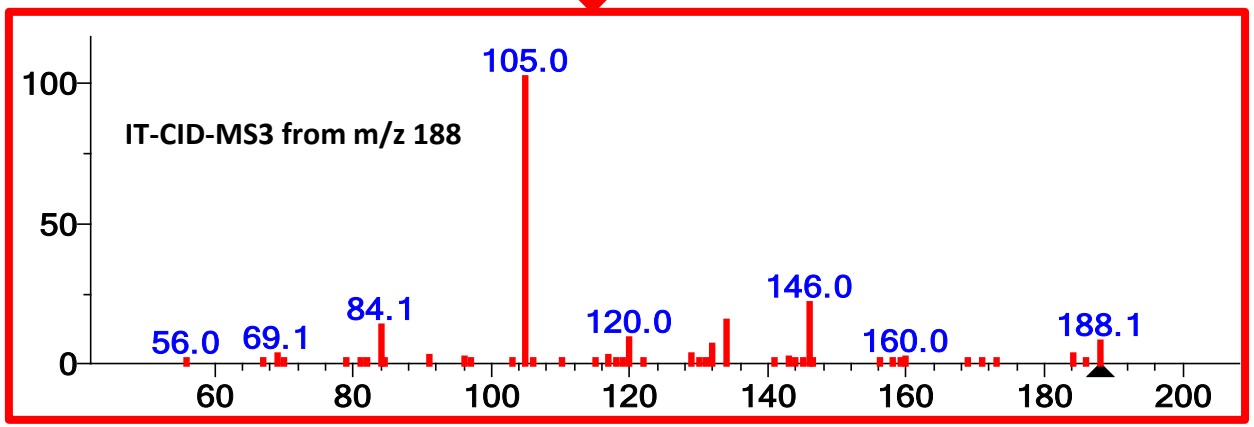
Spectra of Fentanyl (m/z 337 for protonated precursor) in MS library

Ion trap Spectra:

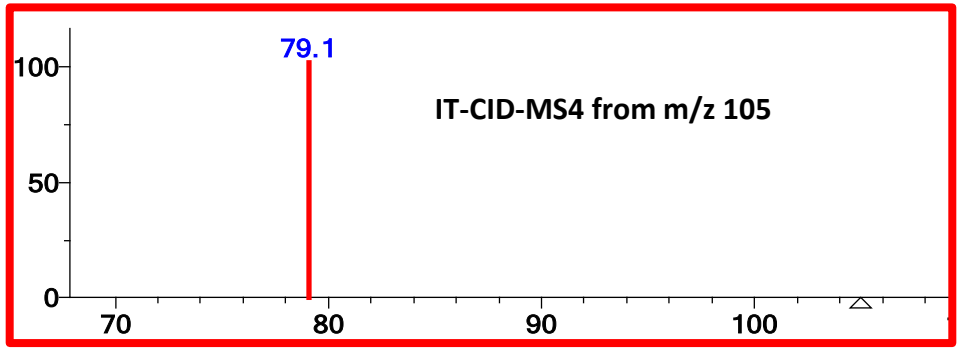




MS2 to MS3

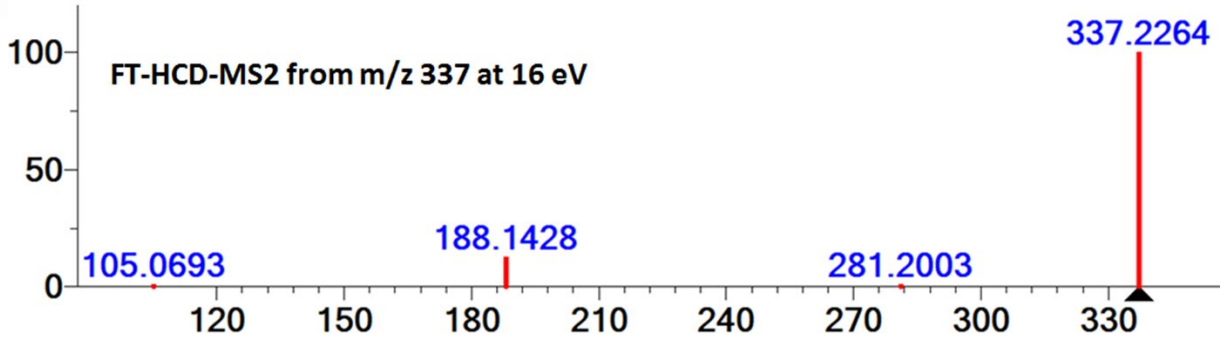


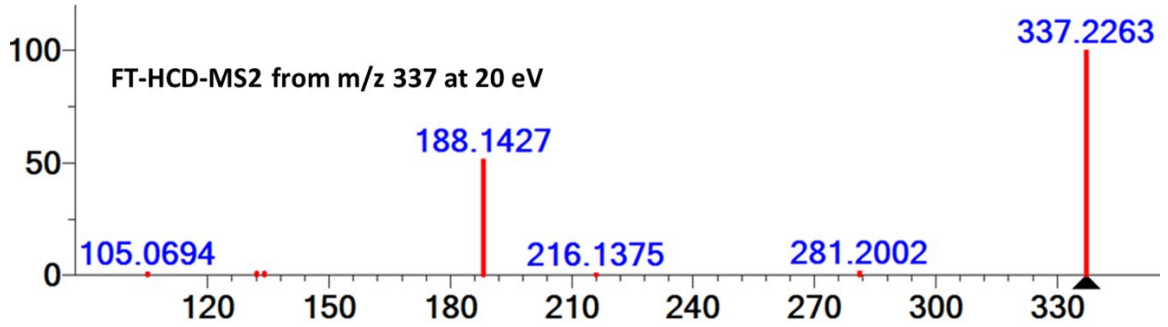
MS3 to MS4

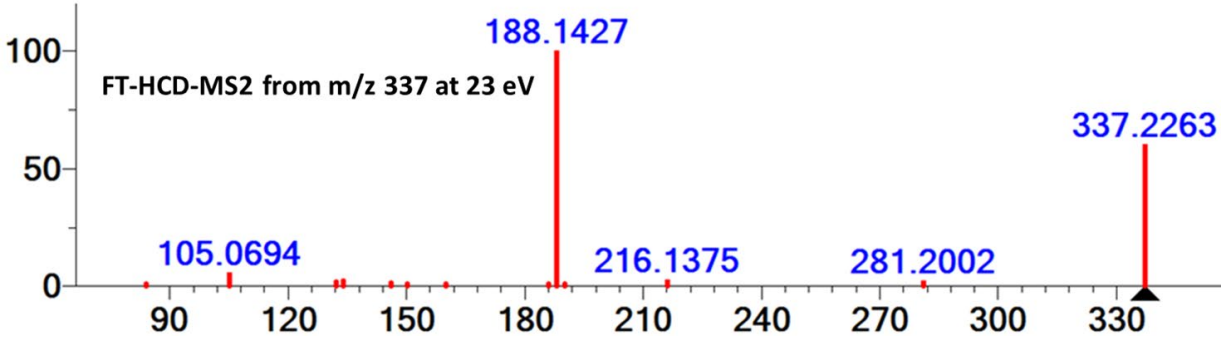


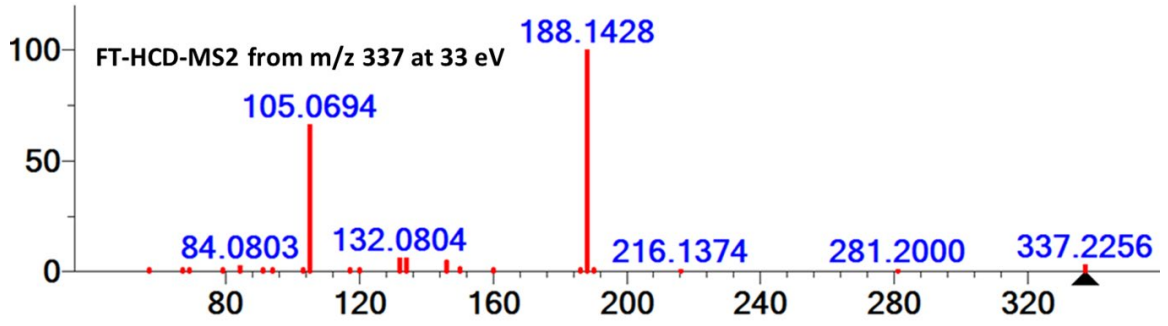
Spectra of Fentanyl (m/z 337 for protonated precursor) in MS library

HCD Spectra with wide range of NCEs:

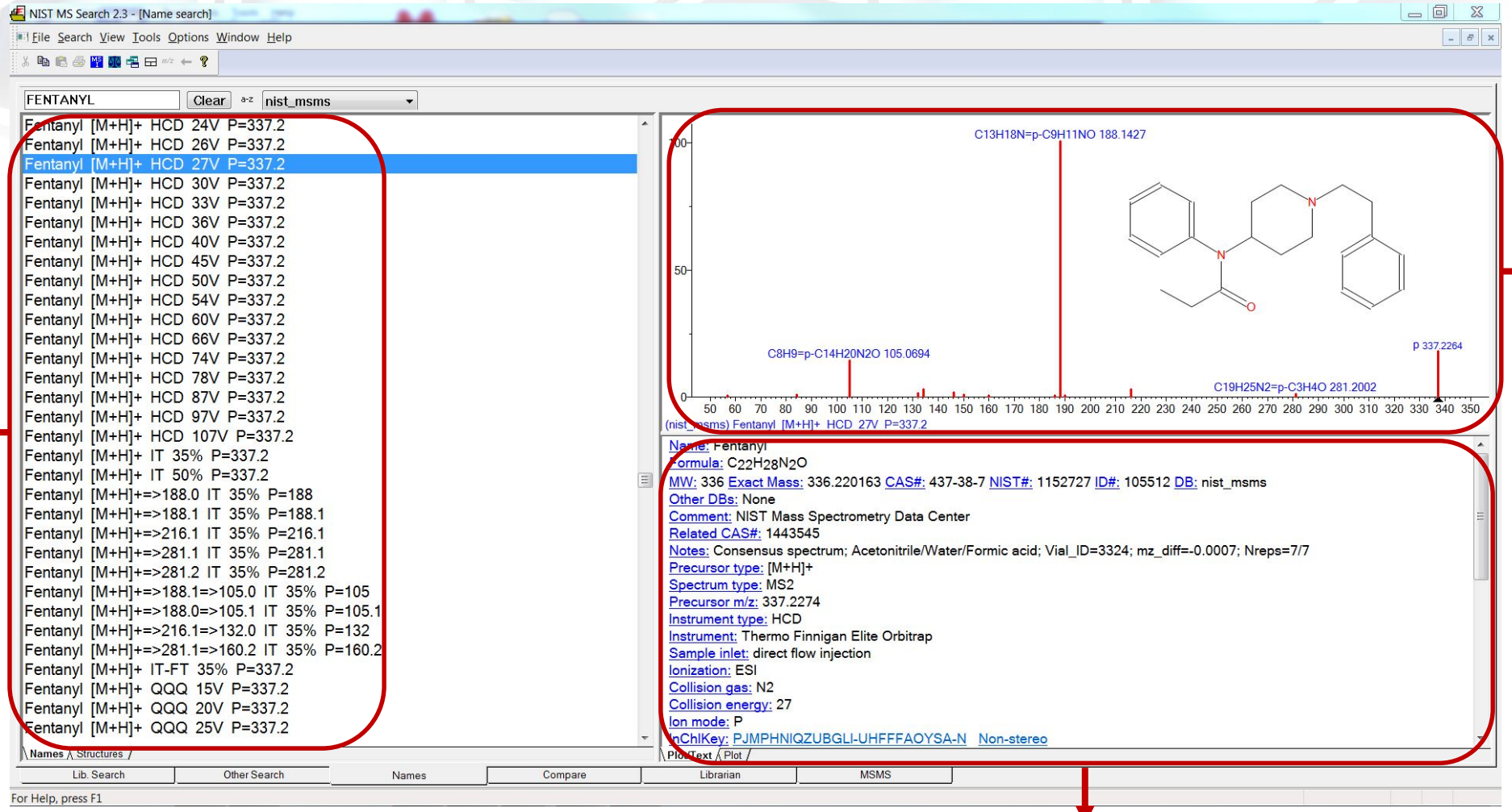








Evaluation of Spectra of Fentanyl with NIST MS Search 2.3

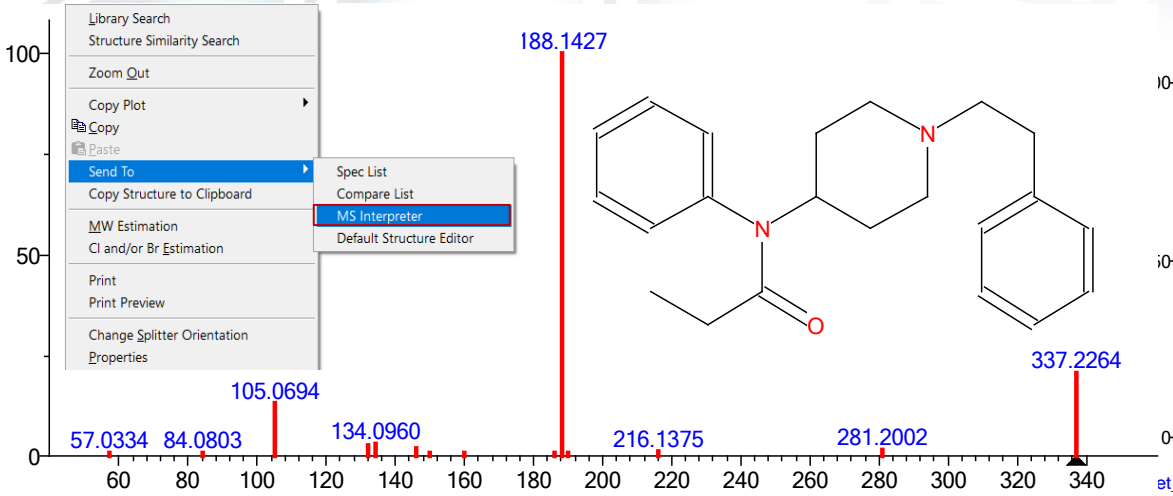


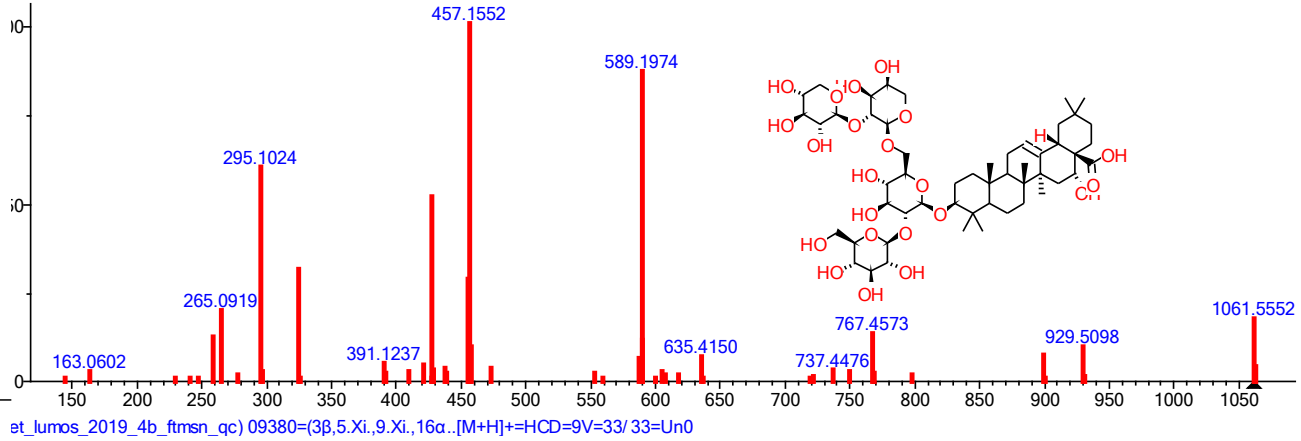
Spectra list

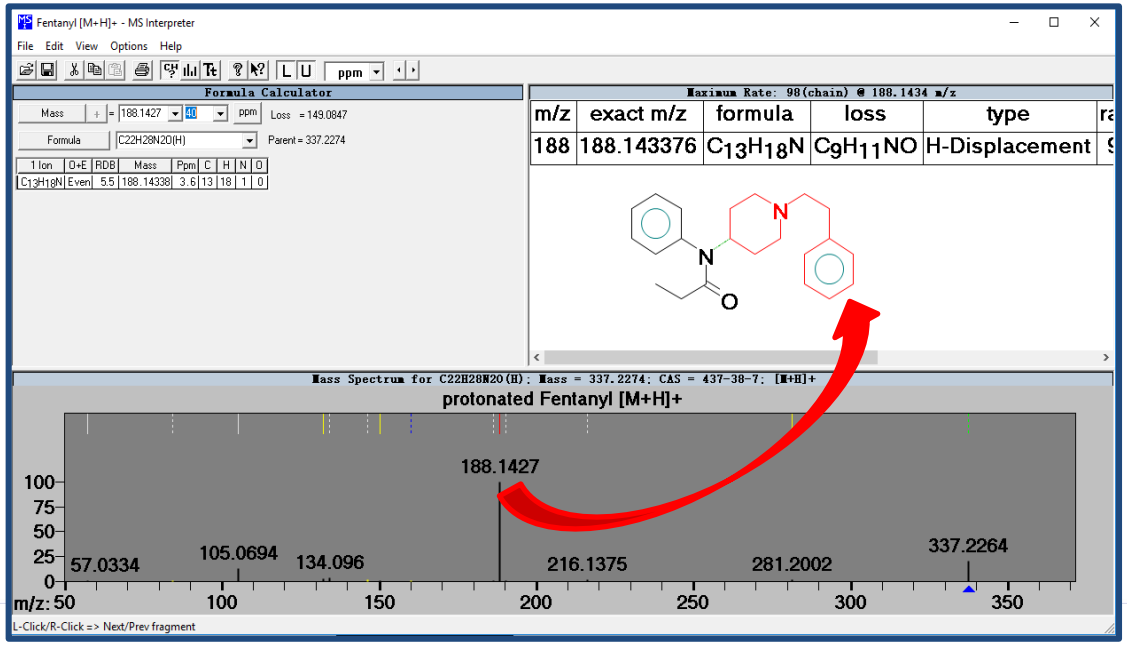
Spectrum

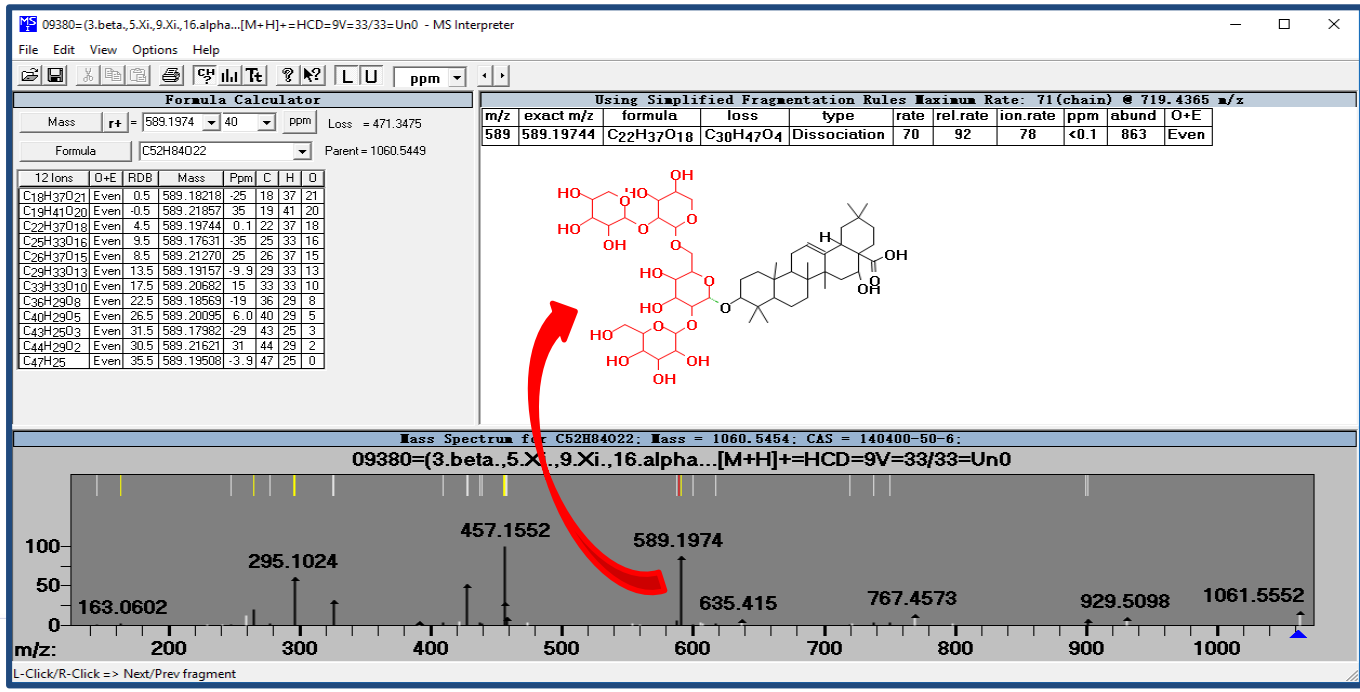
- Peak abundance
- Mass accuracy
- Full MS purity
- The number of spectra replicates

MS Interpreter makes spectrum evaluation much easier !

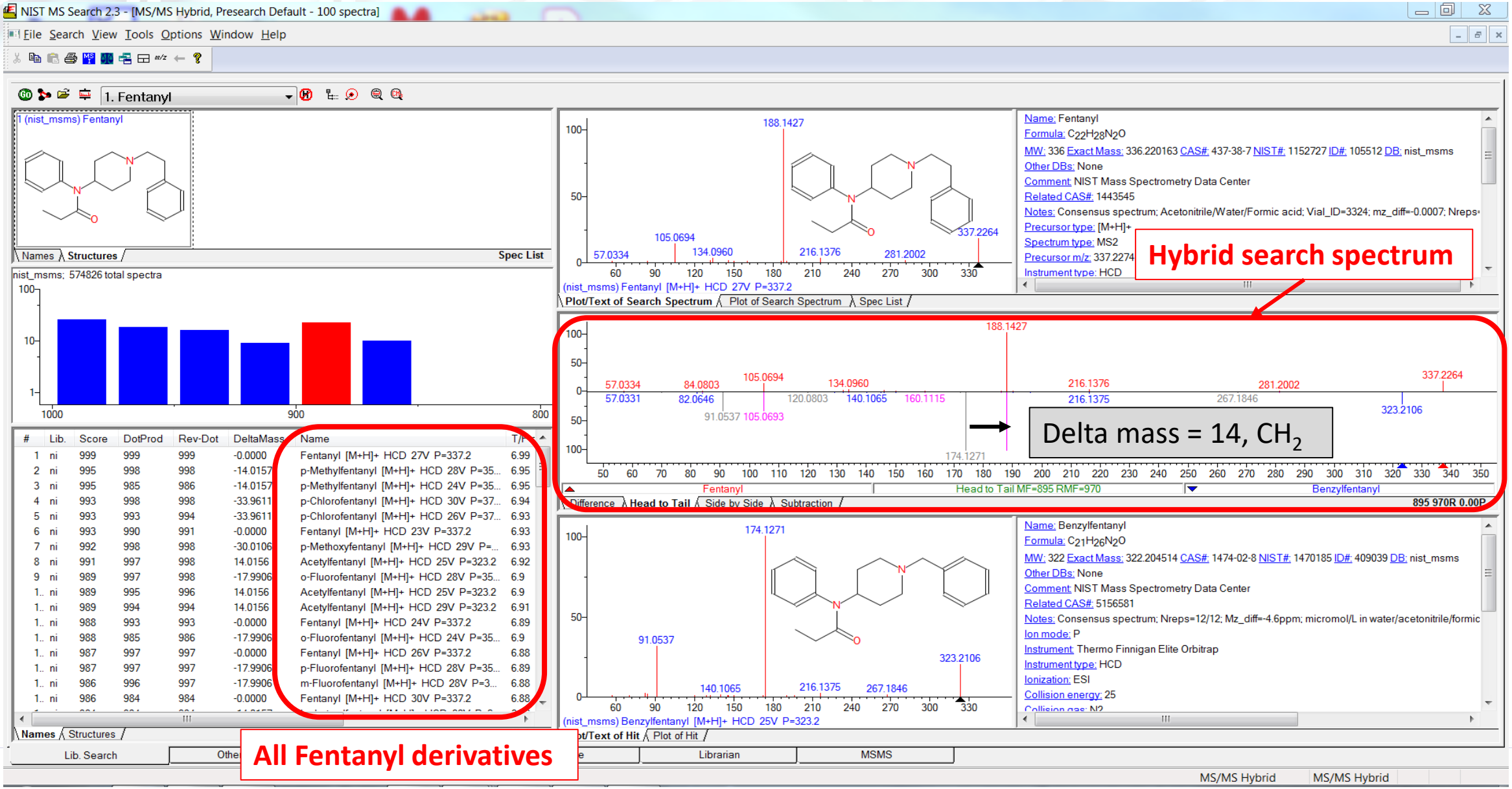




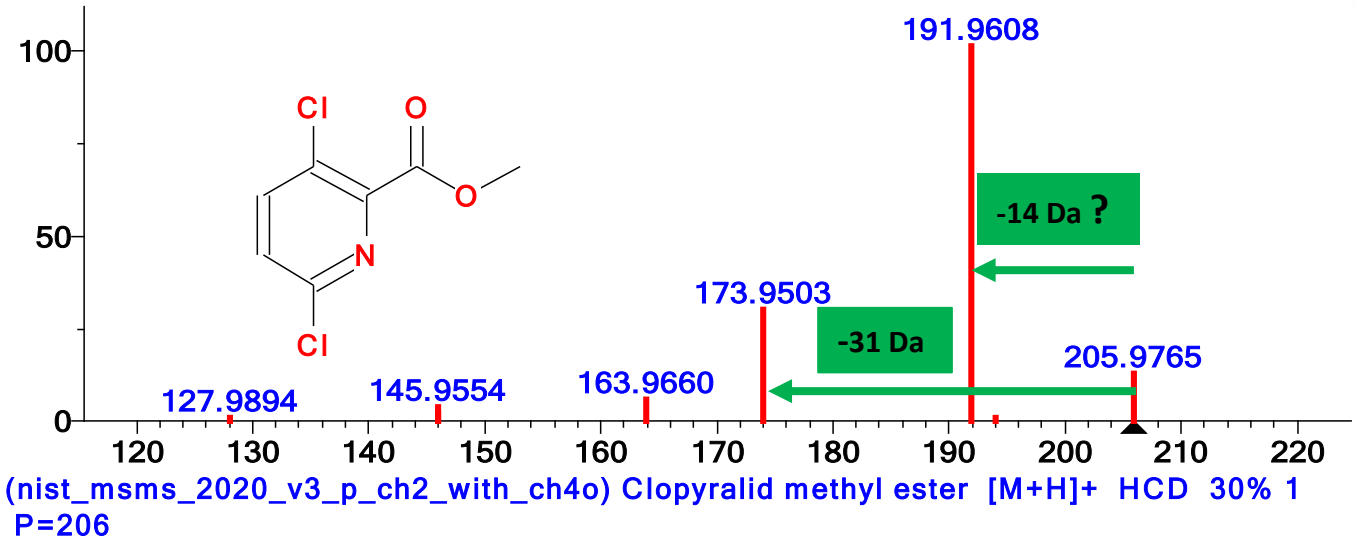




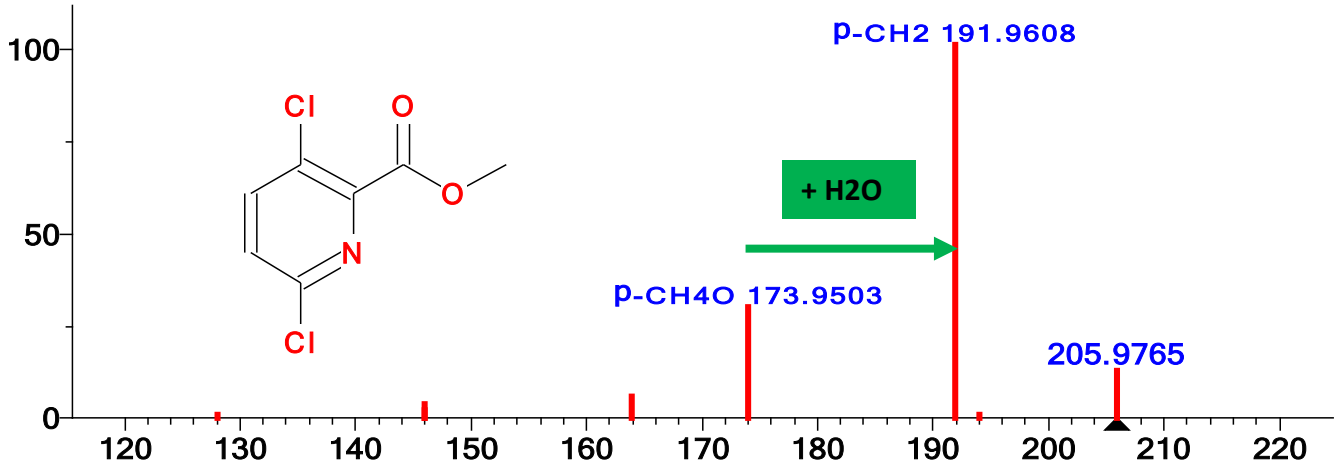
Verification of spectra by Hybrid Search in NIST MS search !

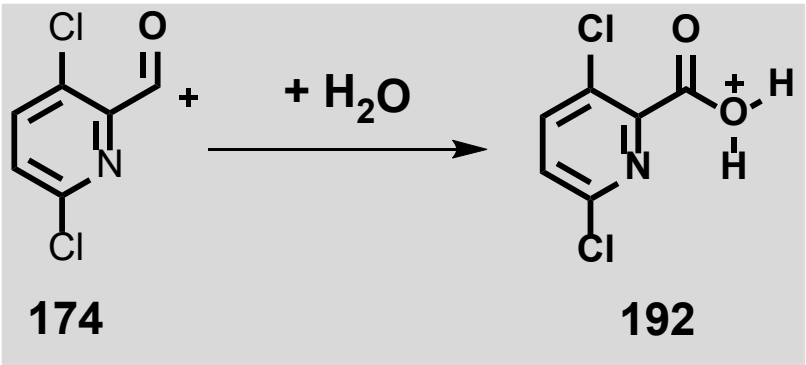


Unexpected peaks in tandem mass spectra due to reaction of product ions with water in collision cell



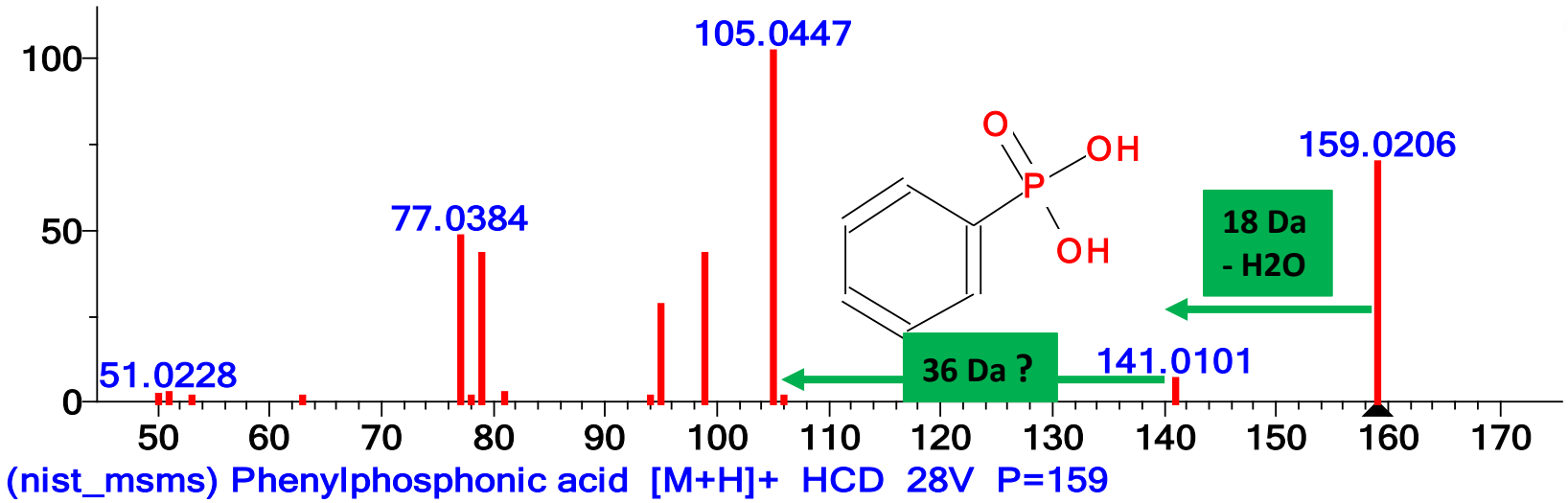
$M\text{-}CH_3OH+H_2O = M\text{-}CH_2$

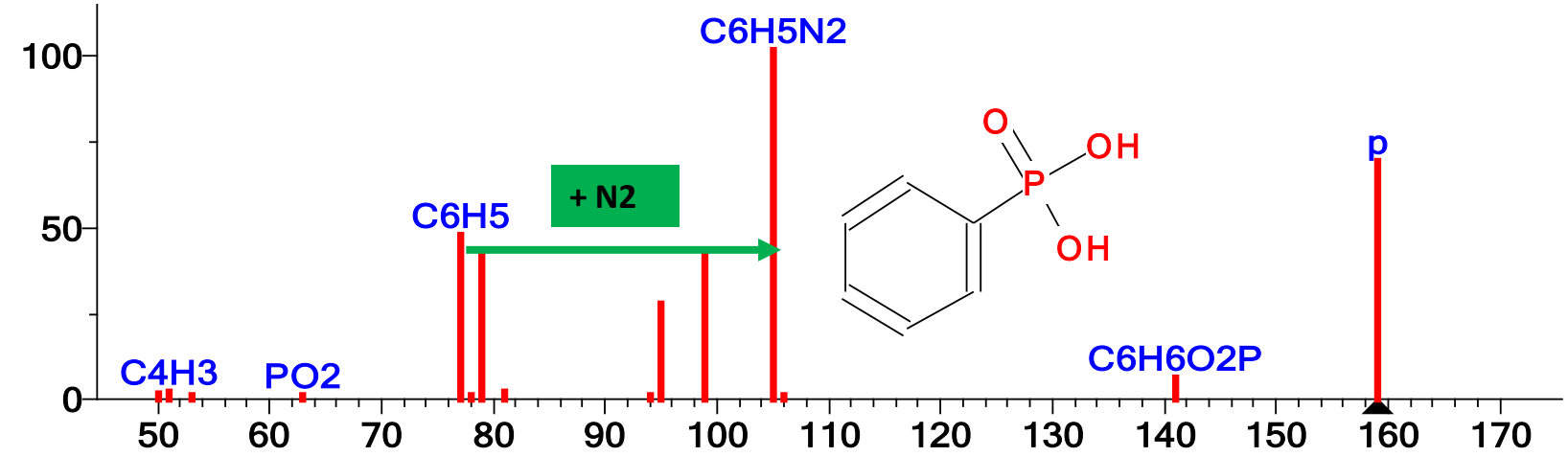


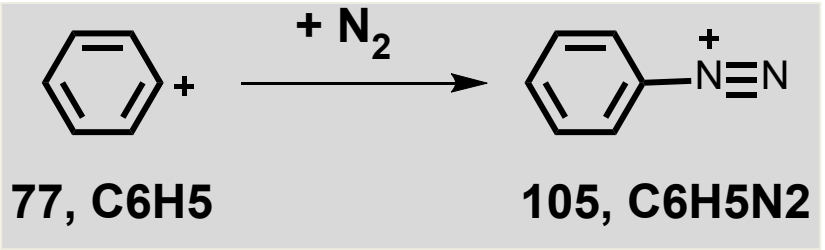


P. Neta, M. Farahani, Y. Simón-Manso, Y. Liang, X. Yang, S. E. Stein. Rapid Commun. Mass Spectrom. **2014**, *28*, 2645.

Unexpected peaks in tandem mass spectra due to reaction of product ions with nitrogen gas in collision cell

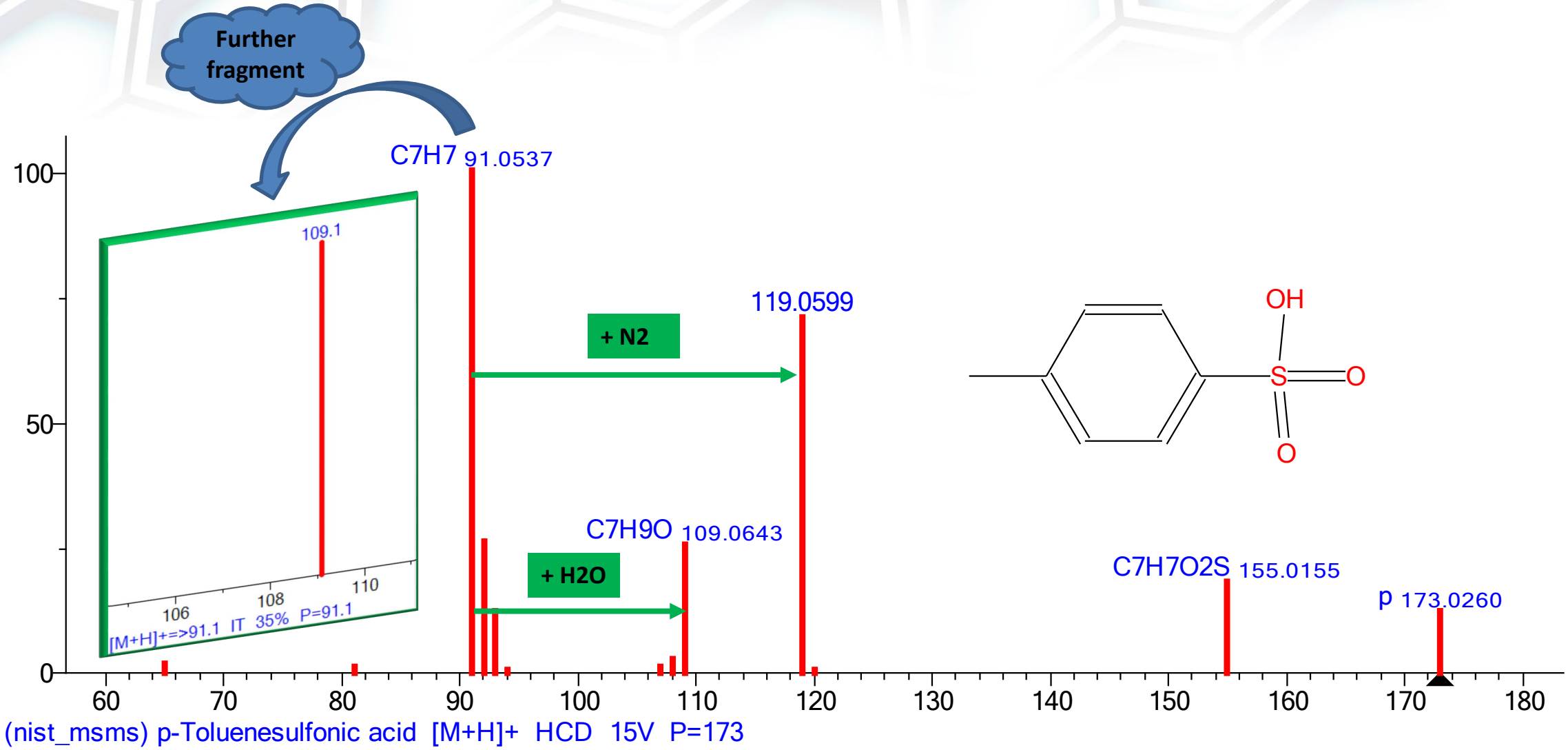




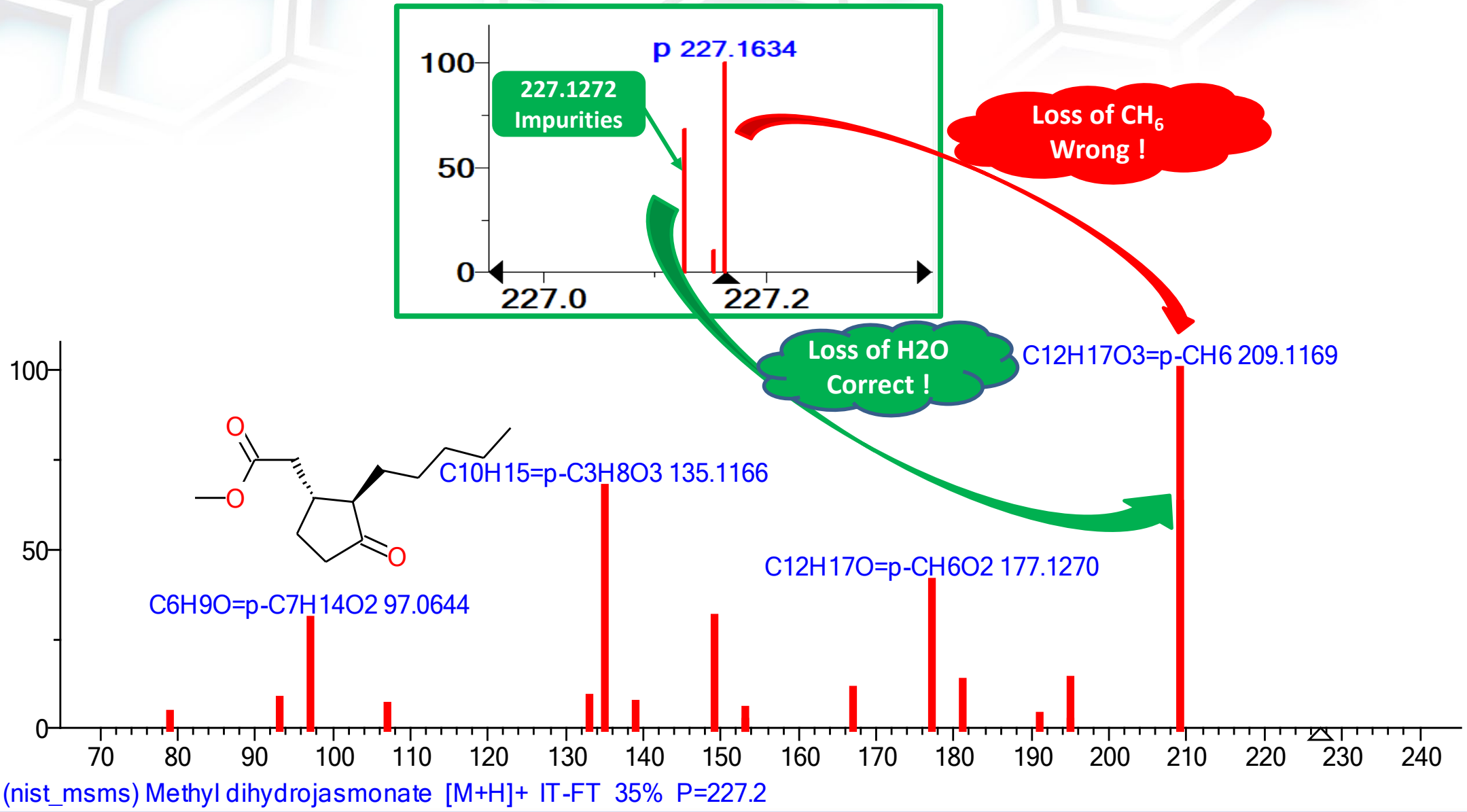


Y. Liang, P. Neta, Y. Simón-Manso, S. E. Stein. Rapid Commun. Mass Spectrom. **2015**, *29*, 629.

Reaction of product ions with water and nitrogen gas in collision cell



Some unexpected peaks come from impurities !



SUMMARY

- The NIST tandem MS library 2020, which provides reference mass spectra for the identification of a wide range of compounds including metabolites, drugs, peptides, glycans, lipids and environmental contaminants, contains 1.3 M tandem mass spectra from >31,000 compounds. This release offers a significant software upgrade and covers more than twice as many compounds as the 2017 version.

- Ion trap (IT-CID and FT-CID and MSn) and HCD spectra were acquired with positive and negative mode. Ion trap spectra comprised of MS2, MS3 and MS4 were acquired at the normalized collision energy of 35 % and high-energy collision induced dissociation (HCD) spectra were acquired with a wide range of normalized collision energies (NCE) (2-180).

- Interpretation of fragmentation ions always plays an important role in examining mass spectra. With aids of NIST developed “MS interpreter” and “hybrid search” software, it is much easier to interpret most of fragmentation ions than before.

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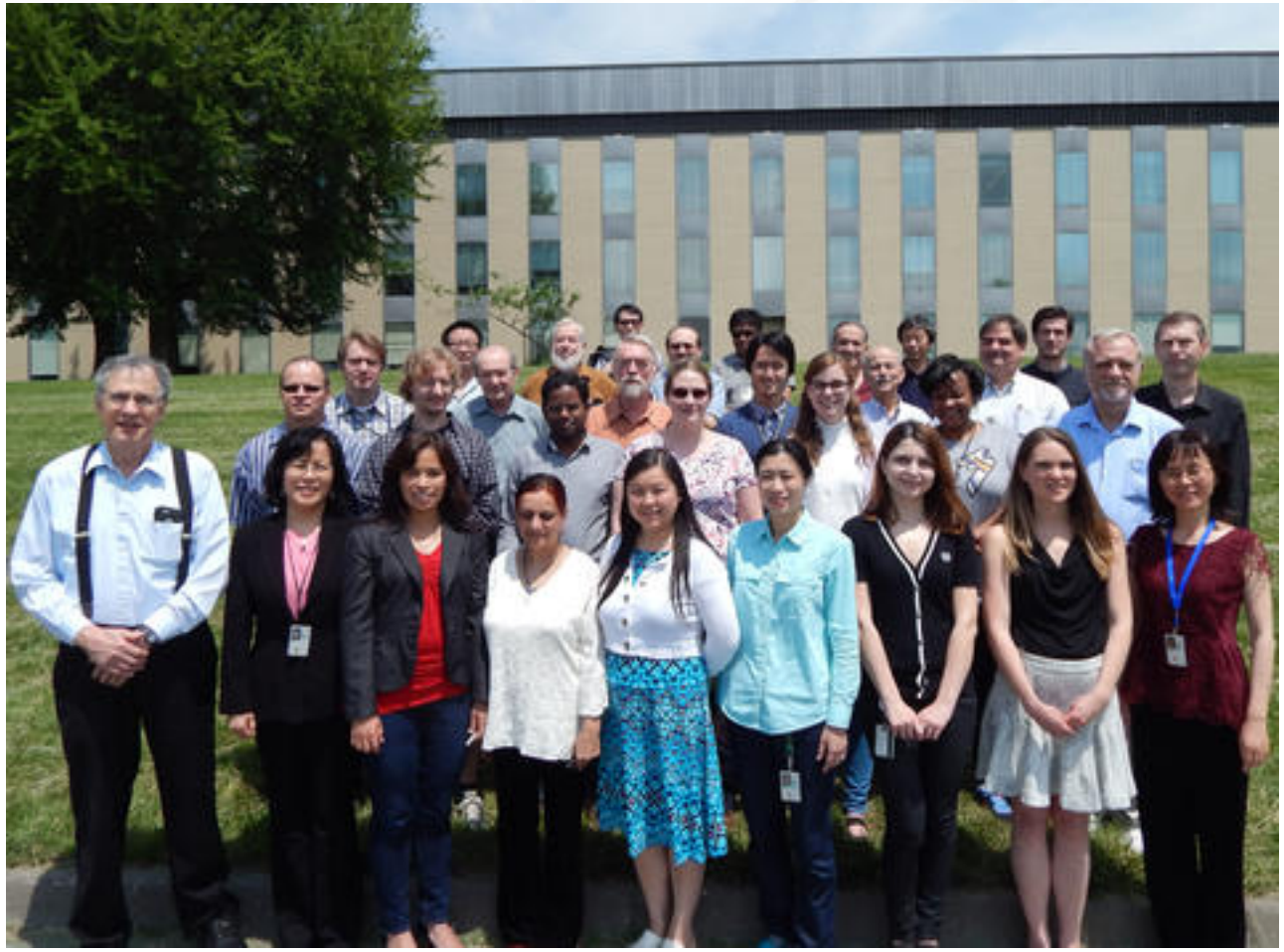

Thank you for your attention!