Investigation of Self-Calibrated, Fine-Isotope-Line Fit Scoring in Combined HPLC-Fourier Transform Orbital Trapping MS for Candidate Formulae Elimination

Yongdong Wang and Ming Gu, Cerno Bioscience, Norwalk, CT

Solving Unknown's Formula – Mass Accuracy Important

BUT – in FTMS At R=250,000 – Fine A+1, A+2, …Isotope "Fingerprint" and Fine Structures Reveal Atom Content too

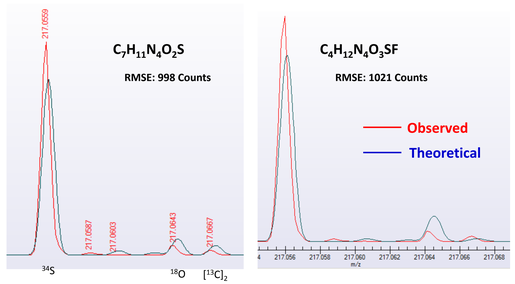
SO - Comparison of Theoretical and Observed Data for Fit Scores when Sorting Atomic Composition "Hit List"

Here: Application of the sCLIPS processing to Data From ***Combined HPLC-FT-Orbital Trapping MS*** is Shown

AND – The Problem of Changing Fine Isotope Structures during HPLC Elution Is Examined

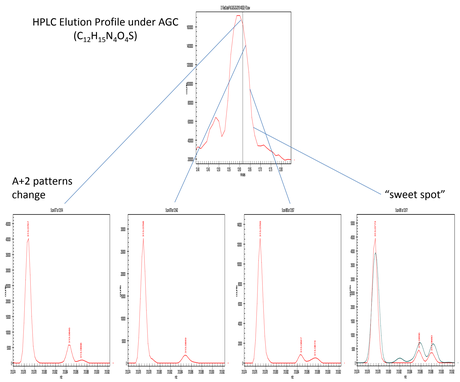
EXAMPLE OF [A+2] ISOTOPE STRUCTURE OF "UNKNOWN"

WHICH ANSWER IS BETTER CURVE FIT? CALCULATE IT AND RANK!
VERY RAPID EVEN FOR LONG LISTS OF "HITS"



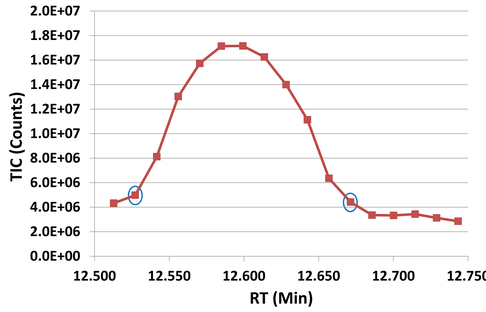
- **Determine** the actual line shape of the monoisotope line, that is, the true instrument function
- **Transform** the actual instrument line function into a mathematically defined function, e.g. , a perfect Gaussian
- **Reapply** this transformation to the higher A+1, A+2, … isotope lines (fine structures)
- **Compute** theoretical mass spec for each candidate on the elemental composition "hit" list derived from mass accuracy, double bond equivalents, and other parameters
- **Compare** each theoretical mass spec to the peak shape calibrated actual MS data – Fitting Residual (RMSE) & Spectral Accuracy
- **Combine** the information on mass error (magnitude and sign) with RMSE or Spectral Accuracy to arrive at correct formula

–> **self-C**alibrated **L**ine shape **I**sotope **P**rofile **S**earch (s**CLIPS**)



Examining A+2 Isotopes on Descending Side of HPLC Peak

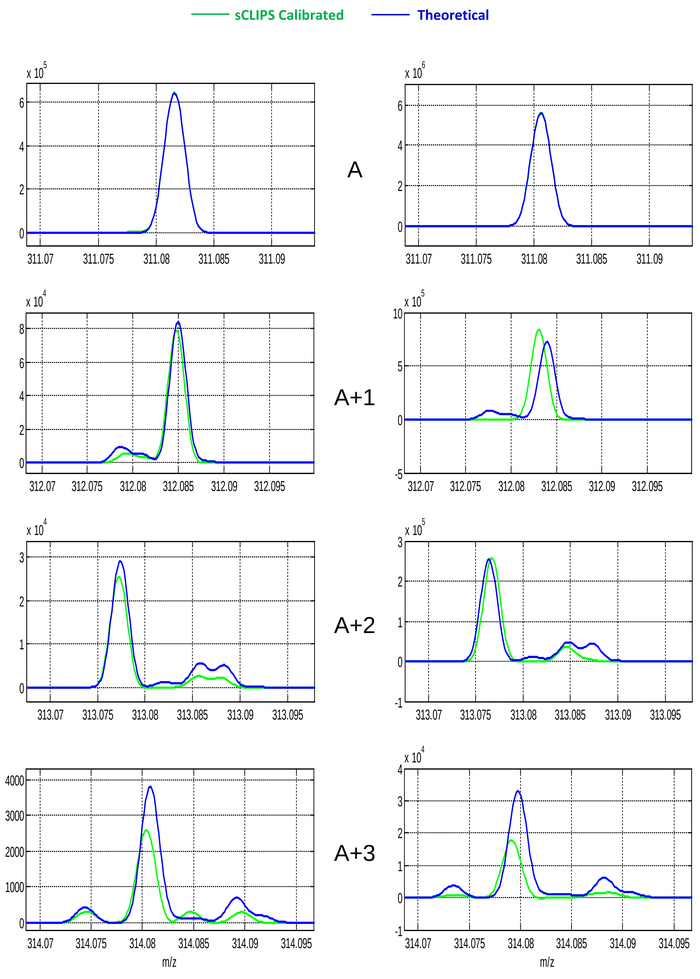
Analyzing scan-by-scan mass spectrum with sCLIPS across the chromatographic peak



Reveals strong scan-to-scan dependency of both spectral and mass accuracy, with **TWO** spectral accuracy "sweet spots" located near but above the bottoms of the chromatographic peak.

Sweet Spot (RT=12.67Min)
Spectral Accuracy: 97.4%

Apex (RT=12.60Min)
Spectral Accuracy: 90.0%



36 hits. Correct formula as
- 21st mass accuracy hit
- 1st spectral accuracy hit

42 hits. Correct formula as
- 9th mass accuracy hit
- 3rd spectral accuracy hit

Wide Open but Reasonable sCLIPS Search Elements:
C [1-25], **H** [0-46], **N** [0-13], **O** [0-13], **S** [0-2], **F** [0-11], **P** [0-6], **I** [0-2]
Other generous condtions (4ppm, DBE etc)

Use of sCLIPS in HPLC / *Orbitrap FTMS*

ID and Ranking of Operational Qualification (OQ) Mixture

Formula – M/Z (nom)	Rank	Spectral Accuracy	Rejected List	*Reasons-Errors
$C_7H_{11}N_4O_2S$ 215	#1	92.70	#2-#3	Error sign / Magnitude
$C_{28}H_{38}N_2O_7$ 556	#1	95.86	# 2-7	Error sign / Mag. / [A+2]
$C_{19}H_{31}N_3O_5$ 381	#1	90.90	# 2-4	Error sign / Magnitude
$C_{37}H_{68}NO_{13}$ 741	#1	95.30	# 2-4	Error sign / Magnitude
$C_{12}H_{15}N_4O_2S$ 311	#1	92.70	# 2-4	Error sign / Mag. / [A+2]
$C_{33}H_{41}N_2O_9$ 609	#1	93.60	# 2-7	Error sign / Mag. / [A+2]
$C_{32}H_{42}NO_2$ 472	#1	93.53	# 2-5	Error sign / Mag. / [A+2]

Orbitrap Errors are SYSTEMATIC and PRECISE..See Strife, R.J. 2011 "Orbitrap High Resolution Applications," Ch. 4, in Characterization of Impurities and Degradants Using Mass Spectrometry, B. Pramanik, M.S. Lee and G. Chen (ed.), John Wiley & sons, Hoboken, NJ 2011

Mathematically possible atom limits, restricted atom set (orthogonal information), Even electron restriction, RDB and ppm windows "wide"

Synthetic Impurities Industrial Application

HPLC / Orbitrap FTMS

sCLIPS Used in the Identification of 8 Synthetic Impurities

Formulas contain C, H, N, O, S, Na – C_{30}-C_{44} range – MW 500-1000 Da

7/8 Compounds Ranked #1- #3 With Others Rejected By Error Sign / Magnitude

1/8 Ranked #4 - Others Rejected By Error Sign / Magnitude and [M+2] Inspection

Findings…

- Measurement variations on all isotopes, A+1, A+2, … All under AGC!
- "Sweet spots" do exist and very useful!
- Sign/magnitude of mass errors useful as well.
- Spectral Accuracy ≥ 98.0%

  → **Unique** Formula!
- More trapping experiment {AGC thresholds, m/z}?

Acknowledgement

1. Dr. Robert Strife from P&G for the experimental data and many fruitful discussions.

2. Dr. Christine Gu from Genentech for interesting discussions.

References

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