IDENTIFICATION OF "UNKNOWNS" – STRUCTURAL CLUES FROM ADVANCED ISOTOPE PEAK MODELING OF MS AND ORTHOGONAL MS/MS DATA

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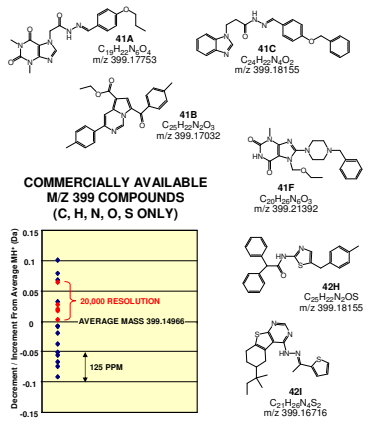
OVERVIEW

- → Evaluate novel approach for determination of elemental composition using CLIPS™ functionality in MassWorks™ software package.
- → Analyze "blind" six nominal m/z 399 compounds with variable C, H, N, O, and S compositions using mass calibrant.
- → Utilize representative MS instrument configurations (e.g., infusion ESI-TOF, UPLC-ESI-TOF, infusion ESI-Quad)
- → Perform proper statistical analysis of processed data – 99% prediction interval (PI) using 6 replicate determinations.
- → Compare identification results.

TEST COMPOUNDS



COMMERCIALLY AVAILABLE M/Z 399 COMPOUNDS (C, H, N, O, S ONLY)

RESULTS

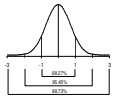
- ★ Compound formulas verified by hybrid LTQ-ICR-FTMS measurements (99% PI = 50 ppb, single composition answers)*
- → TOF results – Directional improvement in correct m/z determination (accuracy and precision) and formula selection.
- → Quad results – 30x improvement in accuracy and 15x improvement in PI width (decreases # of candidates).
- → Quad results – Correct formula (sulfur compounds) always in top 3 hits of candidate list sorted by spectral accuracy / always in top 10 for non-sulfur compounds.

** LTQ-ICR-FTMS data courtesy Vladimir Zabrouskov, Thermo Fisher Scientific*

PROPER STATISTICAL TREATMENT

- → All data has random error – accuracy quotes alone are insufficient! Precision must also be evaluated for m/z and isotope ratios when evaluating potential candidate formulas.
- → S_x provides sample estimate of the standard deviation, σ_x
- → 99% confidence limit expresses coverage of distribution area based on limited number of "n" measurements.

	n – 1	$t_{\alpha,\varphi}$
$t_{\alpha,\varphi}$ multiplier used because σ_x is not known	3	5.84
	4	4.60
	5	4.03
	6	3.71

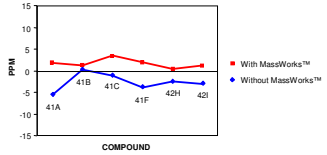


★ Prediction interval expresses future statistical behavior of the measurements.

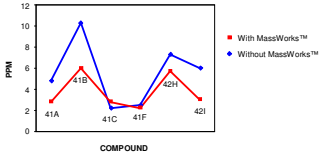
$$PI = \bar{x} \pm s_x (t_{\alpha,\varphi})[1 + 1/n]^{1/2}$$

TOF DATA IMPROVEMENT

- → 99% PI determined from 6 separate runs
- → Precision reduced to ± 9 ppm
- → M+1 / M ratio approaches ± 4%
- → Effect on accuracy:



- → Effect on precision:



QUAD DATA IMPROVEMENT

- → Improved procedure – 0.1 Da = 250 ppm
- → Accuracy ~30x improved
- → PI ~15x narrower
- → Over 400 formula "hits" found (200 hits for C_{15+})
- → Results by compound:

	Accuracy (ppm)	99% PI (ppm)
41A	25	48
41B	15	32
41C	8.5	44
41F	17	40
42H	7	15
42I	32	60

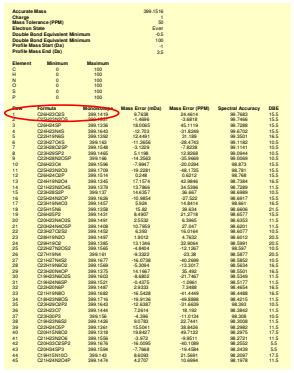
SPECTRAL ACCURACY – A BETTER METRIC FOR FIT RANKINGS

- → Top six candidate formulas for **42I ($C_{21}H_{27}N_4S_2$)** based on 99% PI using TOF data:

WITHOUT MASSWORKS™	WITH MASSWORKS™	SPECTRAL ACCURACY
$C_{19}H_{28}N_4S_2Na$	$C_{21}H_{27}N_4S_2$	99.73
$C_{20}H_{31}O_4S_2$	$C_{20}H_{31}O_4S_2$	99.63
$C_{19}H_{32}N_2OPS_2$	$C_{19}H_{28}N_4S_2Na$	99.61
$C_{21}H_{27}N_4S_2$	$C_{19}H_{32}N_2OPS_2$	99.55
$C_{23}H_{27}O_4S$	$C_{19}H_{32}O_4S_2Na$	99.42
$C_{18}H_{32}O_4S_2Na$	$C_{23}H_{27}O_4S$	99.27

SPECTRAL ACCURACY – A BETTER METRIC FOR COMPOUND ID

- → Elemental composition list for **42H ($C_{25}H_{23}N_2OS$)**
- ★ Rank ordered by spectral accuracy – correct formula is #1 using Quad data.

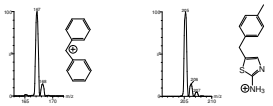


MS/MS DATA FOR ORTHOGONAL COMPOSITION FILTERING

- → Preserve isotope information in fragmentation data by passing entire isotope envelope to collision cell of QQQ system.
- → Possible compositions identified for **42H ($C_{25}H_{23}N_2OS$)** based on spectral accuracy and 99% PI include:

Composition Candidates	Spectral Accuracy
1. $C_{25}H_{23}SON_2$	99.73
2. $C_{24}H_{23}SN_4$	99.71
3. $C_{22}H_{24}SNaO$	99.61
4. $C_{23}H_{23}SNaON_2$	99.21

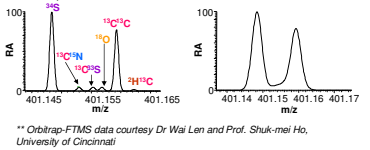
- → Fragment at m/z 381 (loss of water) eliminates candidate #2 – non-oxygen-containing formula.
- → Absence of m/z 376 (loss of sodium) eliminates sodium-containing formulas #3 and #4.
- → Sulfur isotope pattern observed in fragment at m/z 205, but not in fragment of m/z 167.



FUTURE WORK

- → Can partially resolved isotope lines be successfully used for elemental composition determination?

M+2 PEAKS FOR COMPOUND 42H ($C_{25}H_{23}N_2OS$)



*** Orbitrap-FTMS data courtesy Dr Wai Len and Prof. Shuk-mei Ho, University of Cincinnati*