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Combining Accurate Mass from High Resolution and Spectral Accuracy from Lower Resolution towards Unique Elemental Composition Determination

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Introduction

Unknown compound identification benefits greatly from the ever-increasing mass accuracy capabilities on high resolution (HiRes) MS systems. Unfortunately, due to various hardware/firmware/software factors, the holy grail of uniquely determining the elemental composition of a real unknown remains elusive except in limited cases. While high resolution MS is known to readily achieve measurements within 1-5ppm or even better mass accuracy, these instruments have been found to lack spectral accuracy (SA) which could help further eliminate incorrect elemental compositions (Ref 1-2). Paradoxically, low or lower resolution (LowRes) MS lends itself to higher spectral accuracy but is typically associated with lower achievable mass accuracy. In this paper, we demonstrate the feasibility of combining high- and low- resolution measurements into one analysis to achieve unique but also high confident elemental composition determination, all within the same single injection by taking advantage of both HiRes and LowRes scanning capability available on an Orbitrap LC/MS system!

Method

Thermo Orbitrap LC/MS system

Sample and sample preparation

LC column and mobile phase

MS Data Acquisition: ESI+ mode
- Full MS scan, HiRes 120K resolving power (RP), AGC target 1e6, 50-500 Da
- DDA Full MS2 scan: 10 ions, 15K RP, 0.50Da window, AGC target 1e5
- Full MS scan, LowRes 15K RP, AGC target 1e6, 50-500 Da

Thermo Xcalibur Software for Post Processing

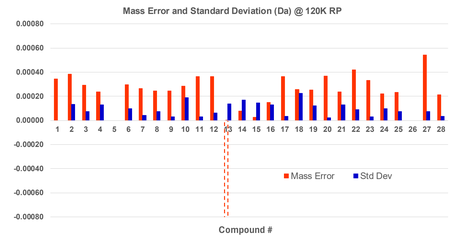
Cerno MassWorks software for sCLIPS (self Calibrating Line-shape Isotope Profile Search) Spectral Accuracy analysis

Results and Discussion

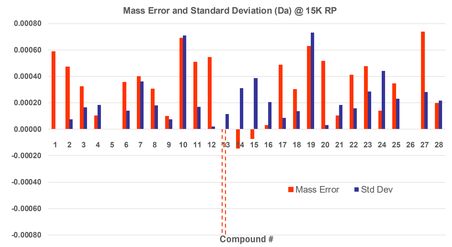
Using the Xcalibur software and the accurate mass XIC confirmed 26 of 28 expected compounds in the run data acquired, with their expected RT location identified as shown in the summary table below along with other analysis results.

Compound #	Name	Elemental Composition (M)	Exact Mass [M+H]+	MS1	MS1 RT (Min)	120K RP Only				120K & 15K RP				ΔSA%	Notes
						SA%	Rank	Total	SA Breakout (%)	SA%	Rank	Total	SA Breakout (%)		
1	Bitertanol	C20H23N3O2	338.18630	☑	14.18, 14.26	98.3	1	33	0.2	99.7	1	33	2.8	1.4	
2	Bromuconazole	C13H12BrCl2N3O	375.96136	☑	12.58	96.3	3	940	-0.5	98.4	1	940	1.3	2.0	
3	Cyproconazole	C15H18ClN3O	292.12112	☑	12.18, 12.30	96.6	1	43	1.5	97.7	1	43	2.0	1.1	
4	Dichlorobutrazol	C15H20ClN3O	294.13677	☑	11.90	83.3	1	31	0.2	94.2	1	31	0.7	10.9	interference
5	Difenoconazole	C19H17Cl2N3O	374.08214	☐											
6	Diniconazole	C15H17Cl2N3O	326.08214	☑	14.49	97.5	1	151	1.1	98.8	1	151	1.6	1.3	
7	Epoxiconazole	C17H13ClFN3O	330.08039	☑	13.15	97.2	1	159	0.3	98.9	1	159	0.1	1.7	
8	Etaconazole	C14H15Cl2N3O2	328.06141	☑	12.95, 13.57	97.2	1	208	0.1	99.2	1	208	2.0	2.0	
9	Ethirimol	C11H19N3O	210.16009	☑	0.80	99.1	1	2	2.4	99.5	1	2	2.4	0.4	
10	Etaxazole	C21H23F2NO2	360.17696	☑	17.24	98.2	1	69	3.4	99.0	1	69	1.5	0.9	
11	Fenarimol	C17H12Cl2N2O	331.03995	☑	12.63	97.8	1	304	0.1	97.9	1	304	1.3	0.1	
12	Fenbuconazole	C19H17ClN4	337.12145	☑	14.09	98.0	1	118	0.3	98.2	1	118	0.6	0.2	
13	Fluquinconazole	C16H8Cl2FN5O	376.01627	☑	13.42	96.2	3	940	-0.8	99.0	1	940	2.2	2.8	
14	Flusilazole	C16H15F2N3Si	316.10761	☑	13.90	93.0	17	89	-3.7	98.9	1	89	0.2	5.9	
15	Flutriafol	C16H13F2N3O	302.10995	☑	9.92	98.6	1	64	0.5	99.2	1	64	0.2	0.5	
16	Fuberidazole	C11H8N2O	185.07094	☑	0.78	99.1	1	12	3.4	99.0	1	12	3.2	-0.1	
17	Hexaconazole	C14H17Cl2N3O	314.08214	☑	14.00	97.3	1	124	0.4	98.5	1	124	2.1	1.3	
18	Ipconazole	C18H24ClN3O	334.16807	☑	15.27, 15.46	97.0	1	47	1.6	99.3	1	47	3.0	2.3	
19	Metconazole	C17H22ClN3O	320.15242	☑	14.04, 14.24	97.2	1	48	1.2	99.1	1	48	2.9	1.9	
20	Nuarimol	C17H12ClFN2O	315.06950	☑	11.26	98.6	1	167	0.4	99.2	1	167	2.5	0.7	tied for top hit
21	Paclobutrazol	C15H20ClN3O	294.13677	☑	11.89	66.6	1	30	1.4	94.2	1	30	0.9	27.6	interference
22	Penconazole	C13H15Cl2N3	284.07158	☑	13.98	98.7	1	82	1.1	98.5	1	82	1.1	-0.2	
23	Propiconazole	C15H17Cl2N3O2	342.07706	☑	14.48, 14.58	97.3	1	206	0.1	99.0	1	206	1.8	1.7	
24	Tebuconazole	C16H22ClN3O	308.15242	☑	13.62	97.2	1	31	7.9	98.6	1	31	3.0	1.5	
25	Tetraconazole	C13H11Cl2F4N3O	372.02881	☑	13.74	98.2	1	565	0.2	99.3	1	565	0.1	1.1	tied for top hit
26	Triadimenol	C14H18ClN3O2	296.11603	☐											
27	Triflumizole	C15H15ClF3N3O	346.09285	☑	13.99	98.1	1	181	0.3	98.8	1	181	0.1	0.8	
28	Triticonazole	C17H20ClN3O	318.13677	☑	12.26	97.5	1	60	1.0	98.4	1	60	1.0	0.9	

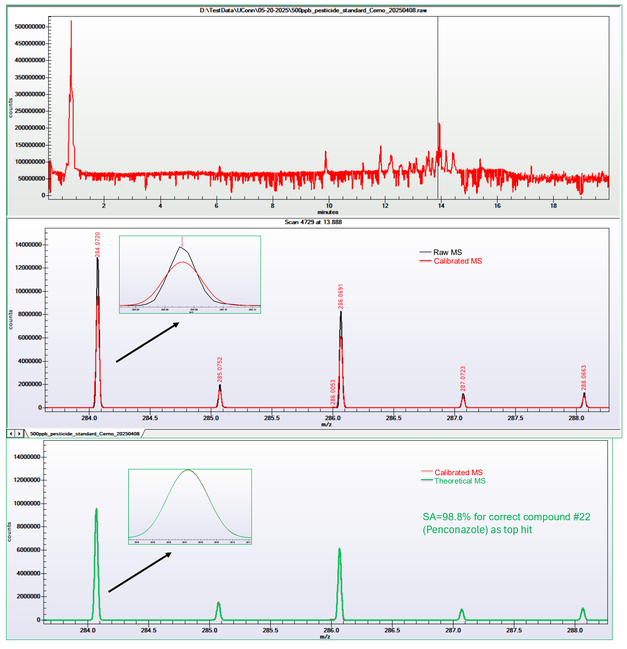
Within each RT window identified, up to 3 most abundant full MS scans at 120K and 15K RP were used to report up to 3 accurate monoisotopic mass values at each RP, from which an average accurate mass was calculated and compared to the exact mass to compute a mass error (bias) along with the corresponding standard deviation at each RP, as shown in the below graphs.



At 120K RP, all mass errors are positive and <0.6 mDa (except for the mistaken compound #13) whereas the standard deviation is 2-3x smaller or <0.2mDa, indicating that the systematic mass error dominates at 120K RP and further improvement is likely limited if not impossible. Nonetheless, this makes it feasible to search for unknown elemental composition within a tight 1mDa mass window, which in and by itself would not be enough to get even close to unique identification (medium # total hits = 86 from the summary table on the left).



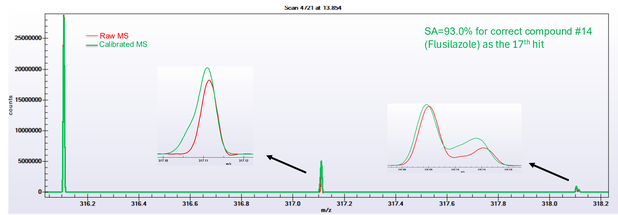
At 15K RP, both the mass error and the standard deviation increase and there are now negative mass errors due likely to the larger standard deviation. It would not be feasible to use the tight 1mDa mass window for confident unknown elemental composition search at 15K RP, unless a more accurate 120K RP mass measurement is available and utilized.



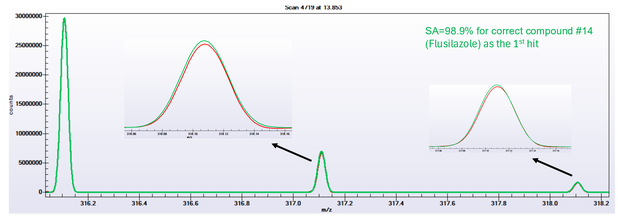
In order to treat these test chemicals as true unknown general organic compounds, a generous elemental composition search conditions were devised to cover m/z up to 500Da, including all reasonably expected elements and using the broadest empirical rules on the possible number of atoms for each element and their ratios (Ref 3), as shown in the table below.

Charge	1	
Mass Tolerance (mDa)	1	
Electron State	Even	
Double Bond Equivalent Minimum	-1	
Double Bond Equivalent Maximum	50	
Profile Mass Start (Da)	-1	
Profile Mass End (Da)	5.5	
Empirical Rules	Enabled	
Empirical Elemental Limits	Wiley	
H/C Ratio	Extended	
Heteroatom Ratios	Extended	
Element	Minimum	Maximum
C	1	40
H	0	73
N	0	20
O	0	21
Cl	0	11
Br	0	5
S	0	11
F	0	17
Si	0	6
P	0	1
Na	0	1

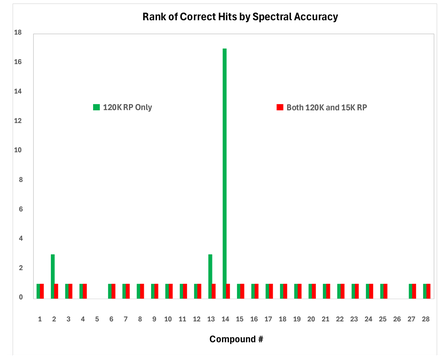
For each compound detected, the most abundant scans from 120K and 15K RP were selected for sCLIPS elemental composition determination. For the 120K scan, its own accurate mass was used whereas for the 15K scan, the accurate mass from the 120K scan was adopted instead to search for all possible elemental compositions, with the list of hits sorted by SA from high to low. The SA for the correct compound, its rank among the total number of hits, and the SA breakout from the 2nd best hit when the correct compound is the top hit are all listed in the summary table on the left. When the correct compound is not the top hit, the SA breakout is calculated as the SA difference between the correct compound and that of the top hit (a negative value). For all compounds, there is a significant increase from 120K to 15K RP scan in SA itself (median 97.4% vs 98.9%) and/or SA breakout (median 0.4% vs 1.6%), resulting in better ranking while increasing identification confidence. It should be noted that 1.0% in SA often means the difference between unique and ambiguous identification.



120K RP Scan



15K RP Scan



Using sCLIPS and SA for unknown elemental composition at 120K RP, 23 out of the 26 compounds are found as the top SA hits, an impressive feat from a median # of 86 hits.

The most remarkable results are obtained by using both 120K and 15K RP scans, where all 26 compounds are determined as the top SA hit with high confidence due to the better SA and/or larger SA breakout, achieving unique unknown elemental composition on a commercially available MS as is.

Conclusion

This study with 26 small molecule compounds demonstrated that it is feasible to achieve unique elemental composition determination of true unknowns by using both HiRes and LowRes scans in the same data acquisition and taking advantage of the high mass accuracy from HiRes scan and high spectral accuracy from the LowRes scan on an Orbitrap MS system.

References

1. Wang SA
3. John Erve
3. T. Kind

Conflict of Interest (COI) Statement