Formula Determination and Relative Quantitation from Overlapping Ion Signals

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Introduction

Comparing to other analytical technologies, mass spectrometry is typically considered of high selectivity, due to its relatively high resolving power, even at the conventional unit mass resolution. Overlapping mass spectral signals, however, can be observed frequently in mass spectrometry, even on higher resolution MS systems. Some examples include:

- The overlap of the two different forms of the same drug metabolites, one with and one without isotope labeling (^{14}C or ^{15}N), resulting in ions offsetting each other by the exact mass of one mass unit with very small mass defects, causing interferences to the two ions involved.
- The fragment ions from EI GC/MS or LC/MS/MS experiments.
- Product oxidation or reduction through the loss or gain of H_2.

This paper will present a novel approach to both identify and quantify these mutually overlapping ions.

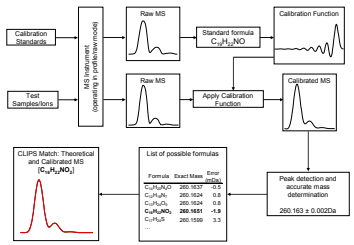
Methods

While it is sometimes possible to move to MS systems of higher and higher resolving power to separate these overlapping mass spectral signals, a different approach will be presented in this paper to mathematically resolve these mutually interfering signals. To accurately deconvolute these overlapping signals, a unique mass spectral calibration has to be performed that calibrates not only m/z axis but, more importantly, mass spectral peak shape. With the Spectral Accuracy achieved through this calibration, the overlapping mass spectral signals can now be resolved reliably and mathematically, leading to both qualitative (e.g., elemental compositions) and quantitative results (e.g., the relative concentration of the overlapping ions), even at unit mass resolution.

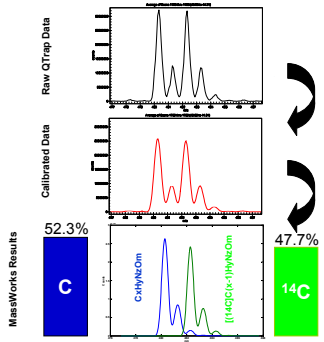
Theory

➢Unlike the conventional MS calibration, a novel calibration involving both m/z and MS peak shape allows for exact isotope modeling and formula determination on even a unit mass resolution system, provided that the mass spectral signal is free from significant interferences arising from coexisting ions.

MassWorks CLIPS Formula ID

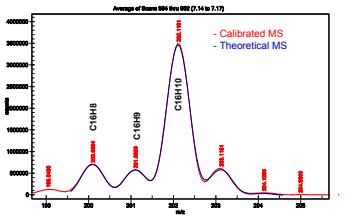


➢In the presence of mass spectral interferences, the Spectral Accuracy and formula ID will be compromised.
➢When the mass spectral interference is from an ion related to the ion of interest, however, the same exact isotope modeling can be expanded to include the interference ions in the Spectral Accuracy and formula ID process, as shown by the example below.



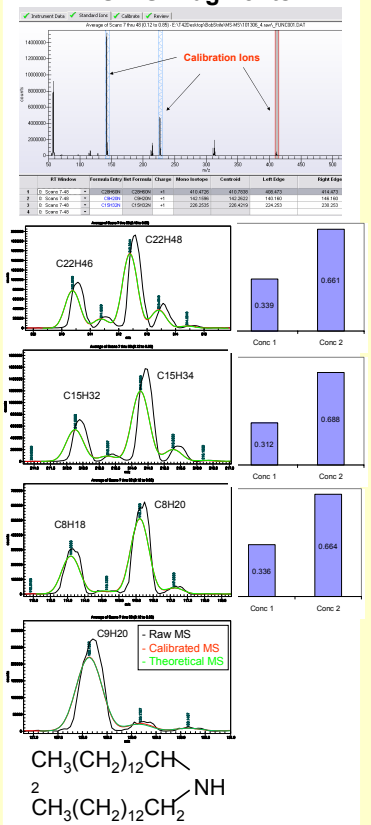
Results and Discussion

EI GC/MS



In GC/MS experiments with EI source, the electron beam can often strike a gas molecule hard enough for it to lose one or more hydrogen atom(s) during the electron impact process. This can happen to both the molecular ion itself or its fragments, especially if the molecular ion is a hydrocarbon. In the above graph, the molecular ion loses both one and two hydrogen atoms, forming a three component mixture in the observed mass spectrum. With the mass spectral calibration performed, all these three components can be mathematically resolved down to the random noise level, allowing for both qualitative formula determination and relative quantitation of all three components involved.

LC/MS/MS Fragments ID

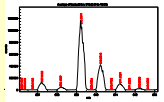


$CH_3(CH_2)_{12}CH$
$CH_3(CH_2)_{12}CH_2$ NH

In this LC/MS/MS experiment with a triple quad from Waters Quattro, the MassWorks calibration is performed using known fragments ions coming from the same precursor ion to achieve a calibration on Q3 which serves as the mass analyzer. Alternatively, one could have used the known fragments of an entirely different precursor ion. The various fragments may or may not overlap with each other but could be separated mathematically for structural elucidation purpose. The isolation window for the MS/MS experiment is set to be open, allowing for all isotopes of the precursor ion to come through for this analysis.

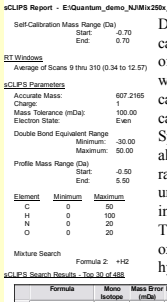
HiRes Quad w/Pharmaceuticals

- A Self Calibration Case Study



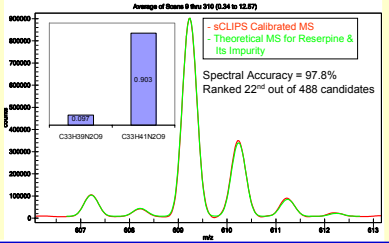
For drug compound reserpine, an impurity is typically observed whose m/z is close enough to become an interference to the standard reference line at 609.2812Da.

While it would have required a calibration process in order to perform both identification and relative quantitation of this impurity, we tested a self calibration process called sCLIPS using higher resolution quadrupole MS data available on a Thermo Quantum instrument here with interesting and useful results.



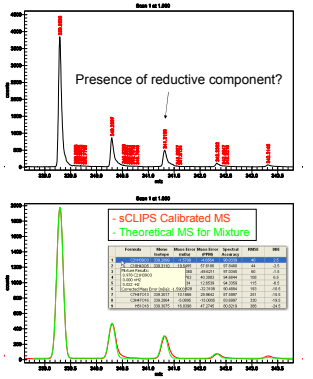
Due to the lack of mass calibration, a large mass tolerance of 100mDa is used to come up with a list of 488 formula candidates. With the peak shape calibration, however, the high Spectral Accuracy of 97.8% allows the correct formula to be ranked as the 22nd hit with unbiased relative quantitative information (9.7% impurity). The results point to possible oxidation via the loss of two hydrogen atoms.

	Formula	Mono Isotope	Mass Error (mDa)	Mass Error (PPM)	Spectral Accuracy	RMSE	DBE
1	C9H27O10	607.1604	-56.0779	-92.3524	98.3322	3.346	22.5
2	C8H30O9	607.2907	74.2080	122.2101	98.3113	3.388	14.5
3	C34H27N2O6	607.1717	-44.8445	-73.8526	98.2531	3.505	22.5
4	C39H19N2O3	607.2080	-8.4580	-13.9308	98.2372	3.537	21.5
5	C34H31N4O7	607.2193	2.7744	4.5690	98.2348	3.542	21.5
6	C34H34N2O8	607.3019	85.4414	140.7099	98.2324	3.546	14.5
7	C39H31O3	607.1998	-19.6924	-32.4306	98.1450	3.722	21.5
8	C33H31N4O6	607.2305	14.0078	23.0688	98.1379	3.736	21.5
9	C34H35N6O5	607.1366	-79.8928	-131.5719	98.1148	3.782	28.5
10	C33H35N6O5	607.1476	-68.6592	-113.0721	98.0914	3.829	28.5
11	C34H30O10	607.2543	37.8525	62.2883	98.0909	3.830	15.5
12	C33H27N4O6	607.1829	-33.6111	-55.3528	98.0857	3.841	22.5
13	C33H31N4O7	607.2132	96.6748	159.2097	98.0863	3.880	14.5
14	C34H33O11	607.1240	-92.4634	-152.2742	98.0547	3.903	23.5
15	C33H19N4O7	607.1254	-91.1260	-150.0717	98.0488	3.915	28.5
16	C34H35N6O5	607.2689	50.3933	82.9906	98.0086	3.996	20.5
17	C33H35N6O4	607.2781	61.6267	101.4904	97.9954	4.042	20.5
18	C32H39N3O4	607.1591	-57.4258	-94.5723	97.9920	4.049	28.5
19	C32H31N6O5	607.2417	25.2411	41.5686	97.9904	4.092	21.5
20	C33H35N4O6	607.2557	39.1599	64.4908	97.9472	4.119	20.5
21	C32H35N3O3	607.2894	72.8600	119.9002	97.9806	4.252	20.5
22	C33H39N2O9	607.2656	40.0699	65.9581	97.8731	4.267	16.5
23	C32H27N6O7	607.1941	-22.3777	-36.8530	97.8938	4.312	22.5
24	C33H29N2O10	607.1383	-81.2300	-133.7744	97.8408	4.332	23.5
25	C39H35N2O7	607.2444	27.0265	45.0910	97.8382	4.398	20.5
26	C33H33N10O3	607.1955	-21.0403	-34.6505	97.8079	4.398	27.5
27	C31H19N12O3	607.1703	-46.1925	-76.0725	97.7994	4.415	28.5
28	C34H23N8O4	607.1842	-32.2737	-53.1503	97.7701	4.474	27.5
29	C32H29N3O6	607.2067	-9.8070	-16.1507	97.7686	4.477	27.5
30	C31H15N9O5	607.1227	-93.8114	-154.4941	97.7685	4.477	29.5



DART AccuTOF Food Analysis

Mono-olein (OOO – (2 x oleic acid +H2O)) in Olive Oil



Conclusions

➢Though the presence of overlapping ion signals may be difficult or even impossible to overcome through either chromatography or higher resolution MS, there exists an elegant mathematical solution.
➢The mathematical solution provides both qualitative results (formulae) as well as quantitative results (relative concentrations).
➢At unit mass resolution, this mathematical solution calls for a comprehensive MS calibration involving both m/z and peak shape through known standards.
➢At higher-than-unit-mass resolution, it is possible to perform both qualitative and quantitative analysis through self calibration, i.e., without the use of any known standards. While a larger mass tolerance window may be needed, which may compromise the performance, the differentiation power of Spectral Accuracy can still produce meaningful results.

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