The Determination of an Optimized FT-ICR Resolving Power to Achieve the Best Spectral Accuracy for Unknown Identification

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Overview

➤ **Spectral accuracy** was evaluated for FT-ICR data on small molecules acquired at different resolving powers.

➤ High resolving power provides distinct isotopic information and greatly facilitates identification of unknown compounds.

➤ Formula determination via spectral accuracy was demonstrated by a search with mass tolerance of 2.5 ppm and possible elements C/H/N/O/F/P.

Introduction

Spectral accuracy is a similarity measurement between the isotope patterns of experimental and theoretical spectra. It is calculated as $(1-RMSE)*100$, where RMSE is the fit error between the calibrated and theoretical spectra.[1] This new metric has been shown to vary as a function of resolving power (RP). In recent studies with Orbitrap data a clear trend of decreasing spectral accuracy with increasing RP was observed. This is likely due to destructive and/or constructive interference at higher RP.[2] Here we report the first investigation on spectral accuracy at various RP with FT-ICR data. This work will provide details on the spectral accuracy behavior towards higher RP as well as optimal RP to achieve the best spectral accuracy for unknown ID.
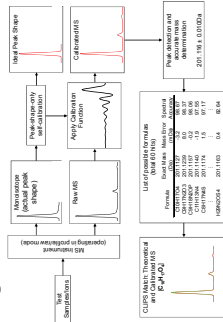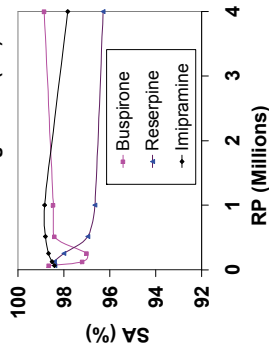
Fig 1. MassWorks sCLIPS Formula ID

Fig 2. Spectral Accuracy (SA) vs. Resolving Power (RP)

Methods

➤ **Data Acquisition:** A mixture of commercially available small molecules including Imipramine, Buspirone, and Reserpine were analyzed on a solariX 12 FT-ICR (Bruker Daltonics, Billerica MA). Data was acquired at varying dataset acquisition sizes (TD), ranging from TD=512k to 4M. This corresponds to RP(m/z 400) =66,000 to 530,000.

➤ **Data Analysis:** All mass spectral data acquired on the FT-ICR were exported as ASCII data and analyzed by **sCLIPS** (self Calibrated Line-shape Isotope Profile Search) through MassWorks (Cerno Bioscience, Norwalk, CT). **sCLIPS** performs a peak-shape-only calibration to transform the actual peak shape function into a known mathematical function by using the measured monoisotope peak itself as a calibration standard. When applied to the whole isotope profile of an unknown ion, it is transformed into a calibrated isotope profile with the same known peak shape function, which is then used in the calculation of the theoretical mass spectrum for any given formula candidate to achieve exact isotope modeling with high Spectral Accuracy (SA).

Results and Discussion

The overall spectral accuracy for the three compounds was found to be fairly consistent at different RP. The worst at 96.3% (m/z 609, @ RP of 64K). All the measurements achieved a spectral accuracy better than 97.0%, except for two data points. Although no clear pattern on improved spectral accuracy vs. increasing RP was observed, the spectral accuracy for the three compounds remained virtually unchanged as TD increased from 518K to 4M. However, at the same dataset size, a spectral accuracy better than 98.0% was obtained for m/z 281 and 386, while the m/z 609 had spectral accuracy about 97.0% or less. The high spectral accuracy at high TD for low mass ions could be tremendously valuable for unknown identification as demonstrated through a case study with Buspirone (Fig 3).

The general strategy for an effective elemental composition determination of unknowns is to utilize as much chemistry knowledge regarding the unknowns as possible including the limits of possible elements and their upper and lower bounds within a given mass tolerance. Such restrictive parameters can be easily available in the applications of metabolite identification and pharmaceutical impurity determination since the unknowns are more or less related to parent drug molecules. As a result, high mass accuracy formula determination is the applications. For example, a search for m/z 386.2548 including only C H N O at mass tolerance of 2.5 ppm resulted in only two possible formulas: $C_{21}H_{32}O_2N_5$ and $C_{20}H_{36}O_6N$. On the other hand, there will be a total of 11 formulas found when the search was conducted with the same mass tolerance of 2.5 ppm but with additional elements of F and P. These two elements are notorious in formula determination because they only have a monoisotope peak. Their addition to a search only produces more formulas and does not provide any unique isotope patterns to help in formula determination.
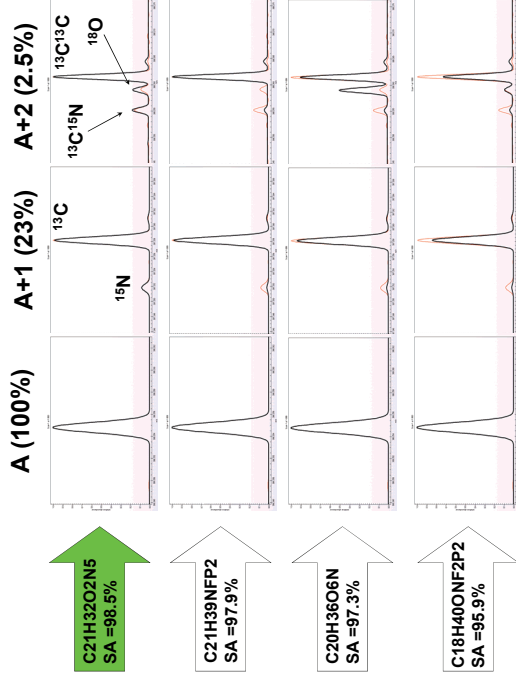
Results and Discussion

Table 1. Elemental Composition Determination for m/z 386

Row	Formula	Mono Isotope	Mass Error (mDa)	Mass Error (PPM)	Spectral Accuracy	RMSE	DBE
1	C21H32O2N5	386.2556	0.80	2.1	98.5	166,684	8.5
2	C21H39NFP2	386.2542	-0.62	-1.6	97.9	236,863	3.5
3	C20H36O6N	386.2543	-0.54	-1.4	97.3	307,286	3.5
4	C18H40ONF2P2	386.2553	0.52	1.3	95.9	467,407	-0.5
5	C17H36ON3F3P	386.2548	0.01	0.0	95.1	556,889	0.5
6	C17H37O7NF	386.2554	0.61	1.6	94.8	590,906	-0.5
7	C16H32ON5F4	386.2543	-0.50	-1.3	94.2	667,573	1.5
8	C15H33ON9P	386.2546	-0.23	-0.6	93.0	804,129	4.5
9	C14H29ON11F	386.2641	-0.74	-1.9	91.8	940,182	5.5
10	C12H34O2N9FP	386.2557	0.91	2.4	89.9	1,158,114	0.5
11	C11H30O2N11F2	386.2552	0.40	1.0	88.7	1,291,217	1.5

Fig 3. Formula Determination Powered by Fine Isotope Structures

In order to closely examine the fine isotope structures of A+1 and A+2 peaks, the plots for the peaks are normalized to the largest peak in their mass range. The spectra in black are theoretically calculated and those in red are calibrated (RP=4M).

A (100%)   A+1 (23%)   A+2 (2.5%)

C21H32O2N5 SA =98.5%

C21H39NFP2 SA =97.9%

C20H36O6N SA =97.3%

C18H40ONF2P2 SA =95.9%

Fortunately this is a typical case where spectral accuracy can make a great difference. As shown in Table 1 and Fig 3, the true formula for the search, Buspirone, has nearly perfect match between its theoretically calculated and calibrated spectra. It achieved the best spectral accuracy (98.5%) and ranked as the number one hit among 11 possible formulas. Buspirone's great SA match was observed not only in the monoisotope peak, but more importantly, in the isotopic peaks of ^{15}N, ^{13}C, $^{13}C^{15}N$, ^{18}O, and $^{13}C^{13}C$. These distinct fine isotope structures carry the unique fingerprint of Buspirone and help to determine the formula of this molecule. In contrast, the other three formulas (Fig 3.) containing only one N have obvious mismatches on both ^{15}N and $^{13}C^{15}N$ peaks. Additional mismatches can be found on ^{13}C, ^{18}O, and $^{13}C^{13}C$ peaks.

With help of spectral accuracy, the requirement of high mass accuracy for unknown identification can be significantly relaxed. This can be demonstrated by formulas of $C_{21}H_{32}O_2N_5$ (Buspirone, m/z 386.2556, #1 in Table1) and $C_{12}H_{34}O_2N_9FP$ (m/z 386.2557, # 10 in table) from the same search. With only 0.1 mD difference in terms of m/z values, it will be extremely challenging to distinguish them according to the m/z values only. But their huge difference, ~9% in spectral accuracy can help to confidently rule out $C_{12}H_{34}O_2N_9FP$ as a possible candidate for the unknown.

Conclusions

➤ Excellent **Spectral accuracy** better than 98% was found for the small molecules as demonstrated through m/z 281 and 386 acquired with RP from 518K to 4M. The combination of high spectral accuracy and high RP fully takes advantages of resolved isotope fine structures of A+1 and A+2 peaks to achieve confident formula determination.

➤ Formula determination with **Spectral Accuracy** can greatly relax high mass accuracy requirements for unknown ID.

➤ Future work will include investigations on spectral accuracy changes with RP of 128K and 256K and additional measurements to confirm the findings through current studies.

References

(1) Y. Wang, M. Gu. The Concept of Spectral Accuracy for MS. Anal. Chem. 2010, 82, 7055.

(2) J.C.L. Erve, M.Gu, Y. Wang, W. DeMaio, R.E. Talaat. Spectral Accuracy of Molecular Ions in an LTQ/Orbitrap Mass Spectrometer and Implications for Elemental Composition Determination. J. Am. Soc. Mass Spectrom. 2009, 20, 2058.