Beyond Mass Accuracy: the Neglected Role of Spectral Accuracy in Mass Spectrometry

Yongdong Wang, Ming Gu and Don Kuehl
Cerno Bioscience, Danbury, CT

Overview

- Accurate mass measurements alone, even to 1ppm, generally do not produce unambiguous elemental composition results (ECD).
- A new, novel algorithm to self-calibrate mass spectrometer line shape (spectral accuracy) provides a powerful metric for ECD.
- Spectral accuracy can enable highly accurate ECD via isotope profile matching greatly enhancing the ability to obtain unambiguous ECD for high resolution MS such as TOF or Orbitrap.

Introduction

Even at 1ppm mass accuracy unambiguous ECD is typically not attainable[1]. It has long been known that the isotope profile for an unknown ion can be used to assist in ECD[2] but previous approaches were limited to simple first order models involving peak ratios or linear fitting[3,4,5]. A novel algorithm which calibrates for both mass accuracy as well as spectral line shape has been previously described which dramatically improves MA on unit resolution instruments[6].

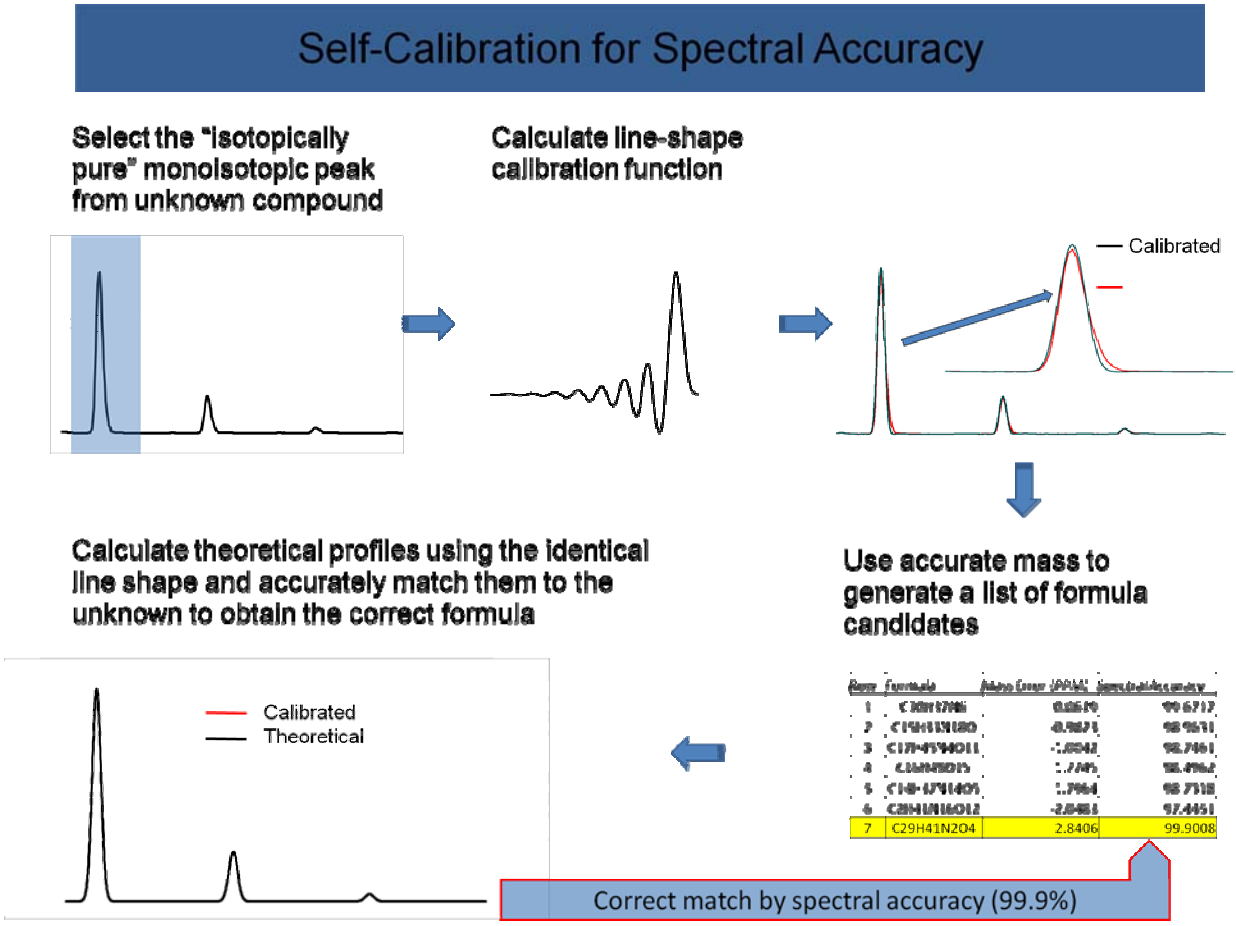
A new method has been developed for high resolution MS which allows for the self-calibration of the spectral line shape. The ability to calibrate the spectral data to a mathematically defined line shape produces well defined spectral profiles of high spectral accuracy. This allows for highly accurate isotope profile matching which can allow for unambiguous ECD.

In this work we will evaluate the use of spectral accuracy for ECD using a variety of high resolution instruments to illustrate the significant advantages of spectral accuracy for ECD.

Methods

Spectra were collected from 3 different types of medium to high resolution instruments including a Finnigan Quantum Ultra (high resolution triple quad), a Bruker micrOTOF (TOF), and a Finnigan Orbitrap.

A variety of small molecule pharmaceuticals ranging in mass from 152 to 734 Daltons were run on the each instrument by LC/MS and by infusion. All data was acquired in positive ionization mode as profile spectra.
The data acquired from the runs was read directly into MassWorks software for calibration, post processing and analysis[7]. For each ion of interest, the self-calibration and search procedure outlined below was applied. The spectral accuracy metric is calculated as (1-RMSE) * 100 where RMSE is the fit error between the calibrated and theoretical spectrum. A pre-determined mass accuracy window, based on the accurate mass capability of each instrument, was used to perform an elemental composition search to obtain a list of candidate formulas for input into the isotope profile search.



Results and Discussion

The results below for 3 different instruments for Self-Calibration Isotope Profile Searching (s-CLIPS) prove to be a powerful metric to enhance compound determination by mass spectrometry. Even for instruments of moderate mass accuracy, provided the monoisotopic peak is resolved, excellent formula ID was obtained. Of all the measurements made, the Spectral Accuracy ranked the correct compound as the #1 correct match 14 out of 15 times.

For example, for Ketoconazole to be uniquely identified using the elements C,H,N,O,Cl, and S in an elemental composition search would require a mass accuracy of better than 200 ppb. Even at a mass accuracy of 1ppm over 40 formula candidates must be evaluated for this compound.
In addition, the self-calibration approach requires no additional instrumental or chemical calibrants as the analyte ion is the calibrant for itself. Furthermore, it is a near ideal calibrant as it is as close in mass position and measurement time as is possible. This minimizes errors due to instrument drift over both time and mass value.

Search results on Quantum Ultra (quad)

Run Type	Infusion
Search window	30ppm
Elements	C,H,N,O,Cl ,S
Nominal FWHM	0.15 Da

Name	Exact Mass	Ion Formula	Number of Formulas	Spectral Accuracy	Rank
Acetaminphen	152.0712	C8H10NO2	11	99.3435	1
Promethazine	285.1425	C17H21N2S	97	99.5952	1
Buspirone	386.2556	C21H32N5O2	147	99.8259	1
Terfenadine	472.3216	C32H42NO2	246	99.8659	1
Loperamide	477.2309	C29H34N2O2Cl	531	99.7809	1
Tyr-Tyr-Tyr	508.2084	C27H30N3O7	694	99.3378	5
Reserpine	609.2812	C33H41N2O9	1123	99.8238	1

Search results on micrOTOF (TOF)

Run Type	Infusion
Search window	5ppm
Elements	C,H,N,O,Cl, S
Nominal FWHM	.06 - .12 Da

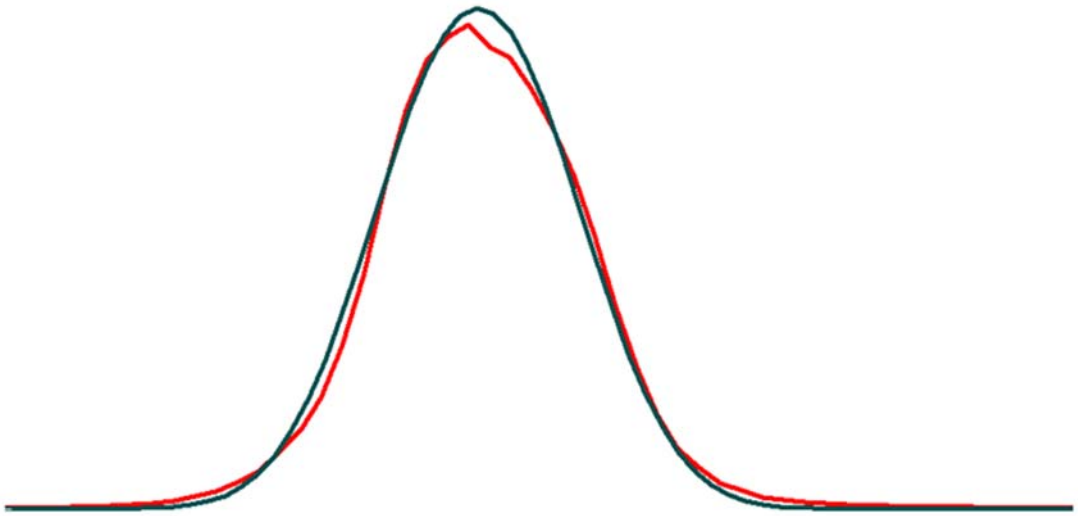
Name	Exact Mass	Ion Formula	Number of Formulas	Spectral Accuracy	Rank
caffeine	195.0876	C8H11N4O2	7	99.9086	1
emetine	481.3060	C29H41N2O4	54	99.9044	1
erythromycin ethyl succinate -water	844.5052	C43H74NO15	280	99.7798	1
erythromycin ethyl succinate	862.5158	C43H76NO16	295	99.8338	1

Search results on Orbitrap

Run Type	LC/MS
Search window	3ppm
Elements	C,H,N,O,Cl, S
Nominal FWHM	0.03 Da

Name	Exact Mass	Ion Formula	Number of Formulas	Spectral Accuracy	Rank
Quinidine	325.1916	C20H25N2O2	13	99.8352	1
Clozapine	327.1376	C18H20ClN4	19	99.7887	1
Ketoconazole	531.1565	C26H29Cl2N4O4	111	99.4985	1
Erythromycin	734.4690	C37H68NO13	100	99.9158	1

Line shape correction before and after self-calibration (quadrupole)



Conclusions

- Even at high mass accuracy (1ppm) the number of formula candidates can be extensive.
- Spectral accuracy can be used as an additional metric to accurately rank formula candidates.
- s-CLIPS relies on a novel self-calibration approach that requires no additional calibration to be performed.
- Isotope patterns are unique for every formula and can relax the need for careful instrument calibration to insure the highest mass accuracy.

References

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