Cerno Application Note

Extending the Limits of Mass Spectrometry

Accurate Mass and Spectral Accuracy Performance of an Agilent LC/MS Single Quadrupole System

Introduction

Single quadrupole LC/MS instruments are the workhorses of analytical labs but fall short for applications that require unknown compound formula identification. On a single quadrupole MS system operating at unit mass resolution, the typical manufacturer specification is for ~0.1-0.5Da mass accuracy relegating it for a rough and quick check of nominal m/z values, but incapable of confident elemental composition determination (formula ID). However, with the advanced calibration techniques available in Cerno's MassWorks™ software, LC/MS single quad instruments can provide up to 100x improvement in mass accuracy, and perhaps more importantly, provide accurate modeling of the compound isotope profile to better than 99% Spectral Accuracy[1]. The enabling combination of the Agilent LC/MS and MassWorks can provide a cost effective solution for formula ID. In this application note we will evaluate the performance of an Agilent LC/MS in terms of achievable mass accuracy and Spectral Accuracy to enable unknown formula ID.

Experimental

The Agilent standard tune solution (G2421-60001) was used as the calibration sample for the performance test. This solution contains a number of calibration ions at mass values from 117Da to 2722Da. The instrument parameters of the Agilent 6120 were set to acquire data in raw (profile) mode with zero ion threshold. The instrument scan range was set from 100 to 1000Da under the Scan mode with Full Scan Data Storage[2]. The test sample for evaluating the performance was Reserpine, a common benchmark standard used in LC/MS with a molecular weight of 608.68 whose molecular formula is C33H40N2O9. 10ul of both the tune solution and the reserpine where loop injected into the LC/MS. The concentration of the reserpine was 100ppm for a total injected amount of 1ug, the equivalent amount on the column in LC analysis. The reserpine was repeatedly injected 10 times to obtain meaningful statistics.

The acquired Agilent MS data of the tune solution is opened directly into the MassWorks software under Calibration mode. The calibration ions are automatically located by simply loading a pre-defined ion list for the Agilent tune mixture. Figure 1 shows some software screenshots of the calibration process. Upon reviewing the results, the calibration file is saved and applied to all the sample runs producing fully calibrated mass spectra.

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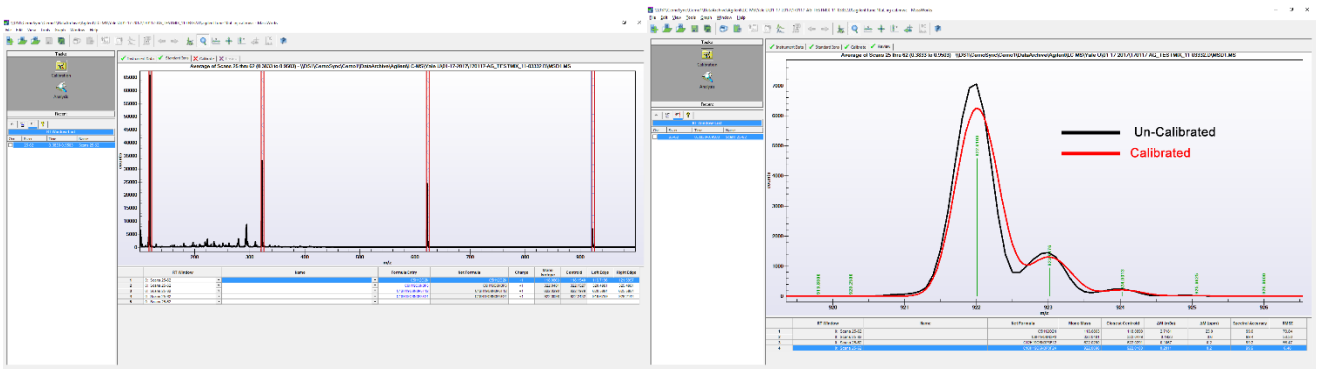

Figure 1. Screen capture shows automatic selection of calibration ions (left) and a review of calibrated and uncalibrated mass spectrum (right).

Results and Discussion

Figure 2 shows the formula search results under the Analysis mode from the calibrated data for one of the repeat measurements. The search was performed to find all formula containing C, H, N, and O within a 10mDa mass tolerance window (i.e., the measured mass after MassWorks calibration is accurate to 0.00xDa), producing 38 possible elemental compositions. Since the whole MS profile including all the major and minor isotopes has also been calibrated to a known lineshape, it is a simple matter to calculate the true mass spectrum based on first principles for each possible elemental composition and compute a corresponding Spectral Accuracy by comparing it to the calibrated mass spectrum. The Spectral Accuracy is a simple mathematical metric that quantitates how well the true and calibrated mass spectrum match. The formula search candidates can then be sorted by Spectral Accuracy to provide extremely high confidence in the formula ID results. In the example below, the Spectral Accuracy is over 99.1% for the correct Reserpine formula, with the next best match falling below 98.7%. This advanced formula ID algorithm (CLIPS- Calibrated Lineshape Isotope Profile Search) combines both mass accuracy and Spectral Accuracy to enable formula ID on these otherwise conventional workhorse LC/MS instruments.

Table 1 below shows the statistical summary of the 10 repeat measurements. The repeatability of the measurements shows that the mass error attainable is within +/- 0.005Da (5mDa), which is approximately 100x better than the uncalibrated instrument specification. In addition, the calibrated mass spectral lineshape provides an additional, and more powerful metric, for formula ID, with a Spectral Accuracy of around 99%. The well-known stability of the Agilent single quadrupole instrument family has been shown to be excellent and can hold calibration for many days[3], making frequent recalibrations unnecessary and improving the ease of use of MassWorks software for formula ID.

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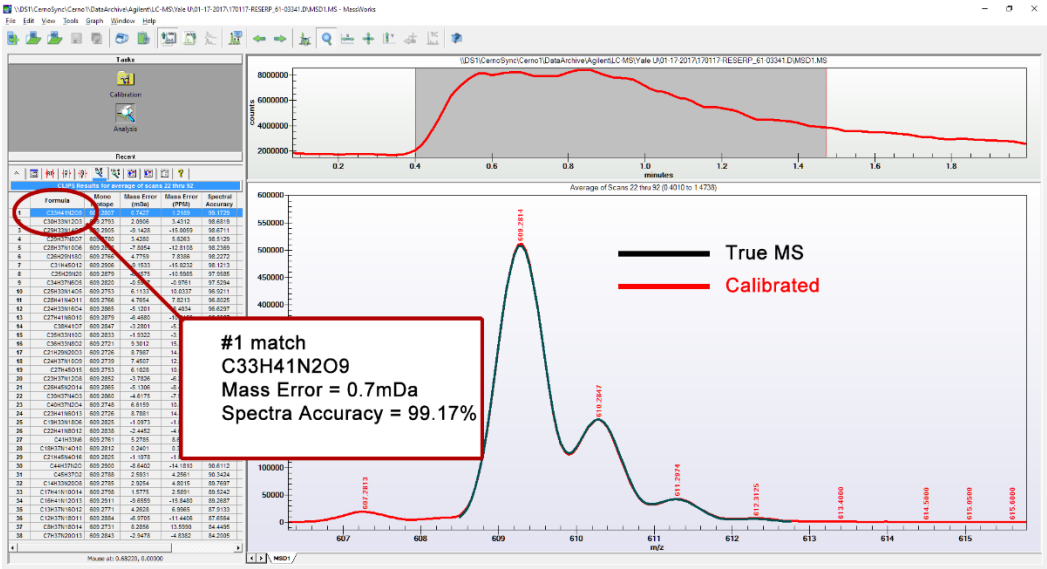

Figure 2. Formula search results for Reserpine showing the #1 match with less than 1mDa mass error and greater than 99% spectral accuracy. The plot shows the spectral overlay of the true mass spec (black) and the calibrated (red) mass spec and how accurate the spectral fit is.

Table 1. Statistical summary of the mass error and Spectral Accuracy from 10 consecutive injections of Reserpine.

Injection	Accurate Mass	Mass Error (mDa)	Mass Error (ppm)	Spectral Accuracy (%)
1	609.2781	-2.6	-4.2	99.06
2	609.2822	1.5	2.5	99.05
3	609.2824	1.7	2.9	99.01
4	609.2840	3.3	5.5	98.71
5	609.2811	0.4	0.7	98.98
6	609.2814	0.7	1.2	99.17
7	609.2802	-0.5	-0.8	98.82
8	609.2800	-0.7	-1.1	99.11
9	609.2792	-1.5	-2.4	98.82
10	609.2805	-0.2	-0.3	99.00
Average	609.2810	0.3	0.4	98.97
Std Dev	0.0017	1.7	2.8	0.15

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Conclusion

The combination of MassWorks with the Agilent LC/MS is shown to provide mass accuracy of better than 5mDa and a Spectral Accuracy of about 99% in repeat measurements of Reserpine. The novel MS calibration and analysis software can transform the workhorse single quadrupole LC/MS into a powerful and cost effective tool for formula ID. This enhanced capability can save both time and money by delivering capabilities usually restricted to more expensive and specialized high resolution instruments to a routine analytical lab.

Acknowledgement

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Reference

[1] "The Concept of Spectral Accuracy for MS", Y.Wang, M.Gu, Anal. Chem. 2010, 82, 7055-7062.
[2] For a detailed guide on setting up the instrument parameters for MassWorks, see the Cerno support notes here.
[3] "The Robustness of Formula Determination on a Single Quadrupole GC/MS", J.Mullis, F.Qiu, Y.Wang, ASMS 2008.

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