Comprehensive Mass Spectral Calibration
to Achieve High Mass Accuracy and Parameter Free Peak Detection

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Introduction

In qualitative MS analysis, there is a growing need for measuring masses at higher and higher mass accuracy (5ppm or even 100ppb) to allow for unambiguous molecular identification in metabolism, biomarker discovery, and proteomics research. In quatitative MS analysis, constant efforts are being made to lower the MS detection limit and increase the quantitative accuracy. Careful examination of MS systems and the overall MS analysis process, however, reveals that the mass accuracy in a given MS system is largely limited by the rudimentary mass calibration schemes used and the detection limit is mostly imposed by the highly heuristic peak detection methods available. This paper will introduce a Highly Accurate Mass Spectral Calibration Approach (HAMSCA) implemented in MSIntegrity™ software to achieve these key objectives in a single-step process (Ref1):

➢ High mass accuracy calibration
➢ Mass Spectral peak shape correction
➢ Noise-filtering without peak distortion
➢ Parameter-free and robust peak picking

Methods

Based on the operating principles of various types of MS systems, a comprehensive instrument calibration is performed with an instrument calibration standard giving multiple ions in the mass spectral range of interest. A continuum mass spectral profile data is collected where each known ion represents a corresponding mass shift and peak shape deviation. A calibration function can then be derived to compensate for both the mass shift and the peak shape deviation and applied to successive MS data to convert raw continuum data into fully calibrated continuum data. The fully calibrated MS data can now be subjected to peak detection through elegant mathematical operations without assuming the usual set of peak-picking parameters.
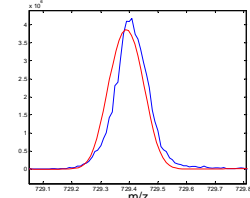
MSIntegrity™ Calibration

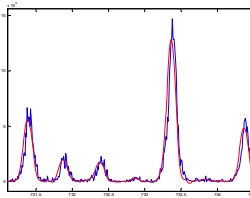

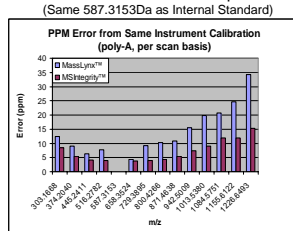## Results and Discussion
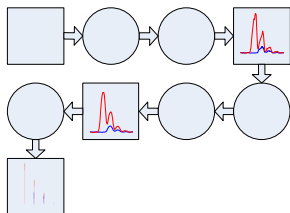
Conventional Calibration on Finnigan TSQ Ultra
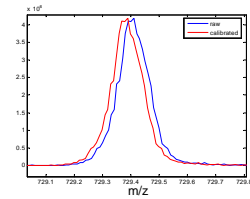
HAMSCA on Finnigan TSQ Ultra

HAMSCA Signal-to-Noise Gain

Mass Error from Waters qTOF II
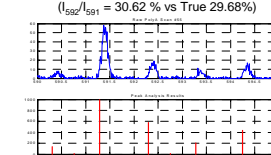(Same 587.3153Da as Internal Standard)

PPM Error from Same Instrument Calibration (poly-A, per scan basis)

Peak Centroiding Results from MassLynx™
(I_{592}/I_{591} = 55% vs True 29.68%)
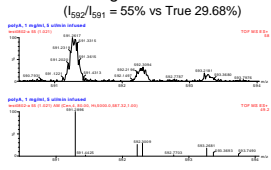
Peak Centroiding Results from MSIntegrity™
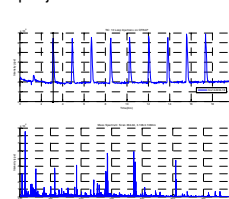(I_{592}/I_{591} = 30.62 % vs True 29.68%)

Single and 20-scan Statistics: MSIntegrity™
(AB/Sciex qStar)

Scan Number	158.9646	430.9142	566.889	702.8638	838.8386	974.8134
340	158.9644	430.9150	566.8880	702.8633	838.8365	974.8140
350	158.9647	430.9148	566.8903	702.8655	838.8365	974.8099
360	158.9649	430.9152	566.8934	702.8626	838.8375	974.8177
370	158.9645	430.9152	566.8892	702.8672	838.8406	974.8143
380	158.9647	430.9139	566.8902	702.8647	838.8384	974.8120
390	158.9648	430.9151	566.8905	702.8673	838.8376	974.8084
400	158.9647	430.9139	566.8920	702.8654	838.8388	974.8167
410	158.9649	430.9140	566.8914	702.8664	838.8410	974.8084
420	158.9648	430.9160	566.8909	702.8670	838.8409	974.8145
430	158.9651	430.9161	566.8918	702.8698	838.8416	974.8156
440	158.9650	430.9153	566.8918	702.8637	838.8387	974.8105
450	158.9647	430.9149	566.8906	702.8666	838.8380	974.8157
460	158.9646	430.9142	566.8911	702.8637	838.8394	974.8150
470	158.9650	430.9160	566.8884	702.8678	838.8397	974.8136
480	158.9646	430.9136	566.8882	702.8611	838.8376	974.8104
490	158.9645	430.9139	566.8908	702.8643	838.8376	974.8084
500	158.9645	430.9147	566.8886	702.8654	838.8364	974.8175
510	158.9643	430.9144	566.8900	702.8680	838.8424	974.8195
520	158.9648	430.9135	566.8887	702.8636	838.8344	974.8209
530	158.9648	430.9148	566.8906	702.8662	838.8387	974.8107
Mean	158.9647	430.9147	566.8899	702.8650	838.8392	974.8131
Single Scan Std Dev (mu)	0.0002	0.0008	0.0018	0.0026	0.0026	0.0032
Single Scan Std Dev (ppm)	1.5	1.8	3.1	3.7	3.1	3.3
20-scan Bias (mu)	0.0001	0.0005	0.0009	0.0012	0.0006	-0.0003
20-scan Bias (ppm)	0.6	1.1	1.6	1.7	0.8	-0.3

Single and 20-scan Statistics: Analyst QS™

Scan Number	158.9646	430.9142	566.889	702.8638	838.8386	974.8134
340	158.9640	430.9157	566.8876	702.8651	838.8327	974.8084
350	158.9642	430.9156	566.8943	702.8626	838.8311	974.8085
360	158.9643	430.9165	566.8943	702.8649	838.8341	974.8212
370	158.9640	430.9161	566.8869	702.8650	838.8348	974.7751
380	158.9640	430.9151				
390	158.9641	430.9155	566.8876	702.8651	838.8327	974.8084
400	158.9639	430.9150	566.8829	702.8682	838.8371	974.8173
410	158.9639	430.9167	566.8871	702.8671	838.8369	974.8042
420	158.9639	430.9172	566.8927	702.8663	838.84	974.8206
430	158.9641	430.9150	566.8914	702.8724	838.8366	974.7819
440	158.9641	430.9153	566.8946	702.8647	838.8365	974.8164
450	158.9641	430.9143	566.8935	702.8696	838.8308	974.8153
460	158.9641	430.9159	566.8901	702.8662	838.8461	974.8254
470	158.9642	430.9157	566.8869	702.8624	838.8373	974.7993
480	158.9638	430.9147	566.8911	702.8662	838.8352	974.9087
490	158.9648	430.9146	566.8901	702.8662	838.8325	974.8075
500	158.9638	430.9146	566.8895	702.8600	838.8373	974.8152
510	158.9633	430.9147	566.8901	702.8731	838.8407	974.8116
520	158.9639	430.9147	566.8902	702.8662	838.8291	974.8050
530	158.9639	430.9148	566.8932	702.8662	838.8339	974.8085
Mean	158.9640	430.9153	566.8907	702.8660	838.8364	974.8076
Single Scan Std Dev (mu)	0.0002	0.0009	0.0034	0.0039	0.0044	0.0135
Single Scan Std Dev (ppm)	1.6	2.0	6.0	5.2	5.3	13.8
20-scan Bias (mu)	-0.0006	0.0011	0.0015	0.0022	-0.0022	-0.0058
20-scan Bias (ppm)	-3.6	2.5	2.7	3.2	-2.6	-5.9

Loop Injection on API 4000 QTRAP

Mass Accuracy on API 4000 QTRAP
(285.1425 and 609.2812Da for Internal Cal)

Calculated Masses for Each Injection and 10-Injection Statistics

| Injection #	285.1425	366.2556	473.3216	477.2309	506.2084	609.2812	716.4385
1	285.1432	366.2495	473.3316	477.2303	506.2004	609.2911	716.4400
2	285.1433	366.2493	473.3170	477.2261	508.1993	609.2911	716.4284
3	285.1432	366.2598	473.3147	477.2261	506.2151	609.2912	716.4400
4	285.1440	366.2559	473.3312	477.2245	506.1969	609.2872	716.4400
5	285.1432	366.2578	473.3210	477.2284	506.2018	609.2974	716.4379
6	285.1430	366.2591	473.3209	477.2461	506.2114	609.2803	716.4406
7	285.1432	366.2368	473.3161	477.2306	506.2042	609.2872	716.4214
8	285.1434	366.2548	473.3225	477.2275	506.2042	609.2812	716.4351
9	285.1436	366.2569	473.3305	508.1982	609.2814	716.4384	
Mean	285.1434	366.2519	473.3174	477.2296	506.2076	609.2873	716.4411
1-Injection STD (mu)	0.0003	0.0059	0.0058	-0.0001	-0.0005	0.0000	-0.0100
10-Injection Bias (mu)	0.0009	-0.0037	-0.0042	-0.0013	-0.0008	0.0000	-0.0174

Mass Accuracy Comparison to Analyst™
(AB/Sciex API 4000 QTrap LC/MS)
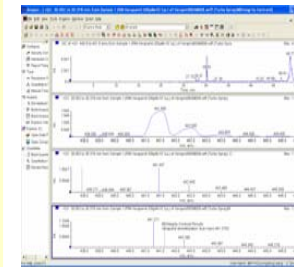
Raw Data Before Alignment
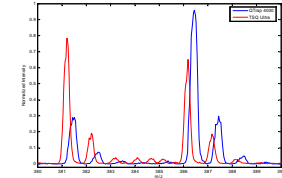
HAMSCA Mass Spectral Alignment
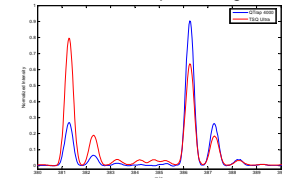
Conclusions

➢ Calibrating for mass alone would not yield the highest possible mass accuracy.

➢ Peak shape correction is as important as mass calibration for mass accuracy.

➢ With the use of HAMSCA in MSIntegrity, the mass error on a unit mass resolution system can be reduced from 0.1-0.2Da to several mDa with a gain factor approaching 100x.

➢ Effective noise filtering of over 3x can be achieved without the usually detrimental peak shape distortion.

➢ Unambiguous and parameter-free peak picking preserves all quantitative information such as isotope ratios while taking advantage of all available ion signals.

➢ HAMSCA also enables quantitative mass spectral alignment, a key requirement in applying multivariate statistics to mass spectral data.

Notes

1. Ming Gu, Yongdong Wang, and Don Kuehl, *Spectroscopy* (*Current Trends in Mass Spectrometry*), 2005, p22-26.