

# Simultaneous Compensation of Peak Shape and m/z for an LC-TOF System to Achieve High Mass Accuracy

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## Overview

- > Novel algorithms to calibrate a TOF mass spectrometer for both mass and peak shape
- > Both external and internal calibration used to compensated for instrument drift
- > Less than 5ppm mass accuracy achieved in an LC-TOF system through MSIntegrity™
- > Tests performed through infusion of drug and peptide mixtures

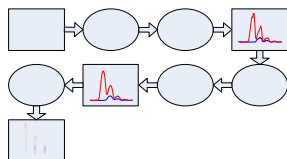
## Introduction

Achieving high mass accuracy of 5ppm or better typically requires a careful mass calibration involving both external and internal standards. What is also required is the elaborate and sometime cumbersome tuning of the mass spectrometer to produce a desirable peak shape with good symmetry and little or no tailing, a practice typically recommended for even high end instruments such as qTOF or high resolution TSQ. In this paper, a very different approach will be taken where the peak shape deviation can be compensated during a comprehensive mass spectral calibration process to achieve high mass accuracy even in the presence of peak asymmetry and significant tailings.

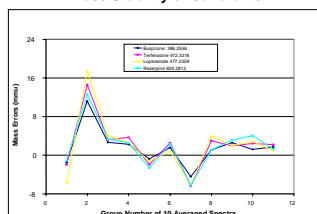
## Methods

- > Infusion of a drug mixture for external calibration
- > A peptide of m/z = 466 used for internal calibration in the peptide mixture
- > Data collected on Paradigm™ MX1 ESI-TOF mass spectrometer with 5,000 mass resolution
- > Data processed by the HAMSCA (Highly Accurate Mass Spectral Calibration Approach) implemented in the MSIntegrity software from Cerno Bioscience

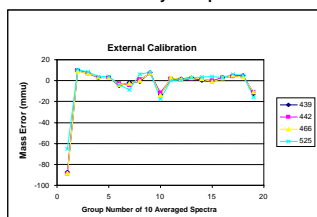
## Calibration Procedures



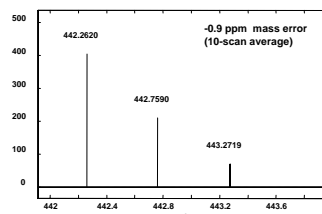
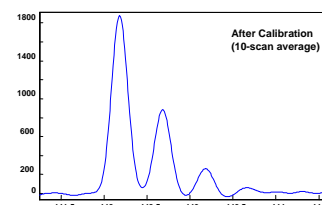
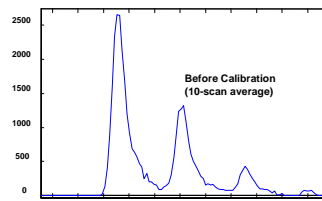
## Mass Stability of Calibrants



## Mass Stability of Peptides

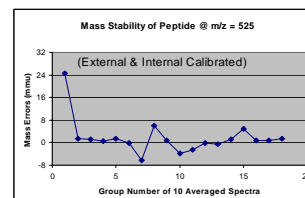
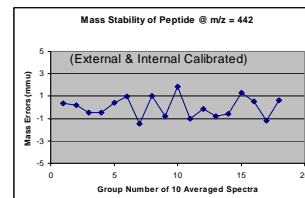
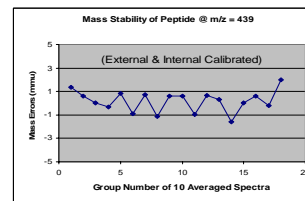


## Peak Shape and Mass Calibration



## Results and Discussion

### Mass Stability w/Internal Calibration



Peptides	Mono isotope m/z
Phe-Gly-Leu-Met-NH <sub>2</sub>	466.259
Arg-Val-Tyr-Val-His-Pro-Ile	442.262
Tyr-Gly-Gly-Phe-Met-Arg-Phe	439.205
Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Arg-Gly-NH <sub>2</sub>	525.735

### Peptide AM Statistics

Group #	True m/z Values			
	439.205	442.262	466.249	525.735
1	439.207	442.262	466.249	525.759
2	439.207	442.262	466.249	525.760
3	439.207	442.262	466.249	525.762
4	439.207	442.261	466.249	525.762
5	439.208	442.262	466.249	525.764
6	439.207	442.263	466.249	525.764
7	439.208	442.261	466.249	525.757
8	439.207	442.262	466.249	525.763
9	439.207	442.261	466.249	525.764
10	439.208	442.263	466.249	525.760
11	439.207	442.262	466.249	525.757
12	439.208	442.262	466.249	525.757
13	439.208	442.261	466.249	525.757
14	439.206	442.261	466.249	525.758
15	439.206	442.262	466.249	525.763
16	439.207	442.262	466.249	525.764
17	439.207	442.261	466.249	525.764
18	439.209	442.262	466.249	525.766
<b>Average</b>	<b>439.207</b>	<b>442.262</b>	<b>466.249</b>	<b>525.761</b>
<b>Std Dev</b>	<b>0.001</b>	<b>0.001</b>	<b>0.000</b>	<b>0.003</b>
<b>Bias</b>	<b>0.002</b>	<b>0.000</b>	<b>0.000</b>	<b>0.027</b>
<b>ppm</b>	<b>4.140</b>	<b>0.441</b>	<b>0.045</b>	<b>50.485</b>

- Notes:
- 10 spectra averaged in each group
  - m/z 466 used as internal standard
  - m/z 525 outside calibration range

## Conclusions

- > Less than 5 ppm mass accuracy can be achieved through MSIntegrity with asymmetrical peak shapes typical of TOF MS.
- > Internal calibration is required to compensate mass drift due to temperature changes and hardware instabilities.
- > Further studies will focus on accurate mass measurements on an LC/MS time scale.