

# Accurate Mass Measurements on Unit Mass Resolution Mass Spectrometers

Ming Gu and Yongdong Wang, Cerno Bioscience, 5 Science Park, Suite 13, New Haven 06511  
Xian-guo Zhao and Zhe-ming Gu, Xenobiotic Laboratories, 107 Morgan Lane, Plainsboro, NJ 08536



## Overview

>Novel algorithms have been developed for mass spectrometry calibration and ultra-fine tuning of peak shapes.

>5ppm mass accuracy has been demonstrated to be achievable on unit mass resolution instruments from Thermo Electron Quantum Discovery MAX, AB/Sciex 4000 Q TRAP, and Waters Quattro Ultima through the use of MSIntegrity™.

>5ppm mass accuracy has been confirmed in different mass spectrometer operational modes including infusion, loop injection, and LC/MS.

## Introduction

With the recent advent of high resolution mass spectrometers such as FTMS, qTOF, and TOF/TOF, accurate mass (AM) measurements are becoming the method of choice for a variety of applications including metabolite identification, peptide sequencing through database search, and confirmation of pharmaceuticals, impurities, and degradation products (Refs 1&2). However, the prohibitive cost and lack of versatile scanning functions of these instruments warrant the development of new accurate mass (AM) measurement technologies applicable to less expensive unit mass resolution mass spectrometers. Through extensive investigation on ion trap, linear ion trap, and triple stage quadrupole instruments, a novel scheme has been developed and demonstrated to perform AM measurements on unit mass resolution mass spectrometers with 5ppm or better mass accuracy.

## Methods

>Data acquired in profile mode and processed by the HAMSCA (Highly Accurate Mass Spectral Calibration Approach) implemented in the MSIntegrity™ software

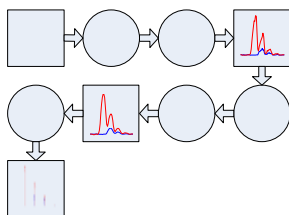
>Infused standards of sodium trifluoroacetate for external calibration combined with internal calibration for highest possible mass accuracy

## Methods (Cont'd)

>Demonstration data including a mixture of terfenadine and loperamide infused on Thermo Electron Discovery MAX, a mixture of diazepam and sulfamethoxazole via loop injection on Waters Quattro Ultima, and LC/MS of rat microsomal (RM) incubation of verapamil acquired on 4000 Q TRAP.

>C18 column with gradient for separation of the metabolites of RM incubation of verapamil

## Calibration Procedures



## Highlights of Results

>5-10 ppm mass accuracy obtained on the drug mixture containing buspirone, terfenadine, an loperamide acquired on API 4000 QTRAP, using external calibration of sodium trifluoroacetate

>5 ppm mass accuracy achieved even with the distorted peak shapes for the drug mixture of terfenadine and loperamide acquired on Quantum Discovery MAX

>5 ppm mass accuracy also achieved on the LC/MS time scale illustrated by the demethylated metabolite of verapamil rat microsomal incubation, acquired on 4000 Q TRAP

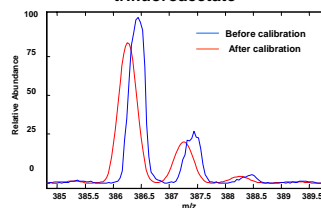
## Results and Discussion

### Fundamental limits

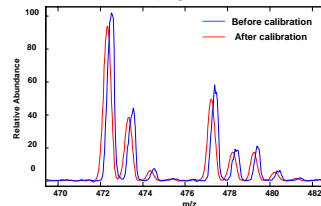
$$\sigma_{ppm} = \frac{10^6}{CRS^{1/2}}$$

R – Resolving power (mass/fwhm)  
S – Signal  
C – Signal unit conversion, algorithms etc

### Buspirone loop injected on 4000 Q TRAP, external calibration with sodium trifluoroacetate

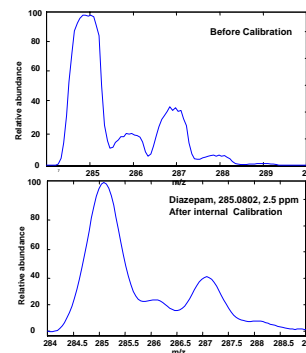


### Terfenadine & Loperamide loop injected on 4000 Q TRAP, external calibration with STFA

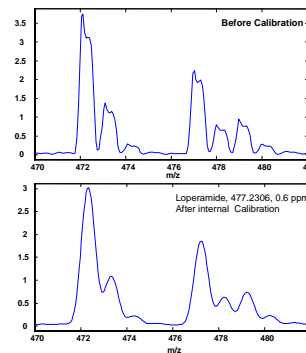


Compounds	True	Measured
Buspirone	386.2556	386.2589
Terfenadine	472.3216	472.3241
Loferamide	477.2309	477.2350

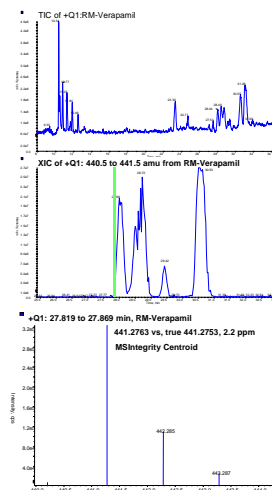
### Diazepam loop injected on Quattro Ultima, internal calibration with Sulfamethoxazole



### Terfenadine & Loperamide infused on Discovery MAX, internal calibration with Terfenadine



### AM of demethylated verapamil on 4000 Q TRAP, external calibration with verapamil



## Conclusions

- > Accurate mass measurements with 5 ppm or better can be achieved on unit mass resolution instruments through MSIntegrity.
- > Mass spectrometry calibration including both mass and peak shape is critical for high mass accuracy.
- > Internal calibration can achieve the better mass accuracy.

### References

- 1) Tyler, A. et al *Anal. Chem.* **1996**, 68, 3561.
- 2) Blom, K. F. *Anal. Chem.* **2001**, 73, 715.

### Acknowledgement

The authors wish to thank Jianyao Wang of Weyth Pharmaceuticals for providing loop injection data.