

# **Factors Affecting Mass Measurement Accuracy in a TOF Mass Spectrometer**

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# INTRODUCTION

Mass measurement accuracy and precision are important for determining elemental composition for an unknown compound. Procedures have been proposed to improve the accuracy the measurement.<sup>1-3</sup> This study investigates a few of the factors that affect accuracy in a Micromass LCT time-of-flight (TOF) mass spectrometer. A careful look is taken at (i) the errors caused by the dead-time characteristics of the detector, (ii) the relative intensity ratios of the analyte and lockmass ions, and (iii) biases observed when using the lockspray feature.

<sup>1</sup>Blom, K.F., *Anal. Chem.*, **2001**, 73, 715.

<sup>2</sup>Liang Z. and Bansal, S., *Proceedings of the 48<sup>th</sup> ASMS Conference*, Long Beach, CA, 2000.

<sup>3</sup>Miller S.A. and Bolgar, M.S., *Proceedings of the 49<sup>th</sup> ASMS Conference*, Chicago, IL, 2001.

## EXPERIMENTAL

The experiments were performed on a Micromass LCT time-of-flight mass spectrometer (3.6 GHz) using MassLynx v3.5 software (update #367). Mixtures of Leucine Enkephalin and Reserpine were introduced into the analyte and lockspray electrospray units using two separate Harvard infusion pumps. By introducing mixtures, it is possible to compare using a lockmass from either the lockspray reference channel (employing the Mass Measure feature) or the analyte channel (employing the Peak Center feature). The spectra were acquired in continuum and 10 scans were averaged together at a time. Experiments were designed to study the effects of ion abundance, lockmass, and Np-value (utilized in the dead-time correction algorithm).

# Mathematical Model

A spreadsheet was generated to model the dead-time effects. The mathematics used in the model are similar to those used in Micromass' dead-time correction algorithm.<sup>4-6</sup> The variables include m/z ratio, number of ions per push, Np-value, dead-time duration, and resolution. The resulting charts help the visualization of the effects.

$$GT = \sum_{r=1}^{\infty} rP(r; \lambda).G(x; \sigma)[1 - P^-(x; \sigma, Dt)]^{r-1}$$

<sup>4</sup>Hoyes, J., "Accurate Mass Considerations on an Electrospray Quadrupole-TOF Instrument", 10<sup>th</sup> Sanibel Conf., 1998

<sup>5</sup>Esposito, F., Spinelli, N. Velotta, R., Berardi, V., *Rev. Sci. Instrum.* **1991**, 62(11), 2822.

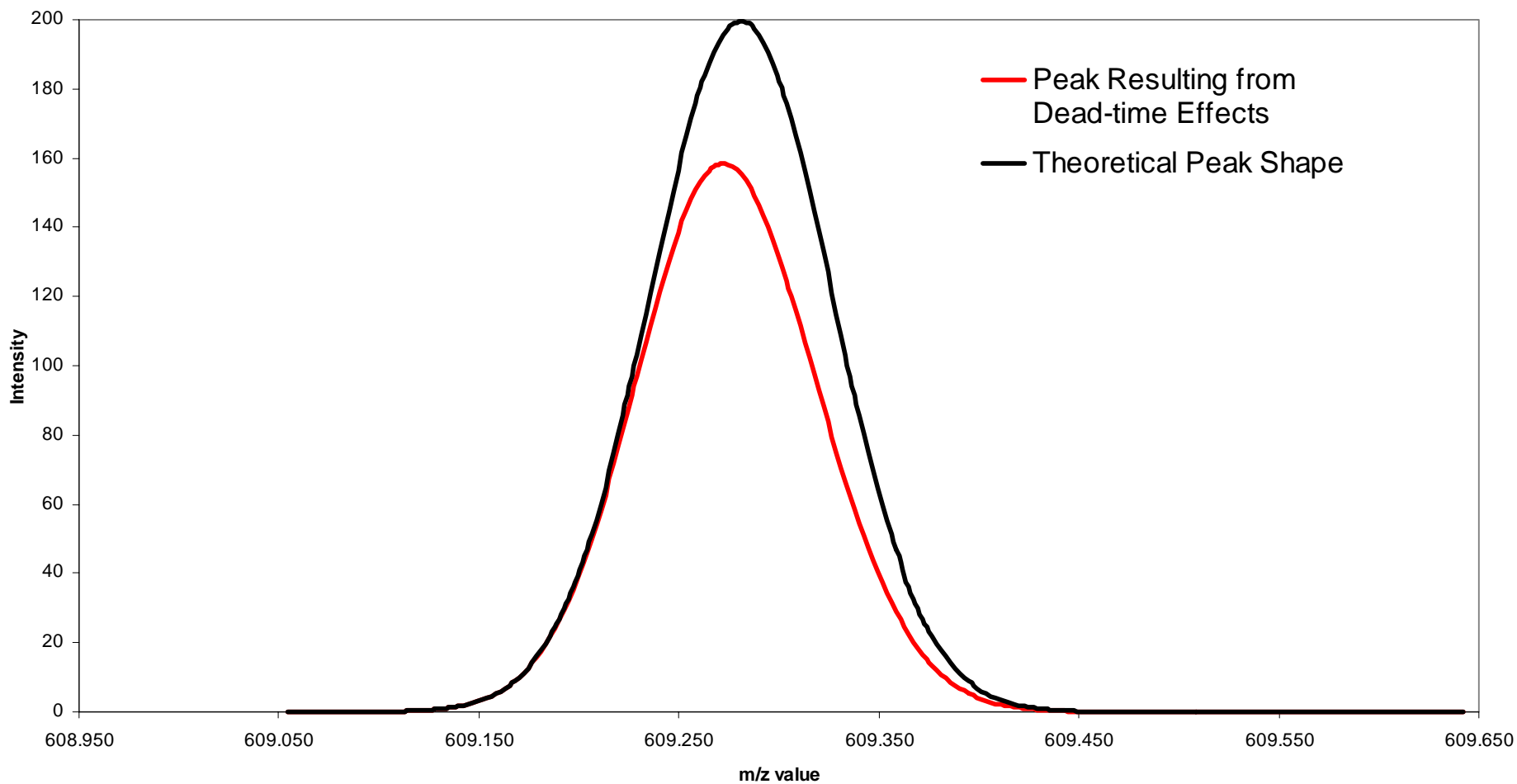
<sup>6</sup>Patent, International Publication Number WO 99/38192.

# RESULTS

## **Dead-time Effects**

Due to the inherent nature of standard ion-counting detectors, a finite amount of post-detection processing time is required before the device is able to detect subsequent ions. Ions arriving during this “dead time” are not detected. It has been well documented that these dead-time effects influence the perceived mass assignment and ion abundance.<sup>4-6</sup> A mathematical model is used to demonstrate this effect in Figure 1.

# Simulated Dead-time Effects



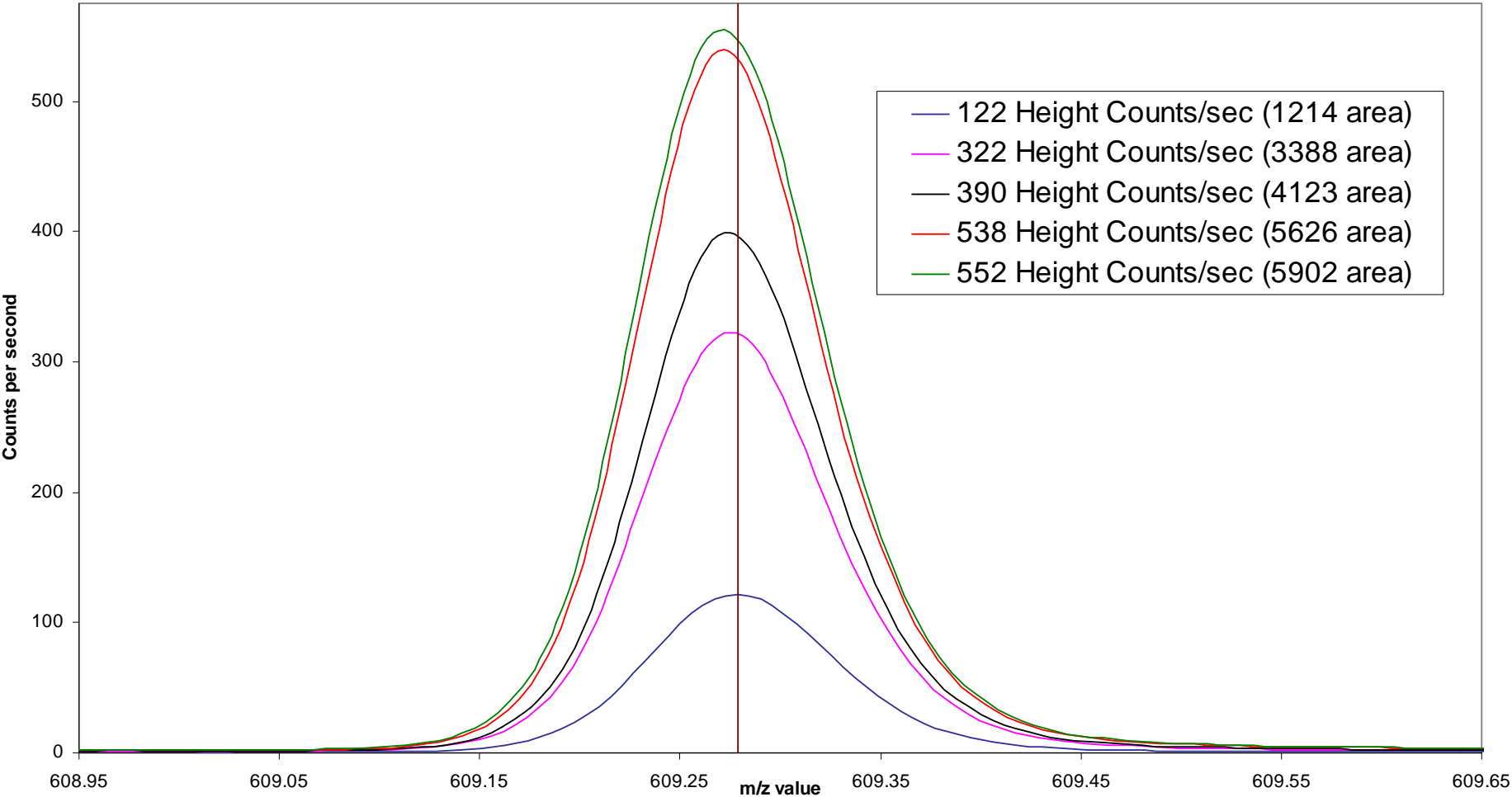
## Dead-time Effects

To study these effects, samples of varying concentrations of Reserpine were sequentially introduced directly into the analyte electrospray. Figure 2 shows a mass shift (to lower mass) as the ion abundance increases. Although it can not be discerned from this figure, more and more intensity is also lost as the ion abundance grows.

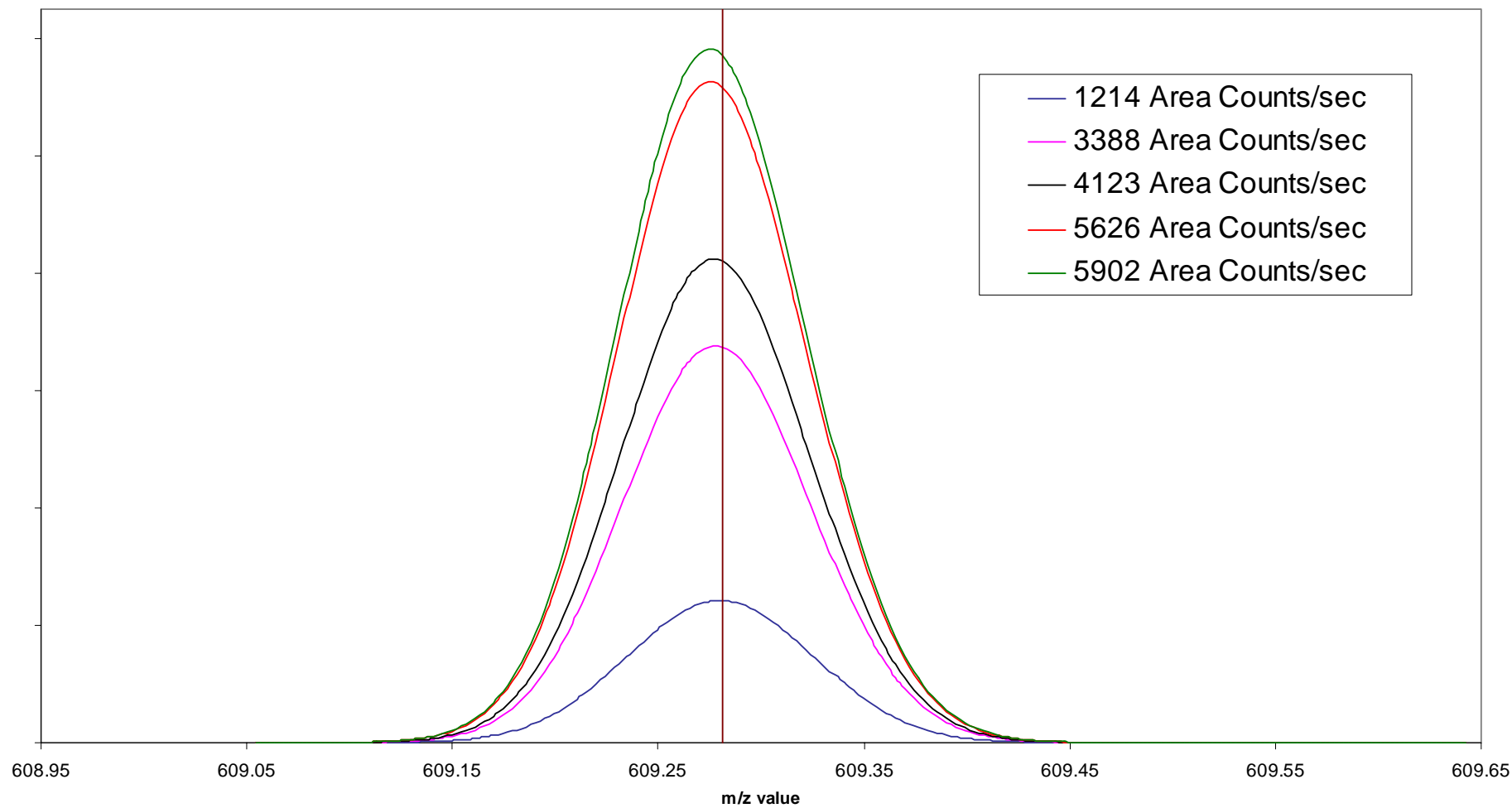
This same experiment was mathematically simulated as shown in Figure 3. Interestingly, the peak shapes remain roughly Gaussian. This is due to the fact that the 5 ns dead time is a significant fraction of the resolved peak. At 609 Da (5700 resolution), 5 ns equates to 0.2 Da. At higher  $m/z$  values, such as  $m/z$  5000, the dead-time effects give more asymmetric peak shapes.

# Reserpine at Increasing Concentrations

(Data from LCT)



# Mathematical Model – Simulated Data



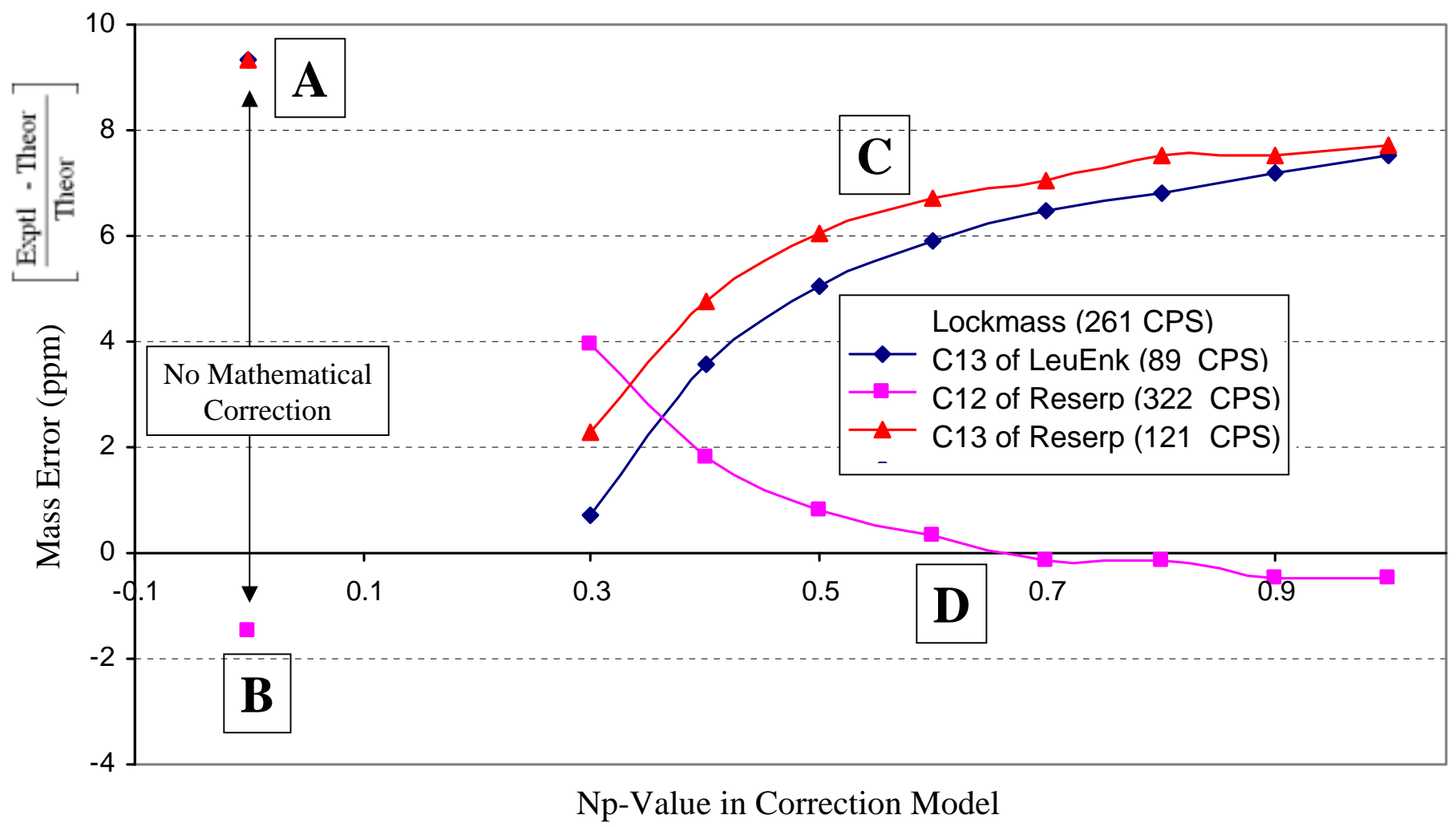
## Effectiveness of Dead-time Correction

With these abundance-dependent mass shifts in mind, several experiments were designed to explore how this might affect calibrations. A mixture of Leucine Enkephalin (lockmass) and Reserpine were introduced into the analyte electrospray.

The mass error is a function of (i) the abundance of the analyte ions, (ii) the abundance of the lockmass ions, and (iii) the  $N_p$ -value of the dead-time correction algorithm. Figure 4 shows the average mass error as a function of the  $N_p$  correction factor for LeuEnk C13, Reserpine C12 and Reserpine C13 ions (using LeuEnk C12 as the lockmass). The HEIGHT counts per second are listed in the legend.

Similar results are shown in Figure 5. In this case, the intensity of the lockmass is greater than the intensities of the analytes.

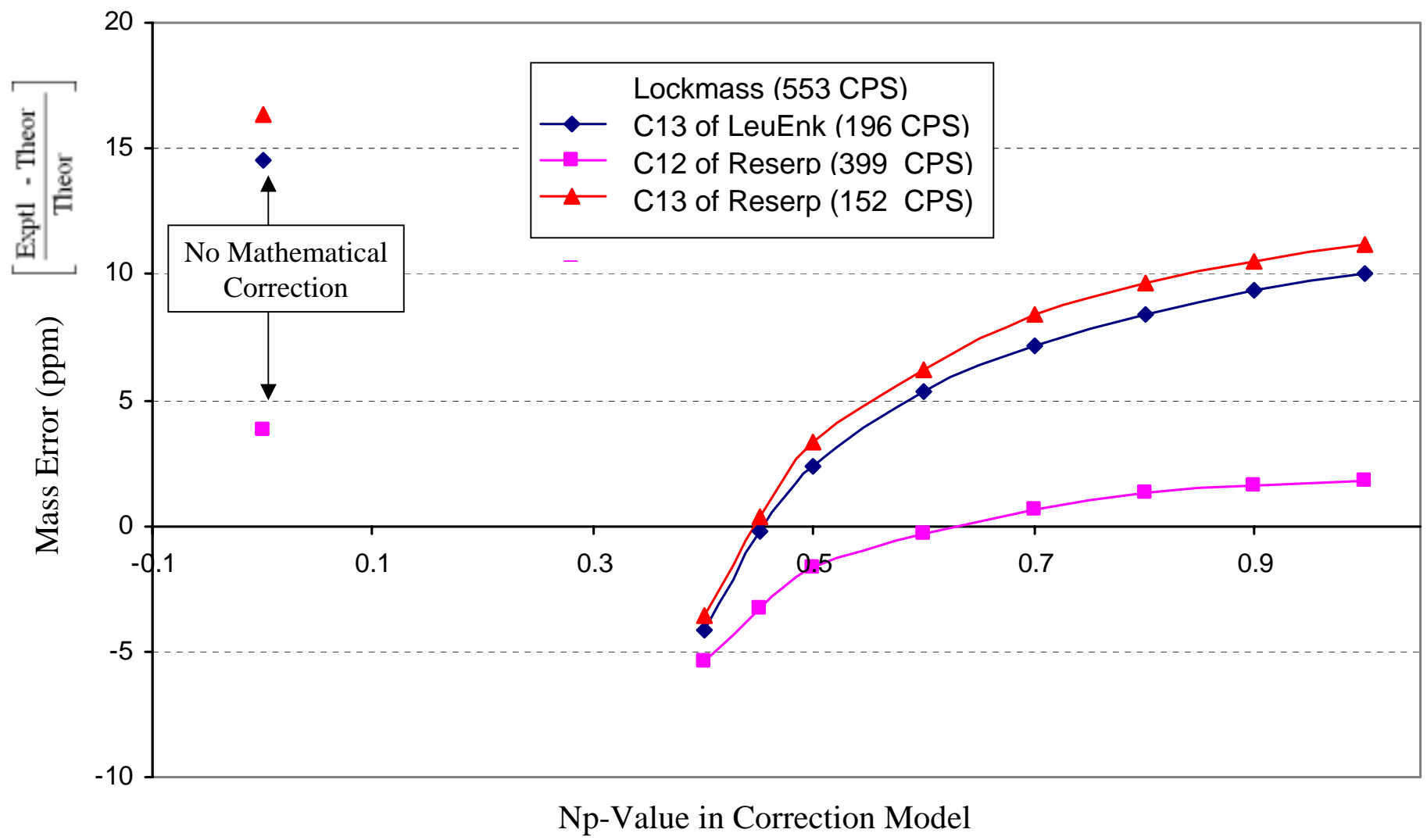
# Mass Accuracy vs. Np-Value for ions of various abundances



## Analysis of Figure 4

With  $N_p=0$ , no dead-time correction is made. Figure 4 shows that the assignments of the C13 isotopes of LeuEnk and Reserpine are about 9 ppm too high. (A) These positive errors can be explained by the differences in peak heights between the lockmass and the analytes. Since the lockmass sets the calibration, its negative mass shift results in a calibration that is over-adjusted for less intense peaks. Conversely, if an analyte is more abundant than the lockmass, as with the C12 of Reserpine, then a negative error occurs (B). The  $N_p$ -value is a sensitivity factor for the dead-time correction algorithm. By lowering the  $N_p$ -value, the magnitude of the correction increases. If the analyte and lockmass have similar intensities, then changes to the  $N_p$ -value have little effect. If they have dissimilar intensities, the most abundant peak will be “corrected” more than the less abundant peak (C & D). The intensity ratios dictate the sign and slope of those curve.

# Mass Accuracy vs. Np-Value for ions of various abundances

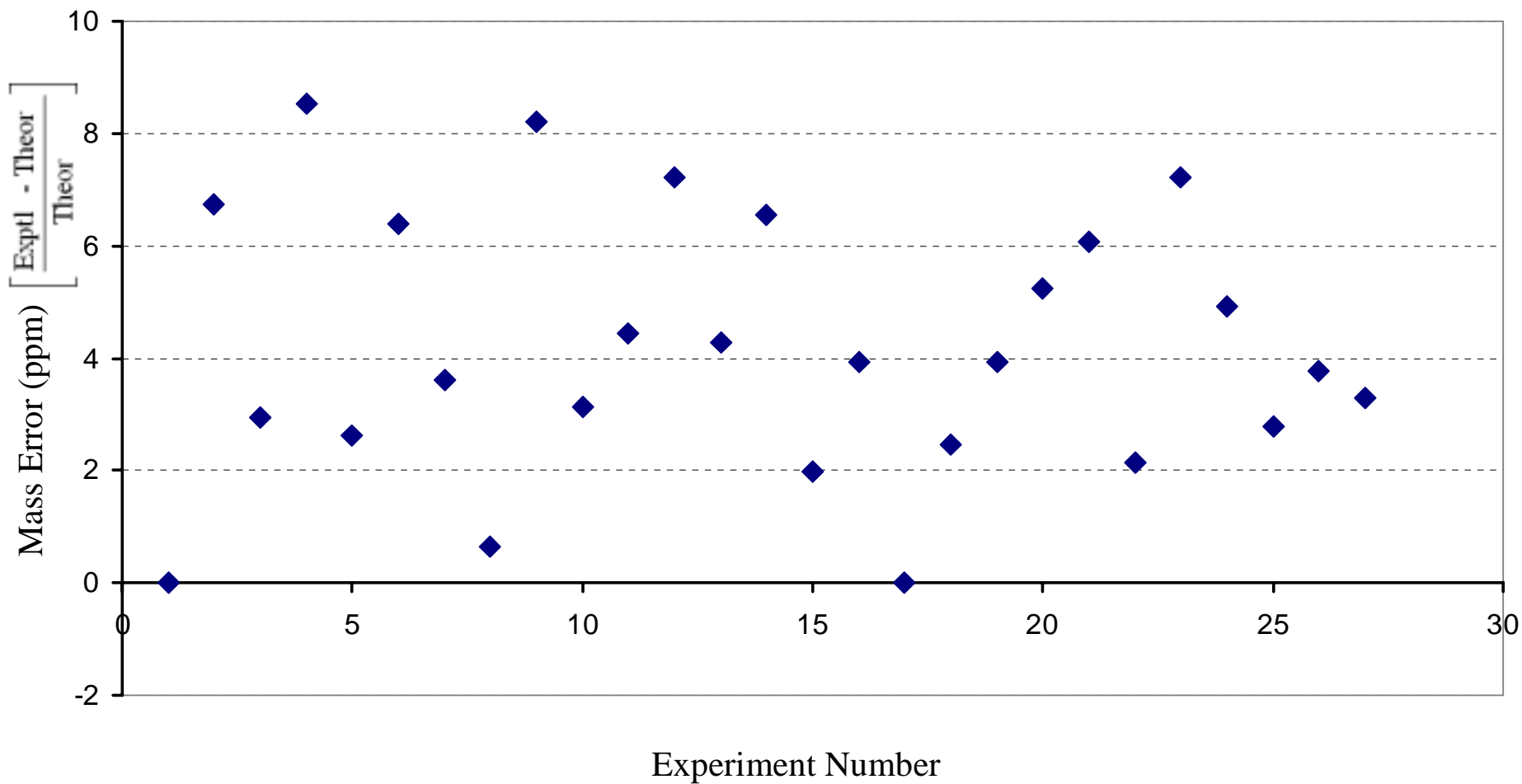


## Bias in Accuracy when using Lockspray

*When using Micromass' Lockspray feature, the precision is often good yet there is a bias in the mass measurement accuracy. Figure 6 clearly demonstrates the issue. This data was collected by introducing Reserpine into both the analyte and lockspray electrospray units using separate infusion pumps.*

*After looking at many possible factors, it was determined that the bias is actually created by a bug in MassLynx v3.5. The bias is introduced because the dead-time correction is applied to the data from the analyte electrospray, but it is not applied to the data from the lockspray. Since the correction is made to the analyte data but not the lockspray data, a bias is introduced.*

# Lockspray Bias with MassLynx v3.5



# Conclusions

- Dead-time issues affect both the intensity and mass assignment.
- Remember that these issues affect both the analyte and the lockmass.
- Don't assume that the correction algorithm is perfect.
- Under typical conditions, the “distorted” peak shape is still roughly Gaussian due to the significant dead time of 5 ns.
- Coupled with Lockspray, the algorithm is not applied correctly in MassLynx v3.5, creating a bias.

# Recommendations

- Limit peak intensities so as to minimize dead-time effects.
- Use similar peak intensities for lockmass and analyte (dead-time effects cancel each other out).
- Use a lockmass that is similar in mass to the analyte (compensates for dead-time effects in calibration file).
- Will investigate using a mixture of components in the lockspray to provide options for various intensities.