

Accurate Determination of Relative Isotopic Abundances Using Folded Flight Path™ Time-of-Flight Mass Spectrometry

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1. Introduction

The ability to accurately measure the contributions provided by isotopes to a mass spectrum is of significant importance. This has been supported often in literature.⁽¹⁻²⁾ Accurate determination of the relative abundance of isotopes provides the ability to more definitively relate a formula to a measured m/z. The ability to define the isotopes is a function of the resolving power of a mass spectrometer and stable intensity measurement. Once adequate resolving power has been achieved, a fingerprint of the isotopes is available. The ability to provide a more quantitative measure and the accuracy of this measurement enables the proposed formula to be robustly monitored and prioritized.

Comparison of the measured and theoretical values for known compounds provides a good evaluation. As a simple test of this capability, Agilent Tune Mix (ATM) was infused into a Citius™ LC-HRT High Resolution Time-of-Flight Mass Spectrometer. The resultant ions were analyzed and the relative isotope abundance compared to that obtained from theoretical calculations for C₁₂H₁₈N₃O₆F₁₂P₃ (m/z 622.02896 for M+H).

2. Experimental

As part of a routine analysis ATM was infused at approximately 10 µL/min into a flow of acetonitrile/water (approximately 50% each) containing 0.1% formic acid at 250 µL/min. Positive ion spectra were acquired using the Citius™ in high resolution mode (R(FWHM) = 50,000) at an acquisition speed of 3 spectra/second in positive ion mode. In addition to normal, precursor ion spectra, fragment ion spectra were also generated at ca. 10 eV collision energy using MSc² (Mass Spectrometry with Comprehensive CID). The energy provided little fragmentation to the calibrant. To achieve the spectra in question a 20 second region of the infusion was summed. The resultant spectrum is shown in Figure 1.

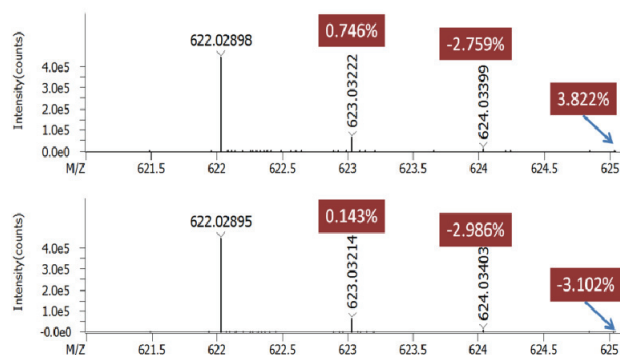


Figure 1. High resolution spectrum from positive ion analysis of Agilent tune mixture on the Citius LC-HRT. The observed resolution at 622 was > 48,000 (FWHM). The relative error in the observed isotopic abundance for each of the detected isotopes is shown in the red boxes.

The relative response of the first three isotopes detected in the spectrum for C₁₂H₁₈N₃O₆F₁₂P₃ (m/z 622.02896 for M+H) was calculated using the mass calculator in the LECO ChromaTOF HRT™ software. Theoretical values were calculated at 50,000 resolution and were normalized to the monoisotopic ion. The observed and theoretical normalized intensities from the precursor and MSc² channels are provided in Tables 1 and 2, respectively. Theoretical values were calculated at 50,000 resolution and normalized to the monoisotopic ion. Comparison of the theoretical and observed values allow for the determination of the relative errors. In both the precursor and product ion channels all relative errors were less than 4% (relative) for even the low level M+3 isotope.

Table 1. Relative Errors in Measured Isotope Abundance for in Precursor Ion (C₁₂H₁₈N₃O₆F₁₂P₃).

Observed m/z	Observed Normalized Intensity	Theoretical Normalized Intensity	% Error (Isotope Abundance)
622.02898	100	100	—
623.03222	14.4255989	14.5340212	0.746
624.03403	2.27804072	2.21687959	-2.759
625.03659	0.21153845	0.21994478	3.822

Table 2. Relative Errors in Measured Isotope Abundance for in Product Ion (C₁₂H₁₈N₃O₆F₁₂P₃) in MSc².

Observed m/z	Observed Normalized Intensity	Theoretical Normalized Intensity	% Error (Isotope Abundance)
622.02898	100	100	—
623.03222	14.4255989	14.5340212	0.143
624.03403	2.27804072	2.21687959	-2.986
625.03659	0.21153845	0.21994478	-3.102

3. Conclusion

In this simple example, the inherent capability of the Citius LC-HRT to accurately measure the relative abundance of isotopes with a low relative error is demonstrated. Errors in relative isotope abundance were less than 4% in both the precursor and product ion channels providing a powerful tool for formula refinement of unknown ions.

4. References

¹A.W.T. Bristow, *Mass Spectrom. Rev.* (2006), 25, 99-111.

²T. Kind and O. Fiehn, *BMC Bioinformatics* (2007), 8, 105- 124.



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