Isotope effects in stability of MS analyte/internal standard ratio measurements

Geoffrey S. Rule[1]; Stephen Jones[2], Silviana Ruci[2], Alan L. Rockwood[1, 3]

[1]ARUP Laboratories, Salt Lake City, UT;

[2]IsoSciences LLC, King of Prussia, PA

[3] Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT

OVERVIEW

- **For certain assays we are interested in reducing requirements for assay calibration. One objection is that instrument analyte to IS ratio may drift over time.**
- **Here we consider instrument (triple quad) contributions to variance in quantitation when using isotopically labeled IS.**
- **Comparisons between ^{13}C and deuterium labeling are made**

INTRODUCTION

Scalability in the high-throughput clinical MS laboratory can be achieved in several ways, one of which is to reduce the number of calibration standards analyzed each day. Such reduction would provide substantial cost savings. Recently, we have shown improvements in data quality (better precision) achieved by use of a weighted historical, single point calibration update strategy [1, 2]. These efforts confirmed what others have demonstrated both in theory [3] and practice [4].

Use of single point calibration strategies appear to be accepted in clinical industry guidelines and checklists [5, 6] although one often hears objections to the concept. It is generally accepted that when an instrument and reagents are demonstrated as stable it is permissible to use less frequent calibration [7] and this approach has been widely adopted in commercial, high throughput, spectrometric clinical assays.

To consider the stability of modern mass spectrometric assays we divided potential sources of assay variation into individual contributions when using isotopically labeled internal standards [8]. Here we focus on a limited number of contributions from triple quadrupole instrumentation with electrospray ionization (panel, far right).

Related Studies

Mirza et al. [9] showed a measureable difference in gas phase proton affinity between a compound and its deuterated analog.

Davies et al. [10] described the effect of temperature, mobile phase, flow rate, and concentration on loss of ring deuterium atoms from substituted indoles during APCI. Zherebker and colleagues [11] showed similar temperature related losses during electrospray of compounds having keto-enol tautomerism.

Allen et al. [12] performed studies with ^{13}C isotope labeling of plant tissues and evaluated fragmentation efficiency of peptides across precursor isotopologues. They found that the lower mass precursors, within an isotopic distribution, were preferentially fragmented during CID and concluded this was a mass dependent effect as opposed to a kinetic isotope effect.

Berg and colleagues [13] observed that it is easier to fragment unlabeled analyte than it is a series of deuterated amphetamine isotopologues. Their work shows a correlation between collision energy required and the extent of labelling. In studies with GC-MS/MS, Tsikas et al. [14] showed similar effects with derivatized ibuprofen and a deuterated isotopolog. They note, specifically, the dependence of peak area ratio on collision energy.

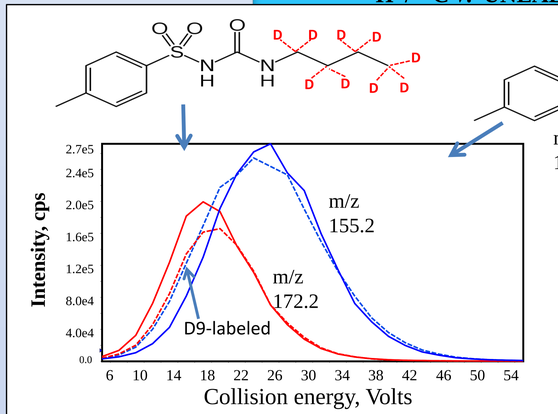
1. Rule, G.S. and A.L. Rockwood, Clin Chem, 2015. **61**(2): p. 431-3.
2. Rule, G.S. and A.L. Rockwood,. Anal Chim Acta, 2016. **919**: p. 55-61.
3. Renman, L. and D. Jagner, Analytica Chimica Acta, 1997. **357**(1–2): p. 157-166.
4. Tan, A., et al., J Chromatogr B Analyt Technol Biomed Life Sci, 2012. **911**: p. 192-202.
5. *Chemistry and Toxicology Checklist*, College of American Pathologists, Northfield, IL. 2014.
6. *Liquid Chromatography-Mass Spectrometry Methods; Approved Guideline*. 2014, Clinical and Laboratory Standards Institute: Wayne, PA.
7. Carreiro-Lewandowski, E., *Basic Principles and Practice of Clinical Chemistry*, in *Clinical Chemistry Techniques, Principles, Correlations*, M.L. Bishop, Fody, E.P., and Schoeff, L. E., Editors. 2010, Lippincott Williams & Wilkins: Baltimore, MD.
8. Rule, G.S., Rockwood, A.L (poster) *Mass Spectrometry: Applications to the Clinical Lab*. 2016. Palm Springs, CA.
9. Mirza, S.P. et al. International Journal of Mass Spectrometry, 2003. **230**(2-3): p. 175-183.
10. Davies, N.W. et al. Rapid Commun Mass Spectrom, 2010. **24**(7): p. 1105-10.
11. Zherebker, A. et al., Analyst, 2016.
12. Allen, D.K., B.S. Evans, and I.G. Libourel,. PLoS One, 2014. **9**(3): p. e91537.
13. Berg, T., et al. J Chromatogr A, 2014. **1344**: p. 83-90.
14. Tsikas, D., et al. J Chromatogr B Analyt Technol Biomed Life Sci, 2016 (e-pub, online).

METHODS

All studies performed on AB Sciex API 5500 triple quadrupole with positive ion mode electrospray infusion at 10 uL/min. Solutions prepared at approximately 200 pg/uL in 1:1, acetonitrile:water, containing 0.1% formic acid, to contain approximately equivalent concentrations of both labeled and unlabeled compound. After initial measurement, concentration was adjusted so that signal intensity of most intense transitions were nearly equivalent. Efforts were made to insure signal intensities were within linear range of detector. Solutions of tolbutamide and 5-hydroxyindole acetic acid, along with corresponding isotopologues, were infused while performing collision energy ramps, or while manually adjusting declustering potential (DP), or CAD gas setting. Data was processed directly within Analyst (1.6.1) or taken into PSI-Plot, (Pearl River, NY) for further processing.

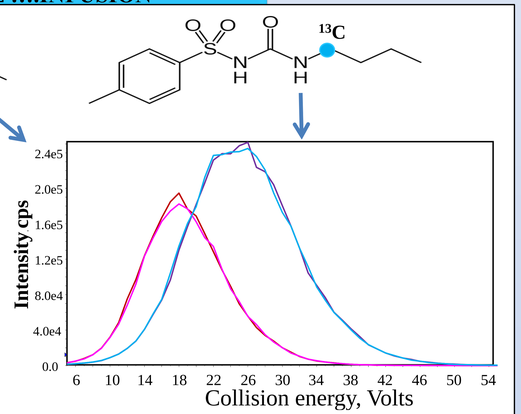
Fractionation during the ionization process and detector effects are not considered here. ^{13}C compounds were from IsoSciences, LLC. Deuterium and unlabeled isotopologues were from multiple suppliers.

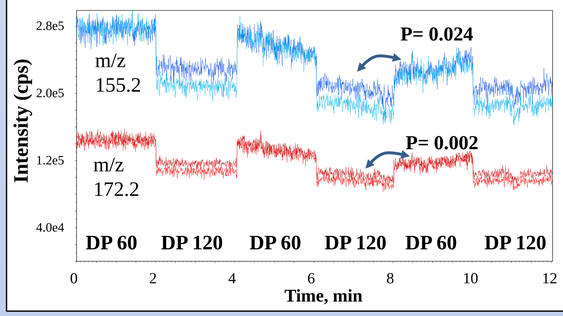
^{2}H / ^{13}C *v.* UNLABELED TOLBUTAMIDE ….INFUSION



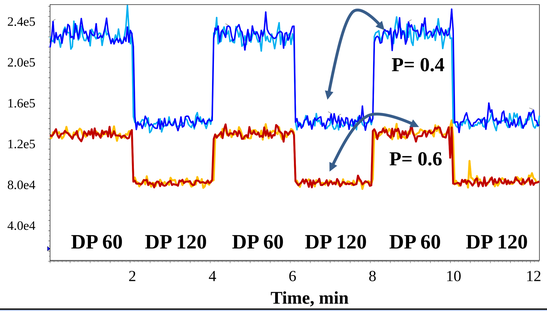
$$E_{com} = \frac{m_G * E_{kin}}{m_i + m_G}$$

Energy for fragmentation related to mass

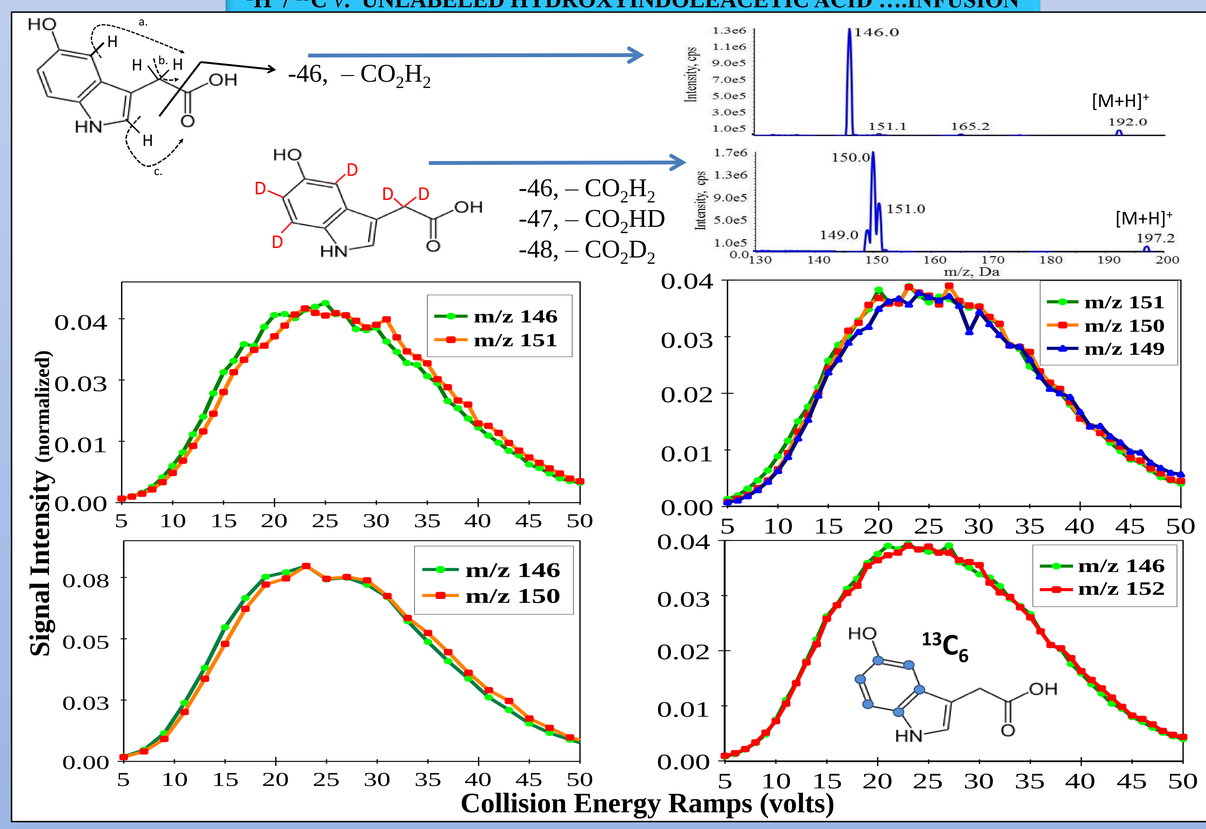




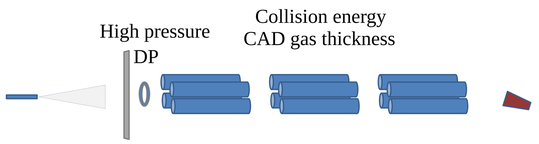
Influence of declustering potential on analyte/ IS ratio



^{2}H / ^{13}C *v.* UNLABELED HYDROXYINDOLEACETIC ACID ….INFUSION



SOURCES OF ERROR



MS sources of error in peak area ratio (PAR) determination might be attributed to the following:

e. Ionization

-fractionation effects? eg, gas phase proton affinity, solvent effects

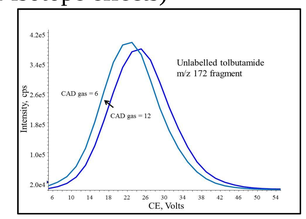
-temperature related effects; eg, scrambling or exchange

f. Mass spec parameters

-declustering potential – "Up-front" CID fragmentation (mass or isotope effects)

-collision energy

-CAD gas thickness



g. Detector performance (linearity)

CONCLUSIONS

- **Aside from well known chromatographic effects of deuterium labeling, it is commonly assumed that isotopically labeled internal standards behave identically to their unlabeled isotopologues.**
- **Our data shows the triple quadrupole may not yield perfectly constant analyte/IS ratios, particularly when deuterium labeling is used.**
- **For utmost assay stability, there may be additional reason to choose alternatives to deuterium when selecting ISs.**
- **Question that remains: To what extent do MS parameters investigated actually drift over time and under varying conditions?**

ACKNOWLEDGEMENTS

Thanks are given to the ARUP Institute for Clinical and Experimental Pathology for making this work possible.