Leveraging Successful Troubleshooting Experiences for the Prevention or Reduction of Internal Standard Response Variations during LC-MS Bioanalysis

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You might have experienced different IS response variations just as I did in the past!

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Internal standard response variations during incurred sample analysis by LC–MS/MS: Case by case trouble-shooting

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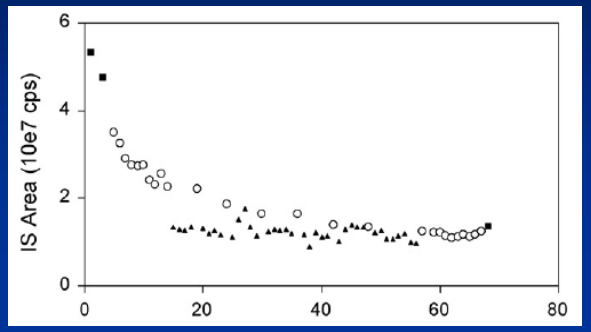
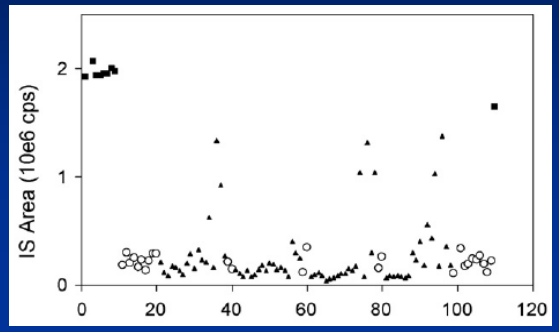
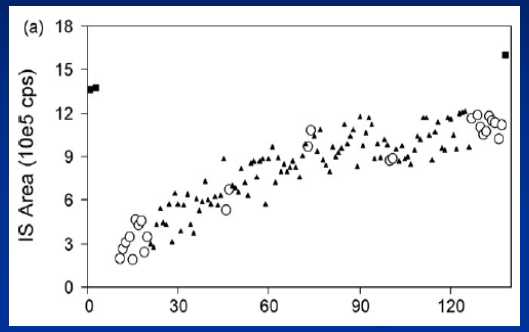
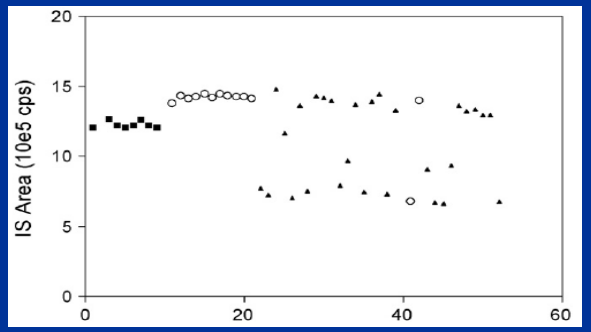
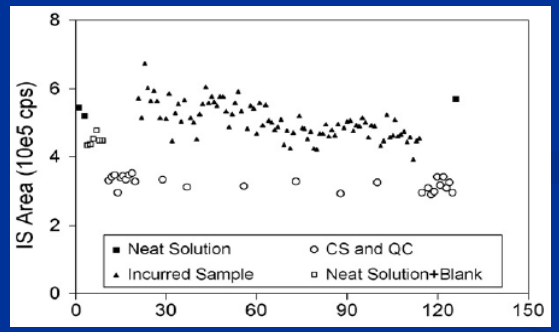
Look familiar?

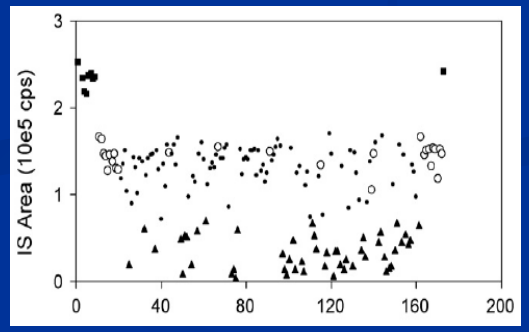


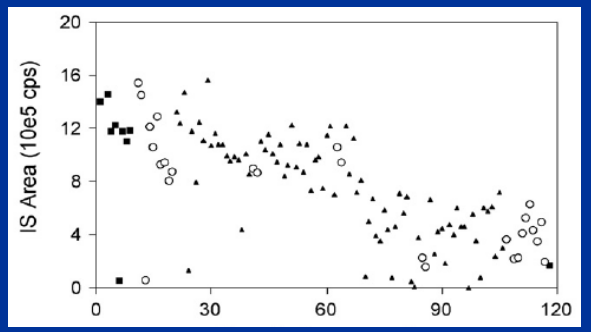


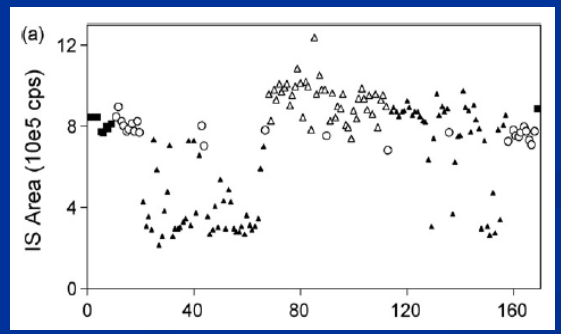


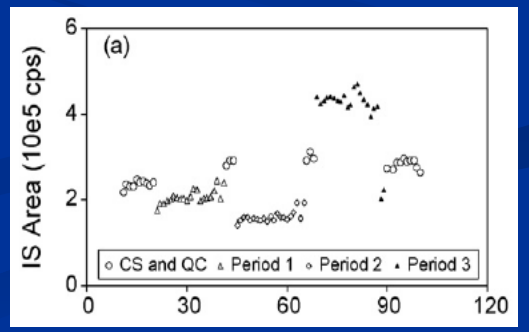












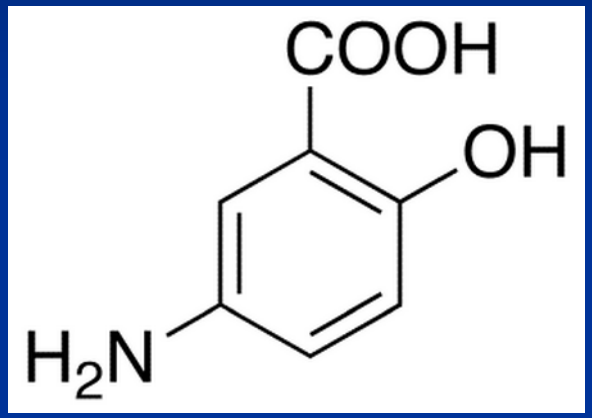
Can we reduce or prevent IS response variations?

Yes, we can!

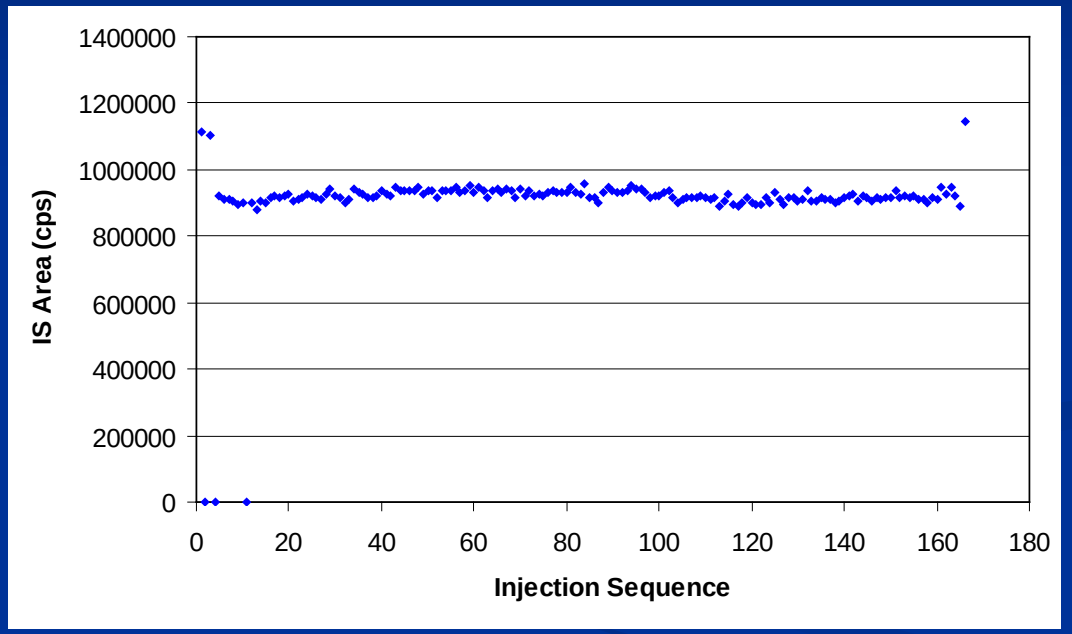
Average % of IS repeats (study-wise)	0.2 (0-1.9)
Average % of IS repeats (sample-wise)	0.2
% Studies without IS repeats	60
% Studies that need investigation	0

Based on 43 studies and a total of 50k incurred samples that were analyzed at BioPharma Services Inc.

A typical batch from mesalamine study



Mesalamine
(Mesalazine or 5-ASA)



Mean: 9.2 E5; Min: 8.9 E5; Max: 9.6 E5

Within ±4% variation in absolute response!

How did we accomplish that?

Many factors can cause IS variations!

IS solution	Stability and/or solubility issue
IS addition	Missed or double addition; imprecise addition
Extraction	Variation in recovery for IS & co-extracted matrix components
Injection	Missed or variable injection vol.
Chromatographic separation	Co-elution with ion-suppression or enhancement component(s); late eluter; column deterioration
MS detection	Ion suppression/enhancement; ionization/detector saturation; inadequate optimization
Others	Wrong materials; leakage; inadequate equipment/instrument use, etc.

1) *A. Tan et al., J. Chromatogr. B, 877 (2009) 3201.*
2) *A. Tan et al. (2012), Internal Standards for Quantitative LC-MS Bioanalysis, in LC-MS in Drug Bioanalysis (eds. Q.A. Xu and T.L. Madden), Springer, New York, USA, p. 1.*

No approach is perfect for monitoring IS variation.

- Setting the upper and lower limits for IS response

  e.g. 50-150% of the mean IS response of known samples (CS & QC)

- Performing a trend analysis on IS responses of known samples to define the acceptable variation for unknown samples

1) *A. Tan et al., J. Chromatogr. B, 877 (2009) 3201.*
2) *M. Jemal et al., Rapid Commun. Mass Spectrom., 17 (2003) 1723.*
3) *R. Bakhtiar, T.K. Majumdar, J. Pharmacol. Toxicol. Methods, 55 (2007), 227.*
4) *Global CRO Council (GCC), Bioanalysis, 3 (2011) 1323.*

But, there is a bottom line…

Summary of IS repeats in our lab

Due to low IS response (%)	70
Due to high IS response (%)	30
Unmatched original and reassay results (due to error in IS addition) (%)	30 (<100)
Overall IS addition error rate (%)	0.06

Based on a total of 50k incurred samples that were analyzed at BioPharma Services Inc.

1) Unmatchable original results must be singled out for reassay;
2) Any IS variation patterns that were not seen in R&D/assay validation should be detected and investigated upon.

Even reproducible results may not be reportable!

- Response vs. concentration relationship might have changed.

  linear for CS → quadratic for samples of abnormal IS responses or vice versa

- Abnormal IS responses may be outside IS linearity range.

  Ionization or detector saturation;

  Analyte & IS may not be simultaneously detected inside MS.

1) *G. Liu, Q.C. Ji, M.E. Arnold, Anal. Chem., 82 (2010) 9671.*
2) *A.K. Hewavitharana, J. Chromatogr. A, 1218 (2011) 359.*
3) *A. Tan and K. Awaiye (2013), Use of Internal Standards in LC-MS Bioanalysis, in Handbook of LC-MS Bioanalysis: Best Practices, Experimental Protocols, and Regulations (eds. W. Li, J. Zhang and F. L.S. Tse), John Wiley & Sons Inc., Hoboken, NJ, USA, p. 217.*

Strategy 1: Choose a good IS and use it properly

- Select the best IS possible;

  ^{13}C and/or ^{15}N > deuterated > structural analogue;

  A stable isotope labeled IS for parent drug is a structural analogue IS for the metabolite!

- One IS for each analyte;
- Determine an appropriate IS concentration;
- Co-elution of analyte and its IS;
- Check working IS solubility & stability;
- Check IS linearity;
- Accurate and reproducible addition of IS.

A. Tan and K. Awaiye (2013), Use of Internal Standards in LC-MS Bioanalysis, in Handbook of LC-MS Bioanalysis: Best Practices, Experimental Protocols, and Regulations (eds. W. Li, J. Zhang and F. L.S. Tse), John Wiley & Sons Inc., Hoboken, NJ, USA, p.217.

Strategy 2: Achieve high & consistent recovery

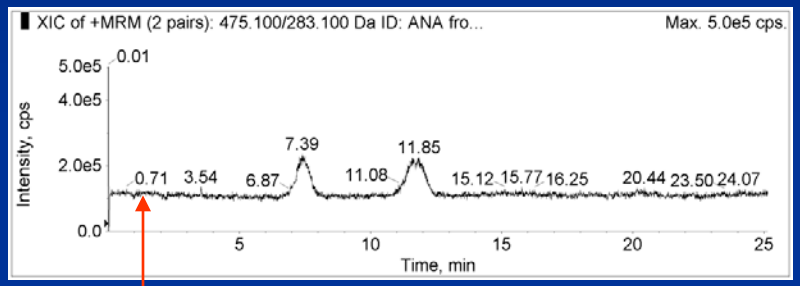
- Determine the best extraction strategy;
- Make sure there is sufficient buffering capacity!
- Adequate transfer volumes;
- Adequate reagent volumes.

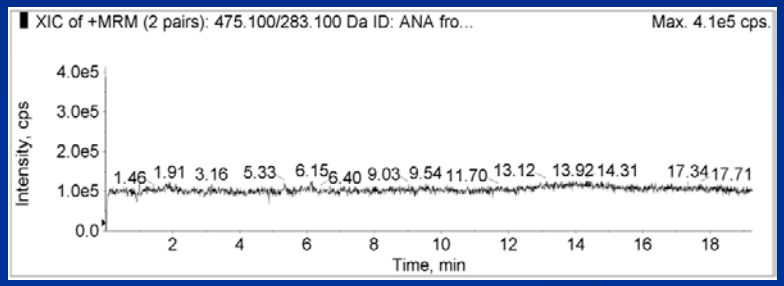
Strategy 3: Don’t stop at successful matrix factor test!

Mobile Phase A

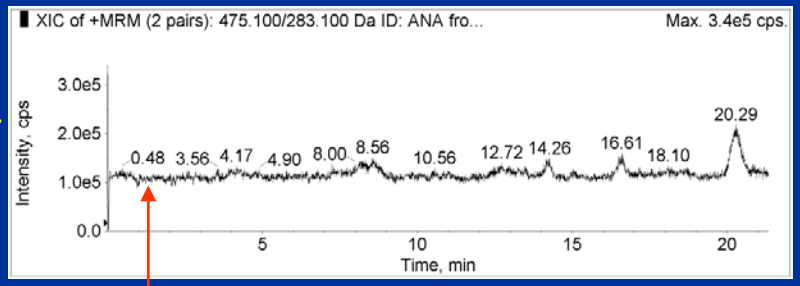
Mobile Phase B

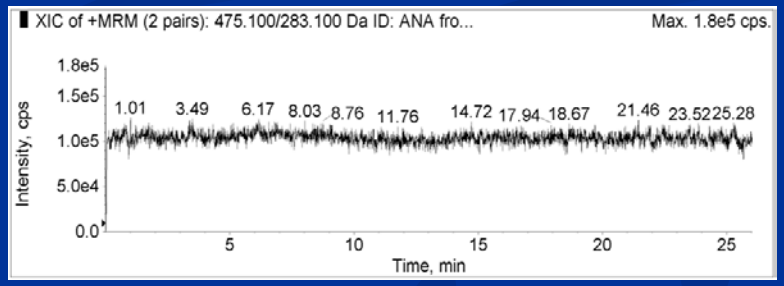
Lipemic blank





Hemolytic blank





Analyte: Sildenafil

Matrix: Human EDTA plasma

Extraction: SLE

Other Strategies

- Test different LC columns and different LC–MS systems;
- Add extra tests as needed;

  *e.g. check autosampler stability for hemolyzed samples if the analyte is a phenolic compound**
- Precise execution of validated assay;
- Close monitoring of assay performance;
- Adequate maintenance of lab equipment and instruments;
- Hire the best research scientists!

** E.-R. Bérubé, M.-C. Lacasse, M. Furtado, F. Garofolo, Bioanalysis, 5 (2013) 1491.*

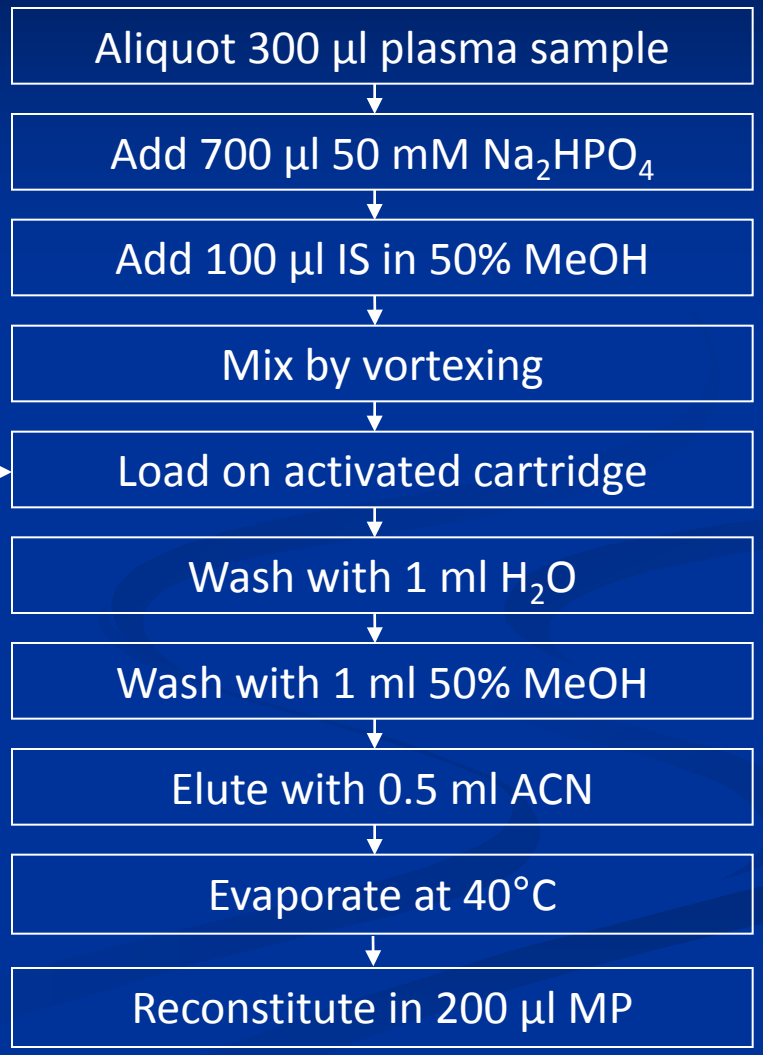
Quiz: What's the cause of IS variation? What could be improved in this method?

Analyte:	Weak base
IS:	D_7
Matrix:	Human K_2EDTA plasma
Conc. range:	15-4000 pg/ml (linear, $1/C^2$)
Extraction:	SPE, Bond Elut-C18 100 mg/1 ml

Activation: 1 ml MeOH & 1 ml H_2O

Scenario:

1) Successful assay validation (analyte recovery 66-71%);
2) Low & variable IS responses mainly with study samples;
3) 20% of IS repeats still had low IS responses.
4) Good agreement between reassay and initial results, including ISR.



Conclusions

- Many different factors can cause IS response variation.
- Once observed, investigation should be done to find the root cause. It is important to demonstrate that the accuracy of quantitation has not been impacted.
- Sometimes, reproducible results may still not be reportable.
- IS response variation is preventable or at least it can be significantly reduced through thoughtful assay development and precise execution of validated assays.

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Thank you for your attention!

Questions or comments?

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