



Identifying Trends and Improving Outcomes from ISR failure Investigations in a Bioanalytical CRO

Min Meng



Scope

- Outline of Tandem Labs ISR SOP
- Statistics of failed ISR studies in Tandem Labs (SL)
(Jan. 2008-Dec. 2009)
- Case Studies (3)
- Summary and Trend Analysis of Failed ISR
- Recommendations
- Acknowledgements



Outline of Tandem Labs ISR SOP

- **Type of studies:** Clinical: all studies; Preclinical: once per method per species
- **Number of ISR samples:** Minimum 20 for small studies, 5-10% for large to mid-size studies, with cap (~200, if applicable)
- **Selection of ISR samples:** >3X LLOQ, mid-range and near the C_{max}
- **Dilution scheme:** same dilution scheme as the original analysis
- **Timing:** “Early and often”; minimize time from original analysis to ISR
- **Acceptance criteria:** Two-thirds (67%) repeat value must be within +/- 20% of the mean of original and ISR value.
- **Corrective Action (CA) and Client notification:** Notify sponsor immediately upon failure; investigation required. Also, investigate ANY sample result > 50% from mean of original and ISR value.



Statistics of Failed ISR Studies in Tandem Labs (Salt Lake City Site)

January 2008 – December 2009

- Over 250 individual analytes tested for ISR
- Nine (9) analytes failed for ISR
- Nine (9) Corrective Action Investigation Reports (CAIR);
(length from 3 pages to 139 pages)
- **What happened in these studies?**
- **What can we learn from these studies?**



Case Study 1

Assay Background

- Single analyte assay
- Validated in both monkey and rat plasma
- Initial use in a sample analysis study

ISR failure - Study Background

- Monkey plasma study
- Study Size: 1140 samples
- ISR Sample Size: 72 samples
- ISR Status: 53 samples failed, all ISR results biased low



Case Study 1

Data evaluation

- Same lot of STD and QC used for all runs.
- An extra peak (early eluter) was observed in chromatography. The size of the extra peak increased in ISR run.
- ISR in a companion rat study met acceptance criteria.
- Similar extra peak was observed in rat, but at much lower levels.

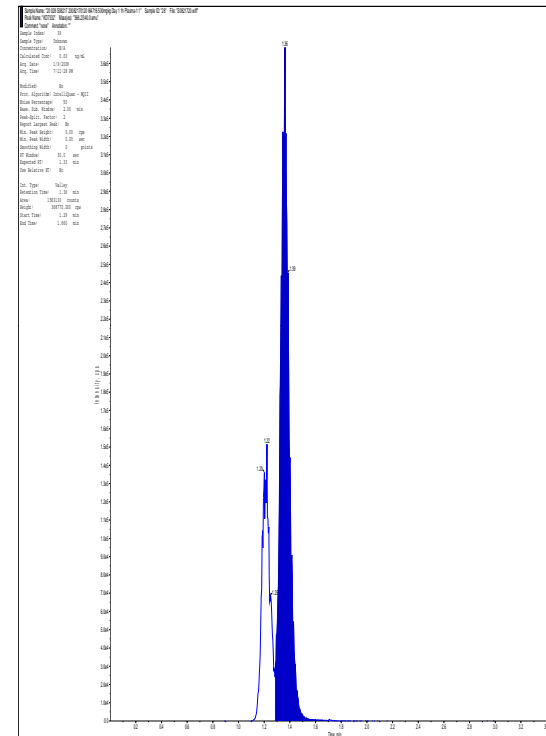
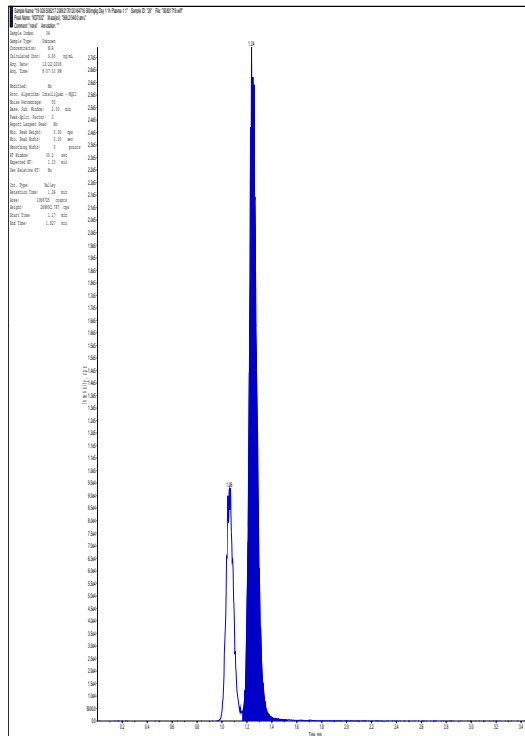
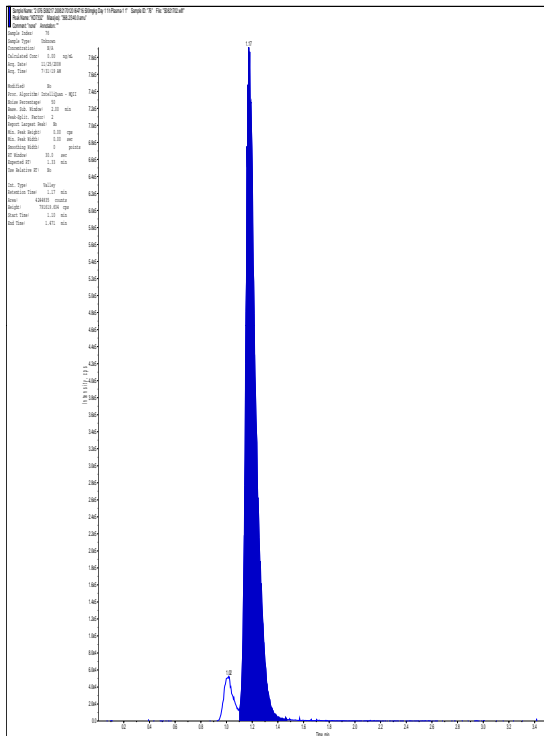
Action

- Reanalyzed the same 72 samples.
- Investigated the peak ratio.

Case Study 1

Mandatory Repeat Report 1st 10 samples			
Sample ID	Original Conc (ng/mL)	1st ISR %Bias	2nd ISR %Bias
500mgkg Plasma-1 Day 1 12h	11200	-19.3	-19.9
500mgkg Plasma-1 Day 23 2h	63700	-31.2	-25.5
500mgkg Plasma-1 Day 23 3h	43500	-27.7	-21.5
500mgkg Plasma-1 Day 1 3h	60300	-34.2	-29.0
500mgkg Plasma-1 Day 23 24h	3810	-21.6	-23.1
500mgkg Plasma-1 Day 1 4h	59600	-28.3	-23.1
500mgkg Plasma-1 Day 23 0h	3650	-23.1	-27.2
500mgkg Plasma-1 Day 23 8h	10300	-22.1	-30.3
500mgkg Plasma-1 Day 1 .25h	11500	-29	-28.8
500mgkg Plasma-1 Day 1 1h	36300	-19.6	-19.8

Case Study 1

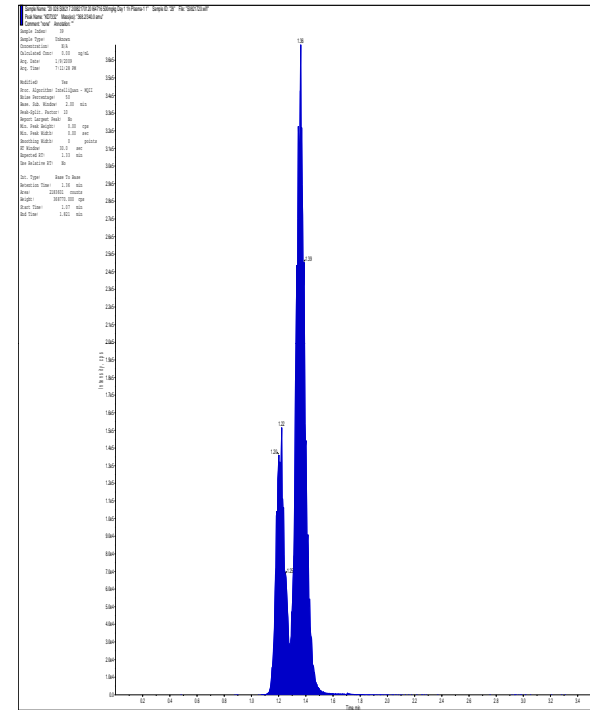
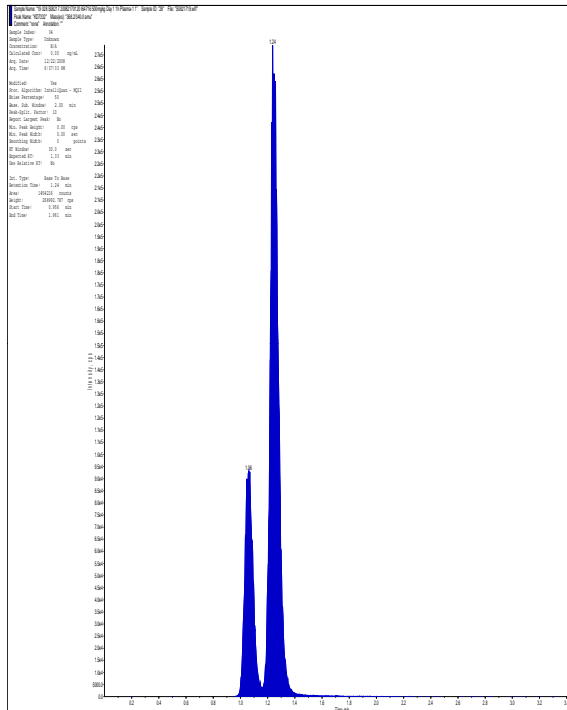
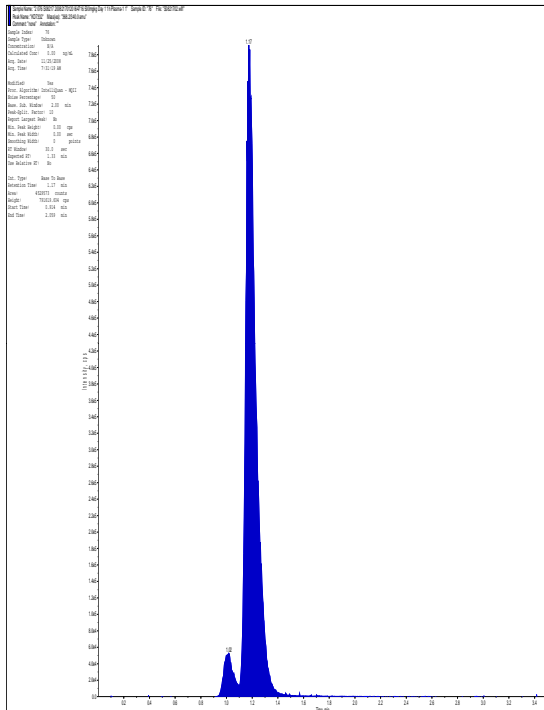


Sample 120 (run 2 Original)
36300 ng/mL

Sample 120 (run 19 1st ISR)
29200 ng/mL (-19.6%)

Sample 120 (run 20 2nd ISR)
29100 ng/mL (-19.8%)

Case Study 1



Sample 120 (run 10 Original)
38800 ng/mL

Sample 120 (run 19 1st ISR)
40200 ng/mL (3.5%)

Sample 120 (run 20 2nd ISR)
41800 ng/mL (6.0%)



Case Study 1 – Analyte Instability; Conversion to Unidentified Metabolite

Conclusion

- Assay performance was acceptable.
- ISR failure was due to the instability of analyte; conversion of analyte to unidentified metabolite.
- Long-Term Stability in spiked QCs was not representative of incurred sample stability.
- Original data was reported “as is” as most accurate reflection of true sample concentrations



Case Study 2

Assay Background

- Single analyte assay
- Validated in rat and dog plasma
- Initial use for dog sample analysis

ISR Failure – Study Background

- Study Size: 54 dog samples
- ISR Status: two-thirds of ISR samples failed



Case Study 2

Data Evaluation

- Same Stds/QCs were used
- Samples analyzed within established LTS
- No chromatographic/IS anomalies observed

Action

- Conducted 1st investigational batch
 - INV#1 - repeated ISR samples only (results still imprecise)
- Examined sample vial and tested mixing
- Conducted 2 more investigational batches
 - INV#2 - repeated entire study (still did not match earlier results)
 - INV#3 - repeated entire study again (matched INV#2)

Case Study 2



Vial on the far right was used; majority of the study samples initially had sample volumes approaching the capacity of the fixed insert.



Case Study 2 - Inadequate Mixing

Conclusion

- Assay performance acceptable
- ISR failure was due to inadequate mixing (non-homogeneity) and the specific container/sample volume.
- The initial analyses (original, first ISR, INV#1) were imprecise and inaccurate.
- The two final investigational runs (INV#2 & #3) were repeated with adequate mixing and agreed with each other
- Results of first full reanalysis (INV#2) were reported.
- However, can't assess the real impact on true study sample concentrations after the initial non-homogeneous sample aliquots were removed.



Case Study 3

Assay Background

- Two analyte assay
- Compounds are endogenous
- Validated in rat and dog plasma
- Parent known to be unstable; 1% Phosphatase inhibitor added at collection site

Study Background

- Analyzed 600 samples (rat) and 1232 samples (dog)
- For both species: ISR failed for parent (all biased low) but passed for metabolite.



Case Study 3

Data Evaluation

- Same Stds/QCs were used; Samples analyzed within established LTS; No chromatographic anomalies observed.
- Because 100% of metabolite ISR passed, execution error was excluded as source of parent ISR failure

Action

- Conducted several investigational experiments; ruled out extract instability
- Re-validated amount/effectiveness of the enzyme inhibitor in- vitro
- Client conducted dosing experiment with additional animals for enzyme inhibition evaluation (using sample collection procedure outlined in protocol)



Case Study 3

Investigational Dosing and Inhibitor Experiment

Dosed Animal Results		
Test Group 1 (control)	Test Group 2	Test Group 3
5% Phos Inhibitor w/NaF	1% Phos Inhibitor	No Phos Inhibitor
0.854601	0.037574	0.03342
0.901422	0.109422	0.206614
0.475743	0.023566	0.021002
0.636688	0.027035	0.038604
0.709535	0.052897	0.046493
% Loss from control	- 93%	- 91%



Case Study 3 – Insufficient Enzyme Inhibition to Prevent Analyte Instability

Conclusion

- Addition of 1% phosphatase inhibitor was not sufficient for incurred samples (despite successful MD/MV evaluations)
- Two possible reasons:
 - Matrix storage stability. Long Term stability was established using surrogate ^{13}C labeled analytes which may not represent the natural ^{12}C analog
 - Addition of 1% Inhibitor addition did not scale effectively in actual study samples (1 mL into 100 mL for STD/QC pools vs. 5 uL into 500 uL for study samples).
- Sponsor determined that sample data for the parent drug for this study was not reliable and would not be reported.



Summary of ISR Failures

- **Type of Matrix**

- Three human plasma assays
- Four animal plasma assays
- Two human urine assays

- **Number of Analytes**

- Four single analyte assays
- Four two analyte assays
- One multiple analyte assays
- Regardless of total number of analytes, only one analyte failed per ISR



Reasons for ISR Failure

- **Incurred sample instability (5)**
 - Conversion to unidentified metabolite (1)
 - Insufficient enzyme inhibition (2)
 - Bench-top matrix instability (1)
 - General matrix storage instability (1)
- **Execution error (3)**
 - Known execution error (mixing/dilution) (2)
 - Unknown execution error (1)
- **Assay Ruggedness (1)**
 - LC column lot variability (1)

Checklist for ISR Investigation

Category	Itemized details	Comments
Study Scope	Total samples:	
	Total ISR samples:	
	Failed ISR samples:	
	Are split samples available?	
Numbers of the analytes:	Total number of analytes:	
	Which ones pass or fail?	
Method history:	1 st time using for SA?	
	Any previous ISR failure or success?	
	Any technical issue during MD?	
	Any known insolubility?	
	Any known instability?	
Sample condition:	Sample Volume:	
	Container type:	
	Aliquot volume:	
Anticoagulant type:	Heparin or EDTA	
Treated matrix:	Inhibitors or additives?	
Raw data evaluation:	IS area plots (original and ISR)	
	Watson Standard and QC report	
	Chromatography (original and ISR)	
Stats of the ISR report:	Watson ISR report	
	Sort by treatment, subject, time-point, concentration and run numbers	



Checklist for ISR Investigation (Cont)

Run #	Std Lot #	QC Lot #	Dilution scheme	Extraction Scientist	MS ID	F/T cycles	Collection time	Extraction time	Analysis time



Recommendations for ISR success

For Method Development Scientists:

BUILD A SOLID METHOD

- Know your molecules (pKa, stability, solubility, matrix effects, additives, anticoagulants).
- Consider others (production, collection site)



Recommendations for ISR success

For Bench Scientists:

MIX, MIX, MIX (homogeneity is key)

- Pay attention to sample volume and container type
- Treat every study sample exactly the same as Standards and QCs



Recommendations for ISR success

For Project Manager or Principle Investigator:

- Use same Standards and QCs for entire study if possible
- Conduct ISR runs “early and often”
- Use checklist to conduct investigation in logical and constructive manner
- Work closely with the sponsor at all times



The future of ISR ???

For the Scientific Community...

- Standardized approach for ISR failure investigation?
- If compound demonstrates instability, what is the best way to stabilize the compound?
 - Inhibition (enzyme inhibitors, ice bath, other additives)
 - Pre-extraction at collection site (addition of MeCN or MeOH 1:4 v/v).
- Re-consider the ISR requirement for urine assays
 - Only for studies with urine data as pK endpoint



Acknowledgments

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