



# Method Development Strategies for Improving ISR Reproducibility – Use of Surfactants for Success

## AUTHORS

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## INTRODUCTION

The FDA recently addressed the issue of bioanalytical method reproducibility by ruling that analytical studies require 10% Incurred Sample Reanalysis (ISR). Reproducible accuracies in an assay are crucial as the ability to generate the same result over time demonstrates the validity of the result being measured. Failure to reproduce a result can be attributed to potentially several method related causes such as stability, matrix effects, non-homogeneity, etc.

This poster will address proactive method development strategies to overcome homogeneity problems that affect reproducibility which manifest in failed ISR during sample analysis studies. This approach investigates the purposeful uses of surfactants.

## REAL LIFE PROBLEM #1

### “RECOVERY ISSUES”

#### OBSERVATION

Stored QC control is initially accurate but slowly becoming inaccurate with low bias.

#### M.O.

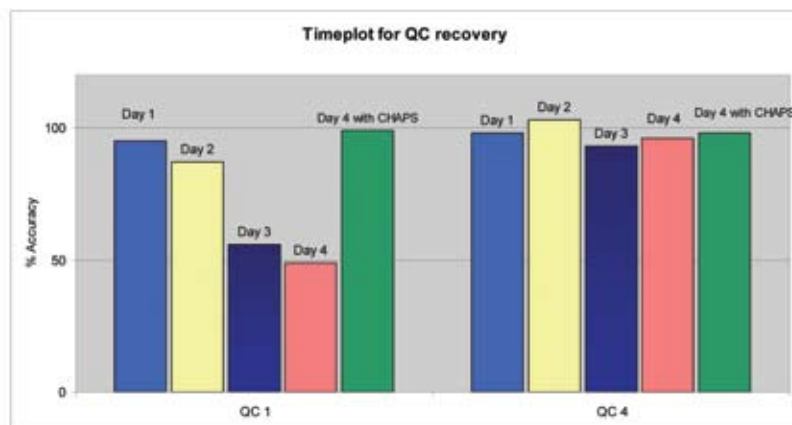
Hides itself as stability problem, characteristically picks on lower concentrations.

## REAL LIFE PROBLEM #1 *continued*

### EVIDENCE-Real life data

TIME POINT	QC 1 0.600 ng/mL	QC 4 40.0 ng/mL
% Accuracy		
DAY 1	95	98
DAY 2	87	103
DAY 3	56	93
DAY 4	49	96
DAY 4 with CHAPS*	99	98

\* CHAPS surfactant added to same QC at a 1% (w/v) concentration.



### RESULT

Lost sample is found!

### THE VERDICT

These samples will be troublesome to reproduce over time without the addition of the surfactant CHAPS. So... add CHAPS.

### Proactive MD approach to avoid this (or rather what I would do)

- Δ Conduct tests for over time for benchtop reproducibility with and without surfactant added and Good Luck.

## REAL LIFE PROBLEM #2

### “ABSORPTION ISSUES”

#### OBSERVATION

A serially diluted (with matrix) curve is concave. The response factors drop with decreasing concentration. Directly spiked QC's have high bias vs a serially diluted curve.

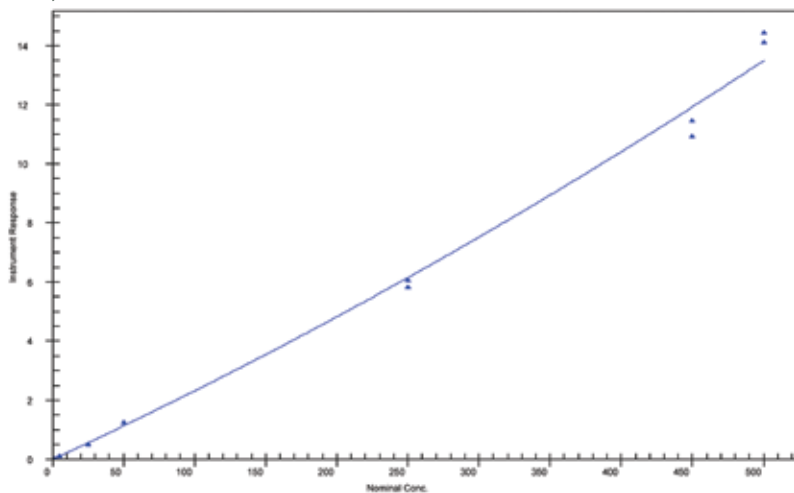
#### M.O.

Seduces you into thinking this is a QC preparation error but it really is an adsorption issue

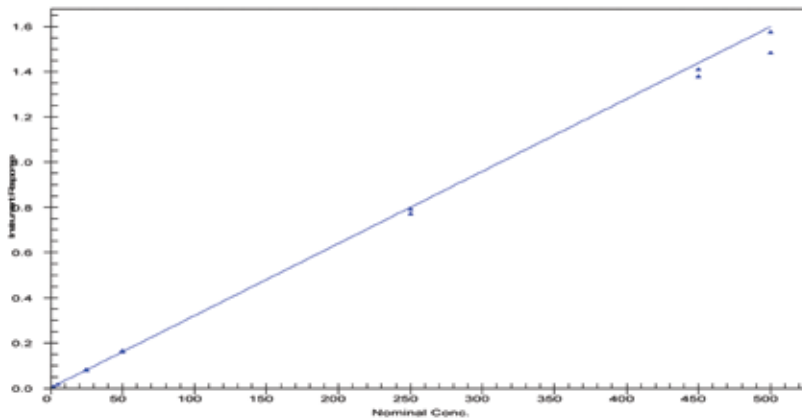
#### EVIDENCE

“Classic” concave curve and dilution QC's look GREAT!

Serially Diluted Curve- “Concave” Curve - No Surfactant Added



Serially Diluted Curve- Surfactant added \*



\*Serially diluted with 1% Triton X-100 (v:v).  
(CHAPS is far too expensive in this economy for use here.)

## REAL LIFE PROBLEM #2 *continued*

### Declining Response Factors without Surfactant

Response Factors vs Concentration			
	Std Conc. (ng/mL)	Response factors	
		Without Triton Added	With Triton Added
<b>Std A</b>	1.00	19.3	29.1
<b>Std B</b>	2.00	18.3	26.6
<b>Std C</b>	5.00	22.7	26.3
<b>Std D</b>	25.0	20.5	26.1
<b>Std E</b>	50.0	25.3	25.2
<b>Std F</b>	250	23.9	25.0
<b>Std G</b>	450	24.9	25.3
<b>Std H</b>	500	28.6	24.7

### "Inaccurate" QC's without Surfactant

Accuracy of QC's with and without Surfactant Added to Standard Curve					
	QC1	QC2	QC3	QC4	Dilution QC 5
<b>Theor. Conc. ng/ mL</b>	3.00	30.0	200	400	10000
<b>No Triton in Std Curve</b>	135	113	126	125	99.7
<b>Triton added to Std Curve</b>	104	100	102	101	101

Notice that the concave curve is gone and QC's are accurate when Triton is added to the standards. The dilution QC's are still great.

## RESULT

With Triton the results are accurate and reproducible as you are no longer at the mercy of adsorption effects. Consideration must be given to the containers the samples themselves arrive in, as well as the sample collection technique. It may turn out that surfactant addition will be into the received sample, causing a dilution, but the dilution is small and is the lesser of two evils.

(The Dilution QC's are accurate for both methods because it adsorbed to the container on the dilution transfer)



## REAL LIFE PROBLEM #2 continued

### VERDICT

Add Triton

### Proactive MD Approach to Ensure Accuracy and Reproducibility

Δ Conduct transfer tests using the materials your analyte will be exposed to during sample processing. If absorption is observed try a surfactant.

*These transfer tests are typically just an exaggeration of the exposure your sample will encounter during sample processing. A typical experiment would be to transfer the QC's low and high, 5-7 times, via pipette from vessel to vessel and compare to non- transferred QC's.*

## REAL LIFE PROBLEM #3

### "HOMOGENEITY ISSUES"

#### OBSERVATION

Sample analysis of QC controls for this cross validation had continually high bias and poor precision.

#### M.O.

Some samples are good others not so good.

#### EVIDENCE

High Bias QC's without Surfactant

QC Accuracy –Surfactant Treated* vs Not Treated					
Sample Treatment	Theor. Conc ng/mL.	QC1	QC2	QC3	QC4
		n=6			
<b>No CHAPS added</b>	%Theoretical	125	119	121	112
<b>CHAPS added</b>	%Theoretical	101	99	100	99
	% Difference	24	21	21	13

\* Treated with 1% CHAPS (w:v)



## REAL LIFE PROBLEM #3 *continued*

### RESULT

This matrix had stratified with heavier components sinking to the bottom. The analyte had no affinity to the bottom “pseudo pellet” layer that resulted in a concentration into the “supernatant” layer. The addition of CHAPS made the pellet soluble in the supernatant i.e. homogeneous, thereby eliminating this concentration effect.

### VERDICT

Add surfactant.

### PROACTIVE MD APPROACH

Analyze QC's in MD, compare centrifuged vs. not centrifuged, vortexed vs. not vortexed & with surfactant vs. without surfactant.

## DISCUSSION

In all three problems the reproducibility of a result was affected resulting in potential ISR failures.

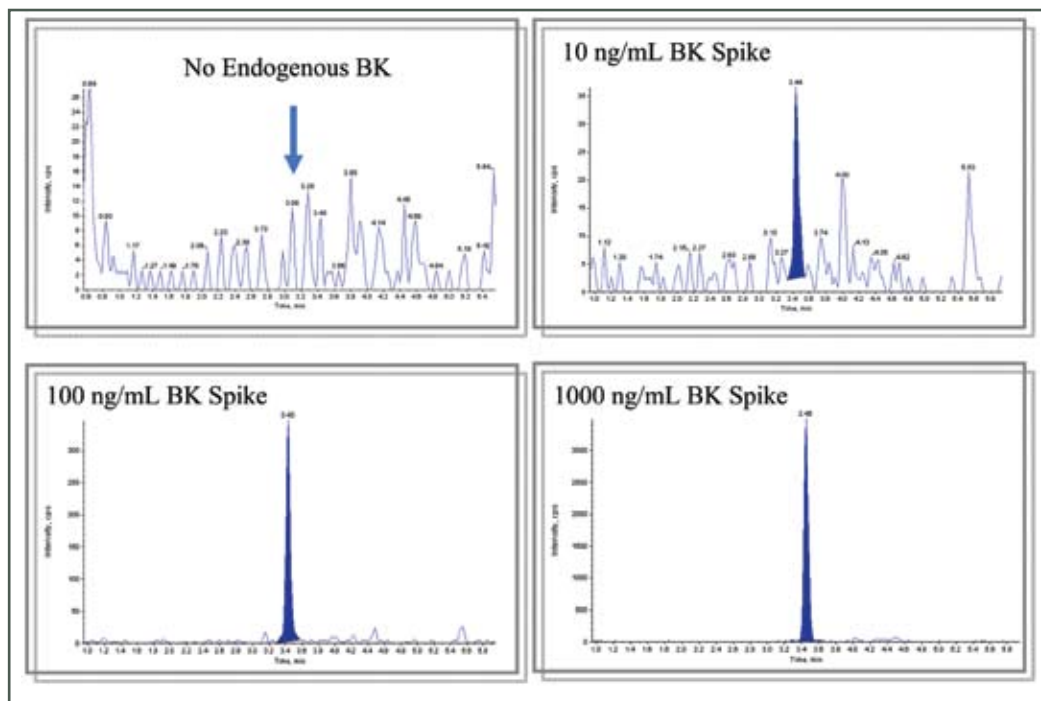
- Δ Problem #1 was due to time related recovery loss, causing a low bias. The addition of a surfactant eliminated this bias, ensuring ISR success.
- Δ Problem # 2 was an adsorption issue; increased exposure due to number of vessels (esp. for dilution samples), surface area contact and time can all affect the results making reproducibility hard to control. Addition of a surfactant eliminated this. ISR success!
- Δ Problem # 3 was an issue of homogeneity. The matrix being stratified resulting in a partition of sample between what I will call a pseudo pellet and a supernatant. Overcoming this by excessive mixing and vortexing can be hard to reproduce. The addition of a surfactant keeps the pseudo pellet in suspension and allows for a homogeneous aliquot. No worries.

## CONCLUSION:

Simple tests, if carried out in method development can go a long way in improving sample result reproducibility. This proactive approach can eliminate costly reassays and ensures that the result being reported is solid. These problems were all detected in MD and were adverted, preventing costly re-work.

## RESULTS AND DISCUSSION *continued*

Mass Chromatograms of BK in 1:1000 Diluted Human Plasma



## CONCLUSION

- Δ The quantification of the endogenous nonapeptide bradykinin (BK) is feasible in human plasma without using an isotope labeled bradykinin (BK-D6).
- Δ The construction of the calibration curve can be achieved using BK indiluted human plasma (with water) or a common proteomic buffer ammonium bicarbonate.
- Δ The precision and accuracy of quantifying BK in human plasma-based QC samples are within 15% (CV) and 80-120%, respectively, in the tested analytical range of 75-750 ng/mL.