

# Viewing the Bigger Picture through a Keyhole: Providing Context to Bioanalytical Challenges

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# Background

## ■ Introduction

- Context to the study/program

## ■ Define problem

- Stored Stability

## ■ Problem solving

- Thought processes and strategies

## ■ Open discussion

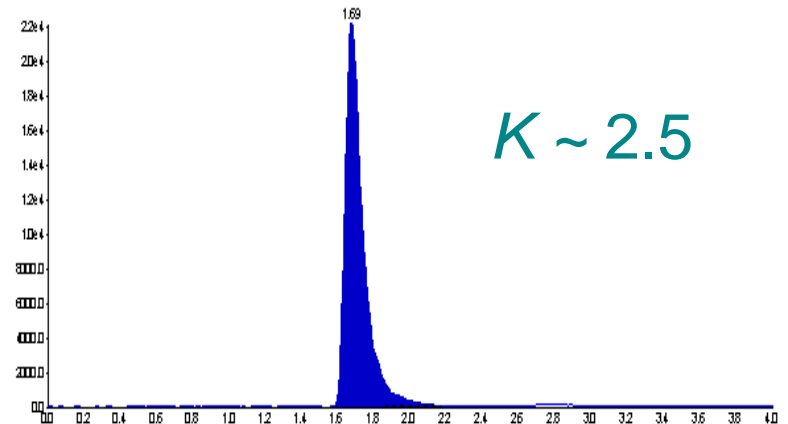
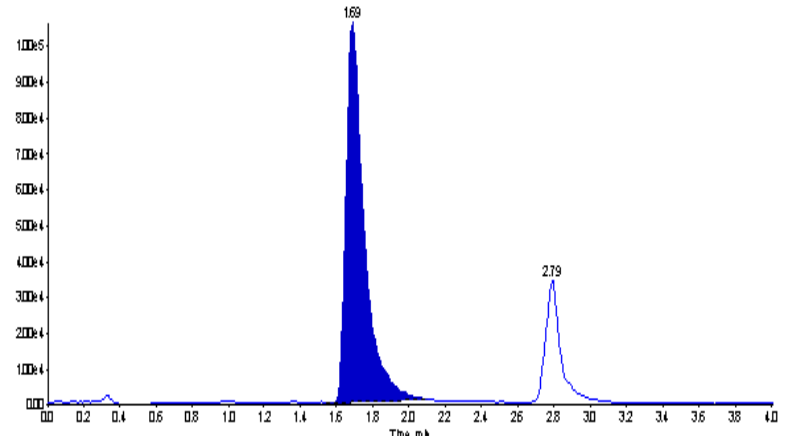
## ■ Conclusions

# Introduction

- Urgent request for sample analysis
- TK support in mouse serum
- Samples previously taken and stored frozen
  - Samples ca 90 days old (-70°C)
- Legacy assay existed at HLS

# Introduction

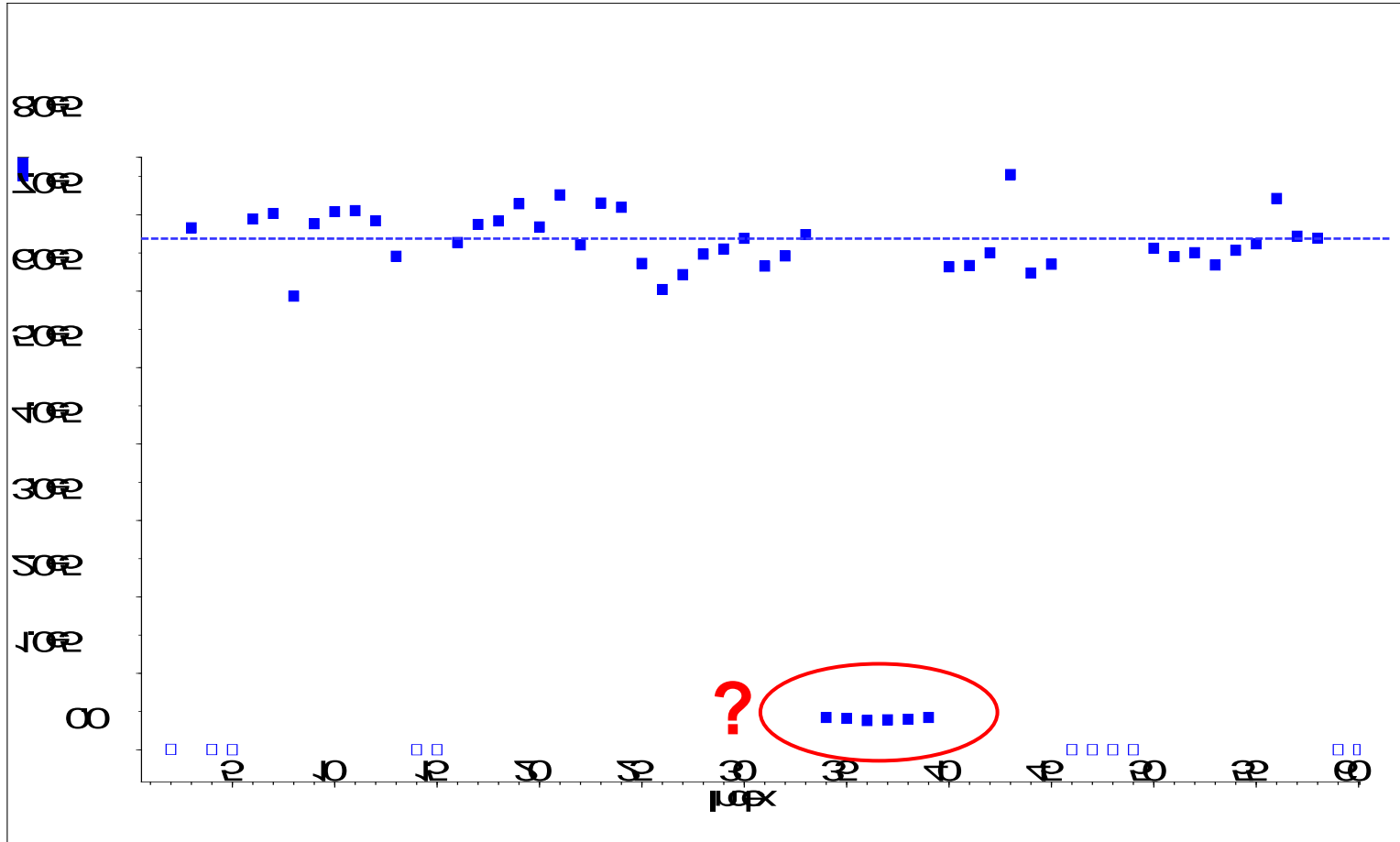
- Legacy assay
  - Analyte: ~450 mw
  - 0.05 - 100 ng/mL
  - $\mu$ SPE sample preparation
  - BioBasic SCX 50 x 3.0 mm
  - Isocratic
  - API 3000 MS/MS
  - $^{13}\text{C}_6$ -labelled IS
  - 282 days stability at (-20°C)



# Problem: Defined

	Concentration (ng/mL)			
	Performance QCs		Stability QCs	
	Low	High	Low	High
	0.150	90.0	0.150	90.0
	0.159	87.1	0.148	57.1
	0.145	89.1	0.117	55.3
	0.150	86.9	0.123	57.2
	0.158	73.3	0.168	58.8
	0.161	86.3	0.129	55.6
	0.142	87.0	0.148	55.8
Mean	0.153	85.0	0.139	56.6
RE (%)	1.7	-5.6	-7.5	<b>-37.1</b>
CV (%)	5.2	6.8	13.9	2.3

# Investigation initiated



# Problem: Defined

	Peak Area (counts)					
	Performance QC High			Stability QC High		
	Analyte	IS	PAR	Analyte	IS	PAR
	1088744	631816	<b>1.723</b>	47728	42233	<b>1.130</b>
	1116489	633457	<b>1.763</b>	44881	41036	<b>1.093</b>
	1117799	650182	<b>1.719</b>	43238	38182	<b>1.132</b>
	1090506	752507	<b>1.449</b>	45560	39165	<b>1.163</b>
	1064982	623661	<b>1.708</b>	43875	39873	<b>1.100</b>
	1093562	635166	<b>1.721</b>	46616	42211	<b>1.104</b>
CV(%)	1.8	7.5	6.8	3.7	4.1	2.4

# Problem(s): Defined

- Why do we observe a stability miss-match between low and high serum QCs stored at  $-70^{\circ}\text{C}$ ?
- Why do we have a stability miss-match in serum when stored at  $-20^{\circ}\text{C}$  vs  $-70^{\circ}\text{C}$  (high QC only)?
- Why do we observe a difference in the  $^{13}\text{C}_6$ -IS behaviour when spiked into serum stored at  $-70^{\circ}\text{C}$  (high QC only)?



# Problem Solving

## ■ High QC failure only

- Inefficient MS/MS source? Solubility?

## ■ MS/MS

- Source API3000 → API4000 and increase ISV

## ■ Solubility

- Water QCs frozen at -70°C
- Frozen low QC over-spiked with high QC working solution

## ■ Further experiments:

- No SPE losses & post column infusion clear

# Questions...

- Why do we observe a stability miss-match between low and high serum QCs stored at  $-70^{\circ}\text{C}$ ?
- Why do we have a stability miss-match in serum when stored at  $-20^{\circ}\text{C}$  vs  $-70^{\circ}\text{C}$  (high QC only)?
- Why do we observe a difference in the  $^{13}\text{C}_6$ -IS behavior when spiked into serum stored at  $-70^{\circ}\text{C}$  (high QC only)?
- Within the context of the study/program – does it matter?

# Conclusions

- Problems encountered or “failures” need not be a barrier to program progression
- Troubleshooting/problem solving should not be done in isolation: shared goals and shared problems
- Align troubleshooting/problem solving effort with context and overall endpoint

# Acknowledgements

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Thank you