TRANSFORMING PROMISING IDEAS INTO COMMERCIAL REALITY

How Problematic Situations Can Become Positive Ones: Case Studies of Bioanalytical Issues

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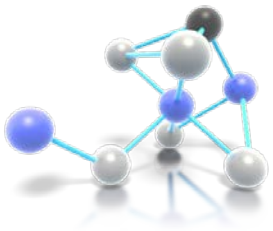
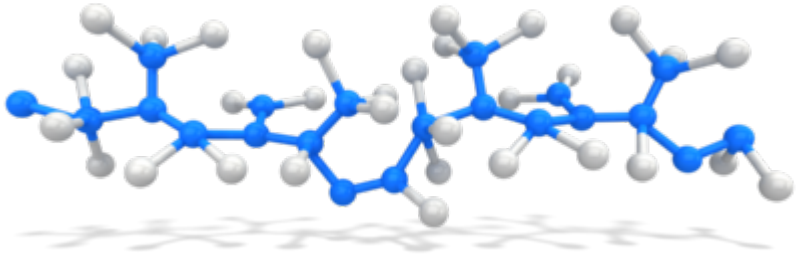
Presentation Content

- Introduction
- Case studies
- Discussion with the audience/suggestions
- Solutions to issues

Introduction

- inVentiv Health Clinical (former Anapharm) is a CRO since 1994
- The bioanalytical laboratory performs more than 1 000 projects per year corresponding to more than 700 000 samples per year
- Analyses mostly done by LC/MS/MS and UPLC/MS/MS (n=36)
- Case studies
  - Clopidogrel (anti-platelet agent) (in publication)
  - Perindopril (ACE inhibitor) and perindoprilat
  - Rosuvastatin (high cholesterol treatment)





Clopidogrel - Issue

- Clopidogrel assay was used for multiple studies without any known issue
- A problem occurred with a study of about 1400 samples
- 43 samples were re-analyzed diluted because their concentration was above the ULOQ
- For 11 of them (less than 1%):
  - When diluted, the reanalyses did not confirm the first extrapolated values
  - They were re-assayed undiluted and the reanalyses confirm neither the first analyses nor the diluted ones
  - % difference with the extrapolated original values ranged from -115 to -44%

Clopidogrel - Analysis Summary

Sample Number	Concentrations (pg/mL)				Percent Difference Between Values 1 and 3 (%)
	Value 1 (extrapolated)	Value 2 (diluted)	Value 3 (undiluted)	Value 4 (undiluted)	
1	5094	3662	2785	2578	-59
2	4986	3944	2914	2944	-52
3	5177	2357	1584	1807	-106
4	7491	3186	2839	2869	-90
5	6594	2855	2164	2149	-101
6	5018	2097	1595	1571	-104
7	6016	2305	1641	1727	-114
8	6447	2707	2012	1904	-105
9	5330	2113	1440	1473	-115
10	5305	4118	3385	3554	-44
11	5310	2228	1667	1639	-104

- Should we investigate when few repeated analyses did not confirm?
- What is next when the root cause is found?

Perindopril and Perindoprilat - Issue

- Perindopril/perindoprilat assay was used for routine analysis
- Deficiency letter to discuss the possible back-conversion of the glucuronide metabolites was received even if nothing was problematic in the study.
- An investigation was initiated and showed that:
  - Perindopril is not affected by the hydrolysis of its glucuronide (bioequivalence criteria were based on perindopril)
  - Perindoprilat glucuronide degrades into perindoprilat in matrix
  - Perindoprilat concentrations were overestimated for sampling times from 0.3 to 3.0 hours post-dose

Perindoprilat - Analysis Summary

Sample Number	% change			
	Subject 1 Perindopril	Subject 1 Perindoprilat	Subject 2 Perindopril	Subject 2 Perindoprilat
0.000	---	---	---	---
0.333	-4.90	---	-4.35	**131.32**
0.500	4.52	**487.92**	-2.77	**120.94**
0.833	2.64	**238.36**	-0.13	**50.41**
1.25	6.39	**101.76**	-3.15	16.82
2.00	5.14	**36.42**	2.14	2.52
3.00	9.59	**29.98**	7.26	2.43
4.00	8.82	1.42	-3.87	1.31
5.00	1.54	14.70	---	0.92
6.00	2.64	4.99	---	1.40
7.00	-1.34	-0.49	---	0.99

- Should we reanalyze the whole study with an improved assay?
- Should we report the results as is, since the perindoprilat values are used as supportive data?

Rosuvastatin - Issue

- Rosuvastatin assay was validated and used for study sample analysis
- A client asked us to verify the potential back-conversion of a lactone. This interference of lactone metabolite was not studied during the validation
- After an investigation, it was found that:
  - A lactone interferes in plasma after 23 hours at room temperature but the interference was not significant at 12 hours
  - The lactone did not interfere at 4°C; the samples should be kept on ice during thawing and extraction

Rosuvastatin - Stability

Stability Period	% Change When Fortified With Lactone		Status
	Low Quality Control	High Quality Control	
12 hours	11.70	13.02	Pass
23 hours	16.62	19.01	Fail

Acceptance criteria: should be within +/-15%.

- Knowing that, should we keep the assay as is (at RT) or modify the thawing temperature for 4°C?

Questions

- Each time an issue occurs, the same questions are asked:
  - Should we systematically reanalyze the whole study when irreproducibility is observed?
  - When the irreproducibility is found only for the metabolite used as supportive data, should we report the results as is or investigate further?
  - In the case of ISR meeting the acceptance criteria of 67%, do we need to investigate the out-of-criteria samples?
  - What can we change in our R&D processes to avoid this kind of issues?
- Did you experience this kind of issues in your laboratory? Do you want to share your experience?

What was done to solve these issues?

Clopidogrel - Solutions

- The investigation showed that a back-conversion of a metabolite in presence of methanol caused the variability
- New improved methanol-free assay was validated
- The study with the issue was reanalyzed
- Sponsors of older studies were notified as well as regulatory agencies

- Solutions:
  - All studies performed with the old assay were reanalyzed or re-dosed and analyzed
  - No study outcome was changed but reliable data is now available

Perindopril and Perindoprilat - Solutions

- The investigation showed that all results for perindopril were reliable and those for perindoprilat too, except for timepoints from 0.3 to 3.0 hours post-dose; the perindoprilat-glucuronide was cleaved and perindoprilat was overestimated for these timepoints
- Sponsors were notified as well as regulatory agencies
- Solutions:
  - Statistical analyses were performed without the timepoints up to 3 hours (inclusively)
  - The outcome of the studies was not changed
  - In parallel, the method was modified to avoid any back-conversion. An in-house study was done to demonstrate that the issue was solved

Rosuvastatin - Solutions

- The investigation showed that a lactone converted into Rosuvastatin after a long period at room temperature

- Solutions:

  - In order to have a more robust method, we re-validated the method at 4 degrees Celsius even if the stability at room temperature was demonstrated for 12 hours

  - Study samples from an in-house study were used to validate the method. ISR was evaluated at 91% during method validation

Method Development Improvement

- These issues led our R&D team to implement new and improved processes for method development and validation
- We now have a team responsible of verifying:
  - All the metabolite pathways
  - The potential stability issues
  - All potential back-conversion of metabolites
  - The right analytical range required for each study

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Thank you

