Road to Recovery

Exploring the challenges in assessing recovery during the validation of an LC-MS/MS method in a rare matrix

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Introduction

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Goal
Validation of an LC-MS/MS method for the determination of a small molecule therapeutic in Human ELF

Analyte
A small molecule therapeutic under development for treatment of pulmonary disease

Internal Standard
Stable isotopically labelled (SIL) internal standard

Matrix
Human Epithelial Lining Fluid (ELF) modified with 2% Tween 80

Extraction methodology
Liquid-Liquid Extraction

Instrumentation and Analytical Range
Waters Xevo TQS, Waters Aqcuity, 10.0 – 10,000 pg/mL

Recovery: An overview

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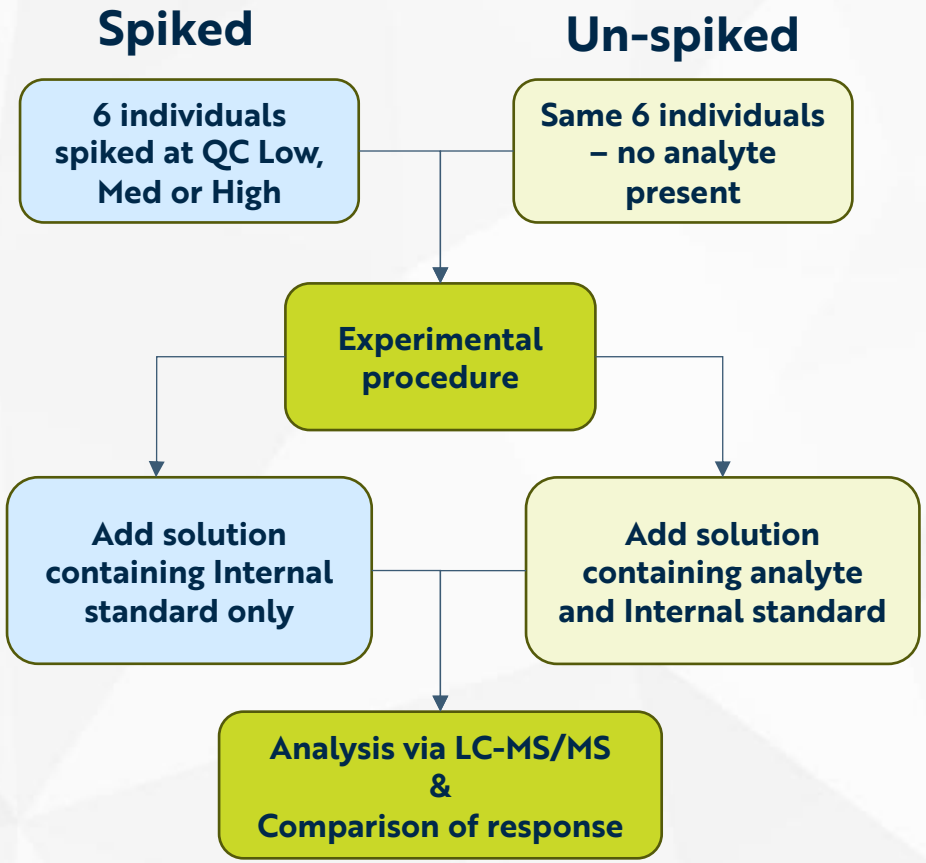
Assessment of the amount of analyte or IS lost (or retained) during the course of sample extraction when compared with un-extracted samples which represent 100% recovery.

- Pre-ICH M10 procedure:
- 2018 FDA Guidance on Bioanalytical Method Validation outlined assessment of Recovery
- At Resolian – Spike 6 individuals at QC Low, Med and High and take them through extraction, adding IS at the end
- Compare response to the same un-spiked individuals, reconstituted with 100% of expected analyte conc at the end of the extraction



There are no set acceptance criteria in terms of % of analyte recovered

Needs to be consistent across analytical range and across individuals (≤15% CV)

Initial Assessment of Recovery

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- Extracted Area Ratio (EA): Individuals spiked with analyte prior to extraction
- Unextracted Area Ratio (UA): Individuals reconstituted with 100% expected analyte concentration at the end of extraction
- %Recovery: How much analyte has been retained during extraction

Level Assessed	Control Matrix ID	Unextracted Area Ratio (UA)	Extracted Area Ratio (EA)	Recovery (%)
QC Med 5000 pg/mL	BA2206154	3.153323	2.103012	66.7
	BA2206155	3.180289	3.127848	98.4
	BA2206156	3.176549	2.974303	93.6
	BA2206157	3.18013	3.152372	99.1
	BA2206158	3.154737	3.511714	111.3
	BA2206159	3.165821	3.014147	95.2
	Mean	-	-	94.1
	S.D.	-	-	14.8
	% CV	-	-	15.7
QC High 7500 pg/mL	BA2206154	4.720149	3.268191	69.2
	BA2206155	4.724442	4.23485	89.6
	BA2206156	4.698951	4.681197	99.6
	BA2206157	4.713524	4.443463	94.3
	BA2206158	4.695822	5.208208	110.9
	BA2206159	4.679441	4.66767	99.7
	Mean	-	-	93.9
	S.D.	-	-	14
	% CV	-	-	14.9

Next steps

- In the initial assessment, internal standard was added to the spiked QCs at the end of the extraction. This does not mirror standard sample extraction.
- Internal standard is used to correct for any analyte loss during extraction.
- The lower %Recovery in individual 01 seen previously may be permissible if we can demonstrate that the internal standard can correct for the loss of analyte.

Investigation Batch:

- All six individuals spiked at the level of QC Low, Medium and High to prepare “Recovery QCs”
- Recovery QCs then extracted in the presence of the internal standard like traditional QCs.
- The calculated concentration of the Recovery QCs then compared to the hypothetical QC concentration.
- Provided they met standard acceptance criteria (≤±15% RE, ≤15% CV), the assessment would be deemed successful.

Re-assessment of Recovery

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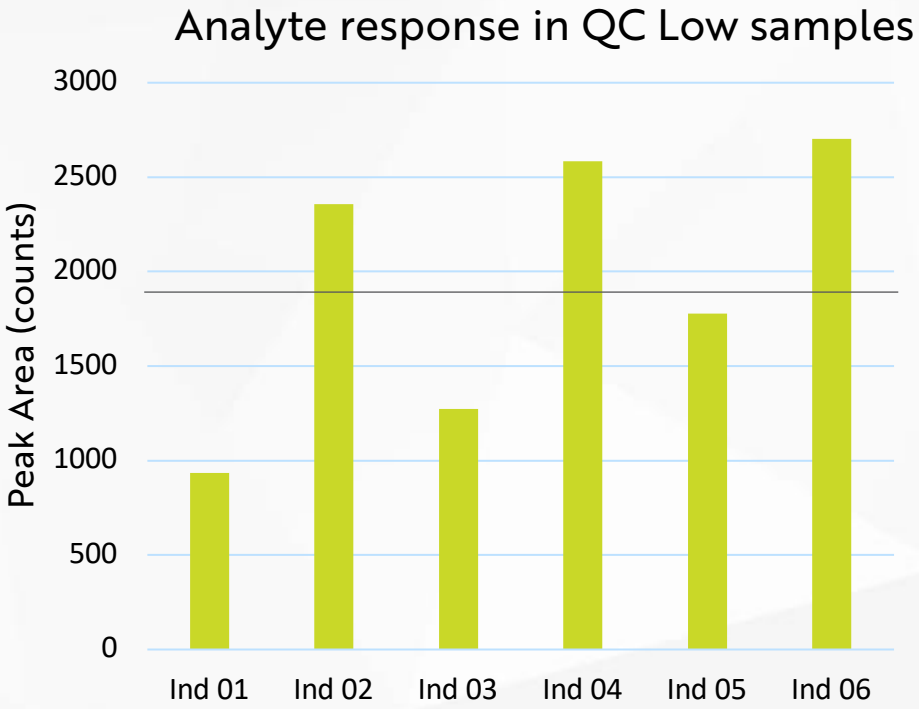
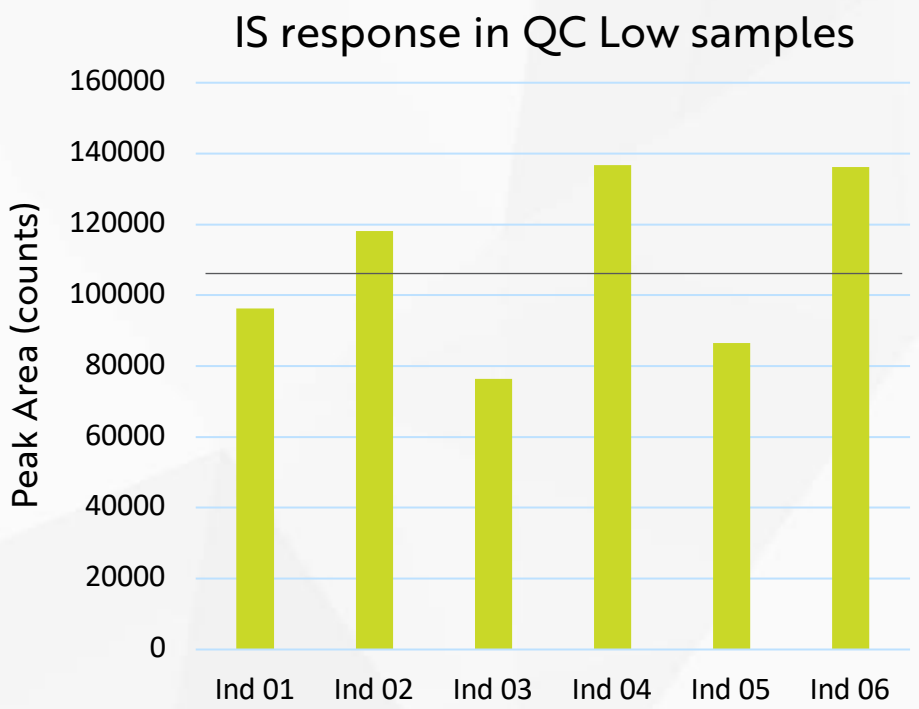
	QC Low (30.0) pg/mL	QC Med (5000 pg/mL)	QC High (7500 pg/mL)
Ind 01	13.5	2440	3680
Ind 02	28.4	4970	7520
Ind 03	23.6	4640	7270
Ind 04	26.8	4930	7680
Ind 05	29.2	5260	7940
Ind 06	28.2	4780	7640
n	6	6	6
Mean	25.0	4503	6955
SD	5.95	1032	1619
%CV	23.8	22.9	23.3
%RE	-16.8	-9.93	-7.27

	QC Low (30.0) pg/mL	QC Med (5000 pg/mL)	QC High (7500 pg/mL)
Ind 01	13.5*	2440*	3680*
Ind 02	28.4	4970	7520
Ind 03	23.6	4640	7270
Ind 04	26.8	4930	7680
Ind 05	29.2	5260	7940
Ind 06	28.2	4780	7640
n	5	5	5
Mean	27.2	4916	7610
SD	2.21	232	244
%CV	8.1	4.7	3.2
%RE	-9.2	-1.68	1.47

Even in the presence of internal standard for the duration of the extraction, Individual 01 does not demonstrate acceptable recovery

Re-assessment of Recovery





Hypothesis – A matrix component present in Individual 01 is impacting the quantitation of our analyte.

A) Binding to the analyte prior to addition of the internal standard

B) Selectively binding or suppressing the analyte not the internal standard.

Deciding on a strategy

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Acquire new individuals and repeat the assessment

- Resolian preferred option
- Rare matrix, lengthy lead time
- No pre-dose samples collected as part of clinical study
- Would lead to delay of sample analysis (samples already collected)

Assess parallelism using study samples

- A linear response in diluted samples would demonstrate a lack of matrix effects impacting quantitation

Think outside the box – sample by sample assessment of recovery

- Analyse sample to obtain reportable value
- Re-analyse on a second occasion after sample spiked with known conc. of analyte, giving a theoretical concentration (X pg/mL)
- Provided theoretical concentration was reached (± a determined %RE) on re-analysis, the original result would be deemed valid and reported

Deciding on a strategy

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Strategy 01 - Acquire new individuals and repeat the assessment

Strategy 02 - Assess Parallelism using study samples

Strategy 03 - Sample by sample assessment of recovery

Lessons learned and recommendations

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Analysing individuals rather than a pool helped to uncover an issue that may have otherwise remained un-identified until sample analysis

Now we are performing matrix effects in spiked individuals (ICH M10 strategy)…
Do we still need to assess recovery during method validation?

- Validate methods prior to collecting samples wherever possible
- Don’t ignore an analytical issue just because it sneaks inside your acceptance criteria
- When determining a solution to a problem, the wider context must be considered (sample numbers, expected concentration, timelines)

Thank you for listening

Are there any questions?

Acknowledgements

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