ICH M10 – Bioanalytical Method Validation
Industry Case Studies

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ICH M10

- Objective of ICH M10? To become a bioanalytical guideline useful worldwide
- How has ICH M10 been developed? By gathering feedback from all professional sectors, across all geographies
- Potential pitfalls - What to avoid to make a truly harmonized and useful guideline?
- Foundation of current guideline & future challenges

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Agenda

- Method Development
- Internal Standard
- Stability
- Matrix Effects
- ISR
- Documentation

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Method Development

- Does not require extensive record keeping
- The applicant should record the changes to procedures, as well as any issues and their resolutions to provide a rationale for any changes made to validated methods
- No formal report
- Reduce validation?
- Experiments during method development?
  - Recovery?
  - WBS?

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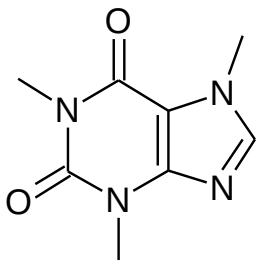
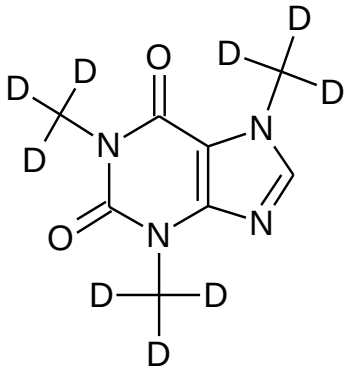
Internal Standard

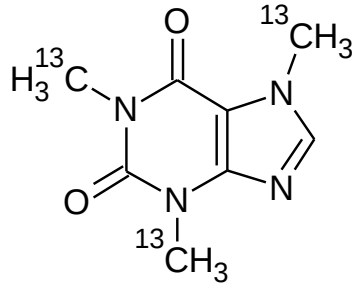
- Recommendation to use Stable Isotopically Labelled Internal Standard
- Test substance added to all samples at a constant concentration to correct for experimental variability
- SIL IS ‘Gold Standard’
- Better correction variability/matrix effects
- Custom synthesis: lengthy and expensive



Caffeine



Caffeine – D_9



Caffeine – $^{13}C_3$

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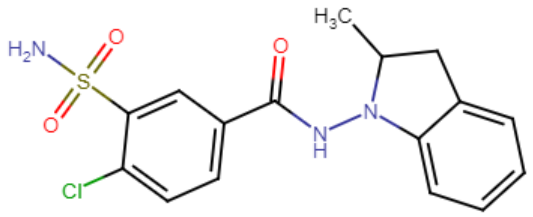
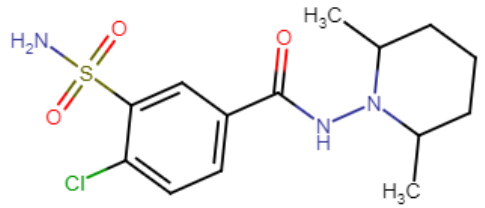
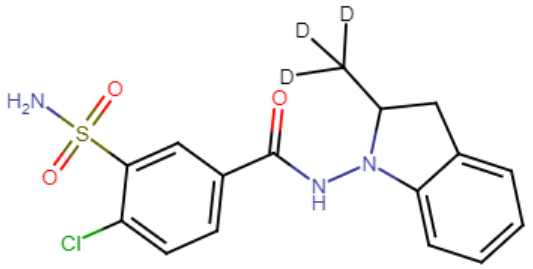
Internal Standard



Indapamide



Clopamide
10 mg $150



Indapamide-d_3
10 mg $1400

- Clopamide:
  - OK for fasted samples
  - High (but acceptable) variability for fed conditions
  - ISR: 70.59%

- Indapamide-d3:
  - Low variability of the assay
  - ISR: 96.05%

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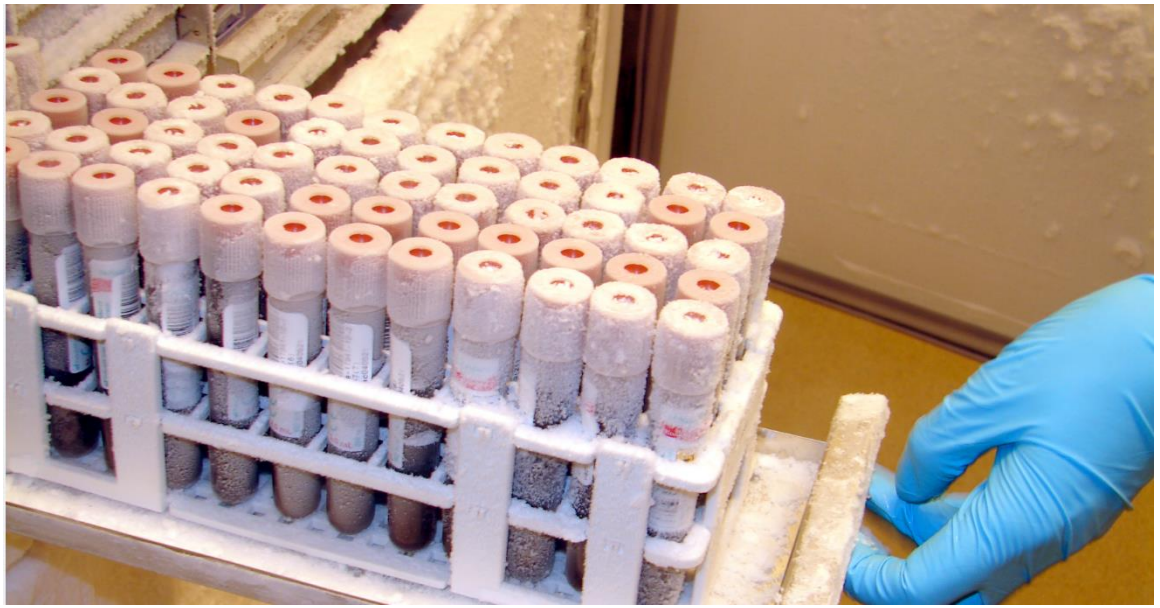
Stability

- Stock and working solutions (IS)
- Matrix:
  - Freeze / thaw in matrix
  - Short term
  - Processed sample
  - Long term
  - Whole blood
- Reinjection reproducibility



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Stability of Stock and Working Solutions

- If the **reference standard** expires, or it is past the retest date, the stability of the stock solutions made previously… are defined by the expiration or retest date established in the stock solutions

- The routine of making stock and working solutions from reference standards solely for extending the expiry date of the reference standards is not acceptable

Exp: 1st May

Prep: 30th Apr
Exp: 30st May

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Stability of Stock and Working Solutions

- Price

Octreotide
300 € /mL

Ramipril - Gluc
242 € /mg

R-Chlorphenyramine
360 € /mg

- Delivery timelines

Eslicarbazepine
34 days

Esketamine
30 days

Moxifloxacin
30 days

Oxycodone
25 days

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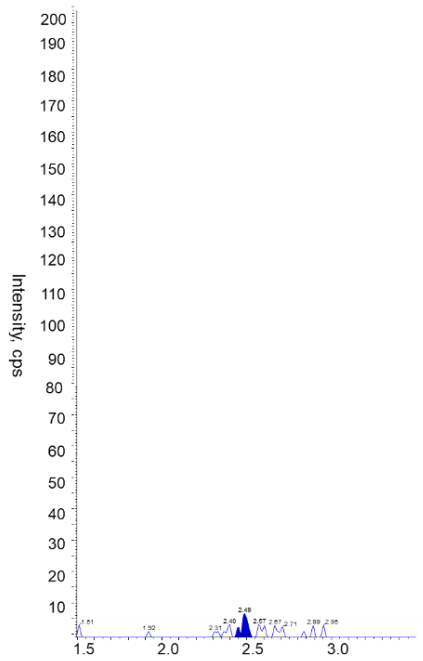
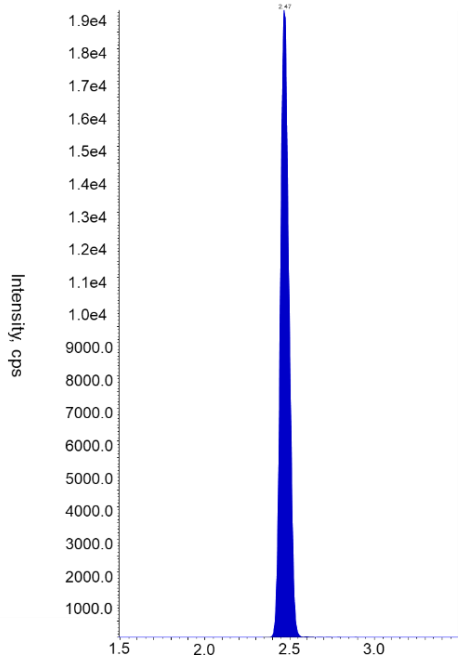
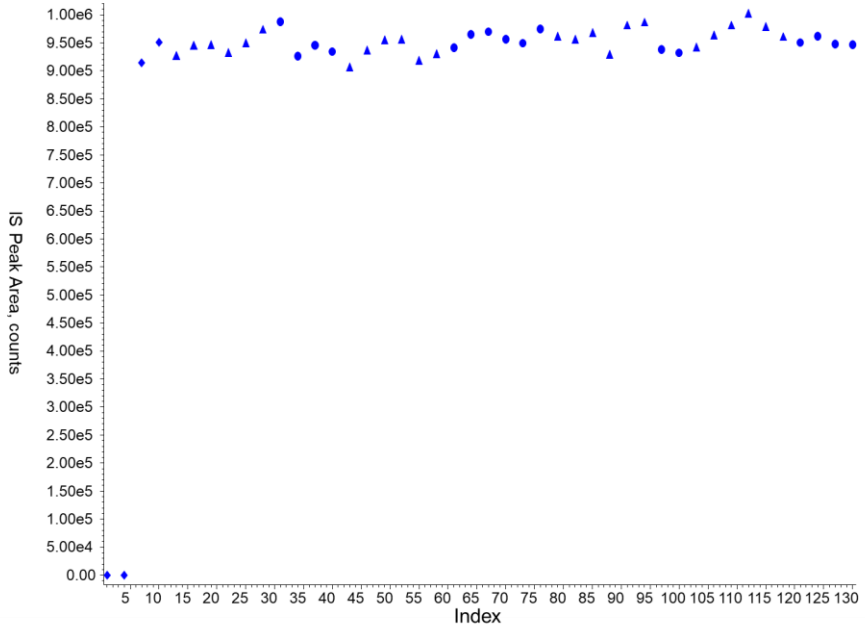
Stability of Internal Standard

- If no isotopic exchange occurs for the SIL IS under the same storage conditions as the analyte, no additional stability assessments for the IS are necessary

- A CoA is not required for the IS as long as the suitability for use is demonstrated







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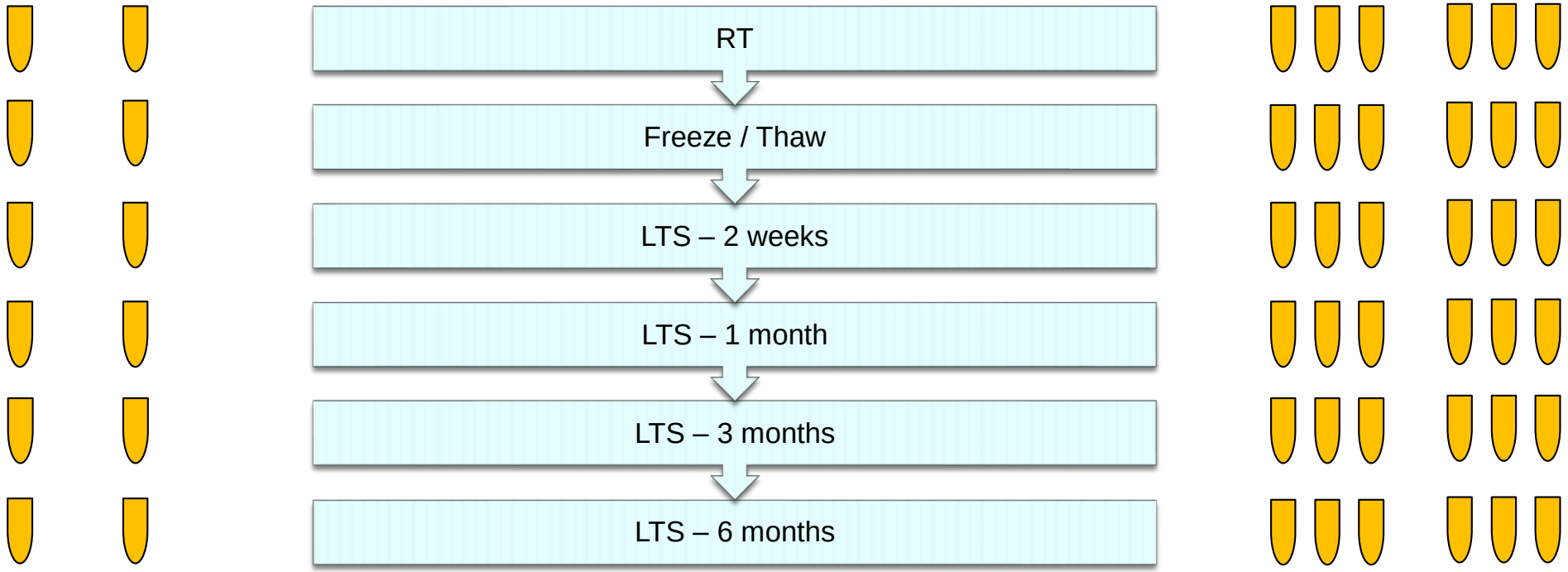
Stability in Matrix

- A minimum of three stability QCs should be prepared and analysed per concentration level/storage condition/timepoint



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Stability with Co-medications

- If multiple analytes are present in the study sample, (studies with a fixed combination or due to a specific drug regime) the stability test of an analyte in matrix should be conducted with the matrix containing all analytes

- Unrealistic
- Co-meds appear as CT progresses
- Co-medication affecting stability?
- Only if co-med affects matrix

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Stability with Co-medications

Fluvoxamine	Pregabalin	Duloxetine	Quetiapine	Vit D_3	Valsartan
Sotalol	Nitrendipine	**Esketamine**	Venlafaxine	Paroxetine	Allopurinol
Lamotrigine	Ibuprofen	Escitalopram	Folic Acid	Anafranil	Duloxetine
Levothyroxine	Venlafaxine	Amlodipine	Fluoxetine	Losartan	Clomipramine
Ursodiol	Atorvastatin	Methotrexate	Bisoprolol	Acetyl Salicylic acid	Metformin

- Capecitabine:
  - >100 Co-meds
  - Co-medication value higher than price of study

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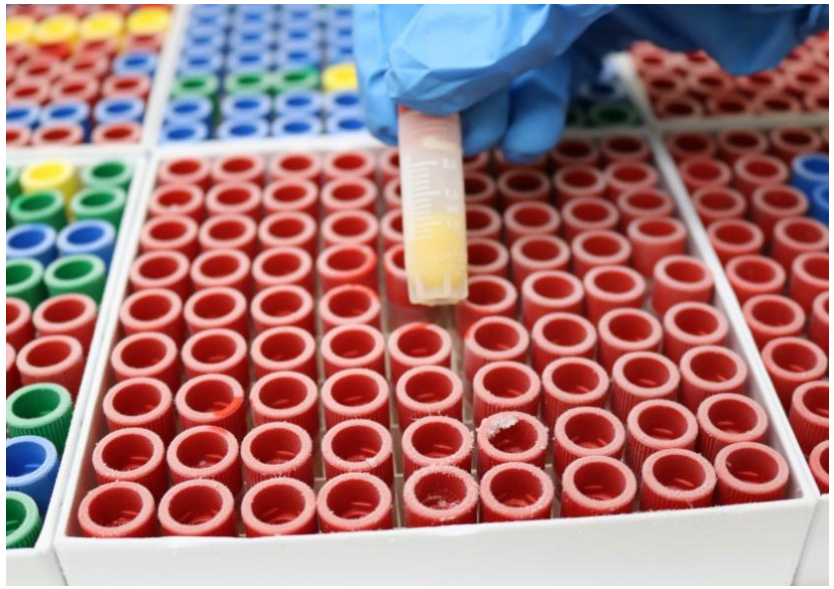
Stability above the ULOQ

- If the concentrations of the study samples are consistently higher than the ULOQ of the calibration range, the concentration of the high stability QC should be adjusted to reflect these higher concentrations

- Impact on drug development timelines
- Sample analysis report not completed
- Stability issues normally seen at lower concentrations
- Scientific reasoning



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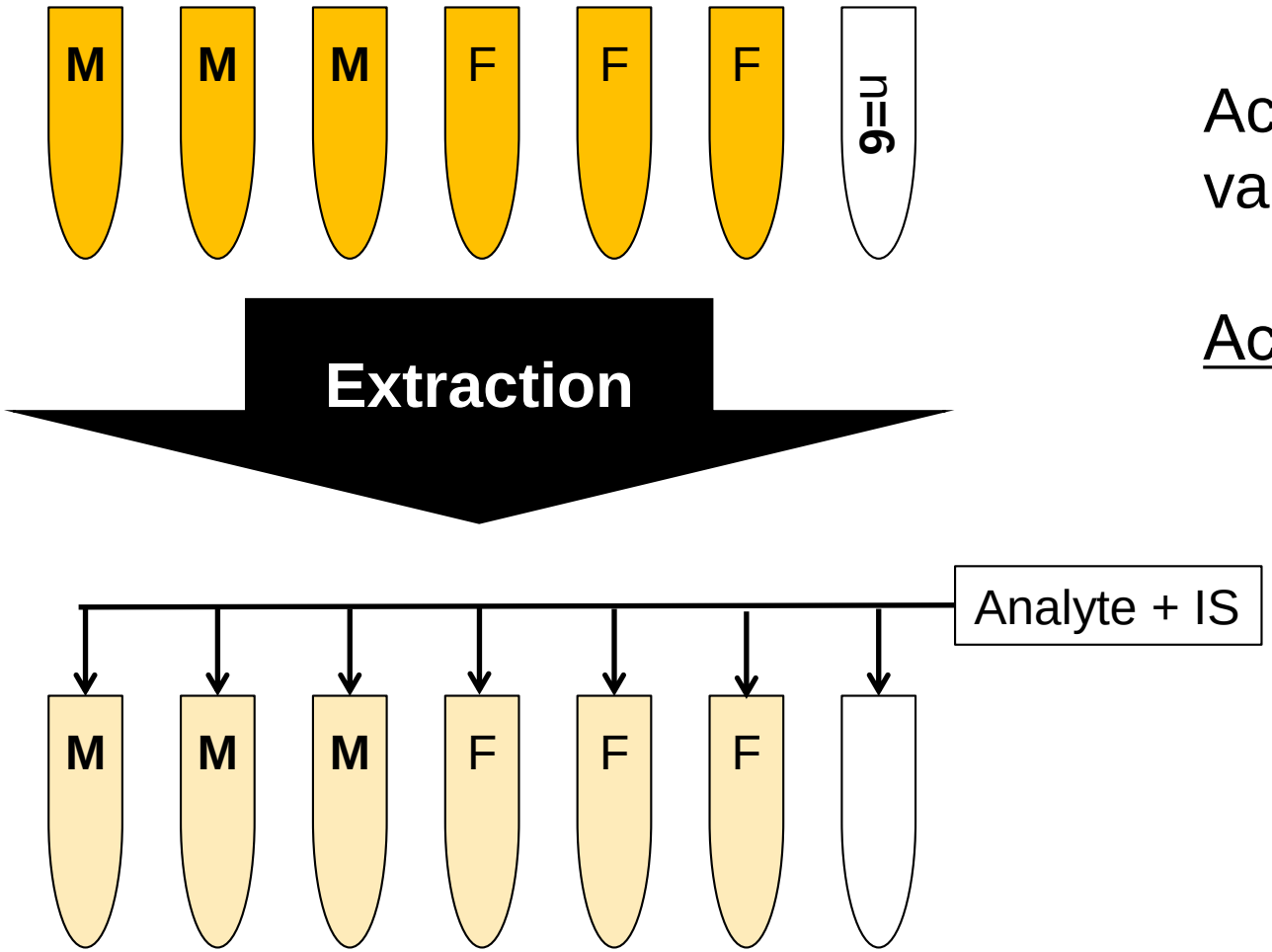
Matrix Effects



Acceptance criteria based on overall variability

Acceptance criteria: CV ≤15%

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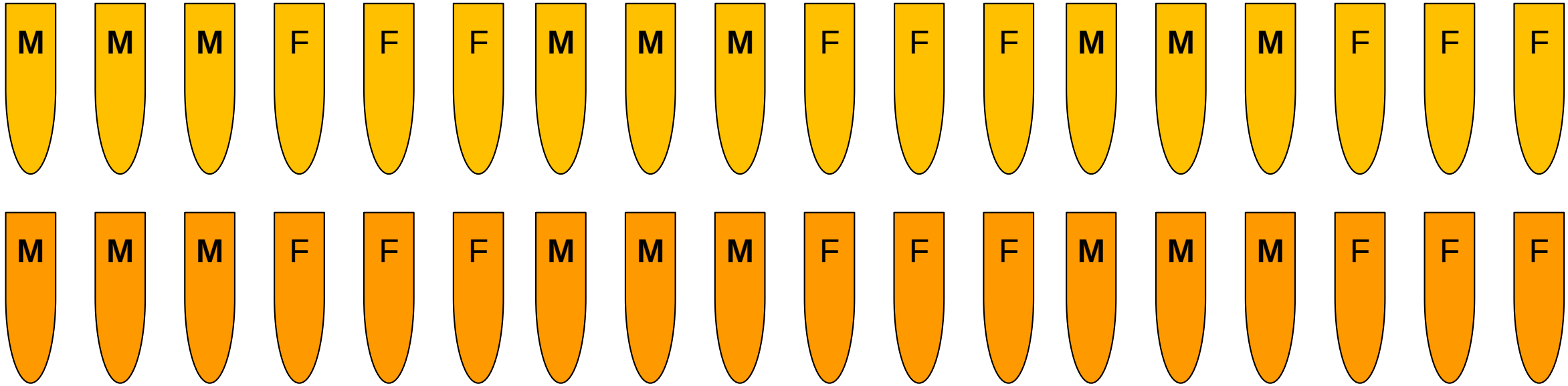
Matrix Effects

- The matrix effect should be evaluated by analysing at least 3 replicates of low and high QCs, each prepared using matrix from at least 6 different sources / lots



- Too much matrix
- Rare matrices? Preclinical studies?

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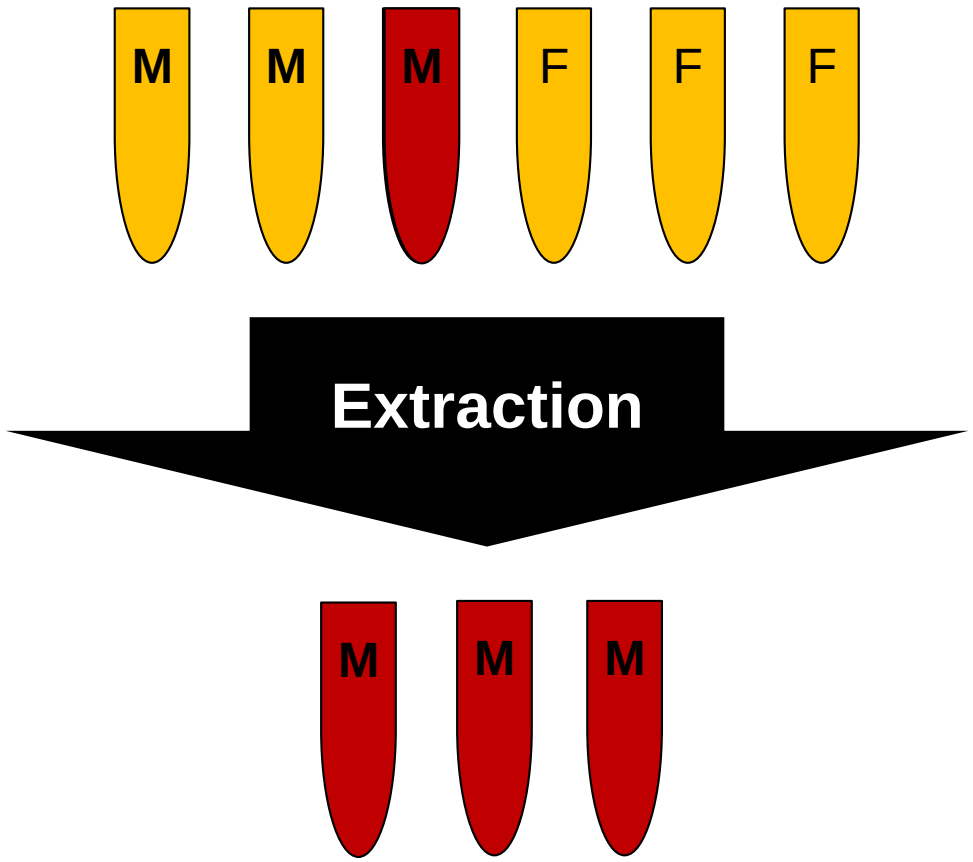
Matrix Effects



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ISR

- Runs on different days using the same bioanalytical method
- Minimum:
  - 10% of first 1000 samples + 5% of rest
  - Objective criteria
  - Coverage of PK profile (Cmax and elimination)
- ISR number?
- Post-validation assessment of reproducibility and robustness
- Very low failure rate (1.1%)
- 5% + cap?

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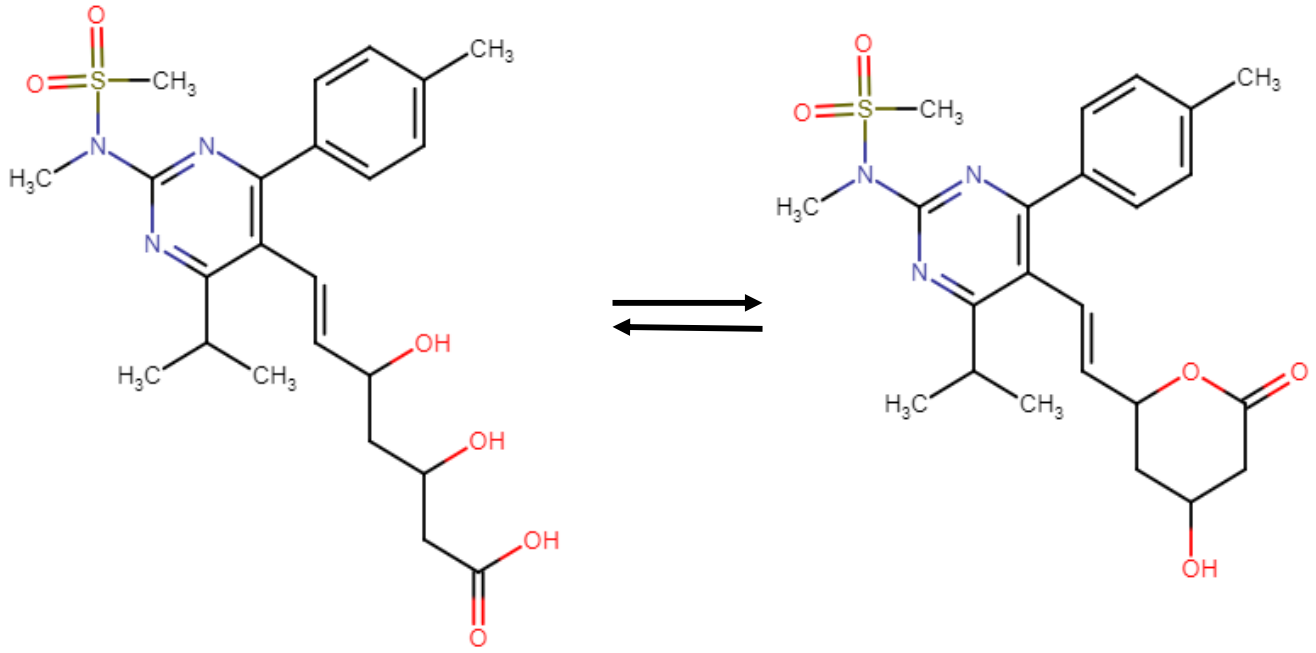
ISR

- Metabolite interaction or interconversion
- Matrix Effects
- Protein binding
- Recovery
- Sample inhomogeneity (urine)



Rosuvastatin

- Bias at elimination stage
- Lactone ↔ COOH
- Observed with fewer samples

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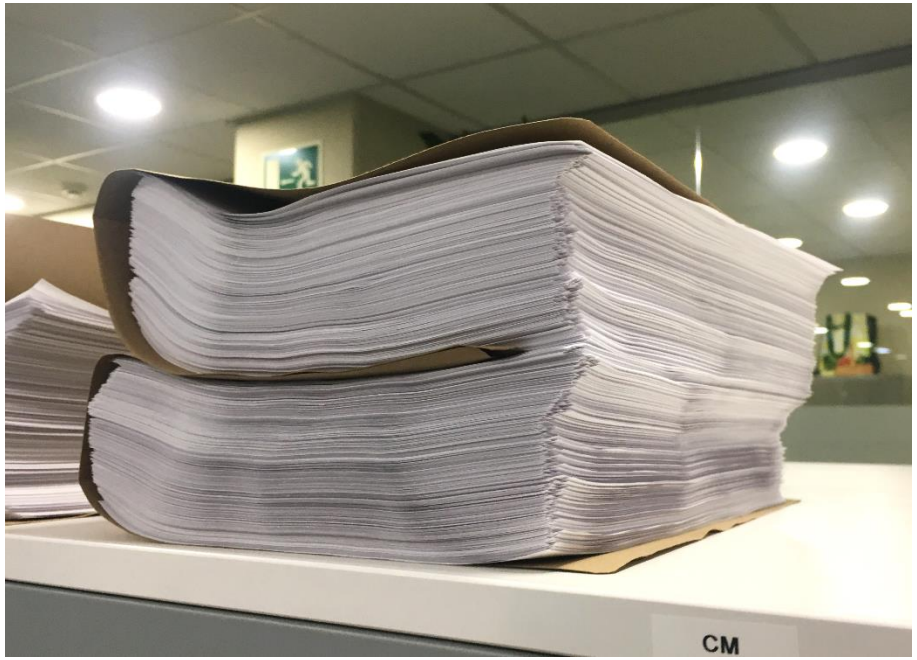
Bioanalytical Report

- IS plots for each analytical run (failed included)
- Original and reintegrated chromatograms and initial and reintegrated results
- Reason for reintegration
- 100% of chromatograms
- SOP for reintegration



- 100% of run summary sheets (accepted and failed), including calibration curve, regression, weighing factor, analyte and IS responses and retention factors and dilution factors if applicable

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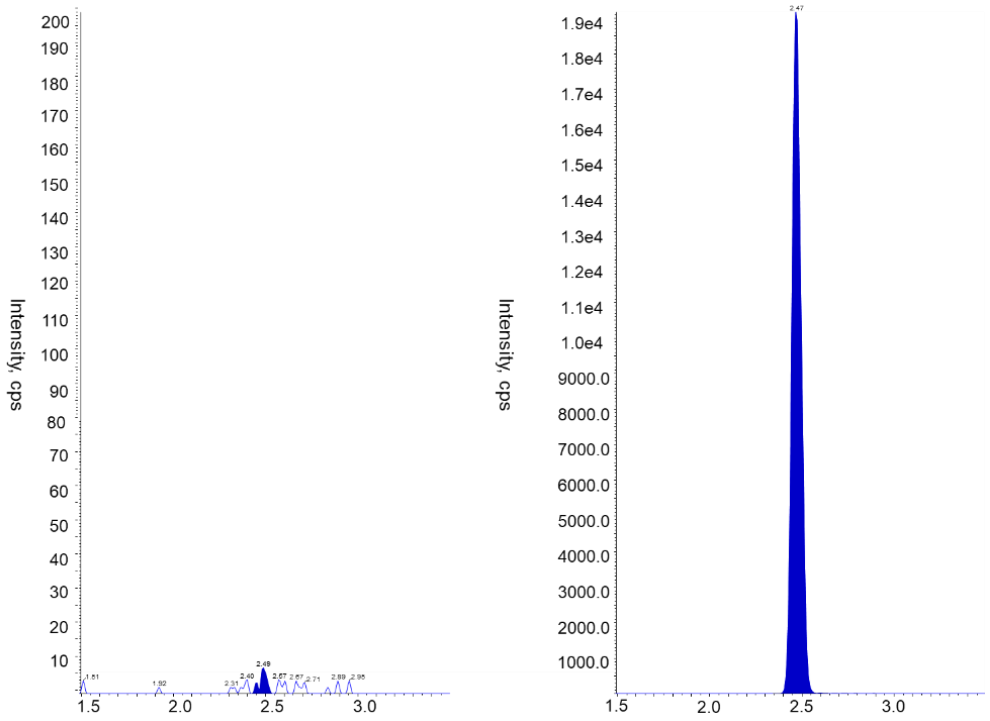
Bioanalytical Report

- Standard study:
  24 subjects, 2 periods, 20 timepoints
  1500 chromatograms (Analyte + IS)

- Multiple analyte:
  Atorvastatin (*o*-OH and *p*-OH)
  24 subjects, 2 periods, 20 timepoints
  4500 chromatograms (Analyte + IS)

- Large studies:
  96 subjects, 2 periods, 20 timepoints
  6000 chromatograms (Analyte + IS)



Original + reintegrated

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Conclusion

- Well written guide
- Allow to streamline processes
- Scientific knowledge
- Points for discussion

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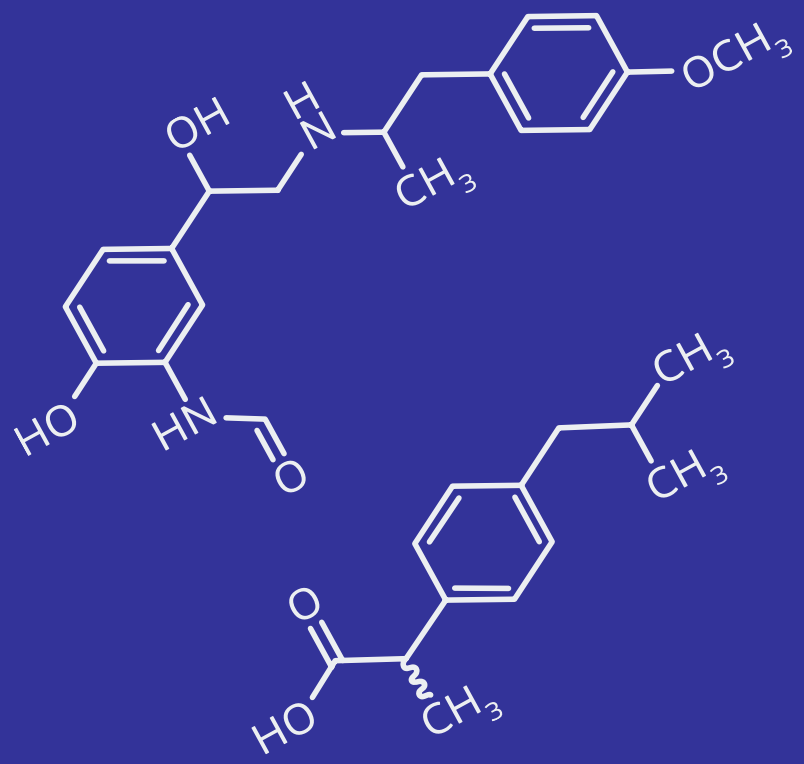

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Many thanks for your attention

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