Principles of method validation

With a focus on LC-MS methods.

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RESEARCH FELLOW

10th December 2025, Rome

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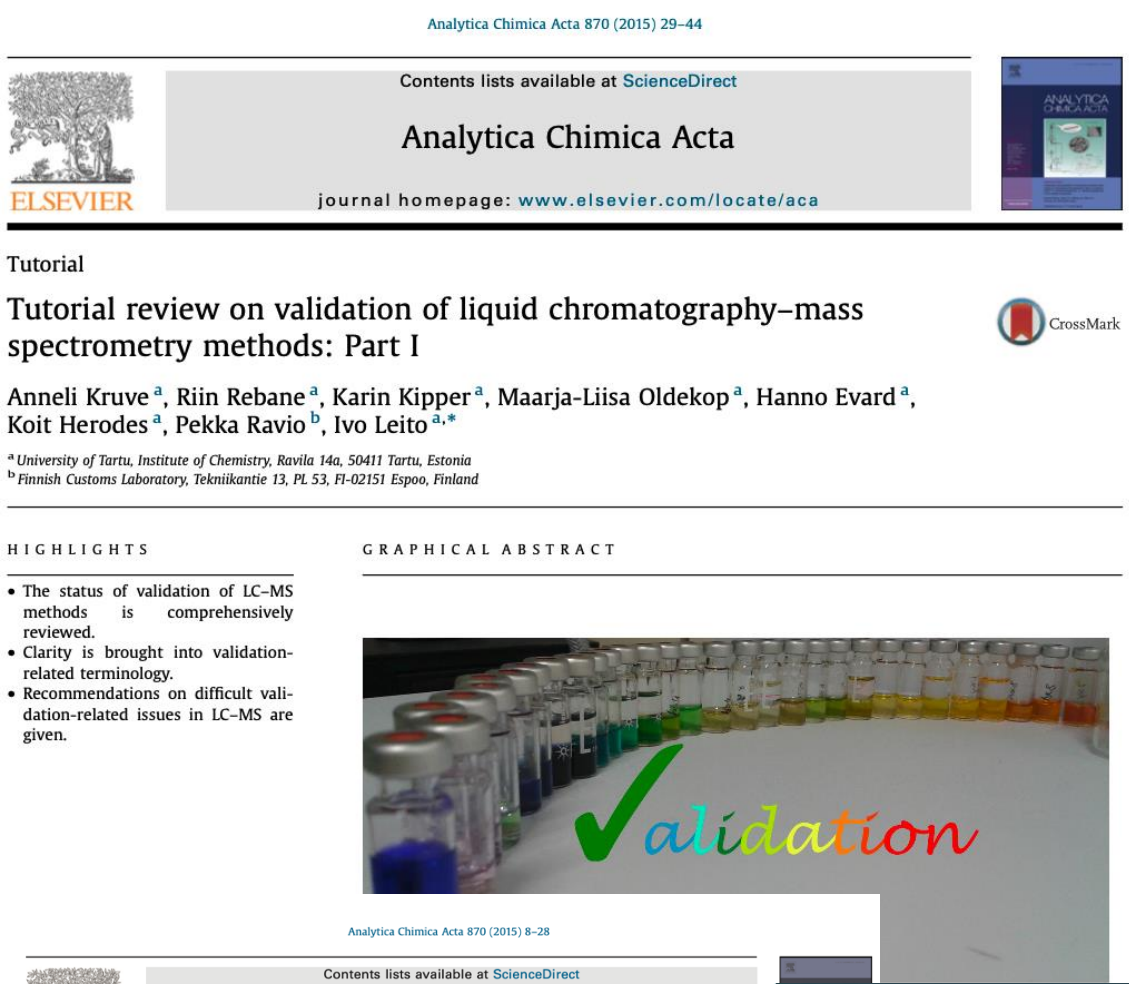
Today's journey

What is method validation?

 *Why do we validate methods?*

 *How should we validate methods?*



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Tutorial

Tutorial review on validation of liquid chromatography–mass spectrometry methods: Part I

Anneli Kruve [a], Riin Rebane [a], Karin Kipper [a], Maarja-Liisa Oldekop [a], Hanno Evard [a], Koit Herodes [a], Pekka Ravio [b], Ivo Leito [a,*]

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HIGHLIGHTS

- The status of validation of LC–MS methods is comprehensively reviewed.
- Clarity is brought into validation-related terminology.
- Recommendations on difficult validation-related issues in LC–MS are given.

GRAPHICAL ABSTRACT

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Tutorial

Tutorial review on validation of liquid chromatography–mass spectrometry methods: Part II

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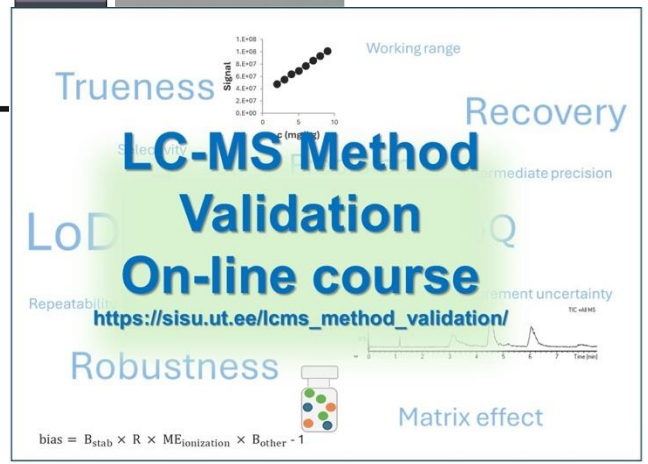

Background

10 years from the publication of a tutorial review.

10 years of LC-MS validation MOOC.

Trainings and personal experiences with LC-MS validation.

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Method validation vs method development

LC-MS methods

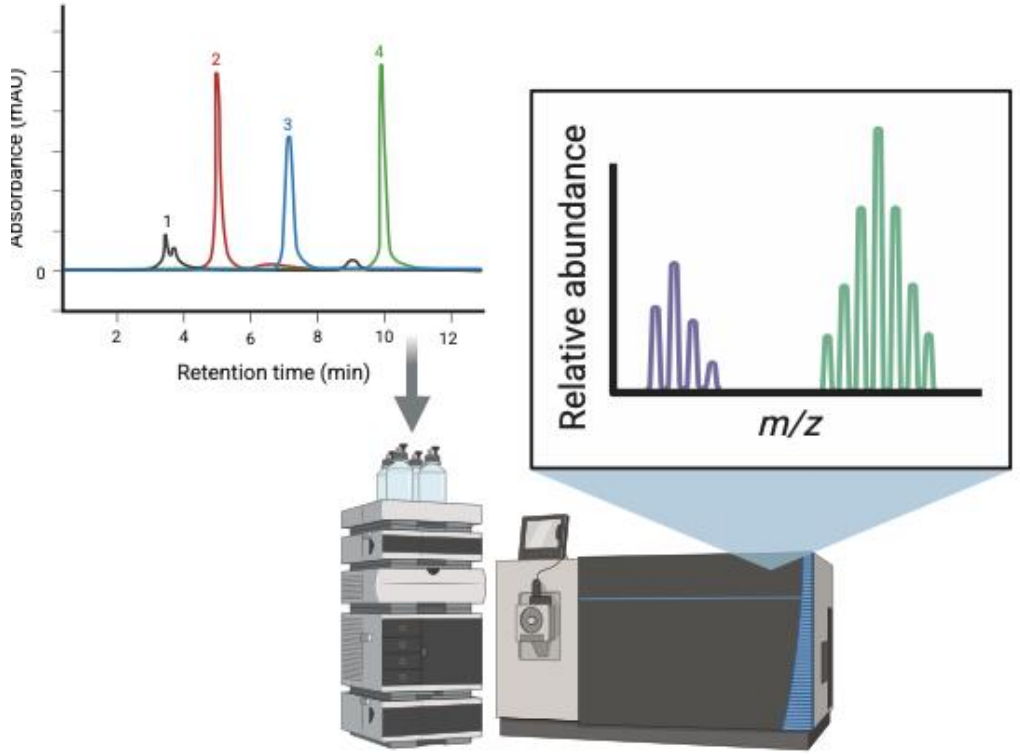
Analytically excellent

but sometimes LoQ levels not low enough

Scientifically relevant

but poor validation parameters (as sometimes seen in scientific publications)

- LC-MS methods are of high sensitivity, therefore also of **high vulnerability**.



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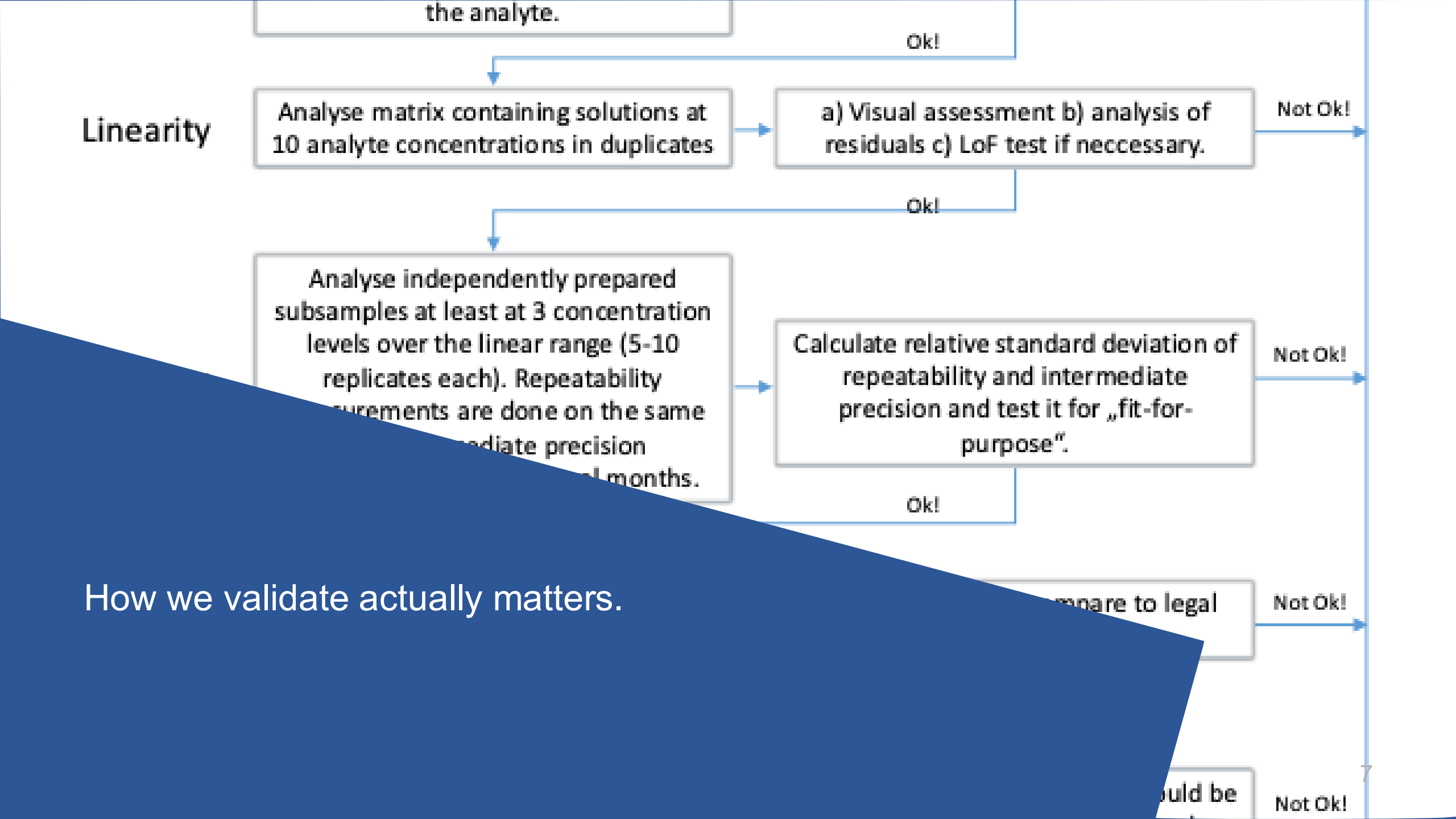
	🛠️ Method Development	✅ Method Validation
Goal	To build a method	Fit-for-purpose evidence.
Focus	Optimization	Testing parameters
Nature	Iterative, experimental	Structured, documented, and systematic
Output	Draft SOP	Validation Report
Risk	Minimizes technical issues of the method.	Minimizes the risks of presenting the wrong result.

With LC-MS methods, we often times go back and forth between them, therefore...



How we validate actually matters.

Validation parameters

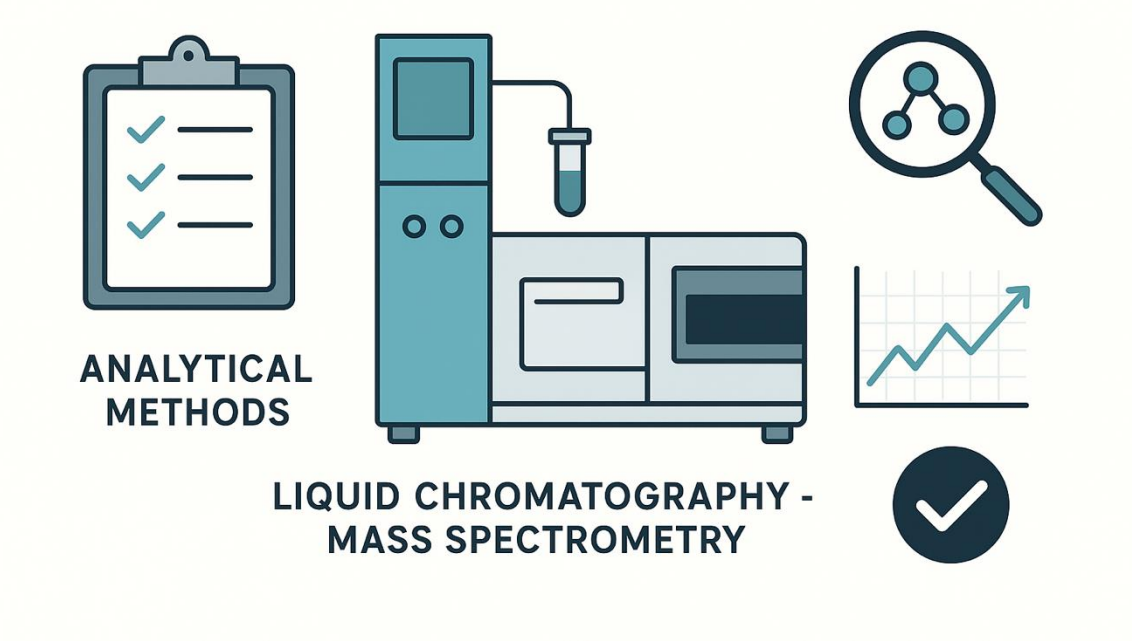
+ Allows to make validation traceable.
+ All crucial aspects of method are then taken into account.

…however…

- Doing validation as a checklist can give false assurance (especially for people with less experience).
- Data can be misleading.

Fitness for purpose

- To measure the right compound
- Assign correct concentrations
- Make sure that results don’t vary over time or conditions
- Assure that results are comparable, traceable and defensible



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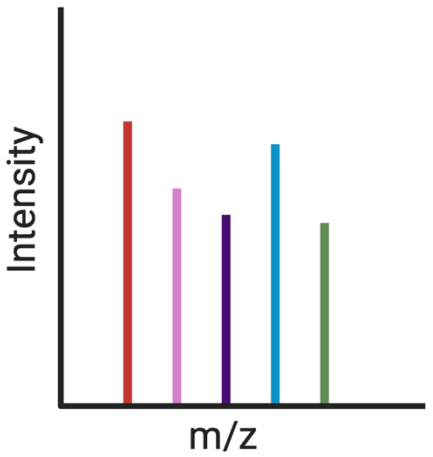
Introducing risks in LC-MS analysis

- Identity risk
- Matrix risk
- Quantification risk
- Variability risk
- Decision risk



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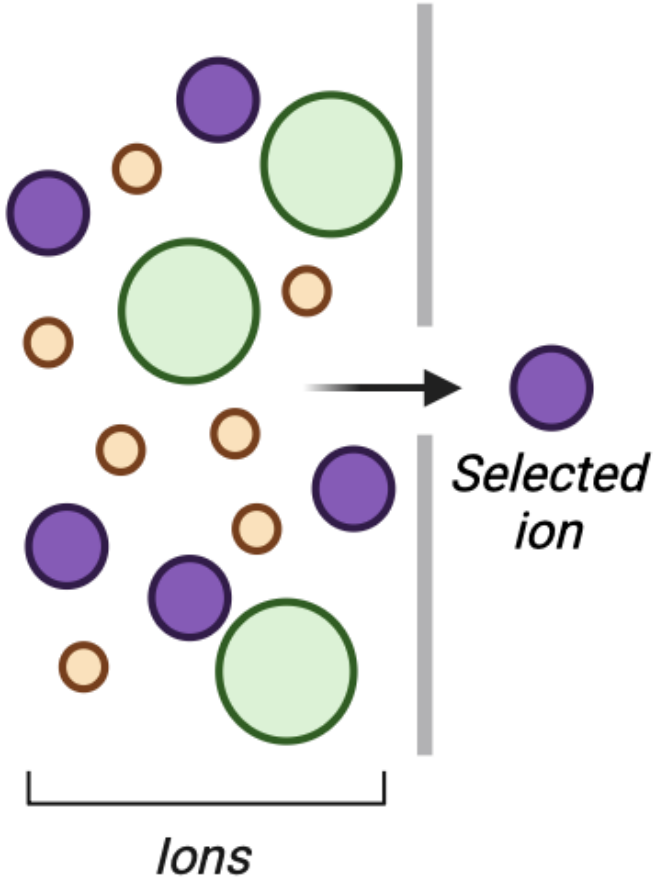
Identity risk 🎯

To measure the right compound



Tools:

- retention time matching
- using MS/MS transitions
- analysing ion ratios

Output: Confidence in molecular assignment

Validation parameter: selectivity, *also stability*

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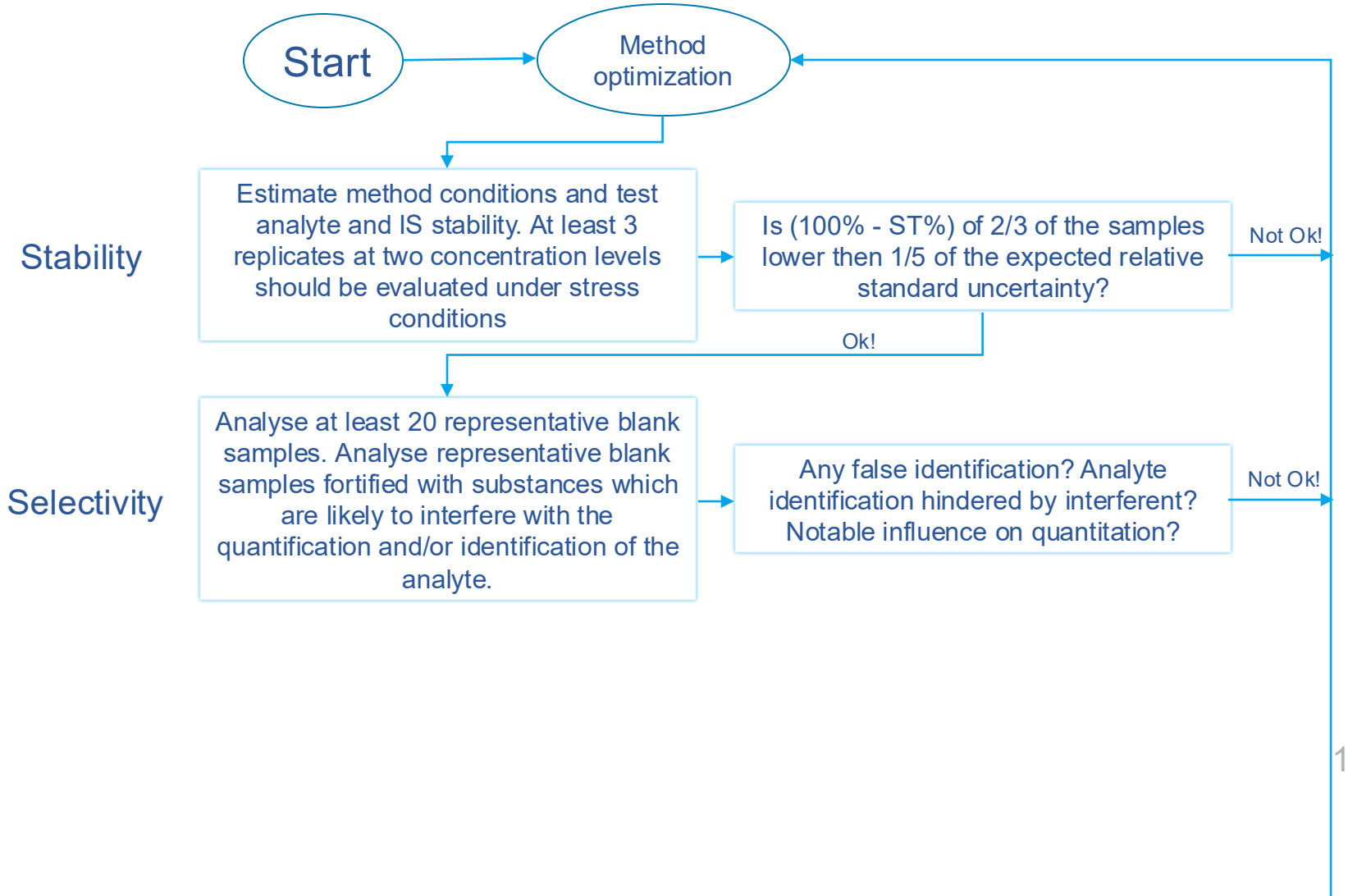
Validation workflow



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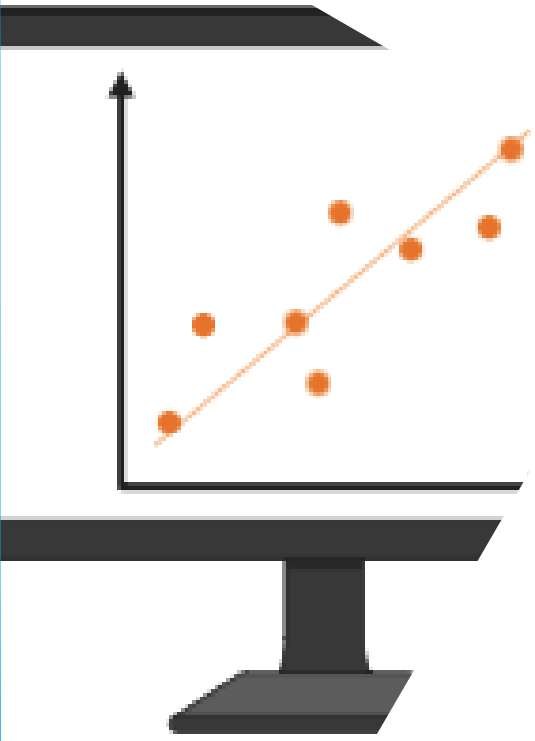
Quantification risk

Assign correct concentrations

Tools	
Calibration curve	Post-column infusion
Post-extraction addition	Matrix variability experiments
Range-specific precision and trueness experiments	Detection capability



Output: Confidence that any reported concentration is accurate within the defined linear and working limits.

Validation parameters: linearity, precision, trueness, LoD/LoQ

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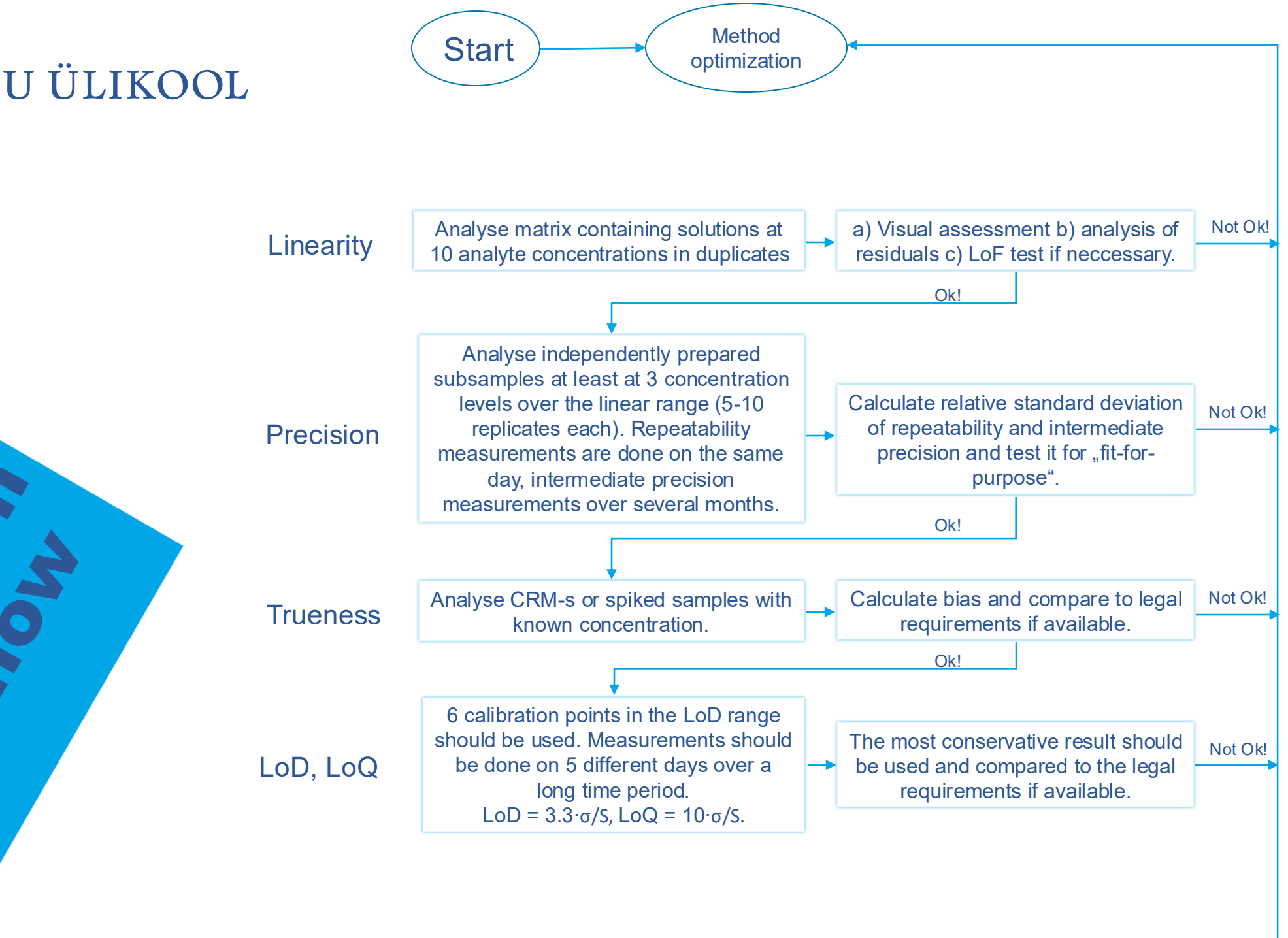
Validation workflow



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Variability risk

Make sure that results don't vary over time or conditions

Tools:

- Replicate analysis
- Analysis under varying conditions (short and long term)
- Quality control replicates

Outcome: Confidence that results are comparable under slightly different (but permitted) conditions.

Validation parameters: precision, stability, robustness

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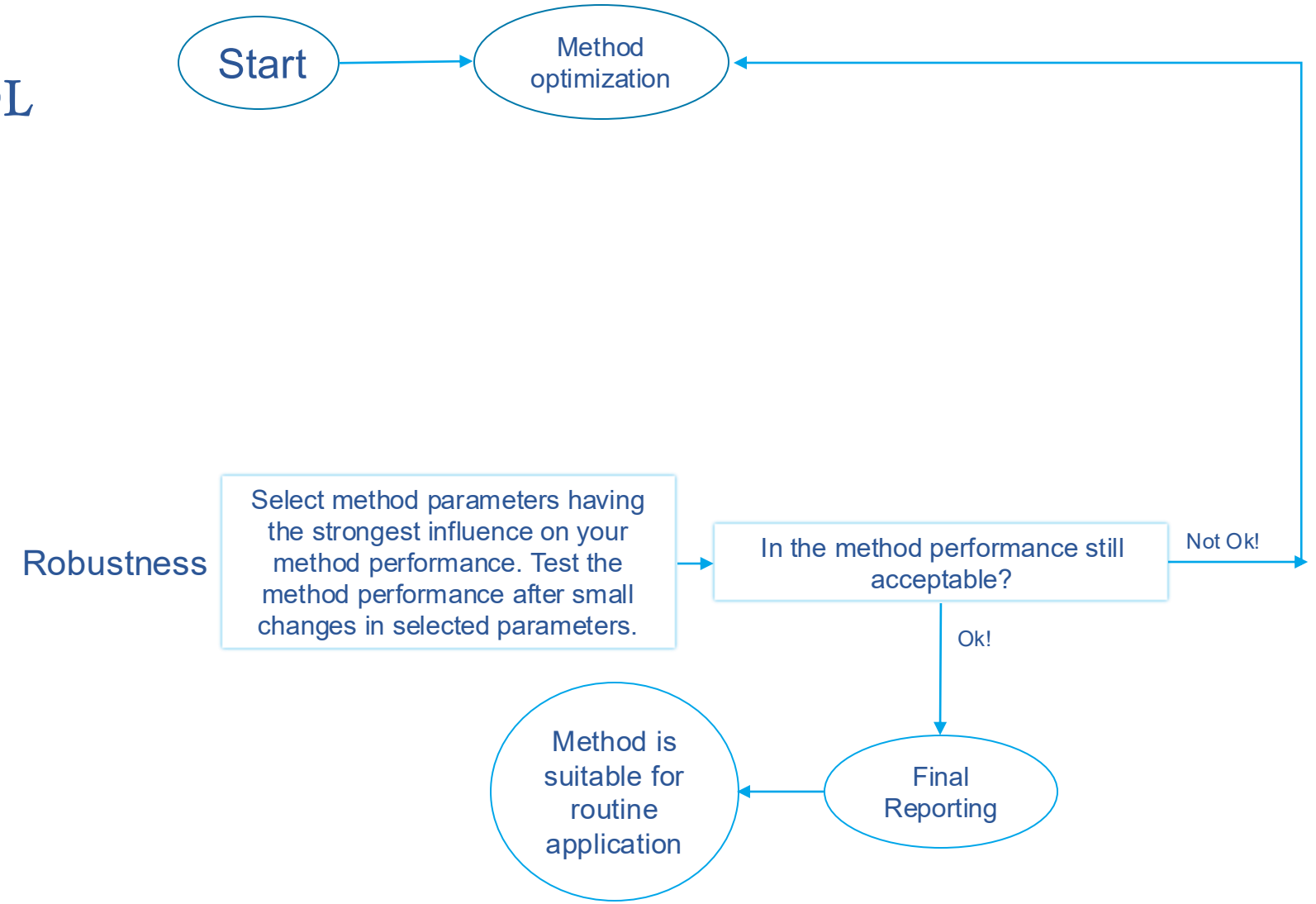
Validation workflow – final stretch



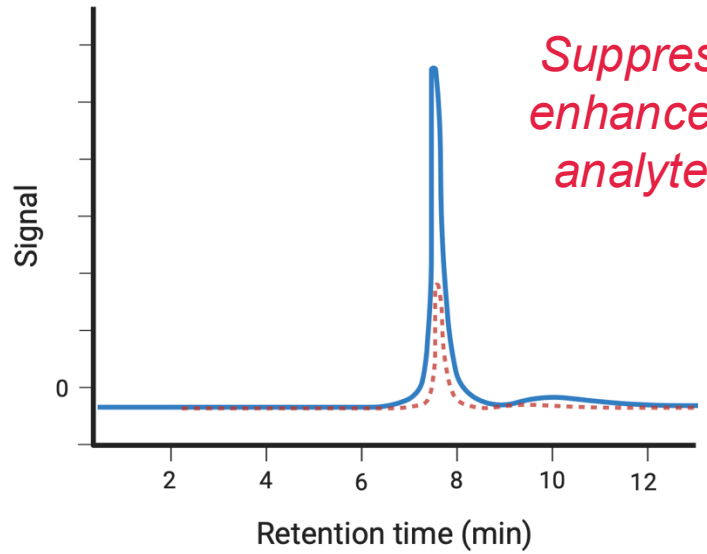
Matrix induced risks

Assign correct concentrations with all the sample matrices

Can contribute to all other risks: identity, quantification and variability.

Output: method will remain accurate for real samples

Validation parameter: trueness



Suppression or enhancement of analyte signal

Matrix variability between sample types or lots

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Decision risk ⚖️

Assure that results are comparable, traceable and defensible

Output: Known confidence allows the user to confidently compare the result to a legal limit or therapeutic threshold, making a risk-informed decision.

Validation parameters: measurement uncertainty

Summary

Checklist Parameter (The "Tool")	The Real-World Risk (The "Goal")
Selectivity	**Identity Risk** (To measure the right compound)
Linearity, LoD/LoQ, Trueness	**Quantification Risk** (Assign correct concentrations)
Precision, Stability, Robustness	**Variability Risk** (Results don’t vary over time or conditions)
(Synthesis of all others)	**Decision Risk** (Assure results are traceable and defensible)

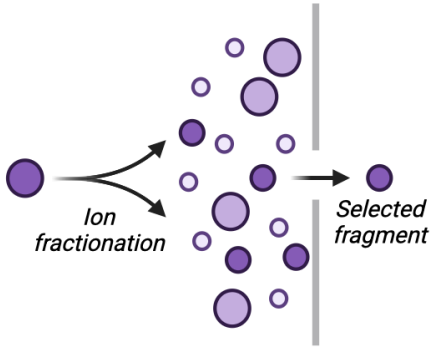
And everything in between…

e.g. where a checklist approach might fail us

Identity risk + matrix

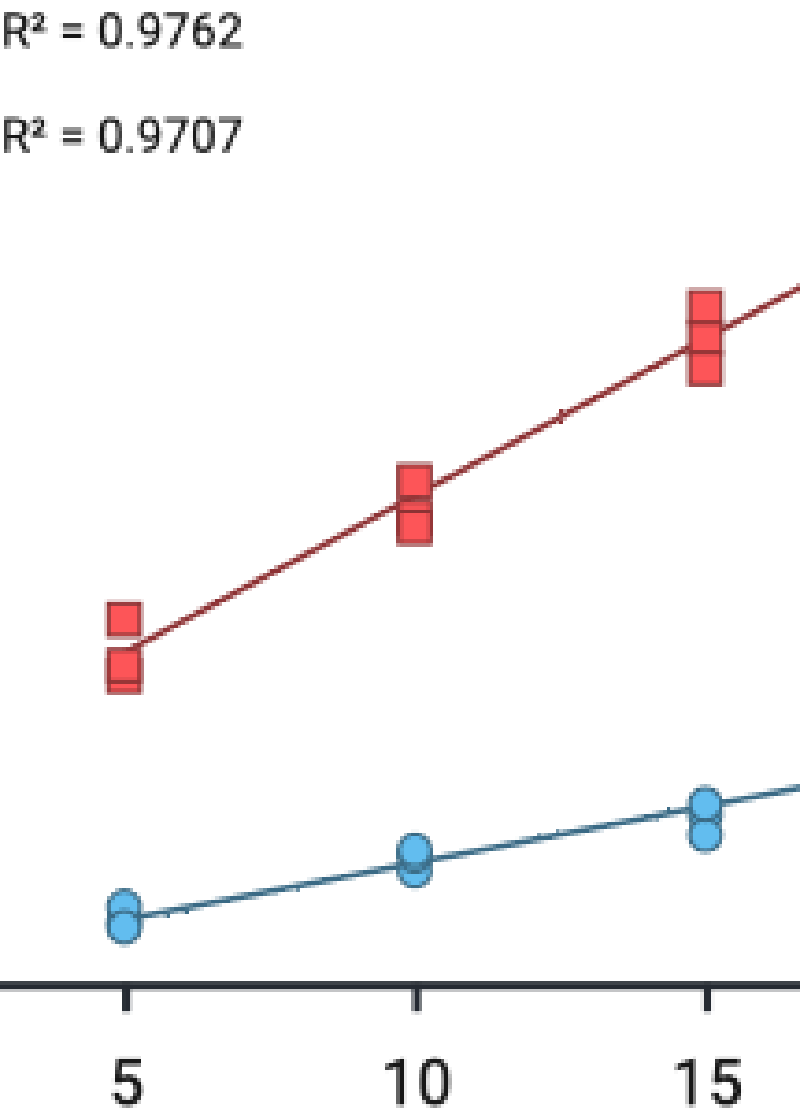
Tools:

- Blank matrices
- Influence checks
- Fragmentation patterns



The matrix can contain compounds that co-elute and share MS/MS transitions, giving a false-positive signal. The ultimate goal is to confirm no co-eluting compounds have an effect on the analysis.

Quantification risk + matrix



Tools:

- Replicate low-level spiked samples in real matrices
- Avoid S/N-based assumptions
- Matrix-matched calibrations

Reliable Limits: Establishing the LoQ based on achievable precision and trueness in the matrix, ensuring reliable results.

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Variability risk + quantification

Tools:

- Quality control replicates
- Inter-day variation experiments
- Proficiency testing

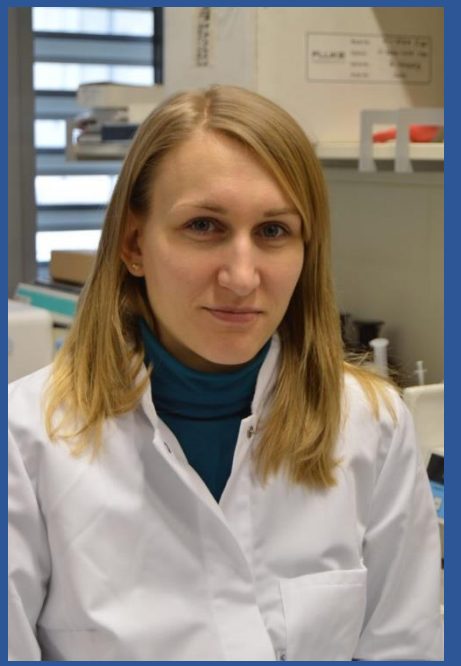
The data is used to estimate the expected uncertainty measurements. This is the quantitative expression of the doubt about the result, essential for the final Decision Risk.

🚀 Take home message:

Validation is not about a checklist – it is about ensuring the method produces results that support real scientific or regulatory decisions with known confidence.







Thank you!



Presentation

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