

Tutorial on estimating the limit of detection using LC- MS analysis

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About the articles

- Two part tutorial
 - Theoretical review of Limit of Detection (LoD)
 - Practical aspects of estimating LoD
- Focusing on estimation methods suggested by different organizations
- Focusing on liquid chromatography mass spectrometry (LC-MS)
 - Analysis methods are not equal

Limit of Detection

Limit of detection (LoD) – smallest amount or concentration of analyte in the test sample that can be reliably distinguished from zero

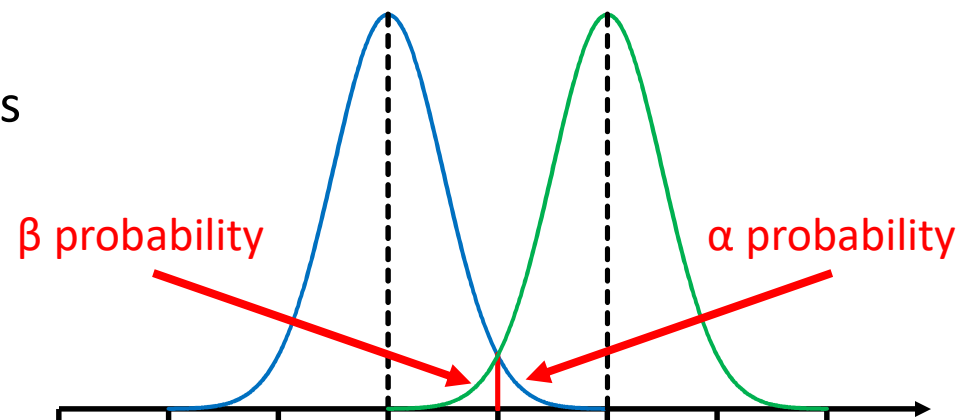
- Answers two separate questions:
 - Is the analyte detected in the sample?
 - How low concentrations can this analytical method detect?

LoD

- The general LoD definition is ambiguous
- Many different approaches to estimate LoD
 - Different standards and guidelines suggest different approaches
- Approaches make assumptions and simplifications

CC_α and CC_β

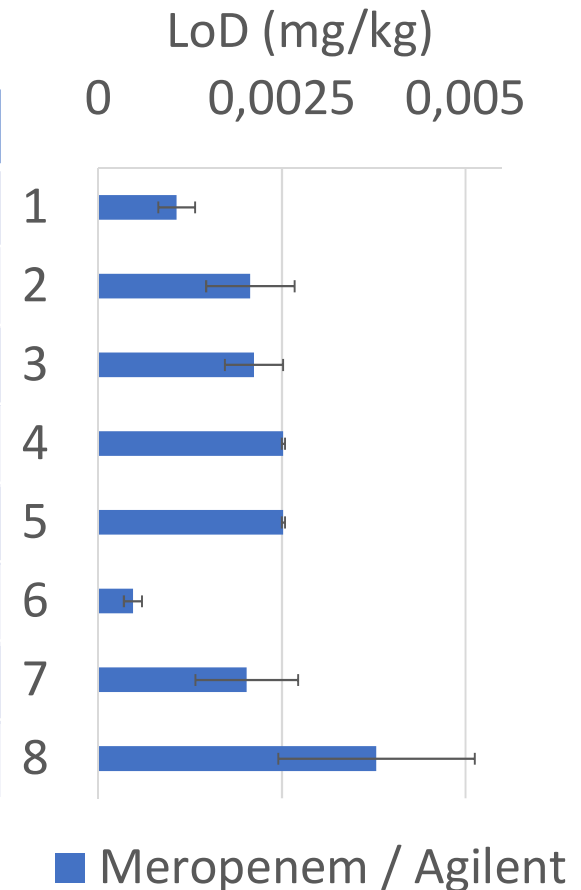
- False positive and false negative results in definition
- Estimation can be statistically complex
- LoD equal to CC_β



**LoD is not appropriate for critical decisions
(detected or not?)**

Differences in approaches

Description	Guideline
$LoD = I(\text{blank}) + 3 \times s(\text{blank})$	AOAC, Eurachem
$LoD = I(\text{blank}) + 4.65 \times s(\text{fortified})$	Eurachem
$S/N \geq 3$	ICH
"Cut-off" approach	Eurachem, NordVal
Visual evaluation	ICH
$LoD = 3.3 \times s(\text{blank}) / \text{Slope}$	ICH
$LoD = 3.3 \times s(\text{intercept}) / \text{Slope}$	ICH
$LoD = 3.3 \times s(\text{residuals}) / \text{Slope}$	ICH

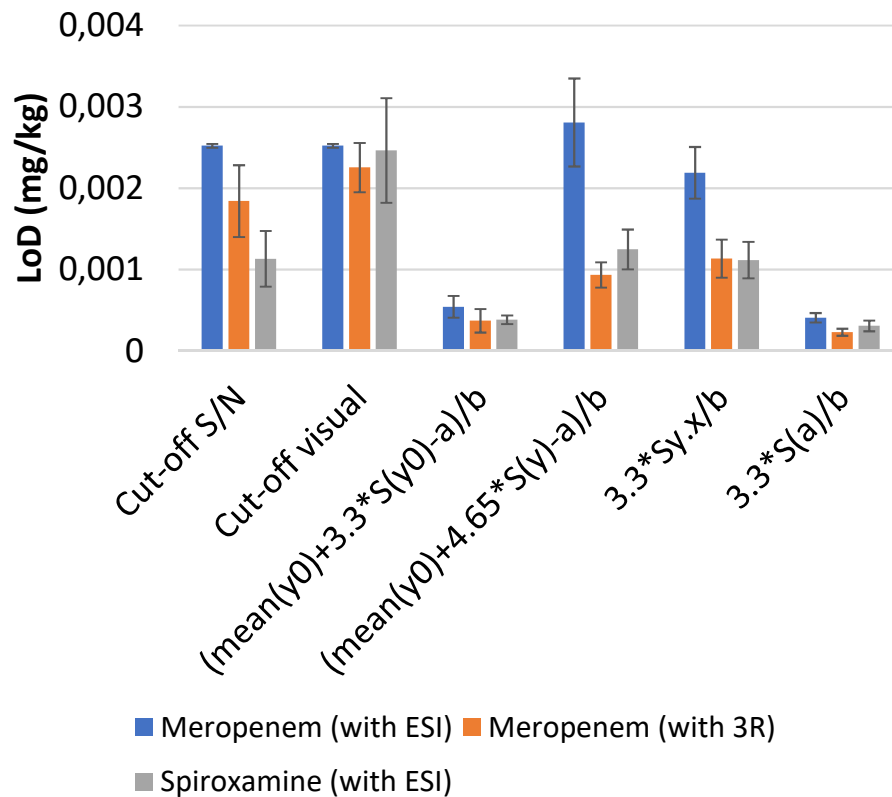


Goals of the experimental work

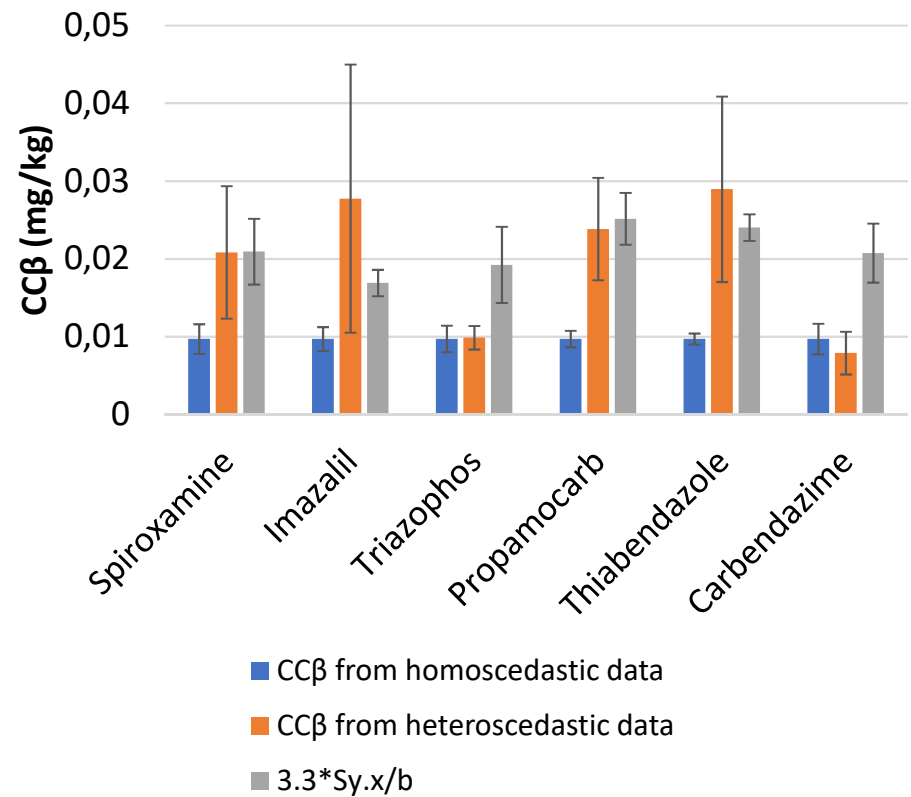
1. Compare LoD to CC_{β}
2. Compare estimates of different approaches
3. Study experimental design for LoD estimation
4. Study subjectivity of relevant tests
5. Change of LoD between days
6. Make practical suggestion for estimating LoD for MS methods

LC-ESI/MS/MS

- Different methods give similar results



- CC_{β} significantly different from LoD in some cases



Simulations, experimental design

- Calibration points below LoD used for estimation
 - Simulations in R with random number generator
 - 1000 calibration lines generated and LoD estimated
 - Standard deviation (stdev) of blank samples is 100 and slope is 5

$$LoD = 3.3 \times \frac{s}{Slope}$$

	CC α = 32.9 & CC β = 65.8	Stdev of slope	Average (LoD, stdev of residuals)	Average (LoD, stdev of intercept)
1	C = (0, 75, 100, 125, 150, 175, 200)	0.60	65 (± 22)	12.8 (± 1.6)
2	C = (0, 35, 60, 85, 110, 135, 160)	0.72	63 (± 23)	12.8 (± 2.0)
3	C = (0, 5, 25, 50, 75, 100, 125)	0.83	65 (± 24)	13.0 (± 2.3)
4	C = (0, 5, 10, 15, 20, 25, 30)	3.75	286 (± 7862)	38.1 (± 1068.2)

Between-days LoD and between-labs LoD

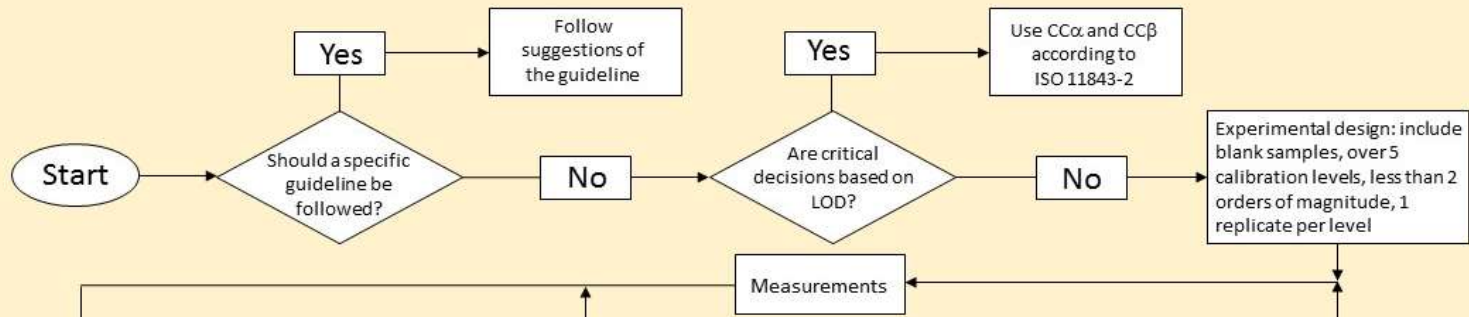
- Systematic approach or solution has not been offered
 - Depends on analytical method

ANOVA	p
Spiroxamine	0.62
Imazalil	0.64
Triazophos	0.21
Propamocarb	0.42
Thiabendazol	0.70
Carbendazim	0.60

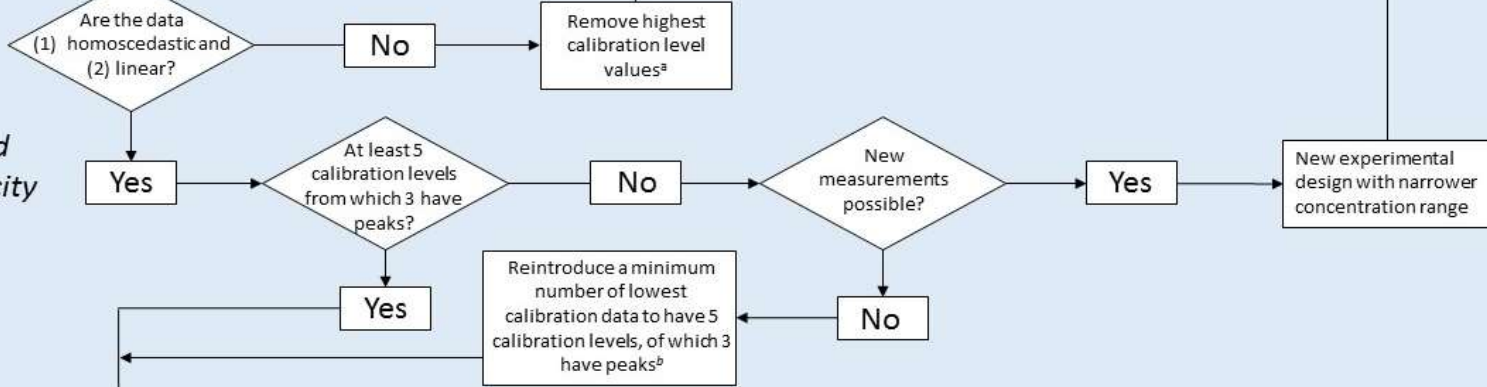
$$LoD_{btw-da} = mean(LoD) + 1.65 \times s(LoD)$$

	Within-day LoD	Between-days LoD	Between-labs LoD
Interpretation of results	1. LoD is critical 2. Variance of LoD between days is large	Suitable	Not suitable
Characterization of analysis method	Not appropriate (only approximately)	Suitable	For comparing analytical methods

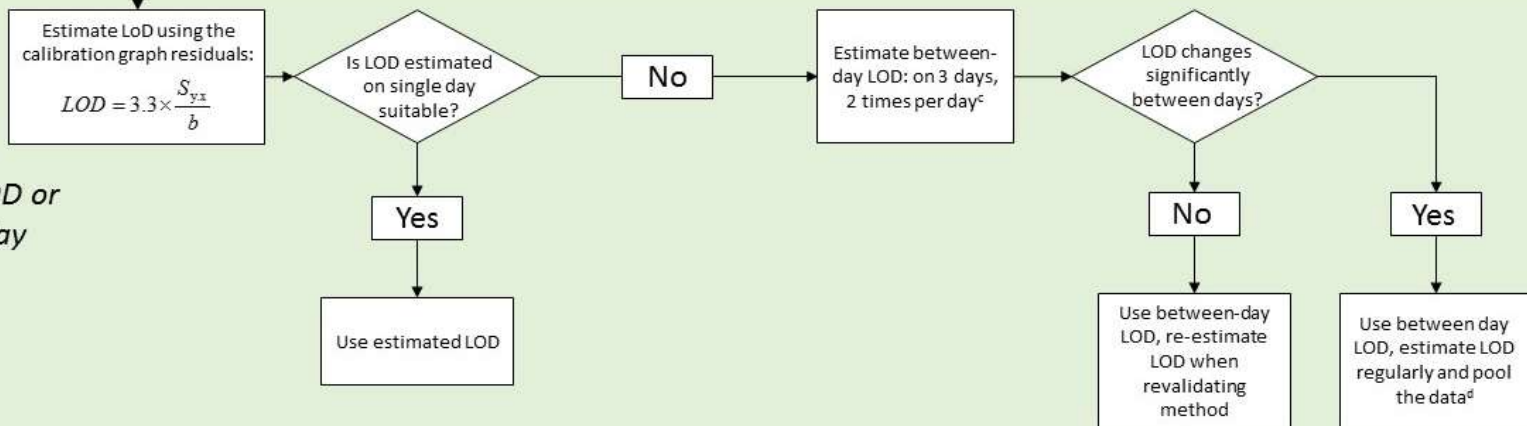
LOD estimation guidelines and experimental design



Linearity and homoscedasticity of data



Single day LOD or between-day LOD



Further issues to consider

- Fragmentation and identification
- Tolerance interval
- Bayesian statistics
- LoD estimation with different analytical methods

- Issues with Limit of Quantitation

- Education of metrology in chemistry

Thank you!