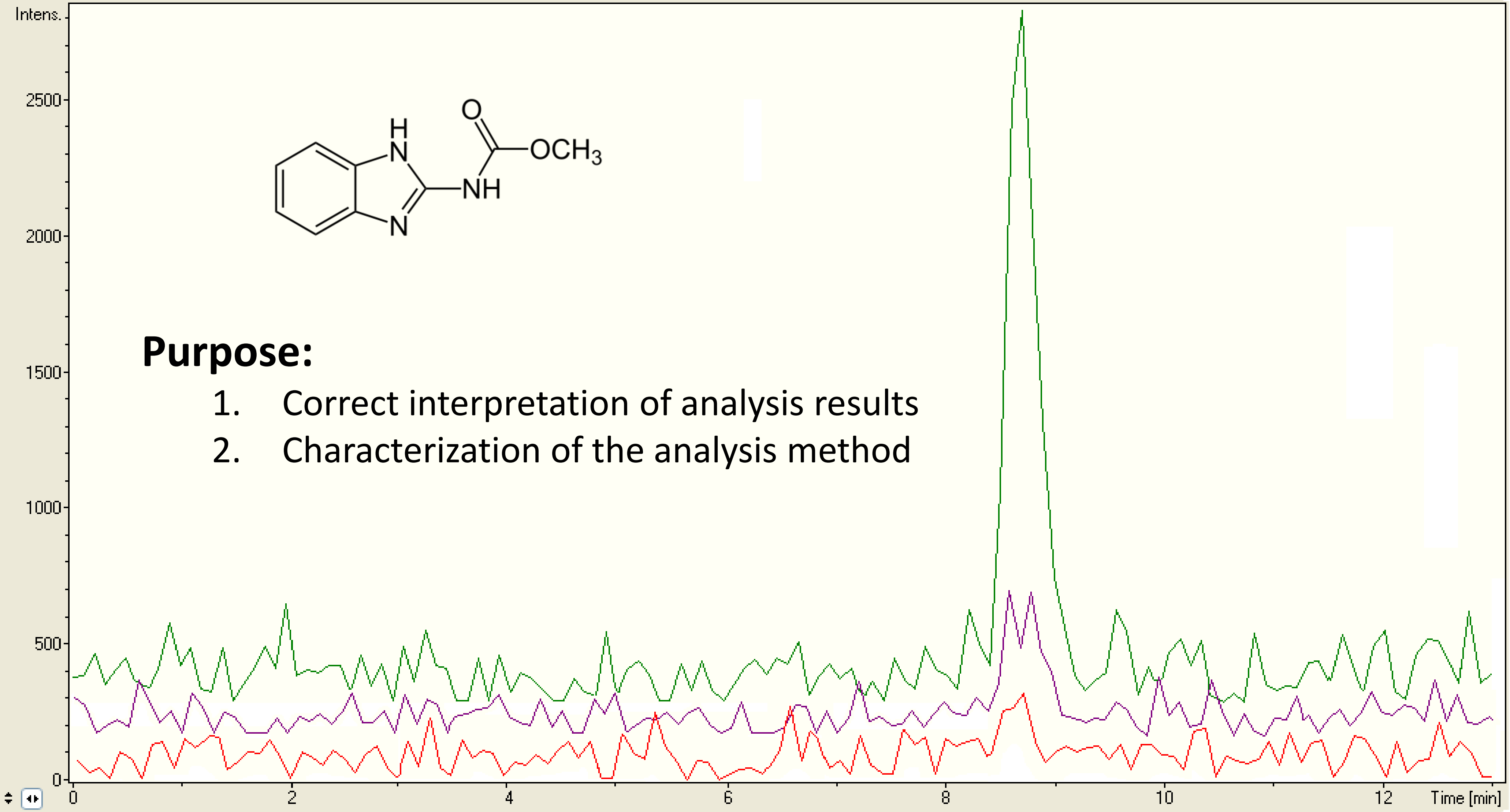
Limit of Detection

LC-MS/MS chromatogram of carbendazim (m/z 192 -> 160)



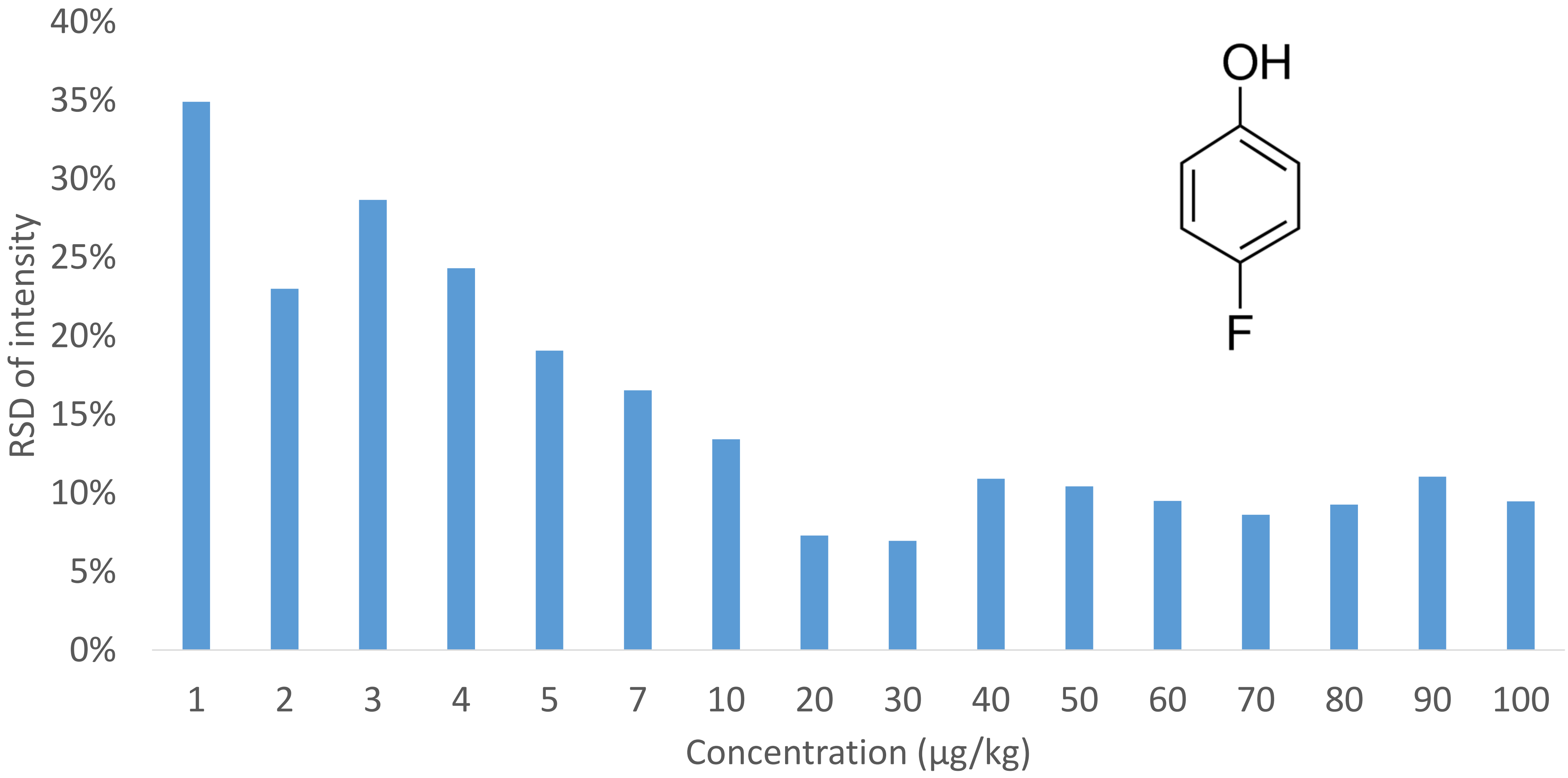
Purpose:

1. Correct interpretation of analysis results
2. Characterization of the analysis method

IUPAC definition

In broad terms, the detection limit (limit of detection) is the smallest amount or concentration of analyte in the test sample that can be **reliably** distinguished from zero.

Limit of Quantitation

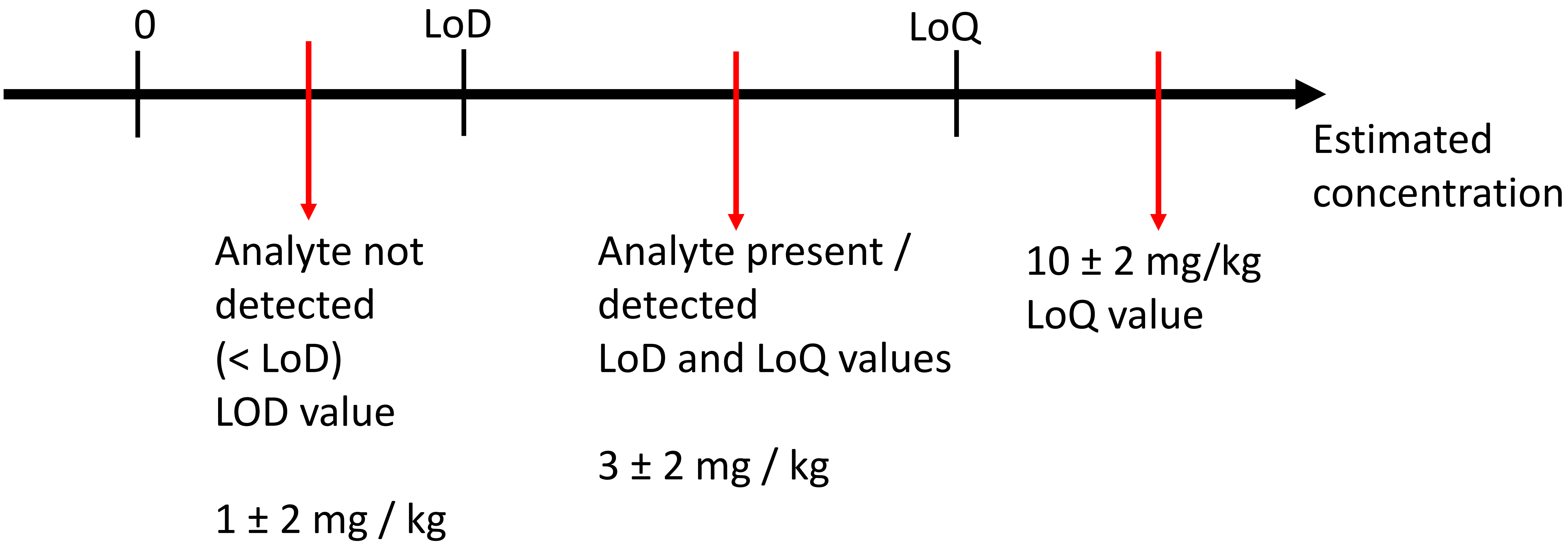

40%
35%
30%
25%
20%
15%
10%
5%
0%
RSD of intensity
1
2
3
4
5
7
10
20
30
40
50
60
70
80
90
100
Concentration (µg/kg)
OH
F

Limit of Quantitation (LoQ)

- Definition by Eurachem:
  - The lowest concentration of analyte that can be determined with an acceptable repeatability and trueness
- Repeatability and trueness limits for LoQ can be set by relevant guidelines or standards
  - For example SANCO demands ≤ 20% repeatability and trueness between 70-120%
- Quantitation below LoQ is possible
  - In range of LoD uncertainty becomes large, comparable to the result

Interpretation of analysis results with LoD and LoQ

Interpretation of analysis results with LoD and LoQ

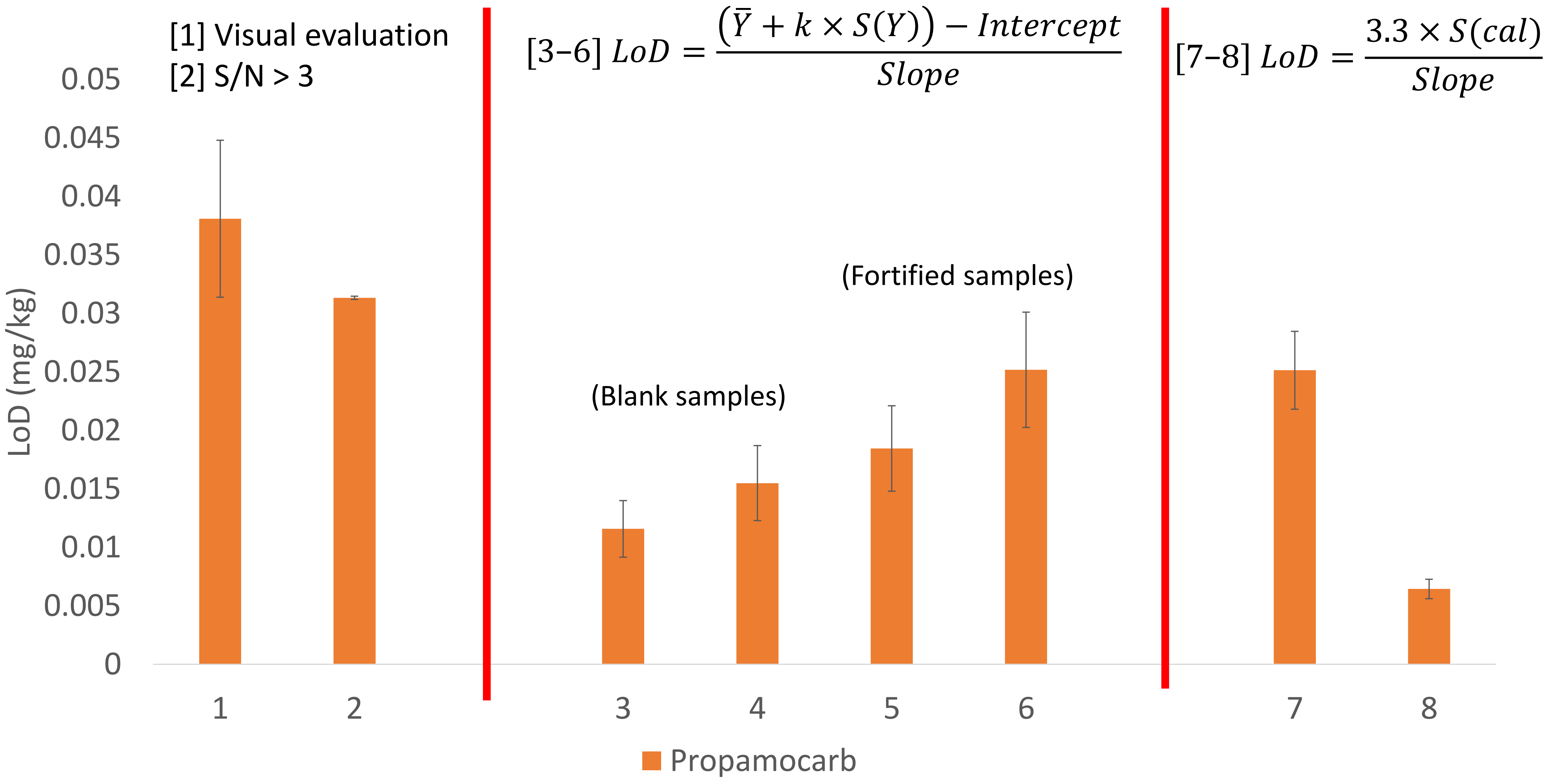


Different approaches to estimate LoD

Important aspects

- Guidelines give different approaches
  - E.g. FDA, IUPAC, Eurachem, NordVal, US EPA, etc.
  - Not all approaches are fitting for all analytical methods

- Different approaches make different assumptions



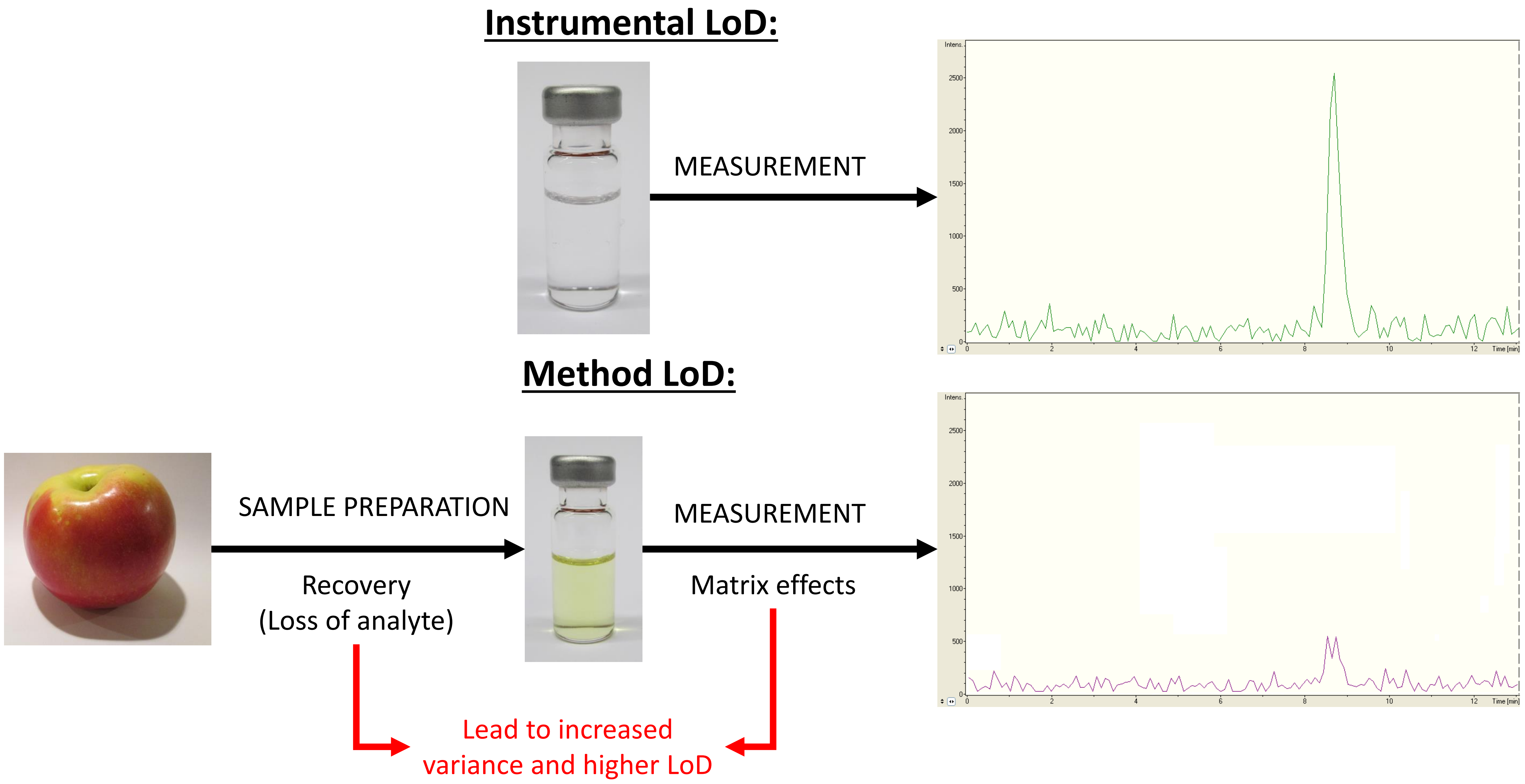
[1] Visual evaluation
[2] S/N > 3
[3–6] $LoD = \frac{(\bar{Y} + k \times S(Y)) - Intercept}{Slope}$
[7–8] $LoD = \frac{3.3 \times S(cal)}{Slope}$
(Fortified samples)
(Blank samples)
LoD (mg/kg)
0.05
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
1
2
3
4
5
6
7
8
Propamocarb

Conclusions

- Results of different approaches are not comparable

- LoD depends on
  - Variance
  - Slope and intercept
  - Only an estimate of LoD can be found

Instrumental LoD and method LoD


Instrumental LoD:
MEASUREMENT
Intens.
2500
2000
1500
1000
500
0
0
2
4
6
8
10
12
Time [min]
Method LoD:
SAMPLE PREPARATION
Recovery
(Loss of analyte)
MEASUREMENT
Matrix effects
Intens.
2500
2000
1500
1000
500
0
0
2
4
6
8
10
12
Time [min]
Lead to increased
variance and higher LoD

Conclusion

- For a whole analysis method instrumental LoD is not suitable
  - Blank matrix matched samples must be used
  - All samples must go through the whole method

- Similar conclusions for LoQ

Decision limit (CC_α) and detection capability (CC_β)

CC_α and CC_β

- The general definition of LoD is ambiguous
  - False positive and false negative results

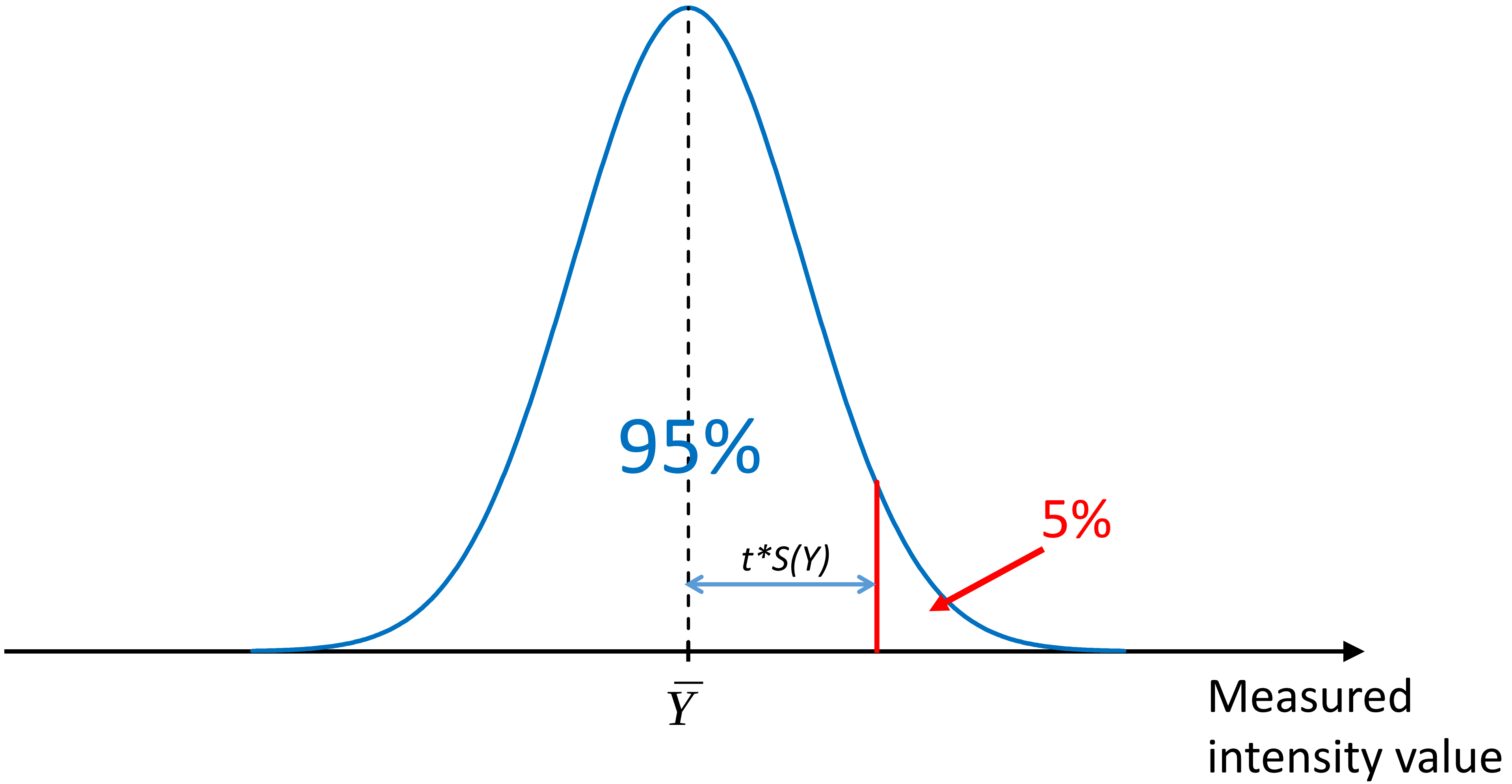
	A positive result is received	A negative result is received
Sample is truly positive	✓	False negative result
Sample is truly negative	False positive result	✓

Definitions

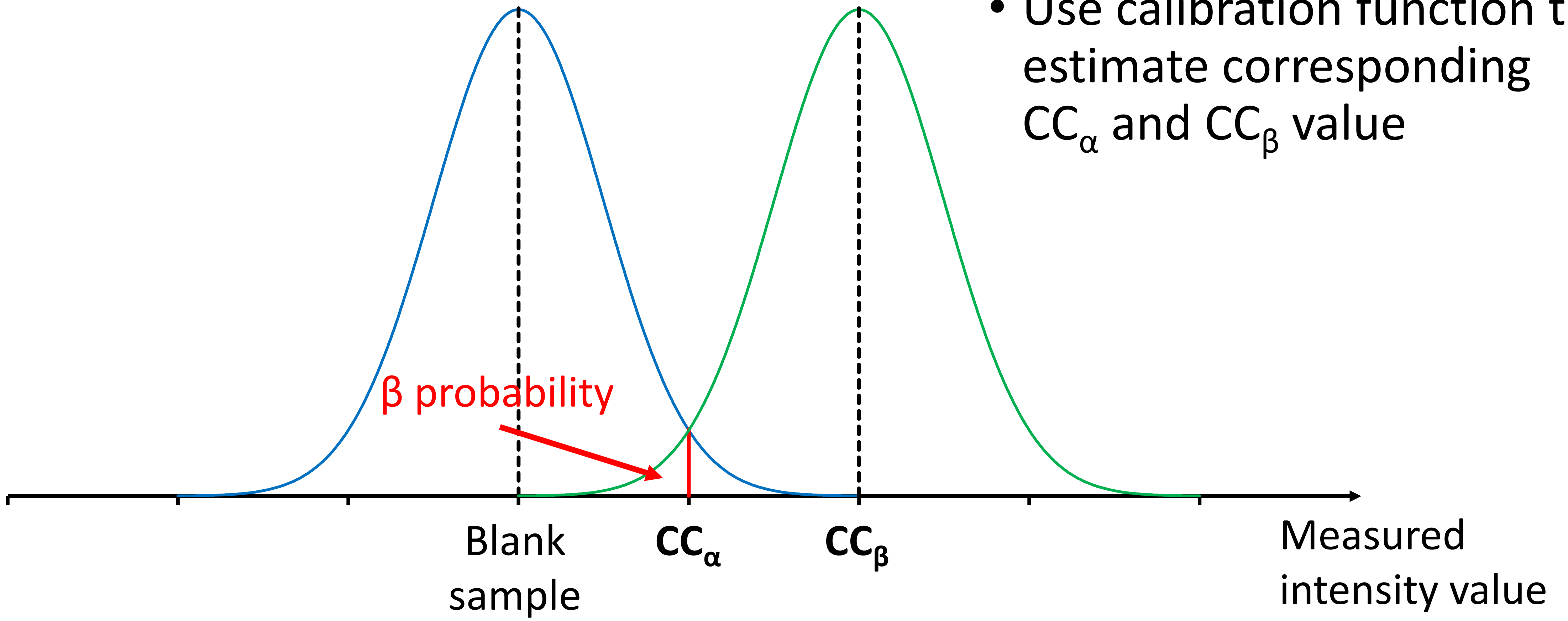
- Decision limit (CC_α) – analyte concentration level above which we can state that the signal is caused solely by the noise with the probability below α
  - α = 5% or 1%

- Detection capability (CC_β) – analyte concentration level in a sample above which there is less than β probability that the result will be randomly below CC_α (and therefore interpreted as a negative result)
  - β = 5% or 1%

Normal distribution of measurement results



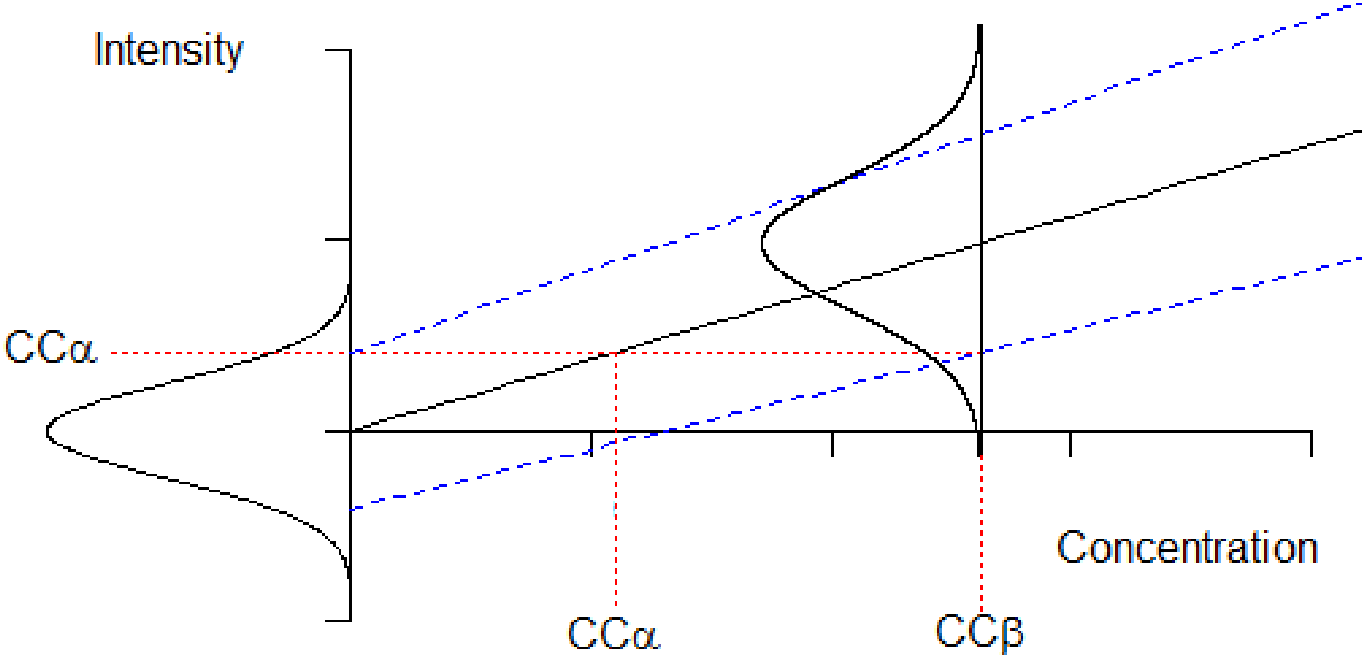
CC_α and CC_β



- Use calibration function to estimate corresponding CC_α and CC_β value

Calculating CC_{α} and CC_{β}

Calculating CC_α and CC_β

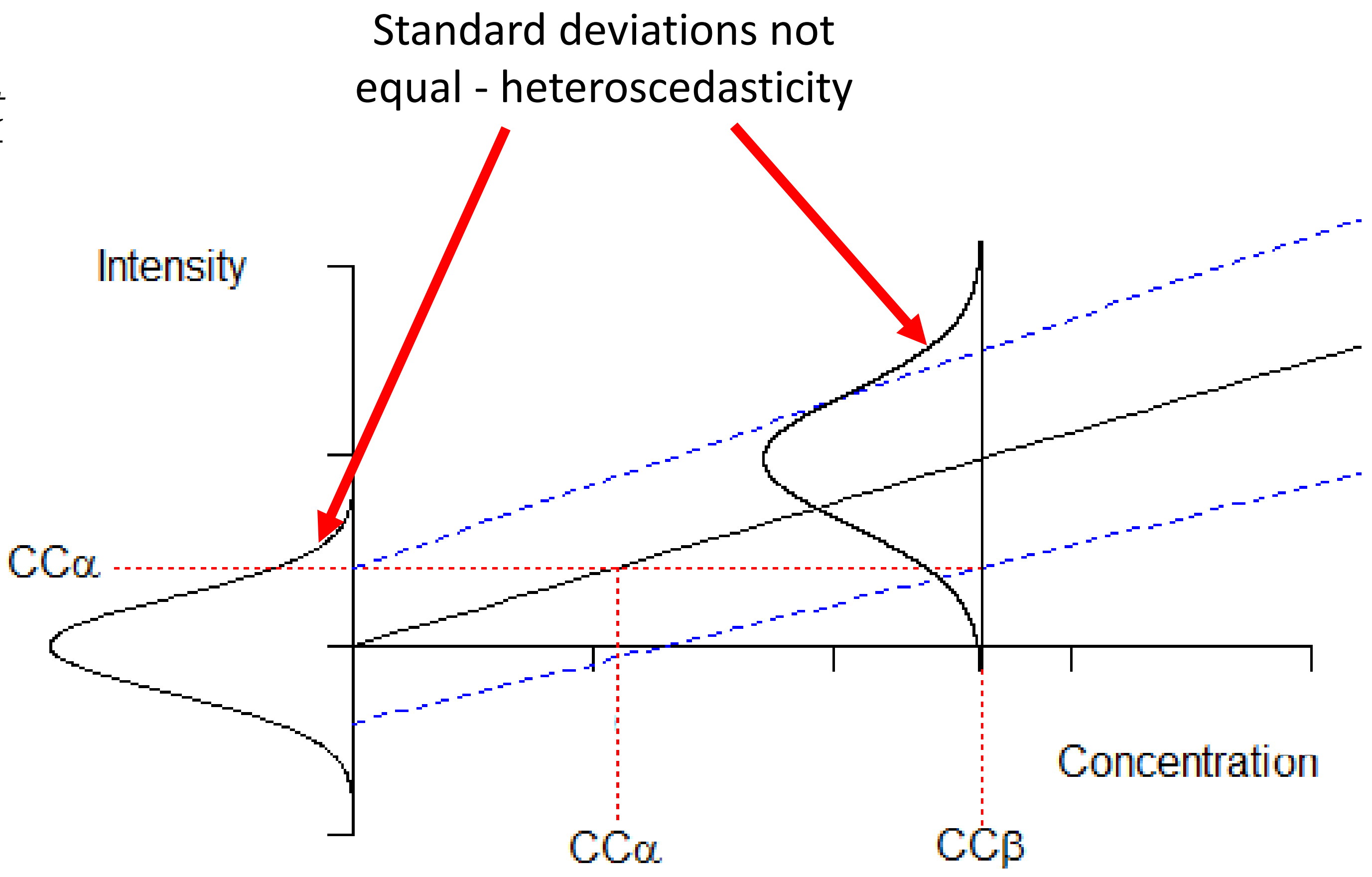


$$CC_{\alpha} = \frac{(\bar{Y}(blank) + t \times S(blank)) - Intercept}{Slope}$$

$$CC_{\beta} = \frac{(\bar{Y}_{CC\alpha} + t \times S(Y_{CC\beta})) - Intercept}{Slope}$$

$$LoD = \frac{(\bar{Y} + k \times S(Y)) - Intercept}{Slope}$$

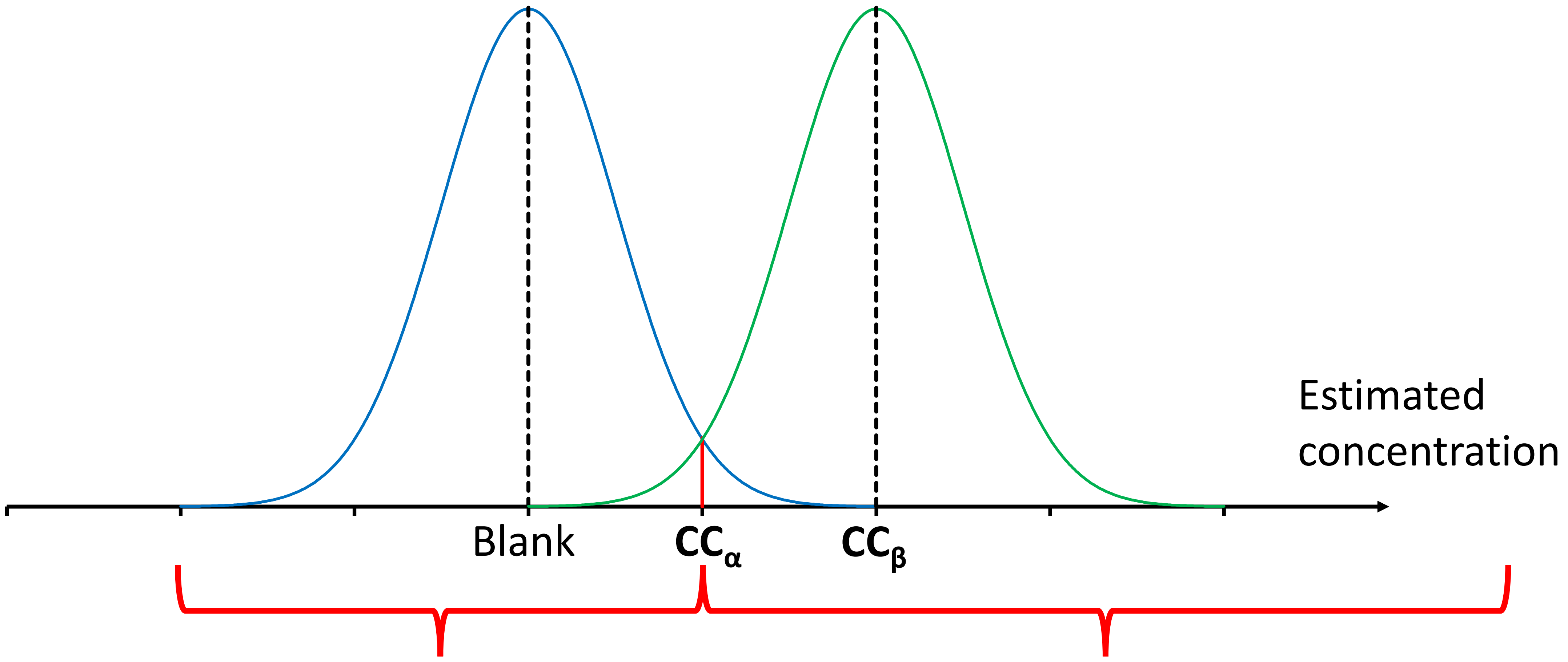
$$LoD = \frac{3.3 \times S(cal)}{Slope}$$



- Complex approaches with less assumptions and simplifications exist
  - ISO 11843-2

Interpreting results with CC_{α} and CC_{β}

Interpreting results with CC_α and CC_β



Below CC_α
Analyte concentration below CC_β

Above CC_α
Analyte detected

Conclusion

- CC_α – for making the decision (analyte detected or not)

- CC_β – for characterizing the approach
  - When analyte is not detected
  - For comparing different analytical methods
  - For comparing a method with a set limit

- With decision also give
  - CC_α and CC_β values
  - Result with uncertainty if necessary

Important aspects of estimating LoD and CC_α, CC_β

Choosing between LoD estimation approaches

1. Is LoD necessary?

2. Should a standard (or guideline) be followed?

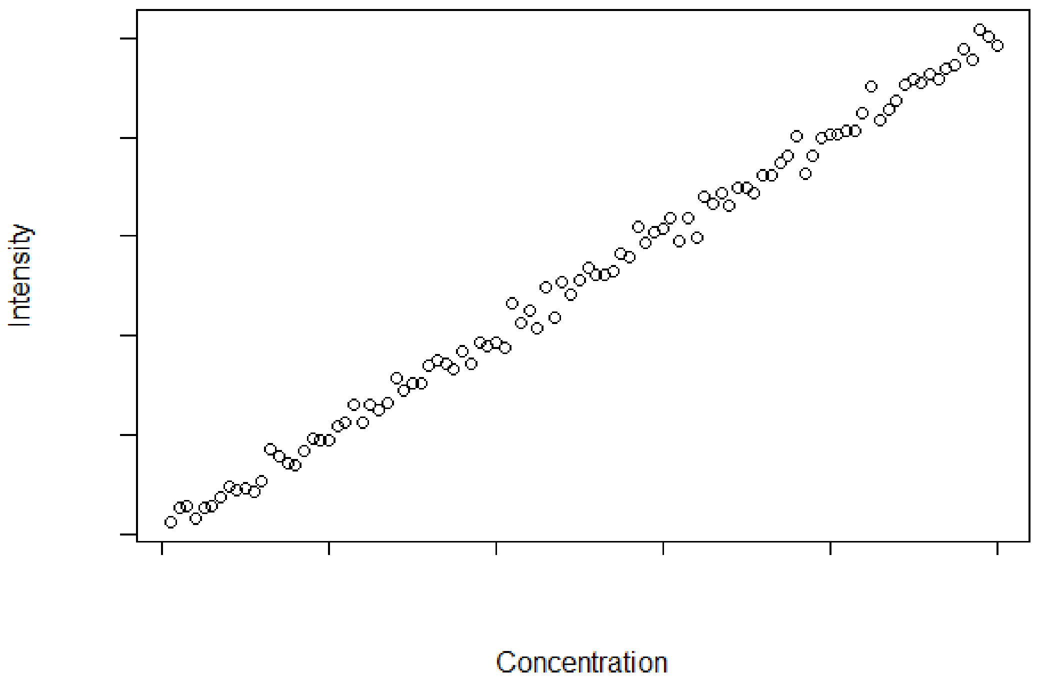
3. Are critical decisions based on LoD?
    - If “Yes” then estimate CC_α and CC_β (procedure given in ISO 11843-2)
    - If “No” using the following equation is suggested:

$$LoD = \frac{3.3 \times S(residuals)}{Slope}$$

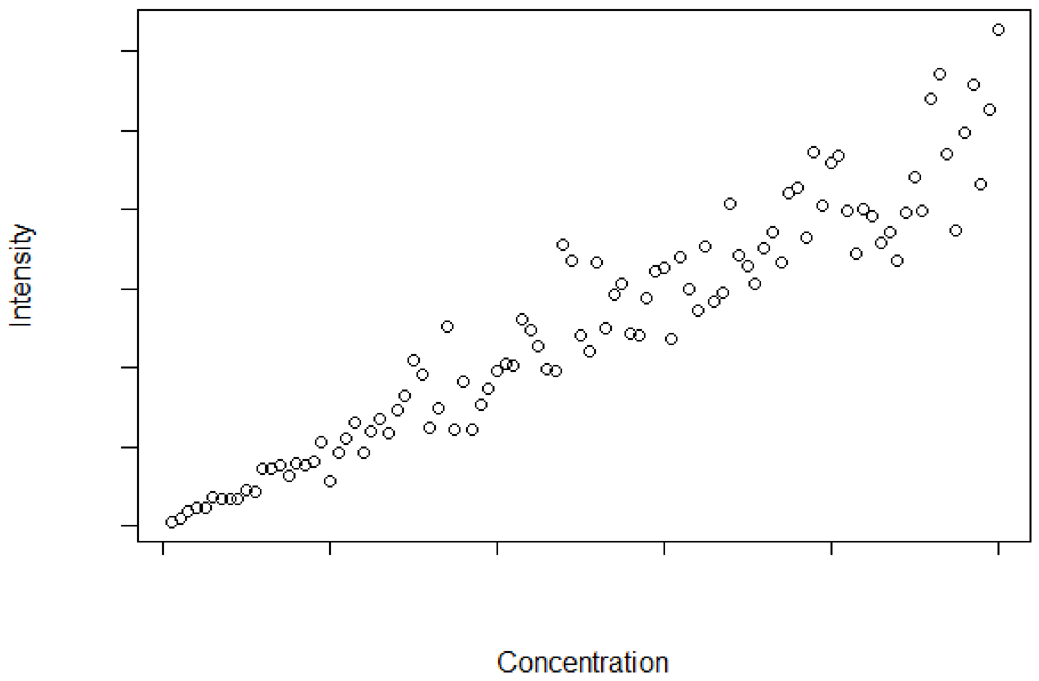
Linearity and scedasticity

- Data should be in range of LoD and linear
- Data should be homoscedastic
  - Use narrow concentration range

Homoscedastic data



Heteroscedastic data



Other important aspects to consider when estimating LoD (CC_α and CC_β)

- LoD varies between measurement series and days
  - Regular reevaluation is recommended
- Not all approaches are appropriate for all analytical methods
  - Integration of blank samples with LC-MS/MS
- Matrix matching of used samples

Different approaches to estimate LoQ

LoQ estimation approaches

1. Trueness and precision at each concentration

- Preferable, but labor-intensive
- Different relevant guidelines or standards set precision and trueness limits
- Can be based on uncertainty at different concentration levels

2. Approaches related to LoD estimation

- Same data for LoQ, LoD
- *k* values different in guidelines
- Trueness and precision are not estimated
- S/N and visual evaluation

$$LoQ = \bar{Y} + k \times S(Y)$$

$$LoQ = \frac{10 \times S(cal)}{Slope}$$

LoQ estimation

- Choice depends on importance of LoQ parameter
  - When necessary specific guidelines must be followed

- Our recommendation:
  - If LoQ is critical use precision and trueness estimation
  - Otherwise use ICH suggested approach

$$LoQ = \frac{10 \times S_{y.x}}{Slope}$$

Important aspects of LoQ estimation

- LoQ is used for:
  1. Is the sample concentration high enough for “fit for purpose” quantitation?
  2. To characterize the analytical method
     - The used LoQ estimation approach must be stated

- When estimating LoQ:
  - Use data in range of LoQ
  - Use matrix matching samples
  - LoQ changes between measurement series and days

- For approaches that use calibration function:
  - Linearity and scedasticity