# Strategies for Linear and Nonlinear Calibrations in Instrumental Analysis taking into account applicable Standards

Karl Molt

Uni Duisburg-Essen, Fakultät für Chemie



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   - Regression calculation
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# Establishment of calibration lines

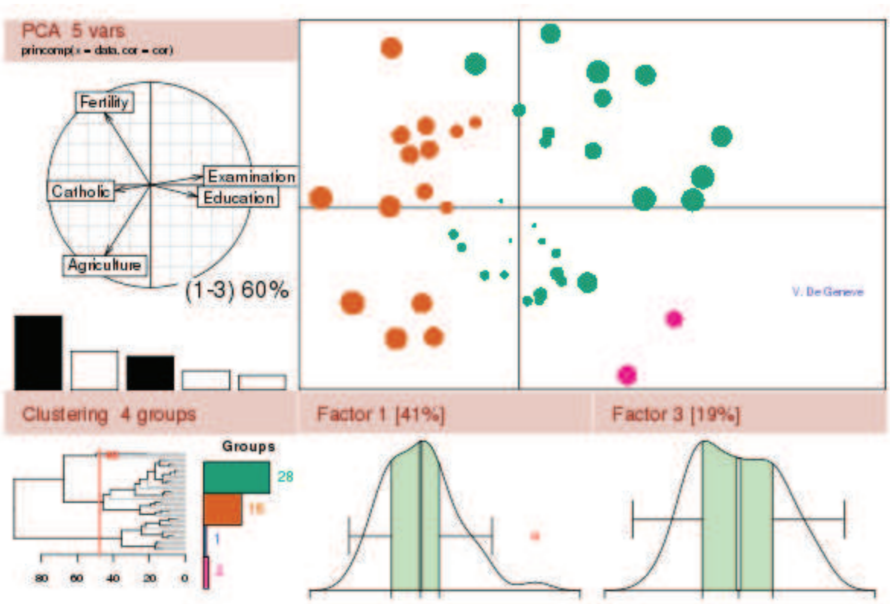
## Performance of calculations

- The calculation of calibration lines is performed by linear regression, which can be carried out by common statistical software.
- Mathematical details can be found in
  - DIN ISO 11095:1996: "Linear calibration using reference materials"
  - ISO/TS 28037:2010: "Determination and use of straight-line calibration functions"
- All calculations and graphics in this presentation were performed with **R**.





www.r-project.org




R is a language and environment for statistical computing and graphics. It is "Free Software" (GNU General Public License) and runs on a wide variety of UNIX platforms and similar systems (including FreeBSD and Linux), Windows and MacOS.

# Linear calibration functions

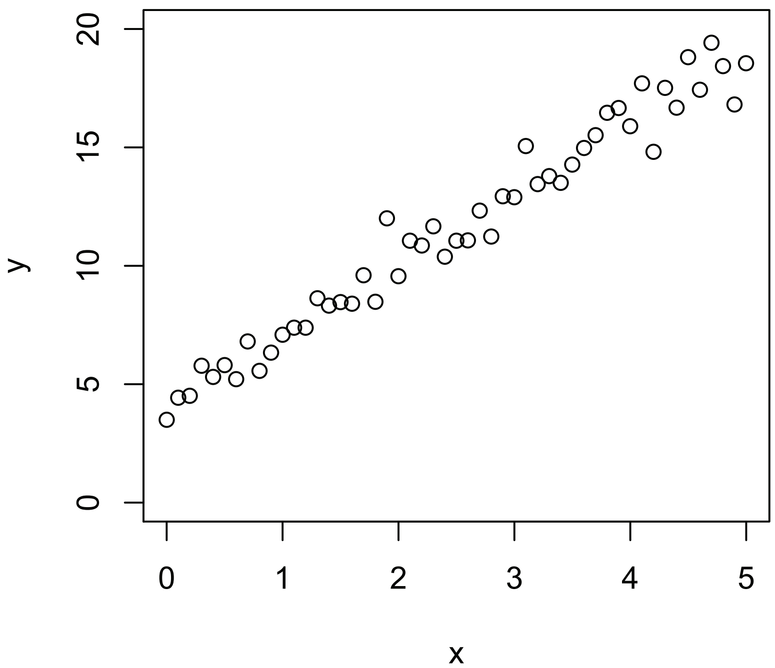
$$y = bx + a$$

| | |
|---|---|
| $x$ | independent variable: content<br>“Gehaltsgröße” |
| $y$ | dependent variable (response): signal<br>“Messgröße” |
| $b$ | slope<br>corresponds to the “sensitivity” of the calibration |
| $a$ | intercept |





# Simulated example




```
> x = seq(0,5,0.1)
> y = 3*x +4
> n = length(x)
> set.seed(100)
> noise = rnorm(n)
> y = y + noise
> plot(x,y)
```

# Regression calculation for linear calibration functions

Data: $(x_i, y_i)$, $i = 1 \ldots n$

$$y = bx + a \quad (1)$$

$$\hat{y}_i = bx_i + a \quad (2)$$

$$\sum_{i=1}^{n} (y_i - \hat{y}_i)^2 \rightarrow \text{minimum} \quad (3)$$

$$\hat{x}_i = \frac{y_i - a}{b} \quad (4)$$





# Regression calculation for linear calibration functions

$y = bx + a$

$$Q_{xx} = \sum_{i=1}^{n} (x_i - \bar{x})^2 = \sum_{i=1}^{n} x_i^2 - \frac{(\sum_{i=1}^{n} x_i)^2}{n}$$

$$Q_{xy} = \sum_{i=1}^{n} (x_i \cdot y_i - \bar{x} \cdot \bar{y}) = \sum_{i=1}^{n} (x_i y_i) - \frac{\sum_{i=1}^{n} x_i \sum_{i=1}^{n} y_i}{n}$$

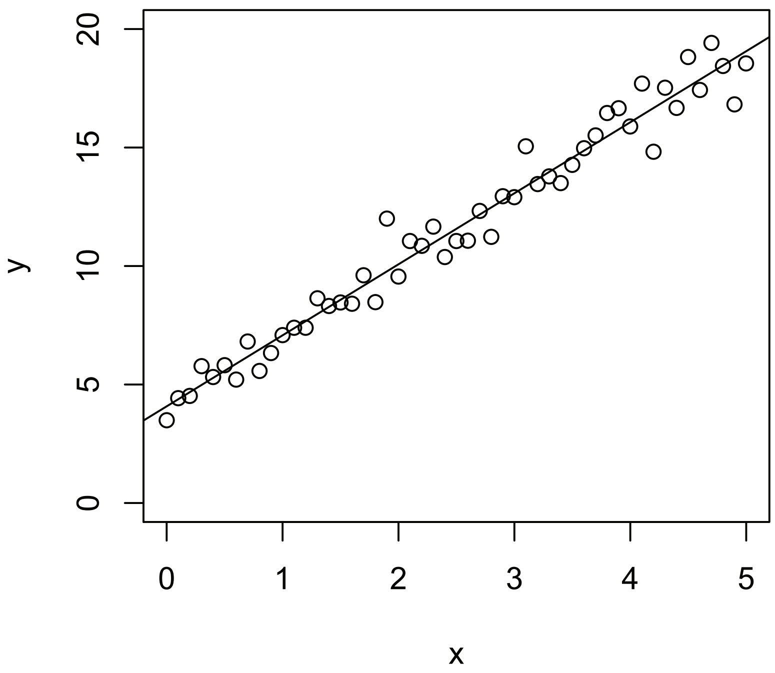
$$b = \frac{Q_{xy}}{Q_{xx}}$$

$$a = \bar{y} - b\bar{x}$$

```
> Q.xx = sum(x^2)-sum(x)^2/n
> Q.xy = sum(x*y)-sum(x)*sum(y)/n
> b = Q.xy/Q.xx
> b
[1] 2.996833
> a = mean(y)-b*mean(x)
> a
[1] 4.079062
```

```
> lm.yx = lm(y~x)
> coef(lm.yx)
(Intercept)           x
   4.079062    2.996833
```

# Simulated example




`> abline(lm.xyx)`



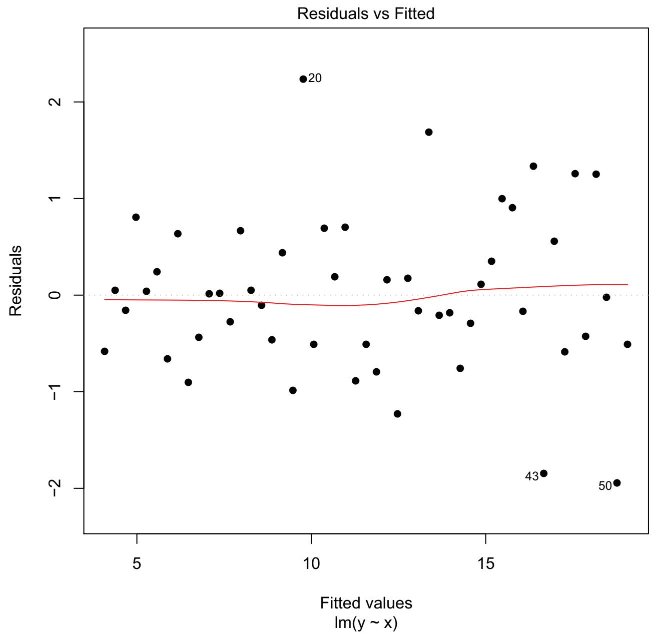


# Determination of performance characteristics

ISO 8466-1: “Water quality: Calibration and evaluation of analytical methods and estimation of performance characteristics; part 1: **statistical evaluation of the linear calibration function**” (1990-03)

This ISO was derived from DIN 38402-51:1986.

# Residual std. dev. and std. dev. of method




$$s_y = \sqrt{\frac{\sum(y_i - \hat{y}_i)^2}{n-2}}$$

$$s_{x0} = \frac{s_y}{b}$$

$$V_{x0} = \frac{s_{x0} \cdot 100\%}{\bar{x}}$$

```
> Summary = summary(lm.yx)
> s.y = Summary$sigma
[1] 0.822403
> s.x0 = s.y/b
[1] 0.2744240
> V.x0 = s.x0*100/mean(x))
> V.x0
[1] 10.97696
```





# Prediction Intervals for $\hat{y}$

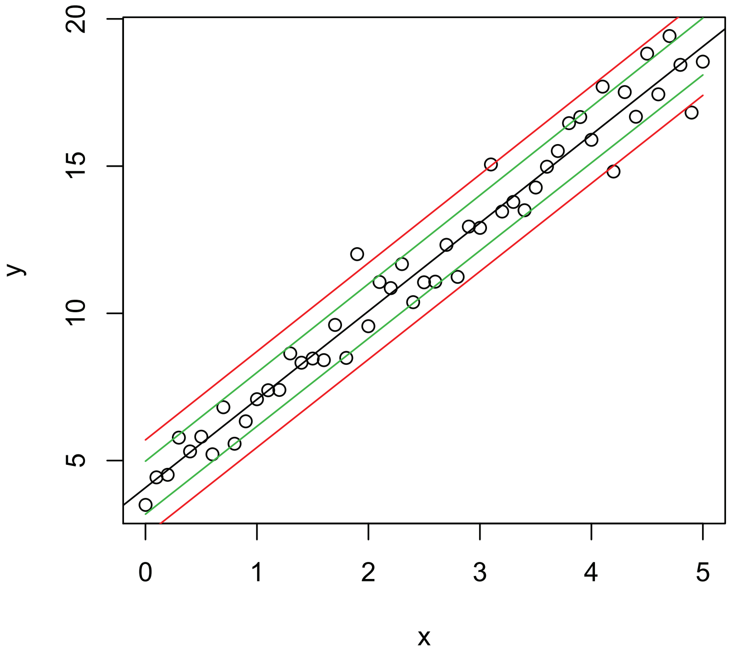
$$\hat{y}_j^{u,l} = (bx_j + a) \pm s_y t_{f,\alpha}^{\mathtt{I}} \sqrt{\frac{1}{n} + \frac{1}{m} + \frac{(x_j - \bar{x})^2}{Q_{xx}}}$$

```
> (t = qt(1-0.025,df=n-2))
[1] 2.009575
> Q.xx = sum(x^2) -sum(x)^2/n
> m = 1 # Single measurement
> y.u = b*x + a + s.y*t*sqrt((1/n + 1/m + (x - mean(x))^2)/Q.xx)
> y.l = b*x + a - s.y*t*sqrt((1/n + 1/m + (x - mean(x))^2)/Q.xx)
> lines(x,y.o,col=2)
> lines(x,y.u,col=2)
# Repeat with m = 3, col=3 for triple measurement
```

$n$ Number of calibration standards
$m$ Number of replicate measurements of the sample

# Prediction band for $y$

Single resp. triplicate measurement of the analysis sample




m=1
m=3

**Replicate measurements enhance the precision of the prediction from the calibration!**





# Prediction Intervals for $\hat{x}$

For the prediction of $x$ from $y$ eq. (5) has to be solved for $x$.

$$\hat{y}_j^{u,l} = (bx_j + a) \pm t^{I}_{f,\alpha} s_y \sqrt{\frac{1}{n} + \frac{1}{m} + \frac{(x_j - \bar{x})^2}{Q_{xx}}} \quad (5)$$

$$\hat{x}_j^{u,l} = \frac{y_j - a}{b} \pm t^{I}_{f,\alpha} \frac{s_y}{b} \sqrt{\frac{1}{n} + \frac{1}{m} + \frac{(\hat{x}_j - \bar{x})^2}{Q_{xx}}} \quad (6)$$

$$\hat{x}_j^{u,l} = \frac{y_j - a}{b} \pm t^{I}_{f,\alpha} s_{x0} \sqrt{\frac{1}{n} + \frac{1}{m} + \frac{(y_j - \bar{y})^2}{b^2 Q_{xx}}} \quad (7)$$

Uncertainty for predicted x-values
"S L R Ellison and A Williams (Eds). Eurachem/CITAC guide: Quantifying Uncertainty in Analytical Measurement, Third edition, (2012) ISBN 978-0-948926-30-3. Available from www.eurachem.org."

Appendix E.4: "Uncertainties from linear least squares calibration"

# Uncertainty for predicted x-values

Eurachem/CITAC guide (2012)

There are four main sources of uncertainty to consider in arriving at an uncertainty on the estimated concentration $\hat{x}_j$:

- Random variations in measurement of $y$, affecting both the reference responses $y_i$ and the measured response $y_j$.
- Random effects resulting in errors in the assigned reference values $x_i$.
- Values of $x_i$ and $y_i$ may be subject to a constant unknown offset, for example arising when the values of $x_i$ are obtained from serial dilution of a stock solution.
- The assumption of linearity may not be valid.





# DIN 32645

Performance characteristics for qualitative and quantitative analysis

"Chemische Analytik – Nachweis-, Erfassungs- und Bestimmungsgrenze unter Wiederholbedingungen – Begriffe, Verfahren, Auswertung"

Translation:
"**Chemical analysis – Decision limit, detection limit and determination limit under repeatability conditions – Terms, methods, evaluation"** Normenausschuss Materialprüfung (NMP) im DIN, November 2008

# DIN ISO 11843-2

DIN ISO 11843-2:2006
"Capability of detection - Part 2: Methodology in the linear calibration case"

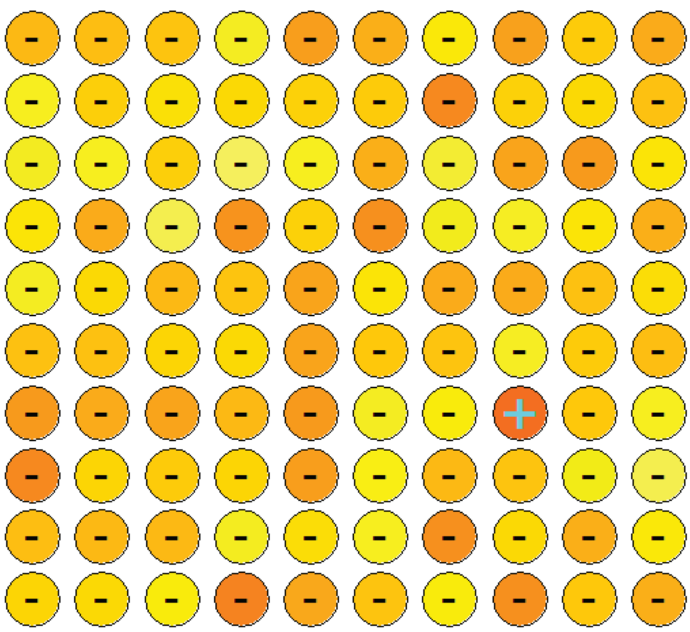




# Qualitative Analysis

## Specificity

$\alpha = 0.01$; Specificity = 99%



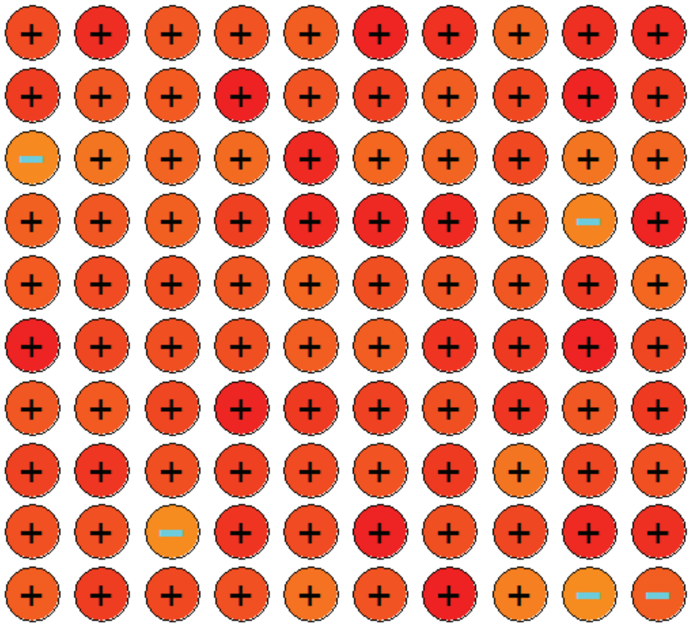
c = 0

$$
\begin{aligned}
Spec &= 1 - \alpha \\
Spec &= 1 - \frac{n_{FP}}{n}
\end{aligned}
$$

# Qualitative Analysis

## Sensitivity

β = 0.05; Sensitivity = 95%




c = LOD

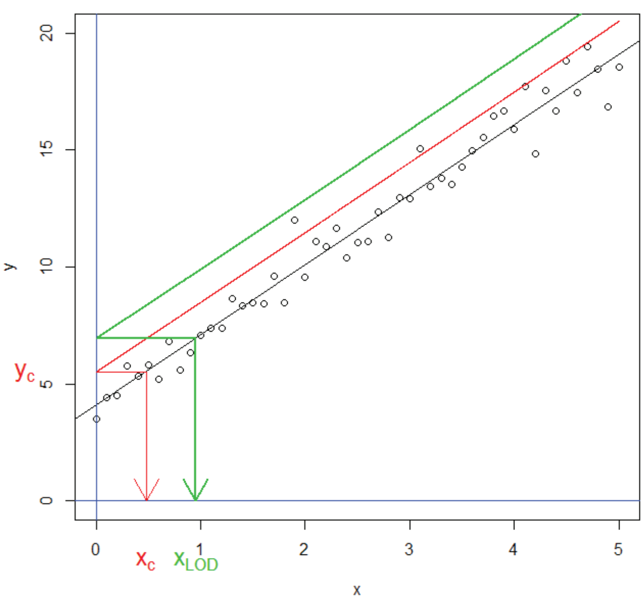
$$Sens = 1 - \beta$$

$$Sens = 1 - \frac{n_{FN}}{n}$$





# Critical values and Limit of Detection




$$y_c = a + s_y t^{\mathsf{T}}_{f,\alpha}\sqrt{\frac{1}{n} + \frac{1}{m} + \frac{\bar{x}^2}{Q_{xx}}}$$

$$x_c = s_{x0} t^{\mathsf{T}}_{f,\alpha}\sqrt{\frac{1}{n} + \frac{1}{m} + \frac{\bar{x}^2}{Q_{xx}}}$$

$$x_{LOD} = x_c + s_{x0} t^{\mathsf{T}}_{f,\beta}\sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(x_{LOD} - \bar{x})^2}{Q_{xx}}}$$

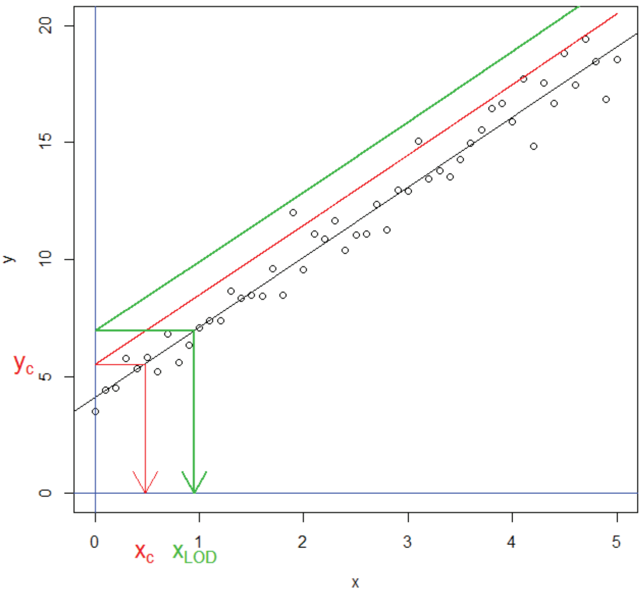
$$x_{LOD} \approx x_c + s_{x0} t^{\mathsf{T}}_{f,\beta}\sqrt{\frac{1}{m} + \frac{1}{n} + \frac{\bar{x}^2}{Q_{xx}}}$$

For $\alpha = \beta$ this simplifies to:

$$x_{LOD} \approx 2 \cdot x_c$$

# Critical values and Limit of Detection

```
> x.c <- s.y/b*t*sqrt(1 + 1/n + mean(x)^2/Q.xx)
```



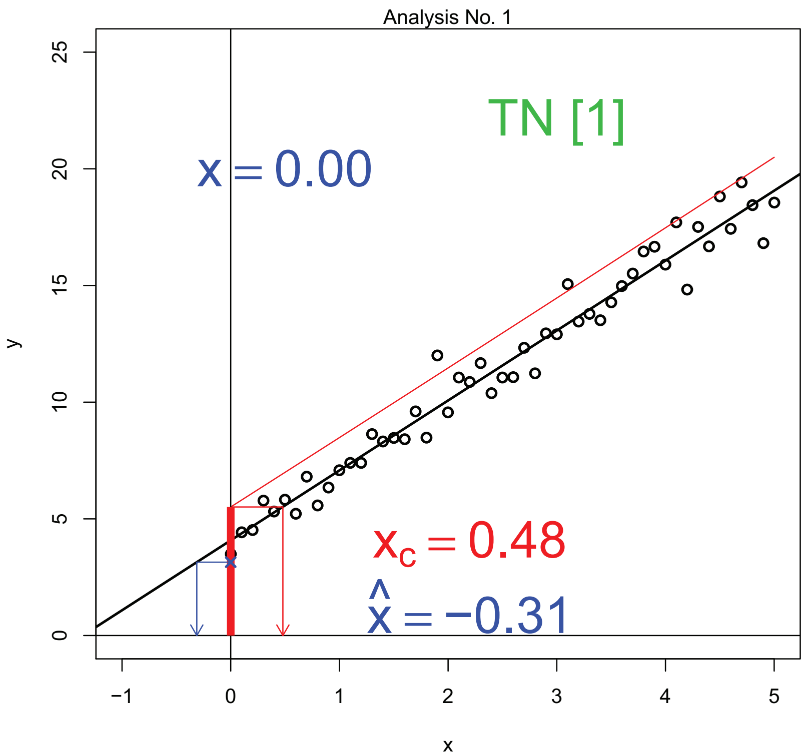

```
> cround(x.c,2)
[1] 0.48
x.LOD <- 2*x.c
x.LOD
[1] 0.96
```





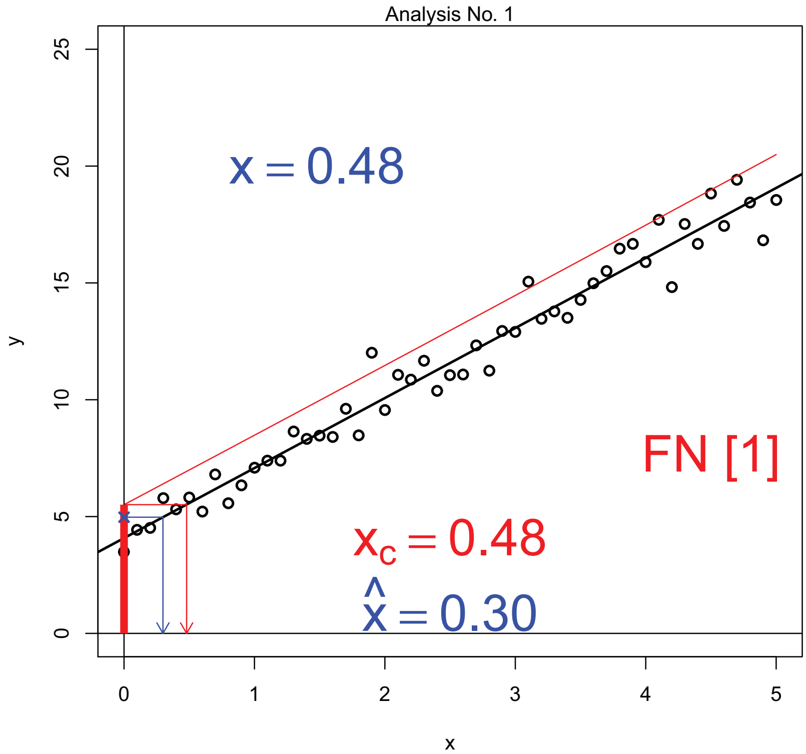
# Execution of analyses

$x = 0$



# Execution of analyses

$x = x_c$

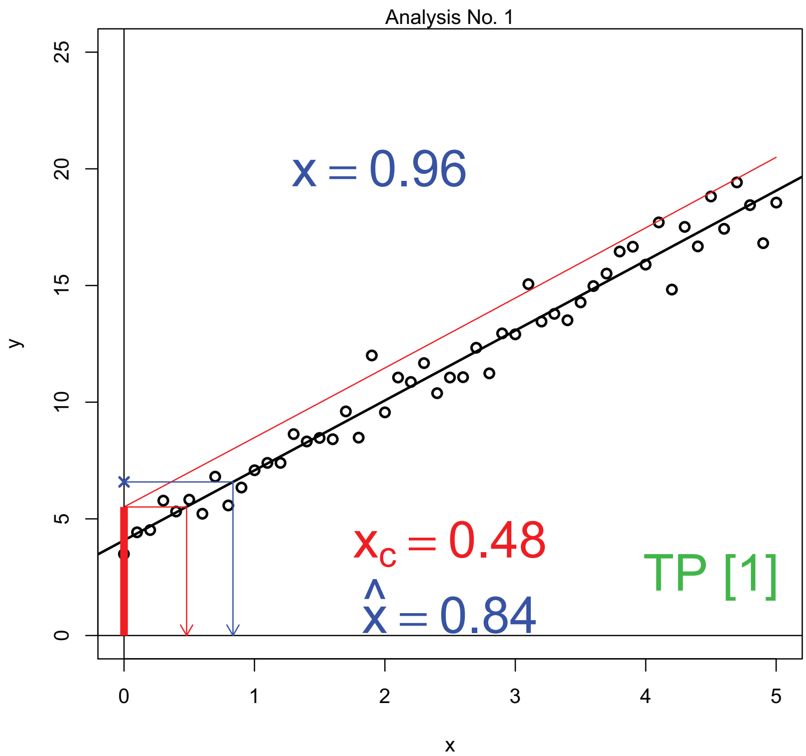







# Execution of analyses

$x = x_{LOD}$



# Limit of Detection

## Purity guaranty

If we get a *negative* anlaysis result we can – with an error probability of $\beta$ – guarantee that the analyte content is less than $x_{LOD}$.

$$x_{LOD} = x_c + s_{x0} t^{\mathsf{T}}_{f,\beta} \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(x_{LOD} - \bar{x})^2}{Q_{xx}}}$$

$$x_{LOD} \approx x_c + s_{x0} t^{\mathsf{T}}_{f,\beta} \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{\bar{x}^2}{Q_{xx}}}$$

For $\alpha = \beta$ this simplifies to:

$$x_{LOD} \approx 2 \cdot x_c \qquad (8)$$





# Limit of Quantification

## for a demanded degreee of precision of $k$

At the Limit of Quantification a degree of precision $\frac{x_{LOD}}{\Delta x_{LOD}} = k$ is demanded[2]. With this we get $x_{LOQ} = k \times \Delta x_{LOQ}$. $\Delta x_{LOQ}$ corresponds to half the *two-sided* prediction interval (in the direction of $x$).

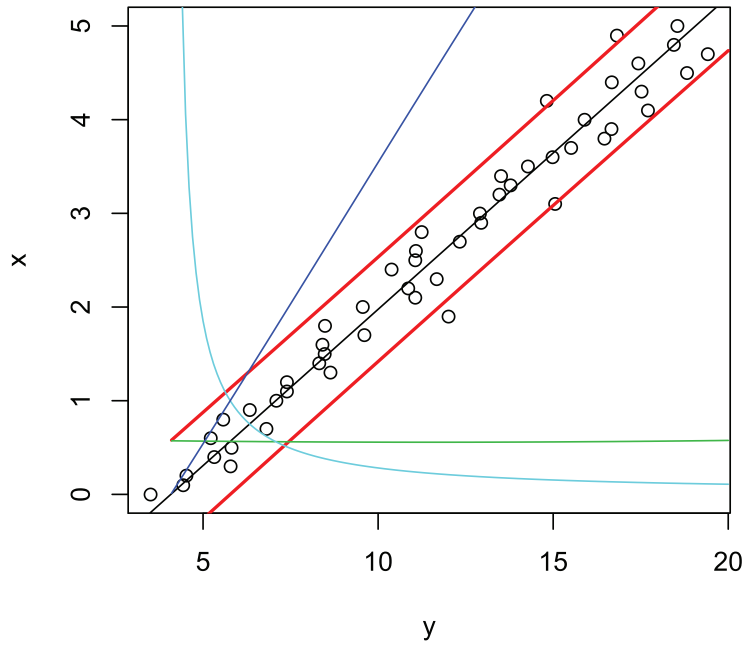
$$x_{LOQ} = k \frac{s_y t^{\mathsf{I}}_{f,\alpha}}{b} \sqrt{\frac{1}{n} + \frac{1}{m} + \frac{(x_{LOQ} - \bar{x})^2}{Q_{xx}}} \qquad (9)$$

DIN 32645 apples an iterative solution. But a closed solution is also possible. To demonstrate the principle the following slides show a graphical solution.

[2] $\frac{1}{k} = \frac{\Delta x_{BG}}{x_{BG}}$ is the relative uncertainty of the result. DIN 32645 sets $k = 3$.

# Limit of Quantification

The uncertainty of the result $\Delta x$ is equal to half the prediction interval for $x$




Annotations:

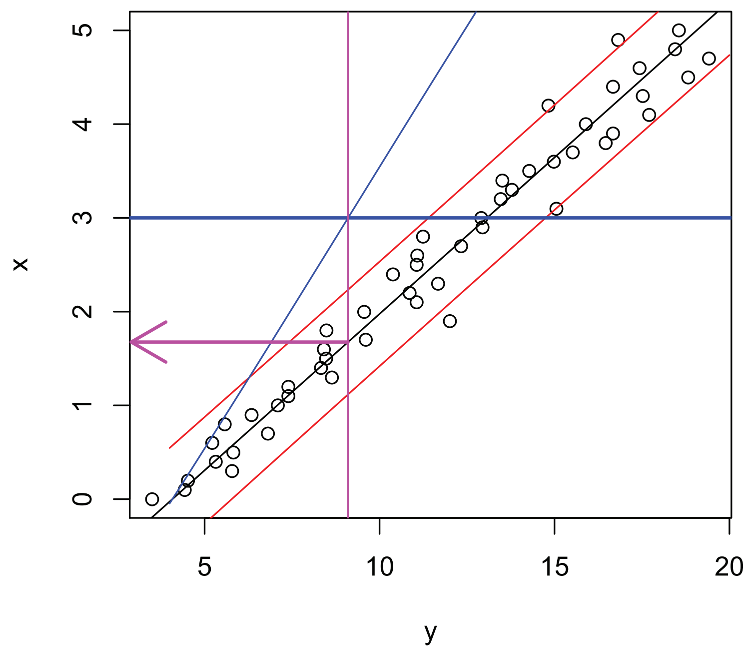
- $\Delta x$: uncertainty of result
- $\Delta x / x$: relative uncertainty of result
- $x / \Delta x$: degree of precision





# Limit of Quantification $x_{LOQ} = 1.68$

Definition: $\left.\frac{x}{\Delta x}\right|_{x=x_{LOQ}} = k, \; k = 3$



# Limit of Quantification from blank values

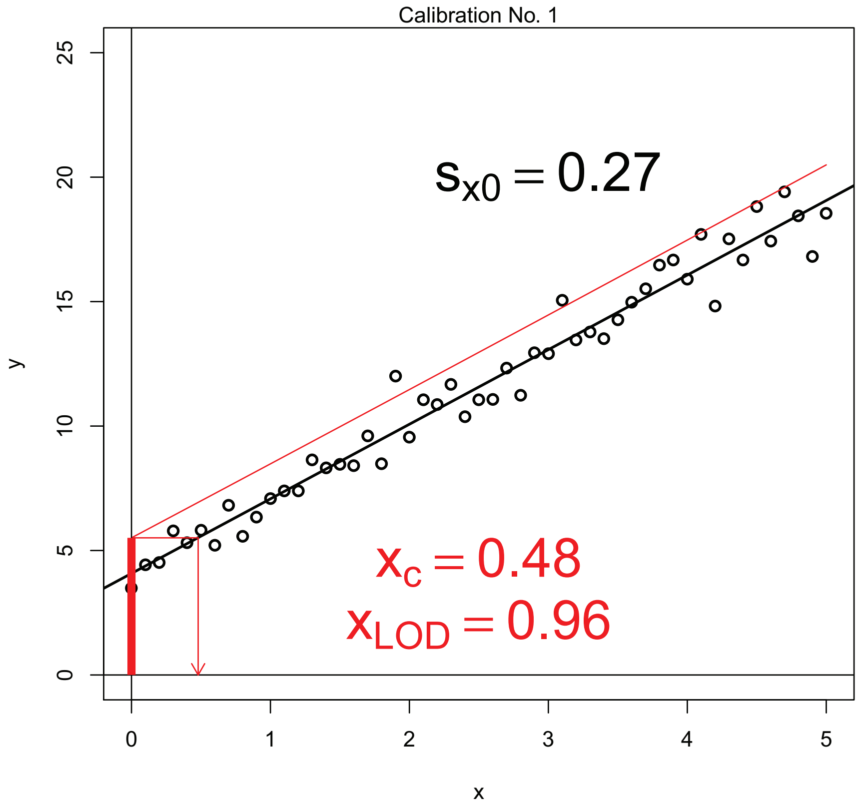
## IUPAC

Lloyd A. Currie, "Nomenclature in evaluation of analytical methods including detection and quantification capabilities", Pure & Appl. Chem., Vol. 67, No. 10, pp. 1699-1723, 1995.



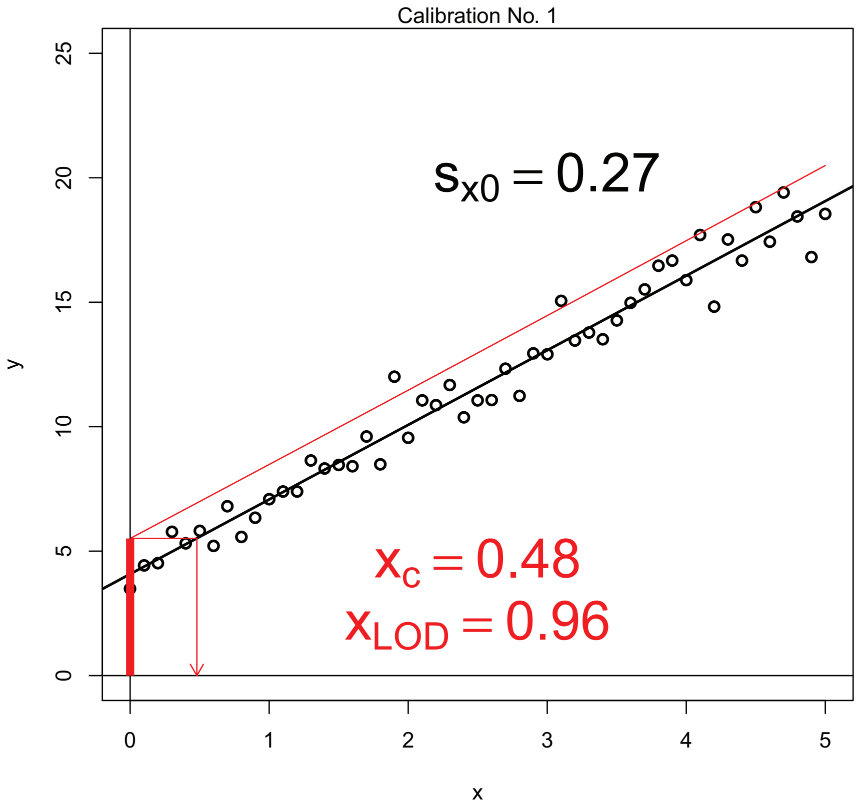
# Repetitions of the calibration

## under the same conditions




The calibration data set can be regarded as a statistical sample.

# Confidence interval for performance characteristics




Due to DIN 32645:

$$\kappa_l = 0.594763$$
$$\kappa_u = 1.246133$$

From this follows:

| | lower l. | upper l. |
|---|---|---|
| $s_{x0}$ | 0.23 | 0.34 |
| $x_c$ | 0.40 | 0.59 |
| $x_{LOD}$ | 0.80 | 1.18 |
| $x_{LOQ}$ | 1.40 | 2.08 |





# Calculation of $\kappa_{l,u}$

```
> df = 1:100
> alpha=0.05
> kappa.l <- sqrt(df/qchisq(1-alpha/2,df))
> kappa.u <- sqrt(df/qchisq(alpha/2,df))
> Tab <- data.frame(df,kappa.l,kappa.u)
> Tab
   df   kappa.l  kappa.u
   1  0.446      31.910
   2  0.521       6.285
   3  0.566       3.729
   5  0.624       2.453
  10  0.699       1.755
  20  0.765       1.444
  30  0.799       1.337
  50  0.837       1.243
 100  0.878       1.161
```

# DIN 32646: Limit of Detection and Quantification as processing parameters

## Estimation in an interlaboratory test

Determination under reproducible conditions (due to DIN 55350-13[3])

- pre-set analytical procedure
- identical sample material (same composition of matrix and no influence of sampling in the different laboratories)
- different laboratories (operators, instruments, labs)

[3]DIN 55350-13:1987-07
"Concepts in quality and statistics; concepts relating to the accuracy of methods of determination and of results of determination"





# DIN ISO 8466-2

Wasserbeschaffenheit – Kalibrierung und Auswertung analytischer Verfahren und Beurteilung von Verfahrenskenndaten – Teil 2: "Kalibrierstrategie für nichtlineare Kalibrierfunktionen zweiten Grades"

Normenausschuss Wasserwesen (NAW) im DIN

ISO 8466-2:2001

Water quality – Calibration and evaluation of analytical methods and estimation of performance characteristics – Part 2: "**Calibration strategy for non-linear second-order calibration functions"**

# Second-order calibration function

## $y = cx^2 + bx + a$

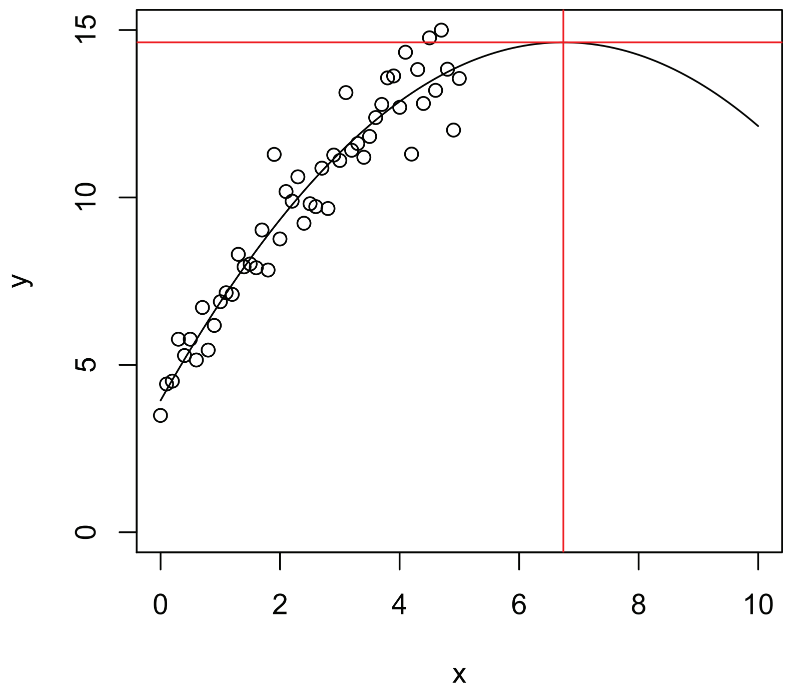
| | |
|---|---|
| $x$ | independent variable: content<br>"Gehaltsgröße" |
| $y$ | dependent variable (response): signal<br>"Messgröße" |
| $b$, $c$ | coefficients |
| $y' = 2cx + b$ | slope<br>corresponds to the "sensitivity" of the calibration |
| $a$ | intercept |





# 2nd degree non-linear calibration function

$y = a + bx + cx^2$




ATTENTION!

$$Max\left(\frac{-b}{2c}, a - \frac{b^2}{4c}\right)$$

# DIN ISO 11843-5

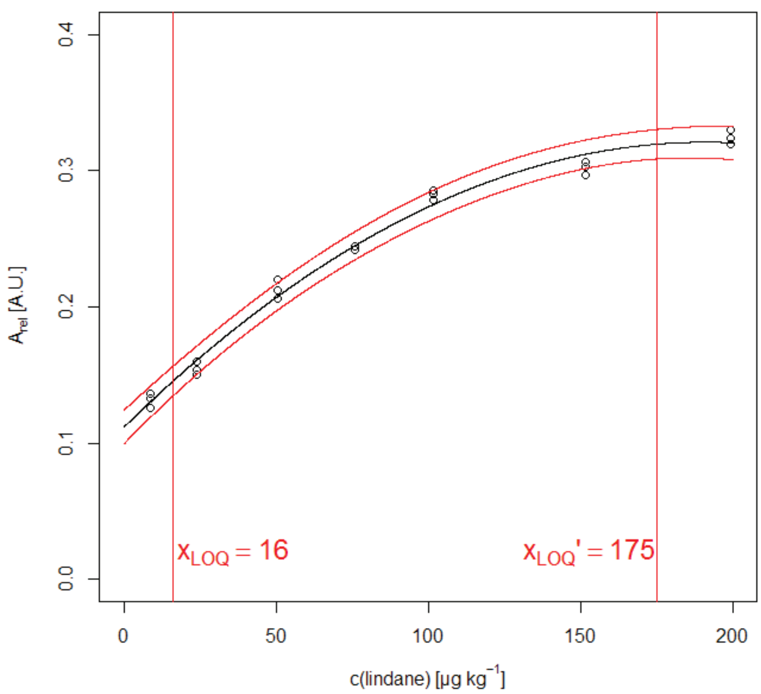
## Qualitative analysis

DIN ISO 11843-5:2010
"Capability of detection - Part 5: Methodology in the linear and non-linear calibration cases"





# TDS-IMS of pesticides in aqueous samples




# Lindane

$$s_{x0} = 3.6\ \mu g/kg$$
$$x_{LOD} = 10\ \mu\text{g/kg}$$

C. Zscheppank, U. Telgheder, K. Molt; International Journal for Ion Mobility Spectrometry (2012): 1-8, May 01, 2012

BSCW

Datei Bearbeiten Ansicht Optionen Anzeigen Hilfe

Gemeinschaften von anonymous ▸ public ▸ Nachweis-, Erfassungs- und Bestimmungsgren

**Nachweis-, Erfassungs- und Bestimmungsgrenzen**

Name

- **Fortbildungsveranstaltungen**
- **Manuskripte**
  Begleitmaterial zum Fortbildungskurs
- **R-Programme**
  Die Ein- und Ausgabe der Daten erfolgt über Excel-Dateien (csv).
- **DIN 32645**
  Die DIN beschreibt die Kalibrierung mit Hilfe einer Regressiongeraden, die Ermittlung der Nach





# http://bscw.uni-due.de/pub/bscw.cgi/12944089

**R-Programme**

Name

- **F-Test (R-Programm)**
  Durchführung eines F-Tests zur Prüfung auf Varianzhomogenität
- **Kalibrierfunktionen ersten Grades (R-Programme)**
  Erstellung von Kalibriergeraden einschließlich Leerwertmethode und Durchführung von Analysen
- **Kalibrierfunktionen zweiten Grades (R-Programme)**
  Erstellung von Kalibrierkurven einschließlich Leerwertmethode und Durchführung von Analysen
- **Anleitung zur Anwendung der R-Programme**
  Die R-Programme können zur Kalibrierung mit Kalibrierfunkionen ersten und zweiten Grades eingesetzt wer
- **Angebot zur Auswertung von Daten**
  Exemplarische Datensätze werden kostenlos ausgewertet.
- **Disclaimer**

# . . . the end.

Thank you for your attention!

E-mail: karl.molt@uni-due.de

| | |
|---|---|
| **Fortbildungsveranstaltung zum Thema Kalibrierung** | **6. November 2012, Essen Haus der Technik** |