

# Uncertainties and errors in quantitative chemical analysis

When you analyze samples,  
are you sure that you get correct results?

# Lecture Plan

General definitions: mean value, standard deviation, etc.

Types of errors and uncertainty

Method validation

Quality assurance

# Mean value

$$\bar{x} = \frac{\sum_i^n x_n}{n}$$

Example: calculate mean value for the following concentrations measurements: 5.32; 5.22; 5.25; 5.35; 5.30; 5.31 (mg/L)

$$\bar{x} = \frac{5.32 + 5.22 + 5.25 + 5.35 + 5.30 + 5.31}{6} = 5.29$$

# Mean value in MS Excel

|    | A | B                                | C       | D |
|----|---|----------------------------------|---------|---|
| 1  |   | <b>Calculation of mean value</b> |         |   |
| 2  |   |                                  |         |   |
| 3  |   | N                                | C, mg/L |   |
| 4  |   | 1                                | 5,32    |   |
| 5  |   | 2                                | 5,22    |   |
| 6  |   | 3                                | 5,25    |   |
| 7  |   | 4                                | 5,35    |   |
| 8  |   | 5                                | 5,30    |   |
| 9  |   | 6                                | 5,31    |   |
| 10 |   | Mean                             | 5,29    |   |
| 11 |   |                                  |         |   |

**CP3HACH(диапазон)**

**or**

**AVERAGE(range)**

# Standard deviation

$$s = \sqrt{\frac{\sum_i^n (x_i - \bar{x})^2}{n - 1}}$$

Example: calculate standard deviation value for the following concentrations measurements: 5.32; 5.22; 5.25; 5.35; 5.30; 5.31 (mg/L)

$$s = \sqrt{\frac{(5.32 - 5.29)^2 + (5.22 - 5.29)^2 + (5.25 - 5.29)^2 + \dots}{5}} = ?$$

# Standard deviation in MS Excel

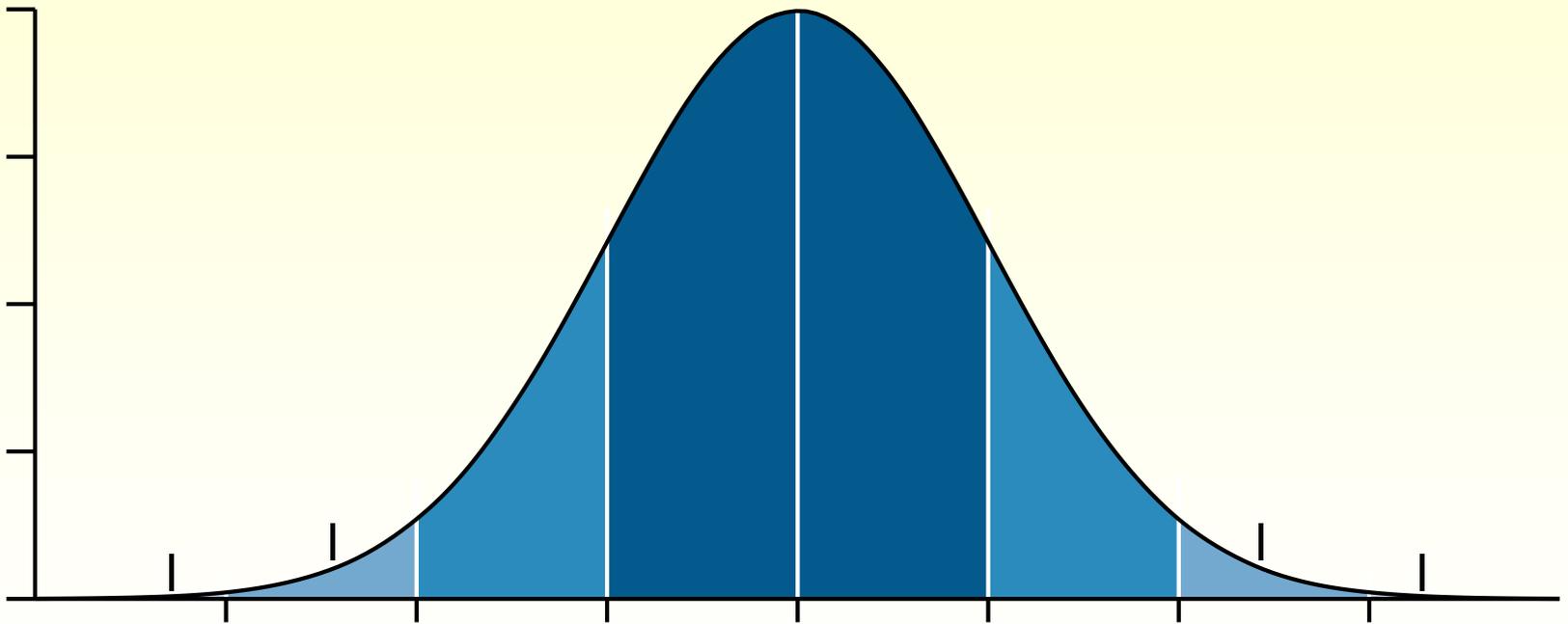
|    | A | B                                | C       | D |
|----|---|----------------------------------|---------|---|
| 1  |   | <b>Calculation of mean value</b> |         |   |
| 2  |   |                                  |         |   |
| 3  |   | N                                | C, mg/L |   |
| 4  |   | 1                                | 5,32    |   |
| 5  |   | 2                                | 5,22    |   |
| 6  |   | 3                                | 5,25    |   |
| 7  |   | 4                                | 5,35    |   |
| 8  |   | 5                                | 5,30    |   |
| 9  |   | 6                                | 5,31    |   |
| 10 |   | Mean                             | 5,29    |   |
| 11 |   | S                                | 0,048   |   |
| 12 |   |                                  |         |   |

**СТАНДОТКЛОН  
(диапазон)**

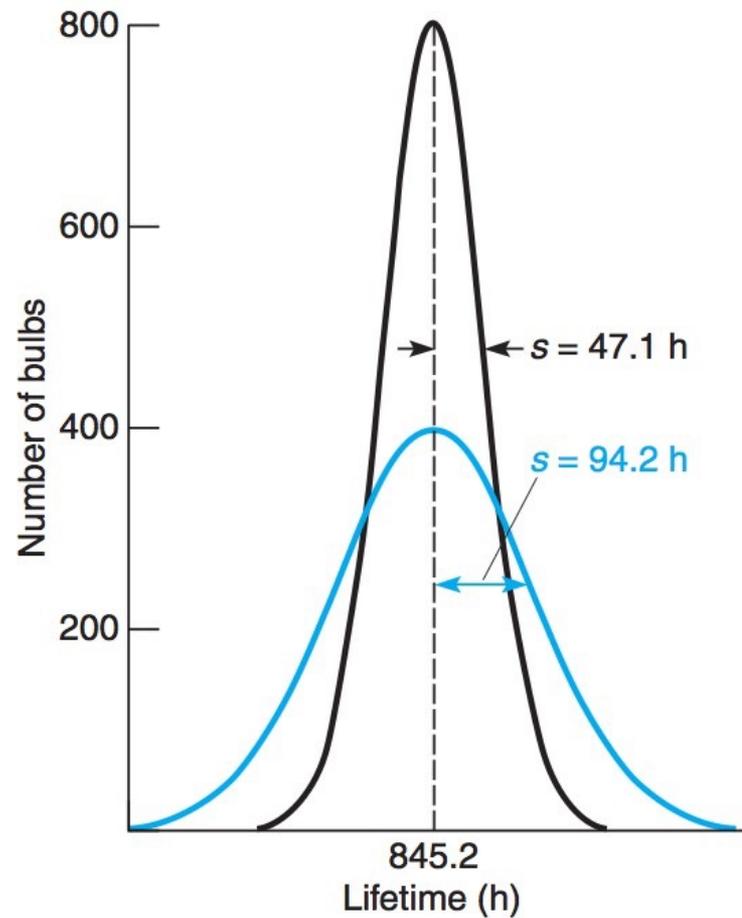
**or**

**STDEV(range)**

# Standard deviation



# Gaussian distribution



# Confidence interval

$$\text{Confidence interval} = \bar{x} \pm \frac{t \times s}{\sqrt{n}}$$

Where:  $t$  – Student's  $t$  coefficient;  $n$  – number of measurements

Example: calculate **confidence interval** (95%) value for the following concentrations measurements: 5.32; 5.22; 5.25; 5.35; 5.30; 5.31 (mg/L)

# Student's t coefficients

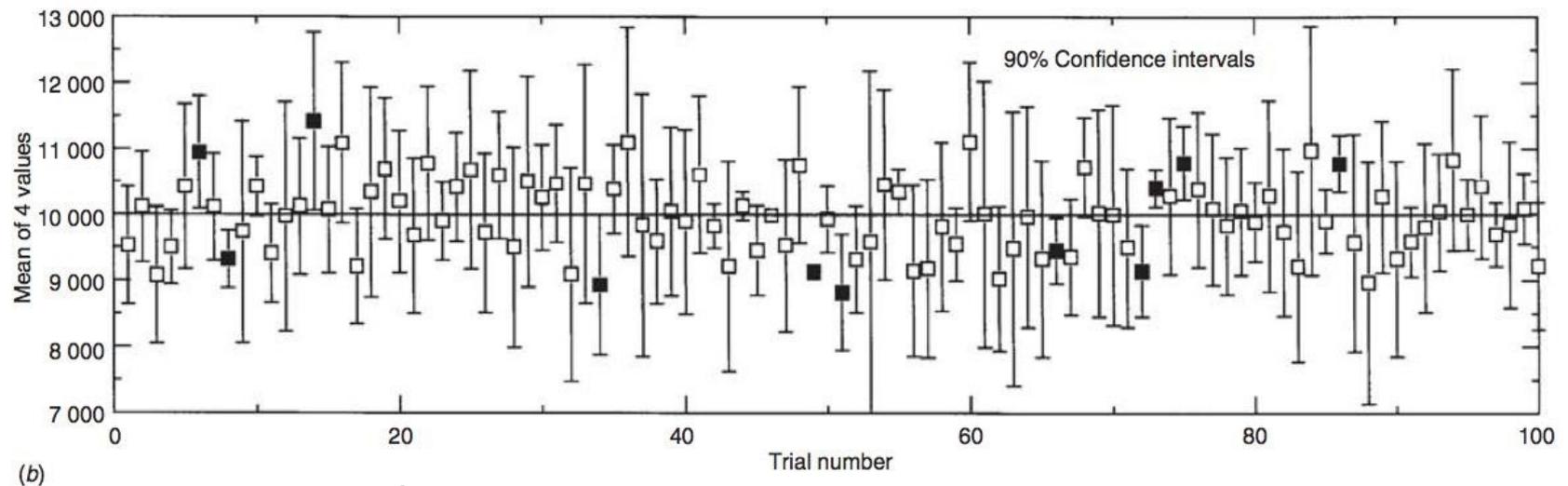
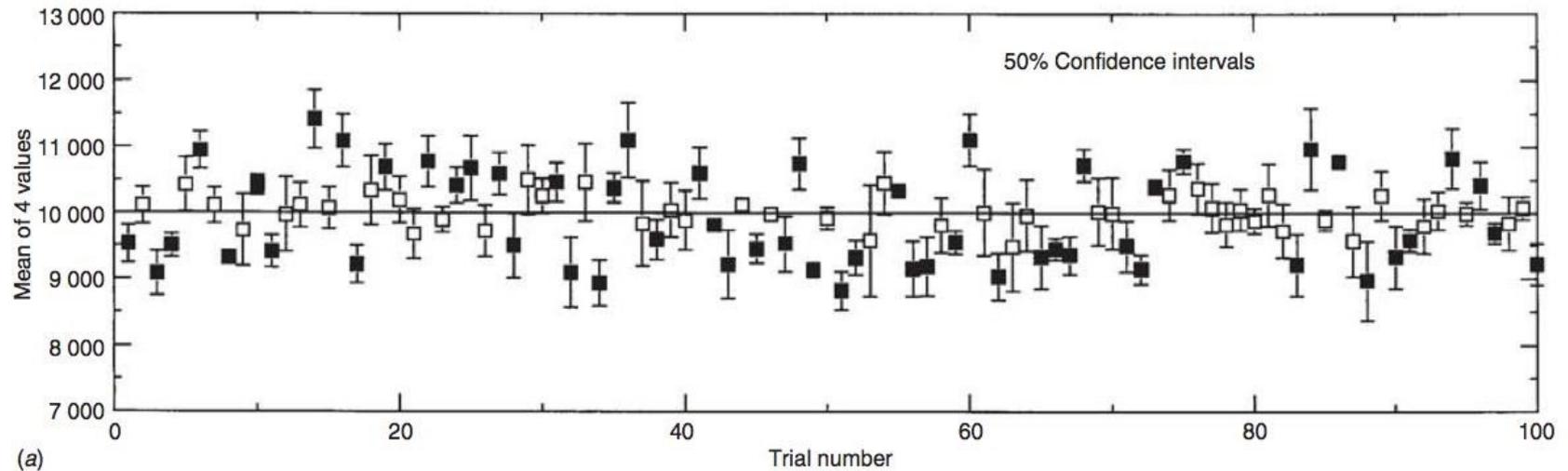
**TABLE 4-2** Values of Student's t

| Degrees of freedom | Confidence level (%) |       |        |        |        |         |         |
|--------------------|----------------------|-------|--------|--------|--------|---------|---------|
|                    | 50                   | 90    | 95     | 98     | 99     | 99.5    | 99.9    |
| 1                  | 1.000                | 6.314 | 12.706 | 31.821 | 63.656 | 127.321 | 636.578 |
| 2                  | 0.816                | 2.920 | 4.303  | 6.965  | 9.925  | 14.089  | 31.598  |
| 3                  | 0.765                | 2.353 | 3.182  | 4.541  | 5.841  | 7.453   | 12.924  |
| 4                  | 0.741                | 2.132 | 2.776  | 3.747  | 4.604  | 5.598   | 8.610   |
| 5                  | 0.727                | 2.015 | 2.571  | 3.365  | 4.032  | 4.773   | 6.869   |
| 6                  | 0.718                | 1.943 | 2.447  | 3.143  | 3.707  | 4.317   | 5.959   |
| 7                  | 0.711                | 1.895 | 2.365  | 2.998  | 3.500  | 4.029   | 5.408   |
| 8                  | 0.706                | 1.860 | 2.306  | 2.896  | 3.355  | 3.832   | 5.041   |
| 9                  | 0.703                | 1.833 | 2.262  | 2.821  | 3.250  | 3.690   | 4.781   |
| 10                 | 0.700                | 1.812 | 2.228  | 2.764  | 3.169  | 3.581   | 4.587   |
| 15                 | 0.691                | 1.753 | 2.131  | 2.602  | 2.947  | 3.252   | 4.073   |
| 20                 | 0.687                | 1.725 | 2.086  | 2.528  | 2.845  | 3.153   | 3.850   |
| 25                 | 0.684                | 1.708 | 2.060  | 2.485  | 2.787  | 3.078   | 3.725   |
| 30                 | 0.683                | 1.697 | 2.042  | 2.457  | 2.750  | 3.030   | 3.646   |
| 40                 | 0.681                | 1.684 | 2.021  | 2.423  | 2.704  | 2.971   | 3.551   |
| 60                 | 0.679                | 1.671 | 2.000  | 2.390  | 2.660  | 2.915   | 3.460   |
| 120                | 0.677                | 1.658 | 1.980  | 2.358  | 2.617  | 2.860   | 3.373   |
| $\infty$           | 0.674                | 1.645 | 1.960  | 2.326  | 2.576  | 2.807   | 3.291   |

# Confidence interval in Excel

| E13 |   | =C12*C11/КОРЕНЬ(B9) |               |   |   |      |
|-----|---|---------------------|---------------|---|---|------|
|     | A   | B                   | C             | D | E | F    |
| 1   | <b>Calculation of confidence interval</b> |                     |               |   |   |      |
| 2   |   |                     |               |   |   |      |
| 3   |   | N                   | C, mg/L       |   |   |      |
| 4   |   | 1                   | 5,32          |   |   |      |
| 5   |   | 2                   | 5,22          |   |   |      |
| 6   |   | 3                   | 5,25          |   |   |      |
| 7   |   | 4                   | 5,35          |   |   |      |
| 8   |   | 5                   | 5,30          |   |   |      |
| 9   |   | 6                   | 5,31          |   |   |      |
| 10  |   | Mean                | 5,29          |   |   |      |
| 11  |   | S                   | 0,048         |   |   |      |
| 12  |   | t                   | 2,57          |   |   |      |
| 13  |   | conf int            | 5,292 ± 0,050 |   |   | mg/L |

# 50% and 90% confidence intervals



# Types of errors

**Systematic** – due to poor design of experiment, equipment or method

**Random** – due to any uncontrolled variables

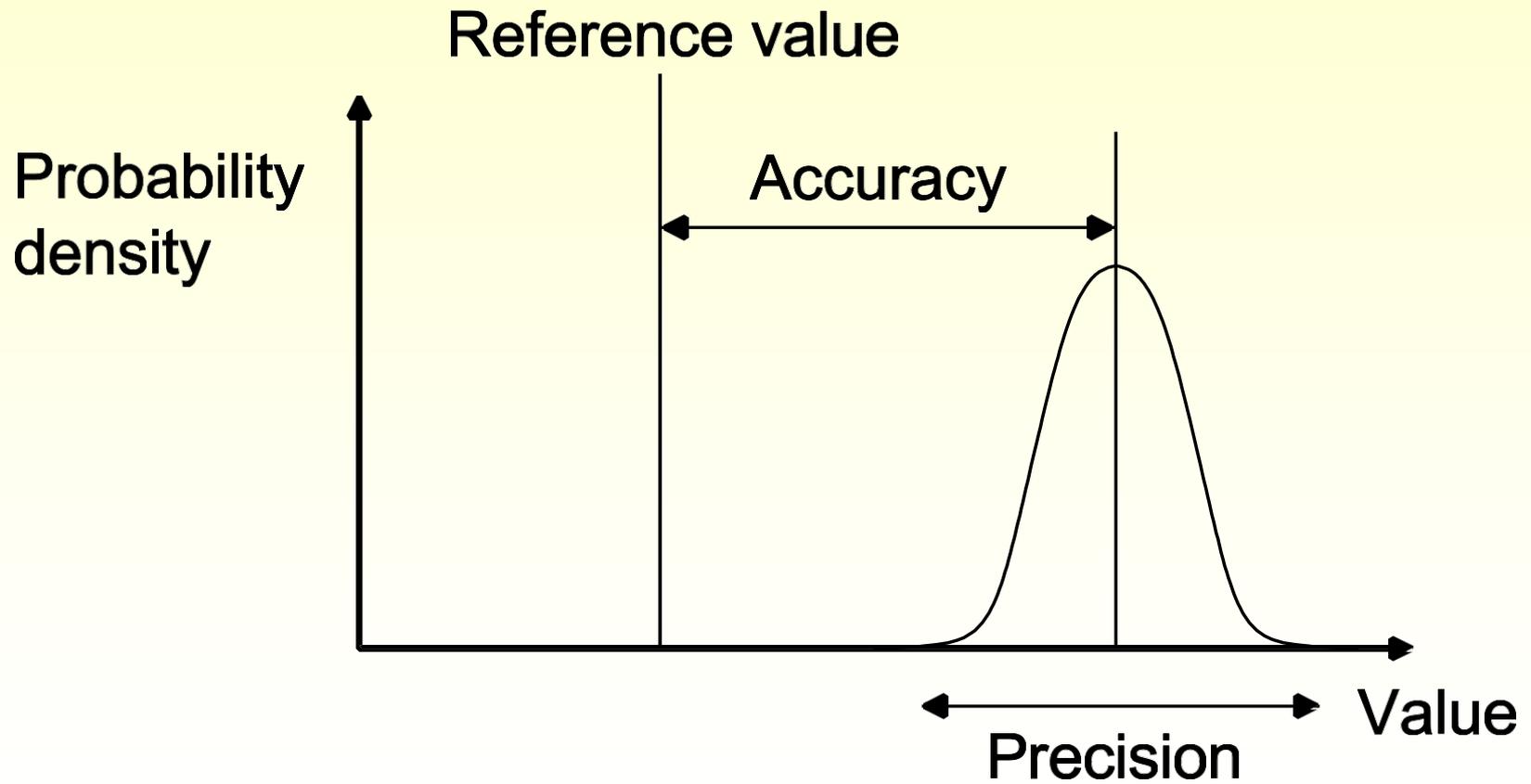
# Accuracy and precision

**Accuracy** shows how close results are to the “real” value

**Precision** shows how reproducible your results are

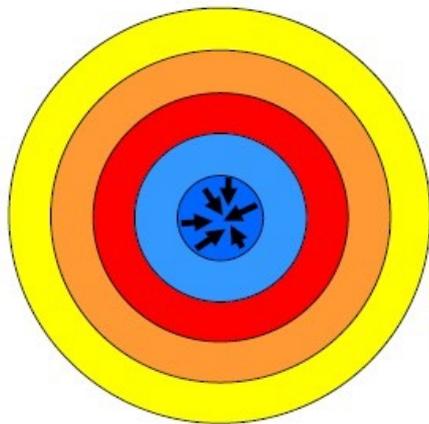
*Reproducibility* – analyses are done several times by different people, in different laboratories, using different equipment, at different conditions, etc.

# Accuracy and precision

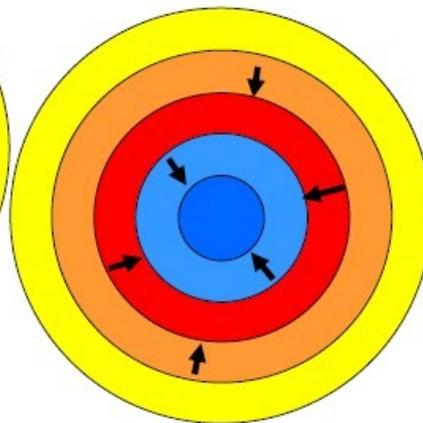


# Accuracy and precision

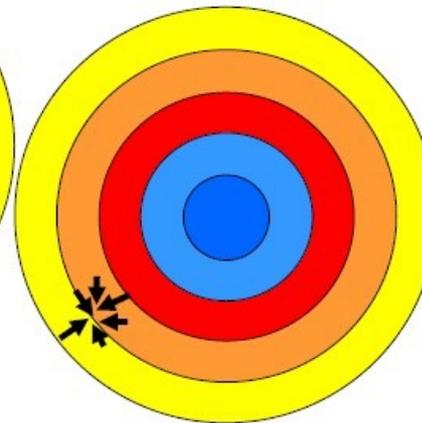
- Accuracy and precision



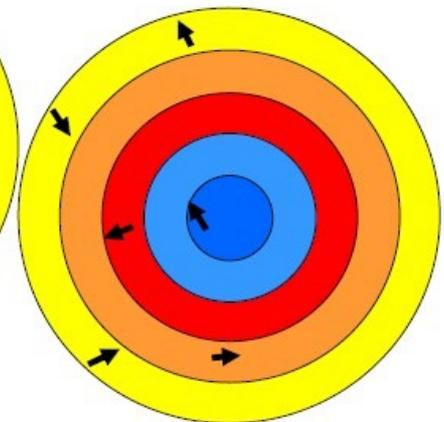
Accurate &  
precise



Accurate &  
imprecise



Inaccurate &  
precise



Inaccurate & imprecise

# Task

Vodka contains 380 mg/L of methanol

You got the following results of analysis (mg/L):

350

370

360

Calculate accuracy and precision. How would you characterize them (high or low)?

# Why do you need accuracy and precision?

## **Accuracy:**

Imagine that your drug must contain certain concentration. A 5% higher concentration will kill you (> lethal dose), but 5% lower will not work as it should (drugs against cancer).

If you perform disease screening, differences in urine and blood concentrations of certain metabolites between sick and healthy patients can be very low. You do not want to be false diagnosed a disease.

## **Precision:**

If the method is imprecise, you will have to analyze many replicates to get accurate results. That will require a lot of extra **time** and **money**

# Uncertainty

Shows the maximum error of chemical analysis

**A “sum” of all systematic and random errors during analysis**

Example:

concentration of quercetin in *Artemisia* plants is  $2.31 \pm 0.05$  mg/kg

Absolute uncertainty is 0.05 mg/kg

Relative uncertainty =  $(0.05 \text{ mg/kg}) / (2.31 \text{ mg/kg}) = 0.022$

Or in %:  $0.022 * 100\% = 2.2\%$

# Summary of rules

**TABLE 3-1 Summary of rules for propagation of uncertainty**

| Function              | Uncertainty                                | Function <sup>a</sup> | Uncertainty <sup>b</sup>   |
|-----------------------|--|-----------------------|--|
| $y = x_1 + x_2$       | $e_y = \sqrt{e_{x_1}^2 + e_{x_2}^2}$       | $y = x^a$             | $\%e_y = a\%e_x$   |
| $y = x_1 - x_2$       | $e_y = \sqrt{e_{x_1}^2 + e_{x_2}^2}$       | $y = \log x$          | $e_y = \frac{1}{\ln 10} \frac{e_x}{x} \approx 0.434\ 29 \frac{e_x}{x}$ |
| $y = x_1 \cdot x_2$   | $\%e_y = \sqrt{\%e_{x_1}^2 + \%e_{x_2}^2}$ | $y = \ln x$           | $e_y = \frac{e_x}{x}$  |
| $y = \frac{x_1}{x_2}$ | $\%e_y = \sqrt{\%e_{x_1}^2 + \%e_{x_2}^2}$ | $y = 10^x$            | $\frac{e_y}{y} = (\ln 10)e_x \approx 2.302\ 6 e_x$                     |
|                       |  | $y = e^x$             | $\frac{e_y}{y} = e_x$  |

a.  $x$  represents a variable and  $a$  represents a constant that has no uncertainty.

b.  $e_x/x$  is the relative error in  $x$  and  $\%e_x$  is  $100 \times e_x/x$ .

# Uncertainty: example

Find the total amount of impurities in gold if concentration of:

- 1) Silver is  $5.21 \pm 0.05\%$ ;
- 2) Nickel –  $1.11 \pm 0.04\%$ ;
- 3) Titanium –  $0.22 \pm 0.02\%$

The total concentration of impurities in gold is  $5.21 + 1.11 + 0.22 = 6.54\%$

**What is uncertainty of this value?**

$$e_{total} = \sqrt{\sum_{i=1}^n e_i^2} = \sqrt{(0.05)^2 + (0.04)^2 + (0.02)^2} = 0.07$$

Relative uncertainty =  $(0.07\% / 6.54\%) \times 100\% = 1.0\%$

# Calculations in MS Excel

D8     $\times$      $\checkmark$      $f_x$     =КОРЕНЬ(D5^2+D6^2+D7^2)

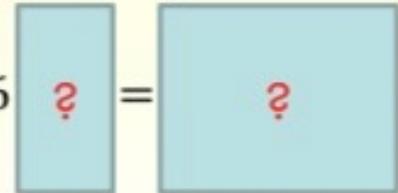
|    | A | B                                 | C           | D           |
|----|---|-----------------------------------|-------------|-------------|
| 1  |   | <b>Calculation of the results</b> |             |             |
| 2  |   | Impurities in gold sample         |             |             |
| 3  |   |                                   |             |             |
| 4  |   |                                   | C           | e           |
| 5  |   | Silver                            | 5,21        | 0,05        |
| 6  |   | Nickel                            | 1,11        | 0,04        |
| 7  |   | Titanium                          | 0,22        | 0,02        |
| 8  |   | Total                             | <b>6,54</b> | <b>0,07</b> |
| 9  |   | Relative e, %                     |             | <b>1,0</b>  |
| 10 |   |                                   |             |             |

# Uncertainty: example 2

Find the concentration of solution if  $10.4 \pm 0.1$  mg of  $\text{BaCl}_2$  (purity  $99.95 \pm 0.05\%$ ) were weighted using analytical balances and dissolved in water to the final volume  $50.00 \pm 0.02$  mL. Find the uncertainty of the concentration of  $\text{BaCl}_2$

The concentration of  $\text{BaCl}_2$  in solution can be found using:

$$C = \frac{m \times \text{Purity}}{V \times 100\%} = \frac{10.4 \text{ mg} \times 99.95 \%}{50.00 \text{ mL} \times 100 \%} = 0.207896$$



**What is the uncertainty of this value?**

$$\text{rel } e_{\text{total}} = \sqrt{\sum_{i=1}^n \text{rel } e_i^2}$$

# MS Excel calculations

E8     $\times$      $\checkmark$      $f_x$     =КОРЕНЬ(E5^2+E6^2+E7^2)

|   | A | B  | C            | D            | E           |
|---|---|--|--------------|--------------|-------------|
| 1 |   | <b>Calculation of the results</b>        |              |              |             |
| 2 |   | <b>Concentration of BaCl<sub>2</sub></b> |              |              |             |
| 3 |   |  |              |              |             |
| 4 |   |  | Value        | e            | rel e (%)   |
| 5 |   | Mass, mg                                 | 10,4         | 0,1          | 0,96        |
| 6 |   | Purity, %                                | 99,95        | 0,05         | 0,05        |
| 7 |   | Volume, mL                               | 50,00        | 0,02         | 0,04        |
| 8 |   | Concentration, mg/mL                     | <b>0,208</b> | <b>0,002</b> | <b>0,96</b> |
| 9 |   |  |              |              |             |

# Task

Calibrated gas sampling bulb ( $V = 250 \pm 2$  mL) was spiked with  $10.0 \pm 0.2$   $\mu\text{L}$  of benzene solution in methanol ( $C = 15.0 \pm 0.2$  ng/ $\mu\text{L}$ ). Calculate the concentration of benzene in the bulb and its uncertainty

$$C_2 = \frac{C_1 V_1}{V_2} = \frac{15 \frac{\text{ng}}{\mu\text{L}} \times 10 \mu\text{L}}{250 \text{ mL}} = \frac{150 \text{ ng}}{250 \text{ mL}} = 0.600 \frac{\text{ng}}{\text{mL}}$$

$$\%e_{C_2} = \sqrt{\%e_{C_1}^2 + \%e_{V_1}^2 + \%e_{V_2}^2}$$

$$\%e_{C_1} = \frac{0.2}{15.0} \times 100\% = 1.33\%$$

$$\%e_{V_1} = \frac{0.2}{10.0} \times 100\% = 2.0\%$$

$$\%e_{V_2} = \frac{2}{250} \times 100\% = 0.80\%$$

# Solution (continued)

$$\begin{aligned}\%e_{C_2} &= \sqrt{(1.33\%)^2 + (2.0\%)^2 + (0.8\%)^2} = \sqrt{1.769\%^2 + 4\%^2 + 0.64\%^2} \\ &= \sqrt{6.409}\end{aligned}$$

$$\%e_{C_2} = \sqrt{6.409\%^2} = 2.53\%$$

$$e_{C_2} = \frac{C_2 \times \%e_{C_2}}{100\%} = \frac{0.600 \frac{\text{ng}}{\text{mL}} \times 2.53\%}{100\%} = 0.01518 \frac{\text{ng}}{\text{mL}}$$

Answer:  $C_2 = 0.600 \pm 0.015$

# Exercise

10.0 g of soil containing N-nitrosodimethylamine (NDMA) was extracted by two portions of acetone (25 mL each). Extracts were combined to the final volume  $42 \pm 2$  mL and evaporated to the final volume  $1.00 \pm 0.02$  mL. Analysis of the evaporated extract by GC-MS showed NDMA concentration  $27 \pm 1$  ng/mL. Calculate NDMA concentration in soil sample and its uncertainty.

# Solution

From the available data, we can calculate the total mass of analyte in concentrated extract:

$$m = V \times C = 27 \frac{\text{ng}}{\text{mL}} \times 1.00 \text{ mL} = 27 \text{ ng}$$

If no analyte was lost during evaporation, this analyte mass was also present in the extract before evaporation. Extraction recovery was not provided. Some portion of solvent remained in soil during filtration. But probably, the mass of NDMA in this part is negligible and recovery is 100%.

$$C = \frac{m_{\text{NDMA}}}{m_{\text{soil}}} = \frac{27 \text{ ng}}{10.0 \text{ g}} = 2.7 \frac{\text{ng}}{\text{g}}$$

# General formula

$$m = V \times C_{ex} = 27 \frac{\text{ng}}{\text{mL}} \times 1.00 \text{ mL} = 27 \text{ ng}$$

$$C_s = \frac{m_{NDMA}}{m_{soil}} = \frac{27 \text{ ng}}{10.0 \text{ g}} = 2.7 \frac{\text{ng}}{\text{g}}$$

$$C_s = \frac{V \times C_{ex}}{m_{soil}} = \frac{27 \frac{\text{ng}}{\text{mL}} \times 1.00 \text{ mL}}{10.0 \text{ g}} = 2.7 \frac{\text{ng}}{\text{g}}$$

# Uncertainty

$$\%e_{c_s} = \sqrt{\%e_{c_{ex}}^2 + \%e_V^2 + \%e_m^2}$$

$$\%e_{c_{ex}} = \frac{1}{27} \times 100\% = 3.7\%$$

$$\%e_V = \frac{0.02}{1} \times 100\% = 2\%$$

$$\%e_m = \frac{0.1}{10.0} \times 100\% = 1\%$$

# Uncertainty

$$\%e_{C_s} = \sqrt{3.7^2 + 2^2 + 1^2} = 4.3\%$$

$$e_{C_s} = 2.7 \frac{\text{ng}}{\text{g}} \times \frac{4.3\%}{100\%} = 0.12 \frac{\text{ng}}{\text{g}}$$

**Answer: NDMA concentration in analyzed soil sample is  $2.70 \pm 0.12$  ng/g**

# Exercise

Brandy sample was analyzed for the concentration of iso-butanol using standard addition method. Standard additions were 0.50; 1.00; 2.00; 5.00; 10.0 and 20.0 mg/L. Peak areas of iso-butanol peaks were 6.23; 7.44; 9.46; 15.5; 25.5 и 45.3 arbitrary units. Calculate the concentration of iso-butanol in the analyzed sample and it's uncertainty.

# Significant figures: Real Rule

$$\frac{0.002364 (\pm 0.000003)}{0.02500 (\pm 0.00005)} = 0.0946 (\pm 0.0002)$$

$$\frac{0.002664 (\pm 0.000003)}{0.02500 (\pm 0.00005)} = 0.1066 (\pm 0.0002)$$

$$\frac{0.821 (\pm 0.002)}{0.803 (\pm 0.002)} = 1.022 (\pm 0.004)$$

Rule: when first number in the answer is 1 or 9, there can be one extra or one lacking significant figure, respectively.

# Significance of difference

Case 1: you analyzed certified reference material (CRM) having known concentration of analyte. Are your results significantly different from the certified value?

Answer:

- 1) analyze sample several times;
- 2) calculate 95% confidence interval for replicate measurements;
- 3) if certified concentration does not lie in your confidence interval, the results are "different".

Example: CRM of coal contains 3.19% of sulfur. You analyzed sample 4 times and got the following concentrations of sulfur: 3.29; 3.22; 3.30 and 3.23%. Does your answer agree with the known answer?

# Solution in MS Excel

| Agreement of data           |               |       |          |
|-----------------------------|---------------|-------|----------|
| CRM of sulfur by new method |               |       |          |
| N                           | C, %          |       |          |
| 1                           | 3.29          |       |          |
| 2                           | 3.22          |       |          |
| 3                           | 3.30          |       |          |
| 4                           | 3.23          |       |          |
| Mean                        | 3.26          |       |          |
| S                           | 0.041         |       |          |
| t                           | 3.18          |       |          |
| conf int                    | 3.260 ± 0.065 |       | %        |
| CRM                         | 3.19          | 0.070 | Mean-CRM |

## Conclusion:

- 1) CRM does not lie in confidence interval;
- 2) Results are significantly different

# Significance of difference

Case 2: comparing replicate measurements.

You and your colleague analyzed sample using 2 different methods.  
Are your results significantly different?

$$t_{\text{calculated}} = \frac{|\bar{x}_1 - \bar{x}_2|}{S_{\text{pooled}}} \sqrt{\frac{n_1 \times n_2}{n_1 + n_2}}$$

$$S_{\text{pooled}} = \sqrt{\frac{S_1^2 (n_1 - 1) + S_2^2 (n_2 - 1)}{n_1 + n_2 - 2}}$$

if  $t_{\text{calculated}} > t_{\text{table}}$ , results are different

# Example in MS Excel

| =КОРЕНЬ(((C15^2)*(C16-1)+E15^2*(E16-1))/(C16+E16-2)) |   |   |                                 |  |
|--|---|---|---------------------------------|--|
| B  | C   | D | E                               |  |
|  | Difference between 2 sets of measurements |   |                                 |  |
|  | Mass of nitrogen isolated from air        |   |                                 |  |
|  | From air (g)                              |   | From chemical decomposition (g) |  |
| 1  | 2.31017                                   |   | 2.30143                         |  |
| 2  | 2.30986                                   |   | 2.29890                         |  |
| 3  | 2.31010                                   |   | 2.29816                         |  |
| 4  | 2.31001                                   |   | 2.30182                         |  |
| 5  | 2.31024                                   |   | 2.29869                         |  |
| 6  | 2.31010                                   |   | 2.29940                         |  |
| 7  | 2.31028                                   |   | 2.29849                         |  |
| 8  |   |   | 2.29889                         |  |

## Conclusion:

1)  $t_{\text{calc}} > t_{\text{table}}$ ;

2) Two sets of measurements are significantly different

# Grubbs test for Outliers

You analyzed your sample several times and need to find an outlier.

$$G_{\text{calculated}} = \frac{|questionable\ value - \bar{x}|}{S}$$

Data points having  $G_{\text{calculated}} > G_{\text{table}}$  are outliers.

**Homework: Understand example on Page 83 (Harris book), section 4-6**

**TABLE 4-5 Critical values of  $G$  for rejection of outlier**

| Number of observations | $G$<br>(95% confidence) |
|------------------------|-------------------------|
| 4                      | 1.463                   |
| 5                      | 1.672                   |
| 6                      | 1.822                   |
| 7                      | 1.938                   |
| 8                      | 2.032                   |
| 9                      | 2.110                   |
| 10                     | 2.176                   |
| 11                     | 2.234                   |
| 12                     | 2.285                   |
| 15                     | 2.409                   |
| 20                     | 2.557                   |

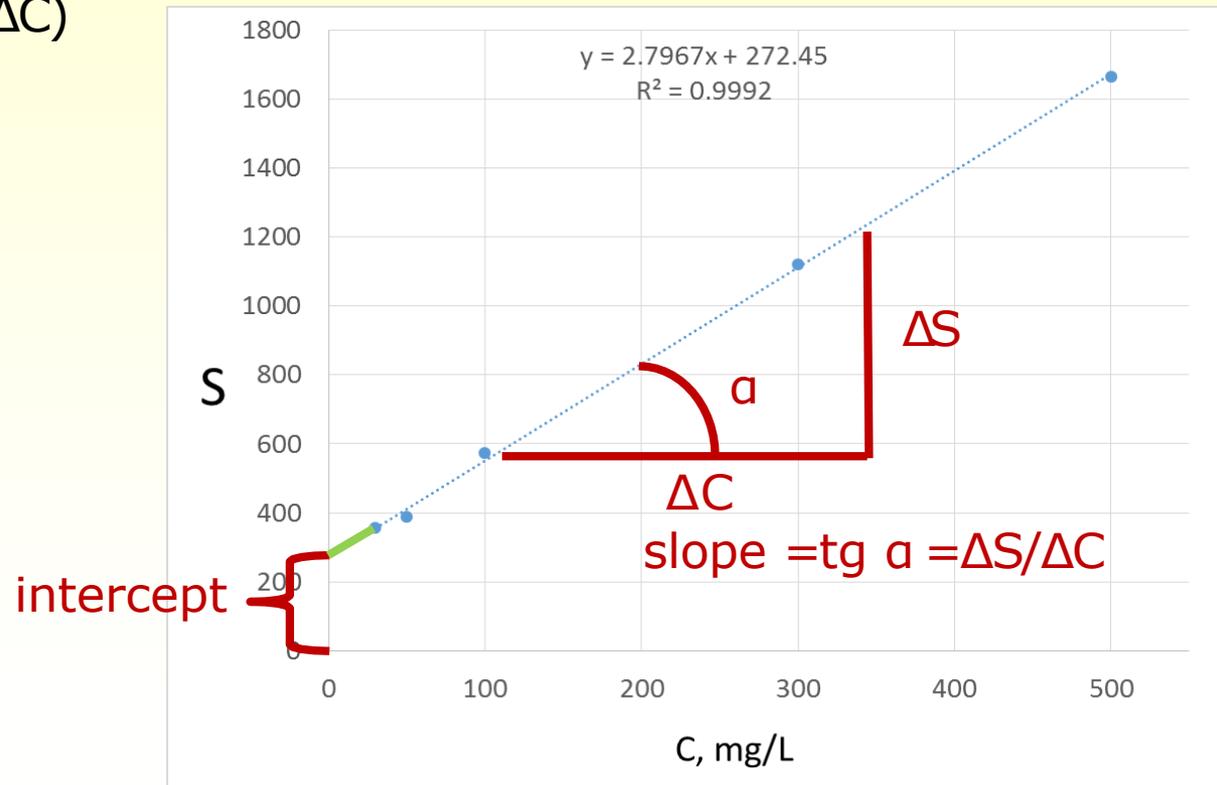
# Least squares method

Used to get linear calibration curve (Signal = f (Concentration)):

$$S = aC + b$$

a – slope ( $\Delta S / \Delta C$ )

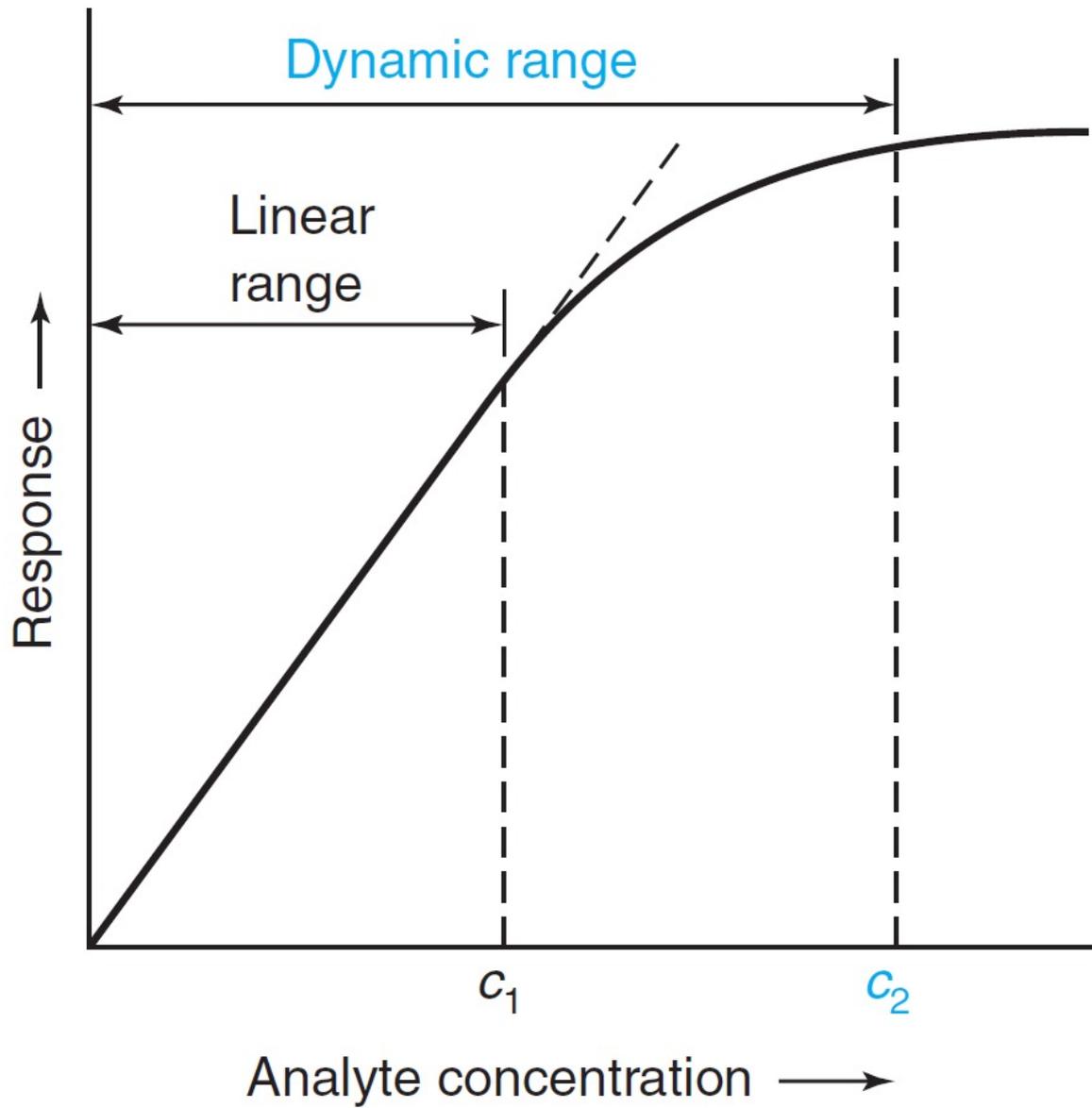
b -intercept



# Linear range of calibration

The **linear range** of an analytical method is the analyte concentration range over which response is proportional to concentration

**Dynamic range** - the concentration range over which there is a measurable response to analyte, even if the response is not linear



# Least squares in MS Excel

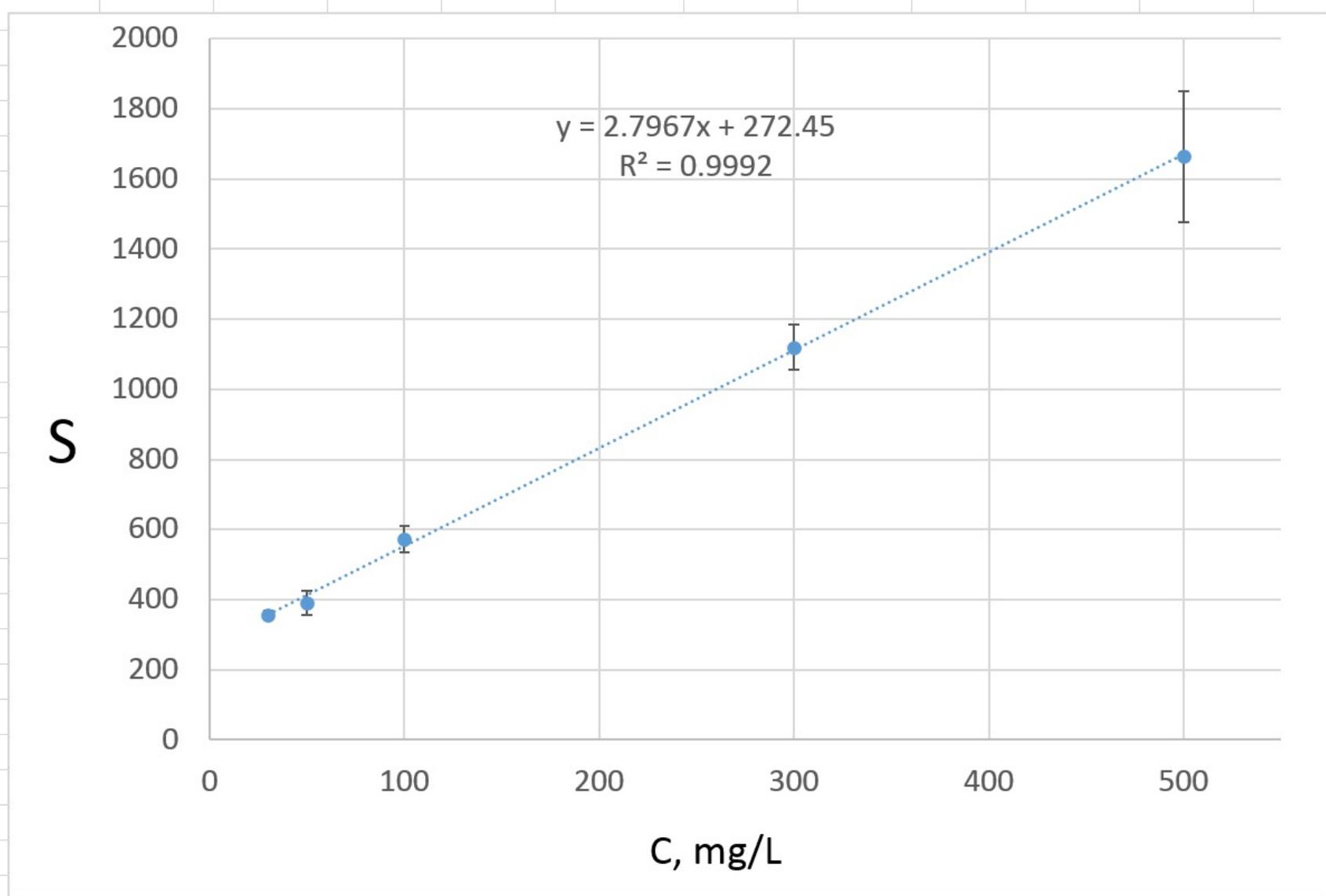
| G3 : X ✓ fx {=ЛИНЕЙН(С3:С7,В3:В7,ИСТИНА,ИСТИНА)} |   |         |        |       |   |           |        |           |  |
|--|---|---------|--------|-------|---|-----------|--------|-----------|--|
|  | A | B       | C      | D     | E | F         | G      | H         |  |
| 1  |   |         |        |       |   |           |        |           |  |
| 2  |   | C, ug/L | S      | SD    |   |           | Slope  | Intercept |  |
| 3  |   | 30      | 356.6  | 13.3  |   | Parameter | 2.797  | 272       |  |
| 4  |   | 50      | 390.1  | 35    |   | SD        | 0.046  | 12        |  |
| 5  |   | 100     | 572.6  | 37.2  |   | R2        | 0.9992 | 19 Sy     |  |
| 6  |   | 300     | 1119.8 | 63.8  |   |           |        |           |  |
| 7  |   | 500     | 1663.9 | 186.7 |   |           |        |           |  |

Use "ЛИНЕЙН" or "LINEST" functions:

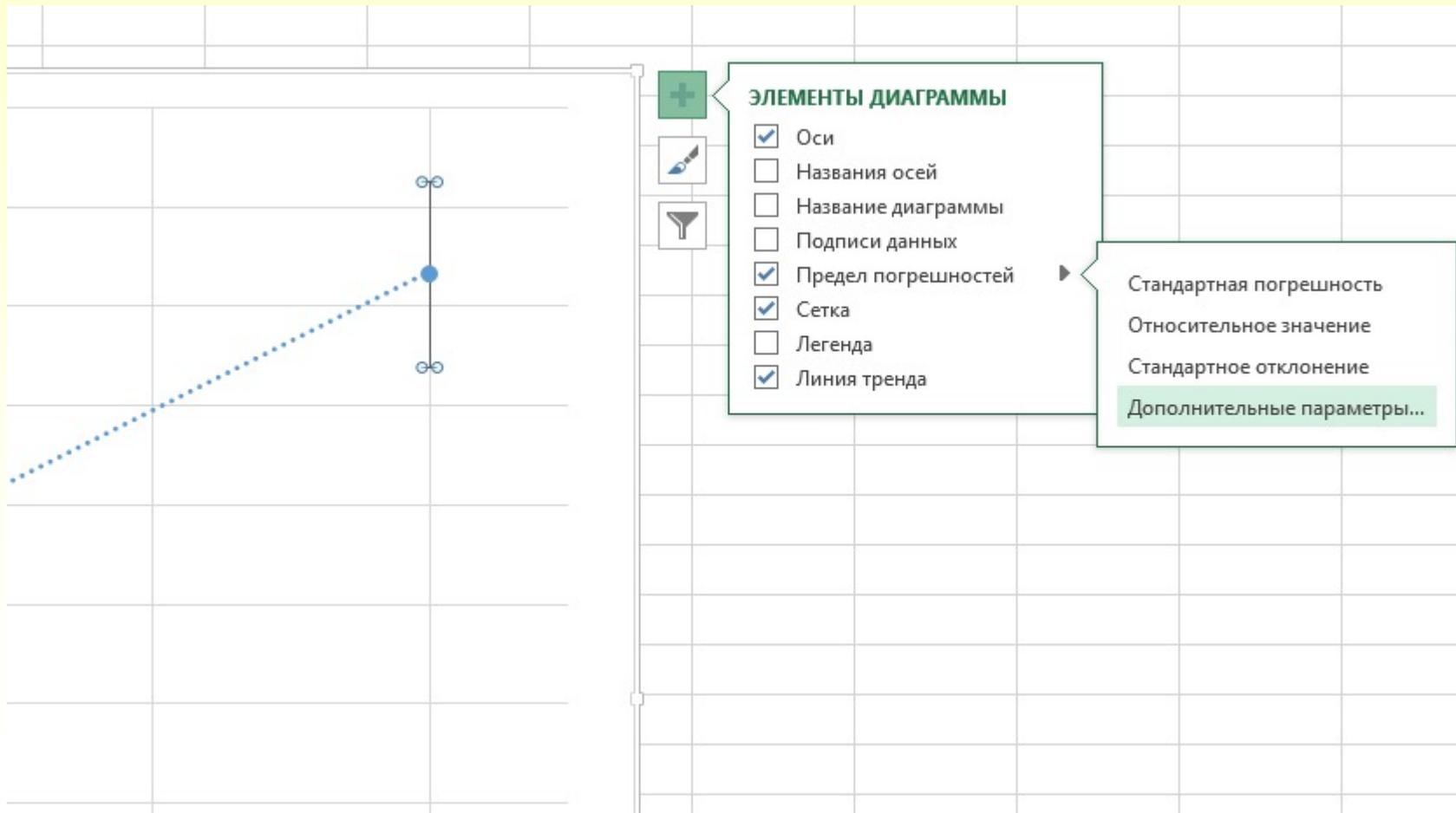
- 1) Select 3x3 cell array;
- 2) Enter the formula, select Y and X ranges (see above);
- 3) Last 2 variables must have "TRUE" and "TRUE" values;
- 4) Hold "SHIFT+CTRL" buttons, press "ENTER" button.

Linear plot equation:  $S = (2.797 \pm 0.046) \times C + (272 \pm 12)$

# Calibration plot with error bars



# Error bars in MS Excel 2013



# Error bars in MS Excel 2013

ПАРАМЕТРЫ ПРЕДЕЛА ПОГРЕШНОСТЕЙ ▾

▲ **ВЕРТИКАЛЬНЫЙ ПРЕДЕЛ ПОГРЕШНОСТИ**

Направление

Все

Минус

Плюс

Стиль края

Без точки

Точка

Величина погрешности:

фиксированное значение

относительное значение  %

стандартное отклонение

стандартная погрешность

пользовательская

|  | C, ug/L | S      | SD    |
|--|---------|--------|-------|
|  | 30      | 356.6  | 13.3  |
|  | 50      | 390.1  | 35    |
|  | 100     | 572.6  | 37.2  |
|  | 300     | 1119.8 | 63.8  |
|  | 500     | 1663.9 | 186.7 |

Select range

# Propagation of uncertainty

$$S_x = \frac{S_y}{|a|} \sqrt{\frac{1}{k} + \frac{1}{n} + \frac{(y - \bar{y})^2}{a^2 \sum (x_i - \bar{x})^2}}$$

k – number of replicate measurements of unknown sample;

n – number of data points in calibration plot;

a – slope;

$\bar{y}$  - mean value of Y for the points of calibration plot;

$\bar{x}$  - mean value of X for the points of calibration plot



# Method validation

Specificity (selectivity)

Linearity

Accuracy

Precision

Concentrations range

Limits of detection and quantification

# Method accuracy check

Analyze certified reference material or spiked sample

Compare your results with certified value or concentration spiked

Check if the differences are significant (see slides above)

# Spike recovery

$$\% \text{ recovery} = \frac{C_{\text{spiked sample}} - C_{\text{unspiked sample}}}{C_{\text{added}}} \times 100\%$$

We established benzene concentration in water sample 10.0 µg/L. A benzene spike of 5.0 µg/L was added to a replicate portion of sample. Analysis of the spiked sample gave a concentration of 14.6 µg/L. Find the percent recovery of the spike

$$\% \text{ recovery} = \frac{14.6 \mu\text{g/L} - 10.0 \mu\text{g/L}}{5.0 \mu\text{g/L}} \times 100\% = 92\%$$

Acceptable recovery depends on the method requirements. For environmental samples, recovery 50-120% is generally acceptable

# Task

Soil sample (10.0 g) having DDT concentration  $5.0 \mu\text{g}/\text{kg}$  was spiked with  $10.0 \mu\text{L}$  of DDT solution in hexane with concentration  $100 \text{ ng}/\mu\text{L}$ . Analysis of the spiked sample gave the DDT concentration  $17 \mu\text{g}/\text{kg}$ . Calculate spike recovery

# Method precision check

**Instrument:** single aliquot, day, instrument, analysts

**Intra-assay:** multiple aliquots, single day, instrument, analyst

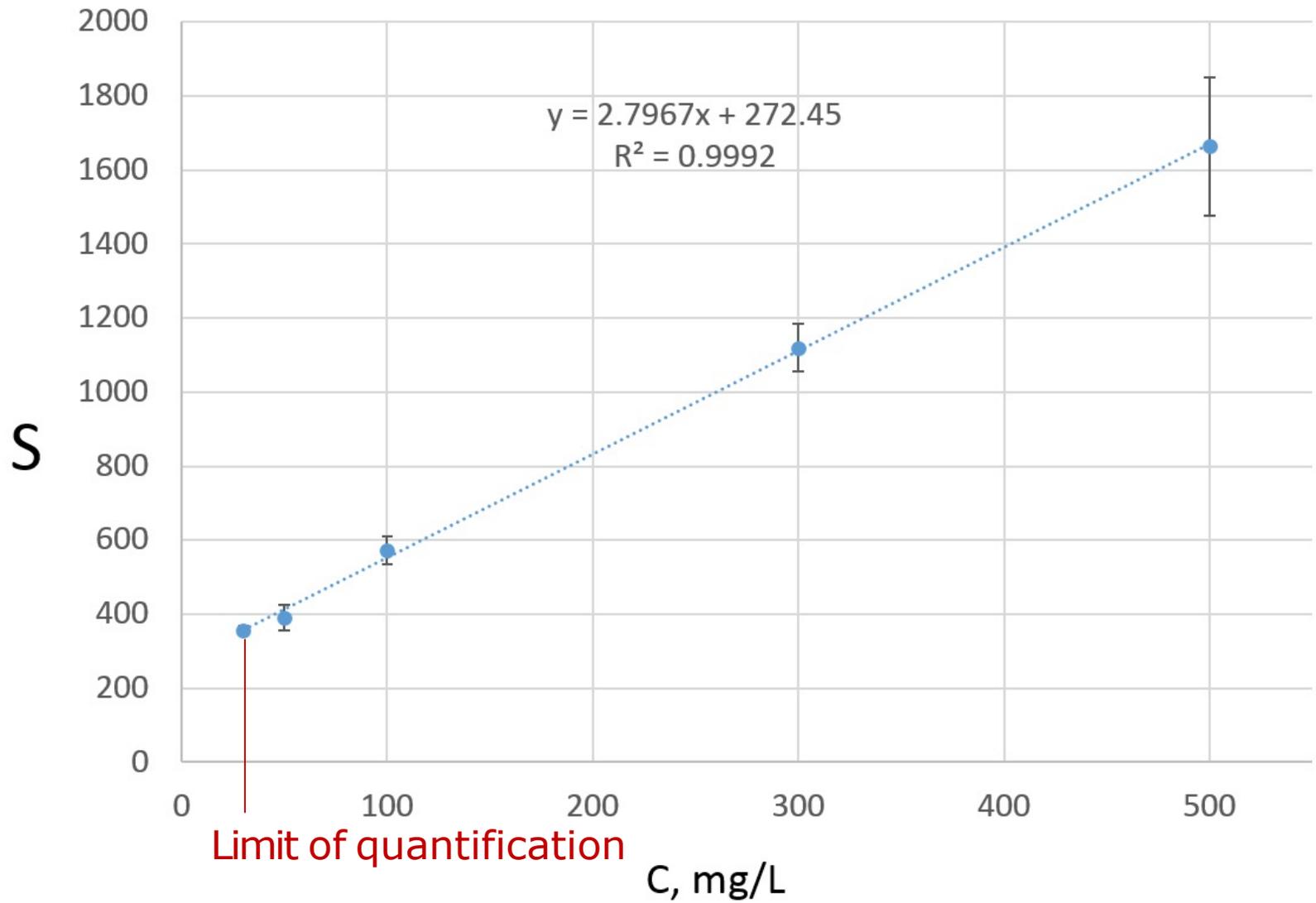
**Intermediate:** multiple people, days, instruments, analysts in the same lab

**Interlaboratory:** multiple people, days, instruments, analysts and laboratories

# Limit of quantification (LOQ)

The minimum concentration of analyte that can be analyzed with a desired error (for example, <10%)

Typically, predicted using calibration curve and standard deviation values of calibration solutions



# Limit of detection (LOD)

Limit of detection is the smallest quantity of analyte that is "significantly different" from the blank

For chromatography, if separation is very efficient and baseline is stable, it may correspond to concentration that gives peak having 3:1 signal to noise ratio

## **Most popular method:**

- 1) Analyze concentration that is close to the smallest concentration on calibration plot in 7 replicates;
- 2) Calculate standard deviation (SD) of concentration determined using calibration plot;
- 3) Multiply SD by t-coefficient (for 99% confidence and 6 degrees of freedom) – 2.896

# Task

Sample with NDMA (N-nitrosodimethylamine) concentration in water close to the limit of quantification was analyzed in 7 replicates. The results are ( $\mu\text{g/L}$ ):

**3.5; 3.1; 4.0; 3.7; 4.2; 3.6; 3.2**

Calculate detection limit for the method of NDMA determination in water

# Quality assurance

Quality assurance is what we (e.g., analytical chemists) do to provide sufficient accuracy and precision of results of chemical analyses

There is no point in spending extra money to obtain a more accurate or more precise answer if it is not necessary

# Laboratory accreditation

**МЕЖГОСУДАРСТВЕННЫЙ  
СТАНДАРТ**

**ГОСТ ИСО/МЭК  
17025-2009**

---

**ОБЩИЕ ТРЕБОВАНИЯ К КОМПЕТЕНТНОСТИ  
ИСПЫТАТЕЛЬНЫХ И КАЛИБРОВОЧНЫХ ЛАБОРАТОРИЙ**

**ISO/IEC 17025:2005**

**General requirements for the competence  
of testing and calibration laboratories**

**(IDT)**

# Quality assurance methods

Analysis of blank (not containing analyte) samples

Spike recovery

Control charts

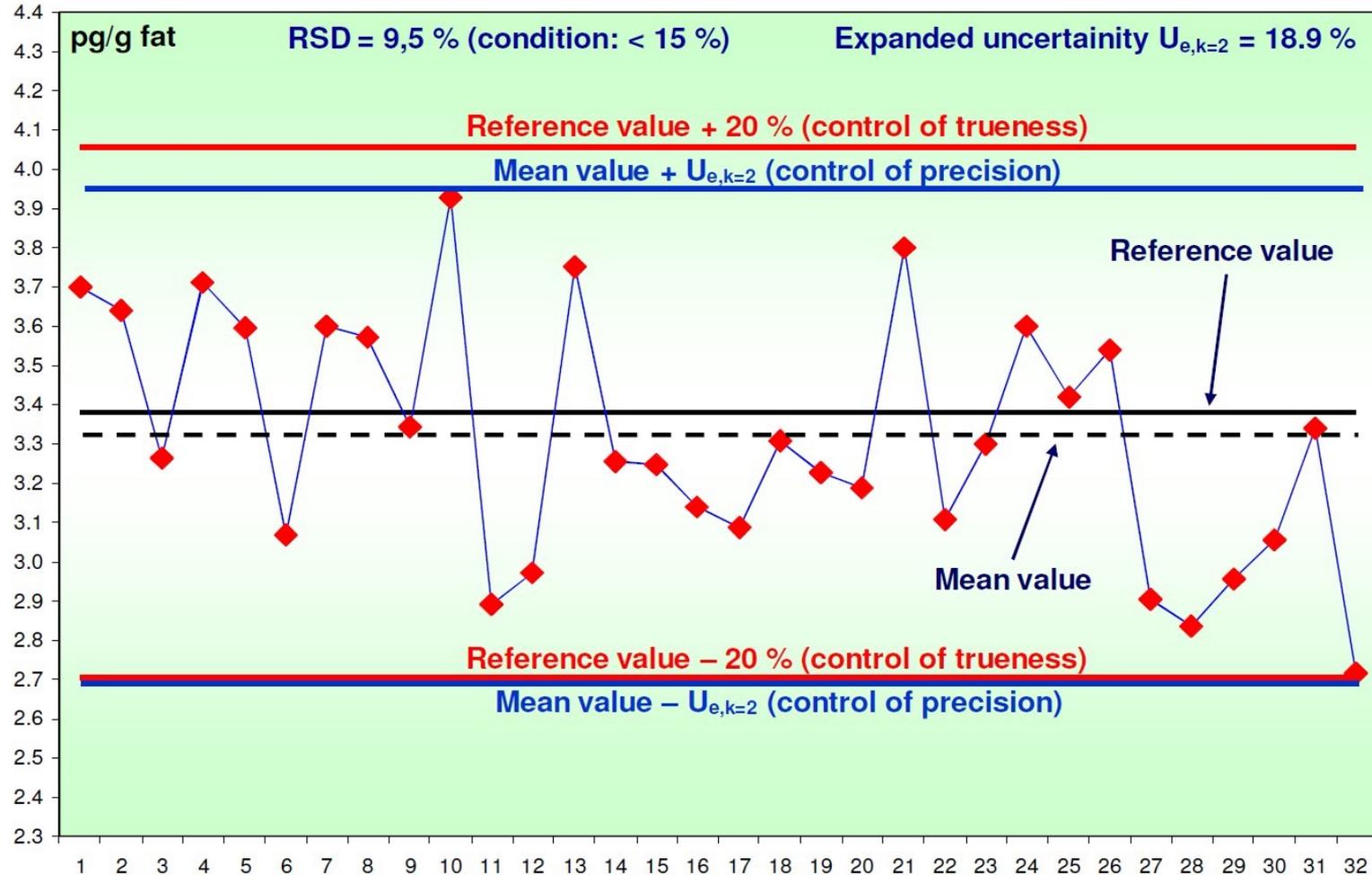
Analyses of performance test samples

Monitoring of equipment and all materials

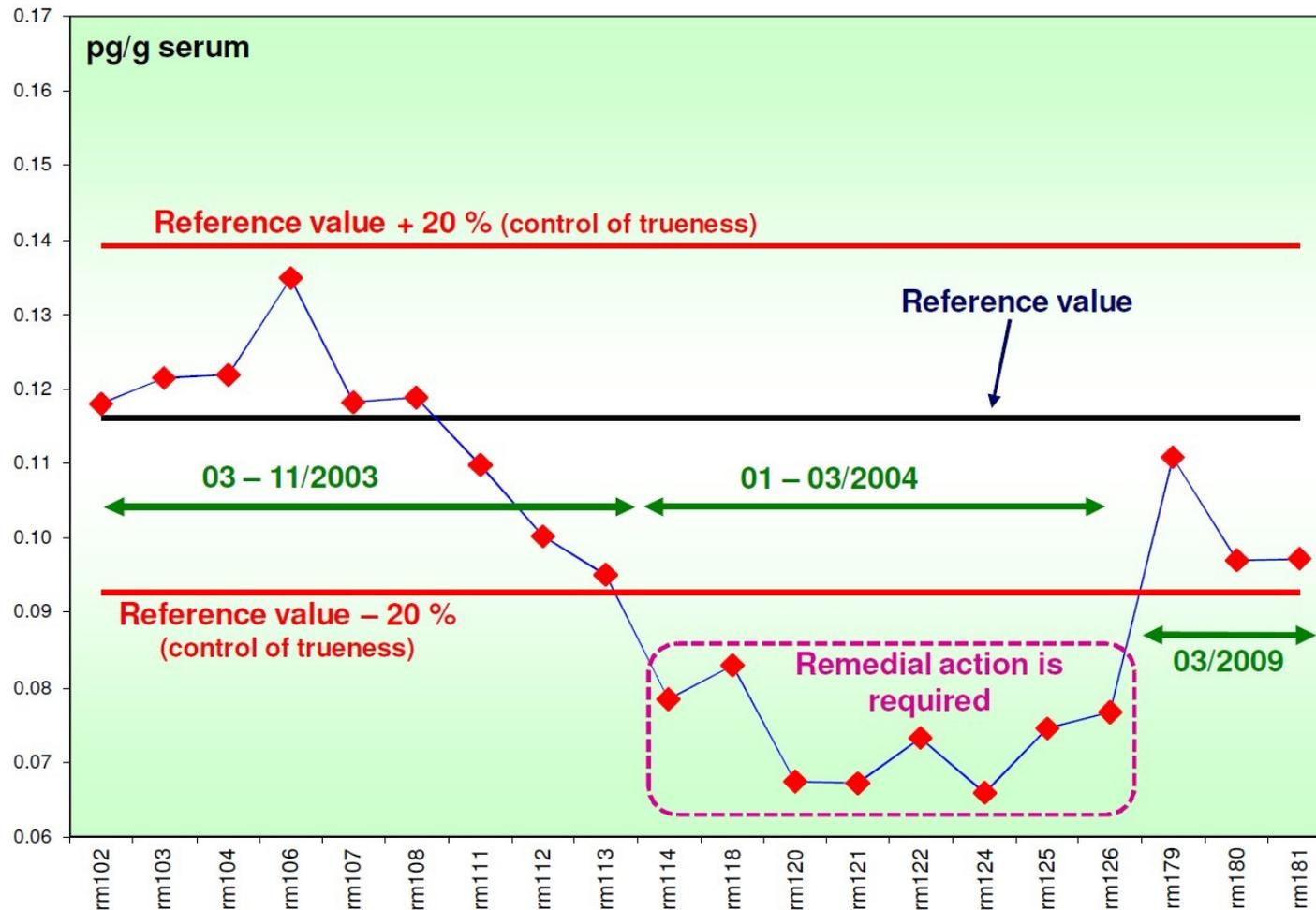
Regular interview and training of laboratory personnel

**These are responsibilities of a Laboratory Manager**

## Control chart for PCDD/F TEQ in a reference material for years 2001–2009 (sunflower oil spiked with 17 congeners)



## Control chart for PCDD/F TEQ in a reference material for years 2003–2009 (pig blood serum)



# Proficiency test on determination of PCDD/Fs, dioxin-like PCBs and indicator PCBs in pork sausage and lard

(organized by the EU Reference Laboratory for Dioxins and PCBs in Food and Feed in 2012)

Pork sausage (1201-PLA)  
WHO-PCDD/F-PCB-TEQ upper bound (reported)

Consensus value: 1.42 pg/g fat

**RECETOX value: 1.40 ng/g fat**

- Meat dried with Na<sub>2</sub>SO<sub>4</sub> anhyd.
- Soxhlet extraction with n-hexane
- Fat spiked with <sup>13</sup>C<sub>12</sub>-labeled surrogates
- Clean-up with H<sub>2</sub>SO<sub>4</sub>/silica, KOH/silica, active carbon
- GC separation on a 60m DB5-MS column
- Quantification by HRMS (10000 resolution)

