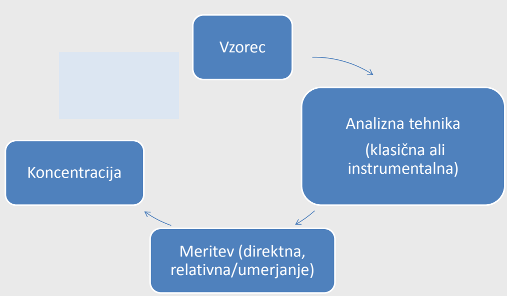
# Intrumentalna analiza

Značilnosti instrumentalnih tehnik

## Kvantitativna analiza




## Značilnosti instrumentalnih tehnik

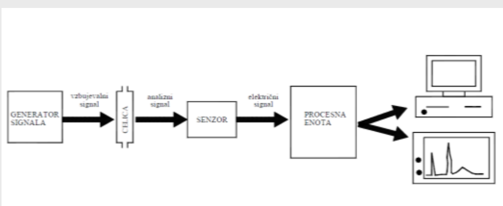
- Temeljijo na merjenju določene fizikalne lastnosti analita
- Prednosti instrumentalnih tehnih pred klasičnimi?
  - Analitika sledov (< µg/L),
  - Majhna poraba vzorca,
  - Analiza velikega števila vzorcev,
  - Hitrost analize,
  - Simultano določanje več analitov,
  - Možnost avtomatizacije,
  - Praviloma enostavna uporaba

# Izbira analizne tehnike

- Masni delež Au (v ‰) v vzorcu zlate žice (Božič, Grabec Švegl, Zbornik SKD 2002, 261)

| Vzorec | Kupelacija (SIST EN ISO 11426) | ICP-AES | ICP-AES (z int. stand.) |
|---|---|---|---|
| A | 752,5 | 755 | 758 |
| B | 752,4 | 760 | 755 |
| C | 752,4 | 760 | 750 |
| D | 751,8 | 768 | 758 |
| E | 752,2 | 762 | 761 |
| F | 752,4 | 755 | 755 |
| **Povprečje** | **752,3** | **760** | **756** |
| **Stand. odmik** | **0,3** | **5** | **4** |

# Konfiguracija analiznega instrumenta




# Instrumentalne komponente

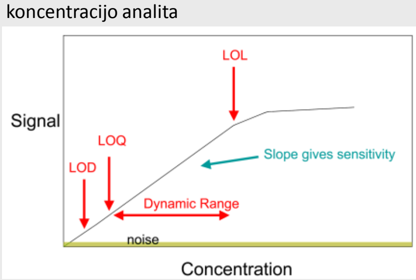
| Detektor | Generator signala | Analizni signal | Senzor |
|---|---|---|---|
| **UV-VIS** | Svetilo (W, D), monokromator | Absorpcija svetlobe | Fotodioda, fotopomnoževalka |
| **Fluorescenca** | Svetilo (Hg, Xe), monokromator | Emisija svetlobe | Fotopomnoževalka |
| **Lomni količnik** | Svetilo (Hg, Na), laser | Uklon, lom svetlobe | Fotodioda |
| **Prevodnost** | Izmenična napetost | Sprememba upornosti | Par elektrod (Pt, Au) |
| **Amperometrija** | Električna napetost | Električni tok | Elektroda (Pt, Au, C) |
| **Kulometrija** | Električna napetost | Naboj (Q) | Elektroda z veliko površino (C, Pt, Au) |

## Pomembne lastnosti analiznih metod

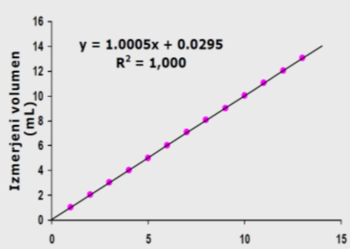
- Pravilnost,
- Natančnost,
- Merilno območje,
- Občutljivost,
- Linearnost,
- Meja zaznave (LOD) in določitve (LOQ),
- Selektivnost/specifičnost,
- Robustnost,
- Hitrost,
- Cena,
- ...

## Umerjanje v analizni kemiji

- Ugotavljanje zveze med merjeno (fizikalno) količino in koncentracijo analita

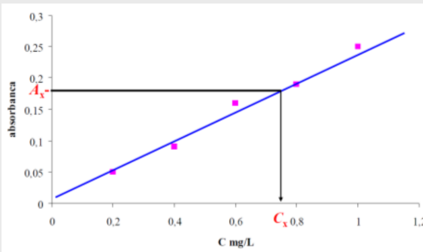



## Kalibracija – primer: Gravimetrična kalibracija 20 mL avtomatske birete



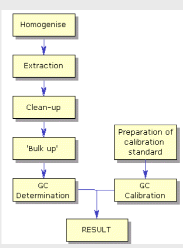
# Kalibracija - eksterna

- Odvisnost merjene količine od koncentracije

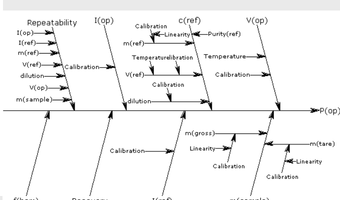



# Merilna negotovost

- Določevanje organofosfornih pesticidov




$$P_{op} = \frac{I_{op} \cdot c_{ref} \cdot V_{op}}{I_{ref} \cdot Rec \cdot m_{sample}} \cdot F_{hom} \cdot 10^6 \quad \text{mg kg}^{-1}$$




# Merilna negotovost pri HPLC

Parameter values for the determination of uncertainty

| Variable | Defined | Value | $\sigma$ | Distribution |
|---|---|---|---|---|
| Instrument parameters | | | | |
| Temperature ($T$) | | 25.00°C | $0.15/\sqrt{3} = 0.0866$°C | Uniform |
| Flow rate ($U$) | | 1.000 ml min$^{-1}$ | 0.3% RSD = 0.003 ml min$^{-1}$ | Normal |
| Absorbency ($A$) | Eq. (11) | | $1.0 \times 10^{-5}$ au | Normal |
| Sample parameters | | | | |
| $\beta_i$ | $\beta_i = \beta_0 \times \exp(-\Delta G_i/RT)$ | 666.7 | $\mathrm{RSD}_\beta = \frac{\Delta G}{RT^2}\sigma_T =$ 0.188%, $\sigma_\beta = 1.3$ | Normal |
| Detector response factor ($R_i$) | Eq. (11) and Section 2.3 | 0.08 (µmol l$^{-1}$)$^{-1}$ | 0 | Constant |
| Retention volume ($V_i$) | | 6.00 ml | | Constant for given column and eluate |
| Retention time ($T_i$) | $T_i = \frac{V_i}{U}$ | 6.0 min | $\mathrm{RSD}_{T_i} = \mathrm{RSD}_U$, $\sigma_T = 0.018$ min | Normal |
| Number of theoretical plates ($N_i$) | $N_i = \beta_i V_i$ | 4000 | $\mathrm{RSD}_{N_i} = \mathrm{RSD}_\beta$, $\sigma_N = 7.5$ | Normal |
| Injection volume ($V_{\mathrm{inject}}$) | | 100.0 µl | 0.5% RSD = 0.5 µl | Normal |
| Injected concentration ($C_{\mathrm{inject}}$) | | 100 µmol l$^{-1}$ | 0 | Normal |
| Injected amount ($M_i$) | $M_i = C_{\mathrm{inject}} V_{\mathrm{inject}}$ | 10 nmol | $\mathrm{RSD}_{M_i} = \sqrt{\frac{\sigma^2_{C_{\mathrm{inject}}}}{C^2_{\mathrm{inject}}} + \frac{\sigma^2_{V_{\mathrm{inject}}}}{V^2_{\mathrm{inject}}}} = \mathrm{RSD}_{V_{\mathrm{inject}}}$, $\sigma_M = 0.05$ nmol | Normal |

## Merilna negotovost – ključni parametri

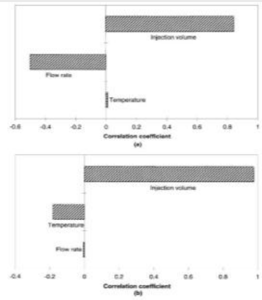



Fig. 3. Sensitivity analysis of contributions to: (a) peak area; and (b) peak height. Bars are proportional to the correlation coefficient between the indicated variable and the output.

**Physico-chemical constants**

| Quantity | Symbol | Value | Unit | Relative std. uncert. $u_r$ |
|---|---|---|---|---|
| Avogadro constant | $N_A, L$ | $6.022\,141\,29(27) \times 10^{23}$ | $mol^{-1}$ | $4.4 \times 10^{-8}$ |
| atomic mass constant | | | | |
| $m_u = \frac{1}{12}m(^{12}C) = 1$ u | $m_u$ | $1.660\,538\,921(73) \times 10^{-27}$ | kg | $4.4 \times 10^{-8}$ |
| energy equivalent | $m_u c^2$ | $1.492\,417\,954(66) \times 10^{-10}$ | J | $4.4 \times 10^{-8}$ |
| | | $931.494\,061(21)$ | MeV | $2.2 \times 10^{-8}$ |
| Faraday constant[1] $N_A e$ | $F$ | $96\,485.3365(21)$ | $C\ mol^{-1}$ | $2.2 \times 10^{-8}$ |
| molar Planck constant | $N_A h$ | $3.990\,312\,7176(28) \times 10^{-10}$ | $J\ s\ mol^{-1}$ | $7.0 \times 10^{-10}$ |
| | $N_A hc$ | $0.119\,626\,565\,779(84)$ | $J\ m\ mol^{-1}$ | $7.0 \times 10^{-10}$ |
| molar gas constant | $R$ | $8.314\,4621(75)$ | $J\ mol^{-1}\ K^{-1}$ | $9.1 \times 10^{-7}$ |
| Boltzmann constant $R/N_A$ | $k$ | $1.380\,6488(13) \times 10^{-23}$ | $J\ K^{-1}$ | $9.1 \times 10^{-7}$ |
| | | $8.617\,3324(78) \times 10^{-5}$ | $eV\ K^{-1}$ | $9.1 \times 10^{-7}$ |
| | $k/h$ | $2.083\,6618(19) \times 10^{10}$ | $Hz\ K^{-1}$ | $9.1 \times 10^{-7}$ |
| | $k/hc$ | $69.503\,476(63)$ | $m^{-1}\ K^{-1}$ | $9.1 \times 10^{-7}$ |
| molar volume of ideal gas $RT/p$ | | | | |
| $T = 273.15$ K, $p = 100$ kPa | $V_m$ | $22.710\,953(21) \times 10^{-3}$ | $m^3\ mol^{-1}$ | $9.1 \times 10^{-7}$ |
| Loschmidt constant $N_A/V_m$ | $n_0$ | $2.651\,6462(24) \times 10^{25}$ | $m^{-3}$ | $9.1 \times 10^{-7}$ |
| molar volume of ideal gas $RT/p$ | | | | |
| $T = 273.15$ K, $p = 101.325$ kPa | $V_m$ | $22.413\,968(20) \times 10^{-3}$ | $m^3\ mol^{-1}$ | $9.1 \times 10^{-7}$ |
| Loschmidt constant $N_A/V_m$ | $n_0$ | $2.686\,7805(24) \times 10^{25}$ | $m^{-3}$ | $9.1 \times 10^{-7}$ |
| Sackur-Tetrode (absolute entropy) constant[2] | | | | |
| $\frac{5}{2} + \ln[(2\pi m_u k T_1/h^2)^{3/2} k T_1/p_0]$ | | | | |
| $T_1 = 1$ K, $p_0 = 100$ kPa | $S_0/R$ | $-1.151\,7078(23)$ | | $2.0 \times 10^{-6}$ |
| $T_1 = 1$ K, $p_0 = 101.325$ kPa | | $-1.164\,8708(23)$ | | $1.9 \times 10^{-6}$ |
| Stefan-Boltzmann constant | | | | |
| $(\pi^2/60)k^4/\hbar^3 c^2$ | $\sigma$ | $5.670\,373(21) \times 10^{-8}$ | $W\ m^{-2}\ K^{-4}$ | $3.6 \times 10^{-6}$ |
| first radiation constant $2\pi hc^2$ | $c_1$ | $3.741\,771\,53(17) \times 10^{-16}$ | $W\ m^2$ | $4.4 \times 10^{-8}$ |
| first radiation constant for spectral radiance $2hc^2$ | $c_{1L}$ | $1.191\,042\,869(53) \times 10^{-16}$ | $W\ m^2\ sr^{-1}$ | $4.4 \times 10^{-8}$ |
| second radiation constant $hc/k$ | $c_2$ | $1.438\,7770(13) \times 10^{-2}$ | m K | $9.1 \times 10^{-7}$ |
| Wien displacement law constants | | | | |
| $b = \lambda_{max} T = c_2/4.965\,114\,231...$ | $b$ | $2.897\,7721(26) \times 10^{-3}$ | m K | $9.1 \times 10^{-7}$ |
| $b' = \nu_{max}/T = 2.821\,439\,372...\ c/c_2$ | $b'$ | $5.878\,9254(53) \times 10^{10}$ | $Hz\ K^{-1}$ | $9.1 \times 10^{-7}$ |

[1] The numerical value of $F$ to be used in coulometric chemical measurements is 96 485.3401(48) [$5.0 \times 10^{-8}$] when the relevant current is measured in terms of representations of the volt and ohm based on the Josephson and quantum Hall effects and the internationally adopted conventional values of the Josephson and von Klitzing constants $K_{J-90}$ and $R_{K-90}$ given in the "Adopted values" table.

[2] The entropy of an ideal monoatomic gas of relative atomic mass $A_r$ is given by $S = S_0 + \frac{3}{2}R \ln A_r - R \ln(p/p_0) + \frac{5}{2} R \ln(T/K)$.

## Načini kalibracije ali kako umerimo instrument?

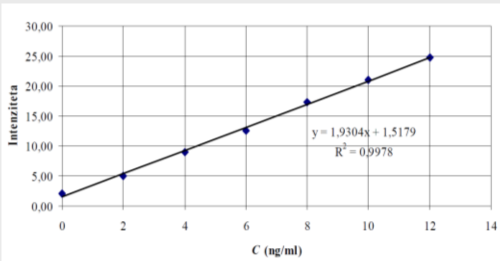
- Umerjanje (kalibracija) je potrebno pri relativnih tehnikah

- Umerjanje s standardi (eksterni standard)
- Standardni dodatek
- Interni standard

# Linearna regresija

- Linearno zvezo med koncentracijo ***x*** in signalom y opišemo z enačbo:

***y = a + bx***




# Koeficient korelacije

- Prileganje eksperimentalnih vrednosti premici podaja korelacijski koeficient ***r***

$$r = \frac{\sum_{i=1}^{n}\left[(x_i - \bar{x})(y_i - \bar{y})\right]}{\sqrt{\left[\sum_{i=1}^{n}(x_i - \bar{x})^2\right]\left[\sum_{i=1}^{n}(y_i - \bar{y})^2\right]}}$$

$$-1 \leq r \leq +1$$

O podrobnostih: J. C. Miller, J. N. Miller: Statistics for Analytical Chemistry, 3rd Ed. Ellis Horwood PTR Prentice Hall, NY 1993.

# Koeficienta premice

- naklon:

$$b = \frac{\sum_{i=1}^{n}\left[(x_i - \bar{x})(y_i - \bar{y})\right]}{\sum_{i=1}^{n}(x_i - \bar{x})^2}$$

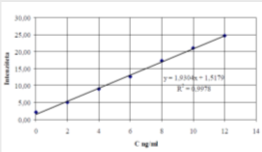
- odsek:

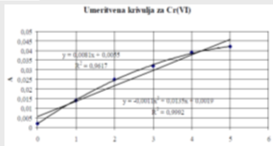
$$a = \bar{y} - b\bar{x}$$

- enačba premice:

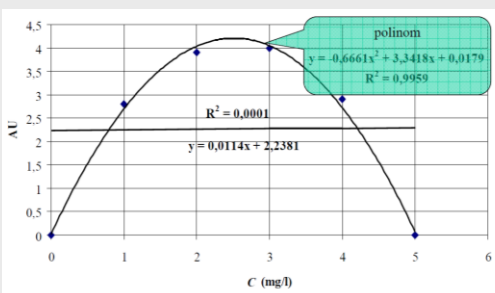
$$\hat{y} = a + bx_i$$

## Umeritev – linearna zveza?






## Umeritev – linarna zveza?

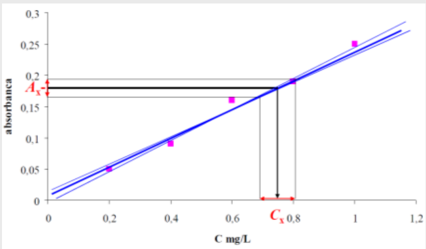



## Negotovost parametrov premice

$$s_{y/x} = \sqrt{\frac{\sum_{i=1}^{n} (y_i - \hat{y})^2}{n - 2}}$$

$$s_b = \frac{s_{y/x}}{\sqrt{\sum_{i=1}^{n} (x_i - \bar{x})^2}} \qquad s_a = s_{y/x} \sqrt{\frac{\sum_{i=1}^{n} x_i^2}{n \sum_{i=1}^{n} (x_i - \bar{x})^2}}$$

## Negotovost koncentracije




## Negotovost koncentracije

- Umeritvena krivulja

$$s_{xo} = \frac{s_{y/x}}{b}\sqrt{\frac{1}{m}+\frac{1}{n}+\frac{(y_o-\bar{y})^2}{b^2\sum_{i=1}^{n}(x_i-\bar{x})^2}}$$

- Standardni dodatek

$$s_{xe} = \frac{s_{y/x}}{b}\sqrt{\frac{1}{n}+\frac{\bar{y}^2}{b^2\sum_{i=1}^{n}(x_i-\bar{x})^2}}$$

## Meja zaznave

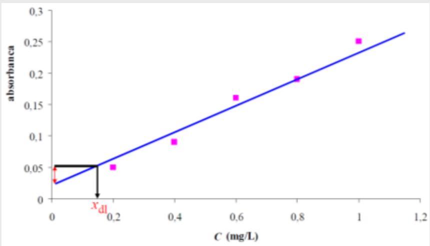
- Najnižja koncentracija $x_{dl}$, ki jo lahko izmerimo z dano stopnjo verjetnosti

- minimalni signal:

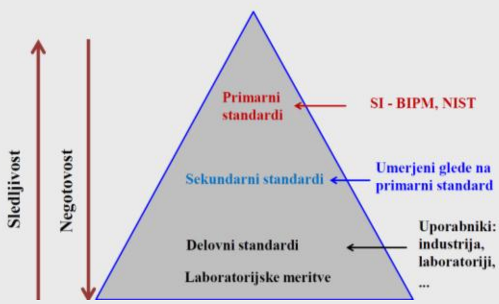
$$y_{dl} = y_{sv} + 3s_{sv}$$

$$y_{dl} = a + 3s_{y/x} \Rightarrow x_{dl} = \frac{y_{dl}-a}{b} = \frac{3s_{y/x}}{b}$$

# Meja zaznave




# Sledljivost laboratorijskih rezultatov




# Priprava standardnih raztopin – merilna negotovost?

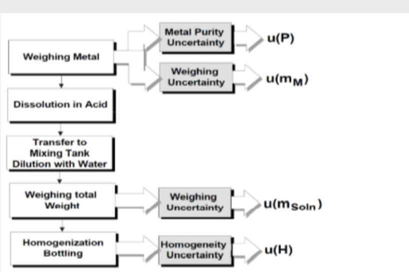



*Fig. 1: Preparation of a monoelemental solution and uncertainty components*

## Stabilnost standardnih raztopin?

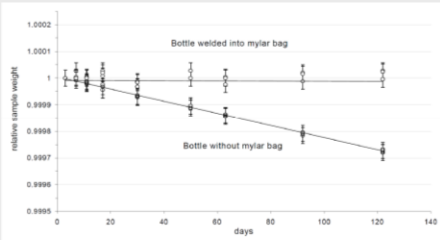
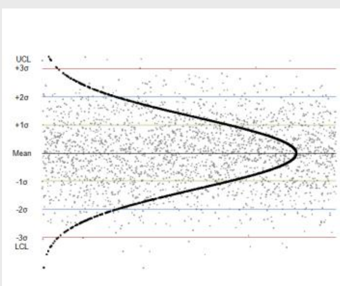
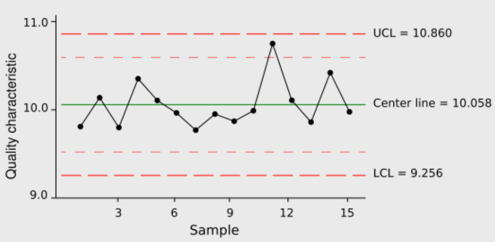



*Fig. 1 Evaporation study of 250 ml HDPP bottles. Without mylar bag, a loss of weight of 0.007% per month was observed.*

## Zagotavljanje kakovosti – kontrolne karte




## Zagotavljanje kakovosti – kontrolne karte

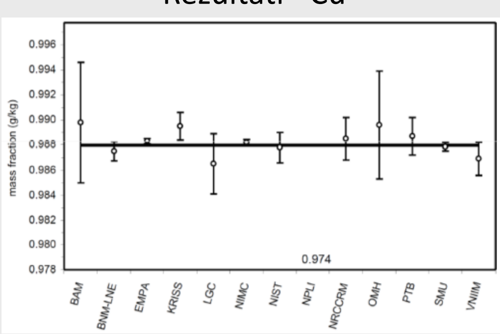


## Zagotavljanje kakovosti – kontrolne karte


## Zagotavljanje kakovosti – medlaboratorijske primerjave (PT)

| Institution | Methods of measurement | | | |
|---|---|---|---|---|
| | Aluminium | Copper | Iron | Magnesium |
| BAM | Titrimetry (EDTA) | ID-TI-MS and Electrogravimetry | Titrimetry (EDTA) | ID-ICP-MS and Titrimetry (EDTA) |
| BNM-LNE | Titrimetry (EDTA, back Zn) | Titrimetry (EDTA) | Titrimetry (EDTA) | Titrimetry (EDTA) |
| EMPA | Titrimetry (EDTA, back $ZnSO_4$) | Titrimetry (EDTA) | Titrimetry (EDTA) | Titrimetry (EDTA) |
| KRISS | ICP-OES (internal standard) | ICP-OES (internal standard) | ICP-OES (internal standard) | ICP-OES (internal standard) |
| LGC | ICP-OES | ID-ICP-MS | ID-ICP-MS | ID-ICP-MS |
| NIMC | Titrimetry (CyDTA, back $Pb(NO_3)_2$) | Titrimetry (EDTA) | Titrimetry (EDTA, back $Bi(NO_3)_3$) | Titrimetry (EDTA) |
| NIST | ICP-OES (internal standard) | ICP-OES (internal standard) | ICP-OES (internal standard) | ICP-OES (internal standard) |
| NPLI | ICP-OES | ICP-OES | ICP-OES | ICP-OES |
| NRCCRM | Titrimetry (EDTA, back Pb) | Controlled potential Coulometry | Controlled potential Coulometry | Titrimetry (EDTA) |
| OMH | Gravimetry | Gravimetry | | Gravimetry |
| PTB | ICP-MS | ID-ICP-MS | ID-ICP-MS | ID-ICP-MS |
| SMU | Titrimetry (EDTA, back Zn) | Titrimetry (EDTA, back Zn) | Titrimetry (EDTA) | Titrimetry (EDTA) |
| VNIIM | Titrimetry (EDTA, back Zn) | Titrimetry (EDTA, back Zn) | Titrimetry (EDTA) | Titrimetry (EDTA) |

## Rezultati - Cu



# Literatura


- D.A. Skoog, F.J. Holler, S.R. Crouch: *Principles of Instrumental Analysis*, 6th Edition, Thomson Brooks/Cole, Belmont, CA, 2007,
  - Introduction, Chapter 1, Appendix I