SIGNALI – tipi analiznih metod

- **Kemija in kemijska analiza**
  kemijska analiza v biomedicini
- **Dokumentacija**
- **Kvalitativna analiza**
- **Kvantitativna analiza**

Definicija:

Analizna kemija **je veda o pridobivanju in uporabniško orientirani interpretaciji informacije o materialnem sistemu s pomočjo znanstvenih metod.**

- **Sodobni trendi**

ANALIZNI SIGNALI

Definicija:

Merjenje **lahko definiramo kot primerjavo nekega merjenca z osnovno enoto.**

Razliko med merjencem in osnovno enoto lahko določimo neposredno z merili ali z uporabo merilnih naprav, instrumentov.

Definicija:

Merjenje je pridobivanje informacije o neki spremenljivki, ki vključuje tudi proces pretvorbe te spremenljivke v zaznaven signal.

- **Izbira fizikalne količine**
  - **specifičnost**
  - **nespecifičnost**
  - **izolacija komponente ali signala**

KLASIFIKACIJA ANALIZNIH METOD

1. Klasične metode (kvalitativna, kvantitativna analiza)

- **Gravimetrija**
- **Volumetrija**

2. Instrumentalne metode

Definicija:

Analitski instrumenti so naprave, ki merijo fizikalne oz. kemične lastnosti proučevane snovi oz. merijo neko lastnost, ki omogoča karakterizacijo snovi.

- **Spektroskopske**
- **Elektroanalitske**
- **Separacijske**
- **Termogravimetrične**

- **Fiziološke metode**

ELEMENTI SPLOŠNEGA INSTRUMENTA ZA ANALIZO

Izvor → detektor → procesor → prikaz

Definiciji:

- Signal definiramo kot odziv instrumenta na določen stimulus.
Ta je običajno fizikalna količina oz. njena sprememba.

- Vsako meritev na instrumentu lahko tudi označimo kot signal.

Table 1: Bioassays using cell lines

Hormone	System used	Parameter measured
Prolactin	Rat lymphoma cells	Cell growth
Interleukin 1 (IL1)	Human myeloma cells	Cell growth
Transforming growth factor β (TGFβ)	Erythroleukaemic cell line	Inhibition of interleukin 5 (IL5) stimulated growth

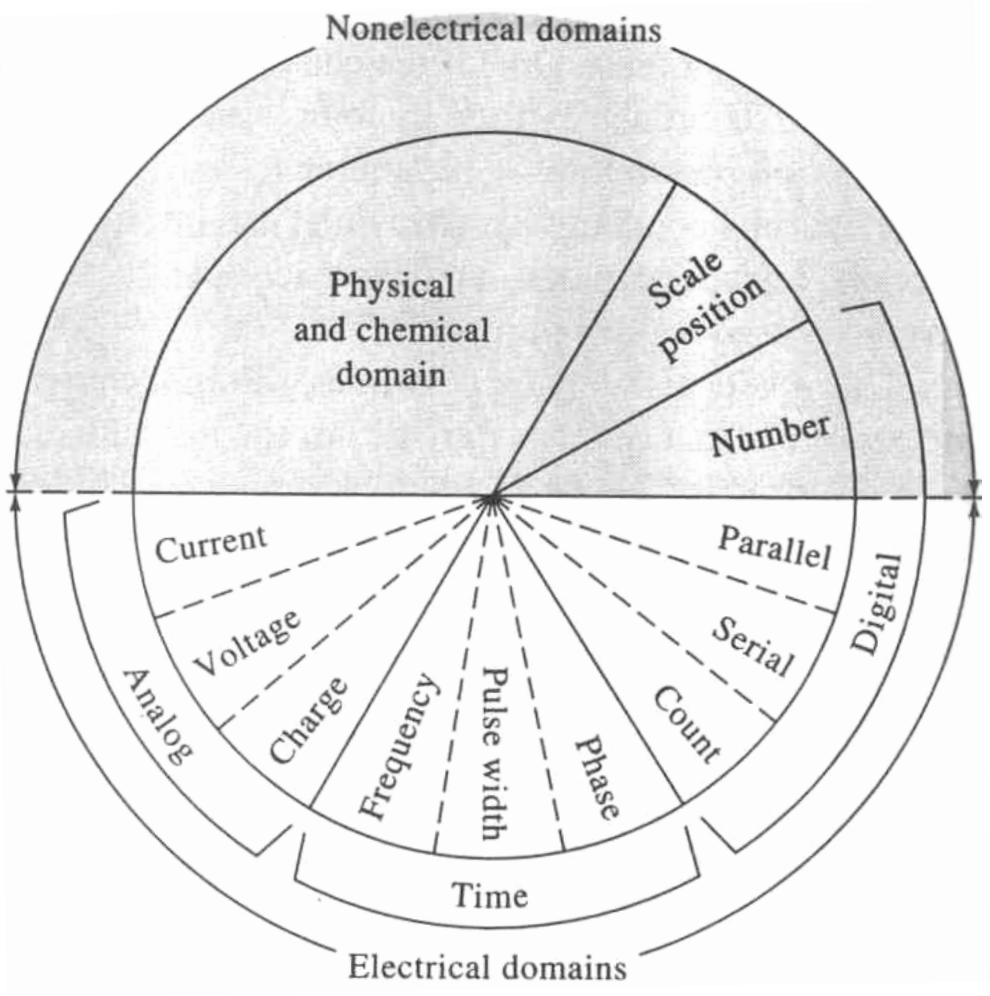


Figure 1: Data domains map. The upper (shaded) half of the map comprises nonelectrical domains. The bottom half is made up of electrical domains. Note that the digital domain spans both electrical and nonelectrical domains.

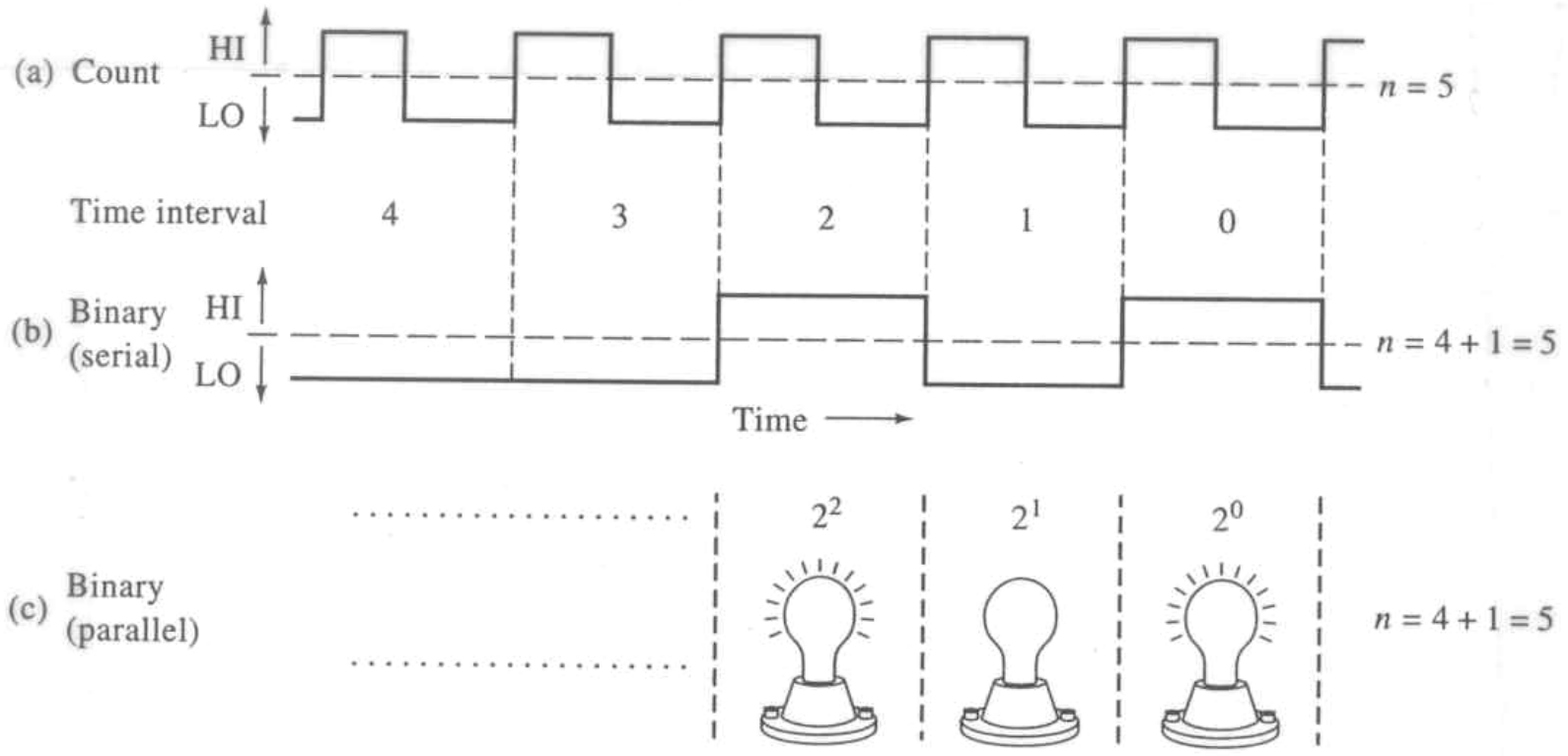


Figure 2: Diagram illustrating three types of digital data: (a) count serial data, (b) binary-coded serial data, and (c) parallel binary data. In all three cases, the data represent the number n = 5

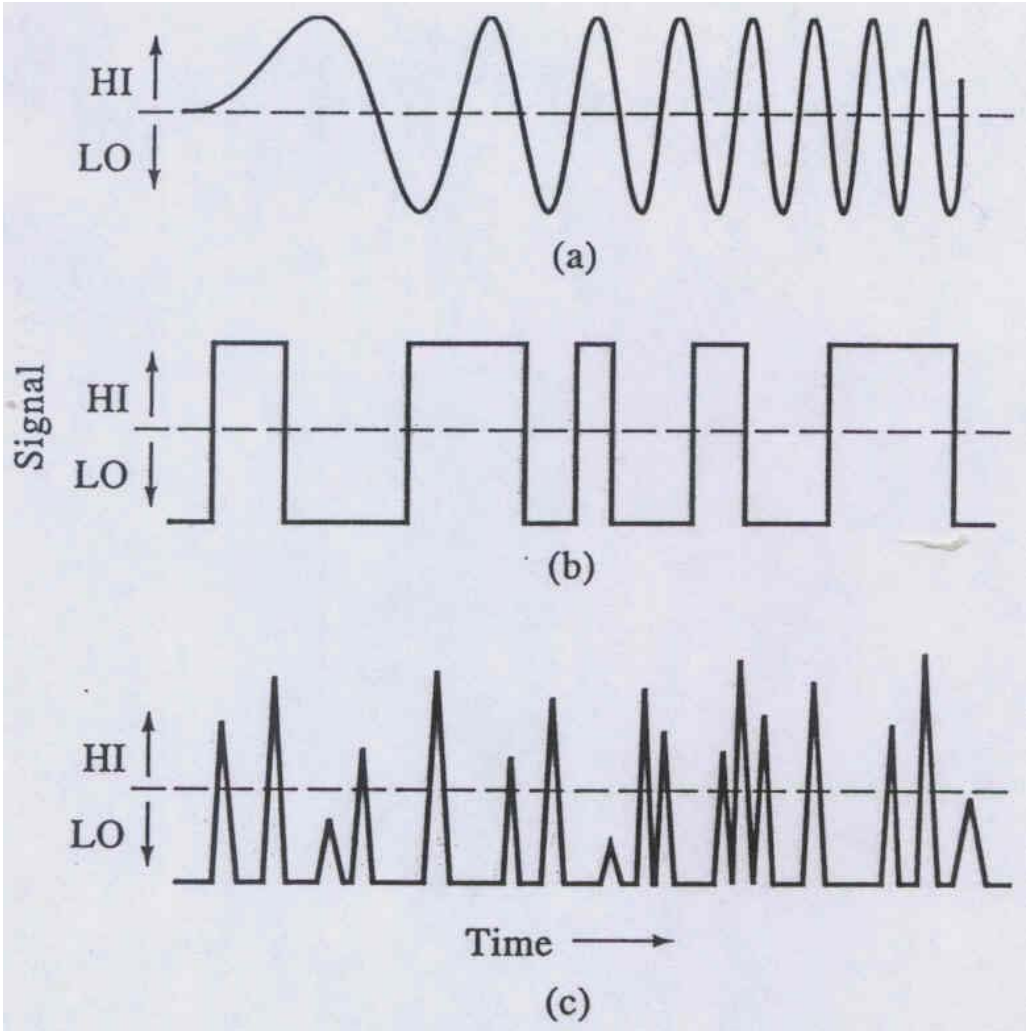


Figure 3: Time - domain signals. The horizontal dashed lines represent signal thresholds. When each signal is above the threshold, the signal is HI, and when it is below the threshold, the signal is LO.

SIGNAL	INSTRUMENTALNE METODE
Sevanje svetlobe	emisijska spektroskopija (X, UV, Vis, elektroni); fluorescenca, fosforescenca, luminiscenca (X, UV, Vis)
Absorpcija svetlobe	spektrofotometrija in fotometrija (X, UV, Vis, IR); fotoakustična spektroskopija; NMR; ESR
Sipanje svetlobe	turbidimetrija; nefelometrija; Raman spektroskopija
Lom svetlobe	refraktometrija; interferometrija
Uklon svetlobe	X in elektronske difrakcijske metode
Rotacija svetlobe	polarimetrija; optična rotacijska disperzija
Električni potencial	potenciometrija
Električni naboj	kulometrija
Električni tok	polarografija; amperometrija
Električni upor	konduktometrija

SIGNAL	INSTRUMENTALNE METODE
Radioaktivnost	radiokemične metode
Razmerje masa / naboj	masna spektrometrija
Termične lastnosti	toplotna prevodnost; absorpcija toplote
Masa	gravimetrija; termogravimetrija
Volumen	volumetrija

Fizikalne lastnosti, ki jih lahko merimo s primerno natančnostjo	Primeri kvantitativnega določanja		
	proteini	svinec	kisik
Masa	+	+	
Volumen			+
Specifična teža	+		
Viskoznost	+		
Površinska napetost	+		
Absorpcija svetlobe	+	+	
Sipanje svetlobe	+		
Električni tok			+
Električni potencial			+

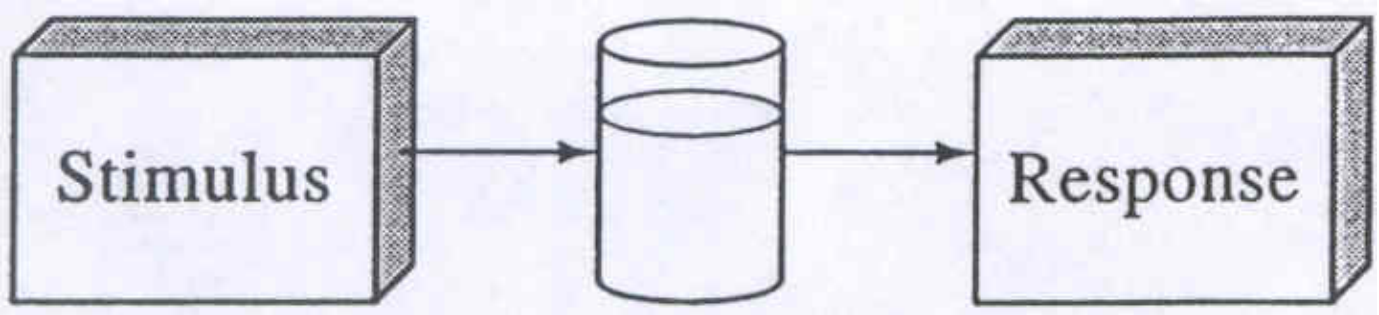


Figure 4: Block diagram showing the overall process of an instrumental measurement.

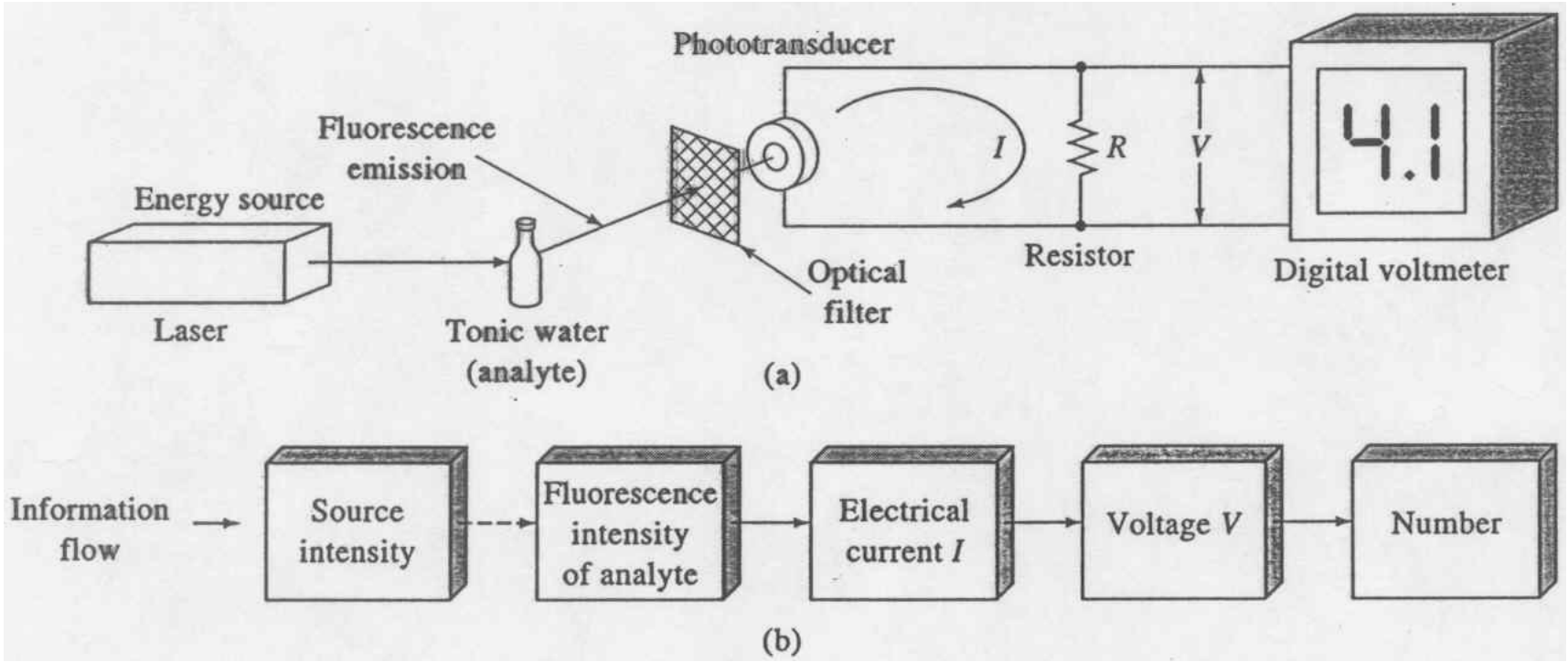


Figure 5: A block diagram of a fluorometer showing (a) a general diagram of the instrument, (b) a diagrammatic representation of the flow of information through various data domains in the instrument, and (c) the rules govering the data domain transformations during the measurement process.

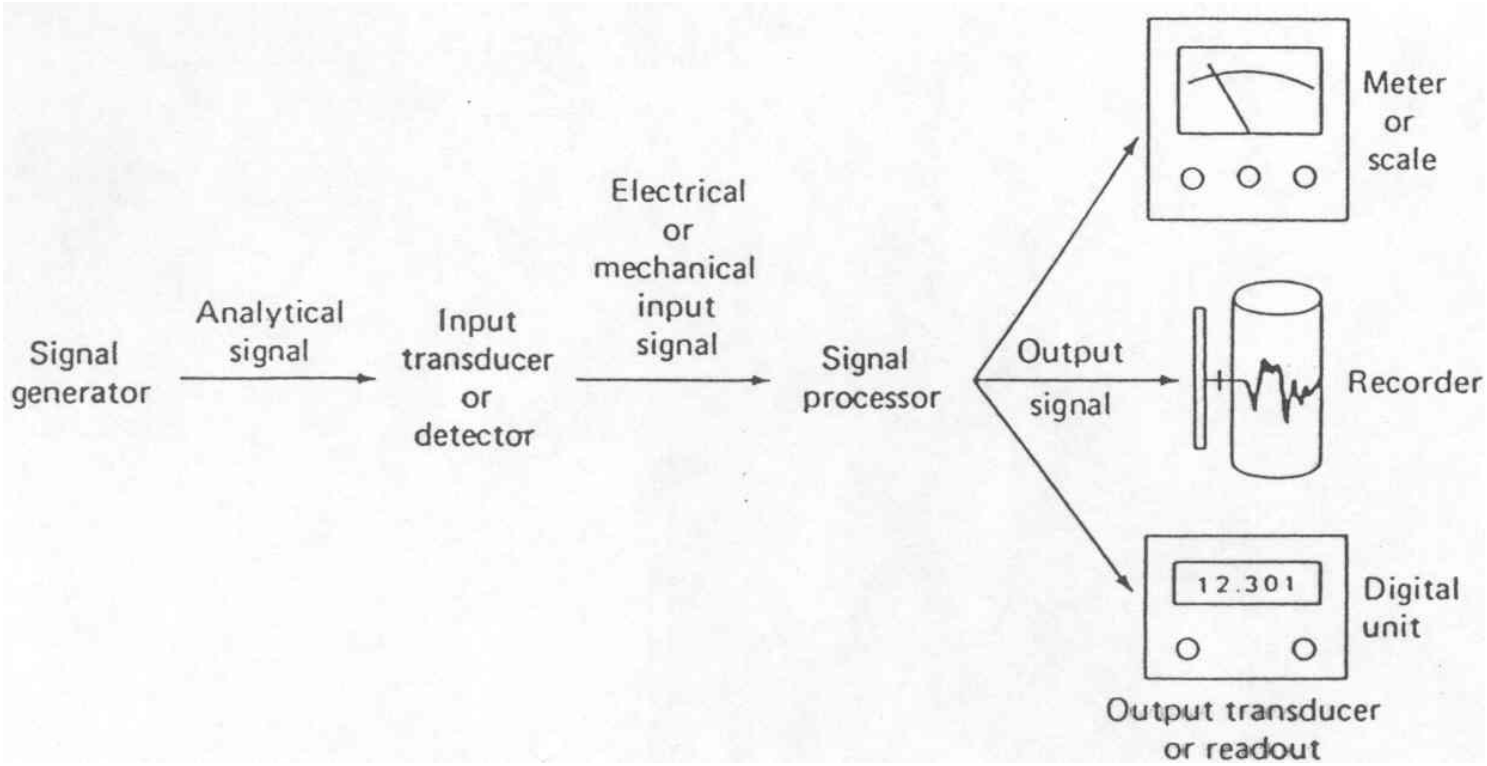
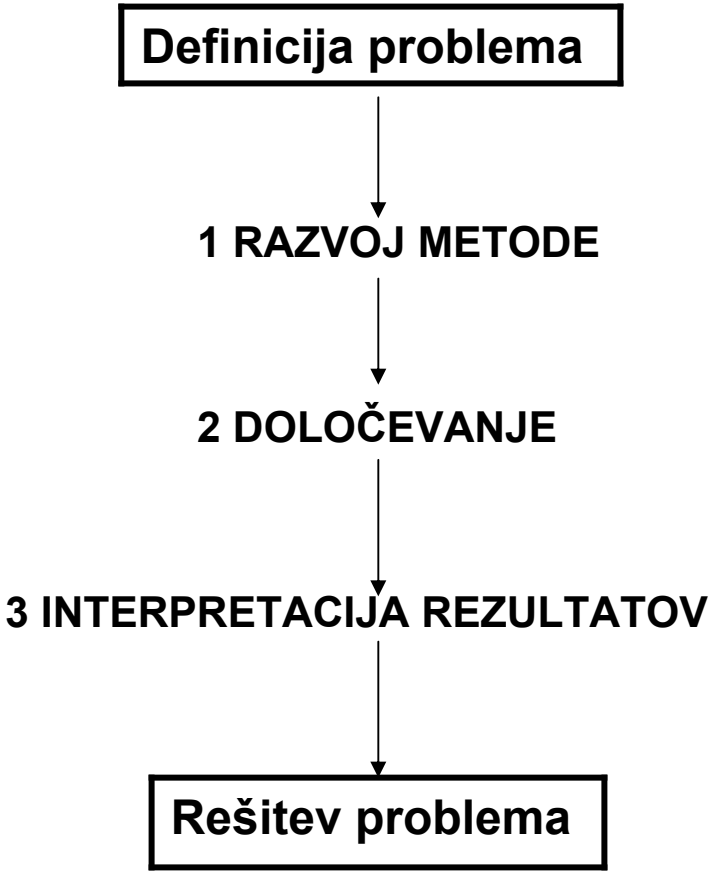


Figure 6: Components of a typical instrument.

1. What accuracy and precision are required?
2. How much sample is available?
3. What is the concentration range of the analyte?
4. What components of the sample will cause interference?
5. What are the physical and chemical properties of the sample matrix?
6. How many samples are to be analyzed?


Definicija problema
1 RAZVOJ METODE
2 DOLOČEVANJE
3 INTERPRETACIJA REZULTATOV
Rešitev problema

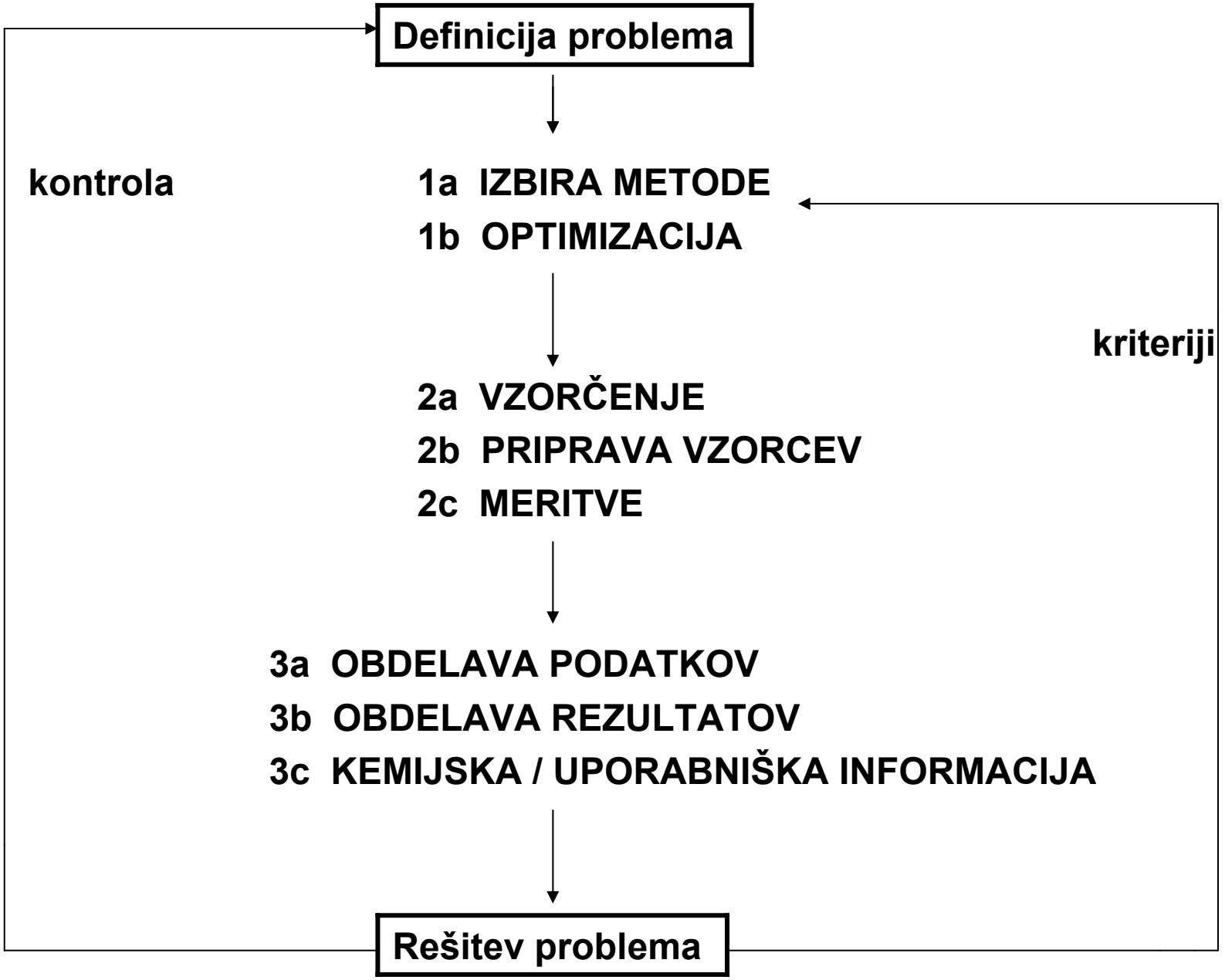

Definicija problema
kontrola
1a IZBIRA METODE
1b OPTIMIZACIJA
kriteriji
2a VZORČENJE
2b PRIPRAVA VZORCEV
2c MERITVE
3a OBDELAVA PODATKOV
3b OBDELAVA REZULTATOV
3c KEMIJSKA / UPORABNIŠKA INFORMACIJA
Rešitev problema

TABLE 2:

Numerical Criteria for Selecting Analytical Methods

Criterion	Figure of Merit
Precision	Absolute standard deviation, relative standard deviation, coefficient of variation, variance
Bias	Absolute systematic error, relative systematic error
Sensitivity	Calibration sensitivity, analytical sensitivity
Detection limit	Blank plus three times standard deviation of a blank
Concentration range	Concentration limit of quantitation (LOQ) to concentration limit of linearity (LOL)
Selectivity	Coefficient of selectivity

TABLE 3:

Other Characteristics to Be Considered in Method Choice

1. Speed
2. Ease and convenience
3. Skill required of operator
4. Cost and availability of equipment
5. Per – sample cost

KRITERIJI ZA IZBIRO ANALIZNE METODE

- **PRECISION (natančnost); randomizirana (slučajna) napaka: s (SD), RSD, s_m, CV, s^2**
- **BIAS – systematic ERRORS (sistemske napake: instrument, oseba, metoda)**

- **SENSITIVITY (občutljivost) – naklon UK, aparat**
  - **calibration sensitivity (= naklon UK)**
  - **analytical sensitivity: $\gamma = m/s_s$**

- **DETECTION LIMIT (meja detekcije)**

$$S_m = \overline{S_{bl}} + \overline{k} \cdot s_{bl} \qquad S = m \cdot c + S_{bl} \qquad \text{(UK)}$$

$$c_m = \frac{S_m - \overline{S_{bl}}}{m} \qquad k = 3\ ?$$

- **KONCENTRACIJSKO OBMOČJE**

LOQ = 10 x s_{bl} **(najnižja koncentracija v UK)**

- **SELEKTIVNOST**

$$S = m_A c_A + m_B c_B + m_C c_C + S_{bl}$$

$k_{B,A} = m_B/m_A$ **selektivnostni koeficient za B glede na A**

$$S = m_A (c_A + k_{B,A} \cdot c_B + k_{C,A} \cdot c_C) + S_{bl}$$

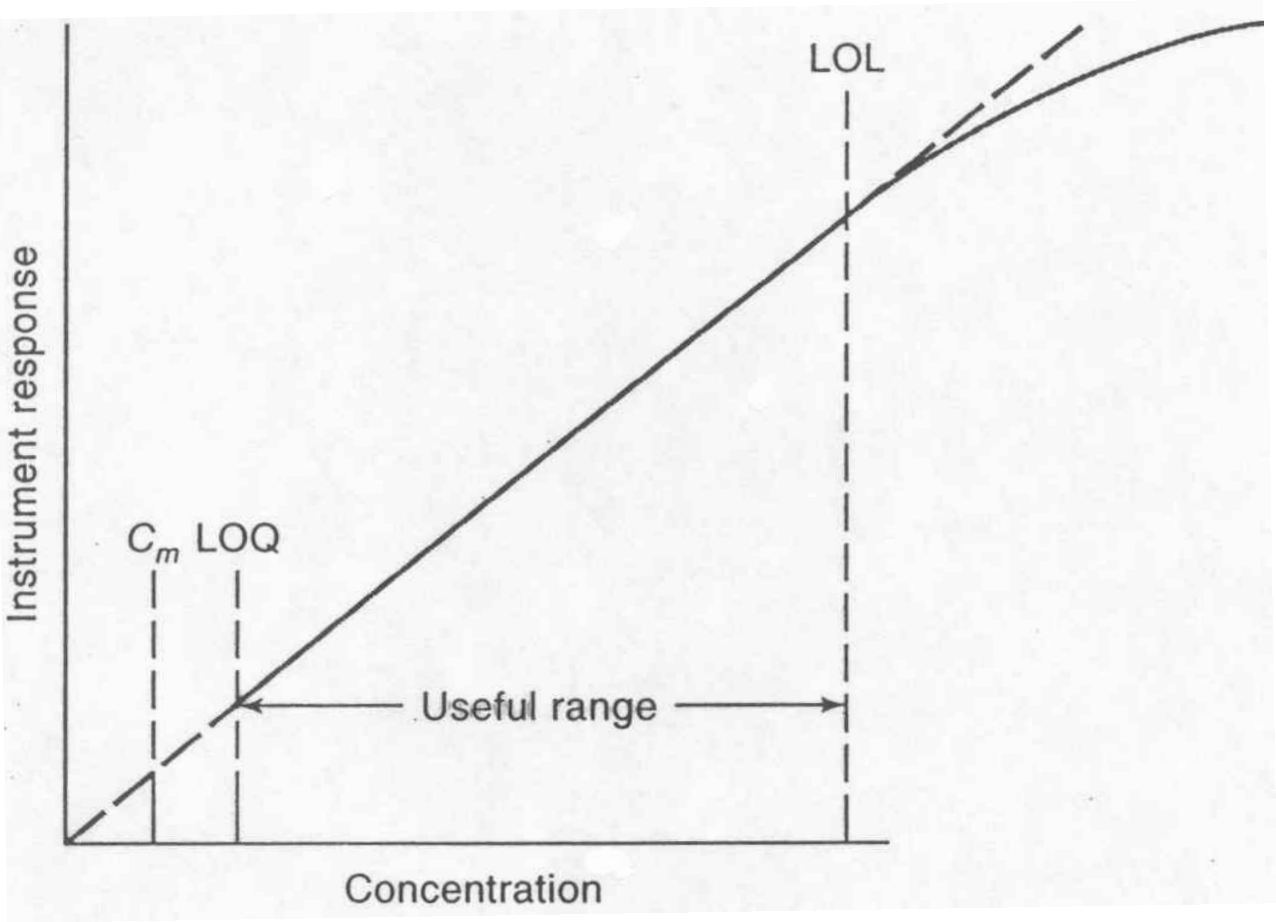


Figure 7: Useful range of an analytical method. LOD – limit of detector; LOQ – limit of quantitative measurement; LOL – limit of linear response.

ICH (International Conference on Harmonization of Tehnical Requirements for Registration of Pharmaceuticals for Human Use)

VALIDACIJA (analitske metode) – prikaz primernosti postopka (analize) za“željeni namen”

Range (območje):
- za vsebnost substance (80 – 120 % testne conc.)
- enakomernost vsebnosti (70 – 130 % testne conc.)
- “disolucijski testi” (± 20 % specificiranega območja)
- določanje nečistot (od “napovedane” stopnje nečistote do 120 % specificirane vrednosti)

Meja detekcije:
- na osnovi “vizualne ocene”
- glede na razmerje signal – šum (cca. 2 – 3)
- glede na SD odziva in naklon UK

DL = 3,3 σ/S (S – slope)
σ oziroma s – iz “slepih vzorcev”, iz UK – linearna regresija, SD y odseka (območje v DL)

Limita (meja) določljivosti (Quantitation Limit):

- vizualno

- razmerje signal : šum (10 : 1)

- glede na SD (QL = 10σ/S)

Robustnost:

- "validity of the analytical procedure is maintained whenever used"

- stabilnost raztopine analita; čas ekstrakcije (analize)

npr. HPLC (spremenljivke): pH v mobilni fazi, sestava MF, različne kolone (dobavitelji, različne serije), T, pretok...

SST (System Suitability Testing):

- oprema, elektronika, analitska metoda in vzorci za analizo sestavljajo "integralni sistem", ki ga evaluiramo v celoti.

GLOSSARY

1. ANALYTICAL PROCEDURE

The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of apparatus, generation of the calibration curve, use of the formulae for the calculation, etc.

2. SPECIFICITY

Specifity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

This definition has the following implications:

- Identification: to ensure the identity of an analyte.

- Purity Tests: to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.

Assay (content or potency):

to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

3. ACCURACY

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

4. PRECISION

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution.

The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

4.1. Repeatability

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termined intra - assay operation.

4.2. Intermediate precision

Intermediate precision expresses within – laboratories variations: different days, different analysts, different equipment, etc.

4.3. Reproducibility

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology).

5. DETECTION LIMIT

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

6. QUANTITATION LIMIT

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and / or degradation products.

7. LINEARITY

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

8. RANGE

The range of an analytical procedure is an interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demostrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

9. ROBUSTNESS

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Type of analytical procedure characteristics	IDENTIFICATION	TESTING FOR IMPURITIES quantitat.	 limit	ASSAY - dissolution (measurement only) - content /potency
Accuracy	–	+	–	+
Precision				
Repeatability	–	+	–	+
Interm. Precision	–	+ (1)	–	+ (1)
Specificity (2)	+	+	+	+
Detection Limit	–	– (3)	+	–
Quantitation Limit	–	+	–	–
Linearity	–	+	–	+
Range	–	+	–	+

– signifies that this characteristic is not normally evaluated

+ signifies that this characteristic is normally evaluated

(1) in cases where reproducibility (see glossary) has been performed, intermediate precision is not needed

(2) lack of specificy of one analytical procedure could be compensated by other supporting analytical procedure(s)

(3) may be needed in some cases