Analytische Qualitätssicherung bei Trenntechniken

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Eurachem

A FOCUS FOR ANALYTICAL CHEMISTRY IN EUROPE

Outline:

- Structures in Chemical Metrology
- Important technical requirements
- General model of chemical measurements (with examples)
- Achieving traceability for chemical results
- The new role of validation

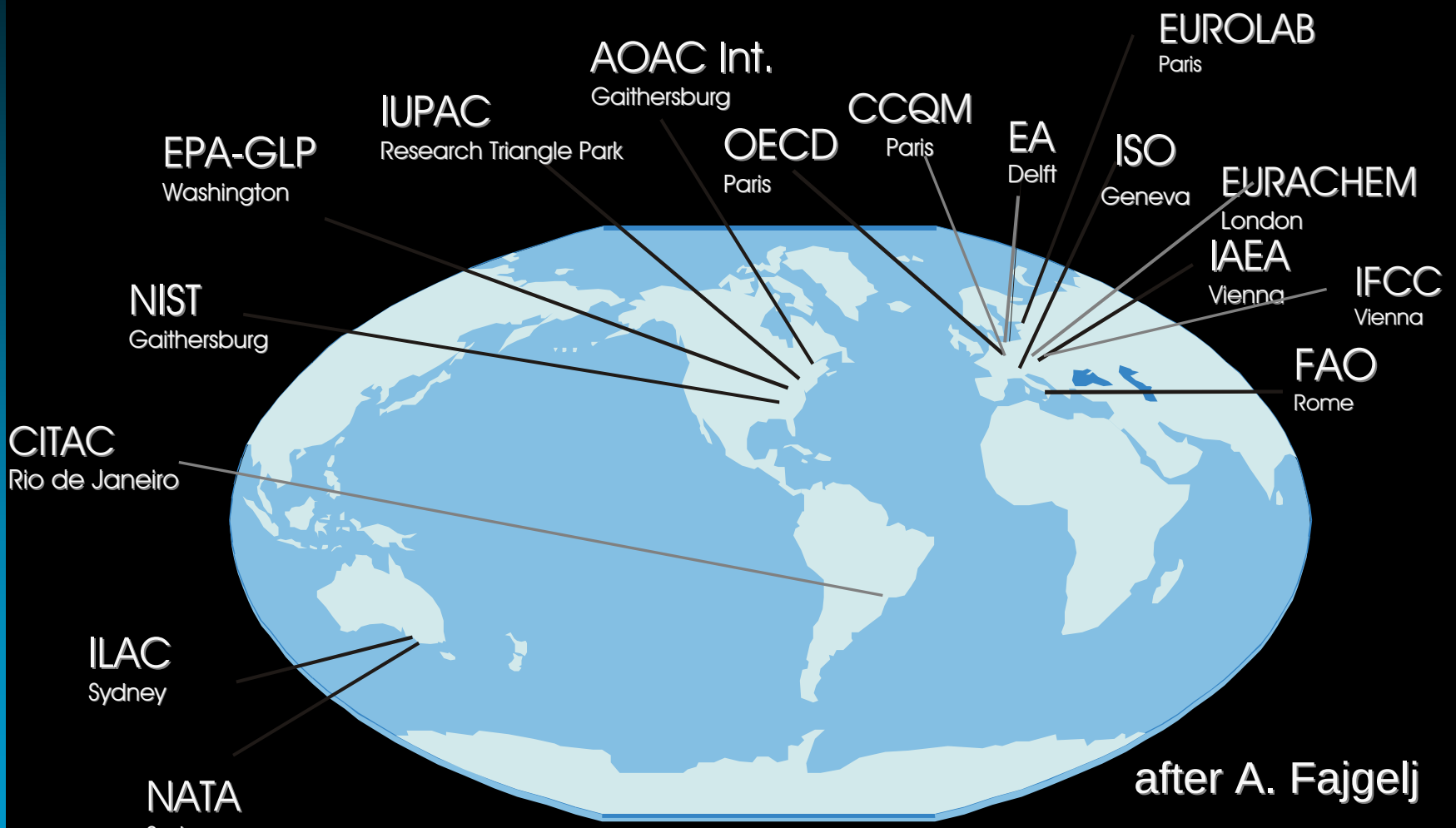
QS in Analytical Chemistry have a flat hierarchy: Reasons

- Operational Reasons
  - innumerable problems to deal with
  - millions of substances
  - close cooperation of synthetic and analytical chemistry
  - impractical to produce ´intermediate´ standards
- Metrological Reasons
  - uncertainty mostly dominated by the practice of a method
  - accuracy ratio must be high
  - no unique hierarchy of methods of measurements

Diversity of Chemical Measurements

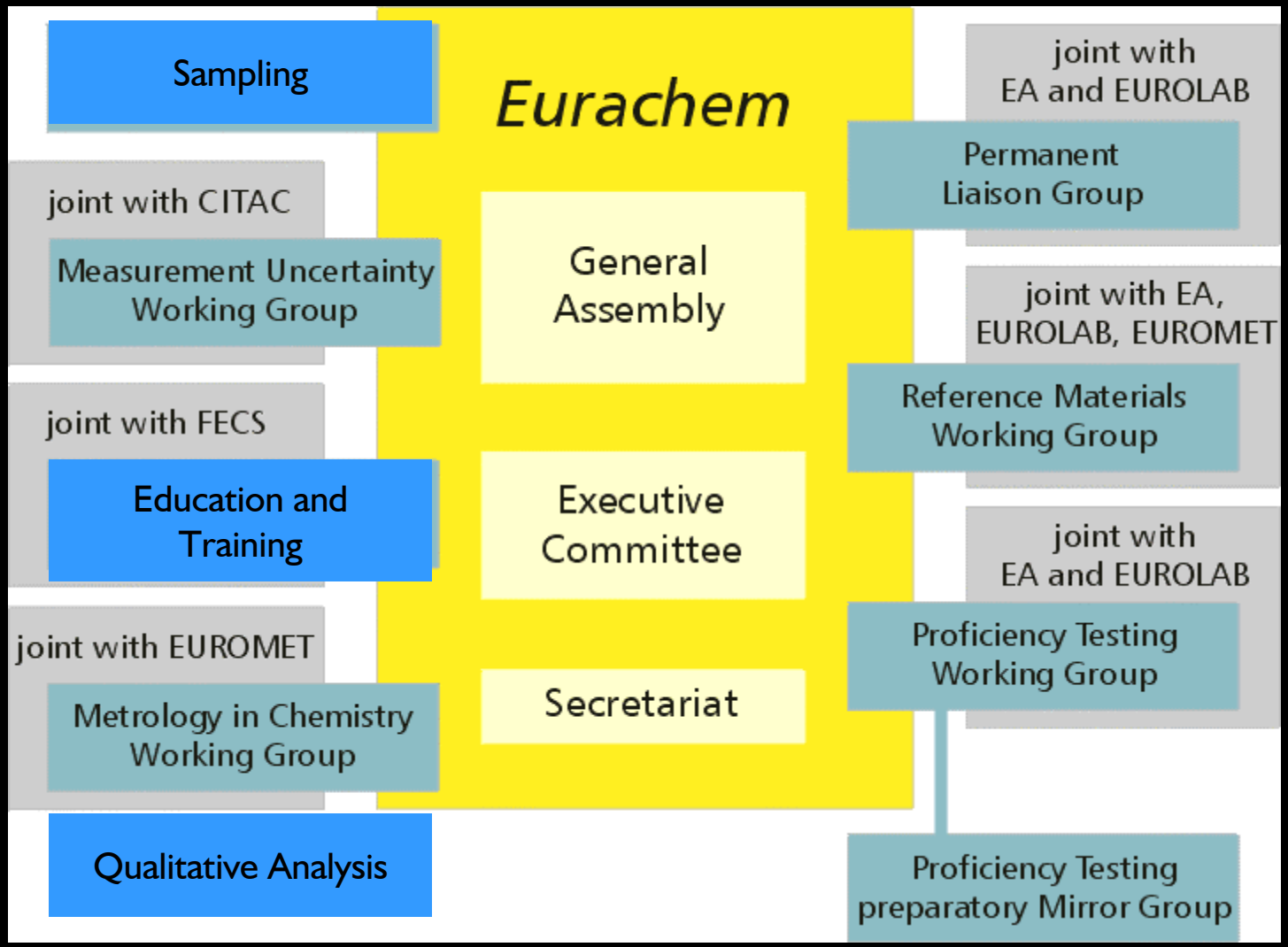
- Food and beverages
- Health commodities
- Clinical chemistry
- Drugs
- Environmental
- Forensic
- Exploration
  - terrestrial, maritime, extraterrestrial
- Biology
  - zoology, botany
- Biotechnology
- Metallurgy
- Construction
- Electronics
- Chemicals
- ....

QS in Analytical Chemistry have a flat hierarchy:



Horizontal structures:

- *Interlaboratory comparisons at all levels:*
  - *Key comparisons*
  - *Regional comparisons: EU-wide by EA, APLAC, …*
  - *National comparisons*
  - *Sectorial comparisons*
  - *Bi-lateral comparisons: customer/supplier*
- *Collaborative development of measurement procedures*
- *Proficiency testing*
- *Training as enabling strategy worldwide*


Eurachem
Sampling
joint with CITAC
Measurement Uncertainty Working Group
joint with FECS
Education and Training
joint with EUROMET
Metrology in Chemistry Working Group
Qualitative Analysis
General Assembly
Executive Committee
Secretariat
joint with EA and EUROLAB
Permanent Liaison Group
joint with EA, EUROLAB, EUROMET
Reference Materials Working Group
joint with EA and EUROLAB
Proficiency Testing Working Group
Proficiency Testing preparatory Mirror Group

Traceability:

Property of the

- result of a measurement or → A posteriori
- the value of a standard → A priori

whereby it can be related to

- stated references, usually national or international standards,

through

- an unbroken chain of comparisons all having stated uncertainties

(current VIM Definition)

Two key components to traceability:

- standards $\pm u_{st}$

- uncertainty $\pm u_{pr}$

---

combined uncertainty:

$$u_x = \sqrt{u_{st}^2 + u_{pr}^2}$$

Uncertainty of a standard:

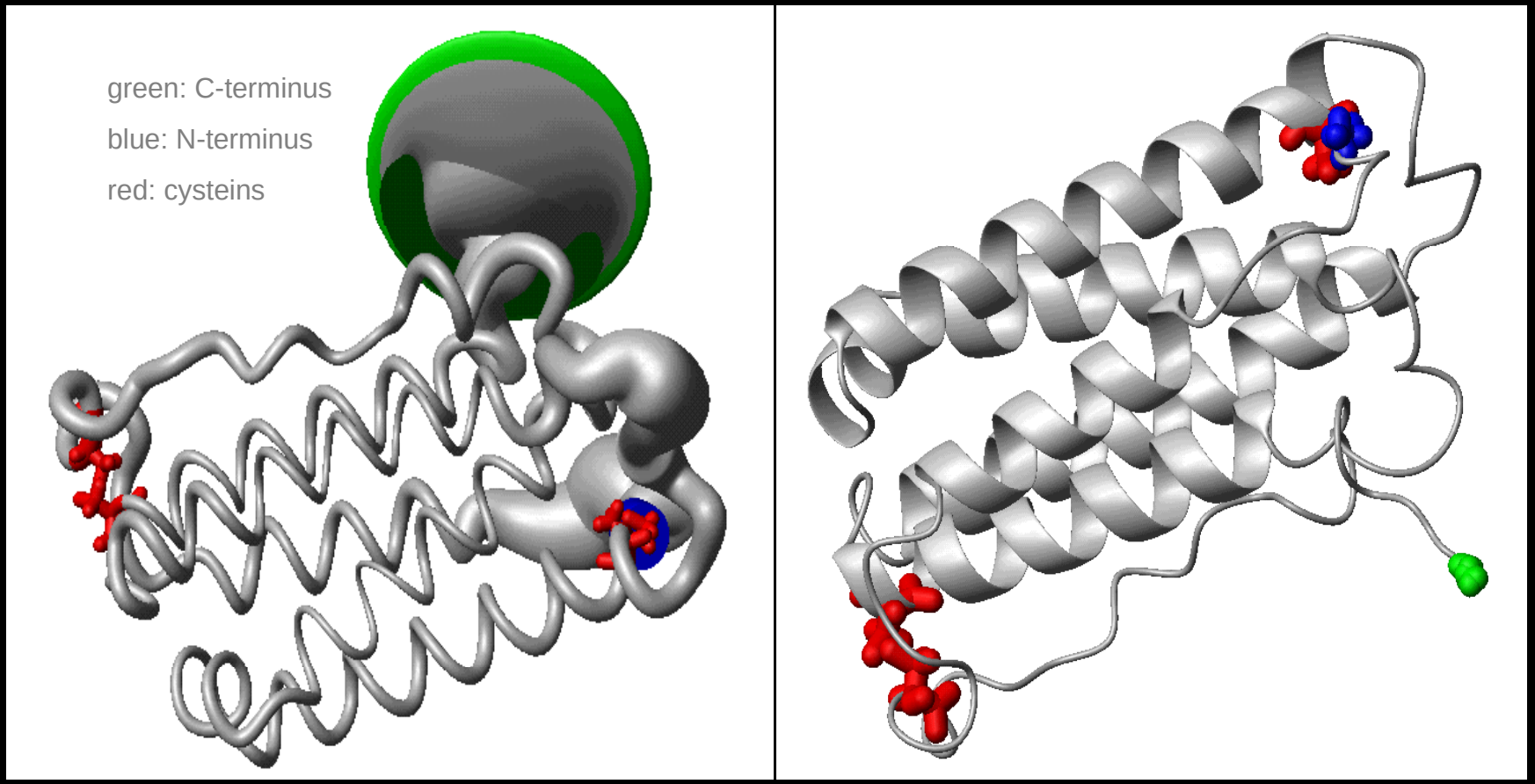
- Identity
- Purity
- Preparation

Understanding Analytical Chemistry: Drug Development

- identity
  - primary sequence
  - secondary structure: folding
  - biological activity
- content: amount of substance
- process dependent contaminations
  - proteins of host cell
  - DNA
  - pyrogens
  - virus
  - process additives
- side products
- stability

from F. Nachtmann, 2000

$^{1}H/^{13}C/^{15}N$-NMR of Interferon Alpha



Overlay structure

Mean structure

calculated from 24 NMR spectra: J. Mol. Biol, 274 (1997) 661-675

W. Wegscheider @ Olten 2006

Contaminations from a Biotechnological Process: Purity of a Standard

- Proteins from host cell
- DNA
- Endotoxins
- Bioburden: Microbiological Assays
- Virus
- Process additives

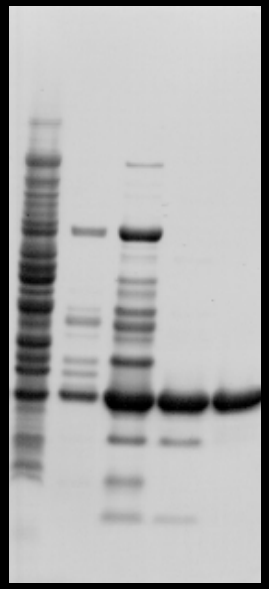
Contaminations from a Biotechnological Process, 2

- Proteins from host cell
  - physico-chemical methods for the determination of proteins
  - Immunological methods
    1. Mock fermentation for production of host cell proteins WITHOUT products
    2. Production of specific antibodies
    3. Development of an immunological method of analysis (e.g. Westernblot, ELISA, Threshold)

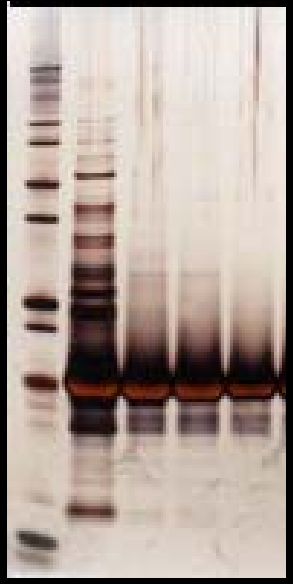
Purification of Product: SDS-PAGE

purification of product

Coomassie



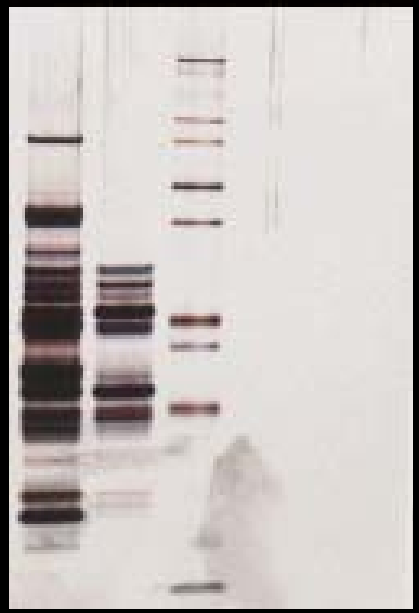
silver



1 2 3 4 5 6 7 8 9 10

mock purification

silver



11 12 13 14 15

purification sequence:	Gel trace:
Fermentation broth	1 (dil.)
Raw extract	2 (dil.)
IEX	3
HIC	4, 7, 11
RPC	5, 8, 12
IEX	9, 14
SEC	10, 15

traces 6 und 13: standards for molecular mass

A general model of measurement and validation in chemistry

x_{ijk} ....result

μ.....true value

$$x_{ijk} = \mu + \varepsilon_{ijk}$$

huge and unknown

for N results:

$$\bar{x}_{ijk} = \mu + \varepsilon'_{ijk}$$

$$u(x_{ijk}) = f(\varepsilon_{ijk})$$

somewhat smaller, but unknown

A general model of measurement and validation in chemistry

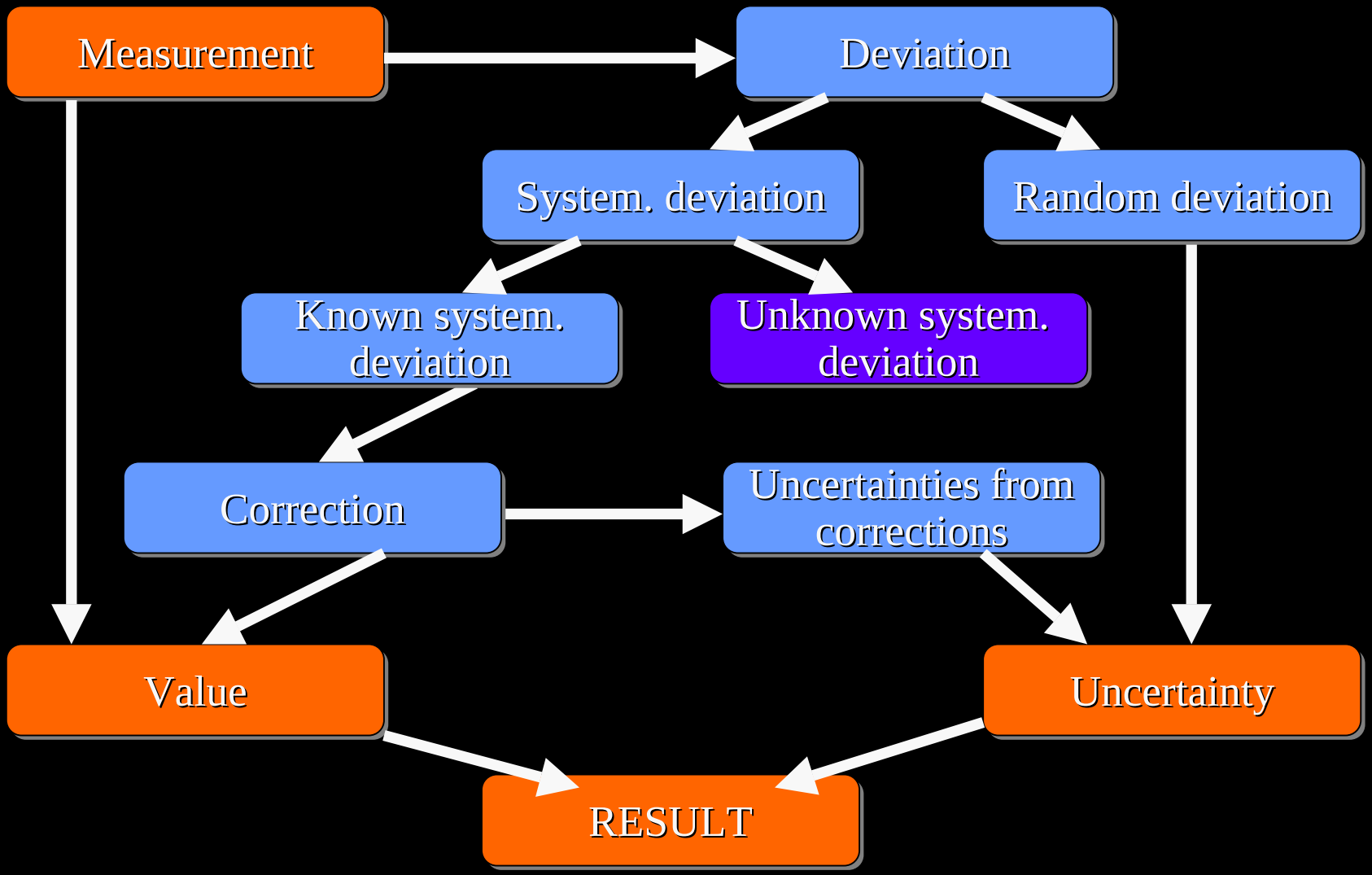
After validation:

$$x_{ijk} = \mu + \delta_i + \delta_j + \delta_k + \varepsilon_{ijk}$$

identified effects on result

small and accessible from precision data

From deviations to uncertainty:



Two (extreme) ways to define the measurand / analyte

A) Careful and complete description of circumstances:
- (exactly) what species
- What (kind of) samples/concomitants
- Which environmental conditions (p, T)

B) Measure as specified and give the analyte a name

Two extreme ways to define the measurand / analyte: MODEL B

A) Measure as specified and give the analyte a name:

$$x_{ijk} = \mu + \delta_i + \delta_j + \delta_k + \varepsilon_{ijk}$$

The „new“ analyte

Accessible through interlab comparisons

$$u(x_{ijk}) = f(\varepsilon_{ijk})$$

The Measurement of pH

IUPAC Working Party on pH

- Notational definition:

$$pH = -\lg a_H = -\lg\left[\frac{m_H \gamma_H}{m^0}\right]$$

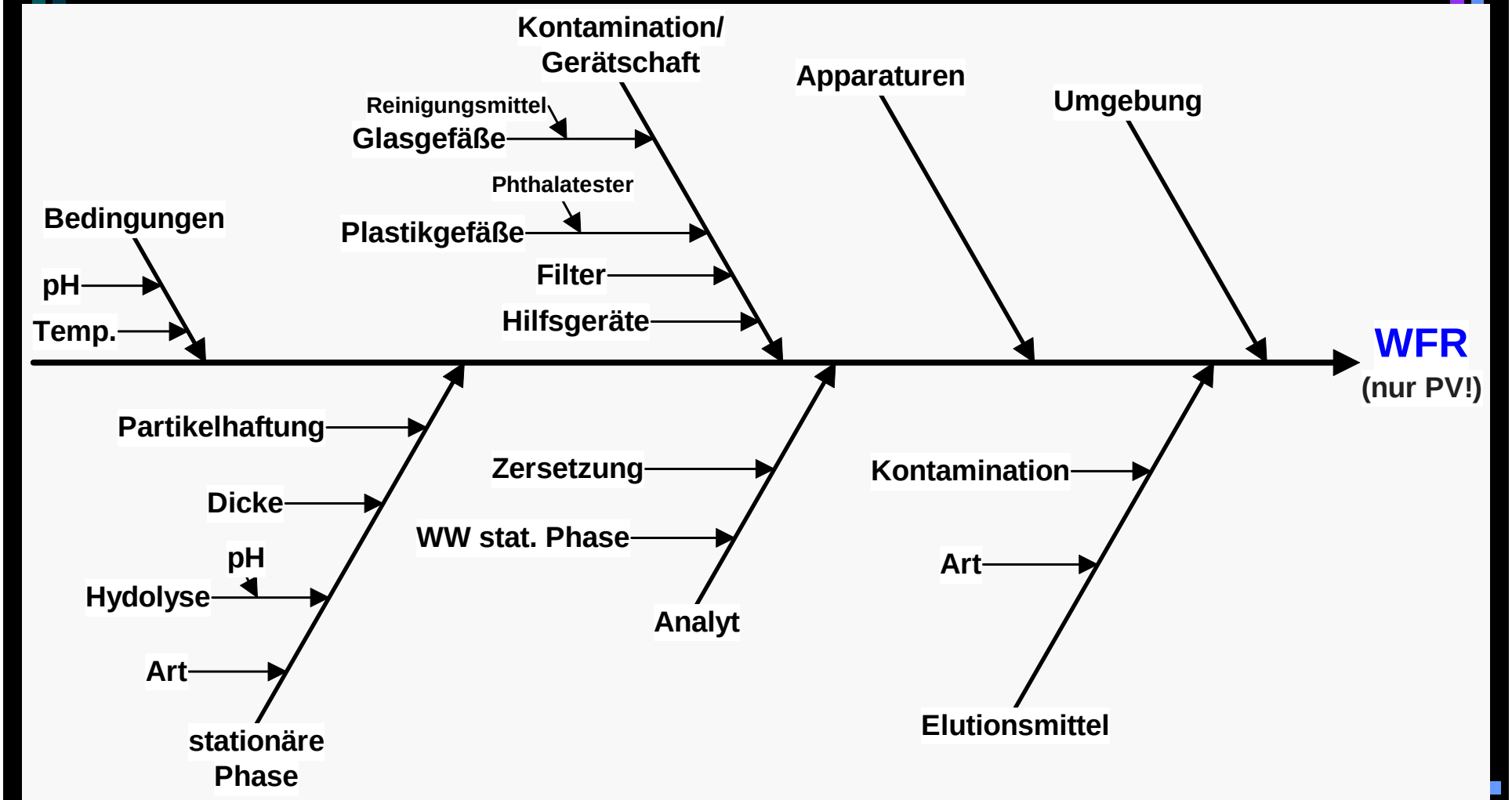
- Operational definition:

$$Pt\left|H_2\right|buffer\ S, Cl^-\left|AgCl\right|Ag$$

Operational Definition of pH

- Debye-Hückel formalism with Bates-Guggenheim convention
- (only) 5 primary buffers
- $3 < pH < 10$
- $I < 0.1$ mol/l
- Aqueous solution

SPE


Kontamination/
Gerätschaft
Reinigungsmittel
Glasgefäße
Phthalatester
Plastikgefäße
Filter
Hilfsgeräte
Apparaturen
Umgebung
Bedingungen
pH
Temp.
WFR
(nur PV!)
Partikelhaftung
Dicke
pH
Hydolyse
Art
stationäre
Phase
Zersetzung
WW stat. Phase
Analyt
Kontamination
Art
Elutionsmittel

Estimation of recovery

indirect:

- Experiments on reference materials
- Comparison with an alternative method
- Spiking

V. Barwick, S. Ellison, Analyst 124 (1999), 981-990

numerical:

$$\overline{\overline{R}} = \overline{R_m} \times R_S \times R_{rep}$$

Methods for estimating recovery /1

Scope of method / availability of reference materials	R_m	R_S	R_{rep}
Single matrix and analyte conc. Representative CRM available	Recovery studies on CRM (-> R_m and $u(R_m)$	N. A.	N. A.
Single matrix and analyte conc. NO representative CRM available	(-> R_m) •spiked repres. matrix •Comp. of typ. sample with standard method •Altern. extraction system •„worst case“ CRM	N.A.	How representative is spiking ?

Methods for estimating recovery /2

Scope of method / availability of reference materials	R_m	R_S	R_{rep}
Multiple matrices a/o concentrations Representative CRM available	Repeated measurements on CRM (-> R_m and $u(R_m)$	spiking $u(R_S)$ from mean recoveries	N.A.
Multiple matrices a/o analyte conc. NO representative CRM available	spiking (-> R_m und $u(R_m)$	Estimation of $u(R_S)$ from R_m data	How representative is spiking ?

Two extreme ways to define the measurand / analyte: MODEL A

B) Careful and complete description of circumstances:

$$x_{ijk} - \delta_i - \delta_j - \delta_k = \mu + \varepsilon_{ijk}$$

The „old“ analyte well defined

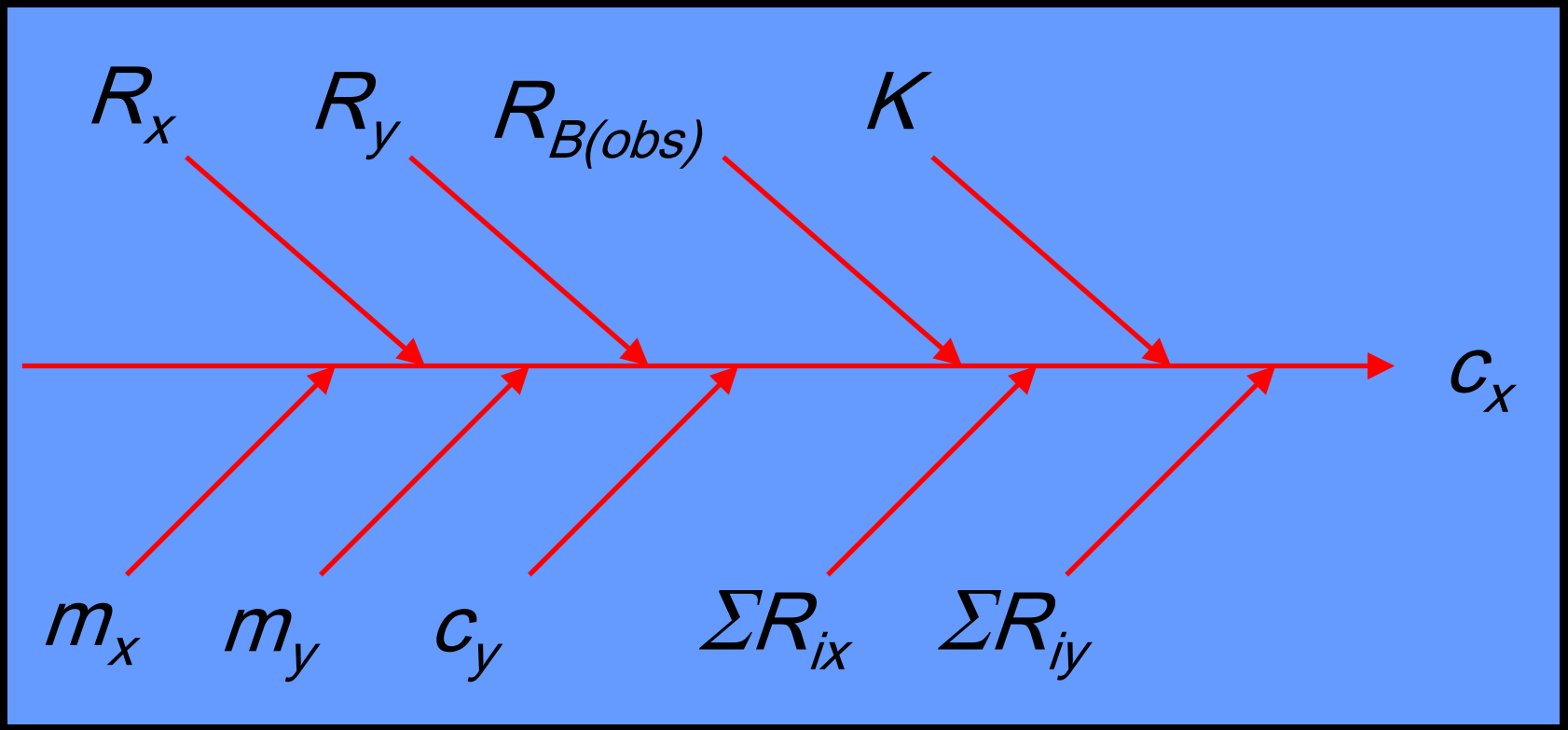
Very small part of uncertainty

$$u(x_{ijk}) = f(\delta_i, \delta_j, \delta_k, \varepsilon_{ijk})$$

MODEL A Uncertainty of a „well understood“ method IDMS

$$c_x = \frac{R_y - K \cdot R_{B(obs)}}{K \cdot R_{B(obs)} - R_x} \cdot \frac{R_x + \sum_{i-1} R_{ix}}{R_y + \sum_{i-1} R_{iy}} \cdot \frac{m_y}{m_x} \cdot c_y$$

$$c_x = \frac{R_y - K \cdot R_{B(obs)}}{K \cdot R_{B(obs)} - R_x} \cdot \frac{R_x + \sum_{i-1} R_{ix}}{R_y + \sum_{i-1} R_{iy}} \cdot \frac{m_y}{m_x} \cdot c_y$$



Everything is fine ? ..... except

$$I_{corr,interference,i} = I_{corr,background,i} - f_{corr} \cdot I_{interference,i}$$

$$R_{B(obs),i} = \frac{I_{corr,interference,M,B,i}}{I_{corr,interference,m,B,i}}$$

$$I_{corr,background,i} = I_{corr,deadtime,i} - \bar{I}_{background}$$

$$c_x = \frac{R_y - K \cdot R_{B(obs)}}{K \cdot R_{B(obs)} - R_x} \cdot \frac{R_x + \sum_{i-1} R_{ix}}{R_y + \sum_{i-1} R_{iy}} \cdot \frac{m_y}{m_x} \cdot c_y$$

$$I_{corr,deadtime,i} = \frac{I_{meas...}}{1 - I_{measured,i} \cdot \tau}$$

Correlations in model

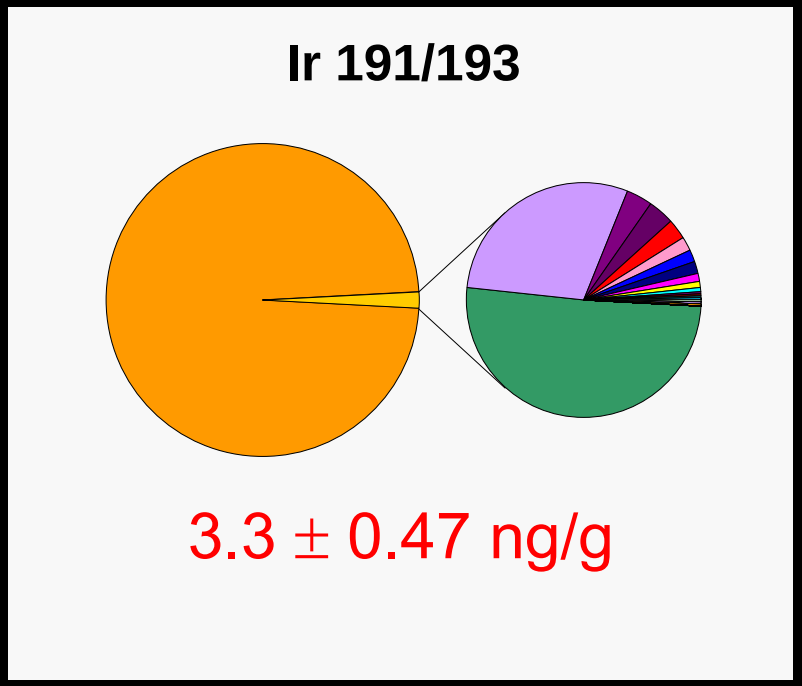
Hidden contributions:

- Dead time
- Serial dilutions
- Identical equipment: balances

Drift:

- Mass calibration
- Sensitivity

Results from isotope dilution mass spectrometry:



More important components of uncertainty :

- Inhomogeneity and preparation ...
- Ratio of blend ...
- Concentration of element standard ...
- Dead Time ...
- Abundances ...

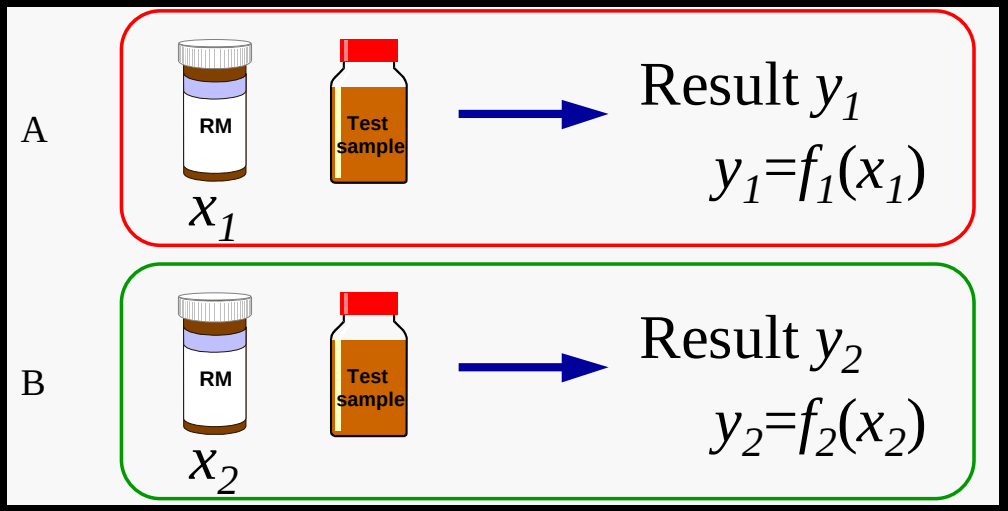
Moser, Wegscheider, Meisel, Fellner ABC 2003

Role of traceability:

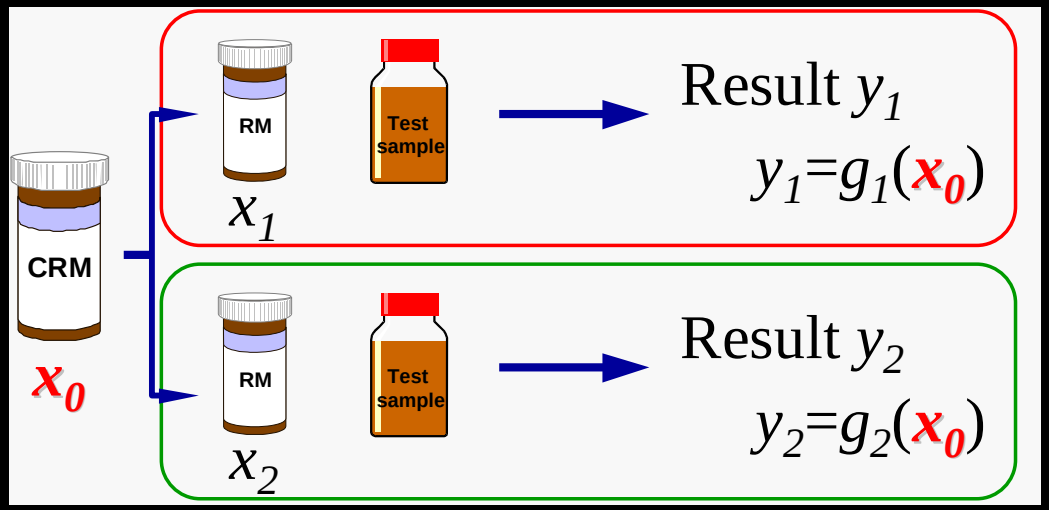
Eurachem/Citac Guide 2003

- Provide a firm and identical base of units worldwide
- Supply this base in a manner stable in time
- Underpin the hierarchy of measurements/procedures/laboratories
- .....

Local vs. Global Traceability



local



global

Practical steps towards traceability

- Follow a clear procedure
- Identify and procure all standards/ references/étalons
- Guesstimate the influence of each on the measurement uncertainty
- Minimize the large contributions to uncertainty
- Optimize: Produce a smaller uncertainty and a better estimate

The nature of „stated references“:

- Time
- Temperature
- Pressure
- Length:
  - Diameter
  - Volume
- Amount of substance: chemical standards
- ....

What is required ?

- A (complete) model of measurement

$$x_{i,p,T,V,m} = f(x_1, x_2, x_3, x_4, \ldots)\Big|_{p,T,V,m} + \varepsilon_i$$

x....*result*

f ...*model*

$x_1, x_2, x_3, x_4, x_{..}$ ...*values of standards*

ε_i ...*random deviation of measurement i*

Two „types“ of stated references:

1. with *little* influence on final result
2. with *large* influence on final result

Ad 2)

- large uncertainty on reference contributes a lot to uncertainty
- poor control to this reference produces a large deviation

A success story of traceability in spectroscopy

- Serum Cholesterol by GC-IDMS
  - addition of cholesterol-$^{13}C_3$
  - hydrolysis, extraction
  - silylation to trimethylsilyl ethers
  - non-polar fused silica capillary GC
  - magnetic sector MS

P. Ellerby et al., Anal. Chem. 61 (1989) 1718

Conditions for making it a success story

- Measurement protocol
- Instrumentation
- Choice of labelled material
- Interference study
- Memory effects
- Standards cross-check
- Confirmatory measurements

Conditions for making it a success story, 2

- Measurement protocol
  - two bracketing standards
  - each standard and sample measured twice
  - intensity ratios agreed to <0.5 %
  - reverse the order of measurement on a 2nd day
- Instrumentation
- Choice of labeled material
- Interference study
- Memory effects
- Standards cross-check
- Confirmatory measurements

Conditions for making it a success story, 3

- Measurement protocol
- Instrumentation
  - ion beam with electrical switching by electrical deflection after mass separation
- Choice of labeled material
- Interference study
- Memory effects
- Standards cross-check
- Confirmatory measurements

Conditions for making it a success story, 4

- Measurement protocol
- Instrumentation
- Choice of labelled material
  - with carbon-13-labelled material instead of deuterium labelled material
  - no separation from unlabelled on GC column
- Interference study
- Memory effects
- Standards cross-check
- Confirmatory measurements

Conditions for making it a success story, 5

- Measurement protocol
- Instrumentation
- Choice of labeled material
- Interference study
  - separation of lathosterol (5a-cholest-7-en-3b-ol)
  - 24 s gap between integration windows
- Memory effects
- Standards cross-check
- Confirmatory measurements

Conditions for making it a success story, 6

- Measurement protocol
- Instrumentation
- Choice of labeled material
- Interference study
- Memory effects
  - test by injection sequence labelled-unlabelled-labelled in extreme ratios
- Standards cross-check
- Confirmatory measurements

Conditions for making it a success story, 7

- Measurement protocol
- Instrumentation
- Choice of labeled material
- Interference study
- Memory effects
- Standards cross-check
  - two independent set of standards
  - compare ID-MS weight ratio to gravimetric weight ratio: mean difference <0.01 %
- Confirmatory measurements

Conditions for making it a success story, 8

- Confirmatory measurements
  - on three sets of SRM 909, 1951, 1952
  - EI fragment at m/z 329/332 on a nonpolar column
  - EI molecular ion at m/z 458/461 on a moderate-polarity column
  - ammonia CI fragment at m/z 386/389 on a nonpolar column
- all differences < +0.18 %; precision 0.22 %

Elements of validation

D. Dadgar et al., J. Pharm. Biomed. Anal. 13 (1995) 89

generally agreed that the key criteria for evaluation of method reliability and overall performance are:

(i) analyte stability;
(ii) method selectivity;
(iii) limit of quantitation;
(iv) accuracy;
(v) precision;
(vi) relationship between response and concentration (e.g. linearity);
(vii) recovery; and
(viii) ruggedness.

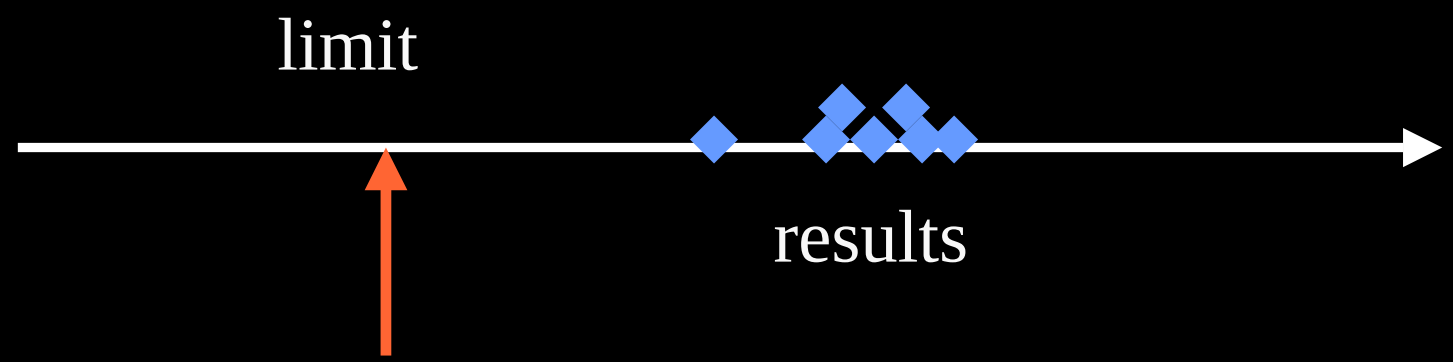
Traditional roles of method validation

- Establish performance characteristics
  - Linearity
  - Limits of detection/determination
  - Precision: repeatability, intermediate reproducibility
  - Effect of concomitants
- Present data for approval of method
- Produce control limits for everyday operation

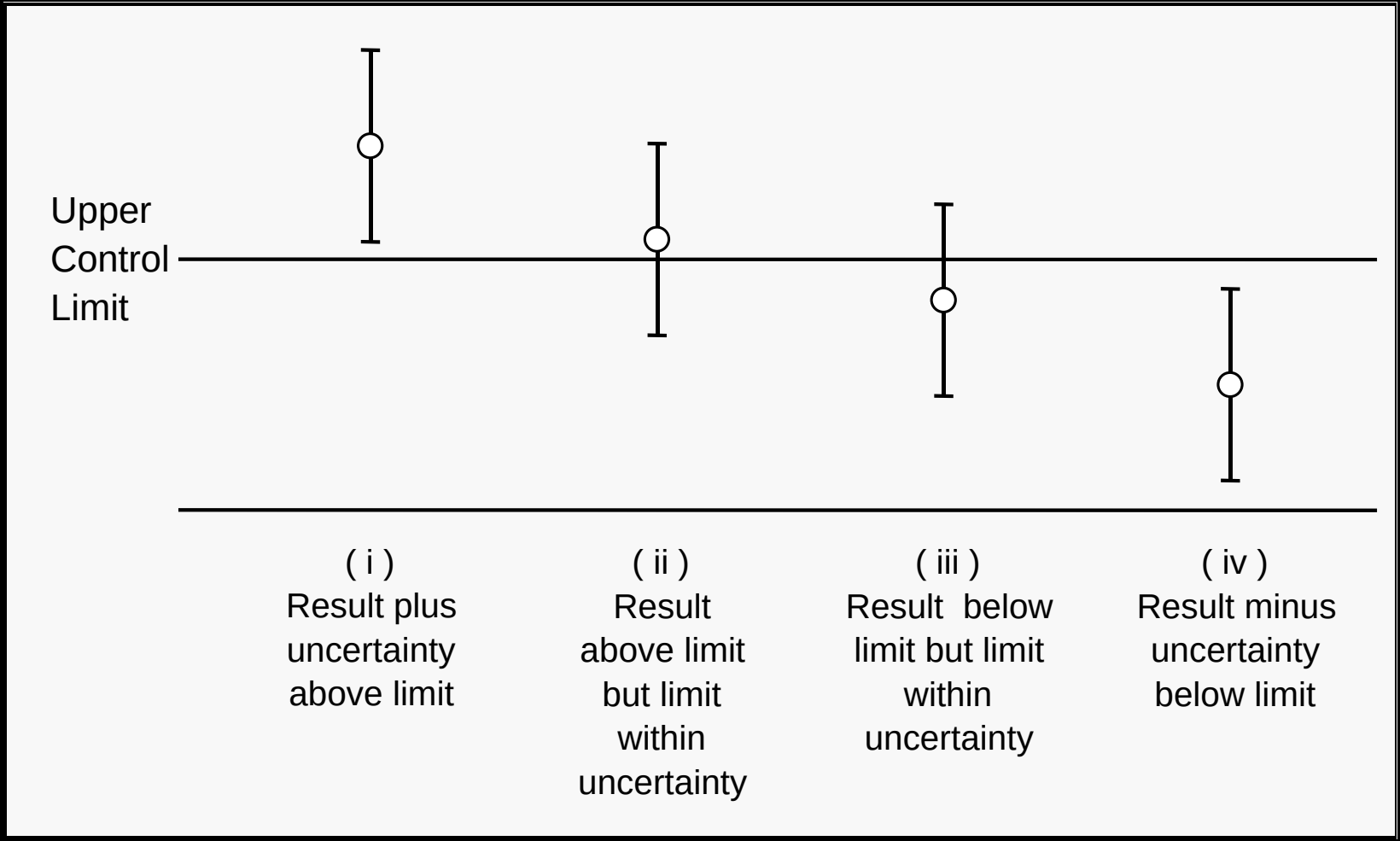
What **should** be the role of validation ?

As analytical chemistry is about making decisions

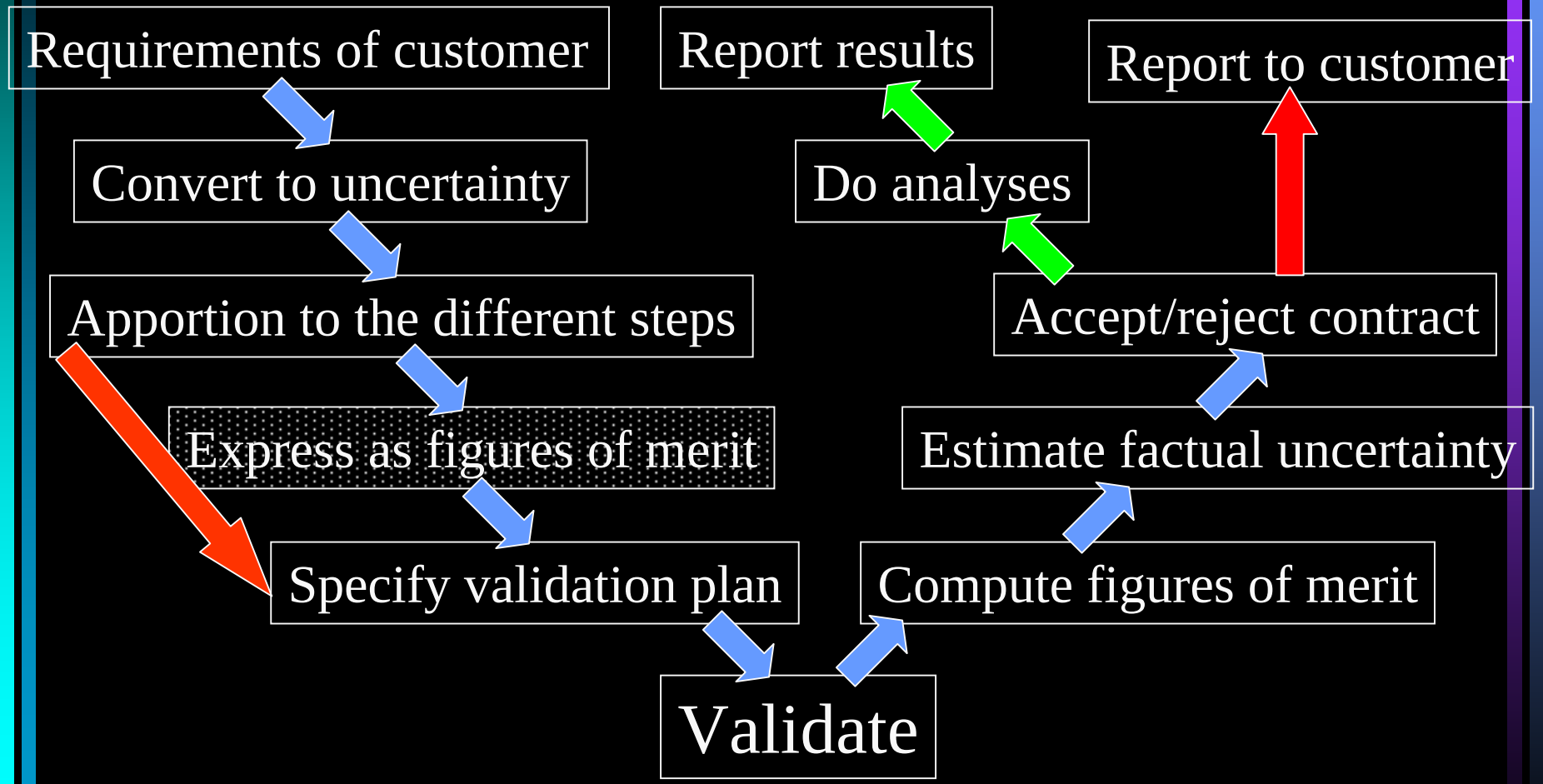
- validation should support the decision making process



Obvious Problem with Credibility of Chemical/Biological Results

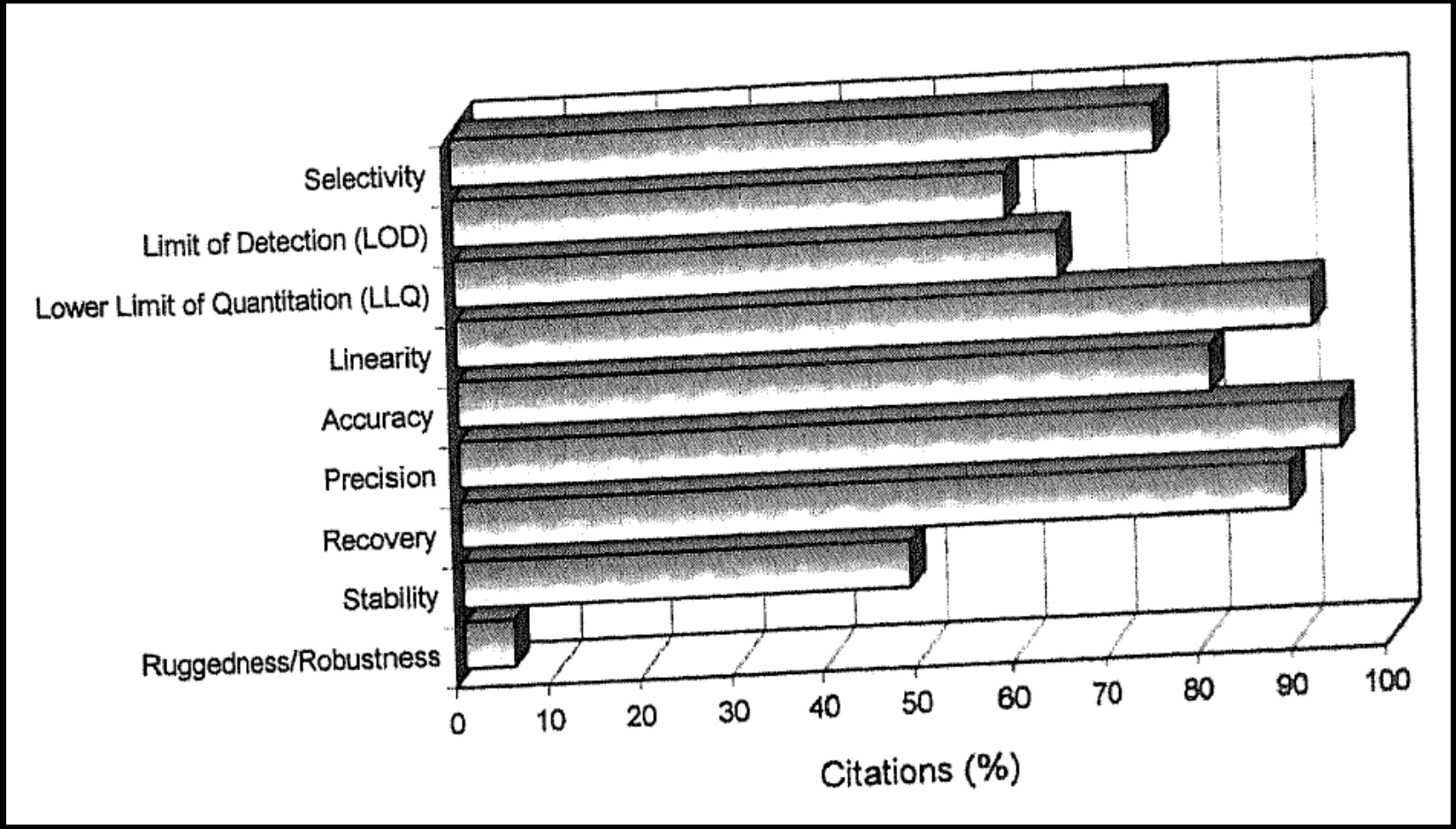


Validation starts out from the customer´s needs

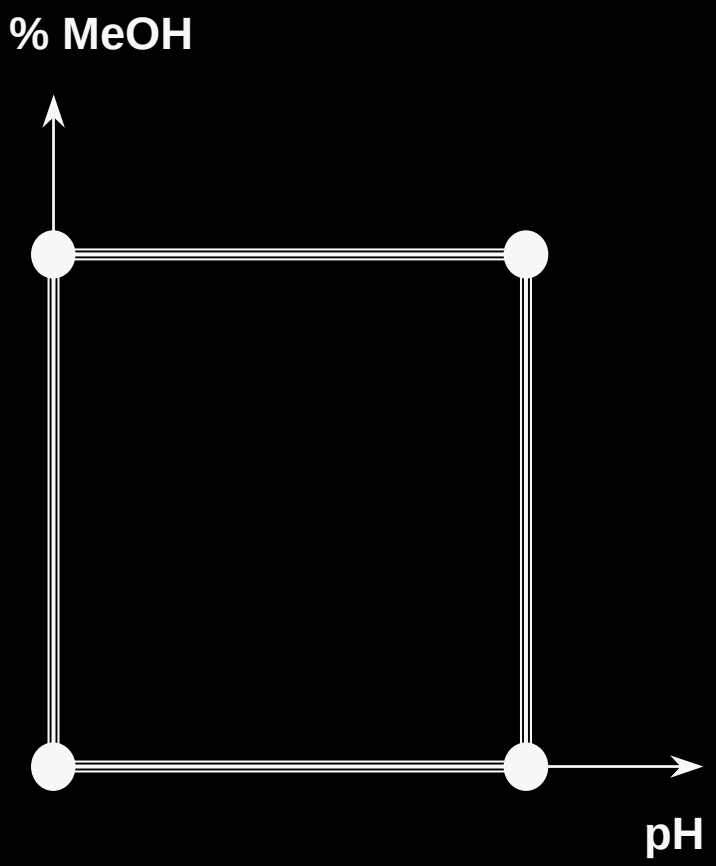


Frequency of validation parameter

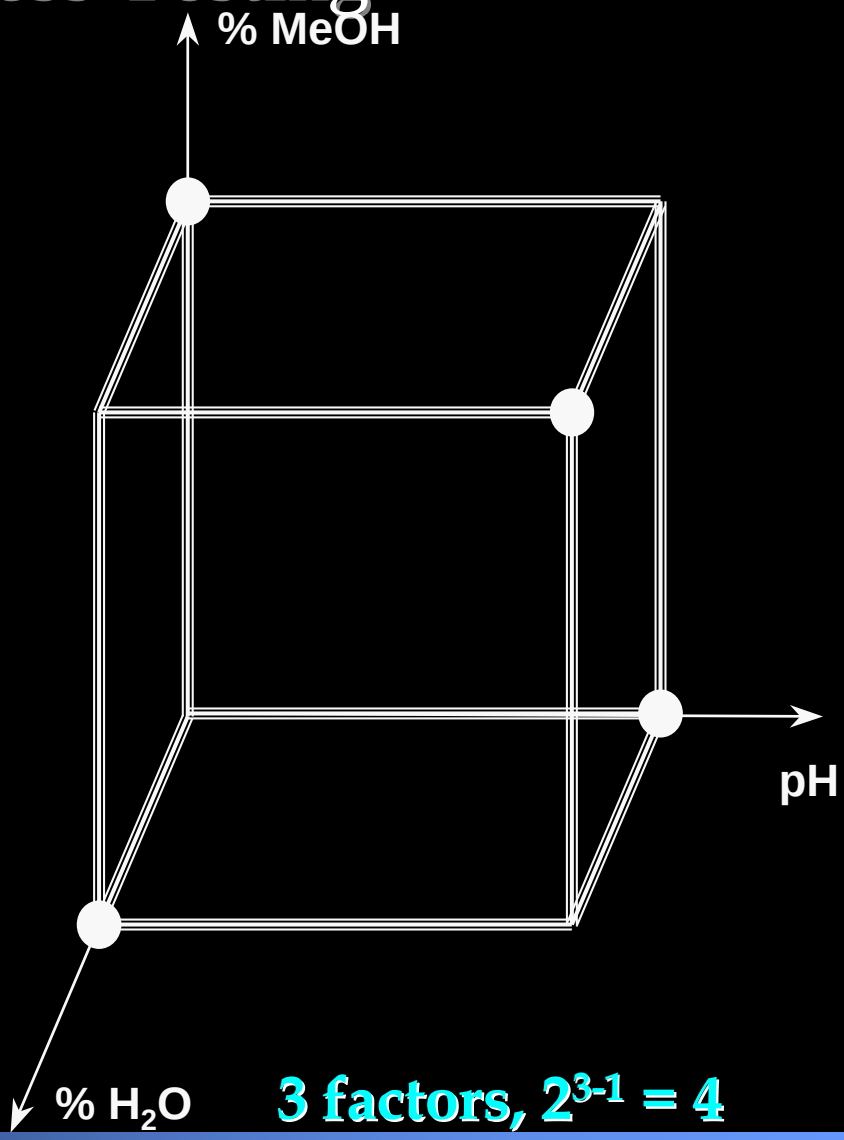
H. Rosing et al., J.Liq.Chrom. 23 (2000) 329



Screening Designs are Well Suited for Ruggedness Testing

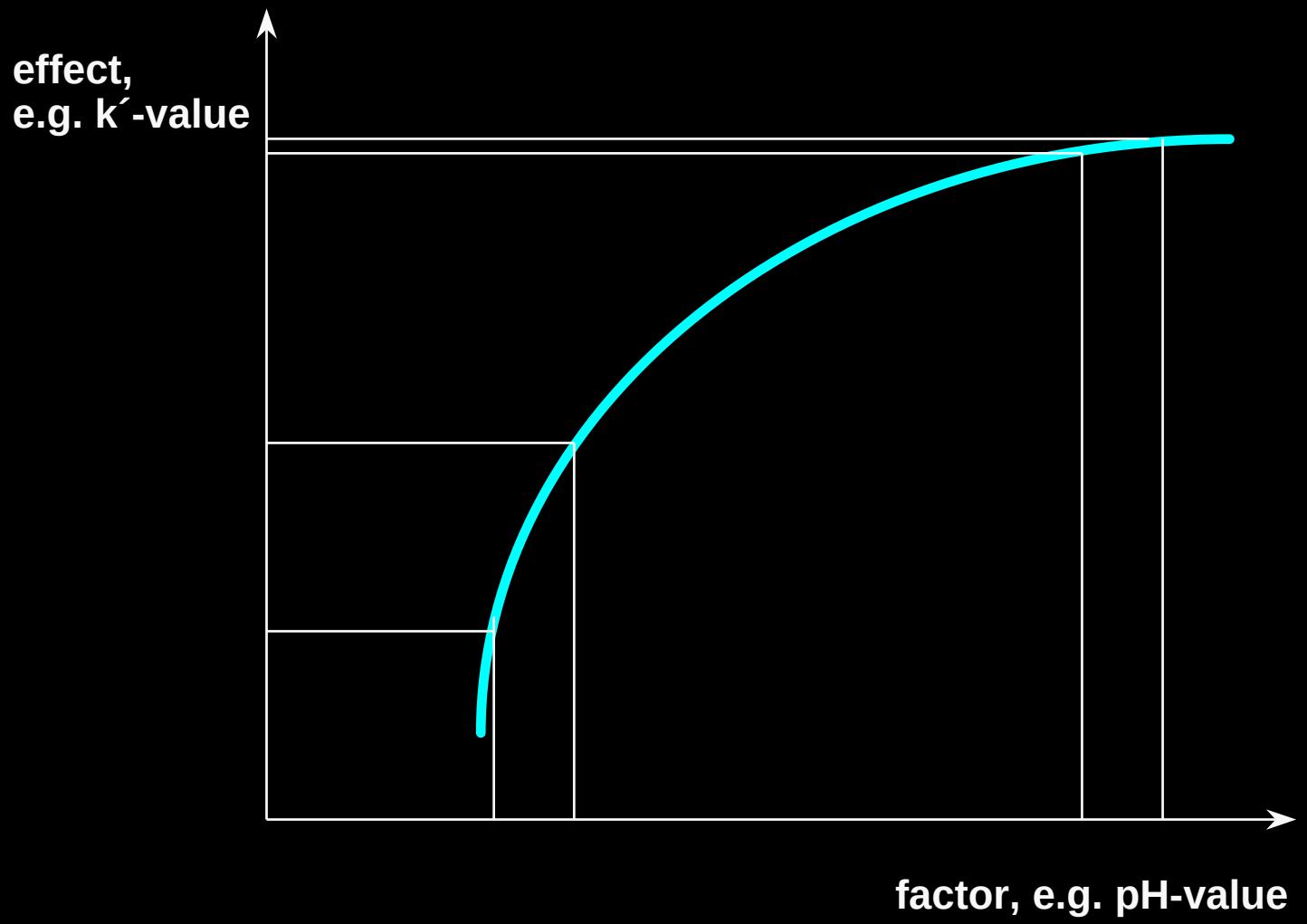


2 factors, $2^2 = 4$



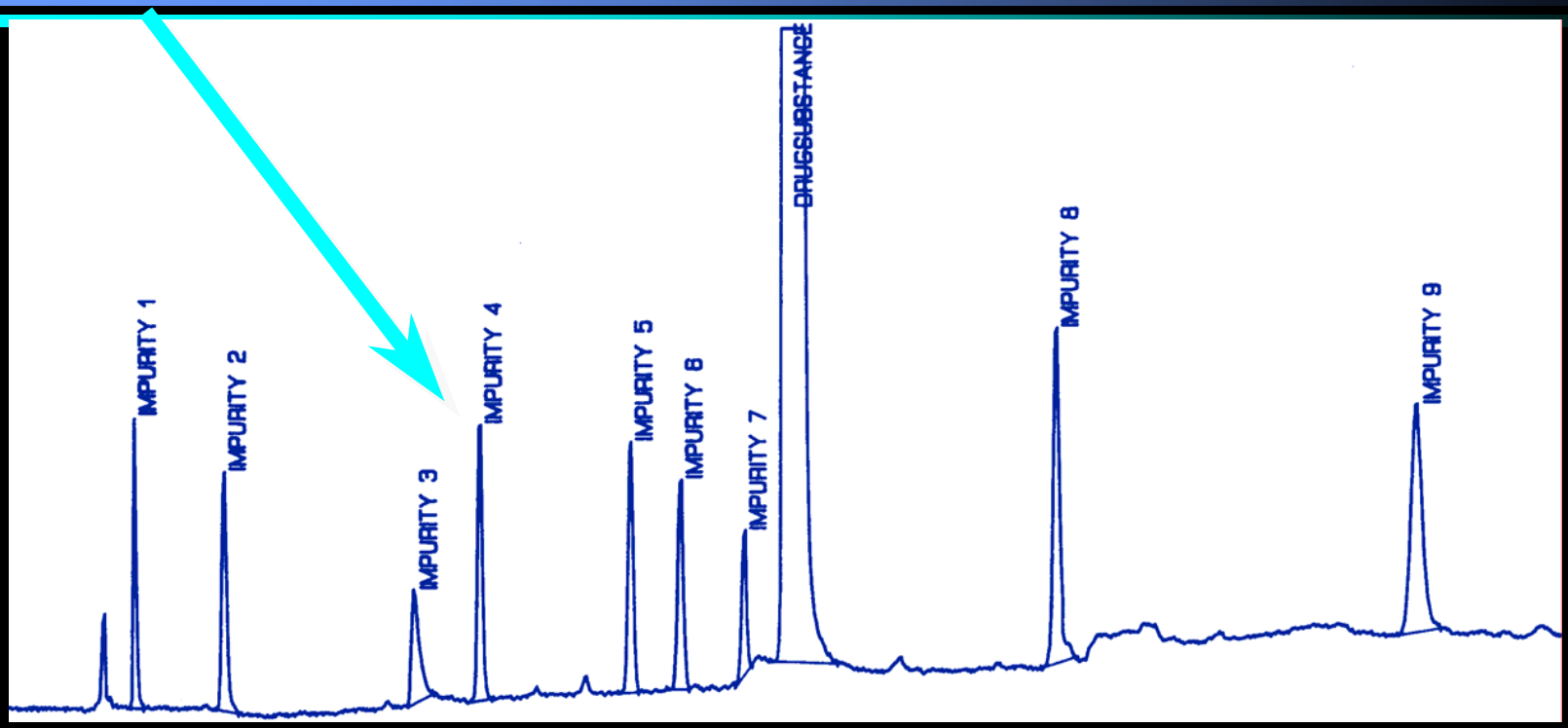
3 factors, $2^{3-1} = 4$

Optimization for Ruggedness



Parameters in Ruggedness Test

Parameter	normal conditions	lower boundary - 1	upper boundary + 1
mobile phase: MeCN from 1		0	2
[%]	to 20	18	22
buffer strength [mol/l]	0.1	0.05	0.15
pH	7.0	6.8	7.2
flow rate [ml/min]	1.5	1.3	1.7
temperature [°C]	35	30	40
detection at [nm]	230	225	235
injection volume [❍l]	5	5	15



Influence of different factors on **resolution** of "impurity 3" and "impurity 4":

molarity = 4.57791 +/- 0.380886

pH = -2.22209 +/- 0.380886

MeCN_start = -0.472091 +/- 0.380886

MeCN_end = -0.697091 +/- 0.380886

flow rate = 0.602909 +/- 0.380886

temperature = -0.597091 +/- 0.380886

inject_vol = 1.04655 +/- 0.399243

wavelength = 0.402909 +/- 0.380886

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EURACHEM / CITAC Guide CG 4

Quantifying Uncertainty in Analytical Measurement

Second Edition

QUAM:2000.1

Eurachem

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Co-Operation on International Traceability in Analytical Chemistry

EURACHEM / CITAC Guide

Traceability in Chemical Measurement

A guide to achieving comparable results in chemical measurement

2003

July 2002 rev02

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Take-home messages:

- Accreditation is about competence
- Competence leads to flexible solutions
- Customer based validation gives uncertainty of measurement
- Design space is defined by boundary conditions in optimization
- EURACHEM Guides give generic advice and specific examples

Acknowledgements:

- Thomas Meisel, Leoben
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- EURACHEM Working Group „*Measurement Uncertainty and Traceability*“