



Setting standards
in analytical science

International Protocols Adopted by CODEX: Collaborative Study, Single Laboratory Validation, Proficiency Testing and Recovery

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Overview

- CODEX Principles
- IUPAC protocols
 - Collaborative Study
 - Single Laboratory Validation
 - Proficiency Testing
 - Recovery
 - Internal Quality Control
- Further developments

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Codex principles

June 1997



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Food Control Laboratories should

- ...comply with the general criteria for testing laid down in ISO/IEC Guide 25:1990... **17025 Accreditation**
- Participate in appropriate proficiency testing schemes for food analysis...
- Whenever available, use methods of analysis which have been validated according to the principles laid down by the Codex Alimentarius Commission;
- Use internal quality control procedures... **IUPAC Guides**
- Use certified reference materials** **ISO REMCO**

**Added subsequently

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Single laboratory Validation

HARMONIZED GUIDELINES FOR SINGLE
LABORATORY VALIDATION OF METHODS OF
ANALYSIS

Pure Appl. Chem., Vol. 74, No. 5, pp. 835–855, 2002

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Why consider single-laboratory validation?



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- Standard methods take a long time to develop
- Technology moves faster than method development
- Some validation within a laboratory is always necessary to show a standard method is working

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Characteristics to be assessed



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- Applicability
- Selectivity
- Calibration
- Trueness and Recovery
- Precision
- Range
- Limits of Detection and Quantification
- Sensitivity (“not useful”)
- Ruggedness
- Fitness-for-Purpose
- Measurement uncertainty

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Extent of validation depends on application



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- The laboratory is to use a fully validated method • **Verify only**
- The laboratory is to use a fully validated method, but a new matrix or new instrument is to be used • **Verify only**
- The laboratory is to use a well-established, but not collaboratively studied, method • **Verify + reproducibility**
- The method is published in the scientific literature together with some analytical characteristics • **Verify + R & r**
- The method is published in the scientific literature; no characteristics given • **Full validation**
- The method has been developed in-house • **Full validation**

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Shortcomings



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- No detailed description of experimental procedures
 - number of materials
 - level of replication
- New IUPAC project approved 2009 to provide supplementary guidance
 - Intended to describe basic experiments with typical replication etc.
 - Intended to allow any experiment of comparable test power, to allow freedom to vary according to conditions whilst guaranteeing the same stringency

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Collaborative Study

PROTOCOL FOR THE DESIGN, CONDUCT
AND INTERPRETATION OF
METHOD-PERFORMANCE STUDIES
Pure & Appl. Chem., Vol. 67, No. 2, pp. 331-343, 1995

Aims of collaborative study

- To identify factors affecting measurement results
 - Within- or between-laboratory?
- To check that a method can be transferred to other laboratories
- To check that the written protocol is clear to new users
- To estimate the precision characteristics of the method in practice

Different precision estimates



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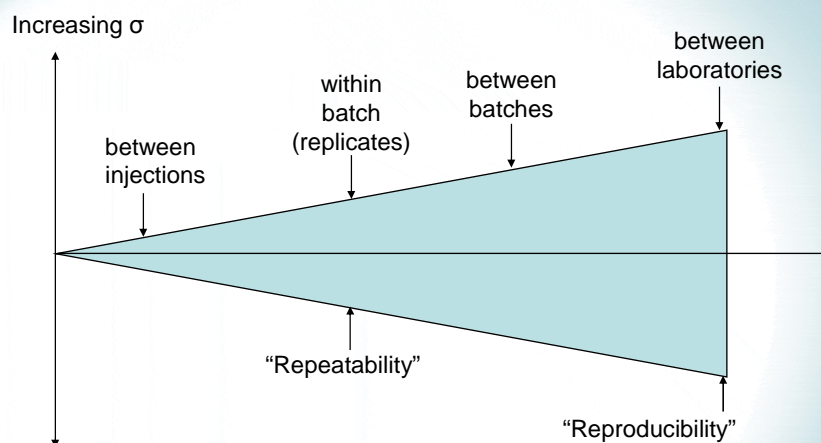
- Repeatability
 - same laboratory, analyst, equipment, short time interval
- Intermediate precision
 - within lab variation - different days, analysts, equipment
 - can be in a number of forms - user defined
- Reproducibility
 - different laboratories, analysts, equipment; short period of time [ISO 3534]

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Effect of varying conditions



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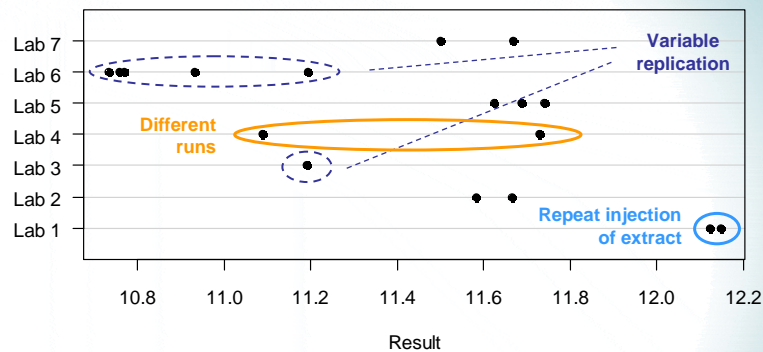
Typical laboratory study format



- Stable, homogeneous materials distributed to several laboratories
- Laboratories undertake replicate analysis
- Results returned to organiser
- Organiser estimates repeatability and reproducibility

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Typical uncontrolled study



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Standards for collaborative study



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- ISO 5725: Precision of test methods
 - Part 2: Basic method for the determination of repeatability and reproducibility

- IUPAC: Protocol For The Design, Conduct And Interpretation Of Method-Performance Studies

CCC-MAS adopted

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IUPAC recommendations



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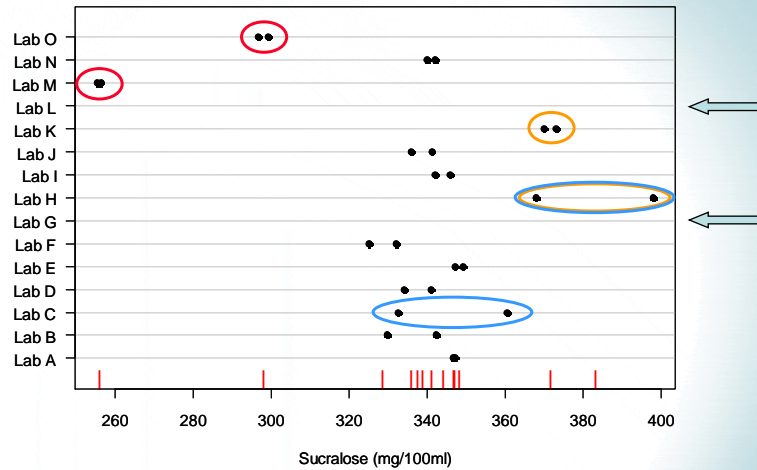
- Minimum of 8 laboratories
 - 5 in exceptional circumstances
- Minimum of 5 test materials
 - 3 under some conditions
- Replication specified in preference order:
 1. Split level (slightly different samples)
 2. Combination blind replicates and split level
 3. Blind replicates (Separate samples, no visual cues)
 4. Known replicates
 5. Independent analyses
 - 1 replicate, repeatability determined separately

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Typical data from standardized study design



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Data treatment recommendations



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- Outlier testing
 - Cochran (for excess variance)
 - Grubbs tests (for extreme mean values and pairs of means)
- Outlier action
 - IUPAC: Remove at 97.5% confidence
 - ISO 5725: Inspect at 95%; Remove at 99%
- Repeated outlier tests
 - Permitted to maximum of 22.2% data set loss (IUPAC)

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Results



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- Processing using 1-way analysis of variance gives:

mean	347.10	
r	26.68	← 2.8 s_r
sr	9.53	
RSDr	2.74	← $s_r/\text{mean} (\%)$
H0r	0.63	←
R	48.64	← 2.8 s_R
SR	17.37	
RSDR	5.00	← $s_R/\text{mean} (\%)$
H0R	0.75	←

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Horwitz expressions



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- Horwitz equation

$$RSD_H (\%) = 2^{(1-0.5\log_{10}(c))}$$

$$s_H = 0.02 c^{0.8495}$$

- Horwitz ratio ("HorRat")

$$HO_R = RSD_R / RSD_H$$

- Repeatability

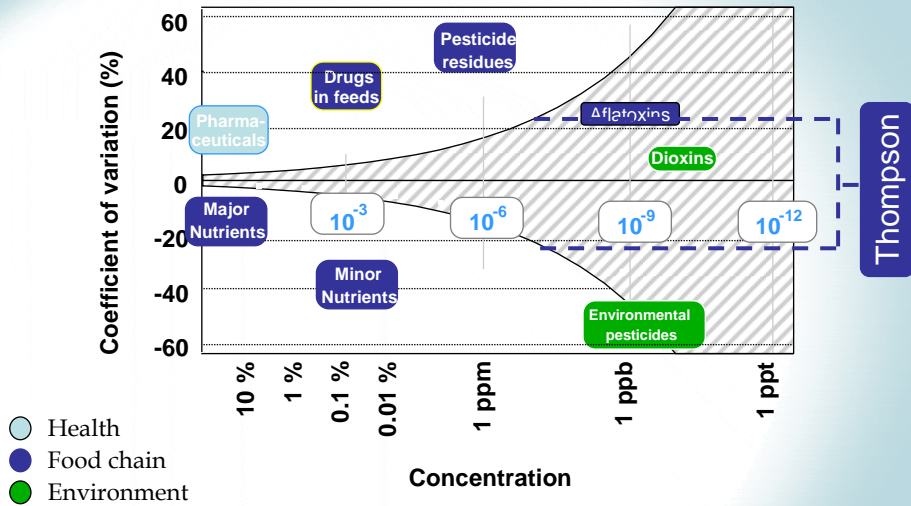
$$s_r \approx 0.66 s_R$$

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Horwitz predicted %RSD



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Results: Interpreted using Horwitz criteria



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mean	347.10
r	26.68
sr	9.53
RSDr	2.74
H0r	0.63
R	48.64
SR	17.37
RSDR	5.00
H0R	0.75

Less than 1
Precise
compared to
typical range

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What does collaborative study tell us?



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- The precision of results after removing outliers
 - Precision when no-one makes a mistake?
- **NOT** the dispersion of all results
- How precision changes with concentration
 - APPROXIMATELY across many methods
- How precision compares with past practice

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Does the Horwitz equation always work?



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- Thompson (Analyst, 2000, 125, 385–386)
 - FAPAS PT data
 - At low concentrations ($c < 0.12$ ppm), RSD stabilises at approximately 22%
 - At high concentrations ($c > 0.138$), s_R tends to follow $0.01c^{0.5}$
- Horwitz (various studies)
 - Pesticide data more precise than predicted
 - Mycotoxin analysis poorer than predicted and less dependent on concentration

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Collaborative study summary



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- IUPAC guidance provides a harmonised method for collaborative study
- Repeatability and reproducibility data can be compared among different methods of analysis
- CODEX often uses Horwitz predictions as a benchmark in addition to the IUPAC protocol

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Recovery

HARMONISED GUIDELINES FOR THE USE OF
RECOVERY INFORMATION IN ANALYTICAL
MEASUREMENT

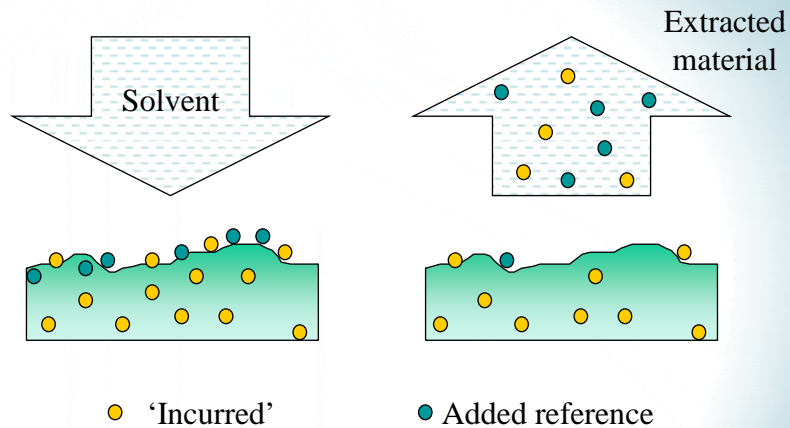
Pure & Appl. Chem., Vol. 71, No. 2, pp. 337–348, 1999

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Extraction processes



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The problem



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- Not all of the analyte is extracted
- Analysts measure recovery
 - $(\text{amount found})/(\text{amount added})$
- Added internal references may be differentially extracted
- Different experiments may give different measured recovery
- Some analysts correct for recovery, and others do not

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IUPAC Recovery protocol



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- Defines terms used
 - “Recovery”, “Surrogate recovery”, “Spiking”...
- Reviews methods of determination
 - Certified reference materials
 - Spiking (addition of analyte and re-extraction)
 - Isotope dilution methods (Isotopically different)
 - Surrogates (related but different material)
- Advises on correction for observed recovery

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Arguments for and against recovery correction



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Pro:

- The true concentration can be estimated only if significantly low recoveries of analyte are corrected.
- Uncorrected bias due to low recovery means that results will not be universally comparable and therefore unfit to support mutual recognition.
- Methods of correction are similar to other corrections used
- Additional uncertainty can be estimated.

Con:

- Estimated recoveries may be inaccurate
- Correction factors may vary among different
- matrices and for different concentrations of analyte.
- Estimated correction factors often have a high relative uncertainty.
- Relatively small deviations from unity in correction factors could arise by chance, making corrected results more variable.
- Some legislation is framed on the understanding that uncorrected results will be used.

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Recovery: Recommendations



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- Quantitative analytical results should be corrected for recovery unless there are specific reasons for not doing so. Reasons against include:
 - the analytical method is regarded as empirical (Type I)
 - a contractual or statutory limit has been established using uncorrected data
 - recoveries are known to be close to unity
- All data, when reported, should
 - show clearly whether or not a recovery correction has been applied
 - if a recovery correction has been applied, state the amount of the correction and the method by which it was derived
- Recovery values should always be established as part of method validation.
- When the use of a recovery factor is justified, the method of its estimation should be specified in the method protocol.
- IQ control charts for recovery should be established during method validation and used in all routine analysis.

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Proficiency testing

THE INTERNATIONAL HARMONIZED
PROTOCOL FOR THE PROFICIENCY TESTING
OF ANALYTICAL CHEMISTRY LABORATORIES

Pure Appl. Chem., Vol. 78, No. 1, pp. 145–196, 2006.

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Scope of 2006 IUPAC protocol



- Only chemical analysis.
- Only results obtained on a fitness-for-purpose basis (*i.e.*, suitable for z-scoring with a pre-set value of σ_p).
- Only results on an interval scale or a ratio scale.
- Primarily scientific aspects
 - minimal administrative details
 - no criteria for assessment or accreditation of laboratories or PT schemes.

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2006 HP Scoring



- Focuses on the z-score

$$z = (x - \hat{\mu}_{rob}) / \sigma_p \quad \text{where} \quad \sigma_p \equiv u_f$$

- 'Fit-for-purpose' scoring basis

$$\sigma_p \equiv u_{ffp}$$

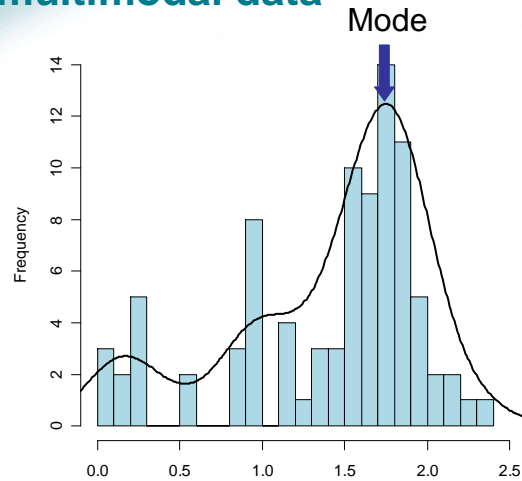
- Robustified against extreme values and informative about fitness for purpose.
- The protocol **is not restricted to consensus values**

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Adds method for handling multimodal data



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- Generate kernel density ($h=0.75\sigma_p$)
- Minor modes large
- Largest mode deemed 'correct'
- Use Kernel Density Mode

* If not, abandon scoring and investigate further

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Describes test material homogeneity testing procedure



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- Comminute and mix bulk material.
- Split into distribution units.
- Select $m > 10$ distribution units at random.
- Homogenise each one.
- Analyse 2 test portions from each in random order, with high precision, and conduct one-way ANOVA on results.
- Test for **sufficient** homogeneity
- Requires up to 1 within-unit outlier rejection

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2006 protocol: Fearn-Thompson test



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- Test $H_0 : \sigma_{sam}^2 < \sigma_{all}^2$ (usually $0.3\sigma_p$)

- Reject only when

$$s_{sam}^2 > \frac{\sigma_{all}^2 \chi_{m-1}^2}{m-1} + \frac{s_{an}^2 (F_{m-1,m} - 1)}{2}$$

- Less likely to reject at random

Ref: *Analyst*, 2001, **127**, 1359-1364.

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Summary of PT guidance



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- Emphasis on fitness-for-purpose in scoring
- Clear acceptance of continued use of consensus values
 - with advice on implementation
- Testing for statistical evidence of insufficient homogeneity instead of fixed value
- Does not recommend that the organiser provide scores based on participant uncertainties
 - DOES control uncertainties in assigned value
 - Provides methods for participants to assess their own uncertainty and fitness for purpose

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Internal Quality Control

HARMONIZED GUIDELINES FOR INTERNAL
QUALITY CONTROL IN ANALYTICAL CHEMISTRY
LABORATORIES

Pure & Appl. Chem., Vol. 67, No. 4, pp. 649-666, 1995

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AQC provisions

- Definitions (few and now well known)
- Quality Assurance Practices And Internal Quality Control
 - Quality assurance, Choice of analytical method, IQC and PT
- Internal Quality Control Procedures
 - General approach - **statistical control**
 - Internal quality control and fitness for purpose
 - The role of certified reference materials
 - Preparation of control material
 - ... etc

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Application of IQC guidance



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- Required by CODEX principles
- Internal QC monitored by accreditation bodies
- Accreditation bodies in food control laboratories should be checking consistency with IUPAC guidance
 - IUPAC provisions are consistent with ISO 17025 requirement

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Conclusions



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- Harmonised protocols are produced by external professional scientific bodies
- IUPAC guidance directly addresses the CODEX principles
- Adoption has allowed harmonisation of practice based on sound technical principles
- Further guidance proposed to clarify single-laboratory method validation

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