



VAM SEMINAR: CURRENT ISSUES IN METHOD VALIDATION

**WHERE WE ARE WITH RESPECT TO METHOD
REQUIREMENTS AND VALIDATION IN THE FOOD SECTOR**

Roger Wood, Food Standards Agency, UK
c/o Institute of Food Research,
Norwich Research Park, Colney, Norwich NR4 7UA



Will cover:

Quality Standards of Laboratories in the EU and in
Codex

Criteria for Acceptable Methods of Analysis

Measurement Uncertainty - The Relationship
Between the Analytical Result and the Specification
in Legislation

Sampling Uncertainty



**GUIDELINES FOR THE ASSESSMENT OF THE
COMPETENCE OF TESTING LABORATORIES
INVOLVED IN THE IMPORT AND EXPORT
CONTROL OF FOOD**

Adopted by the Commission at Step 8 in June 1997



SCOPE

1. These guidelines provide a framework for the implementation of quality assurance measures to ensure the competence of testing laboratories involved in the import and export control of foods.
2. These guidelines are intended to assist countries in the application of requirement for trade in foodstuffs in order to protect the consumers and to facilitate fair trade.



REQUIREMENTS

3. The following criteria shall be adopted by laboratories involved in the import and export control of foods:
 - Compliance with the general criteria for testing laboratories laid down in ISO/IEC Guide 25: 1990 "General requirements for the competence of calibration and testing laboratories"; [i.e. effectively accreditation].
 - Participation in appropriate proficiency testing schemes for food analysis which conform to the requirements laid down in "The International Harmonised Protocol for the Proficiency Testing of (Chemical) Analytical Laboratories", Pure and Applied Chemistry 65 (1993) 2132-2144; [already adopted for Codex purposes by the CAC at its 21st Session in July 1995]



- Whenever available, use methods of analysis which have been validated according to the principles laid down by the CAC, and
 - Use internal quality control procedures, such as those described in the "Harmonised Guidelines for Internal Quality Control in Analytical Chemistry Laboratories", Pure and Applied Chemistry 67 (1995) 649-666
4. The bodies assessing the laboratories referred to above should comply with the general criteria for laboratory accreditation, such as those laid down in the ISO/IEC Guide 58:1993: "Calibration and testing laboratory accreditation systems - General requirements for operation and recognition".



**REGULATION (EC) No 882/2004 OF THE EUROPEAN
PARLIAMENT AND OF THE COUNCIL
of 29 April 2004**

on official controls performed to ensure the verification
of compliance with feed and food law, animal health and
animal welfare rules.



Article 11

Methods of sampling and analysis

1. Sampling and analysis methods used in the context of
official controls shall comply with relevant Community
rules or,
 - (a) if no such rule exist, with internationally recognised
rules or protocols, for example those that the European
Committee for standardisation (CEN) has accepted or
those agreed in national legislation; or



- (b) in the absence of the above, with other
methods fit for the intended purpose or
developed in accordance with scientific
protocols



2. Where paragraph 1 does not apply, validation of methods of analysis may take place within a single laboratory according to an internationally accepted protocol.
3. Wherever possible methods of analysis shall be characterised by the appropriate criteria set out in Annex III.



4. The following implementing measures may be taken in accordance with the procedure referred to in Article 62(3):
 - (a) methods of sampling and analysis, including the confirmatory or reference methods to be used in the event of a dispute;



- (b) performance criteria, analysis parameters, measurement uncertainty and procedures for the validation of the methods referred to in (a); and
 - (c) rules on the interpretation of results



Article 12
Official laboratories

1. The competent authority shall designate laboratories that may carry out the analysis of samples taken during official controls.



2. However, competent authorities may only designate laboratories that operate and are assessed and accredited in accordance with the following European Standards:
 - EN ISO/IEC 17025 on "General requirements for the competence of testing and calibration laboratories";



- EN 45002 on "General criteria for the assessment of testing laboratories";
 - EN 45003 on "Calibration and testing laboratory accreditation system – General requirements for operation and recognition",
- taking into account criteria for different testing methods laid down in Community feed and food law.



3. The accreditation and assessment of testing laboratories referred to in paragraph 2 may relate to individual tests or groups of tests.
4. The competent authority may cancel the designation referred to in paragraph 1 when the conditions referred to in paragraph 2 are no longer fulfilled.



ANNEX III: CHARACTERISATION OF METHODS OF ANALYSIS

- Methods of analysis should be characterised by the following criteria:
- accuracy;
- applicability (matrix and concentration range);
- limit of detection;
- limit of determination;
- precision;



- repeatability;
- reproducibility;
- recovery;
- selectivity;
- sensitivity;
- linearity;
- measurement uncertainty;
- other criteria that may be selected as required.



- The precision values referred to in 1(e) shall either be obtained from a collaborative trial which has been conducted in accordance with an internationally recognised protocol on collaborative trials (eg ISO 5725:1994 or the IUPAC International Harmonised Protocol) or, where performance criteria for analytical methods have been established, be based on criteria compliance tests. The repeatability and reproducibility values shall be expressed in an internationally recognised form (eg the 95% confidence intervals as defined by ISO 5725:1994 or IUPAC). The results from the collaborative trial shall be published or freely available.



4. In situations where methods of analysis can only be validated within a single laboratory then they should be validated in accordance with eg IUPAC Harmonised Guidelines, or where performance criteria for analytical methods have been established, be based on criteria compliance tests.

IUPAC harmonised guidelines for single-laboratory validation of methods of analysis (Michael Thompson, Stephen L R Ellison and Roger Wood, Pure Appl. Chem., 2002, 74(5), 835-355) now accepted in EU and Codex.



WHAT IS THE CRITERIA APPROACH TO METHODS OF ANALYSIS?

WHY INTRODUCE IT?



Traditional Approach (prescribing a specific method of analysis) means:

- The analyst is denied freedom of choice and thus may be required to use an inappropriate method in some situations;
- The procedure inhibits the use of automation; and
- It is administratively difficult to change a method found to be unsatisfactory or inferior to another currently available.



Criteria Approach (prescribing performance characteristics) means:

- This “criteria” approach gives greater flexibility than the present procedure adopted by organisations such as Codex and the EU
- In some areas of food analysis there are many methods of analysis which are available, which meet requirements as regards method characteristics, but which are not considered by Codex or the EU because of time constraints.



EU Tin Performance Criteria*

A: Specific Parameters Approach

Specific methods for the determination of tin contents are not prescribed. Laboratories should use a validated method that fulfils the performance criteria indicated [in Table 3]. The validation should ideally include a certified reference material in the collaborative trial test materials.

[* from EU Tin Sampling and Analysis Directive]



Table 3: Performance criteria of methods for tin analyses

Parameter	Value/Comment
Applicability	Foods specified in Regulation (EC) No.../2003
Detection limit	No more than one 5 mg/kg
Limit of quantification	No more than one 10 mg/kg
Precision	HORRAT, or HORRAT _R values of less than 1.5 in the validation collaborative trial
Recovery	80% - 105%
Specificity	Free from matrix or spectral interferences



Horwitz Values

The precision values are calculated from the Horwitz equation, i.e.:

$$RSD_R = 2^{(1-0.5\log C)}$$

where:

RSD_R is the relative standard deviation calculated from results generated under reproducibility conditions [(s_R / \bar{x}) x 100]

C is the concentration ratio (i.e. 1 = 100g/100g, 0.001 = 1,000 mg/kg)



The mathematical form of the function

The functional form of the Horwitz relationship is more easily perceived if the traditional trumpet is replaced by a mathematically equivalent relationship between predicted reproducibility standard deviation σ_H and concentration c , namely

$$\sigma_H = 0.02c^{0.8495}$$



This is a generalised precision equation which has found to be independent of analyte and matrix but solely dependent on concentration for most "routine" methods of analysis.



HORRAT Values

Hor The HORRAT value for repeatability is the observed RSD_r divided by the RSD_r value estimated from the Horwitz equation using the assumption $r=0.66R$.

HoR The HORRAT value for reproducibility is the observed RSD_R value divided by the RSD_R value calculated from the Horwitz equation.



B: Uncertainty Function Approach

However, an uncertainty approach may also be used to assess the suitability of the method of analysis to be used by the laboratory. The laboratory may use a method which will produce results with a maximum standard uncertainty given by the following formula:

$$Uf = \sqrt{(CL/2)^2 + (0.1C)^2}$$

where: Uf is the maximum standard uncertainty
 CL is the detection limit of the method
 C is the concentration of interest

Results with an uncertainty less than that stipulated above will be produced by a method which is equivalent to one meeting the performance characteristics given in Table 3.



- The adoption of a more generalised approach would ensure that such methods are brought into the legislative system and does not disadvantage developments being undertaken elsewhere in the analytical community.



THE RELATIONSHIP BETWEEN THE FINAL ANALYTICAL RESULT AND THE SAMPLING, THE MEASUREMENT UNCERTAINTY AND THE RECOVERY FACTOR USED TO OBTAIN THAT RESULT



These factors affect the relationship between the final analytical result and the provisions in legislation

Decisions taken by those responsible for the enforcement of legislation directly affect decisions as to whether a lot is in compliance with that legislation.



SCIENTIFIC CO-OPERATION TASK 9.1

“PREPARATION OF A WORKING DOCUMENT IN SUPPORT OF THE UNIFORM INTERPRETATION OF LEGISLATIVE STANDARDS AND THE LABORATORY QUALITY STANDARDS PRESCRIBED UNDER DIRECTIVE 93/99/EEC”

was initiated to identify differences amongst Member States.

14 participated. Final Report is now published.



MAJOR ISSUES IDENTIFIED

The basic principles of the sampling procedures used by The Member States, the treatment of analytical variability (normally known as the measurement uncertainty) in the interpretation of an EU specification, and the use of recovery corrections when calculating and reporting analytical results.



The effect of different countries taking different approaches for each of the issues identified are described. It must be appreciated that there may be other enforcement issues which have a similar effect.

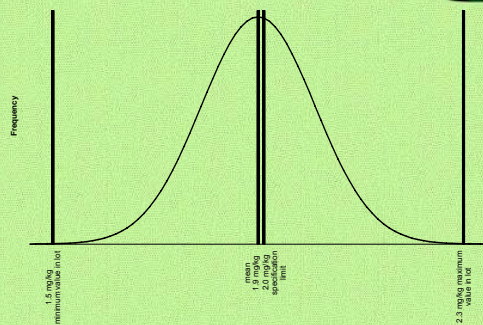


At the present time there is no common interpretation of analytical results across the EU in the food sector so significantly different decisions may be taken after analysis of the "same sample". Material for which there is a statutory limit of, say, $4\mu\text{g}/\text{kg}$ for a contaminant (e.g. total aflatoxins) may be interpreted as containing $3\mu\text{g}/\text{kg}$ on analysis in one country but $8\mu\text{g}/\text{kg}$ in another. This is because some countries correct analytical results for recovery, others do not; some countries use an "every-item-must-comply" sampling regime, others may use an "average of a lot" regime, some make an allowance for measurement uncertainty, others do not.



It is essential that interpretation of analytical results is similar if there is to be equivalence across the EU; without it there is no uniform interpretation of legislation.

Some of these points now explained.





Two countries may have different national rules for the interpretation of results from lots.

Country A requires: that each and every item in the lot meets the specification. In this example it means that all 1,000 units, if analysed separately, would have to be less than 2.0 mg/kg. Here a significant number of units are greater than 2.0 mg/kg so the lot would be deemed to be in non-compliance with the legal specification and so would be rejected, but Country B requires: that the mean value of the characteristic in the lot is to be less than the legal specification. In this case the mean value is 1.9 mg/kg so the lot would be deemed to be in compliance with the legal specification.



Consequence: the two countries A and B will make different judgements as to compliance with a legal specification on essentially the same lot. This is unacceptable and can only be avoided if the sampling procedures are elaborated at the same time as the commodity standard is elaborated. In addition it should also be noted that the number of units to be analysed also influences the decision on compliance.



REPORTING OF RESULTS WITH RESPECT TO THEIR MEASUREMENT UNCERTAINTY

All analytical results should be reported in the form " $a \pm b$ " where "a" is the best estimate of the true value of the concentration of the measurand (the analytical result) and "a-b" to "a+b" is the range within which the true value is estimated, with a given probability, to fall. The value of "b" is known as the "measurement uncertainty" and may be estimated by the analyst in a number of different ways.



The estimation of the value of “a” is dependent on: the accuracy of the method of analysis used, how well the analyst uses that method, i.e. whether the analytical system is “in control”.



The value of the measurement uncertainty “b” is dependent on:

- the inherent precision of the method of analysis used
- the number of analytical replicates that are carried out.

The more replicates the less the value of the measurement uncertainty.



REPORTING OF RESULTS BY FOOD CONTROL ANALYSTS

The procedure adopted by some food control analysts is to report samples as containing “not less than “a” – “b”” in situations where the statutory limit is a maximum permissible concentration. Thus, in any enforcement situation the maximum benefit is given to the food producer. This is consistent with the requirement to prove **beyond reasonable doubt** that a limit has been exceeded, if the case should come to Court. This does mean that the effective enforcement limit is, in such countries, not identical to the numerical value given in legislation.



Other food analysts may report the value “a” without taking into account any measurement uncertainty considerations.



Similar considerations identified in Codex Alimentations Commission

Section on
“The Use of Analytical Results: Sampling, Relationship Between the Analytical Results, the Measurement Uncertainty, Recovery Factors and the Provisions in Codex Standards”
to be included in Procedural Manual



ISSUES INVOLVED

There are a number of analytical and sampling considerations which prevent the uniform implementation of legislative standards. In particular, different approaches may be taken regarding sampling procedures, the use of measurement uncertainty and recovery corrections.

At present there is no official guidance on how to interpret analytical results across the Codex Community. Significantly different decisions may be taken after analysis of the “same sample”. For example some countries use an “every-item-must-comply” sampling regime, others use an “average of a lot” regime, some deduct the measurement uncertainty associated with the result, others do not, some countries correct analytical results for recovery, others do not. This interpretation may also be affected by the number of significant figures included in any commodity specification.



It is essential analytical results are interpreted in the same way if there is to be equivalence across the Codex Community.

It is stressed that this is not an analysis or sampling problem as such but an administrative problem which has been highlighted as the result of recent activities in the analytical sector, most notably the development of International Guidelines on the Use of Recovery Factors when Reporting Analytical Results and various Guides prepared dealing with Measurement Uncertainty.



RECOMMENDATIONS

It is recommended that when a Codex Commodity Committee discusses and agrees on a commodity specification and the analytical methods concerned, it states the following information in the Codex Standard:



1. Sampling Plans

The appropriate sampling plan to control conformity of products with the specification. This should state:

- whether the specification applies to every item in a lot, to the average in a lot or the proportion nonconforming;
- the appropriate acceptable quality level to be used;
- the acceptance conditions of a lot controlled, in relation to the qualitative/quantitative characteristic determined on the sample.



2. Measurement Uncertainty

That an allowance is to be made for the measurement uncertainty when deciding whether or not an analytical result falls within the specification. This requirement may not apply in situations when a direct health hazard is concerned, such as for food pathogens.



3. Recovery

Where relevant and appropriate the analytical results are to be reported on a recovery corrected basis and that the recovery should be quoted in any analytical report. Analytical results are to be expressed on a recovery corrected basis where appropriate and relevant, **and when corrected it has to be so stated.**
In all cases it has to be stated when the result is corrected for recovery.



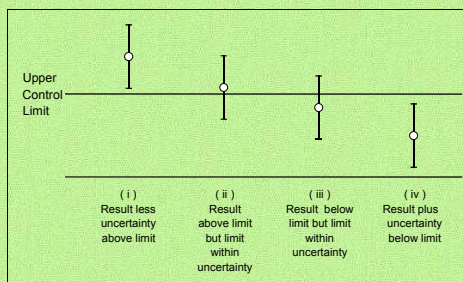
If a result has been corrected for recovery, the method by which the recovery was taken into account should be stated. The recovery rate is to be quoted **wherever** possible.

When laying down provisions for standards, it will be necessary to state whether the result obtained by a method used for analysis within conformity checks shall be expressed on an recovery-corrected basis or not.



4. Significant Figures

The units in which the results are to be expressed and the number of significant figures to be included in the reported result.





This means that the legal specification and enforcement limit are different.

This should be appreciated when specification is being set.



UNCERTAINTY OF SAMPLING

Draft EURACHEM Guide

Codex paper



UNCERTAINTY OF SAMPLING

Example 1 – Nitrate concentration in glasshouse lettuce

Mean = 4408 mg kg⁻¹

Analytical uncertainty: 168 mg kg⁻¹

Sampling uncertainty: 319 mg kg⁻¹

Measurement uncertainty: 361 mg kg⁻¹



**Example 2 – Infant wet meals (retail survey)
(Cadmium)**

Mean = 7.575 µg kg⁻¹

Analytical uncertainty: 1.100 µg kg⁻¹

Sampling uncertainty: 1.235 µg kg⁻¹

Measurement uncertainty: 1.654 µg kg⁻¹



Example 3 – Moisture in wholesale butter

Mean = 15.754 % (m/m)

Analytical uncertainty: 0.0421 % (m/m)

Sampling uncertainty: 0.1947 % (m/m)

Measurement uncertainty: 0.1992 % (m/m)



FUTURE

Codex looking at the dispute situation

IUPAC looking at qualitative analysis

Inter-Agency Members (ISO, CEN etc) looking at making more method validation information available.

National Reference Laboratories to be designated in a number of areas.



CONCLUSIONS

The analyst must:

- Work in a “quality environment”
- Decide what is an acceptable method.
- Assess individual performance characteristics.
- Consider the role of validation of methods within a single laboratory.



- Need to appreciate that sampling and its uncertainty will become a real issue

But what is the cost to him?

It will be essential for him to develop and appreciate statistical skills in order to be able to use this new-found freedom effectively.
