# Method validation and verification

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# Method Validation

- What is it?
- When is it required?
- Why is it necessary?
- What are required?
- How much is adequate?
- How should it be done?

# Method Validation – what are required

Validation of methods (cl. 5.4.5.3 )

The range and accuracy of the values obtainable from validated methods (e.g.

- Uncertainty of results
- Detection limit
- Selectivity
- Linearity
- Repeatability
- Reproducibility
- Robustness/cross-sensitivity

# HOKLAS SC No. 37 “Food” Test Category – Chemical Testing

- Being commented by AAB WP on Food (today is the last day for comment)
- Will be published very soon
- Contains criteria specific to food testing
- With a view to assist labs
- In particular, method validation and sample preparation

# HOKLAS SC No. 37 ***Section 5.3 Validation of methods***

- Screening methods: <5% false compliant rate at level of interest
- More stringent may be required for some applications

# Confirmation of identity

- Mass spectrometric detection

| Relative intensity (% of base peak) | EI-GC-MS (relative) | CI-GC-MS, GC-$MS^n$, LC-MS, LC-$MS^n$ |
|---|---|---|
| >50% | ±10% | ±20% |
| >20% to 50% | ±15% | ±25% |
| >10% to 20% | ±20% | ±30% |
| <10%% | ±50% | ±50% |

# Limit of Detection

$x_{LD} = 3s_O + x_{Bl}$

Where

$x_{LD}$ is the limit of detection

$s_O$ is the SD of the outlier free result of a matrix blank sample. should be based on at least 10 independent complete determinations of analytes concentration in a typical matrix blank or low-level material, with no censoring of zero or negative results.

$x_{Bl}$ is the mean concentration of the matrix blank

# Limit of Quantification

The limit of quantification represents a concentration of the determinand that can reasonably be determined with an acceptable level of accuracy. Usually it is arbitrarily taken as a fixed multiple of the detection limit.

# Limit of Quantification

*Estimated by:*

$$x_{\mathrm{LQ}} = 3x_{\mathrm{LD}}$$

The factor k=3 corresponds to a relative result uncertainty of approximately 33%.

# Limit of Quantification

AOAC

- smallest amount of analyte in a test sample that can be quantitatively determined with suitable precision and accuracy under previously established method conditions.
- It is often taken as the blank value plus 10 times the std dev.

## Reporting limits

- Set at a level at which quantitative results may be obtained with a specified degree of confidence
- Limits of detection and reporting shall be verified.

# Reporting limits

## *ISO/TS 13530:2009*

- RL is a specific concentration at or above the limit of quantification that is reported to the client with a certain degree of confidence. It is often defined on a project-specific basis. If the RL is set below the limit of quantification by the client, method modification is required.

## Reporting limits

***ISO/TS 13530:2009***

- For verification of limit of detection and limit of quantification, spiked blank matrix samples at these concentration levels and blank matrix samples shall be analysed in the same manner as real samples.

## Reporting limits

***ISO/TS 13530:2009***

- If the uncertainty of results for the samples spiked at the limit of quantification level is smaller than or equal to the relative precision corresponding to the factor *k*, the limit of quantification is verified.

# Reporting limits

***ISO/TS 13530:2009***

$$\frac{\Delta x}{x_{LQ}} = \frac{1}{k}$$

where

$\Delta x = t \,.s/\sqrt{n}$

*k* is the factor for calculating the limit of quantification as a multiple of detection, here: *k*=3

# Reporting limits

- ***In essence, the laboratory needs to provide supporting data that, at the reporting limit, the measurement uncertainty (including bias and precision) meets the acceptance criteria.***
- ***The direct method is to find the recovery and RSD at the reporting limit and compare them with the acceptance criteria.***

# Recommended LOD and LOQ

| | |
|---|---|
| LOD | For ML ≧ 0.1 mg/kg, LOD ≦ML X 1/10<br>For ML < 0.1 mg/kg, LOD ≦ML X 1/5 |
| LOQ | For ML ≧ 0.1 mg/kg, LOD ≦ML X 1/5<br>For ML < 0.1 mg/kg, LOD ≦ML X 2/5 |

ML= specified maximum and/or minimum level

# Recommended Recovery

| | Unit | Recovery (%) |
|---|---|---|
| Recovery (R) | 100%(100g/100g) | 98-102 |
| | ≧ 10% (10g/100g) | 98-102 |
| | ≧ 1% (1g/100g) | 97-103 |
| | ≧ 0.1% (1mg/g) | 95-105 |
| | 100mg/kg | 90-107 |
| | 10mg/kg | 80-110 |
| | 1mg/kg | 80-110 |
| | 100µg/kg | 80-110 |
| | 10µg/kg | 60-115 |
| | 1µg/kg | 40-120 |

# Precision

AOAC Official Methods of Analysis (2005)
Appendix E: Laboratory Quality Assurance
Common values of within lab precision

| Concentration | $RSD_r$ |
|---|---|
| 100% | 1 |
| 1% | 2 |
| 0.01% | 5 |
| 1ppm | 10 |
| 10ppb | 20 |

# Estimation of standard deviation by mean range

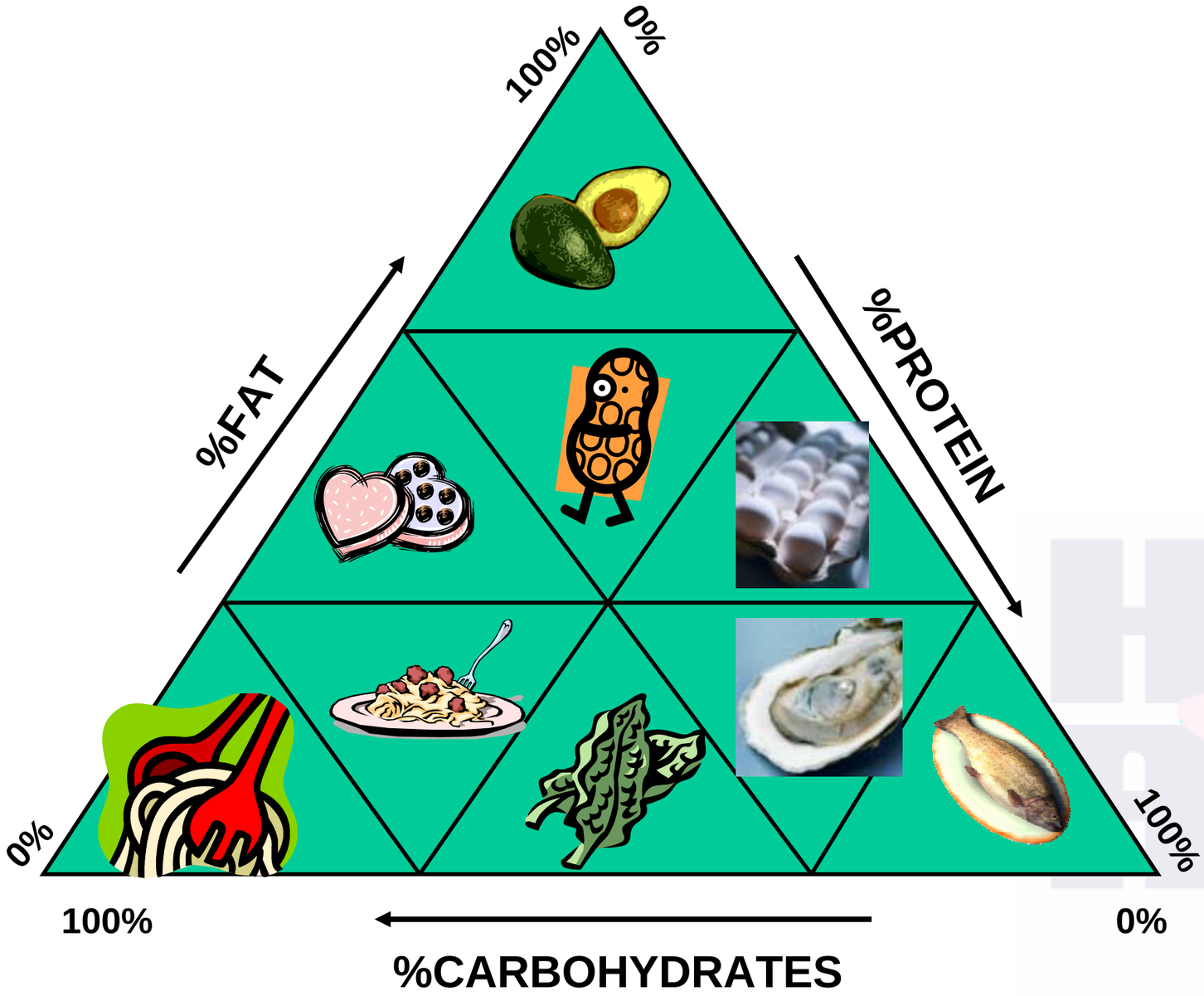
$$s = R/d_2$$

$d_2$ is 1.128 for duplicate

$R$ is the mean range of the duplicates

# Range of food matrices

- If a method is to be accredited under “general foodstuffs”, satisfactory validation data shall be obtained for at least five different food matrices (protein, carbohydrate, oil, dietary fibre and water) with at least three food types representative of each food matrix.




J. AOAC, 83 (2), 413 (2000)
"The Referee", AOAC Int'l, July 1993

**Annex 1.**

*Selection of representative matrices*[7]

**Vegetables, fruits and cereals**

| Commodity Categories | Commodities included in this category | Typical representative commodities |
|---|---|---|
| High water content | Pome fruit | Apples, pears |
| | Stone fruit | Apricots, cherries, peaches, |
| | Bulb vegetables | Bulb onion |
| | Fruiting vegetables/cucurbits | Tomatoes, peppers, cucumber, melon |
| | Brassica vegetables | Cauliflower, Brussels sprout, cabbage, broccoli |
| | Leafy vegetables and fresh herbs | Lettuce, spinach |
| | Stem and stalk vegetables | Leek, celery, asparagus |
| | Forage/fodder crops | Wheat and barley forage, alfalfa |
| | Fresh legume vegetables | Fresh peas with pods, petit pois, mange tout, broad bean, runner bean, dwarf French bean |
| | Leaves of root and tuber vegetables | |
| | Sugar cane | Sugar beet and fodder beet tops |
| | Fresh green tea | |
| | Fungi | |
| High oil content | Tree nuts | Walnut, hazelnut, chestnut |
| | Oil seeds | Oilseed rape, sunflower, cotton, soybean, peanut |
| | Oil | |
| | Olives | |
| | Avocados | |
| | Hops | |
| | Cacao beans | |
| | Coffee beans | |
| | Spices | |
| High protein content or high starch content | Dry legume vegetables/Pulses | Field bean, dried broad bean, dried haricot bean (yellow, white/navy, brown, speckled) |
| | Cereal grain | Wheat, rye, barley and oat grain; maize, rice |
| | Roots of root and tuber vegetables | Sugar beet and fodder beet roots, carrot |
| | Starchy root crops | Potato, sweet potato |
| | Bread | Wholemeal white, crackers |
| | Confectionary products | Cakes, biscuits, breakfast cereals |
| | pasta | Spaghetti, etc. |
| High acid content | Citrus fruit | Lemon, mandarin, tangerine, orange |
| | Berries | Strawberry, blueberry, |
| | Currants | |

[7] OECD Environment, Health and safety Publications, Series on Testing and Assessment , No72 and Series on Pesticides No. 39

| Commodity Categories | Commodities included in this category | Typical representative commodities |
|---|---|---|
| | Grapes<br>Kiwifruit<br>Pineapple<br>Rhubarb | raspberry<br>Black currant, red currant, white currant |
| "Difficult or unique commodities" * | | Hops<br>Fermented cacao, coffee and Tea<br>Spices |

*"Difficult commodities" should only be fully validated if they are frequently analysed. If they are only analysed occasionally, validation may be reduced to just checking the reporting levels using spiked blank extracts.

**Products of animal origin**

| Commodity Categories | Commodities included in this category | Typical representative commodities |
|---|---|---|
| Meat | Red meat<br>White meat<br>Fish<br>Offal *)<br>fat from meat | Beef, pork, lamb, game, horse<br>Chicken, duck, turkey<br>Cod, haddock, salmon, trout,<br>Liver, kidney |
| Milk and milk products | Milk<br>Cheese<br>Yogurt<br>Cream<br>Butter | Cow, goat and buffalo milk<br>Cow, goat cheese |
| Eggs | Eggs | Chicken, duck, quail, goose eggs |
| Honey | Honey | |

*) Offal (liver, kidney) should be validated separately, if necessary

## Range of food matrices

- The range of matrices shall also in line with those listed in relevant regulations. Due consideration shall also be taken for the food matrices with potential interferences, e.g. high chloride effect on the ICP-MS determination.

# Range of food matrices

- “General foodstuffs” ≠ all foods
- Test procedure shall document the food matrices used in the validation studies
- Labs need to assess and determine the applicability of their methods to the food samples received.

# Method verification

- Released early 2008

- http://www.aoac.org/al
  acc_guide_2008.pdf

# How should it be done?

The following are some examples of useful references

- *Harmonised guidelines for single-laboratory validation of method of analysis* (IUPAC Technical Report), 2002
- *How to meet ISO 17025 Requirements for Method Verification*, AOAC, 2007
- **ISO/TS 21748:2004** *Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty estimation*

# How should it be done?

CRMs

- *How many replicate results are required for proper comparison of the certified reference value and the actual analysis result?*

- *Is it necessary for the mean of test result for a CRM to lie within the uncertainty range of the certified value?*

- *Is a result outside the uncertainty range acceptable ?*

# How should it be done?

Useful references

- **ISO Guide 33: 2000**

  *Uses of certified reference materials*

# How should it be done?

comparison of results with other methods

(ISO 5725-6:1994 clause 8)

- comparison of precision of the two methods

$$F = \frac{S_r^2 B}{S_r^2 A}$$

where $S_r^2 B$= repeatability standard deviation of method B

$S_r^2 A$= repeatability standard deviation of method A

if $F<F(95\%)$ ($\nu_r A, \nu_r B$): statistically no significant difference

# How should it be done?

Comparison of results with another laboratory

(ISO 5725-6:1994 clause 7.2.4.3)

- the means of the two laboratories are compared

if $\left|\overline{y_1} - \overline{y_2}\right| \leq 2\sqrt{2}\sqrt{\delta_R^2 - \frac{\delta_r^2}{2}}$ ,

then statistically no significant difference

where $\delta_r^2$ = repeatability standard deviation

$\delta_R^2$ = reproducibility standard deviation

# How should it be done - verification

Worked examples of method verification are available from AOAC

- *Determination of Total, Saturated, and Monosaturated Fats In Foodstuffs by Hydrolytic Extraction and Gas Chromatographic Quantitation: Collaborative Study*
- *Determination of Low-Level Glucose and Fructose in Raw and Refined Crystalline Sugar by High-Performance Anion Exchange Chromatography*

# Common deficiencies

- Number of food matrices used not adequate
- Number of food types for each matrix not adequate
- Concentration levels used not adequate
- Reporting limits estimated but not verified
- Food samples chosen not representative of the food matrices

# Common deficiencies

- Confirmation of identity technique not available or adequate
- Validation/verification data analyses not done correctly
- Measurement uncertainty not available/not estimated correctly
- Equipment and/or test procedures not conforming to the test std requirements

# Common deficiencies

- Lack of or inappropriate sampling procedure
- QC plan acceptance limits not appropriate

# Thank you