

PROTOCOL

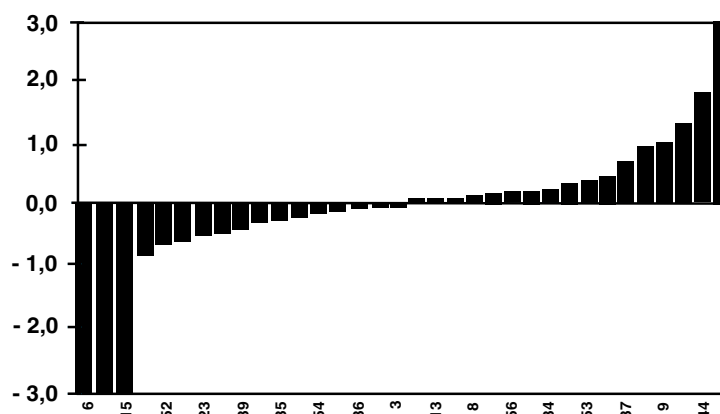
– Policy, Organisation and Statistics –

Food Chemistry

Nutritional Components of Food

Trace Elements in Food

Vitamins in Food



**LIVSMEDELS
VERKET**

NATIONAL FOOD
ADMINISTRATION, Sweden

First edition – September 2002
Second edition – December 2004
Third edition – September 2006
Fourth edition – August 2007
Fifth edition – June 2008
Sixth edition – June 2010

Publisher: Head of Chemistry Division 2

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1. Introduction

The quality of analytical data produced by laboratories involved in public analysis, is an important aspect in the general control of the quality of foods. The National Food Administration (NFA), as a regulatory authority, has an interest in promoting good performance of the analytical laboratories in the food area through the organisation of proficiency testing programmes.

The Swedish National Food Administration (NFA) started a Proficiency Testing (PT) programme for chemical food analysis in 1980, as a part of a quality assurance programme. The aim of the programme was to enhance the quality in major nutrient analysis of the Swedish laboratories. In 1997 a PT programme for trace elements in foodstuffs was initiated, primarily to fulfil the need in the Nordic countries. In 2003 a programme for vitamins in food was introduced. Today the Food Chemistry Programme has a worldwide participation.

The purpose of this protocol is to help the participants obtain an understanding of the general policy and practical aspects about organisation, requirements and statistical calculations of the different programmes. It will thus help the participating laboratories to take full advantage of their involvement in the programmes.

The Food Chemistry Programme is part of a wider PT-programme provided by the NFA (appendix 1).

2. Organisation

The Swedish National Food Administration is the central administrative authority for matters relating to food, including drinking water.

2.1 The programmes

The three chemistry programmes are:
Nutritional Components of Food (NUT)
Trace Elements in Foods (TEF)
Vitamins in Food (VIT)

The procedures, organisation and evaluation of the PT programmes have been elaborated on the basis of the established international criteria for interlaboratory studies. Special consideration has been given to the AOAC/ISO/ IUPAC Harmonised Protocol (1).

The chemistry programmes are accredited according to applicable parts of the current versions of ISO Guide 43, International Standard ISO/IEC 17025 and ILAC-G13 (2, 3, 4).

2.2 The Technical Advisory Group (TAG)

Members of the Technical Advisory Group (TAG) are, the head of Chemistry division 2, the programme coordinators, the division quality coordinator, the statistic coordinator and a secretary. External consultants may be called upon when required.

The TAG provides technical advice on the operation of the chemistry PT-programmes. It is also the task of the advisory group, in consultation with the coordinator, to plan and organise internal audits as required by the schedule. The TAG shall convene when required.

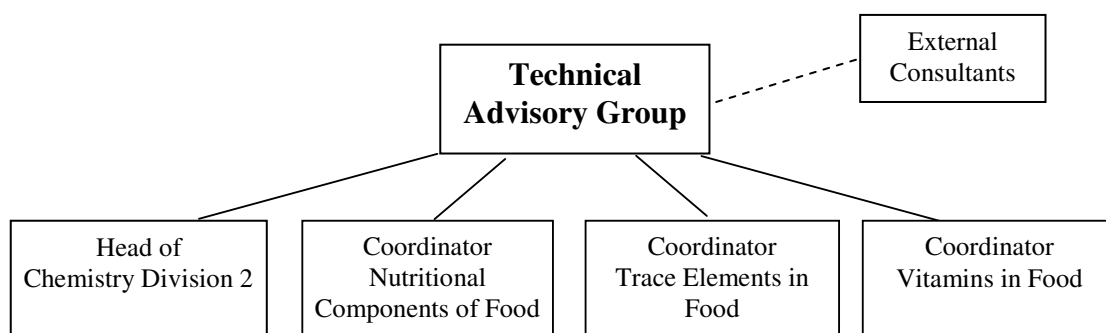


Fig.1 Organisation of the technical advisory group.

Coordinator, the person who is responsible for coordinating all of the activities involved in the operation of this each programme

2.3 Collaborators

The accredited laboratories, contracted to carry out the preparation and testing for homogeneity of test material, are referred to as collaborators.

3. The Food Chemistry Proficiency Testing Programmes

The coordinators will make every possible effort to keep the time schedule. However, if the planned test material is not ready according to the timetable (e.g. due to suspected inhomogeneity), the coordinator reserves the right to delay the round one month. The participants will be notified in advance.

3.1 Nutritional Components of Food

Two rounds are organised annually, one in March-April and one in September-October. In each round the participating laboratory receives one test material.

3.1.1 Test materials

Type of test materials

In order to simulate the measurement process as closely as possible, two matrix materials are used as test materials:

Dry powdered foodstuffs

A homogeneous mixture of some of the following ingredients: cereal flour (e.g. corn, wheat, rye, oats, etc) mixed with milk powder, vegetable oil, sugar, minerals and vitamins. Labelled **K(n)**.

Meat-based foodstuffs

A sample consisting of meat or meat products (e.g. pork, chicken, sausages, pâté, etc.) mixed with some other foods and/or additives. Labelled **K(n+1)**.

Note: n is the consecutive number of the test materials.

Test material preparation

The dry-powdered foodstuff is purchased in a local retail, blended mechanically and transferred by hand into the plastic containers.

The meat-based foodstuff is homogenised, transferred into a suitable container and autoclaved (+110°C, 10 minutes).

3.1.2 Analytes

The participant should analyse in one or both samples some of the following analytes: ash, moisture, nitrogen, fat, sodium, potassium, calcium, iron and phosphorus.

3.1.3 Time schedule

Round March-April

Sample distribution	First week of March
Deadline for report of results	First week of April
Distribution of final report for the round	Last week of April

Round September-October

Sample distribution	First week of Sept.
Deadline for report of results	First week of Oct.
Distribution of final report for the round	Last week of Oct.

3.2 Trace elements in Food

Two rounds are carried out each year. Each round contains one test material that may be analysed for 4-5 analytes.

3.2.1 Test materials

Type of test materials

The test materials consist of a freeze-dried powder or a preserved product on fresh weight, based on products of animal or vegetable origin.

Test material preparation

The test materials are procured from industry or retail, homogenised if necessary, transferred to plastic containers of suitable size and tested for homogeneity.

3.2.2 Analytes

Lead and cadmium are the core metals, which are determined in each round. In addition, two to three other elements that are decided for each round may be determined (selected from a list of elements that reflects the current interest)

3.2.3 Time schedule

After shipment of the test material, the participant has two months in which to return the results. After the deadline for reporting of results the report will be issued within one month.

3.3 Vitamins

One round is carried out once a year. Each round contains one or two test materials that may be analysed for 4-6 analytes.

3.3.1 Test materials

Type of test materials

The test materials consist of a freeze-dried powder or a preserved product on fresh weight, based on products of animal or vegetable origin. They can either be fortified or have a natural content of vitamins.

Test material preparation

The test materials are procured from industry or retail and tested for homogeneity. If necessary the test material is homogenised and transferred to plastic containers of suitable size.

3.3.2 Analytes

Both fat-soluble and water-soluble vitamins are analysed in each round. The fat-soluble vitamins alpha-tocopherol and/or retinol will be included in each round, complemented by e.g. vitamin D₃, vitamin K₁, and beta-carotene.

Folate and/or ascorbic acid will be part of each round along with some of the other water-soluble vitamins niacin, thiamine, riboflavin, vitamin B₆, vitamin B₁₂, biotin or pantothenic acid.

3.3.3 Time schedule

After shipment of the test material the participant has two months in which to return the results. After the deadline for reporting of results the report will be issued within one month.

4. Test for homogeneity and stability

4.1 Test for homogeneity

The homogeneity of each test material is assessed by duplicate analyses of ten units selected at random. The test materials are analysed for several constituents chosen on the basis of established chemical or physical criteria. The results are tested for outlying pairs by the Cochran test. Pairs detected as outliers at the 99 % significance level may be excluded before further statistical treatment.

The sampling variance is calculated using one-way analysis of variance. The sampling variance is compared to a critical value. The critical value is based on the target standard deviation, analytical standard deviation and table values. If the sampling variance doesn't exceed the critical value, the material can be considered sufficiently homogeneous. An example is given in Appendix 2.

Homogeneity can only be guaranteed down to a certain minimum sample weight, usually 0.2 – 10.0 gram depending on the method.

The results of the homogeneity tests are included, and if necessary commented on, in the report.

4.2 Stability of the test materials

The test materials are usually selected among materials, for which stability is not a problem during the short period of time from the homogeneity testing to the analysis by the participants. If a test material is considered to have a stability problem, the participants are notified.

5. Transport

The test materials are shipped in a package that ensures the integrity of the samples. If the test material is temperature sensitive, it will be dispatched in an appropriate refrigerated condition.

6. Instructions to the participants

The participants are asked to report one result with three significant figures for each analyte. Results reported as “not detected” or “less than” will not be included in the calculation of the z-score.

The participants will be informed if the results should be reported on a dry or a wet weight basis. Reporting the measurement uncertainty is presently not requested.

7. Analytical methods

Participants may use the analytical methods of their choice. However, it is greatly recommended to use the laboratory routine methods.

Likewise, it is recommended to treat the test material in the same manner as routine samples.

8. Statistics

8.1 Assigned value

The assigned value (x_a) is the best available estimate of the measurand (the “true value” of the concentration of the analyte). The consensus value of the laboratories results may be established in two ways, using the mode of kernel density or using the robust mean after removal of non-valid values. The procedure described below will generally be used, in some cases other statistical methods may be used if considered necessary. The procedure used will be described in each specific report. A special case is when a certified reference material is used. Then the consensus of the laboratories may not be used, see 8.1.3.

8.1.1 Step 1: Kernel density

Kernel density plots can be used to estimate the mode of a distribution. It is especially useful for strongly tailing distributions or in cases of multi modality. The Kernel density is a computer-intensive method, which involves smoothing the data while retaining the overall

structure. The kernel plot and the graph of the overall dispersion of the results are presented to facilitate the visual inspection of the distribution of the results, and do not necessarily contain all results. A main advantage is that the plot gives a graphical view of the results that can be easily understood by the participants.

The kernel density plot is examined and classified according to the different cases below:

Case A. If the kernel density plot shows a near normal distribution the robust mean is used, see point 8.1.2.

Case B. If the distribution is tailing the mode of the kernel density is used as assigned value.

Case C. If the distribution shows several modes great consideration should be given to how to set the assigned value. In some cases there may be arguments in favour of choosing one of the modes. In other cases it may be inappropriate to set an assigned value at all. In these cases consultation with the technical advisory group can be needed.

8.1.2 Step 2: Use of robust statistics (Case A in 8.1.1)

The robust statistic approach changes the emphasis from outlier detection and rejection, to outlier accommodation. It is a convenient modern method of handling results when they are expected to follow a near-normal distribution and it is suspected that they include a small proportion of outliers. Clearly outlying results may be excluded before calculation. An exclusion of results outside of e.g. $\pm 50\%$ of the median can normally be used. (1).

8.1.3 Use of certified reference materials (CRM)

If a CRM is used as test material it will be treated in such a way that the participants are not able to recognise it as a CRM. The mean of the certificate may then be used as the assigned value.

8.1.4 Estimation of uncertainty of the assigned value

The uncertainty of the assigned value can be estimated by the robust standard deviation after exclusion of clearly outlying results. The uncertainty, $u(x_a)$, is calculated as:

$$u(x_a) = \frac{\hat{\sigma}_{rob}}{\sqrt{n}}$$

8.2 Standard deviation for proficiency assessment

The performance criteria, (σ_p), for standard deviation for the proficiency assessment is a fitness for purpose criterion defining the acceptable variation between laboratories. Three possible approaches are considered for determining this value.

8.2.1 Performance criteria predicted by the Horwitz equation.

The modified Horwitz equation has the following mathematical form:

$$\begin{aligned} \sigma_p &= 0.02c^{0.8495} && \text{if } 1.2 \times 10^{-7} \leq c \leq 0.138 \\ \sigma_p &= 0.01c^{0.5} && \text{if } c > 0.138 \\ \sigma_p &= 0.22c && \text{if } c < 1.2 \times 10^{-7} \end{aligned}$$

Where c is concentration and σ_p the predicted reproducibility standard deviation. Both c and σ_p are mass ratios, (e.g. $\% = 10^{-2}$, $\text{mg/kg} = 10^{-6}$).

8.2.2 Performance criteria based on a value from a validated method

The standard deviation of a validated method that is relevant for a particular round and/or analyte.

8.2.3 Performance criteria based on fitness for purpose

In some cases other criteria may be used. The basis for that could be legislation.

8.3 Treatment of outliers

8.3.1 Outlier elimination

Clearly non-valid results are excluded before calculation of the robust mean. An exclusion of results outside of e.g. $\pm 50\%$ of the median can normally be used (1). The outlying results are marked in the report.

8.4 z-scores

The approach used to evaluate the individual analyte from one laboratory is by transforming the results reported to the corresponding value of z in the *standard normal distribution* or *unit normal distribution*, as it is sometimes called. The standard normal distribution has a mean of 0 and a standard deviation of 1.

Thus, the z-score is a measurement of the deviation (in standard deviation units) of the result from the assigned value. The z-score is given by the following equation:

$$Z = \frac{x - x_a}{\sigma_p}$$

Where:

x = the reported value of analyte concentration in the test material

x_a = the mean of all values, outliers excluded

σ_p = the standard deviation for proficiency assessment

8.5 Interpretation of z-scores

The z-score of results is interpreted as:

- $|z| \leq 2$ the result is considered satisfactory (approximately 95% of results reported)
- $2 < |z| < 3$ the result is considered questionable (will occur in about 5% of results reported)
- $|z| \geq 3$ the result is considered unsatisfactory (approximately 0.3% of results reported)

Also, $2 < |z| < 3$ can be considered as a warning value, i.e. a revision of the result is desirable. $|z| > 3$ should be considered as an action value, i.e. a revision of the result is necessary.

Since the z-score is standardised, it can be compared independently of the test-material, the method of analysis, and the concentration of the analyte. A graphical presentation of the z-scores is shown in Appendix 3.

9. Reporting of results and confidentiality

The programme secretary sends a report to the participants with information about the results, analytical methods used and performance of each laboratory. Code numbers are assigned to the participants in each round.

The Swedish Act of Secrecy guarantees the anonymity of the participants.

10. Survey of earlier performances

When a laboratory has been participating regularly, a summary of the z-scores of consecutive rounds may be added to the report. This allows the participating laboratory to compare its performance at a particular time with its performance in the past.

Up to now, there is no common criterion about the recommended frequency and regularity of participation in proficiency testing programmes. However, the TAG considers that three consecutive good performances can be used to demonstrate convincing competence in performing the analysis.

On the other hand, it is not clear that three poor performances demonstrate incompetence. However, such unsatisfactory performance should lead to adequate corrective action of the participant

The laboratories are identified by the codes assigned to the reported rounds.

11. Complaints and participant's feed-back

A record is kept over complaints and the corrective actions taken by the coordinator. Complaints are treated individually if only the performance of a particular laboratory has been affected, but in the case that a complaint casts doubt upon the correctness of the total operation of the programme (e.g. inhomogeneity or instability of the test material), this will be notified to all participants.

Comments on a participant's performance and technical advice on the interpretation of the statistical analysis may be provided by the coordinator upon request to participants. Suggestions and comments that could help improving the quality of the programmes are highly appreciated by the coordinators.

12. Archiving of results

All forms and results received by the organiser will be archived for a minimum of four years after the completion of the proficiency test round.

13. Cost of participation

Participants will be invoiced for each round independently (no refunds will be made once test materials are distributed). The participant can also be invoiced for its participation in the annual programme.

The aim is to keep the cost for the participants as low as reasonable.

14. Surplus test materials

Although a test material of a proficiency testing used as secondary reference material has a lower metrological level than Certified Reference Materials, its use for internal quality control purpose is highly recommended.

After a completed round the remaining test materials are at the participants' disposal for a small fee.

15. The Protocol

This protocol will be periodically reviewed and if necessary reedited to ensure continuing compliance with the new applicable international requirements. It is distributed free of charge to all participants.

16. References

1. The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories (2006). *Pure Appl. Chem.*, 78, 145-196.
2. ISO Guide 43-1. Proficiency testing by interlaboratory comparison. Part 1: Development and operation of laboratory proficiency testing schemes.
3. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories.
4. ILAC-G13. Guidelines for the requirements for the competence of providers of Proficiency Testing Schemes.

Appendix 1. Proficiency testing programmes provided by the NFA

Programme Frequency per year	Samples per round / Matrix	Analyses / Components	Coordinator
Food chemistry			
Nutritional components 2 rounds	1 Meat-based or cereal-based	Ash, water, nitrogen, fat, sodium, potassium, calcium, iron, phosphorus	Leonardo Merino leme@slv.se
Trace elements 2 rounds	1 Food	Lead, cadmium in each round, plus 2-3 other metals (varying between rounds)	Lars Jorhem lajo@slv.se
Vitamins 1 round	1-2 Food	Fat- and water-soluble vitamins	Anders Staffas anst@slv.se
Microbiology			
Drinking water 2 rounds	3-4 Freeze-dried material, to be dissolved	Cultivable micro organisms (total count) Faecal indicator bacteria Coliform bacteria <i>Pseudomonas aeruginosa</i> Micro fungi	Tommy Slapokas tosl@slv.se
Food 3 rounds	3-4 Freeze-dried material, to be dissolved	Total count of aerobic micro organisms Indicators among Entero bacteriaceae Food borne pathogenic bacteria Other groups of micro organisms	Christina Normark chrn@slv.se

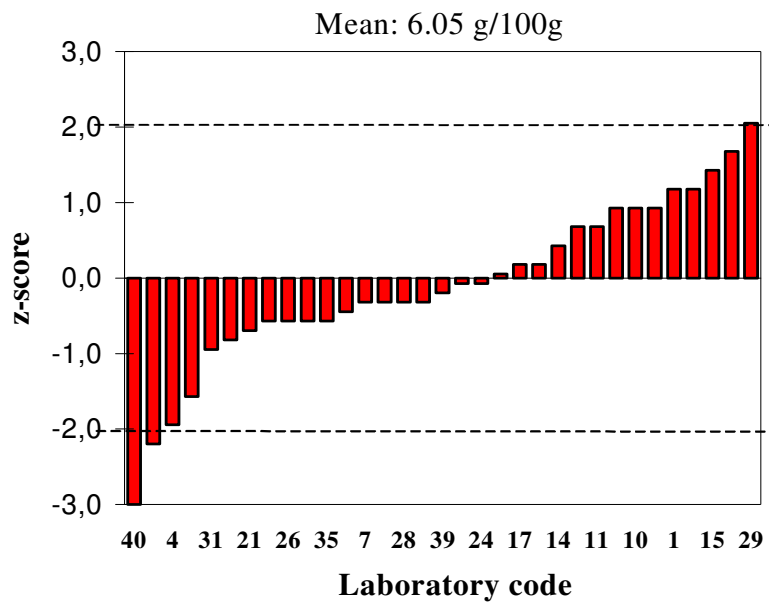
Appendix 2. Example of homogeneity testing of test materials

Homogeneity test of vitamin B₁₂ in fish pâté

Unit ug/100g							
Bottle	A	B		D ²	OL	S	Mean
1	0.565	0.506		0.00348		1.071	0.5355
2	0.550	0.547		0.00001		1.097	0.5485
3	0.581	0.596		0.00023		1.177	0.5885
4	0.567	0.529		0.00144		1.096	0.5480
5	0.548	0.545		0.00001		1.093	0.5465
6	0.497	0.578		0.00656		1.075	0.5375
7	0.516	0.470		0.00212		0.986	0.4930
8	0.538	0.542		0.00002		1.080	0.5400
9	0.600	0.627		0.00073		1.227	0.6135
10	0.507	0.540		0.00109		1.047	0.5235
Mean, n	0.547	20	SSD	0.0157	Vs	0.004393656	
S _{an}	0.028		D ² max	0.0066	MSB	0.002196828	
S _{sam2}	0.0007		C	41.85	MSW	0.00078395	
S _{all2}	0.0033		C critical	65.5	m	10	
Critical	0.0070		Significance	No	F1	1.88	
Suff. Homog.	Yes		Horw unit	1.00E-08	F2	1.01	
Target s	0.1918						
Ratio S _{an} ² /σ ²	0.0213						
<hr/>							
D ²	square of difference (A and B) SSD						
OL	Cochran outlier			D ² max			
S	standard deviation A and B			C			
Mean	mean of A and B			C critical			
Mean, n	total mean			Significance			
S _{an}	analytical standard deviation			Horw unit			
S _{sam2}	sampling standard deviation			Vs			
S _{all} ²	square of standard deviation			MSB			
Critical	critical value for sampling variation			MSW			
Suff. Homog.	evaluation of sampling variation			m			
Target s	standard deviation according to Horwitz			F1			
Ratio S _{an} ² /σ ²				F2			

Appendix 3. An example of a bar-chart of z-score for ash

Fig 1. z-Score for ash in mix-powder food (K72)



Proficiency Testing Programme



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