

MAFF VALIDATED METHODS FOR THE ANALYSIS OF FOODSTUFFS¹

No. V0

INTRODUCTION, GENERAL CONSIDERATIONS AND ANALYTICAL QUALITY CONTROL

Introduction

The European Community (EC) is laying down rules for food analysis which will aid the operation of the Single Market. Firm guidelines have been adopted which define the criteria to be considered when a method of analysis is to be included in a foodstuffs Directive⁽¹⁾. These criteria include specificity, accuracy, precision, sensitivity, practicability and applicability; they are similar to those required by the Codex Alimentarius Commission⁽²⁾. In particular, precision parameters such as repeatability (r) and reproducibility (R) are required, and these must be determined by collaborative trial. General agreement on many aspects of the conduct of such trials has now been reached as a result of the development of the collaborative trial protocol of the International Union of Pure and Applied Chemistry (IUPAC)⁽³⁾; the way now lies open towards recognition of analytical protocols and hence one element in the acceptance of analytical results across the EC.

Other elements include demonstration of laboratory quality standards; the availability of accepted methods of analysis will not diminish the need for individual laboratories to demonstrate their competence on a continuing basis via accreditation and participation in proficiency testing schemes.

Before a food analysis procedure can be acceptable for legislative purposes, an appropriate method must be selected, its protocol standardised and its performance characteristics established. In order to promote harmonisation, the Ministry of Agriculture, Fisheries and Food (MAFF) has developed a collaborative trial programme to validate methods of analysis for food. The reports of these collaborative trials are normally published in the scientific literature; in addition methods successfully validated will be published within the Journal of the Association of Public Analysts.

This Series may also include methods of particular interest which have been validated by a collaborative trial not carried out under the direct auspices of the MAFF collaborative trial programme.

The performance characteristics of the methods, and guidance as to their interpretation, are presented in the validated methods series separately

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from the protocol text and in the form recommended by IUPAC⁽⁴⁾. General procedural considerations and principles of Analytical Quality Control are given in Protocol V0 of the series and also the introductory booklet, and apply to all methods: specific applications are covered individually with each method.

Although methods validated in this Series will not have any specific legal status in food law enforcement, their reliability and authority will make them invaluable to both the food industry and to enforcement authorities.

In addition, a further series of booklets is being prepared, "Statutory Methods" (S1, S2, etc.), which will give the principle and references for those methods of analysis and examination which are already part of UK legislation for foodstuffs.

General Considerations

1 Preparation of the Analysis Sample

1.1 The mass of the sample presented to the laboratory for analysis shall be sufficient to enable all the determinations required of the sample to be carried out.

1.2 Mixing

The sample for analysis shall always be mixed thoroughly before any test portion is weighed out. Samples in powder or paste form shall be removed from the container, any lumps broken down, the sample mixed in an appropriate manner and placed in a suitable container. Samples in liquid form shall be mixed by stirring.

1.3 Containers

The sample shall always be kept in an airtight and moisture-tight container, at a temperature which ensures that no change takes place which would affect the result of the analysis.

2 Reagents

2.1 Water

Wherever mention is made of water for solution, dilution or washing purposes, distilled water, or demineralised water of at least equivalent purity, shall be used.

2.2 Solvent

Wherever reference is made to "solution" or "dilution" without further indication, "solution in water" or "dilution with water" is meant.

2.3 Chemicals

All chemicals shall be of recognised analytical reagent grade quality except where otherwise specified.

3 Equipment

The lists of apparatus contain only those items with a specialised use and items with a particular specification. "Analytical balance" means a balance capable of weighing to a precision of at least 0.1 mg.

4 Expression of Results

The result stated in the analytical report shall be the mean value obtained from at least two determinations, the repeatability of which is satisfactory. It shall not contain more significant figures than are justified by the precision of the method of analysis used. Except where otherwise specified, the result shall be calculated as a percentage by mass of the sample.

5 Test Report

The test report shall identify the method of analysis used (with references where appropriate) as well as the results obtained. In addition, it shall mention all details of procedure, not specified in the method of analysis, or which are optional, as well as any circumstances that may have influenced the results obtained.

The test report shall give all the information necessary for the complete identification of the sample.

ANALYTICAL QUALITY CONTROL

GENERAL PRINCIPLES

1 Repeatability

When a check on the precision of analysis within a laboratory is required, two test results for each sample must be obtained under conditions of repeatability, i.e. conditions where independent observed values or test results are obtained with the same method on identical test material, in the same laboratory by the same operator using the same equipment, within the shortest feasible interval of time. Each protocol gives observed values of repeatability, r , for the method it describes, and suggests a practicable target for the repeatability, r .

The term 'single test result' shall be the value obtained when the standardised test method is applied fully and once to a single sample.

2 Acceptability of Test Results

When two test results are obtained under conditions of repeatability, the final quoted result is their mean (e.g. arithmetic for chemical analysis, geometric for food examination procedures), provided that the conditions for acceptable repeatability are met. If this is not the case, the cause

should be investigated; if there is no obvious cause, further results must be obtained until the overall repeatability becomes acceptable.

2.1 Procedure for assessing acceptability

2.1.1 If the absolute difference between two test results x_1 and x_2 is less than r , the precision is acceptable, and the quoted result is their mean. If not, the procedure is continued (2.1.2).

2.1.2 A third result x_3 is obtained. If the absolute difference between the highest and lowest values of x is less than $1.2r$, the precision is acceptable, and the quoted result is the mean of all three values of x . If not, the procedure is continued (2.1.3).

2.1.3 A fourth result x_4 is obtained. If the absolute difference between the highest and lowest values of x is less than $1.3r$, the precision is acceptable, and the quoted result is the mean of all four values of x . If not, the precision is questionable but the quoted result should be the median of the four values of x , i.e. the mean of the middle two values.

3 Reproducibility

When a check on the precision of analysis in two or more laboratories is required, two test results for each sample must be obtained under conditions of reproducibility, i.e. conditions where observed values or test results are obtained with the same method on identical test material in different laboratories with different operators using different equipment. Each protocol gives observed values of R for the method it describes, and suggests a practicable target for the reproducibility, R . It should be noted that the values of reproducibility (R) apply in the particular case where a single test result from each laboratory is compared. If it is desired to compare final quoted test results, which are the mean results from two or more "single test results" carried out by each laboratory on identical laboratory samples, the appropriate values for R may be calculated as outlined in ISO 5725 Part 6 Section 2.

4 Trueness (Bias)

When a check on the trueness of the test results is required, one or more reference materials (certified or prepared in-house), of similar matrix and analyte concentration to that of the test samples, must be analysed in parallel with the test samples. A decision is then made as to whether the difference, if any, between the expected value(s) for the reference material(s) and that obtained by analysis of the latter is statistically significant. With certified reference materials, confidence limits are cited; prepared in-house reference materials need plotted data such as those on control charts. In the latter case, a test result obtained for the prepared reference material which falls within ± 2 units of the standard deviation (S) of the accepted mean value for the analyte of interest may be considered acceptable. Here, the accepted mean value and standard deviation would be calculated from the results obtained from not less than

ten analyses of the prepared in-house reference material. If the difference between the test result and the mean value exceeds $\pm 3S$, the test result must be rejected.

In appropriate cases, a check on consistent method and individual laboratory bias may be made by spiking and determining the recovery of the added analyte.

5 Limit of Detection

Recommendations have been made by various organisations on the determination of detection limits (e.g. by the Analytical Methods Committee of the Royal Society of Chemistry)⁽⁵⁾. In this series of papers the lower limit of precise determination, or of absolute detection, can only be estimated by analysing samples with known low levels of analyte. In the absence of such data, the limiting concentration has been taken to be the value of the repeatability, r , extrapolated to such levels.

6 Interpretation of Observed Levels

Each protocol tabulates the statistical parameters observed in the collaborative trial of the method: these include the mean observed level of analyte, the repeatability (r), the reproducibility (R), the corresponding standard deviations S_r and S_R , and the corresponding relative standard deviations (coefficients of variation), RSD. The following relationships are employed: $R = 2.8S_R$; $r = 2.8S_r$; $RSD = 100S/\text{mean}$.

7 COSHH

No information has been given on COSHH (Control of Substances Hazardous to Health) aspects of the methods. Analysts must make the appropriate hazard and risk assessments required by COSHH before using the methods in their own laboratories.

8 Key to the Symbols and Definitions of the Statistical Parameters used in the Protocols

Symbol	Definition
$\bar{x}$	Overall mean value
S_r	The standard deviation of repeatability
RSD_r	The relative standard deviation of repeatability, expressed as a percentage of the mean (coefficient of variance of repeatability CV_r)
r	Repeatability
S_R	The standard deviation of reproducibility
RSD_R	The relative standard deviation of reproducibility, expressed as a percentage of the mean (coefficient of variance of reproducibility CV_R)
R	Reproducibility

FOOD SAFETY DIRECTORATE

References

1. Council Directive 85/591/EEC of 20th December, 1985, O. J. L 372, 31.12.85
2. Codex Alimentarius Commission Procedural Manual, Seventh Edition
3. "Protocol for the Design, Conduct and Interpretation of Collaborative Studies", Pure and Applied Chemistry, 1988, 60(6), 855-664
4. "Harmonized Protocols for the Adoption of Standardized Analytical Methods and for the Presentation of their Performance Characteristics", Pure and Applied Chemistry, 1990, 62(1), 149-162
5. "Recommendations for the Definition, Estimation and Use of the Detection Limit", Analytical Methods Committee, Royal Society of Chemistry, The Analyst, 1987, 112(2), 199