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CURRENT PROBLEMS IN METHOD VALIDATION AND DATA QUALITY IN THE NOVEL FOODS AREA

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The European Union and Codex Alimentarius Commission quality criteria for methods of analysis in the food laboratory are described, as is their application in the novel foods area, where the determination of GMOs is taken to be representative of the sector. Particular aspects of the introduction of the criteria approach and of measurement uncertainty requirements are discussed, and how the latter considerations demonstrate the large variability in precision that should be expected from methods of analysis in the novel foods area. Results that reinforce these considerations are presented.

INTRODUCTION

It is important that in the novel foods area the same quality criteria for methods of analysis are applied as for food analysis generally. In order to achieve this, it is important to appreciate the general requirements for food analysis. Here a validated methods of analysis may be described as one for which we know the applicability, the reliability and the performance characteristics. These will be described in greater detail below.

In addition we also need assurance that the laboratory is proficient in the use of any method of analysis. This aspect is becoming of increasing importance given the requirements for food control laboratories in the European Union and for laboratories concerned with the import/export of foodstuffs within the Codex Alimentarius system. These are also described in greater detail below.

Official Control of Foodstuffs Directive (OCF) 1989

The Council Directive on the Official Control of Foodstuffs (OCF) which was adopted by the Community in 1989 [Council Directive 89/397/EEC] looked forward to the establishment of laboratory quality standards, by stating that "In order to ensure that the application of this Directive is uniform throughout the Member States, the Commission shall, within one year of its adoption, make a report to the European Parliament and to the Council on the possibility of establishing Community quality standards for all laboratories involved in inspection and sampling under this Directive" (Article 13).

Additional Measures concerning the Official Control of Foodstuffs (AMFC) Directive 1993

Following that the Commission, in September 1990, produced a Report which recommended establishing Community quality standards for all laboratories involved in inspection and sampling under the OCF Directive. Proposals on this were adopted by the Community in the 1993 Directive on Additional Measures Concerning the Official Control of Foodstuffs (AMFC) [EU Council Directive 93/99/EEC].

In Article 3 of the AMFC Directive it states:

"1. Member States shall take all measures necessary to ensure that the laboratories referred to in Article 7 of Directive 89/397/EEC [EU Council Directive 89/397/EEC] comply with the general criteria for the operation of testing laboratories laid down in European standard EN 45001 [ECS, 1989a] supplemented by Standard Operating Procedures and the random audit of their compliance by quality assurance personnel, in accordance with the OECD (Organisation of Economic Co-operation and Development) principles Nos. 2 and 7 of good laboratory practice as set out in Section II of Annex 2 of the Decision of the Council of the OECD of 12 Mar 1981 concerning the mutual acceptance of data in the assessment of chemicals [OECD, 1981].

2. In assessing the laboratories referred to in Article 7 of Directive 89/397/EEC Member States shall: (a) apply the criteria laid down in European standard EN 45002 [ECS, 1989b]; and (b) require the use of proficiency testing schemes as far as appropriate.

Laboratories meeting the assessment criteria shall be presumed to fulfil the criteria referred to in paragraph 1.

Laboratories which do not meet the assessment criteria shall not be considered as laboratories referred to in Article 7 of the said Directive.

3. Member States shall designate bodies responsible for the assessment of laboratories as referred to in Article 7 of Directive 89/397/EEC. These bodies shall comply with the general criteria for laboratory accreditation bodies laid down in European Standard EN 45003 [ECS, 1989c].

4. The accreditation and assessment of testing laboratories referred to in this article may relate to individual tests or groups of tests. Any appropriate deviation in the way in which the standards referred to in paragraphs 1, 2 and 3 are applied shall be adopted in accordance with the procedure laid down in Article 8."

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and in Article 4, it states:

"Member States shall ensure that the validation of methods of analysis used within the context of official control of foodstuffs by the laboratories referred to in Article 7 of Directive 89/397/EEC comply whenever possible with the provisions of paragraphs 1 and 2 of the Annex to Council Directive 85/591/EEC of 23 December 1985 concerning the introduction of Community methods of sampling and analysis for the monitoring of foodstuffs intended for human consumption." [EU Council Directive 85/591/EEC].

As a result of the adoption of the above Directives, legislation is now in place to ensure that there is confidence not only in national laboratories but also those of the other Member States. As one of the objectives of the EU is to promote the concept of mutual recognition, this has been achieved in the laboratory area by the adoption of the AMFC Directive.

In addition it is important that there is dialogue and co-operation by the laboratory with its customers. This is also required by virtue of the EN 45001 Standard at paragraph 6, and will be emphasised even more in future revised versions of EN 45001 and ISO/IEC Guide 25 [1990].

This Directive is currently undergoing revision, but it is not expected that the laboratory requirements will be any less stringent than in the current legislation.

CODEX ALIMENTARIUS COMMISSION

The decisions of the Codex Alimentarius Commission (CAC) are becoming increasingly important because of the acceptance of Codex Standards in World Trade Organisation (WTO) Agreements. They can be regarded as being semi-legal in status. Thus, on a world-wide level, the establishment of the WTO and the formal acceptance of the Agreements on the Application of Sanitary and Phyto-sanitary Measures (SPS Agreement) and Technical Barriers to Trade (TBT Agreement) have dramatically increased the status of Codex as a body. As a result, Codex Standards are now seen as *de facto* international standards and are increasingly being adopted by reference into the food law of both developed and developing countries.

Because of the status of the CAC described above, the work that it has carried out in the area of laboratory quality assurance must be carefully considered. One of the CAC Committees, the Codex Committee on Methods of Analysis and Sampling (CCMAS) has developed criteria for assessing the competence of testing laboratories involved in the official import and export control of foods. These were recommended by the Committee at its Twenty-first Session in March 1997 [Codex Alimentarius, ALINORM 97/23A] and adopted by the Codex Alimentarius Commission at its Twenty-second Session in June 1997 [Codex Alimentarius, ALINORM 97/37]; they are intended to assist countries in their fair trade in foodstuffs and to protect consumers. They mirror the EU recommendations for laboratory quality standards and methods of analysis.

The criteria for laboratories involved in the import and export control of foods, now adopted by the Codex Alimentarius Commission are: (1) to comply with the general criteria for testing laboratories laid down in ISO/IEC (The International Electrotechnical Commission) Guide 25: 1990 "General requirements for the competence of calibration and testing laboratories" [ISO/IEC Guide 25, 1990] (*i.e.* effectively accreditation); (2) to participate 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" [IUPAC, 1993] (already adopted for Codex purposes by the CAC at its 21st Session in July 1995); (3) to use, whenever available, methods of analysis which have been validated according to the principles laid down by the CAC; and (4) to use internal quality control procedures, such as those described in the "Harmonised Guidelines for Internal Quality Control in Analytical Chemistry Laboratories" [IUPAC, 1995].

In addition, the bodies assessing the laboratories 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" [ISO/IEC Guide 58, 1993].

Thus, as for the European Union, the requirements are based on accreditation, proficiency testing, the use of validated methods of analysis and, in addition, the formal requirement to use internal quality control procedures which comply with the Harmonised Guidelines. Although the EU and Codex Alimentarius Commission refer to different sets of accreditation standards, the ISO/IEC Guide 25: 1990 and EN 45000 Series of Standards are similar in intent. It is only through these measures that international trade will be facilitated and the requirements to allow mutual recognition to be fulfilled will be achieved. They both aim to facilitate international trade by enabling mutual recognition of efficient analytical laboratories. However, all of these Standards have effectively been replaced by the ISO/IEC Standard 17025 [1999].

It is important to recognise that an over arching requirement in both sets of requirements are that laboratories comply with the general criteria for testing laboratories as laid down in the ISO/IEC Standard 17025: 1999, *i.e.* that they are accredited, and that they participate in appropriate proficiency testing schemes, use methods of analysis that have been validated according to the principles laid down by the EU and by Codex and used in appropriate internal quality control procedures.

METHODS OF ANALYSIS

Methods of analysis criteria

Methods of analysis criteria were initially laid down in Directive 85/591/EEC7 but are currently being revised within the EU. The general requirements are that methods should be assessed for the following criteria: accuracy; applicability (matrix, concentration range and preference to be given to "general" methods); linearity; precision (*i.e.* repeatability and reproducibility); detection/determination limits if appropriate for the determination being considered; recovery; selectivity (interference effect, *etc.*); sensitivity; other criteria that may be selected as required.

It is also commented that the precision values referred to above shall be obtained from a collaborative trial which is being conducted in accordance with the internationally

recognized protocol on collaborative trials, *e.g.* ISO 5725 [1994] or the IUPAC Harmonized International Harmonized Protocol [Horwitz, 1988].

The repeatability and the reproducibility values shall be expressed in internationally recognized form (*e.g.* 95% competence intervals as defined by ISO of IUPAC). The results from the collaborative trial shall be published or freely available.

Methods of analysis which are applicable uniformly to various groups of commodities shall be given preference over methods which only apply to individual commodities.

Single laboratory validation

It is also stated that in situations where methods of analysis can only be validated within a single laboratory, then they should be validated in accordance with the IUPAC Harmonized Guidelines [Thomson *et al*., 2002].

Criteria approach

The EU is increasingly introducing a performance based approach rather than a prescribed method approach for methods of analysis in legislation. Thus, a food control laboratory may use any method provided that: (1) the laboratory is proficient; (2) the method meets defined performance characteristics; (3) the measurand is rational rather than empirical – *i.e.* the value obtained is not directly dependant on the method of analysis used – alternative methods may be used which give equivalent results.

In order to achieve all of this, it is necessary to have method performance information as well as laboratory proficiency testing, all of which is aided by the use of certified reference materials (CRMs). This also helps in enabling the measurement uncertainty of a result to be estimated.

Thus, analytical chemists are now more than ever coming under increased pressure to be able to demonstrate the quality of their results by giving a measure of the confidence based on a particular result to demonstrate its fitness for purpose. This included the degree to which the method would be expected to agree with other results irrespective of the method used. "Measurement Uncertainty" (MU) is a useful parameter which gives this information, and one that is increasingly discussed and being used in the Food Analysis Community.

MEASUREMENT UNCERTAINTY

All quantitative results may be reported to the customer in the form of "a+-b" where "a" is the best estimate of the true value of the concentration of the measureand (the analytical results), and "2b" is the range within which the true value is estimated, with a given probability (normally 95%), 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. Even though this terminology is considered suspect by some, it is now internationally accepted.

The estimation of the value of "a" is dependant on: the accuracy of the method used; and how well the analyst uses that method, *i.e.* whether the analytical system is "in control".

The value of the measurement uncertainty "b" is dependant on: the inherent precision of the method of analysis used; and the number of analytical replicates that are carried out. The more replicates the less the value of the measurement uncertainty.

APPLICATION TO NOVEL FOODS

All of these issues discussed above apply not only to methods of analysis generally, but also to analysis in the novel food sector. For the purposes of this paper, the activities within the genetically modified organism sector (GMOs) will be taken as being representative of the issues that confront method validation and data quality in the novel foods area generally. In the GMO sector there are a number of activities currently being undertaken in some Commission and Codex Working Groups and Committees.

Fora in which analytical methodology for GMOs is currently being discussed

GM methodology and associated issues are currently being discussed in an EU wide analytical network of control laboratories and Competent Authorities. That activity is being coordinated through the Joint Research Centre ISPRA. It has a website (www.engl.jrc.it) where its activities are described.

Methods of analysis to determine and detect food derived from biotechnology are also being considered within the Codex Alimentarius Commission, and specifically in the Codex Committee on Methods of Analysis and Sampling. There an international working group, co-chaired by Germany and the UK, will consider such methods and try to develop appropriate criteria. Some 21 countries and organizations are involved in this activity, *i.e.* Argentina, Australia, Brazil, Canada, Egypt, France, Iran, Ireland, Italy, Japan, Malaysia, Netherlands, Philippines, United States, European Commission, AOAC, AOCS, EUROPABIO and ISO.

Collaborative trials in the GM area

There have been some very recent collaborative trials completed which demonstrate that methods with acceptable precision for determination of GM soya in soya are available. As an example, an international collaborative trial coordinated by the Federal Institute for Health Protection in Consumers and Veterinary Medicine (BGVV) Germany, gave the results presented in Table 1.

Proficiency testing

There have been a number of proficiency testing exercises carried out in the GMO area. Here, the results have not been so reassuring as has been the case with the recent collaborative trials. As an example, a known sample of bread containing 4.5% GM soya in soya was sent to a number of "expert" laboratories to assess the mean value obtained together with the standard deviation of the individual results obtained using 10 replicas. The results are given in Table 2.

The same laboratories were also asked to determine a cake containing 1.5% GM soya in total soya and there the results obtained, are also variable. These results are given in Table 3.

It is possible to express these results as measurement uncertainties at the 1% threshold value, *i.e.* the values whereby the concentration at which the analyst may be

TABLE 1. Results from an international collaborative trial for the determination of GM soya in soya.

"Expected" [%]*	0.1	0.5	1	2	5	2
Mean value [%]	0.11	0.49	1.00	2.26	4.91	1.71
Repeatability standard deviation S_r	0.02	0.12	0.17	0.20	0.56	0.39
Repeatability limit r ($r = 2.8 \times S_r$)	0.05	0.35	0.47	0.55	1.56	1.08
Reproducibility standard deviation S_R	0.02	0.12	0.27	0.60	0.95	0.48
Reproducibility limit R ($R = 2.8 \times S_R$)						

*All values refer to % GM Soya flour in Flour.

TABLE 2. Results of a "mini collaborative trial" with 5 laboratories each analysing a sample of bread containing 4.5% GM soya in soya, ten times in replicate.

Laboratory	Mean	Standard deviation
1	2.2	0.54
2	3.1	1.13
3	4.8	1.57
4	1.6	1.62
5	2.6	0.22

TABLE 3. Results of a "mini collaborative trial" with 5 laboratories each analysing a sample of cake containing 1.5% GM soya in total soya, ten times in replicate.

Laboratory	Number of replicates	Mean	Standard Deviation
1	11	2.0	0.28
2	10	3.3	1.08
3	10	2.5	0.90
4	14	1.5	1.00
5	10	2.2	0.14

reliably said to exceed the 1% threshold is given in the table below. In effect, the values given are the „enforcement limits that would be used by the analyst before he would be prepared to say that the value obtained is beyond reasonable doubt that it exceeds the 1% threshold (Table 4).

TABLE 4. Results found in the "mini collaborative trials" expressed as measurement uncertainties at the 1% threshold.

Laboratory no	Cake% 1%+MU	Bread% 1%+MU
1	1.6	2.1
2	3.2	3.3
3	2.8	4.1
4	3.0	4.2
5	1.3	1.4

Problems with GM and novel food analysis

The main problems with GM analysis are that very low concentrations of an analyte are being determined in what is a hostile matrix. It would be useful to express the concentration for method comparative purposes at the percentage of GM DNA in total DNA extracted, which for the bread sample described above is in the region of 0.015% (150 ppm) and for the cake is in the region of 0.05% (500 ppm). These figures are calculated on a dry weight basis, taking into account those ingredients that do not contain any DNA, *i.e.* materials such as sugar and fat.

It is also true that in food analysis generally and the GMO sector specifically the number of reference material available are few in number and have been subject to many sample preparation difficulties. They have been found to be non-homogeneous when prepared and also unstable. Great effort is now being undertaken by a number of institutes to develop and make available to analysts suitable reference materials.

HOW DO WE IMPROVE ANALYTICAL PERFORMANCE?

In the GM area, which we may take as being representative of the novel food area, it is clear that we need to improve the quality of analysis. In particular we need:

(1) Much more information on the performance of methods in the sector. At present, there are few fully validated methods of analysis and also few which have been characterised within a single laboratory.

(2) More effort being expended in the preparation of reference materials which are both well characterised and stable. The use of reference materials in analysis, particularly in an accredited environment, is becoming of increasing importance.

(3) More effort being expended in the preparation of internal quality control (IQC) materials The use of (IQC) materials are of increasing importance in the food sector. It is clear that in all areas of food analysis, the availability of such materials would be much appreciated by many analysts to aid their routine quality control procedures. Such materials could be characterised through proficiency testing or collaborative trial exercises.

(4) It is also clear that developments within this sector could be much enhanced if there were to be greater cooperation and pooling of information between analysts. This is probably best achieved through informal networks such as ENGL.

(5) It must be recognised that, analytically, this is fast moving sector with both new methods of analysis being developed and their application still be optimised.

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