Online Training Curriculum

Confirmation Methods for Food contaminants

General Background on food contaminants and food regulatory management

"Fit for purpose determination"

Jérémie Théolier, Ph.D.

Research Associate, Laval University

parera
Food Risk Analysis and Regulatory Excellence Platform

INAF INSTITUTE OF NUTRITION AND FUNCTIONAL FOODS

UNIVERSITÉ LAVAL
Faculty of Agriculture and Food Sciences

Overview

Fit for purpose determination

As part of their responsibility for verifying that the relevant requirements of food law are fulfilled by food business operators at all stages of production, processing and distribution, regulatory agencies carry out monitoring of food on the national market, including imported food.

The monitoring of food products is a key part of risk analysis to ensure compliance to food regulations but also to allow the tenure of risk assessment. In the same way, robust validated analytical methods have a pivotal role to play in maintaining both the quality and safety of foodstuffs entering the supply chain and ultimately safeguarding the consumer. The consequences of inaccurate data can have wide reaching consequences which can be measured in a variety of terms (economic, legal and consumer safety).

There are numerous sources providing guidance on validation procedures and performance criteria including:

- papers in scientific literature
- guidance issued by scientific bodies
- guidance from international organisations involved in the establishment of harmonized standards such as the AOAC International, the Codex Alimentarius Commission (CAC) and the European Committee for Standardisation
- regulatory organisations such as the European Commission (EC) and the U.S. Food and Drug Administration (FDA) and independent expert committees such as Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives

The extent of guidelines for validation requirements provided by different organizations varies. However, there is a shared common objective which is to achieve “fit for purpose” analytical results.

Using a “fit for purpose” approach relies on the assessment of method performance to define the suitability of a given methodology to provide an answer to a given requirement. Such an approach helps to ensure that not only does the methodology address the requirement it is being used for, but that a range of technologies and facilities can be used to achieve the desired outcome.

There are some fundamental issues to consider, it is essential to know precisely what the regulatory and performance requirements that must be achieved are to ensure the method is fit for purpose. Those issues include:

- sufficient information on the compound of interest including the metabolic or degradation pathways,
- the matrices that will be included in the testing program and
- the concentration range to be targeted in accordance with any established regulatory limits
- the intended use of the method (qualitative or semiquantitative screening, quantitative and/or confirmatory analysis). For example, if an operator wants to quantify mycotoxins in a specific sample, there is a need to define what does it mean by mycotoxins (all the known mycotoxins, all the regulated mycotoxins, all the mycotoxins that are usually being found in a specific sample)
- the performance criteria applicable for the technology used must also be considered.

Choice of the adequate analytical method

The nature of the sample and the specific reason for the analysis commonly dictate the choice of analytical methods. Speed, precision, accuracy, robustness, specificity, and sensitivity are often key factors in this choice. Validation of the method for the specific food matrix being analyzed is necessary to ensure usefulness of the method.

The Codex Alimentarius develop a guideline to selection the adequate method for a large number of food commodities and food contaminants [1]. A method is the application of a technique for a specific analyte in a specific matrix. However, in order to reach quality assurance optimum, a procedure explaining how to apply a method to a particular sample, including information on obtaining samples, handling interferents, and validating results, are needed. In the same way, a protocol, which is a set of stringent guidelines specifying a procedure that must be followed if an agency is to accept the results, have to be create. Protocols are common when the result of an analysis supports or defines public policy.

The requirements of the analysis determine the best method. According to the Codex guidelines, official methods of analysis elaborated by international organizations occupying themselves with a food or group of foods should be preferred. In choosing a method, preference should be given to methods of analysis the reliability of which have been established in respect of the following criteria:

- Accuracy,
- Precision, repeatability intra-laboratory (within laboratory), reproducibility inter-laboratory (within laboratory and between laboratories)
- Limit of detection (LoD) and limit of quantification (LoQ)
- Sensitivity,
- Selectivity,
- Robustness,
- Ruggedness,
- Practicability and applicability under normal laboratory conditions
  - scale of operation,
  - analysis time,
  - availability of equipment, and
  - cost.

Other performance characteristics to be considered are:

- analyte stability,
- linearity and calibration curve,
- analytical range,
- recovery,
- measurement uncertainty (MU),
- sample stability.

However, it is important that data generated from food monitoring yields acceptable trueness and precision for use in complex risk assessment calculations and statistical analyses. In the same way, regulators who enforce maximum levels or maximum residue levels must have confidence that the reported analytical results represent the true residue concentrations. In quality assurance and research activities, a precise estimate of the true contaminant concentration of a lot becomes important and the absence of a well-designed sampling plan will result in the collection of unrepresentative samples which will invalidate the results of subsequent contaminant determination.

The success of any analytical method relies on the proper selection and preparation of the food sample, carefully performing the analysis, and doing the appropriate calculations and interpretation of the data. Methods of analysis developed and endorsed by several non-profit scientific organizations allow for standardized comparisons of results between different laboratories and for evaluation of less standard procedures. Such official methods are critical in the analysis of foods, to ensure that they meet the legal requirements established by regulatory agencies.

Sampling and sampling plan

In analyzing food samples, all results depend on obtaining a representative sample and converting the sample to a form that can be analyzed. A sampling plan is defined by a test procedure and a defined acceptation/rejection limit. The sampling step specifies how the sample will be selected or taken from the primary sample to the analytical sample. In the end, the measured contaminants concentrations in the sample are used to estimate the food contaminants concentration in the bulk lot or compared to a defined accept/reject limit that is usually equal to a legal limit. Comparing the measured concentration to an accept/reject limit is often called acceptance sampling, because the measured concentration value is not as important as whether the measured concentration (and thus the lot concentration) is above or below a legal limit.

Because of the uncertainty associated with a sampling plan, the true food contaminant concentration of a bulk lot cannot be determined with 100% certainty; in the same way, it is impossible to correctly classified lots into good and bad categories with 100% accuracy. There are generally two types of uncertainties associated with sampling procedures, accuracy and precision.

- Accuracy is defined as the closeness of measured values to the true value. Precision is defined as closeness of measured values to each other. Accuracy is associated with a bias. Biases have the potential to occur in the sample selection process and in the quantification process. Biases are the easiest to control and eliminate but are the most difficult to measure because of the difficulty in knowing the true concentration of the lot. Sample selection equipment and analytical methods are continuously performance tested to minimize any biases.

- Precision is associated with variability. Variability can occur with each step of the mycotoxin test procedure and is usually associated with the pesticide distribution among contaminated particles in the lot. Variability is reduced when the sampling size is important [2,3].

Regulatory agencies, including the Codex Alimentarius, wrote some guidelines to perform sampling for the determination of contaminants in food and feed. The European Union (EU) has established sampling plans for a variety of commodities in accordance with the Codex guidelines. Both guidelines can be found below:

CA: http://www.fao.org/fileadmin/user_upload/livestockgov/documents/1_CXS_193e.pdf

EU: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32002L0063

Although novel detection technologies will continue to be sought to improve the efficiency of analysis, for the food analyst, the upstream portions of the procedure, which means those involving sampling and food sample preparation, also needs the greatest attention.

Sample preparation

Sample preparation is one of the main steps of food analysis**.** Direct analysis of several compounds in foodstuff is very difficult without any sample preparation methods. Ideally, methods for preanalytical sample preparation of foods should accomplish one or all of the following functions:

- separate target molecules from the food,
- increase their concentration,
- purify them from extraneous material,
- achieve volume reduction in bulk samples,

- produce a homogeneous sample, and
- exclude inhibitory substances.

In food analysis, traditional methods for sample preparation are laborious, time consuming and usually involve large amounts of solvents, which are expensive and generate considerable waste. In addition, usually more than one clean-up stage prior to detection is required. As a result, modern sample preparation procedures have been developed or improved to overcome the drawbacks of the traditional approaches. Modern concerns regarding food safety require that more and more samples have to be tested. Consequently, interest in procedures that are fast, accurate, precise, with few or without solvent, inexpensive and amenable to automation is ongoing [4].

Special focus should always be put on sample preparation procedures, including for methods that are already well understood. While testing procedures are generally straightforward and well described, preparation methods will sometimes be based on the judgment or personal experience of the operator, especially for new foods. Regardless, sample preparation should always be scientifically validated. Unfortunately, food matrices are often a tough challenge, which limits or defeats many method applications. Foods are nearly unlimited in variety, so that a universal preparative method is virtually impossible to develop. Despite best efforts to prepare the matrix for detection, residual, food-associated compounds frequently interfere with the detection assay.

References

1. Codex Alimentarius (CXS 234). Recommended Methods of Analysis and Sampling. Available at http://www.fao.org/fao-who-codexalimentarius/codex-texts/list-standards/en/

2. Ambrus & Soboleva, 2004. *Journal of AOAC*, 87(6): 1368-1379.

3. Farkas et al., 2015. *Journal of Agricultural and Food Chemistry*, 63: 4409-4417.

4. Beyer & Biziuk, 2008. *Food Chemistry*, 108(2): 669-680.