VALIDATION OF ANALYTICAL METHODS FOR COMPLIANCE OF FOOD CONTACT MATERIALS

Maria Rosaria MILANA [1], Veruscka Mannoni[1], Giorgio Padula[1]

[1] Dept. Environment and Connected Primary Prevention, Unit Exposure and risk from Materials
Istituto Superiore di Sanità, Roma, Italy; mariarosaria.milana@iss.it

Abstract – Quality requirements for Food Contact Materials analysis are the same applicable to Foods. The migration testing is a two steps process for which different sources of uncertainties can be characterized. Schemes for validation are available and shown.

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1. INTRODUCTION

Food Contact Materials and articles (FCM) belong to the subjects covered by the umbrella of the EU Food Law. (Reg. (EC) 178/2002). In this context, the Framework Regulation on FCM is the Reg. (EC) n. 1935/2004. The same basic criteria laid down in Official Food and Feed Control (Reg. (EC) 882/2004) must be considered for FCM, including the quality criteria for the analytical controls. This strongly influences the choices of the Official Laboratories of the Competent Authorities in Italy and more in general in the EU.

2. EXPERIMENTAL

2.1. Official Controls for FCM and quality requirements

The role of Official Control Laboratories (OCL) for Enforcement of FCM safety criteria is to monitor samples at border level and on the national market.

The safety criteria and parameters may be expressed as general requirements or as composition or migration limits to be respected. In both the cases analytical skill of the laboratory is necessary. This does not mean only good structure, equipment and personnel, but also implementation of quality requirements in analytical testing, to ensure reliability of the analytical results, on which legal decisions will be taken.

According to articles 11, Annex III Reg. (EC) n. 882/2004, defined criteria for characterization of methods of analysis are listed. These criteria are applicable also to FCM field. It must be said that this Regulation is currently under revision and these requirements will be probably amended. For the timebeing, to fulfil these criteria, methods should be get ready performing an unavoidable series of steps: developing, in house validation, ISO/IEC/ 17025 accreditation, intercalibration, verification etc.

2.2 Validation of analytical methods for FCM: an open issue

In few cases, (some monomers, overall migration methods) the experimental test methods to determine migration were internationally developed and validated at CEN level and therefore OCLs must demonstrate to perform to the published criteria for reproducibility and repeatability. In other cases (the majority) the OCL must develop, validate and accreditate a proper method.

There are two possibilities: FCM already into contact with foods (food products) or material/article intended to be used into contact with foods.

In the first case methods to search a substance migrated from materials into the foods should be get ready, with the same criteria used for food analysis of additives, contaminants etc.

In the second case, proper contact test shall be developed, to simulate the real interaction of the material/article with the food in its entire shelf or service life. This is the case that is considered in this paper and the discussion will follow on that.

In the sequence of physicochemical events that lead to migration of a substance from a material/article to a matrix (food/food simulant) two main different steps may be identified:

- *Migration*: contact of an article or a specimen with food or food simulants, for defined time and temperatures. In this period a series of phenomena occur leading to migration events. Schematically these phenomena can be identified as diffusion of the migrant into the material, interaction of the contact medium with the material, transfer of the migrant from interface material/medium to the contact medium. These phenomena are regulated by the characteristics of the three actors of the migration process (material, contact medium, migrant molecule) and by the contact conditions (temperature, time, contact surface).The combination of favourable conditions (eg high T, long time, affinity of the migrant for the medium, low resistance to diffusion of the material) would lead to high migration.
- *Measurement*: this step deals with the analytical determination of the substance migrated into the food or simulant. This step should be seen as a typical quantitative analysis to determine an analyte in a matrix. The analyte is the migrant, the matrix is the food or the food simulant. This requires, in general, a sample work up part to extract the analyte (to concentrate it, or to remove from interfering substances) and an instrumental determination of the extracted analyte. Depending of the characteristic of the analyte and the food/simulant, the migrated amount, and the eventual legal limit, the sample work up will require more or less handling and the instrumental techniques will be different (eg. GC, LC, MSD etc).

When the two steps are completed and the amount of migrated substances is determined, for each result to be dealt with from a legal point of view uncertainties should be calculated. The process to calculate the uncertainty should take into account all the sources contributing to uncertainty of the final result. However, the main sources are different for the two different steps (migration part, measurement part) of the migration experiment and not always the two contributions may be estimated and joined in a unique final figure to calculate an overall uncertainty including both the two parts.

The following outline, of course not exhaustive, and the discussion presented in the successive paragraphs, are intended to better address this issue.

Outline of the sources of uncertainties for Migration part

Sample: measurement of the contact surface

Contact conditions: temperatures and time to be kept during the contact

Volumes: Glassware, dilutions, aliquoted volumes, devices, etc.

Outline of the sources of uncertainties for Measurement part

Sample work up: volumes, glassware, dilutions, aliquotted volumes, devices, etc.

Analytical/instrumental method: instruments, linearity, sensitivity, calibrations, reference standards.etc

In theory, all the factors and the describing parameters should be kept under control and their impact on uncertainty could be calculated, but, actually, all the measurements should be finally compared with the expected result from a Certified Reference Material (CRM) for migration, under certain conditions, from a specific material, with a specific composition. In other words a certified material from which a predetermined migration is to be expected. Differently from other sectors, for FCM this kind of CRM is not available. Just one reference polyamide for overall migration from plastic (total immersion) is available as CRM and is regularly offered by FAPAS to the laboratories that apply the official EN 1186 method for OM. But this is just a tiny part with respect to the hundreds (or thousands..) of the substances that can be used in FCM in the different materials and for a variety of contact conditions. For instance, at the EU level there is a list of substances authorised for plastics FCM and a variety of specific migration limits, but there are also a number of EU Member States that have specific national legislations with migration limits, to be respected, as well. Therefore there was, and still there is, the necessity to set up and to keep a uniform approach to this field, to get uncertainty figures.

Another point must be underlined. To perform validation of a method, a number of replicated tests must be performed and the results must be averaged to calculate repeatability and/or reproducibility and the related figures.

This is possible and correct only for specimens obtained from the same sample, i.e. when from a sheet, a roll, several subsamples are cut. Only in this case the results could be considered to be averaged as results of effective replicated tests. In fact in this case it is reasonable to think that adjacent parts of the same sample have negligible intrinsic variability in their composition and morphological features. In the case of final articles (e.g containers to be tested by filling with simulants) the eventual differences between the results from different replicates would not be derived only from the variability sources in the test but also from the variability intrinsic to the manufacturing process. This factor is out of the control of the laboratory and, on a consequence, out of its responsibility. Averaging all the results would mean to overcharge the laboratory of additional uncertainties, extraneous to the performance of the laboratory. Therefore each article should be treated as a unique article, without the possibility to average the replicates. This is another important and unavoidable obstacle to the calculation of the uncertainties of the whole procedure (migration + measurement).

In view of what above, in absence of CRMs for FCM, and having not knowledge of the industrial variability in the commercial FCM, the solution to afford this situation is to consider only the part that is under the control and the responsibility of the OCL. This means to consider the measurement part and to calculate uncertainty for this part.

Therefore when dealing with articles, the results must not be averaged, but considered separately and each one should be compliant with the applicable limit. Deviations from the legal limits of one of the tested articles should be discussed as in contrast with GMP Regulation (EC) 2023/2006. A number of accredited laboratories all over the EU already have followed this unique way.

Schemes for single laboratory in house validation, for full validation (method destined to be an international/official one) and verification (to keep accreditation, to verify competence and performance of the laboratory) are available from several sources. However, a milestone for the OCLs of the EU Member States in the specific field of FCM is the document "*Guidelines for performance criteria and validation procedures of analytical methods used in controls of food contact materials*" published in 2009 by the EURL for FCM [1]. The document was developed in cooperation among the EURL and a Task Force of Experts from NRLs on FCM, including the Italian NRL.

The EURL/NRL Guidelines are endorsed by the European Union official Network of National Reference Laboratories and approved by the EU Commission competent service DG SANCO.

Four different validation schemes are presented, depending on the scope of the activity:

a) "Full" single laboratory validation protocol
b) "Standard level" of single laboratory validation
c) "Basic level" of single laboratory validation
d) Method verification

a) "Full" single laboratory validation protocol

To be performed for new methods to get ready its performance characteristics, to be used for standardization purposes and/or official controls. In general, the performance parameters are mostly relevant only for the validating laboratory.

b) "Standard level" of single laboratory validation

This is the working standard agreed by the OCLs for FCM. It indicates the minimum requirements to establish non-compliance of a material or article intended for food contact.

c) *"Basic level" of single laboratory validation*

The starting point for all the labs for a minimum level of harmonization. Nowadays it is obsolete, but it is still indicated as useful for emergency or occasional case situations

e) Method verification

To be used to verify the performance of the lab with respect the parameters of an already validated method, official or not, not used from long time or regularly used but not checked from long time. Frequency at least once a year

Useful applicative tables for the above four schemes are indicated in the EURL/NRL Guidelines.

For the daily life of the OCLs in the field of FCM, the scheme b) the so called "*Standard level" of single laboratory validation"* is indicated as suitable in the EURL/NRL Guidelines as effective FCM working standard. (see Figure 1)

First of all, it is to note that to calculate bias, no CRM or Reference Materials (RM, as defined in EURACHEM, 2014) [2] are available. For some

analytes (migrants) collaborative trials or PT are offered by EURL or may be bought from private providers. In most of the situations, to calculate uncertainty the laboratory has to use the recovery data obtained from the so called "spiked sample" approach.

The scheme for this approach and the number of necessary replicates have been developed by the EURL/NRL group in order to set up an analytical plan that, at the end, would ensure to have the necessary data to allow an appropriate number of degrees of freedom to calculate precision from repeatability and within laboratory reproducibility data. The plan was designed with the purpose to produce and to use the available data without redundancy, but using replicated analyses produced for other set from the plan. ANOVA test is suggested to elaborate the Precision data combining the results from Repeatability with those from Reproducibility.

However it is possible in the daily life to go ahead also without ANOVA test and the following worked example is suggested in the EURL/NRL Guideline to better clarify what in the Figure 1.

In synthesis, the number of analyses to be performed are so outlined:

Samples: A total of 31 analyses had to be performed so distributed along 3 days. First day : 1 concentration (legal limit, LL) in 7 replicates, two other concentration levels (0.2LL and 2LL) in 3 replicates; Day 2 and Day 3 the 3 concentration levels (0.2LL, LL and 2LL) in 3 replicates by different operator.

Therefore, for spiked samples 13 analyses in day 1, 9 analyses in day 2, 9 analyses in day 3.

3 calibration curves:

For inorganic analyses: A total of 12 analyses had to be performed (3 times 3 concentrations+ blank) in days 1, 2, 3 by three different operators

For organic analyses: A total of 18-21 analyses had to be performed (3 times 5-6 concentrations+ blank) in days 1, 2,3 by different operators.

In total:

43 analyses by 3 different operators in 3 days for inorganic analyses

49-52 analyses by 3 different operators in 3 days for organic analyses

Trueness can be calculated from 3 replicates of spiked analyte in the food simulant for 3 different concentrations within the working range. The working range is set around the Legal Limit (LL) to be respected. Therefore the working range is 0.2LL, LL, 2LL (or 5LL when there is the so called reduction factor, applicable to migration from plastics).

These data can be collected from the data produced from the precision studies. Acceptability criteria for trueness are described in the mentioned EURL/NRL Guidelines where more extensive information can be found.

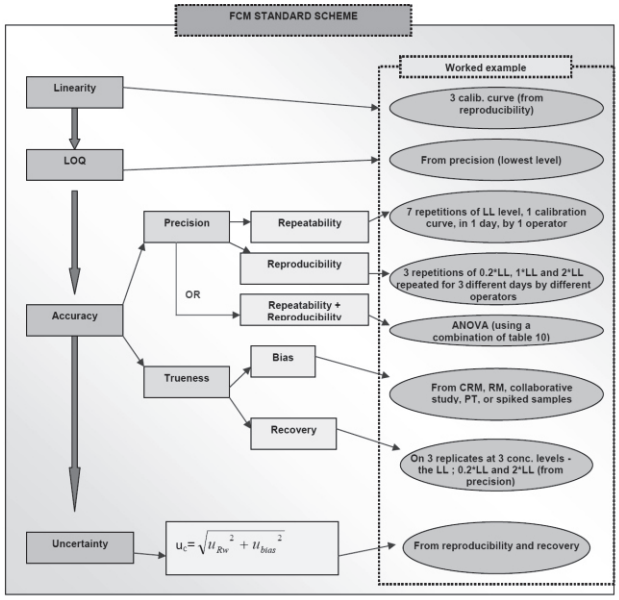

Fig. 1 effective working standard for FCM (from "Guidelines for performance criteria and validation procedures of analytical methods used in controls of food contact materials", Stefanka Bratinova, Barbara Raffael, Catherine Simoneau.EUR 24105 EN - 1st edition 2009)

To calculate expanded Uncertainty (U) to be associated to the final results, the combined standard uncertainty (u_c) is multiplied by a factor k= 2 that associates to the uncertainty a 95% level of confidence for the effective degrees of freedom

$$U = k\, u_c$$

Combined standard Uncertainty from the "spiked samples" can be calculated through the approach illustrated in the FCM Standard Scheme in Fig.1. The contribution from random errors is got from the standard deviation of the Within laboratory reproducibility study (U_{Rw} in the scheme). The contribution from systematic errors may be obtained from recovery data (at least 6 recovery experiments) where U_{bias} consists of the averaged systematic biases from recovery

assessment and the uncertainty of the standard addition (spike) $u_{add:}$ according to the following formulas :

$$u_c = \sqrt{u_{Rw}^2 + u_{bias}^2}$$

where $u_{bias} =$

$$\sqrt{\sqrt{\frac{\sum_{i=1}^{n} bias_i^2}{n}} + u_{add}^2}$$

All the necessary data are already available from the produced data required in the FCM Standard Scheme. Therefore, the scheme here illustrated is a workable and agreed scheme to be taken into account as valid, especially in the presence of indications developed in a EURL/NRL network and published in guidelines approved by the EU Commission.

The applied scheme demonstrates the importance to get ready a proper validation data set and internal quality control plan. Proficiency or ILC data are a powerful tool, too. This is also addressed in international publications [2] [3], where useful indications are reported.

3. CONCLUSIONS

The migration test is a two step process for which different sources of uncertainties can be characterized. The migration part has different sources of uncertainties depending on sample type, contact type, contact conditions. The analytical part may be in common with different FCM and therefore it is easier to be standardized and the uncertainties well characterized. An agreed approach in this direction among the OCL in EU allowed getting a more homogeneous behaviour of the laboratories in different EU Member States and the publication of these criteria ensured transparency in the official controls.

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