MEASUREMENTS OF FORMALDEHYDE MIGRATED FROM FOOD CONTACT TABLEWARE INTO SIMULANTS FOR ACIDIC FOODS: CALCULATION OF UNCERTANTIES OF THE "CHROMOTROPIC ACID" METHOD

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Abstract – The amount of Formaldehyde migrated in food simulant from food contact tableware must be measured to verify compliance to the legal limit. "Chromotropic acid" method is frequently used. Expanded uncertainties must be calculated taking into account the contributions of the different steps of the method.

Keywords: food contact materials, formaldehyde, chromotropic acid, uncertainty, food safety

1. INTRODUCTION

The migration of formaldehyde (FA) from plastic food contact materials should not exceed 15 mg/kg food, according to Regulation (EU) 10/2011 on plastic food contact materials and articles. The limit must be enforced in control activities in the Member States of the European Union. From the result of the analytical controls, legal actions may be put in place, in the case of non compliance. The results must be associated with their uncertainties and, at the enforcement level, the comparison with the legal Specific Migration Limit (SML) must be done after subtracting the expanded uncertainty. This paper presents a scheme for the calculation of uncertainty associated to the determination of formaldehyde migrated in food simulants from plastic tableware. It is important to point out that the International Technical Standard that is used (see next Chapter) deals with the determination of formaldehyde already migrated in a liquid solution. This is consistent with the approach to consider the compliance tests for FCM as composed by two steps:

- Exposure to a liquid food simulant, in predetermined contact conditions (surface, time, temperature). During this contact test, physicochemical interactions between the FCM and the simulant would occur leading to eventual migrations of substances from FCM to the simulant.
- Measurements of the amount of the substance migrated into the simulant. This step is typically an analytical step in which, when necessary there is a sample work up phase and then an instrumental quali-quantitative measurement of the detected migrant.

Generally, replicated tests are performed on at least 3-4 samples, when the method is already validated or verified in the laboratory. In this case the results could be given as mean of the different results. However, this cannot be done when the test is performed on finished FCM articles, because the variability of the manufacturing process in producing articles is not known. Averaging is indeed possible when the test is performed on specimen got from the same sample (subsamples).

Therefore, uncertainty is calculated only for the analytical phase, starting from the liquid simulant containing the migrated substances, that is, in this case, formaldehyde.

It is worth to note that also the exposure phase should be taken under control with respect the parameters that might significantly impact on the results. These parameters, to be monitored and controlled by the laboratory, are mainly the contact temperature, the contact time, the filling volumes. On the other hand, a laboratory that performs official analyses on FCM in the frame of EC Regulation 882/2004 has to ensure quality criteria not only for ISO/17025 accredited methods, but also for the good analytical practice in all the other activities relevant for Official Controls.

In the next Chapters it is presented a scheme to be used for validation of the method by single laboratory and the calculation of the expanded uncertainty of the analytical phase.

2. EXPERIMENTAL

The food contact article is put into contact with the simulant for predefined times and temperatures. Aqueous simulants, especially if acidic, are worst case for migration of formaldehyde. The determination of formaldehyde migrated into the simulant after contact may be performed using a CEN normalized method, that is UNI CEN/TS 13130-23 2006. The method is based on the development of an azo-colorant due to the reaction between formaldehyde and chromotropic acid, in the presence of sulphuric acid. A violet color of the migration solution reveals the presence of formaldehyde.

The amount of migrated formaldehyde may be determined by UV-VIS spectrophotometry reading the absorbance at 574 nm. Multilevel calibration is performed by external standard. The method uses two repeated calibration curves and calculates their linear regression individually. Because no reference material for migrated formaldehyde is commercially available, standardisation of the reagents to calibrate the solutions is necessary.

3. METHOD VALIDATION

3.1 REPEATABILITY

Repeatability was determined at 3 different concentration levels (Level 1, 2 3) within the field of application of the method (working range 0,15– 28 mg/l). To this aim, 10 spiked FA solutions in 3% acetic acid for each concentration level were prepared and analysed.

In the case of results obtained from the analysis of one or two aliquots of simulant repeatability is calculated as shown in Tables 1and 2.

Table 1: Calculation of repeatability for determination on one aliquot of the simulant

1 determination	Level 1	Level 2	Level 3
FA Mean(mg/l)	1,8	9,7	19
Standard deviation (s) (mg/l)	0,062	0,16	0,21
Relative standard deviation (CV%)	3,4%	1,7%	1,1%
n	10	8	10
t-Student [p=0.05]	2,26	2,36	2,26
Repeatability limit (r) (mg/l)	0,20	0,55	0,67

Table 2: Calculation of repeatability for determination on two aliquots of the simulant

2 determinations	Level 1	Level 2	Level 3
FA Mean (mg/l)	1,8	9,7	19
standard deviation (s) (mg/l)	0,044	0,12	0,15
relative standard deviation (%)	2,4%	1,2%	0,8%
n	10	8	10
t-Student [p=0.05]	2,26	2,36	2,26
Results from 2 determinations Repeatability limit (r) (mg/l)	0,14	0,39	0,47

From the obtained results it is easy to observe that, as expected, 2 replicated determinations gave better repeatability as shown in Figure 1 where the profile of repeatability limit is visualized. The solid line is the profile from the double replicated determinations (Table 2) while the dashed line is that from a single determination (Table 1). The profile of the relative standard deviation (%) for the two-replicated determinations (Table 2) is shown in Figure 2.

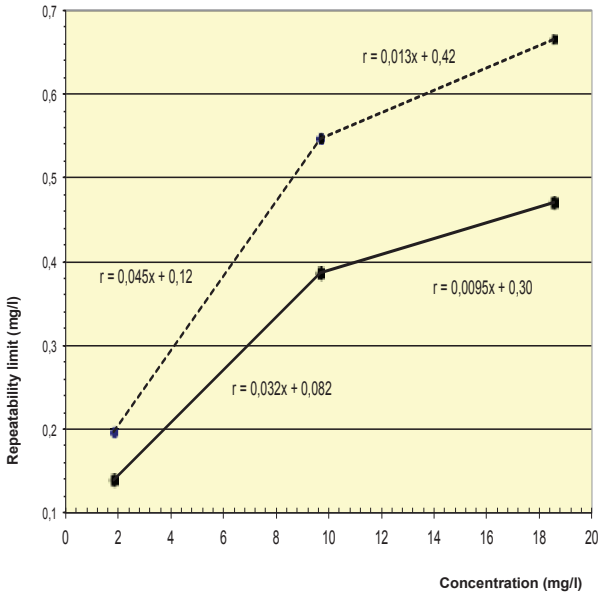

Figure 1: Profile of repeatability limits obtained from the data in Table 1 (single determination, dashed line) and from Table 2 (two replicated determinations, solid line)

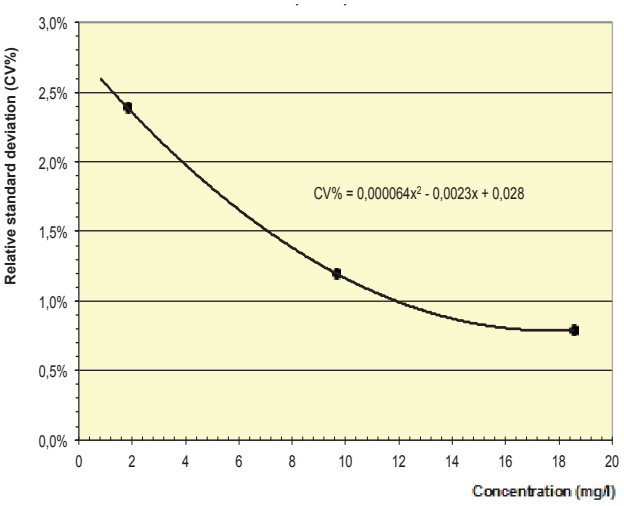

Figure 2: Relative standard deviation for the two-replicated determinations (Table 2)

2.3. LINEARITY

Linearity of the method is firstly demonstrated in the investigated working range with a of linear correlation coefficient > 0.9999. A typical equation for linear regression is corresponding to: Y= 0.00105 + 0.0071 x, but the slope could obviously change depending on the instrumental response.

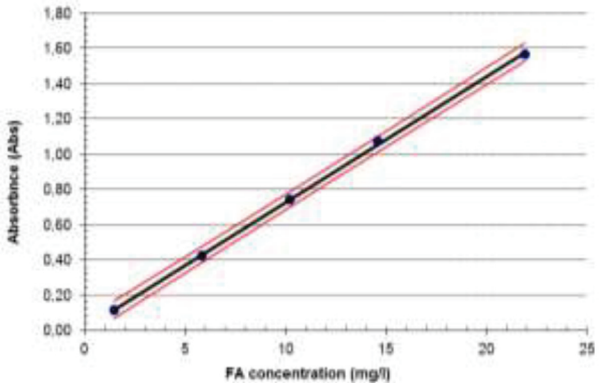

Figure 3: Linear regression plot in the working range

Then, linearity of the method is furtherly investigated in the relevant working range by considering the residues in the plot. Also this verification was successful, being the residues in the plot (Fig. 4) both positive and negative for each investigated concentration level.

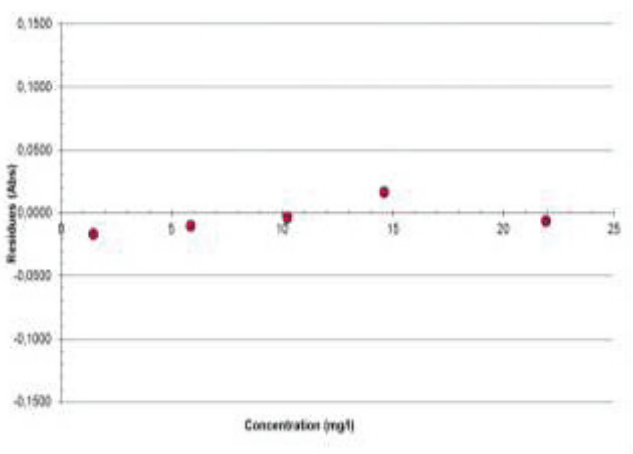

Figure 4: Plot of residues

2.5. TRUENESS

In absence of a certified reference material (CRM) for the determination of FA, trueness was estimated on 3 spiked samples replicated for 5 times. According to the UNI CEN/TS 13130-23 2006, FA concentration should be predetermined indirectly through Sodium Thiosulphate. Therefore, FA used to spike the simulants for trueness test was different from that used to prepare stock solutions of FA for calibration curves.

The results obtained from the tests on the spiked samples demonstrated non significant deviations from the expected results for recovery.

Furthermore, the Laboratory participated several Interlaboratory comparisons provided by the EURL (ISPRA, JRC) and Ring Tests provided by FAPAS ® on determination of Formaldehyde and used the Chromotropic acid method. The laboratory obtained always Z scores very satisfactory (<<1) thus confirming the high performance of the laboratory in this determination.

4. UNCERTAINTY

To calculate uncertainty, two different contributions are considered:

- Repeatability (u_r)
- Calibration (u_t)

The uncertainty contribution from *repeatability* was calculated based on the 3 sets of 10 replicated samples, each one set being spiked to get 3 different concentration levels of formaldehyde.

The uncertainty affecting *calibration* was calculated taking into consideration the contribution derived

from the uncertainties of the two regression curves and the contribution due to the references solutions used to calibrate the reagents.

The contribution due to the references solutions used to calibrate the reagents includes: uncertainty of the title (N) of Sodium Thiosulfate as reference material; uncertainty in use of the dispensers of volume (burettes, pipettes, etc.) used to determine the concentrations of the stock solutions of formaldehyde; uncertainty in use of the containers and dispensers of the volume (flasks, micropipettes, etc.) used to dilute the stock solutions of formaldehyde. These contributions were calculated and summed up, and amounted to around 2%.

The combined contributions are suitable to give the final expanded uncertainties that characterize the application of the method. An uncertainty profile may be obtained.

4.1. Application of the scheme to calculate uncertainty

The calculation of uncertainty is schematically shown in Table 3.

The uncertainty from calibration (u_t) is calculated from the uncertainty obtained from the calibration line estimated in the step of the verification of the method in the laboratory. The uncertainty from repeatability (u_r)was derived as previously discussed When the contribution to uncertainty derives from a linear regression for the calibration line, the least squares method must be applied to get the relevant linear regression equation.

$$y(q) = a + bq \qquad (1)$$

where:
y(q)=absorbance
a= intercept
b= slope
q= concentration

From the regression equation of least squares the differences between the calculated Y with respect the experimental Y share used to verify the residues. Uncertainty of linear regression can be calculated from the commonly used equations in the statistical manuals [1]

Table 3: Input figures used to calculate Expanded Uncertainty at 3 levels of FA concentration .

	Level 1	**Level 2**	**Level 3**
FA Concentration level (*mg/l)*	**1.8**	**9.7**	**19**
relative (%) standard uncertainty			
relative (%) standard uncertainty for repeatability (u_r)	**2,4%**	**1,2%**	**0,8%**
degrees of freedom (u_r)	9	7	9
relative (%) standard uncertainty for calibration (u_t)			
relative (%) standard uncertainty of linear regression	**7,8%**	**1,3%**	**0,69%**
degrees of freedom	6	6	6
relative (%) standard uncertainty of the reference solutions	**2.0%**	**2.0%**	**2.0%**
degrees of freedom	inf.	inf.	inf.
relative (%) standard combined uncertainty (u_c)	**8,4%**	**2,7%**	**2,3%**
degrees of freedom (effective)	7	64	315
coverage factor (k)	2,36	2,00	1,97
relative Expanded uncertainty (U%)	**20%**	**5,3%**	**4,4%**
Expanded Uncertainty (U) (*mg/l*)	**0,36**	**0,52**	**0,83**

From the results shown in Table 3 on the Expanded Uncertainty at 3 different concentration levels it is possible to describe the uncertainty profile of the method.

Figure 5 shows the change of uncertainty in function of the concentration of FA.

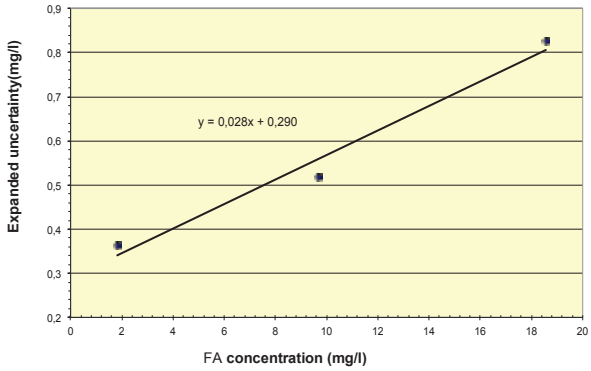

Figure 5: Expanded Uncertainty profile

5. CONCLUSIONS

The analytical and calculation plan used for this method may be suggested as a useful scheme, from the National Reference Laboratory for food contact materials (FCM) in Italy to the Official Control Laboratories.

The obtained expanded uncertainties, relevant for the investigated working range were not impacting significantly the results, at levels relevant for the Specific Migration Limit (15 mg/kg). This confirms the good performance of the method when carried out by trained laboratories.

It is advised to prepare calibration levels not too high with respect to the specific migration limit of formaldehyde (15 mg/kg food approximated by law to 15 mg/l simulant), to prevent deviations from linear response of the method.

It is worth to note that, as for other cases of FCM, the part of the method it is dealt with for these calculations is the measurement part (FA in the simulant) and not the previous part of contact test, according to the reasons above discussed.

This is consistent with the approach agreed for FCM in the network of European reference laboratories and national reference laboratories for FCM and extensively discussed in the specific published guideline [2].

REFERENCES

[1] V J Barwick and S L R Ellison VAM Project 3.2.1 (ver. 5.1) Development and harmonisation of measurement uncertainty principles - Part (d): Protocol of uncertainty evaluation from validation data January 2000 LGC/VAM/1998/088 Version 5.1

[2] Bratinova S, Raffael B, Simoneau C, Guidelines for performance criteria and validation procedures of analytical methods used in controls of food contact materials. EUR 24105 EN – 1st edition 2009.

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