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Minutes

Second meeting of the task force group on analytical methodologies for the determination of acrylamide from food

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Agenda of 22 June 2004

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- Work planned by JRC and HEATOX project

- Methods to be validated

- Samples to be included

- Selection of laboratories

Participants

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Opening and general issues

E. Anklam welcomed the participants of the task force on analytical methodology for the determination of acrylamide (AA) from food at the EC-DG JRC-IRMM. She stated that analytical achievements were obtained and several events such as workshops have happened recently on this subject.

H. Chin gave a short overview on the AA workshop, that was jointly organised by the US Food and Drug Administration (FDA) and JIFSAN (13-15 April 2004, Chicago). He reported that about 150 participants, of which were about one-third from Europe, attended the meeting. After a general session, the workshop consisted of special thematic working groups such as mechanisms of formation and methods for mitigation, analytical methodology, exposure and biomarkers, toxicology and metabolic consequences, risk communication and finally risk characterisation. H. Chin chaired the working group on analytical methods. He reported also on two projects that were conducted recently in the USA. Promising results were shown regarding mitigation, but the new techniques have not yet been proven in commercial applications. In another project dealing with the toxicology of AA, studies of the metabolites of AA in urine and blood were performed. Regarding the results of new exposure studies it was stated, that they do not differ much of the assessment two years ago. In addition there would not be a dramatic decrease in the AA exposure, if one food group would be withdrawn from the nutrition.

Results of JIFSAN proficiency test

H. Chin reported the results of a recently carried out JIFSAN proficiency test. This was carried out in course of the preparation of the above described US FDA JIFSAN workshop on acrylamide (April 2004), an aqueous solution of acrylamide as a quality control sample, two blind replicates of a cereal sample, a peanut butter sample (from NIST), a chocolate sample (from NIST) and a coffee powder sample were sent to 20 laboratories in Europe (8) and North America (12). All of them could be classified as experienced in the analysis of AA in food. A number of 18 laboratories reported results, of which one was excluded from the evaluation due to a systematic overestimation of the AA content of the samples and the absence of data of replicate measurements. The majority (12) of the remaining 17 laboratories applied LC/MS/MS techniques for the analysis, followed by GC/MS including derivatisation of the analyte (3). The other two laboratories applied GC/MS analysis of the native analyte and liquid chromatography coupled to a single stage mass spectrometer. The results were evaluated in applying Gaussian statistics, meaning that the assigned value and the target standard deviation were derived from the arithmetic mean and the standard deviation of the results of the participants.

The results for the quality control sample (nominal value 20 ppb) were 18.5 ± 6.7 ppb. A big standard deviation (74.3 ppb) compared to the assigned value (103.3 ppb) was retrieved for the peanut butter sample. After elimination of one result, the mean value and the standard deviation decreased to 85.6 ± 13.0 ppb. The respective results for coffee powder and chocolate were 157.8 ± 42.0 ppb and 120.5 ± 48.9 ppb. z-Scores were calculated as performance indicators and plotted against the laboratory code (laboratory identity was coded). The distribution of the z-scores was found to be quite narrow. In case of the coffee sample, just one laboratory performed not satisfactory. The mean values and standard deviations for the blind duplicates of the cereal sample were 19.6 ± 14.5 ppb (16.5 ± 2.3 ppb after elimination of high values) and 27.5 ± 29.5 ppb (respectively 18.2 ± 5.5 ppb). In comparing the z-scores for all samples, it was stated that the performance of many laboratories was similar for all analyses, which came evident from a narrow distribution of the z-scores. A broad distribution of z-scores was found for a number of 4 laboratories. In general, the distribution of z-scores for the chocolate sample was broader than for the other samples. Regarding analysis methods it was found

that LC/MS/MS as well as GC/MS with derivatisation are suitable for this type of analysis, if performed thoroughly.

The participants of this task force group discussed intensively the expression of the laboratory performance in terms of z-scores, since the latter do not give any indication of the absolute deviation of the results of a particular laboratory from the assigned value, as long as the assigned value and the target standard deviation is not given. Several members of the task force supported this opinion. K.E. Hellenäs said that a satisfactory performance of a laboratory should be questioned if the absolute difference to the assigned value exceeds 200 % of the assigned value, as it was the case for one laboratory in the analysis of AA from one of the crispbread samples. The large differences for the chocolate sample were also discussed. Although this sample was a NIST reference material (but not certified for acrylamide), it could not be excluded that the large range of results (21 ppb to 216 ppb) could be caused by sample inhomogeneity.

Results of 2nd JRC proficiency test

This second proficiency test offered by the DG JRC in March 2004 was a follow-up of a first study that was organised in July 2003. T. Wenzl presented the design of the study and the composition of the participating laboratories in terms of analytical methodology and extraction techniques and the evaluation of the results. Twenty of 42 participants applied LC/MS/MS, 11 participants carried out GC/MS without derivatisation and 8 participant performed GC/MS with derivatisation. Aqueous extraction was most popular (26 participants). Only two laboratories used pure organic solvents (n-propanol). The others applied mixtures of water and methanol. The test samples were three crisp bread matrices with AA levels of 46, 413 and 497 µg/kg.

Both the first and second JRC test were evaluated in applying robust statistics for the determination of the assigned value and the Horwitz function for calculation of the corresponding target standard deviation. The laboratory performance was expressed by z-scores that were determined according to international guidelines. Seven of 40 laboratories that reported results for the crispbread sample with low AA content performed not satisfactory. The proportions of not satisfactory performing laboratories were for the other two crispbread samples 4 respectively 8 of 42. Some laboratories that got z-scores above an absolute value of two for the low-level crispbread in the first JRC proficiency test could enhance their performance (6), while others did not improve (4). The similarity of performance of the laboratories participating in both trials was demonstrated by plotting the z-scores of one trial against those of the other. The same picture was retrieved for the two crispbread samples with higher AA content of the second study, concluding that the deviations from the assigned value are rather systematic than coincidental. In comparing the absolute deviations of the reported results from the assigned values of the crispbread samples with the deviations of the results for standard solutions with the standard concentration, it became evident that some laboratories had severe problems with instrument calibration. Finally, it was concluded that a training effect could be identified from the results of some participants, but other participants could not improve their performance. z-Scores above an absolute value of 2 were attributed for at least one crispbread sample to 12 of 42 laboratories. Although it was shown on the basis of the sample preparation protocols of well performing laboratories, that extraction and sample clean-up have just little influence on the results of analysis for this type of food, it was suggested to validate analytical methods (based on GC/MS with derivatisation and LC/MS/MS) in a collaborative trial, in order to set a standard for method performance characteristics.

Results of recent FAPAS proficiency tests

L. Castle presented an overview of six FAPAS proficiency tests that were performed since 2002 up to now. He stated that the interest in the tests was higher in the first rounds and that the number of participants decreased from about 40 at the beginning to about 25 in the last study. The number of laboratories that do not apply isotopic labelled internal standards decreased from round to round. In recent studies, nearly all laboratories applied either ^{13}C -labelled or deuterated AA for internal standardisation. A difference in the results gained by the application of one or the other labelled substance was not found. Neither was a clear tendency in the performance of the different methods deducible. However, the z-scores of laboratories that apply LC/MS/MS indicated more often underestimation of the AA content of the samples compared to GC/MS with bromination, for which a uniform distribution was presented. He showed also that the pattern of the performance indicators did not change very much between the first and recent studies. Looking at the absolute difference between the lower and upper border values for satisfactory performance, he stated that the currently applied method of calculating z-scores is probably too generous in indicating satisfactory performance.

Laboratories that were accredited explicitly for the analysis of AA from food, performed in general well in the FAPAS studies. This was in contradiction to some findings of the JRC. In the second JRC test, the laboratory with the largest deviations from the assigned values was especially accredited for acrylamide analysis.

Finally, L. Castle gave an overview on planned FAPAS proficiency tests on the determination of AA from food. The next study will start in July on AA in coffee powder.

The JRC acrylamide level monitoring database

T. Wenzl presented briefly an overview on the EU-JRC database on acrylamide levels in food. He showed the evaluation of the 3500 accepted data in terms of distribution characteristics as well as plots of the acrylamide content of certain food classes against the production respectively expiry date of the product. It was said that the classification was quite rough for some types of food, e.g. fine bakery ware covers different kinds of biscuits. Therefore, for those products potential mitigation could have been lost by the classification. For others, e.g. potato crisps classification was narrow. However, a trend in the acrylamide content of the investigated food groups could not be identified. The database is available on the website of DG JRC-IRMM.

Feasibility studies on CRMs prepared in JRC and BAM

F. Ulberth reported on the work that is performed so far on the preparation of certified reference materials (CRM). He stated that there will be two reference materials, one produced at IRMM (crispbread, AA content between 300 and 500 $\mu\text{g}/\text{kg}$) and the other produced by the German Federal Institute for Materials Research and Testing (BAM) (crispbread, AA content between 800 and 1000 $\mu\text{g}/\text{kg}$). The homogeneity studies are finalised. Right know stability studies are ongoing. It was found that the material is stable at -20°C and $+4^\circ\text{C}$, but not at 40°C . The stability at room temperature is currently evaluated. F. Ulberth gave an overview on the planned characterisation study of the two reference materials, which will probably start in September and asked the experts to participate in it.

Discussion on future validation studies

During the discussion, the experts raised many topics as summarised in the following.

The task force members discussed intensively the limit of quantification and its determination. From the method descriptions of the participants of the JRC study, it was deduced that the LOQ was determined in many laboratories by automatic software routines and based on just the most intensive ion transition. It was felt that the measurement of just one transition could lead to wrong results due to interferences.

Concerning bad calibration practised in laboratories, it was discussed to set-up guidelines for the preparation of calibration standards. F. Ulberth reported that according to his experiences stock standard solutions are stable for at least three month. Other task force members confirmed this.

In general, the application of the Horwitz function for the determination of the target standard deviation of proficiency tests was questioned. Applying isotope dilution mass spectrometry (IDMS) should reduce the variability of the results much more. An indication for too generous attribution of performance indicators was found in the second JRC study, where the adjusted mean absolute deviation (MADe) of the results of the participants was lower than the respective Horwitz standard deviation. R. Stadler stated that methods have to be more precise to allow detection of mitigation. Otherwise, this will be lost in the variability of the analytical results. F. Ulberth reported that a standard measurement uncertainty of less than 1 % could be achieved in applying an extended IDMS approach. However, the definition of an appropriate target standard deviation is not possible due to a lack of knowledge which performance characteristics could be achieved in best case with one particular method.

It was explained by T. Wenzl that a standard method should be set and that as long as this standard does not exist, questions toward the reliability of analytical results will not stop. In addition, authorities of the EU Member States are waiting for a fully validated method. R. Stadler stated that methods should be validated for all relevant matrices. T. Wenzl suggested starting first with not too difficult, but relevant matrices, e.g. potatoes. R. Gatermann remarked that the relevance of a particular food regarding AA exposure of the population depends on nutritional habits and therefore very much on the country. E. Anklam proposed to validate a method on 3 to 4 different matrices according to the IUPAC protocol. She said that the need of fully validated methods was expressed at the Chicago workshop too. H. Chin commented that participants of the Chicago meeting passed this issue to this task force. S. Lalljie remarked that a good quality system is always based on the interaction of different elements, being good standard materials, methods and inter-comparison studies.

K.E. Hellenäs reported that HEATOX is going to investigate and to optimise two different methods (LC/MS/MS and GC/MS with derivatisation) for four food matrices, in particular potato chips, potato crisps, coffee and cereals. It is planned to validate these methods according to the IUPAC guidelines in collaboration with the JRC. Furthermore, the complexity of some food matrices (e.g. chocolate and gingerbread) and matrix related analytical problems were discussed. From the discussion, it could be concluded that one universal analytical method would be too complex and too laborious for applying it for simple food matrices such as crispbread. So it would be pointless to fully validate an analytical method that shows limited application in daily business, due to one of the before mentioned reasons. Just to give an example, FDA already described a general method, but in fact, none of the participants of the JRC proficiency test applied it. K.E. Hellenäs said that one SPE cartridge for the method (currently investigated at the Swedish National Food Administration) costs about 10 Euros.

Regarding the supply of appropriate test materials for the validation study, R. Stadler proposed to put this request to the Confederation of the Food and Drink Industries of the EU (CIAA), which could forward it to its members. Regarding the relevancy of food matrices it was stated that chocolate is not so relevant in terms of AA intake. Therefore, no urgent need for the validation of methods for the determination of AA in chocolate was seen. A severe problem was identified in the absence of suitable blank material to be included in the validation study. For that reason, it was proposed to reduce the number of concentration levels per food matrix being investigated in a collaborative trial from four to two.

Many concerns were expressed toward the applicability of one method with different generations of LC/MS/MS instruments. R. Gatermann informed that the sensitivity of instruments could be lowered if the mobile phase would be changed.

Finally, a consensus about the conditions for a method validation was not fully reached in this meeting.

It was agreed to wait for the outcome of the characterisation study for the certification of the reference materials and to design afterwards the validation study of the collaborative trial. Meanwhile results of the HEATOX partners on robustness of analysis methods would be available and a joint JRC-HEATOX method validation study will be carried out in near future. The JRC will work out a design for this validation and will circulate this to the members of the task force group for comments.

The next meeting of this task force group is envisaged to be held after the preparation of the CRMs and a potential other proficiency test organised by the JRC on coffee and cocoa powder.