JRC Scientific and Technical Reports

Proficiency Test on the Determination of Acrylamide in Potato Crisps

Final Report

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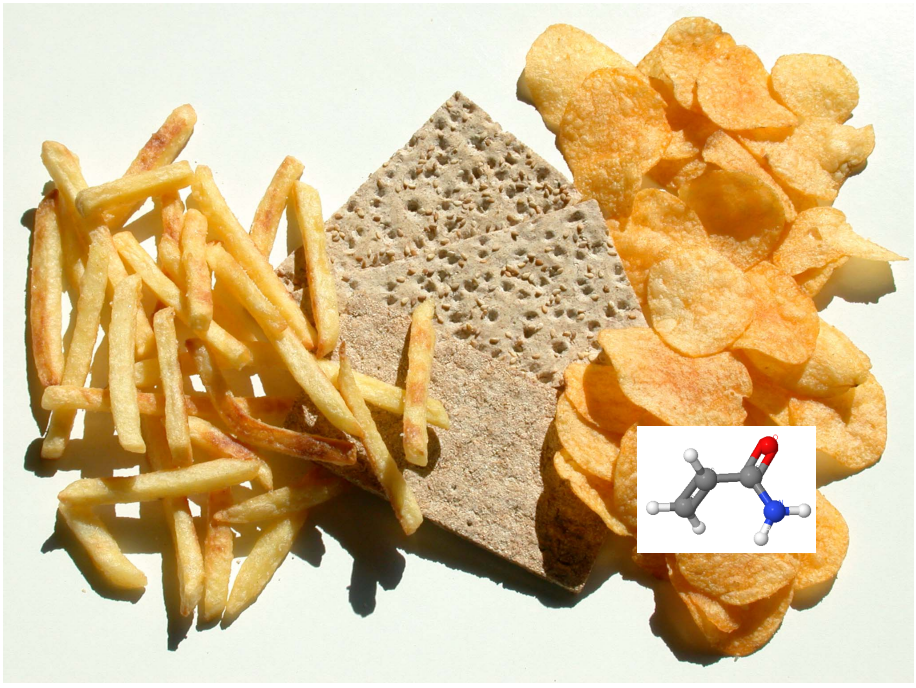

EUR 23276 EN - 2008

JRC EUROPEAN COMMISSION

irmm Institute for Reference Materials and Measurements

The mission of the IRMM is to promote a common and reliable European measurement system in support of EU policies.

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Joint Research Centre
Institute for Reference Materials and Measurements

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Summary

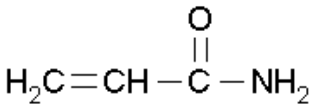
The Institute for Reference Materials and Measurements (IRMM) of the European Commission's Joint Research Centre (JRC) was requested by the Directorate General Health and Consumer Protection (DG SANCO) to organise a proficiency test on the determination of acrylamide in potato products in 2007. The aim of this test was to support the implementation of the acrylamide monitoring Recommendation 2007/331/EC.
The organisation of the study as well as the evaluation of the results was done in accordance with "The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories". The potato crisps used for the preparation of the test material was produced in a pilot plant of the German Federal Research Centre for Nutrition and Food (Detmold, Germany). The study was free of charge for the participants.
Altogether forty two laboratories from 16 EU Member States subscribed for participation in the study. The participants were asked to determine the acrylamide content in the test sample by application of their usual in-house analysis methods. The laboratories were requested to report the results via a web-interface into a secured databank.
In total, 36 result data sets were reported to the organisers of the study. Details regarding the applied analytical methods were requested from the participants. Thirty one participants filled in and returned the questionnaire with the method details back to the organisers.
An assigned value for the acrylamide content of the test material was established by an isotope dilution HPLC-MS/MS method. The target standard deviation was calculated according to a proposal of Thompson, which applies a concentration dependent modification of the Horwitz equation. The performance of laboratories was expressed by the z-score. They are considered satisfactory if the values of $|z| \leq 2$. Twelve laboratories of 36 (33.3%) reported results $|z| > 2$.
The percentage of successful laboratories is lower in comparison to previous proficiency tests organised by IRMM as well as to the last FAPAS® round. Reasons for this might be a more complex food matrix and/or the application of improper methods. However, the study showed the importance of continuous participation in proficiency testing schemes in order to achieve comparability of results.

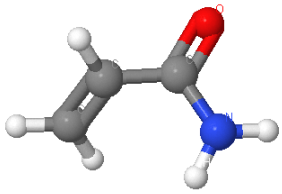
Contents

1. Introduction

Acrylamide (AA) is a substance that has found widespread application in industry, e.g. for the purification of drinking water and as a polymer in food packaging. Due to its toxicological properties, legal limits have been set for both drinking water and for migration into food [1-5]. Since the finding of elevated levels of acrylamide in heat-treated potato products and other foods was reported by the Swedish National Food Authority in April 2002, concerted efforts have been made to investigate the nutritional intake of this substance by monitoring its content in different kinds of food [6, 7].

Figure 1: Structure of acrylamide: a) structural formula, b) 3-D molecule model

a) 

b) 

The Institute for Reference Materials and Measurements (IRMM) of the European Commission's Joint Research Centre (JRC) organised three comparison tests among European laboratories on the determination of acrylamide in 2003 - 2004 [8-10]. From the first test, it became clear that additional training efforts would be necessary to improve the performance for a significant number of laboratories. Therefore, a second and third trial were organised by the JRC - IRMM to evaluate the progress of the laboratories.

The JRC - IRMM was requested by the Directorate General Health and Consumer Protection (DG SANCO) to organise a fourth trial in 2007. The aim of the fourth proficiency test was to support the implementation of the acrylamide monitoring Recommendation 2007/331/EC [11].

The inter-laboratory comparison test was free of charge for the participants, as in the previous cases. The organisation of the study as well as the evaluation of the results was done in accordance with "The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories", further-on denoted as "Harmonised Protocol" [12]. It was announced via DG SANCO to the competent authorities of EU Member States and EU Candidate Countries. Additionally all participants nominated by the competent authorities were informed by e-mail. Information concerning the application procedure for the study was

also made available on the homepage of the JRC-IRMM. Registration of participants was carried out via a special web-interface.

Altogether 42 laboratories from 16 EU Member States subscribed for participation in the study. Receipt of the test samples was confirmed by the participants via the sample receipt form (see Annex 2).

The participants were asked to determine the acrylamide content in the test sample by application of their usual in-house analysis methods. The laboratories were requested to report the results via the web-interface into a secured databank:

http://www.irmm.jrc.be/imepapp/jsp/loginResult.jsp

2. Test Material

2.1 Preparation

The potato crisp test material was produced in a pilot plant of the German Federal Research Centre for Nutrition and Food (Detmold, Germany). In order to prevent the introduction of heterogeneity in the samples by the grinding process, e.g. due to fatty particles that stick together, all samples were frozen in liquid nitrogen prior to processing and kept at temperatures of at least - 20 °C throughout grinding. The material was filled in portions of approximately 10 g in 20 mL clear glass vials capped with aluminium caps with silicone/PTFE septa and stored at -20 °C. Each vial was uniquely numbered. The homogeneity and stability of the samples were tested as it is described below.

2.2 Homogeneity of samples

Homogeneity was tested according to the Harmonised Protocol [12].

Ten randomly selected packages of test sample were analysed in duplicate applying a method based on isotope dilution high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). This method was previously validated in a collaborative trial organised by JRC-IRMM in 2005-6 [13].

Portions of 2.0 g of homogenised sample were placed into 50 ml Falcon tubes and extracted after addition of 400 ng of internal standard (d_3-acrylamide) solution with 40 ml of water by shaking intensively for 15-30 s on a Vortex shaker. Samples were defatted by addition of 2

mL of *n*-hexane. Afterwards they were put for 60 min on a horizontal shaker adjusted to maximum sample-extractant agitation speed (100 rpm) and centrifuged in a cooled centrifuge (10 °C, 4000 rpm, 20 min). A layer of *n*-hexane was removed.

An aliquot (10 mL) of the supernatant was passed through a preconditioned Isolute Multimode® solid phase extraction cartridge (6 mL, 500 mg) placed on a vacuum manifold. The eluate from the column was collected and then loaded on a preconditioned Isolute ENV+® solid phase extraction column (6 mL, 500 mg). The eluate was discarded and acrylamide was eluted with 3 mL of 60% methanol, which was evaporated from the extract by a gentle stream of nitrogen. The block temperature of the evaporator was set to maximum 40°C. The final volume of the extract was approximately 500 µL.

The quantification of acrylamide was performed by HPLC-MS/MS with electrospray ionisation in positive mode. Acrylamide was identified by selected reaction monitoring (SRM) set to record transitions m/z 72>72, 72>55 and 72>44. Monitored transitions for the internal standard were m/z 75>58 and 75>44. Quantification was performed by internal standardisation, monitoring the SRM transitions m/z 72>55 (acrylamide) and 75>58 (d_3-acrylamide).

The homogeneity of the test samples was proved by subjecting the results of the duplicate measurements obtained on ten different vials of the test material to one-way analysis of variance (ANOVA). As the variation of the acrylamide content between the ten different sample containers was not significantly larger than the variation within the containers (=method repeatability), it was concluded that the test material is sufficiently homogeneous as shown in Annex 3.

2.3 Stability of samples

The acrylamide content of the test material was monitored, using the above mentioned protocol, at the beginning of the study, during the study as well as after receipt of the results of the participants. Test samples were kept frozen for the period of the study. No statistically significant differences in the results were observed by ANOVA.

2.4 Dispatch of samples

All samples were sent via express mail in polystyrene boxes, together with approximately 1 kg of dry ice. The samples were received frozen, mostly within 24 hours after dispatch.

3. Statistical evaluation of the results

3.1 Assigned value

An assigned value for the acrylamide content of the test material was established by isotope dilution HPLC-MS/MS using the "bracketing technique" for calibration. The bracketing calibration method is frequently used for the establishment of reference values for the analyte contents of reference materials [14-15].

The isotope labelled acrylamide was added to the sample at a level close to that of the naturally present acrylamide level in the test material, which was roughly estimated in a preceding analysis. Two standard solutions containing native acrylamide were prepared in parallel:

Standard A: AA concentration level between 10 and 20 % lower than roughly estimated acrylamide content of sample

Standard B: AA concentration level between 10 and 20 % higher than roughly estimated acrylamide content of sample

The standards and the sample contained labelled AA at the same concentration level, which was close to the level of the assigned value. The sample and the standards were analysed in the following sequence: Standard A – Sample - Standard B - Standard B -Sample - Standard A - Standard A – Sample - Standard B - Standard B – Sample - Standard A. The measurement scheme was repeated on a second day with freshly (starting from the pure substances) prepared standards. The assigned value corresponds to the average value of all sample measurements of the two days, with the boundary condition that the average result of day one and the average result of day two had to agree within 3 %.

The acrylamide content of the sample was calculated for each standard-sample-standard triplet according to equation 1:

$$C = \left[\frac{(I_S - I_A) * (W_B - W_A)}{(I_B - I_A)} + W_A \right] * \frac{M_{Lab}}{M_S} \qquad \text{Equation 1}$$

C: AA content of the test sample (µg/kg)
I_S: ion intensity ratio of unlabelled/labelled AA measured in the test sample
I_A: ion intensity ratio of unlabelled/labelled AA measured in Standard A
I_B: ion intensity ratio of unlabelled/labelled AA measured in Standard B
W_A: mass ratio of unlabelled/labelled AA measured in Standard A
W_B: mass ratio of unlabelled/labelled AA measured in Standard B
M_{Lab}: mass of the labelled AA added to the sample (ng)
M_S: weight of the sample (g)

The combined uncertainty of the assigned value was estimated from the standard uncertainties of the different sources (weighing, purity of standards, and repeatability of measurements).
To validate the chosen analytical approach a certified reference material ERM®-BD273, acrylamide in toasted bread, was repeatedly analysed by the above mentioned method. The results did not differ from the certified value at the 95 % confidence level.
Results of the determination of the assigned value by isotope dilution HPLC-MS/MS are shown in Annex 4.

3.2 Performance indicator and target standard deviation

The performance of an individual laboratory *i* is expressed by the z_i-score, which is calculated according to equation 2:

$$z_i = \frac{x_i - \overline{X}}{\sigma} \qquad \text{Equation 2}$$

z_i: z-score of laboratory *i* for the respective sample; x_i reported result of laboratory *i* for that sample, expressed as the mean of duplicate determinations; $\overline{X}$: assigned value for the respective sample, σ: target standard deviation

The target standard deviation was calculated according to a proposal of Thompson, which applies a concentration dependent modification of the Horwitz equation [16]. Below an assigned value of 120 µg/kg, the target standard deviation is set to 22 % of the assigned value. Above that border value, it is calculated according to equation 3, which includes the assigned value, expressed as dimensionless mass ratio (1 µg/kg ~ 1 ppb = 10^{-9}):

$$\sigma = 0.02 \frac{\left(\overline{X} \times 10^{-9}\right)^{0.8495}}{10^{-9}} \qquad \text{Equation 3}$$

σ: target standard deviation; $\overline{X}$: assigned value (µg/kg)

z-Scores were calculated for the sample. The acceptability of a laboratory's performance was evaluated according to the following generally accepted limits [12]:

|z| ≤ 2.0	satisfactory
2.0 < |z| < 3.0	questionable
|z| ≥ 3.0	unsatisfactory

4. Data evaluation

4.1 Overview

In total, 36 result data sets were reported to the organisers of the study. The deadline for the reporting of results was extended twice on request of the participants to 14 November 2007.
In order to maintain confidentiality, the identities of the laboratories were coded by a unique number between 10 and 100.
Details regarding the applied analytical methods were requested from the participants. Thirty one participants filled in and sent the questionnaire with the method details back to the organisers. The details of applied methods are given in Annex 5.

Laboratories that reported measurement result for the acrylamide content of the samples were considered in the statistical evaluation of the results. A summary of the statistical evaluation is presented in table 1.
The distribution of the results was checked by kernel density estimation. This analysis is also capable of determining multimodality [12]. In general the results of analysis were not normally distributed and the respective kernel density plot showed several modes (figure 2). Consequently, a reference value estimated by a validated isotope dilution HPLC-MS/MS measurement procedure using bracketing for calibration was assigned to the test material.
Proportional representation of methods applied by the participants is shown in figure 3 and the numbers and percentages of the results with $|z| > 2$ related to the application of each particular method are presented in table 2.

Table 1: Summary statistics for potato crisps

Number of results		36
Range of results	µg/kg	34 to 758
Median	µg/kg	367
Huber H15	µg/kg	385
Major mode	µg/kg	375
Assigned value (isotope dilution HPLC-MS/MS)	µg/kg	344
Target standard deviation (Horwitz equation)	µg/kg	64
Number (percentage) of results of $\|z\| > 2.0$		12 (33.3 %)

Figure 2: Kernel density plot of the participants' results distribution

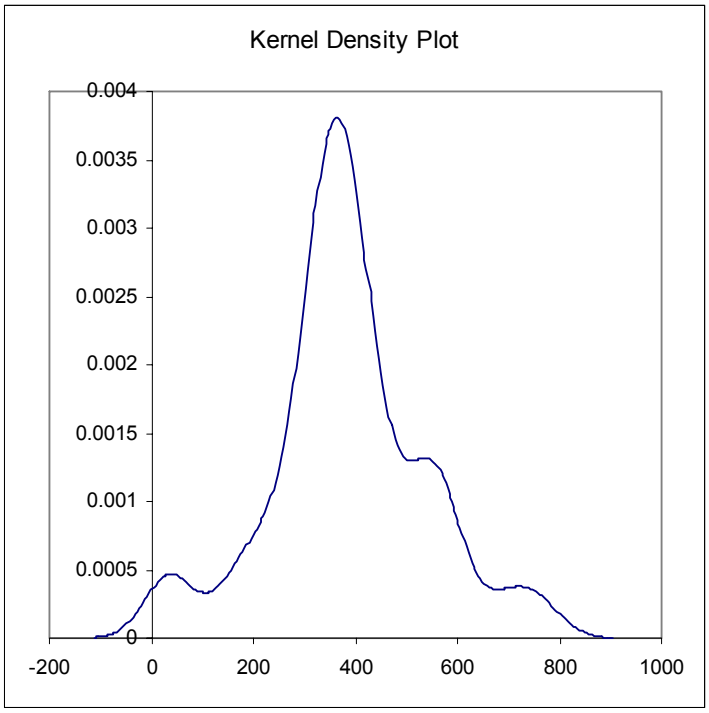
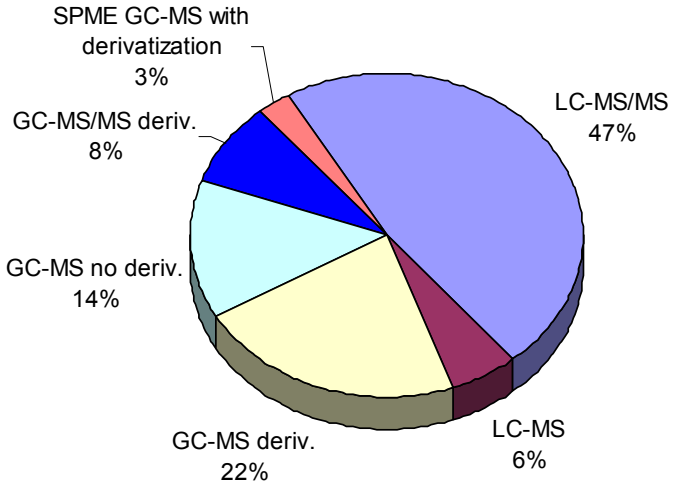


Table 2: Comparison of methods applied by the participants

method	number of participants	percentage of use	number of results with |z|>2	percentage of results with |z|>2 per method	percentage of total results with |z|>2
LC-MS/MS	17	47	4	23.5	11.1
LC-MS	2	6	1	50.0	2.8
GC-MS with derivatisation	8	22	2	25.0	5.6
GC-MS without derivatisation	5	14	3	60.0	8.3
GC-MS/MS with derivatisation	3	8	1	33.3	2.8
SPME GC-MS with derivatisation	1	3	1	100.0	2.8
total	36	100	12		33.3

Figure 3: Proportional representation of the analytical methods applied by the participants



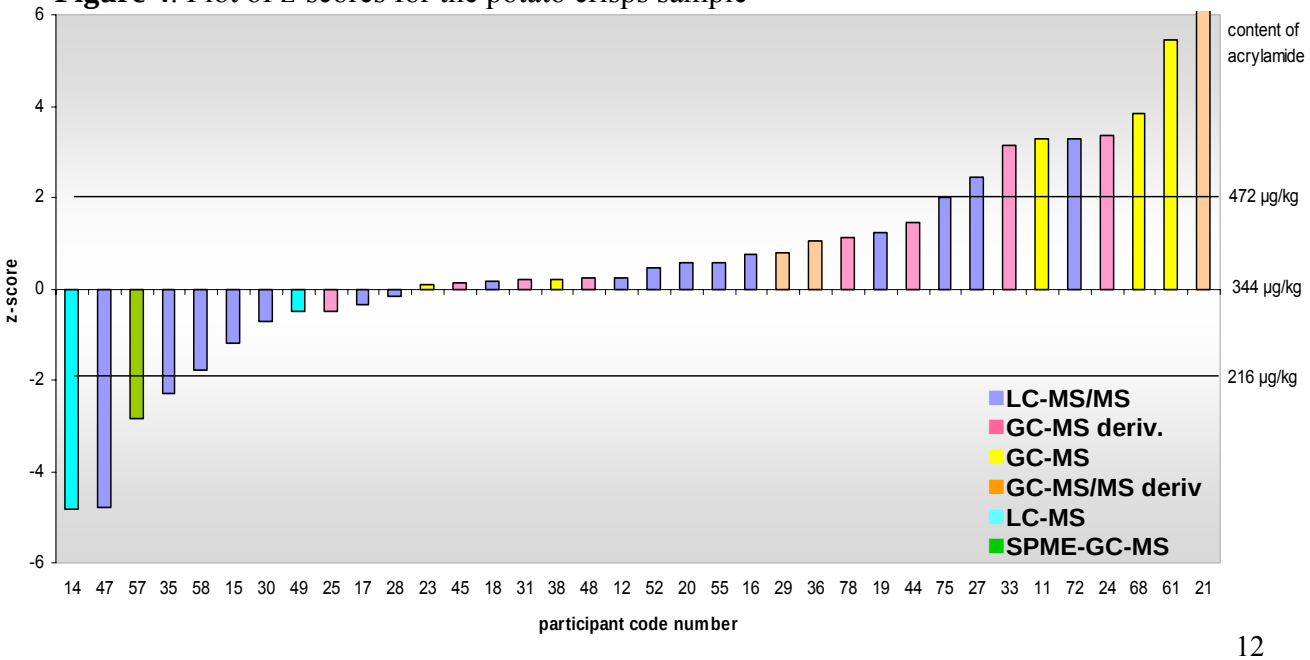
4.2 z-Scores of the participants

Twelve laboratories out of 36 (33.3%) reported results with |z|>2. Mean values of the determinations of acrylamide in the potato crisps sample are tabulated with the corresponding z-score in table 3. Figure 4 shows the plot of z-scores in ascending order.

Table 3: Results of analysis and z-scores for the potato crisp test samples; bold printed z-scores mark results outside the satisfactory range

Lab Number	reported result [µg/kg]	z - score	Lab Number	reported result [µg/kg]	z - score
11	554	**3.3**	**33**	545	**3.1**
12	360	0.2	**35**	198	**-2.3**
14	34.2	**-4.8**	**36**	412	1.1
15	268	-1.2	**38**	357	0.2
16	393	0.8	**44**	436	1.4
17	321	-0.4	**45**	352	0.1
18	354	0.2	**47**	37.0	**-4.8**
19	424	1.2	**48**	359	0.2
20	380	0.6	**49**	312	-0.5
21	758	**6.4**	**52**	374	0.5
23	350	0.1	**55**	381	0.6
24	559	**3.3**	**57**	161	**-2.8**
25	313	-0.5	**58**	230	-1.8
27	501	**2.4**	**61**	695	**5.5**
28	333	-0.2	**68**	591	**3.8**
29	395	0.8	**72**	556	**3.3**
30	298	-0.7	**75**	472	2.0
31	356	0.2	**78**	415	1.1

Figure 4: Plot of z-scores for the potato crisps sample



5. Conclusions

Sixty seven percent of participants reported results that were rated according to international guideline satisfactory, which means that a z-score ≤ |2| was attributed. However, this percentage is smaller than in previous proficiency tests organised by IRMM. It is also lower compared to the last FAPAS® proficiency test on the determination of acrylamide in crispbread (Test 3015, 88% of satisfactory z-scores) [17]. The more complex food matrix could have caused the higher percentage of underperforming laboratories.

Other potential reasons for unsatisfactory performance might be:

- A lack of experience with this type of analysis: A number of laboratories stated that they just stepped into this field.
- The application of improper methods: Three out of 12 not satisfactorily performing laboratories did not apply internal standardisation or did not apply an isotopic labelled internal standard. One participant applied an analysis method based on solid phase micro extraction (SPME) and gas chromatography mass spectrometry (GC-MS), which seems to be not suitable for the analysis of acrylamide in food.
- Additionally calculation/reporting errors might have led to unsatisfactory performance.

However, the study showed the importance of continuous participation in proficiency testing schemes in order to achieve comparability of demonstrated results.

6. References

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Annex

Annex 1: Announcement of Study

EUROPEAN COMMISSION
DIRECTORATE GENERAL JRC
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
IRMM

Geel, 28.08.2007
D08/TW/bk/ (2007) D 19996

Dear Madame/Sir,

The inter-laboratory comparison study on the determination of acrylamide in potato crisps has started today with the dispatch of sample.

Please be prepared to receive the sample within the next 3 days.

Please **store the sample frozen** (below -10 °C) in order to maintain sample integrity!

The sample shall be analysed in duplicate applying a method of your choice. The mean value of the duplicate analyses will be applied for calculation of performance indicators.

Results have to be reported via the web-interface:
http://www.irmm.jrc.be/html/interlaboratory_comparisons/acrylamide_in_crisps/index.htm

Deadline for reporting of results is: 05 October 2007

Mr. Karasek (Lubomir.Karasek@ec.europa.eu; Tel.: +32-14-571-301) and myself are at your disposal for any clarification you may wish!

With best regards

Thomas Wenzl

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Annex 2: Sample receipt form

EUROPEAN COMMISSION
DIRECTORATE GENERAL JRC
JOINT RESEARCH CENTRE
Institute for Reference Materials and Measurements
IRMM

Geel, 28.08.2007

Interlaboratory comparison test
on the anlysis of acrylamide from potato crisps

SAMPLE RECEIPT FORM

Name of Participant	
Organisation	
Address	

Please check if the sample has been received undamaged.

Date of sample receipt	
The sample has been received undamaged	Yes ☐ / No ☐

Please store the sample at below -10 °C!

Please return the completed form by email to: Lubomir.Karasek@ec.europa.eu

or by fax to: +32-14-571-343

Annex 3: Homogeneity data

Table 3.1: Homogeneity data for the potato crisps sample

sample id	rep 1	rep 2	count	sum	square	average	variance
1	327.8619	342.1146	2	670.0	448868.4	334.9882	101.5700
2	338.0581	339.1136	2	677.2	458561.6	338.5859	0.5570
3	318.6878	305.7427	2	624.4	389913.5	312.2153	83.7888
4	350.9954	318.3398	2	669.3	448009.6	334.6676	533.1973
5	334.8518	357.7374	2	692.6	479679.8	346.2946	261.8744
6	339.4386	338.0889	2	677.5	459043.4	338.7637	0.9108
7	329.4142	304.9035	2	634.3	402359.0	317.1589	300.3869
8	327.9289	320.4762	2	648.4	420429.2	324.2026	27.7716
9	324.2374	336.3184	2	660.6	436334.1	330.2779	72.9754
10	356.1713	324.8385	2	681.0	463774.3	340.5049	490.8705

mean	sd	cv
331.77	14.49	4.37

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2113.499	8	264.187314	1.5508066	0.24502441	2.94799
Within Groups	1873.903	11	170.354779			
Total	3987.401	19				

Sufficient Homogeneity

target σ	*F<Fcrit?*	s_s/σ
62.704	**ACCEPT**	0.109 *critical* $s_s/\sigma = 0.3$

Annex 4: Results of isotope dilution HPLC-MS/MS with bracketing calibration

	Day 1	Day2
Standard A [ng/mL]	312	319
Standard B [ng/mL]	422	439
D_3 - AA [ng/mL]	420	
$^{13}C_3$ - AA [ng/mL]		376
IS amount [ng]	420	376
Sample 1 [µg/kg]	371	336
Sample 2 [µg/kg]	342	369
Sample 3 [µg/kg]	324	347
Sample 4 [µg/kg]	320	346
average per day [µg/kg]	339	349
average [µg/kg]		**344**
uncertainty (k=2) [µg/kg]		14

Annex 5: Analytical methods applied by the participants

The method details are tabulated as they were reported by the participants. Not tabulated information was not submitted. It should be noted that the authors do not claim completeness of the given method details.

The following abbreviations are used:

AA	Acrylamide
AcN	Acetonitrile
CI	Chemical ionisation
EI	Electron ionisation
ESI+	Electrospray ionisation in positive mode
APCI	Atmospheric pressure chemical ionisation
EtAc	Ethyl acetate
I.D.	Internal diameter
LOD	Limit of detection
LOQ	Limit of quantitation
m/z	Mass/charge ratio
MeOH	Methanol
MP	Mobile phase
PCI	Positive chemical ionisation
RT	Room temperature
t-BME	tert-butyl methyl ether
SPME	Solid phase micro extraction
PLE	Pressurised liquid extraction

Table 5.1: LC-MS/MS - Standardisation and Extraction

Participant	**units**	**12**	**15**	**16**	**17**	**18**	**19**	**20**	**27**	**28**	**30**	**35**	**47**	**55**	**58**	**72**	**75**
Internal Standardisation		Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes
External Standardisation												Yes					
Internal Standard		D3-AA	D3-AA	D3-AA	D3-AA	D3-AA	13C3-AA	D3-AA	D3-AA	D3-AA	D3-AA		D3-AA	D3-AA	D3-AA	D3-AA	D3-AA
Equilibration of internal standard with sample		yes	yes	Yes	No	yes	No	no	Yes	yes	No			Yes	Yes	yes	No
Equilibration time	min	45	60	2		30			10	15				10	15	5	
Weight-in quantity *	g	0.7	2	1.0	2	1.0	2	1.0	5	2	2.00	0.2234 and 0.3950	1.0	1.0	2.0	2.0	1.0
Extraction solvent		Water	Water	Water acetone (1:4)	Water	Water	Water	Water	Water	Water acetonitrile 1:1	water	5% MeOH, 95% water with 0.2% of formic acid	1 mL water with 0.1% FA, 5 mL acetone	Water	Water	Water	water, 2M NaCl
Solvent volume	mL	40	40	15	40	40	20	5	100	100	40	25		10	20	15	10
Extraction temp	°C	25	RT	20	21	40	21	22	40	60	25	30		RT	60	22	ambient
Extract. time	min	45	60	15	60	30	1	10	10	5	60	30		20	30	3	5
Maceration time	min																
Sample / solvent ratio *	g/mL	0.02	0.05	0.07	0.05	0.03	0.10	0.20	0.05	0.02	0.05	0.10		0.10	0.10	0.13	0.10

* Figures are given as they have been reported.

Table 5.2: LC-MS/MS - Sample clean-up

Participant		**12**	**15**	**16**	**17**	**18**	**19**	**20**	**27**	**28**	**30**	**35**	**47**	**55**	**58**	**72**	**75**
Freezing after extraction																	
Defatting				Yes		Yes	Yes	Yes	Yes		No		Yes		Yes	Yes	
Defatting solvent				*n*-pentane		*n*-hexane 20 mL + butylmethyl ether (95+5,v+v)	*n*-hexane 10 mL	Dichloro methane	*Iso*hexane, *t*-butylmethyl ester				*n*-pentane, 10 mL		*n*-hexane	*n*-hexane, 4 mL	
Centrifugation of extract		Yes	Yes		Yes		Yes	Yes			Yes	Yes	Yes	Yes		Yes	
Ultrafiltration																	
Carrez precipitation						Yes			Yes	Yes					Yes	Yes	
Volumes of Carrez solutions I + II	mL + mL					1 + 1				2 + 2						1 + 1	
SPE		Yes	Yes	Yes	Yes	Yes	Yes, dispersive SPE	Yes	Yes		Yes		Yes	Yes		Yes	Yes
Cartridges		Isolute M-M 1g	Isolute M-M 1g	Oasis 180 mg	Isolute M-M 1g	Isolute MFC 18, 500 mg	magnesium sulphate	Oasis HLB	Isolute MFC 18		Isolute M-M 1 g		Bakerbond C18, 200 mg	Oasis HLB 200 mg/ 6 mL		Isolute M-M 300 mg	Waters HLB
		Isolute ENV+ 1g	Isolute ENV+ 1g		Isolute ENV+ 1g	OASIS HLB 200mg / 6mL	basic aluminium oxide				Isolute ENV+ 1 g			Bond Elut Accucat (200 mg/3 mL)			Waters MCX
Liquid/liquid extraction							change of solvent ACN to water			Yes, EtAc					addition of acetonitrile		
no special clean-up												yes					
Filtration				Yes		Yes		Yes		yes		yes	Yes	Yes			

Table 5.3: HPLC conditions

Participant		**12**	**15**	**16**	**17**	**18**	**19**	**20**	**27**	**28**	**30**
Inj. Vol	µL	10	10	20	10	50	20	100	10	10	10
Sample amount / injection	g/mL	0.20	1g of dry sample/mL		0.25	0.04	0.10	0.20			0.25
Column supplier		Thermo Fisher	Thermo Fisher	Waters	Thermo Fisher	Merck	Waters	1: Thermo Fisher 2: Waters	Thermo Fisher	Phenomenex	Thermo Fisher
Type		Hypercarb	Hypercarb	Atlantis C18	Hypercarb	Purospher RP 18e	Atlantis dC18	1: Hypercarb 2: Atlantis dC18	Hypercarb	Luna	Hypercarb
Length	mm	50	50	4.1	50	250	100	1: 100; 2: 150		150	50
I.D.	mm	2.1	2.1	2.1	2.1	4.6	3.0	1: 2.1; 2: 2.1		3.0	2.1
Particle size	µm	5	5		5	5	3	1: 3; 2: 5		3	5
Mobile phase		0.1% v/v acetic acid in water	0.1% v/v acetic acid in water	0,1% acetic acid, 0.5% MeOH	0.1% FA in water	water: methanol: formic acid (90:10:0.9)	5% water, 95% acetonitrile	methanol/acetic acid 1% (5+95)	0.2% formic acid, MeOH	90% water, 10% MeOH	0.1% FAc in water
MP flow	mL/min	0.40	0.40	1.00	0.40	0.40	0.30	0.25	0.20	0.30	0.40
Column temp	°C	ambient	20	20	25	60	35	30	25	60	20
Net-retention time	min	2.20	2.00	8.10	1.80	8.70	3.80	5.31	4.00	5.00	1.80
Ionisation		ESI+	ESI+	ESI+	ESI+	ESI+	ESI+	APCI+	APCI	ESI+	ESI+

Table 5.3: HPLC conditions - continued

Participant		**35**	**47**	**55**	**58**	**72**	**75**
Inj. Vol	µL	100	5	50	20	20	20
Sample amount / injection *	g/mL	0.008936 and 0.0158		0.005	0.050	0.13	0.20
Column supplier		Chrompack	Zorbax	Phenomenex	Merck	Phenomenex	Waters
Type		Spherisorb 5 ODS2	Eclipse XDB-C18	Synergi Hydro RP-80A	Lichrospher 100 CN	Polar RP	Atlantis C18
Length	mm	250	150	250	250	150	100
I.D.	mm	4.60	2.1	2	4.00	3.00	2.1
Particle size	µm	5		4.0		3	5
Mobile phase		95% water with 0.1% FA and 0.01 mM Ac acid, 5% MeOH	water:MeOH:ACN: FA 1% (80:10:5:5) to (0:90:5:5)	0.1% acetic acid and 0.5% MeOH	50%ACN in 1%AcA for 5 min, rinsing with 100% ACN 5 min, recond. 50%ACN in 1%AcA for 10 min	water, methanol	0.1% FA in water
MP flow	mL/min	0.60	0.20	0.20	0.70	0.30	0.15
Column temp	°C	40	25	RT	25	40	25
Net-retention time	min	6.20	2.80	7.60	5.90	4.00	3.50
Ionisation		ESI+	ESI+	ESI+	ESI+	ESI+	ESI+

* Figures are given as they have been reported.

Table 5.4: MS/MS conditions

Participant		12	15	16	17	18	19	20	27	28	30
Recorded transitions		72>55 72>54 75>58	72>55 75>58 72>54 72>44	72>55 72>72 72>44 72>55	72>55 72>44 75>58	72>55 72>44 75>58	72>55 75>58 72>54 72>57	72>55 75>58	72>55 75>58	72>55	72>55 72>54 75>58
LOD	µg/kg	< 5	10	30.00	7.5	20	15	16	8	5	1.20
LOQ	µg/kg	5	20	30	15	60	40	32	24	15	2.4

Table 5.4: MS/MS conditions - continued

Participant		35	47	55	58	72	75
Recorded transitions		quant. 44 qual. 54, 55	72>55 75>58	72>55 72>44	72>55 72>72	72>55 72>44	72>55 72>44
LOD	µg/kg	10	5	20.00	10	15	25
LOQ	µg/kg	50	10	40	30	30	50

Table 5.5: GC-MS with derivatisation - Standardisation and Extraction

Participant		**25**	**33**	**44**	**45**	**48**	**78**
Internal Standardisation		Yes	Yes	Yes	Yes	Yes	Yes
External Standardisation							
Internal Standard		D3-AA	D3-AA	D3-AA	13C3-AA	D3-AA	13C3-AA
Equilibration of internal standard with sample		No		No	Yes	No	Yes
Equilibration time	min				25		20
Weight-in quantity	g	5 to 20	2.0	5.0	2.0	2.5	3.77
Extraction solvent		Water	Water	Water	Water/MeOH (1:1)	Water	Water
Volume	mL	200	8	100	50	50	100
Extraction temp	°C	80	25	ambient	ambient	70	60
Extract. time	min	60		30	85	30	20
Addition of amylase		yes, 0.5 mL					

Table 5.6: GC-MS with derivatisation - Sample clean-up

Participant		**25**	**33**	**44**	**45**	**48**	**78**
Defatting			Yes			yes	
Defatting solvent			*n*-hexane			*n*-hexane, 2-3 mL	
Centrifugation		Yes			Yes		Yes
Carrez precipitation		Yes		yes		yes	
Volumes of Carrez solutions	mL + mL	1 + 1				0.5 + 0.5	
Derivatisation		bromination		bromination	bromination		
Reaction time					over night		
Reaction temp	°C						
Extraction solvent		EtAc	EtAc	EtAc	EtAc		
Extraction solvent volume	mL + mL	50		2	4 + 4		
SPE					silica C18	Strata-X-C 30 µm (200 mg/6mL)	
Liquid/liquid			Yes				
other treatment							
Final volume	mL				0.5 - 1		

Table 5.7: GC-MS with derivatisation - Chromatographic parameters

Participant		**25**	**33**	**44**	**45**	**48**	**78**
Inj. Vol		2	1	3	2	1	1
Sample amount / injection	g/mL	2.5 - 10		0.25		0.025	0.038
Injection technique		Splitless, 0.4 min	Splitless	splitless pulsed	Splitless	Splitless, 0.25 s	Split
Column supplier		SGE	Equity	J&W	J&W	J&W	J&W
Type		BPX50	Equity-5	FFAP	DB-17 MS	DB-5 MS	DB-WAX
Length	m	30	30	30	30	30	30
I.D.	mm	0.25	0.25	0.25	0.25	0.25	0.25
Film thickness	µm	0.25	0.25	0.20	0.15	0.25	0.25
Mobile phase		He		He		He	He
MP flow	mL/min	1.00	0.88	1.00	1.00	0.80	1.54
Temp. Program				60/1-20-100/0-5-185/0-20-230/5	110/2-8-180/0-30-320/0	80/1-15-170/0-22-250/5	50 - 150
Net-retention time	min	9.00		16.00	8.24	13.18	14.11
Ionisation		EI	EI	EI	EI	EI	EI
Recorded ions	m/z	150, 155; 106; 133	106, 153, 149, 110, 137	149, 133, 106	150, 106,108, 152	150, 155, 152, 153	149, 150, 152, 154
LOD	µg/kg		10	3	2	0.6	5
LOQ	µg/kg		30	40	10	1.7	15

Table 5.8: GC-MS without derivatisation - Standardisation and Extraction

Participant		**11**	**23**	**38**	**61**
Internal Standardisation		Yes	Yes	Yes	Yes
External Standardisation					
Standards		Methacrylamide	D3-AA	D3-AA	D3-AA
Equilibration of internal standard with sample		Yes	Yes	No	Yes
Equilibration time	min	2	15		20 - 80
Weight-in quantity	g	2.0	2.5	5.0	1.0
Extraction solvent		water	n-propanol	Water	2-propanol
Volume	mL	60	50	50	40 - 50
Extraction temp	°C	60	20	50	60
Extract. time	min	45	2	30	3*4 min
PLE					yes

Table 5.9: GC-MS without derivatisation - Sample clean-up

Participant		**11**	**23**	**38**	**61**
Defatting		Yes	Yes	by deep freezing	Yes
Defatting solvent		*n*-hexane	*n*-hexane, 2*2 mL		*n*-hexane
Centrifugation		Yes	Yes	Yes	Yes
SPE		Chromabond XTR	DON/MycoSep-cartridges (Romer)		
Carrez		Yes		Yes	Yes
Volumes		1 + 1		3 + 3	5.5
Liquid/liquid				3 * 20 mL EtAc	yes

Table 5.10: GC-MS without derivatisation - Chromatographic parameters

Participant		11	23	38	61
Inj. Vol	µL	1.5	1	2	1
Sample amount/injection	g/mL	5.00	0.25	5.00	0.20
Injection technique		Splitless	Splitless	Splitless	Splitless
Column supplier		J&W	Phenomenex	Varian	SGE
Type		FFAP	ZB WAX	CP WAX 52CB	SolGelWax
Length	m	30	60	60	60
I.D.	mm	0.25	0.25	0.25	0.25
Film thickness	µm	0.25	0.25	0.25	0.25
Mobile phase		He	He	He	He
MP flow	mL/min	1.00	1.00	30 cm/s	1.20
Temp. Program	°C	67/1-10-250/15	70/2-20-220/0-30-240/20	80/2-10-220/10	70/2-20-220/0-6-270/5
Net-retention time	min	15.70	11.90	16.25	8.00
Ionisation		PCI	CI	EI	PCI
Reactant gas		ammonia	methane		ammonia
Recorded ions	m/z	89, 103, 72, 106, 120	75,55	75, 72	89, 92, 72
LOD	µg/kg	10	30	30	10
LOQ	µg/kg	30	100	50	30

Table 5.11: GC-MS/MS - Standardisation and Extraction

Participant	**21**	**29**	**36**
Internal Standardisation	Yes	Yes	Yes
External Standardisation			
Internal Standard	D3-AA	D3-AA	13C3-AA
Equilibration of internal standard with sample	Yes	No	Yes
Equilibration time	1 - 2		2
Weight-in quantity	3.00	1.0	1.00
Extraction solvent	Water	Water	Water
Volume	20	50	2*30
Extraction temp	60	24	70
Extract. time	120	10	2*10

Table 5.12: GC-MS/MS – Derivatisation and sample clean-up

Participant	**21**	**29**	**36**
Defatting	yes		yes
Defatting solvent	hexane, 2*10 mL		hexane, 2*2 mL
Centrifugation	Yes	Yes	Yes
Carrez precipitation			Yes
Volumes of Carrez solutions			1 + 1
Derivatisation	bromination	bromination	bromination
Reaction time		1 hour	
Reaction temp			
Extraction solvent		EtAc	EtAc
Extraction solvent volume			
SPE			LiCrolut EN - 200 mg/3 mL
Liquid/liquid			
other treatment	filtration (PVDF)		
Final volume			1

Table 5.13: GC-MS/MS - Chromatographic parameters

Participant	**21**	**29**	**36**
Inj. Vol	1	1	2
Sample amount / injection	30	0.20	1
Injection technique	splitless	on-column, cool	splitless
Column supplier	J&W	Varian	Restek
Type	DB-35MS	CP Sil 24 CB	Rtx-5MS
Length	30	30	30
I.D.	0.25	0.25	0.25
Film thickness	0.25	0.25	0.25
Mobile phase	He	He	He
MP flow	40 cm/s	1.00	1.00
Temp. Program	65/1-15-250/10	55/2-17.5-220/2-30-270/3	55/1-10-270/15
Net-retention time	10.50	10.76	11.70
Ionisation		EI	EI
Recorded ions	152, 135, 155, 137	152>135, 152>109,	152>135, 150>133, 153>136, 155>138
LOD	11	7	5
LOQ	40	21	10

Table 5.14: LC-MS - Standardisation and Extraction

Participant	**units**	**14**	**49**
Internal Standardisation			Yes
External Standardisation		yes	yes
Internal Standard			13C3-AA
Equilibration of internal standard with sample			yes
Equilibration time	min		10
Weight-in quantity	g	1	0.450
Extraction solvent		Water	Water
Solvent volume	mL	10	5
Extraction temp	°C	RT	25
Extract. time	min	60	20
Maceration time	min		
Sample / solvent ratio	g/mL	0.10	0.09

Table 5.15: LC-MS - Sample clean-up

Participant		**14**	**49**
Freezing after extraction			
Defatting			
Defatting solvent			
Centrifugation of extract			yes
Ultrafiltration			
Carrez precipitation			yes
Volumes of Carrez solutions I + II	mL + mL		0.75 + 0.75
SPE		Yes	Yes
Cartridges			Oasis HLB, 30 mg, 1mL
Liquid/liquid extraction			
no special clean-up			
Filtration			

Table 5.16: LC-MS - Chromatographic parameters

Participant		**14**	**49**
Inj. Vol	µL	5	60
Sample amount / injection	g/mL		0.07
Column supplier		Supelco	GLC Sciences
Type		Discovery C18	ODS - Inertsil ODS3
Length	mm	250	25
I.D.	mm	4.6	4.6
Particle size	µm		5
Mobile phase		0.1% v/v acetic acid in water + 0.5% MeOH	0.2% v/v formic acid in water
MP flow	mL/min	1.00	0.60
Column temp	°C	RT	RT
Net-retention time	min	2.00	9.0 - 9.6
Ionisation		ESI+	ESI+
Recorded Ions		72	72.1
			75.1
LOD	µg/kg		12
LOQ	µg/kg		45

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Abstract

The Institute for Reference Materials and Measurements (IRMM) of the European Commission's Joint Research Centre (JRC) was requested by the Directorate General Health and Consumer Protection (DG SANCO) to organise a proficiency test on the determination of acrylamide potato products in 2007. The aim of this test was to support the implementation of the acrylamide monitoring Recommendation 2007/331/EC.
The study was a dedicated collaborative trial and was free of charge for the participants. The organisation of the study as well as the evaluation of the results was done in accordance with "The International Harmonised Protocol for the Proficiency Testing of Analytical Chemistry Laboratories".
Altogether 42 laboratories from 16 EU member states subscribed for participation in the study. The participants were asked to determine the acrylamide content in the test sample by application of their usual in-house analysis methods. In total, 36 data sets were reported to the organisers of the study.
The performance of laboratories was expressed by the z-score.
The percentage of successful laboratories is lower in comparison to previous proficiency tests organised by IRMM. Reasons for this might be a more complex food matrix and/or the application of improper methods. However, the study showed the importance of continuous participation in proficiency testing schemes in order to achieve comparability of results.

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.

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