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Proficiency test on the determination of furan in baby food

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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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Summary

The Institute for Reference Materials and Measurements (IRMM) of the European Commission's Joint Research Centre (JRC) was requested by the Directorate General for Health and Consumers (DG SANCO) to organise a proficiency test on the determination of furan in baby food in 2008. The aim of the proficiency testing (PT) was to support the implementation of Commission Recommendation 2007/196/EC which recommends Member States to monitor the presence of furan in foodstuffs that have undergone heat treatment.
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 test material was a commercial baby food consisting of carrot and potato puree.
Altogether 25 laboratories from 16 EU Member States subscribed for participation in the study, which was free of charge for the participants. They were asked to determine the furan content in the test samples by application of their usual in-house analytical methods. The laboratories were requested to report the results via a web-interface into a secured database.
In total, 22 data sets were reported to the organisers of the study. Details regarding the applied analytical methods were requested from the participants too.
The assigned value for the furan content of the test material was established by an isotope dilution GC-MS method. The target standard deviation was calculated according to the Thompson-modified Horwitz equation. The performance of laboratories was expressed by z-scores, which are considered satisfactory if $|z| \leq 2$. 16 out of 22 (73%) participants reported results that were rated satisfactory. Six laboratories (27 %) reported results $|z| > 2$. In two cases, underperforming might be due to calculation mistakes, since the reported values deviated from the assigned value by more than a factor of 10. The complexity of the food matrix, the high volatility of furan, and the application of improper methods might have contributed as well.
The results from the PT:

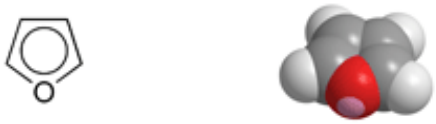
- demonstrate that the majority of the laboratories participating in the PT are capable of analysing furan in food matrices;
- confirm that laboratories with more experience in analysing furan on a regular basis perform better than those less experienced (cf. z-scores);
- show that both types of methods: the static headspace extraction gas chromatography mass spectrometry (HS-GC-MS) and the headspace solid phase micro-extraction gas chromatography mass spectrometry (HS-SPME-GC-MS) are equally suitable for analysis of furan in food matrices.

Contents

1. Introduction

The presence of furan (Fig. 1), which can be formed during the Maillard reaction, in food is a cause of public concern, since this substance was classified by the International Agency for Research on Cancer (IARC) [1] as possibly carcinogenic to humans (2B). Analytical methods for the determination of furan in food are most frequently based on headspace gas chromatography mass spectrometry (HS-GC-MS) [2, 3, 4].

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



a) b)

In 2008 the JRC - IRMM was requested by the Directorate General for Health and Consumers (DG SANCO) to organise in 2008 a proficiency test (PT) on the determination of furan in baby food. The aim of the PT was to support the implementation of Commission Recommendation 2007/196/EC of 28 March 2007 [5] which recommends Member States to monitor the presence of furan in foodstuffs that have undergone heat treatment. 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" [6]. 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 (see Annex 1). 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. The inter-laboratory comparison test was free of charge for the participants.

Altogether 25 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 furan content in the test sample by application of their usual in-house validated analytical method. The laboratories were requested to report the results via the web-interface into a secured database:

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

2. Test Material

2.1 Preparation

The samples of commercially available baby food were purchased from local retailers in Belgium in 2007. Experiments have shown that the furan content in different jars of the same batch of baby food varies. Therefore, the content of 12 jars was mixed and analysed. In order to prevent furan losses during the sample preparation special focus was put on maintaining the temperature of samples below 10 °C. The content of 12 jars of carrot/potatoes baby food cooled to a temperature of 3 °C to 4 °C was transferred into a 2.0 dm^3 pre-cooled Dewar flask. The baby food was then mixed for 60 seconds using a hand blender (Robert Bosch GMBH, Slovenia). Afterwards it was filled into 22 ml crimp cap glass vials (about 2/3 of vial volume), containing a magnetic stirrer bar in order to facilitate homogenisation of the content during the thawing procedure, and closed with aluminium/silicone crimp caps. The samples were labelled according to the sequence of bottling and stored frozen.

2.2 Homogeneity of samples

Homogeneity was tested according to the Harmonised Protocol [6]. Ten samples were randomly selected covering the whole sequence of bottling, and duplicate analysis by static headspace extraction gas chromatography mass spectrometry (HS-GC-MS) was performed on each sample within 24 hours. Previously homogenised (using the magnetic stirrer bar) portions of 1 g of baby food were weighed into 22 mL headspace vials, which were immediately crimped after addition of 13 ml of saturated aqueous potassium sulphate solution. Internal standardisation with isotope labelled furan (d4-furan, 99.0 % purity, Dr. Ehrenstorfer GmbH, Augsburg, Germany), was applied for quantification.

The instrument setup consisted of a GC-MS (6890 GC and 5973 MS both from Agilent Technologies, Diegem, Belgium) and a static headspace sampler (TurboMatrix 40, PerkinElmer, Zaventem, Belgium). The headspace sampler parameters were as follow: oven temperature 40 °C, sample loop temperature 60 °C, transfer line temperature 60 °C, equilibration time 30 min, cycle time 50 min, vial pressurization time 30 s, loop fill time 18 s, loop equilibration time 6 s. Helium was used as carrier gas. A Supel-Q^{TM} PLOT (Supelco, Bellafonte, PA, USA) capillary column (30 m length and 0.32 mm internal diameter) was used for chromatographic separation. The GC oven was heated from 50 °C to 225 °C with a heating rate of 10 °C/min and held at 225 °C for 12.5 min. The total run-time was 30 min. The GC inlet temperature was 200 °C. The mass spectrometer was operated in electron

ionization, and single ion monitoring mode. Furan was determined by monitoring of the ion m/z 68, and the identity was confirmed by fragment ion m/z 39. The recorded molecular ion of the labelled internal standard was m/z 72.

Samples were analysed in random order. The homogeneity of the test samples was evaluated 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 furan content between the ten different sample vials was not larger than the variation within the sample vials (=method repeatability), it was concluded that the test material is sufficiently homogeneous (data shown in Annex 3).

2.3 Stability of samples

The furan 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 changes in furan content were observed.

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 furan content of the test material was established by isotope dilution HS-GC-MS using the bracketing technique for calibration. The bracketing calibration method is frequently used for the establishment of reference values and value assignment of reference materials [7-8].

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

Standard A: Furan mass ratio of about 40 µg/kg

Standard B: Furan mass ratio of about 60 µg/kg

The standards and the sample contained labelled furan at very similar concentration levels, which was on day one about 38 µg/kg and on day two about 45 µg/kg. 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.

The furan 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: furan content of the test sample (µg/kg)

I_S: ion intensity ratio of unlabelled/labelled furan measured in the test sample

I_A: ion intensity ratio of unlabelled/labelled furan measured in Standard A

I_B: ion intensity ratio of unlabelled/labelled furan measured in Standard B

W_A: mass ratio of unlabelled/labelled furan measured in Standard A

W_B: mass ratio of unlabelled/labelled furan measured in Standard B

M_{Lab}: mass of the labelled furan added to the sample (ng)

M_S: mass 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).

The repeatability of measurements was estimated form pooled bracketing data. The expanded uncertainty of the assigned value was calculated by multiplying the combined standard uncertainty of the assigned value by a factor of two, which corresponds to a confidence level of approximately 95 %.

Results of the determination of the assigned value by HS-GS-MS are shown in Annex 4.

The assigned value was 44.2 µg/kg with an expanded uncertainty (k=2) of 2.1 µg/kg.

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:

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

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; $\bar{X}$: 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 [9]. Below an assigned value of 120 µg/kg, the target standard deviation is set to 22 % of the assigned value. Hence the value of target standard deviation was σ=9.7 µg/kg (see Table 1).

The acceptability of a laboratory's performance was evaluated according to the following generally accepted limits [6]:

$\lvert z \rvert \leq 2.0$	Satisfactory
$2.0 < \lvert z \rvert < 3.0$	Questionable
$\lvert z \rvert \geq 3.0$	Unsatisfactory

4. Data evaluation

4.1 Overview

In total, 22 results were reported to the organisers of the study. They are listed in Table 3 together with the corresponding performance indicators. The identities of the laboratories were randomly coded by a number between 1 and 22.

The distribution of the results was checked by kernel density estimation. This analysis is capable of determining multimodality [6]. In general the results of analysis were not normally distributed and the respective kernel density plot of all data showed two modes (Figure 2a). The mode at the higher concentration was caused by an outlier. For better illustration, Figure 2b contains the kernel density plot with a narrower concentration scale. Robust statistics was applied for the estimation of the mean, employing a macro for Microsoft Excel that can be downloaded form the homepage of the Analytical Methods Committee of the Royal Society of Chemistry [10]. The values of different estimators: median, A15 mean and H15 mean, major mode and assigned value, are presented in Table 1. All of them are not significantly different form each other. However taking into account the limited number of participants, which could impact on the consensus value calculated by robust statistics from the results of the participants [11], a reference value established by isotope dilution HS-GC-MS using bracketing calibration was assigned to the test material and used for the calculation of z-scores.

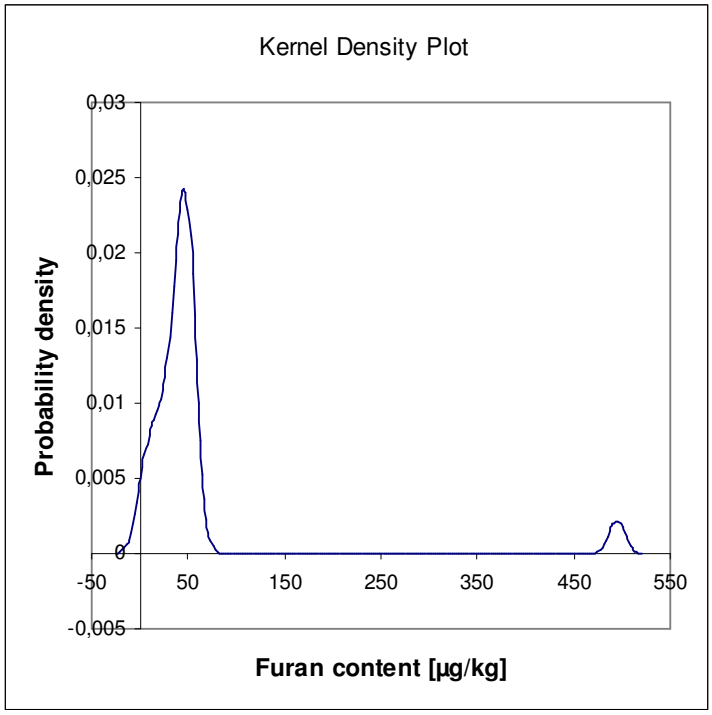
Table 1: Summary statistics for furan

Estimate		Estimate value
Number of results		22
Range of results	[µg/kg]	0.32 to 495
Median	[µg/kg]	43.4
A15 mean	[µg/kg]	39.2
H15 mean	[µg/kg]	38.3
Major mode	[µg/kg]	45.9
Assigned value (isotope dilution HS-GC-MS)	[µg/kg]	44.2

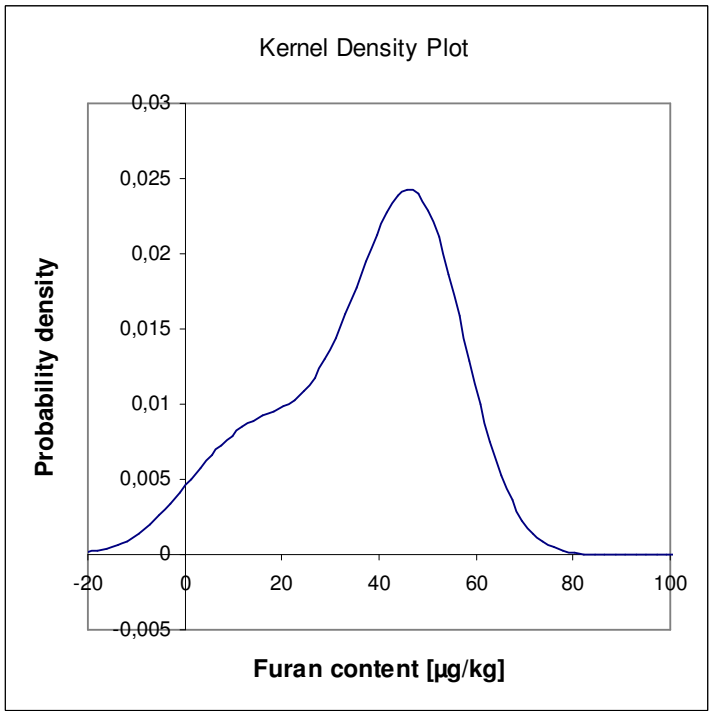
Selected details regarding the applied analytical methods were requested from the participants. 22 participants filled in and returned the questionnaire on method details to the organisers. They are compiled in Annex 5.

Figure 2: Kernel density plot of the participants' results distribution: a) full scale; b) narrow scale

a)



b)



The Box-and-Whisker Plot of the reported results according to the analytical methods applied by the participants is shown in Figure 3. The numbers and percentages of the results with |z|>2 related to the application of the respective method are presented in Table 2. About 70% of laboratories applied static HS-GC-MS for the determination of furan in baby food. The rest employed headspace solid phase micro-extraction gas chromatography mass spectrometry (HS-SPME-GC-MS) for that purpose. However, the performance of the methods was comparable. Bias inherent to the analytical method could not be identified.

Figure 3: Box-and-Whisker Plot of the reported results according to the analytical methods applied by the participants.

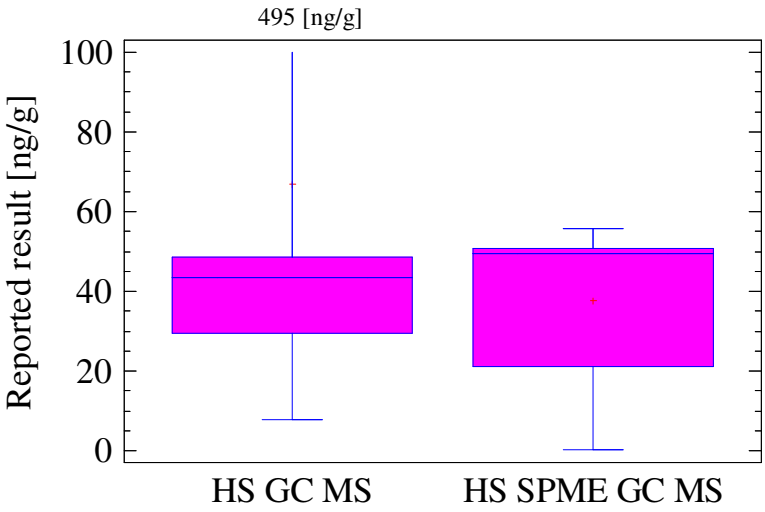


Table 2: Comparison of methods applied by the participants

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
static HS-GC-MS	15	68	4	27	18
HS-SPME-GC-MS	7	32	2	29	9
Total	22	100	6	-	27

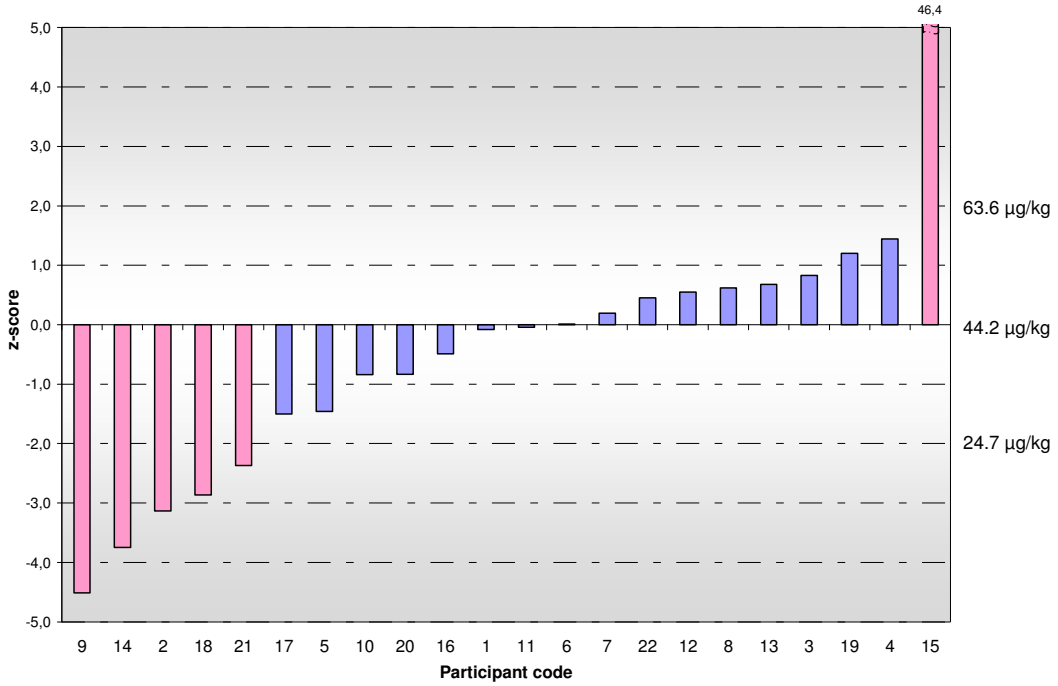
4.2 z-Scores of the participants

Of the 22 laboratories 16 (~73%) reported results with |z|<2. Mean values of the determinations of furan in the baby food samples are tabulated with the corresponding z-score in Table 3. Figure 4 shows the plot of z-scores in ascending order. Pink bars indicate results outside the satisfactory performance range.

Table 3: Results of analysis and z-scores for the baby food test samples; bold printed z-scores mark results outside the satisfactory range; number of significant figures of results are given as reported

Laboratory code	Reported result [µg/kg]	z-Score		Laboratory code	Reported result [µg/kg]	z-Score
9	0.32	**-4.5**		11	43.75	0.0
14	7.75	**-3.7**		6	44.25	0.0
2	13.7	**-3.1**		7	46.05	0.2
18	16.35	**-2.9**		22	48.55	0.5
21	21.15	**-2.4**		12	49.50	0.6
17	29.55	-1.5		8	50.15	0.6
5	30.00	-1.5		13	50.75	0.7
10	36.00	-0.8		3	52.20	0.8
20	36.05	-0.8		19	55.80	1.2
16	39.40	-0.5		4	58.17	1.4
1	43.35	-0.1		15	495.00	**46.4**

Figure 4: Plot of z-scores for the baby food sample (pink bars indicate z-scores outside the satisfactory performance range)



5. Conclusions

The results from this PT demonstrate that 16 out of 22 (~ 73%) participants are capable of analysing furan in food matrices and reported results that were rated satisfactory according to international guidelines. Underperformance of about 27% of laboratories could be due to the complexity of the food matrix and the high volatility of furan. The reasons for unsatisfactory performance of two participants might be due to calculation/reporting errors (outliers). Also the lack of experience appears to have an effect since 50% of laboratories stated that they analysed less than 10 samples per month; this ratio was even higher (five out of six) for the laboratories with z-score > |2|. Concerning analytical methodology, two types of methods HS-GC-MS and HS-SPME-GC-MS were applied by the participants and both of them are equally suitable for analysis of furan in food matrices.

The results of this proficiency test are similar to the FAPAS® proficiency test on furan in baby food (round 3017) organised in 2007 [12] where 68% (19 out of 28 participants) got satisfactory z-scores results.

The study showed the importance of frequent participation in PT in order to achieve comparability of results. Considering the fact that 22 laboratories from only 15 (out of 27) European Union countries participate in this PT further steps should be considered in order to check the implementation of Commission Recommendation 2007/196/EC of 28 March 2007 [5] in the countries which were not represented in this PT.

Acknowledgements

We thank the following internal reviewers for their comments: Donata Lerda, Anne-Mette Jensen and Franz Ulberth.

6. References

[1] International Agency for Research on Cancer IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, **63,** (1995), Lyon, France

[2] Report of the CONTAM Panel on provisional findings on furan in food. (2005) http://www.efsa.europa.eu/en/science/contam/contam_documents/760.html

[3] Becalski A., D. Forsyth, V. Casey, B. P.-Y. Lau, K. Pepper, S. Seaman. Development and validation of a headspace method for the determination of furan in food. Food Additives and Contaminants, **22**, (2005), 535-540

[4] Goldmann T., A. Perisset, F. Scanlan, R. H. Stadler. Rapid determination of furan in heated foodstuffs by isotope dilution solid phase micro-extraction-gas-chromatography-mass spectrometry. Analyst, **130**, (2005), 878-883

[5] Official Journal of the European Union L 88, 29.03.2007, p. 56

[6] M. Thompson, S.L.R. Ellison, R. Wood. The International Harmonized Protocol For The Proficiency Testing of Analytical Chemistry Laboratories. Pure Appl. Chem., **78**, (2006), 145-196

[7] M. Sargent, R. Harte, C. Harrington, Guidelines for Achieving High Accuracy in Isotope Dilution Mass Spectrometry (IDMS). RSC Analytical Methods Committee, LGC Limited, 2002

[8] CCQM-K6: Key Comparison on the Determination of Cholesterol In Serum, Final Report, NIST, 2001

[9] M. Thompson. Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing. Analyst, **125**, (2000), 385-386

[10] http://www.rsc.org/Membership/Networking/InterestGroups/Analytical/AMC/Software/kerneldensities.asp

[11] M. Belli, S. L. R. Ellison, A. Fajgelj, I. Kuselman, U. Sansone, W. Wegscheider. Implementation of proficiency testing schemes for a limited number of participants. Accred Qual Assur, **12**, (2007), 391-398

[12] FAPAS® Proficiency Test 3017, Furan, June -July 2007, Report, CSL, Joanne Croucher, York, UK

Annex

Annex 1: Announcement of the Study

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

Geel, 11.04.2008
D08/FSQ/bk/D(2008) 9415

Dear Madame/Sir,

The inter-laboratory comparison study on the determination of furan in baby food will start next week (14-18 April 2008) with the dispatch of samples.

Please be prepared to receive the samples in the course of next week.

Please **store the samples (two 22 ml vials) frozen** (at below -10 °C) in order to maintain sample integrity! Both vials contain the same carrot baby food test material, which was prepared in one batch.

Please analyse the content of each vial once, applying a method of your choice. The mean value of the analyses will be applied for calculation of performance indicators.

Results have to be reported via the web-interface:
https://irmm.jrc.ec.europa.eu/ilc/ilcReporting.do
using your password key: EGUC6228827

Deadline for reporting of results is: 22 May 2008

Mr. Kubiak (aleksander.kubiak@ec.europa.eu; Tel.: +32-14-571-313) and myself are at your disposal for any clarification you may wish!

With best regards

Thomas Wenzl

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Tel.: +32-(0)14-571 211 - Direct line: 320 •Fax: +32-(0)14-584 343; Email: Thomas.Wenzl@ec.europa.eu
http://www.irmm.jrc.be

Annex 2: Sample receipt form

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

Geel, 15.04.2008

Interlaboratory comparison study
on the on the determination of furan in baby food

SAMPLE RECEIPT FORM

Name of Participant	
Organisation	
Address	

Please check if the sample (consisting of two 22 mL headspace vials) has been received undamaged.

Date of sample receipt	
The sample has been received undamaged.	Yes ☐ / No ☐
There was still dry ice in the package	Yes ☐ / No ☐

Please store the sample at below -10 °C!

Please return the completed form by email to: **aleksander.kubiak@ec.europa.eu**

or by fax to: +32-14-571-783

Retieseweg 111, B-2440 Geel, Belgium
Tel.: +32-(0)14-571 211 - Direct line: 320 •Fax: +32-(0)14-584 783; Email: Thomas.Wenzl@ec.europa.eu
http://www.irmm.jrc.be

Annex 3: Homogeneity data

Table 3.1: Homogeneity data for the furan samples

sample id	rep 1	rep 2	count	Sum	square	average	variance
1	**51.9**	**49.2**	2	101.1	10221.2	50.6	3.6
2	**57.6**	**53.1**	2	110.7	12254.5	55.4	10.1
3	**46.2**	**48.0**	2	94.2	8873.6	47.1	1.6
4	**49.7**	**50.8**	2	100.5	10100.3	50.3	0.6
5	**48.0**	**45.5**	2	93.5	8742.3	46.8	3.1
6	**51.6**	**51.1**	2	102.7	10547.3	51.4	0.1
7	**54.0**	**55.3**	2	109.3	11940.3	54.6	0.9
8	**54.3**	**56.3**	2	110.6	12232.4	55.3	2.0
9	**51.8**	**49.7**	2	101.5	10302.3	50.8	2.2
10	**44.5**	**46.9**	2	91.4	8354.0	45.7	2.9

mean	sd	cv
50.8	3.6	7.2

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	224.9	9	25.0	9.2	0.0	3.0
Within Groups	27.2	10	2.7			
Total	252.1	19				

target σ	*F<Fcrit?*	s_s/σ
12,7		0,26
		critical s_s/σ ***= 0.3***
	no->ss	**ACCEPT**

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

	Day 1	Day2
Standard A [ng/g]	39.7	39.5
Standard B [ng/g]	59.5	59.6
D4 [ng/g]	**37.4**	
D4 [ng/g]		**45.2**
IS amount [ng]	**37.4**	**45.2**
Sample 1 [µg/kg]	49.3	42.7
Sample 2 [µg/kg]	46.3	44.1
Sample 3 [µg/kg]	41.9	43.1
Sample 4 [µg/kg]	45.8	39.9
Day average [µg/kg]	**45.8**	**42.5**
average [µg/kg]		**44.2**
uncertainty (k=2) [µg/kg]		**2.1**

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 participants do not claim completeness of the given method details.

Table 5.1: Sample storage temperature and special measures during sample handling ("X" means applicable, "-" not applicable)

Laboratory number #	Sample storage temperature			Sample handling temperature		Special measures during sample handling
	< 0 °C	0-10 °C	> 10 °C	Homogenisation	Subsampling	
1	X	-	-	4-10 °C	4-10 °C	The sample remained frozen until required for analysis. In this case during homogenisation the sample remained in the sealed headspace vial. Weighing of chilled sample was carried out as quickly as possible.
2	X	-	-	4 °C	10 °C	None
3	X	-	-	4 °C	4 °C	Headspace vials were stored in a cooled headspace-vial-rack.
4	X	-	-	<10 °C	<10 °C	Sample weighted into cold headspace vials, diluted each test portion with 10 ml water (<10 °C)
5	X	-	-	2-8 °C	2-8 °C	Cooling, doing handling as fast as possible.
6	X	-	-	25 °C	2 °C	The sample vial was chilled in a ice bath before opening. A subsample was drawn quickly using a plastic tubing (with the same diameter as vial opening) fit to a syringe, added to the headspace vial with cold water placed on a balance and then vial was sealed directly.
7	X	-	-	6 °C	20 °C	Weighing at ambient room temperature but sample cooled with cooler bags, shaking in cold room (6 °C)
8	X	-	-	4 °C	4 °C	During handling, the samples were maintained at 4 °C (ice/water bath) in a closed vial. The transference of the sample from the original container to the analysis vials was performed very quickly to avoid possible losses of furan.
9	X	-	-	>5 °C	not measured	The sample was homogenized immediately after being taken out from the fridge where it was stored overnight. While weighing, the sample was stored in an ice bath.
10	X	-	-	around 10 °C	15 °C	Minimum time for handling. Recipients for sub-sampling immersed in water and ice.
11	X	-	-	5 °C	5 °C	Sample handling, IStd Handling (Furane-d4) and standard addition handling as cool as possible (refrigerator temperature).
12	X	-	-	0-4 °C	0 -4 °C	Avoidance of keeping at room temperature (as much as possible), storage on ice when out of fridge, use of cold solutions, keeping the vials closed (as much as possible).
13	X	-	-	20 °C	4 °C	Working at 4 °C, quickly transfer the sample (after opening the vial) and close the analysis vial.
14	X	-	-	5-10 °C	5-10 °C	Prevent open handling, only opening for weighing 5 g into another headspace vial and adding internal standard solution. After closing, opening the next sample.
15	X	-	-	5 - 10 °C	5 - 10 °C	Try to keep everything as cold as possible. After weighing directly closing the vials. Handling of the sample as fast as possible.
16	X	-	-	0 °C	0 °C	Cooling, rapid weighting.

Table 5.1continued

Laboratory number	Sample storage temperature			Sample handling temperature		Special measures during sample handling
	< 0 °C	0-10 °C	> 10 °C	Homogenisation	Sub-sampling	
17	X	-	-	room temperature	chilled before opening vial	All equipment that will come into contact with samples/standard spikes should be chilled at 3 °C before use. This includes headspace vials, syringes, spiking standards, deionised water for dilution and samples. Always ensure headspace vials are crimped tightly (cap should not twist on vial once crimped).
18	X	-	-	ice bath	4 °C	Cooling of sample.
19	X	-	-	4 °C	4 °C	Thawing of sample in an ice bath (approx. 4 hours), keeping the sample, vials, NaCl solution and gastight syringes at 4 °C, standard solution addition to the sample through the septum of the vial.
20	X	-	-	0 °C	0 °C	Constant temperature (0 °C) during handling (weighing), using chilled syringes, headspace vials, chemicals, and new septum (not pierced).
21	X	-	-	<10 °C	< 10 °C	The vial was put into the ice bath.
22	X	-	-	4 °C	4 °C	None

Table 5.2: Sample homogenisation methods ("X" means applicable, "-" not applicable)

Laboratory number #	Sample homogenisation method					
	Stirring	Shaking	Ultra Turrax	Blender	Other techniques	No homogenisation
1	X	X	-	-	-	-
2		X	-	-	-	-
3	X	X	-	-	-	-
4	X	X	-	-	-	-
5		X	-	-	-	-
6	X	X	-	-	-	-
7		X	-	-	-	-
8	X	X	-	-	-	-
9		X	-	-	-	-
10	X	-	-	-	-	-
11	-	X	-	-	-	-
12	X	X	-	-	-	-
13	X	X	-	-	-	-
14	X	X	-	-	-	-
15	-	-	-	-	X	-
16	X	-	-	-	-	-
17	-	X	-	-	-	-
18		-	X	X	-	-
19	X	-	-	-	-	-
20	X	-	-	-	-	-
21	X	-	-	-	-	-
22	-	-	-	-	X	-

Table 5.3: Calibration methods ("X" means applicable, "-" not applicable)

Laboratory number		Calibration method		
	Matrix matched standards	External calibration	Standard addition	Internal standardisation
1	-	X	-	-
2	-	-	-	X
3	X	-	X	-
4	X	-	X	-
5	-	-	-	X
6	-	-	-	X
7	-	-	-	X
8	-	-	-	X
9	-	-	-	X
10	X	-	-	X
11	X	-	X	-
12	-	-	-	X
13	-	-	X	-
14	X	-	-	X
15	X	-	-	X
16	X	-	X	-
17	-	-	-	X
18	-	-	-	X
19	X	-	X	-
20	X	-	-	X
21	X	-	-	X
22	-	-	X	-

Table 5.4: Extraction method and GC-MS parameters ("X" means applicable, "-" not applicable)

Laboratory number	Extraction method						GC-MS parameters	
	Static HS-GC-MS	HS-SPME-GC-MS	SPME fibre type	Other method	Equilibration /Extraction time (min)	Equilibration temp (°C)	Column type	Column dimensions (l (m) x i.d. (mm) x df (µm))
1	X	-	-	-	25	50 °C	HP PLOT Q	15 m x 0.32 mm
2	X	-	-	-	30	50 °C	HP PLOT Q	15 m x 0.32 mm
3	X	-	-	-	30	50 °C	HP-PLOT Q	15 m x 0.32 mm
4	X	-	-	-	25	60 °C	HP PLOT Q	15 m x 0.32 mm
5	X	-	-	-	30	50 °C	HP-PLOT Q	15 m x 0.32 mm
6	X	-	-	-	30	50 °C	Varian CP-PoraBOND Q, 5µm	25 m x 0.32 mm
7	X	-	-	-	30	50 °C	Supelco Q Plot	30 m x 0.32 mm
8	-	X	CAR/PDMS 75 µm Carboxen/Polydimethyl siloxane fiber	-	5/20	30 °C	BPX-VOLATILES (SGE Europe, Villebon, France) 1.4µm film thickness	60 m x 0.25 mm
9	-	X	65 µm PDMS/DVB	-	15/5	35 °C	DB wax	60 m x 0.25 mm
10	X	-	-	-	30	60 °C	DB 23 (50%cyanopropyl) Methyl polysiloxano	60 m x 0.25 mm
11	X	-	-	-	30	50 °C	HP PLOT Q	25 m x 0.32 mm
12		X	CAR/PDMS 75 µm (Supelco)	-	20	45 °C	HP-PLOT/Q (J & W Scientific)	30 m x 0.32 mm
13	-	X	Carboxen-PDMS	-	26	4 °C	PoraBond Q	25 m x 0.32 mm
14	X	-	-	-	5	60 °C	SPB 624 1.4µm	30 m x 0.25 mm
15	X	-	-	-	30	50 °C	SP SIL 88 chemical bonded	100 m x 0,25 mm
16	X	-	-	-	30	50 °C	Varian PoraBOND Q	25 m x 0.32 mm
17	X	-	-	-	20	35 °C	Heliflex Capillary AT-Q (Alltech, Deerfield, IL)	30 m x 0,32 mm
18	X	-	-	-	30	50 °C	CP-SIL 13CB (Varian)	50 m x 0.25 mm
19	-	X	PDMS/CX/DVB	-	10/5	50/50 °C	VOCOL	60m x 0.32 mm
20	-	X	CAR/PDMS	-	15	50 °C	VOCOL	60 m x 0.32 mm
21	-	X	50/30 µm DVB/CAR/PDMS 23GA	-	5/15	50 °C	SPB 624	30 m x 0.25 mm
22	X	-	-	-	30	50 °C	HP PLOT Q	27.5 m x 0.32 mm

Table 5.5: Method performance ("X" means applicable, "-" not applicable)

Laboratory number		Method performance data			
	Number of analysed samples per month	LOD	LOQ	Lower limit	Upper limit
1	from 10 to 50	2µg/kg	5 µg/kg	4 µg/kg	83 µg/kg
2	<10	0.002 mg/kg	0.005 mg/kg	-	-
3	<10	1.3 µg/kg	4.8 µg/kg	4 µg/kg	50 µg/kg
4	<10	2µg/kg	4 µg/kg	-	-
5	<10	-	25 µg/kg	25 µg/kg	1500 µg/kg
6	<10	not established	not established	10 ng/vial	1000 µg/vial
7	from 10 to 50	0.5 µg/kg	2 µg/kg	2 µg/kg	500 µg/kg
8	>50	0.01 µg/kg	0.03 µg/kg	0.03 µg/kg	6 µg/kg
9	<10	5 µg/kg	10 µg/kg	n/a	n/a
10	<10	2 ng/g	4 ng/g	4 ng/g	122 ng/g
11	<10	2 µg/kg	3 µg/kg	2 µg/kg	200 µg/kg
12	<10	1,9 µg/kg	4 µg/kg	2 µg/kg	94 µg/kg
13	from 10 to 50	0.1 µg/kg	0.5 µg/kg	0.5 µg/kg	150 µg/kg
14	<10	0.1 µg/kg	5 µg/kg	5 µg/kg	200 µg/kg
15	<10	5 µg/kg	10 µg/kg	10 µg/kg	40 µg/kg
16	<10	2µg/kg	7 µg/kg	0.95 µg/kg	19 µg/kg
17	from 10 to 50	0.07 ng/g	0.2 ng/g	2 µg/L	200 µg/L
18	from 10 to 50	0.5 µg/kg	1 µg/kg	0.5 ng/ml	25 ng/ml
19	from 10 to 50	0.1 µg/kg	0.3 µg/kg	0.3 µg/kg	500 µg/kg
20	<10	2µg/kg	5 µg/kg	2 µg/kg	5000 µg/kg
21	<10	2 µg/kg	6 µg/kg	2 µg/kg	25.3 µg/kg
22	from 10 to 50	0.6 µg/kg	1.9 µg/kg	1.9 µg/kg	6500 µg/kg

European Commission

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Title: **Proficiency test on the determination of furan in baby food**

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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 for Health and Consumers (DG SANCO) to organise a proficiency test on the determination of furan in baby food in 2008. The aim of the proficiency testing (PT) was to support the implementation of Commission Recommendation 2007/196/EC which recommends Member States to monitor the presence of furan in foodstuffs that have undergone heat treatment.

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 test material was a commercial baby food consisting of carrot and potato puree.

Altogether 25 laboratories from 16 EU Member States subscribed for participation in the study, which was free of charge for the participants. They were asked to determine the furan content in the test samples by application of their usual in-house analytical methods. The laboratories were requested to report the results via a web-interface into a secured database.

In total, 22 data sets were reported to the organisers of the study. Details regarding the applied analytical methods were requested from the participants too.

The assigned value for the furan content of the test material was established by an isotope dilution GC-MS method. The target standard deviation was calculated according to the Thompson-modified Horwitz equation. The performance of laboratories was expressed by z-scores, which are considered satisfactory if $|z| \leq 2$. 16 out of 22 (73%) participants reported results that were rated satisfactory. Six laboratories (27 %) reported results $|z| > 2$. In two cases, underperforming might be due to calculation mistakes, since the reported values deviated from the assigned value by more than a factor of 10. The complexity of the food matrix, the high volatility of furan, and the application of improper methods might have contributed as well.

The results from the PT:

- demonstrate that the majority of the laboratories participating in the PT are capable of analysing furan in food matrices;
- confirm that laboratories with more experience in analysing furan on a regular basis perform better than those less experienced (cf. z-scores);
- show that both types of methods: the static headspace extraction gas chromatography mass spectrometry (HS-GC-MS) and the headspace solid phase micro-extraction gas chromatography mass spectrometry (HS-SPME-GC-MS) are equally suitable for analysis of furan in food matrices.

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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