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Methods for the determination of furan in food

Outcome of a survey conducted among EU food control laboratories

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Rationale

Commission Recommendation 2007/196/EC of 28 March 2007 [1] recommends Member States to perform monitoring on the presence of furan in foodstuffs that have undergone heat treatment. The data collected will be used for a risk assessment by the European Food Safety Authority (EFSA) at a later stage. During the discussions on the Commission Recommendation, Member States have emphasised that the analytical determination of furan remains difficult due to the volatility of this substance.

In response to this, the Institute for Reference Materials which is part of the European Commission's Joint Research Centre (JRC-IRMM) conducted a survey among European food control laboratories on analytical methods applied for the determination of furan in food. The survey was conducted in order to evaluate comparability of the analysis protocols, to highlight potential pitfalls and to provide support to laboratories that are new in that field.

General

Physico-chemical properties and safety considerations of furan

Furan is highly toxic by inhalation, ingestion and through skin contact. It is classified as potentially carcinogenic to humans [2]. It may be fatal if swallowed, inhaled, or absorbed through skin. It irritates skin and eyes.

Personal protective equipment such as safety glasses, gloves, and laboratory coats shall be used when handling furan.

CAS No:	110-00-9
Appearance:	colourless liquid
Melting point:	-85.6 °C
Boiling point:	31.4 °C
Density:	0.936 g/mL at 25 °C
Vapour pressure:	493 mm Hg at 20 °C; 1672 mm Hg at 55 °C
Vapour density:	2.35 (air = 1)
Flash point:	-35 °C
Explosion limits:	2.3% - 14.3%
Molecular Weight:	68.07 g/mole
Solubility	Easily soluble in acetone. Soluble in methanol, diethyl ether. Very slightly soluble in cold water.

Results of the questionnaire

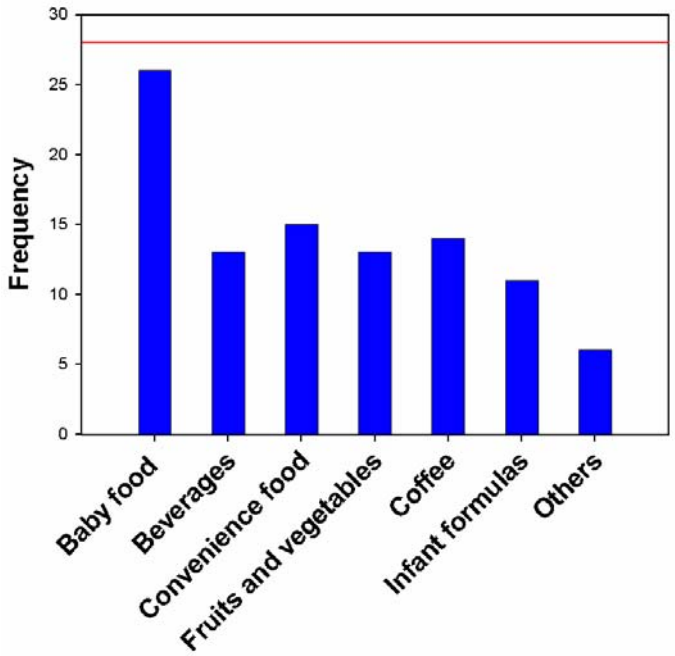
Overview on data providers and food matrices covered

The JRC-IRMM prepared a questionnaire on details of methods of analysis applied for the determination of furan in food. The questionnaire was distributed by DG SANCO end of June 2007 to the EU Member States. Upon request by the participants, the initial deadline for submission of the questionnaires was prolonged until beginning of November 2007.

Information on analytical methods for the determination of furan in food was provided by 28 laboratories from 12 EU Member States. They reported details of 35 analysis methods applied for a very narrow to a broad range of different types of foods. Figure 1 shows the different matrices tested routinely by the laboratories surveyed in this study. Most laboratories are investigating the furan content of baby food. Other food categories are analysed in about half of the laboratories responding to this questionnaire.

Food commodities such as malt, cacao, plum butter, potato crisps/chips, bread, and rusk are investigated by only a few laboratories.

Figure 1: Number of laboratories that investigate the furan content of specific types of food. The coloured horizontal line indicates the total number of laboratories that participated in the survey.



Details of the respective methods are presented and discussed in the following sections.

Sample storage, and test sample preparation

Due to the high volatility of furan, proper handling and storage of samples is crucial for obtaining reliable data on the furan content of food. Laboratories that participated in the survey mainly store samples between 0 °C and 10 °C, although some keep them below 0 °C. In the latter case increased attention has to be paid to sample homogeneity after thawing of samples. The thawing of samples should be performed under controlled conditions at low temperature (e.g. in an ice bath). Sample storage above 10 °C was hardly reported.

Sample preparation too needs a lot of attention, since furan might be lost during sample handling. However, only two laboratories reported analysis of baby food samples without prior sample homogenisation. All other laboratories apply one or the other homogenisation technique, which is of course adjusted to the properties of the investigated food matrix. Laboratory blenders are most frequently employed, followed by homogenisation using an ultra turrax. For samples with low viscosity shaking, stirring, or mixing with a vortex mixer might be sufficient. The US Food and Drug Administration (US FDA) proposes in its web-published method of analysis different strategies for sample homogenisation depending on the physical state of the sample and other specific properties such as the fat content [3].

However, all analysis procedures agree in the conditions that are applied during sample handling. The surveyed laboratories pointed out that samples need to be chilled during sample handling and that sample handling has to be done as quickly as possible. This is also highlighted in the FDA method. Many laboratories keep the samples in an ice bath during homogenisation and sub-sampling. This procedure is also successfully applied in our own laboratories where we focus on the determination of furan in baby food. Sample homogeneity of such pulpy matrices can be reached by mixing with an ultra turrax for only a few seconds. The mixing rod shall be chilled for this operation. Cooled stainless steel Dewar vessels are applied if composite samples from different baby food containers have to be produced. Several food control laboratories investigating the furan content of solid samples add dry ice for cooling during grinding of samples with a food processor. Becalski et al. blend chilled food with cold water and an aliquot of the internal standard for one minute prior to sub-sampling [4].

To summarise this section:

- Samples shall be stored refrigerated or frozen. Thawing of frozen samples shall be performed at low temperatures, best in an ice bath or in the refrigerator.
- Homogenisation of samples is done by applying appropriate techniques keeping samples and equipment at temperatures below 10 °C.
- All sample manipulations shall be organised in a way that handling time is minimised.

Standard preparation and calibration

Standard stock solutions and working standard solutions shall be prepared in closed systems. A short outline of a frequently applied method for the preparation of furan standard stock solutions comprises the pipetting of twenty millilitres of methanol into a tared headspace vial, which is then tightly sealed with a PTFE coated septum and an aluminium crimp cap. Furan is added to the solvent with a chilled gastight syringe through the septum of the headspace vial. Both the amount of solvent and amount of furan added are determined by differential weighing and the furan concentration is calculated from the respective masses. The same procedure is applied for the preparation of the stock solution of the labelled internal standard ($^2H_4$-furan). Of particular importance is that the headspace in the vials containing the standard solutions is kept as small as possible, in order to keep partitioning of furan into the gas phase low. Details on the preparation procedure and on standard concentrations have been published [3]. The shelf life of the standard stock solutions is quite short due to the volatility of furan. Becalski et al. recommend to store standard stock solutions for no longer than four weeks. The US FDA limits shelf life to even two weeks [3, 4]. The standard stock solutions are pipetted with chilled gastight syringes through the septa in order to avoid losses of furan to the surrounding atmosphere. However, it is strongly recommended to replace pierced septa daily [3, 4].

Working standard solutions are prepared from the standard stock solutions applying the same procedure as for the standard stock solutions, with the exception of using water as the solvent instead of methanol. Working standards have to be prepared on a daily basis.

The applied calibration methods are less uniform. Many laboratories seem to follow the US FDA approach which is based on standard addition. However, labelled furan ($^2H_4$-furan) is added to the test portion prior to equilibration in the US FDA method too, which is obviously used to compensate for variations in instrument response. Hence, analysis results are calculated according to the standard addition method with the speciality that the peak area ratio native furan / labelled furan is plotted against the spiking level. The furan content of the native test sample is determined by extrapolating the linear relationship between the peak area ratios and the amount of spiked furan towards a peak area ratio of zero. The amounts of native furan and labelled furan that are added to in total seven test portions of a particular sample is adjusted to the furan content of the native sample, which has to be roughly estimated in one preceding analysis. Twelve out of 34 protocols reported are based on that principle. Conventional standard addition without addition of labelled standards to the test portion is described in one single method.

Fifteen analysis protocols apply calibration by internal standardisation, without matching the matrix of the calibration standards with that of the test samples. Matrix matched standards in combination with internal standardisation is employed in 6 reported methods.

Hence matrix effects on the analysis results are considered in 20 of 35 reported methods, either via standard addition or by matrix matched calibration standards.

A significant decrease of the furan response of aqueous standard solutions after addition of a low percentage of corn oil to the standards was reported by Becalski et al [4]. This indicates that matrix effects could severely bias analysis results, if not taken into account. However, there are food samples where matrix matched calibration and non-matrix matched calibration (with aqueous standards) do not show statistically significant differences, thus allowing conventional calibration by internal standardisation, which is more time efficient than matrix matched calibration. Information on food matrices that might fall under one or the other category is omitted intentionally, since this could lead to misinterpretations. The influence of the matrix on the analysis results has to be evaluated on a case-by-case basis.

- In merging the information reported by the surveyed laboratories with that given in recent publications it can be concluded and recommended that standards shall be frequently prepared from scratch, always applying closed systems and low temperature.
- Attention has to be paid to avoid losses of the target analytes by evaporation.
- Matrix effects have to be taken into account. Absence of matrix effects shall be proven when non-matrix matched calibration is intended to be applied.

Preparation of samples for analysis

An aliquot of the cooled test sample is weighed into an empty chilled headspace vial. The great majority of laboratories use a test portion size of five grams for analysis. However a range between 0.5 and 10 grams was reported. Most laboratories add to the test portion between 5 and 10 millilitres of water. Water is replaced by a sodium chloride solution or a strongly alkaline solution (e.g. sodium hydroxide solution) (to eliminate precursors of furan) in a small number of laboratories.

The addition of water reduces the viscosity of many samples, which is favourable for equilibration, and changes the gas phase to sample phase ratio. Sodium chloride solution (proposed by US FDA) as well as sodium sulphate solutions might reduce the solubility of furan in the aqueous phase and hence lead to more intensive signals. This was reported by Becalski et al., who experienced an increase of the furan signal intensity by a factor of 1.5 when adding sodium sulphate to aqueous standard solutions [4]. We made ourselves similar observations when we tested the influence of water, saline and strongly alkaline solutions on the headspace extraction of furan from baby food samples. Both the saline and

alkaline solutions gave higher signal intensities compared to water. However the relative signal intensities of furan and labelled furan did not statistically significantly change.

Depending on the analysis procedure, labelled, or both labelled and native furan standard solutions are added to the samples. A majority of the 28 questioned laboratories add furan at this stage of the sample preparation, directly to the test portion. The two other laboratories add the labelled standards already prior to sample homogenisation. However the content of labelled furan added to the test portions varies depending on the respective laboratory between 10 µg/kg and 500 µg/kg. The US FDA recommends adjusting the content of labelled furan to the content of native furan in the test sample.

Native furan has to be added to the test sample when applying the standard addition approach. Most laboratories seem to follow the concept of US FDA which specifies the highest spiking level to two times the furan content of the native test sample.

One general remark concerns the handling of standard solutions. Some laboratories indicated the handling of very low standard solution volumes, e.g. of one microlitre, in the preparation of the samples for analysis. Taking into account the volatility of both the solvent and the analyte (labelled as well as native furan), it should be pointed out that handling of such small volumes could easily lead to severe bias. In this particular case, gravimetrical evaluation of the added amount of analyte by differential weighing is not anymore meaningful since the mass of the handled stock standard solution is less than one milligram and the total mass of the test portion (water, and headspace vial) amounts to about 30 grams. Much more convenient and much less prone to error is the handling of at least fifty µL volumes for the addition of native and/or labelled standard solutions, which can easily be weighed on an analytical balance.

Summary of the preparation of the sample for analysis:

- Keeping the sample temperature as low as possible during sample handling is of utmost importance to limit losses of the analyte by evaporation as much as possible. Hence sub-sampling and preparation of the samples for headspace analysis has to be fast, well designed and optimised with respect to time.
- Most laboratories add water or a saline solution to the samples to modify sample viscosity and the phase ratio.
- Almost all laboratories compensate variations of the analyte signal by the addition of labelled furan to the samples. In that respect it shall be highlighted that the amount of labelled standard shall be adjusted to the amount of native furan in the sample.

Measurement method

Twenty-six out of twenty-eight laboratories apply static headspace extraction combined with gas chromatography mass spectrometry (HS-GC-MS) for the determination of furan in food. One laboratory employs a static headspace extraction system with integrated trap (HS-trap) for analyte enrichment and another laboratory uses headspace-solid phase micro extraction (HS-SPME) for the isolation of the analyte from the food matrix, followed in both cases by gas chromatographic mass spectrometric detection. The equilibration parameters vary between (minimum) 10 minutes at 30 °C and (maximum) 30 minutes at 90 °C. The majority of laboratories apply about 30 minutes equilibration time and 50 °C to 60 °C as equilibration temperature. The effects of equilibration conditions on the results of analysis were studied by several groups.

Senyuva and Gökmen investigated the formation of furan during sample equilibration [5]. They observed an exponential increase of furan response with increasing equilibration temperature, concluding that a matrix matched calibration curve shall be prepared for each particular food sample. US FDA confirmed the findings and consequently modified its analysis method, recommending an equilibration temperature of 60 °C instead of 80 °C in the updated version [6]. Hasnip et al. also studied the formation of furan during sample incubation and found 50 °C applicable for a broad variety of food matrices [7]. The formation of furan was not detected at that temperature.

Lower incubation temperatures are not only beneficial with regard to artefact formation; they also prevent co-extraction of large amounts of less volatile substances. The required time for reaching equilibrium at a given equilibration temperature depends very much on the food matrix and the size of the test portion. Hence suitable equilibration conditions need to be identified for each type of food individually.

The headspace extraction parameters of the HS-trap-GC-MS methods are more or less in line with the parameters applied by the other laboratories. The shortest incubation time was specified for the HS-SPME-GC-MS method, which employs a divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS) fibre for the extraction of furan from the headspace. However, it is not know in particular which food commodity was analysed with these parameter settings, and whether or not the analysis was based on the equilibrium between the three phases.

Porous layer open tubular (PLOT) GC columns are most frequently used for the separation of furan from co-extractives (in 22 of 28 laboratories). The column length of the applied PLOT columns is typically 15 meters, but also longer columns are used. Some laboratories apply instead of PLOT columns capillaries that were tailor made for EPA method 624, which deals with the analysis of purgeable organics from water. Stationary phases based on phenyl-methylpolysiloxane only are rarely used for the determination of furan from food.

The mass spectrometers are commonly operated in selected ion monitoring (SIM) mode, focusing on the mass charge ratios (m/z) of 39 and 68 for furan, and 42 and 72 for d4-furan. A minority of laboratories measures furan in scan mode, covering the m/z range of 35 to 100. However, most of these laboratories quantify furan based on single m/z ratio peaks that are extracted from the scan spectrum.

Data on the working range, limit of detection (LOD) and limit of quantification (LOQ) are given for information only. The working ranges of the reported methods greatly vary. The smallest value for the lower level of the working range was specified to 0.1 µg/kg, whereas the highest value for the upper level of the working range was 6000 µg/kg. The broadness of the working range very much depending on the range of food products covered. Similarly diverging data were reported for LOD and LOQ. However, the working range as well as LOD and LOQ need to be put into perspective with regard to the scope of the analysis method. The values for LOD and LOQ might be additionally influenced by applying different estimation methods. Therefore, they are hardly applicable for judging the quality of a particular analysis procedure. Nevertheless they are listed in the Annex for information purposes.

Two to three replicate determinations are usually performed per test sample. Howsoever, up to ten replicate analyses per test sample were reported.

Repeatability of the reported analysis methods varied to a large extent. The laboratories reported repeatability relative standard deviations in the range between 1 % and more than 40 %. The repeatability values of 90 to 110 %, which were reported for method 14, are exceptionally high and might be confused with recovery values. The majority of laboratories stated repeatability relative standard deviations in the range of 5 % to about 15 %, which is in line with our own experiences. High repeatability standard deviations might be caused either by insufficient homogeneity of the test sample or analyte losses during sample preparation. In either case it is advisable to reconsider the sample preparation procedure. The number of replicate analyses should be increased when high repeatability standard deviations cannot be avoided due to e.g. a difficult sample matrix, in order to increase confidence in the analysis result.

Quality control

Only a few laboratories use quality control (QC) materials for monitoring of the stability of the analytical process. They consist of either spiked or naturally contaminated food samples, or standard solutions. The furan content level of the applied quality controls materials is different in all laboratories, which is not an issue, since the only requirement on the QC material with regard to analyte content level is that it is representative for the analyte content range of the analysed food samples and that the QC material used is homogeneous and stable over the time it is in use. The matrix of the QC material ideally agrees with the matrix of the investigated food samples. However the number of QC materials, their matrix composition, and the analyte content level needs to be balanced if a broad range of food matrices has to be covered. The availability of a suitable certified reference material would be desirable (as expressed by one respondent), but will probably be impeded by the volatility of furan.

Nevertheless, experiments in our laboratory revealed that the production of QC materials which are stable for at least eight weeks is feasible. Such material enables the analyst to monitor the stability of the analysis method at least throughout temporarily limited analysis campaigns. The respective QC material consisted of five jars of commercial carrot baby food that were pooled, homogenized and immediately filled in 22 mL headspace vials. The vials were filled up to the rim, and tightly sealed with PTFE-coated septa and aluminium crimp caps. They were stored at -20 °C and unfrozen prior to subsampling in an ice bath. This thawing procedure consumes some time, but has the advantage that the cold chain is not interrupted.

Proficiency tests (PT) are organised by commercial PT providers on a regular basis. Twenty eight laboratories reported results in a PT that was conducted in 2007, of which nineteen were rated satisfactory [8]. Eight of nine laboratories that were outside the satisfactory range reported analysis results which were too low, which underpins the importance of careful sample handling and thorough monitoring of the stability of method performance.

Summary

This report summarises details of 35 methods of analysis for the determination of furan in food as applied in 28 European food control laboratories. Information retrieved from recent scientific publications as well as own experiences were merged with the surveyed method details.

At first sight, the analysis methods for the determination of furan from food seem to be quite trivial. However, in practise many questions related to sample handling need to be addressed in order to avoid losses of the analyte. Most important is that the cold chain is not broken, meaning that samples are kept cooled throughout the whole sample preparation process. Standard preparation needs to be well designed as well. Errors in standard and sample preparation are more likely if small volumes/quantities are handled. Gravimetric control of pipetted standard volumes is advisable. The shelf life of standard preparations is in general low. Stock standard solutions were reported to be stable for two weeks, whereas working standard solutions need to be prepared daily.

The majority of laboratories apply static headspace extraction GC-MS for the determination of furan in food. Very few analysis procedures are based on headspace-solid phase micro extraction GC-MS. However, there is no indication that one or the other method would lead to biased results. From a recently conducted proficiency test it can be concluded that both analysis principles give satisfactory results, if applied correctly.

In that respect quality control of the analysis procedure is very important. To monitor stability of the method performance laboratories shall identify and prepare suitable quality control materials that shall then be included in the analysis sequences.

Additionally participation in proficiency tests is considered vital to demonstrate the agreement of own analysis results with results from other laboratories.

Definitions:

Base peak (BP): Peak in the mass spectrum with the greatest intensity [9].

Qualifier ion (QI): Ion monitored in the mass spectra for identification purpose.

Laboratory sample: The sample or subsample(s) received by the laboratory [9].

Test sample: Laboratory sample ready to take test portion for analysis.

Test portion: The amount of test sample taken for analysis [9]

References

[1] OJ L 88, 29.3.2007, p. 56

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

[3] US FDA: http://www.cfsan.fda.gov/~dms/furan.html

[4] Becalski A, Forsyth D, Casey V, Lau BPY, Pepper K, Seaman S (2005) Food Addit Contam 22:535-540

[5] Şenyuva HZ, Gökmen V (2005) Food Addit Contam 22:1198-1202

[6] US FDA: http://www.cfsan.fda.gov/~dms/furan2.html

[7] Hasnip S, Crews C, Castle L (2006) Food Addit Contam 23:219-227

[8] FAPAS® Report on Proficiency Test 3017, 2007

[9] International Union of Pure and Applied Chemistry (IUPAC) Compendium of Chemical Terminology, Electronic version, http://goldbook.iupac.org/S05451.html

Annex 1: Details of the particular method

Table 1: Scope of the method ("X" means applicable, "-" not applicable)

Method #	Food matrices covered							
	Baby food	Beverages	Convenience food	Fruits and vegetables	Coffee	Infant formulas	Others	Others specification
1	-	-	-	-	X	-	-	brewed
2	-	-	-	-	-	-	X	Bread
3	-	-	-	-	X	-	-	Coffee powder
4	X	X	X	X	-	-	-	
5	X	-	X	X	X	X	-	
6	X	-	-	X	-	X	-	
7	X	X	X	X	X	-	-	
8	X	-	-	-	-	-	-	
9	X	X	X	X	X	X	-	
10	-	-	-	-	X	-	-	
11	X	-	X	-	-	-	-	
12	X	X	-	-	-	X	-	
13	X	X	-	X	X	-	-	
14	X	X	-	X	X	-	X*	
15	X	-	-	-	-	-	-	
16	X	-	X	X	-	X	-	
17	X	-	X	-	X	-	-	
18	X	X	X	X	-	X	-	
19	X	X	X	-	-	X	X	Crisps
20	X	-	X	-	-	-	-	
21	X	-	-	-	-	-	-	
22	-	-	-	-	X	-	-	
23	X	X	-	-	X	X	-	
24	X	X	X	X	X	X	-	
25	-	X	-	-	-	X	-	
26	X	-	-	-	-	-	-	
27	X	X	X	X	X	-	-	
28	X	-	-	-	-	-	-	
29	X	-	-	-	-	-	-	
30	X	X	X	X	X	X	X	All foods
31	-	-	-	-	X	-	X	
32	-	-	-	-	-	-	X	Potato crisps/chips
33	X	-	X	X	-	-	-	
34	-	-	-	-	-	-	-	Soy sauce
35	X	-	X	-	-	-	-	

* tea, beer, malt, cacao, alcoholic beverages, plum butter, chips, bread, zwieback, coca cola, coffee-milk

Table 2: Sample storage temperature and sample homogenisation ("X" means applicable, "-" not applicable)

Method #	Sample storage temperature			Sample handling temperature		Special measures during sample handling	
	0°C	0-10°C	> 10°C	Homogenisation	Subsampling	Special measures	Specification special measures
1	-	-	X	0°C	0°C	-	
2	X	-	-	4°C to 8°C	4°C to 8°C	-	
3	-	-	X	4°C to 8°C	4°C to 8°C	-	
4	X	X	X	4°C to 8°C	4°C to 8°C	-	
5	-	X	-	0°C to 10°C	0°C to 10°C	X	Handle sample cold (between 0°C and 10°C).
6	X	-	-	below 0°C	below 0°C	X	Ice bath.
7	-	-	X	4°C	chilled	X	Sample is chilled before opening according to the USFDA method June 2005.
8	-	X	-	0°C	0°C	-	
9	-	X	-	-4°C	6°C	X	Samples which are homogenised will be kept in freezer for several hours before homogenisation. Sample vials are sealed immediately after weighing.
10	-	X	-	< 10°C	< 10°C	-	
11	-	X	-	4°C	4°C	X	Samples cooled with ice.
12	-	X	-	ca 3°C to 10°C	ca 3°C to 10°C	X	The sample homogenisation is done on an ice bath.
13	-	X	-	0°C	0°C	-	
14	X	X	-	0°C - 10°C	0°C - 10°C	X	Sample is kept cool.
15	-	-	X	5°C to 8°C	5°C to 8°C	X	Time for sample handling is kept as short as possible.
16	-	-	X	< 0°C	< 0°C	X	Handing of samples as cool as possible.
17	-	X	-	< 10°C	not specified	X	Keep time between homogenisation and closing of the headspace vial short (about 10 min).
18	X	-	-	ca. 8°C	ca. 8°C	X	Keep the food samples cool and work quickly.
19	X	-	-	-78°C	-18°C	X	All used materials are cooled prior to use.
20	-	X	-	4°C to 8°C	4°C to 8°C	-	
21	-	X	-	8°C	0°C	-	
22	X	-	-	-20°C	0°C	X	After preparation of the sample with a coffeemaker, the sample is chilled rapidly with ice water.
23	-	X	-	< 10°C	< 10°C	-	
24	-	X	-	4°C	4°C	X	Food container is refrigerated for at least 4 hours prior to homogenisation. The contents are transferred to a beaker immersed in an ice bath and then homogenised as quickly as possible. The sample is kept chilled until sealed in the headspace vial
25	-	X	-	25°C	25°C	-	
26	-	X	-	< 4	< 4	-	
27	X	-	-	4°C	4°C	X	The samples are filled in vials on an ice bath.

Table 2 continued

Method #	Sample storage temperature			Sample handling temperature		Special measures during sample handling	
	0°C	0-10°C	> 10°C	Homogenisation	Subsampling	Special measures	Specification special measures
28	-	X	-	0°C	0-4ºC	X	Application of an ice bath.
29	-	X	-	0°C	5ºC	X	The samples were handled on an ice bath.
30	X	-	-	0°C - 4°C	0°C - 4°C	X	Liquid samples are homogenised shortly (30 secs) on an ice bath. They are weighed after cooling in an ice bath. Solid samples are mixed and blended at temperatures below 4 °C. Where possible samples are milled in dry ice and weighed frozen.
31	-	-	X	< 4°C	> 20°C	X	All equipment that come into contact with the sample/standards is chilled to <4°C before use e.g. syringes, vials, water for homogenisation/dilution.
32	-	-	X	< 4°C	> 20°C	X	
33	-	-	X	< 4°C	> 20°C	X	
34	-	-	X	< 4°C	> 20°C	X	
35	-	X	-	4°C	4°C	-	

Table 3: Sample homogenisation and sample composition ("X" means applicable, "-" not applicable)

Method #	Sample Homogenisation						Sample composition		
	Stirring	Shaking	Ultra Turrax	Blender	Other techniques	No homogenisation	Sample intake	Solvent addition	Solvent added
1	-	-	-	-	-	X	10 ml	-	
2	-	-	-	X	-	-	2 g	X	Water
3	X	-	-	-	-	-	0.5 g	X	Water
4	X	-	-	X	-	-	5 g	X	Water
5	X	-	-	-	-	-	1g - 5g	X	Water and internal standard solution
6	-	-	-	X	-	-	5g	X	Water and saturated NaCl solution if necessary
7	X	-	-	-	-	-	5g	-	
8	-	-	-	X	-	-	10g	X	10 ml of 20 % NaCl in water and internal standard (170 µl in water)
9	X	-	-	X	-	-	1.5g - 10g	X	In case of a sample with high fat content saturated NaCl solution is used, otherwise water is used for diluting.
10	-	-	-	-	Lab. mixer Grindomix	-	5g	X	NaCl-solution
11	-	-	X	-	-	-	5g	X	NaCl-solution
12	-	X	X	-	-	-	5g	X	40 µl internal standard solution and 5 ml water
13	-	-	-	X	-	-	0.5g - 5g	X	Water as solvent, internal standard Furan-d4
14	-	-	X	X	mill	-	1g	X	Sample + water, ->pH 10 with KOH
15	X	-	-	-	-	-	5g	X	Water (5 ml) with ISTD
16	-	-	-	X	-	-	5g	X	Distilled (purified) water
17	-	-	X	-	-	-	5g	X	Furan-d4 solution
18	X	X	X	X	-	-	5g	X	5 ml of water, 40 µl of internal standard solution
19	-	-	-	X	with dry ice	-	1g	X	Furan-d4 and water
20	X	X	-	X	-	-	5g	X	5.0 ml water as solvent, eliminate precursors: add NaOH (pH~10)
21	X	-	-	-	-	-	5g	X	Water, Internal standard Furan-d4, Furan standard solution (Standard-Addition)
22	-	-	-	X	-	-	0.5g	X	Sample is in "water"
23	-	-	-	-	Technique depends on sample matrix	-	5g	X	Water in case test material is not homogenised.
24	-	-	-	X	-	-	5g	X	Water
25	-	X	-	-	-	-	10g	-	
26	-	X	X	-	-	-	5g - 10g	X	5 mL water
27	-	-	X	-	-	-	5g	X	Water purified by water purification system (Millipore)

Table 3 continued

Method #	Sample Homogenisation						Composition of test sample		
	Stirring	Shaking	Ultra Turrax	Blender	Other techniques	No homogenisation	Sample intake	Solvent addition	Solvent added
28	-	-	-	-	-	X	5g	X	Water
29	-	-	-	-	-	X	5g	X	5g water
30	-	-	X	X	-	-	5g	X	Internal standard and spike solutions
31	-	-	-	-	Beans ground. Ground samples vortex mixed<4°C	-	2g	X	Water
32	-	-	-	X	Vortex mixing	-	1g - 5g	X	Water
33	-	-	-	X	Vortex mixing of blended samples in sealed HS vials	-	5g	X	Water
34	-	-	-	-	Vortex mixing	-	5g	X	Water
35	-	-	X	X	-	-	5g	X	ISTD in Methanol, water

Table 4: Calibration and quantitation ("X" means applicable, "-" not applicable)

Method #		Calibration method			Details on internal standard				Solvent for standard preparation
	Matrix matched standards	External calibration	Standard addition	Internal standardisation	Internal standard	Amount ISTD	Addition prior homog.	Addition after homog.	Solvent for standard preparation
1	-	-	-	X	Furan-d4	250 ug/l	-	X	Methanol, water
2	-	-	-	X	Furan-d4	1250 µg/kg	-	X	Methanol, water
3	-	-	-	X	Furan-d4	5000 µg/kg	-	X	Methanol, water
4	-	-	-	X	Furan-d4	500 µg/kg	-	X	Methanol, water
5	X	-	X	X	Furan-d4	60 µg/kg	-	X	Methanol
6	-	-	-	X	Furan-d4	40 ul of 5 µg/ml furan-d4	-	X	Stock standard solution-methanol (HPLC grade), working standard solution-water (HPLC grade)
7	-	-	X	X	Furan-d4	20 µg/kg	-	X	Methanol
8	-	-	-	X	Furan-d4	50,541 µg/kg	-	X	Water
9	-	X	-	X	Furan-d4	11,6 µg/kg	-	X	Methanol, water
10	X	-	X	X	Furan-d4	0,24 µg/kg	-	X	Methanol
11	X	-	X	X	Furan-d4	0,24 µg/kg	-	X	Methanol
12	X	-	X	X	Furan-d4	40µl of a solution of 5 ng/µl ==> 40 µg/kg	-	X	Stock solution: methanol Working solutions: the stock solution is diluted with water
13	X	-	X	X	Furan-d4	10 µg/kg	-	X	Water
14	-	-	X	X	Furan-d4	variable, dependent on the content of furan in the sample	-	X	Methanol diluted with water
15	-	-	X	X	Furan-d4	20 µg/kg	-	X	Methanol, water
16	X	-	-	X	Furan-d4	30 µg/kg	-	X	Methanol
17	-	-	-	X	Furan-d4	120 µg/kg	-	X	Stock solution in methanol, standards diluted in water
18	X	-	X	X	furan-d4 (working solution in water)	approx. twice the expected concentration of furan	-	X	Methanol (stock solution), water (working solution)
19	X	-	-	X	Furan-d4	200 µg/kg	-	X	Methanol, water
20	X	-	X	X	Furan-d4	working IS concentration at the estimate of furan	-	X	5,0 ml water
21	X	-	X	X	Furan-d4	ca. 45 µg/kg	-	X	Methanol and water

Table 4 continued

Method #		Calibration method			Details on internal standard				Solvent for standard preparation
	Matrix matched standards	External calibration	Standard addition	Internal standardisation	Internal standard	Amount ISTD	Addition prior homog.	Addition after homog.	
22	X	X	X	-	Furan-d4	not specified	-	-	Methanol
23	-	X	-	X	Furan-d4	1000-1300 µg/kg	-	X	Methanol in the fist dilution step, then water for further dilutions
24	-	X	-	X	Furan-d4	50 µg/kg	-	X	Water
25	X	X	-	X	Furan-d4	5,0 µg/kg	-	X	Methanol
26	X	-	-	X	Furan-d4	2 Xo (estimated concentration by preliminary analysis)	-	X	Methanol
27	X	-	-	X	Furan-d4	1ul from 2,38 mg/ml	X	-	Methanol
28	X	-	-	X	Furan-d4	50-60 µg/kg	X	-	Stock solutions in methanol, working solution in water
29	-	X	-	X	Furan-d4	30-50 µg/kg	-	X	Methanol stock solutions , water for working solutions
30	X	-	X	X	Furan-d4	Typically	-	X	Methanol
31	-	-	-	X	Furan-d4	250 µg/kg	-	X	Methanol used for stock and intermediate standards, water used for calibration standard solutions
32	-	-	-	X	Furan-d4	500 µg/kg	-	X	Methanol used for stock and intermediate standards, water used for calibration standard solutions
33	-	-	-	X	Furan-d4	100 µg/kg	-	X	Methanol used for stock and intermediate standards, water used for calibration standard solutions
34	-	-	-	X	Furan-d4	100 µg/kg	-	X	Methanol used for stock and intermediate standards, water used for calibration standard solutions
35	X	-	X	X	Furan-d4	25 µg/kg	-	X	Methanol, water

Table 5: Extraction method and GC-MS parameters ("X" means applicable, "-" not applicable)

Method #	Extraction method						GC-MS parameters		
	static HS-GC-MS	HS-SPME-GC-MS	SPME fibre type	Other method	Equilibration time (min)	Equilibration temp (°C)	Column type	Column dimensions (l (m) x i.d. (mm) x df (µm))	Recorded m/z ratios
1	X	-	-	-	30	50	HP-PLOT Q	15 m x 0.32 mm x 20 µm	68, 72
2	X	-	-	-	30	50	HP-PLOT Q	15 m x 0.32 mm x 20 µm	68, 72
3	X	-	-	-	30	50	HP-PLOT Q	15 m x 0.32 mm x 20 µm	68, 72
4	X	-	-	-	30	50	HP-PLOT Q	15 m x 0.32 mm x 20 µm	68, 72
5	-	-	-	Static headspace-trap GC-MS	40	80	BPX5	60 m x 0.25 mm x 1.0 µm	MS method: full scan between m/z 35 and 100, m/z used for quantification: 68 for furan, 72 for D4-furan
6	X	-	-	-	30	90	HP-PLOT Q	15 m x 0.32 mm x 20 µm	m/z 35 to 150
7	X	-	-	-	30	80	Vocol	60 m x 0.25 mm x 1.50 µm	
8	X	-	-	-	10	30	HP-PLOT Q	30 m x 0.32 mm x 20 µm	Furan: m/z 68 and 39; Furan-d4: 72 and 42
9	X	-	-	-	30	60	Supel-Q PLOT	30 m x 0.32 mm	m/z 39, m/z 68, m/z 42, m/z 72
10	X	-	-	-	30	80	HP-PLOT Q	15 m x 0.32 mm x 20 µm	Furan: 68-39-38 100%-83,9%-16,2% d4-Furan: 72
11	X	-	-	-	30	80	HP-PLOT Q	15 m x 0.32 mm x 20 µm	Furan: 68-39-38 100%-83,9%-16,2% Furan-d4: 72
12	X	-	-	-	30	50	HP-PLOT Q	30 m x 0.32 mm x 20 µm	Furan 68; 39; 29 Furan-d4 72; 42; 30
13	X	-	-	-	30	50	Varian PoraBond Q	25 m x 0.32 mm x 5 µm	Full Scan m/z=35-99, Quantification Mass Furan m/z=68, Furan-d4 m/z=72
14	X	-	-	-	30	50	HP-PLOT Q	30 m x 0.32 mm x 20 µm	Scan 35 – 75
15	X	-	-	-	30	50	Cpsil5CB-MS	60m x 0,32 mm x 1 µm	39 / 68 / 72
16	X	-	-	-	30	50	HP-PLOT Q	25 m x 0.32 mm x 0.2 µm	68, 72
17	X	-	-	-	5	60	Supelco SPB-624	30 m x 0,25 mm x 1,4 µm	m/z 35-75
18	X	-	-	-	30	50	HP-PLOT Q	15 m x 0.32 mm x 20 µm	Furan: m/z 68 and 39; Furan-d4: m/z 72 and 42
19	X	-	-	-	30	60	HP-PLOT Q	15m x 0.32 mm x 20 µm	68, 72

Table 5 continued

Method #	Extraction method						GC-MS parameters		
	static HS-GC-MS	HS-SPME-GC-MS	SPME fibre type	Other method	Equilibration time (min)	Equilibration temp (°C)	**Column type**	**Column dimensions (l (m) x i.d. (mm) x df (µm))**	**Recorded m/z ratios**
20	X	-	-	-	30	50	HP-PLOT Q	15 m x 0.32 mm x 20 µm	Furan: m/z 68, 39 Furan-d4: m/z 72, 42
21	X	-	-	-	20	not specified	HP-PLOT Q	30 m x 0.32 mm x 20 µm	Furan: 39, 68 Furan-d4: 72
22	-	-	-	-	20	40	Varian VFS 624 ms	60 m x 0.32 mm	m/z 68
23	-	-	-	HS-GC-MS with agitation (autosampler)	30	50	HP-PLOT Q	15 m x 0.32 mm x 20 µm	68, 72
24	X	-	-	-	25	50	HP-PLOT Q	15 m x 0.32 mm x 20 µm	m/z 68 (furan) and m/z 72 (furan-d4), m/z 39 (furan - qualifier ion)
25	X	-	-	-	30	60-80	HP - PLOT Q	15 m x 0.32 mm x 20 µm	m/z = 39 - 68 - 72
26	X	-	-	-	15	50	HP-PLOT Q and particle trap	0.32 mm	Furan: m/z 68, 39; Furan-d4: m/z 72, 42
27	X	-	-	-	30	80	Polystyrene/divinyl benzene	15 m x 0.32 mm x 20 µm	Furan m/z 68; Furan-d4 m/z 72
28	X	-	-	-	30	80	HP-PLOT-Q	15 m x 0.32 mm, 20 µm	68, 72
29	X	-	-	-	20	50	HP PLOT Q	15 m x 0.32 mm x 20 µm	Furan m/z 68, m/z 39. Furan-d4 m/z 72
30	X	-	-	-	20	not specified	Heliflex AT-Q	30 m x 0.32 mm	68 & 39 (Furan); 72 & 42 (Furan-d4)
31	X	-	-	-	20	not specified	Heliflex AT-Q	30 m x 0.32 mm	68 & 39 (Furan); 72 & 42 (Furan-d4)
32	X	-	-	-	20	not specified	Heliflex AT-Q	30 m x 0.32 mm	68 & 39 (Furan); 72 & 42 (Furan-d4)
33	X	-	-	-	20	not specified	Heliflex AT-Q	30 m x 0.32 mm	68 & 39 (Furan); 72 & 42 (Furan-d4)
34	X	-	-	-	30	60	HP-PLOT Q	15 m x 0.32 mm x 20 µm	Furan: m/z 39, 68; Furan-d4: m/z 42, 72
35	-	X	5-/3- um DVB/CAR/PDMS 23GA	-	5/15	60	SPB 624	30 m x 0.25mm x 1.4um	Furan: m/z 39, 68; Furan-d4: m/z 42, 72

Table 6: Method performance and quality control ("X" means applicable, "-" not applicable)

Method #		Method performance data			Quality control	
	Replicate analyses per sample	LOD	LOQ	Repeatability	Application of QC material	QC materials specifications
1	2	-	5 ug/l	3 %	-	
2	2	-	25 µg/kg	3 %	-	
3	2	-	100 µg/kg	3 %	-	
4	2	-	10 µg/kg	3 %	-	
5	2	3 µg/kg	6 µg/kg	10 %	X	1) % RSD between two duplicate analyses < 5%; - 2) Three standard additions < 10% or 15% (depending of concentration in sample)
6	3	5 µg/kg	10 µg/kg	12 % - 18 %	-	
7	2	2 µg/kg	7 µg/kg	10 %	-	Not yet, we have just begun the analysis and now we are going to order. There is a need for suitable CRM
8	2-3	1 µg/kg	3,3 µg/kg	At the level of 4,91 ppb CV% is 33,5 and 51,5 ppb CV% is 15,3 and 103 ppb CV% is 19,7	-	
9	2	0,5 µg/kg	2 µg/kg - 5 µg/kg	<10 %	X	The control sample is made in saturated NaCl solution or sample matrix. Concentration is 10 - 50 µg/kg.
10	2	0,1 µg/kg	0,3 µg/kg	16,4 %	-	
11	2	0,1 µg/kg	0,3 µg/kg	16,4 %	-	
12	2	1 µg/kg	3 µg/kg	1 % to 8 % (3 µg/kg to 1 µg/kg)	-	
13	1	2 µg/kg	7 µg/kg	-	-	
14	10	2 µg/kg	5 µg/kg	90- 110 %	X	Baby food, coffee, chips, condensed milk, external reference solution (from collaborative trial)
15	2	0,4 µg/kg	1 µg/kg	ca. 10 %	-	
16	3	2,0 µg/kg	3,0 µg/kg	15 %	X	Participation in FAPAS proficiency test; e.g. baby food, 25 µg/kg furan.
17	depends on relevance	0,1 µg/kg	5 µg/kg	10 %	-	
18	ca. 7	0,5 µg/kg	1,0 µg/kg	20 %	-	
19	3	0,5 µg/kg	1,0 µg/kg	-	-	
20	2	0,9 µg/kg	4,4 µg/kg	nb	-	
21	2 x 5	1 µg/kg	3 µg/kg	-	-	
22	1	-	-	15 %	-	
23	3	2 µg/kg	5 µg/kg	-	-	

Table 6 continued

Method #		Method performance data			Quality control	
	Replicate analyses per sample	LOD	LOQ	Repeatability	Application of QC material	QC materials specifications
24	A sample is analysed in duplicate with every batch run or with any new matrix or as necessary.	1 µg/kg	3 µg/kg	41.08 %	X	A spiked sample is analysed with every batch run or with any new matrix or as necessary. Spiking level is typically 50 µg/kg.
25	3	-	-	-	-	
26	3x	<3 µg/kg	<10 µg/kg	-	X	We used the standard addition in negative sample about 10 ng/ml
27	2	1,3 µg/kg	4 µg/kg	3,1 % - 11 % (100 µg/kg - 4 µg/kg)	-	
28	3	1,3 µg/kg	4 µg/kg	1,5 %	-	
29	2	1 µg/kg	2 µg/kg	7 %	X	Tomato sauce containing 25 µg/kg
30	2	<0.1 µg/kg	<1 µg/kg	9 %	-	None available
31	2	<0.1 µg/kg	<1 µg/kg	9 %	-	None available for crisps/chips
32	2	<0.1 µg/kg	<1 µg/kg	9 %	-	None available
33	2	<0.1 µg/kg	<1 µg/kg	9 %	-	None available
34	3	1.5 µg/kg	5 µg/kg	8.6 %	-	
35	3	0,15 µg/kg	20 µg/kg	15,0 %	-	Water

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Abstract
Commission Recommendation 2007/196/EC of 28 March 2007 recommends Member States to perform monitoring on the presence of furan in foodstuffs that have undergone heat treatment. The data collected will be used for a risk assessment by the European Food Safety Authority (EFSA) at a later stage. During the discussions on the Commission Recommendation, Member States have emphasised that the analytical determination of furan remains difficult due to the volatility of this substance.
In response to this, the Institute for Reference Materials which is part of the European Commission's Joint Research Centre (JRC-IRMM) conducted a survey among European food control laboratories on analytical methods applied for the determination of furan in food. The survey was conducted in order to evaluate comparability of the analysis protocols, to highlight potential pitfalls and to provide support to laboratories that are new in that field.
This report summarises the information supplied by 28 laboratories identifies common features and issues that need special attention.

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.