In-house validation of a simple headspace gas chromatography-mass spectrometry method for determination of furan levels in food

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Summary

A method for determination of furan using a headspace gas chromatography-mass spectrometry technique was validated for routine application in food control. Validation was performed by evaluating the following characteristics: precision, trueness, recovery, limit of detection, limit of quantification, operating range and calibration. Uncertainty statements obtained for tomato ketchup (limit of detection 0.9 μg.kg^{-1}; limit of quantification 2.9 μg.kg^{-1}; recovery 103, 107 and 115%, respectively; relative standard deviation 4, 5 and 8%, respectively) confirm that the mentioned method is suitable for determination of furan in this food matrix. The method was also used for determination of furan in such food as baby food, canned meat and vegetables, liquid seasoning, sauces and coffee. As found, levels ranged from lower than the detection limit up to 920 μg.kg^{-1}.

Keywords

furan; headspace; gas chromatography-mass spectrometry; validation; food contaminant; tomato ketchup

Furan and its derivatives were identified in a small number of heat-treated foods, such as coffee, canned meat, bread, cooked chicken, fish protein concentrate and caramel back in 60's and 70's [1, 2]. They belong to a great group of substances associated with the flavour of foods which are formed during the Maillard reactions [3]. In 2004, US Food and Drug Administration published a report on the occurrence of unsubstituted "parent" furan in a wide number of thermally treated foods [4]. After this announcement, the concern of many analytical laboratories has been addressed to the analysis of this substance classified as possibly carcinogenic to human (Group 2B) since 1995 under the classification of the International Agency for Research on Cancer (IARC) [5].

Due to the high volatility of furan, it is very important to find a suitable analytical methodology. Headspace sampling seems to be the most appropriate method for the analysis of very volatile compounds [6, 7]. This is a relatively simple and well-proven methodology in which a food sample in a liquid or slurry form is heated in a sealed vial to achieve equilibrium partition between the liquid phase and the gaseous headspace. The headspace gas is sampled and the vapour injected into a gas chromatograph. Detection can be performed by non-selective means such as flame ionization detector (FID) or by a selective detection such as mass spectrometry. A simple headspace method for determination of furan in food was developed by US FDA and described as follows: five grams of a test portion of semi-solid or solid foods are diluted with water, fortified with internal standard (d_4-furan), and sealed in a headspace vial. Similarly, ten grams of a test portion of liquid foods are fortified with d_4-furan and sealed in a headspace vial. Automated headspace sampling followed by gas chromatography-mass spectrometry (GC-MS) analysis is used to detect furan and d_4-furan in selected-ion monitoring mode (SIM). Furan is quantified using a standard additions curve, where the concentration of furan in the fortified test samples is plotted versus the furan/d_4-furan response factors using the following ions: m/z 68 and 39 for furan and m/z 72 and 42 for d_4-furan, respectively [8]. This method has the advantage that there is no need for sample purification and it can be automated for high sample throughput. However, impact of various food matrices has to be eliminated for a correct determination of such volatile compound with low mass number as furan is. Method validation is required for any new method which is intended for routine analysis to demonstrate that

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a defined method protocol, applicable to a specified type of test material and to a defined concentration range of the analyte, is fit for a particular analytical purpose [9].

For an accurate quantitation of furan, matrix-matched calibration curve should be prepared for each particular food sample being analysed applying the same headspace sampling conditions. In this study, method validation in tomato ketchup matrix is described. To validate the headspace-GC-MS method for determination of furan in food in our laboratory, we evaluated a series of method-performance characteristics, such as precision, trueness, recovery, limit of detection (*LOD*), limit of quantification (*LOQ*), operating range and calibration. Afterwards, we performed a screening determination of furan in foods with high consumption rates for adults and infants.

MATERIALS AND METHODS

Reagents

Methanol (HPLC grade) was obtained from Sigma Aldrich (Steinheim, Germany). Water was prepared by HP 340 Deionizer (Purite, Thame, Oxfordshire, United Kingdom). All other reagents were of analytical grade. Furan 99+% was from Fluka Chemie (Buchs, Switzerland) and d_4-furan, isotopic purity 98 atom % D, was from Sigma Aldrich.

Apparatus

- Headspace system - G1888 Network Headspace Sampler (Agilent Technologies, Santa Clara, California, USA);
- GC-MS system - AT 6890N gas chromatograph with a AT 5973 mass selective detector (Agilent Technologies);
- Column - capillary column 19091P-Q04 HP-PLOT Q, 30 m × 0,32 mm × 20 μm, with particle trap 5181-3351 (Agilent Technologies);
- Homogenizer - hand blender XB982, 200 W (Tesco Stores SR, made in China);
- Syringes - 10, 50, 100, 500 and 1000 μl gas-tight syringes (Hamilton, Bonaduz, Switzerland);
- Headspace vials – flat bottom, 20 ml, with silicon septa, aluminium crimp seals, hand crimper, and decapper (Agilent Technologies).

Operating Conditions

Headspace operating conditions - equilibration temperature 60 °C, equilibration time 15 min, shaken low, volume of headspace gas sampled 500 μl.

GC-MS operating conditions - carrier gas helium, constant flow 1.0 ml.min^{-1}, oven temperature profile: initial 50 °C (1 min), rate 10 °C.min^{-1} to 230 °C, hold 11 min; 150 °C injector; injection mode splitless, purge 0.25 min; MS ionization mode 200 eV EI+; source temperature 230 °C; scan mode: selected ion monitoring (SIM); ions (*m/z*): 39, 68 (furan), 42, 72 (d_4-furan); dwell time 100 ms each ion.

Standards

All stock furan and d_4-furan solutions were prepared in methanol and stored at –18 °C for no longer than four weeks. Preparation of stock solutions: By using a volumetric pipette, 20.0 ml of methanol were placed in a headspace vial and the vial was sealed. The sealed vial was weighed to the nearest 0.1 mg (*W1*). By using a chilled 50 μl syringe, 50 μl of furan or d_4-furan were transferred through the septum of the vial containing methanol and shaken vigorously. The sealed vial was reweighed to the nearest 0.1 mg (*W2*). *W1* was subtracted from *W2* to determine the weight of furan transferred (*W3*). The stock standard concentration equals *W3* divided by the total volume (20.05 ml).

Commercial food

Samples (jars, canned foods and coffee) for the study were purchased from retail food stores in the Bratislava area (Slovakia).

Preparation of the test portion

For liquids with high water content, test portions of 10 g were used. For semisolid foods, test portions of 5 g were used, each diluted with 5 g of water. For foods that are not homogeneous, such as beef goulash, samples were homogenized as follows: the unopened container was chilled at 4 °C in a refrigerator for approx. 4 h, the sample was transferred to a beaker immersed in an ice bath, and the sample was homogenized with a hand blender. Portions of 5 g of the homogenates were used diluted with 5 g of water. All samples were capped immediately with teflon-lined crimp seals.

Calibration standards and spiking experiments

Tomato ketchup was stirred at 35 °C for 1 h. After cooling, 5 g of the homogenates were placed in a vial, diluted with 5 g of water and spiked at 0.4, 1, 1.5, 2, 2.5, 5, 10, 50 and 100 ng levels with a native furan solution in methanol for matrix calibration standards, and at 10, 50 and 100 ng levels (10 replicates for all concentration levels) for spiking experiments. The internal standard (d_4-furan) concentration was 250 μg.kg^{-1}.

RESULTS AND DISCUSSION

Determination of *LOD* and *LOQ*

There is no term in analytical chemistry or parameter for which there is a greater variety in terminology than *LOD* and *LOQ*. In general, *LOD* is understood as the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value. *LOQ* then corresponds to the amount of analyte, which can be quantified with a variation coefficient not higher than 10% [10].

In this study, *LOD* and *LOQ* were determined using calibration standards in a tomato ketchup matrix with furan concentrations of 0.4, 1.5, 2, 2.5 and 5 μg.kg^{-1}. All data were subjected to linear regression analysis where x equals the amount [ng] of furan added to the test portion and y equals the integrated area for m/z 68 divided by m/z 72. Fig. 1 shows a calibration line. The slope (A) and intercept (B) of the calibration line were determined. Response ratio (RR) for 10 unspiked ketchup samples was measured. *LOD* and *LOQ* values were calculated on the basis of the standard deviation for RR (SD_{RR}) and the slope of the linear regression (A) as follows:

$$LOD\ [\mu g.kg^{-1}] = 3\frac{SD_{RR}}{A} = 3\frac{0.0012}{0.0041} = 0.9$$

$$LOQ\ [\mu g.kg^{-1}] = 10\frac{SD_{RR}}{A} = 10\frac{0.0012}{0.0041} = 2.9$$

Limit of detection and limit of quantification in the tomato ketchup matrix determined in our laboratory are comparable with literature. NYMAN et al. [11] reported *LOD* = 0.9 and *LOQ* = 2.9 μg.kg^{-1} for furan in peanut butter. *LOD* and *LOQ* values for furan in apple juice, chicken broth, infant formula and green beans determined by the same authors are equal to or slightly lower than *LOD* and *LOQ* values for furan in tomato ketchup.

Trueness

Trueness is expressed in terms of bias or percentages of error. Bias is the difference between the mean value determined for the analyte of interest and the accepted true value or known level actually present. It represents the systematic deviation of the measured result from the true result. Method trueness is also an indicator of utility and applicability of that method with real samples [12]. The easiest way to determine the trueness is analysing reference materials. If no reference materials are available, a blank sample matrix of interest can be spiked with a known amount of a pure and stable in-house material. Recovery is then calculated as the percentage of the measured spike in the matrix sample relative to the measured spike in the blank control, or the amount of spike added to the sample. The smaller is the recovery %, the larger the bias that is affecting the method and thus the lower the trueness.

For trueness determination, calibration standards in tomato ketchup with furan concentrations of 1.5, 2, 2.5, 5, 10, 50 and 100 μg.kg^{-1} were prepared and analysed. All data were subjected to linear regression analysis where x equals the amount (ng) of furan added to the test portions and y equals the integrated area for m/z 68 divided by m/z 72. Fig. 2 shows linear calibration curve for furan in tomato ketchup (R^2 = 0.999), which was used for calculation of recovery % at fortification levels of 10, 50 and 100 μg.kg^{-1}. All these values as well as standard deviation and relative standard deviation are summarized in Table 1.

Recovery of native furan after tomato ketchup spiking at 10, 50 and 100 μg.kg^{-1} was 107, 103 and 115%, respectively. These results are comparable with results reported by BECALSKI et al. [13], who

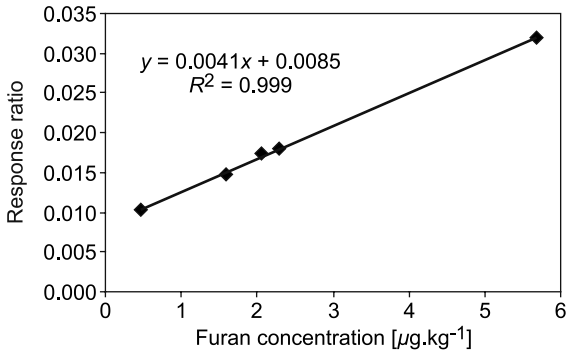

Fig. 1. Calibration curve for furan in tomato ketchup (furan concentration range 0.4–6.0 μg.kg^{-1}).

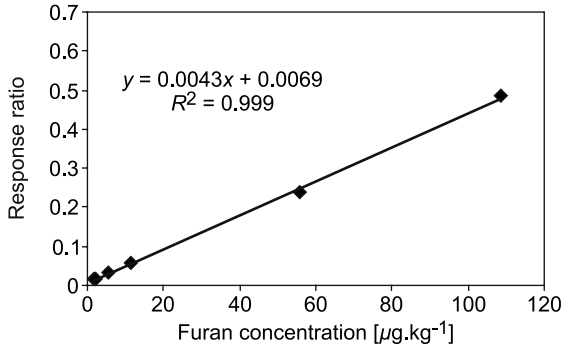

Fig. 2. Calibration curve for furan in tomato ketchup (furan concentration range 1.5–110 μg.kg^{-1}).

Tab. 1. Standard deviation (*SD*), relative standard deviation (*RSD*) and recoveries values obtained from analyses of tomato ketchup.

Fortification level [μg.kg^{-1}]	*SD*	*RSD* [%]	Recovery [%]
10	0.96	7.93	107
50	2.47	4.29	103
100	6.26	5.03	115

SD and percentage of *RSD* determined with 10 replicates.

determined recoveries of 110, 92 and 122%, respectively, for chicken broth (baby food) spiked at 25, 50 and 250 μg.kg^{-1}. The authors attributed this phenomenon to a slower equilibration and re-distribution of furan during homogenization of samples in a blender. This slower equilibration and the partitioning of furan between water and fat constituents of the sample combined with volatility of furan may impart errors of magnitude in the determination of furan in denser matrices [13]. However, certain levels of furan were found during the headspace analyses of raw foods [14] causing a suspicion that it is formed under mild thermal conditions. If furan is formed in the food matrix during headspace analysis, the past and the formed furan will be measured together during GC-MS analysis.

Determination of furan in food products

For a screening determination of furan in food, 15 food products with high consumption rates were analysed in duplicates, namely baby food, coffee, canned ready to eat meals, ketchup and seasonings. The results obtained are summarized in Table 2.

CONCLUSIONS

The results from the validation study demonstrate that the headspace-GC-MS method is fit for purpose for furan determination in thermally treated foods and show that the method will reliably detect and quantify furan in foods at μg.kg^{-1} levels. The method was used for screening determination of furan in food products commonly consumed in Slovakia. The furan levels found ranged from lower than the detection limit to 920 μg.kg^{-1}.

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Tab. 2. Furan concentrations in foods from the Slovak market.

Sample type	Sample description (country of origin)	Furan concentration [μg.kg^{-1}]
Baby food	Vegetable soup 1 (Czech Republic)	64.6
	Vegetable soup with chicken meat (Czech Republic)	28.5
	Vegetable soup 2 (Czech Republic)	20.7
	Vegetable soup with veal meat (Czech Republic)	16.4
Coffee	Coffee powder, 100% Arabica	918
	Coffee drink	86.7
Sauces	Ketchup (Czech Republic)	11.8
	Sauce with green pepper (Slovak Republic)	26.1
Liquid seasoning	Soya sauce (Slovak Republic)	122
	Hydrolysed vegetable protein (Slovak Republic)	below *LOQ*
Canned meat and vegetables	Beef goulash (Czech Republic)	78.2
	Sauce for pasta (Czech Republic)	59.2
	Pork goulash with sauerkraut (Slovak Republic)	36.8
	Baked beans with sausage (Czech Republic)	58.4
	Baked beans with sausage (Slovak Republic)	51.1

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