



RECYCLED PAPER MADE FROM 20% POST CONSUMER CONTENT



**U.S. Food and Drug Administration****CENTER FOR FOOD SAFETY AND APPLIED NUTRITION**

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CFSAN/Office of Plant and Dairy Foods

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Determination of Furan in Foods

1. SCOPE OF APPLICATION

This method is for the determination of furan in foods.

2. PRINCIPLE

Five gram test portions of liquid, semi-solid, or solid foods are diluted with water, fortified with internal standard (*d4*-furan), and sealed in headspace vials. Automated headspace sampling followed by gas chromatography/mass spectrometry (GC/MS) analysis is used to detect furan and *d4*-furan in the scan mode. Furan is quantified by using a standard additions curve, where the concentration of furan in the fortified test portions is plotted versus the furan/*d4*-furan response factors.

3. REAGENTS

High purity standards and analytical grade reagents should be used.

- 3.1 Furan minimum purity 99% (Aldrich, CAS# 110-00-9, density = 0.936 g/mL). Store in -20°C freezer.
- 3.2 *d4*-Furan minimum purity 99% (Aldrich, CAS# 6142-90-1, density = 0.991 g/mL). Store in -20°C freezer.
- 3.3 Water, HPLC grade or purified by water purification system such as Milli-Q
- 3.4 Methanol, HPLC grade

4. STANDARDS

4.1 Preparation of Furan Stock Standard (*ca.* 2.50 mg/mL): By using a volumetric pipet, place 20.0 mL methanol in a headspace vial and seal the vial. Weigh the sealed vial (W1) to the nearest 0.1 mg. By using a chilled 50 μ L syringe, transfer 50 μ L of furan through the septum of the vial containing the methanol and shake vigorously or vortex. Reweigh the sealed vial and record the weight (W2) to the nearest 0.1 mg. Subtract W1 from W2 to determine the weight of furan transferred (W3). The stock standard concentration equals W3 divided by the total volume (20.05 mL).

The stock standard should be stored in a 4°C refrigerator to minimize loss of furan by evaporation. Once the septum on the stock standard has been pierced, it should be replaced daily. The furan stock standard is stable for two weeks.

4.2 Preparation of Furan Working Standard (*ca.* 30.9 µg/mL): By using a syringe, transfer 250 µL of the *ca.* 2.50 mg/ml furan stock standard to a sealed headspace vial containing 20 mL water and shake vigorously. The working standard concentration equals *ca.* 625 ng divided by 20.25 mL. Prepare daily.

Note: A *ca.* 30.9 µg/mL furan working standard may not always be appropriate; the concentration may need to be adjusted depending on the target quantitation level.

4.3 Preparation of *d4*-Furan Stock Internal Standard (IS, *ca.* 2.50 mg/mL): By using a volumetric pipet, place 20.0 mL methanol in a headspace vial and seal the vial. Weigh the sealed vial (W1) to the nearest 0.1 mg. By using a chilled 50 µL syringe, transfer 50 µL of *d4*-furan through the septum of the vial containing the methanol and shake vigorously or vortex. Reweigh the sealed vial and record the weight (W2) to the nearest 0.1 mg. Subtract W1 from W2 to determine the weight of *d4*-furan transferred (W3). The stock standard concentration equals W3 divided by the total volume (20.05 mL).

The IS should be stored in a 4°C refrigerator to minimize loss of *d4*-furan by evaporation. Once the septum on the IS has been pierced, it should be replaced daily. The *d4*-furan IS is stable for two weeks.

4.4 Preparation of *d4*-Furan Working IS (*ca.* 30.9 µg/mL): By using a syringe, transfer 250 µL of the *ca.* 2.50 mg/ml *d4*-furan stock standard to a sealed headspace vial containing 20.0 mL water and shake vigorously or vortex. The concentration equals *ca.* 625 ng divided by 20.25 mL. Prepare daily.

Note: A *ca.* 30.9 µg/mL *d4*-furan working IS may not always be appropriate and may need to be adjusted depending on the target quantitation level.

5. PREPARATION OF TEST PORTIONS

Quantification is based on a standard additions curve. The amounts of furan and/or *d4*-furan added to the test portions are based on an estimate (x^0) of the furan concentration in the food.

5.1 Estimate of x^0 : A one-point estimate of the concentration of furan in the food can be made by diluting a 5 g test portion of the food with 5 mL water, fortifying with working IS, and analyzing by headspace GC/MS as described in Section 6. By using the integrated response ratio for furan/*d4*-furan (*m/z* 68/72) and the ng amount of IS, the ng amount of furan in the food can be estimated.

5.2 Homogenization of Foods: For foods that are not homogenous, chill the entire container of food in a refrigerator for *ca.* 4 hr. Transfer contents to a beaker immersed in an ice bath. Homogenize using a tissumizer or similar benchtop homogenizer. Quickly transfer test portions to tared headspace vials. To avoid loss

of furan, it is essential that the homogenization be done as quickly as possible, and that the sample be kept chilled until test portions are transferred to headspace vials.

5.3 Preparation of Test Portions for Standard Additions Based on Internal Standard: Transfer seven 5.00 g portions of food to tared headspace vials. Dilute each test portion with 5 mL water. Fortify all seven vials with *d4*-furan working IS at *ca.* x^0 . Seal the first three vials. Fortify the remaining four vials with furan working standard as follows: two vials at *ca.* $0.5x^0$, one vial at *ca.* x^0 , and one vial at *ca.* $2x^0$.

5.4 Example of Standard Addition Fortification: If the estimated concentration of furan in the food, x^0 , is *ca.* 20 ng/g:

5.4.1 Prepare furan and *d4*-furan working standards at *ca.* 5 $\mu\text{g/mL}$ by transferring, with a 50 μL syringe, 40 μL of *ca.* 2.50 mg/mL stock standards into individual sealed headspace vials containing 20.0 mL of water. The resulting furan and *d4*-furan working standard concentrations equal *ca.* 100 ng divided by 20.04 mL.

5.4.2 Accurately weigh 5.00 g portions of the food into seven headspace vials and add 5 mL water. Using two 50 μL syringes, fortify each vial with the furan and *d4*-furan working standards as indicated in the following table:

5 g test portion	μL 5 $\mu\text{g/mL}$ furan	μL 5 $\mu\text{g/mL}$ <i>d4</i> -furan	ppb furan added
$0x^0$	-	40	0
$0x^0$	-	40	0
$0x^0$	-	40	0
$0.5x^0$	10	40	10
$0.5x^0$	10	40	10
$1x^0$	20	40	20
$2x^0$	40	40	40

6. APPARATUS

6.1 Refrigerator at 4°C.

6.2 Freezer at -20°C.

6.3 Top pan balance capable of weighing to nearest 0.01 g.

- 6.4 Analytical balance capable of weighing to nearest 0.1 mg.
- 6.5 Dynamic headspace autosampler (Perkin Elmer Turbo Matrix 40, or equivalent).
- 6.6 GC/MS (Agilent 6890N GC with Agilent 5973N MSD, or equivalent).
- 6.7 GC column: HP-Plot Q, 15 m, 0.32 mm I.D., 20 μ m film.
- 6.8 20 mL headspace vials with aluminum crimp seals and Teflon-faced silicon septa. Store vials in 90°C forced-air oven until ready for use.
- 6.9 Syringes (gas-tight syringes are recommended)
 - 6.9.1 Two, 50 μ L syringes
 - 6.9.2 Two, 100 μ L syringes
 - 6.9.3 One, 200 μ L syringe
 - 6.9.4 Two, 1 mL syringes
- 6.10 Hand crimper for sealing vials
- 6.11 Hand de-crimper for removing vial seals
- 6.12 Homogenizer equipped with 30 x 150 mm open slotted generator (Telemar Tissumizer or equivalent)

7. HEADSPACE GC/MS ANALYSIS

- 7.1 Headspace sampling
 - 7.1.1 Temperatures
 - Needle - 100°C
 - Transfer Line - 130°C
 - Oven - 80°C
 - 7.1.2 Timing
 - Injection - 0.2 minutes
 - Pressurization - 0.5 minutes
 - Withdrawal - 0.2 minutes
 - Thermo Equilibration - 30 minutes
 - Cycle Time - 48 minutes
 - 7.1.3 Options

Vial Vent - On
Water Trap - Off
Shaker - On
Operation Mode - Constant
Injection Mode - Time
Hi PSI Injection - On

7.1.4 Programmed Pneumatic Control

Inject - 20.0 psi
Column/Vial Head Pressure - 10.0 psi

7.2 GC Conditions

GC Oven - 50°C, 10°C/min to 225°C and hold 12.5 min. Run-time, 30 min.
GC Column flow, 1.7 mL/min helium (constant flow).
GC inlet temperature, 200°C.
Split ratio - 2:1
Gas saver - off.
Under these conditions, retention times of furan and *d4*-furan are *ca.* 6-7 min

7.3 MS parameters

MS source temperature - 230°C
MS quad temperature - 150°C
MS transfer line - 225°C
Scan range - m/z 25 to 150
Scan time - 2.5 to 25 min
Threshold - 100 counts
Samples - n=3
Scans/sec - 5.56

7.3.1 MS Confirmation of Furan

Determine the integrated response for m/z 39 and 68 for the test portions and calibration standards and calculate the response ratio of m/z 39 divided by m/z 68. The response ratio for the test portions should agree with the average of the response ratios for the calibration standards by ± 10 percent, and the retention time (RT) for the test portions should agree with the average RTs for the calibration standards by ± 2 percent.

8. CALCULATIONS

8.1 Determine the integrated responses for m/z 68 for furan and m/z 72 for *d4*-furan and calculate the response ratio, m/z 68 divided by m/z 72.

8.2 Standard Additions Calibration Curve with Internal Standard: For each sample, subject all test portions ($0x^0$) and calibration standards ($0.5 x^0$, $0.5 x^0$, x^0 ,

and $2x^0$) to a linear regression analysis where the x equals the ng amount of furan added to the test portion and the y equals the response ratios. Determine the slope and intercept of the calibration curve and solve for $0x^0$ at y equal to zero. Divide $0x^0$ by the test portion amount in grams to determine the ppb amount of furan in the sample.

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