APPLICATION NOTE

GC-Mass Spectrometry
and Headspace Sampling

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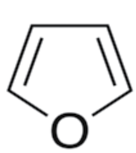
# Determination of Furan in Food by Gas Chromatography-Mass Spectrometry and Headspace Sampling

## Introduction

Furan is naturally occurring at low levels in many foods and drinks.[1] Furan consumption is of concern because it has been classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans, based on studies with laboratory animals. The U.S. FDA has recently published a report on the occurrence of furan in a large number of thermally processed foods, especially canned and jarred foods, including baby foods and infant formulas. The primary source of furan in food is considered to be thermal degradation of carbohydrates, such as glucose, lactose and fructose.

Of all the foods tested in various papers, coffee contained the largest amount of furans.[1] Furan is a colorless, volatile and lipophilic organic compound. It has a molecular weight of 68 and a low boiling point (31 °C). Due to its high volatility, furan levels in foods are easily determined, with high accuracy, by headspace methods.

This application note will demonstrate a rapid method for the identification and quantification of furan in food samples, using gas chromatography with headspace sampling and mass spectrometry. In addition to method optimization and standard analysis, we will analyze a number of food samples for furan. We chose to test coffee containing drinks, sauces, and canned foods, as previous studies demonstrated high levels of furan in these foods. The samples were randomly collected from the local market.



Synonyms: furfuran, oxole, tetrole, divinylene oxide, oxacyclopentadiene

Formula: $C_4H_4O$

MW: 68.07

MP: -85.6 ºC

BP: 31 ºC

*Figure 1.* Structure and physical properties of furan.

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## Experimental

The PerkinElmer® Clarus® 680 Gas Chromatograph, Clarus 600 C Mass Spectrometer and a TurboMatrix™ HS-40 system were used for this application. Table 1 presents the detailed operating parameters of the GC/MS and the HS system. The instrument interaction, data analysis and reporting was completed with the PerkinElmer TurboMass™ data system.

*Table 1.* **Detailed Instrument Conditions Used in the Determination of Furans.**

| | | |
|---|---|---|
| **Instrument Details:** | **Clarus 680 Gas Chromatograph** | |
| Analytical Column | PerkinElmer Elite™-624 N9316204 (60 meter, 0.32 mm i.d., 1.8 µm df) | |
| GC Column Flow | 1.4 mL/min helium at constant flow mode | |
| GC Inlet Temperature | 200 ˚C | |
| Split Ratio | 2:1 | |
| Oven Temperature Program | 40 ˚C hold for 6.0 min, 20 ˚C/min to 110 ˚C and hold for 1.0 min, 70 ˚C/min to 250 ˚C and hold for 3.5 min; runtime is 20 min | |
| **MS Parameters:** | **Clarus 600 C Mass Spectrometer** | |
| MS Source Temperature | 230 ˚C | |
| MS Interface Temperature | 225 ˚C | |
| Scan Range | m/z 35-150 | |
| Scan Time | 2.5-25 min | |
| Multiplier | 500 V | |
| Scans/Sec | 5.56 | |
| **Headspace Parameters:** | **TurboMatrix HS-40** | |
| Temperatures | Thermostatting Oven | 60 ˚C |
| | Needle | 100 ˚C |
| | Transfer Line | 130 ˚C |
| Time | Injection | 0.2 min |
| | Pressurization | 0.5 min |
| | Withdrawal | 0.2 min |
| | Equilibration | 20 min |
| | Cycle | 20 min |
| Options | Vial Vent | ON |
| | Shaker | ON |
| | Operation Mode | Constant |
| | Injection Mode | Time |
| | Hi Psi Injection | ON |
| PPC | Inject | 35 psi |
| | Column/ Headspace Pressure | 25 psi |

Headspace is a perfect technique for sample introduction in furan analysis due to the ease of sample preparation and the limited interaction of the instrumentation with the sample matrix. Caution must be taken when setting the vial oven temperature; a high temperature can result in furan formation in the sample during analysis. To reduce this risk the method presented here uses a low incubation temperature.

**Stock Solution:** A stock solution of 1000 µg/mL of furan and furan-$d_4$ was used as the starting point for all standard solutions (SPEX CertiPrep®).

**Standard Preparation:**

10 µL of the stock furan solution was diluted to 10 mL in methanol to give a solution of 1 µg/mL. 20 µL of the stock furan-$d_4$ solution was diluted to 10 mL in methanol to give a solution of 2 µg/mL.

**Calibration Curve:** The volume of 1 µg/mL furan was diluted in water to achieve the final standard concentration presented in Table 2. 100 µL of furan-$d_4$ from 2 µg/mL stock was added to each headspace vial containing 10 mL of water resulting in an internal standard concentration of 0.02 µg/mL (20 ppb). 4 g of NaCl was added to each of the vials to decrease the miscibility of furan in water.

**Preparation of Solutions:**

*Table 2.* **Scheme Used for the Creation of a Five Level Calibration.**

| Calibration Level No. | Concentration of Furan in ppb | Std Solution Added in µL | Final Vol. (mL) |
|---|---|---|---|
| 1 | 1 | 10 | 10 |
| 2 | 2 | 20 | 10 |
| 3 | 10 | 100 | 10 |
| 4 | 20 | 200 | 10 |
| 5 | 40 | 400 | 10 |

*4 gm of NaCl was added to each of the headspace vials.

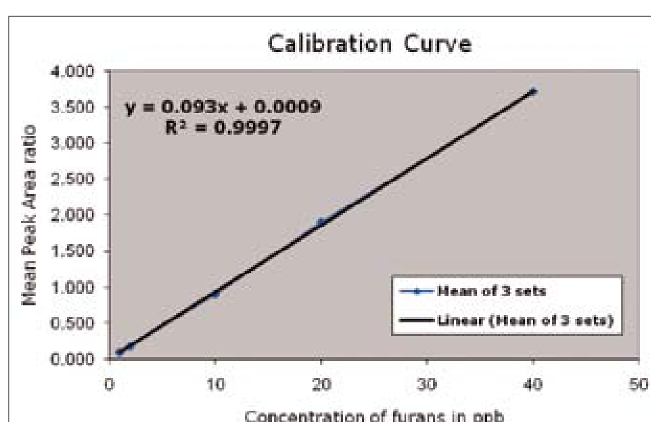


*Figure 2.* Calibration curve for furan.

**Calibration:** The MS was calibrated across the range of 1.0 to 40 ng/mL and each calibration point was run in triplicate to demonstrate the precision of the system. The average coefficient of determination for a line of linear regression was 0.9997 for furan. The calibration curve for furan is depicted in Figure 2.

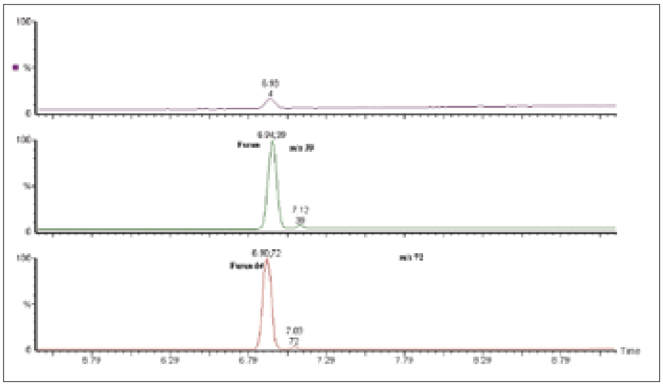

*Figure 3.* Example chromatogram of 40 ppb furan standard showing the total and extracted ion chromatograms as well as the extraction ion chromatogram for the furan-$d_4$ internal standard.

Also in Table 3 is the percent relative standard deviation (%RSD) for each calibration point (n=3). The precision of the system across the calibration range is excellent. The chromatograms and the spectrum from the analysis of standard material are shown in Figure 3.

***Table 3.*** **% RSD's for Three Sets of Linearity Experiment.**

| Sr. Number | Number of Levels | Mean Peak Area Average Relative Response (n=3) | %RSD |
|---|---|---|---|
| 1 | 1 | 0.098 | 10.046 |
| 2 | 2 | 0.184 | 8.012 |
| 3 | 10 | 0.904 | 1.475 |
| 4 | 20 | 1.900 | 0.435 |
| 5 | 40 | 3.709 | 1.627 |

The precision of the method was measured at both 0.5 and 1 ppb. The detection limit of this method is approximately 0.5 ppb (Table 4).

***Table 4.*** **RSD Values for Detection Limit and Quantification Level.**

| Sr. No. | Conc. of Furan in ppb | Furan/IS Area Ratio | Conc. of Furan in ppb | Furan/IS Area Ratio |
|---|---|---|---|---|
| 1 | 0.5 | 0.035 | 1 | 0.102 |
| 2 | 0.5 | 0.031 | 1 | 0.097 |
| 3 | 0.5 | 0.031 | 1 | 0.106 |
| 4 | 0.5 | 0.021 | 1 | 0.103 |
| 5 | 0.5 | 0.021 | 1 | 0.096 |
| 6 | 0.5 | 0.022 | 1 | 0.093 |
| **Mean** | | **0.03** | | **0.1** |
| **S.D.** | | **0.01** | | **0.0** |
| **%RSD** | | **23.75** | | **4.78** |

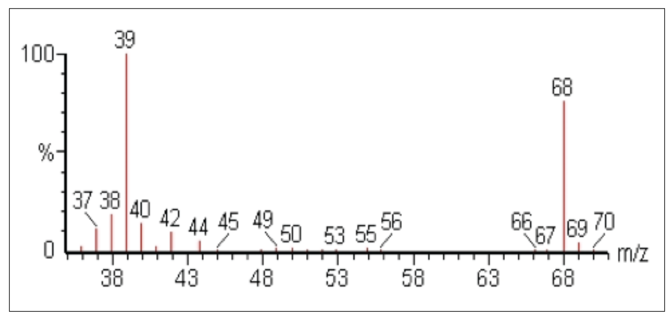


*Figure 4.* Full scan mass spectrum obtained experimentally for furan.

***Table 5.*** **Method Validation Summary.**

| | |
|---|---|
| Linearity: | 1.0 ppb to 40 ppb of furan |
| RSD for Replicate Analysis: | for 1.0 ppb 4.78% |
| Detection Level: | 0.5 ppb |
| Quantification Level: | 1.0 ppb |
| Recovery Study: | at three levels for all the samples within 80-120% |

**Sample Preparation:** Samples were collected from the local market. The samples included: coffee, milk, canned foods, sauces, peanut butter and apple juice (Table 6). All the samples were refrigerated before analysis. 10 mL of sample was transferred into a headspace vial; 4 g of NaCl was added to it. Milk and other viscous samples were diluted with water (1:2 or 1:4). The semi-solid samples were ground and 5 g of sample was added to headspace vials with 5 mL of saturated salt (NaCl) solution. Coffee powder was dissolved following directions on the package, and then treated like a non-viscous liquid sample.

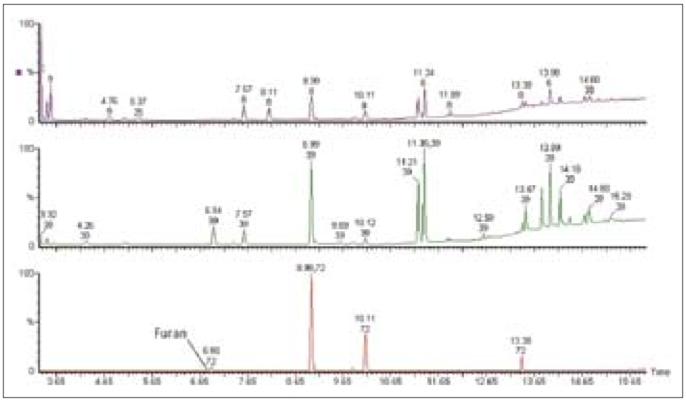


*Figure 5.* Experimental chromatogram from the analysis of espresso coffee with furan peak visible at 6.9 minutes.

***Table 6.* Sample Analysis Results.**

| Sample No. | Sample Details | Amt. of Furan Found in ppb |
|---|---|---|
| Sample 1 | Lab Coffee | 0.67 |
| Sample 2 | Chocolate Flavored Milk (AKCF) | 1.67 |
| Sample 3 | Espresso Coffee | 45.18 |
| Sample 4 | Coffee Flavored Milk (AKC) | 10.87 |
| Sample 5 | Cocoa Flavored Milk (AKK) | 1.76 |
| Sample 6 | Energy Drink (milk based) (NAEM) | 13.21 |
| Sample 7 | Brewed Coffee | 36.59 |
| Sample 8 | Filtered Coffee | 253.99 |

### Method Validation:

The recovery of the method was tested with the analysis of the brewed coffee sample spiked at three different levels: 2, 5, 10 µg/L. The measured amount was 2.03, 5.44, 9.54 µg/L demonstrating that the headspace technique is quantitative in its extraction of furan from an aqueous matrix.

## Results

Eight samples of common beverages were analyzed using the HS-GC/MS method developed here. The samples were chosen because they had been shown to have detectable levels of furan in the literature. Of the samples analyzed, brewed coffee was demonstrated to have the highest levels of furan, at 250 µg/L. The remaining sample results are demonstrated in Table 6.

## Conclusion

This application presents a method for the determination of furans in beverages using headspace sample introduction. Headspace GC is fast, reliable and can be used for the quantification of furans in common beverages. The internal standard calibration of furan across 1-40 µg/L responded linearly. Beverages were analyzed and the level of furan determined. The furan was identified by both the retention time and the MS fragmentation pattern. The method was validated at several levels and coffee matrix recovery values were between 95-101%.

## References


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